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**INDONESIAN FOOD AND NUTRITION
PROGRESS**

Development of the Traditional *Tape Ketan* into Probiotic Drink with the Supplementation of Lactic Acid Bacteria

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Abstract

Probiotics are living microorganism which give health benefit when consumed. Probiotic needs a 'vehicle' for their specialized actions in gastrointestinal tract. In this research, *tape ketan* was used as 'vehicle'. The objective of this research was to produce probiotic *tape ketan* powder as an ingredient of functional beverage. *Tape ketan* used in this research was made by fermentation of glutinous rice with *ragi* and supplemented with *Lactobacillus plantarum* Dad 13 (10^7 CFU/g glutinous rice). Probiotic powder was obtained using spray dryer with inlet temperature of 90°C with the addition of 35% maltodextrin. The powder was reconstituted into water with addition of pectin and sucrose. The results showed that viability of lactic acid bacteria and *L. plantarum* decreased after drying (0.82 and 0.90 log cycle respectively) with viable count from 1.29×10^{10} CFU to 1.96×10^9 CFU for lactic acid bacteria and from 1.04×10^{10} CFU to 1.32×10^9 CFU for *L. plantarum*. To obtain probiotic *tape ketan* beverage, 20% *tape ketan* powder (w/v), 0.5% pectin (w/v) and 4% sucrose (w/v) were reconstituted. *Tape ketan* powder which was supplemented with probiotic *Lactobacillus plantarum* Dad 13 is potential as an ingredient of functional beverage (from the viability after drying).

Keywords: *Tape ketan*; probiotic; powder; *Lactobacillus plantarum* Dad 13; spray dryer.

Introduction

Tape ketan is one of the Indonesian traditional fermented food made from the fermentation of glutinous rice at 25-30°C for 2-4 days. It has sweet tastes yet slightly alcoholic, with a pleasant, fragrant aroma. It has been consumed for a long time, with many ways to consume it, including: consumed directly, mixed with hot water into drinks (*wedang tape*), or with cold water (*es tape*, *tape ice*). *Tape ketan* usually packed with banana leaves or just a container (Steinkraus, 1989).

Tape ketan has a short shelf life because the fermentation continues even after the glutinous rice reaches its optimum stage and makes the taste very sour. Thus it has to be consumed immediately, but if it is chilled, it may be kept for almost 2 weeks (Steinkraus, 1989). Sour taste in *tape ketan* is caused by microorganisms which produced lactic acid. It will decrease sensory score of the product. Besides, the acid and alcohol which is produced during further fermentation will lead to the cell death. Drying and make it into powder is an

alternative way to improve the shelf life of *tape ketan*. Spray drying is usually applied to produce powder from milk or fruit juice (Naknean, 2011).

Nowadays, people consume foods not only to eliminate hunger or to fulfill nutrient intake but also to improve their health. This refers to the concept of functional food, that is processed foods containing one or more functional components which is based on scientific assessment, has a specific physiological function, not endanger and beneficial to the health (BPOM, 2005). So, the need for functional food increased. Probiotic is one of the functional food product which has been developing.

Probiotic is life microorganisms which when consumed in adequate amounts confer a health benefit on the host (FAO/WHO, 2001) by improving their intestinal balance (Fuller, 1989 in Lee and Salminen, 2009). Most of the probiotic products are dairy-based products. Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used. Viability is important in the probiotic product to get some beneficial effects. In clinical studies, the dosage of probiotics needed to be able to attribute the health benefits are in excess of 10^8 - 10^9 viable cells per day. Therefore, food regulatory/advisory bodies are generally stipulate that foods containing probiotic organisms need to have $>10^6$ - 10^7 CFU/g at the time of consumption (Lee and Salminen, 2009).

In Indonesia, probiotic products began to appear after Yakult (milk fermented product with *Lactobacillus casei* Shirota strain as probiotic) was produced in Indonesia, in 1991. After that there have been other probiotic products such as Vitacharm and Activia but still use imported probiotic agents. In Indonesia, scientists have isolated and screened lactic acid bacteria from local fermented product for probiotic agents but

have not used optimally. One of them is *Lactobacillus plantarum* Dad 13 which was isolated from dadih, fermented product made from buffalo milk, originated from West Sumatra. *Lactobacillus plantarum* Dad 13 has an ability to assimilate cholesterol, conjugate bile salt, grow on bile salt and low pH medium (Ngatirah, 2000).

Utilization of this isolate should be incorporated with food as 'vehicle'. One of fermented food which could be developed as 'vehicle' was *tape ketan* because nondairy probiotic products are also required to provide probiotic products for people with lactose intolerance. Therefore probiotic *tape ketan* was developed.

Tape ketan is a sugar-rich product. Drying of sugar-rich products into powder is difficult, mainly due to the low molecular weight sugar, such as fructose, glucose, and sucrose. Due to their low molecular weights, the molecular mobility of the materials are high when the temperature is just above the glass transition temperature (Tg) (Jaya and Das, 2004). To overcome this problem, addition of drying aid such as maltodextrin can be used to get non sticky powder. Maltodextrin is a starch hydrolysis product. It consists of β -D-glucose unit links mainly by glycosidic bonds (1, 4) and are usually classified according to their dextrose equivalency (DE) (Gabas et al., 2007). It has several properties including rapid dispersion, high solubility and capability to form a film, low hygroscopic properties, low browning, and capable to inhibit crystallization (Sriharni et al., 2010).

The general objective of this research was to produce probiotic *tape ketan* powder as an ingredient of functional beverage. The specific objectives were to determine the fermentation time for producing *tape ketan* with the supplementation of *Lactobacillus plantarum* Dad 13 as co-inoculum, the

minimum amounts of maltodextrin which was added into *tape* extract before drying, to determine the viability of lactic acid bacteria and *Lactobacillus plantarum* in the probiotic *tape ketan* powder, formulation probiotic *tape ketan* powder with addition of pectin and sucrose for making beverage, and to know the potency of *tape ketan* powder which was supplemented with probiotic bacteria *Lactobacillus plantarum* Dad 13 as an ingredient of functional beverage (from the viability after drying).

Materials and Methods

Materials

Glutinous rice and ragi (yeast) 'NKL' were obtained from local traditional market, maltodextrin was obtained from CV. Chemix Pratama, *Lactobacillus plantarum* Dad 13 was obtained from FNCC (*Food and Nutrition Culture Collection*) Pusat Studi Pangan dan Gizi Universitas Gadjah Mada Yogyakarta, commercial pectin was obtained from PT. Ekacita Dian Persada, distilled water, sucrose (sugar) and mineral water 'aqua' were obtained from local supermarket Mirota Kampus.

The media used for plate count enumeration were MRS agar for lactic acid bacteria (LAB), Rogosa agar for *Lactobacillus*, and LPSM (*Lactobacillus plantarum* Selective Medium) for *L. plantarum* (Bujalance et al., 2006).

The chemicals were used include 0.1 N NaOH, indicator phenolphthalein, anhydrous glucose, Nelson reagents A and B, and arsenomolybdate.

Preparation of Culture

Cultures production (*L. plantarum* Dad 13) was carried out using MRS broth medium and then incubated at 37° C for 18 hours (Nur, 2010). Cells were harvested by centrifugation at 3000 rpm for 20 minutes at

4°C, then wet pellets were separated from the supernatant and washed with 0.1% sterile peptone water.

Preparation of *tape ketan*

Glutinous rice was washed, then soaked in water for 12 hours, then removed water. After that, the sticky rice was steamed for ±12 minutes and moistened by washed it with water. After moistened, the sticky rice was steamed for ±15 minutes, then the rice was cooled in room temperature before inoculation. Next step, the rice was inoculated with 0.2% ragi 'NKL' and supplemented with *L. plantarum* Dad 13 at amount 10⁷CFU/g glutinous rice that produced previously. Fermentation was conducted at room temperature (27-30°C) for three days. The number of lactic acid bacteria and *Lactobacillus* during four days fermentation was evaluated.

Preparation of *tape ketan* Powder

Tape ketan was chopped. The pulp was diluted with distilled water by 1:1 (w/v) and then filtered using filter cloth to obtain the extract. Maltodextrin was added into the *tape* extract with various concentrations (15, 20, 25, 30, 35% w/v) and dried using a spray dryer (SD LabPlant-05) with inlet temperature of 90°C ± 2°C and outlet temperature 54°C ± 2°C.

Formulation of *tape ketan* Powder

Determination of powder's concentration reconstituted into the water was carried out by dissolving the powder, starting from low concentrations to higher concentrations, then the intensity of sour taste was evaluated. After that, determination of the concentration of pectin (as stabilizer) which must be added was carried out. Probiotic *tape ketan* powder and pectin with various concentrations dissolved in water and

then the suspension stability was observed visually, whether the separation occurs or not.

Sensory evaluation was carried out by the hedonic scale test by 24 untrained panelists. There were four samples, *tape ketan* powder with an addition of 2%, 4%, and 6% (w/v) sucrose, and *tape ketan* beverage which made by dissolved *tape ketan* with an addition of 4% sugar (control). Panelists were asked to evaluate the appearance, flavor, mouthfeel, and overall assessment. The scale which was used between 1 and 7, 1 means that the panelists dislike very much and 7 means that panelists like very much.

Analysis Methods

The media used for plate count enumeration were MRS agar (+ 1% CaCO₃ and sodium azide) for lactic acid bacteria, Rogosa agar for *Lactobacillus*, and LPSM (*Lactobacillus plantarum* Selective Medium) for *L. plantarum* (Bujalance et al., 2006). Analysis of water content and total solid used method of thermogravimetry (AOAC, 1990), titratable acidity content used method of titration with 0.1N NaOH, and reducing sugar content used method of Nelson-Somogyi (AOAC,1990).

Results and Discussion

Fermentation of *Tape ketan* with Supplementation of *Lactobacillus plantarum* Dad 13 as Co-inoculum

Supplementation of *L. plantarum* Dad 13 was carried out at the same time with the addition of ragi, in the fermentation process. The amount of biomass was 10⁷ CFU/g of glutinous rice. Fermentation was conducted at room temperature (28-30°C) for 2, 3, and 4 days. During fermentation, sensory evaluation was conducted to evaluate the sour taste, sweet taste, and alcoholic flavor. The results showed that sour taste and alcoholic flavor increased with increasing fermentation time. At the day 2 of fermentation, sour taste was

the most dominant. At the day 3, sweet taste and alcoholic flavor increased. Whereas at the day 4, sour taste and alcoholic flavor were the most dominant, even appeared slightly bitter taste.

Increasing of sour taste was caused by increasing of lactic acid which was produced by lactic acid bacteria in the *tape ketan*. Tenagy (2011) showed that during fermentation of probiotic *tape ketan* until days 7 titratable acidity increased, from < 0.01% to 0.48%. Increasing of alcoholic flavor was caused by increasing of ethanol content. Ethanol was produced by yeast metabolism, which break down the glucose. Ethanol content increased, from not detected at day 0 to 2.33 % at day 7 (Tenagy, 2011). At the day 2 to day 3 sweet taste increased. The increasing of sweet taste was caused by increasing of glucose. Molds hydrolyzed the starch and produced glucose and maltose. Then at day 4, the sweet taste decreased due to glucose had metabolized by lactic acid bacteria and yeast. This was consistent with the increasing of reducing sugar from day 0 to day 3, from not detected to 13.41% and after that the reducing sugar decreased (Tenagy, 2011).

Sweet taste, sour taste, and alcoholic flavor appeared because of the role of different types of microbes that exist in the ragi and from probiotic supplementation. Ragi *tape* 'NKL' was used. It contains various types of microbes, molds, yeast, and bacteria. From the research, showed that the number of lactic acid bacteria in ragi 'NKL' was 6.75 x 10⁵ CFU/g. In accordance with Sujaya et al. (2008), they showed that the number of lactic acid bacteria in ragi 'NKL' was 2.4 x 10⁵ CFU/g, whereas Siebenhandl et al. (2001) showed that the number of molds was 1.80 x 10⁵ CFU/g and yeast 6.05 x 10⁶ CFU/g. The number of lactic acid bacteria and *Lactobacillus* during four days fermentation was evaluated and shown in **Figure 1**.

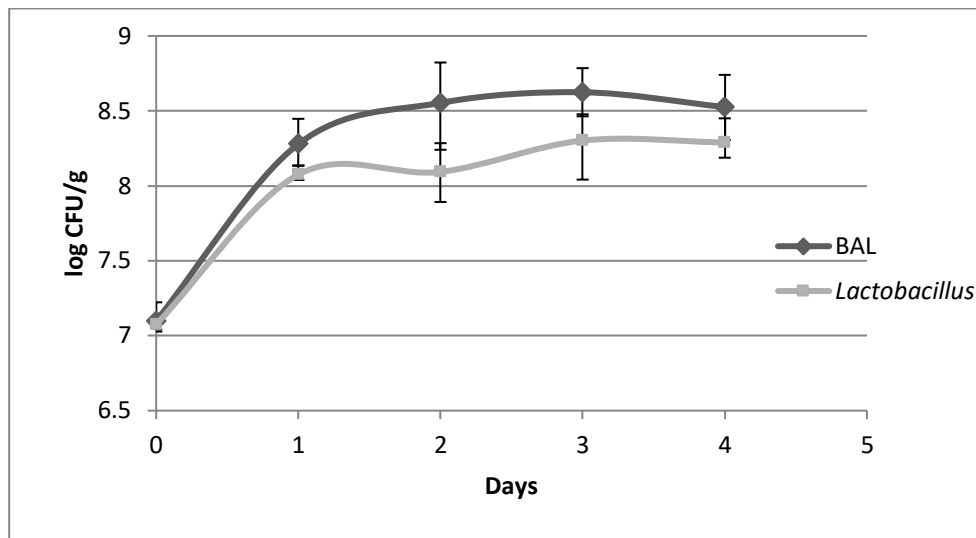


Figure 1. Changes the number of lactic acid bacteria (LAB) and *Lactobacillus* during 4 days fermentation

Figure 1 showed that the number of LAB and *Lactobacillus* increased during fermentation. The number of LAB at the beginning of fermentation was 1.28×10^7 CFU/g and increased to 4.40×10^8 CFU/g at day 3 and then decreased to 3.64×10^8 CFU/g at day 4. Whereas the number of *Lactobacillus* increased from day 0 to day 3, 1.20×10^7 CFU/g to 2.18×10^8 CFU/g respectively, and then decreased to 2.02×10^8 CFU/g at day 4. The increasing in number of *Lactobacillus* during fermentation showed that *Lactobacillus plantarum* Dad 13 which was supplemented had the ability to grow with the others microbes from ragi. Ragi tape contains yeast, filamentous fungi, and bacteria. The major lactic acid bacteria found in ragi tape are *Pediococcus pentosaceus*,

Enterococcus faecium, *Lactobacillus curvatus*, *Weissella confusa*, and *W. paramesenteroides* (Sujaya et al, 2001).

Making Tape ketan Powder Using Spray Dryer

Drying *tape ketan*, which is sugar-rich products need a drying aid, such as maltodextrin. The results of drying *tape ketan* with addition various concentration of maltodextrin shown in **Table 1**.

Table 1 showed that the use of maltodextrin up to concentration of 30% (w/v) had not been able to produce *tape ketan* powder. The materials obtained were sticky, cohered to wall chamber and cyclone. *Tape ketan* powder was obtained by maltodextrin addition with concentration of

Table 1. Drying *tape ketan* with addition various concentration of maltodextrin (w/v)

Maltodextrin concentration (% w/v)	Results
15	Sticky
20	Sticky
25	Sticky
30	Sticky
35	Form powder, not sticky

Note:

Concentration: weight of maltodextrin/ volume of *tape* extract

35 % (w/v) or 35 g maltodextrin per 100 ml *tape* extract. The *tape ketan* powder had moisture content of 4.99%, viable count of LAB was 3.76×10^7 CFU/g, and viable count of *L. plantarum* was 2.43×10^7 CFU/g. With the number of *L. plantarum* $> 10^6$ - 10^7 CFU/g, *tape*

ketan powder can be categorized as probiotic foods.

The number of LAB and *L. plantarum* during fermentation and drying changed. The changes shown in **Figure 2**.

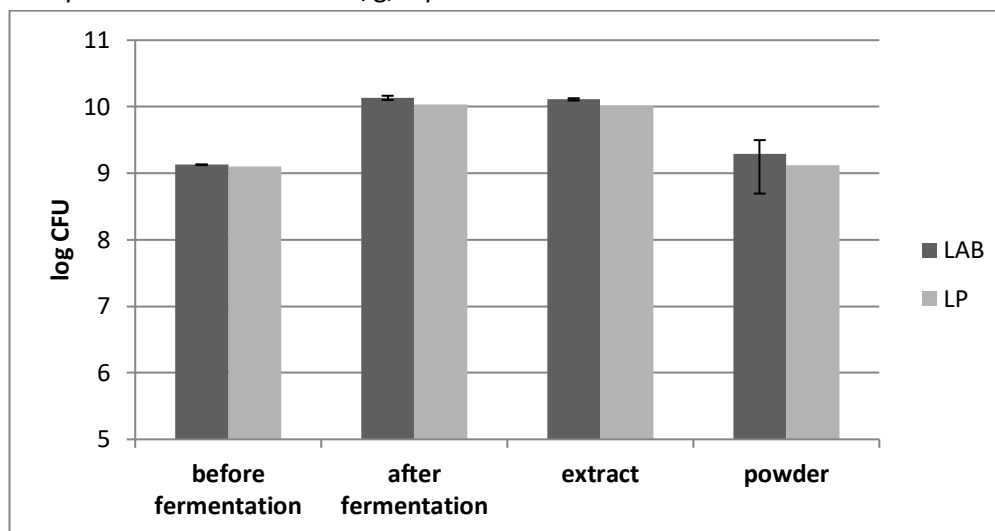


Figure 2. The number of LAB and *L. plantarum* in probiotic *tape ketan*, before and after fermentation, in *tape* extract, and in probiotic *tape ketan* powder

Figure 2 showed that the number of total LAB and *L. plantarum* before fermentation were 1.35×10^9 CFU and 1.27×10^9 CFU and then increased after 3 days into 1.35×10^{10} CFU and 1.07×10^{10} CFU respectively. Before dried by spray dryer, extraction with distilled water was carried out, filtered and then the extract was taken. The number of total LAB and *L. plantarum* in the *tape* extract before addition of maltodextrin were 1.29×10^{10} CFU and 1.04×10^{10} CFU respectively. Whereas the number of total LAB and *L. plantarum* after drying were 1.96×10^9 CFU and 1.32×10^9 CFU. From these data it could be seen that the number of total LAB and *L. plantarum* decreased (0.90 and 0.82 log cycle respectively).

The loss of viability appears to be related to damage on the cell membrane, cell wall, and DNA (Teixeira et al., 1995). Besides that, also due to the loss of cells residing in

the solid of *tape ketan*, given the loss of $\pm 50.23\%$ solid occurred during drying. Nur (2010) showed that the loss of viability of *Lactobacillus plantarum* Dad 13 after spray drying with inlet temperature 90°C was 2 log cycles. The research used 5% maltodextrin as the protective material. The other research was done by Harmayani et al., (2001). They made dried cultures of *Lactobacillus plantarum* Dad 13 by spray dryer with 10% skim milk as the protective material. The results showed that the viability of the cell decreased, 2.5 log cycles after drying. In this research, the decrease in the number of cells less than the others. This was caused by in the drying process of *tape ketan* required 35% maltodextrin. US Patent (2010) explained that maltodextrin was one type of saccharides that can be used to protect cells during drying. Besides that, in the *tape ketan*, there was 13.41% (wb) reducing sugar. It also protects

the cells. Santivarangkna et al. (2009) conducted a study of drying cells of *L. delbrueckii ssp. bugaricus* by vacuum drying with temperature 70°C. The results showed that with the addition of trehalose, sucrose, and maltose, higher cell viability could be obtained compared to drying without addition of sugar. The authors said that the use of sugar as a heat protection can protect the cells from damage during drying.

Formulation of *Tape ketan* Powder

Formulation of *tape ketan* powder was carried

out because the final purpose of making this powder was used as a functional beverage. Therefore concentration of the powder which was reconstituted into water must be known. Besides that, when the powder was reconstituted into water for 30 minutes, there was separation in the beverage. The physical appearance of a beverage greatly affects the acceptance by consumers. The concentration of powder reconstituted into water in order to obtain a taste of *tape ketan* is shown in **Table 2**.

Table 2. Taste of *tape ketan* beverage produced from reconstituted the powder into water at various concentrations.

Powder concentration (% w/v)	Sour taste	Titrateable acidity content (%)	pH of beverage
5	Tasteless	0,09	4,36
10	Sour taste +1	0,21	3,82
15	Sour taste +3	0,39	3,67
20	Sour taste +5	0,47	3,61
25	Sour taste +6	0,54	3,58

Note: the higher +, the higher sour taste

Powder concentration of 5% (w/v) obtained tasteless beverage. Then the powder concentration was increased every 5% (w/v) and the sensory evaluation was carried out. Sensory properties of *tape ketan* beverage were sour taste, typical as *tape ketan*. Sour taste typical *tape ketan* began to be accepted at the powder concentration of 20% (w/v), with pH of beverage was 3.61 and titrateable acidity content was 0.47%. For the powder concentration of 25% (w/v), a sour taste was not much different with powder concentrations of 20% (w/v). So it was decided to use a concentration of 20% (w/v).

Pectin was used as stabilizer in this research. Addition of stabilizer was necessary because when the powder was reconstituted into water for 30 minutes, there was

separation in the beverage and precipitate formed. With the addition of pectin the beverage was expected to be more stable. The results of visual observations shown in **Table 3**.

Table 3 showed that addition 0.1% pectin, at the minutes 60 there was separation. Whereas addition of 0.3% pectin, at minutes 90 was also showed separation. From these results, it can be concluded that the minimum concentration of pectin to obtain stable *tape ketan* beverage until minutes 120 was 0.5%. The separation in the juice is caused by cloud particles. The cloud particles consist inter alia of proteins, lipids, neutral polysaccharides, pectin, and other substances like minerals. The cloud particles contain a core consisting inter alia of protein

Table 3. Stability of *tape ketan* beverage with various concentration of pectin (0.1%, 0.3%, 0.5% w/v).

Pectin concentration (% w/v)	Stability of beverage (at minutes-)				
	0	30	60	90	120
0.1	+	+	-	-	-
0.3	+	+	+	-	-
0.5	+	+	+	+	+

Note: + : no separation

- : separation

which is positively charged. This positively charged core is able to build a complex with negatively charged pectin (Benitez et al, 2007). To overcome instability in the *tape ketan* beverage, pectin was added. According to Phuangsinoun et al, 2008, the clouding fruit juice can be stabilized by adding pectin because of its viscosity properties. Increasing viscosity of fruit juice inhibits the movement of the particles, so the agglomeration followed by sedimentation can be prevented.

Addition of sucrose into *tape ketan* powder was carried out to decrease sour taste

in the beverage. The concentration of 2%, 4%, and 6% (w/v) was used. *Tape ketan* beverage with 20% (w/v) powder, 0.5% (w/v) pectin, and with various concentration of sucrose were tested by hedonic scale test. It was carried out to measure the beverage preference by panelists. Extract *tape ketan* beverage made by dissolving probiotics *tape ketan* into the water and added by 4% sucrose was used as a control. The results of hedonic test shown in **Table 4**.

Table 4. Hedonic test for *tape ketan* beverage, made by reconstituting the powder with addition various concentration of sucrose (2%, 4%, and 6% w/v) and control.

Samples (Sucrose concentration (%))	Attributes			Overall
	Appearance	Taste	mouth feel	
2%	5.09 ^b	3.00 ^a	4.83 ^a	3.61 ^a
4%	4.83 ^b	4.43 ^b	4.87 ^a	4.52 ^{ab}
6%	4.78 ^b	4.78 ^b	5.09 ^a	4.95 ^b
control	3.83 ^a	4.43 ^b	4.39 ^a	4.52 ^{ab}

Note: Different superscript sign letter in one column, showed significant different Hedonic test:

(1) Dislike very much (7) Like very much

Table 4 showed that panelists prefer the appearance of sample with the addition of 2%, 4%, and 6% (w/v) sucrose than control. From those, could be known that panelists prefer the beverage that no separation. The separation in the sample control happened since there was no addition pectin as stabilizer. For the taste, panelists prefer the beverage with addition 4%, 6% (w/v) sugar, and control. The results showed that panelists prefer the sweeter beverage. For the mouth feel, the score between samples were not significantly different. The addition of sucrose and pectin did not affect the score of the mouth feel. For overall, there was no significantly different on the score of the samples with addition 2%, 4% (w/v) sucrose, and control. Whereas for samples with addition 4%, 6% (w/v) sucrose and control, the score was also no significantly different.

Conclusion

Probiotic *tape ketan* was successfully made by fermentation of glutinous rice with ragi and *Lactobacillus plantarum* Dad 13 (10^7 CFU/g glutinous rice). *Tape ketan* powder was obtained by spray drying with inlet temperature 90°C and addition of maltodextrin into *tape* extract at concentration 35% (w/v). The viability of lactic acid bacteria and *L. plantarum* decreased after drying by 0.82 and 0.90 log cycle, respectively, with viable count from $1.29 - 1.96 \times 10^9$ CFU for lactic acid bacteria and from 1.04×10^{10} CFU to 1.32×10^9 CFU for *L. plantarum*. The highest score of sensory test was obtained by reconstituting *tape ketan* powder 20% (w/v), 0.5% (w/v) pectin, and 4% (w/v) sucrose with water to produce probiotic *tape ketan* beverage. *Tape ketan* powder which was supplemented with probiotic *Lactobacillus plantarum* Dad 13 is a potential ingredient of functional beverage.

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