International Food Research Journal 24(3): - (June 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Survivability of immobilized *Lactobacillus plantarum* cells within bacterial cellulose in mamao juice

Phromthep, K. and *Leenanon, B.

Department of Food Technology, Faculty of Technology, Khon Kaen University, 40002, Khon Kaen, Thailand

Article history

Received: 16 February 2016
Received in revised form: 19 May 2016
Accepted: 20 May 2016

Keywords

Survivability
Probiotics
Cells Immobilization
Bacterial cellulose
Mamao

Abstract

This study was aimed to apply mature coconut water and mamao fruit residues as substrates for bacterial cellulose production via fermentation with *Acetobacter xylinum* TISTR 893. Selected probiotic strain identified as *Lactobacillus plantarum* was immobilized within suitable bacterial cellulose cubes. The survivals of free *L. plantarum* cells (control) and immobilized *L. plantarum* ones within different bacterial cellulose cubes in mamao juice samples were determined on day 0, 4, 8, 12, 16, 20, 24 and 28 during storage at 4°C. It was found that mamao juice samples supplemented with free *L. plantarum* cells (control) had rapidly declined in numbers whereas the ones supplemented with immobilized *L. plantarum* cells had slowly declined during storage. Interestingly, it was found that after 28 days of storage, free *L. plantarum* cells in mamao juice samples were greatly decreased in numbers to 0.76 log CFU/ml while the immobilized *L. plantarum* ones within bacterial cellulose cubes from mature coconut water and mamao fruit residues were slowly decreased to 6.41 and 6.32 log CFU/ml respectively which were not significantly different ($p>0.05$). Moreover, total phenolic content, total anthocyanin content and antioxidant activity by DPPH assay of the mamao juice samples were significantly different between day 0 and 28 of storage period ($p\leq0.05$). Sensory evaluation was eventually conducted by a panel of 30 untrained members on a 9 point hedonic scale. The results showed that all mamao juice samples were not significant different in appearance, odor, acidity and sweetness ($p>0.05$) whereas the one supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from mamao fruit residues had the texture and overall liking scores higher than free *L. plantarum* cells treatment ($p\leq0.05$) but it was not significantly different from the one supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from mature coconut water ($p>0.05$).

Introduction

The application and development of functional foods are interesting presently. Several food products could be used to improve the health and well-being of the consumers. For commercial applications, probiotic lactic acid bacteria are the most favorite and popular bacteria applied in food products (Wedajo, 2015). Probiotics can be defined as a food product that contains a sufficient number of viable microorganisms to alter the microflora of the host and has the potential for beneficial health effects. Also, it can be defined as live microorganisms that when administered in adequate amounts confer a health benefit on the host (Suresh *et al*., 2013).

Most of the commercially available probiotics are in the genera of Gram-positive bacteria and mostly members of genus *Lactobacillus* and *Bifidobacterium* which are part of normal healthy gastrointestinal flora. The other strains such as *Lactococcus* sp. and *Streptococcus* sp. are also included (Ishibashi and Yamazaki, 2001; Tuohy *et al*., 2003). The many clinical benefits of probiotics have been proposed for the risk reduction of illnesses such as reduction of cancer risk, improvement of heart health, stimulation of immune system, decrease of menopause symptoms, improvement of gastrointestinal health, maintenance of urinary tract health, anti-inflammatory effects, reduction of blood pressure, maintenance of vision, antibacterial and antiviral activities, reduction of osteoporosis and antiobese effects (Saarela *et al*., 2000; Goktepe *et al.*, 2006; Granato *et al*., 2010).

Bacterial cellulose is a final product of carbon metabolism synthesized by a strain of acetic acid bacteria group also called *Gluconobacter xylinus* (formerly called *Acetobacter xylinum*) (Chawla *et al.*, 2009). The various properties of bacterial cellulose such as purity, degree of crystallinity, density, water binding capacity, and surface area are better than plant cellulose, thus bacterial cellulose

*Corresponding author.
Email: *boris394135@gmail.com*

has been brought to apply in the processed food area including thickener, stabilizer, snack (nata de coco in the Philippines), texturizer and calorie reducer etc (Ross *et al*., 1991; Okiyama *et al.*, 1992 Skinner and Cannon 2000; Chawla *et al*., 2009).

Mao or Mamao fruit (*Antidesma thwaitesianum* Mull.Arg.) is a kind of medicinal plants in Thailand which has a little bit sweet and sour taste and a purplish-red or dark-purple color appearance when it ripens. Mamao products widely consumed among Thai people are jelly, jam, drinking juice, concentrated juice and wine. Mamao is a new potential source of natural antioxidants and could be used for healing symptoms of heart disease, reducing platelet aggregation, anti-oxidant, anti-cancer and anti-inflammation (Butkhup and Samappito, 2008; Samappito and Butkhup, 2008; Puangpronpitag *et al*., 2011).

Many useless seeds and marcs from mamao after juice extraction are regarded as waste products or mamao fruit residues. Thus, some authors tried to extract some phytochemicals from these products and found that the extracts of mamao seeds and marcs had strong antioxidant properties of polyphenolic compounds (Puangpronpitag *et al*., 2011). Thus, the application using these waste products as substrates for bacterial cellulose production would be interesting because they are enriched and low-cost substrates for bacterial cellulose synthesis. Generally, the production of bacterial cellulose from food wastes was conducted using substrates from sugar cane (juices and molasses), coffee cherry husk and pineapple peel (Keshk and Sameshima, 2006b; Castro *et al*., 2011; Usha-Rani and Anu-Appaiah, 2013).

The adverse environmental conditions found in food products may lead to a decrease in survival of probiotic bacteria during storage and passage through the human gastro-intestinal system. Therefore, an approach to extend the viability of probiotic living cells and the application of immobilized cells with polymer to protect the probiotic cells with a physical barrier against adverse environmental conditions are currently receiving considerable interest.

The advantages of cell immobilization are for example, good stability, easy recycling, durability, high functional efficiency, as well as the resistance to the detrimental environmental factors such as temperature, pH, and toxic substances. For the recent study, the immobilization of cells has wide application and developmental potential in the fields of food products, medicine, biology, and environmental protection (Tsen *et al*., 2007). There are many immobilized supporting materials applied to entrap the probiotic cells and then supplemented in food products. Some investigations were reported and implemented using the materials from polysaccharide derivatives, gelling agent, fruit pieces, etc., while mostly, probiotic survival number is higher than minimum probiotic count in probiotic foods (~5 log CFU/mL) after long period storage (Plessas *et al.*, 2005; Kourkoutas *et al.*, 2005, 2006a, 2006b; Ding and Shah, 2008; Moayednia *et al.*, 2009; Doherty *et al*., 2010; Phuapaiboon *et al*., 2013).

This study was aimed to apply the bacterial cellulose produced from different substrates including mature coconut water and mamao fruit residues by fermentation with Acetobacter xylinum TISTR 893 for probiotic immobilization and determine the probiotic survivability in mamao juice during refrigerated storage along with the product sensory analysis.

Materials and Methods

Microorganisms

Probiotic bacterial strain used in this study was *Lactobacillus plantarum* which was isolated from raw mamao fruits provided from Sakon Nakhon province, Thailand. The strain was identified using API 50 medium and interpreted as *Lactobacillus plantarum* via the APILAB plus program (Bio-Merieux, France).

Acetobacter xylinum TISTR 893 was used for bacterial cellulose production which was obtained from Microbiological Resource Center (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand.

Preparation of bacterial cellulose

Acetobacter xylinum was inoculated into the sterile glucose medium (Hestrin and Schramm, 1954). The medium consisted (w/v) of 2.0% glucose, 0.5% yeast extract, 0.5% peptone, 0.115% citric acid and 0.27% K2HPO4. The pH of medium was adjusted to 4.2 with glacial acetic acid. The inoculated medium was then incubated at 37°C for 7 days before scaling up.

The scaling up media used in this study were mature coconut water and mamao fruit residues. For mature coconut water preparation, it was collected from several mature coconuts and stored at 4°C before used for the experiments. For mamao fruit residues, they were prepared using the method reported by Rani and Appaiah (2013). Mamao fruit residues were collected from Sakon Nakhon province, Thailand. Then, they were cleaned, dried and ground to 50 mesh size. Mamao dried powder

was then mixed with distilled water at a ratio of 1:10 (w/v) using stirrer for 30 minutes and the slurry was filtered through muslin cloth. The mamao filtrate was stored under refrigeration (below 4°C) before used for the experiments.

The substrates were then supplemented with 0.5% (w/v) ammonium sulfate and the medium pH was adjusted to 4.2 with glacial acetic acid. The total soluble solids of the medium were adjusted to 10° Brix using hand held refractometer and all substrates were sterilized using autoclave at 121°C for 15 minutes. They were subsequently used for bacterial cellulose production. The starter (10%, v/v) was added to scaling up substrates and they were then incubated for 14 - 21 days at 30°C.

After incubation, the bacterial cellulose pellicles were formed about 1.0 cm. thick and these pellicles were harvested and washed by distilled water to eliminate sour taste and cut into small cubes of equal dimension as 1.0 cm^3. The bacterial cellulose cube samples were washed again to remove the medium or bacterial residues and the cubes were sterilized using autoclave at 121°C for 15 minutes before used for the experiments.

Preparation of free and immobilized probiotic cells

The probiotics were inoculated into Man Rogosa and Sharpe (MRS) broth and incubated at 37°C for 24 hours. The cells were centrifuged at 3,000×g for 10 minutes. The pellet cells were washed three times with sterile saline solution (0.85%, w/v). These probiotic cells were used as free cells and immobilized cells within supports.

Probiotic immobilization was achieved by resuspending the cells in sterile saline solution. Then, the prepared bacterial cellulose cubes (1.0 cm^3) were mixed with probiotic cells suspension and incubated at 37°C for 12 hours or overnight without agitation. At the end of the incubation period, the immobilized bacterial cellulose cubes were aseptically removed and washed by sterile saline solution for three times and the samples were kept under cold storage (4°C) before further investigations.

Probiotics in mamao juice

For mamao juice preparation, the mamao fruits were purchased from Sakon Nakhon province, Thailand. The mamao juice was extracted from fresh mamao fruits and the total soluble solids were adjusted to ~16 °Brix. After that, they are pasteurized at 80°C for 20 minutes and immediately cooled down and stored in refrigerator at 4°C before use. The probiotic cells were resuspended in pasteurized mamao juice to achieve 10% (v/v) probiotics (with cell viability more than 8 log CFU/ml) in mamao juice. The different immobilized cubes at about 15% (w/v) were added to sterile glass bottle (320 ml) of pasteurized mamao juice and then stored at 4°C for 4 weeks (Champagne and Gardner, 2008).

Probiotics enumerations

The counts of probiotic survival cells were determined following method reported by Jagannath *et al*. (2010). Ten grams of immobilized Nata cubes were blended with 90 ml of 0.85% (w/v) sterile saline solution in a stomacher for 2 minutes and serially diluted in saline solution. The suitable diluted samples were pipetted on plates and MRS agars were poured and incubated at 37°C for 48 hours under aerobic conditions. For free probiotic cells enumerations (control treatment), 10 ml of mamao juice samples were mixed with 90 ml of 0.85% (w/v) sterile saline solution for 2 minutes and serially diluted in saline solution. The MRS agar was poured in plates and incubated at 37°C for 48 hours under aerobic conditions. The survivals of probiotic cells were determined during storage every 4 days for 28 days. The bacterial counts were expressed as mean log colony forming units (log CFU) per gram or ml of sample.

Determinations of total soluble solids, % titratable acidity, and pH value

The total soluble solids were determined with a handheld refractometer at 20°C, pH value was measured with a pH meter and % titratable acidity (expressed as citric acid) was determined by diluting each 5 ml aliquots of mamao juice samples in 95 ml of distilled water and then titrating to pH 8.2 with 0.1 mol/L NaOH (Ayala-Zavala *et al*., 2004).

Determination of total phenolic content, anthocyanin content and antioxidant activities

The total phenolic content (TPC) of the methanolic extracts was determined using the Folin-Ciocalteu reagent (Butkhup and Samappito, 2011a; 2011b). Fruit juice sample (200 ml) was dissolved in the Folin–Ciocalteu reagent (1,000 ml) and distilled water (1,000 ml) and then mixed thoroughly for 3 minutes. After that, 7.5% (w/v) sodium carbonate solution (0.8 ml) was added. The mixtures were agitated with a vortex mixer and allowed to stand for a further 30 minutes in the dark. The absorbances of extracts and a prepared blank were set and measured at 765 nm using a spectrophotometer. The measurement was compared to a standard curve of prepared gallic acid solutions (0–250 mg/l range), and their absorbances were recorded at 765 nm and

expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight basis, which was determined from known concentrations of gallic acid standard prepared similarly.

Total anthocyanin contents in mamao juice samples were determined using the pH differential method (Butkhup and Samappito, 2011a; 2011b). The absorbance was measured by spectrophotometer at 523 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{523} - A_{700})$ pH 1.0 - $(A_{523} - A_{700})$ pH 4.5] with molar extinction coefficients of cyanidin-3-glucoside (26900) for fruit sample. The results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of dry weight.

Antioxidant activity was determined using DPPH assay or free-radical scavenging activity using the free radical DPPH• assay described by Puangpronpitag *et al.* (2008). The mamao juice samples were previously diluted in deionized water in order to get a good result, owing to the high antioxidant capacity of each one. One half ml of either ma-mao juice samples (0.02 to 10 µg/ml) was added to 0.5 ml of a 0.1 mM 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol. The reaction mixture was mixed and left to stand for 30 minutes in the dark. Absorbance at 517 nm was determined against a blank. The concentrations of trolox and mamao juice samples tested were 0.1 to 10 and 0.02-2 µg/ml, respectively. Results were expressed as µg/mL trolox equivalent per gram dry weight.

Sensory evaluation

Sensory assessment of mamao juice samples was also investigated following method reported by Walkling-Ribeiro *et al.* (2010). The mamao juice samples were refrigerated, randomly coded and served at 15°C in aliquots of 20 ml together with non-salted table biscuits and plain water to panelists placed separately in booths for unbiased evaluation of sensory attributes. Sensory evaluation was assessed by a panel of 30 untrained members (Majumdar *et al*., 2011) and comparisons among mamao juice samples were carried out to estimate product-specific appearance, texture, odor, acidity, sweetness and overall liking on a 9 point hedonic scale.

Statistical analysis

The study of probiotic survivability and physiochemical properties had been statistically planned as completely randomized design (CRD) whereas the sensory analysis had been statistically planned as randomized complete block design (RCBD). The experiments were repeated three times and mean value ± standard deviations were presented. Data analyses were performed by ANOVA (analysis of variance) and computed by SPSS statistical software program for Windows version 19 (SPSS, Chicago, Ill., U.S.A.). Duncan's new multiple range test (DMRT) was used to determine significant differences among results and statistical significance was accepted at 95% probability ($p \leq 0.05$).

Results and Discussion

Probiotic survivability

Selected probiotic strain based on its highest survivability in mamao juice during storage at 4°C for 14 days and subsequently identified as *L. plantarum* was used to immobilize within suitable bacterial cellulose cubes produced from mature coconut water with its pH, total soluble solids (°Brix) and % titratable acidity (as citric acid) of 5.5, 4.5 and 0.48 respectively and mamao fruit residues. The survivals of *L. plantarum* in mamao juice samples with free cells (control) and immobilized cells within different bacterial cellulose cubes from mature coconut water and mamao fruit residues were determined every four days during storage at 4°C and the results were shown in Figure 1. From Figure 1, mamao juice samples supplemented with free *L. plantarum* cells (control) had rapidly declined in numbers during storage whereas the ones supplemented with immobilized *L. plantarum* cells had slowly declined in numbers.

According to Table 1, the numbers of free *L. plantarum* cells and immobilized ones within bacterial cellulose cubes produced from mature coconut water and mamao fruit residues in mamao juice samples were 8.14, 8.35 and 8.29 log CFU/ml respectively on day 0 of storage. Interestingly, it was found that after 28 days of storage, free *L. plantarum* cells in mamao juice samples were greatly decreased in numbers to 0.76 log CFU/ml while the immobilized ones within bacterial cellulose cubes produced from mature coconut water and mamao fruit residues were slowly decreased to 6.41 and 6.32 log CFU/ml respectively which were not significantly different ($p>0.05$) and still higher than the minimum therapeutic dose (5 log CFU/ml).

Lee and Salminen (1995) noted that the probiotic properties of desirable bacteria were largely dependent on their ability to remain viable and to colonize the surface of human intestinal cells. Also, sufficient numbers of viable bacteria must be present at the time of consumption. A concentration of $1x10^5$ CFU per g or ml of the final product has been suggested as the "therapeutic minimum".

L. plantarum cells could attach and live within or onto the fibrous structure of bacterial cellulose

Table 1. Changes in viable count, pH, % titratable acidity and total soluble solids of mamao juice samples supplemented with free and immobilized *L. plantarum* cells within bacterial cellulose from coconut water and mamao fruit residues stored at 4°C, on day 0 and 28

Analysis	Day	Mamao juice samples		
		free *L. plantarum* cells	*L. plantarum* immobilized within BC from mature coconut water	*L. plantarum* Immobilized within BC from mamao fruit residues
Viable count (log CFU/ml)	0	8.14±0.10^{a,y}	8.35±0.49^{a,x}	8.29±0.05^{a,x}
	28	0.76±0.10^{b,y}	6.41±015^{b,x}	6.32±0.06^{b,x}
pH value	0	3.13±0.11^{a,x}	3.15±0.12^{a,y}	3.22±0.01^{a,z}
	28	3.11±0.01^{a,x}	3.18±0.01^{a,y}	3.20±0.01^{a,z}
%Titratable acidity (as citric acid)	0	2.28±0.04^{a,x}	2.04±0.05^{a,y}	2.05±0.06^{a,y}
	28	2.07±0.01^{b,x}	1.91±0.02^{b,y}	1.97±0.00^{b,y}
Total soluble solid (°Brix)	0	16.53±0.15^{a,x}	15.80±0.10^{a,y}	15.83±0.15^{a,y}
	28	16.87±0.12^{a,x}	15.87±0.12^{a,y}	16.07±0.12^{a,y}

Values represent the mean of triplicate±SD.
Values with different letters (a-b) within the same column differ significantly ($p \leq 0.05$).
Values with different letters (x-z) within the same row differ significantly ($p \leq 0.05$).

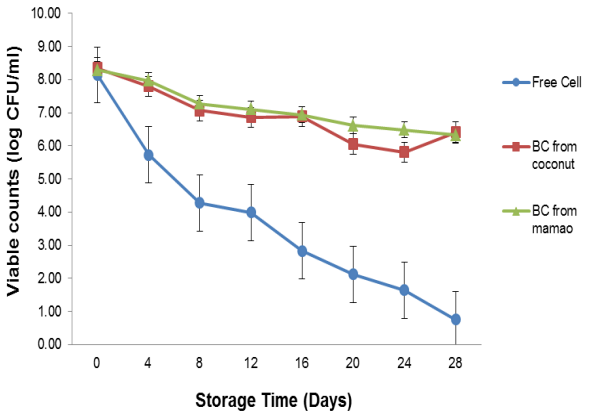

Figure 1. Viable counts of *L. plantarum* in mamao juice samples supplemented with free and immobilized *L. plantarum* cells within bacterial cellulose (BC) from coconut water and mamao fruit residues during 28 days storage at 4°C

without chemical bonding or physical adsorption with carrier support. The cellulose ribbons were overlapped, inter twisted and parallel formed but disorganized and had the potential to hold the bacterial cells in the spaces and also on the surface (Jagannath *et al.*, 2010) to enhance their survivability.

Kylä-Nikkilä *et al*. (2010) described that cellulose is an excellent matrix for immobilization purposes because it does not require chemical modifications and is commercially available in many different forms at low price. Jagannath *et al.* (2010) noted that immobilization technique within bacterial cellulose could be used as a supporting material for probiotic bacteria and it had a good potential as a cryoprotectant. Nata or bacterial cellulose with its fibrous structure could be a physical barrier to deleterious freezing effects and provided an attachment matrix for the lactic acid bacteria.

Saarela *et al*. (2006) described that various dietary fiber had been suggested that they can protect the probiotic cells by a mechanism involving the physical cells immobilization onto the fiber during processing and storage of food. Kourkoutas *et al.* (2006b) reported that the pieces of apple and pear had ability to improve probiotic survivability especially *L. casei* by using immobilization technique because the cellulosic structures of these fruits were not digested and could resist to improper condition in cheese product during making and ripening processes. In addition, Kourkoutas *et al.* (2006a) had proved a possible effect of these fruit pieces to protect probiotic during delivery through the intestinal tract to the colon.

Phuapaiboon *et al.* (2013) reported that the segments of fruit, tuber crop segments of pineapple, tuber crop segments of yam bean, and dietary fiber (jerusalem artichoke) were inexpensive material used for probiotic cells immobilization and they could support growth and viability of probiotic microorganisms. The immobilized *Lactococcus lactis* cells within pineapple segments, jelusalem artichoke (JA) powder, and yam bean segments were increased in viability when compared to free cells treatment during storage of yogurt with the cells viability decrease as 43.77%, 63.62%, 80.11%, and 87.14% respectively after storage at 4°C for 35 days.

pH, % titratable acidity and total soluble solids

The pH, % titratable acidity and total soluble solid values of mamao juice samples stored at 4°C on day 28 were shown in Table 1. In addition, their original pH, % titratable acidity (as citric acid) and total soluble

solid (°Brix) were 3.14, 1.91 and 9.80 respectively. According to Table 1, pH values of the mamao juice samples were not significantly different between day 0 and 28 of storage period. After 28 days of storage, pH value of mamao juice sample supplemented with free probiotic cells had the lowest value when compared to immobilized probiotic cells within bacterial cellulose cubes produced from mature coconut water and mamao fruit residues which were 3.12, 3.18 and 3.20 respectively ($p \leq 0.05$).

Ding and Shah (2008) had determined the physical changes in the fruit juice samples supplemented with free probiotic cells and encapsulated ones and monitored each physical parameter such as pH, total soluble solid (^{o}Brix), and organic acid content before and after the addition of probiotic organisms during six weeks of storage. The results showed that the pH of the apple and orange juice were reduced when tested with the probiotic microorganisms in a free or encapsulated state and after the end of the six weeks storage period, the juice sample with encapsulated probiotics had higher pH value than the samples inoculated with free probiotic bacteria.

The viable or non-viable probiotic cells may utilize or release the enzymes for hydrolyzing carbohydrates especially sugars in the fruit juice and produce amounts of organic acids for lowering the pH of product during storage. These results also showed that the juice samples had a more stable environment when they were supplemented with encapsulated probiotic organisms.

Pereira *et al.* (2013) had studied the stability of probiotics in cashew apple juice during cold storage (4°C) for 42 days and determined the product quality such as sugars, organic acids, color and antioxidant activity of the sweetened and non-sweetened probiotic juice. In case of the changes of pH value, it was reported that the pH value of the probiotic cashew apple juices was reduced since *L. casei* was able to utilize some sugar in fruit juice and produced acid even at low temperatures.

Ying *et al*. (2013) described that the pH of the apple juice with spray dried microencapsulated *Lactobacillus rhamnosus* GG changed a little when stored at 4°C for 5 weeks. Antunes *et al.* (2013) studied the physicochemical changes (pH, °Brix and organic acid content) of a microencapsulated probiotic *(B. animalis)* in cellulose acetate phthalate, added to acerola nectar stored at 5°C for a total of 35 days of storage. Due to the wall material of the microparticles, the samples supplemented with microencapsulated probiotics showed higher pH values when compared to both control (no added probiotic) and free cells treatments and the pH values were 3.60± 0.02, 3.44±0.02 and 3.44±0.02 respectively ($p \leq 0.05$).

Rodrigues *et al.* (2012) investigated the chemical stability of fruit juices (orange and peach) supplemented with free cells and encapsulated *Lactobacillus paracasei* L26 stored at 5°C over 50 days. The results showed that pH of both fruit juice samples with free cells and encapsulated *L. paracasei* L26 were decreased during 50 days of storage and the pH of peach juice decreased more than the one of orange juice. In addition, the pH in both fruit juice samples supplemented with free cells of *L. paracasei* L26 decreased more than the samples with encapsulated *L. paracasei* L26.

Nualkaekul *et al.* (2012a) studied the effect of multi-layer coating of alginate beads on the survival of encapsulated *Lactobacillus plantarum* in simulated gastric solution and pomegranate juice during storage at 4^{o}C. The results showed that the coating beads with chitosan could increase probiotic protection and pH value of the juice sample. In case of the pH value, the samples with uncoated, single coated and double coated alginate beads had increased in the pH value from 3.2 to 3.4 and 3.6 respectively. The pH values of the samples were a bit changed during storage for 6 weeks.

Moreover, % titratable acidity (as citric acid) in mamao juice samples was slightly declined with significant difference between day 0 and 28 of storage ($p \leq 0.05$). Also, % titratable acidity of mamao juice samples supplemented with free probiotic cells had higher value when compared to immobilized probiotic cells within bacterial cellulose cubes from mature coconut water and mamao fruit residues which were 2.07, 1.91 and 1.97 respectively ($p \leq 0.05$) after 28 days of storage.

Ding and Shah (2008) had demonstrated the quantity changes of organic acid (malic acid) in orange and apple juices supplemented with free and encapsulated probiotic bacteria by HPLC method and the results showed that small quantities of malic acid were produced by probiotic bacteria and the juice sample supplemented with encapsulated probiotic bacteria had malic acid less than free probiotic bacteria treatment.

Antunes *et al.* (2013) described that after 35 days of storage, the total titratable acidity was not significantly different for acerola nectar supplemented with free and microencapsulated *Bifidobacterium animalis* cells and control (no probiotic added) and the mean results were 0.28± 0.00, 0.29±0.01 and 0.28±0.01 g 100 g^{-1} respectively. Pereira *et al.* (2013) had reported that some of the organic acid from sugar utilization such as lactic acid could increase in

Table 2. Changes in total phenolic content, anthocyanin content and antioxidant activities of mamao juice samples supplemented with free and immobilized *L. plantarum* cells within bacterial cellulose from mature coconut water and mamao fruit residues stored at 4°C, on day 0 and 28

Analysis	Day	Mamao juice samples		
		L. plantarum free cells	*L. plantarum* Immobilized within BC from mature coconut water	*L. plantarum* Immobilized within BC from mamao fruit residues
Total phenolic content (gallic acid (mg/g))	0	7.22±0.19 ^{a,x}	6.30±0.18 ^{a,x}	6.48±0.17 ^{a,x}
	28	5.50±0.05 ^{b,x}	4.51±0.11 ^{b,x}	4.59±0.09 ^{b,x}
Total anthocyanin content (TAC (mg/100ml))	0	37.91±0.67 ^{a,x}	32.87±0.91 ^{a,y}	33.19±0.88 ^{a,y}
	28	8.34±0.59 ^{b,x}	7.10±0.18 ^{b,y}	7.11±0.11 ^{b,y}
Antioxidant activity (DPPH trolox (mmol/g))	0	20.98±0.54 ^{a,x}	17.90±0.37 ^{a,y}	18.54±0.11 ^{a,y}
	28	19.36±0.02 ^{b,x}	16.41±0.34 ^{b,y}	16.75±0.29 ^{b,y}

Values represent the mean of triplicate±SD.
Values with different letters (a-b) within the same column differ significantly ($p \leq 0.05$).
Values with different letters (x-y) within the same row differ significantly ($p \leq 0.05$).

cashew apple juices supplemented with *L. casei* after 42 days of storage.

For total soluble solids, there were no significant differences between day 0 and 28 of storage ($p>0.05$). After 28 days of storage, total soluble solids of mamao juice sample supplemented with free probiotic cells had the highest value when compared to the ones supplemented with immobilized probiotic cells within bacterial cellulose cubes produced from mature coconut water and mamao fruit residues which were 16.87, 15.87 and 16.07 respectively ($p \leq 0.05$).

Ding and Shah (2008) reported that after 6 weeks of storage, the final total soluble solids (oBrix) of apple and orange juice samples with encapsulated probiotic bacteria were higher than the juice sample with free probiotic bacteria. On the contrary, the juice sample without probiotic bacteria had a little bit change in the total soluble solid only a shift of 0.1% oBrix and this shift occurred in the late stages of storage period. This study suggested that both of juice samples with free probiotic bacteria could more readily utilize some of the sugars in the juice samples when compared to juice samples with encapsulated probiotics.

Nualkaekul *et al.* (2011a) had studied the survival of Bifidobacterium longum NCIMB 8809 during refrigerated storage for 6 weeks in model solutions and the results were described that the chemical changes such as pH and citric acid in the juice samples were not significantly changed ($p>0.05$) because the juice samples produced small amounts of lactic acid (from 0.1 to 0.8 g/l) and acetic acid. In addition, the total sugar concentration (expressed as the sum of sucrose, glucose and fructose concentrations) increased in most cases between 1 and 13 g/l. However, smaller decreases were observed for the inoculated juice samples compared to their respective non inoculated juice samples.

Antunes *et al.* (2013) reported that the soluble solids contents of acerola nectar had remained constant for samples supplemented with free and microencapsulated Bifidobacterium animalis cells and control (no probiotic added) as a mean value of 11.9±0.08°Brix after 35 days of storage. Pereira *et al.* (2013) described that some of sugar contents in the probiotic cashew apple juice (glucose, fructose, and sucrose) was decreased while *L. casei* viability was increased along the whole storage period for 42 days. Ding and Shah (2009) reported that the encapsulated cells probably limited the diffusion of sugars into the capsule by the membrane especially the capsule with double coating.

Total phenolic content, anthocyanin content and antioxidant activities

The phytochemicals of mamao juice samples were analyzed such as total phenolic and total anthocyanin contents and antioxidant activities which were analyzed on day 0 and 28 of storage and the results were shown in Table 2. According to Table 2, mamao juice samples with free *L. plantarum* cells and the ones supplemented with immobilized probiotic cells within bacterial cellulose cubes produced from mature coconut water and mamao fruit residues were not significantly different in total phenolic content ($p>0.05$). However, there was a significant difference in total phenolic content ($p \leq 0.05$) between day 0 and 28 of storage period.

Gyawali and Ibrahim (2012) reported that various functional components such as phenolic compounds, antioxidants, and micronutrients promoted the growth of probiotics (growth-promoting factors)

Table 3. Sensory assessment of mamao juice samples supplemented with free and immobilized *L. plantarum* cells within bacterial cellulose from mature coconut water and mamao fruit residues using a 9 point hedonic scale

Sensory Attributes	Mamao juice samples		
	free *L. plantarum* cells	*L. plantarum* Immobilized within BC from mature coconut water	*L. plantarum* Immobilized within BC from mamao fruit residues
Appearance	7.07±1.14[a]	7.23±1.19[a]	6.87±1.53[a]
Texture	6.03±1.71[b]	6.57±1.73[ab]	6.90±1.42[a]
Odor	6.37±1.56[a]	6.03±1.71[a]	6.37±1.49[a]
Acidity	5.70±2.09[a]	6.16±1.55[a]	6.37±1.43[a]
Sweetness	5.47±2.03[a]	5.80±1.88[a]	6.10±1.60[a]
Overall liking	5.83±1.5[b]	6.33±1.69[ab]	6.87±1.25[a]

Values with different letters (a-b) within the same row differ significantly ($p \leq 0.05$).

by creating suitable growth environment for these bacteria. For examples, the phenolic compounds and anthocyanin pigments which are present in berry fruits have been shown to enhance growth of the probiotic (lactobacilli and bifidobacteria). The phenolic compounds could be metabolized by probiotic bacteria especially *Lactobacillus* sp. and the stimulating effect could favor the greater growth rate and get more cell densities.

In case of total anthocyanin content, mamao juice samples with free *L. plantarum* cells had total anthocyanin content more than the ones supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from mature coconut water and mamao fruit residues ($p \leq 0.05$). Also, there were significant differences in total anthocyanin content of mamao juice samples between day 0 and day 28 of storage period ($p \leq 0.05$). However, the total anthocyanin content of mamao juice samples with free *L. plantarum* cells was reduced more than the ones supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from mature coconut water and mamao fruit residues ($p \leq 0.05$). Free cells were freely exposed to anthocyanin; thus, they could metabolize anthocyanin much more than the immobilized ones. In addition, mamao juice samples with free *L. plantarum* cells had the value of antioxidant activity by DPPH assay more than the ones supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from coconut water and mamao fruit residues ($p \leq 0.05$). Free cells were exposed to phenolic compounds freely so they could metabolize those compounds to others with antioxidant activity much more than the immobilized ones. Also, there were significant differences in values of antioxidant activity by DPPH assay of mamao juice samples between day 0 and day 28 of storage period ($p \leq 0.05$). It was noted that antioxidants favored the growth of probiotic bacteria as growth-promoting factors; therefore, their content was significantly reduced after 28 days storage (Gyawali and Ibrahim, 2012).

Nualkaekul *et al.* (2012b) described that there were various factors affecting the probiotic cell survivability in fruit juice such as water activity and pH but not include citric acid, dietary fiber and total phenolic concentrations which did not have any significant effect on probiotic cells in fruit juice. Pereira *et al.* (2013) reported that the antioxidant properties of cashew apple juice fermented with *L. casei* was increased and preserved when compared to nonfermented juice along the storage period because the total phenolic content had a similar trend with antioxidant activities and there was a positive correlation between total phenolic content and antioxidant activity.

Sensory evaluation

Sensory evaluation was performed by a panel of 30 untrained members (Majumdar *et al*., 2011) and a comparison among mamao juice samples were carried out to estimate product-specific appearance, texture, odor, acidity, sweetness and overall liking on a 9 point hedonic scale (Table 3). According to Table 3, all mamao juice samples were not significant different in appearance, odor, acidity and sweetness ($p > 0.05$). In addition, mamao juice samples supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from mamao fruit residues and mature coconut water were also not significantly different in texture and overall liking scores ($p > 0.05$) but these scores were significantly higher than those of the one supplemented with free cells ($p \leq 0.05$).

Conclusion

The bacterial cellulose produced from mamao fruit residues via fermentation by Acetobacter xylinum could be used as supporting materials for *L. plantarum* cells immobilization similar to the one from coconut water. Also, immobilized *L. plantarum* cells could survive better than free *L. plantarum* cells during storage at 4°C for 28 days and the numbers of *L. plantarum* were still higher than the minimum therapeutic dose (≥5 log CFU/ml) at the end of storage. Moreover, all mamao juice samples were not significant different in appearance, odor, acidity and sweetness ($p>0.05$). Surprisingly, mamao juice samples supplemented with immobilized *L. plantarum* cells within bacterial cellulose cubes from mamao fruit residues and mature coconut water were not significantly different in texture and overall liking scores ($p>0.05$) but these scores were significantly higher than those of the one supplemented with free cells ($p\leq0.05$). Thus, it is very likely to apply bacterial cellulose produced from agricultural byproducts via fermentation as the supporting materials for commercial probiotic immobilization which could be one of the alternative choices for probiotic or functional foods.

Acknowledgments

I gratefully acknowledge Post harvest Technology Innovation Center Research Fund (scholarships for general support, Doctoral Degree), Department of Agricultural Engineering, Faculty of Engineering, Khon Kaen University, Thailand and another support fund from School of Graduate Studies, Khon Kaen University, Thailand (scholarships for general support, Doctoral Degree).

References

Antunes, A. E. C., Liserre, A. M., Coelho, A. L. A., Menezes, C. R., Moreno, I., Yotsuyanagi, K. and Azambuja, N. C. 2013. Acerola nectar with added microencapsulated probiotic. LWT - Food Science and Technology 54: 125-131.

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y. and González-Aguilar, G. A. 2004. Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. LWT - Food Science and Technology 37: 687-695.

Butkhup, L. and Samappito, S. 2008. An analysis on flavonoids contents in mao luang fruits of fifteen cultivars *(Antidesma bunius)*, grown in northeast Thailand. Pakistan Journal of Biological Sciences 11(7): 996-1002.

Butkhup, L. and Samappito, S. 2011a. Change in physico-chemical properties, polyphenol compounds and antiradical activity during development and ripening of mao luang (*Antidesma bunius* Linn.) fruits. Journal of Fruit and Ornamental Plant Research 19(1): 85-99.

Butkhup, L. and Samappito, S. 2011b. Phenolic constituents of extract from mao luang seed and skin-pulp residue and its antiradical and antimicrobial capacities. Journal of Food Biochemistry 35(6): 1671-1679.

Castro, C., Zuluaga, R., Putaux, J. L., Caro, G., Ĩnaki, M. and Ga˜nan, P. 2011. Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes. Carbohydrate Polymers 84(1): 96–102.

Champagne, C. P. and Gardner, N. J. 2008. Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses. Food Research International 41: 539-543.

Chawla, P. R., Bajaj, I. B., Survase, S. A. and Singhal, R. S. 2009. Microbial cellulose: fermentative production and applications. Food Technology and Biotechnology 47(2): 107–124.

Ding, W. and Shah, N. P. 2008. Survival of free and microencapsulated probiotic bacteria in orange and apple Juices. International Food Research Journal 15: 219-232.

Ding, W. K. and Shah, N. P. 2009. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. Journal of Food Science 74: M53-M61.

Doherty, S. B., Gee, V. L., Ross, R. P., Stanton, C., Fitzgerald, G. F. and Brodkorb, A. 2010. Efficacy of whey protein gel networks as potential viability-enhancing scaffolds for cell immobilization of *Lactobacillus rhamnosus* GG. Journal of Microbiological Methods 80(3): 231-241.

Goktepe, I., Juneja, V. K. and Ahmedna, M. 2006. Probiotics in food safety and human health. London: Taylor and Francis Group.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F. and Shah, N. P. 2010. Probiotic dairy products as functional foods. Comprehensive Reviews in Food Science and Food Safety 9(5): 437–599.

Gyawali, R. and Ibrahim, S. 2012. Impact of plant derivatives on the growth of foodborne pathogens and the functionality of probiotics. Applied Microbiology and Biotechnology 95: 29-45.

Hestrin, S. and Schramm, M. 1954. Synthesis of cellulose by Acetobacter xylinum. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. Biochemical Journal 58: 345-352.

Ishibashi, N. and Yamazaki, S. 2001. Probiotics and safety. American Journal of Clinical Nutrition 73(Suppl. 2): 465S-470S.

Jagannath, A., Raju, P. S. and Bawa, A. S. 2010. Comparative evaluation of bacterial cellulose (nata) as a cryoprotectant and carrier support during the freeze drying process of probiotic lactic acid bacteria. LWT - Food Science and Technology 43: 1197-1203.

Keshk, S. and Sameshima, K. 2006a. Influence of

lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. Enzyme and Microbial Technology 40(1): 4–8.

Keshk, S. and Sameshima, K. 2006b. The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. Applied Microbiology and Biotechnology 72(2): 291–296.

Kourkoutas, Y., Xolias, V., Kallis, M., Bezirtzoglou, E. and Kanellaki, M. 2005. *Lactobacillus casei* cell immobilization on fruit pieces for probiotic additive, fermented milk and lactic acid production. Process Biochemistry 40(1): 411–416.

Kourkoutas, Y., Bosnea, L., Taboukos, S., Baras, C., Lambrou, D. and Kanellaki, M. 2006a. Probiotic cheese production using *Lactobacillus casei* cells immobilized on fruit pieces. Journal of Dairy Science 89(5): 1439-1451.

Kourkoutas, Y., Kanellaki, M. and Koutinas, A. A. 2006b. Apple pieces as immobilization support of various microorganisms. LWT - Food Science and Technology 39: 980-986.

Kylä-Nikkilä, K., Alakuijala, U. and Saris, P. E. J. 2010. Immobilization of *Lactococcus lactis* to cellulosic material by cellulose-binding domain of Cellvibrio japonicus. Journal of Applied Microbiology 109: 1274-1283.

Lee, Y. K. and Salminen, S. 1995. The coming of age of probiotics. Trends in Food Science and Technology 6: 241-245.

Majumdar, T. K., Wadikar, D. D., Vasudish, C. R., Premavalli, K. S. and Bawa, A. S. 2011. Effect of storage on physico-chemical, microbiological and sensory quality of bottlegourd-basil leaves juice. American Journal of Food Technology 6(3): 226-234.

Moayednia, N., Ehsani, M. R., Emamdjomeh, Z., Asadi, M. M., Mizani, M. and Mazaheri, A. F. 2009. The effect of sodium alginate concentrations on viability of immobilized *Lactobacillus acidophilus* in fruit alginate coating during refrigerator storage. Australian Journal of Basic and Applied Sciences 3(4): 3213-3226.

Nualkaekul, S., Deepika, G. and Charalampopoulos, D. 2012b. Survival of freeze dried *Lactobacillus plantarum* in instant fruit powders and reconstituted fruit juices. Food Research International 48: 627-633.

Nualkaekul, S., Lenton, D., Cook, M. T., Khutoryanskiy, V. V. and Charalampopoulos, D. 2012a. Chitosan coated alginate beads for the survival of microencapsulated *Lactobacillus plantarum* in pomegranate juice. Carbohydrate Polymers 90: 1281-1287.

Nualkaekul, S., Salmeron, I. and Charalampopoulos, D. 2011a. Investigation of the factors influencing the survival of *Bifidobacterium longum* in model acidic solutions and fruit juices. Food Chemistry 129: 1037-1044.

Okiyama, A., Motoki, M. and Yamanaka, S. 1992. Bacterial cellulose II. Processing of the gelatinous cellulose for food materials. Food Hydrocolloids 6(5): 479–487.

Pereira, A., Almeida, F., de Jesus, A., da Costa, J. and Rodrigues, S. 2013. Storage stability and acceptance of probiotic beverage from cashew apple juice. Food and Bioprocess Technology 6: 3155-3165.

Plessas, S., Bekatorou, A., Kanellaki, M., Psarianos, C. and Koutinas, A. A. 2005. Cells immobilized in a starch–gluten–milk matrix usable for food production. Food Chemistry 89(2): 175–179.

Phuapaiboon, P., Leenanon, B. and Levin, R. 2013. Effect of *Lactococcus lactis* immobilized within pineapple and yam bean segments, and jerusalem artichoke powder on its viability and quality of yogurt. Food and Bioprocess Technology 6: 2751-2762.

Puangpronpitag, D., Areejitranusorn, P., Boonsiri, P., Suttajit, M. and Yongvanit, P. 2008. Antioxidant activities of polyphenolic compounds isolated from *Antidesma thwaitesianum* Müll. Arg. seeds and marcs. Journal of Food Science 73: C648-C653.

Puangpronpitag, D., Yongvanit, P., Boonsiri, P., Suttajit, M., Areejitranusorn, P., Na, H. K. and Surh, Y. J. 2011. Molecular mechanism underlying anti-apoptotic and anti-inflammatory effects of mamao (*Antidesma thwaitesianum* MÜll. Arg.) polyphenolics in human breast epithelial cells. Food Chemistry 127(4): 1450–1458.

Rani, M. U. and Appaiah, K. A. A. 2013. Production of bacterial cellulose by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk. Journal of Food Science and Technology 50: 755-762.

Rodrigues, D., Sousa, S., Gomes, A., Pintado, M., Silva, J., Costa, P., Amaral, M., Rocha-Santos, T. and Freitas, A. 2012. Storage stability of *Lactobacillus paracasei* as free cells or encapsulated in alginate-based microcapsules in low pH fruit juices. Food and Bioprocess Technology 5: 2748-2757.

Ross, P., Mayer, R. and Benziman, M. 1991. Cellulose biosynthesis and function in bacteria. Microbiology Reviews 55(1): 35-88.

Saarela, M., Mogensen, G., Fonde´n, R. and Mattila-Sandholm, J. M. 2000. Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology 84(3): 197–215.

Saarela, M., Virkajärvi, I., Nohynek, L., Vaari, A. and Mättö, J. 2006. Fibres as carriers for *Lactobacillus rhamnosus* during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. International Journal of Food Microbiology 112: 171-178.

Samappito, S. and Butkhup, L. 2008. An Analysis on organic acids contents in ripe fruits of fifteen mao luang *(Antidesma bunius)* cultivars, harvested from dipterocarp forest of phuphan valley in northeast Thailand. Pakistan Journal of Biological Sciences 11(7): 974-981.

Skinner, P. O. and Cannon, R. E. 2000. *Acetobacter xylinum*: An inquiry into cellulose biosynthesis. The American Biology Teacher 62(6): 442-444.

Suresh, K. S., Krishnappa, S. and Bhardwaj, P. 2013. Safety concerns of probiotic use: A review. IOSR Journal of Dental and Medical Sciences 12(1): 56-60.

Tsen, J. H., Huang, H. Y., Lin, Y. P. and King, V. A. E. 2007.

Freezing resistance improvement of *Lactobacillus reuteri* by using cell immobilization. Journal of Microbiological Methods 70(3): 561–564.

Tuohy, K. M., Probert, H. M. Smejkal, C. W. and Gibson, G. R. 2003. Using probiotics and prebiotics to improve gut health. Drug Discovery Today 8(15): 692-700.

Usha-Rani, M. and Anu-Appaiah, K. A. 2013. Production of bacterial cellulose by *Gluconacetobacter hansenii* UAC09 using coffee cherry husk. Journal of Food Science and Technology 50(4): 755-762.

Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Lyng, J. G. and Morgan, J. 2010. Shelf life and sensory attributes of a fruit smoothie-type beverage processed with moderate heat and pulsed electric fields. LWT - Food Science and Technology 43(7): 1067-1073.

Wedajo, B. 2015. Lactic acid bacteria: benefits, selection criteria and probiotic potential in fermented food. Journal of Probiotics and Health 3(2): 1-9.

Ying, D., Schwander, S., Weerakkody, R. and Sanguansri, L. 2013. Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. Journal of Functional Foods 5: 98-105.