

Application of Plantaricin as an Antimicrobial Substrate in the Milking Process to Maintain Milk Quality in Smallholder Dairy Farm

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ABSTRAK

Wahyuningtyas AN, Arief II, Taufik E. 2021. Aplikasi plantarisin sebagai substrat antimikroba dalam proses pemerahan untuk mempertahankan kualitas susu pada peternakan sapi perah rakyat. *JITV* 26(2): 65-73. DOI. <http://dx.doi.org/10.14334/jitv.v26i2.2718>.

Cemaran bakteri patogen yang terdapat pada susu sapi segar dapat disebabkan karena manajemen pemerahan yang kurang baik. Proses pemerahan secara tradisional ini memungkinkan susu terkontaminasi dari bakteri dan kotoran. Pencelupan puting sapi perah menggunakan antiseptik komersial adalah upaya umum yang dapat dilakukan untuk mencegah mastitis. Pencelupan puting dapat dilakukan setelah pemerahan dengan menggunakan bahan antiseptik sintetik seperti povidone iodine dan chlorine. Namun penggunaan antiseptik sintetik sebenarnya dapat menimbulkan efek iritasi ringan dan alergi serta meninggalkan residu. Oleh karena itu, penggunaan antiseptik berbahan dasar alami diharapkan dapat menggantikan antiseptik sintetik. Salah satu antiseptik berbahan dasar alami yang dapat digunakan adalah bakteriosin. Penelitian ini bertujuan untuk menganalisis aplikasi plantarisin IIA-1A5 sebagai pengganti antibakteri sintetik selama proses pemerahan sapi perah terhadap kualitas susu sapi yaitu kualitas mikrobiologi, fisikokimia, dan pengukuran pH. Penelitian dilakukan dengan menggunakan Rancangan Acak Kelompok (RAK) dengan tiga ulangan. Rancangan perlakuan terdiri dari kontrol (tanpa pencelupan), plantarisin 0,0074%, dan povidone iodine 0,2%. Hasil penelitian menunjukkan aplikasi plantarisin IIA-1A5 sebagai pencelupan puting sebelum pemerahan dapat menurunkan populasi Total Plate Count, *Escherichia coli*, dan *Staphylococcus aureus*. Penggunaan plantarisin IIA-1A5 sebagai pencelupan puting tidak mengubah nilai pH dan kualitas fisikokimia (lemak, SNF, laktosa, dan protein) yang berada di bawah Standar Nasional Indonesia (SNI) tentang susu segar. Kemampuan ini sebanding dengan iodine, antibakteri sintetik yang banyak digunakan peternak kecil di Indonesia. Disimpulkan bahwa plantarisin IIA-1A5 dapat digunakan sebagai pengganti antibakteri sintetik iodine untuk pencelupan puting susu sebelum pemerahan.

Kata Kunci: *L. Plantarum* IIA-1A5, Plantarisin IIA-1A5, Susu segar

ABSTRACT

Wahyuningtyas AN, Arief II, Taufik E. 2021. Application of plantaricin as an antimicrobial substrate in the milking process to maintain milk quality in smallholder dairy farm. *JITV* 26(2): 65-73. DOI. <http://dx.doi.org/10.14334/jitv.v26i2.2718>

Pathogenic bacterial contamination found in fresh cow's milk can be caused by poor milking management. This traditional milking process allows the milk to be contaminated from bacteria and dirt. Dyeing dairy cows using a commercial antiseptic is a common measure that can be done to prevent mastitis. Nipple immersion can be done after milking using synthetic antiseptic agents such as povidone iodine and chlorine. However, the use of synthetic antiseptics can actually cause a slight irritation and allergic effect and leave a residue. Therefore, it is hoped that the use of natural-based antiseptics can replace synthetic antiseptics. One of the natural based antiseptics that can be used is bacteriocin. This research aimed to analyze the application of the plantaricin IIA-1A5 as a substitute for synthetic antibacterial for teat dipping before milking namely microbiological tests, physicochemical tests, and pH measurements. The study was conducted using a randomized block design (RBD) with three replications. The treatment design consisted of control (without immersion), plantaricin 0.0074%, and povidone iodine 0.2%. Results showed application of plantaricin IIA-1A5 as teat dipping before milking can reduce the Total Plate Count, *Escherichia coli*, and *Staphylococcus aureus* population. The use of plantaricin IIA-1A5 as teat dipping did not change pH value and physicochemical quality (fat, SNF, lactose, and protein), which is below the Indonesian National Standard (SNI) about fresh milk. This ability is comparable to the iodine group, a synthetic antibacterial widely used by smallholder breeders in Indonesia. It is concluded that plantaricin IIA-1A5 can be used as a substitute for synthetic antibacterial (iodine group) for teat dipping before milking.

Key Words: Fresh milk, *L. Plantarum* IIA-1A5, Plantaricin IIA-1A5

INTRODUCTION

Pathogenic bacterial contamination found in fresh cow's milk can be caused by poor milking management (Prihutomo et al. 2015). Dairy farming in Indonesia still uses traditional milking methods or does not use machines. This traditional milking process exposes the milk for contamination of bacteria and dirt.

Dyeing dairy cows using a commercial antiseptic is a common measure that can be done to prevent mastitis. Nipple immersion can be done after milking using synthetic antiseptic agents such as *povidone iodine* and *chlorine* (Tomita et al. 2008). However, the use of synthetic antiseptics can actually cause a slight irritation and allergic effect and leave a residue (Flachowsky et al. 2014). Therefore, it is proposed that the use of natural-based antiseptics can be an alternative to replace the synthetic antiseptics. One of the natural based antiseptics that can be used is bacteriocin.

Bacteriocin is a peptide compound produced by lactic acid bacteria and has antimicrobial activity. These bacteriocins are non-toxic to humans, stable to changes in pH and temperature, and safe for food preservatives because they are easily digested by digestive enzymes (Hata et al. 2010). Therefore, bacteriocins can be used as a biopreservative in fresh and processed food products (Soenarno et al. 2020). *Lactobacillus plantarum* is a bacteriocin-producing lactic acid bacterium known as plantaricin. *L. plantarum* IIA-1A5 is a strain of indigenous lactic acid bacteria from local Indonesian beef that was identified using polymerase chain reaction (PCR) and 16s rRNA sequence analysis (Arief et al. 2012). The utilization of bacteriocins such as plantaricin IIA-1A5 as natural preservatives that contains antimicrobial compounds is expected to destroy and kill pathogenic bacteria, such as *Staphylococcus aureus* in fresh dairy milk.

Several studies have been conducted to determine the function and characteristics of plantaricin. Plantaricin is degraded by the trypsin protease enzyme, survive at temperatures of 80 °C and 121 °C for 30 and 15 minutes respectively, remains active in the pH range of 4 to 9 (Arief et al. 2013), and is proven to be able to inhibit the growth of pathogenic bacteria such as *Escherichia coli*, *Salmonella Typhimurium*, *Bacillus cereus* and *Staphylococcus aureus* (Arief et al. 2013). It is suggested that Plantaricin IIA-1A5 inhibits the growth of pathogenic bacteria by damaging the cell membranes of the bacteria. Furthermore, plantaricin IIA-1A5 can be used as a biopreservative for fresh and processed food products (Soenarno et al. 2019).

This study's objectives were to analyze the application of the plantaricin IIA-1A5 as a substitute for synthetic antibacterial for teat dipping before milking

on the milk quality, by testing its value on microbiological tests, physicochemical tests, and pH measurements.

MATERIALS AND METHODS

This research was carried out at dairy farms in Kawasan Usaha Peternakan (KUNAK) Cibungbulang District, Bogor Regency, and at the Integrated Laboratory, Animal Product Technology Division, Department of Animal Production and Technology, Faculty of Animal Science, IPB University. This research was conducted for four months, starting from June to October 2020.

Whey making

Following the procedures of (Soenarno et al. 2019) and (Fatmarani et al. 2018), fresh cow's milk was pasteurized at a temperature of 75 °C for 15 minutes, and then cooled down to a temperature of 37 °C. The rennet was then inoculated into pasteurized milk at a concentration of 0.02 g L⁻¹ of milk. The milk coagulated after 60 minutes during the inoculation process and formed a curd. Curd was used to make cheese, and the liquid by-product (whey) was used as a medium for growing *L. Plantarum* IIA-1A5.

Production and purification of plantaricin IIA-1A5

The production of plantaricin IIA-1A5 from *L. plantarum* IIA-1A5 culture was performed according to Arief et al. (2015). The medium used for the growth of *L. plantarum* IIA-1A5 was 8 L of whey sterilized at 115 °C for 3 minutes. It was then inoculated with 10% (v/v) of *L. plantarum* IIA-1A5 culture (10⁸-10⁹ CFU mL⁻¹). Incubated for 20 hours at 37 °C and centrifuged (Himac CR21G) at 10.000 x g for 20 minutes at 4°C. The supernatant obtained from centrifugation was filtered with a filter membrane (0.20 µm Millipore Sartorius) and pH was neutralized to 5.8-6.2 with 1 N NaOH. The supernatant was evaporated using a Heidolph VV micro evaporator at temperature of 40-45 °C until the volume is half of the previous volume.

Partial purification using ammonium sulfate (NH₄)₂SO₄ was performed to produce protein deposits, with gradual saturation (20%, 40%, 60%, and 80%), homogenized slowly at 4 °C, and put in refrigerator for 24-48 hours. The precipitate formed from the saturation process was separated by centrifugation (Himac CR21G) at 20.000 x g for 20 minutes at 4°C. The crude plantaricin deposits obtained were then subjected to dialysis.

Dialysis

Dialysis was carried out using a dialysis membrane (cellulose) with a diameter of 3.5 μm and immersed in a phosphate buffer (KH_2PO_4 and K_2HPO_4) 20 mM and pH of 6.8 for 24 hours at 4 °C. The phosphate buffer was then replaced four times every 6 hours (Hata et al. 2010).

Antimicrobial test of plantaricin IIA-1A5 against pathogenic bacteria

Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 25922 were selected as representatives of Gram positive and Gram negative pathogenic bacteria according to Arief et al. (2013). Pathogenic bacteria were rejuvenated 2 times. The culture was inoculated in 0.85% NaCl medium so that the concentration was 10^8 CFU mL^{-1} (compared to the McFarland standard solution). The same dilution was carried out again to obtain a bacterial concentration of 10^6 CFU mL^{-1} . 20 mL of MHA (Muller Hinton agar, oxoid) medium was poured into a sterile petri dish. A total of 100 μl of pathogenic bacteria were spread on the MHA media surface, which had hardened in the petri dish. A sterile paper disc was placed on the surface of the MHA media that had been inoculated with pathogenic bacteria, and 50 μl of plantaricin IIA-1A5 solution was dropped onto a sterile paper disc. The plates were incubated for 24 hours at 37 °C. The antimicrobial activity of plantaricin was characterized by the formation of a clear zone around the paper disc and the diameter was measured.

Application of plantaricin IIA-1A5 as a natural preservative for cow teats

Teat dipping was done before and after milking in the morning for 5 seconds. Three dairy cows Frisian Holstein were randomized into three teat dipping treatments. Teat dipping treatments were control, plantaricin 0.0074% (74 ppm), and *povidone iodine* (0.2%). The milk was stored at room temperature for 6 hours after 1 hour of milking and observed every 2 hours, at 1, 3, 5, and 7 hours of room temperature storage for microbiological, chemical, and pH measures.

Microbiological characteristics of fresh milk

The microbiological characteristics of fresh cow's milk include analysis of Total Plate Count (TPC) and the presence of *E. coli* and *S. aureus* bacteria was measured according to the procedures of Arief et al. (2012). 25 mL of fresh cow's milk was put in 225 mL of sterile Buffered Peptone Water (BPW) solution.

Dilutions were carried out to 10^{-4} , 10^{-5} , and 10^{-6} for TPC and to 10^{-1} , 10^{-2} , and 10^{-3} for *E. coli* and *S. aureus*. Plate Count Agar media (PCA), Eosin Methylene Blue Agar medium (EMBA), and media Baird-Parker agar (BPA) added with potassium tellurite was poured into 20 mL petri dishes and homogenized. The frozen petri dishes were incubated upside down for approximately 24 hours at 37 °C. Aerobic bacterial colonization was indicated by the appearance of white color while *E. coli* colonization was indicated by the appearance of purple color when exposed to light. Colony counts were calculated based on the number that is feasible to count (25-250 colonies) (Maturin & Peeler 2001).

Chemical characteristics of fresh milk

Chemical characteristics (fat content, Solid Non-Fat (SNF), lactose, protein content) was measured using the Lactoscan tool. 25 mL of fresh milk were taken, and poured into a cuvette (25 mL). The cuvette was inserted into the space provided in the Lactoscan. Lactoscan results appeared in 10 minutes, and the results will be automatically printed.

pH analysis

Ten mL of milk samples were taken for pH measurement using pH meter that had previously been calibrated at pH 4 and 7. The pH value of milk was read and recorded.

Experimental design and statistical analysis

All data were statistically analyzed by analysis of variance (ANOVA) with Duncan as a post hoc test (Steel & Torrie 1996). For this purpose, completely randomized block design (RBD) using 1 control, 2 treatments with 3 replications was applied. Groups were based on different sampling weeks.

RESULTS AND DISCUSSION

Characteristics of crude plantaricin IIA-1A5

The data in Figure 1 shows that the molecular weight of crude plantaricin IIA-1A5 determined by SDS-PAGE is 9 kDa and classified as IIA.

Arief et al. (2015) reported that the molecular weight of crude plantaricin IIA-1A5 was less than 10 kDa and was classified in the IIA classification. The plantaricin IIA-1A5 was successfully purified using cation exchange chromatography, and had a molecular weight of 9.65 kDa (Soenarno et al. 2020). This is

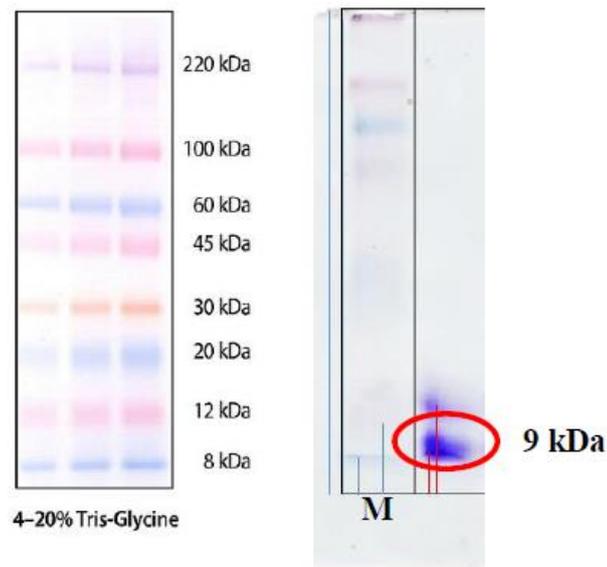


Figure 1. SDS-PAGE of crude plantaricin IIA-1A5

similar to the study of Arifin et al. (2020), that plantaricin IIA-1A5 was successfully purified from ammonium sulfate, and cation exchange chromatography and had a molecular weight of 9.4 kDa. Fatmarani et al. (2018) also researched the production of plantaricin IIA-1A5 from whey cheese, and found a molecular weight of crude plantaricin extract of 9.5 kDa. Lower molecular weights of bacteriocin produced by plantarum IIA-1A5 (6.41 kDa) and by *L. plantarum* FGC12 (4.1 kDa) were reported by Arief et al. (2015b) and Lv et al. (2017). Despite having different molecular weights, these plantaricins were classified as group IIA and relatively heat stable (Zacharof & Lovitt 2012). Another bacteriocin produced by *L. plantarum* in the study of Hu et al. (2013) include plantaricin 163 with a molecular weight of 3.5 kDa, and plantaricin K25 with a molecular weight of 1.7 kDa (Wen et al. 2016). This difference in molecular weight was caused by *L. plantarum* Strain. Kia et al. (2015) suggest that different *L. plantarum* strains greatly affect the characteristics of plantaricin and protein concentrations produced in the SDS-PAGE electrophoresis calculations.

Antimicrobial activity

The diameter of inhibition zone of crude plantaricin to pathogenic bacteria was presented in Table 1. The antimicrobial activity shown by the inhibition zone's diameter in crude plantaricin IIA-1A5 against *E. coli* and *S. aureus* was not significantly different. The values were less than 3 mm and so the antimicrobial activity was categorized as weak.

These low inhibition zone could be influenced by the storage of bacteriocin plantaricin IIA-1A5 at room temperature. It could also be caused by the largest bacteriocin component (Karpinski & Szkaradkiewicz 2013). Todorov et al. (2016) reported that the media's low inhibitory activity could be caused by reduced bacteriocin antimicrobial activity due to the role of organic acids.

The antimicrobial character of plantaricin IIA-1A5 against Gram positive and Gram negative bacteria were closely related to bacterial strains (Arief et al. 2013). According to Arief et al. (2015), that *L. plantarum* IIA-1A5 grown on commercial MRSB media, plantaricin had good antimicrobial activity against *E. coli* ATCC 25922, *S. thymurium* ATCC 14028, and *S. aureus* ATCC 25923 ranged from 6.86-12.38 mm. Gram positive bacteria were more sensitive, while Gram negative bacteria were more resistant (Fatmarani et al. 2018). Based on the research results of Soenarno et al. (2020), that plantaricin IIA-1A5 has a broad spectrum antimicrobial ability against Gram positive and Gram negative pathogenic bacteria.

Microbiological characteristics

Good milk is produced from healthy milking and clean cows udders, normally containing 10^6 CFU mL⁻¹ milk (SNI 3141.1: 2011). Temperature control is very important to prevent changes in milk quality associated with bacterial growth. The average total of fresh milk microbes during storage at room temperature is presented in Table 2.

Table 1. Diameter of inhibition zone of crude plantaricin to pathogenic bacteria

	Protein Yield	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)
Crude Plantaricin	96707.77	0.1±0.00 ^a	0.1±0.00 ^a

Means in the same row with different superscript differ significantly (P<0.05).

Table 2. Population of *E.coli* and *S.aureus* and TPC in fresh cow milk during storage at room temperature

Parameter	Treatment	Population in n-hours (log CFU mL ⁻¹)			
		1	3	5	7
TPC	Control	5.46±0.03 ^b	6.81±0.06	8.01±0.06 ^b	8.27±0.02 ^b
	<i>Povidone Iodine</i>	5.43±0.02 ^a	6.25±0.65	6.86±0.02 ^a	8.25±0.03 ^a
	Plantaricin	5.42±0.03 ^a	5.87±0.66	6.83±0.05 ^a	8.25±0.02 ^a
<i>E. coli</i>	Control	2.44±0.01 ^b	2.59±0.02 ^b	3.68±0.00 ^b	3.79±0.07 ^b
	<i>Povidone Iodine</i>	2.41±0.01 ^a	2.45±0.00 ^a	2.45±0.01 ^a	2.48±0.01 ^a
	Plantaricin	2.40±0.01 ^a	2.44±0.02 ^a	2.44±0.03 ^a	2.45±0.01 ^a
<i>S. aureus</i>	Control	0.00±0.00	0.00±0.00	1.43±0.04 ^b	1.93±0.05 ^b
	<i>Povidone Iodine</i>	0.00±0.00	0.00±0.00	0.00±0.00 ^a	1.41±0.09 ^{ab}
	Plantaricin	0.00±0.00	0.00±0.00	0.00±0.00 ^a	1.02±0.08 ^a

Means in the same column with different superscript differ significantly (P<0.05).

Total plate count

Plantaricin IIA-1A5 and *povidone iodine* application to dip the teats suppress bacterial growth significantly (P <0.05) at 1 and 5 hours of storage, compared to controls. At 7 hours of storage, the TPC population trends in plantaricin were comparable to *povidone iodine*.

Plantaricin, which was produced by local Indonesian lactic acid bacteria, had been researched and could be effectively used as a preservative in fresh and processed meat products, such as meatballs and sausages (Arief et al. 2012; Arief et al. 2017). Plantaricin IIA-1A5 produced using commercial media deMann Rogosa Sharp Broth (MRSB) maintained the shelf life of fresh meat for 15 hours at room temperature storage (Sihombing et al. 2015). Application of 0.3% plantaricin could maintain the quality of beef meatballs for 20 hours at room temperature storage (Kia et al. 2015) and effective as a preservative in beef sausage products for six days inside the cold storage (Arief et al. 2017).

Soenarno et al. (2020) stated that plantaricin IIA-1A5 and nisin to fresh milk reduced the increase in the amount of TPC in the first two hours compared to controls. In terms of safety, these results indicated that iodine and plantaricin IIA-1A5 can act as antimicrobials to slow down the total microbial population in fresh milk during the milking process. Although TPC results show a safe microbial population from fresh milk up to

5 hours of storage, TPC was a non-selective medium that may include some pathogenic bacteria that were very harmful to humans, even with low populations. In this experiment, selective media was used to investigate the presence of pathogenic bacteria in fresh milk, including *E. coli* and *S. aureus*. Lactic acid bacteria were known to have the potential for antimicrobial compounds such as bacteriocins that inhibit pathogenic bacteria (Le et al. 2019).

Escherichia coli

Escherichia coli found in control milk was 2.44 log CFU mL⁻¹ at 1 hour of storage at room temperature. This population of *E. coli* increased to 3.79 log CFU mL⁻¹ after 7 hours of storage. However, with plantaricin IIA-1A5 as a natural antibacterial agent for the milking process, the *E. coli* colonies that grew up to 7 hours of storage at room temperature were successfully reduced by 2.44 log CFU mL⁻¹. Whereas, for 7 hours of storage in fresh milk with *povidone iodine*, *E. coli* was observed to have a population of 2.48 log CFU mL⁻¹, which was still lower than the maximum population allowed for consumption 3.0 log CFU mL⁻¹ (SNI 3141.1 2011).

The maximum *E. coli* population allowed in fresh milk products was 3.0 log CFU mL⁻¹ (Badan Standardisasi Nasional 2011). Although *povidone iodine* and plantaricin IIA-1A5 resulted in a lower *E. coli*

Table 3. Chemical characteristics and pH analysis in fresh milk during storage at room temperature

Storage Time (Hour)	Parameter	Treatment		
		Control	<i>Povidone Iodine</i>	Plantaricin
1	Fat (%)	3.78±0.03	3.81±0.06	3.79±0.05
	SNF (%)	7.57±0.14	6.73±0.35	7.03±0.55
	Lactose (%)	4.17±0.07 ^b	3.72±0.22 ^a	3.76±0.15 ^a
	Protein (%)	2.77±0.05 ^b	2.49±0.14 ^a	2.48±0.03 ^a
	pH	6.72±0.02 ^b	6.61±0.05 ^a	6.67±0.02 ^{ab}
3	Fat (%)	3.79±0.03	3.82±0.06	3.80±0.05
	SNF (%)	7.68±0.14 ^b	6.95±0.35 ^a	7.18±0.06 ^{ab}
	Lactose (%)	4.22±0.08 ^b	3.81±0.20 ^a	3.92±0.02 ^{ab}
	Protein (%)	2.80±0.05 ^b	2.55±0.15 ^a	2.64±0.02 ^{ab}
	pH	6.67±0.09 ^b	6.57±0.05 ^{ab}	6.55±0.02 ^a
5	Fat (%)	3.80±0.03	3.83±0.06	3.81±0.05
	SNF (%)	7.48±0.22 ^b	6.89±0.45 ^a	7.28±0.17 ^{ab}
	Lactose (%)	4.11±0.12 ^b	3.78±0.25 ^a	4.05±0.04 ^{ab}
	Protein (%)	2.73±0.08 ^b	2.53±0.17 ^a	2.67±0.06 ^{ab}
	pH	6.63±0.1	6.49±0.03	6.59±0.03
7	Fat (%)	3.81±0.03	3.84±0.06	3.83±0.04
	SNF (%)	7.54±0.15 ^b	7.21±0.05 ^a	7.04±0.08 ^a
	Lactose (%)	4.17±0.11 ^b	3.95±0.02 ^a	3.86±0.05 ^a
	Protein (%)	2.74±0.04 ^b	2.69±0.08 ^{ab}	2.58±0.02 ^a
	pH	6.61±0.07	6.56±0.07	6.58±0.06

Means in the same row with different superscript differ significantly (P<0.05).

population than the maximum population allowed by the standard, these results suggested that plantaricin IIA-1A5 inhibits *E. coli* much more strongly than *povidone iodine*. The ability of plantaricin IIA-1A5 to inhibit the growth of *E. coli* is in line with (Arief et al. 2012, Arief et al. 2013)). *Escherichia coli* are known to cause the putrefaction of bacteria in food. Plantaricin IIA-1A5 is effective in inhibiting the growth of *E. coli* bacteria from forming a bacterial inhibition zone. Bacteriocins can damage bacterial cell walls, causing the death of *E. coli*. The higher the plantaricin percentage, the larger the inhibition zone produced. This shows that *E. coli* bacteria are inhibited by plantaricin activity. The highest inhibition zone was found in the highest plantaricin percentage, namely 50% (Siswara et al. 2019).

Staphylococcus aureus

Staphylococcus aureus populations in control milk ranged from 0.00 to 1.93 log CFU mL⁻¹ for 1-7 hours of storage at room temperature. The presence of *povidone*

iodine was significantly inhibited the growth of *S. aureus* compared to the control milk. The population of *S. aureus* for 7 hours of room temperature storage after *povidone iodine* was added 0.00-1.41 log CFU mL⁻¹. Interestingly, plantaricin IIA-1A5 demonstrated the ability to inhibit *S. aureus* populations similar to use iodine. Colonies were observed for 1-7 hours of storage at room temperature with a population of 0.00-1.02 log CFU mL⁻¹. The population of *S. aureus* in fresh milk using plantaricin or *povidone iodine* was lower than the maximum level allowed by the standard (2 log CFU mL⁻¹).

Fresh milk that is safe for consumption has a maximum *S. aureus* population of 2 log CFU mL⁻¹ with the risk requirements of *S. aureus* for consumption after storage for up to 6 hours at room temperature after 1 hour of milking (Badan Standardisasi Nasional 2011). These results show that up to 7 hours of storage at room temperature, fresh milk with iodine or plantaricin IIA-1A5 is quite safe for consumption. It should be noted that the ability of plantaricin IIA-1A5 to inhibit *S. aureus* is comparable to that of *povidone iodine*,

suggesting the potential use of plantaricin as an *povidone iodine* substitute. Following Arief et al. (2012), plantaricin has antimicrobial activity so that it can be used as a biopreservative in meatball products achieved by inhibiting the total growth of microbes and *E.coli*. The plantaricin used in this study is crude plantaricin, where crude plantaricin has been shown to suppress microbial growth, such as TPC, *E. coli*, and *S. aureus*.

Plantaricin IIA-1A5 with *povidone iodine* has differences in suppressing the number of *S. aureus* bacteria, because the composition of the active or antibacterial substances in both of them is different in reducing the number of these bacteria. Plantaricin IIA-1A5 has antimicrobial properties from organic materials, while *povidone iodine* from inorganic materials.

Chemical characteristics and pH analysis

Results of measurements on the physicochemical quality of fresh milk using the teat dipping method at storage 1, 3, 5, and 7 hours. Results of pH meter measurements of fresh milk using the teat dipping method at 1 and 3 hours storage was significantly different ($P < 0.05$). Plantaricin as a natural preservative applied in teat dipping before the milking process does not make any differences in the fat and SNF content. The mean chemical quality and pH analysis resulting from plantaricin and *povidone iodine* treatment was presented in Table 3. Chemical characteristics

Chemical characteristics in fresh milk during storage of 1 to 7 hours at room temperature. Plantaricin as a natural preservative in teat dipping does not make a difference in the fat, and SNF content. In the measurement of fat, SNF, and protein content, all treatments and controls showed values below the SNI 3141.1:2011 level, namely $>3\%$ (fat content), $>7.8\%$ (SNF), and $>2.8\%$ (protein content). Application of plantaricin IIA-1A5 and *povidone iodine* on teat dipping had a higher trend than control. Lactose levels in the plantaricin treatment at 5 hours increased. This shows cause increase of lactose in milk is due to changes in the composition of fat-protein-lactose so that the lactose content in milk has increased.

pH analysis

The three treatments' pH values during storage of 1 to 7 hours at room temperature. The three treatments' pH value was significantly influenced by the treatment and storage time up to 2 hours ($P < 0.05$). In general, there was no change in pH for control or teat dipping treatment with *povidone iodine* and plantaricin for 5 to 7 hours. This is because *povidone iodine* contains 14 Polyvinylpyrrolidone active zinc, a strong acid, where

the active substance is very useful in coating the nipple hole and can kill bacteria that enter the nipple hole by destroying the metabolism of cells in the cytoplasm to the cell nucleus, so that acidification of milk caused by bacterial activity can be avoided and maintained the pH of the milk at normal. The process of acidifying milk was caused by the fermentation of *Streptococcus lactis* against lactose, which significantly reduce the pH value (Mahardhika et al. 2012). The conversion of lactose caused an increase or decrease in pH into lactic acid by microorganisms and enzymatic activity (Mirdhayati et al. 2008). Marsh et al. (2014) stated that a fermented product's pH was influenced by the buffering capacity with different amounts and different types of protein. The growth of good lactic acid bacteria occurred at pH 6, and the growth rate decreased if the extracellular media became acidic. The decrease in pH resulted from acid accumulation from lactic acid bacteria.

CONCLUSION

Application of plantaricin IIA-1A5 as teat dipping before milking can reduce Total Plate Count, *Escherichia coli*, *Staphylococcus aureus* population and did not change pH value and physicochemical quality (fat, SNF, lactose, and protein). This ability is comparable to the iodine group, a synthetic antibacterial widely used by smallholder breeders in Indonesia. It is concluded that Plantaricin IIA-1A5 can be used as a substitute for synthetic antibacterial (iodine group) for teat dipping before milking.

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