

Improvement of Viability of *Lactobacillus casei* and *Bifidobacterium longum* with Several Encapsulating Materials using Extrusion Method

Widaningrum¹, Miskiyah¹, Indrasti D², Hidayat HC²

¹Indonesian Centre for Agricultural Postharvest Research and Development, Bogor, Indonesia

²Department of Food Technology, Bogor Agricultural University

E-mail: widaningrum.adnan@gmail.com

(received 23-08-2018; revised 06-12-2018; accepted 07-12-2018)

ABSTRAK

Widaningrum, Miskiyah, Indrasti D, Hidayat HC. 2018. Peningkatan viabilitas bakteri *Lactobacillus casei* dan *Bifidobacterium longum* dengan beberapa materi enkapsulasi menggunakan metode ekstrusi. JITV 23(4): 189-201. DOI: <http://dx.doi.org/10.14334/jitv.v23i4.1547>

Lactobacillus casei dan *Bifidobacterium longum* merupakan bakteri probiotik yang umum digunakan sebagai *starter* kering pada sistem pangan. Proses pengeringan pada produksi *starter* kering dapat mengurangi jumlah probiotik, sehingga probiotik sangat perlu untuk dienkapsulasi. Tujuan penelitian ini yaitu untuk memperoleh bahan pengkapsul terbaik untuk melindungi kedua probiotik tersebut. Teknik enkapsulasi yang digunakan dalam penelitian ini yaitu ekstrusi pada kombinasi maltodekstrin-alginat, pati sagu-alginat, pati jagung-alginat, dan kontrol (susu skim-alginat) yang digunakan sebagai bahan pengkapsul. Keempat kombinasi bahan pengkapsul tersebut mempengaruhi nilai sintasan probiotik, efisiensi enkapsulasi, jumlah sel pada manik-manik basah dan manik-manik kering, jumlah sel hidup selama proses pengeringan, dan jumlah sel terenkapsulasi pada kondisi simulasi asam dan keberadaan garam empedu di lingkungan hidupnya. Berdasarkan sintasan *L. casei* dan *B. longum*, sifat matriks manik-manik, jumlah sel pada manik-manik basah dan kering, serta jumlah sel yang bertahan selama proses pengeringan, kombinasi maltodekstrin-alginat memberikan hasil yang lebih baik dibandingkan dengan kombinasi pati sagu-alginat dan pati jagung-alginat, tetapi tidak sebaik susu skim-alginat (kontrol) dalam mengenkapsulasi probiotik. Jumlah sel *L. casei* dan *B. longum* pada manik-manik kering maltodekstrin-alginat masing-masing yaitu 4.69 ± 0.08 log CFU/g dan 5.32 ± 0.21 log CFU/g, sedangkan jumlah sel *L. casei* dan *B. longum* pada manik-manik kering susu skim-alginat lebih tinggi yaitu masing-masing 5.08 ± 0.07 log CFU/g dan 6.20 ± 0.16 log CFU/g. *L. casei* lebih tahan terhadap lingkungan asam (pH rendah). Dalam keberadaan 0.3% garam empedu, *L. casei* dan *B. longum* terenkapsulasi susu skim-alginat meningkat masing-masing sebanyak 2.75 ± 0.02 dan 1.61 ± 0.04 siklus log.

Kata Kunci: Enkapsulasi, Ekstrusi, Probiotik, Ketahanan Sel, Sintasan

ABSTRACT

Widaningrum, Miskiyah, Indrasti D, Hidayat HC. 2018. Improvement of viability of *Lactobacillus casei* and *Bifidobacterium longum* with several encapsulating materials using extrusion method. JITV 23(4): 189-201. DOI: <http://dx.doi.org/10.14334/jitv.v23i4.1547>

Lactobacillus casei and *Bifidobacterium longum* are probiotics commonly applied as dry starter for food system. Drying process in the production of dry starter can reduce the number of probiotics, therefore they are necessary to be encapsulated. Aim of this research was to obtain best encapsulating material for both probiotics. Encapsulation technique used in this research was extrusion with maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and control of treatment skim milk-alginate (w:w) as encapsulating materials. The four encapsulating materials significantly affected the value of viability, encapsulation efficiency, number of cell in wet beads and dry beads, number of survival cell during drying process, and number of survival encapsulated cell in simulated acid and bile salt conditions. Based on viability of *L. casei* and *B. longum*, beads matrix characteristic, number of cell in wet beads and dry beads, and number of survival cells during drying process, maltodextrine-alginate was better than sago starch-alginate and corn starch-alginate, but was not as good as skim milk-alginate (control of treatment) as encapsulating material. Viability (number) of *L. casei* and *B. longum* in dry beads of maltodextrine-alginate were 4.69 ± 0.08 log CFU/g and 5.32 ± 0.21 log CFU/g, while number of *L. casei* and *B. longum* in dry beads of skim milk-alginate were higher 5.08 ± 0.07 log CFU/g and 6.20 ± 0.16 log CFU/g. *L. casei* more resistant than *B. longum* against acidic (low pH) environment. In the presence of 0.3% bile salt, *L. casei* and *B. longum* encapsulated with skim milk-alginate increased as much as 2.75 ± 0.02 and 1.61 ± 0.04 log cycles, respectively.

Key Words: Encapsulation, Extrusion, Probiotics, Cell Resilience, Viability

INTRODUCTION

Probiotics are living microorganisms actively able to improve human health by balancing microflora in gastrointestinal tract if they are consumed in sufficient number (Fuller 1992). Probiotic consumption, in some ways, is important to maintain gastrointestinal tract (GIT) health and to improve host immune system. According to Gibson & Robertfroid (1995), enough number of bacteria in the body could improve immune system thus increase body's ability against diseases. A number of genus of bacteria currently consumed as probiotic are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, *Enterococcus*, *Lactococcus*, and *Streptococcus* (Shah 2007), yet, the most developed probiotic come from *Lactobacillus* and *Bifidobacterium*.

Of two among species of *Lactobacillus* and *Bifidobacterium* classified as probiotic are *Lactobacillus casei* and *Bifidobacterium longum*. *Lactobacillus casei* or *L. casei* naturally lies on human's small intestine (Reid 1999), whereas *Bifidobacterium longum* dominantly lies on human's colon (Arbolea et al. 2016). Both bacteria are able to give health advantage for human body because they could inhibit growth of harmful bacteria and keep the balance of gastrointestinal tract (Holzapfel & Schilinger 2002).

Application of *L. casei* and *B. longum* in food generally in the form of starter of probiotic. Probiotic starter could be in the liquid or dried form. Nevertheless, usage of probiotic starter in the dried form recognized as more benefited since it easier to be used and to be packaged. In addition, dried starter could prolong the shelf life of starter (Krasaekoopt et al. 2003). Nonetheless, drying process in the production of dry starter could decrease probiotic number in the final product (starter). Thus, optimizing (and maintaining) high number of probiotic in the dry form is of fundamental importance. Increasing number of probiotic in starter can be done by encapsulating probiotic in the initial step, then dry them by the appropriate drying method. Dry starter resulted could be having probiotic bacteria in the high number. According to Tamime et al. (2006), minimum concentration of probiotic in the food product is 106 CFU/g of product.

Encapsulation is a coating process of core of a material using certain encapsulates. Core material in this case is probiotic bacteria. Purpose of encapsulation is to maintain viability of probiotic bacteria and protect them from damages caused by undesirable environment condition (Frazier & Westhoff 1998). Extrusion is encapsulation technique that done with the way of adding probiotic microorganisms into hydrocolloid of natrium alginate, then being dropped into hardening

solution (CaCl_2) until beads containing bacteria cells (microcapsules) were being formed. Microcapsule or bead systems using various biopolymers are very easy to prepare on a lab-scale with any encapsulated ingredients. Alginate is on top among other ingredients. Nevertheless, calcium alginate beads tend to be very porous which allows fast and easy diffusion of water and other fluids in and out the matrix (microcapsules) (Anal & Singh 2007). This has to be overcome by using other potential degradable materials which may address the porosity issue. Starch is one of the biopolymers that have the potential to be investigated as encapsulating materials since it is available abundantly and relatively cheap in cost. Exploring starch as bio-encapsulating material would be worthed particularly if it is aimed to be used in the industrial scale. As extrusion technique marked as easier, cheaper, and simpler thus able to protect probiotic cells viability (Krasaekoopt et al. 2003), this technique was being chosen as encapsulating technique in this research.

Mixing of starch and alginate were used in this research. Type of starch used was maltodextrine, sago starch, and corn starch. Encapsulation of *L. casei* and *B. longum* with the mixing of starch and alginate hopefully could maintain viability of both probiotic bacteria thus dry starter of probiotic bacteria with high number of bacteria could be obtained. Besides, usage of maltodextrin, sago starch and corn starch as encapsulating material have not been developed yet, thus they can be made as new candidates for new encapsulating material. This research purposed to determine the best encapsulating material and to study the effects of encapsulating material toward viability of probiotic bacteria, encapsulation efficiency, and resilience of dry beads *L. casei* and *B. longum* against low pH and bile salt.

MATERIALS AND METHODS

Preparation of starch-alginate solution

Freeze dried isolates of probiotic bacteria *L. casei* FNCC 0090 and *B. longum* ATCC 15707 were obtained from Food and Nutrition Collection Center Gadjah Mada University Indonesia, Na-alginate, skim milk, maltodextrin, sago starch, corn starch were obtained from Yoek Shop, MRSA (de Man Rogosa Sharpe Agar, MERCK), MRSB (de Man Rogosa Sharp Broth, MERCK) for bacterial growth. Chemical used were aquadest, CaCl_2 , NaCl , PBS, NaOH , HCl , and bile salt.

This stage initiated by preparing 20 ml of encapsulate suspension which is consist of starch-alginate with comparison 1 : 3 (1% : 3%) (w:w) from each starch, and 1 : 2 for skim milk-alginate as control of treatment. Number of total solid as much as 4%. Usage of 4% total solid refers to Mandal et al. (2006)

which reported that viability of *L. casei* NCDC 298 on low pH (1.5) had increased in line with the increase of alginate concentration. Besides, the highest viability of *L. casei* obtained from the use of 4% alginate. Skim milk-alginate as encapsulating material with composition of 1 : 2 (1.3% of skim milk : 2.7% of alginate) was used as control of treatment.

Usage of skim milk-alginate as control of treatment refers to research report of Adrianto (2011) which reported that *L. casei* encapsulated with skim milk and alginate at a ratio of 1 : 2 had higher percentage of cell resilience compared to those without skim milk addition (4% alginate) after being dried using oven on 40°C for 6 hours. Dried skim milk : alginate encapsulated-*L. casei* had 58.4% cell resilience, whereas without addition of skim milk (4% alginate) the value was just less than 22.1%. Before being used for encapsulation process, all of encapsulating material suspension was subsequently sterilized in autoclave at 121°C for 15 minutes. After cooling, the suspension was then dropped into a solution of CaCl₂ 0.1 M by a 10 mL syringe to form *beads* (Krasaekoopt et al. 2003). The bead was filtered using whitman filter paper and washed by sterile 0.85% NaCl.

Encapsulation of *L. casei* and *B. longum* (Krasaekoopt et al. 2003)

Encapsulation technique used was extrusion technique adopted from Krasaekoopt et al. (2003). There were four types of encapsulating materials used to encapsulate *L. casei* and *B. longum*, i.e. maltodextrine-alginate, sago starch-alginate, corn starch-alginate (with the ratio of starch to alginate was 1% : 3%) and skim milk : alginate (as control of treatment with the ratio 1% : 2%). Encapsulation process started by mixing 1% of probiotic cultures (in MRSB) into sterilized encapsulating material, then homogenized for 40 minutes. The mixture of suspension was placed into sterile syringe and dropped into 0.1 M CaCl₂ with the drop distance of ± 1 cm while stirring gently using magnetic stirrer at 200-350 rpm). Hardening time in 0.1 M CaCl₂ solution was ± 30 minutes. Subsequently, obtained beads were being screened and washed using 0.85% NaCl then being drained for 2 minutes and placed in sterile petridish. Observed parameters in this step including yields, total plate count (pouring method), encapsulation efficiency and cell viability according to method of Sheu & Marshal (1993).

Calculation of encapsulation efficiency

Methods of encapsulation process of *L. casei* and *B. longum* and way of preparation of beads for measuring total plate count based on following way: alginate was

mixed with either skim milk or maltodextrin or sago starch or corn starch, then aquadest was added into the formula. The formula then being sterilized at 121°C for 15 minutes, thus cooled at ambient temperature. After that, 1% of *B. longum* and or *L. casei* were mixed each into the formula then homogenized for 40 minutes. Subsequently, the formula was being dropped into sterilized 0.1 M CaCl₂ and drained and washed with 0.85% NaCl until beads of *B. longum* and *L. casei* were resulted. Cell number then measured, and the beads then being dried at temperature of 40°C for 7 hours. At the end of the process, dry beads of *B. longum* and *L. casei* were resulted and again the cell number was measured.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Probiotic population (CFU/g beads)}}{\text{Total probiotic in suspension (CFU/g)}} \times 100$$

Calculation of cell viability

Cell viability was calculated using equation:

$$\text{Viability (\%)} = \frac{\text{Log}_{\frac{\text{cfu}}{\text{g}}}^{\text{cfu}} \text{ of wet beads}}{\text{Log}_{\frac{\text{cfu}}{\text{g}}}^{\text{cfu}} \text{ of biopolymer suspension}} \times 100$$

Calculation of yield of dried beads

Drying process aimed to dry *L. casei* and *B. longum* beads thus dry probiotic beads can be obtained. Beads drying done on 40°C using blower oven. Drying using blower oven marked as easier and cheaper compared to spray drying or freeze drying. Temperature of 40°C was used regarding the range of *L. casei* growth temperature (15-40°C) and *B. longum* (37-41°C) thus there still possibility that both probiotic bacteria still alive. Drying time was determined by measuring mass of beads during drying process until the constant masses were obtained. Observed parameters were moisture of dry beads and yield of dry beads.

$$\text{Yield of dry beads (\%)} = \frac{\text{Mass of dry beads (g)}}{\text{Mass of wet beads (g)}} \times 100$$

Calculation of cell resilience

Total plate count (pouring method), and percentage of cell resilience after drying process. Yield of dry beads and percentage of cell resilience can be counted by following formula:

$$\text{Cell resilience (\%)} = \frac{\text{Log}_{\frac{\text{CFU}}{\text{g}}}^{\text{CFU}} \text{ of beads after being dried}}{\text{Log}_{\frac{\text{CFU}}{\text{g}}}^{\text{CFU}} \text{ beads before being dried}} \times 100$$

Resilience testing of probiotic dry beads toward low pH and bile salt (modification of Lin et al. 2006)

Resilience testing of probiotic bacteria toward low pH and bile salt aimed to study the ability of both probiotic bacteria to remain stable on gastrointestinal track environment, and to study the ability of encapsulating materials in protecting probiotic bacteria on low pH and availability of bile salt. Low pH and bile salt testing was done continually according to modification method of Lin et al. (2006). Resilience testing of probiotic bacteria toward low pH was done by adding 1 g of dry beads into 9 ml PBS (pH 2.0) arranged by using HCl 0.1 N, and then incubated for 3 hours on 37°C. After being incubated, cell number on dried beads measured by pouring method using MRSA and incubated on 37°C for 48 hours.

Resilience testing of probiotic bacteria toward bile salt was done by re-mixing (re-suspended) dry beads of bacteria after being incubated on pH 2.0 treatment into MRSB contained 0.3% of bile salt. Before being resuspended, firstly beads must be washed using PBS on pH 7.2, after that medium containing beads can be incubated on 37°C for 36 hours and the cell number was being counted (pouring method) using MRSA.

Moisture content analysis (Oven method, SNI 01-2891-1992)

Moisture content analysis was done to obtain moisture content of dry beads from each encapsulating materials. Firstly, empty cups were being dried in oven at 105°C for 15 minutes. Cups then being cooled in desiccator then being weighed (W2). Subsequently, sample were being into cups as much as 0.5 g. Dish then being dried by oven drying at 105°C for 3 hours or more until constant weight were reached. Finally, cups contained sample were being cooled in desiccator then being weighed (W2). Moisture content was measured using following formula:

$$\text{Moisture content (g/100 g dried samples)} = \frac{w - (w1 - w2)}{W1 - w2} \times 100$$

Statistical analysis

Statistical analysis was used to process obtained data from stages: encapsulation and drying of *L. casei* and *B. longum* beads and resilience testing of probiotic dry beads toward low pH and bile salt. Statistical analysis aimed to obtain information whether the two factors (encapsulating materials and probiotic type) had significant effect or not towards obtained data from every stage of research. Those data then processed

using Analysis of Variance (ANOVA) and further testing Duncan on 95% significance level.

Experimental design

Experimental design used in this research was completely randomized design. There were two factor i.e. type of encapsulating materials (combination of maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and skim milk-alginate as control of treatment) and type of probiotic (*L. casei* and *B. longum*). Data were obtained in four replications.

RESULTS AND DISCUSSION

Encapsulation of *L. casei* and *B. longum*

Viability of *L. casei* and *B. longum* can be seen on Table 1. The higher viability, the lower decrease on cell probiotic number after being encapsulated. According to statistical data (analysis of variance/anova), probiotic type and type of encapsulating material affected ($P < 0.05$) viability of probiotic bacteria cells. The highest viability of *L. casei* and *B. longum* obtained from control treatment of encapsulating material (skim milk-alginate) i.e. $99.55\% \pm 0.37$ on *L. casei* and $97.48\% \pm 0.22$ on *B. longum*, yet, viability of both probiotic encapsulated with three others encapsulating material based on starch-alginate i.e. maltodextrine-alginate, sago starch-alginate, and corn starch-alginate also had high number which reached more than 90%. It showed there was a little decrease in cell numbers of *L. casei* and *B. longum* during encapsulation process. Jownonski et al. (1997) reported that alginate-starch capsules had ability to encapsulate *Lactobacillus acidophilus* without decreasing bacteria viability and its ability to ferment. Likewise, Adrianto (2011) reported that encapsulation of *L. casei* with encapsulating material based on protein-alginate such as skim milk, whey and sodium caseinate produced viability as much as 95% suggesting starch-alginate may have ability to encapsulate probiotic bacteria as good as material-based protein (skim milk)-alginate.

Encapsulation efficiency of *L. casei* and *B. longum* can also be seen on Table 1. According to statistical data (anova), encapsulation efficiency of *L. casei* and *B. longum* from four types of encapsulating materials were significantly different ($P < 0.05$). Between the three alginate based-encapsulating materials, the lower efficiency of encapsulation obtained by sago starch-alginate ($12.02\% \pm 2.10$) on *L. casei* and $12.62\% \pm 2.29$ on *B. longum*, whereas the higher encapsulation efficiency obtained by skim milk-alginate (control of treatment). Encapsulation efficiency was affected by yield and bacteria cell number presence on beads. The highest bacteria cell number presence on skim milk-

Table 1. Properties of *L. casei* and *B. longum* wet beads

Parameters	Skim milk-Alginate (1:2) (control of treatment)	Maltodextrine-Alginate (1:3)	Sago starch-Alginate (1:3)	Corn starch-Alginate (1:3)
<i>Lactobacillus casei</i>				
Cell population in encapsulating material suspensions (log CFU/g)	8.12±0.04 ^a	8.25±0.13 ^b	8.19±0.02 ^{ab}	8.11±0.05 ^a
Cell population in beads (log CFU/g)	8.08±0.03 ^a	7.83±0.09 ^a	7.50±0.07 ^a	7.71±0.07 ^a
Viability (%)	99.55±0.37 ^e	94.81±0.49 ^c	91.50±0.88 ^a	95.11±0.49 ^c
Encapsulation efficiency (%)	45.45±2.42 ^e	22.87±2.46 ^c	12.02±2.10 ^a	25.61±2.40 ^c
Diameter of beads (mm)	2.15±0.11	2.59±0.10	2.72±0.17	3.00±0.13
Yield of wet beads (%)	49.40±0.78 ^a	61.17±0.86 ^c	59.13±0.52 ^b	63.64±0.58 ^d
<i>Bifidobacterium longum</i>				
Cell population in encapsulating material suspensions (log CFU/g)	8.24±0.25 ^a	8.63±0.28 ^b	8.61±0.09 ^b	8.36±0.06 ^{ab}
Cell population in beads (log CFU/g)	8.03±0.22	8.06±0.26	7.94±0.05	7.94±0.09
Viability (%)	97.48±0.22 ^d	93.40±0.39 ^b	92.15±0.89 ^a	95.02±0.44 ^c
Encapsulation efficiency (%)	30.60±1.61 ^d	16.59±1.45 ^b	12.62±2.29 ^a	24.47±1.77 ^c
Diameter of beads (mm)	2.15±0.11	2.59±0.10	2.72±0.17	3.00±0.13
Yield of wet beads (%)	49.32±0.71 ^a	61.43±0.58 ^c	59.18±0.44 ^b	63.62±0.61 ^d

Value on the table were average ± standar of deviation with n=4. Value with different character showed the significant different (p<0.05) based on Duncan post hoc tests

alginate, therefore value of encapsulation efficiency resulted was higher than other encapsulating material. Encapsulation efficiency of *L. casei* and *B. longum* from skim milk-alginate were 45.4%±2.42 and 30.60%±1.61. Lactose content on skim milk as one of carbon source for growing lactic acid bacteria caused yield and bacteria cell number higher than other treatment.

Therefore, the percentage of the highest viability and encapsulation efficiency of *L. casei* and *B. longum* went to skim milk-alginate (control of treatment), indicating skim milk-alginate had better performance than the three others encapsulating materials. Those high percentages were caused by higher cells number of *L. casei* and *B. longum* in beads containing skim milk-alginate compared to the number in the three others encapsulating materials. These results can be explained that skim milk and alginate could form better beads matrices thus the occurrence of cell number decrease was lower and cell number can be more encapsulated compared to those with the three others encapsulating materials. Skim milk is easier to dissolve than other

starch. Solubility of encapsulating materials in the preparation of suspension might a factor which affected beads matrices. Skim milk-alginate was easy to dissolve in the water-based material, whereas sago starch-alginate was the most difficult. Therefore, matrices of skim milk-alginate beads more compact compared to sago starch-alginate beads. According to Castilla et al. (2010), characteristics of beads formed inter encapsulating materials would affect the successful of encapsulation.

Viability of probiotic was affected by several factors, such as nutrient availability, strain types, presence of growth *promotor* or inhibitor, O₂ solubility, and number or inoculation level (Oliviera & Damin 2003). According to those factors, one of ways that can be done to increase number and probiotic viability on beads was to increase the level of bacteria inoculation. Increase of cell number in suspension could increase cell numbers in beads and finally it might increase viability or bacteria encapsulation efficiency (Mortazavian et al. 2007).

Drying of *L. casei* and *B. longum* Beads

Drying of *L. casei* and *B. longum* done at 40°C using blower oven. Before doing drying, optimum drying time must be determined first. Drying time was determined by measuring beads mass during drying until constant mass was reached. Curve of beads mass decrease from each of encapsulating material can be seen on Figure 1.

During drying process, there had been a decrease in beads mass. All types of encapsulating materials (skim milk-alginate, maltodextrine-alginate, sago starch-alginate, and corn starch-alginate) seem underwent weight decrease constantly across time course. In hour 5 and 6, mass beads seem got stable, yet the condition of beads from all encapsulating materials still adhere on petridish and difficult to be released. Therefore, 7 hrs drying time was chosen either on beads of: skim milk-alginate, maltodextrine-alginate, sago starch-alginate, or corn starch-alginate has reached constant mass and did not adhere to the dishes. Dry beads resulted had flat-circled and brown in color. Beads appearance after being dried can be seen on Figure 2.

Characteristics of *L. casei* and *B. longum* dry beads can be seen on Table 3. According to statistical data

(anova), probiotic types did not affect moisture and yield of dry beads, because the two parameters just were affected by encapsulating materials. Encapsulation of *L. casei* and *B. longum* with maltodextrine-alginate resulted the lowest yield and moisture (4.58% with 12.50%±0.45 moisture content on *L. casei* dry beads and 4.85% with 12.56±0.465% moisture content on *B. longum* dry beads), whereas the highest yield and moisture content resulted by encapsulating material skim milk-alginate, they were 5.16% with moisture content 11.78%±0.32 on *L. casei* dry beads and 5.29% with moisture content 11.95 ± 0.28% on *B. longum* dry beads.

Dry beads moisture content showed margin of beads before and after drying process. During drying process, water evaporation might be happened, thus decrease mass of beads would occur. Moisture content of dry beads was affected by moisture content of encapsulating material and was not affected by number of cells and bacterial cell resilience. Bacterial cell resilience much more affected by beads matrices which was formed by encapsulating material, due to beads matrices would give protection during drying process and affected cell numbers on beads after being dried.

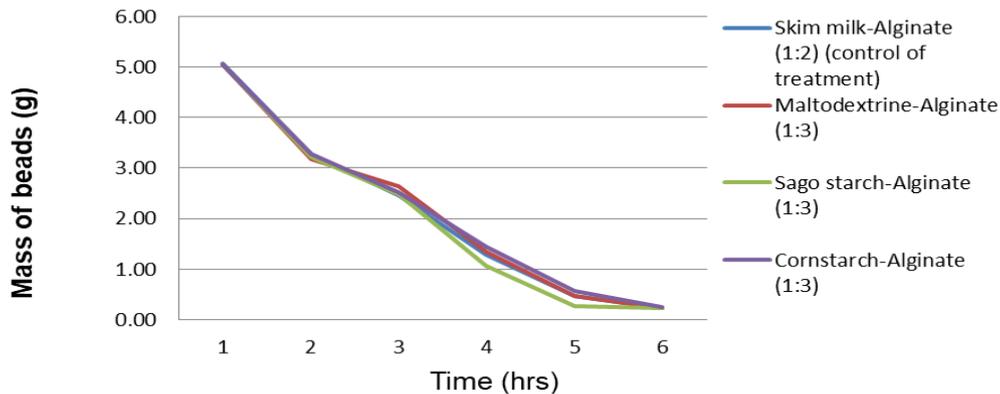


Figure 1. Curve of mass beads decrease during drying process.

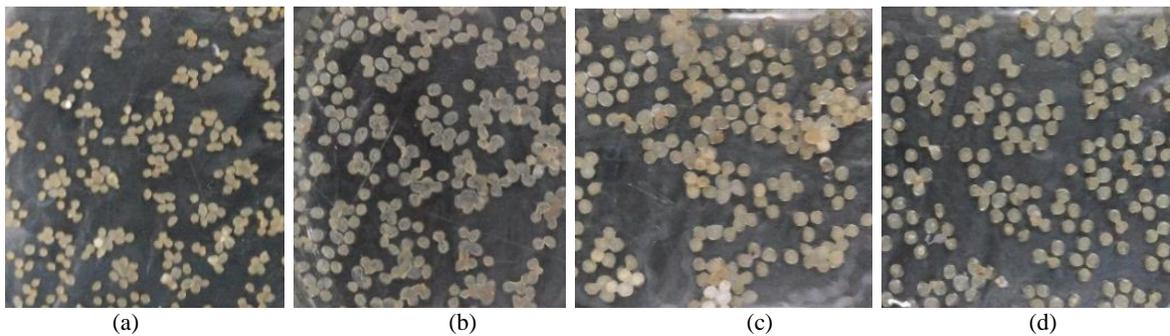


Figure 2. Appearance of dry beads made from following encapsulating materials : (a) skim milk-alginate, (b) maltodextrine-alginate, (c) sago starch-alginate, (d) corn starch-alginate.

Table 2. Properties of *L. casei* and *B. longum* dry beads

Lactobacillus casei				
Parameters	Skim milk-Alginate (1:2) (control of treatment)	Maltodextrine- Alginate (1:3)	Sago starch- Alginate (1:3)	Corn starch-Alginate (1:3)
Cell population in dry beads (log CFU/g)	5.08 ± 0.07 ^a	4.69 ± 0.08 ^b	3.71 ± 0.02 ^c	3.49 ± 0.03 ^d
Cell resilience (%)	63.84 ± 0.75 ^d	59.98 ± 0.81 ^c	49.56 ± 0.43 ^b	45.28 ± 0.82 ^a
Mass of dry beads (g)	2.55 ± 0.19	2.80 ± 0.27	3.05 ± 0.15	3.28 ± 0.13
Yield of dry beads (%)	5,16	4,85	5,16	5,15
Moisture (%)	11.78 ± 0.32 ^a	12.50 ± 0.45 ^b	13.25 ± 0.48 ^c	13.28 ± 0.22 ^c
Color	Brown	Brown	Dark brown	Brown
Bifidobacterium longum				
Parameters	Skim milk-Alginate (1:2) (control of treatment)	Maltodextrine- Alginate (1:3)	Sago starch- Alginate (1:3)	Corn starch-Alginate (1:3)
Cell population in dry beads (log CFU/g)	6.20 ± 0.16 ^d	5.32 ± 0.21 ^b	4.83 ± 0.09 ^a	4.83 ± 0.06 ^a
Cell resilience (%)	77.20 ± 0.46 ^f	65.98 ± 0.70 ^e	60.78 ± 0.84 ^c	60.84 ± 0.87 ^c
Mass of dry beads (g)	2.61 ± 0.09	2.98 ± 0.24	2.96 ± 0.22	3.16 ± 0.13
Yield of dry beads (%)	5,29	4,85	5,00	4,97
Moisture (%)	11.95 ± 0.28 ^a	12.56 ± 0.46 ^b	13.25 ± 0.29 ^c	13.30 ± 0.24 ^c
Color	Brown	Brown	Dark brown	Brown

Value on the table were average ± standar of deviation with n=4. Value with different character showed the significant different ($p < 0.05$) based on Duncan post hoc tests

Value of dry yield was affected by resulted beads mass. Among four encapsulating materials, skim milk-alginate (control of treatment) had the lowest beads mass (Table 1). Yield of dry beads seems to be affected by moisture content of the beads materials, thus it led to result lowest yield than other treatment. The moisture content of maltodextrine, skim milk, sago starch, corn starch and natrium alginate were 6% (Blancard & Katz 1995), 3% (Tamime & Robinson 1989), 12-21% (Wattanachant et al. 2002), 12-21% (Wattanachant et al. 2002), and 5-20% (Winarno 2008), respectively.

Probiotic population on dry beads less than those on wet beads. It is showed that during drying process, there was a decrease in bacterial cell number which presence in the beads. During drying process, *L. casei* and *B. longum* which were encapsulated with maltodextrine-alginate had lower bacteria cell decrease, compared to those with sago starch and corn starch. Encapsulation of *L. casei* with maltodextrine-alginate had decreased as much as 3.13 ± 0.07 log cycles, whereas *B. longum* had lower decrease (2.74 ± 0.07 log cycle). If it is compared with control of treatment, decrease on cell number of

maltodextrine-alginate was still higher. Cell number of skim milk-alginate encapsulated-*L. casei* had decreased as much as 2.92 ± 0.06 log cycles, whereas decrease of *B. longum* cell number was 1.83 ± 0.07 log cycle for the same encapsulating material. Percentage of decrease of *B. longum* and *L. casei* cell number after being dried can be seen on Figure 3 and 4.

If both bacteria are compared, decrease of *L. casei* bacterial cell number after being dried was higher than decrease of *B. longum* on all encapsulating materials. The difference was allegedly caused by the drying temperature factor. According to Heller (2001), *L. casei* grow in the temperature range of 15-40°C with 30°C as the optimum temperature, whereas *B. longum* could grow in the temperature range of 37-41°C with 37°C as optimum temperature (Holt et al. 1994). Temperature used in the drying process of *L. casei* and *B. longum* beads was 40°C. Margin of drying temperature and optimum temperature of *B. longum* growth was lower than those on *L. casei*. Therefore, death cell number of *B. longum* during drying process was lower than *L. casei*.

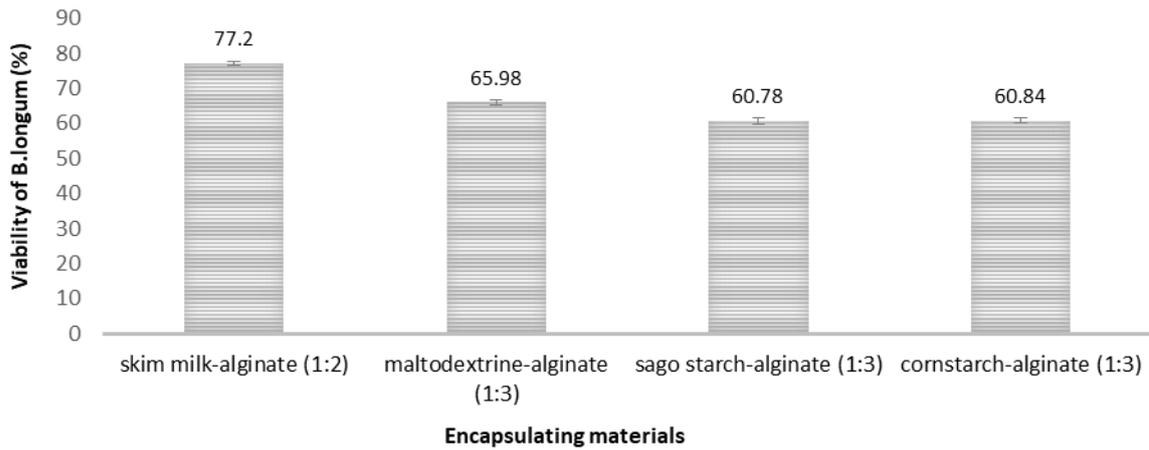


Figure 3. End population of *B. longum* after drying process (let the initial population in the wet beads = 100%).

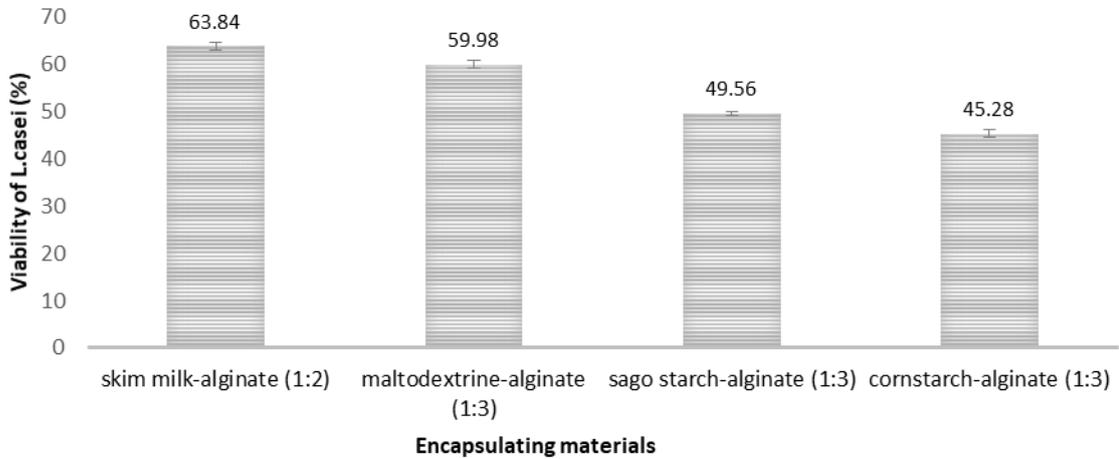


Figure 4. End population of *L. casei* after drying process (let the initial population in the wet beads = 100%).

Aside from temperature factor, decrease in *L. casei* and *B. longum* cell numbers during drying process might be caused by the loss of free water which act as important component of cells as well as the availability of oxygen. Free water is needed by bacteria to be used in metabolism process such as to synthesize cellular components, to help transport of nutrition, and to mediate other biochemical reactions (Rahayu & Nurwitri 2012). Therefore, decrease in free water number during drying process could decrease bacterial cell number. Oxygen is a sort of poison for lactic acid bacteria which may lead damages in bacterial membrane (Talwakar & Kailasapathy 2004). As long as drying process, beads were exposure to oxygen comes from air that presence in the oven, if oxygen reacts with bacterial cell it would lead oxidation and cell death.

Probiotic drying process either by oven, spray drying or freeze drying would lead decrease in bacterial cell number. According to Texeria et al., (1995), the loss of cell viability during spray drying was related to damages of cell components, membrane cell, cell walls and bacterial DNA because of high temperature that was used. Meanwhile on freeze drying, the presence of cell and medium cooling step to reach freezing point, forming of intra and extracellular ice, thawing process and reducing water in drying process leading the bacterial cell number decrease (Johnson & Etzel 1995). Whereas on drying process using oven, the main cause of decrease in bacterial cell number during drying process were temperature, water loss and the presence of oxygen.

The value of bacterial cell number decrease after being dried affected toward percentage of cell bacterial resilience. The higher the decrease of cell number, the lower its resilience. According to statistical data (anova) probiotic types and encapsulating material types significantly affected ($P < 0.05$) the percentage of cell resilience after being dried. The values of *L. casei* and *B. longum* resilience from each encapsulating materials can be seen on Table 3.

Among of all starch based-encapsulating materials used in this research, percentage of the highest cell resilience was obtained from *L. casei* and *B. longum* encapsulation with maltodextrine-alginate which reached $59.98\% \pm 0.81$ and $65.98\% \pm 0.70$. Nevertheless, if it is compared with control of treatment, percentage of cell resilience resulted by skim milk-alginate was still higher ($63.84\% \pm 0.75$ and $77.20\% \pm 0.46$). According to Lian et al. (2003), protein-based material such as skim milk was marked better as wall in protecting cell from heat (act as thermo protectant) compared to gelatin, soluble starch, and gum arab thus it was common to be used in drying process.

Castilla et al. (2010) reported that beads characteristics formed inter encapsulating materials affected the successful of probiotic encapsulation. The more compact beads matrices formed inter encapsulating material, the more it gave protecting toward probiotic cells. Beads compactness was affected by encapsulating material solubility when suspension was being made. Among three starch based-encapsulating materials that used, maltodextrine was the most soluble material with alginate and water compared to sago and corn starch. Although sago starch and corn starch can be dissolved after sterilization process, but clumps of solids still can be found in the suspension suggesting the starch did not dissolve completely in alginate, different from maltodextrine that could dissolve completely in alginate. According to Blancard & Katz (1995), maltodextrine had high soluble properties and strong bond power. Similar to maltodextrine, skim milk also could well dissolve when it was mixed with water and alginate. The good solubility of encapsulating material would result more compact beads because maltodextrine and skim milk will fill the porous spaces formed at natrium alginate matrices. Those will reduce direct contact between cell and outer environment for example with air (oxygen) and heat during drying process. Therefore, encapsulation of *L. casei* and *B. longum* with skim milk-alginate and maltodextrine-alginate resulted higher cell resilience compared to sago starch-alginate and corn starch-alginate.

According to the percentage of cell resilience and decrease of cell numbers during drying process, encapsulation of *L. casei* and *B. longum* with maltodextrine-alginate was better than sago starch-

alginate and corn starch-alginate, nonetheless, it had not better yet than those which was encapsulated with skim milk-alginate (control of treatment). Cell number of *L. casei* and *B. longum* on dry beads from encapsulating material maltodextrine-alginate was 4.69 ± 0.08 and 5.32 ± 0.21 log CFU/g. This number was fewer than those encapsulated with skim milk-alginate (control of treatment) which the number reached 5.08 ± 0.07 and 6.20 ± 0.16 log CFU/g. According to Tamime et al. (2005), minimum concentration of probiotic on food products was 10^6 CFU/g. Referring to that, dry beads from skim milk-alginate encapsulated *B. longum* seems fulfill the requirement of minimum probiotic concentration. Aim of encapsulation in this research was to produce probiotic dry starter. On its application, starter will be added to milk, then during fermentation time it will be increase in bacterial cell number which was generally will be followed with its increasing viscosity and milk acidity. Adrianto (2011) reported that application dry encapsulated *L. casei* as starter for cow dadih led to the increase of viscosity and milk acidity and the increase of *L. casei* cell number as much as 3 log cycles after 48 hours fermentation time.

Resilience testing of probiotic dry beads against low pH and bile salt

This test aimed to study ability of both probiotic bacteria to survive and their endurance and persistency in gastrointestinal environment, and to study ability of encapsulating materials in protecting both probiotic bacteria in low pH condition and the presence of bile salt. According to Schmid et al. (2006), resilience testing against low pH and bile salt can be done to test matrices of certain material in protecting probiotic cells. Gastrointestinal is main place which can affect probiotic bacteria viability in the human body. Gastrointestinal track started from mouth, esophagus, gastric, small intestine, colon, and end up in rectum. Gastric and small intestine is a critical location when in these places occur pH reduction and bile salt secretion. Therefore, to get small intestine, probiotic must be able to stay alive at low pH and in the presence of bile salt (Sahadeva et al. 2011).

Testing the resilience of *L. casei* and *B. longum* against low pH and the presence of bile salts was carried out continuously. According to the results, incubation of dry beads from the four encapsulating materials on PBS at pH 2.0 led the decrease of *L. casei* and *B. longum* bacterial cells. Decrease in *L. casei* and *B. longum* bacterial cells was significantly different ($P < 0.05$) on each encapsulating materials. Decrease of *L. casei* and *B. longum* from encapsulating material skim milk-alginate (control of treatment) was 0.19 ± 0.08 and 0.93 ± 0.18 log cycles. The decrease was lower than those of *L. casei* and *B. longum* encapsulated with the

three others encapsulating materials starch-alginate. Graph of decrease of the number of *L. casei* and *B. longum* led by treatment of pH can be seen on Figures 5 and 6.

Decrease in *L. casei* and *B. longum* during incubation process was caused by dissociated of HCl (hydrochloric acid) that resulted proton which led pH decrease in the outer cell media (extracellular pH). In a very acidic condition such as pH 2.0, pH of cell cytoplasmic (intracellular pH) could decrease, and the damages of bacterial cell outer membrane could occur leading the death of cell (Hutkins & Nannen 1993). Degree of cell resilience against low pH was varying on each bacteria. Based on decrease of *L. casei* and *B. longum* cell number after being incubated at pH 2.0, it is known that decrease of *L. casei* cell number from all

types of encapsulating materials was lower than those on *B. longum*. It is shown that genus of *Lactobacillus* more resistant against acidic condition rather than *Bifidobacterium*. Widodo (2003) reported that genus of *Lactobacillus* could grow at the range of pH 3.5-6.8, whereas genus of *Bifidobacterium* could grow at the narrower pH range (5.5-7.0). Bacterial tolerance against low pH due to their bacterial could maintain intracellular pH (cytoplasmic pH) to be stable at alkaline pH against extracellular pH. Although, decrease in intracellular pH will still keep continuing in agreement with the decrease of extracellular pH. Therefore, although probiotic genus like *Lactobacillus* tolerant to acidic condition, the decrease of cell numbers would still happen.

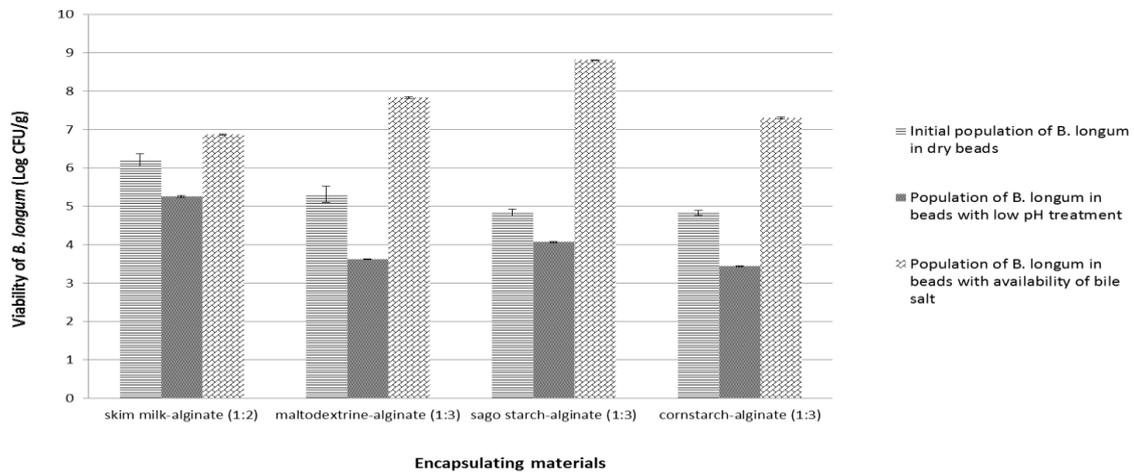


Figure 5. Graph changes on cell bacterial number (*B. longum*) due to low pH treatment and availability of bile salt.

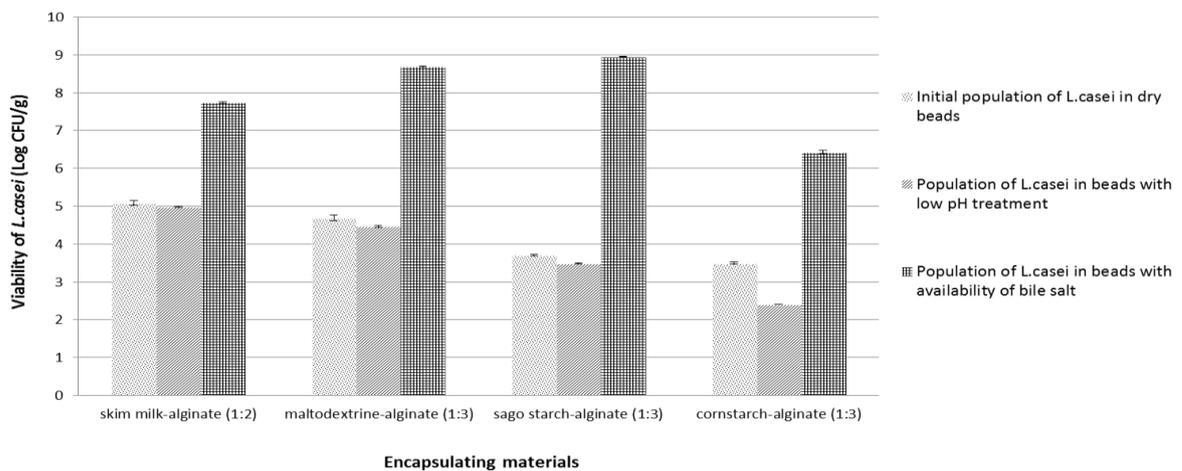


Figure 6. Graph changes on cell bacterial number (*L. casei*) due to low pH treatment and availability of bile salt.

Sultana et al. (2000) reported that testing the resilience of Hi-maize resistant starch encapsulated-*L. acidophilus* and *B. infantis* against low pH led the decrease of cell numbers as much as 5 and 3 log cycles after being incubated for 3 hours at pH 2.0. This was also happened on this research, compared to bacterial cell number on dry beads, cell number on beads which were incubated at the same condition also decreased. The decrease of *L. casei* and *B. longum* cell number was fewer than 2 log cycles. If it is compared with research results of Sultana et al. (2000), the decrease of bacterial cells caused by low pH in this research was lower. It is shown that encapsulation of *L. casei* and *B. longum* with maltodextrine-alginate, sago starch-alginate, corn starch-alginate, and skim milk-alginate (control of treatment) could protect bacterial cells on low pH.

Testing resilience of *L. casei* and *B. longum* dry beads against low pH and bile salt was carried out continuously. After being incubated for 36 hours on MRSB contained 0.3% of bile salt, there was an increase in the cell number of *L. casei* and *B. longum* compared to bacterial cell number that could survive at the low pH. The bacterial cell increase can be seen on Figure 8. Statistically, the increase of bacterial cell number after bile salt treatment was significantly different ($P < 0.05$) on each encapsulating materials. After incubation process, cell number of *L. casei* from encapsulating material maltodextrine-alginate, sago starch-alginate, and corn starch-alginate increased successively as much as 4.22 ± 0.03 , 5.47 ± 0.02 and 4.03 ± 0.04 log cycles, whereas *B. longum* cell number were 4.21 ± 0.03 , 4.74 ± 0.02 , and 3.87 ± 0.01 log cycles for the consecutive encapsulating materials. If compared with these three types of encapsulating materials, increase of cell number of *L. casei* and *B. longum* with encapsulating materials skim milk-alginate (control) was lower. Cell number of *L. casei* and *B. longum* from encapsulating material skim milk-alginate had increased 2.75 ± 0.02 and 1.61 ± 0.04 log cycles after 36 hrs incubation.

Jacobsen et al. (1999) reported that 0.3% bile salt was representative to test bacterial resilience against bile salt. Bacterial incubation in media containing bile salt generally would reduce bacterial cell number. This is caused by ability of bile salt to reduce lipid which was substance that arrange bacterial cell membranes. As a result, damages occurred on the bacterial cell membranes followed by leakages and cell lysis (Hill 1995). Different from testing of *L. casei* and *B. longum* dry beads against bile salt in this research, incubation of *L. casei* and *B. longum* in MRSB containing bile salt may have increased the bacterial cell number. This increase highly likely caused by the resilience of both bacteria against bile salt and use MRSB which has function as source of nutrition and medium of growth. According to Djide & Wahyudin (2008), lactic acid

bacterial isolates are able to grow in the medium, although bile salt has been added into the medium. Lin et al. (2006) also showed that there was increase some types of lactic acid bacterial as much as 1-4 log cycles after being incubated in MRSB containing 0.3% bile salt for 36 hours. Besides using MRSB, some resilience testing against bile salt also use sterile solution which was mixed with bile salt (Castilla et al. 2010; Lee & Heo 2000). Usage of those sterile solutions can be done to avoid increase of bacterial cell number which might be caused by the availability of nutrition from media.

Increase of *L. casei* and *B. longum* bacterial cell number after being incubated from encapsulating materials based on starch (maltodextrine-alginate, sago starch-alginate, and corn starch-alginate) was higher than those with skim milk-alginate (control of treatment). This was possible due to matrices of encapsulating materials. On encapsulating materials maltodextrine-alginate, sago starch-alginate, and corn starch-alginate, higher alginate concentration (3%) compared with skim milk-alginate (2.67%). Skim milk-alginate composition used in this research was the best result from previous study, while for the treatment were from previous optimization steps, thus presumably led beads from those encapsulating materials become porous.

According to Rokka & Rantamäki (2010), matrices of alginate were very porous so as to cause water diffusion in and out of beads. During incubation time, MRSB diffused into maltodextrine-alginate beads, sago starch-alginate beads, corn starch-alginate beads, and skim milk-alginate beads thus cause bacterial colonization. The highest increase of *L. casei* and *B. longum* cell number went to sago starch-alginate, whereas to skim milk-alginate (control of treatment), the increase of *L. casei* and *B. longum* were the lowest. Meanwhile on skim milk-alginate, it was allegedly that skim milk could fill more much alginate pores thus resulted in more solid beads matrices and formed barrier of media diffusion into beads. According to Castilla et al. (2010), solidity of encapsulating material matrices would affect ability of material to absorb and protect bacteria from acid and bile salt effects. Although more solid beads matrices could increase of lower bacterial cell number, dry beads with compact encapsulating material matrices hopefully could more protect probiotic from outer environment during handling and storage (Frazier & Westhoff 1998).

CONCLUSION

Maltodextrine-alginate was the best treatment compared to other encapsulating material (sago starch and corn starch) to viability, beads matrices characteristic, cell number on wet and dry beads, and

percentage of resilience cell of *L. casei* and *B. longum* during drying process.

Cell number of maltodextrine-alginate-encapsulated *L. casei* and *B. longum* in the form of dry beads was 4.69 ± 0.08 log CFU/g and 5.32 ± 0.21 log CFU/g, whereas cell number of skim milk-alginate-encapsulated *L. casei* and *B. longum* in the form of dry beads were higher, they are 5.08 ± 0.07 log CFU/g and 6.20 ± 0.16 log CFU/g.

Resilience of both encapsulated probiotics was different against low pH and availability of bile salt, whereas *L. casei* more resistant than *B. longum* against acidic (low pH) environment due to its tolerance to a wider range of pH compared to *B. longum*.

In the presence of 0.3% bile salt, *L. casei* and *B. longum* encapsulated with skim milk-alginate increased as much as 2.75 ± 0.02 and 1.61 ± 0.04 log cycles, respectively.

REFERENCES

- Adrianto A. 2011. Enkapsulasi *Lactobacillus casei* dengan teknik ekstrusi sebagai starter untuk pembuatan dadiah susu sapi [Script]. [Bogor (Indones)]: Bogor Agricultural University.
- Anal A, Singh H. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *J Trends Food Sci Technol*. 18:240-251.
- Arbolea S, Watkins C, Stanton C, Ross P. 2016. Gut bifidobacteria populations in human health and aging. *Front Microbiol*. 7:1-9.
- Blancard PH, FR Katz. 1995. Starch hydrolysis in food polysaccharides and their application. New York (USA): Marcell Dekker.
- Castilla OS, Calleros CL, Galindo HSG, Ramirez JA, Carter EJ. 2010. Textural properties of alginate-pectin beads and survivability of entrapped *Lb. casei* in simulated gastrointestinal condition and in yoghurt. *J Food Res Int*. 43:111-117.
- Djide MN, Wahyudin E. 2008. Isolasi bakteri asam laktat dari air susu ibu dan potensinya dalam penurunan kadar kolesterol secara in vitro. *Majalah Farmasi dan Farmakologi*. 12:73-78.
- Frazier WC, Westhoff DC. 1998. Food microbiology. 4th ed. Singapore (Singapore): McGraw-Hill Book Co.
- Fuller R. 1992. Probiotics: The scientific basis. New York (USA): Chapman & Hall.
- Gibson GR, Robertfroid MB. 1995. Dietary modulation of the human colonic microbiota-Introducing the concept of prebiotics. *J Nutr*. 125:1401-1412.
- Heller KJ. 2001. Probiotic bacteria in fermented foods: Product characteristics and starter organisms. *Am J Clin Nutr*. 73:374-379.
- Hill MJ. 1995. Role of gut bacteria in human toxicology and pharmacology. New York (USA): Taylor & Francis.
- Holt JG, Krieg NR, Sneath PHA, Staley JL, Williams ST. 1994. Bergey's manual of determinative bacteriology. 9th ed. Baltimore (USA): Williams and Wilkins Co.
- Holzappel WH, Schillinger U. 2002. Introduction to pre- and probiotics. *J Food Research Int*. 35:109-116.
- Hutkins EW, Nannen NL. 1993. pH homeostasis in lactic acid bacteria. *J Dairy Sci*. 76:2354-2365.
- Jacobsen CN, Nielsen VR, Hayford AE, Moller PL, Michaelsen KF, Erregaard AP, Sandstorm B, Tvede M, Jacobsen M. 1999. Screening of probiotic activities of forty seven strains of *Lactobacillus* spp. By in vitro techniques and evaluation of the colonization ability of five selected strains in human. *J Appl Environment Microbiol*. 65:4949-4956.
- Johnson JAC, MR Etzel. 1995. Properties of *Lactobacillus helveticus* CNRZ-32 attenuated by spray drying, freeze drying of freezing. *J Dairy Sci*. 78:761-768.
- Jownonski T, Zialinska M, Wyskakoska A. 1997. Encapsulation of lactic acid bacteria with alginate/strach capsule. *J Biotechnol Techn*. 13:3-13.
- Krasaekoopt WB, Bhandari H, Deeth H. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt. *Int Dairy J*. 13:3-13.
- Lee K, Heo T. 2000. Survival of *Bifidobacterium longum* immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. *J Appl Environment Microbiol*. 66:869-873.
- Lian WC, Hsiao HC, Chou CC. 2003. Viability of microencapsulated bifidobacteria in simulated gastric juice and bile solution. *Int J Food Microbiol*. 86:293-301.
- Lin WH, Hwang CF, Chen LW, Tsen HY. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *J Food Microbiol*. 23:78-81.
- Mandal S, Puniya AK, Singh K. 2006. Effect of alginate concentrations on microencapsulated *Lactobacillus casei* NCDC-298. *Int Dairy J*. 16:1190-1195.
- Mortazavian A, Razavi SH, Ehsani RM, Sohrabvandi S. 2007. Principles and methods of microencapsulation of probiotic microorganisms. *Iranian J Biotechnol*. 5:1-18.
- Oliviera MN, Damin MR. 2003. Efeito do teor de solidos e da concentrao de sacarose na acidificaca, fermeza e viabilidade de bacterias do iogurte e probioticas em leite fermentado. *Ciencia e Tecnologia de Alimentos*. 23:172-176.
- Rahayu WP, Nurwitri CC. 2012. Mikrobiologi pangan. Bogor (Indones): IPB Press.
- Reid G. 1999. The scientific basis for probiotic strains of *Lactobacillus*. *Appl Environment Microbiol*. 65:3763-3766.

- Rokka S, Rantamäki P. 2010. Protecting probiotic bacteria by microencapsulation: Challenges for industrial application. *European J Food Res Technol.* 231:1-12.
- Sahadeva RPK, Leong SF, Chua KH, Tan CH, Chan HY, Tong EV, Wong SYW, Chan HK. 2011. Survival of commercial probiotic strains to pH and bile. *Int Food Res J.* 18:1515-1522.
- Schmid K, Schlothauer RC, Friedrich U, Staudt C, Apajalahti J, Hansen EB. 2006. Development of probiotic food ingredient. In: Goktepe I, Junena VK, Ahmedna M, editors. *Probiotics in food safety and human health.* New York (USA): CRC Press.
- Shah NP. 2007. Functional culture and health benefits. *Int Dairy J.* 17:1262-1277.
- Sheu TY, Marshall RT. 1993. Microentrapment of *lactobacilli* in calcium alginate gels. *J Food Sci.* 58:557-561.
- SNI 01-2891-1992. Jakarta (Indones): Badan Standarisasi Nasional.
- Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K. 2000. Encapsulation of probiotics bacteria with alginate starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int J Food Microbiol.* 62:47-55.
- Talwakar A, Kailasapathy K. 2004. The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium* spp. *J Current Issues Intestinal Microbiol.* 5:1-8.
- Tamime AY, Saarela M, Sondergaard AK, Mistry VV, Shah NP. 2006. Production and maintenance of viability of probiotic micro-organism in dairy product. In: Tamime AY, editor. *Probiotic dairy products.* Oxford (UK): Blackwell Publishing.
- Tamime AY, Robinson AK. 1989. *Yoghurt science and technology.* Oxford (UK): Pergamon Press Ltd.
- Texeria PC, MH Castro, FX Malcata, RM Kirby. 1995. Survival of *Lactobacillus delburgi ssp. Bulgaricus* following dray drying. *J Dairy Sci.* 78:1025-1031.
- Wattanachant S, Muhammad SKS, Hashim DM, Rahman RA. 2002. Suitability of Sago Starch as a Base for Dual-Modification. *J Sci Technol.* 24:431-438.
- Widodo. 2003. *Bioteknologi industri susu.* Cetakan Pertama. Yogyakarta (Indones): Lacticia Press.
- Winarno FG. 2008. *Kimia pangan dan gizi.* Edisi Terbaru. Bogor (Indones): M-Brio Press.