Analysis of The Best Method in Produced of Synbiotics Products for Shrimp Using Microencapsulation Techniques

Dian Eka Ramadhani[1*], *Wida* Lesmanawati[1], *Erni* Sulistiawati[2], and *Widanarni* Widanarni[3]

[1]Production Technology and Management of Aquaculture Study Program, School of Vocational Studies IPB, Kumbang St. No. 14 Bogor, 16151, West Java.
[2]Veterinary Paramedics Study Program, School of Vocational Studies IPB, Kumbang St. No. 14 Bogor, 16151, West Java.
[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Raya Dramaga St., Bogor, 16680, West Java.

Abstract. This research was conducted to find the best combination of coating material and microencapsulation method for synbiotics. This research consists of 3 chapters. Chapter 1, was conducted to obtain the best combination of 3 types of coating materials (whey protein, skim milk, and maltodextrin) with 3 doses of coating material (0%, 10%, and 20%). Chapter 2, was conducted to find the best microencapsulation method that can maintain viability, the density of probiotic bacteria, and physiological and biochemical properties of bacteria. The probiotic bacteria used was *Pseudoalteromonas piscisida* with the prebiotic *mannan-oligosaccharide* (MOS). A total of 9 treatments in Chapter 1 was microencapsulated using spray- and drum-drying methods. Chapter 3, was conducted to determine the economic value of the synbiotic microcapsule products. The best result from Chapters 1 and 2 is synbiotics coated with a combination of 20% skim milk and 20% maltodextrin (treatment 6) with the spray drying method compared to other treatments. Treatment 6 is produced the highest percentage of product, the physical characteristics of which were good white powder when stored in cold (4^{o}C) and room (25-29^{o}C) temperatures. The best result in Chapter 3 is treatment 6 that showed profitable, highest R/C ratio and B/C ratio than other treatments. However, even though the quantity of the product is profitable, the resulting product can not live. Our suggestion is to use another method namely, freeze-drying for microencapsulation of heat-resistant bacteria such as the bacteria used in this study.

1 Introduction

Various disease prevention in aquaculture commodities including shrimp have been carried out, one of which is the use of microorganisms known as probiotics. Probiotics are live microbes that, when administered in sufficient quantities, can have a beneficial effect on host immunity and can improve microbial balance in the digestive tract, feed efficiency, and environmental quality [1,2]. Recent studies reported that probiotic bacteria *Pseudoalteromonas piscicida* has the potential to be applied in aquaculture, this bacteria is isolated from Pacific white shrimp nauplii [3]. However, not many types of potential aquaculture probiotics from research results can be produced on an industrial scale because most probiotics are not resistant to high temperatures, resulting in cell viability that drops dramatically during the manufacturing process [4]. Therefore, the types of probiotics sold in the market are relatively limited. Commonly used probiotics in aquaculture include *Bacillus* sp., *Lactobacillus* sp., *Bifidobacteria* sp., and *Saccharomyces cerevisiae* [2,4].

Probiotics are often applied together with prebiotics to enhance the role of these probiotics. Prebiotics are food ingredients that cannot be digested and it have a beneficial effect on the host by stimulating the growth and activity of a number of bacteria in the digestive tract of the host [5]. Materials that are often used as synthetic prebiotics include mannan-oligosaccharides (MOS), fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin while natural prebiotics can be obtained from honey, fruits, tubers, seeds, and so on. The combination of prebiotics with probiotics is called synbiotics, which have been shown to have a synergistic effect on the host [6]. Several studies reported that the administration of synbiotics could improve growth performance, survival, and immune response of Pacific white shrimp [7, 8, 9].

The potential of synbiotics is one of the way for disease prevention in aquaculture commodities, it is important to find methods or techniques that can be used to maintain cell viability in the minimum range of 10^{6}-

* Corresponding author: dianeka06@apps.ipb.ac.id

10^7 CFU/g of feed according to the recommended dose [2,10]. Microencapsulation is a new technology for packaging biomolecules or cells in encapsulated membranes to reduce cell loss during the manufacturing process and their release in the host digestive tract. Microencapsulation techniques that are widely used are spray-drying, freeze-drying, drum-drying, and so on. This method is most commonly used in the food, drug, and other feed additive industries [11,12]. In the microencapsulation process, the type of coating material that is often used as a coating material for probiotic bacteria is a combination of whey protein, skim milk, and maltodextrin. Several studies have reported that whey protein, skim milk, and maltodextrin can protect probiotic bacteria during the microencapsulation process [7, 13, 14, 15]. The right combination of coupling material is needed to obtain maximum viability of probiotic bacteria after passing through the microencapsulation process.

This research was conducted to find the best combination of coating material and the best microencapsulation method for probiotic bacteria *Pseudoalteromonas piscicida* mixed with MOS prebiotics so that synbiotic microcapsules with maximum viability can be produced to be applied in shrimp farming activities. The research is divided into 3 chapters. Chapters 1 was conducted to obtain a combination of coating materials that gave maximum results on the viability of probiotic bacteria in the synbiotic microencapsulation process. Chapter 2 was conducted to obtain the best method for the synbiotic microencapsulation process to produce synbiotic microcapsules. Chapters 3 research is to evaluate the economic value of the resulting synbiotic microcapsule product.

2 Method

2.1 Time and Place

This research was carried out from August-November 2020 at Laboratory of Microbiology, IPB Sukabumi Campus and SEAFAST (Southeast Asian Food & Agricultural Science & Technology) Center, Bogor Agricultural University.

2.2 Research Material

The materials needed in this research include cow's milk, rennet pills, skim milk powder, maltodextrin, $CaCl_2$, rifampicin, PBS (phosphate-buffered saline; 0,8 g NaCl, 0,02 KH_2PO_4, 0,15 g Na_2HPO_4, 0,02 g KCL, and aquadest) , bacto agar, and SWC media (seawater complete; 0,5 g bacto peptone, 0,1 g yeast extract, 0,3 mL glycerol, 75 mL seawater and 25 mL aquadest), probiotic bacteria *Pseudoalteromonas piscicida* 1 Ub from the Laboratory of Fish Health and Management, Department of Aquaculture and prebiotics mannan-oligosaccharide (MOS) (Alltech Inc., KY USA) with a minimum content of 30% crude protein, minimum 1,4% crude fat and maximum 13% crude fiber.

2.3 Research Design

This research is divided into 3 Chapters. Chapter 1, a combination of 3 types of coating material (whey protein, skim milk and maltodextrin) was used with 3 doses of coating material (0%, 10% and 20%), so there were 9 treatments in total (Table 1). Parameters observed were viability and density of probiotic bacteria before and after coating with various treatments. Chapter 2, the best result from Chapter 1 encapsulated with 2 microencapsulation methods, for a total of 18 treatments (Table 2). The encapsulation methods used are spray-drying and drum-drying. Parameters observed were viability and density of probiotic bacteria after passing through the microencapsulation process. Chapter 3, was conducted to determine the economic value of the three synbiotic microcapsules products so that the best and economical method could be determined to be applied in the field. Observation parameters in the form of profit R/C ratio and B/C ratio.

Table 1 Treatment in stage 1 research: the best combination and dose of coating material

No	Treatment	Description
1	PS 10	Synbiotic coating with whey protein + 10% skim milk
2	PM 10	Synbiotic coating with whey protein + 10% maltodextrin
3	SM 10	Synbiotic coating with 10% skim milk + 10% maltodextrin
4	PS 20	Synbiotic coating with whey protein + 20% skim milk
5	PM 20	Synbiotic coating with whey protein + 20% maltodextrin
6	SM 20	Synbiotic coating with 20% skim milk + 20% maltodextrin
7	PSM 10	Synbiotic coating with whey protein + 10% skim milk + 10% maltodextrin
8	PSM 20	Synbiotic coating with whey protein + 20% skim milk + 20% maltodextrin
9	K	Synbiotic coating with PBS only (Control)

Table 2 Treatment in Chapter 2: the best microencapsulation method

Combination coating material	Microencapsulation method	
	Spray-Drying (S)	Drum-Drying (D)
PS 10	PS 10-S	PS 10-D
PM 10	PM 10-S	PM 10-D
SM 10	SM 10-S	SM 10-D
PS 20	PS 20-S	PS 20-D
PM 20	PM 20-S	PM 20-D
SM 20	SM 20-S	SM 20-D
PSM 10	PSM 10-S	PSM 10-D
PSM 20	PSM 20-S	PSM 20-D
K	K-S	K-D

2.4 Procedures

2.4.1 Preparation of Synbiotic

Bacterial preparation was started by culturing the bacteria on SWC-agar media which already contained the antibiotic rifampicin 50 g/mL as a marker. Then the bacteria were cultured in 50 mL of SWC-liquid media and incubated in a water bath shaker at a temperature of 29-30ºC for 18-24 hours at a speed of 140 rpm, then continued up scaling (1:10). After that, the bacteria were harvested and ready to be centrifuged 1 time and rinsed using PBS. The prebiotic that will be used is Bio-MOS. Synbiotic is made by combining probiotics and prebiotics in the same container.

2.4.2 Microencapsulation of Synbiotic

The first stage begins with the preparation of synbiotics and coating materials. According to the treatment, the coating material is prepared in a separate container. Whey protein coating is obtained by boiling 1L of cow's milk at a temperature of 50-60ºC for 15 minutes and constantly stirring. Then wait until the temperature is around 30ºC and give rennet pills to form lumps and then let stand at room temperature for 3-4 hours. Furthermore, it is filtered with sterile mori filter cloth and the resulting liquid (whey protein) is then stored in a refrigerator or can be used directly. After that, the coating material and synbiotic were mixed. The ratio of synbiotics, whey protein and maltodextrin is 1:1:0,1 (v/v/w) [16]. Furthermore, the synbiotic was dried using a sprayer with an inlet temperature of 50ºC and an outlet temperature of 90ºC. The results of the microencapsulation are then put in a closed container and stored in the refrigerator. For the drum-drying method, the sample is put into the drum dryer and the results are placed in a container and stored in the refrigerator.

2.4.3 Parameters

The product percentage parameter was calculated by comparing the synbiotic volume (fresh-culture) with the dry product produced after the encapsulation process (v/w). Parameters of viability and density of probiotic bacteria were calculated after coating, temperature incubation, before and after spray-drying and drum-drying as well as during storage. Measurement of bacterial viability was carried out using the spread plate method (TPC; total platting count) [17]. A sample of 1 g was homogenized in 0,9 mL of PBS then serially diluted. Samples were then taken as much as 50 µL and spread on SWC-agar + antibiotic rifampicin. Then incubated in an incubator at a temperature of 29-30º C for 18-24 hours. Then the bacterial colonies were counted one by one and added together. The formula for counting bacteria is below:

$$\sum \text{bacteria (CFU/g)} = \text{number of bacterial colonies (CFU)} \times \frac{1}{\text{dilution}-\text{n}} \times \frac{1}{spread\ volume\ (\mu L)}$$

The economic analysis parameters measured include profit, R/C ratio and B/C ratio, with the following calculation formula:

Profit = total revenue – total cost

$$\text{R/C ratio} = \frac{total\ revenue}{total\ cost}$$

$$\text{B/C ratio} = \frac{total\ profit}{total\ cost}$$

2.4.4. Data Analysis

The research data was tabulated in Microsoft Excel 2016 and data was performed using table and graph with descriptive analysis.

3 Results and Discussion

3.1 Product percentage

The results of calculating the percentage of probiotic bacteria products contained in synbiotics include: 1) the percentage of products after the spray and drum drying process; 2) the percentage of viability of probiotic bacteria after the spray and drum drying process. The following is the result of calculationg the percentage of the product (Figure 1).

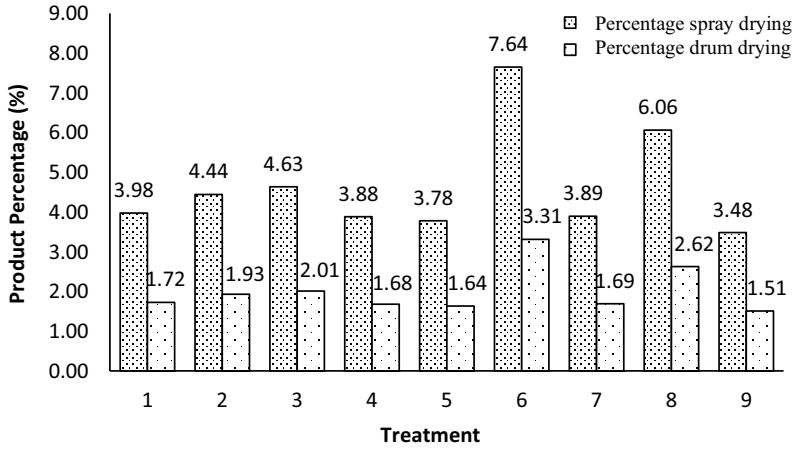

Fig. 1 Percentage of product after spray- and drum-drying

The results showed that the percentage of product in the spray drying method was found in treatment 6 (coating material skim milk 20% and maltodextrin 20%) which was 7,6% higher than other treatments. Meanwhile, the lowest product percentage was found in treatment 9 (Control), which was 3,4%. The results showed that the percentage of product in the drum-drying method was found in treatment 6 (coating material skim milk 20% and maltodextrin 20%) which was 3,31% higher than other treatments. While the lowest product percentage is in treatment 9 (control) which is 1,51%. The following is the viability of 1Ub probiotic bacteria after being coated with a coating material (Table 3). The results showed that the viability of probiotic bacteria in each treatment after being given the coating material still grew with a density range of 10^8-10^{10} CFU/mL, but after drying with the spray drying and drum drying methods, the viability of the bacteria became 0 meaning it did not grow at all.

Table 3. Density of 1Ub probiotic bacteria cells after being coated with various treatment materials

Treatment	Probiotic bacteria cell density (CFU/mL)
1	1,50 x 10^{10}
2	4,61 x 10^9
3	5,80 x 10^8
4	2,00 x 10^8
5	4,80 x 10^9
6	4,46 x 10^9
7	8,40 x 10^8
8	6,32 x 10^9
9	4,00 x 10^9

3.2 Synbiotic microcapsule physical quality

At the end of the in-vitro test, there was a change in the physical form of each treatment including a change in color and the presence of clumping. Table 4 shows the physical quality of dry synbiotic cultures from spray drying and drum drying during 1 month storage.

Table 4 Physical quality of dry synbiotic culture from spray drying and drum drying during 1 month storage.

Treatment	Storage	Physical form of spray drying		Physical form of drum drying	
		Day 1	Day 30	Day 1	Day 30
1	Room temperature (25-29ºC)	Powder, white, slightly cream	Powder, white, lumpy	Creamy brown, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white,	Creamy brown, slightly lumpy	Creamy brown, slightly lumpy
2	Room temperature (25-29ºC)	Powder, white,	Powder, white,	Creamy, slightly lumpy	Creamy, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white,	Creamy, slightly lumpy	Creamy, slightly lumpy
3	Room temperature (25-29ºC)	Powder, white,	Powder, white, slightly cream	Creamy, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white,	Creamy, slightly lumpy	Creamy brown, slightly lumpy
4	Room temperature (25-29ºC)	Powder, white,	Powder, white, slightly cream	Creamy, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white, slightly cream	Creamy, slightly lumpy	Creamy brown, slightly lumpy
5	Room temperature (25-29ºC)	Powder, white,	Powder, white, slightly lumpy	Creamy brown, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white, slightly lumpy	Creamy brown, slightly lumpy	Creamy brown, slightly lumpy
6	Room temperature (25-29ºC)	Powder, white,	Powder, white, slightly cream	Creamy, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white,	Creamy, slightly lumpy	Creamy, slightly lumpy
7	Room temperature (25-29ºC)	Powder, white,	Powder, white,	Creamy brown, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white,	Creamy brown, slightly lumpy	Creamy brown, slightly lumpy
8	Room temperature (25-29ºC)	Powder, white,	Powder, white,	Creamy, slightly lumpy	Creamy brown, slightly lumpy
	Cold temperature (4ºC)	Powder, white,	Powder, white,	Creamy, slightly lumpy	Creamy, slightly lumpy
9	Room temperature (25-29ºC)	Powder, white, slightly cream	Powder, white, watery, yellowish white and lumpy	Brown, slightly lumpy	Brown, slightly lumpy, sticky
	Cold temperature (4ºC)	Powder, white, slightly cream	Powder, white, watery, yellowish white and lumpy	Brown, slightly lumpy	Brown, slightly lumpy,

The results of microencapsulation (spray-drying) showed that the percentage of product in treatment 6 (addition of 20% skim milk coating material and maltodextrin) resulted in the highest percentage of product compared to other treatments. The higher composition of skim milk in the encapsulating material, the lower the water content of the microencapsulated produced. The water content of skim milk ranges from 3-7% [18]. According to [19] the range of good moisture content for microencapsulated products obtained from spray drying is 2-6%. Furthermore, carbohydrates such as starch, maltodextrin, are good coating materials because of their low viscosity at high solids and high solubility properties [20]. Microencapsulation using the drum-drying method showed the same results in treatment 6 (coating material skim milk 20% and maltodextrin 20%). This treatment produced the highest percentage of product compared to other treatments.

The concentration of addition of skim milk combined with maltodextrin can affect the viability of bacterial cells. This is in accordance with the research conducted by [21] which showed that the higher the coating concentration, the higher the encapsulation efficiency, the better and stronger the shell. This statement was again proven in the research of [22], encapsulation treatment with 10% skim milk and 20% maltodextrin resulted in bacterial cell viability of 97,76%. These results were significantly different from the other three treatments. The better the coating material used, it can protect the core material well and protect volatile substances when the drying process takes place, which results in increased retention of the core material.

The choice of coating material that will protect the core material is the most important factor to maintain the viability of bacterial cells in the microencapsulation process. According to [23] optimal efficiency can be obtained from the protein and carbohydrate matrix as a microencapsulated wall. The coating materials used in this study were whey protein, skim milk, and maltodextrin. Referring to the results of the study, the two encapsulation ingredients in the form of 20% skim milk and 20% maltodextrin (Treatment 6) were the best ingredients. The microencapsulated wall consisting of two encapsulated materials is able to provide good protection because the use of these two materials results in higher efficiency than one encapsulated material as a filler. This is due to the ability of the encapsulant to interact to form granules that can coat the encapsulated components better [22].

3.3 Economic Analysis

The economic analysis used includes profit, business feasibility analysis (R/C) ratio, and (B/C) ratio. The results of the economic analysis of the resulting product are shown in Table 5.

Table 5 Results of the economic analysis of synbiotic microcapsules using the spray drying and drum drying methods

Treatments	*Spray drying method*			*Drum drying method*		
	Benefit	R/C Ratio	B/C Ratio	Benefit	R/C Ratio	B/C Ratio
1	(335,444,444)	0.74	-0.256064461	(371,745,941)	0.72	-0.283775527
2	(221,111,111)	0.83	-0.168787108	(261,671,442)	0.80	-0.199749193
3	(174,833,333)	0.87	-0.13346056	(217,117,479)	0.83	-0.165738533
4	(359,944,444)	0.73	-0.274766751	(395,333,333)	0.70	-0.30178117
5	(384,444,444)	0.71	-0.293469042	(418,920,726)	0.68	-0.319786814
6	562,888,889	1.43	0.429686175	493,125,119	1.38	0.376431389
7	(356,541,667)	0.73	-0.272169211	(621,542,980)	0.53	-0.47446029
8	174,291,667	1.13	0.133047074	(238,247,851)	0.82	-0.181868589
9	(457,944,444)	0.65	-0.349575912	(489,682,904)	0.63	-0.373803743

Based on economic analysis, the most profitable treatment and has business feasibility is treatment 6. This can be seen in the results of the percentage of synbiotic products produced which have a greater value than the others. R/C ratio analysis is an analysis used to see the relative advantages that will be obtained in a business. Basically, a business will be said to be feasible to run if the R/C ratio value obtained is greater than 1. This can happen because the higher the R/C ratio value of a business, the higher the profit level to be obtained [24]. The results of the R/C ratio analysis showed that with the spray drying method, treatments 6 and 8 had values of 1,4 and 1.13, respectively. While in the drum drying method, the treatment with the highest R/C ratio value resulted from treatment 6. The next analysis is the B/C ratio, where the B/C ratio is the ratio between positive net benefits and negative net benefits in a business. In the application of the B/C ratio, a business/investment can be said to be feasible if the B/C value >1 is obtained, while a business is said to be unfeasible if the B/C value <1. In its application to a business, the analysis of the B/C ratio against a situation is an analysis that is needed to see

to what extent the comparison between the value of benefits and the value of costs is seen in the present value condition. The results of the analysis of the B/C ratio on the spray drying and drum drying methods in all treatments showed a B/C value <1, but in treatment 6 the resulting value was still positive and if further improvements were made it could provide maximum business feasibility.

Conclusions and Suggestions

Synbiotic coated with a combination of 20% skim milk and 20% maltodextrin (Treatment 6) with spray drying and drum drying methods was the best result in this study compared to other treatments. Treatment 6 on the spray drying method resulted in the highest percentage of product, physical characteristics in the form of a white fine powder when stored at cold temperatures (4ºC) or at room (25-29ºC). Meanwhile, in the drum drying method, the characteristics are brownish beige and there are few lumps. The results of the economic analysis also show that treatment 6 is more profitable and from the analysis of the R/C ratio along with the B/C ratio, treatment 6 has business feasibility compared to other treatments. Research on drying methods that can maintain the viability of 1Ub bacteria needs to be done, one of which is the freeze drying method, but it needs a combination that matches so that the resulting product has high viability and the method used can be more efficient to use.

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References

1. [FAO] Food and Agriculture Organization. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria.Food and Agriculture Organization of the United Nations and World Health Organization Expert ConsultationReport(Availableat: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf. Last Accessed 19.03.134) (2001)
2. S. K. Nayak. Immunol, *Probiotics and imunity: a fish perspective*, **29**: 2-14. (2010)
3. Widanarni, I. Tepu, Sukenda, S. Mia, J Ris Akuak, *Seleksi bakteri probiotik untuk biokontrol vibriosis pada larva udang windu, Penaeus monodon menggunakan cara kultur bersama*, **4**: 95-105 (2009)
4. P. Chitprasert, P Sudsai, A Rodklongtan, Carbohydrate Polymers, *Aluminum carboxymethyl cellulose–rice bran microcapsules: Enhancing survival of Lactobacillus reuteri KUB-AC5*, **90**: 78–86 (2012)
5. R. Cerezuela, J. Meseguer, M. A. Esteban, J Aquac Res Develop. *Current knowledge in synbiotic use for fish aquaculture*, 2155 - 9546 (2011)
6. T. G. Huynh, Y. L. Shiu, T. P. Nguyen, Q. P. Truong, J. C. Chen, C. H. Liu, Fish Shellfish Immunol, *Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review*, **64**: 367-382 (2017)
7. D. E. Ramadhani, Widanarni, Sukenda. Jurnal Akuakultur Indonesia, *Microencapsulation of probiotics and its application with prebiotic in Pacific white shrimp larvae through Artemia* sp. **18**(2): 130-140 2019
8. Hamsah, Widanarni, Alimuddin, M. Yuhana, M. Z. Junior, Res J Microbiol, *Bacterial population, activity of enzymes and growth rate of pacific white shrimp larvae administered Pseudoalteromonas piscicida and Mannanoligosaccharide through bio-encapsulation of Artemia* sp., **12** (2): 128-136 (2017)
9. D. Febrianti, Yuhana M, Widanarni, J Fish Aquac Sci, *Dietary synbiotic microcapsule influence the immune responses, growth performance and microbial populations to white spot syndrome virus in pacific white shrimp (Litopenaeus vannamei)*, **11**(1): 28-42 (2016)
10. J. W. Collins, R. M. La Ragione, M. J. Woodward, L. E. J. Searle, Springer-Verlag, *Application of prebiotics and probiotics in livestock,* 57-88 (2009)
11. M. J. Martin, F. Lara-Villoslada, M. A. Ruiz, M. E. Morales, Innov Food Sci Emerg Technol, *Microencapsulation of bacteria: a review of different technologies and their impact on the probiotic effects.* **27**: 15-25 (2015)
12. K. Sultana, G. Godward, N. Reynolds, R. Arumugaswamy, P. Peiris, K. Kailasapathy, International Journal of Food Microbiology, *Encapsulation of probiotic bacteria with alginate–starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt*, **62**: 47–55 (2000)
13. D. Y. Ying, M. C. Phoon, L. Sanguansri, R. Weerakkody, I. Burgar, M. A. Augustin, Journal of Food Science, *Microencapsulated Lactobacillus rhamnosus GG powders: Relationship of powder physical properties to probiotic survival during storage*, **75**: 588–595 (2010)

14. C. B. Fritzen-Freire, E.S. Prudêncio, R. D. M. C. Amboni, S. S. Pinto, A. N. Negrâo-Murakami, F. S. Murakami, Food Research International, *Microencapsulation of Bifidobacteriaby spray drying in the presence of probiotics*, **45**: 306–312 (2012)
15. R. Paéz, L. Lavari, G. Vinderola, G. Audero, A. Cuatrin, N. Zaritzky, J. Reinheimer, Food Research International, *Effect of heat treatment and spray drying on lactobacilli viability and resistance to simulated gastrointestinal digestion*, **48**: 748–754 (2012)
16. W. Munaeni, M. Yuhana, Widanarni*, Microbiologi Indonesia*, *Effect of micro-encapsulated synbiotic at different frequencies for luminous vibriosis control in white shrimp* (*Litopenaeus vannamei*). **8**(2): 73-80 (2014)
17. J. Reynolds, M. Farinha, Richard College, *Counting bacteria*, (2005)
18. D. Setyaningsih, D Kurniawan, N. Muna, IOP. Conf. Series: Earth and Environmental Science, *Encapsulation of ginger oleoresin with a combination of maltodextrin and skim milk powder as wall material,* **472**: 012016 (2020)
19. M. I. Re, Drying Technology, *Microencapsulation by spray drying,* **16**(6): 1195-1236 (1998).
20. P. Balasubramani, P. T. Palaniswamy, R. Visvanathan, V. Thirupathi, A. Subbayaran, J. P. Maran, International Journal of Biological Macromolecules, *Microencapsulation of garlic oleoresin using maltodextrin as wall material by spray drying technology,* **72**: 210-217 (2014)
21. N. V. N. Joythi, P. M. Prasanna, S. N. Sakarkar, K. S. Prabha, P. S. Ramaiah, G. Y. Srawan, Journal of Microencapsulation, *Microencapsulation techniques, factors influencing encapsulation efficiency,* **27** (3): 187-197 (2010)
22. A. P. Cahya, M. Syaflan, Ngatirah, Indonesian Food and Nutrition Progress, *Probiotic (Lactobacillus casei) encapsulation the method of spray drying with combined prebiotic from iles-iles (Amorphopallus oncophyllys) and protectant agent (skim milk, gum arabic, maltodextrin),* **15** (2): 0854-6177 (2018)
23. C. C. Lin, S. Y. Lin, L. S. Hwang, J. Of Food Sci, *Microencapsulation of Squid Oil with Hydrophilic Macromolecules for Oxidative and Thermal Stabilization*, **6** (1): 36-39 (1995)
24. J. Odeck, A, Kjerkreit, Transportation Research Part A, *The accuracy of benefit-cost analyses (BCAs) in transportation: An ex-post evaluation of road projects,* **120**: 277-294 (2019)