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Abstract. Encapsulation aimed to protect and stabilize cells during the production process and applications in food products. Carrageenan extrusion techniques do not cause damage to cells so that viability is maintained. The research aimed to get the highest yield and viability from various concentrations of carrageenan coating material. Encapsulation of probiotic *Lactobacillus paracasei* ssp. *paracasei* Ml3 using carrageenan extrusion technique with a concentration of 1%, 2%, 3% and 4%. The parameters observed were microcapsule yield, diameter, sensory test and cell viability of probiotic. The results showed that the highest yield was obtained at a 3% carrageenan concentration of 43.2% with a diameter of 3.98 mm, round-oval shape, yellowish white color. The highest viability of microcapsules at 1% carrageenan concentration of 10.44 log CFU / g, 2% of 10.89 log CFU / g, and 3% of 10.11 log CFU / g was not significantly different between treatments, whereas at the lowest 4% concentration of 8.25 log CFU / g.

1. Introduction

Probiotic encapsulation technologies could stabilize the cell viability during the production process and minimized the negative effect of processing so that the effect on health can be maintained. The health effects of probiotics will be retrieved when consuming probiotics 10^6-10^7 colony/g [1]. Probiotic bacteria can be protected using the encapsulation coating method.

Encapsulation is a process of coating probiotic bacteria form the inner matrix-like capsule that protects core materials. Microencapsulate is the process of the formation of a layer with a matrix inside a wall of capsules [2]. Encapsulation of bacteria is capable of protecting the microbe from unfavorable conditions, such as heat and chemicals or the worst environment, thus maintain viability and shelf life [3]. Encapsulation can be made by some techniques like emulsion and extrusion [1]. The technique of extrusion is done by adding probiotic microorganisms into the protective solution, then melted into $CaCl_2$ solution as hardener using by syringe to form the beads. This technique is low damage cells of the probiotic because it does not use high temperature, viability remains high, it can be done on the condition of aerobic and anaerobic.

Commonly used encapsulating materials are polysaccharides and proteins of various types, such as; starch, alginate, gum arabic, gelatin, carrageenan, lipids (such as wax) albumin, soybeans, whey, and

casein. Encapsulation materials have different characteristics; therefore, it is necessary to select the right one, so that it matches the core material to be encapsulated [4]. Carrageenan is a polysaccharide extracted from brown seaweeds Eucheuma cottonii and Eucheuma red spinosums. Carrageenan has the important role of stabilizer (balance control), thickener (thickening), forming a gel, emulsifiers.

Carrageenan is much applied in food and pharmaceuticals, non-toxic and does not cause irritation. Carrageenan is widely used as supplementary material to fix the food texture. Carrageenan is a polymers gel-forming; thus, the composition of the form allows the Helix gel to be perfect. According to Rowe, et al. [5]. The use of carrageenan on encapsulation is 0.02-2.0% [5]. Gel form depends on temperature changes, at a temperature of 40-45°C and gelation occurs after cooling to room temperature.

The research aimed to find the right concentration of carrageenan using encapsulation material extrusion method, with respect to the characterization of the resulting beads.

2. Materials and Method

The materials used were isolated *Lb. paracasei* ssp. *paracasei* M13-obtained from dadih, fermented buffalo milk from West Sumatera. Carrageenan (Bratacahem), $CaCl_2$ (Merck), MRS broth (Oxoid), Bacto Agar (Oxoid), KH_2PO_4, 90% alcohol, methylated spirit, and aquades. The tools used are incubator, autoclave, sterilization ovens, laminar flow, colony counters, microscope, caliper, micropipette, water bath, analytic scales, vortex, magnetic stirrer, test tubes, petri dish, Erlenmeyer flask, baker glass, bunsen, and microscope.

2.1. Research Design

This research consisted of 1) preparation of isolate, 2) encapsulation of the extrusion with 4 treatments concentration of carrageenan i.e. 1%, 2%, 3%, and 4%. The experiments were repeated four times, and the results are presented in the value of the standard deviation (SD) using SPSS 20. When the results of the ANOVA shows a distinction in treatment, then continued with a real difference test Duncan with the level of $P<0.05$.

2.2. Research Procedure

2.2.1. The making of microcapsule carrageenan. Making microcapsules refers to the modified Le-Tien, Millette, Mateescu, and Lacrox [6] and Rokka and Rantamaki (2010) methods. Bacterial culture *Lb. paracasei* ssp. *paracasei* M13 as much as 10% refreshed, then centrifuged at a speed of 4500 rpm for 15 minutes. Then washed with sterile water and re-centrifuged at 3000 rpm for 10 minutes. Pellets are dissolved with sterile aquades to form a suspension with a concentration of 10%. Carrageenan was prepared according to treatment that is 1%, 2%, 3% and 4%, the solution was shaken until homogeneous then sterilized at 121°C for 10 minutes. The capsule solution is cooled to 45°C, then a culture suspension is mixed with a ratio of 1: 4 (10 ml of probiotic culture mixed with 40 ml of the capsule solution). The solution is stirred then the mixture is put into syringe No. 30 G in a volume of 50 ml. The mixture is dropped into a 4% CaCl hardener solution and left for 120 minutes in a refrigerator to form a capsule. Microcapsules were washed with saline was repeated twice with a 15 minutes washing interval. Microcapsules are stored in sterile bottles.

2.2.2. The yield of the microcapsule. The microcapsule yield is calculated by weighing all the microcapsules produced. The yield is the result of reducing the initial weight of the carrageenan suspension with the final weight of the microcapsules produced, then divided by the initial weight of the suspension and multiplied by 100%.

2.2.3. Measurement of microcapsule the diameter. The diameter of the microcapsules was measured using Li-Tien method [6] by measuring the diameter of the microcapsules of 15 pieces at random, then measured using a digital caliper 150×6/0.01 mm (Krisbow, Indonesia).

2.2.4. Sensory testing microcapsule. The sensory observation was carried out by looking at the differences in color, shape, and smell of microcapsules, the scoring method used to provide sensory quality value at the level of quality scale or score—numeric scale one as the lowest and three being the highest.

2.2.5. Viability of Lb. paracasei ssp paracasei M13. A number of cells that persist in a microcapsule after extrusion refers to the method [6] and [7] being modified. Microcapsules weighed as much as 1g dissolved in 9 ml of sterile diluent solutions, shaken with Vortex until crushed, and then silenced for an hour. Further dilution series until seven, then as much as 1 ml is inserted into Petri dish, then poured the media MRS Agar. Incubation for 48 hours at 370C, the colony grew by the method of Total Plate Count (TPC) [8].

3. Result and Discussion

3.1. Yield of Microcapsule

The average yield of microcapsule produced with different concentrations of carrageenan is presented in Figure 1.

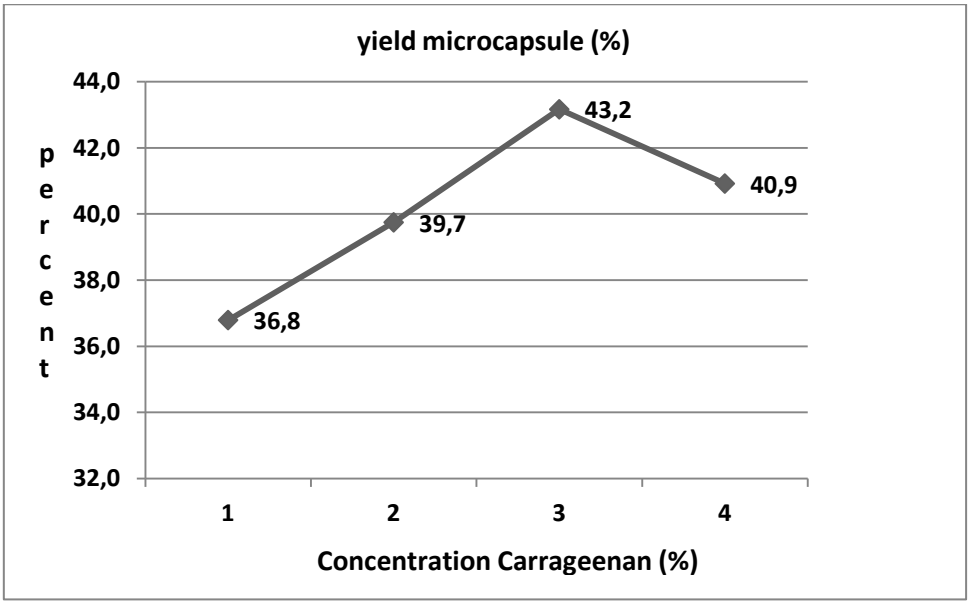

Figure 1. The yield of Microcapsule on Various Concentrations of Carrageenan

The highest yield was obtained at a 3% carrageenan concentration of 43.2%, the yield increased with increasing levels of the encapsulated material, and at a concentration of 4% it decreased to 40.9%. The movement of carrageenan suspension droplets with bacterial cells will increase with increasing intensity, thereby reducing the possibility of sticking between microcapsule droplets and microcapsules that form more compact when dripped into CaCl2 solution, as a result, the yield increases to a concentration of 3%. Conversely, the lower the level of the more dilute suspense resulted in an encapsulated droplet movement more quickly and result in micro-capsules accumulate and sticky at the bottom of the glass and the yield of the microcapsules to be down.

The lower the concentration, the more water trapped in suspension encapsulation then decline the strength of mechanical micro-capsules that generated so much more tender [9]. The increasing number of beads generated is affected by an increase in $CaCl_2$ solution, so it will improve the efficiency of the absorption of molecules at the time of the printing solution of carrageenan [10].

3.2. Diameter of Microcapsule

Figure 2 shows the difference in the width of the microcapsules with increasing concentrations of carrageenan. A High carrageenan level at 4% treatment is 4.4 mm. It results in a stronger bond between the gels so that the gel becomes more compact, and the size of the microcapsule diameter becomes larger.

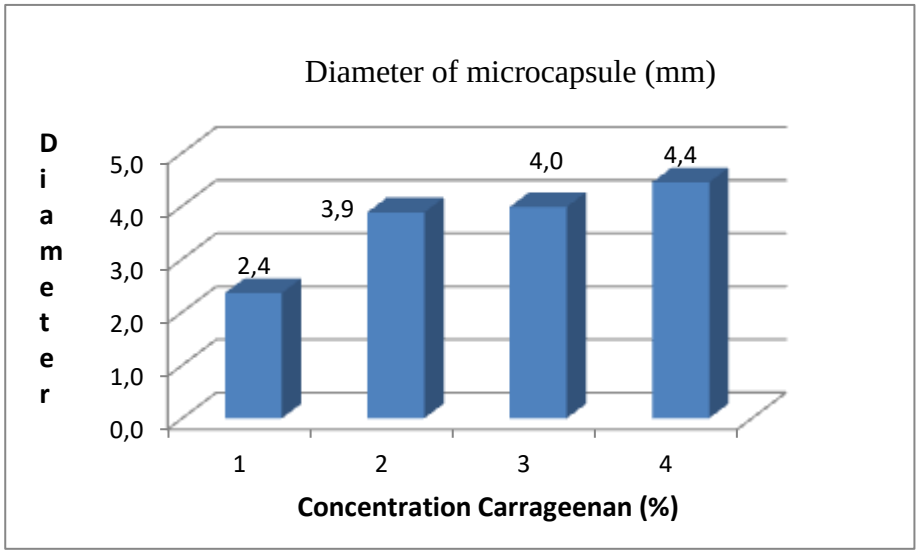

Figure 2. The diameter of Microcapsule on Various Concentrations of Carrageenan

Water molecules will surround carrageenan with a more hydrophilic polymer. As a result, IM mobilization and the solution will be more condensed along with an increase in the concentration of suspense. It further impacts the size of the droplets at the time of the formation of micro-capsules [11]. While the size of the diameter of the microbead capsule due to the difference in the value of viscosity [1]. Microbead, diameter size capsule produced four concentrations above still acceptable micro-capsule size extrusion method, i.e., range 0.1 – 10 mm [12].

3.3. Sensory Testing Microcapsule

Microcapsules were made using the carrageenan extrusion method; the size of the microcapsules is more uniform than the emulsion method. The results are as in Table 1 below. The results are as in Table 1 below.

Table 1. Assessment Sensory Test Microcapsule Carrageenan at Different Concentration

Carrageenan concentration (%)	Sensory Test		
	Form	Colour	Odor
1	Perfectly round	White	Odorless
2	Perfectly round	White	Odorless
3	Round-oval	White yellowish	Odorless
4	Elongated	White yellowish	Odorless

As shown in Table 1, the perfect round form of Microcapsule was obtained at a lower carrageenan concentration level. Vice versa, with a higher viscosity, the more viscous the microcapsule is. Hence, it is increasingly difficult for dripping out from the suspense of needles because the microcapsules are not spherical. The same is also true with regards to the color, and it becomes more yellowish with higher carrageenan concentration. But, the odor is not affected.

3.4. Viability of Lb. paracasei spp. Paracasei M13

The results of the analysis of the viability of cells of microcapsules *Lb paracasei* ssp *paracasei* M13-with different kinds of material encapsulation carrageenan concentration can be seen in Table 2.

Table 2. Viability of *Lb. paracasei* spp. *paracasei* M13

Carrageenan concentration (%)	Free Cells of *Lb. paracasei* spp. *paracasei* M13 (log cfu/g)	*Lb. paracasei* spp. *paracasei* M13 Encapsulated (log cfu/g)
1	10.41	10.45
2	10.41	10.89
3	10.41	10.11
4	9.15	8.96

Table 2 shows that there is no change in cell viability before and after encapsulation, except in the 4% level, there log decreased. Wherein the coating material, carrageenan can protect the cells during the extrusion process. One of the factors causing the decline of the cells after encapsulation is lost cells in a solution of $CaCl_2$ and lose viability in microencapsulation of cells with microorganisms; resilience engineering extrusion reached 85-95% [1]. The encapsulation material is a protector that protects the bacteria from the external influences so that the process of trapping takes place. Added by Ding and Shah (2009), carrageenan is encapsulating that it is able to protect probiotic bacteria as effectively from the influence of pressure and environmental conditions [13].

4. Conclusion

Encapsulated cell protection delivers carrageenan probiotics *Lb. paracasei* ssp *paracasei* M13 are perfect so that all cells are added to the suspension would be trapped in a matrix of carrageenan. The highest concentrations were obtained until the viability of carrageenan 3% i.e. reaching 10^{10} colonies/g (log 10), whereas at concentrations of 4% occur slightly decreased from 10^9 colonies/ml be 10^8 colonies/g (8.9 log). The highest yield was obtained at concentrations of 3% carrageenan as much as 43.2.08% with a microcapsule diameter of 3.98 mm. Characteristics of micro-capsules that generated the odorless, white to yellowish white and to rounded capsule form until a little bit elongated.

5. Acknowledgment

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References

[1] Krasaekoopt W, Bhandari B, and Deeth H 2003 Evaluation of Encapsulation Techniques of Probiotics for Yoghurt. *Int. Dairy J.* **13** 3-13 doi: 10.1016/S0958-6946(02)00155-3.

[2] Corona–Hernandez RI, ´Alvarez–Parrilla E, Lizardi–Mendoza J., Islas–Rubio AR, Rosa LA, and Wall–Medrano A 2013 Structural Stability and Viability of Microencapsulated Probiotic Bacteria *Comprehensive Reviews in Food Science and Food Safety* **12(6)** 614–628.

[3] Wu W, Roe WS, Gimino VG, Seriburi V, Martin DE, and Knapp SE 2000 Low Melt Encapsulation With High Laurate Canola Oil *US. Patent* **6153 326**.

[4] Desmond C, Stanton C, Collins GFK, and Ross RP 2002 Improved survival of *Lactobacillus paracasei* NFBC 338 in spray dried powders containing gum acacia *J of Appl Microbiol* **93** 1003-1012.

[5] Rowe, Raymond C, Sheskey PJ, and Quinn ME 2009 *Handbook of Pharmaceutical Excepient* 6th edition. USA: **Pharmaceutical Press.**

[6] Le-Tien C, Millette M., Mateescu MA., Lacrox M 2004 Modified algnate and chitosan for lactic acid bacteria mmobilzation *Botechnol Appl Biochem* **39** 347 – 354.

[7] Ivanovska, Tanja Petrusevska-Toji L, Kostoska MKD, Geskovski N, Grozdanov A, Stain C, Stafilov T, Mladenovska K 2012 Microencapsulation of Lactobacillus casei in chitosan-Ca-alginat microparticles using spray drying method *Maced J Chem Chem Eng* **31** 115-123.
[8] Aneja KR 2013 *Experimen in Microbiology, Plant Phatology and Biotechnology* India: **New Age International Publisher**.
[9] Chan SW, Mirhosseini SH, Farah ST, Tan CP 2011 Comparative study on physical properties of K-carrageenan extracted from Eucheuma cottonii in Tawau, Sabah and commercial k-carrageenan. *LSP* **18** 310-317.
[10] Homayouni A, Azizi A, Ehsani MR, Yarman MS, Razavi SH 2008 Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of symbiotic ice cream *Food Chemistry* **111** 50-55.
[11] William RB, Harper AR 2010 Carrageenan In ImersonA (ed) Food Stabilizer, Thickner and Gelling Agent 73-88. **Whey-Blackwell Publshng Ltd**,Oxford England.
[12] Solanki HK, Pawar DK, Shah DA, Prajapati VD, Jani GK, Mulla AM, and Thakar PM 2013 Development of Microencapsulation Delivery System for Long-Term Preservation of Probotics as Biotheraupetics Agent *Biomed Research Journal*.
[13] Ding WK, and Shah NP 2009 Effect of various encapsulating materials on the stabilty of probiotic bacteria *Journal of Food Science* **74 (2)** M1000-M107.