Combination Activity of Lactic Acid Bacterial Culture to Improve Quality of Honey Pineapple Yoghurt Enriched with Seaweed *Eucheuma spinosum*

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Abstract. Yogurt, substituting other ingredients, such as honey pineapple, will affect the quality of the final quality produced. Yogurt quality will be determined by organoleptic parameters, product acidity, and the number of probiotics contained in yogurt. This study aimed to determine the effect of using a combination of starter cultures in improving the quality of honey pineapple yogurt. This study is an experimental study using a completely randomized design consisting of 1 factor, namely the combination of lactic acid bacteria culture *L. bulgaricus*: *S. thermophilus*: *L. acidophilus* (1:1:0 [K1], 1:1:1 [K2], 2:2:1 [C3], 1:1:2 [C4]). The data were evaluated using an analysis of variance (Kruskall Wallis) with a significance level of 5%, and the significantly different data were further tested using an additional test of a Mann U whitney using IBM SPSS 26. The results showed that the combination of K4 culture was the best treatment because it produced yogurt with a high amount of LAB and had the highest viability of live bacteria. In addition, the resulting pH is also close to a good yogurt pH classification, which is 4, and is not significantly different from the pH in the K1 treatment. The organoleptic parameters in taste and aroma were also not significantly different from other treatments with the criteria of moderate liking.

Keywords: Pineapple Yoghurt, Combination of Culture, LAB.

1 Introduction

1.1 A Subsection Sample

Pineapple is one of the local fruits that is always available throughout the year. One of the superior products of pineapple that is often found in Lombok is the type of honey pineapple. People, in general, consume pineapple as fresh cut. In NTB, in particular, very little is found processed from pineapple. Pineapple, as a tropical fruit with a fresh taste, can be used as raw material for various kinds of preparations. One of them is drinking. Processed drinks that are very likely to be combined with pineapple are yogurt.

Yogurt is a dairy product obtained through fermentation with the addition of lactic acid bacteria culture as a starter [1]. Making yogurt uses a mixed culture of lactic acid bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [2]. Adding lactic

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acid bacteria will convert the lactose in milk into lactic acid, which causes the milk protein to coagulate to form a thick yogurt texture with a distinctive aroma and taste. Adding pineapple to yogurt will give it a distinctive taste. Kusumawati's research [3] showed that adding honey pineapple to as much as 60% gave the most preferred organoleptic response.

The taste, texture, and aroma will determine the quality of the yogurt and the number of probiotics contained in the yogurt. Some treatments that can be done to improve the quality can be done by adding a stabilizer in the form of seaweed. The results showed that adding seaweed *E. spinosum* to yogurt significantly affected pH, syneresis, and yogurt viscosity. The addition of 5% seaweed provides the most preferred taste, texture, preference, and aroma by panelists [4]. The addition of seaweed as a stabilizer resulted in smaller syneresis in yogurt. In addition, the type of culture will also greatly affect the quality of yogurt. Yogurt with pineapple substitution will reduce the lactose content, which produces a distinctive aroma of yogurt. The work activity of the added starter culture will also be different from the manufacture of yogurt, which only uses milk as its raw material.

The microbial culture used in making yogurt belongs to the type of lactic acid bacteria (LAB). The fermentation process can be carried out using single cultures or mixed cultures. Each added culture will usually give its own characteristics to the resulting yogurt. Lactic acid bacteria commonly used are *L. bulgaricus*, *S. thermophylus* and *L. acidopylus*. The use of mixed cultures of *L. bulgaricus* and *S. thermophylus* in a symbiotic mutualism will generally produce short amino acids and peptides that will form the distinctive characteristics of yogurt.

Meanwhile, *L. acidophilus* culture will act as a probiotic that will play an essential role in digestive health. The addition of *L. acidophilus* in the manufacture of yogurt can reduce the level of lactose consumption [5]. Combining the starter culture of yogurt with adding *L. acidophilus* is expected to produce good-quality pineapple yogurt. Therefore, it is necessary to research: The Effect of a Combination of Lactic Acid Bacterial Culture on the Quality of Honey Pineapple Yoghurt Enriched with Eucheuma spinosum Seaweed.

2 Material and Methods

2.1 Material

Equipments used in this study were basins, plates, UC bottles, spoons, glass cups, and, as well as an autoclave (Hirayama, Japan), an analytical balance (ABJ, Germany), a blender (Philips, Netherlands), a vortex (Heidolph, Germany), a pH meter (Schott, Germany), an incubator (Memmert, Germany), a measuring cup, a volume pipette, and a micropipette Honey pineapple were obtained from the Kebon Roek market in Mataram; dried *E. spinosum* seaweed was obtained from Seriwe Village, Jerowaru District, East Lombok Regency; water minerals were obtained from Narmada, Indonesia; skim milk, granulated sugar, and starter cultures *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus*, De Man Rogosa and Sharpe Broth (MRSB) (Oxoid, UK) dan media De Man Rogosa and Sharpe Agar (MRSA) (Oxoid, UK).

2.2 Methods

Yogurt Making Process. The process of making honey pineapple yogurt refers to the yogurt making process carried out by [6]. With modifications to the combination treatment the starter culture used.

Analytical Methods. The design used in this study was a completely randomized design (CRD) with a single factor experiment, the combination of yogurt culture which consisted of 4 levels combination of culture (*L.bulgaricus*: *S. thermophilus*: *L. achidophylus*) namely K1 = 1:1:0; K2 = 1:1:1; K3 = 2:2:1; K4 = 1:1:2. Each treatment was performed in three replicates to achieve a total of twelve experimental units. IBM SPSS Statistics 26 software was used to analyze observational data using analysis of variance (Analysis of Variance) by Kruskall Wallis Test with a significance threshold of 5%. If a substantial difference exists, an additional test of Mann U Whitney is performed. Chemical, physical, microbiological and organoleptic characteristics were all observed in this investigation.

3 Result and Discussion

3.1 Total Titrated Acid

Figure 1. shows the total amount of titrated acid contained in pineapple yogurt with the use of various combinations of types of cultures. The addition of a probiotic bacterial culture of *L. achidophilus* showed a higher amount of titrated acid in pineapple yogurt than the use of two cultures without the addition of a probiotic culture. Although the numbers are not significantly different. The results of [7] showed that partial substitution of yogurt-making cultures with probiotic bacterial cultures of *L. acidophilus* and *L.plantarum* resulted in a greater amount of titrated acid compared to yogurt cultures without probiotic bacteria. This can be caused by pineapple honey yogurt made from pineapple juice which contains D-glucose which will be broken down better by *L. achodopylus* to be broken down into organic acids in honey pineapple yogurt. According to [8], the sugar, namely lactose, is the main basis for the formation of yogurt, which is actively transported across the membrane of lactic acid bacteria through the mediation of the enzyme galactoside permease. The enzyme P-galactosidase hydrolyzes lactose into glucose and galactose. Furthermore, glucose is metabolized to pyruvate via the Emden-Meyerhof-Parnas (EMP) pathway and lactate dehydrogenase converts pyruvate to lactic acid. This is also supported by the opinion of [9], that the decrease in pH value was associated with an increase in the total acid titrated by the activity of microorganisms in yogurt.

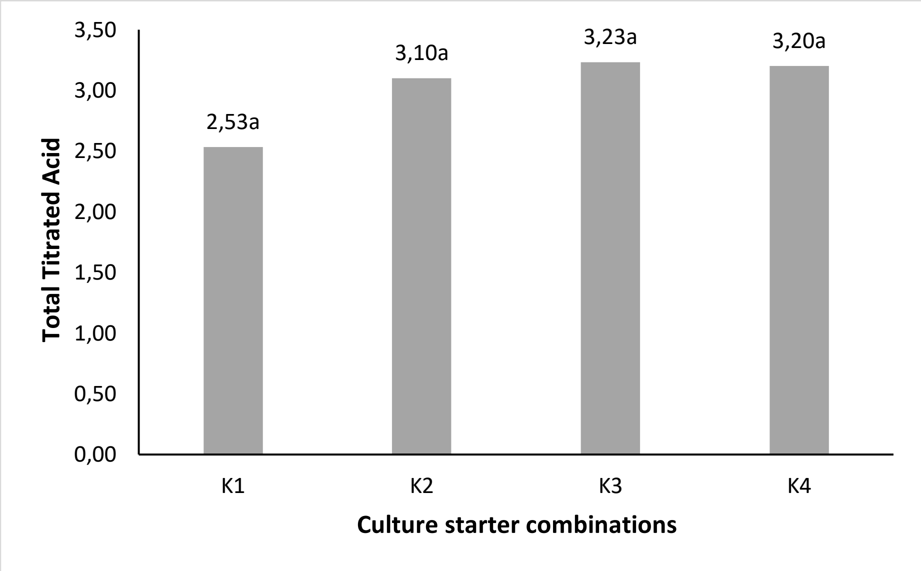

Fig. 1. The Effect of The Combination of Yogurt Culture On The Total Titrated Acid of Yogurt

3.2 pH

Figure 2. shows the combination of yogurt fermentation culture affects the pH of the resulting honey pineapple yogurt. Yogurt without the addition of *L. achidopylus* culture showed the highest pH value of 4.10. while the combination of the number of balanced cultures 1:1:1 showed the lowest pH, which was 3.92. The pH value of honey pineapple yogurt with various combinations of these cultures is still in the appropriate pH for yogurt. According to [10], the pH of a good fermented milk product is in the range of 3.8-4.6. The low pH is influenced by high acid levels also in yogurt products with the use of *L. achydophilus* culture compared to without *L. achydophilus* culture. The data shows that the addition of *L. achidopylus* probiotic culture causes the pH of yogurt to decrease. *L. achidophilus* can change the media containing fructose into organic acids better so as to lower the pH of the medium. Honey pineapple is a fruit that contains fructose so that it can be used by *L. achydopilus* to produce more organic acids.

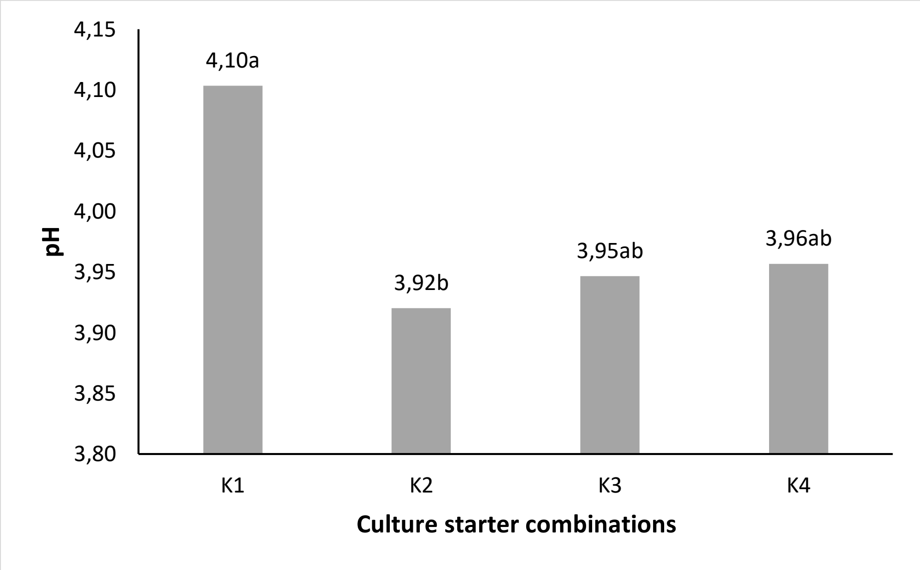

Fig. 2. The Effect of The Combination of Yogurt Culture On pH of Yogurt

3.3 Viscosity

Viscosity of yogurt from various culture starter combinations are summarized in Figure 3. There is no significant difference of viscosity in all yogurt samples. K1 yogurt has the highest viscosity, 31,47 cP followed by K2 yogurt 29,33 cP and K3 yogurt 28,00 cP. The least viscosity showed by K3 yogurt, 28,00 cP. These results were also reported in another study which uses pineapple extract to produce yogurt (29,33 cP) by using *L. bulgaricus*, *S.thermophilus*, and *L.achidophylus* starter [6]. The viscosity of yogurt in this research is related to its water content [11]. Pineapples have a great amount of water content 82,86-85,3 percent [12] thus it has low viscosity.

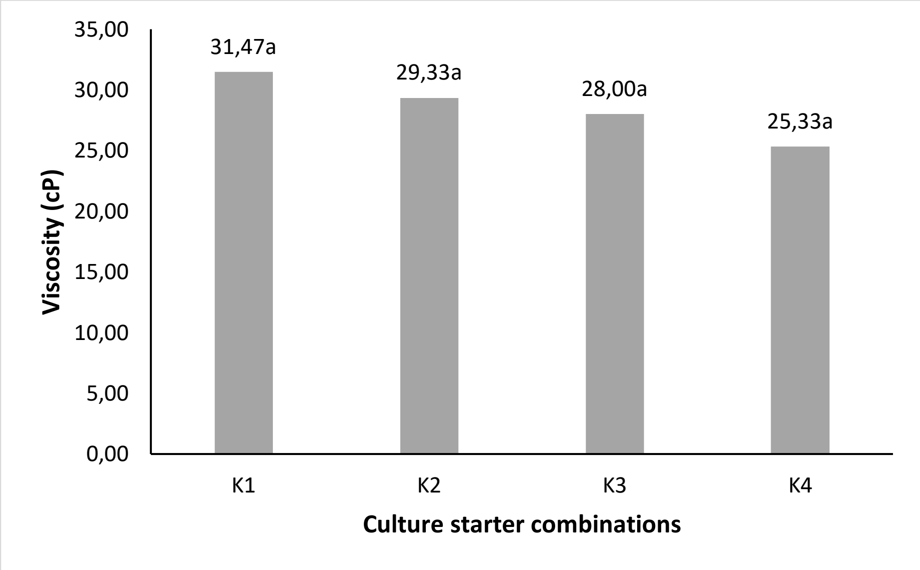

Fig. 3. The Effect of The Combination of Yogurt Culture On pH of Yogurt

3.4 Total Lactic Acid Bacteria

Figure 4 shows the number of total LAB in yogurt from various culture starter combinations. The yogurt produced from K1 combination has the highest number of total LAB (11.05 log CFU/ml) while K4 combination yogurt has the lowest number of total LAB (10.48 log CFU/ml). Overall, there is no significant effect on total LAB number in yogurt made from various culture starter combinations. This means all the starter bacteria can grow well and fermented pineapple extract to produce yogurt. Similarly, [6] used pineapple extract to produce yogurt using *Lactobacillus bulgaricus*, *S. thermophilus*, and *Lactobacillus acidophilus* starter and got the total number of LAB growth in yogurt as 10,66 Log cfu/ml. The high amount of total LAB in these yogurt can be caused by the addition of *E. spinosum* seaweed as a natural stabilizer. Total LAB in yogurt become higher following the more *E. spinosum* concentration added [13]. The cell wall of the seaweed *E. spinosum* composed of carrageenan, a polymer consists of a variety of carbohydrates, including oligosaccharides. As already known, oligosaccharides are categorized as prebiotics because they feed and support the growth of beneficial bacteria in the intestines [14]. The total number of LAB from K1, K2, K3 and K4 achieve the required standards of SNI 2981:2009, which states that yogurt should contain at least 10^7 CFU/ml or 7 log CFU/ml of total LAB.

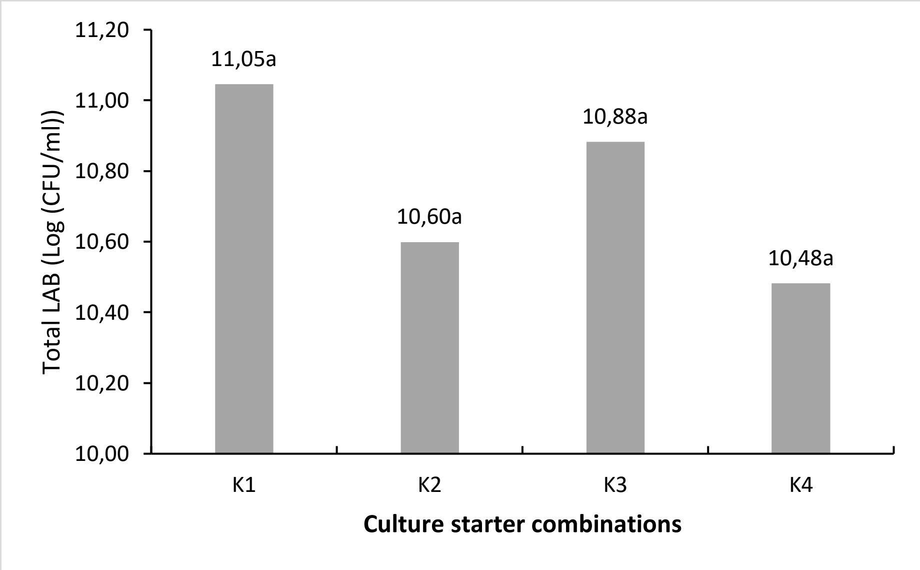

Fig. 4. The Effect of The Combination of Yogurt Culture on Total LAB of Yogurt

3.5 Viability

The number of bacteria endured after incubation in bile salts are shown in Table 1. Result shows all the samples have high numbers of bacteria even after contact with bile salt, ranging between 8,45 log CFU/ml (K2) and 9,55 log CFU/ml (K3). Despite this, all the samples have decreased numbers of LAB bacteria from theirs initial number, varying from the least as 0,02 log CFU/ml (K3) to the greatest decrease as 0,94 log CFU/ml (K2), but the number of surviving cells are still in accordance with the WHO's viability criterion of 7 log CFU/g while passing gastric acid. The survival rates of LAB are 9,55 log CFU/ml, 9,39 log CFU/ml, 9,15 log CFU/ml, and 8,45 log CFU/ml respectively for K3, K1, K4, and K2. K2 has the lowest initial number of colonies and even has the highest decrease number, as a result it has the lowest number of bacteria after incubation with bile salts. All the starter bacteria combinations used to produce yogurt in this research were able to remain alive while passing through intestinal tract that contains bile salt and stomach acid. [15] also reported the resistance of *Lactobacillus acidophilus* in fermented pineapple juice against stress factor 0,6% bile salts. Some authors also reported the resistance of fresh probiotic bacteria against gastric stresses [16] & [17].

Table 1. The Effect of The Combination of Yogurt Culture on Bacterial Viability of Yogurt

Culture starter combinations	Number of initial colonies (Log CFU/ml)	Final colony count after incubation with bile salts (Log CFU/ml)	Decreased viability of Lactic Acid Bacteria (Log CFU/ml)

K1	9,91	9,39	0,52
K2	9,39	8,45	0,94
K3	9,57	9,55	0,02
K4	9,67	9,15	0,52

3.6 Organoleptic

Table 2. shows that the combination of culture treatments did not have a significantly different effect on the taste and aroma of hedonic honey pineapple yogurt. The hedonic values obtained are 2.74-3.00 for taste and 2.61-3.04 for aroma with the criteria of somewhat liking. This is due to the taste and aroma of the resulting yogurt tends to be slightly sour. This shows that the use of *L. acidophilus* culture causes the yogurt to taste more sour than yogurt without the addition of *L. acidophilus* culture. The sour taste that arises in the manufacture of yogurt is caused by the fermentation process of lactic acid bacteria. According to [18], the distinctive taste that arises from yogurt is usually due to the presence of lactic acid, acetic acid, carbonyl, acetaldehyde, acetone, acetoin, and diacetyl. The results of [19] research showed similar results. The combination of *L. bulgaricus*, *S.thermophilus* and *L. plantarum* cultures did not have a significant effect on the characteristics of the assessment and the preference for the aroma of yogurt. *L. bulgaricus* bacteria are more involved in the formation of aroma, while *S. thermophilus* is more involved in the formation of flavor [20]. The results of [7] showed that partial substitution of yogurt-making cultures with probiotic bacterial cultures of *L. acidophilus* and *L.plantarum* resulted in a greater amount of titrated acid compared to yogurt cultures without probiotic bacteria. The total titrated acid showed a difference, but statistically the panelists did not care about the intensity of the sour taste in the resulting yogurt.

Table 2. The Effect of The Combination of Yogurt Culture on Organoleptic Preference of Yogurt

Culture starter combinations	Number of initial colonies (Log CFU/ml)	Final colony count after incubation with bile salts (Log CFU/ml)	Decreased viability of Lactic Acid Bacteria (Log CFU/ml)
K1	9,91	9,39	0,52
K2	9,39	8,45	0,94
K3	9,57	9,55	0,02
K4	9,67	9,15	0,52

4 Conclusion

Yogurt with the use of a combination of K3s (2:2:1) cultures was the best treatment because it was able to produce yogurt with a high amount of LAB and had the highest viability of live bacteria. In addition, the resulting pH is also close to a good yogurt pH classification, which is 4 and is not significantly different from the pH in the K1 treatment. The organoleptic parameters in terms of taste and aroma were also not significantly different from other treatments with the criteria of moderate liking.

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