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# MICROENCAPSULATION OF *Lactobacillus acidophilus* (La-5), ITS EVALUATION AND APPLICATION IN THE YOGHURT

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*Lactobacillus acidophilus La-5* is one of the most studied probiotic strains and often used in dairy product formulations. However, the survivability of La-5 was affected by environmental factors. To improve its survivability, *lactobacillus acidophilus* La-5 was microencapsulated using mixture of whey protein and polysaccharides (sodium alginate, λ-carrageenan, inulin, lentinan and glucose) as wall materials and was used in yoghurt making. The encapsulated La-5 only decreased from $2.8 \times 10^6$ to $1.2 \times 10^5$ in simulated gastric fluid. Yoghurts added with or without microencapsulated La-5 were analyzed for probiotic survivability, physico-chemical and textural properties. All samples showed similar trends in decrease of pH and increase of the titratable acidity during fermentation. Samples showed higher hardness values when compared with samples with non-microencapsulated cultures. Microencapsulation application in yoghurt resulted in a significant increase ($p<0.05$) in all textural parameters analyzed except springiness. The microencapsulated La-5 was more resistant to acid than the control, and survived better as well in yoghurt during storage.

**Keywords:** Microencapsulation, probiotic cultures, lactic acid bacteria, La-5, yoghurt, physico-chemical characteristics, texture profile.

## INTRODUCTION

Probiotic cultures mainly lactic acid bacteria (LAB) are beneficial to human being due to their various functions. Lactic acid bacteria (LAB) have high viability while passing the gastro-intestinal tract, resulting in mucosal defense against pathogenic microbes (FAO/WHO, 2001; Boylston *et al.,* 2004). These bacteria can be contained in diet for oral administration. In order to produce therapeutic benefits, food products containing such live bacteria should contain at least $10^6$ live cells microorganisms. $g^{-1}$ or $mL^{-1}$ of the product at the time of consumption (Shah, 2000). It has been well reported that probiotic live bacteria have a number of beneficial effects, but their low survivability and stability in various food products particularly in yoghurt is still a problem (Sultana *et al.,* 2000; De Vos *et al.,* 2010). Studies on survival and viability of LAB in fermented products were focused on selection of the durable strain (Maria *at al.,* 2015); supplementing the micronutrients to the milk such as amino acids and peptides (Dave and Shah, 1998).

Microencapsulation is a technology which protects the sensitive ingredients from the deteriorative reactions or adverse conditions by entrapping the ingredients in a biopolymer (encapsulating matrix/membrane), and serves as a barrier to release core materials. As for LAB, it also protects it from the potential cell injury and improves the viability (Picot *et al.,* 2004). The materials being used for microencapsulation are quite large in number; however, the lack of the interfacial functionality limits the application of the carbohydrates as a wall material (Kim and Morr, 1996). The blends of carbohydrate and protein to protect bacteria with improved encapsulating properties have been studied (Rajam, 2012). The food grade biopolymers such as whey protein and alginate have been used to protect the acid sensitive bacteria with varying successes (Lee *et al.,* 2004; Reid, 2007). Whey protein, inulin, and glucose are common stabilizers used in the stirred yoghurts to limit the syneresis (Kailasapathy, 2006). This study aimed to investigate the effects of mixture of sodium alginate, whey protein concentrate (WPC) and others as encapsulation materials on survivability of *Lactobacillus acidophilus* (La-5) and their application in yoghurt to get desired characteristics.

## MATERIALS AND METHODS

***Materials*:** Sodium alginate, λ-carrageenan, lentinan were purchased from Biological Technology Co., Ltd. (Gansu, China). Whey protein concentrate (protein concentration ≥ 80%) was provided by Hilmar (CA, USA). Glucose (purity ≥ 99%), trypsin and pepsin were from Sigma Chemical Co. (St. Louis, MO, USA). Skim milk powder was purchased from Fonterra (New Zealand). R-704 starter and *Lactobacillus acidophilus* La-5 was provided by Chr. Hansen (Milwaukee,

WI, USA). Materials for the preparation of MRS culture were provided by AoBoXing Co., Ltd. (Beijing, China).

Phosphate Buffered Saline (PBS): NaCl 8 g, KCl 0.2 g, $NaH_2PO_4$ 1.44 g, $KH_2PO_4$ 0.24 g, pH 7.4 with the 1 mol.$L^{-1}$ HCl at 25°C.

Simulated gastric fluid (SGF): 16.4 mL HCl (0.1 mol/L) and 10 g pepsin were dissolved in 1000 mL, and the pH was adjusted to 2.

Simulated intestinal fluid (SIF): 6.8 g $KH_2PO_4$ and 10 g trypsin were dissolved in 1000 mL, and the pH was adjusted to 6.8 with 0.1 M NaOH.

***Microencapsulation of Lactobacillus acidophilus (La-5)*:** *Lactobacillus acidophilus* (La-5) was sub-cultured in sterilized skim milk for several times until stable at 37°C. Then the culture was incubated in 100 mL sterilized skim milk at 37°C until pH reached to 4.6, collected by centrifugation (4000 rpm, 4°C, 15 min), rinsed with sterilized saline solution (0.85% NaCl) twice, mixed with 40 mL WPC (10%, w/v, heated at 85 °C for 30 min), and then held at 4°C overnight. The mixture was then mixed with 80 mL sodium alginate solution (2%, w/v), 10 mL inulin solution (5%, w/v), 20 mL glucose solution (5%, w/v), 10 mL λ-carrageenan solution (1%, w/v), and 10 mL lentinan solution (5%, w/v). The solutions used above were sterilized at 121°C for 15 min before use. The resulted cell suspension was injected into sterilized $CaCl_2$ solution (0.1 M) through a 0.1 mm needle. The beads were allowed to stay 30 min for gelification, and then filtered, rinsed with $CaCl_2$. The beads were dried by a freeze drier (ALPHA1-2, CHRIST, Germany) (Favaro-Trindale *et al.*, 2002). Microencapsulation of La-5 with WPC as the only wall material was prepared as the control.

***Bacterial enumeration*:** Enumeration of microencapsulated La-5 with mixture of whey protein and polysaccharide or whey protein concentrate and enmicroencapsulated ones were conducted every week for 4 weeks. To destroy the beads, 1 g encapsulated beads were dispersed in 99 mL sterilized PBS solution (pH 7.4) and then gently shaked at 180 rpm for 2 h. The cells were enumerated on MRS agar at 37°C for 48 h under aerobic conditions. All of the enumerations were performed in triplicates.

***Survivability of microencapsulated cells in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)*:** Determination of the survivability of microencapsulated cells in SGF and SIF was performed according to the method described by Krasaekoopt *et al.* (2004). The viable cells of were enumerated in triplicate. The prepared beads (1 g) were placed into 10 mL artificial SIF and incubated with shaking at 37°C, 180 rpm, for 30, 60, 90 and 120 min. After incubation, the beads were collected and rinsed with PBS solution. One mL aliquot of dissolved beads was removed and enumerated in triplicates on MRS agar.

***Yoghurt preparation*:** Protocol for yoghurt preparation has been given in the Figure 1.

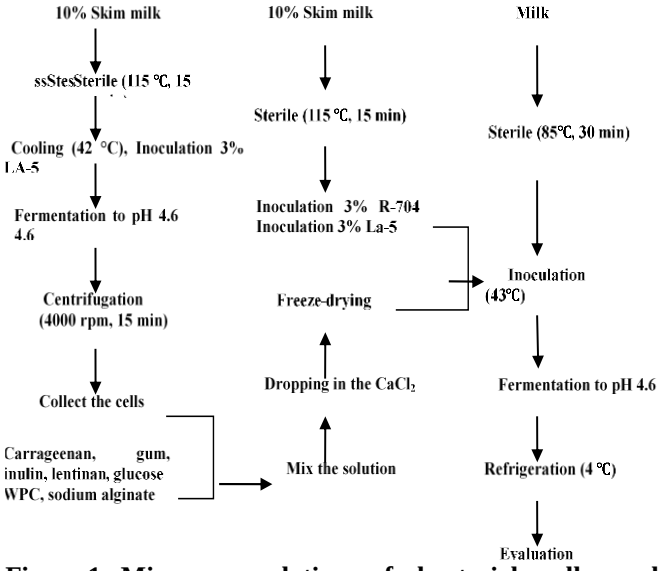


**Figure 1. Microencapsulation of bacterial cells and protocol for manufacture of yoghurts from microencapsulated *Lactobacillus acidophilus* La-5.**

The milk was heated to 85°C for 30 min and cooled down to 42°C. Five yoghurt samples were made and each inoculated with different combinations of starter cultures as shown in the Table 1: sample 1 with 3% yoghurt culture; sample 2 with 2% yoghurt culture and 1% non-microencapsulated La-5; sample 3 with 2% yoghurt culture and 0.35 g.100 $mL^{-1}$ microencapsulated La-5; sample 4 with 2% yoghurt culture and 0.20 g.100 $mL^{-1}$ microencapsulated La-5; sample 5 with 3% non-microencapsulated La-5 only. They were all incubated at 42°C until the pH reached to 4.6. The fermented samples were stored at 4°C.

**Table 1: The formulations of the experimental yoghurts.**

| Sample | Yoghurt culture (R-704) | Non-micro-encapsulated La-5 | Micro-encapsulated La-5 |
|---|---|---|---|
| Sample 1 | 3% | | |
| Sample 2 | 2% | 1% | |
| Sample3 | 2% | | 0.35 g.100 $mL^{-1}$ |
| Sample 4 | 2% | | 0.20 g.100 $mL^{-1}$ |
| Sample 5 | | 3% | |

***Measurement of pH and the titratable acidity*:** The pH values and titratable acidity during incubation were monitored on hourly basis. The titratable acidity of yoghurt was assessed with 0.1 M NaOH and using phenolphthalein as indicator. Approximately 10 g of yoghurt was diluted with 20 mL

distilled water (boiled and then cooled to evacuate the air) before titration. Titratable acidity was expressed as the consumption of 0.1 M NaOH multiplied by 10.

***Syneresis*:** The syneresis index of different yoghurt samples was determined according to the method described by Farnsworth *et al.* (2006) and Gauche *et al.* (2009) with some modifications. Briefly, after 3 days of storage, the samples (15 g) were centrifuged at altered centrifugal force (1400 g) and time (40 min) at 4°C in triplicate.

Syneresis (%) = Weight of whey (g)/Weight of yoghurt sample (g) × 100

***Apparent viscosity*:** Yoghurt samples were kept at room temperature for 2 h before being loaded into the viscometer (DV- lll , Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). The apparent viscosity of stirred yoghurt samples was measured at $10s^{-1}$ for 1 min under a constant rate of 10 rpm. The apparent viscosity of stirred yoghurt samples stored for 0, 7, 14, 21 days.

***Texture profile analysis*:** For texture analysis, the yoghurt samples were kept in 80 mL plastic containers (45 mm in diameter) during storage. The samples remained at room temperature for 2 h before analyses. The texture analysis was carried out using a texture analyzer (CT-3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a cylindrical probe (2.0 cm diameter; 3.81 cm high). The parameters were as follows: pre-test speed: 2.0 $mm.s^{-1}$, test speed: 2.0 $mm.s^{-1}$, post-test speed: 2.0 $mm.s^{-1}$, distance covered in the sample: 20 mm. The samples were tested in duplicate for 0, 7, 14, 21 and 28 days.

***Statistical analysis*:** All analyses except texture profile (tested in duplicate) were carried out in triplicates. Statistical analyses were performed with SPSS 16.0. Significant differences between treatments were tested by ANOVA.

## RESULTS AND DISCUSSION

***Survivability of microencapsulated La-5 during storage*:** The population of microencapsulated La-5 with mixture of whey protein and polysaccharide or whey protein concentrate and microencapsulated ones were shown in Figure 2. Compared with microencapsulated ones (reduced 3 log during 28 days), microencapsulated La-5 with whey protein concentrate as the only wall materials exhibited protective effect and its population decreased only 1.5 log. However, when whey protein concentrate was mixed with various polysaccharides as the wall materials, the microencapsulated La-5 showed better survivability with reduction of only 0.7 logs during 28 day storage. Sodium alginate and whey protein concentrate are biocompatible carriers for oral administration of sensitive bacteria (Chen and Subirade, 2006). Sodium alginate and whey protein concentrate microspheres were formed by emulsification/internal gelation technique with W/W/O double emulsion, which could give double protective effect for La-5. λ-carrageenan, an anionic polysaccharide may interact with whey protein concentrate through electrostatic reaction, resulting a possible coat on the surface of microspheres (Klein *et al.*, 2006). Inulin, glucose and lentinans may provide necessary growth nutrients for La-5.

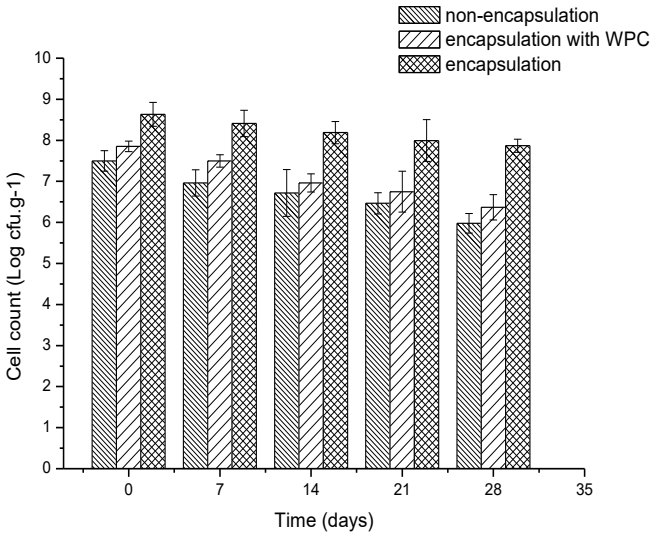


**Figure 2. The viability of cells in non-encapsulated, encapsulated and encapsulated with WPC.**

***Survivability of La-5 in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)*:** Survivability of *Lactobacillus acidophilus* La-5 in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF) were shown in Figure 3. *Lactobacillus acidophilus* La-5 was not resistant to low pH and could be damaged by the gastric fluid.

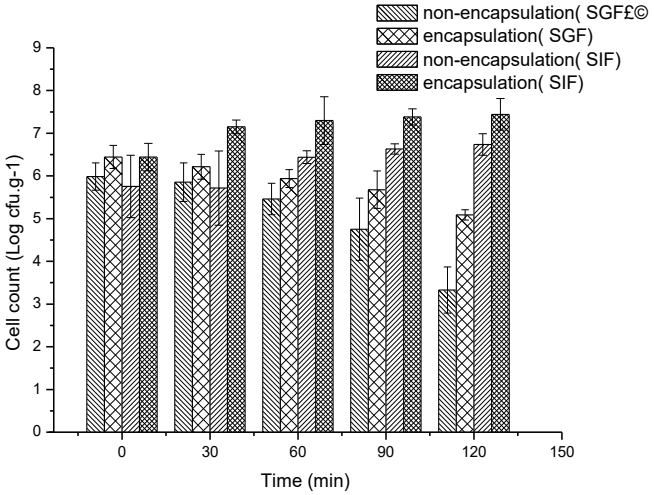


**Figure 3. Survival of non-encapsulated and encapsulated *Lactobacillus acidophilus* La-5 after 2h exposure to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).**

**Table 2: The survival of La-5 in yoghurt smples.**

| Storage time (days) | $Log_{10}$ colony forming units [$CFU.g^{-1}$] | | | | |
|---|---|---|---|---|---|
| | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** |
| 1 | $6.75 \pm 0.70^{a}$ | $6.57 \pm 0.43^{a}$ | $7.14 \pm 0.07^{b}$ | $7.10 \pm 0.12^{b}$ | $6.50 \pm 0.44^{a}$ |
| 5 | $6.72 \pm 1.25^{a}$ | $6.35 \pm 0.31^{a}$ | $6.98 \pm 2.55^{b}$ | $6.45 \pm 1.05^{a}$ | $6.15 \pm 0.21^{c}$ |
| 10 | $6.62 \pm 1.95^{a}$ | $6.28 \pm 0.08^{c}$ | $6.87 \pm 1.06^{b}$ | $6.36 \pm 1.14^{a}$ | $5.82 \pm 0.82^{d}$ |
| 15 | $6.59 \pm 0.40^{a}$ | $5.98 \pm 0.62^{d}$ | $6.63 \pm 0.40^{b}$ | $6.12 \pm 0.30^{a}$ | $5.18 \pm 0.26^{e}$ |
| 20 | $6.36 \pm 0.50^{a}$ | $5.91 \pm 1.95^{d}$ | $6.28 \pm 0.18^{f}$ | $5.78 \pm 0.39^{g}$ | $4.93 \pm 1.59^{h}$ |
| 25 | $5.51 \pm 0.34^{j}$ | $5.31 \pm 1.85^{j}$ | $5.97 \pm 0.55^{f}$ | $5.46 \pm 0.36^{k}$ | $3.88 \pm 0.75^{l}$ |
| 30 | $5.19 \pm 0.05^{m}$ | $5.15 \pm 1.95^{m}$ | $5.80 \pm 0.09^{f}$ | $5.32 \pm 0.10^{k}$ | $2.76 \pm 0.85^{n}$ |
| 35 | $4.77 \pm 0.60^{o}$ | $4.67 \pm 4.90^{p}$ | $5.36 \pm 0.18^{q}$ | $5.16 \pm 0.25^{k}$ | $2.26 \pm 0.09^{r}$ |

Values in the same row with different superscript letters indicates significant difference ($P < 0.05$) relative to sample 1; values in the same column with different superscript letters indicates significant difference ($P < 0.05$) relative to day 1

The non-microencapsulated La-5 had a 3 log reduction after incubation in the gastric solution after 2 h. Microencapsulated La-5 population was slightly decreased from $2.8 \times 10^6$ to $1.2 \times 10^5$ CFU/mL. The results indicated the protective effect of microencapsulation using mixture of whey protein and polysaccharide as wall materials. Population of La-5 increased after 2 h treatment by simulated intestinal fluid regardless of microencapsulation or not. However, compared with the non-encapsulated cells, encapsulated cells survived far better in SIF. Similar results were reported by Mandal *et al*. (2006).

***Survivability of microencapsulated La-5 in yoghurt*:** The survivability of La-5 in yoghurt samples during storage was shown in Table 2. La-5 in samples inoculated with microencapsulated cells had better survivability than other samples. Sample 3 with 0.35 g/100mL microencapsulation had a reduction of 1.78 Log during 35-day-storage, while there was a decrease about 2 Log in the control. The lower population in the yoghurt with non-microencapsulated La-5 might be due to the intolerance of La-5 to acid environment. Sample 3 had less reduction than sample 4 might be due to higher addition level of microencapsulated La-5, which could protect the La-5 from the acid environment.

***pH and titratable acidity*:** All samples showed similar trends of changes in pH and titratable acidity during fermentation time of 5 h. Titratable acidity increased from 20 °T to 105 °T while pH decreased from 6.5 to 4.6 (Fig. 4, 5). At the first 1-2 h, the pH of all samples increased slowly, and followed by a sharply decrease during 2-4 h, and then finally reached the pH of 4.6 at about 5 h. Sample 3 and sample 4 had a slightly higher titratable acidity.

***Syneresis*:** Syneresis is a common defect in fermented dairy products and refers to the appearance of liquid on the milk gel surfaces resulting gel shrinkage and loss of milk whey and soluble constituents (Lucey and Singh, 2004). The syneresis of sample 1 and sample 5 were 65.5% and 63.6%, respectively, which is 10.6% and 8.7% higher than that of sample 3, which showed the lowest syneresis (54.9%) with the highest concentration of microencapsulated La-5. The reducing syneresis may due to the protein used for microencapsulation (Lorenzen *et al*., 2002).

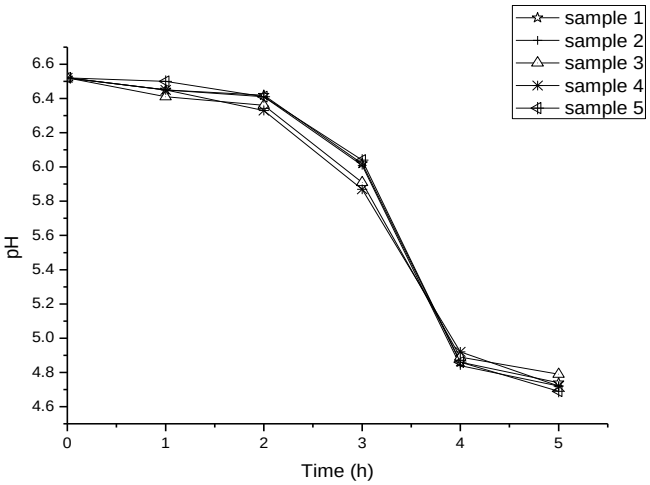


**Figure 4. The pH change during the fermentation.**

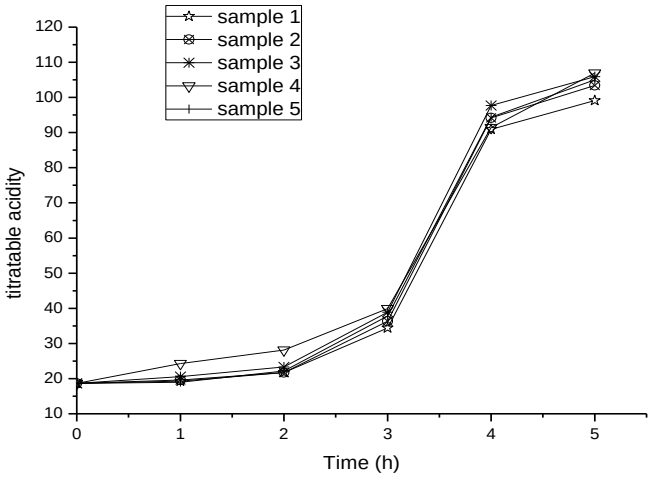


**Figure 5. The titratable acidity change during the fermentation.**

**Table 3: The texture profile analysis of yoghurt samples during the one month storage with an interval of 7 days.**

| Day | Texture parameters | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|---|---|
| 0 | | 132.7 ± 25.6$^{a}$ | 128.5 ± 15.2$^{a}$ | 135.7 ± 27.1$^{a}$ | 129.9± 56.4$^{a}$ | 132.0± 38.1$^{a}$ |
| 7 | | 171.9 ± 31.4$^{b}$ | 171.1 ± 39.8$^{b}$ | 171.6 ± 38.1$^{b}$ | 173.9 ± 37.1$^{b}$ | 169.8 ± 20.5$^{b}$ |
| 14 | H | 185.1 ± 45.7$^{c}$ | 176.3 ± 41.7$^{c}$ | 184.9 ± 49.2$^{c}$ | 186.9 ± 46.5$^{c}$ | 177.2 ± 19.4$^{c}$ |
| 21 | | 188.5 ± 37.8$^{c}$ | 198.3 ± 27.9$^{d}$ | 221.2 ± 26.7$^{d}$ | 201.2 ± 36.8$^{d}$ | 176.7 ± 17.6$^{c}$ |
| 28 | | 186.8 ± 47.9$^{c}$ | 181.8 ± 35.4$^{e}$ | 196.3 ± 19.8$^{f}$ | 173.1 ± 16.4$^{g}$ | 175.8 ± 18.3$^{c}$ |
| 0 | | 25.9 ± 9.68$^{a}$ | 26.8 ± 3.98$^{a}$ | 28.6 ± 3.48$^{a}$ | 26.8 ± 6.87$^{a}$ | 26.1 ± 2.32$^{a}$ |
| 7 | | 57.0 ± 12.56$^{b}$ | 54.4 ± 8.64$^{b}$ | 61.1 ± 9.67$^{b}$ | 57.4 ± 8.67$^{b}$ | 40.8 ± 4.56$^{c}$ |
| 14 | Co | 67.8 ± 7.69$^{d}$ | 63.1 ± 7.94$^{d}$ | 62.0 ± 6.31$^{b}$ | 61.5 ±7.68$^{b}$ | 52.8 ± 9.67$^{e}$ |
| 21 | | 67.0 ± 8.64$^{d}$ | 76.1 ± 5.61$^{f}$ | 89.0 ± 8.64$^{g}$ | 72.9 ±8.99$^{h}$ | 61.9 ± 8.31$^{i}$ |
| 28 | | 57.5 ± 9.82$^{j}$ | 62.7 ± 10.35$^{k}$ | 70.7 ± 9.67$^{l}$ | 57.1 ±6.71$^{m}$ | 58.3 ± 7.68$^{i}$ |
| 0 | | 4.7 ± 0.98$^{a}$ | 4.5 ± 0.67$^{a}$ | 5.0 ± 1.23$^{b}$ | 4.3 ± 0.67$^{a}$ | 4.6 ± 1.20$^{a}$ |
| 7 | | 11.1 ± 1.29$^{c}$ | 10.6 ± 0.98$^{c}$ | 11.7 ± 1.37$^{c}$ | 11.2 ± 1.16$^{c}$ | 7.1 ± 1.69$^{d}$ |
| 14 | A | 12.3 ± 2.69$^{e}$ | 11.8 ± 3.57$^{e}$ | 11.5 ± 2.19$^{e}$ | 10.6 ± 3.59$^{f}$ | 10.8 ± 4.25$^{f}$ |
| 21 | | 11.8 ± 3.68$^{e}$ | 12.4 ± 4.37$^{e}$ | 14.1 ± 3.24$^{g}$ | 11.9 ± 4.65$^{h}$ | 11.1 ± 4.28$^{h}$ |
| 28 | | 10.6 ± 4.05$^{i}$ | 10.9 ± 3.97$^{i}$ | 11.0 ±4.06$^{i}$ | 9.5 ± 2.71$^{j}$ | 9.0 ± 4.19$^{k}$ |
| 0 | | 17.8 ± 2.34$^{a}$ | 18.0 ± 3.05$^{a}$ | 17.6 ± 5.61$^{a}$ | 17.7 ± 6.28$^{a}$ | 17.5 ± 7.01$^{a}$ |
| 7 | | 16.5 ± 4.67$^{b}$ | 16.5 ± 7.61$^{b}$ | 16.3 ± 4.29$^{b}$ | 16.5±7.26$^{b}$ | 16.8 ± 9.13$^{b}$ |
| 14 | S | 16.0 ± 3.19$^{c}$ | 16.0 ± 4.34$^{c}$ | 15.9 ± 3.27$^{b}$ | 16.1 ± 8.25$^{b}$ | 15.9 ± 4.82$^{c}$ |
| 21 | | 15.1 ± 5.02$^{d}$ | 15.9 ± 5.06$^{c}$ | 15.3 ± 4.69$^{c}$ | 15.4 ± 7.61$^{c}$ | 15.5 ± 7.34$^{c}$ |
| 28 | | 14.5 ± 5.24$^{d}$ | 15.0 ± 6.23$^{c}$ | 15.0 ± 9.27$^{c}$ | 14.4 ± 3.44$^{d}$ | 14.8 ± 6.96$^{d}$ |
| 0 | | 56.2 ± 9.38$^{a}$ | 57.4 ± 10.35$^{a}$ | 56.6 ± 10.65$^{a}$ | 59.0 ± 11.39$^{a}$ | 58.8 ± 5.47$^{a}$ |
| 7 | | 56.7 ± 10.36$^{a}$ | 58.3 ± 12.39$^{a}$ | 57.9 ± 13.97$^{a}$ | 60.7 ± 16.24$^{a}$ | 59.2 ± 67.89$^{a}$ |
| 14 | G | 60.6 ± 9.52$^{a}$ | 60.3 ± 20.67$^{a}$ | 62.1 ± 19.67$^{b}$ | 67.7 ± 15.68$^{b}$ | 63.9 ± 10.38$^{b}$ |
| 21 | | 73.3 ± 10.65$^{c}$ | 74.9 ± 28.58$^{c}$ | 74.2 ± 19.34$^{c}$ | 74.8 ± 24.69$^{c}$ | 65.0 ± 15.65$^{b}$ |
| 28 | | 72.0 ± 12.36$^{c}$ | 69.2 ± 15.38$^{c}$ | 74.1 ± 17.36$^{c}$ | 63.9 ± 19.35$^{d}$ | 55.2 ± 18.42$^{e}$ |
| 0 | | 9.8 ± 1.95$^{a}$ | 10.1 ± 2.68$^{a}$ | 9.8 ± 0.97$^{a}$ | 10.3 ± 3.29$^{a}$ | 10.1 ± 1.65$^{a}$ |
| 7 | | 9.9 ± 0.99$^{a}$ | 10.5 ± 2.32$^{a}$ | 9.8 ± 1.45$^{a}$ | 10.8 ± 1.24$^{a}$ | 10.7 ± 5.93$^{a}$ |
| 14 | Ch | 11.0 ± 1.31$^{b}$ | 11.1 ± 3.15$^{b}$ | 10.7 ± 2.39$^{b}$ | 11.7 ± 1.92$^{b}$ | 11.0 ± 1.94$^{b}$ |
| 21 | | 12.3 ± 3.67$^{c}$ | 11.7 ± 2.02$^{c}$ | 11.2 ± 4.57$^{c}$ | 12.0 ± 3.76$^{c}$ | 11.6 ± 1.83$^{c}$ |
| 28 | | 11.7 ± 3.16$^{c}$ | 10.9 ± 3.91$^{c}$ | 11.6 ± 2.39$^{c}$ | 12.3 ± 2.64$^{c}$ | 11.4 ± 2.97$^{c}$ |

H = hardness; Co = cohesiveness; A = adhesiveness; S = spring; G = gumminess; Ch = chewiness (mean values ± SD obtained of three replicates). Values in the same row with different superscript letters indicates significant difference ($P < 0.05$) relative to sample 1; values in the same column with different superscript letters indicates significant difference ($P < 0.05$) relative to day 1

***Apparent viscosity*:** Apparent viscosity of yoghurt was affected many factors such as protein crosslinking which could increases the strength of the gel (Lauber *et al.*, 2000). Changes in apparent viscosity of the yoghurt samples during storage was shown in Figure 7. At the beginning, all samples showed relatively high apparent viscosity between 3000-4000 mPas, and a sudden decrease occurred at day 7. All samples reached the highest apparent viscosity at day 21 (Fig. 7). Compared with other samples, there was no considerable difference in viscosity for sample with microencapsulated La-5, indicating that microencapsulated mixed culture may have no effect in short time. Sample 2 showed significantly higher viscosity values than other samples ($p<0.05$), which might be the reason that mixed culture in the yoghurt could contributed to an increase in the consistency.

***Texture profile analysis*:** All samples had a consistency texture and all the texture parameters had their highest values at day 28 (Table 3). At day 0, compared with other samples, yoghurts with microencapsulated La-5 showed significantly increased adhesiveness ($p<0.05$). At day 21, hardness, cohesiveness, adhesiveness, gumminess and chewiness of samples with mixed culture (samples 2, 3 and 4) showed higher values than those of samples with only culture (sample 1 and 5), especially the texture parameters of hardness, cohesiveness and adhesiveness. The mixed culture had an intense cross-linking of protein chains, which resulted in the higher values than others in all texture parameters. However, at day 28, samples with microencapsulated La-5 exhibited higher values in all parameters than other samples ($p<0.05$). The more microencapsulated La-5 added the higher parameters they had. *Lactobacillus acidophilus* La-5 and

fermented starter probably had synergetic effect on proteolytic casein micelles, especially owing to the application of microencapsulation in yoghurt. The rate and extend of acidification may have a major impact on the texture via demineralization of casein micelles (Flávia *et al.*, 2005). Sample 5 had lower values than the sample 1 in most of the texture parameters, when only non-microencapsulated La-5 used.

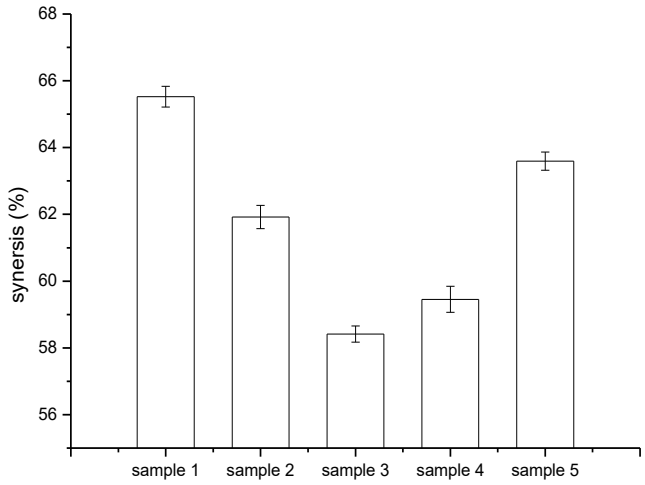


**Figure 6. Syneresis of the yoghurt samples with different cultures combinations.**

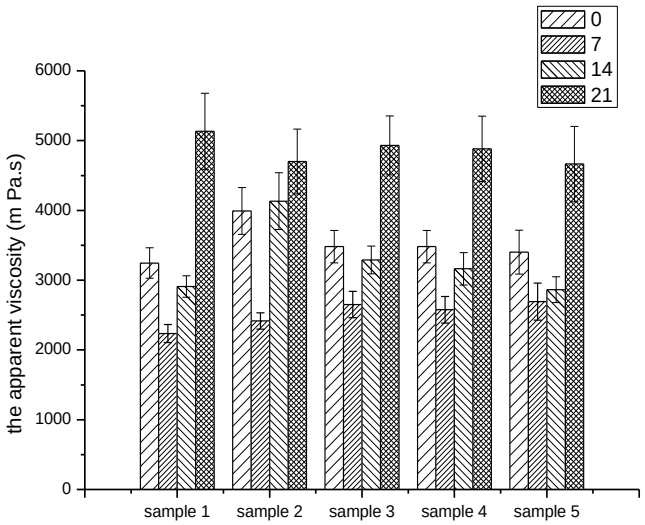


**Figure 7. The apparent viscosity of yoghurt samples at an interval of 7 days.**

***Conclusions*:** La-5 could be microencapsulated using mixture of whey protein and polysaccharides as wall materials. The La-5 microencapsule remained stable during storage of 28 days and had better survivability in SGF and released in SIF. Yoghurt inoculated with the microencapsulated La-5 showed high population above $10^5$ log CFU/mL after storage of 35 days. There was a significant increase in the texture parameters and apparent viscosity when mixed culture and microencapsulated La-5 were used in the yoghurt.

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