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Pediocin N6 Powder Production by Isolates *Pediococcus pentosaceus* Strain N6 and Storage Effect of Antimicrobial Activity against Bacterial Pathogens

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Abstract: Pediocin N6 powder derived from Pediocin N6 liquid produced by isolate`*Pediococcus pentosaceus* Strain N6. These isolated from a hot spring of Rimbo Panti West Sumatra. Pediocin N6 powder could potentially be used as a natural preservative in food processing industry which involves heating. The purpose of this research is to investigate the Pediocin N6 powder produced in various combinations capsule formulation that has the highest antimicrobial activity by spray dryer and can be stored in a longer period of time. This test using a completely randomized design (CRD) factorial consisting of three factors and repeated twice. A factor, material capsule formulations are: R1 = Maltodextrin, R2 = Maltodextrin: Skin milk (83.33%: 16.67%), R3 = Maltodextrin: Skin milk (66.73%: 33.33%). Factor B, Pediocin N6 pure liquid composition is: S1 = 10%, 20% and S2 = S3 = 30%, factor C. temperature factor inputs, namely: T1 = T input 130°C, T2 = T input 150°C and 170°C T3 = T input. Effect of storage on the activity of the antimicrobial Pediocin N6 powder tested using factorial experiment in a completely randomized design with three replications 2x2. The first factor, namely: storage time consists of 6 and 12 weeks. Factor II is composed of 4°C storage temperature and 27°C. The results showed that the Pediocin N6 powder production which influence significantly different ($P<0.05$) increase antimicrobial activity contained in $R_2S_2T_2$ treatment (83,33% maltodextrin: 16, 67% Skin milk, 20% Pediocin liquid N6 and the input temperature 150°C), with an area of ??inhibition zone of 50.2 mm (39172.13 AU/ml) against the bacterium *L. monocytogenes*, 42.5 mm (27965.6 AU/ml) against *E. coli O157: H7* and 37.3 mm (21450.8 AU/ml) against *S. thyphimurium* bacteria. The antimicrobial activity of liquid Pediocin N6 before encapsulation lower the inhibition zone diameter of 37.4 mm (21568.03 AU/ml) against the bacterium *L. monocytogenes,* 32.3 mm (15987.1 AU/ml) against *E. coli O157: H7* and 29.2 mm (12993.9 AU/ml) against the bacterium *S. thyphimurium*. Results of analysis of variance (ANOVA) showed that the powder storage Pediocin N6 for 6 and 12 weeks at 4°C to have an influence significantly different ($P<0.01$) in improving the antimicrobial activity than storage for 6 and 12 weeks at 27 °C to bacterial pathogens like *E. coli O157: H7, S. thyphimurium and L. monocytogenes.*

Key words: Pediocin N6 Powder · Mikrocapsulation · Antimicrobial activity

INTRODUCTION

Bacteriocin is a protein compound (such as peptides) that is bactericidal against microbes (bacteria), if the terms of the filogeniknya have close relations with the bacteriocin-producing microbes [1]. Furthermore, according to [2], bacteriocins production by Lactic Acid Bacteria (LAB) are beneficial for human health are included in the GRAS Brazilians is a new approach to control pathogens in food stuffs. Bacteriocins are a protein or peptide molecules extracellular which have bactericidal or bacteriostatic action against bacteria that have a close kinship. The bacteriocins can be degraded by proteases in the digestive tract. Bacteriocins are irrevesibel, easy to digest, positive effect on health and active at low concentrations.

Bacteriocins can be produced by lactic acid bacteria such as *Pediococcus, Leuconostoc* and *Lactobacillus spp*. Bacteriocins of *L. brevis* bacteria can inhibit the growth of *E. coli, Salmonella typhimurium*

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and *Listeria monocytogenes*, as well as bacteriocins from *Leuconostoc.* Bacteriocins of *L. plantarum* and *L. sakei* able to inhibit the growth of *L. monocytogenes* bacteria found in cooked meat. Bacteriocins of *L. plantarum* is able to inhibit the growth of *E. coli*. In addition bacteriocins have advantages over antibiotics for bacteriocins can be destroyed by digestive enzymes. This shows that the consumption of this compound will not interfere with digestion and also will not pose a risk to health such as the use of antibiotics in general [3].

Encapsulation an entrapment process sensitive substances or core material by a protective polymer as an agent capsulation. Enkapsulation generally done with a spray dryer technique. The microcapsules are a small space with a layer of uniform wall around it. Material contained in microcapsules the core material while the surrounding material called shells or membranes. Enkapsulation generally use a mixture of several ingredients capsule which is a mixture of maltodextrin and skim milk [4].

Mikrocapsulation is a means to modify components within the liquid into solid particles and protects the material from environmental influences. Patron given by capsule material can prevent the degradation of the core material due to the influence of light or oxidation and can slow down the evaporation. Encapsulation other than to maintain the composition of the material is also possible to do repairs encapsulated material properties that can slow down time damage to the product at a certain point. Bacteriocins which have been dried can be stored longer without decomposition reaction product at appropriate storage conditions [5].

The process of encapsulation by spray drying (spray dryer) has the advantage of this technology is already controlled so easily obtained, capable of producing capsules in large quantities, types of coating materials suitable for spray drying is also feasible as a foodstuff and the coating material used is water soluble so it can be release the core material in the absence of the deposited coating material [6]. According to [7], spray drayer commercially inexpensive method for large-scale bacteriocin production and easy to apply. Spray drayer used to manufacture dairy ingredients in large quantities, can be transported cheaply and can be stored for long periods and remain stable.

MATERIALS AND METHODS

Materials used 100 ml isolate BAL thermophilic bacteriocin production, MRSB, Nutrient Broth (NB), Nutrient Agar (NA), medium combinations of carbon and nitrogen source best, bacteriocins liquid, capsule material namely the combination of maltodextrin and skim milk with the provision of capsule materials (50 g or 20% w/w) consists of maltodextrin and skim milk in the ratio (1: 5), the media used MRSB, pathogenic bacteria *E. coli O157: H7, S. thyphimurium* and *L. monocytogenes* and distilled water and enzyme papain purchased from IPB.

Production of Liquid Pure Pediocin N6 in 8 Liter MRSB: Method of [8], inoculated culture isolates 10 ml in 90 ml MRSB then isolates fermented in an incubator at 50°C for 24 hours. Pediocin N6 Production in 8 liters of medium MRSB, propagation is done by 2 stages: first, inoculating 80 ml BAL cultures in 720 ml MRSB media, fermentation process is carried out in an incubator shaker speed of 150 rpm, 50° C for 24 hours. Both inoculation of 800 ml bacterial culture of lactic acid in 7200 ml of medium MRSB pH 5, then fermented in an incubator shaker speed of 150 rpm, 50°C for 9 hours (time of production). Purification is done with ammonium sulfate and dialysis.

Antimicrobial Activity Test Liquid Pediocin N6: Agar diffusion method [9], a single colony growing on MRS agar was grown in MRS broth and then incubated aerobically at 50°C for 48 hours. After the culture was centrifuged at 10,000 rpm for 20 min at 4°C to obtain a supernatant. Prepared media MHA 15 ml of sterile (autoclaved). After a rather cold and then poured into a petri dish. After that freeze, created the well using the blue tip that cut edges so large 5 mm diameter wells. Cultivated bottom of the well is not perforated in order to isolate LAB added perk not go everywhere. A sterile cotton (cotton bath) dipped in one type of pathogenic bacteria inoculum density therein with 10^8 CFU/ml and rotated several times. Pressed on the inside wall of the tube to remove excess fluid inoculum, then inoculated the entire surface of the medium MHA. The procedure was repeated while turning petri dish to ensure even distribution of inoculum. Thereafter it was incubated for 24 h at 37°C. A total of 50 mL of the supernatant of lactic acid bacteria is dripped into the well in the media MHA and subsequently allowed to stand for 15-20 minutes. The petri dishes were incubated at 37°C for 48 hours. Each petri dish evaluated diameter of inhibitory zone including diameter wells that, measured at six points using calipers.

Pediocin N6 Powder Production and Determination of the Best Capsule Formulations: Method [5], pure liquid bacteriocins are the core materials to be encapsulated. Capsule materials (50 grams or 20%) consists of maltadekstrin and skim milk with a ratio of A1 (1: 0), A2 (1: 5) and A3 (1: 2) was dissolved in distilled water 190

g (B1), 180 g (B2) and 170 g. After soluble mixture was homogenized with a speed of 4000 rpm for 30 minutes and then stored in the refrigerator for 12-24 hours. Bacteriocins as much as 10 g (B1), 20 g (B2) and 30 g (B3) is added to the mixture and homogenized for 15 minutes at the same speed. The mixture is dried with a spray dryer with a temperature of 130°C input (T1), 150 °C (T2) and 170°C (T3) and as well as the feed flow rate of 20 ml/min.

Testing Activity of Antimicrobial Pediocin N6 Powders: Methods [10], Pediocin N6 used in testing inhibitory activity against pathogenic bacteria by 50 mL with a concentration Pediocin N6 solid in it amounted to 0.00049 g (w/w) is equivalent to 9.7 mL of the extract Pediocin N6 is not encapsulated. Pediocin N6 powder with a concentration of 10% can be directly tested by adding powder Pediocin N6 as much as 0.045 g/well, Pediocin N6 9 mL of liquid is used as the reference solution. For Pediocin N6 powder with a concentration of 20% dissolved 1.1 ml of sterile water and the concentration of 30% reconstituted with 2.2 ml of sterile water.

The data obtained in this test is processed by using a completely randomized design (CRD) 3x3x3 factorial repeated twice. Factors studied consisted of:

Factors A. capsule formulation factors, namely:

- R1 = Maltodextrin
- R2 = Maltodextrin: Skin milk (83.33%: 16, 67%)
- R3 = Maltodextrin: Skin milk (66.73%: 33, 33%)

Factor B. The composition of pure liquid Pediocin N6 namely:

- S1 = 10% and S2 and S3 = 20% = 30%

Factors temperature input factors, namely:

- T1 = T input 130 °C, 150 °C input T2 = T, T3 = T input 170°C

Parameter: The antimicrobial activity of Pediocin N6 powder, moisture content and solubility.

Effect of Storage Time and Temperature Pediocin N6 Powder Antimicrobial Activity Test: Effect of storage time and temperature on the Pediocin N6 powder antimicrobial activity conducted by examining the back of antimicrobial activity after Pediocin N6 powder stored for 6 and 12 weeks at 4°C and 27°C. The design used in this test using a factorial experiment in a completely randomized design 2 x 2 with three replications. The first factor, namely: storage time consists of 6 and 12 weeks. Factor II are: composed of 4°C storage temperature and 27°C. Data processing results of research conducted with SPSS version 20.

Measured Variables: The antimicrobial activity of Pediocin N6 powder after storage.

RESULTS AND DISCUSSION

Comparison of Antimicrobial Activity Pediocin N6 Pure Liquid and Pediocin N6 Powder: The results of comparative testing antimicrobial activity Pediocin N6 pure liquid and Pediocin N6 powder can be seen in given Fig. 1.

E. coli O157: H7: Results of analysis of variance there is interaction significantly different ($P<0.05$) inhibitory activity against Pediocin N6 powder on the bacterium *E. coli. O157: H7* between capsule materials, the percentage Pediocin N6 and temperature inputs, as well as between capsule material and the percentage Pediocin N6, between capsule materials and temperature inputs as well as between the percentage Pediocin N6 and temperature inputs. Based on the test results up to the Post Hoc Tests showed a significantly different effect increases the antimicrobial activity of Pediocin N6 powder at treatment $R_3S_2T_2$ (capsule formulation 66.67% maltodextrin, 33, 33% skim milk), 20% Pediocin N6 liquid and 150 °C temperature input) with inhibitory activity of 42.5 mm (27965.6 AU / ml). The capsule formulation has a value of water content and solubility of 2.14 and 38.20%.

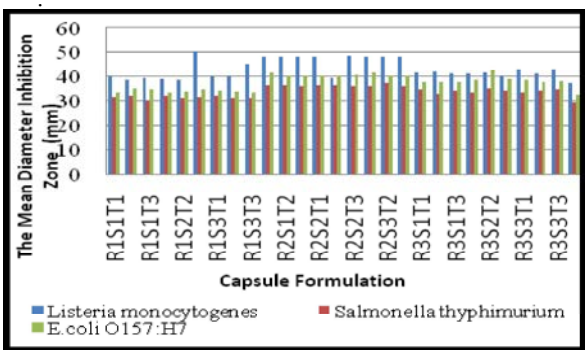

Fig. 1: Comparison of Antimicrobial Activity Pediocin N6 Pure Liquid and Pediocin N6 Powder Against Bacterial Pathogens

Interaction capsule materials, the percentage Pediocin N6 and temperature inputs generate higher antimicrobial activity than other treatments against bacteria *E. coli O157: H7* because capsule material formulations with a combination of 66.67% maltodextrin and 33.33% skim milk, maltodextrin amounted to a doubling of skim milk, maltodextrin has the ability to bind with either skim milk to form a capsule which better protects the core material in it so can enhance the antimicrobial activity of the N6 Pediocin powder. Appropriate according to [11], encapsulation using skim milk can protect well the material encapsulated therein, thus inhibitory activity bacteriocins work more optimum powder. The use of 20% Pediocin N6 liquid combined with temperature inputs 150°C produces antimicrobial activity higher than other treatments because of the concentration of Pediocin N6 is a concentration of the best to kill pathogenic bacteria and can be dissolved completely in the capsule formulation these, as well as the temperature input which is used to produce solubility and good water levels and improve antimicrobial activity. Appropriate according to [12], said that the ability of antimicrobial compounds to inhibit or kill microbes is determined by: 1) the concentration of antimicrobial compounds, 2) the type, number and background of living microorganisms, 3) temperature and contact time and 4) the physical and chemical properties of media (pH, water content, the type and amount of solute).

Salmonella thypimurium: Results of analysis of variance there is interaction significantly different ($P < 0.05$) against the antimicrobial activity of the Pediocin N6 powder in bacteria *Salmonella thypimurium* between capsule materials, the percentage Pediocin N6 and temperature inputs, as well as between the capsule material and percentage Pediocin N6, between capsule materials and temperature inputs as well as between the percentage Pediocin N6 and temperature inputs. Based on the test results up to the Post Hoc Tests showed a significantly different effect increases the antimicrobial activity of Pediocin N6 powder at treatment $R_2S_3T_2$ (capsule formulation 83.33% maltodextrin: 16, 67% skim milk, 30% Pediocin N6 liquid and 150°C temperature input) with activity antimicrobial of 37.3 mm (21450.8 AU /ml). The capsule formulation has a value of water content and solubility of 2.75% and 49.22%.

Interaction capsule materials, the percentage Pediocin N6 and temperature inputs generate higher antimicrobial activity than other treatments against bacteria *Salmonella thypimurium*, this suggests that the interaction of the three capsule formulations Pediocin N6 affect antimicrobial activity that has been encapsulated. It appears that the material composition capsule best treatment against bacteria *S. thypimurium* namely the treatment capsule R2, where in the composition is comprised of maltodextrin capsule 83.33 and 16.67% skim milk with the highest average activity. Capsule materials combined with the percentage of Pediocin N6 and temperature inputs above are a good combination produces the highest inhibitory activity. Appropriate according to [13], factors that affect the activity of each bacteriocins difference between the target organism's responses to environmental factors that can change shape/structure of the cell wall. Drying temperature conditions are known to influence the activity of bacteriocins and broad-spectrum inhibitory.

Different capsule material composition of each treatment, causing each has different capacities to capsulation Pediocin N6 liquid. Besides the temperature factor inputs also have a significantly different effect ($P<0.05$) against the antimicrobial activity of Pediocin N6 powder. The input temperature determines the viability Pediocin N6 which remain stable or decrease of viability before encapsulated. Temperature inputs also affect the temperature of the resulting product output, where in the output temperature affects the water content of the product.

***Listeria monocytogenes*:** Results of analysis of variance there is interaction significantly different ($P < 0.05$) against the antimicrobial activity of the Pediocin N6 powder on the bacteria *Listeria monocytogenes* between capsule materials, the percentage Pediocin N6 liquid and temperature inputs, as well as between the capsule material and percentage Pediocin N6, between capsule materials and temperature entries as well as the percentage Pediocin N6 and temperature inputs. Based on the test results up to the Post Hoct Tests showed a significantly different effect increases the antimicrobial activity of Pediocin N6 powder at treatment $R_1S_2T_3$ (100% maltodextrin, liquid N6 Pediocin 20% (w/w) and a temperature of 1700C input values ??inhibitory activity against *L. monocytogenes* bacteria amounting to 50.2 mm (39172.13 AU / ml). Pengkapsul formulations have a value of water content and solubility of 1.55% and 45.22%.

Interaction materials pengkapsul, the percentage Pediocin N6 and temperature inputs that produce antimicrobial activity higher than other treatments against bacteria *Listeria monocytogenes* as the substance pengkapsul form of maltodextrin without coupled with

skim milk has been able to protect well the core material at a concentration of Pediocin N6 liquid by 20% and input temperature 170°C. The input temperature is the temperature of the highest among the other treatments, but still can produce high levels of water and good solubility thus increasing inhibitory activity Pediocin powder N6. This input temperature does not affect the inhibitory activity Pediocin N6 for Pediocin N6 liquid is a class IIa bakteriocin that is heat resistant, work optimally at a temperature of 100°C. According [14], that thermophilic LAB producing heat resistant class IIa bacteriocins containing peptides that have very strong anti Listeria. Furthermore [15], expressed class II bacteriocins have two disulfide bonds C terminal so as to maintain the overall structure of the temperature rise. Changing the structure of the helical regions in an increase in temperature causes a loss of activity of the peptide. Terminal hydrophobic residues on one side of the helical amphipathic the class IIa bacteriocins important for receptor recognition and specific to a particular organism.

The above results indicate that the antimicrobial activity of powder Pediocin N6 highest produce inhibition zone diameter of 50.2 mm (39172.13 AU/ml) against the bacterium *L. monocytogenes*, 42.5 mm (27965.6 AU/ml) against *E. coli O157: H7* and 37.3 mm (21450.8 AU /ml) against the bacterium *S. thyphimurium.* While antimicrobial activity Pediocin liquid N6 produce inhibition zone diameter of 37.4 mm (21568.03 AU/ml) against the bacterium *L. monocytogenes*, 32.3 mm (15987.1 AU/ml) against *E. coli O157: H7* and 29,2 mm (12993.9 AU/ml) against the bacterium *S. thyphimurium*. The antimicrobial activity Pediocin dienkapsul N6 liquid before and after dienkapsul (powder Pediocin N6) does not decline even increased antimicrobial activity. This is due to the material used in addition to protecting pengkapsul Pediocin N6 from environmental influences, can also improve the nutritional content of antimicrobial activity. Appropriate research [16], the spray drying process does not affect the inhibitory activity of bacteriocins produced by Carnobacterium divergens, *Lactobacillus salivarius* and *Lactobacillus sakei* before and after spray drying. Furthermore, according to [17], that the advantages of powder besides bacteriocins are durable and lightweight powder bacteriocins results encapsulation is more resistant to external conditions and more active. The resulting inhibition zone diameter of powder Pediocin N6 can be seen in given Fig. 2.

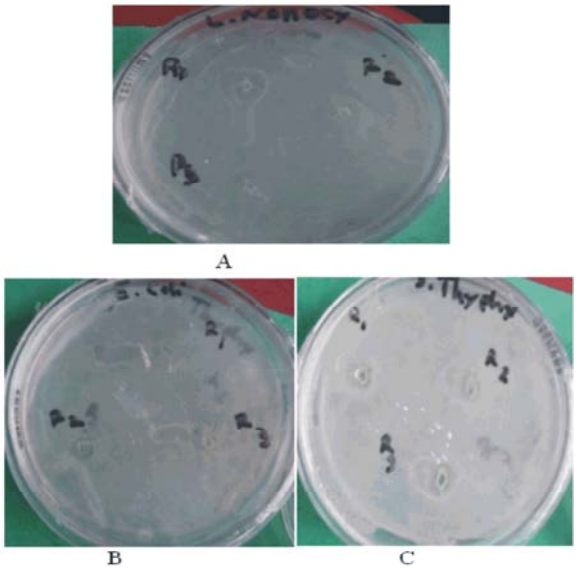

Fig. 2: Inhibition zones were formed from Pediocin N6 powder Against Bacterial Pathogens A: *L. monocytogenes*, B: *E. coli O157: H7* and C: *S. thyphimurium*

[11], stating encapsulation using skim milk can protect well-encapsulated material, so that the inhibitory activity bacteriocins work more optimum powder. As capsule material, the main component of skim milk has lactose and whey protein having a real influence for their protection and the remaining proteins are not degraded. Furthermore, according to [18], testing of meat products that encapsulation Pediocin AcH in limposom derived from phosphatidyl choline showed anti-Listeria activity better than the Pediocin AcH without encapsulation.

Effect of Storage Time and Temperature Pediocin N6 Powder: The mean value of the antimicrobial activity of the Pediocin N6 powder after deposit against pathogenic bacteria *E. coli O157: H7* can be seen in Table 1.

Table 1: Mean activity of antimicrobial Pediocin N6 powder after storage against bacterial pathogens *E. coli O157: H7*

	Temperature (°C)	
	4	27
Long Time (week)	Diameter inhibition zone (mm)	
6	41.8^{aa}	11.8^{bb}
12	41.6^{aa}	11.6^{bb}

Description: Score with superscript in the same row and column shows the effect did not differ significantly (p> 0.01) in. While the numbers with different superscripts indicate significant influence (P<0:01).

Results of analysis of variance showed that there were highly significant interaction (P<0.01) in between the storage time and temperature on the antimicrobial activity of the Pediocin N6 powder on pathogenic bacteria *E. coli O157: H7*, as well as the influence of storage time and the temperature alone. Further test results with Post Hoc Tests, 4°C storage of 6 weeks showed highly significant interaction (P<0.01) in the 6 weeks storage temperature of 27°C. Pediocin N6 powder inhibitory zone diameter of 41.8 mm at treatment week 4°C storage 6 and 11.8 mm at 6 weeks storage temperature of 27°C. Storage 12 weeks 4 °C showed highly significant interaction (P<0.01) in the 12 weeks storage temperature of 27°C. Inhibition zone diameter of 41.6 mm at 12 weeks of storage temperature 4 °C and 11.6 mm at 12 weeks storage temperature of 27°C. Old storage 6 and 12 weeks 4 °C show the influences that are not significantly different (P>0:01). Storage 6 and 12 weeks of 27°C show the influences that are not significantly different (P>0:01). The storage temperature of 4 °C to 27°C shows highly significant effect (P <0:01). The mean value of antimicrobial activity after storage Pediocin N6 powder against pathogens *S. thyphimurium* can be seen in Table 2.

Results of analysis of variance showed that there were highly significant interaction (P<0.01) in between the storage time and temperature on the antimicrobial activity of the powder P ediocin N6 bacterial pathogens *S. thyphimurium*, as well as the influence of storage time and the temperature alone. Further test results with Post Hoc Tests, 4°C storage of 6 weeks showed highly significant interaction (P<0.01) in the 6 weeks storage temperature of 27°C. Inhibitory zone diameters of 36.8 mm Pediocin N6 Powder at treatment week 4°C storage 6 and 10.63 mm in 6 weeks storage temperature of 27°C. Storage 12 weeks 4°C showed highly significant interaction (P<0.01) in the 12 weeks storage temperature of 27°C Inhibition zone diameter of 36.7 mm at 12 weeks of storage 4°C and 10.73 mm at 12 weeks storage temperature of 27°C. Old storage 6 and 12 weeks 4°C show the influences that are not significantly different (P>0:01). Storage 6 and 12 weeks of 27°C show the influences that are not significantly different (P>0:01). The storage temperature of 4°C to 27°C shows highly significant effect (P<0:01).

The mean value of the antimicrobial activity of the Pediocin N6 powder after the deposit of the pathogenic bacteria *Listeria monocytogenes* can be seen in Table 3.

Table 2: Mean activity of antimicrobial Pediocin N6 powder after storage against bacterial pathogens *S. Thyphimurium*

	Temperature (°C)	
	4	27
Long Time (week)	Diameter inhibition zone (mm)	
6	36.8^{aa}	10.63^{bb}
12	36.7^{aa}	10.73^{bb}

SE: 0.61779

Table 3: Mean activity of antimicrobial Pediocin N6 Powder after storage against bacterial pathogens *L. monocytogenes.*

	Temperature (°C)	
	4	27
Long Time (week)	Diameter inhibition zone (mm)	
6	49.5^{aa}	13.8^{bb}
12	49^{aa}	10.4^{bb}

SE: 1.20531

Description: Score with superscript in the same row and column shows the effect did not differ significantly (p> 0.01) in. While the numbers with different superscripts indicate significant influence (P<0:01).

Results of analysis of variance showed that there were highly significant interaction (P<0.01) in between the storage time and temperature on the antimicrobial activity of the Pediocin N6 powder bacterial pathogens *Listeria monocytogenes*, as well as the influence of storage time and the temperature alone. Further test results with Post Hoc Tests, 6 weeks storage 4 °C shows the interaction is highly significant (P<0.01) in the 6 weeks storage temperature of 27°C. Pediocin N6 powder inhibitory zone diameter 49.5 mm at treatment week 4 °C storage 6 and 13.8 mm at 6 weeks storage temperature of 27°C. Storage 12 weeks 4°C showed highly significant interaction (P<0.01) in the 12 weeks storage temperature of 27°C. Inhibition zone diameter of 49.0 mm at 12 weeks of storage 4°C and 10.4 mm at 12 weeks storage temperature of 27°C. Old storage 6 and 12 weeks 4 show the influences that are not significantly different (P>0:01). Storage 6 and 12 weeks of 27°C show the influences that are not significantly different (P>0:01). The storage temperature of 4°C to 27°C shows highly significant effect (P<0:01).

Pediocin N6 powder storage for 6 and 12 weeks at 4°C gave a very different effect significantly improves the antimicrobial activity than storage for 6 and 12 weeks at 27°C to bacterial pathogens like *E. coli O157: H7, S. thyphimurium* and *L. monocytogenes*. This shows that the Pediocin N6 powder stored at 6 and 12 weeks at 27°C

decreased antimicrobial activity, this is due to the Pediocin N6 powder his physical condition is not good (watery) because it is hygroscopic and is unable to protect the protein Pediocin N6 to the influence of environmental temperature, Protein Pediocin N6 at 27°C remain employed so long as the storage 6 and 12 weeks suffered damage and decrease the activity of the antimicrobial Pediocin N6 powder. Appropriate research [16], that the inhibitory activity of bacteriocins spray after storage for three months at 4°C and 18°C does not decline. According to [19], the storage temperature can affect the ability of antimicrobial bacteriocins powder such as nisin. According to [20], the rate of change of temperature affect the quality of a product is associated with the occurrence of chemical reactions or biochemical products. The higher the ambient temperature affecting the system, then the chemical or biochemical reactions that trigger the faster product damage incurred. The chemical reaction rate generally increases twice as high as any temperature rise as much as 10°C.

Storage at 6 and 12 weeks at 4°C there is an interaction that is not significantly different (P>0.01) in decrease antimicrobial activity means that the Pediocin N6 powder storage at 4°C to 12 weeks did not affect antimicrobial activity against pathogenic bacteria *E. coli O157: H7, S. thyphimurium* and *L. monocytogenes*. This indicates that the antimicrobial activity Pediocin N6 powder at 4°C storage remains stable, because the Pediocin N6 powder still good physical condition so as to protect the protein against the influence of environmental temperature. In addition, protein Pediocin N6 at 4 °C is off work so long as 6 and 12 weeks of storage are not damaged. According to [21], some proportion of nisin will be lost due to long storage time. The loss rate depends on the temperature and product storage period. The loss rate would be relatively small and stable during storage at low temperature (<10°C).

Pediocin N6 powder produced in this study has the ability to inhibit the antimicrobial activity of the bacteria *E. coli O157: H7* and *S. thyphimurium* (Gram negative) because, according to [22], the bacteriocins is highly hydrophobic so it can bind to the outer membrane of *E. coli O157: H7* and *S. thyphimurium* and a peptide capable of binding of Mg^{2+} in the outer membrane of Gram-negative bacteria. The binding of Mg^{2+} ions causes a loss of integrity of the coating and damage lipopolysaccharide outer membrane of the bacteria. According to [23], these capabilities are not owned nisin, nisin is not able to inhibit the Gram-negative bacteria outer membrane of Gram-negative because of protective measures to prevent the trajectory of molecules exceeds 700 Da, thus nisin size 3353 Da unable to reach the place of action.

Pediocin N6 powder produced in this study has the ability to inhibit the antimicrobial activity of *L. monocytogenes* bacteria (gram-positive), because the bacteria Pediocin is a Gram-positive N6 also suitable according to [24]; [25];[26] and [27], bacteriocins produced by lact. sakei CTC 494 (Sakacin K) and *lact. salivarius* CTC 2197 has inhibitory activity against gram-positive bacteria are *Listeria spp* and *Staphylococcus aureus*. According to [28], bacteriocins are generally active against species closely related to the bacteria at similar ecological conditions. According to [29], sensisitas powder against *L. monocytogenes* indicate bacteriocin powder had an alliance with the bacterium *L. monocytogenes* phylogeny. Filogenic located on the base of DNA/DNA homologous, for example 16Sr RNA as well as some of the physiological and biochemical characteristics such as being able to ferment glucose, are catalase negative, Gram-positive and do not form spores.

Pediocin N6 powder generated in this study means to have antimicrobial activity against Gram positive and Gram negative bacteria, according to [30], bacteriocins have properties capable of inhibiting bacterial Gram-positive and Gram-negative can also inhibit the growth of pathogenic bacteria in filogenik close kinship with the producing bacteria. The process of inhibition of bacteriocins according to [31], bacteriocins first entry into the cell target passes through the wall or cytoplasmic membrane in order to enter and distributed into cells targeted to inhibit bacteria, bacteriocins form pores in the cell membrane that is sensitive and lowers the potential or gradient pH cause damage to cellular material.

According to [1], bacteriocins as food biopreservatif must meet the criteria which have bactericidal and bacteriostatic activity against the Gram-positive and Gram negative and uniformly distributed within the food system. Furthermore, [32], the mechanism of bactericidal (killing) of bacteriocins, namely: 1) molecule bacteriocins having direct contact with the cell membrane, 2) contact process is capable of disrupting the membrane potential in the form destabilitas cytoplasmic membrane sehingg cells become stronger and 3) the instability of the membrane is able to provide the impact of the formation of holes or pores in the cell membrane through the process of disruption to PMF (Proton Motive Force). Furthermore, according to [33], membrane leakage impact on the cellular pH gradient. This resulted in the release of intra-cellular

molecules and the influx of extra cellular substances or environments. The effect is inhibited cell growth and produces cell death process in which sensitive to bacteriocins.

Pediocin N6 powder produced in this study has antimicrobial activity for bacteriostatic and bactericidal. Appropriate according [30], the model inhibition (bacteriostatic) and murder (bactericidal) of bacteriocins against sensitive cells begins with the placing of Pediocin N6 receptor cytoplasmic membrane so that the membrane secrete intracellular material, through lysis of cells and pathogenic bacteria eventually die. Other mechanisms also explained that bacteriocins adsorbed on specific receptors, microbes are susceptible further changes so that the permeability of cell membrane integrity loses its ability to divide and lyse. According to [34], most of bacteriocins require specific membrane receptors or non-specific in order to bind to the target cell membrane, but there are several types of bacteriocins such as nisin no need at all to membrane receptors bind to the target cells. Their inhibitory effect bacteriocins produced in this study to the three pathogenic bacteria signify the Pediocin N6 able to bind to the cell membrane of the three types of bacteria and disrupt the cell permeability.

CONCLUSIONS

The results showed that there was an interaction that is significantly different in material capsule formulations $R_2S_2T_2$ (83.33% maltodextrin: 16.67% Skin milk, 20% Pediocin liquid N6 and the input temperature 150°C), resulting in the highest antimicrobial activity with broad zones of inhibition amounting to 50.2 mm (39172.13 AU/ml) against the bacterium *L. monocytogenes,* 42.5 mm (27965.6 AU/ml) against *E. coli O157: H7* and 37.3 mm (21450.8 AU /ml) against the bacterium *S. thyphimurium.* The antimicrobial activity of liquid Pediocin N6 before encapsulation lower the inhibition zone diameter of 37.4 mm (21568.03 AU/ml) against the bacterium *L. monocytogenes*, 32.3 mm (15987.1 AU /ml) against *E. coli O157: H7* and 29.2 mm (12993.9 AU/ml) against the bacterium *S. thyphimurium*. Results of analysis of variance showed the storage Pediocin N6 powder for 6 and 12 weeks at 4°C gave a very different effect significantly improves the antimicrobial activity than storage for 6 and 12 weeks at 27°C to bacterial pathogens like *E. coli O157: H7, S. thyphimurium* and *L. monocytogenes*

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