Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 1 January 2024

Research Article

Formulation and Characterization of Instant Probiotic Powder from Orange Sweet Potato (*Ipomoea batatas* L) Using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* Bacterial Cultures

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DOI: 10.31080/ASMI.2024.07.1329

Received: November 27, 2023

Published: December 13, 2023

Abstract

Probiotic yoghurt is a functional food product that boosts the immune system from a combination of fermented yoghurt and milk with probiotic bacterial cultures. The bacteria used to manufacture probiotics are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Orange sweet potato is rich in beta-carotene and can be used as an alternative ingredient for making probiotic drinks. Instant powder drink is an easy food product to serve; just add hot or cold water and stir until it dissolves in water. This research aims to make an instant probiotic drink from orange sweet potato pollen (Ipomoea batatas L) with the bacterial culture *L. bulgaricus* and *S. thermophilus*. The method used is laboratory experimental. The first stage is making an orange sweet potato probiotic yoghurt drink. The second stage is making an instant probiotic drink from orange sweet potato pollen with the addition of maltodextrin and characterization of the instant probiotic drink from orange sweet potato pollen. The results of the study showed that the instant probiotic drink of orange sweet potato pollen with the addition of maltodextrin had a water content of 2.97%-3.35%, flow rate of 0.38–0.62 gram/second, angle of repose between 20.39°- 29.83°, compressibility index in the range 32.28%-38.14%, and chemical quality with acidity (pH) results ranging between 3.38-3.68 and total lactic acid namely 0.97% -2.57%; The total BAL value ranges from 3.40×10^4 CFU/mL – 5.95×10^4 CFU/mL. The orange sweet potato juice probiotic drink can be formulated into powder with good physical quality testing (water content, flow rate, angle of repose, and compressibility index) and good chemical quality (pH and total acid), but the total BAL value does not meet the requirements SNI 7552: 2009.

Keywords: *Ipomoea batatas* L; Instant Probiotic Drink; Maltodextrin; Freeze-drying

Introduction

Functional food naturally contains one or more compounds with specific physiological functions that are beneficial for health [1]. Probiotics are live microbial food supplements that benefit the host by improving the balance of intestinal microbes. LAB probiotics belong to the genera *Lactobacillus*, *Bifidobacterium, Enterococcus, Lactococcus, Streptococcus, and Leuconostoc*. Probiotics are consumed as yoghurt, fermented milk, or other fermented foods. This product containing live microorganisms has been used traditionally to restore intestinal health [2]. Yoghurt results from milk fermentation with lactic acid bacteria such as *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*. These bacteria support each other's growth in the fermentation process and play a role in forming the texture and taste of yoghurt and influencing the quality of yoghurt [3]. However, of the several species of bacteria, there are two bacteria which are the best combination of cultures for making yoghurt because there is mutual symbiosis during the yoghurt fermentation process. The two bac-

teria are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, where *L. bulgaricus* will produce amino acids and short peptides that can trigger the growth of *S. thermophilus* and *S. thermophilus* itself produces formic acid, which can help the growth of L. *bulgaricus* [4].

Sweet potatoes are a food ingredient that contains nutrients that are beneficial for health. Until now, its use has been limited to flour, sauce, chips or traditional processing. Compared to other food commodities such as cassava or corn, the nutritional content of sweet potato allows for the development of more valuable products, namely as ingredients for making probiotic drinks. Sweet potatoes with orange tuber flesh have a high β-carotene content. This compound is an antioxidant compound and provitamin A. The orange flesh of the tuber is rich in anthocyanins, also antioxidant compounds. Sweet potato extract containing antioxidant compounds can be used as a fermentation medium for probiotic bacteria, which are beneficial for digestive health. Combining several beneficial nutrients can make a reliable, functional food product. Based on previous research, the best formula for probiotic drinks is orange sweet potato juice with a ratio of probiotic bacterial culture: orange sweet potato juice, which is 50:50 [5].

Fresh yoghurt products stored at room temperature have a shelf life of around 1-2 days when packaged in glass bottles. Making yoghurt powder is an alternative to processing yoghurt so that it has a longer shelf life and can be stored at room temperature. Yoghurt powder is a product from fermented milk, which is then processed further through drying. Drying is the removal of the water content of a material using the principle of the difference in humidity between the drying air and the food being dried. It can inhibit or stop the development of microorganisms and enzyme activities that can cause spoilage, thereby reducing damage to the material and producing new products that can provide convenience in transportation [6].

Orange sweet potato powder probiotic yoghurt is a probiotic yoghurt product made from orange sweet potato, which is fermented with L. bulgaricus and S. thermophilus bacterial cultures and has been drying. Drying of probiotic yoghurt aims to extend shelf life and reduce product volume to facilitate handling, packaging, and distribution. Previous studies have shown that the shelf life can be maintained for more than 30 days in the form of powdered yoghurt, but it still has problems with low acceptability due to poor texture and solubility [7].

The human need for functional food ingredients is a consideration in developing probiotic yoghurt products, especially in the form of presentation of probiotic yoghurt products so that they can provide choices to the public in consuming these products.

Materials and Methods

Materials

The materials used in this research were the bacterial starter culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, sucrose, skim milk, maltodextrin, distilled water, MRS agar medium, phenolphthalein indicator 1%, NaOH 0.1 N and 0.9% NaCl.

Tools

The tools used in this study were autoclaves (Labtech), incubators (Memmert), biosafety cabinets (Biobase), digital pH meters (Hanna), colony counters (MRC), analytical balances (MRC), digital thermometers, room thermometers, hotplates (Favorites), micropipettes (Thermo Scientific), Erlenmeyer (Pyrex), measuring cups (Pyrex), test tubes (Pyrex), burettes (Pyrex), beakers (Pyrex), clamps, states, Petri dishes, loop needles, bunsen, stirring rod, Durham tube, microtip, watch glass, glass bottle, dropper pipette, spatula, blender, and filter cloth.

Method

Sample preparation

The sample used in this study was orange sweet potato tubers obtained from Pasir Batang Village, Manonjaya District, Tasikmalaya Regency, West Java. Collect the orange sweet potato tubers, peel the skin, and then wash the tubers in running water. Cut the tubers into several pieces, then puree and strain.

Making a bacterial starter

The manufacture of bacterial starter *Lactobacillus bulgaricus* and *Streptococcus thermophillus* comes from powder starter, which is temporarily inactivated, so it is necessary to reactivate the starter. The way that can be done is to prepare 500 ml of pure milk, pasteurize the milk at 80°C for 15 minutes, and let it cool down. Then, stir the milk with 500 mg powder in a sterilized jar. Place the jar in the incubator at 37°C for 24 hours [5].

Making orange sweet potato probiotic drink

The manufacture of the orange sweet potato probiotic drink begins by mixing 15% orange sweet potato extract with 3% sucrose and 7% skim milk and then mixing it with aquadest. Then pasteurize for 15 minutes at 80°C, then wait until it cools down; add 4% of the bacterial starter and stir until homogeneous. Place in a bottle and incubate at 37°C for 24 hours [5].

Making instant probiotic drink from orange sweet potato powder

The manufacture of the orange sweet potato powder instant probiotic drink begins with the manufacture of dry yoghurt using orange sweet potato juice probiotic drink, which is obtained then dried using a freeze dryer method. Probiotic powder formulations are made using a variety of filler ingredients. The complete formula can be seen in Table 1.

Table 1: Formula of probiotic yoghurt granules from orange sweet potato juice.

Ingredient	F1 (gram)	F2 (gram)	F3 (gram)	Function
Sweet potato yoghurt powder	10.0	20.0	30.0	Active ingredient
Sucralose	0.2	0.2	0.2	Sweetener
Maltodextrin	89.8	79.8	69.8	Filler

Testing the characteristics of instant probiotic drink orange sweet potato powder

Testing the characteristics of the orange sweet potato powder instant probiotic drink included organoleptic tests, pH, total lactic acid bacteria, and total lactic acid.

- Organoleptic tests of orange sweet potato juice probiotic powder include shape, odour, taste and consistency. Take a sufficient sample, place it in a watch glass, and observe the sample organoleptically.
- The pH test is used to determine a product's acidity or alkalinity level by first calibrating the pH meter with a buffer solution of pH 7 and 10. The tip of the electrode of the pH meter is dipped in the sample, and then looking at the results of the numbers printed on the tool.
- The total lactic acid bacteria (LAB) test used de Man Rogosa and Sharpe (MRSA) media. 1 mL of the sample was taken and put into a test tube, then 9 mL of distilled water was added and stirred until smooth. 0.1 mL of the dilution was born, and 9.9 ml of distilled water was added. This dilution was carried out until 10^8. From the dilution results in the last test tube, 1 mL was taken, spread over the MRSA medium, and then incubated for 24 hours at 37°C in an inverted position. The number of existing colonies is counted using a colony counter.
- The total lactic acid test was carried out by weighing 10 mL of the sample and then putting it into an Erlenmeyer, dissolving it using CO2- free distilled water two times the sample volume. Add two drops of 1% Phenolphthalein indicator and titrate with 0.1 N NaOH solution until a pink colour forms.

Powder evaluation includes five test parameters: water content, flow rate, angle of repose, determination of and compressibility index.

The water content was determined by heating the cup in the oven at 105°C for 15 minutes, then weighing it. Weigh the sample 2-3 grams and put it in a dried cup, and find the weight in the oven at 105°C for 3 hours. Transfer the sample containing the sample to the desiccator, cool it for 15 minutes and weigh the final weight. Calculate dry weight / dry base and wet/wet base weight [25]:

The flow speed and angle of repose tests were carried out by slowly inserting a 100-gram powder sample into a funnel placed on a stand and closing the hole. After the powder enters, the funnel hole is opened until all the powder passes through the funnel. Record the time, height of the pile and diameter of the pile formed from the granules. Calculate the angle of repose with the following formula [26]:

The test for determining the compressibility index The natural specific gravity was obtained by placing a sample of 20 grams in a measuring cup with a tilt angle of 45°, and then the measuring cup was straightened. Note the resulting volume and replicate it three times. Natural specific gravity is calculated based on the following formula [26]:

Incompressible specific gravity is obtained by placing a 20-gram granule sample in a measuring cup and then tapping at 50 to 200 times intervals. Record the volume produced from each knock. The following formula calculates incompressible specific gravity [26]:

After getting the natural specific gravity and compressed specific gravity, it can calculate the compressibility index with the following formula [26]:

Results and Discussion

Organoleptic testing of instant probiotic drink orange sweet potato powder

The organoleptic observations of the instant probiotic drink orange sweet potato powder showed that all formulas were organoleptically stable and had an orange-white colour. However, the powder produced in F3 was the brightest in colour because the concentration of F3 sweet potato yoghurt powder was the highest (30g) and 69.8g of maltodextrin (Figure 1). This results in the colour of the powder becoming brighter orange because yoghurt powder is orange, and maltodextrin is white [8]. All formulas have a slightly sweet taste due to sucralose, which has a sweet taste. The difference between maltodextrin and sweet potato yoghurt powder does not affect the taste of probiotic drink powder. According to the research results from Gabriela et al., maltodextrin will not change the sweet taste or make the product sweeter. So, in making the average product, there will be no difference in taste or, in other words, the treatment has no real influence. All instant probiotic drink formulas with orange sweet potato powder have a distinctive aroma from sweet potatoes and are the result of LAB metabolism in the fermentation process, and this is due to the use of sweet potato yoghurt powder. The orange sweet potato pollen instant probiotic drink can be seen in Figure 1.

Figure 1: Orange sweet potato pollen instant probiotic drink (a) Formula 1 (b) Formula 2 (c) Formula 3.

Chemical quality testing (pH and TAT) and microbiological quality (Total BAL)

The results of testing total lactic acid bacteria (LAB), total acid, and pH values in the instant probiotic drink orange sweet potato pollen with the addition of maltodextrin are presented in Table 2.

The degree of acidity (pH) is an essential factor affecting the quality of fermented beverages. The pH changes during the fermentation process of orange sweet potato juice into a fermented drink of orange sweet potato juice. Table 2 shows that orange sweet po-

Table 2: Test Results for Total Acid, pH and Total LAB with the Addition of Maltodextrin.

Evaluation	Formula 1	Formula 2	Formula 3	Category
pH	3.68 ± 0.04	3.53 ± 0.03	3.38 ± 0.06	-
Total acid (%)	0.97 ± 0.05	1.71 ± 0.03	2.57 ± 0.02	0.5-2% good category (SNI 01-2981-1992)
Total LAB (CFU/mL)	$3.40 \times 10^4 \pm 0.42$	$4.60 \times 10^4 \pm 0.42$	$5.95 \times 10^4 \pm 0.35$	$> 1 \times 10^6$ CFU/mL (SNI 7552: 2009)

tato juice variations do not affect the pH of the orange sweet potato pollen probiotic drink produced. The degree of acidity (pH) of the orange sweet potato instant probiotic drink ranges from 3.38 to 3.68. Based on the observation results, it is known that there is a decrease in pH in each formula. The decrease in pH is caused by hydrogen ions, which come from the breakdown of carbohydrate compounds in the fermented orange sweet potato juice drink into acidic compounds, which cause an accumulation of acid originating from BAL [9]. Treatment with adding more variations of orange sweet potato juice will increase the growth of lactic acid bacteria so that the total acid increases and the pH value will be lower. Orange sweet potatoes are high in sugar. This sugar plays a role in lactic acid bacteria in creating an acidic atmosphere by making these nutrients into food to become lactic acid and lowering the pH [10]. The availability of nutrients will increase the number of bacterial cells and impact the maximum breakdown of sugar so that the total acid will increase and the pH will decrease [11].

The amount of lactic acid produced in the fermentation process affects the pH value because it can dissociate into $CH3CHOHCOO^-$ and H^+ ions. The more H^+ ions produced, the more the pH value goes down [12]. This causes the total titrated acid (TAT) produced to increase. Total titrated acid is the amount of lactic acid formed during the fermentation process, which results from the breakdown of lactose by lactic acid bacteria. The titrated acid produced in this study was 0.97%-2.57%. This follows the lactic acid standard for yoghurt according to SNI 01-2981-1992, which is 0.5-2%. The sugar in orange sweet potato juice belongs to the monosaccharide group. Monosaccharides are more easily utilized by lactic acid bacteria than milk sugar lactose. Lactic acid is the final product produced from the metabolic process, resulting in an increase in acidity in the orange sweet potato pollen instant probiotic drink.

The total BAL produced continues to increase in the orange sweet potato juice variation. The total BAL of the orange sweet potato pollen instant probiotic drink produced ranged from 3.40×10^4 CFU/mL – 5.95×10^4 CFU/mL. An excellent total LAB in a fermented beverage product based on SNI 7552: 2009 is more significant than 1×10^6 CFU/mL. So, the BAL values in the three formulas decreased from the initial probiotic drink before the powder was made. The decrease in the viability of the two LAB can occur due to freezing using low temperatures in the freeze-drying process [13].

Physical quality testing of orange sweet potato pollen instant probiotic drink

Evaluation	Formula 1	Formula 2	Formula III	Category
Water content (%)	2.97 ± 0.02	3.17 ± 0.04	3.35 ± 0.05	3% good category (SNI 01-4320-1996)
Flow speed (g/s)	0.38 ± 0.00	0.52 ± 0.00	0.62 ± 0.00	< 4 g/detik good category (14)
Angle of respose (°)	29.83 ± 0.54	21.98 ± 0.27	20.39 ± 0.37	<20-30 good category (15)
CompresibilityIndeks (%)	34.06 ± 2.84	38.14 ± 4.76	32.28 ± 1.04	10-38% good category(16)

Table 3: Evaluation results of the physical properties of instant probiotic drink orange sweet potato pollen.

Water content is the main parameter in determining the quality of dry products; low water content can prevent the growth of destructive microorganisms, such as bacteria and fungi, which can damage the product (Fiana R). With a high water content value, the product will quickly be damaged due to destructive microbial and internal biological activity [17]. Water content plays a critical role in the characteristics of the powder because if the water content is high, it will disrupt the stability of the product, and it will clump during storage. Based on the data in Table 3, all samples have a water content value of 2.97% -3.35%. The water content value in all products is outstanding because, according to SNI 01-4320-1996, it is required that the water content of instant powdered drinks is no more than 3%. The concentration of maltodextrin is inversely proportional to the water content of the powder on F1-F3. The higher the concentration of maltodextrin, the lower the water content of the powder. In the F1-F3 formula, increasing the concentration of maltodextrin decreased the percentage of water content. This is because maltodextrin can increase the amount of free water that can be absorbed, thereby increasing the amount of water vapour that is evaporated during drying [18].

The flow properties test results of the instant probiotic drink with orange sweet potato pollen showed results of 0.38 – 0.62 grams/second, which means the powder can flow well. These re-

sults are by the literature on a good flow time of 4-10 grams/second. The flow speed will be related to the flow properties, and if the powder has large cohesiveness between particles, it will form large lumps, making it difficult to flow. If the powder has difficulty flowing, the flow speed will decrease. Powders with poor flow properties will affect the preparation process. Flow properties are influenced by the size and shape of the particles; more significant and rounder particles show better flow [19].

The compressibility index test, also called Carr's index, can be used to determine flow properties. A low compressibility index value of a material usually indicates better flow properties than a high compressibility index value. A compressibility index value of less than 10% indicates excellent flow, while a compressibility index of more than 38% indicates inferior flow. The compressibility test aims to determine whether the flow properties can form a stable mass. The results of the compressibility test of the orange sweet potato pollen instant probiotic drink had an average compressibility index value that was different in the range of 32.28% -38.14%, so the percentage compressibility index of all formulas was in a suitable category according to Carr's index. Formula 3 had the best flow properties because of the lowest compressibility index (32.28%) [20].

The angle of repose is a fixed angle between a pile of cone-shaped particles and a horizontal plane when a certain amount of powder or granules is poured into a measuring device. The shape, size and humidity of the powder/granule influence the size of the angle of repose [21]. A powder/granule has a perfect angle of repose if it is less than 30° and will flow freely, so it can be said that the orange sweet potato pollen instant probiotic drink has good flowing properties. The angle of repose test results for all formulas averaged a range of 20.39°- 29.83° (Table 3). F3 owns the lowest angle of repose. For this reason, the flow speed increases and the angle of repose decreases.

Conclusion

Orange sweet potato pollen instant probiotic drink can be formulated into powder with good physical quality with a water content of 2.97% -3.35%, flow properties of 0.38 – 0.62 grams/second, angle of repose between 20.39°- 29.83°, compressibility index in the range of 32.28%-38.14%, and good chemical quality with the resultant degree of acidity (pH) ranging from 3.38-3.68 and total acid lactate, namely 0.97%-2.57%; The total BAL value ranges from 3.40×10^4 CFU/mL – 5.95×10^4 CFU/mL, but does not comply with the requirements of SNI 7552: 2009.

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