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RESEARCH ARTICLE

VIABILITY AND SURVIVAL RATE OF PROBIOTIC *LACTOBACILLUS PARACASEI* SSP. *PARACASEI* MI3 IN CARRAGEENAN-SKIM MILK BIO-CAPSULES

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Abstract

The ingredients of coated carrageenan which mixed with skim milk as the physical immersion material will able to stabilize the cell viability along the production process. It could reduce the negative impact of the processes, and decrased of resistance inside gastrointestinal due to the release of gastric acid. This study aimed to compare of Carrageenan-skim against bio-capsule viability, probiotic survival, bio-capsule viability at low pH 2.0 and the synthesis of probiotics in bio-capsules pH 2.0. The microencapsulation procedure with extrusion technique were performed with different composition of coated ingredients and dropped into a sterile 3% KCL solution. The respective composition are 1:1, 2:1 and 3:1.Carrageenan-skim coating can significantly protect the probiotic cells ($P \leq 0.05$) with high visibility and durability of the pH 2.0 as well as bio-capsule probiotic synthesis and bio-capsule synthesis at pH 2.0 in various comparisons. The comparison of 2:1 was obtained the highest viability of 1.97 x 10^9 CFU/G and suffered a decrease in visibility during extrusion of 0.7 Log CFU/G, with a synthesis in bio-capsules of 93.3%. Resistance to pH 2.0 after 3 hours incubation is 2.6 x 10^8 with a decrease after extrusion 0.89 Log CFU/G and the survival rate in bio-capsule pH 2.0 is 82.94%.

Introduction:-

Consumers are more often interested to choices their food having positive impact and improve their health.This type of functional food that take advantage of health beyond offering a nutrition.Functional food are the simplest by consumed as natural ingredient, even modified through fortification, enrichment or enhancement.Example of functional food are probiotic (1).

The probiotics must survive in the acidic conditions in stomach and keep counting the food. Consumption probiotics have health benefit if viable bacteria delivered to the intestines around 10^6-10^9CFU/ml (2). One of probiotics from local food is *Lactobacillus paracasei*(*Lb. paracasei*) *spp. paracasei* MI3. Unfortunately, the viability of probiotic in food products is sensitive to the manufacturing process and storage conditions.Exposing *the Lb. paracaseispp. paracasei* MI3 to oxygen, high temperatures, acidity condition or light, might be risky to viability of viable cells(3).

The most commonly method for against stress environment are encapsulation. This is can improve viability and survival rate of probiotic viable cells. The encapsulation materials include alginate, carrageenan, xanthan, pectin,

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and chitosan.There are three most popular types of carrageenan, that is mono-sulfated κ-carrageenan, bi-sulfated ι-carrageenan and tri-sulfated λ-carrageenan, despite κ-carrageenan can make gels. The κ-carrageenan hydrogels have been used for bio-capsule of bioactive components like probiotics (3)

Coating material used to encapsulation can make with carrageenan suspension, than added with skim milk. Combination skim milk and maltodextrin can improve performance of beverage containing probiotic(4). When probiotic cells were encapsulated in alginate beads amended with skim milk, higher survival rates were observed compared to either free cells or cells immobilized in alginate alone(5).The objective of this study was to evaluate the viability and survival rate on acidity condition by different concentration of bio-capsule, carrageenan and skim.

Materials and methods:-

The materials used were isolate *Lb. paracaseissp. paracasei* MI3 obtained from dadih, namely fermented buffalo milk that came from West Sumatera. Carrageenan (Bratachem), $CaCl_2$ (Merck), MRS broth (Oxoid), Bacto Agar (Oxoid), KH_2PO_4, 90% alcohol, methylated spirit, and sterile water. The tools used are incubator, autoclave, sterilization ovens, laminar flow, colony counters, microscope, caliper, micropipette, waterbath, analytic scales, vortex, magnetic stirrer, test tubes, petri dishes, Erlenmeyer, baker glass, bunsen, and microscope.

Research Design:

This research consisted of 1) preparation of isolate, 2) encapsulation of the extrusion with 3 treatments composition of carrageenan-skim milk ie 1:1, 2:1, and 3:1. All the experiments were repeated four times, and the results are presented in the value of the standard deviation (SD) using SPSS. When the results of the ANOVA showed a distinction in treatment then continued with a real difference test Duncan with level of $P<0.05$.

The Making of Carrageenan-Skim Milk Bio-Capsule:

Making microcapsules refers to the modified(6) and Rokka and (7)methods. Bacterial culture *Lb. paracasei ssp. paracasei*MI3 as much as 10% was refreshed into MRS-Broth media, and 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 water to form a suspension with a concentration of 10%. Carrageenan-Skim milk was prepared according to treatment that is 1:1, 2:1, and 3:1, the solution was shaken until homogeneous then sterilized at 121℃ for 10 minutes. The capsule solution is cooled to 45℃, and 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 volume of 50 ml. The mixture is dropped into a 3% KCl 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.

Viability of*Lb. paracasei ssp. paracasei*MI3:

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 petridish, then poured the media MRS Agar. Incubation for 48 hours at 37^0C, the colony grew by the method of Total Plate Count (TPC) (8).

Results:-

The Viability of Bio-Capsule:

Viabilitas of *Lb. paracasei ssp. paracesi* MI3 with several composition carrageenan and skim are found in Table 1.

Table 1:- Viability of Visible Cells and Cel in Bio-Capsule at Different of Composition.

Composition of Coating	Viability of Visible Cell (CFU/G)	Viability in Bio-Kapsul (CFU/G)	Viability Celllog CFU/G)
1:1	6.3×10^9	1.5×10^9	0.5^a
2:1	1.3×10^9	2.0×10^9	0.72^a
3:1	1.8×10^9	7.5×10^8	1.38^b

The average with different subscription letter are significant at devisiation 5%.

Table 1.shows at composition 1:1 wasnot decrease of viability cell to before and after bio-capsule yet, but a decrease occurred in the 2:1 and 3:1 composition. The more decrease occurred at 3:1 which is 1.38 Log CFU/G.

The larger beat of bio-capsule cause softer texture of beat. This is due to is absorbed to be absorbed in the cell. Beside that, it will be able to absorb microbial cell in high number. More then this, the larger bio-capsule look worst appearance to fermented milk product (9).Encapsulation material is a coating to protect of bacteria cell from external factor (10).Carrageenan was coating of probiotics from stress and environment (11).

Survival Rate of Encapsulated Probiotic:

The Effect of difference bio-capsule to survival rate showen in Figure 1.Based on number of viability probiotic found that the higher survival rate of *Lb. paracasei spp. paracasei*MI3 are 1:1 thus 2:1 and 3:1 respectively.Based on analysis of variance showed that composition of 1:1 and 2:1 wasnot significant. But both of composition is significant with 3:1 sample. The increasing of carrageenan in the bio-capsule, will be the lower porosity of beads. This is effect of shelf life of probiotics to oxygen demand. This phenomena cause of the higher skim milk can lower activity water that negatively for bacteria.

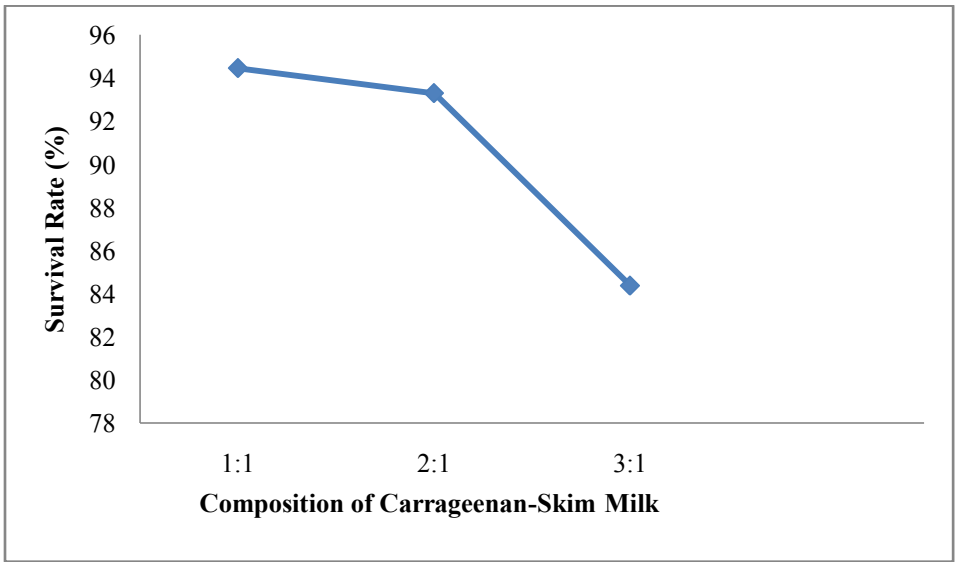

Figure 1:- Survival Rate of Encapsulated Probiotics in Bio-capsule With Difference of Composition.

The high skim milk was contributed to*Lactobacillus acidophilus* and *Bifidobacteriumanimalis ssp. lactis* growth (12). Combining of bio-capsule by 3% carrageenan and 3% tofu pulp can increase of porosity up to 68% (13).

The Viability Cell at pH 2.0:

The viability *of Lb. paracasei spp. paracesei*MI3 and at coating of carrageenan-skim milk at pH 2.0 found in Table 2.

Table 2:- Viability of Visible Cells and Cel in Carrageenan-Skim Milk Bio-Capsule at pH 2.0.

Composition of Coating	Viability of Visible Cell, Incubated 0 Hour	Viability at Bio-Capsule Incubated 3 Hour(CFU/G)	Viability Cell (log CFU/G)
1:1	1.5×10^9	1.6×10^7	1.97^a
2:1	2.0×10^9	2.6×10^8	0.89^b
3:1	7.5×10^9	2.5×10^6	2.48^c

The average with different subscription letter are significant at devisiation 5%.

The lower viability after incubated 3 hours, pH 2.0 found at each treatment. The highest found in 3:1 was 2.48 Log CFU/G.Thus, 1:1 and 2:1 which 1.97 dan 0.89 Log CFU/G respectively. The viability of bio-capsule *Lactobacillus casei* by carrageenan was reduced more than 4.45 log cycles after 120 min when exposed to acid condition [13]. The cell suspensions were incubated in acid conditions simulated the major factors influencing the survival of the ingested bacteria in the gastrointestinal tract. At pH 2.0 has considered influence of acid gastric stress (14).

Survival Rate of Probiotic at pH 2.0:
Differences in synthesis of bio-capsule *Lb. paracasei ssp. paracasei* MI3 in several consentratetreatment at pH 2.0 are presented in Figure 2.

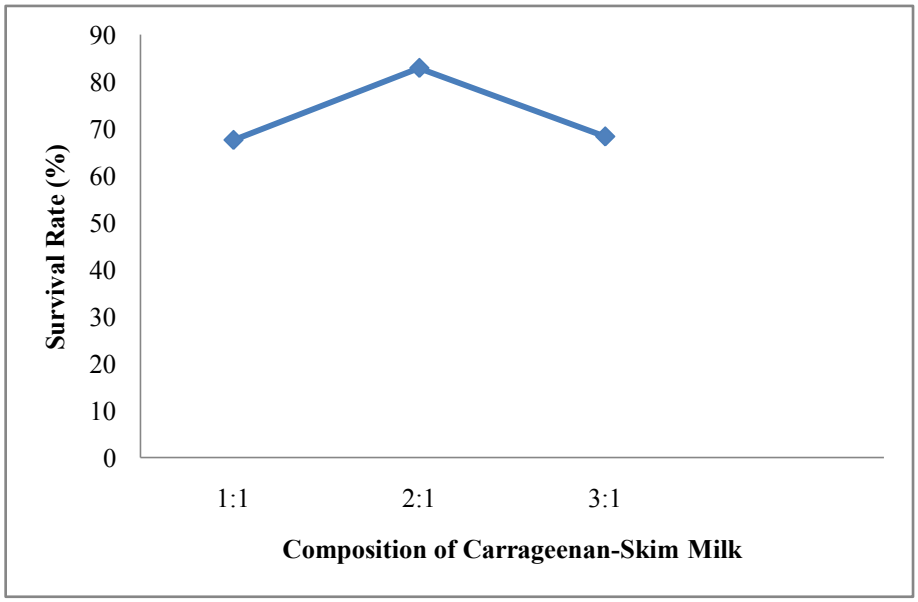

Figure 2:-Survival Rate of Encapsulated Probiotics in Bio-capsule at pH 2.0

The survival rate of *Lb. paracaseispp. paracasei*MI3 ranged 67.60-82.94% in all treatment. Data also show that 2:1 coated bio-capsule is higher rate.Theself life of probiotics in bio-capsule at pH 2.0 influenced by material of coating.

The capsule size determines the size of the capsule air cavity. Rare texture makes it easier to form air voids. In this study, the 1:1 composition has a liquid texture, making easier to form air when making bead. In addition 1:1 composition has an irreversible size, so the number of cell in the capsule will be different.As same as with 3:1 composition, the higher carrageenan used, the surface of the capsule is rougher and pores. Variate of capsule size effected distribution of cell in capsule. The air cavity will reduce the capsule ability to protect of probiotic cells at pH 2.0. The porosity of alginate makes it reaches lower pH in 24 hours than carrageenan (13).

Conclutions:-

Result of this study suggest the comparison of 2:1 was obtained the highest viability of 1.97 x 10^9 CFU/G and suffered a decrease in visibility during extrusion of 0.7 Log CFU/G, with a synthesis in bio-capsules of 93.3%. Resistance to pH 2.0 after 3 hours incubation is 2.6 x 10^8 with a decrease after extrusion 0.89 Log CFU/G and the synthesis in bio-capsule pH 2.0 is 82.94%.

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