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Stability of probiotics encapsulated with Thai herbal extracts in fruit juices and yoghurt during refrigerated storage

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ABSTRACT

This study intended to investigate the impact of alginate encapsulation with Thai herbal extracts including cashew flower, pennywort and yanang on viability of probiotic bacteria suspended in fruit juices (i.e. mulberry, maoberry, longan and melon juices) and stirred yoghurt during storage at 4 °C. The strains of probiotics used in this work were *Lactobacillus casei* 01, *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* Bb-12. The results displayed that the surviving population of *L. casei* 01 cells entrapped with 0.05% (w/v) cashew flower extract were significantly higher than those encapsulated with pennywort and yanang extracts, after storage for 30 days. Accordingly, this level of cashew flower extract was selected to immobilize the cultures before inoculation into fruit juices and yoghurt, in order to comparison with green tea extract (a standard plant extract). Upon storage, cashew flower and green tea extracts noticeably improved the stability of probiotic beads in all the products, as compared to the controls. Encapsulated *L. casei* 01 and *B. lactis* Bb-12 showed better survival than encapsulated *L. acidophilus* LA5. The diminution of pH values in each product was also observed.

1. Introduction

Over the last decade, development of new foods that promote health for consumers is one of the key research priorities of the food industry. The acceptance of probiotic products has increased due to their health benefits (Najgebauer-Lejko, 2014). Probiotic bacteria, such as *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium lactis*, are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). The health-promoting effects of these bacteria, i.e. alleviation of gastrointestinal complaints, reduction of lactose intolerance, lowering serum cholesterol level, anti-carcinogenic activity, anti-tumor property, reduction of allergic symptoms, stimulation of immune system and prevention of diarrhea, are recently updated and presented by Sanders et al. (2013). Nevertheless, in order to exert the beneficial health impacts, the level of probiotics in food products that serve as delivery systems needs to be high, suggesting minimum level of live probiotic cells should be at least 10^6–10^7 CFU/ml or CFU/g before consumption (Nualkaekul, Lenton, Cook, Khutoryanskiy, & Charalampopoulos, 2012). Therefore, preserving of probiotic cultures in the products during storage is very important.

Encapsulation has demonstrated to be an alternative technique for the protection of probiotics from unfavorable environments (Chaikham et al., 2013; Chaikham, Apichartsrangkoon, George, & Jirarttanarangsri, 2013; Ribeiro et al., 2014). Sodium alginate is mainly used for this purpose because of its low cost, simplicity and biocompatibility (Krasaekoopt, Bhandari, & Deeth, 2003). Several researchers established that alginate incorporated with other materials, for instance, gelatin (Chaikham et al., 2013), chitosan, pectin, glucomannan (Chávarri et al., 2010; Nualkaekul, Cook, Khutoryanskiy, & Charalampopoulos, 2013), hi-maize starch (Sultana et al., 2000), inulin, galactooligosaccharides and fluctooligosaccharides (Krasaekoopt & Watcharapoka, 2014; Sathyabama, Ranjith kumar, Bruntha devi, Vijayabharathi, & Brindha priyadharisini, 2014), can be used to increase the survivability of various probiotic strains in food products during storage and under gastrointestinal environments.

Presently, there is no information on the impact of alginate combined with plant extracts for enhancing survival of probiotics in foods and beverages. Maisuthisakul, Suttajit, and Pongsawatmanit, (2007) and Siriwatanametanon, FiebichEfferthPrieto, and Heinrich (2010) reported that some Thai indigenous plants exhibited a potential for use as natural antioxidants, since their extracts contained high contents of total phenolic compounds and flavonoids. Thus, the purpose of this research was to find out the impact of alginate encapsulation with some Thai plant extracts on stability of probiotic bacteria including *L. casei* 01, *L. acidophilus*

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LA5 and *B. lactis* Bb-12 in fruit juices and stirred yoghurt during refrigerated storage for 30 days.

2. Materials and methods

2.1. Preparation of plant extracts

Thai herbal plants, such as cashew flower (*Anacardium occidentale* L.), yanang (*Tiliacora triandra*), pennywort (*Centella asiatica* (Linn.) Urban) and green tea (*Camellia sinensis* L.), were freshly harvested from the orchards in Chiang Mai and Lamphun provinces, Thailand. All plants were dehydrated using a tray dryer (Mammert, Germany) at 65 °C for 3–8 h before powdering. Afterward, 100 g of powder were extracted with 500 mL distilled water by stirring (60 rpm) at 70 ± 5 °C for 1 h and continuously filtered through Whatman® No. 1 filter paper (Whatman, Spain). All filtrates were then lyophilized using a freeze dryer (SciQuip, Germany) at −50 °C for 15–20 h.

2.2. Probiotic cultures

Commercial probiotic bacteria including *L. casei* 01, *L. acidophilus* LA5 and *B. lactis* Bb-12 were purchased from Chr. Hansen (Hørsholm, Denmark). Cell pellets of *L. acidophilus* LA5 and *L. casei* 01 were prepared following the procedures of Chaikham et al. (2013), Chaikham, Apichartsrangkoon, et al. (2013). For *B. lactis* Bb-12, the freeze-dried culture was incubated in sterile MRS broth (Hi-Media, India) containing 0.05% (w/v) *L*-cysteine hydrochloride (Sigma, Australia) at 37 °C for 20 h. All cultures were harvested by centrifugation at 3000 rpm for 20 min and then washed twice with 0.1% (w/v) sterile peptone water (Hi-Media, India). The cell pellets of all strains were diluted to provide the concentration of 10^{11} CFU/mL by 0.85% sterile saline water before encapsulation.

2.3. Encapsulation of probiotics

Probiotic capsules were produced according to the procedure of Chaikham, Apichartsrangkoon et al. (2013) with some modifications. Cell pellets (20%, v/v) and different levels of plant extracts (0.05–0.2%, w/v) were mixed with sterile sodium alginate solution (Sigma-Aldrich, UK). The mixture was injected through a 0.5 mm sterile needle (Nipro, Japan) into 0.1 M sterile calcium chloride solution (Merck, Germany) and then held for 30 min for gelation. Afterward, the beads were washed with 0.85% (w/v) sterile saline water and kept at 4 °C prior to use.

2.4. Enumeration of immobilized probiotics

In brief, one gram of probiotic beads was transferred into 99 mL of 0.1 M sterile phosphate buffer (pH 7) (Merck, Germany) and stomached for 10 min (Krasaekoopt & Watcharapoka, 2014). Subsequently, the decimal dilutions were made with 0.1% (w/v) sterile peptone water (Hi-Media, India) before plating on MRS agar. All plates were anaerobically incubated at 37 °C for 24–72 h before counting of colonies.

2.5. Viability of probiotic encapsulated with Thai herbal extracts during storage

Ten grams of immobilized *L. casei* 01 with herbal extracts were vacuum- or normal-packed in a laminate bag and then kept at 4 °C for 30 days. To quantify the survival numbers, the samples were taken every 5 days. Subsequently, the optimal dilutions of each sample were made and poured on MRS agar before incubating at 37 °C for 24–72 h.

2.6. Survival of encapsulated probiotics in fruit juices and yoghurt during storage

To evaluate the viability of encapsulated probiotics in fruit juices and yoghurt, 10 g of encapsulated probiotics, which were chosen from the above experiment (Section 2.5) were aseptically inoculated into 90 mL of pasteurized mulberry, maoberry, longan and melon juices or 90 g of yoghurt before storing at 4 °C. Afterward, the samples were withdrawn on days 5, 10, 15, 20, 25 and 30 for quantifying the survivors in MRS agar (Hansen, 2001; Chaikham, Apichartsrangkoon et al., 2013). Changes of pH values in the products were measured using a pH meter (Sartorius PB-20, Germany).

2.7 Data analysis

The results are presented as mean ± standard deviation. Analysis of variance (ANOVA) was carried out using SPSS version 11.5 for Windows (SPSS Inc., USA). The determination of significant differences among treatment means was done by Duncan's multiple range tests ($P \leq 0.05$).

3. Results and discussion

3.1. Viability of *L. casei* 01 encapsulated with Thai herbal extracts during storage

To our knowledge this is the first study on the feasibility of employing Thai herbal extracts for improving probiotic stability during refrigerated storage. Thai herbs including cashew flower, pennywort and yanang mostly contain high levels of bioactive components and antioxidant activities (Chaikham, Chunthanom, & Apichartsrangkoon, 2013; Chaikham & Prangthip, 2015; Chunthanom, Chaikham, & Intaket, 2013). In this work, the attenuation of probiotic cells encapsulated with and without herbal extracts during storage under normal atmosphere and vacuum environments was observed (Table 1). The results noticeably displayed that 0.05% cashew flower extract could increase the survival rate of *L. casei* 01 cells when compared to control and other levels ($P \leq 0.05$). For pennywort and yanang, their extracts had no impact on the improving survival of probiotic cells. It was emphatically seen that the quantities of cell loss increased with raising the extract levels, in particular 0.2% yanang extract showed the inhibitive effect on this strain. Similar effects of these extracts on viability of *L. casei* 01 were observed between both packaging conditions. However, the entrapped cells kept under vacuum condition still displayed a significantly higher survivability than under normal atmosphere environment ($P \leq 0.05$). Analogous findings were obtained by Rodrigues et al. (2011) with encapsulated *L. acidophilus*, *Lactobacillus paracasei* and *Bifidobacterium animalis* during storage at 5 and 22 °C for 6 months. To and Etzel (1997) demonstrated that during storage at 4 °C amounts of probiotic powders markedly diminished in the presence of oxygen, but they were relatively stable in the absence of oxygen. Furthermore, Hsiao et al. (2004) observed that the surviving population of encapsulated bifidobacteria kept in glass bottle was found to be higher than in PET bottle. They concluded that oxygen permeability of PET during storage affected the lower viability of bifidobacteria. Overall, this can explain that oxygen is an important factor that affects the stability of probiotic bacteria. Korbekandi et al. (2011) suggested that oxygen can affect probiotics in three ways; (i) it is directly toxic to some cells, (ii) certain cultures produce toxic peroxides in the presence of oxygen and (iii) free radicals produced from the oxidation of components are toxic to probiotic cells.