

Stability of black grass jelly (*Mesona chinensis*) probioticated by encapsulation of *Lactobacillus plantarum* Mar8 with agar and gum Arabic

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Abstract. Wulandari NF, Yulinery T, Suharna N, Nurhidayat N. 2019. Stability of black grass jelly (*Mesona chinensis*) probioticated by encapsulation of *Lactobacillus plantarum* Mar8 with agar and gum Arabic. *Nusantara Bioscience* 11: 84-88. Black grass jelly [*Mesona chinensis* (Benth)] is one of the traditional food ingredients that can be used as the encapsulation material. However, syneresis easily occurs so that becomes a problem during storage. This study was conducted to evaluate the effect of type of stabilizers (agar and gum Arabic) and storage temperature (cold/4°C and ambient temperature/37°C) on black grass jelly stability and the viability of encapsulated *Lactobacillus plantarum* Mar8 in probiotication. The concentrations of stabilizers were 0.5%; 1.0%; 1.5% and 2.0% (w/v). The result showed that the syneresis rate was stable up to 2% of agar concentration at low storage temperature, while gum Arabic in the concentration of >1% resulted in increasing syneresis rate. Ambient temperature storage increased the syneresis rate. This study showed that agar or gum Arabic was supporting the probiotication as good encapsulant. Moreover, it was suitable for stabilizing or gelling agent at 0.5%-1% concentration to produce black grass jelly as RTD.

Keywords: Black grass jelly, probiotic, storage, syneresis

INTRODUCTION

The term probiotics are technically defined as living microorganisms which after being consumed in certain quantities provide health benefits beyond inherent general nutrition (FAO/WHO 2002; Mustafa 2016; Seddik et al. 2017). Guidelines for evaluating probiotics in food compiled by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) define the term probiotics as "living microorganisms that, when given in sufficient quantities, provide health benefits" (FAO/WHO 2002). This guide evaluates prospective probiotics with *in vitro* tests including resistance to acidity, bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, anti-microbial activity against pathogenic bacteria, ability to reduce pathogenic adhesion to the surface, hydrolase salt activity bile, and resistance to spermicide. Probiotics are also usually characterized by traits such as acid resistance, tolerance to bile salts, antimicrobial activity, antibiotic resistance, intestinal epithelial cell adhesion, resistance to simulated gastric-intestinal fluid and lysozyme (O'Hara and Shanahan 2006; Booijink et al. 2007). *Lactobacillus plantarum* Mar8 is one of the selected potential probiotics originated from tropical fruit, i.e., passion fruit (*Passiflora edulis*) (Nurhidayat 2012; Yulinery and Nurhidayat 2015). Previous study showed that this strain was able to produce plantaricin A (Yulinery and Nurhidayat 2015) and a stable mannose-specific adhesion (Nurhidayat 2012). Based on these

properties, this strain was ideal as a probiotic for intestinal health.

Its application as a probiotic supplement on the black jelly is very interesting especially as a ready to drink (RTD). This is certainly closely related to the nature of black grass jelly which has an impact on health, especially if consumed daily. According to Handayani et al. (2017), this jelly was known as healthy food or drink because it contained high concentration of soluble fiber and low-calorie. High soluble fiber had been proven to reduce lipid profiles (Ramos et al. 2011; Wahyono et al. 2015; Handayani et al. 2017). The Indonesian Ministry of Health's Nutrition Directorate (year of 1992) stated that 100 grams of black jelly contained 6.23 grams of crude fiber. Daily consumption of black jelly will be able to meet daily fiber requirements (30 g). Its high phenol content in black jelly might be related to high antioxidant activity, so that it might prevent DNA damage in human lymphocytes due to exposure to free radicals such as hydrogen peroxide and UV radiation (Tasia and Widyaningsih 2014; Handayani et al. 2017). In addition, black jelly was found to have anti-dyslipidemia activity in rats fed with high cholesterol (Handayani et al. 2017).

However, in the probiotication of black grass jelly drinks preconditions are needed to support the viability and stability of probiotics, including encapsulation. Encapsulation must be suitable to be applied to black grass jelly as an RTD. In a previous study, black grass jelly with a concentration of 4% had not been able to maintain viability or stability of *L. plantarum*, so that a suitable

stabilizer was needed. In this study, commercial agar and gum Arabic were studied for their suitability for both as stabilizer and encapsulant for probiotic.

Gum Arabic is collected from the branches of acacia species and is usually used in the food industrial sector as a food additive (Bertolini et al. 2001; Motlagh et al. 2006). The gum originated from *Acacia senegal* is known as the foremost gum used for commercial purposes because it has boosted emulsification properties when linked to the gum collected from *A. seyal* (Elmanan et al. 2008). Gum Arabic use is been allowable as an unthreatened compound by the joint FAO/WHO Expert Committee on Food Additive (Anderson and Eastwood 1987). Gum Arabic is also known as a primary coating component owing to its emulsifying properties (Maqbool et al. 2011; Valiathan and Athmaselvi 2018).

The purpose of this study was to obtain a jelly stabilizer and its suitability for encapsulation of *L. plantarum* applied to black jelly drink as RTD.

MATERIALS AND METHODS

Encapsulation of probiotic bacteria

Cultivation

One ose of *L. plantarum* Mar8 isolate from oblique agar media was inoculated in a 200 ml sterile *Lactobacillus* MRS Broth media (Himedia RefM369-500g) and incubated for 24 hours. This culture was used as the master culture. 200 μ L of master culture were taken out and put into 200 ml of MRS broth media and incubated for 48 hours at (37°C). This culture was prepared for pellet collection.

Pellet collection

The bacterial biomass was collected by centrifugation at 10,000 rpm (revolutions per minute) for 15 minutes. The pellets were then collected.

Jelly stabilization and probiotic encapsulation

Four grams of jelly were mixed in 1 L of boiling water and stirred carefully. Each stabilizers (encapsulants) i.e.: commercial agar and gum Arabic at different concentrations (0.5%; 1%; 1.5%; 2%) were mixed with jelly by three replications. After reaching ambient temperature (37°C), 1 gram of the bacterial pellets was added to each jelly and mixed carefully. After being hardened (25°C), each of the jelly mixtures was stored in 2 different storage temperature (ambient temperature/37°C) and cold temperature/4°C).

Viability test after encapsulation

The encapsulation products were stored in sterile container. After stabilization and encapsulation were complete, each mixture of black grass jelly containing stabilizer and probiotics were stored at ambient temperature (37°C) and cold temperature (4°C). The total plate count was performed in series dilutions of 10^{-4} , 10^{-5} and 10^{-6} . Colony counting was carried out after 36-48 hours of incubation period at ambient temperature and cold temperature.

Physical and organoleptic analysis

Syneresis and pH were measured at 37°C and 4°C. Syneresis was measured by calculating the weight loss during storage compared to the initial weight (Latimer, 2012). Flow rate, syneresis, texture, breaking strength were observed and measured directly at cold temperature (4°C). Breaking strength was measured using the Lloyd instrument, pH was determined by pH meter.

RESULTS AND DISCUSSION

Results

The results showed that the rate of syneresis was lower at cold storage temperature (4°C) compared to ambient temperature storage (37°C). At ambient temperature storage (37°C), the rate of syneresis was faster shown in the mixture with 2% gum Arabic than the mixture with agar. The syneresis rate at cold temperature storage (4°C) was 37 and 30. 2 at the mixture of gum Arabic 0.5% and 1% respectively, but they were not statistically different. The syneresis rate in the mixture with gum Arabic at the concentration of 1.5 and 2% were almost in similar rate but increases twice when compared to the gum arabic mixture in concentrations of 0.5 and 1% (Figure 1).

The initial pH of all jelly mixtures before storage was 7. However, storing the mixture at ambient temperature caused the pH decreased to 4. While storing in cold temperature (4°C), the pH of both jelly mixtures with agar or gum Arabic were stable at 7 (Table 1).

Flow rates at cold temperature storage in the mixture of 2% agar or 0.5% gum Arabic were similar to control. However, it increases in the mixture of agar at a concentration of 0.5, 1 and 1.5%. Flow rate also increased very rapidly in the mixtures of gum Arabic at concentrations of 1.5% and 2% (Fig. 2).

Breaking strength of the mixture with 0.5 and 1% gum Arabic almost the same rate i.e.: 28.56 and 29.9 respectively, while breaking strength of controls were 29.31 and 29.78 points for agar and gum Arabic respectively. On the contrary, the mixture with 1.5% and 2% of gum Arabic decreased breaking strength to 24.04 and 19.04 respectively.

There were similarities in the texture of the mixture with agar and gum Arabic at concentrations of 0.5 and 1%, respectively. However, the mixture with agar at the concentrations of 1.5 and 2% caused the jelly mixture to become harder. Texture of the mixture with gum Arabic at concentrations > 1.5% were soft and flabby (Table 1).

Both types of encapsulants or stabilizers could maintain the stability and high viability of probiotics. Viability of *L. plantarum* Mar8 was better in the agar mixture (9.7×10^8 cfu/mL) compared to the gum arabic mixture (6.7×10^8 cfu/mL) (Table 2).

Discussion

The results of this study indicated that the storage temperature had an effect on syneresis of the jelly that might also be related to the pH change of the jelly. The initial pH of all mixtures were 7 but it decreased after

stored at ambient temperature (37°C) to 4. At this pH value, syneresis increases. This might be due to the low pH changing the chemical structures due to acidic conditions. Organic acids may react to the structure and chemical composition of the mixed mixture. Black jelly is known to contain organic acids which influence the decrease in pH (Yulianto and Widyaningsih 2013).

Jelly mixture with agar or gum Arabic had an impact on syneresis. The syneresis might lower if the concentration of agar or gum Arabic was higher. Gum Arabic at a

concentration of 1.5% and 2% underwent a drastic change of its syneresis compared to 0.5% and 1%.

The results of the measurement of "breaking strength", show that only by adding a 1% gum Arabic to the grass jelly were similar to that of control, it means that the addition of gum Arabic up to 1% has no effect. Whereas the addition of agar reduces "breaking strength" even though the concentration increases further. Conversely, the addition of gum Arabic above 1% resulted in decreasing breaking strength.

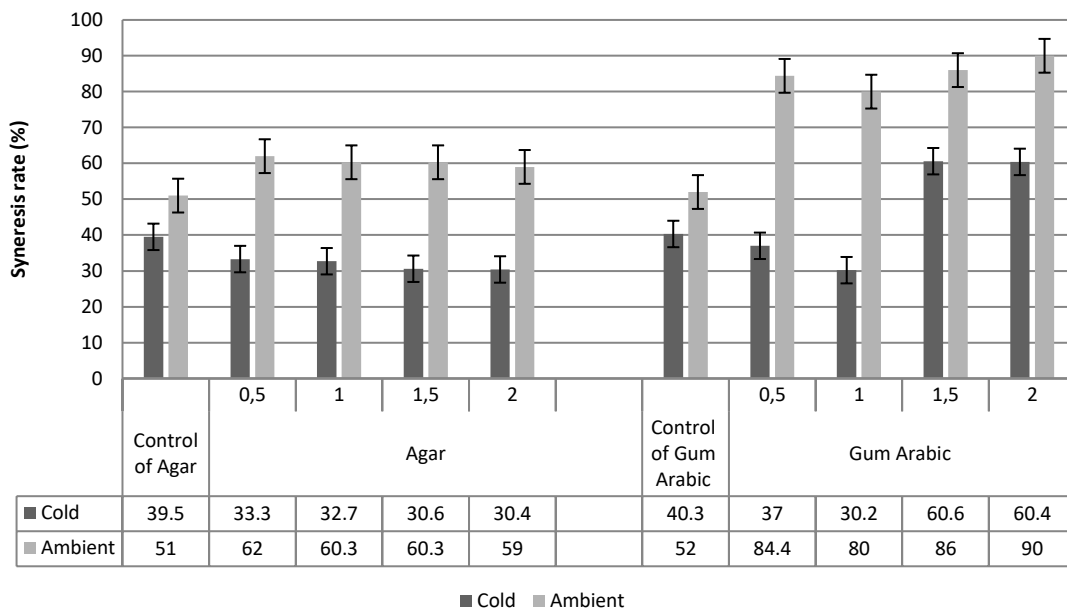


Figure 1. Syneresis rate (%) of black grass jelly in mixture with Agar or gum Arabic at cold temperature storage (4°C) and ambient temperature storage (37°C) with 4 different concentrations of agar or gum Arabic (0.5, 1, 1.5 and 2%).

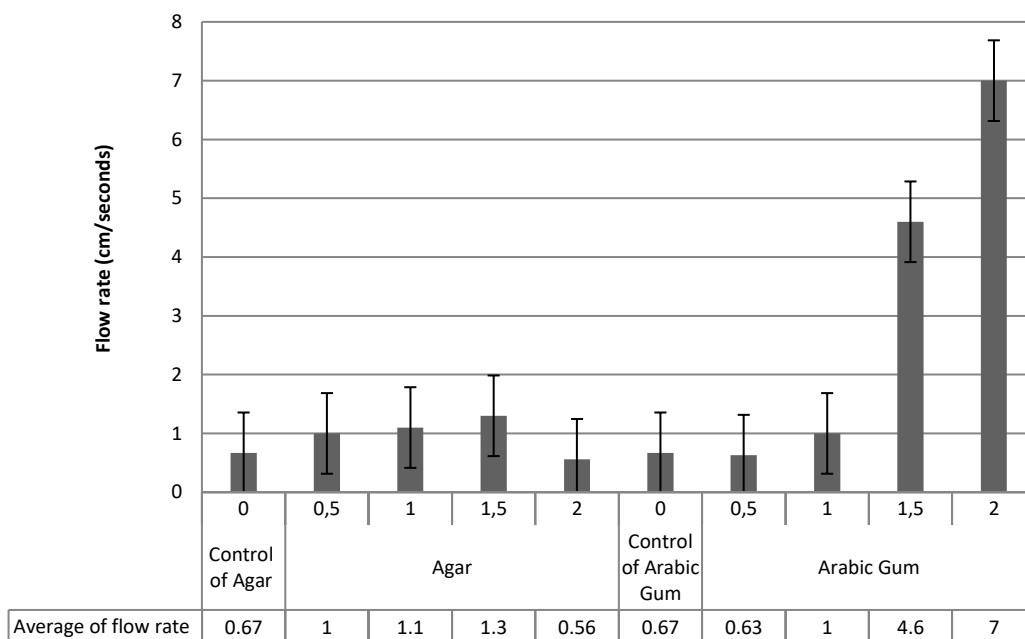


Figure 2. Flow rate of the jelly mixture with agar and gum Arabic at cold temperature.

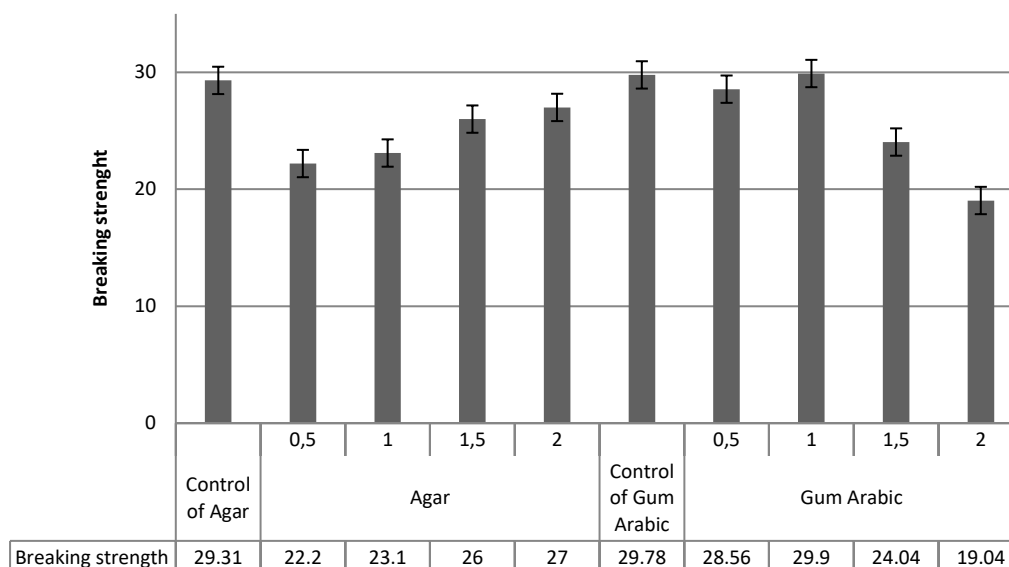


Figure 3. Breaking strength of jelly mixture with agar or gum Arabic with various concentrations stored at cold temperature

Table 1. The pH and texture of the jelly mixture with stabilizers.

	pH			Texture
	Before	Cold	Ambien	
Control of agar	7	7	4	
Agar	0.5	7	7	4 Chewy (+)
	1	7	7	4 Chewy (+)
	1.5	7	7	4 Chewy and rather hard (++)
	2	7	7	4 Chewy and rather hard (++)
Control of gum Arabic	7	7	4	
Gum Arabic	0.5	7	7	4 Chewy (+)
	1	7	7	4 Chewy (++)
	1.5	7	7	4 Soft and flabby (+)
	2	7	7	4 Soft and flabby (+++)

Table 2. Viability of *Lactobacillus plantarum* Mar8 in jelly mixture with encapsulants after stored for two weeks at 4°C.

Mixture of black grass jelly and encapsulants (stabilizer)	Cell count of <i>L. plantarum</i> Mar8 (CFU/mL)	
	Before stored	After stored
Agar (1.5 %)	9.7 x 10 ⁸	18.8 x 10 ⁸
Gum Arabic (1.5%)	6.7 x 10 ⁸	9.5 x 10 ⁸

Changes in the characteristics of the jelly mixture with agar or gum Arabic are closely related to the differences from their properties. In order to be as "gelling agent", the higher the concentration, so the higher the "gelling" effect will be. Gum Arabic is more of an "emulsifier".

This study shows that at the concentrations up to 1%, gum Arabic functions as stabilizer, but at the higher concentration gum Arabic functions more as an emulsifier. This is affecting breaking strength, so that the mixture becomes soft and flabby. Another consideration of the use

of "gelling agent" is the compatibility of the pH and texture of the gelling agent itself. A typical jelly agent texture will indicate a good gelling agent. The criteria for a good jelly drink are soft gel texture so that when smoked to use the help of easy gel straws destroyed, but the shape of the gel is still felt in the mouth. Gel texture criteria for jelly drink this can be achieved by addition gelling agent in the jelly processing process drink (Vaniaa et al. 2017).

Syneresis is known to be influenced by several factors such as pH, temperature, set pressure, and the properties of the chemical composition of the solvent (Aurand and Woods 1973; Costa et al. 2017). The pH value is also important to keep jelly drinks good for RTD drinks. At lower pH values, the rate of syneresis is increased because the gel becomes a higher ability of contractions (Jovanović et al. 2004).

Syneresis is an important factor to be considered because it is affecting the viability of encapsulated probiotics in the jelly. Probiotics encapsulations with agar or gum Arabic can maintain high viability of probiotics. The presence of probiotic cells with density reaching 10⁸-10⁹ cfu/mL indicated that the probiotication has effectively functioned as a probiotic.

This study showed that the use of agar at a concentration of 0.5% or 1% was suitable for probiotication in jelly mixture, which indicated by its physical and organoleptic properties. This is agar has a hydroxyl group that is hydrophilic due to the nature of the agar which has a hydroxyl group that is hydrophilic. Therefore, agar has the ability to bind water strongly which result in the difficulty of water to evaporate (Widyaningsih and Safitri 2014). Another advantage of using agar result in increasing the total solids of the mixture. Moreover, its fillings can maintain a good texture of jelly (Widyaningsih and Safitri 2014).

The similar result also showed that the use of gum Arabic up to 1% was ideal for the use as the stabilizers of

the Jelly and encapsulant of *L. plantarum* Mar8. This might be caused by its supporting characters as hydrocolloid (Bertolini et al. 2001; Rizqiati et al. 2009). Gum Arabic is recognized as an ordinary salt composed of calcium, magnesium and potassium ions (Prakash et al. 1990). Its hydrocolloid characteristic is very soluble in hot and cold water, so it will form a solution with low viscosity, but it is not soluble in alcohol and other organic solvents. The advantage of gum Arabic in food or drinks application was that it can retain the flavor of food dried by spray drying method because this gum can form layers that can protect flavor from oxidation, absorption, and evaporation (Bertolini et al. 2001). Because of its low viscosity, no taste and color, so adding gum Arabic in certain quantities does not interfere with the organoleptic properties of food products (Mosilhey 2003; Rizqiati et al. 2009).

It can be concluded that agar and gum Arabic can be used as an encapsulant that maintains high viability of probiotics and good stability. The concentration of 0.5% or 1% agar or gum Arabic were ideal or suitable for use both as the stabilizers and encapsulant of *L. plantarum* Mar8.

This study used a higher concentration of agar or gum Arabic for *Lactobacillus*'s viability test rather than the suitable concentration (0.5-1%) of those encapsulants. However, the result would be similar if that concentration was also applied. Rizqiati et al. (2009) reported that the use of gum Arabic was suitable for microencapsulation of *L. plantarum*. This study showed that agar or gum Arabic was supporting the probiotication as good encapsulant. Moreover, it was also suitable for stabilizing or gelling agent at 0.5%-1% concentration to produce black grass jelly as RTD.

Probiotication was successfully applied with ideal cell numbers ($\sim 10^9$ cfu/mL) so that this can be applied as probiotic for health food or drink supplement as RTD beverage. This study revealed a new upgrading of Jelly drinks to be healthier as RTD beverages by using suitable encapsulant to bring probiotication meet the prerequisites as probiotic for health supplement.

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