ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2020.12.04.358

Research Article

Diammonium Phosphate and Sucrose Enrichment on Fermentation for Anti-bacteria Activity by Lactic Acid Bacteria

DAVID YUDIANTO[1]*, ELLYAS ALGA NAINGGOLAN[2], DHINA APRILIA NURANI WIDYAHAPSARI[1], ANDITA SAYEKTI[3], JONI KUSNADI[4]
[1]Quality Assurance of Food Industry, Politeknik AKA Bogor, Kota Bogor West Java, Indonesia
[2]Department of Bioprocess Engineering, Faculty of Biotechnology, Institut Teknologi Del. Toba Samosir, North Sumatra, Indonesia
[3]Department of Management, Faculty of Economics and Management, IPB University, Bogor, Indonesia
[4]Department of Food Technology, Faculty of Agricultural Technology, Brawijaya University, Malang, East Java, Indonesia
*Corresponding Author
Email ID: davidyudianto.se@gmail.com
Received: 20.07.20, Revised: 10.08.20, Accepted: 05.09.20

ABSTRACT
Cheese manufacturing produces a large number of liquid whey byproduct that could contaminate the environment. Nevertheless, this cheese byproduct (whey) which could increase biological oxygen demand has ability to be consumed by bacteria as it contains high of lactose and protein. *Lactobacillus plantarum* B2 and *Lactobacillus bulgaricus* were conducted in this study as the aim to evaluate their ability for antibacterial agent production during fermentation in cheese whey by product. Diammonium phosphate and sucrose were used for substrates enrichment in the media. Data was collected from analysis and measurement by triplicate using completely randomized design as experimental design. Data was conducted to analysis of variance. The comparison of means was conducted by Duncan's multiple range test. The result showed that the addition of diammonium phosphate (0.1%, 0.3%, and 0.5%) and sucrose (6%, 8%, and 10%) influenced total sugar ($P<0.05$), pH ($P>0.05$), total Lactic Acid Bacteria (LAB) ($P>0.05$), and anti-bacteria activity on *Escherichia coli* and *Staphylococcus aureus* ($P>0.05$). The best combination treatment from cheese whey fermented drink was 0.3% diammonium phosphate and 8% sucrose proportion which had evaluation result for total LAB 6,926 log CFU/mL, pH 3.8, total sugar 7.65%, anti-bacteria activity on *Escherichia coli* as indicator bacteria 4,255 cm^2, and anti-bacteria activity on *Staphylococcus aureus* 5.335 cm^2. Based on this study, whey as the byproduct of cheese industry has potential to be used as the raw material of fermented drink which has antibacterial activity from LAB towards *Escherichia coli* and *Staphylococcus aureus* significantly.

Keywords: Anti-bacteria, Cheese Whey, Diammonium Phosphate, Lactic Acid Bacteria, Sucrose

INTRODUCTION

Whey is a byproduct from cheese industry that contain about 20% of total milk protein. Whey consists of several different proteins such as β-lactoglobulin (β-LG), α-lactalbumin (α-LA), the heavy-and light-chain immunoglobulins (Igs), bovine serum albumin (BSA), lactoferrin (LF), lactoperoxidase, and glycomacropeptide (GMP). Whey also consists of proteose-peptone component, 20 amino acid and all nine essential amino acids [1]. In 2015, world cheese production increased by 2% annually and reached around 22 million MT. This sector produced approximately 8-9 L of whey per Kg of cheese produced or 180-190 milion ton/year in global estimation [2]. Whey has negative effect to the environment due to its biological oxygen demand ability. Whey rich source of microorganism that cause its fermentation or decomposition if it is not treated or processed immediately [3]. Considering the composition of whey such as lactose and the presence of some essential nutrient for microorganism growth makes the whey one of the potential substrates for production of different bioproduct throught biotechnological means. The production lactic acid production through lactic acid bacteria could be an alternative processing route for whey lactose utilization [4]. *Lactobacillus plantarum* B2 and *Lactobacillus bulgaricus* are LAB that could produce organic acid as antibacteria agent toward pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*. One factor that influences the growth of LAB is the presence of nutrients. Enrichment of nutrient is expected to enhance antibacteria agent production by LAB. Diammonium phosphate is one of inorganic nitrogen source that has high solubility, stable, high phosphorus and nitrogen compounds. [5], stated that the addition of peptone and diammonium phosphate increased the growth of *Lactobacillus lactis* RM 2-24 with maximum D-lactic acid

production. [6], reported the effective diammonium phosphate for lactate fermentation by a *B. coagulans* strain. The objective of this study was evaluation of proportion for diammonium phosphate and sucrose in cheese whey media fermentation by *Lactobacillus plantarum* B2 and *Lactobacillus bulgaricus*. Addition of both compounds were expected to enhance the antibacterial activity toward pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*).

METHODOLOGY

Materials

Mozzarella byproduct (whey) was obtained from CV. An Nur, Koperasi Mitra Bhakti Makmur, Junrejo, Batu. *Lactobacillus plantarum* B2, *Lactobacillus bulgaricus*, *Escherichia coli*, and *Staphylococcus aureus* were obtained from Microbiology Laboratory, Department of Agricultural Product Technology, Brawijaya University. MRSA, MRSB, diammonium phosphate, and glucose were purchased from Sigma-Aldrich.

Whey Media Preparation

Fresh whey was carried by icebox from the industry to Department of Agricultural Product Technology, Brawijaya University. Whey was pasteurized at 85°C for 15 minutes as soon as possible. Separation of curd was done by filter cloth directly after pasteurization. Every experiment always used fresh whey from industry.

Fermentation

Hot whey (85°C) was prepared for 100 ml in every bottle and was added by diammonium phosphate (0.1%, 0.3%, and 0.5%) and sucrose (6%, 8% and 10%). Cooling was carried out at room temperature until the temperature reached 37°C. Inoculation was done by addition of 2% *Lactobacillus plantarum* B2 and 2% *Lactobacillus bulgaricus* starters. Fermentation was conducted in anaerob condition at 37°C for 42 hours.

Determination of Total LAB, pH and Total Sugar

Total LAB was calculated by Total Plate Count method on the MRS Agar media. The pH value measurement was conducted by pH meter (Satorious, USA) at ambient temperature that was calibrated by standard of pH 4.0 and 7.0. Total sugar determination was done by Lane and Eynon volumetric method. This method used titration by Fehling reagents that expressed as grams of total sugar per 100 mL of sample.

Antibacterial Activity Measurement

Antibacterial activity of cheese whey fermented drink was conducted by preparing nutrient agar media with *Escherichia coli* and *Staphylococcus aureus*, respectively, as indicator bacteria. Two wells were created per media petridisk and gave each of sample in the well. Evaluate the formation of clear zone around the well and calculated the area as the capability of antibacterial activity produced by LAB.

Statistical Analysis

Data was collected from analysis and measurement by triplicate. Experimental design was completely randomized design. Data was conducted to analysis of variance. The comparison of means was conducted by Duncan's multiple range test.

RESULTS AND DISCUSSION

Total LAB

The higher diammonium phosphate and sucrose lead to have high total LAB ($P>0.05$). This condition accordanced with [5] that addition of diammonium phosphate in the production medium could increase *Lactobacillus lactis* RM2-24 growth and lactic acid production (69 g/l) with 2.3 g/l/h productivity. [7] Stated that amount of reduction sugar in yogurt can support the growth of LAB and stimulate activities LAB in producing lactic acid. [8] Confirmed that the population of LAB in goat yogurt and goat milk co-fermented with yeast and LAB increased during fermentation and exceeded the minimum bacteria population (10^6 cfu/mL).

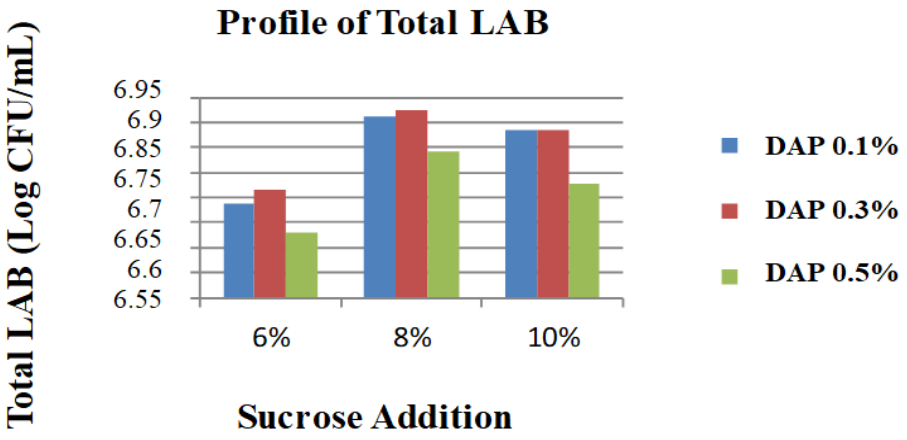

Fig 1: Effect of diammonium phosphate and sucrose addition on lab's growth. DAP means diammonium phosphate

Figure 1 shows that total LAB in whey cheese fermented drink by addition of 0.5% diammonium phosphate was lower than addition of 0.1% and 0.3% diammonium phosphate at the end of fermentation (P>0.05). According to [9], ammonium had bactericidal property at 1% concentration and bacteriostatic at 0.5% concentration. The mechanism of ammonium in bacterial growth inhibition was by damaging the cell membrane after reacting with phosphate in phospholipids on microbial cell membrane. Moreover, total LAB on Figure 1 shows that at 8% sucrose addition showed high bacteria growth compared to 6% and 10% (P>0.05). In 10] explain that increased of sugar addition could inhibit the bacteria growth. Table 1 shows that the combination of diammonium phosphate and sucrose addition at 0,3% and 8%, respectively gave the highest LAB growth in cheese whey media (P>0.05). In [11] stated that during fermentation, LAB growth reached the exponential phase occurred at 0-16 hours of incubation, while stationary phase occurred at 16-48 hours of incubation. Along with the growth of LAB, there was change in pH which the pH decreased then at stationary phase tends to be stable. According to [5], addition diammonium phosphate into fermentation mediaum increased LAB growth, lactic acid concentration and lactic acid productivity. In addition, the appropriate sucrose addition will provide carbon source as nutrient for rapid growth of bacteria. In [12] stated that the nutrient requirements of lactobacilli are complex and either strain or species dependent in the fermentation of carbohydrates, nitrogen sources, fatty acids, and growth factor.

Table 1: Effect of Diammonium Phosphate and Sucrose Addition on LAB's Growth.

Treatment		LAB's Growth (Log CFU/mL)		
DAP (%)	Sucrose (%)	Before Fermentation	After Fermentation	Changes
0.1	6	5.452	6.739	1.287
	8	5.442	6.913	1.471
	10	5.686	6.884	1.198
0.3	6	5.480	6.765	1.284
	8	5.406	6.926	1.519
	10	5.586	6.884	1.298
0.5%	6	5.473	6.678	1.205
	8	5.527	6.842	1.314
	10	5.380	6.778	1.397

DAP means Diammonium Phosphate

pH

Decreasing in pH value was occurred during fermentation process. Decreasing of pH value was caused by H+ ions organic acid compounds from the metabolism of LAB. In [8] stated that decreasing in pH value was due to the accumulation of organic acids from LAB. In [13] explained that lactic acid (CH3CH(OH)CO2H), with a pKa value of 3.85, was in equilibrium with its ionized form ($CH3CH(OH)CO2^-$) when the medium pH was equal to its pKa. The lactic acid accumulation decreased rapidly the pH value at early-stage fermentation then lactic acid dissociated to H^+ and ($CH3CH(OH)CO2^-$). The higher lactic acid production leading to dissociate high H^+ ions released in the media. Table 2 shows that combination of 0.1% and 8% concentration for diammonium phosphate and sucrose, respectively gave the highest decreasing of pH value (P>0.05). It was presumed from high amount of organic acids which was produced from metabolism of LAB in media. Enhancement of total LAB lead to produce high lactic acids causing decrease in pH value. In [11] explained that when the LAB was in phase of exponential growth, the LAB count increased rapidly so that the metabolic activity in the cells becomes higher. The LAB metabolic activity produced organic acids, one of which was lactic acid. The percentage of lactic acid increases in the medium and decreasing in pH value at the same time. Concentration of 0.5% diammonium phosphate had high pH value and a little change of pH value compared to all (P>0.05). This phenomenon was suitable with explanation of [9] that ammonium was bacteriostatic at a concentration of 0.5%. This condition has possibility for bacteria growth inhibition.

Table 2: Effect of Diammonium Phosphate and Sucrose Addition on pH value.

Treatment		pH		
DAP (%)	Sucrose (%)	Before Fermentation	After Fermentation	Changes
0.1	6	5.233	3.843	1.39
	8	5.243	3.816	1.426
	10	5.216	3.81	1.406
0.3	6	5.176	3.843	1.333
	8	5.18	3.81	1.37
	10	5.16	3.803	1.356
0.5%	6	5.12	3.88	1.24
	8	5.116	3.853	1.263
	10	5.11	3.843	1.266

DAP means Diammonium Phosphate

Based on Figure 2, the higher sucrose concentration addition leading to get lower pH value ($P>0.05$). It was predicted from the availability of sucrose as the carbon source. Although the total LAB for 10% addition of sucrose was lower than 8% ($P>0.05$), the 10% of sucrose addition was still give the need of carbon source for bacteria resulting on low pH value profil after fermentation process. In [7] stated addition date extract into yogurt drink added up the amount of reduction sugar significantly and support LAB to produce lactic acid. The more lactic acid of yogurt, the lower of pH value.

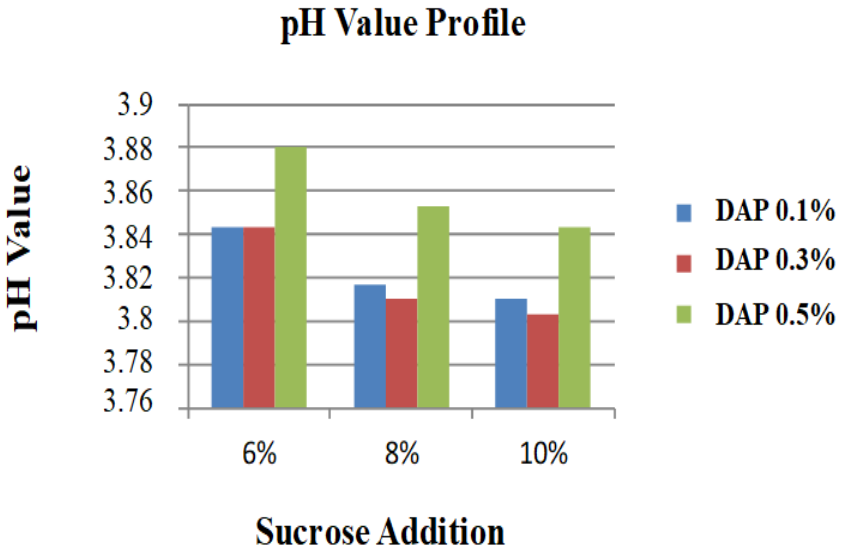

Fig.2: Effect of diammonium phosphate and sucrose addition on change of ph value. DAP means diammonium phosphate

The duration of fermentation will affect the sugar convertion into latic acid by LAB. LAB used sugar as an energy source, growth and produce metabolites in the form of lactic acid during the fermentation process. The total sugar profile is showed on the Figure 3 that had higest total sugar content at the end of fermentation process for 10% sucrose addition ($P<0.05$). This was followed by 8% and 6% sucrose addition in the cheese whey fermented drink. The tendency of total sugar on sucrose addition was similar that follow the content of sugar added in the media before fermentation process. However, the addition of 0.3% diammonium phosphate gave the lowest total sugar compared to 0.1% and 0.5% ($P<0.05$). It means that in this condition, the LAB used both diammonium phosphate and sucrose well during fermentation. This tendency was similar with the total LAB profile which had highest LAB growth at 0.3% diammonium phosphate addition.

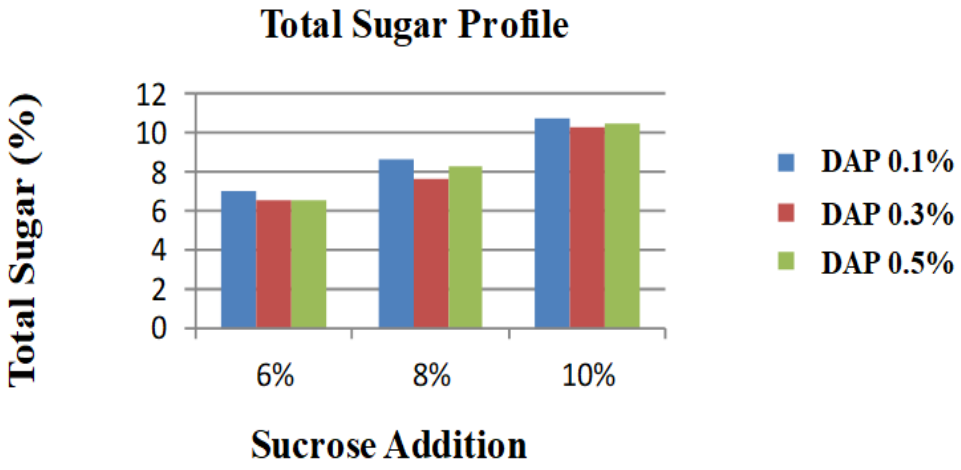

Fig 3: Effect of diammonium phosphate and sucrose addition on change of total sugar. DAP means Diammonium Phosphate

The change of total sugar could be found in Table 3. Decreasing in total sugar was due to LAB activity that used sucrose as carbon source to be converted into organic acids. In [5], addition diammonium phosphate into fermentation mediaum increased LAB growth, lactic acid concentration and lactic acid productivity. The enhancement of total LAB affected the use of nitrogen compound for metabolism needs including the sugar (sucrose/lactose) conversion into lactic acid. However, at the concentration of 0.5% diammonium phosphate had high total sugar because the growth of LAB begins to be inhibited by ammonium compound. This had been explained in the total LAB explanation according to [9], the concentration of 5% ammonium phosphate compounds can inhibit bacteria growth (bacteriostatic). This was suitable with the highest decreasing of total sugar during fermentation which used 0.3% of diammonium phosphate because at this concentration LAB had high of growth lead to consume high sugar as carbon source for their metabolism and growth. According to [14], during fermentation of rice beverages, pH decreased and, at the same time, total acidity increased as a consequence of lactic and acetic acid production. Fermentation also led to a decrease in glucose and fructose concentrations due to their consumption as a source of energy for growth and metabolism of lactic acid bacteria. In [15] stated when the sucrose content is increased during fermentation, the lactic acid content of the lactic acid bacteria fermentation is also increased, so that the pH value is lowered and the total acid content is also increased.

Table 3: Effect of Diammonium Phosphate and Sucrose Addition on Change of Total Sugar.

Treatment		Total Sugar (%)		
DAP (%)	Sucrose (%)	Before Fermentation	After Fermentation	Changes
0.1	8	9.81	8.62	1.19
	10	11.58	10.73	0.85
	6	8.30	6.52	1.78
0.3	8	9.54	7.65	1.89
	10	11.56	10.26	1.29
	6	7.97	6.58	1.39
0.5%	8	9.04	8.30	0.74
	10	11.23	10.50	0.73
	8	9.81	8.62	1.19

DAP means Diammonium Phosphate

Antibacterial Activity (*Escherichia coli* Indicator Bacteria)

Evaluation of antibacterial activity was conducted to *Escherichia coli* and *Staphylococcus aureus* based on the clear zone forming in the agar plate media. Figure 4 shows the profile of antibacterial activity toward viability of *Escherichia coli*. The higher concentration of diammonium phosphate and

sucrose, the greater clear zone area (P>0.05). Clear zone formation was influenced by antimicrobial metabolites produced by LAB such as H_2O_2, diacetyl, reuterin, and bacteriosin [16]. The high total number of LAB lead to enhance the ability of lactic acid production. Lactic acid produced by LAB will be secreted out of the cell accumulating in fermented liquids as explained by [17] that *Lactobacillus plantarum* BL011 produced lactic acid in large amount in liquid acid protein residue of soybean during fermentation. The clear zone formation was related to the total LAB and pH value (P>0.05). The higher total LAB and the lower pH value lead to be greater the clear zone area because more metabolites were produced. This was match with the lowest of total LAB after fermentation which used 0.5% of diammonium phosphate because at this concentration ammonium had bacteriostatic property causing the contribution of *Escherichia coli* inhibition [9].

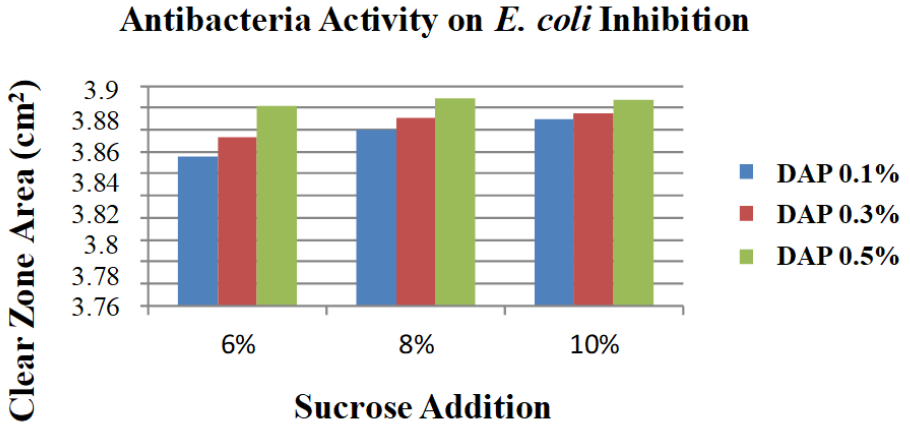

Fig.4: Antibacteria activity toward E*scherichia coli* Inhibition. DAP means Diammonium Phosphate

Table 4 shows the clear zone area at pre- and after fermentation which inform quiet big of clear zone area by the highest concretation of both diammonium phosphate and sucrose. *Escherichia coli* belongs to the class of Gram negative bacteria. Gram-positive bacteria may have a stronger defence system due to the presence of a thicker cell wall. Moreover, the cell wall of gram-negative bacteria possesses a stronger negative charge than gram-positive bacteria due to the presence of lipopolysaccharides (LPS) [18]. The performance of lactic acid to inhibit the bacteria growth was also explained that the lactic acid which was not dissociate could enter through bacterial lipid bilayers and released protons in cytoplasm leading to cause acid environment [19]. Cells will try to maintain their internal pH by releasing the incoming protons that cause the lack of cell's energy and growth. While, the acid concentration of extracellular was high, cell energy was not enough to remove all protons from the cell leading to decrease the pH of cytoplasm. This condition affects the cells which can no longer continue to survive [20]. Increasing the number of protons caused the enzyme denaturation and disrupt the cell membrane permeability and metabolism ending by the dead of bacteria [19].

Table 4: Effect of Diammonium Phosphate and Sucrose Addition on *Escherichia coli* Inhibition. DAP means Diammonium Phosphate

Treatment		Clear Zone Area (cm^2)	
DAP (%)	Sucrose (%)	Before Fermentation	After Fermentation
0.1	6	0	3.383
	8	0	3.993
	10	0	4.221
0.3	6	0	3.823
	8	0	4.255
	10	0	4.380
0.5%	6	0	4.545
	8	0	4.728
	10	0	4.694

Antibacterial Activity (*Staphylococcus aureus* Indicator Bacteria)

Antibacterial activity toward *Staphylococcus aureus* has similar trend with *Escherichia coli* as indicator bacteria on clear zone formation after fermentation. Figure 5 shows the profile of antibacterial activity toward viability of *Staphylococcus aureus*. The higher concentration of diammonium phosphate and sucrose, the greater clear zone area (P>0.05). As explained by [21], clear zone formation was influenced by lactic acid, hydrogen peroxide, proteolytic activity and other antimicrobial compounds produced by LAB.

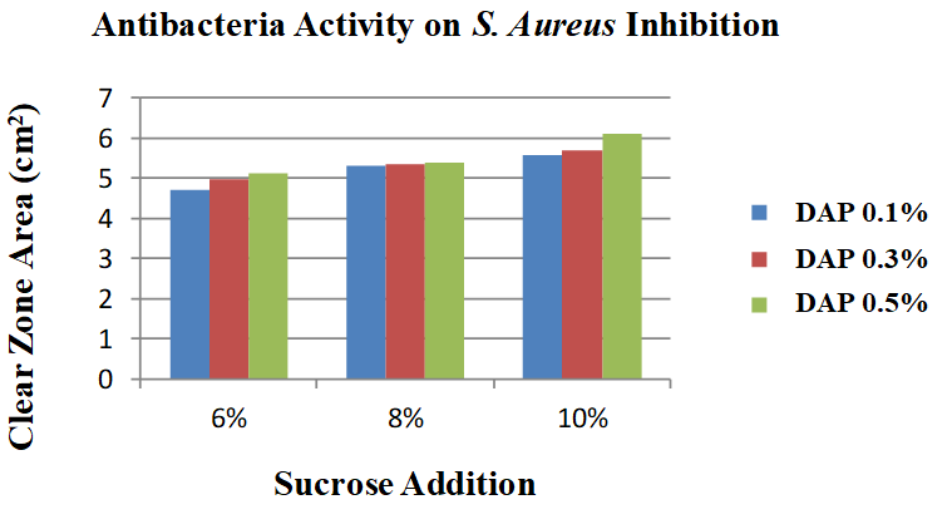

Fig.5: Antibacteria activity toward *Staphylococcus aureus* Inhibition. DAP means Diammonium Phosphate

The antibacterial performance was similar with the explanation to *Escherichia coli* inhibition namely high total LAB cause enhancement of lactic acid production. This lactic acid will be secreted out of the cell and be accumulated in media [17] thus decrease the pH value [8]. Seeing on Figure 1, Figure 2, and Figure 5, clear zone formation was related to the total LAB and pH value (P>0.05). The higher total LAB and the lower pH value lead to be greater the clear zone area because more metabolites were produced (P>0.05). This was appropriate with the lowest of total LAB after fermentation which used 0.5% of diammonium phosphate (P>0.05) because at this concentration ammonium had bacteriostatic property causing the contribution of *Staphylococcus aureus* inhibition [9].

Table 5: Effect of Diammonium Phosphate and Sucrose Addition on *Staphylococcus aureus* Inhibition. DAP means Diammonium Phosphate

Treatment		Clear Zone Area (cm^2)	
DAP (%)	Sucrose (%)	Before Fermentation	After Fermentation
0.1	6	0	4.726
	8	0	5.312
	10	0	5.563
0.3	6	0	4.974
	8	0	5.335
	10	0	5.691
0.5%	6	0	5.139
	8	0	5.382
	10	0	6.097

In [19] stated that the antibacterial effectiveness of lactic acid increased by the decreasing of pH value. Undissociated lactic acid penetrates into cell membrane and enters to high pH value cytoplasm. In high pH value cytoplasm, lactic acid dissociates and produces protons which tend to reduce the cytoplasm pH. The cell will try to maintain its internal pH by neutralizing or forcing out the proton. This effort will desrease bacterial growth because the energy for growth is used to remove

protons. If the external pH is low and the concentration of extracellular acid is high, the cell is quiet hard to do normal activities due to the low pH of cytoplasm. This condition is too hard for log phase of cell growth leading to die while it is occurred.

Comparison of Inhibition toward *Escherichia coli* dan *Staphylococcus aureus*

Based on the Table 6, the inhibition of antibacterial compounds was higher toward *Staphylococcus aureus* compared to *Escherichia coli* ($P>0.05$). This difference in antimicrobial activity between *Esherichia coli* and *Staphylococcus aureus* microorganisms is often attributed to the structure of their different cell walls. *S. aureus* as gram-positive bacteria may have a stronger defence system due to the presence of a thicker cell wall. Moreover, the cell wall of *Escherichia coli* as gram-negative bacteria possesses a stronger negative charge than gram-positive bacteria due to the presence of lipopolysaccharides (LPS) [18]. Moreover, *Lactobacillus plantarum* B2 and *Lactobacillus bulgaricus* are facultative anaerobes. During the fermentation process, it was predicted that there was oxygen around the environment. This condition lead LAB to produce hydrogen peroxide in a state of oxygen excess. Hydrogen peroxide will cause enzyme denaturation and lipid membrane peroxidation which increase the membrane permeability. Hydrogen peroxide was also an antibacterial free radical precursor such as superoxide and hydroxyl which damages DNA. In [22] also explained that LAB can noted the inhibition of *Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, and Pseudomonas aeroginos*a by hydrogen peroxide of LAB strains which contribute to their inhibitory activity against other microorganisms. In [23] also explained that *L. plantarum* exhibited 67% hydrogen peroxide production. That information was predicted to explain the higher antibacterial activity toward *Staphylococcus aureus* than *Escherichia coli*.

Table 6: Comparison of *Escherichia coli* dan *Staphylococcus aureus* Inhibitions from Antibacteria Agent in Fermented Drink

Treatment		Clear Zone Area (cm^2)	
DAP (%)	Sucrose (%)	*Escherichia coli*	*Staphylococcus aureus*
0.1	6	3.383	4.726
	8	3.993	5.312
	10	4.221	5.563
0.3	6	3.823	4.974
	8	4.255	5.335
	10	4.380	5.691
0.5%	6	4.545	5.139
	8	4.728	5.382
	10	4.694	6.097

CONCLUSION

Whey as the by-product of cheese industry has potential to be used as the raw material of fermented drink due to its composition on lactose and protein. Diammonium phosphate and sucrose could be used to enhance the nitrogen and carbon substates, respectively, in the byproduct media to enhance the growth of bacteria. The addition of three different concentrations of diammonium phosphate and sucrose did not affect pH value, total LAB and antibacterial activity to both *Escherichia coli* dan *Staphylococcus aureus* significantly, so that we could use the lowest both diammonium phosphate and sucrose concentrations for efficieny cost.

CONFLICT OF INTEREST

None

REFERENCES

1. B. B. Solak and N. Akin. (2012). Health benefits of whey protein: A review. Journal of Food Science and Engineering, 2, 129-137.
2. E. El-Tanboly, M. El-Hofi and Khorshid. (2017). Recovery of cheese whey, a by-product from the dairy industry for use as an animal feed. Journal of Nutritional Health & Food Engineering, 6(5), 148-154.
3. J. S. S. Yadav, S. Yan, S. Pilli, L. Kumar, R. D. Tyagi, R. Y. Surampalli. (2015). Cheese whey: A potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. Biotechnology Advances, 33(6), 756–774.
4. P. S. Panesar, J. F. Kennedy, C. J. Knill, M. Kosseva. (2010). Production of L (+) lactic acid using lactobacillus casei from whey. Brazillian Archives of Biology and Technology, 53(1), 219-226.
5. M. Singhvi., A. Jadhav and D. Gokhale. (2013). Supplementation of Medium with Diammonium

Hydrogen Phosphate Enhanced the D-Lactate Dehydrogenase Levels Leading to Increased D-Lactic Acid Productivity. Bioresource Technology, 146, 736-739.
6. L. Sun, Y. Li, L. Wang, Y. Wang, B. Yu. (2016). Diammonium phosphate stimulates transcription of l-lactate dehydrogenase leading to increased l-lactate production in the thermotolerant bacillus coagulans strain. Applied Microbiology and Biotechnology, 100, 6653-6660.
7. I. Hartati, Y. B. Pramono, and A. M. Legowo. (2012). Lactose and reduction sugar concentrations, ph and the sourness of date flavored yogurt drink as probiotic beverage. Journal of Applied Food Technology, 1(1), 1-3.
8. Z. Huang, L. Huang, G. Xing, X. Xu, C. Tu, M, Dong. (2020). Effect of Co-Fermentation with Lactic Acid Bacteria and K. marxianus on Physicochemical and Sensory Properties of Goat Milk. Foods, 9(3), 299-312.
9. M. J. Pelczar, R. D. Reid and E. C. S. Chan. (1077). Microbiology. McGraw-Hill Publ. Co. Ltd, New Delhi.
10. M. M. Lobete, M. Baka, E. Noriega, E. Jooken, A. Monballiu, S, Beurme, B. Meesschaert, J. F. V. Impe. (2017). Stevia-based sweeteners as a promising alternative to table sugar: The effect on *Listeria monocytogenes* and *Salmonella typhimurium* growth dynamics. International Journal of Microbiology, 245, 38-52.
11. Desniar, I. Rusmana, A. Suwanto, N. R. Mubarik. (2014). Organic acid produced by lactic acid bacteria from bekasam as food biopreservatives. IOP Conf. Series: Earth and Environmental Science, 414-422.
12. W. H. Holzapfel and B. J. B. Wood. (2014). Lactic Acid Bacteria–Biodiversity and Taxonomy. John Wiley & Sons Ltd, West Sussex.
13. S. D. Nsogning, S. Fischer, and T. Becker. (2018). Investigating on the fermentation behavior of six lactic acid bacteria strains in barley malt wort reveals limitation in key amino acids and buffer capacity. Food Microbiology, 73, 245-253.
14. M. Magala, Z. Kohajdová, J. Karovičová, M. Greifová, J. Hojerová. (2015). Application of lactic acid bacteria for production of fermented beverages based on rice flour. Czech Journal Food Science, 33(5), 458-463.
15. M. Sui, Y. Zhang, J. Rong, K. Zhu, M. Wei. (2019). Study on fermentation process of lactic acid bacteria inoculated with kimchi. IOP Conf. Series: Materials Science and Engineering, pp. 612-618.
16. V. M. Dillon. (2014). Encyclopedia of Food Microbiology. Elvesier, Liverpool.
17. C. Coghetto, C. B. Vasconcelos, G. B. Brinques, M. A. Z. Ayub. (2016). Lactobacillus plantarum BL011 cultivation in industrial isolated soybean protein acid residue. Brazilian Journal of Microbiology, 47, 941-948.
18. M. Bonnet, C. Massard, P. Veisseire, O. Camares, K. O. Awitor. (2015). Environmental toxicity and antimicrobial efficiency of titanium dioxide nanoparticles in suspension. Journal of Biomaterials and Nanobiotechnology, 6, 213-224.
19. S. Naidu and R. A. Clemens. (2000). Natural Food Antimicrobial. CRC Press, LCC.
20. M. R. Adams and M. O. Moss. (2000). Food Microbiology. Royal Society of Chemistry, Cornwall).
21. E. Dincer and M. Kivanc. (2019). Characterization of Lactobacillus plantarum strains isolated from Turkish pastırma and possibility to use of food industry. Food Science and Technology, 40(2), 498-507.
22. L. González, A. F. Cuadrillero, J. M. Castro, A. Bernardo, M. E. Tornadijo. (2015). Selection of lactic acid bacteria isolated from San Simón Da Costa cheese (PDO) in order to develop an autochthonous starter culture. Advance Microbiology, 5, 748-759.
23. Sanni, C. Franz, U. Schillinger, M. Huch, C. Guigas, W. Holzapfel. (2013). Characterization and technological properties of lactic acid bacteria in the production of "Sorghurt," a cereal-based product. Food Biotechnology, 27, 178–198.