Emulsion Encapsulation of *Bifidobacterium animalis* subsp. *lactis* BB12 with the Addition of Lecithin

Ivana LISOVÁ[1], Šárka HORÁČKOVÁ[2], Renata KOVÁČOVÁ[2], Vojtěch RADA[3] and Milada PLOCKOVÁ[2]

[1]Dairy Research Institute Ltd., Prague, Czech Republic; [2]Department of Milk, Fat and Cosmetic Science, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Prague, Czech Republic; [3]Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

Abstract

Lisová I., Horáčková Š., Kováčová R., Rada V., Plocková M. (2013): **Emulsion encapsulation of *Bifidobacterium animalis* subsp. *lactis* Bb12 with the addition of lecithin**. Czech J. Food Sci., **31**: 270–274.

The commercial probiotic strain *Bifidobacterium animalis* subsp. *lactis* Bb12 was encapsulated using emulsion encapsulation into milk protein matrix without and with the addition of 0.5% w/w lecithin into the oil. Different agitation speeds were used during the encapsulation process. The examination of microcapsules was carried out by optical microscope and fluorescence *in situ* hybridisation. The particle size distribution as volume based median $d_{0.5}$ was evaluated by the laser diffraction method. In the case of no lecithin addition, the agitation speed did not influence significantly the size of the microcapsules. The addition of 0.5% (w/w) of lecithin into the oil caused a decrease of $d_{0.5}$ value from 196 ± 37 μm to 79 ± 3 μm at an agitation speed of 500 rpm, and from 193 ± 24 μm to 39 ± 3 μm at 1200 rpm. It can improve the sensory properties of the products with the added microcapsules.

Keywords: microencapsulation; bifidobacteria; emulsifier; partical size distribution

The research on probiotic microorganisms and their application to different types of products have become one of the trends of recent years. Previously, probiotic microorganisms were only included in dairy products and food supplements. The possibilities of probiotics application in a larger number of products such as fruit juices, cereals and non-food products for preventive and also therapeutic purposes are now fully developed (Heller 2001; Crittenden 2009). The application of probiotics in sufficient quantities is important for positive probiotic effects in the gastrointestinal tract of humans. Products containing probiotic microorganisms should include 10^6 probiotic viable cells per 1 g at the time of expiration in fermented dairy products (Picot & Lacroix 2004). The viability of probiotic cells is reduced by a number of factors during the production and storage of the products, and also in the gastrointestinal tract of the consumer. These factors include low pH, high temperature, aerobic environment, concentrated bile salts, digestive enzymes, and others. The encapsulation of probiotic microorganisms is one way to protect the cells from these stress factors (Adhikari *et al.* 2000; Gbassi *et al.* 2009). Nevertheless, some authors did not find any protective effect of encapsulation against gastric juice for encapsulated cells (Brinques & Ayub 2011).

Supported by the Ministry of Agriculture of the Czech Republic, Program VAK 2011 and Project No. QI111B053.

The encapsulation was developed from the immobilisation technology of cell cultures used by the biotechnology industry (Heidebach *et al.* 2009). The process of encapsulation is a physico-chemical or mechanical process that leads to the production of particles ranging in size from several nanometres to several millimetres (Burgain *et al.* 2011). Materials such as alginate, κ-carrageenan, chitosan, starch, gelatine, or milk proteins are primarily used to encapsulate probiotic cells (Picot & Lacroix 2004). Calcium alginate is most often used for the encapsulation of probiotics as it is nontoxic, biocompatible, and inexpensive (Burgain *et al.* 2011). However, its disadvantage is its low resistance to acidic pH and high porosity which can be compensated by combining with other polymers such as starch (Hansen *et al.* 2002; Vidhyalakshmi *et al*. 2009). Natural κ-carrageenan is another commonly used polymer in the food industry. The main disadvantage of carrageenan microcapsules is their fragility (Burgain *et al.* 2011). Resistant starch, which is not split by pancreatic enzymes in the small intestine and which allows the release of probiotic cells in the large intestine, is also used for encapsulation (Charalampopoulos *et al.* 2002). Milk proteins possess good structural and physico-chemical properties (low viscosity, nondescript flavour, ability to form gels) which promote them as ideal encapsulation matrices (Doherty *et al.* 2011). Emulsion, extrusion, and spray drying are the most commonly used techniques for the microencapsulation of probiotics (Heidebach *et al.* 2009; Burgain *et al.* 2011).

Emulsion encapsulation of probiotic cells into a milk protein matrix was successfully carried out in the past by Heidebach *et al.* (2009). It is based on the principle of cold renneting of milk containing the probiotic cells, followed by emulsification with oil. The method was later modified by replacing rennet with transglutaminase which allows cross-linking between milk proteins (Heidebach *et al.* 2010).

The aim of this study was to modify the above mentioned method by the addition of lecithin and to evaluate the effect of the lecithin added as well as the influence of the speed of agitation during the procedure on the capsule size and size distribution.

MATERIAL AND METHODS

Microorganisms. Freeze-dried commercial probiotic strain *Bifidobacterium animalis* subsp. *lactis* Bb12 from Christian Hansen (Hoersholm, Denmark) was used in this study.

Encapsulation procedure. The strain was encapsulated using the method of Heidebach *et al.* (2009) with some modification. Briefly, 30 g of 35% (w/w) reconstituted skimmed milk (skimmed-milk powder Laktino (PROMIL, Nový Bydžov, Czech Republic) was mixed with 1 g of lyophilised probiotic culture. After 15 min of agitation, 400 µl of rennet Naturen™ PREMIM 145 (Christian Hansen, Nienburg, Germany), diluted with distilled water in ratio 1:4 (w/w), was added. The mixture was stirred at a speed of 500 rpm at 5°C for 1 h and subsequently 180 µl of 10% (w/w) $CaCl_2$ was added. Then, the whole mixture was transferred to 150 ml of sunflower oil (Vegetol; Setuza, Ústí nad Labem, Czech Republic) at 5°C without the addition of soya lecithin or with the addition of 0.5, 1.0, 5.0% (w/w) of soya lecithin SOLEC F-10 (Solae, Le Grand-Saconnex, Switzerland) into the resulting mixture while the agitation speed was adjusted to 500 rpm or 1200 rpm. After 5 min agitation, the mixture was heated to 40°C and the aggregation and formation of a gel matrix were observed. The encapsulated cells were separated from the oil by gentle centrifugation (500 *g*, 2 min, 4°C), washed by distilled water, and stored in refrigerator at 4°C for further analysis. A scheme of the encapsulation procedure is shown in Figure 1.

Optical examination. Both native and fixed preparations were used to check the encapsulated cells by optical microscope (Leica MD LF; Leica Camera AG, Solms, Germany). Furthermore, the fluorescence hybridisation *in situ* was used to visualise the encapsulated cells by an epifluorescence microscope Nikon E800 (Nikon, Tokyo, Japan)

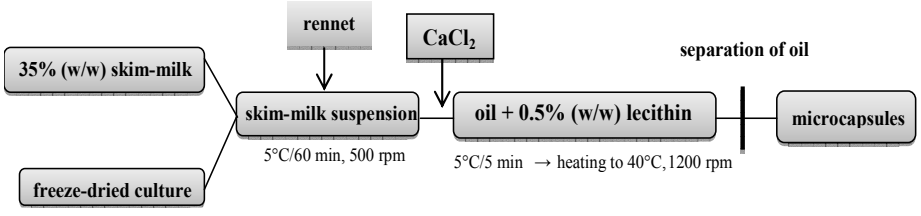

Figure 1. Scheme of emulsion encapsulation of probiotic microorganism with the addition of lecithin

with the filter 495/520 nm excitation/emission. To bind the fluorescent probe, FISH-kit™ for *Bifidobacterium* spp. stained with FITC (Ribo-Technologies, Groningen, Netherlands) was used.

Particle size determination. Laser diffraction, based on the optical properties of dispersions, was used to determine the size of the microcapsules. The capsules were re-suspended in demineralised water with the refractive index 1.33 and the mean size distribution was measured using Mastersizer 2000 with the dispersion unit Hydro G (both Malvern, Worcestershire, UK). A refractive index of 1.45 and absorption coefficient of 0.001 were selected for a sample of microcapsules. The measurement was performed at 25°C. The results are the means from two independent encapsulation procedures, both parallel encapsulated cell samples were analysed three times. The mean of volume based median ($d_{0.5}$) (i.e. 50% of total volume is composed of microcapsules with diameters equal or lower than $d_{0.5}$), and 90% fractiles ($d_{0.9}$) were calculated.

RESULTS AND DISCUSSION

First, the capsules with *B. animalis* subsp. *lactis* Bb12 cells were prepared without the lecithin addition and at a constant agitation speed of 500 rpm. An optical microscope and fluorescence hybridisation *in situ* (FISH) were used for the visual characterisation of the capsules (Figure 2). It was concluded that the microcapsules were mostly of a globular shape of different sizes and were not fully covered by a compact film of oil on their surface (Figure 2A). FISH method confirmed the presence of cells inside the microcapsules (Figure 2B).

Table 1. Volume based fractiles ($d_{0.5}$; $d_{0.9}$) of microcapsules prepared at different agitation speed and with or without the addition of lecithin

Encapsulation process		$d_{0.5}$	$d_{0.9}$
Agitation speed (rpm)	lecithin addition* % (w/w)	(μm)	
500	0.0	196 ± 37[a]	519 ± 85[a]
1200	0.0	193 ± 24[a]	450 ± 39[b]
500	0.5	79 ± 3[b]	245 ± 12[c]
1200	0.5	39 ± 3[c]	85 ± 6[d]

*lecithin addition into the oil; different letters in the same column indicate statistically significant differences ($P < 0.05$)

In contrast to the result of Heidebach *et al.* (2009) who found the volume based median diameter $d_{0.5}$ of only 68 ± 5 μm and $d_{0.9}$ below 93 μm, the results of this study showed much higher values. As shown in Table 1, $d_{0.5}$ value for the microcapsules of *B. animalis* subsp. *lactis* Bb12 was 196 μm. The size of microcapsules is one of the most important parameters for their further use in food because it can significantly influence the sensory characteristics of the product. The emulsion technique usually creates smaller beads (25 μm to 2 mm) compared to the extrusion method (2–5 mm) as reported by Burgain *et al.* (2011). The applications of microcapsules prepared by different methods of encapsulation in dairy products have been described by many authors (Hansen *et. al*. 2002; Picot & Lacroix 2002; Kosin & Rakshit 2006). The size of microcapsules reported to have no potential

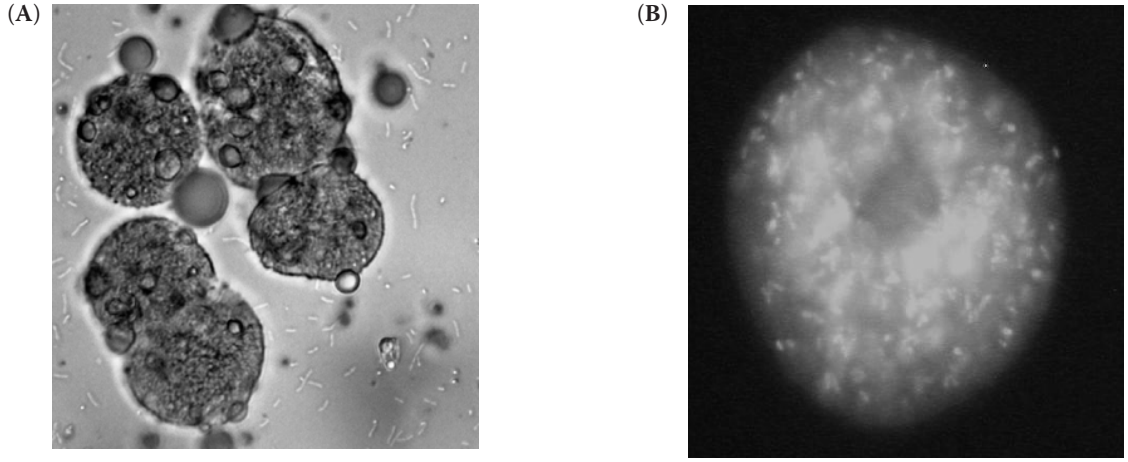

Figure 2. Optical visualisation of microcapsules with *B. animalis* subsp. *lactis* Bb12: (**A**) native preparation (80×); (**B**) FISH method preparation (1000×)

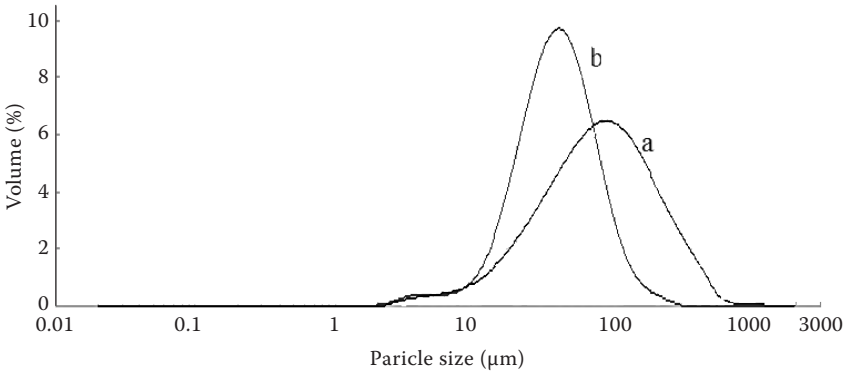

Figure 3. Particle size distribution curves of microcapsules prepared without (line a) and with (line b) the addition of 0.5% (w/w) lecithin into the oil at 1200 rpm agitation speed

sensory defect was approximately 250 μm (Heidebach *et al.* 2009). Therefore, in further experiments the agitation speed was adjusted to 1200 rpm and the particle size was measured again to check the influence of this parameter on the particle size distribution. As shown in Table 1, the increase of the agitation speed did not significantly affect $d_{0.5}$ or $d_{0.9}$ values.

Hence the addition of 0.5, 1.0, and 5.0% (w/w) of lecithin into the oil was applied in order to help the preparation of an emulsion with smaller particles. The addition of 1.0 and 5.0% (w/w) lecithin into the oil caused a significant colour change of the microcapsules. The microcapsules of light brown colour were evaluated as undesirable for the application in dairy products and therefore only 0.5% (w/w) addition of lecithin into the oil was chosen for further experiments. Lecithin addition led to a decrease of $d_{0.5}$ value by a factor of 5 in the case of 1200 rpm agitation. However, when the agitation speed of 500 rpm was used, both $d_{0.5}$ and $d_{0.9}$ values decreased by only a factor of 2 (Table. 1). Also the microcapsules' surface was fully covered by oil layer (picture not shown). Figure 3 shows an example of the particle size distribution curves for the microcapsules of *B. animalis* subsp. *lactis* Bb12 prepared without (line a) or with (line b) lecithin at 1200 rpm. A number of other authors have observed a decrease in the bead size when an emulsifier such as sodium lauryl sulphate and Tween 80 was used during the emulsification process of calcium alginate beads (Rokka & Rantamäki 2010). Above that, the application of lecithin brings several other positive effects as it is non-toxic, well tolerated by humans, and many scientific studies have confirmed its contribution to lowering the cholesterol level (Jimenez *et al.* 1990; Wilson *et al.* 1998).

To conclude, the lecithin application for the preparation of microcapsules with probiotics offers the possibility to decrease the particle size and to enrich food in which the microcapsules will be used by a nutritionally important substance.

References

Adhikari K., Mustapha A., Grün I.U., Fernando L. (2000): Viability of microencapsulated bifidobacteria in set yogurt during refigerated storage. Journal of Dairy Science, **83**: 1946–1951.

Burgain J., Gaiani C., Linder M., Scher J. (2011): Encapsulation of probiotic living cells: From laboratory scale to industrial applications. Journal of Food Engineering, **104**: 467–483.

Brinques G.B., Ayub M.A.Z. (2011): Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration and yogurt. Journal of Food Engineering, **103**: 123–128.

Charalampopoulos D., Wang R., Pandiella S. (2002): Application of cereals and cereal components in functional foods: a review. International Journal of Food Microbiology, **79**: 131–141.

Crittenden R. (2009): Incorporating probiotics into foods. In: Lee Y.L., Salminem S. (eds): Handbook of Probiotics and Prebiotics. 2nd Ed. Wiley, New Jersey: 58–75.

Doherty S.B., Gee V.L., Ross R.P., Stanton C., Fitzgerald G.F., Brodkorb A. (2011): Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection. Food Hydrocolloids, **25**: 1604–1617.

Gbassi G.K., Vandamme T., Ennahar S., Marchioni E. (2009): Microencapsulation of *Lactobacillus plantarum* spp. in an alginate matrix coated with whey proteins. International Journal of Food Microbiology, **129**: 103–105.

Hansen L.T., Ian-Wojtas P.M., Jin Y.T., Paulson A.T. (2002): Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastrointestinal conditions. Food Microbiology, **19**: 35–45.

Heller K.J. (2001): Probiotic bacteria in fermented foods: product characteristics and starter organisms. American Journal of Clinical Nutrition, **73**: 374–379.

Heidebach T., Forst P., Kulozik U. (2009): Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocolloids, **23**: 1670–1677.

Heidebach T., Forst P., Kulozik U. (2010): Influence of casein-based microencapsulation on freeze-drying and storage of probiotic cells. Journal of Food Engineering, **98**: 309–316.

Jimenez M.A., Scarino M.L., Vignolini F., Mengheri E. (1990): Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats. Journal of Nutrition, **120**: 659–667.

Kosin B., Rakshit S.K. (2006): Microbial and processing criteria for production of probiotics: a review. Food Technology and Biotechnology, **44**: 371–379.

Picot A., Lacroix C. (2004): Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. International Dairy Journal, **14**: 505–515.

Rocca S., Rantamäki P. (2010): Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. European Food Research and Technology, **231**: 1–12.

Vidhyalakshmi R., Bhakyaraj R., Subhasree R.S. (2009): Encapsulation "The future of probiotics" – a review. Advances in Biological Research, **3**: 96–103.

Wilson T.A., Meservey C. M., Nicolosi R.J. (1998): Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate. Atherosclerosis, **140**: 147–153.

Received for publication May 3, 2012
Accepted after corrections July 2, 2012

Corresponding author

Ing. Šárka Horáčková, CSc., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav mléka, tuků a kosmetiky, Technická 5, 166 28 Praha 6, Česká republika; E-mail: sarka.horackova@vscht.cz