Release Activity of Encapsulated *Lactobacillus plantarum* NBRC 3070 in Optimum Alginate - *Aloe vera* Matrices during Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) Exposure

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ABSTRACT— ***Probiotic encapsulation approach has the potential to protect microorganisms and to deliver them into the gut. Because of the promising preclinical and clinical results, probiotics have been incorporated into a range of products. However, there are still many challenges to overcome with respect to the encapsulation process and the conditions prevailing in the gut. Thus in this study, the release activity of encapsulated L. plantarum NBRC 3070 and Aloe vera gel within alginate coated chitosan matrices during simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) exposure were investigated. There were four groups of beads prepared in this study: 1) Encapsulated probiotic and Aloe vera within alginate beads (chitosan coated), 2) Encapsulated probiotic within alginate beads (chitosan coated), 3) Encapsulated probiotic and Aloe vera within alginate beads (uncoated) and 4) Encapsulated probiotic alone within alginate beads (uncoated). Encapsulation process was carried out using extrusion method. The optimized composition of alginate matrix (1.34% w/v) and Aloe vera gel (1.99% w/v) were used. In order to investigate their release activity, all beads were exposed in Simulated Gastric (SGF) at pH 2.5 and Simulated Intestinal Fluids (SIF) at pH 6.5 for 120 min and 270 min, respectively. Based on the findings, alginate-Aloe vera beads with chitosan coated was able to protect L. plantarum NBRC 3070 during SGF exposure with only 1 log_{10} cfu/mL reduction. The presence of Aloe vera gel in the beads improved the survivability of the cells. Encapsulated cells were observed successfully slow released of cells from the beads after exposure in SIF. Scan Electron Microscope (SEM) result had shown that cross link activity of the optimum alginate-Aloe vera with chitosan coating resulted in better survival of cells after simulated gastro and able to deliver sufficient probiotic dose to intestinal region. The combinations were able to improve encapsulated cells survivability during low acidic environment passage and release activity into the intestinal target region.***

Keywords—Alginate, *Aloe vera* gel, encapsulation, *Lactobacillus plantarum*

1. INTRODUCTION

The advantages of probiotic bacteria have attracted many researchers to uncover the secrets that are behind these delicate creatures. In fact, current evidence shows that probiotics can treat or prevent various diseases and disorders and syndromes [1] and this has attracted many people to take it as their daily food supplement. This is in accordance with the definition given by Food and Agriculture Association of the United Nations (FAO) and World Health Organization (WHO) which probiotic bacteria can be define as "live microorganisms that when consumed in adequate amount can give beneficial effects to the host health" [2,3] . However, there are difficulties in maintaining the viability of the probiotic cells during processing, storage and exposure along the gastrointestinal tract due to highly acidic condition in the stomach, bile acid elements in intestinal fluids and some others [4].

Prebiotic termed as food for probiotic bacteria which they are dietary fibers helps probiotics to act with better and more effectively and promotes growth of the bacteria [5]. Thus, to achieve this level, prebiotic must able to withstand the digestive process and cannot be digested by the host and can be utilized by probiotic bacteria [6]. In this study, *Aloe vera*

gel has been selected to be a prebiotic source because it has been proven that *Aloe vera* contain high level of carbohydrate which lead to be a potential prebiotic source and this have been proven by Nazirah et al., 2013 [7]. Hence, combination of both probiotic and prebiotic (synbiotic) can help maintain the life of the bacteria and as an increase to precautions taken when they travel along the gastrointestinal tract (GUT). The major problem is maintaining the cells viability during transit through gastrointestinal tract with low acidic environment before reaching to the target area. Thus, encapsulation approach was commonly used [8].

As mentioned earlier, prebiotics act as a food however, cannot protect the probiotic bacteria. Hence, the encapsulation process acts as a delivery tool for the bacteria to the desired place within the GUT. The most basic technique (extrusion technique method) for encapsulation process was used using alginate as the main supporting material. Alginate has the benefits of being non-toxic to the cells and it is accepted for food additive.

The aim of this work was to determine the effect of optimized co-encapsulation matrix between alginate, *L. plantarum* NBRC 3070 and *Aloe vera* gel on the survival and release activity of the encapsulated cells during simulated gastrointestinal tract exposure. The concentration of the optimized beads was used based on our previous studied [9].

2. MATERIALS AND METHODS

2.1 Microorganism Preparation

Microorganism, *Lactobacillus plantarum* NBRC 3070 was used in this study. The inoculum was prepared using de man Rogosa and Sharpe (MRS) broth (LabM Limited, Lancashire, United Kingdom) and cultivated at 37^0C for 24 h under anaerobic condition. The resulting cultures (50% v/v) were then transferred into 5 mL new MRS broth and incubated for 18 h under the same condition to obtain a cell density of about 10^8 colony forming units (CFU)/mL. Culture were harvested by centrifugation at 6000 rpm at 4^0C for 15 min and then the cells suspensions were mixed with new 5 mL MRS broth for further experiment.

2.2 Encapsulation of L. plantarum NBRC 3070

The capsules were prepared by using extrusion technique as described by Krasaekoopt et al. 2003 [10] with a modification using response surface methodology (RSM). Sodium alginate (1.34% w/v) was mixed with 24.4 mL in distilled water and sterilized at 121°C for 15 min. After cooling, cell suspension with volume of 5 mL in MRS broth was mixed in alginate solution together with *Aloe vera* gel at the concentration of 2.0% (v/v) in order to make a final volume of the mixture up to 30 mL. During the encapsulation process, the mixture was left stirred to ensure all compositions were well mixed. Then by using syringe (21G) this mixture was extruded into 200 mL of hardening solution, $CaCl_2$ to form capsule beads. The beads formed were then allowed to harden for 1 h before transferred into 100 mL of 1.0% (w/v) chitosan which act as coating material. The beads were then harvested, filtered and washed with sterile distilled water and ready for further analysis. *L. plantarum* NBRC 3070 encapsulated in alginate and coated chitosan was used as a positive control.

2.3 Encapsulation yield (%)

Enumeration of encapsulated *L. plantarum* NBRC 3070 from both matrices (with chitosan coating and without chitosan coating were carried out. They were enumerated on the de Man Rogosa Sharpe (MRS) agar by using pour plate technique. To determine encapsulation yield (EY) i.e. the number of bacterial cells that survived the process and encapsulated inside the microcapsules was calculated as follows:

$$EY = (N/N_0) \times 100$$

Where N_0 is the number of viable bacteria in CFU/mL of culture and N is the number of viable bacteria in CFU/g of microcapsules.

2.4 Preparation of Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF)

Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were prepared manually as described by Annan *et al*., 2008 [11]. For SGF preparation, 0.1 g of HCl mixed with 0.16 g of pepsin in 50 mL of distilled water and the pH was adjusted to 2.5 with hydrochloric acid. While for SIF, 0.225 g of bile salt been added to 0.05 g of pancreatin in 50 mL of distilled water and NaOH was used to adjust pH to 6.5. Both solutions were filtered with 0.45µm pore size of filter paper to give a sterile solution.

2.5 Survival of L. plantarum NBRC3070 in SGF and SIF

Approximately 1 g beads were placed in universal bottle containing SGF and incubated anaerobically at 37°C. Beads were harvested every 30 min for 2 h of incubation period. Subsequently, those 1 g beads were continue transferred into SIF and further incubated for 4 h. Sampling of beads from SIF was carried out every 1 h of incubation period. Survival

of encapsulated bacteria was determined by spread plate count on MRS agar after 48 h of incubation.

The shape and surface characteristics of the beads are determined by scanning electron microscope (SEM) in conditions: 1) after 2 h exposure in SGF and 2) after 2 h exposure in sequential incubation from SGF to SIF.

2.6 Statistical Analysis

The statistical analysis was performed using MINITAB version (Minitab Inc., PA, and United States). One-way ANOVA was used to examine significant differences between the normally distributed data.

3. RESULTS AND DISCUSSION

Entrapments of *L. plantarum* NBRC 3070 in four combination encapsulating matrices (alginate, *Aloe vera* and chitosan) were determined. Based on the results obtained, it can be observed that all encapsulating matrices were able to entrap the cells ranging from 66.1 to 73.7% (Table 1) and there were no significant differences ($p>0.05$) between coated and uncoated beads in encapsulation yield. Our findings indicated that the average yield of all samples after encapsulation and coating was 70.79%. These findings were in agreement with the results of Ling *et al*., in 2013 [12] by using extrusion method.

Table 1: The entrapment of *L. plantarum* NBRC 3070 (log_{10} cfu/mL) in *Aloe vera* and alginate (with and without chitosan coated) before SGF and SIF exposure.

	Initial *L. plantarum* NBRC 3070 concentrations (prior to encapsulation)	**Entrapped *L. plantarum* NBRC 3070 concentrations**	**Entrapment of *L. plantarum* NBRC 3070 concentration (%)**
L. plantarum NBRC 3070 entrapped within *Aloe vera* + alginate + coated chitosan	*12.67 ± 0.06[a]	9.18 ± 0.98[a]	72.5 [a]
L. plantarum NBRC 3070 entrapped within alginate + coated chitosan	12.39 ± 0.84[a]	8.78 ± 0.20 [a]	70.8 [a]
L. plantarum NBRC 3070 entrapped within *Aloe vera* + alginate (without coated chitosan)	11.99 ± 1.30[a]	8.84 ± 0.11 [a]	73.7 [a]
L. plantarum NBRC 3070 entrapped within alginate (without coated chitosan)	12.79 ± 0.11[a]	8.45 ± 0.44 [a]	66.1[a]

Note: *Mean in the same column followed by different lowercase letters are significantly different ($p<0.05$). The symbols ± indicate the standard deviation based on duplicate experiments.

The viability of entrapped *L. plantarum* NBRC 3070 within alginate and *Aloe vera* encapsulating matrices (coated and uncoated chitosan) during 120 and 270 minutes of incubation in the simulated gastric (SGF) and simulated intestinal (SIF), respectively were shown in Figure 1. As illustrated, the survival of entrapped *L. plantarum* NBRC 3070 was decreased throughput 120 min of SGF exposure. The survival of the entrapped cells within alginate-*Aloe vera*- chitosan coated beads was slightly decreased from 8.80 log_{10} cfu/mL to 7.12 log_{10} cfu/mL after 120 min in SGF. Meanwhile, 2 log_{10} cfu/mL cells reduction can be observed from both, uncoated alginate-*Aloe vera* beads and alginate coated beads. Exposure to SGF resulted in a considerable decreased in the total number of entrapped *L. plantarum* NBRC 3070 within uncoated alginate beads. Findings from this study, shows that the presence of *Aloe vera* in encapsulating matrices can play an important rule to sustain the survivability of the cells within beads. This might be due to prebiotic effect of *Aloe vera* as it is rich in acemanan and able to improve probiotic populations in gastrointestinal region [13]. Besides, researchers also reported that entrapped cells in beads are better protected in the presence of prebiotics [14, 15]. Moreover, encapsulation in chitosan coated able to protect the cells in low acidic environment as alginate alone did not effectively protect the entrapped cells due to porosity of alginate in acidic environment [16].

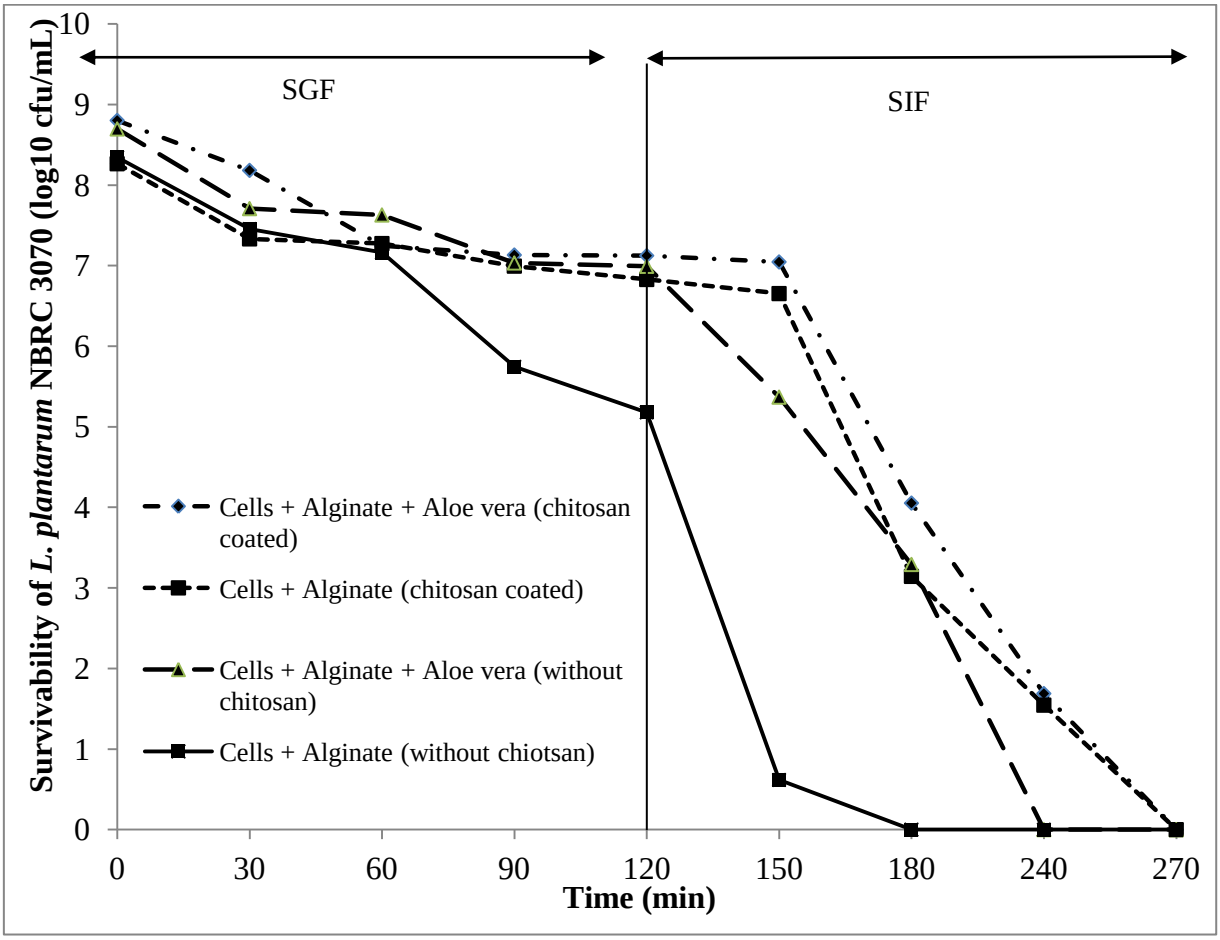

Figure 1: The survival of *L. plantarum* NBRC 3070 (log_{10} cfu/mL) in alginate-*Aloe vera* beads (coated chitosan and uncoated) during exposure in SGF and SIF.

The effect of the SIF on the viability of the entrapped *L. plantarum* NBRC 3070 is presented in Figure 1. The numbers of cells were declined significantly as the incubation time increased. The rate of cells decrease was significantly greater in the uncoated alginate beads and fully released of cells occurred at 180 min of SGF exposure. Slow release of cells from encapsulating matrices, alginate-*Aloe vera* (coated chitosan and uncoated) were observed and occurred between 240 to 270 min of intestinal exposure. Although cells from all four types of beads were successfully deliver to intestinal region, the cells population initially reaching to the region is important in order to ensure the sufficient to boost health benefits to the host. According to researchers [17, 18], the range of the cells reaching to intestinal region is recommended to be more than 10^6 cfu/mL in order to be efficient in their health benefits effect.

Scanning electron microscopy showed the evidence of the cross linking activity of the matrices after expose to SGF and SIF. As shown, the cells remain intact within alginate-*Aloe vera* – chitosan coated beads (Figure 2a) and alginate beads chitosan coated (Figure 2c) after 120 min SGF exposure. In contract, the porosity of alginate-*Aloe vera* uncoated beads (Figure 2b) and alginate uncoated beads (Figure 2d) in SGF can be observed.

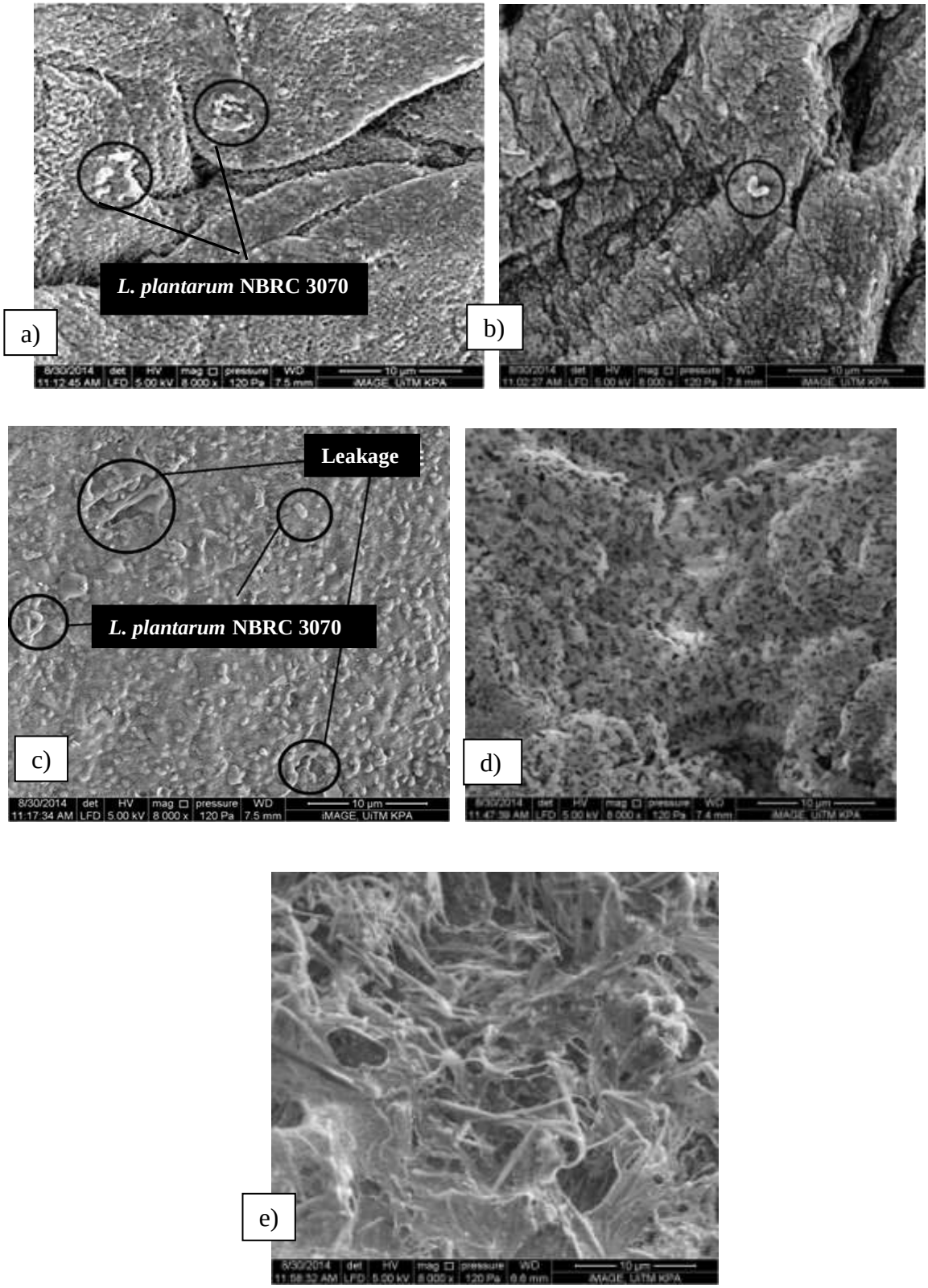

Figure 2: Scan electron microscopy images of (a) alginate-*Aloe vera* – chitosan coated beads after 120 min in SGF, (b) alginate-*Aloe vera* uncoated beads after 120 min in SGF, (c) alginate beads chitosan coated after 120 min in SGF and (d) alginate uncoated beads after 120 min in SGF and e) alginate-*Aloe vera* – chitosan coated beads after 270 min in SIF.

After 270 min SIF exposure, the cross linking activity of the matrices become weaker and this is might be the reason of cells were start to be released in the intestinal region and the beads were started to disintegrate (Figure 2e). This observation give an indication that the relaxation of cross linked activity between alginate-*Aloe vera* chitosan coated

had occurred, suggesting that the cells started to be released into the intestinal region.

4. CONCLUSIONS

Encapsulation of *L. plantarum* NBRC 3070 in alginate-*Aloe vera* with chitosan coating resulted in better survival of cells after simulated gastro and able to deliver sufficient probiotic dose to intestinal region. Moreover the beads were also able to control the release activity of the cells upon reaching to intestinal region. Therefore the applied approach in this study might prove beneficial for the delivery of probiotic cultures to the simulated human gastro-intestinal tract. Chitosan coating provided the best protection of cells in the presence of gastric juice due to its low acid resistance.

5. ACKNOWLEDGEMENT

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6. REFERENCES

[1] Goldin, B. R., "Probiotics and Health Claims: From History to Future; Probiotics and Health Claims", First Edition, Blackwell Publishing Ltd, United Kingdom, 2011.
[2] Sanders, M. E., "Probiotics: Definition, Sources, Selection, and Uses", Clinical Infectious Diseases, 46 (Supplement 2), pp. S58-S61, 2008.
[3] Kotzampassi, K., E. J. Giamarellos-Bourboulis, "Probiotics for infectious diseases: More drugs, less dietary supplementation", International Journal of Antimicrobial Agents, vol. 40, no 4, pp. 288-296, 2012.
[4] Chen, S., Q. Zhao, "Development of a novel probiotic delivery system based on microencapsulation with protectants", Applied Microbiology and Biotechnology, vol. 93, no. 4, pp. 1447-1457, 2012.
[5] Vernazza, C. L., Rabiu, B. A., Gibson, G. R., "Human colonic microbiology and the role of dietary intervention: Introduction to prebiotics", Prebiotics: Development and application, Chichester: John Wiley & Sons Press, pp. 1–2, 2006.
[6] N. P. Shah, A. G. d. Cruz and J. d. A. F. Faria, "Probiotic and Prebiotic Foods: Technology, Stability and Benefits to Human Health", New York, Nova Science Publisher, Inc. pp. 25-39, 2011.
[7] Nazirah, M. Y. H., S. N. Syahirah, "Screening of *Aloe vera* medium with different carbon and nitrogen sources for *Lactobacillus acidophilus* cultivation using fractional factorial design (FFD)", Proceedings - 2013 IEEE 3rd International Conference on System Engineering and Technology, ICSET, 2013.
[8] Moore, S and Kailasapathy, K., "Microencapsulation: Science and technologies for probiotics and prebiotics. In: Probiotic and prebiotic Foods: Technology, Stability and Benefits to the Human Health", Nova Science Publishers, NY. ISBN. 978-1-61668-842-4, 2011.
[9] Syahirah, S. N., Y. Anida, "Optimization of *Aloe vera* and alginate as encapsulating matrices for *Lactobacillus acidophilus* using FCCD-RSM approach", Proceedings - 2013 IEEE 3rd International Conference on System Engineering and Technology, ICSET. 2013
[10] Krasaekoopt, W., B. Bhandari, "Evaluation of encapsulation techniques of probiotics for yoghurt", International Dairy Journal, vol. 13, no.1, pp. 3-13, 2003.
[11] Annan, N. T., A. D. Borza, "Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic *Bifidobacterium adolescentis* 15703T during exposure to simulated gastro-intestinal conditions", Food Research International, vol. 41, no.2, pp.184-193, 2008.
[12] Ling-Xia Pan, Xiu-Juan Fang, Zhen Yu, Yang Xin, Xiao-Ying Liu, Lu-E Shi, Zhen-Xing Tan., "Encapsulation in alginate–skim milk microspheres improves viability of *Lactobacillus bulgaricus* in stimulated gastrointestinal conditions", Journal International Journal of Food Sciences and Nutrition, vol.64, no. 3, 2013.
[13] Gullón B1, Gullón P, Tavaria F, Alonso JL, Pintado M., "*In vitro* assessment of the prebiotic potential of *Aloe vera* mucilage and its impact on the human microbiota", Food Function, vol. 6, no. 2, pp 525-31, 2013.
[14] Zanjani MAK, Tarzi BG, Sharifan A, Mohammadi N, Bakhoda H and Madanipour MM., "Microencapsulation of *Lactobacillus casei* with calcium alginate-resistant starch and evaluation of survival and sensory properties in cream-filled cake", African Journal of Microbiology Research, vol. 6, pp. 5511-5517, 2012.
[15] Homayouni A, Azizi A, Ehsani MR, Yarmand MS and Razavi SH., "Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream", Food Chemistry, vol. 111, pp. 50-55, 2008.
[16] Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ., "Degradation of partially oxidized alginate and its potential application for tissue engineering", Biotechnology Progress, vol. 17, pp. 945–950, 2001.
[17] Borchers A.T., Selmi C., Meyers F.J., Keen C.L., Gershwin M.E., "Probiotics and immunity", Journal of Gastroenterology, vol. 44, pp. 26–46, 2009.
[18] Elizabeth C. Verna, "Use of probiotics in gastrointestinal disorders: what to recommend?", Therapy Advance in Gastroenterology, vol. 3, no. 5, pp. 307–319, 2010.