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Original Article

Survival of encapsulated potentially probiotic *Lactobacillus plantarum* D6SM3 with bioemulsifier derived from spent yeast in simulated gastrointestinal conditions

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Abstract

The effect of encapsulation with three kinds of emulsifier (Tween 80, gum arabic and bioemulsifier extracted from spent yeast) on the survival of *Lactobacillus plantarum* D6SM3 in simulated gastrointestinal tract during storage at 4°C and room temperature was investigated. The survival of all encapsulated cells treated in simulated gastric juice was higher than free cells at both pH 2.5 and 3.0. The viability of the free and encapsulated cells showed a gradual decline throughout the storage period at 4°C. However, the viability rapidly declined at room temperature. In addition, the droplet size distribution of encapsulated cells was compared between those with and without an emulsifier by using the laser diffraction method. The particle size and polydispersity value of encapsulated cells were controlled better in emulsion with emulsifier added. The surface of encapsulated cells with emulsifier exhibited smoother characteristics than those without emulsifier.

Keywords: bioemulsifier, spent yeast, encapsulation, probiotic, *Lactobacillus plantarum*

1. Introduction

Probiotics have been defined as "live microbial feed supplements that have beneficial effects on the host by improving their intestinal microbial balance" (Fuller, 1989). In order to bring about positive health effects, lactic acid bacteria (LAB) have to resist gastric juice and bile salts. However, studies have indicated that the bacteria may not survive in sufficient numbers after the LAB pass through the gastrointestinal tract (Hamilton-Miller, 1999). International standards recommended that probiotics should be at the level of 10^6-10^7 CFU/g in the product at the time of consumption (Ouwehand and Salminen, 1998). However, many products fail to meet these standards when they are consumed (Shah *et al.*, 1995).

Attempts have been made to improve the survival of LAB. Technologies that can protect the viability of probiotics during storage and gastrointestinal transit are highly sought after. Encapsulation in alginate beads is one method of improving their viability by retaining probiotics within a polymer membrane or matrix to reduce cell injury or cell loss. Two widely used methods of encapsulation are the extrusion and emulsion techniques. Emulsion technique has a smaller encapsulation cell, and provides advantages with regard to minimizing adverse impacts on texture and how they feel in the mouth when incorporated into food. The size of the beads is controlled by the speed of agitation (Krasaekoopt *et al.*, 2003). In some cases emulsifiers are added to form a better emulsion, because the emulsifier lowers the surface tension and results in smaller spheres. This can also provide

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more control over the particle size and good polydispersity (Adamson, 1982). The most common emulsifier used is Tween 80 (Sheu *et al.*, 1993). Moreover, the type of emulsifier has been reported to influence the shape of capsules (Krasaekoopt *et al.*, 2003).

The yeast strain *Saccharomyces cerevisiae* is normally used for alcohol fermentation. Generally, local Thai producers directly distill palm sugar wine without separate yeast cells to obtain spirit. After distillation a huge amount of waste containing yeast cells is discharged. This causes environmental problems. Bioemulsifier extracted from *S. cerevisiae* has been shown to be an effective emulsifier (Dikit *et al.*, 2010a, b; Dikit *et al.*, 2012). Bioemulsifier with hydrophilic glucose polymers covalently attached to the protein backbone provides the bioemulsifier with the amphiphilic structure common to surface active agents and effective emulsifier (Cooper and Goldenberg, 1987). A bioemulsifier obtained as a by-product of the wine or brewer's industry, is readily available, has a biodegradable nature and is not toxic and large scale production is possible. It makes it possible to produce value-added by-products (Torabisadeh *et al.*, 1996). Since *S. cerevisiae* is edible and is used in the manufacture of food and beverage products, it is assumed that a bioemulsifier would be non-toxic and generally recognized as safe (GRAS) (Cameron *et al.*, 1988).

The study reported in this paper evaluated the efficacy of a bioemulsifier extracted from spent yeast obtained from Thai traditional liquor distillation as an emulsifier. It was compared with commercial emulsifiers to assess whether it increased the survival rate of encapsulated potentially probiotics LAB strain, *Lactobacillus plantarum* D6SM3 when exposed to simulated gastrointestinal conditions. In addition, the survivability of the encapsulated cells during storage over 28 days at refrigerated and room temperatures was also evaluated.

2. Materials and Methods

2.1 Bacterial strain, growth condition and preparation of cell suspensions

Potentially probiotic LAB strain *L. plantarum* D6SM3 isolated from a fermented shrimp (Hwanhlem *et al.*, 2010) was the best strain that is resistant to simulated gastric juice at pH 2.5 and 3.0 and also resistant to 3 $mg.ml^{-1}$ of pancreatin and ox bile salts at pH 8.0. This strain was cultivated in MRS broth (Labscan Asia Co., Ltd., Thailand) at 37°C. It was checked for purity and maintained in MRS broth supplemented with 25% (v/v) glycerol at -20°C. For routine analysis, the strain was sub-cultured twice in MRS broth for 24 h at 37°C.

2.2 Extraction and partial purification of bioemulsifier

Distillate residues were obtained from local distillery in Songkhla Province, Thailand. It was centrifuged (SCR20B, Hitachi, Japan) at 6000×*g* for 10 min at 4°C. The yeast cells were washed twice in normal saline. Twenty percent (w/v) yeast cells were suspended in distilled water containing 0.1 M potassium citrate and 0.02 M potassium metabisulfite. The pH of the suspension was adjusted to 7 with 1M NaOH. The cell suspension was autoclaved (121°C) for 30 min (Dikit *et al.*, 2012). The resulting suspensions were centrifuged at 6,000×*g* for 10 min at 4°C.

The supernatant was retained and mixed with five times the volume of chilled ethanol, and incubated overnight at 4°C for complete precipitation. The suspension was centrifuged at 6,000×*g* for 10 min at 4°C. After centrifugation, the supernatant was discarded and the precipitate was washed twice with chilled ethanol. The precipitate was dried by rotary evaporator and freeze dried (Barriga *et al.*, 1999). Crude bioemulsifier (10g) from spent yeast was dissolved in 50 ml distilled water and dialysed (8,000 dalton molecular weight cut-off) against distilled water overnight. Finally, the partial purified bioemulsifier was lyophilized.

2.3 Encapsulation and enumeration of encapsulated *L. plantarum* D6SM3

The 8% starter culture of *L. plantarum* D6SM3 was inoculated into 50 ml MRS broth and incubated at 37°C for 24 h to obtain a cell density of about 10^{10} $cfu.ml^{-1}$. Harvesting of the cells was done by centrifugation at 6,500×*g* for 20 min at 4°C. Cell pellets were washed twice with sterile normal saline. Washed cells were then suspended in two mixtures of 1 ml of sterile normal saline, one without and one with 0.2% emulsifier (bioemulsifier, Tween 80 and gum arabic) and stored at 4°C until used.

L. plantarum D6SM3 was encapsulated in sodium alginate mixture by using a modified method of Sheu *et al.* (1993). Washed cells were prepared for encapsulation by the emulsion technique. A washed cell suspension was added to 20 ml of 2% sodium alginate and the mixture was then emulsified into 20 ml of palm oil (Morakot, Morakot Industry Co. Ltd., Thailand). The emulsion was produced through vigorous stirring for 5 min until it was creamy. A solution of 0.1 M calcium chloride (100 ml) was then added quickly along the side of the beaker and the emulsion was vigorously stirred for 5 min. The mixture was left for 30 min until the sodium alginate beads were separated and settled at the bottom of the calcium chloride layer. The oil layer was then removed and the beads were collected by centrifugation at 6,500×*g* for 20 min at 4°C. The beads were then rinsed with sterilized distilled water and filtered by sterile filter paper and stored in peptone saline (1 $g.l^{-1}$ peptone, 8.5 $g.l^{-1}$ sodium chloride) pending further analysis.

The encapsulated *L. plantarum* D6SM3 was enumerated as described by Annan *et al.* (2007). The encapsulated *L. plantarum* D6SM3 in the microcapsules were released using 1.0 g of a filtered microcapsule and were re-suspended in 9.0 ml of PBS buffer (pH 7.6) in a sterile plastic bag. It was homogenized for 10 min to allow complete

release of the bacteria from alginate capsules. The homogenized samples were diluted to appropriate concentrations and plated on MRS agar (Labscan Asia Co., Ltd., Thailand). The plates were incubated for 24 h at 37°C and the encapsulated bacteria were enumerated as cfu.ml^{-1}.

2.4 Survival assay of free and encapsulated *L. plantarum* D6SM3 in simulated gastric and small intestinal juices

Simulated gastric juice was prepared by dissolving pepsin (Fluka, USA) in phosphate buffer to a final concentration of 3 mg.ml^{-1} and the pH adjusted to 2.5 and 3.0 with 6M HCl. The mixture was sterized by membrane filtrate through a membrane (0.45 µm, Sartorius, Germany) (Maragkoudakis *et al.*, 2006).

Simulated small intestinal juice was prepared based on the method of Krasaekoopt *et al.* (2004). Pancreatin (Sigma, Germany) and ox bile salts (Difco, USA) were suspended in phosphate buffer to a final concentrations of 3 mg.ml^{-1}and 3 mg.ml^{-1}, respectively. The mixture was adjusted to a pH of 8.0 with 6 M NaOH and then sterile filtered.

Washed cell suspensions of *L. plantarum* D6SM3 (1.0 ml) or 1 g of encapsulated cells were added to either 9 ml of tempered (37°C) simulated gastric juice or simulated small intestinal juice. They were incubated at 37°C for 4 h (Maragkoudakis *et al.*, 2006).

The survival of the free cells and encapsulated *L. plantarum* D6SM3 before and after exposure to simulated gastric juice and simulated small intestinal juice was determined by plating in MRS agar. The plates were incubated at 37°C for 24 h modified from Madureira *et al.* (2005)

2.5 Survival assay of free and encapsulated *L. plantarum* D6SM3 after sequential incubation in simulated gastric juice and small intestinal juice

One gram of the encapsulated *L. plantarum* D6SM3 and 1 ml of non-encapsulated probiotic sample were incubated in 9 ml of simulated gastric juice (3 mg.ml^{-1} pepsin, pH 2.5) at 37°C for 4 h. Encapsulated beads and non-encapsulated *L. plantarum* D6SM3 in simulated gastric juice were then centrifuged at 6,500×*g* at 4°C for 20 min and washed with 0.85% sodium chloride. The free cells and capsules obtained were re-suspended in 9 ml of simulated small intestinal juice (3 mg.ml^{-1} pancreatin, 3 mg.ml^{-1} ox bile salts, pH 8.0) at 37°C for 4 h. The survival of the free cells and encapsulated *L. plantarum* D6SM3 before and after exposure to small intestinal juice for 4 h was determined by plating in MRS agar. Plating was incubated at 37°C for 24 h modified from Madureira *et al.* (2005).

2.6 Viability of free and encapsulated *L. plantarum* D6SM3 during storage

One milliliter of free and 1 g of encapsulated *L. plantarum* D6SM3 in 9 ml of peptone saline were stored at room temperature and 4°C. They were assessed periodially for 0, 1, 2, 3 and 4 weeks to determine viability during the storage period. The enumeration of the viable probiotic bacteria in each experiment was performed as previously described.

2.7 Analysis of size and morphology of microcapsules

The droplet size distributions (DSD) of the microcapsules was determined by using a laser diffraction method (Mastersizer, 2000) as described by Hayati *et al.* (2007). Distilled water was used as the dispersant for the determination of the size distribution. The software used a reflective index of dispersant RI 1.33 (water) to calculate the Dispersion Index (Span) by span = d(90)-d(10)/d(50). The d(10), d(50) and d(90) values are size values corresponding to the cumulative distribution at 10%, 50% and 90%, respectively. Thus, the d(10) represents a size value below which 10% of the cumulative distribution is present. Microcapsules were introduced into the sample until the concentration reached the optimum one, as indicated by the instrument.

The morphology of the external structure of the encapsulated *L. plantarum* D6SM3 was observed by using the scanning electron microscope (SEM, JSM-5800LV, JEOL, Tokyo, Japan). This was analyzed at 10 kV acceleration voltage.

3. Results and Discussion

3.1 Survival of free and encapsulated *L. plantarum* D6SM3 in simulated gastric juice

Some properties of probiotics enabling them to survive and establish within the human gastrointestinal tract, including their ability to resist the very acidic pHs (pH 2.5 - pH 3.5) of the stomach (Hwanhlem *et al.*, 2010), should be studied. The survival of the free and encapsulated *L. plantarum* D6SM3 in simulated gastric juice after being kept for 4 h is shown in Figure 1. Food remain in the stomach for 2 to 4 h, while liquids can remain for only approximately 20 min (Annan *et al.*, 2007). The pH of the simulated gastric juice affected the survival of both free cells and the encapsulated *L. plantarum* D6SM3. The encapsulated *L. plantarum* D6SM3 survived well in simulated gastric conditions compared to the free bacterial cells because a densely cross-linked gel structure was formed. The alginate mixture stays structurally stable in low acid environments. As the pH is falls below the pKa values of mannuronic (3.6) and guluronic acid (3.7) the alginate is converted to alginic acid with the release of calcium ions and formation of a more dense gel (Doumeche *et al.*, 2004).

This suggested that better protection of bacterial cells was achieved with the encapsulated bacterial cells than with the free cells. The encapsulation technique protected the cells from the effect of both the pH and pepsin enzyme in simulated gastric juice. The survival of *L. plantarum* D6SM3

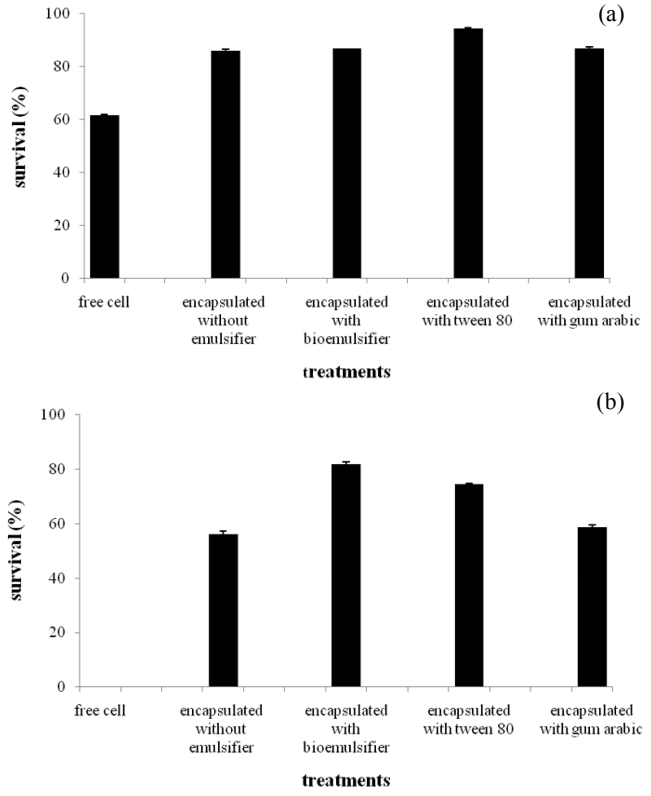

Figure 1. Survival of free and encapsulated probiotic *Lactobacillus plantarum* D6SM3 after exposure to simulated gastric juice kept at 37°C, and incubated at (a) pH 3.0 and (b) pH 2.5 for 4 h. Bars indicate the standard deviation from triplicate determinations.

in all encapsulated treatments was higher than in the free cells at the tested pH (Figure 1). The survival of *L. plantarum* D6SM3 in simulated gastric juice at pH 3.0 was higher than that at pH 2.5. There was about a 4-log decrease in viable free cells in pH 3.0 and no survival was observed when the pH of simulated gastric juice decreased to 2.5.

In addition, it was found that among the three types of emulsifier, Tween 80 exhibited higher survival than other types of emulsifier at pH 3.0. At pH 2.5, adding emulsifier resulted in remarkably higher survival than that of encapsulated cells without emulsifier. It also shown that bioemulsifier and Tween 80 were better than gum arabic at pH 2.5 as shown in Figure 1b. Bioemulsifier gave a similar result with Tween 80 because bioemulsifier not only acts as an emulsifier in the system but also may form extramembrane with alginate. Both properties of bioemulsifier could enhance stability of the bead in acid condition like Tween 80. Despite the suitability of alginate as the encapsulation matrix material, gel encapsulation in alginate has some limitation. A cross-linked alginate matrix system at very low pH is reported to undergo a reduction in alginate molecular weight causing a faster degradation and release of active ingredients (Krasaekoopt *et al.*, 2006). Therefore, special treatment, such as coating the bead with extramembrane, could be applied in order to improve the properties of encapsulated beads. Coated beads not only prevent cell release but also increase mechanical and chemical stability (Krasaekoopt *et al.*, 2003). This result suggested that the encapsulation technique could protect *L. plantarum* D6SM3 in the gastro environment. This result was in agreement with previous studies showing that encapsulation provided better protection of cells in an acid environment (Lee *et al.*, 2004; Musikasang *et al.*, 2009). As a result, alginate encapsulation may provide significant protection of cells from the acidic condition of simulated gastric juice, as alginate gels are stable in low pH solution.

3.2 Survival of free and encapsulated *L. plantarum* D6SM3 in simulated small intestinal juice

Bile salt tolerance is considered to be one of the essential properties for LAB to survive in the small intestine. Survival of free cells and encapsulated *L. plantarum* D6SM3 after exposure to 0.3% bile salt for 4 h, reflecting the time of food spent in the small intestine, is shown in Figure 2. It was found that the survival of non-encapsulated and encapsulated *L. plantarum* D6SM3 did not show a significant difference ($p>0.05$). It was noted that the survival of the non-encapsulated and encapsulated *L. plantarum* D6SM3 was reduced to some extent. They were resistant to pancreatin and oxgall bile salts. Even after 4 h of exposure to the small simulated intestinal juice at pH 8.0 they retained their viability with a little reduction (less than 1.0 log cfu.ml^{-1}). Bile salts did not affect *L. plantarum* D6SM3 since this strain was screened previously for probiotic properties, such as acid and bile salt tolerance (Hwanhlem *et al.*, 2010) except that using gum arabic as emulsifier reduced the viability about 2 log cfu.ml^{-1} due to the chemical properties of gum arabic. The quality of gum arabic available to act as an emulsifier becomes limiting. Gum arabic is also composed of a complex mixture of calcium, magnesium and potassium salts of arabic acid that cause instability of the bead (Kim *et al.*, 1996). Because calcium alginate is chemically unstable when

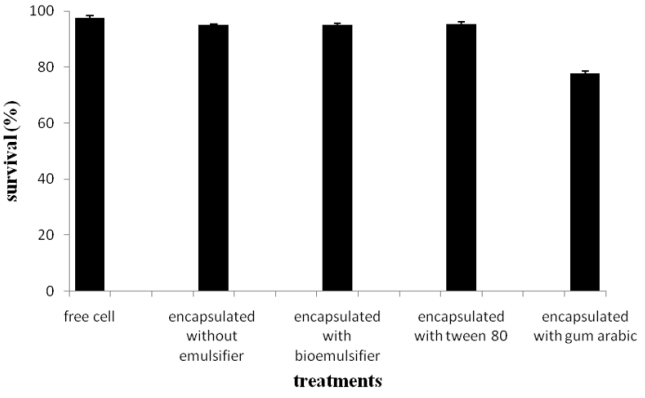

Figure 2. Survival of free and encapsulated *Lactobacillus plantarum* D6SM3 after exposure to simulated intestinal juice kept at 37°C, and incubated at pH 8.0 for 4 h. Bars indicate the standard deviation from triplicate determinations.

chelators such as phosphate and citrate and non-gelling cations such as sodium or magnesium ions are present (Lee *et al.*, 2004), the structure of the bead is weakened resulting in rapid release of the cell.

3.3 Survival assay of free and encapsulated *L. plantarum* D6SM3 after sequential incubation in simulated gastric juice and small intestinal juice

Several studies on acid and bile tolerance have been reported. An important difference in this present study is that bacterial cells were passed through acid and bile salt consecutively. This imitated the real situation in the gastro-intestinal condition. The survival of free cells and encapsulated *L. plantarum* D6SM3 in simulated small intestinal juice after being incubated with simulated gastric juice is shown in Figure 3. The same results are shown in either an acid or bile salt condition. The survival of *L. plantarum* D6SM3 in simulated gastric condition at pH 3.0 was higher than that at pH 2.5. There was about a 4-log decrease in the viable cells with free cells at pH 3.0, and no survival was observed when pH decreased to 2.5. However, encapsulated cell using Tween 80 as emulsifier showed the highest survival owing to its having higher stability of the bead. A number of factors would likely affect the ability of the material to function as emulsifier, i.e., emulsifier concentration, proportion of dispersed and dispersion phases, processing conditions and properties of the material to be encapsulated. These factors control their ability to interact via hydrogen bonding, van der Waals forces, dipole and electrostatic interactions, hydrophobic association and formation of covalent disulfide bonds are believed to affect their emulsification properties (Kim *et al.*, 1996). Tween 80 is the most common emulsifier used and may show higher stabilize after sequential incubation in simulated gastric juice and in simulated small intestinal juice. The encapsulation technique protected the cells from harsh conditions, that is an acidic condition. Sequential incubation affected the survival of free cells in both conditions and even encapsulated cells in exposed to simulated gastric juice at pH 2.5 (Figure 3). We can improve the stability of the bead using bioemulsifier as an emulsifier by optimizing the conditions used in the cell encapsulation process, such as proportion of dispersed and dispersion phases, co-encapsulation with other material that could help stabilize the bead. These may sufficient to produce stable bioemulsifier emulsions.

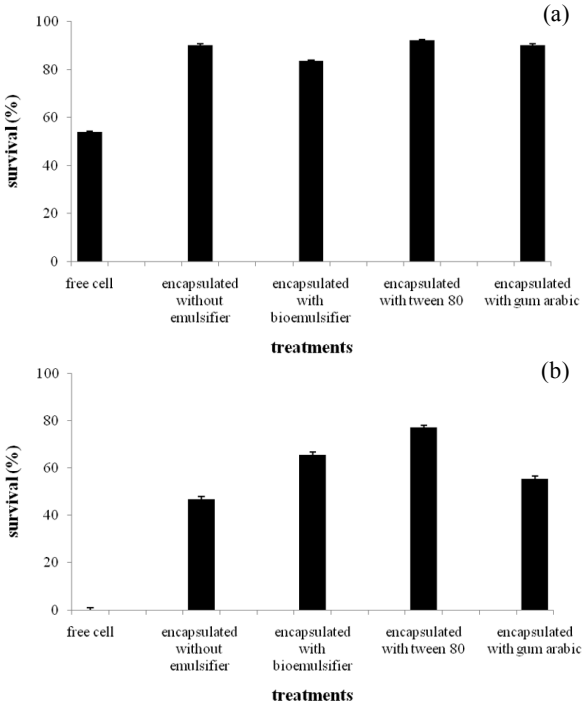

Figure 3. Survival of free and encapsulated *Lactobacillus plantarum* D6SM3 after exposure to simulated intestinal juice after being sequentially incubated with simulated gastric juice kept at 37°C, and incubated (a) pH 3.0 (b) pH 2.5 for 8 h. Bars indicate the standard deviation from triplicate determinations.

3.4 Viability of free and encapsulated *L. plantarum* D6SM3 during storage

Encapsulation of probiotic bacteria has been a common practice for expanding their shelf life (Krasaekoopt *et al.*, 2003). This study evaluated the stability of *L. plantarum* D6SM3 both free and encapsulated in sodium alginate during 4 weeks of storage in refrigerated and room temperature. The numbers of viable free and encapsulated cells declined gradually throughout the storage period at 4°C. The decrease was from 10 log cfu.ml^{-1} to about 8 log cfu.ml^{-1} after 4 weeks of storage (Figure 4a). When free cells and four kinds of emulsifier added to encapsulated cell were compared, it was found that the survival of the encapsulated cells was higher than that of the free cells. However, at room temperature the number of viable free and encapsulated cells declined more rapidly than at 4°C throughout the storage period. The decrease was from 10 log cfu.ml^{-1} to about 4 log cfu.ml^{-1} after 4 weeks of storage (Figure 4b).

In addition, the survival of the encapsulated cells was much higher than that of free cells except when gum arabic was added as an emulsifier. The survival rate of the encapsulated cells with gum arabic after 2 weeks was not different from that of free cells. The survival was rapidly reduced by adding bioemulsifier as an emulsifier after storage for 4 weeks. It was found that the survival of encapsulated cells with both gum arabic and bioemulsifier, was lower than others after 4 weeks of storage. This was because the emulsifiers used have different properties. Gum arabic and bioemulsifer are oil-in-water emulsion whereas Tween 80 is a water-in-oil emulsion. Proportion of oil used in the process affects the quality of gum arabic and bioemulsifier available to provide the structure of the encapsulated bead to function as an emulsifier. The content of the oil may not be suitable for oil-in-water emulsion of gum arabic and bioemulsifier. Accordingly, it could affect to the stability of the emulsion after 4

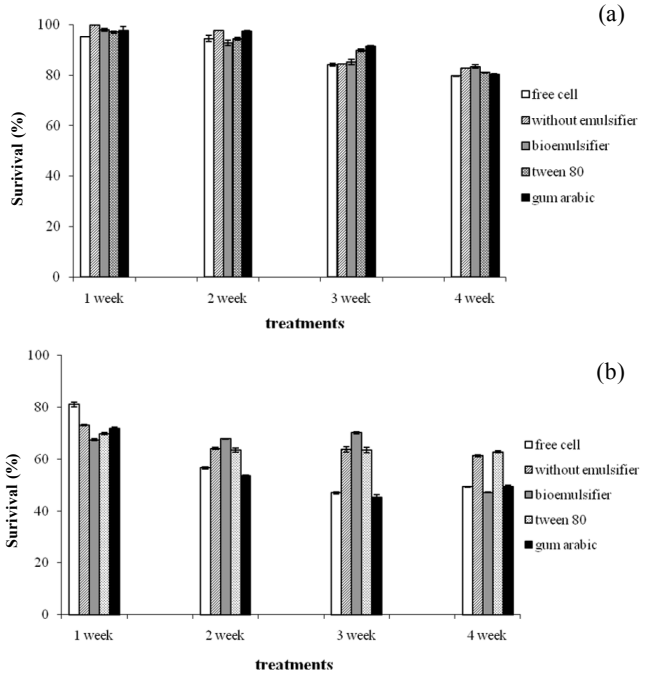

Figure 4. Temperature storage for survival rate of encapsulated *Lactobacillus plantarum* D6SM3 before and after exposure to simulated gastric juice (a) at 4°C (b) at room temperature. Bars indicate the standard deviation from triplicate determinations.

weeks of adding gum arabic and bioemulsifier as emulsifier. However, several studies have shown that the survival of encapsulated bacteria was improved in alginate microparticles compared with non-encapsulated bacteria during the storage period (Hansen *et al.*, 2002; Lee *et al.*, 2004). Moreover, several environmental factors, i.e., temperature, pH, ionic composition and ionic strength could affect the ability of the material to function as an emulsifier.

3.5 Physical examination of encapsulated *L. plantarum* D6SM3

Variations in the size of calcium alginate are caused by polydisperse emulsions. Microscopic polydisperse emulsions may not be visible to the naked eye. A Mastersizer™ 2000 can be used to measure particles ranging between 0.02 μm and 2000 μm by passing the particles through a laser beam. A particle size distribution diagram is generated, which illustrates the size range of calcium alginate beads. However, due to the sensitivity of a Mastersizer™ probiotic organisms may be recorded as particles which may influence the particle size distribution. The droplet mean diameters of d(10), d(50), d(90) and the span is presented in Table 1. The droplet size of encapsulated beads with and without emulsifiers were compared. There were 3 indications of the distribution. The d(10) indicates that there are about 10% of smaller droplets (μm) in the distribution. The d(50) shows that there are half of all droplets (μm) in the distribution. The d(90) shows that there are about 90% of smaller droplets (μm) in the distribution. In addition, the span indicates the width of the distribution regardless of the median size (Palazolo *et al.*, 2004). The particle size where the cumulative distribution is 50% is known as the median droplet diameter ($d_{v,0.5}$). The beads without emulsifier added had a $d_{v,0.5}$ with 50% of the particles under 237.65 μm. This must be compared with those using Tween 80, gum arabic and bioemulsifier added as emulsifier as these showed sizes of 358.59 μm, 338.91 μm and 241.72 μm respectively. This showed that the smallest particle size was obtained from the beads without emulsifier. However, it displayed the largest span of particles size. The wide span of the peak represents the lack of uniformity in diameters of the beads. Variations in the diameters of these beads may have resulted from the polydisperse emulsion that was formed during agitation by magnetic stirring. Although an additional emulsifier coating applied to the calcium alginate beads did not significantly reduce the bead size, narrowest spans were recorded for the beads produced using Tween 80 as emulsifier. Accordingly, additional emulsifier coating applied to the calcium alginate beads represents greater uniformity in diameters of the beads than without emulsifier.

The size of the beads formed by this technique was in the same range as that of Krasaekoopt *et al.* (2003), which were 25 μm to 2 mm. However, the size of the beads using the emulsion method depended on the speed of agitation and the type of emulsifier used. The smallest bead size was obtained when the mixture without emulsifier was used.

Table 1. Droplet mean diameters and Dispersity Index (Span) of encapsulated beads

Samples	d(10)* μm	d(50)* μm	d(90)* μm	Span
Without emulsifier	57.86	237.65	524.80	1.97[a**]
Added tween80	100.75	358.59	698.19	1.67[c]
Added gum arabic	81.02	338.91	699.53	1.83[b]
Added bioemulsifier	39.09	241.72	501.78	1.91[a]

* The experiments were done in triplicate and results are reported as the average from triplicate determinations.

** Different letter in the same column indicate significant differences ($p<0.05$).

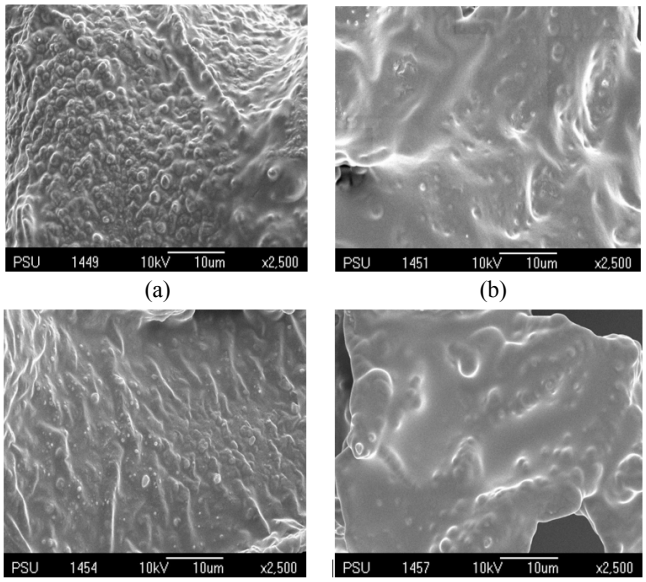

Figure 5. SEM image of encapsulated cells. (a) without emulsifier (b) with added Tween 80 (c) with added gum arabic and (d) with added spent yeast bioemulsifier.

Better emulsion resulted when emulsifier was used since the emulsifiers lower the surface tension, which results in smaller spheres.

A morphological analysis of the freeze-dried encapsulated LAB was undertaken by scanning electron micrographs. This showed different appearance of the one without emulsifier compared with the three with different emulsifiers. It revealed that the surface of those with emulsifiers added had microcapsules that were smoother than the one without emulsifier (Figure 5). The one without emulsifier showed microcapsules with an irregular surface. It showed that emulsifiers could emulsify the oil droplets better than if emulsifier was not added. Consequently, better emulsion capsules formed when emulsifier was used since the emulsifiers lowered the surface tension.

However, the texture of freeze-dried microcapsules was changed. Kwok *et al.* (1991) reported that the wrinkled surface was formed due to the loss of water content during the freeze-drying process. Moreover, SEM observation indicated that the droplets of microcapsules with emulsifiers were much more flocculated than those without emulsifiers. SEM of microcapsules showed that *L. plantarum* D6SM3 remained trapped within the alginate material (data not shown). This was because the encapsulated ones were washed before analysis. *L. plantarum* D6SM3 cells were not visible on the surface of the capsules. However, compared with those without emulsifier, addition of emulsifier not only affects the form of microcapsules, but also increases the survival of *L. plantarum* D6SM3. From the results, bioemulsifier from spent yeast showed the better effect on microcapsules than as synthetic emulsifiers and its safety is already known (Torabisadeh *et al.*, 1996). Therefore, the bioemulsifier can be an alternative emulsifier to be used in the food industry, instead of conventional synthetic emulsifiers.

4. Conclusions

Encapsulation through emulsion techniques is an effective way to increase the survival of probiotics bacteria as they pass through the gastrointestinal conditions of the human stomach. Bioemulsifier from spent yeast could be used as an emulsifier in the encapsulation process. Adding emulsifiers when using encapsulation techniques can improve the survival of probiotic bacteria.

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