Preparation and application characteristics of microencapsulated *Lactobacillus acidophilus* as probiotics for dogs

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Abstract: In this article, preparation and application characteristics of microencapsulated *Lactobacillus acidophilus* were investigated. Results indicated that the optimum condition for preparation of micro encapsulation were 10% (w/v) wall material and the temperature of 20°C, respectively. Many micropores in the porous starch micro particles was also observed by Scanning Electron Microscope. Furthermore, the released cell counts were increase from 2.43 log cfu/g to 9.17 log cfu/g for the time prolong to 3h in the simulated colonic pH solution. On the other hand, the visible cells of *Lactobacillus acidophilus* in the dog feces on the 10th day after the probiotics feeding was improve about 34.8% compare to the before feeding, which was decrease about 24.6% for *Escherichia coli*. Furthermore, the content of is ovaleric acid, indole and 3-methylindole, putrefactive substances in dog feces, before feeding were reduce 24%, 16% and 45% in dog feces on the 10th day after feeding compared to that before feeding, respectively. Micro encapsulation can be considered a useful technology to provide the protection for *Lactobacillus acidophilus* and better application effective.

Keywords: *Lactobacillus acidophilus*, probiotics, release property, application effect.

INTORDUCTION

In recent years, probiotics showed the increasing attention on its application for improving the intestinal microbial balance of the human and animal, which was defined as ‘‘living microorganisms” (Adhikari *et al.*, 2000; Chandramouli *et al.*, 2004; FAO/WHO, 2006; Pedroso *et al.*, 2012). *Lactobacillus acidophilus* is frequently used in food products, which have been reported to suppress the pathogens growth, improve lactose utilization and stabilize the digestive system (Ouwehand *et al.*, 1998; Kopp-Hoolihan, 2001; Kaur *et al.*, 2002; Kim *et al.*, 2008). However, micro encapsulation was developed as the technology to improve the stability and viability of free cells due to their sensitivity to the acidic media and oxygen (Fung *et al.*, 2011; Nag *et al.*, 2011; Pedroso *et al.*, 2012). Moreover, the higher cell viable should be remained because they should pass through the stomach and intestine to provide beneficial effects (Chandramouli *et al.*, 2004).

Micro encapsulation has always been used for providing the controlled release property for cell in the coating materials (Dembczynski *et al.*, 2002; Lee *et al.*, 2004; Picot *et al.*, 2004; Ma *et al.*, 2014; Xing *et al.*, 2014). As reported by Chandramouli *et al.* (2004), the condition for protecting *Lactic acid bacteria* was optimized. Picot *et al.* (2004) also found that the viable cell counts of *Bifidobacterium breve R070* and *Bifidobacterium longum R023* in micro encapsulation using the whey protein as the coating materials was increased during storage at the low temperature. As reported by Mandal *et al.* (2006), the survival of coated *Lactobacillus casei* was better than that for free cells at heat treatment. Moreover, according to Ding *et al.* (2009), during both processing and storage micro encapsulation could improve the survival of microorganisms. Micro encapsulation of bifidobacteria was also prepared by spray drying (Carlise B. Fritzen-Freire *et al.* 2012). The investigation reported by Pedroso *et al.* (2012) was to produce micro particles containing *Lactobacillus acidophilus*. According to the investigation of Xing *et al.* (2014) and Ma *et al.* (2014), *Lactobacillus acidophilus* was also embedded with porous starch as the coating material. Therefore, microencapsulated cell is drawing more and more interesting of many researchers in order to improve its stability during application (Semyonov *et al.*, 2010).

Therefore, the objective in this investigation was to demonstrate the effect of different factors and conditions on the encapsulation efficiency of *Lactobacillus acidophilus* during the processing of micro encapsulation. The morphology and thermo gravimetric analysis of micro encapsulation containing *Lactobacillus acidophilus* was also evaluated. The release property and application effect for feeding dogs was also investigated.

MATERIALS AND METHODS

Materials

Porous starch was provided by Liaoning Lida Bio-Technology Co. Ltd. in Jinzhou of China. The free cell of

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Lactobacillus acidophilus was purchased from China Center of Industrial Culture Collection (No. 6075) was used as the core material for the preparation of micro encapsulation. The chemicals used in this investigation were purchased from Chengdu Ruifeng Lier Technology Co. Ltd, Chengdu of China.

Preparation of micro encapsulated Lactobacillus acidophilus

Microencapsulated *Lactobacillus acidophilus* was prepared as the method reported by Mandal *et al.* (2006), Xing *et al.* (2014) and Ma *et al.* (2014). The freeze-dried *Lactobacillus acidophilus* was activated at 35°C-37°C for 24h-28h and then reactivated in MRS broth by transferring three times. The cells were harvested by centrifugation at 5°C with 3000*g*×10min. The obtained cells were washed twice and adjusted to the concentration of 10^9-10^{10} cfu/mL. Then, the 50mL cell suspension and porous starch were transferred into a sterilized beaker and shocked by ultrasonic wave. After absorption, sodium alginate with 2% concentration and soybean oil (10mL) containing 0.2% Tween 80 were added drop wise in the above solution during stirring. After 5min, in order to harden the coating material of microcapsules, 0.1M calcium chloride (100mL) was added into the obtained solution. After centrifuging with 450g for 10min at 5°C, the obtained capsules were washed with distilled water for twice and precooled in a refrigerator (-40°C) for 5 h, and then freeze drying under -58°C by freeze drying machines for 24h-28h.

Morphology of micro particles and thermo gravimetric analysis

The samples were placed on the Scanning Electron Microscope stubs using a two-sided adhesive tape, and then observed using a JSM-7500F Scanning Electron Microscope at a voltage of 5 kV acceleration after Pt sputtering. The analysis of thermo gravimetry/derivative thermo gravimetry were carried by the TG/DTA-6300 thermo balance. Sample of micro encapsulation (7 mg) on the alumina pans was heated from 30°C to 300°C at a rate of 10°C min^{-1} with the flow of N_2 atmosphere at 100 mL/min.

Release of Lactobacillus acidophilus cells from micro encapsulation

The release of *Lactobacillus acidophilus* cell from micro encapsulation particles in the solution with the simulated colonic pH was investigated according the method reported by Mandal *et al.* (2006) and Xing *et al.* (2014). Samples of micro encapsulation (1g) were transferred into the simulated solution with 10mL (0.1M KH_2PO_4, pH 7.4±0.2) and homogenized for 12 min. The cell counts were evaluated on MRS agar after incubated at 37°C for 3h (Grosso *et al.*, 2004; Ma *et al.*, 2014; Xing *et al.*, 2014).

Application effect of microencapsulated Lactobacillus acidophilus

The 26 medium sized dogs, no history of dietary change or probiotic supplementation, antimicrobial administration, were chose in order to investigate application effect of microencapsulated *Lactobacillus acidophilus*. Moreover, tests were carried out in the same environment and the management of feeding demanded consistent. The counts of *Lactobacillus acidophilus* and *Escherichia coli* and the content of isovaleric acid, indole and 3-methylindole in dog feces were evaluated before the trial, the 10th day and of 20th day probiotic feeding, the 10th day after probiotic feeding.

The microbiological analysis was used the method of Rojas-Graü *et al.* (2008) and Xing *et al.* (2012). 10 g Fresh dog feces with 90mL sterile solution were blended by the stomacher for 60 s. 1mL of obtained solution with appropriate concentration was placed on MRS and EMB incubated at 37°C for 24h for *Escherichia coli* and *Lactobacillus acidophilus*, respectively. Moreover, the analysis method of isovaleric acid, indole and 3-methylindole in fresh dog feces was developed from the reported by Adiguzel *et al.* (2009). 3g of fresh dog feces were constantan to 5mL by the mixture of chloroform and acetone with the ratio of 1:1. The above mixture was treated by ultrasound for 35 min and then centrifuged at 3,000*g* for 6 min. The temperatures of injector were set at 200°C. Nitrogen at a flow rate of 3.0mL/min was used as the carrier gas in the system. The analysis temperature was programmed at the rate of 8°C/min from 80°C to 200°C. 1.0mL diluted sample was injected and identification of sovaleric acid, indole and 3-methylindole was confirmed by comparing their relative retention time, respectively.

STATISTICAL ANALYSIS

The tests were carried out in triplicate and experimental dates were analyzed by SPSS 13.0 software (SPSS Inc.). The one-way analysis of variance procedure with Student-Newman-Keuls test was used to determine the significant difference ($p<0.05$).

RESULTS

Effect of wall material and temperature on the encapsulation efficiency

During the preparation processing of micro encapsulation, effects of wall material concentration and the processing temperature is critical for the encapsulation efficiency. This is because that it is designed to increase the coating efficiency and viable of free cells during processing and application (Pedroso *et al.*, 2012; Ma *et al.*, 2014; Xing *et al.*, 2014). So, influence of wall material concentration and the temperature on the encapsulation efficiency was investigated. As shown in fig. 1a, there was no effect of

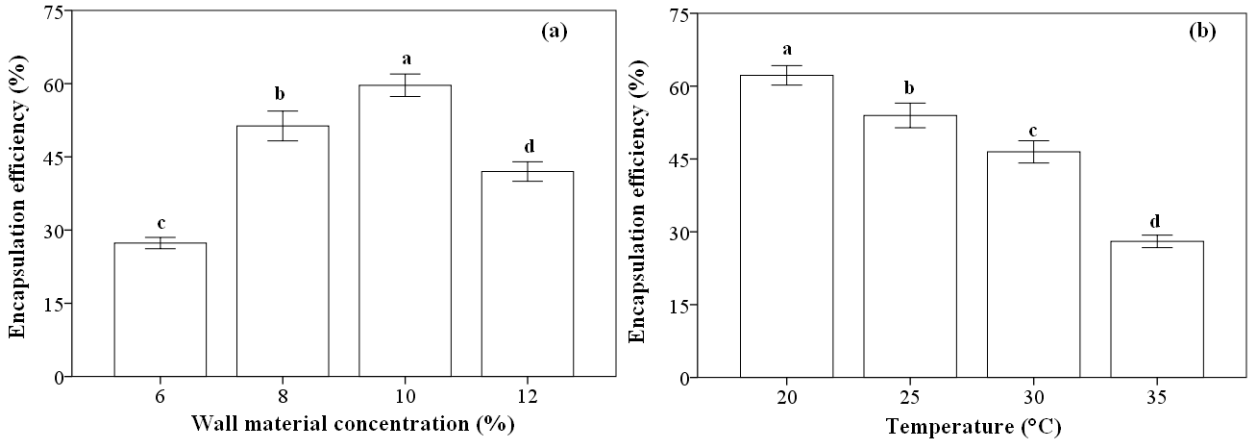

Fig. 1: Effect of different wall material concentration and temperature on the encapsulation efficiency of *Lactobacillus acidophilus*. Mean bars with different letters (a-d) at same temperature for different heating period differ significantly $p<0.05$.

Lactobacillus acidophilus cell load on the retention at high concentrations of wall material, but the retention of cell increased when the wall material concentration increased from 6% to 10%, which is the highest with the concentration at 10%. There was decrease in coated number of cells when the concentration was further increased to12%. During the prepared process, the temperature also influences the *Lactobacillus acidophilus* survival. As shown in fig. 1b, effect of the prepared temperature on the encapsulation efficiency of free cell was also observed, which decrease with increasing the temperature. The highest encapsulation efficiency was observed when the temperature was at 20°C, which was 61.2%. There was significant effect of the temperature during process on the retention of *Lactobacillus acidophilus* cell load, but the encapsulation efficiency of cell decreased when the temperature during the prepared process was 25°C. It was significant decrease in encapsulation efficiency when the wall material concentration was further decreased to 35°C.

Morphological characterization and thermo gravimetry analysis

Morphological characterization of microencapsulated *Lactobacillus acidophilus* was observed by Scanning Electron Microscope. Scanning Electron Microscope photo in fig. 2 revealed that many micropores were observed in the particles of porous starch. The external surfaces showed many micropore, which is fundamental for guaranteeing higher protection. The micropores on the surface may be one of the important reasons for the increase in the retention of *Lactobacillus acidophilus* cell, correlated with an increased presence of cell load. On the other hand, the thermal property of micro encapsulation containing cell can be found from the curves of thermo gravimetry, as indicated in fig. 3. This results domenstarted that from the analysis of thermo gravimetric curves, the different of two representative stages were observed. The first mass loss might occurred between 30°C and 100°C, which refers to moisture loss of micro encapsulation particles. The second loss was occurred between 237°C and 264°C, which indicated the breakdown of the fructose chains in microcapsules and mass loss might be responded to the process of decomposition.

Fig. 2: Morphology of microencapsulated *Lactobacillus acidophilus* observed by Scanning Electron Microscope

Release of encapsulated cells in simulated colonic pH solution

The release cells from microcapsules is essential to evaluate in order to understand the colonization and growth property of probiotics in the action site (Manda *et al.*, 2006). The release characteristics of cells from micro particles in simulated solution of colonic pH at 37°C was investigated. The result fig. 4 showed that the released cell counts could increase to 9.17log cfu/g from the initial count 9.75log cfu/g in the tested micro encapsulation for the exposure time prolong to 3h. These results indicated

that the cells release was increased at the first 60 min with increased incubation time. However, there was no significant change after 60 min evaluated.

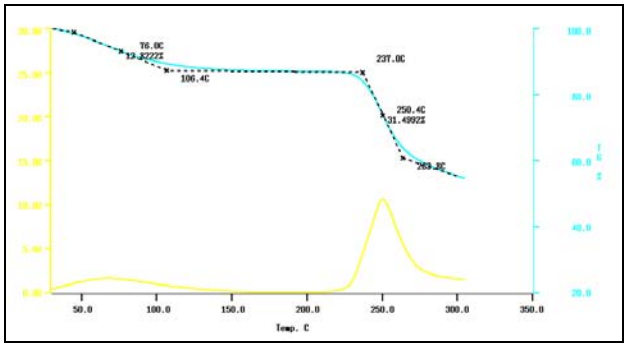

Fig. 3: Thermo gravimetry analysis of microcapsules containing *Lactobacillus acidophilus*

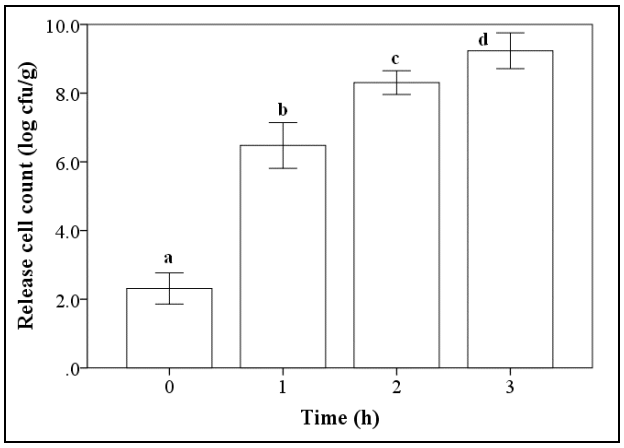

Fig. 4: Release of encapsulated cells in simulated colonic pH solution. Mean bars with different letters (a-d) at same temperature for different heating period differ significantly $p<0.05$.

Application effect of micro encapsulated Lactobacillus acidophilus

Probiotics cell could provide the beneficial effects for human by improving the intestinal microbial balance in the digestive system (Adhikar *et al.*, 2000; FAO/WHO, 2006; Wang, 2009). In recent years, increasing interest has been observed on their application in human health and animal growth (Chandramouli *et al.*, 2004). The results about the counts of *Lactobacillus acidophilus* and *Escherichia coli* and the content of isovaleric acid, indole and 3-methylindole in dog feces could observe in fig. 5. The results showed that, the visible cells of *Lactobacillus acidophilus* for the before feeding and on the 10th day after feeding were 3.97×10^6 cfu/g and 5.35×10^6, respectively, which was improve about 34.8% (fig.5a). However, the visible cells of *Escherichia coli* for the before feeding and 10^{th} days after feeding were 2.24×10^6 cfu/g and 1.69×10^6, respectively, which was decrease about 24.6%. On the other hand, the content of isovaleric acid, indole and 3-methylindole in dog feces before feeding were 0.42mg/g, 0.057mg/g and 0.056mg/g, respectively, which were 0.32mg/g, 0.048mg/g and 0.031mg/g on the 10^{th} day after feeding (figs. 5b-d). Results indicated that the content of isovaleric acid, indole and 3-methylindole was reduce 24%, 16% and 45% on the 10th day after feeding compared to that before feeding in dog feces , respectively.

DISCUSSION

The cell embedded capacity depended on the concentration of wall material and the processing temperature. Result indicated that the effect of wall material concentration on the encapsulation efficiency was existed. Wall material with 10% concentration showed higher *Lactobacillus acidophilus* retention than the other concentrations. This was consisted with the result reported for liquid flavors by other researchers (Soottitantawat *et al.*, 2005; Xing *et al.*, 2014; Ma *et al.*, 2014). As reported by Lee *et al.* (2000), the death rate of *B. longum* coating by alginate decreased with increasing its concentration. As reported by Xing *et al.* (2014) and Ma *et al.* (2014), the concentration of wall material significant influenced the encapsulation efficiency and the visible rate of free cells. This result may be due to the micropores in the porous starch, which was also can be shown in fig. 2. Higher wall material concentration could provide the higher numbers of micropores for free cell load. On the other hand, the temperature during the prepared processing was significant influenced the free cell loaded. This result was also reported by other researchers (Mandal *et al.* 2006; Ma *et al.*, 2014; Xing *et al.*, 2014).

Many micropores were observed clearly by Scanning Electron Microscope. This result was also observed by Saénz *et al.* (2009), Pedroso *et al.* (2012), Carlise B. Fritzen-Freire *et al.* (2012), Xing *et al.* (2014) and Ma *et al.* (2014). However, the *Lactobacillus acidophilus* cells were not visible clearly by Scanning Electron Microscope photomicrography (Xing *et al.*, 2014; Ma *et al.*, 2014). The investigation conducted by Pedroso *et al.*, (2012) reported that the *B. lactis* and *Lactobacillus acidophilus* cells were also not visible on the surface of the micro particles by MEV photomicrography. The similar result about the morphology observation of micro encapsulation enriched with *Lactobacillus acidophilus* cell was also reported by Ma *et al.* (2014) and Xing *et al.* (2014). Furthermore, the thermo gravimetric characteristics of micro encapsulation particles is very important for its application after storage. According to the investigated by Macêdo *et al.* (1997), the decomposition reactions could be occurrence during the processing of thermal analysis for the microcapsules. Moreover, Bohm *et al.* (2005) also investigated thermal degradation of inulin. Their results showed that a consequence breakdown of the fructose chains in micro encapsulation particles was found, which

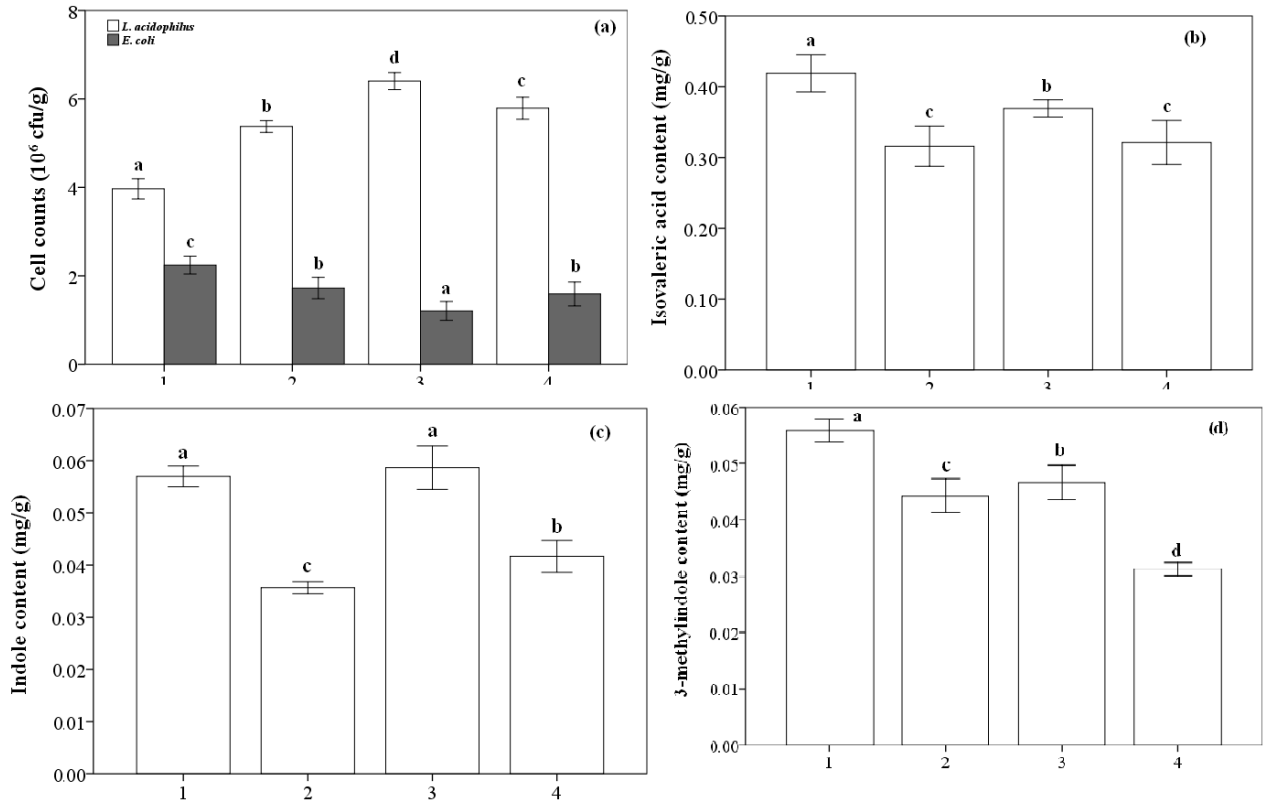

Fig. 5: Counts of *Lactobacillus acidophilus*, *Escherichia coli* and contents of isovaleric acid, indole and 3-methylindole in dog feces. Mean bars with different letters (a-d) at same temperature for different heating period differ significantly $p<0.05$.

might occurred at the temperature of between 213°C and 223°C.

The release property of cells from micro encapsulation is one of the important purpose (Suita-Cruz *et al.*, 2001). Result obtained in this investigation indicated that the cell stability release with prolong the storage time at the temperature of 37°C. This was consisted with the result observed by Picot *et al.* (2004) and Xing *et al.* (2014), in simulated intestinal solutions, the consistent release of viable cells from micro particles was also observed during the thermal period. The application effective is also important for the preparation of micro encapsulation. Result indicated that feeding of microencapsulated *Lactobacillus acidophilus* on the dogs could increase the count of probiotic and reduce the cell count of Harmful microorganism such as *Escherichia coli*. Moreover, the contents of putrefactive substances in dog feces, such as isovaleric acid, indole and 3-methylindole, were also decreased after feeding. This is because that *Lactobacillus acidophilus* could provide beneficial effects on animal and human health (Chandramouli *et al.*, 2004). More importantly, the cell of *Lactobacillus acidophilus* could stabilize and control the growth of pathogens in the digestive system (Ouwehand *et al.*, 1998; Kim *et al.*, 2008). Micro encapsulation technology were always applied to improve the survival and stability of probiotic cells in products (Adhikari *et al.*, 2000; Picot *et al.*, 2004; Chandramouli *et al.*, 2004; Lee *et al.*, 2004).

CONCLUSIONS

Microencapsulated *Lactobacillus acidophilus* was produced in order to protect the probiotics. The concentration of wall material and the temperature were significant influence the encapsulation efficiency of cell. The result of Scanning Electron Microscope photos indicated that many micropores were existed in the particle of porous starch, which was used as the carrier materials. Furthermore, the released cell counts were increase prolong time to 3h. The visible cells of *Lactobacillus acidophilus* in the dog feces on the 10th day after the probiotics feeding was improve but decrease for *Escherichia coli*. Furthermore, the content of isovaleric acid, indole and 3-methylindole in dog feces before feeding were reduce compared to that before feeding. Micro encapsulation could provide the protection for *Lactobacillus acidophilus* and better effective for its application.

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REFERENCES

Adhikari K, Mustapha A, Grun I U and Fernando L (2000). Viability of micro encapsulated bifidobacteria in set yogurt during refrigerated storage. *J. Dairy Sci.*, **83**: 1946-1951.

Adiguzel A, Ozer H, Sokmen M, Gulluce M, Sokmen A, Kilic H, Sanhin F and Bariso O (2009). Anti-microbial and antioxidant activity of the essential oil and methanol extract of Nepeta cataria. *Pol. J. Microbiol.* **58**: 69-76.

Bohm A, Kaiser I, Trebstein A and Henle T (2005). Heat-induced degradation of inulin. *Eur. Food Res. Technol.*, **220**(5-6): 466-471.

Chandramouli V, Kailasapathy K, Peiris P and Jones M (2004). An improved method of micro encapsulation and its evaluation to protect *Lactobacillus* spp. in simulated gastric conditions. *J. Microbiol. Meth.*, **56**(1): 27-35.

Dembczynski R and Jankowski T (2002). Growth characteristics and acidifying activity of *Lactobacillus rhamnosus* in alginate/starch liquid-core capsules. *Enzyme Microb. Technol.*, **31**(1-2): 111-115.

Ding WK and Shah NP (2009). Effect of various encapsulating materials on the stability of probiotic bacteria. *J. Food Sci.*, **74**(2): M100-M107.

FAO/WHO, Probiotics in food (2006). Health and nutritional properties and guidelines for evaluation FAO Food and Nutrition Paper No. 85, World Health Organization and Food and Agriculture Organization of the United Nations, Rome, Italy. Pp1-50.

Fritzen-Freire Carlise B and Prudêncio *et al* (2012). Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. *Food Res. Int.*, **45**(1): 306-312.

Fung WY, Yuen KH and Liong MT (2011). Agrowaste-based nanofibers as a probiotic encapsulant: Fabrication and characterization. *J. Agr. Food Chem.*, **59**(15): 8140-8147.

Grosso CRF and Fávaro-Trindade CS (2004). Stability of free and immobilized *Lactobacillus acidophilus* and *Bifidobacterium lactis* in acidified milk and of immobilized B. lactis in yoghurt. *Braz. J. Microbiol.*, **35**(1): 151-156.

Kaur IP, Chopra K and Saini A (2002). Probiotics: Potential pharmaceutical applications. *Eur. J. Pharm. Sci.*, **15**(1): 1-9.

Kim SJ, Cho SY, Kim SH, Song OJ, Shin IS, Cha DS and Park HJ (2008). Effect of micro encapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. *LWT-Food Sci. Technol.*, **41**(2): 493-500.

Kopp-Hoolihan L (2001). Prophylactic and therapeutic uses of probiotics: A review. *J. Am. Diet. Assoc.*, **101**(2): 229-241.

Lee JS, Cha DS and Park HJ (2004). Survival of freeze-dried *Lactobacillus bulgaricus* KFRI 673 in chitosan-coated calcium alginate microparticles. *J. Agr. Food Chem.*, **52**(24): 7300-7305.

Lee KY and Heo TR (2000). Survival of bifidobacterium longum immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. *Appl. Environ. Microb.* **66**(2): 869-873.

Ma Y, Xing Y, Wang T, Xu Q, Cai Y, Che Z, Wang Q and Jiang L (2014). Microbiological and Other Characteristics of Micro encapsulation Containing *Lactobacillus acidophilus* (CICC 6075). *J. Pure Appl. Microbiol.*, **8**(2): 1693-1699.

Macêdo RO, Moura OM and Souza AG *et al* (1997). Comparative studies on some analytical methods: Thermal decomposition of powder milk. *J. Therm. Aanl. Calorim.*, **49**(2): 857-862.

Mandal S, Puniya AK and Singh K (2006). Effect of alginate concentrations on survival of micro encapsulated *Lactobacillus casei* NCDC-298. *Int. Dairy J.*, **16**(10): 1190-1195.

Nag A, Han KS and Singh H (2011). Micro encapsulation of probiotic bacteria using pH-induced gelation of sodium caseinate and gellan gum. *Int. Dairy J.*, **21**: 247-253.

Ouwehand A C and Salminen S J (1998). The health effects of cultured milk products with viable and non-viable bacteria. *Int. Dairy J.*, **8**(9): 749-758.

Pedroso D L, Thomazini M and Heinemann R J *et al* (2012). Protection of *Bifidobacterium lactis* and *Lactobacillus acidophilus* by micro encapsulation using spray-chilling. *Int. Dairy J.*, **26**(2): 127-132.

Picot A and Lacroix C (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *Int. Dairy J.*, **14**(6): 505-515.

Saénz C, Tapia S and Chávez J *et al* (2009). Micro encapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica). *Food Chem.*, **114**(2): 616-622.

Savolainen M, Khoo C and Glad H *et al* (2002). Evaluation of controlled release polar lipid micro particles. *Int. J. Pharm.*, **244**(1-2): 151-161.

Semyonov D, Ramon O, Kaplun Z, Levin-Brener L, Gurevich N and Shimoni E (2010). Micro encapsula-

tion of *Lactobacillus paracasei* by spray freeze drying. *Food Res. Int.*, **43**: 193-202.

Soottitantawat A, Takayama K and Okamura K *et al* (2005). Micro encapsulation of l-menthol by spray drying and its release characteristics. *Innov. Food Sci. Emerg. Technol.*, **6**(2): 163-170.

Suita-Cruz P and Goulet J (2001). Improving probiotic survival rates. *Food Tech.*, **55**: 36-42.

Wang Y (2009). Prebiotics: Present and future in food science and technology. *Food Res. Int.*, **42**(1): 8-12.

Xing Y, Xu Q, Li X, Che Z and Yun J (2012). Anti-fungal activities of clove oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium citrinum in vitro* and in wounded fruit test. *J. Food Safety*, **32**(1): 84-93.

Xing Y, Xu Q, Ma Y, Che Z, Cai Y and Jiang L (2014). Effect of porous starch concentrations on the microbiological characteristics of micro encapsulated *Lactobacillus acidophilus*. *Food Funct.*, **5**(5): 972-983.

Xu Q, Xing Y, Che Z, Guan T, Zhang L, Bai Y and Gong L (2013). Effect of chitosan coating and oil fumigation on the microbiological and quality safety of fresh-cut pear. *J. Food Safety*, **33**(2): 179-189.