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Probiotication of black grass jelly [*Mesona chinensis* (Benth.)] by encapsulated *Lactobacillus plantarum* Mar8 for a ready to drink (RTD) beverages

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Abstract *Lactobacillus plantarum* is an importantly probiotic bacteria for intestinal microbiota. The use of encapsulated the probiotic *Lactobacillus plantarum* Mar 8 by agar, carrageenan, Arabic gum, konjac (*Amorphophallus konjac* K.Koch), and black jelly [*Mesona chinensis* (Benth.)] for its application for RTD packaged beverages product was investigated. The probiotic bacteria was prepared by cultivation, biomass collection by suspension, and encapsulation. The result showed that 2% of carrageenan was the best encapsulant for the probiotic based on its suitable elasticity of the black jelly for RTD use. The probiotic was maintained at 8-9 log cfu/ml. This result would be potential for the application of encapsulated probiotic for RTD jelly black beverages.

Keywords: beverages, microcapsule, probiotic, storage, viability

Introduction

The black jelly grass is known as a healthy food or drinks (Handayani *et al.*, 2017). This jelly has a beneficial effect on health because that this jelly contains a high concentration soluble fiber and low-calorie composition. High soluble fiber has been proven to lower lipid profiles (Ramos *et al.*, 2011; Wahyono *et al.*, 2015; Handayani *et al.*, 2017). The Indonesian Nutrition Directorate Health Department confirms that 100 grams of grass jelly gel contains 6.23 grams of crude fiber. Hence, daily consumption of black grass jelly along with fruits and vegetables will fulfill personal needs in daily fiber (30 g). The black grass jelly also showed antioxidant activity due to its phenol content. This component has been reported to prevent DNA damage in human lymphocytes when it is exposed to free radicals such as hydrogen peroxide and UV radiation (Tasia and Widyaningsih, 2014; Handayani *et al.*, 2017).

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Recently, black grass jelly (*Mesona palustris* Bl) was reported to have anti-dyslipidemia in high cholesterol diet-fed rats (Handayani *et al*., 2017).

Although black grass jelly is good as a healthy food or drink, adding more beneficial effect on health such as by a probiotication is one of best choice supplements. Probiotics are live microorganisms, which when ingested in sufficient amount, could exert a health benefit on the host (FAO/WHO, 2001). Its benefits of health is as a result of the effect of their properties such as antimicrobial (bacteriosin) activity (Seddik *et al*., 2017; and adherent ability to specific mannose (*L. plantarum* in particular) (Adlerberth *et al*., 1996; Pretzer *et al*., 2005) to compete against harmful bacterial guts. Lactobacillus is grouped in to Lactic acid bacteria (LAB) which constitute a diverse group of Gram-positive, catalase-negative bacteria producing lactic acid as the main end-product of carbohydrate fermentation (Felis and Dellaglio, 2007). Lactobacillus has more than 220 valid species (http://www.bacterio.net/lactobacillus.html) definitely make this genus the main and diverse LAB group. Isolation of Lactobacilli has been made from different ecological niches, and diverse studies show diversities at their genetic and physiological levels (Seddik *et al.*, 2017; Liu *et al.*, 2018). *L. plantarum* is one of the potential probiotics which have a similar beneficial effect on health. *L. plantarum* fulfils the qualified presumption of safety (QPS) status from the European Food Safety Authorities (EFSA) and has been confirmed as the generally recognized as safe (GRAS) status from the US Food and Drug Administration (US FDA). This species also has been related with history of food use (Mogensen *et al.*, 2002; Seddik *et al.* 2017). With regard to a well-characterized bacterium with documented safety, *L. plantarum* is offering various applications along with which its use as probiotic. This highly versatile microorganism is found in wide range environments. This versatility is reflected in its genome plasticity. At present, numerous evidences are constituted on the role of *L. plantarum* in medical cases as diarrhea prevention, cholesterol lowering, and reduction in the IBS symptoms. More benefits value of *L. plantarum* comes from strains with capability to produce plantaricins, which are bacteriocins with specific structural and functional diversities. These bacteriocins present a wealth panel of applications in medical, veterinary, and food domains (Seddik *et al.* 2017).

Our potential *L. plantarum* Mar8 was originated from a tropical fruit and this strain showed to produce plantaricin A (Yulinery and Nurhidayat, 2015). This strain also had showed a stable mannose specific adhesin (MSA) (Nurhidayat, 2012). Based on these properties, this strain was ideal for use as a probiotic to deliver for gut health.

We continue to a new our assessment on another potential probiotic, *L. plantarum* Mar8 to apply as a probiotic supplement for black grass jelly as

RTD beverage. To meet with the condition that will affect the viability and stability of the probiotic, an encapsulation should be made to prolong span life of probiotic during processing or storage of the drink product. An encapsulation of probiotic is aimed at more resistance of the probiotic to such bad environment (very low pH and saline condition) and come to the gut environment at its adequate density for gut health.

We studied potential encapsulant which was suitable one for its application for RTD beverages, such as carrageenan, gum arabic, konjac and agar. The objectives of the research was to get the best microencapsulant for *L. plantarum* Mar 8 which was suitable for the black grass jelly RTD beverages.

Materials and methods

Cultivation of Probiotic Bacteria

One ose of isolate of *Lactobacillus plantarum* Mar8 from culture was inoculated on 200 ml of sterile *Lactobacillus* MRS Broth (Himedia RefM369-500g) as mother stock. After incubated for 24 hours, 200 µL were added to the 200 ml MRS Broth.

Biomass collection by suspension

The pellet of *L. plantarum* Mar8 were centrifugated at 10,000 rpm (round per minutes) for 15 minutes. The pellet were collected.

Encapsulation by Cincau and Stabilizer with Various Concentrations

Four grams of cincau are mixed in 1 L boiling water and stirred carefully. Encapsulants (konjac, carrageenan, gum arabic, commercial order) with different concentrations (0.5; 1; 1.5; 2) were added to the encapsulation process with *L. plantarum* Mar8 in ambient (37^oC) and cold temperature (4^oC).

Measurement of Syneresis Rate, Breaking Strength, Flow rate average, texture, pH

L. plantarum Mar8 which was encapsulated with grass jelly and varied encapsulant was then calculated the syneresis rate, breaking strength, flow rate average, texture and pH. Syneresis was measured by calculated the loss of weight during storage compared to initial weight (Latimer, 2012). The breaking was measured by using Lloyd instrument, pH by pH meter, flow rate by time counting on syneresis, and texture by direct observation.

Viability test after Encapsulation

The encapsulation results were stored in pyrex, glass cups or microwavable plastic bowls so that they remain sterile. After the encapsulation were complete, the cincau/jelly mix were store ambient (37^{o}C) and cold temperature (4^{o}C). The Total Plate Count were conducted in serial dilution 10^{-4}, 10^{-5} and 10^{-6}. Colony counting were done after 36-48 hours of incubation period at room temperature and cold temperature.

Results

Rate of Syneresis

Figure 1 showed various rate of syneresis of encapsulants. Under ambient temperature the rate of syneresis was much lower compared to ambient temperature. Carrageenan and konjac showed lowest rate of syneresis compared to control, agar, Arabic gum and konjac.

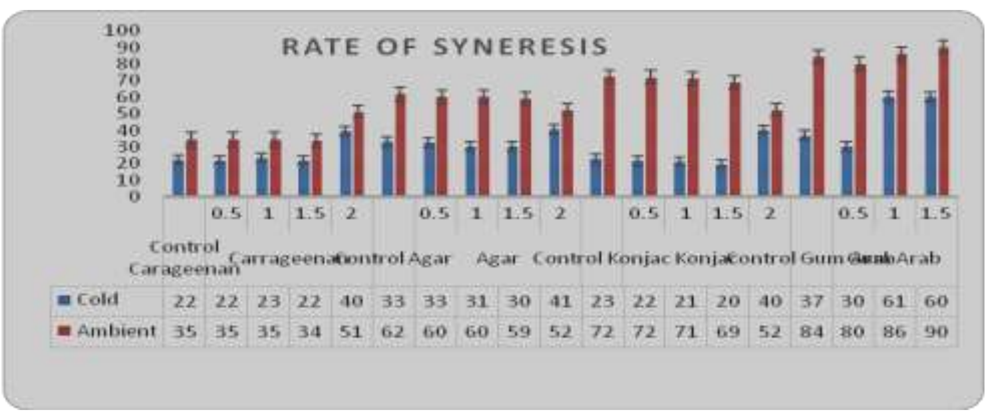

Figure 1. Rate of Syneresis of Various Encapsulants

Flow Rate of Syneresis

Figure 2 showed the average flow rate from concentration 0.5, 1.0, 1,5 and 2% of each encapsulants. Konjac has zero rate of syneresis but at 2% at 0.67, followed by carrageenan by increase from 0.2 to 0.67, agar by decrease from 1.1 to 0.56 and Arabic gum by increase from 1 to 7.

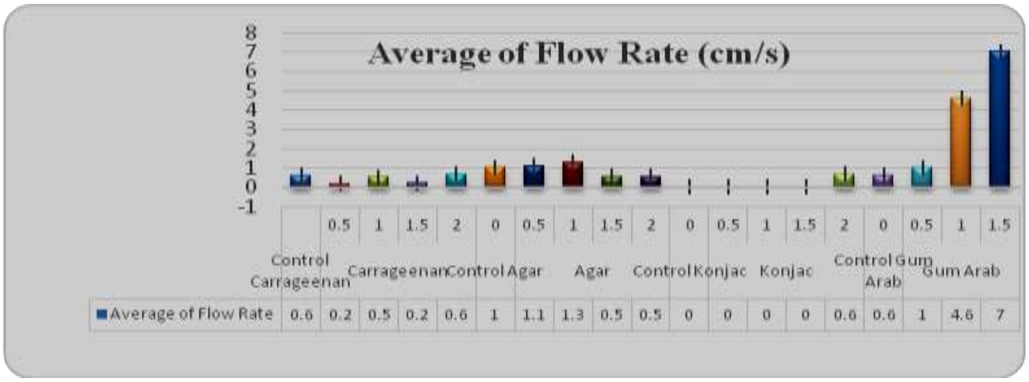

Figure 2. Average of Flow Rate of Various Encapsulants

Breaking Strength

Figure 3 showed that konjac was the highest value of breaking strength, followed by carrageenan, Arabic gum and agar. Konjac and carragenaan were almost the same breaking strength. But, Arabic gum showed decrease of its value at higher concentration. Arabic gum and agar have similar value of its breaking strength.

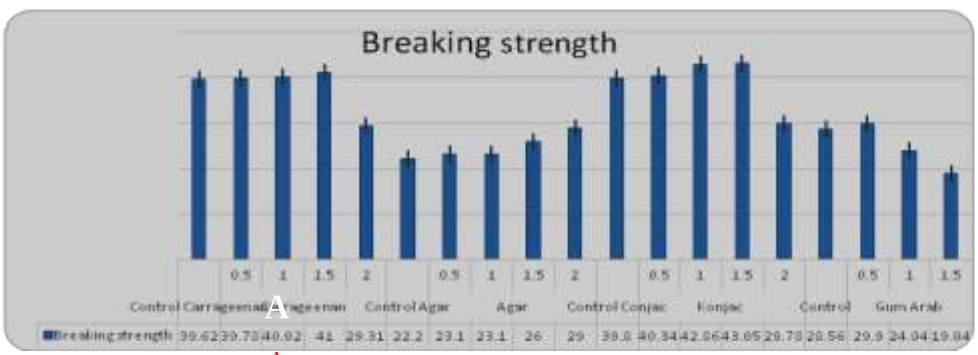

Figure 3. Breaking Strength of Various Encapsulants

Table 1 showed the profile of jelling agent of various encapsulants. All encapsulant have no differences of their pH value. But changes were occurred at ambient temperature to all jelling agent at every concentration, all pH value was decreased to 4. The textures were vary among the encapsulant. In general, carrageenan was the best jelling agent, followed by agar, konjac and Arabic gum. Similar jelling agent texture was showed by 0.5-1% concentration of each encapsulants except for konjac. At 1.5-2% concentration, only carrageenan had stable jelling, konjac showed its texture to become stickier, while Arabic gum become easy to break down.

Table 1. Texture and pH value of encapsulants as jelling agent

Jelling agent (Encapsulant)		pH			Textures	
Treatment	**%**	**Before**	**Cold**	**Ambient**	**Cold**	**Ambient**
Control of carrageenan	0	7	7	4	-	-
carrageenan	0,5	7	7	4	j (+)	j (+)
	1	7	7	4	j (++)	j (++)
	1,5	7	7	4	j (+++)	j (+++)
	2	7	7	4	j (++++)	j (++++)
Control of Agar	0	7	7	4	-	-
Agar	0,5	7	7	4	j (+)	j (+)
	1	7	7	4	j (+)	j (+)
	1,5	7	7	4	J, ss (++)	J, ss (++)
	2	7	7	4	J, ss (++)	J, ss (++)
Control of konjac	0	7	7	4	-	-
konjac	0,5	7	7	4	s, d (+)	s, y (+)
	1	7	7	4	s, d (++)	s, y (++)
	1,5	7	7	4	s, d (+++)	s, y (+++)
	2	7	7	4	s, d (++++)	s, y (++++)
Control of Arabic Gum	0	7	7	4	-	-
Arabic Gum	0,5	7	7	4	j (+)	j (+)
	1	7	7	4	j (++)	j (++)
	1,5	7	7	4	b (+)	b (+)
	2	7	7	4	b (+++)	b (+++)

Note: j=jelly; s=solid; ss= somewhat solid; b= soft and easy breakdown; y=sticky; - = not measured; +=good; ++=better; +++= very good; ++++=excellent

Table 2 showed that all encapsulant were very good to keep the probiotic viable and stable. This result showed that viability was high (9 log cfu/ml). The best encapsulant was the mixture of cincau (black grass jelly) and agar (0.97 x 9 log cfu/ml) and followed respectively by cincau and konjac (0.84 x 9 log cfu/ml), cincau and carrageenan (0.70 x 9 log cfu/ml) and cincau and Arabic gum (0.67 x 9 log cfu/ml).

Table 2. The Viability of *Lactobacillus plantarum* Mar8 in Black Jelly Grass (Cincau) after Two Weeks of Storage at 4 ^{o}C

Cincau and Encapsulants	The Number of *Lactobacillus plantarum* Mar8 (CFU/ml)
Cincau and Konjac	0.84 x 9 log
Cincau and Carrageenan	0.70 x 9 log
Cincau and Gum Arab	0.67 x 9 log
Cincau and Agar	0.97 x 9 log

Discussion

The viability of microencapsulated probiotic is essential for probiotication as this state is the key point for the effectiveness of probiotication for an RTD beverage. The result showed a good indication that all encapsulant was suitable for the probiotic viability. At high density, 9 log cfu/ml, probiotication use was met with the effectiveness prerequisite for delivery to the gut. Probiotic has been known as all organic and inorganic food complexes, in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. The term probiotic is technically defined as live microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition (FAO/WHO, 2001; Mustafa, 2016; Seddik *et al.*, 2017). The definition of probiotics term has been in Guidelines for the evaluation of probiotics in food drafted by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Working Group as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). The guidelines give general *in vitro* tests to evaluate probiotic prospective, include resistance to acidity, bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, anti-microbial activity against potentially pathogenic bacteria, ability to decrease pathogen adhesion to surfaces, bile salt hydrolase (BSH) activity, and resistance to spermicides (appropriate to probiotics for vaginal use). In generally published reports, probiotics are commonly characterized by assessing acid resistance, bile salt tolerance, antimicrobial activity, antibiotic resistance, adhesion to intestinal epithelial cells, resistance to simulated gastric-intestinal juice and lysozyme. The population density of intestinal microorganisms elevate along the gastrointestinal (GI) tract: there are about 10^3 microorganisms/ml of luminal content in the duodenum, 10^8 microbes/g of ileum content, and 10^{12} microorganisms/g of colon content (O'Hara and Shanahan, 2006; Booijink *et al.*, 2007). Lactobacilli contribute the foremost probiotics in the human intestine, significantly playing a role by competing against pathogenic bacteria via adherence to and replication in the intestinal tract. Several characters were regarded as probiotic characters, such as resistant to acid, bile salt, simulated gastric and/or intestinal transit, and antibiotics, anti-microbial activity, ability of adhesion to intestinal epithelial cells, and inhibition of pathogen adhesion (FAO/WHO, 2002). Lots of data based on the probiotic criteria are fulfilled by *L. plantarum*. The high tolerance to acid and bile salt enables *L. plantarum* to transit through stomach and the upper gastrointestinal tract, while adherent ability to intestinal epithelial cells increase colonization in the lower gastrointestinal tract, and improve in competitive inhibition of pathogens (Liu *et al*., 2018). Therefore, it is important to make sure that the viable probiotic must be alive and present in high number. In general, more than 10^9 cells is ingested daily dose (Vasudha and Mishra, 2013). Blurueanua *et al.* (2012) proposed that the minimal number of live probiotic minimally ranged from 10^5 to 10^6 CFU/ml or gram of the product as a functional food. While, other references showed adequately stimulation on the human health by consumption of 10^7-10^{10} live cells in a day (Kailasapathy and Chin, 2000; Arup and Shantanu, 2013). Certainly, every probiotic product should specify the minimum daily amount required for particular health benefit(s) (Vasudha and Mishra, 2013, FAO/WHO, 2002). More over, a study on the safety of two *L. plantarum* strains in Wistar rat model by a short-term oral toxicity assay was already reported. Rats have treated a normal dosage (about 9×10^9 CFU/kg/ day) or a high dosage (about 4.5×10^{10} CFU/kg/day) for 28 days. No adverse effect was observed after those periods, based on general behavior, growth, feed and water consumption, hematology, clinical chemistry indices, organ weights, or histopathologic analysis in the rats that have treated both dosages (Tsai *et al.*, 2014). Previously, investigation by Daniel *et al.* (2006) on mouse colitis model confirmed the safe trait of *L. plantarum* Lp-115 and its inability to translocate to extra intestinal organs. More recent, a study report of AB-LIFE® formulation containing three *L. plantarum* strains (CECT 7527, 7528, and 7529) stated its safety based on a 90-day repeated-dose study in rats. There was no adverse effect showed even after a high dose intake (1.85×10^{11} CFU/kg/day) (Mukerji *et al.*, 2016). These reports showed the safety of Lactobacillus consumption as probiotic even at very high dose (Seddik *et al.*, 2017). Numerous reports have exposed the effects of gut microbiota on cardiometabolic regulation and microcirculatory disturbances, and associating these with metabolic disorders such as hypercholesterolemia, obesity, diabetes, stroke and heart failure. (Turnbaugh *et al.*, 2006; Wang *et al.*, 2011; Mulders *et al.*, 2018). The use of probiotics ultimately alters gut environment and gut microbiota profiles (Liu *et al.*, 2018).

Syneresis is an important point to regard with before the encapsulant was applied that it might act as a jelling agent as well. The jelly could form different properties after applying the encapsulant, such as syneresis. Syneresis is related to water flow out from the substrate which caused by the presence of the pores. Syneresis is affected by several factors such as pH, temperature, set pressure, and properties of chemical composition of its solvent (Aurand and Woods, 1973), As the encapsulated probiotic which was trapped in the jelly which absorbed water, the syneresis will affect the presence of the encapsulated probiotic in the jelly; the probiotic will also come out from the jelly. The higher syneresis occurs at the jelly, the higher losses of the viable probiotic. The use konjac or carrageenan as the encapsulant for probiotic was also good as the gelling agent as well. Konjac showed as the best gelling agent as it can keep water inside which means the probiotic might remain inside as well. While, the use of dextrin as an encapsulant for the *L. plantarum* Mar8 strain was already reported (Nurhidayat, 2012). However, this encapsulated probiotic was not suitable since this encapsulant will be damaged and discolored the drinks that are not desired besides the loss of its function as an encapsulant.

The physical effect of its encapsulant on the jelly grass will need consideration so as to make encapsulation and probiotication are ideal or meet with the prerequisite as probiotic for its application for RTD beverages. Therefore, the addition of the encapsulant must support the character of the jelly drink. The strength of the jelly is important to keep the jelly as in its unbreakable form. The breakdown of the jelly will also reduce the quality of jelly including the viability of the probiotics as they can come out causing decreasing of their viability. Carrageenan or konjac showed its good function to maintain the jelly in the good form as a result from its elasticity. Agar was not suitable for keeping the gel from breakdown. But the use of Arabic gum showed lower value in breaking strength of the gel compared to carrageenan or konjac. Another consideration of suitable jelling agent is the pH and texture of the jelling agent. The good jelling agent will show by the texture of a typical jelly agent. The pH is also essential for keeping the jelly drink well for RTD beverages. Carrageenan was the best jelling agent. The higher the concentration, the higher the value of the jelly agent was reached. Binding water capability of carrageenan in large amounts causes the space between particles to become tighter so that more water is bound and trapped to make the solution hard. The addition of carrageenan concentrations in rosella-sour sop jelly drink caused the level of caffeine to decrease due to the formation of a strong double helix structure so that it can capture and bind water so that the water molecules in the gel are not easily released which will reduce the occurrence of syneresis (Agustin and Putri, 2014).

The same value of pH was observed at all treatment indicated that cold condition was the best condition for keeping the jelly drink from pH changes. The changes of pH to a low value will reduce the quality of drinks such as by chemical reaction of an ionic condition that makes the jelly will be easy to break down or liquefaction. Black grass jelly contains organic acids that will contribute to lower pH (Yulianto and Widyaningsih, 2013). It might be correlated with elevation of syneresis by warm temperature. Agar was also a good was also a good jelling agent and become more solid at higher concentration. Arabic gum was also good jelling agent but at higher concentration, it was not suitable as a jelling agent as their textures became soft and easy breakdown. Although konjac could make jelling well, but it was sticky that it made changed the jelly form besides undesirable for the jelly drink as RTD beverages.

Black grass jelly has 122 calories and 6 grams protein for each 100 grams. Therefore, its beneficial will follow if this jelly is consumed daily engaged. The healthy effect of the jelly is from its high fiber and low-calorie composition (Wahyono *et al.,* 2015). Not only does it contain fiber, the black grass jelly also contains active polyphenol. This component has been reported to prevent DNA damage in human lymphocytes when it is exposed to free radicals such as hydrogen peroxide and UV radiation. Black grass jelly extract has high antioxidant activity due to its phenol content (Tasia and Widyaningsih, 2014; Handayani *et al*., 2017). Black grass jelly can elevate antioxidant level because of their phenolic compounds (Chusak *et al.,* 2014; Lai *et al.*, 2001). Other studies have shown that black grass jelly prevents AGE (Advanced Glycation End Products) formation and protein oxidation, processes associated in diabetes (Adisakwattana *et al*., 2014). Decrease of lipid peroxidation with malondialdehyde (MDA) concentration was also reported (Lai *et al*., 2001).

Conclusion

Probiotication together with its microencapsulation by carrageenan showed a potential application as a health supplement for black grass jelly drink as RTD beverage. This application highly improved the status of healthy black grass jelly drink as RTD beverage after probiotication by encapsulated *L. plantarum* Mar8. Carrageenan was the suitable and ideal for microencapsulation of the probiotic, *L. plantarum.* The amount of live bacterium (9 log cfu/ml) was reached that it was ideal density to ingest so as to take the effect on gut health. The carrageenan has a function to stabilize the jelly and also hold the viability and stability of the probiotic and prevent it from coming out from the jelly.

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