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Study of Pineapple Subgrade Substitution and Fermentation Time on the Physicochemical and Microbiological Characteristics of Cowpea-Based *(Vigna unguilata)*-Based Probiotic Yoghurt

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A B S T R A C T

Yoghurt is pasteurised animal milk, then fermented with lactic acid bacteria until the desired acidity, smell, and taste are achieved. The main ingredients for making yoghurt in this study are cowpea and pineapple. Cowpea (Vigna unguiculata L.) is a high-protein local food, ranking after soybeans. Subgrade pineapple is a smaller pineapple fruit with a length ≤ 9.9 cm and a weight ≤ 0.4 kg, which is rich in antioxidants and has a high vitamin C content. The purpose of this study was to analyse the effect of subgrade pineapple substitution and fermentation time on the physicochemical and microbiological characteristics of cowpea probiotic yoghurt, and then determine the best treatment combination of these variations. The method used was a Completely Randomised Design (CRD) factorial pattern, with Factor I being subgrade pineapple juice substitution (10%, 20%, and 30%) and Factor II being fermentation time (15 hours, 20 hours, and 25 hours). The data obtained were analysed using ANOVA followed by DMRT, further tests at the 5% level. The results showed a significant interaction ($p \leq 0.05$). Yoghurt with 30% subgrade pineapple substitution and 20 hours fermentation time treatment is the best treatment that produces yogurt with a total acid value of 1.10%, pH value of 3.53, viscosity of 462.2 mPa.s., vitamin C 37.75 mg/100ml, total LAB 8.44 log CFU/ml, antioxidant 33.90 mg AEAC/g, protein 0.70%.

Contribution to Sustainable Development Goals (SDGs)
SDG 2 – Zero Hunger
SDG 3 – Good Health and Well-being
SDG 12 – Responsible Consumption and Production
SDG 9 – Industry, Innovation, and Infrastructure

1. INTRODUCTION

1.1. Research Background

Technological developments and changes in consumer diets have led to an increased demand for yoghurt, thereby encouraging the production of more varied yoghurt, both in terms of type, texture, aroma, and taste. The concept of probiotic foods is believed to have a beneficial effect on consumers in terms of nutrition and health [1]. In general, the basic ingredient in making yogurt is cow's milk. But nowadays, the use of animal-based cow's milk can be replaced with several other types of milk, such as vegetable milk. The advantage of the main vegetable ingredients themselves is that they are easily available and the price is cheaper than animal ingredients. However, yogurt processing with vegetable raw materials is not yet widely on the market.

Cowpea is a type of legume that serves as a source of vegetable protein and is widely cultivated in Indonesia. Cowpea has the opportunity to become a raw material for vegetable yogurt

because cowpea contains a lot of nutritional content, even in 100 grams of cowpea material, it contains 24.4 g protein, 56.6 g carbohydrates, 1.9 g fat, 481 mg calcium, 399 mg phosphorus, 481 mg calcium and 2.68 g phytic acid [2].

In the manufacture of vegetable-based yoghurt, fruit substitution is useful for improving the taste, colour, and increasing the nutritional content of the final product. Pineapple fruit has a sweet and sour fresh taste that is favored by the public. Pineapple contains several micronutrients, including vitamin C [3], and is rich in antioxidants [4]. Subgrade pineapple is a smaller pineapple that is not included in the SNI grade pineapple standard. Subgrade pineapple is characterized by a size of about 200 to 400 grams [5], usually available 10-15% of the total harvest [6].

Fermentation time is the time required by a microorganism to break down materials into simpler ones. The length of fermentation will affect the pH, total bacteria, lactic acid, and final yoghurt yield [7].

1.2. Literature Review

Yoghurt is a type of drink with a sour taste and thick texture. Yoghurt contains beneficial lactic acid bacteria that can support a healthy body when consumed regularly. Generally, the raw materials used in making yogurt are animal milk such as cow's milk and goat's milk. Animal milk yogurt has several disadvantages. These weaknesses include low antioxidant levels, high milk prices, and high cholesterol levels of around 9.2-9.9% [8]. Probiotics are live microorganisms that, when consumed in adequate amounts, provide health benefits to their hosts. Probiotics are typically found in fermented foods, such as yoghurt, kefir, tempeh, and kimchi, as well as in supplement form [9]. Probiotic drinks should contain a minimum of 106 to 109 CFU per mL or per g at the time of consumption. This amount is considered sufficient to provide significant health benefits [10].

Cowpea is a type of legume that serves as a source of vegetable protein and is widely cultivated in Indonesia. The protein content of cowpea is 22.90% while soybean is 34.90% and mung bean is 22.20%. This data shows that cowpea is the second-highest-protein bean after soybean [11]. According to the Indonesian Nutritionists Association (2010), cowpea contains many nutrients. Even in 100 grams of cowpea material, it contains 24.4 g of protein, 56.6 g of carbohydrate, 1.9 g of fat, 481 mg of calcium, 399 mg of phosphorus, and 2.68 g of phytic acid. In addition to having a fairly high nutritional content, cowpea also offers other advantages, including a low fat content, which can minimise the negative effects of consuming fatty food products [12].

Pineapple is one type of tropical fruit that is widely consumed by domestic and foreign communities. Based on pineapple varieties, several types of pineapple groups are cultivated, including the Cayenne group, Queen group, Spanish group, and Abacaxi group [13]. Pineapple contains several micronutrients, including vitamin C [14], and is rich in antioxidants [15]. This pineapple fruit can be processed into various processed food ingredients [16]. Subgrade pineapple is a smaller pineapple that is not included in the SNI pineapple grade standard. Subgrade pineapple is characterised by a size of around 200 to 400 grams [5], and is usually available at 10-15% of the total harvest [6]. Referring to the Directorate of Fruit Crop Cultivation (2010), Queen pineapple is divided into 4 quality standard grades, namely: super, A, B, and C, where the distinguishing factor between these grades is only the size and weight of the fruit, namely grade B is 10-13.9 cm long and weighs 0.4-0.69 kg, while grade C is ≤ 9.9 cm long and weighs ≤ 0.4 kg.

1.3. Research Objective

The purpose of this study was to investigate the effect of pineapple subgrade substitution and fermentation time on the physicochemical and microbiological characteristics of cowpea probiotic yoghurt, and to determine the optimal treatment combination of these variations.

2. MATERIALS AND METHODS

2.1. Material

The ingredients used to make cowpea probiotic yogurt are cowpea (vigna unguilata) obtained from the soponyono traditional market, Surabaya, pineapple variety queen grade C obtained from Ngancar Village, Kediri Regency, NZNP brand skim milk, sucrose, Lacticaseibacillus casei bacteria, Lactobacillus bulgaricus, Streptococcus thermophilus obtained from Food Nutrition Culture Collection, Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Bacterial growth media namely MRS (Man Rogosa Soyprotein) Agar and MRS (Man Rogosa Soyprotein) brooth for Lacticaseibacillus casei FNCC-090 growth media. Materials for analysis include distilled water, NaOH, HCI, FeCl3.6H_2O, MRS Agar, NaCl, phosphate (0.2 M pH6.6), Na K. tartarate 1%, Folin-Ciocalteau, Bovine Serum Albumin Solution, Acetate Buffer Solution Ph 3.6, TPTZ, FeCl.6H20, Phenolphthalein Indicator, 1% Starch Solution, 0.01 N iodine standard solution.

2.2. Research procedures

The experimental design used in this study was a completely randomized design (CRD) factorial pattern consisting of 2 factors, namely subgrade pineapple substitution and fermentation time , each consisting of 3 levels of differences in subgrade pineapple substitution (10%, 20%, and 30%) and fermentation time (15 hours, 20 hours, and 25 hours) and 2 replications. The data obtained were analyzed using ANOVA (Analysis of Variance) with a 5% confidence level/interval to determine the presence of a real interaction between treatments. If there is a significant difference in the treatment, then a further test using the DMRT (Duncan's Multiple Range Test) is carried out at the 5% level.

2.3. Preparation of cowpea juice

The cowpeas are washed thoroughly. Soak the cowpeas in a water : cowpea ratio (2:1) for 8 hours. The soaked beans are skinned and separated. The cowpeas are pulverized using a blender with a ratio of water: beans (4:1). Then the cowpea is filtered to extract the filtrate. The cowpea juice was obtained.

2.4. Making Subgrade pineapple juice

The subgrade pineapple was peeled and the skin and eyes removed. The subgrade pineapple was weighed, washed, and cut into approximately 2x2x1.5 cm pieces. The pineapple pieces were then blended for 10 minutes with a 2:1 ratio of water to subgrade pineapple. The resulting subgrade pineapple juice was then heated to 70 °C for 5 minutes.

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2.5. Making starter

Lactobacillus bulgaricus bacteria were taken from Evendoff, then inoculated into 5 mL of MRS Broth, and incubated at 37 °C for 24 hours.

2.6. Preparation of cowpea probiotic yogurt

Cowpea juice with 100 ml pineapple subgrade (10%, 20%, and 30%), 5% sucrose and 5% skim milk were mixed in a pot and stirred well. The mixture was pasteurized at 80-85ºC for 15 minutes and cooled to room temperature and transferred to a sterile container. Inoculated with 10% stater and stirred well. Incubated for (15 hours, 20 hours, and 25 hours) at 37 ºC. The yoghurt that had been formed was then analysed for the number of lactic acid bacteria, total lactic acid, pH, protein content, antioxidant activity, vitamin C content, and viscosity.

3. RESULT AND DISCUSSION

3.1. Physicochemical and microbiological characteristics of cowpea probiotic yogurt

Table 1. Analysis results of total Lactic Acid Bacteria (LAB), total acid, pH, viscosity of cowpea probiotic yogurt

Treatment Pineapple: Fermentation	Total LAB (log CFU/ml)	Total acid (%)	pH	Viscosity (mPa.s.)
10% : 15 h	8.20	0.85	4.24	438.3
10% : 20 h	8.38	0.98	3.78	451.5
10% : 25 h	8.33	0.93	3.92	444.1
20% : 15 h	8.30	0.87	4.21	439.7
20% : 20 h	8.42	1.01	3.69	454.8
20% : 25 h	8.36	0.95	3.89	453.2
30% : 15 h	8.36	0.92	4.07	442.3
30% : 20 h	8.43	1.1	3.53	462.2
30% : 25 h	8.36	1.07	3.60	457.3

Total LAB showed that the higher the substitution of pineapple subgrade, the higher the total LAB value of cowpea probiotic yogurt. The longer fermentation can also increase the total LAB value until the 20th hour, but the total LAB at the 25th hour decreased. Substitution of pineapple subgrade with high concentration can increase the total LAB value. The addition of fruit pulp or juice can enhance the viability of probiotics in yoghurt due to the presence of natural sugars (such as glucose and fructose), vitamins, minerals, and polyphenols, which can support microbial growth and activity during fermentation and storage [17]. The value of total LAB increased along with the increase in fermentation time. The increase in total LAB occurred because nutrients used as energy sources for LAB growth in the fermentation process were still abundant. However, in the treatment with a 25-hour fermentation time, the total LAB count decreased. This is because the longer the fermentation, the lower the availability of nutrients in the substrate, which inhibits the growth of lactic acid bacteria and creates competition for nutrients among microbes due to limited resources [18].

The total acid results showed that the higher the substitution of pineapple subgrade, the higher the total acid value of the cowpea probiotic yoghurt. The longer fermentation can also increase the total acid value until the 20th hour, but the total LAB count decreases at the 25th hour. The higher the concentration of pineapple juice added to soybean juice, the greater the increase in acidity. This is because pineapple contains various organic acids, such as citric acid, which accounts for 78% of the total acid, as well as other acids, including oxalic acid, malic acid, and ascorbic acid [19]. Increasing the length of fermentation will cause changes in chemical properties that affect the increase in the number of microbes, enabling them to produce more lactic acid [20]. The treatment of the 25th hour of fermentation decreased the total acid. The decrease that occurred during the 25-hour fermentation period was attributed to the decline in total lactic acid bacteria, resulting in a decrease in total acid [21].

The pH results showed that the higher the substitution of pineapple subgrade, the lower the pH value of the cowpea probiotic yoghurt. The longer fermentation can also reduce the pH value until the 20th hour, but the total pH increases at the 25th hour. This is because there is a negative correlation between total LAB and lactic acid content, as well as between lactic acid content and the pH of yoghurt. This means that a decrease in the amount of LAB results in a reduction of lactic acid production, which in turn causes an increase in the pH of yoghurt [21].

Viscosity results showed that the higher the substitution of pineapple subgrade, the higher the viscosity of the cowpea probiotic yoghurt. The longer fermentation can also increase viscosity until the 20th hour, but the viscosity decreases at the 25th hour. The increase in yoghurt viscosity is caused by the lower pH of the yoghurt, which leads to protein clumping [22]. In addition, several factors affect the viscosity of yoghurt, including pH, protein content, strain culture type, incubation time, and total milk solids [23].

Table 2. *Analysis Results of Vitamin C, antioxidant, and soluble protein of cowpea probiotic yogurt*

Treatment Pineapple: Fermentation	Vit C (mg/100ml)	Antioxidant (mg AEAC/g)	Soluble Protein (%)
10% : 15 h	31.41	29.0	0.73
10% : 20 h	31.94	30.1	0.76
10% : 25 h	31.67	29.3	0.75
20% : 15 h	34.58	31.2	0.67
20% : 20 h	35.10	31.7	0.73
20% : 25 h	34.84	31.5	0.71
30% : 15 h	36.95	32.5	0.65
30% : 20 h	37.74	33.9	0.69
30% : 25 h	37.22	33.5	0.66

Vitamin C showed an increase along with the increase of subgrade pineapple substitution. This is because the vitamin C contained in pineapple is higher than the vitamin C contained in cowpea so that vitamin C will increase along with the increasing concentration of subgrade pineapple substitution. The treatment of fermentation time did not have a significant effect on the vitamin C content of yoghurt. This is because Lactic Acid Bacteria (LAB) do not produce vitamin C (ascorbic acid) as the main result of fermentation.

The study showed that the higher the substitution of pineapple in the subgrade, the higher the antioxidant content of cowpea probiotic yoghurt. The longer fermentation can also increase antioxidants until the 20th hour, but antioxidants decrease at the 25th hour. This is because subgrade pineapple has a high content of vitamin C and phenolic compounds, which contribute to antioxidant capacity [24]. A longer fermentation time will provide sufficient time for bacteria to produce bioactive compounds, including phenolic components, which increase the antioxidant potential of yoghurt [26]. However, at the 25th fermentation time the antioxidants decreased. This is because

fermentation can increase the bioavailability and effectiveness of antioxidants; however, prolonged fermentation times can cause degradation of bioactive compounds [25].

Soluble protein shows that the higher the substitution of subgrade pineapple, the lower the soluble protein of cowpea probiotic yogurt. While the longer fermentation can increase the soluble protein until the 20th hour, but the soluble protein at the 25th hour decreased. This is because cowpea has a higher protein content than subgrade pineapple, so the proportion of cowpea affects the amount of soluble protein in yoghurt. The increase in soluble protein, along with the increase in fermentation time, occurs because the number of bacterial cells influences protein levels. An increase in the number of bacterial cells will increase proteolytic activity and protein synthesis, including the production of protease enzymes [27]. However, there was a decrease in soluble protein at the 25th hour of fermentation. This is because fermentation by lactic acid bacteria can increase protein availability, but excessive fermentation can reduce protein solubility due to denaturation [28].

4. CONCLUSION

There was a significant interaction between the treatment of subgrade pineapple substitution and fermentation time on the treatment of protein content, antioxidant content, total lactic acid, pH, total LAB, and viscosity of cowpea probiotic yogurt. However, there was no significant interaction on the vitamin C content of cowpea probiotic yogurt. Probiotic cowpea yogurt with 30% subgrade pineapple substitution and 20 hours fermentation time treatment is the best treatment that produces yogurt with a total acid value of 1.10%, pH value of 3.53, viscosity of 462.2 mPa.s., vitamin C 37.75 mg/100ml, total LAB 8.44 log CFU/ml, antioxidant 33.90 mg AEAC/g, and protein 0.70%.

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