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Microbia, Chemical Characteristics and Nutritional Value of Mango Probiotics Drink by Biocapsules *Lactobacillus paracasei* ssp. *paracasei* Ml.3

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Abstract. This study aimed to investigate microbia amount, chemical properties and nutritional value of mango probiotics drink by biocapsules Lb. paracasei ssp. paracasei Ml.3. The production of biocapsules Lb. paracasei ssp. paracasei Ml.3 by extrusion carregenan-skim (2:1) of 12 bio-capsule. The result indicated that the exponential phase of the bio-capsule Lb. paracasei ssp. paracasei Ml3 at 17 hours of incubation required 2.09/hours of generation time and 8.12 generation number to reach 10.81 log CFU/ mL. The chemical characteristics indicated that the moisture content was 82.57%, ash content 0.12%, protein content 1.35%, fat content 0.2%, organic acid content 0.5%, vitamin C content 9.73% and crude fiber content 0.04%. Additionaly, this product contains $1.7 x 10^8$ CFU/ mL Lb. paracasei ssp. paracasei Ml 3. According to the analysis of nutritional value (diet 2000 kkal) considerable high nutritional value and could be developed for functional food. This nutritional value showed protein were 0.949 kkal (0.47%), fat 1,71 kkal (0.43%), carbohydrate 74.373 kkal (5.31%) and total energy 77.032 kkal.

Keywords. chemical characteristics, microbia, nutritional value, probiotic drink

I. Introduction

The health products which grow rapidly today are probiotic drinks in various forms that involve Lactic Acid Bacteria (LAB) in the manufacturing process, which have a positive effect on health. These products are able to colonize so that they can reach the required amount while in the digestive tract by improving the balance of microflora in the intestine. The viability of bacterial cells in probiotic products may decrease during processing, storage and while in the digestive tract. This is due to environmental factors that are less favorable for its survival, including low pH conditions due to the secretion of gastric acid and bile salts in the digestive system. Krasaekoopt, et al. (2003) showed that the health effects of probiotics would be obtained if you consume probiotics with a total of 10^6-10^7 colonies/G. Probiotic bacteria can be protected using a protective or coating material using the encapsulation method.

Encapsulation methods are one of technologies to be applied in the food to maintain viability of probiotics bacteria. According to the result of research Wu *et al*, (2000), encapsulation is a process of coating bacteria to form an inner matrix like a capsule that protects the core material using an encapsulated material. Bacterial encapsulation can provide conditions capable of protecting microbes from unfavorable environmental influences during processing, such as heat, food additives and extreme environments.

Lactobacillus paracasei ssp. *paracasei Ml3* is superior bacteria obtained from *dadih* (traditional fermented food from West Sumatera Province). These bacteria meet the criteria as a probiotic in the digestive tract lowering/anti-cholesterol. These isolates have advantages such as their ability to live at low pH, tolerance to bile salts, and high viability 10^7-10^{10} colonies/mL, and the coaggregation value of 36% - 74%. Organic acids of lactic acid that produced high concentration from 1.04% - 1.2% to inhibit the growth of gram-negative gastrointestinal pathogens *E.coli*, gram-positive *E. coli*, gram-positive *Listeria monocytogenes*, *S. areus* and *B.cereus* (Elida *et al*., 2013). The in vivo test results showed that giving the probiotic suspension Lactobacillus paracasei ssp paracasei Ml3 in mice can reduce cholesterol on day 21 (Elida and Ermiati, 2016). Encapsulation of Lactobacillus paracasei ssp paracasei Ml3 using 3% carrageenan extrusion gave the highest viability to 10.11 log CFU/G in the encapsulated bio-capsules (Elida *et al*., 2020a). The results of the study (Elida *et al*., 2020b) showed a comparison of skim: carrageenan is 2 : 1 had the highest wet bio-capsule viability of 1.97 x 10^9 CFU / G.

This study used skim and carrageenan encapsulation (2:1), using a modified extrusion method by a syringe to trapping Lactobacillus paracasei ssp paracasei Ml3 cells with a carrageenan gel matrix and skim filler which was dropped into KCL so that it was a bio-capsule formed. Bio-capsules are used as a starter for production of mango juice probiotic drinks, so they can provide more health benefits for all ages. This study aimed to obtain the fermentation time of encapsulated *Lactobacillus paracasei* ssp. *paracasei Ml3* in the form of a bio-capsule for the making of mango juice probiotic drinks to investigate microbiological and chemical properties and nutritional value.

II. Materials and Methods

II.1 Materials

The raw materials used are mango juice and pasteurized cow's milk, and the fermentation starter is the probiotic bacteria *Lb paracasei* ssp. *paracasei Ml3* encapsulated.

II.2 Methods

1. Preparation of Probiotic Biomass (Harmayani, et al., 2001) Modified Method.

Lb paracasei ssp. *paracasei Ml3* was subcultured and incubated at 37°C, then its was harvested by centrifuge at 4500 rpm for 15 min. Then it was washed with sterile water twice and centrifuged again at 3000 rpm for 10 minutes (Elida, et al, 2020).

2. Bio-capsules Production

The bio-capsule preparation used the modified methods of Le-Tien, et al., (2004), and Rokka & Rantamaki (2010), the resulting biomass is made into a suspension up to a concentration of 10%. Furthermore, a sterile 3% carrageenan was prepared and a pasteurized skim solution. To the suspension was added to the bacterial biomass solution and the mixture was put into a syringe and dropped into a sterile 3% KCL solution and stored at 100C for 2 hours (Tsen, et al, 2003). The bio-capsules formed were rinsed using a physiological saline solution and filtered.

3. Production of mango juice probiotic

Starter preparation using 12 bio-capsules was crushed and grown in sterile MRSB media for 24 hours. Then from the parent culture was inoculated as much as 4% (v / v) into 10% skim media and 5% (w / v) sucrose, and incubated for 17 hours at 28^0C to obtain a working culture. The production process refers to the modified Rizal, et al (2013) method, mangoes are crushed in a blender then added with water (2: 1), after that as much as 10% (w/v) skim milk and glucose 8% (w/v)) is added to the mango juice, then pasteurized 70-75°C for 15 minutes, then cooled to a temperature of 37°C. Inoculate a working culture of 5% (v/v) encapsulated Lactobacillus paracasei ssp paracasei ML3 and incubated at 25°C – 30°C for 17 hours.

4. Mango Probiotic Drink Production

The probiotic fermented mango juice was homogenized to make a probiotic drink by adding a solution of sugar and water with a ratio of 1; 2; 2.

5. Analysis technique

Observations were made on total lactic acid bacteria (Fardiaz, 1992) by Total Plate Count (TPC). Chemical characteristics include moisture content, ash content, protein content, fat content, total carbohydrates (AOAC, 2000). Calculation of the Nutritional Adequacy Rate (RDA) by calculating the amount of carbohydrates, protein, fat, and energy contained in each package, based on a 2000 kcal diet.

III. Results And Discussion

III.1 Characteristics of Microbiology

1. Growth curve of free and encapsulated cells

The growth characteristics of isolate-free cells can be seen in Figure 1a and after encapsulation in Figure 1b. *Lb paracasei* ssp. *paracasei Ml3* has a fast growth phase at 13 hours of fermentation time, which is 10.64 log CFU/G. Whereas after the log phase encapsulation is achieved at 17 hours of fermentation on MRS Broth medium of 10.86 log CFU/G. The fermentation time is faster when compared to the study by Sulistiani et al (2020), on the NP44 Lb paracasei isolate isolated from Papuan palm sap showed a fast growth pattern at 22 hours fermentation time of $1.65x10^7$ CFU / mL on GYP broth media.

Based on result of research showed that the growth pattern after encapsulation is longer when compared to free cells. This is due to the coating on the cells so that it takes time to adapt again and the cells are in the lag phase longer. Therefore, it takes a longer time for bacteria to release into the fermentation medium and carry out fermentation activities. In this condition, according to Maier (2009), protein synthesis induced by mRNA is still low and the exoenzymes released from cells are still at an early stage, consequently the time to reach the exponential phase is longer.

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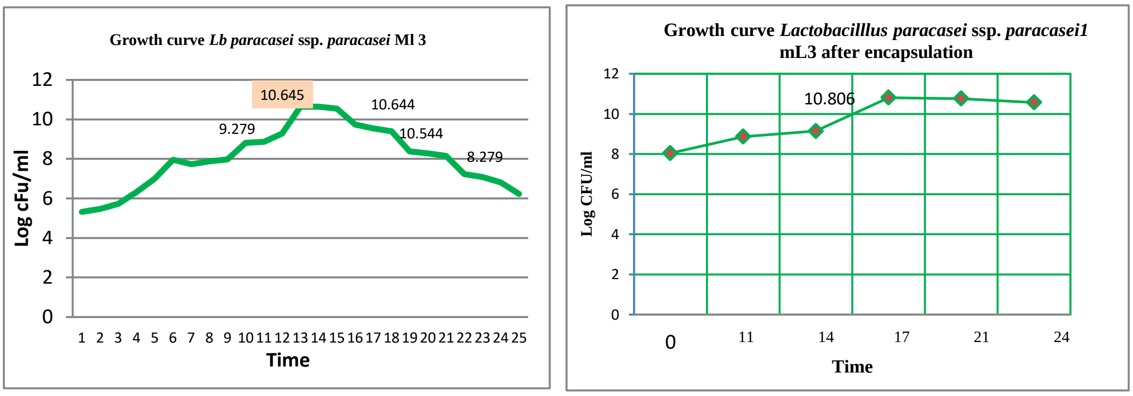

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Figure 1. Growth curve *Lb paracasei* ssp. *paracasei Ml3* free cells (a) and after encapsulation (b)

According to Kailasaphati (2002), encapsulation uses a encapsulating material, to protect bacteria from the environment before they release into the environment. Encapsulation serves as a barrier for bacteria in it not to be released out so that the bacteria may not work optimally to ferment lactose into lactic acid. Added by Sultana *et al*. (2000) stated that encapsulated cells took a longer time than free cells to reach the same pH. The fast growth of 17 hours fermentation is used as the fermentation time for the manufacture of mango juice probiotic drinks.

2. Time of generation of free cells and encapsulated cells

Figure 2a shows that to produce *Lb paracasei* ssp. *paracasei Ml3* free cells in the 13-hour exponential phase, 10.54 Log CFU/mL is produced with the number of generations 3.83 generations, it takes 3.39 hours with a generation rate of 0.29 generations/hour. In Figure 2b, the 17 hour exponential phase to produce 10.81 Log CFU/mL takes 2.09 hours of generation time, with a generation rate of 0.47 and the number of generations of 8.12. Lactic acid bacteria (LAB) in the form of free cells and after being encapsulated have different generation times so that the ability of the adaptation and log phase of bacteria is different.

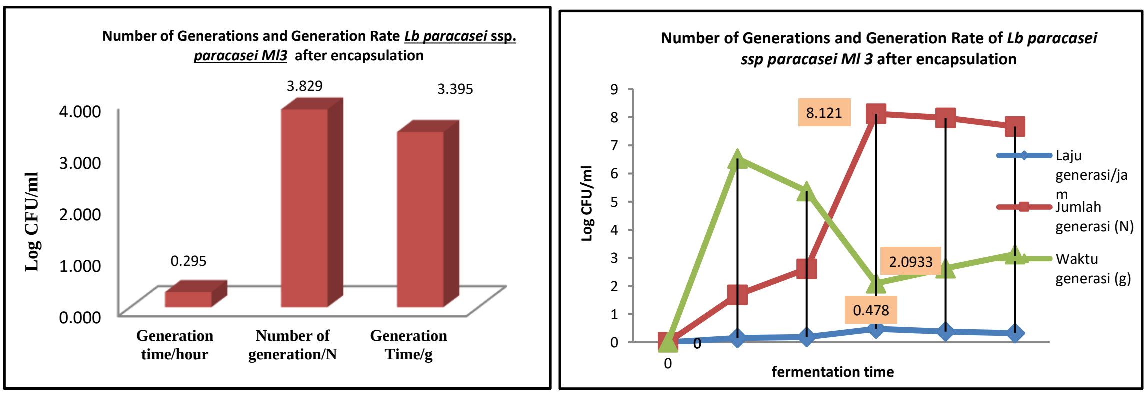

Figure 2. Generation rate, number of generations and generation time of *Lb paracasei* ssp. *paracasei Ml3* in the form of free cells and cells after encapsulation

3. Total Lactic Acid Bacteria (LAB)

The total lactic acid bacteria (LAB) of the mango juice lactic fermented drink with three replications ranged from 8.230 log CFU/mL or equivalent 1.7 x 10^8 CFU / mL (Table 1). The results of analysis of variance and LSD test showed no significant effect on the total LAB of mango juice lactic fermented drinks. The total LAB from each mango juice lactic fermented drink was influenced by the availability of the substrate in the medium and the length of fermentation. The growth of LAB colonies is influenced by the addition of sugar in the mango juice as a carbon source, so that the sugar content in the manga and the addition of glucose can trigger the rapid growth of LAB colonies in large numbers. However, because all factors in the fermentation medium are the same, the LAB growth in the fermentation medium during incubation also does not seem to show different activities, so the amount of LAB in the product is the same.

Table 1. Total LAB mango juice probiotik drink

Replications	Total LAB (log CFU/ml)
1	8.176 a
2	8.255 a
3	8.279 a
Average	8.230 or (1.7x10^8)

Note: The same letter notation shows an insignificant difference between treatments (at the 1% level)

The results of the total LAB analysis of the lactic fermented mango juice drink with addition of *Lb paracasei* ssp. *paracasei Ml3* encapsulated after homogenization and dilution (Table 1) showed a range of 8,176 log CFU/mL to 8,279 log CFU/mL or equivalent to 10^8 CFU/mL (8,230 Log CFU /mL). Rizal *et el* (2016) showed that the amount of LAB in pineapple probiotic fruit juice drinks using *Lactobacillus casei* free isolates without homogenization was 10.04 log CFU /mL. These results were better than the study by Rini *et al* (2019), the amount of LAB in Dutch eggplant probiotic drinks using *Lactobacillus sp* F213 free isolates after three days of cold storage was 1.61x10^6 CFU/mL, and 8.20x 10^5 CFU/mL, and in orange juice 1.25x10^5 CFU/mL. Based on the research results, the total value of LAB of mango juice lactic fermented drink has suitable of the standard of lactic fermented drink. The Indonesian National Standard SNI 7552: 2009 states the minimum requirement for a good total LAB value is 10^6 colony / mL.

III.2 Chemical Charateristics

The result indicated that the moisture content was 82.57%, ash content 0.12%, protein content 1.35%, fat content 0.2%, and total carbohydrates15.76% (Fig 3). This indicates that this product is a liquid product (high water content). Based on this results, this product can be used as a source of energy for consumers. However, the amount is not suitable for all the nutritional needs of humans as a whole. Therefore, this product cannot be used as a staple food, only as a side product. Energy contribution to consumers can be seen in the RDA discussion results.

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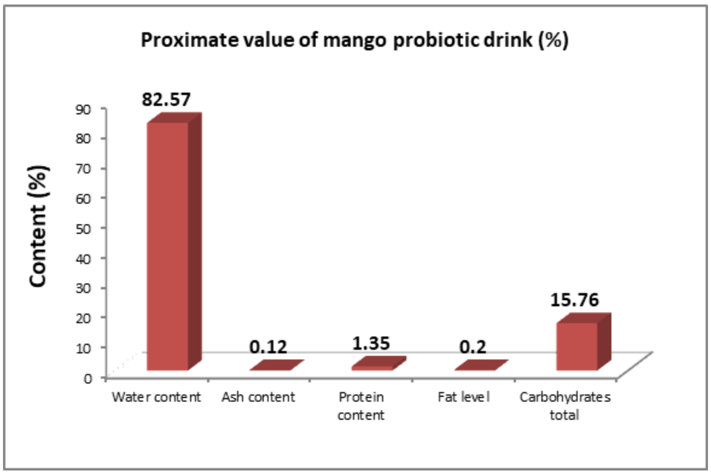

Figure 3. Proximate mango juice probiobitk drink

Qualitatively, this product has the same proximate content as mango juice. However, it is quantitatively different. this is due to the differences in the mango varieties used and the product processing technology used. This is in accordance with the results of research by Ubwa *et al.*, (2014) that the results of the proximate analysis of several varieties of mangoes differ quantitatively.

III.3 Nutritional Adequacy Rate (RDA)

One package of mango juice probiotic drink (80 ml/serving), obtained 77.03 kcal of energy. it has suitable with calories from protein as much as 0.47% of the 200-300 kcal requirement, from fat 0.43% of the total energy requirement of 400-600 kcal. Meanwhile, the nutritional adequacy rate contributed by carbohydrates has met the calorie needs of 5.31% of the 1200 kcal requirement. Referring to the research of Febriyani *et al.*, (2012), this mango juice probiotic drink is classified as a low-calorie drink, which is <100 k.cal, so it is good to use for a low-calorie diet, but contains live bacteria that are beneficial for health.

IV. Conclusions

Microbiological characteristics of mango juice probiotic drink containing LAB 1.7×10^8 CFU/mL (8.230 Log CFU/ mL) using *Lb paracasei* ssp. *paracasei Ml3* encapsulated and suitable for the standards of lactic fermented drinks according to SNI and FDA standards. Mango juice probiotic drink is classified as a low calorie drink < 100 kcal, which is potentially a low calorie diet.

V. Acknowledgments

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