

THE USE OF BACTERIOGIN POWDER FROM BACTERIA *Lactobacillus plantarum* AS BIOPRESERVATIVE AGENT OF CASSAVA GETUK

Fransisca Maria Khilda Azka Krisnani, Ekawati Purwijantiningih, Sinung Pranata

Fakultas Teknobiologi,
Universitas Atma Jaya Yogyakarta,
Jl. Babarsari No. 44, Sleman, Yogyakarta,
e-mail : frsiscamkak@live.com

ABSTRACT

Getuk is an Indonesian traditional food made from cassava. Getuk has short shelf life, which is 12-15 hours caused by the high water content in getuk that is make getuk easily contaminated by microbes. Biopreservation is a safe solution to overcome this problem, one of which is by using bacteriocin. Bacteriocin is safe to use as a biopreservative because it is not toxic and can be digested by the protease enzyme so it will not affect the microbiota in the intestine. Bacteriocin used in this study is bacteriocin produced by *Lactobacillus plantarum* because it is a safe microbial and included in GRAS (Generally Recognized as Safe) and has broad spectrum properties, which can kill gram-positive, gram-negative, and pathogenic bacteria. This research will discuss further about the ability of bacteriocin from *Lactobacillus plantarum* as a biopreservative agent in getuk. Bacteriocin from *Lactobacillus plantarum* will be microencapsulated using spray drying before adding to getuk. The treatments taken are variations in the addition of bacteriocin to getuk and variations in storage time. The variation of bacteriocin addition in getuk is 0% (control), 2,5%, 5%, and 7,5%, the variation of getuk storage time is 0-3 days. Cassava getuk will be tested physically, chemically, and microbiologically during the storage time. The results obtained in this study are the addition of 7,5% bacteriocin powder can increase getuk shelf life for 1 day. On storage day 1, the result of microbe in getuk is still within the national standard safe limits and getuk does not smell sour and there is no yeast that grows on getuk.

Keywords : getuk, bacteriocin powder, *Lactobacillus plantarum*

ABSTRAK

Getuk adalah makanan semi basah dari ubi kayu atau singkong yang memiliki masa simpan relatif pendek, yaitu 12-15 jam. Hal ini disebabkan oleh kadar air getuk singkong yang tinggi sehingga mudah terkontaminasi mikroba. Biopreservasi merupakan solusi yang aman untuk mengatasi masalah ini, salah satunya yaitu dengan menggunakan bakteriosin. Bakteriosin aman untuk digunakan sebagai biopreservatif karena tidak bersifat toksik dan dapat dicerna oleh enzim protease sehingga tidak akan atau sedikit berpengaruh terhadap mikrobiota di dalam usus. Bakteriosin yang akan digunakan pada penelitian ini adalah bakteriosin yang dihasilkan oleh *Lactobacillus plantarum* karena merupakan mikrobia yang aman atau termasuk dalam GRAS (Generally Recognized as Safe) dan memiliki sifat broad spectrum, yaitu dapat membunuh bakteri gram positif, gram negatif, dan juga bakteri patogen. Pada

*penelitian ini akan dibahas lebih lanjut tentang kemampuan bakteriosin dari *Lactobacillus plantarum* sebagai agen biopreservatif pada getuk. Bakteriosin dari *Lactobacillus plantarum* akan dimikroenkapsulasi menggunakan alat spray drying sebelum ditambahkan pada getuk. Perlakuan yang dilakukan yaitu variasi penambahan bakteriosin pada getuk dan variasi lama penyimpanan. Variasi penambahan bakteriosin pada getuk yaitu 0% (kontrol), 2,5%, 5%, dan 7,5%, variasi lama penyimpanan getuk yaitu 0-3 hari. Getuk singkong akan diuji secara fisik, kimia dan mikrobiologis selama masa penyimpanan. Hasil yang diperoleh pada penelitian ini yaitu penambahan serbuk bakteriosin sebanyak 7,5% dapat menambah masa simpan getuk singkong selama 1 hari. Pada penyimpanan hari ke 1, hasil ALT dan AKK getuk singkong masih dalam batas aman SNI serta secara sensori getuk singkong tidak berbau asam dan tidak tumbuh khamir.*

*Kata Kunci : getuk, serbuk bakteriosin, *Lactobacillus plantarum**

INTRODUCTION

Getuk is a semi-wet food made from cassava or cassava (Murni, 2009). The shelf life of getuk is relatively short, namely 12-15 hours if stored at room temperature (Budiono, 2008). This is because getuk is a semi-wet food so that it contains a moisture content of 10-40% and has an aw value of 0.6-0.9 (Basuki et al., 2013). The addition of biopreservatives using bacteriocins can overcome this problem. Bacteriocin is a compound produced by bacteria and can kill other bacteria (Todorov, 2009). Bacteriocin is safe to use as a biopreservative because it is not toxic and can be digested by the protease enzyme so that it will not or have little effect on the microbiota in the intestine (Woraprayote et al., 2016). Bacteriocins that will be used in this study are bacteriocins produced by *Lactobacillus plantarum* because they are safe microbes or are included in GRAS (Generally Recognized as Safe) (Brinques et al., 2010). The bacteriocin produced by *L. plantarum* has broad spectrum properties, which can kill Gram-positive, Gram-negative, and also pathogenic bacteria. Therefore, the application of bacteriocin from *L. plantarum* as a biopreservative agent needs to be done, especially for getuk.

RESEARCH METHODS

The research was conducted at the Food Technology Laboratory and Production Laboratory of the Faculty of Biotechnology, Atma Jaya University, Yogyakarta. The study was conducted from September 2018 to April 2019. This research was conducted using a completely randomized factorial design with a 4 x 4 pattern using 2 factors. The first factor is the difference in the addition of biopreservatives and the second factor is the storage time of getuk at room temperature of 27°C. Repetition was carried out 3 times for each treatment. The stages of this research include the cultivation of *Lactobacillus plantarum* isolates, characterization of *L. plantarum* (Gram stain, motility test, and catalase test), bacteriocin extract production, bacteriocin microencapsulation, testing the inhibitory activity of liquid bacteriocin extract and bacteriocin powder, manufacture of getuk, and storage of getuk. The test of the quality of getuk carried out were sensory tests, moisture content, protein content, carbohydrate content, pH measurements, texture measurements, and microbiological tests (total plate count and mold and yeast levels). Data analysis was performed using ANOVA to determine whether there was a significant difference between treatments. If there is a significant difference, it will be continued with the Duncan Multiple

Range Test (DMRT) test with a 95% confidence level using SPSS version 15.0 software.

RESULT AND DISCUSSION

Lactobacillus plantarum characterization

Lactic acid bacteria *L. plantarum* used in this study went through the characterization stage, namely the Gram stain test, motility test and catalase test. The results of the characterization of *L. plantarum* bacteria can be seen in Table 1.

Based on the Gram stain of *L. plantarum*, it showed a purple color and a short stem cell shape and there were several bacterial cells that seemed to stick to one another like forming a chain. This is in accordance with Pederson's (1936) theory, which states that *L. plantarum* bacteria are Gram positive, rod-shaped and generally single or in short chains with rounded ends. The

results of the motility test on *L. plantarum* showed that growth was only at the puncture site so that *L. plantarum* was non-motile and the results of the catalase test on *L. plantarum* indicated that this bacterium was catalase negative. This is in accordance with the theory of Al-Madboly and Abdullah (2015) which states that *L. plantarum* bacteria are non-motile and catalase negative.

Inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* bacteria

Bacteriocin extract and bacteriocin powder were tested for their inhibitory activity against the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. The liquid bacteriocin extract and bacteriocin powder were tested to compare better or worse bacteriocin inhibition after microencapsulation. The results of the inhibition zone test for liquid bacteriocin extract and bacteriocin powder on *Escherichia coli* bacteria can be seen in Table 2.

Table 1. Result of *L. plantarum* characterization

Parameters	Characterization Results
Gram staining	Blueish purple
Motility	Non motil
Catalase activity	Negative (no bubble)

Table 2. Inhibition zone test results for bacteriocin powder and liquid bacteriocin extract against *Escherichia coli* bacteria

Treatments	Inhibition Zone (mm ²)
Bacteriocin powder	303,79 ^a
Bacteriocin fluid	129,59 ^b

Note: Numbers with the same letter code in the same column show no significant difference with the 95% confidence level in the DMRT test

Table 3. Inhibition zone test results for bacteriocin powder and liquid bacteriocin extract against *Staphylococcus aureus* bacteria

Treatments	Inhibition Zone (mm ²)
Bacteriocin powder	240,79 ^a
Bacteriocin liquid	136,92 ^b

Note: Numbers with the same letter code in the same column show no significant difference with the 95% confidence level in the DMRT test

Based on the results of the inhibition zone test, it can be seen that the average area of the inhibition zone of bacteriocin powder against *E. coli* is 303.79 mm², while the average area of inhibition zone of liquid bacteriocin extract against *E. coli* is 129.59 mm². The area of the bacteriocin powder inhibition zone and the liquid bacteriocin extract was significantly different. Bacteriocin powder and liquid bacteriocin extract have inhibitory activity against *E. coli*, and the inhibitory activity of bacteriocin powder is greater than liquid bacteriocin extract. The results of the inhibition zone test for *S. aureus* bacteria can be seen in Table 3.

Based on these results, it can be seen that the area of the bacteriocin powder inhibition zone is larger and significantly different from the liquid bacteriocin extract. The area of the bacteriocin powder inhibition zone was 240.79 mm², while the liquid bacteriocin extract was 136.92 mm². Bacteriocin powder and liquid bacteriocin extract have inhibitory activity against *S. aureus*. The inhibitory activity by bacteriocin powder and liquid bacteriocin extract against *E. coli* and *S. aureus* is caused by bacteriocins which have antimicrobial activity or can kill other bacteria (Arthur et al., 2014). Bacteriocins are bactericidal, the mechanism is by direct contact between bacteriocins and cell membranes. Bacteriocin will cause holes in the target cells to form, resulting in cell leakage. This will lead to cell death (Marwati et al., 2018). Bacteriocin powder has a larger area of inhibition zone because there is a encapsulating material that surrounds the bacteriocin. The encapsulating material can be a carrier and protector for bacteriocins to contact the target (Kaliaspathy, 2002). Bacteriocin

will interact with microbes through diffusion through the encapsulating material, bacteriocin diffuses slowly and controlled so that it can kill *E. coli* and *S. aureus*. Liquid bacteriocin will immediately diffuse in the medium so that it kills *E. coli* and *S. aureus* cells. After the diffusion is complete, there is no more inhibitory activity of liquid bacteriocin. In bacteriocin powder, the powder will diffuse in the medium so that first the bacteriocin will diffuse through the encapsulating material slowly so that the inhibitory effect will be longer and more effective. The results of the bacteriocin inhibition zone test against *E. coli* and *S. aureus* can be seen in Table 4.

Based on these results it can be seen that the results of the inhibition zone area of *E. coli* and *S. aureus* were not significantly different, namely 216.69 mm² and 188.86 mm². *E. coli* is Gram negative bacteria, and *S. aureus* is Gram positive bacteria. The difference between Gram positive and negative bacteria is that Gram positive bacteria have a cell wall with a thick peptidoglycan layer, whereas Gram negative bacteria only have a thin peptidoglycan layer with lipopolysaccharides and lipoproteins (Budin et al., 2012).

The area of bacteriocin inhibition zone for *E. coli* and *S. aureus* was not significantly different. This shows that the differences in the peptidoglycan layer on the cell wall do not affect the effectiveness of the bacteriocin antimicrobial activity of *L. plantarum* bacteria. According to Sabo et al. (2014), the bacteriocin activity of *L. plantarum* is broad spectrum or can kill Gram positive and negative bacteria, one of the Gram positive and negative bacteria

whose growth can be inhibited is *S. aureus* and *E. coli*.

Tabel 4. Inhibition zone test results for bacteriocin powder and liquid bacteriocin extract against *Escherichia coli* dan *Staphylococcus aureus*

Bacteria	Inhibition Zone (mm ²)
<i>Escherichia coli</i>	216,69 ^a
<i>Staphylococcus aureus</i>	188,86 ^a

Note: Numbers with the same letter code in the same column show no significant difference with the 95% confidence level in the DMRT test

Table 5. Result of sensory test of cassava getuk with and without biopreservative administration during storage

Treatments	Parameters	Storage (days)			
		0	1	2	3
Control	Colour	5	4	4	4
	Flavour	5	3	2	1
	Texture	5	3	2	1
Bacteriocin 2,5%	Colour	5	4	4	4
	Flavour	5	4	2	1
	Texture	5	3	2	1
Bacteriocin 5%	Colour	5	4	4	4
	Flavour	5	4	2	1
	Texture	5	3	3	3
Bacteriocin 7,5%	Colour	5	4	4	4
	Flavour	5	5	4	3
	Texture	5	4	3	3

Note :

Colour : 1 (brownish yellow), 2 (light yellow), 3 (pale yellow), 4 (yellowish white), 5 (white as getuk)

Flavour : 1 (very sour), 2 (sour), 3 (some sour), 4 (minimum sour), 5 (fresh as getuk)

Texture : 1 (very hard, slimy), 2 (hard, slimy), 3 (less hard, slimy), 4 (very less hard), 5 (chewy as getuk)

Sensory Testing

The results of the sensory test for cassava getuk can be seen in Table 5. Based on these results it can be seen that on the 0th day of cassava getuk, all treatments were white getuk, the aroma was fresh, typical getuk, and the texture was chewy. The taste of cassava getuk on day 0 for all treatments was the same, namely the typical sweet getuk. This shows that the

addition of bacteriocin powder does not affect the color, aroma and texture of the cassava juice. Bacteriocin will not affect the sensory quality of food when added to food (Zacharof et al., 2013). All treatments of cassava getuk experienced changes on day 1, especially the texture of getuk in the control treatment, 2.5% and 5% which turned into a bit hard and slimy. Cassava getuk treatment 7.5% on day 1 has no slimy texture, fresh aroma and slightly yellowish white color. This shows that the

quality of treated getuk 7.5% is still sensory good while the quality of other treatment getuk on the first day is not

suitable for consumption because it is slimy.

Table 6. Water content (%) of cassava getuk with and without biopreservative during storage

Treatments	Days				Average
	0	1	2	3	
Control	41,87 ^{bc}	41,77 ^{bc}	41,58 ^b	40,59 ^a	41,45 ^A
Bacteriocin 2,5%	43,96 ^{def}	43,52 ^{de}	43,33 ^d	42,49 ^c	43,32 ^B
Bacteriocin 5%	45,00 ^{gh}	44,63 ^{fg}	44,22 ^{ef}	42,12 ^{bc}	43,99 ^C
Bacteriocin 7,5%	47,14 ⁱ	46,78 ⁱ	45,72 ^h	45,26 ^{gh}	46,22 ^D
Average	44,49 ^X	44,17 ^X	43,71 ^Y	42,61 ^Z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Water Content Test

The results of measuring water content can be seen in Table 6. The addition of bacteriocin powder can affect water content, the more bacteriocin powder added, the more moisture content in the cassava getuk. The water content for all treatments on day 0 was significantly different and the water content increased. This is due to the maltodextrin in bacteriocin powder which is hygroscopic or has the ability to absorb water (Yuliawaty and Susanto, 2015). Therefore, the addition of bacteriocin powder can

increase the moisture content of the cassava root. Based on these results it can also be seen that in all treatments during the storage period the water content continued to decline. The decrease in water content is due to the fact that the cassava getuk has a high enough water content, so that to achieve a balance with the humidity of the environment, during the storage period the water content in the cassava extract evaporates into the environment.

Table 7. Protein level (%) of cassava getuk with and without addition of biopreservatif during storage

Treatments	Days		Average
	0	3	
Control	1,07 ^{ab}	0,99 ^a	1,03 ^A
Bacteriocin 2,5%	1,06 ^{ab}	1,10 ^{ab}	1,08 ^A
Bacteriocin 5%	1,05 ^{ab}	1,10 ^{ab}	1,08 ^A
Bacteriocin 7,5%	1,45 ^{bc}	1,73 ^c	1,59 ^B
Average	1,16 ^Z	1,23 ^Z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Protein Level Measurement

The results of the protein content test can be seen in Table 7. Based on these results, it can be seen that the protein content for

the control treatment, 2.5% bacteriocin and 5% bacteriocin tended to increase but not significantly different, while the protein content for bacteriocin treatment was 7.5%

higher. from other treatments and significantly different. This is due to the 7.5% bacteriocin treatment is the treatment with the most addition of bacteriocin powder. Bacteriocin powder contains bacteriocin and skim milk as one of the encapsulating materials. Bacteriocin is a protein and skim milk also contains protein as much as 3.7% (Buckle et al., 1987). Therefore, the addition of bacteriocin powder will increase the protein content in cassava extract. In the control treatment, bacteriocin 2.5%, 5% and 7.5% protein content between day 0 and day 3 were not significantly different. This is caused by the growth of microbes during storage and also damage to the

protein in the cassava extract. During the storage period, these microbes will also grow and multiply, and there is a possibility that the measurement of protein levels will also measure the protein from these microbes so that protein levels will increase (Alim, 2016). Protein content in cassava getuk during storage has decreased but is proportional to the number of microbes that grow. Whereas in the control treatment the protein content decreased because there was no addition of bacteriocin so that the decrease in protein content due to hydrolysis by microbes was not proportional to the increase in microbes in the control treatment.

Table 8. Carbohydrate level (%) of cassava getuk with and without addition of biopreservatif during storage

Treatments	Days		Average
	0	3	
Control	64,85 ^e	53,99 ^d	59,42 ^A
Bacteriocin 2,5%	53,44 ^d	50,99 ^{bcd}	52,21 ^B
Bacteriocin 5%	52,04 ^{cd}	48,88 ^{ab}	50,46 ^B
Bacteriocin 7,5%	49,23 ^{abc}	47,58 ^a	48,41 ^C
Average	54,89 ^Y	50,36 ^Z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Carbohydrate Level Measurement

The results of carbohydrate testing can be seen in Table 8. The carbohydrate content of the control treatment was the highest and significantly different from the 2.5% and 5% bacteriocin treatment and also significantly different from the 7.5% bacteriocin treatment. This is because the addition of bacteriocin powder is also done by reducing the amount of cassava used. Cassava is the main ingredient and source

of carbohydrates for cassava getuk so that if the amount of cassava used is reduced, the carbohydrate content will also decrease. The average carbohydrate content for day 0 and day 3 decreased and was significantly different. This is because the longer it is stored, the more carbohydrates are degraded by microbes because microbes have a greater opportunity to degrade carbohydrates into organic compounds (Fardiaz, 1992).

Table 9. pH of cassava getuk singkong with and without addition of biopreservatif during storage

Treatments	Days				Average
	0	1	2	3	
Control	6,45 ^h	6,05 ^{ig}	5,92 ^e	5,49 ^b	5,98 ^A
Bacteriocin 2,5%	6,47 ^h	6,06 ^{ig}	5,86 ^e	5,56 ^{bc}	5,99 ^A
Bacteriocin 5%	6,56 ^{hi}	5,99 ^{et}	5,72 ^d	5,61 ^{bcd}	5,97 ^A

Bacteriocin 7,5%	6,65 ⁱ	6,13 ^g	5,66 ^{cd}	5,25 ^a	5,92 ^A
Average	6,53 ^w	6,06 ^x	5,79 ^y	5,48 ^z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Test of pH

The pH test results of cassava getuk can be seen in Table 9. In all treatments, the pH of cassava getuk decreased during the storage period. The decrease in pH in the product can be caused by the growth of microbes in the cassava juice. Microbes will degrade nutrients such as carbohydrates to become acidic so that the

pH decreases during storage (Saramban, 2018). High microbial growth can accelerate the process of breaking down carbohydrates into acids, thus affecting the pH value. The more carbohydrates that can be broken down by microbes, the total acid will increase (Okudu and Ene-Obong, 2015).

Table 10. Texture (*hardness*) (N/mm²) of cassava getuk singkong with and without addition of biopreservatif during storage

Treatments	Days				Average
	0	1	2	3	
Control	182,67 ^d	206,17 ^e	209,50 ^{ef}	216,83 ^{fg}	203,79 ^A
Bacteriocin 2,5%	145,17 ^c	187,17 ^d	216,33 ^{fg}	224,00 ^g	193,17 ^B
Bacteriocin 5%	108,00 ^b	110,33 ^b	113,17 ^b	114,50 ^b	111,50 ^C
Bacteriocin 7,5%	95,33 ^a	106,50 ^b	113,50 ^b	117,00 ^b	108,08 ^C
Average	132,79 ^w	152,54 ^x	163,12 ^y	168,08 ^z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Testure Testing (*Hardness*)

The results of the cassava getuk texture test can be seen in Table 10. Based on these results it can be seen that the texture of cassava getuk for the control treatment was significantly different from the 2.5% bacteriocin treatment and also significantly different from the 5% and 7.5% bacteriocin treatment. This is related to the moisture content of the cassava getuk, the water content of the cassava increases along with the addition of bacteriocins so that the texture value or hardness of cassava getuk decreases. The higher the water content, the softer the texture will be and the texture value will decrease (Faiz et al., 2014). This is also influenced by one of the properties of maltodextrin, which is hygroscopic and tends to absorb water,

causing the water content of cassava getuk to increase along with the addition of bacteriocin powder (Yuliawaty and Susanto, 2015). During the storage period, the texture value increases even more. This is related to the water content that continues to decrease during storage. The texture value will decrease if the water content increases and vice versa, if the water content decreases, the texture value will also increase (Faiz et al., 2014). This also happened in the study of Ji et al. (2007), there were changes in moisture content and texture during storage of MiGao for 2 days. MiGao's moisture content decreased from 39.5% to 28.4% and the texture has also become harder.

Table 11. Total plate count (log CFU/gram) of cassava getuk singkong with and without addition of biopreservatif during storage

Treatments	Days				Average
	0	1	2	3	
Control	4,70 ^c	7,95 ^{de}	8,82 ^{ef}	8,31 ^{def}	7,45 ^A
Bacteriocin 2,5%	3,04 ^b	8,34 ^{def}	9,09 ^{ef}	9,27 ^f	7,44 ^A
Bacteriocin 5%	0 ^a	7,23 ^d	8,81 ^{ef}	9,21 ^{ef}	6,31 ^B
Bacteriocin 7,5%	0 ^a	5,79 ^c	8,42 ^{def}	8,48 ^{def}	5,67 ^C
Average	1,94 ^X	7,33 ^Y	8,79 ^Z	8,82 ^Z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Total Plate Count

The results of the ALT test for cassava getuk can be seen in Table 11. Based on these results it can be seen that the addition of bacteriocin powder can reduce the ALT yield. Based on SNI, the total plate value of cassava getuk is a maximum of 1x10⁶ CFU / gram or 6 log CFU / gram. On day 0, the ALT results of all cassava getuk treatments were still in accordance with the SNI because cassava getuk had just been made so that the cassava getuk was not contaminated by microbes. On the first day, the treated cassava getuk was 7.5%, the ALT value was 5.79 log CFU / gram and was still in accordance with the SNI but on the 2nd and 3rd day for all treatments were not in accordance with the SNI. This shows that the addition of 2.5% and 5% bacteriocin cannot extend the shelf

life of cassava getuk while the addition of 7.5% bacteriocin can only prolong the shelf life of cassava getuk for 1 day. The ability of bacteriocin powder can extend the shelf life of cassava getuk because bacteriocin has bacteriocidal properties or can kill other bacteria. When there is direct contact between bacterial contamination of cassava extract and bacteriocin powder, an interference process will occur with the Proton Motive Force (PMF) on the cell membrane. This causes membrane instability, membrane potential damage, the formation of holes in the cell membrane and results in the death of these bacterial cells (Marwati et al., 2018). The treatment of addition of 7.5% bacteriocin powder was the addition of the highest amount of bacteriocin powder than other treatments, so that the results were better than the 2.5% and 5% treatments.

Table 12. Yeast mold rate of cassava getuk singkong with and without addition of biopreservatif during storage

Treatments	Days				Average
	0	1	2	3	
Control	3,68 ^b	6,18 ^c	6,32 ^{cd}	7,49 ^{cde}	5,92 ^A
Bacteriocin 2,5%	2,48 ^b	6,25 ^c	7,53 ^{cde}	8,13 ^e	6,09 ^A
Bacteriocin 5%	2,34 ^b	7,25 ^{cde}	7,92 ^{de}	8,16 ^e	6,42 ^A
Bacteriocin 7,5%	0 ^a	0 ^a	6,43 ^{cd}	7,77 ^{cde}	3,55 ^B
Average	2,12 ^W	4,92 ^X	7,05 ^Y	7,89 ^Z	

Note: Numbers with the same letter code in the same column and same row show no significant difference with the 95% confidence level in the DMRT test

Yeast Mold Rate Count

The Yeast Mold Rate (*Angka Kapang Khamir/AKK*) test results for cassava getuk during storage can be seen in Table 12. Based on these results it can be seen that the addition of bacteriocin powder can reduce the AKK results. The number of yeast fungi for cassava getuk according to SNI is a maximum of 1×10^4 CFU / gram or 4 log CFU / gram. The AKK results for cassava getuk were in accordance with the SNI, namely on day 0 for all treatments and on day 1 for 7.5% bacteriocin treatment. On day 0, the results of AKK getuk for all treatments according to SNI because cassava getuk has just been made so that there is no mold and yeast contamination. On day 1, the results of AKK getuk cassava treatment of 7.5% bacteriocin according to SNI while the 2.5% and 5% bacteriocin treatment was not in accordance with the SNI. This shows that the addition of 7.5% bacteriocin powder can reduce the amount of mold and yeast contamination in cassava extract until it is still in accordance with SNI and safe for consumption. On the 2nd and 3rd day of storage, the AKK results for cassava getuk for all treatments were not in accordance with SNI and not safe for consumption. This shows that the addition of 2.5% and 5% bacteriocin cannot extend the shelf life of cassava getuk while the addition of 7.5% bacteriocin can only prolong the shelf life of cassava getuk for 1 day. Bacteriocins from *L. plantarum* bacteria can inhibit the growth of molds and yeasts (Barbosa et al., 2016). The mechanism of bacteriocin in killing other microbes begins with direct contact with other microbial cells. After that there will be an interference process with Proton Motive Force (PMF) on the cell membrane. Microbial cells will experience membrane potential damage and also form holes. This causes the cells to leak and intracellular molecules will leave and extracellular substances will enter the cells, this can result in cell death (Marwati et al., 2018).

CONCLUSSIONS

Based on the research that has been done, the following conclusions are obtained: 1) Bacteriocin powder from *L. plantarum* can inhibit the growth of *E. coli* and *S. aureus* bacteria. 2) Bacteriocin powder from *L. plantarum* can be used as a biopreservative agent in cassava extract because it can extend the shelf life of cassava getuk for 1 day. 3) The addition of bacteriocin powder from *L. plantarum* had a significantly different effect on parameters of moisture content, texture (hardness), protein content, carbohydrate content, number of microbes (ALT), number of mold and yeast (AKK), and had no effect on pH parameters. and sensory.

ACKNOWLEDGEMENTS

This research was funded by PT. Indofood Sukses Makmur, Tbk. through research funding assistance for the final project of the Indofood Nugraha Research 2017/2018 program.

REFERENCES

- Alim, L. B. 2016. **Aplikasi edible coating dari pati tapioka dan air perasan jeruk nipis (*Citrus aurantifolia*) pada bakso.** *Naskah Skripsi S-1.* Fakultas Teknobiologi Universitas Atma Jaya Yogyakarta, Yogyakarta.
- Al-Madboly, L. A. dan Abdullah, A. K. 2015. **Potent antagonistic activity of Egyptian *Lactobacillus plantarum* against multiresistant and virulent food-associated pathogens.** *Frontiers in Microbiology* 6 (347) : 1-11.
- Arthur, T. D., Cavera, V. L., Chikindas, M. L. 2014. **On bacteriocin delivery systems and potential**

- application.** *Future Microbiology* 9 (2) : 235-248.
- Barbosa, M. S., Todorov, S. D., Ivanova, I. V., Belguesmia, Y., Choiset, Y., Rabesona, H., Chobert, J. M., Haertle, T. dan Franco, B. D. G. M. 2016. **Characterization of two-peptide plantaricin produced by *Lactobacillus plantarum* MBSa4 isolated from Brazilian salami.** *Journal Food Control* 60 : 103-112.
- Basuki, W. W., Atmaka, W., Rahadian, D. dan Muhammad, A. 2013. **Pengaruh penambahan berbagai konsentrasi gliserol terhadap karakteristik sensoris, kimia dan aktivitas antioksidan getuk ubi jalar ungu (*Ipomoea batatas*).** *Jurnal Teknosains Pangan* 2 (1) : 115-123.
- Brinques, G. B., do Carmo Peralba, M., Ayub, M. A. Z. 2010. **Optimization of probiotic and lactic acid production by *Lactobacillus plantarum* in submerged bioreactor systems.** *Journal of Industrial Microbiology and Biotechnology* 37 (2) : 205-212.
- Buckle, K. A., Edwards, R. A., Flett, S. H. dan Wootton, M. 1987. *Ilmu Pangan*. UI Press, Jakarta.
- Budin, G., Chung, H. J., Lee, H. dan Weissleder, R. 2012. **A magnetic Gram stain for bacterial detection.** *Angewandte Chemie International Edition in English* 51 (31) : 7752-7755.
- Budiono, G. 2008. **Efektivitas proses (steaming dan radiasi microwave-oven) serta metode pengemasan terhadap umur simpan “Getuk Modern Wiling”.** *Naskah Skripsi S-1*. Fakultas Teknologi Pertanian Universitas Katolik Soegijapranata, Semarang.
- Faiz, H., Thohari, I. dan Purwadi. 2014. **Pengaruh penambahan sari temulawak (*Curcuma xanthorrhiza*) terhadap total fenol, kadar garam, kadar lemak dan tekstur telur asin.** *Jurnal Ilmu-Ilmu Peternakan* 24 (3) : 38-44.
- Fardiaz, S. 1992. *Mikrobiologi Pangan I*. Gramedia Pustaka Utama. Jakarta.
- Ji, Y., Zhu, K., Qian, H. dan Zhou, H. 2007. **Microbiological characteristics of cake prepared from rice flour and sticky rice flour.** *Journal Food Control* 18 : 1507-1511.
- Kaliasapathy, K. 2002. **Microencapsulation of probiotic bacteria : technology and potential applications.** *Current Issues in Intestinal Microbiology* 3 : 39-48.
- Marwati, T., Cahyaningrum, N., Widodo, S., Januarsyah, T. dan Purwoko. 2018. **Inhibitory activity of bacteriocin produced from *Lactobacillus* SCG 1223 toward *L. monocytogenes*, *S. thypimurium* and *E. coli*.** *IOP Conf. Series : Earth and Environmental Science* 102 : 1-9.
- Murni, M. 2009. **Pengaruh penambahan sorbitol dan waktu pengovenan terhadap daya simpan getuk**

- pisang oven.** *Jurnal Teknologi Pangan* 3: 62–69.
- Okudu, H. O., & Ene-Obong, H. N. (2015). **Evaluation of the effect of storage time and temperature on some physicochemical properties of juice and jam developed from two varieties of monkey kola (*Cola parchycarpa*, *Cola lepidota*).** *African Journal of Food Science and Technology*, 6(7), 2141–5455.
- Pederson, C. S. 1936. **A study of the species *Lactobacillus plantarum* (Orla-Jensen)** Bergey et al. *Journal of Bacteriology* 31 (3) : 217-224.
- Sabo, S., Vitolo, M. dan Dom, M. 2014. **Overview of *Lactobacillus plantarum* as a promising bacteriocins producer among lactic acid bacteria.** *Food Research International* 64 : 527–536.
- Saramban, M. D. 2018. **Perbedaan jumlah mikrobial, viskositas, pH dan total asam selai papaya pada suhu ruang dan suhu refrigerator selama penyimpanan.** *Naskah Skripsi S-1*. Fakultas Ilmu Kesehatan Universitas Muhammadiyah Surakarta, Surakarta.
- Todorov, S. D. 2009. **Bacteriocins from *Lactobacillus plantarum* – production, genetic organization and mode of action: produção, organização genética e modo de ação.** *Brazilian Journal of Microbiology* 40 (2) : 209–221.
- Woraprayote, W., Malila, Y., Sorapukdee, S. dan Swetwathana, A. 2016. **Bacteriocins from lactic acid bacteria and their applications in meat and meat products.** *Meat Science* 120 : 118–132.
- Yuliawaty, S. T. dan Susanto, W. H. 2015. **Pengaruh lama pengeringan dan konsentrasi maltodekstrin terhadap karakteristik fisik kimia dan organoleptik minuman instan daun mengkudu (*Morinda citrifolia* L.).** *Jurnal Pangan dan Agroindustri* 3 (1) : 41-52.
- Zacharof, M. P., Coss, G. M., Mandale, S. J. dan Lovitt, R. W. 2013. **Separation of lactobacilli bacteriocins from fermented broths using membranes.** *Process Biochemistry* 48 (8): 1252–1261.