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CHEMICAL CHARACTERISTICS DURING THE FERMENTATION PROCESS OF SIAM KINTAMANI ORANGE PEEL (*Citrus nobilis)* PROBIOTIC DRINK

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Abstract: Functional foods containing bacteria that may improve digestive function are called probiotic beverages. The fruit is rich in vitamins, minerals, antioxidants, and bioactive substances that benefit the body's health. Therefore, it can be used to make non-dairy probiotic drinks. Kintamani is a province in Bali that produces fruit, namely Siam oranges. In 2021, citrus fruit production in Kintamani, Bangli, is the second highest, at 104,528 per ton. However, there are still damaged fruits due to poor post-harvest processing and management. Orange peel is one of the citrus wastes that is not utilized, but this waste can still be processed into food with good nutritional value. The skin of Siam oranges contains secondary metabolites composed mainly of flavonoids, phenols, steroids, and triterpenoids. Furthermore, the Kintamani orange peel has better antioxidant potential than its seeds. Metabolites derived from Siam Kintamani orange peel have the potential to be used as probiotic beverages. A SCOBY (Symbiotic Culture of Bacteria and Yeasts) starter produces Siam orange peel probiotic drink. SCOBY is a gel layer formed from a mixture of bacteria and yeast. The desired outcome of this study was to assess the total acid, degree of acidity, pH, vitamin C, and total reducing sugar of Siam Kintamani orange peel as a result of fermented probiotic drinks. This is an experimental study with an entirely randomized approach. The treatment in this study was a variation in fermentation length, including several days (0, 3, 6, 9, 12, 15, and 18 days), with 4 repetitions. ANOVA was used to analyze the data, followed by a multiple-range test based on Duncan's equation, the fermentation time had a substantial impact on the total acid, pH, vitamin C, and total flavonoids of the Siam Kintamani orange peel probiotic drink (*Citrus nobilis*) ($p<0.05$). The time of the fermentation process influences the entire amount of acid, pH, vitamin C levels, and quantity of flavonoids in the Siam Kintamani orange peel probiotic drink. The longer the fermentation process, the more the total acid value increases while the pH decreases. The highest content of vitamin C and total flavonoids was produced on the ninth day of fermentation, at 70455.33 mg/100 ml and 25.46 mg QE/100 g, respectively.

Keywords: *Fermentation, Orange Peel, Probiotic Drink, Beneficial Food*

INTRODUCTION

The growing public interest in improving health and well-being requires the food industry to produce natural-based food products. Along with this, the challenge for the food industry is to develop food made from new raw materials that have a variety of phytochemical content and are beneficial to health. One of the beverage products currently being developed is probiotic food and drink. Probiotic beverages are functional foods with beneficial bacteria that can help digestive health [1].

Most probiotic drinks produced are made from milk and its derivatives. However, there are still many people who cannot consume probiotics. They have a history of milk allergies and lactose intolerance. Currently, what is being developed is a new approach to probiotics, which is a fruit-based, non-dairy drink [2]. Fruit has refreshing benefits and contains many nutrients for health. As a result, it is extensively utilized as a component in non-dairy probiotic beverages. Fruit-based beverages are a good supply of vitamins, minerals, antioxidants, and bioactive substances that have compounds beneficial to the body's health. The fermentation process during the production of fruit probiotic drinks can increase the shelf life of drinks, improve the nutritional and functional characteristics, and benefit health [3]. Fruits used as probiotic drinks are soursop juice, Balinese salak, guava, pineapple, and others.

Kintamani is a province in Bali that produces fruit, namely Siam oranges. In 2021, citrus fruit production in Kintamani, Bangli, is the second highest, 104.528 per tonne [4]. However, there are still damaged fruits due to improper processing and poor post-harvest management. In addition, types of organic waste that are not utilized in processed oranges include peels, pulp, and seeds [5]. Meanwhile, the waste can still be processed into food with good nutritional value. The utilization of waste on Siam oranges has been developed by utilizing Siam Kintamani orange peels as tea bags [6].

Siam Kintamani orange peel contains secondary metabolites such as flavonoids, phenols, steroids, and triterpenoids. The content of metabolite compounds in Siam orange peel has the potential to be used as a probiotic drink. In this study, the manufacture of a probiotic drink from Siam orange peel was carried out by a fermentation process using a

SCOBY (Symbiotic Culture of Bacteria and Yeasts) starter. SCOBY is a gel layer formed from a mixture of bacteria and yeast that live in the same colony. Bacteria present in SCOBY include acidic bacteria such as *Komagataeibacter, Gluconobacter*, and *Acetobacter*) [7], lactic acid bacteria such as *Lactobacillus, Lactococcus*) [8], and yeasts such as *Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata*, and *Saccharomyces cerevisiae* [9]. The presence of microbes that play a role in the beverage fermentation process can change the nature of the material so that it will produce fermented products that can be useful [10].

Secondary metabolites in Siam Kintamani orange peel include flavonoids, phenols, steroids, and triterpenoids. Siam orange peel contains metabolite chemicals that can be utilized as a probiotic drink. In this work, a probiotic drink was made from Siam orange peel utilizing a fermentation procedure and a SCOBY (Symbiotic Culture of Bacteria and Yeasts) starter. SCOBY is a gel layer generated by bacteria and yeast living in the same colony. Acidic bacteria such as *Komagataeibacter, Gluconobacter*, and *Acetobacter*) [7], lactic acid bacteria such as *Lactobacillus, Lactococcus*) [8]. Moreover, yeasts such as *Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata*, and *Saccharomyces cerevisiae* [9] are all found in SCOBY. The presence of microbial organisms that play a part in the beverage fermentation process might alter the character of the material, resulting in valuable fermented products [10].

The Siam Kintamani orange peel has been used as a natural pesticide, antifungal, and tea. Very few companies develop probiotic drinks based on local natural product waste. This study aimed to examine the chemical properties of fermented probiotic beverages such as entirety acid, grade of acidity (pH), vitamin C, and flavonoids of Siam Kintamani orange peel (*Citrus nobilis*).

RESEARCH METHODS

This is an experimental study that uses a fully randomized design. In this investigation, the treatment varied fermentation duration, specifically 0 days, 3 days, 6 days, 9 days, 12 days, 15 days, and 18 days, with 4 repeats.

Production of Siam Kintamani Orange Peel Probiotic Drink

A total of 1000 ml of water was pasteurized for 15 minutes at 80^0C, then added 10% (w/v) granulated sugar until dissolved, added 2.5% (w/v). Siam orange peel juice, and allow to stand for 5 minutes while stirring. Then it is cooled to 37^0C, and the SCOBY starter is put into the Siam orange peel drink, tied tightly using a sterile napkin, and left to stand at room temperature. In Siam orange peel juice, a cellulose layer will form, and this layer will be separated from the fruit juice. Siam orange peel extract is fermented for 18 days and harvested every 3 days by taking SCOBY and filtering it. Furthermore, the probiotic drinks were subjected to chemical tests.

Total Acid Test

To perform the total acid test, the probiotic drink sample was taken in as much as 10 mL and put into an Erlenmeyer flask to be titrated with 0.1 N NaOH. The indicator used was phenolphthalein 1%, with a color change from colorless to pink.

pH Test

pH testing was carried out using a standardized pH meter using a buffer solution at pH 4.01 and 6.86. ± 20 mL of drink samples were prepared, and the pH meter was dipped into the probiotic drink. The pH meter is set aside until it shows a stable number [11].

Test Vitamin C Levels

A reagent solution was prepared by mixing 500 ml of 0.6 M sulfuric acid with 5.322 g of sodium phosphate and 2.471 g of ammonium molybdate. 3 ml of reagent was mixed with 0.3 beverage samples and incubated at 95^0C for 90 minutes in a water bath. After incubation, it was cooled in water for 5 minutes before absorbance was measured at 695 nm. Results are expressed as ascorbic acid equivalents in mg/g [12].

Flavonoid Total Test

Determining total flavonoids using a spectrophotometer with the $AlCl_3$ method refers to [13]. According to the procedure, 0.01 g of extract was diluted into 5 ml of phosphate buffer citrate. In total, 4 ml of distilled water and 0.3 ml of NaNO2 solution (10%) were added to 1 ml of the sample. After adding 0.3 ml of $AlCl_3$ solution (10%) and 2 ml of NaOH solution (1%), it was then incubated for 5 minutes. After that, it was examined right away using a spectrophotometer at a wavelength of 510 nm. The flavonoid concentration in the test sample was calculated as C x V x FP W 37 from a calibration standard prepared using quercetin and expressed as quercetin equivalent in mg QE/g extract.

Data analysis

Analysis of variance (ANOVA) will be used to examine the findings of this study [14]. Do the Duncan's Multiple Range Test (DMRT) if the findings indicate a difference ($p < 0.05$).

RESULTS AND DISCUSSION

Total Acid and pH of Probiotic Drink Siam Kintamani Orange Peel (*Citrus nobilis*)

It takes 18 days for the Siam Kintamani orange peel probiotic drink to ferment. The total acid and pH test results are shown in Table 1 below.

Table 1. Total Acid and pH Test Results, Probiotic Beverages Siam Kintamani Orange Peel (*Citrus nobilis*)

Fermentation Day	Total acid (%)	pH
0	30.47 ± 0.09a	4.15 ± 0.05g
3rd	35.48 ± 0.07b	3.55 ± 0.05f
6th	38.38 ± 0.01c	3.30 ± 0.08e
9th	39.24 ± 0.01d	3.05 ± 0.15d
12th	44.45 ± 0.05e	2.77 ± 0.05c
15th	47.14 ± 0.03f	2.55 ± 0.12b
18th	52.06 ± 0.04g	2.12 ± 0.15a

Description: Different letters on treatment showed significantly different results ($p < 0.05$).

It is common for fermented beverages to undergo changes in chemical characteristics, such as total acid, during fermentation. The time of fermentation significantly influences the total acid of the Siam Kintamani orange peel probiotic drink ($p < 0.05$), as shown in Table 1. The presence of this substance in the form of acid will give probiotic drinks a particular flavor. During the fermenting phase, the total acid generated by the probiotic drink is made from Siam Kintamani orange peel. On fermentation days 0, 3, 9, and 12, the percentages were 30.47%, 35.48%, 38.38%, and 39.24%, respectively. On the 12th, 15th, and 18th days of fermentation, total acids of 44.45%, 47.14%, and 52.06% were generated. The larger increase in acid is assumed to be due to the bacteria in the probiotic drink going through a logarithmic growth phase.

In contrast, the bacteria synthesizing alcohol become more acidic, increasing the overall acid generated [15]. In addition to the duration of the fermentation period, the lactic acid bacteria present in probiotic beverages will have plenty of time to overhaul the nutrients in the substrate, allowing for the buildup of organic acids such as lactic acid in high quantities in probiotic drinks [16]. This causes the average total acid in the Kintamani Siam orange peel probiotic drink to rise as the fermentation process progresses. This, however, affects the pH of the drink. The more lactic acid the drink creates, the more H^+ ions will be released. This is because lactic acid is broken down into H^+ ions during fermentation, causing the pH of probiotic beverages to decrease [17].

The average pH value of the Siam Kintamani orange peel probiotic drink declined until the 18th day of fermentation, according to the data in Table 1. The fermentation period significantly affected the pH of the probiotic drink ($p < 0.05$), and the first day of fermentation had the highest average pH value of 4.15. The lowest average pH value recorded was 2.12 on the 18th day of fermentation. The pH will fall as the fermentation process continues. This is due to lactic acid bacteria breaking down carbohydrates into lactic acid [18]. Bacterial activity occurs, and acids are formed, which might induce a pH fall [19]. The pH decreased significantly from 4.15 to 3.55 during days 0 and 3 of fermentation. Because of the length of the fermentation, the microorganisms in the probiotic beverages are in the adaption and logarithmic phases. Microbes experience rapid development during the logarithmic phase by converting the nutrients in probiotic beverages into lactic acid and other organic acids, reducing pH [20]. As a result, the longer the fermentation process, the sourer the drink's taste.

Flavonoid Total Test and Vitamin C Levels of Probiotic Drink Siam Kintamani Orange Peel (*Citrus nobilis*)

The results of the vitamin C and flavonoid tests of the probiotic drink from the Siam Kintamani orange peel are shown in Table 2 below.

Table 2. Test Results for Flavonoids and Vitamin C for Probiotic Beverages Siam Kintamani Orange Peels (*Citrus nobilis*)

Fermentation Day	Total of Flavonoid (mg QE / 100g)	Vitamin C (mg/100 ml)
0	15.44 ± 0.02a	50789.41 ± 0.47a
3rd	21.84 ± 0.02b	51687.33 ± 0.06b
6th	22.59 ± 0.04c	55713.41 ± 0.08c
9th	25.46 ± 0.02g	70455.33 ± 0.12d
12th	24.85 ± 0.04f	48074.51 ± 0.20e
15th	24.50 ± 0.24e	43672.94 ± 0.03f
18th	23.85 ± 0.03d	42242.90 ± 0.06g

Description: Different letters on treatment showed significantly different results ($p < 0.05$).

Flavonoids are a kind of secondary metabolite generated by plants that function in free radical capture [21]. According to the research findings, total flavonoids rose until the ninth day of fermentation, ranging from 15.44 mg QE / 100g to 25.46 mg QE / 100g, as shown in Table 2. Polyphenolic chemicals in Siam orange peels are biodegraded into more simple molecules, and enzymes released by yeast and bacteria in SCOBY can boost flavonoid levels [22]. Furthermore, a rise in the quantity of phenolic compounds might occur due to sugar hydrolysis by yeast and enzymes produced by lactic acid bacteria during fermentation [23]. Total flavonoids began to decrease after the ninth day of fermentation. The flavonoids varied from 24.85 mg QE / 100g to 23.85 mg QE / 100g from the 12th to the 18th day of fermentation.

Vitamin C is a metabolic product and a natural antioxidant in probiotic drinks. According to the data in Table 1, vitamin C levels in the probiotic drink made from Siam Kintamani orange peel increased from 0 to the ninth day of fermentation. The optimal vitamin C concentration was 70455.33 mg/100 ml on the 9th day of fermentation and declined to 48074.51 mg/100 ml on the 12th day of fermentation until the

18th. Unstable vitamin C content in probiotic drinks can be caused by a too-acidic pH, which can reduce vitamin C content [24]. Vitamin C is stable in acidic conditions with a pH range of 3-6 [25].

In fermented beverages, *Saccharomyces cerevisiae* is involved in converting glucose to alcohol. If no oxygen is present, this facultative anaerobic bacterium will create ethanol as the ultimate result of a fermented beverage. These organisms, however, will convert sucrose to water and carbon dioxide if oxygen is available. Ascorbic acid (Vitamin C) is formed when carbon dioxide reacts with water. Because the bacteria have run out of food, the longer the fermentation lasts, the more the sugar content is reduced, leading the vitamin C level to decline after reaching the optimal point [26].

CONCLUSION

The time of the fermentation process influences the total acid, pH, vitamin C levels, and total flavonoids of the Siam Kintamani orange peel probiotic drink. The total acid value increases as the fermentation process continues, but the pH lowers. The maximum levels of vitamin C and total flavonoids were formed on the ninth day of fermentation, with 70455.33 mg/100 ml and 25.46 mg QE/100g, respectively.

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