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Inulin characterization from tuber of Musa balbisiana Colla as an alternative source of prebiotics

Karakteristik inulin dari bonggol Pisang Batu (Musa balbisiana Colla) sebagai alternatif sumber prebiotik

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Abstract

Inulin is a polysaccharide composed of 2-60 fructose monomers linked by β-(2,1) glycosidic bonds, with a glucose end group. The tuber of Musa balbisiana Colla (batu banana) is a part of the M. balbisiana Colla plant that contains dietary fiber 6,20%. The polysaccharide content allowed the batu banana tubers to contain inulin. The aim of this study was to determine the characteristics of inulin in batu banana tubers. The research method used was the experimental method, which was analyzed descriptively with two replicates at the Universitas Padjadjaran Laboratory of Jatinangor – Sumedang from May until October 2023. The parameters were inulin content, degree of polymerization (DP), reducing sugar content, moisture content, pH, solubility, water activity (a_w), color L*, a*, and b*, viscosity, and sensory analysis using a descriptive method. The results showed that B. tuber inulin had an inulin content of 3,58%, DP of 2,8, reducing sugar content of 2,03%, moisture content of 8,47%, pH of 6,31, solubility of 21,70% (90°C), a_w of 0,432, L* of 66,76, a* of 8,28, b* of 15,90, and viscosity of 2068 mPas (90°C). In conclusion, sensory analysis showed that batu banana tuber inulin has a darker color, bitter taste, stronger flavor, and softer texture than the commercial inulin.
Keywords: Batu banana tuber, degree of polymerization, inulin

Abstrak

Inulin merupakan polisakarida yang tersusun atas 2 – 60 monomer fruktosa yang diikat oleh ikatan glikosidik β-(2,1) dengan molekul akhir glukosa. Bonggol pisang batu (*Musa balbisiana* Colla) merupakan salah satu bagian tanaman pisang batu yang mengandung serat pangan sebesar 6,20%. Kandungan polisakarida tersebut memungkinkan bonggol pisang batu berpotensi mengandung inulin. Penelitian ini bertujuan untuk mengkarakterisasi inulin dari bonggol pisang batu. Metode penelitian yang digunakan adalah metode eksperimental dengan analisis deskriptif yang terdiri dari dua kali ulangan secara duplo yang dilakukan di Laboratorim Universitas Padjadjaran Jatinangor Sumedang dari bulan Mei – Oktober 2023. Parameter yang diteliti meliputi, kadar inulin, derajat polimerisasi (DP), kadar gula pereduksi, kadar air, pH, kelarutan, aktivitas air (a_w), warna L*, a*, dan b*, dan viskositas yang diuji secara eksperimental, data yang diperoleh dihitung rata-rata dan standar deviasi. Analisis sensori dengan metode deskriptif dimana inulin komersial ditetapkan sebagai R (control/pembanding). Hasil analisis sensori menunjukkan bahwa inulin bonggol pisang batu memiliki warna lebih gelap, rasa lebih pahit, aroma lebih kuat, dan tekstur lebih halus dibandingkan dengan inulin komersial.
Kata Kunci: Bonggol pisang batu, derajat polimerisasi, inulin

Introduction

Inulin is an indigestible carbohydrate composed of 2-60 fructose monomers but can be broken down by probiotic bacteria in the large intestine (Khuenpet et al., 2020) as a prebiotic, thus producing secondary metabolites of bacteriocin that have health effects on the body (Apolinário et al., 2017). In general, inulin is used in the food industry as a dietary fiber and gel-former, improves organoleptic properties, and can increase product viscosity. In dairy products, inulin is used as a fat substitute (El-Kholy et al., 2020). The most commonly used commercial source of inulin is chicory tubers which contain 65-79% inulin (Gupta et al., 2019). However, chicory remains difficult to find and develop in Indonesia. One of the local raw materials that has the potential to be a source of inulin is stone banana tubers (Sumanti 2017).

Banana weevils are often considered waste by people and are simply discarded. In fact, banana weevils contain high carbohydrates of 66,20% (Rohmani dan Yugatama, 2019). According to Sumanti (2017), banana weevil flour contains 6,20% dietary fiber, 14,56 mg isoflavones, 5,09% inulin, and 2,98% fructo-oligosaccharides (FOS). The crude inulin content in fresh stone banana humps is 20,12% (Hanidah, 2023).

The application of inulin in food products is influenced by its characteristics, such as the degree of polymerization, reducing sugar content, solubility, viscosity, and water content (Li et al., 2015) (Apolinário et al., 2017) (Zeaiter et al., 2019). However, the characteristics of inulin from banana weevils are not yet known. The characteristics of inulin can be used as recommendations for the amount consumed and for determining the product formulation as a functional food. Therefore, it is necessary to conduct further research on the characteristics of banana hump inulin to provide information on alternative sources of inulin that can be applied as a functional food.

Methods

All research was carried out in the Jatinangor District, Sumedang Regency, West Java Province at the Food Processing Technology Laboratory, Testing Services Laboratory, Food Chemistry Laboratory, Food Processing Engineering Laboratory, Food Industry Technology Department, Padjadjaran University from May to October 2023. Samples of stone banana tubers were washed, sliced, dried, and reduced to 100 mesh. Next, the inulin content, degree of polymerization, reducing sugar content, water content, pH, solubility, water activity, color, viscosity, and sensory analysis were carried out.

The research was conducted using experimental methods and analyzed descriptively with two repetitions, and each parameter was analyzed in duplicate. The data were calculated as average and standard deviation. Sensory analysis was performed using descriptive tests, and the data were presented in the form of a spider web diagram.

Materials and Tools

The main research materials were stone banana tubers obtained from local farmers in Sumedang. The materials used for the characteristic analysis of banana hump inulin include $CaCO_3$, 0,1 N HCl, 1,5 M H_2SO_4, 0,1% anthrone solution, DNS solution, fructose, 10% NaOH, and $Pb(C_2H_3O_2)_2$.

The tools used in making inulin include a 100 mesh sieve, stirring rod, beaker glass, grinder, filter cloth, pan, knife, centrifuge, cutting board, and a thermometer. The tools used for characteristic testing included glassware, aw meter (Aqualab Lite), cup, chromameter (Spectrophotometer CM-5), desiccator, hotplate, oven, pH meter (Lutron PH-201), Rapid Visco Analyzer (RVA Starchmaster 2 Perten), spectrophotometer (Rayleigh UV-9200), vortex, and a water bath.

Making Stone Banana Weevil Inulin

The inulin stone banana weevil (IBW) is made from 1500 g of fresh stone banana heads reduced in size with a chopper and then blanched for 5 min to eliminate browning enzymes. The samples were then dried in a cabinet oven at a temperature of 60°C for 7 h, reduced in size with a grinder, and sieved through a 100 mesh sieve to obtain rock banana hump flour. Inulin extraction was carried out by adding distilled water 1:15 (w/v) between banana weevil flour and distilled water, heating at 90°C for 40 min in a water bath, and filtering with a filter cloth. This process was performed 2 times. The collected filtrate was centrifuged at 1500 rpm for 15 min. The precipitate was dried in a cabinet oven at 50°C for 24 hours to obtain IBW (Li et al., 2015).

Measurement of Inulin Levels

Inulin levels were obtained from the difference between total sugar and inulin-reducing sugar levels (Arrizon et al., 2010). Measurement of total sugar content was carried out using the Anthrone method (Apriyantono et al., 1989) and reducing sugar using the DNS method (Dubois et al., 1956).

Measurement of Degree of Polymerization (DP)

The degree of polymerization was calculated as total sugar (%) per reducing sugar content (%) (Paseephol et al., 2007).

Measurement of Reducing Sugar Levels

The IBW sample was weighed at 0.2 gram in a centrifuge tube, and 10 mL of 1,5 M H2SO4 was added. The mixture was heated for 20 min. The solution was cooled in a container filled with water and 10% NaOH was added. Next, the banana tuber inulin sample solution was filtered into a 100 mL volumetric flask and determined. The sample solution (0,5 mL into a 25 mL volumetric flask. Next, 3 mL of DNS reagent was added, heated in a water bath at a temperature–95-100°C for 5 min, and then cooled in a container filled with water. After cooling, the solution was adjusted with distilled water and homogenized. The solution was incubated for 5 min and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 550 nm. Then plotted on the fructose standard curve.

Water Content Measurement

Water content measurements were performed using the thermogravimetric method (AOAC, 2005). IBW (1 g) was weighed in a constant aluminum cup and heated in an oven at 105°C for 3 h. The cup containing the sample was then placed in a desiccator for 15 min and weighed. This procedure was performed until a constant sample weight was obtained.

pH Measurement

The pH is measured using a pH meter. A total of 5 g of the IBW sample was dissolved in 10 ml of distilled water, and the pH was measured with a pH meter. Before use, the pH meter is first neutralized with a pH 4 and pH 7 buffer solution (AOAC, 2005).

Solubility Testing

Inulin solubility was tested by dissolving 1 g of IBW in 20 ml of distilled water at a temperature of 90°C. Then, it was filtered through a filter paper. The filter paper was then dried at 105°C for 3 h, cooled in a desiccator, and weighed. The samples were dried until a constant weight was obtained. Before use, filter paper was dried in an oven at 105°C until a constant weight was obtained (AOAC, 1995). The solubility of inulin was determined using the following equation:

$$Solubity(\%) = 1 - \left(\frac{w_3 - w_1}{w_2}\right) \times 100\%$$

Information:

w_1 = weight of filter paper (g)

w_2 = weight of sampel (g)

w_3 = weight of the filter paper + sample after drying (g).

Measurement a_w

The a_w meter (Aqualab Lite) was turned on and calibrated using NaCl solution. Subsequently, the a_w meter was opened and an a_w meter container containing the IBW sample was inserted. The device was closed and measurements were taken automatically. Wait for the process until the tool alarm sounds and the a_w value appears on the screen.

Color Measurement

The IBW color was measured using a chromameter. The IBW was placed in a special cup to cover the bottom of the cup. The L* value for the degree of lightness or darkness was measured (L* = 0 for perfect black and L* = 100 for perfect white); a* green red (a+ = 0 – 100 for red and a-=0 – (-80) for green). b* blue-yellow (b+ = 0 – 70 for yellow and b- = 0 – (-70) for blue) (Suyatma, 2009; Siagian et al., 2020). ΔE was then calculated and compared with that of commercial inulin.

Viskosity Measurement

Viscosity measurements were performed using a Starchmaster 2 Perten Rapid Visco Analyzer (RVA). An IBW with known water content is weighed according to a predetermined formula. The aquades were weighed according to a predetermined formula. Then, the distilled water and sample were placed in a special container and installed in the RVA tool, and the start button was pressed.

Sensory Analysis

Sensory analysis was carried out using a descriptive method of color, aroma, texture and taste (Meilgaard et al., 1987). The panelists comprised 20 semi-trained panelists.

Commercial inulin and IBW were used in the test. Panelists were asked to rate each sample for the attributes of color, aroma, texture, and taste on the assessment paper provided. The assessment of the sample's sensory attributes used a horizontal straight line scale, 15 cm long, with a value of 0 as the lowest value. The largest scale for the color attribute indicates that the sample has a white color, and vice versa. The largest aroma scale indicated that the sample had a stronger aroma, and vice versa. The largest scale for texture indicates that the sample has a finer texture, and vice versa. The largest taste attribute scale showed that the sample had a sweet taste. The results of the panelists' assessments for each sensory attribute were averaged and presented in the form of a spider web graph.

Result and Discussion

Inulin levels

Inulin levels are shown in Table 1. The inulin level in stone banana hump inulin was 3,58%. In a study by Mangunwidjaja et al. (2014), the inulin content of the banana hump produced was 1,16%, which was a lower value. When compared with dahlia tuber inulin, the tuber inulin content is smaller than dahlia tuber inulin with levels of 36,45% (Petkova et al., 2018).

Factors that influence inulin levels include plant type and variety, cultivation practices, geographic location, harvest age, and extraction process (Redondo-Cuenca et al., 2021). Hartati et al. (2023) showed that different types of inulin produced different levels of inulin. The results of extracting inulin from honey sweet potatoes (cilembu) were 6,472%, purple sweet potatoes of 6,073%, white sweet potatoes of 4,243%, and stone banana tubers of 3,580%, respectively (Table 1).

Table 1. Characteristics of Banana Tuber Inulin (IBW) and Dahlia Tuber Inulin (DTI) (*Hilman et al., 2021; **Petkova et al., 2018)

Characteristics	IBW	DTI
Inulin levels (%)	3,58±0,98	36,45**
DP	2,8±0,39	21**
Reducing Sugar (%)	2,03±0,09	4,6**
Water content (%)	8,47±0,22	7,11*
pH	6,31±0,18	6-7**
Solubility (%) (90^{o}C)	21,70	97,97*
a_w	0,432	0,333**
Color	Coklat	Putih**

Degree of Polymerization

The degree of polymerization (DP) is the number of monomer units that make up a compound or material. The DP value can be calculated by comparing the total amount of sugar with that of the reducing sugar. The greater the total sugar value, the higher the DP value of a material or compound (Marvie et al., 2022). Based on Table 1, the value of the degree of polymerization of banana weevil inulin is 2.8. Therefore, banana hump inulin is included in the short-chain inulin group (DP≤10) and is used as a prebiotic. Inulin as a prebiotic functions as a dietary fiber which is fermented by the intestinal microflora to produce short chain fatty acids which are able to lower intestinal pH and inhibit the growth of pathogenic bacteria and increase the activity of good bacteria in the intestine (Wan et al., 2020). The application of short-chain inulin to dairy products such as yoghurt can increase the microbial growth in the product. Research by Canbulat dan Ozcan (2015) found that adding 2% (w/w) inulin to yoghurt increased the viability of Lactobacillus rhamnosus bacteria more than yoghurt without inulin.

Banana tuber inulin had a DP of 2,8, which was smaller than that of dahlia tuber inulin with a DP of 21. According to a study by Bach et al. (2015), The DP of inulin is influenced by several factors, such as plant source, growing climate, harvest time, harvest age, and post-harvest handling. Rubel et al. (2014) showed that differences in the shelf life of Jerusalem artichoke tubers produce inulin with different DP values, which is caused by differences in the sugar or carbohydrate content of the tubers. Tubers stored for eight months (DP = 8,3) had a smaller DP value than tubers stored for two months (DP = 9,8).

Reducing Sugar Levels

IBW reducing sugars, such as fructose, are a class of carbohydrates with a free aldehyde or ketone group at the end of the chain to reduce electron-accepting compounds. (Ridhani et al. 2021). As shown in Table 3, the reducing sugar content of banana hump inulin was 2,03%. The reducing sugar content of banana tuber inulin is smaller than dahlia tuber inulin at 4,6% (Petkova et al., 2018).

The difference in reducing sugar levels is probably caused by the Maillard reaction occurring during the extraction process of stone banana tuber inulin so that the reducing sugar content is smaller than dahlia tuber inulin. The Maillard reaction is a non-enzymatic browning reaction due to the reaction between reducing sugars and amino groups during heating to form melanoidin (brown) pigment (Setiawan et al., 2023). This is proven by the color difference between banana tuber inulin, which is brown, and dahlia tuber inulin, which is white. The brown color of banana tuber inulin indicates that a Maillard reaction occurred during the extraction or drying process of inulin, so that the reducing sugar content was smaller than that of dahlia tuber inulin.

Fructose is a lipogenic monosaccharide that is known to be one of the main causes of inulin resistance. Regular low fructose consumption can reduce fasting blood glucose levels, hemoglobin A1c, triglycerides, and high-density lipoprotein cholesterol (Jalilvand et al., 2020) and can control the glycemic index in patients with diabetes (Jalilvand et al., 2018).

The low value of reducing sugar in IBW is new information that IBW can be used as a functional food and diet product for diabetes sufferers.

Water content

The IBW water content was 8,47%, which is greater than that of dahlia tuber inulin, as shown in Table 1. Yudhistira et al. (2020), The standard chicory inulin water content is ±5%. This shows that the water content of rock banana hump inulin is greater than standard chicory inulin. The difference in water content occurs because inulin is hygroscopic or easily absorbs water. During the storage and transfer process, inulin comes into direct contact with air; thus, it absorbs water vapor, which causes the water content to increase.

The water content of a food is an important parameter because it influences the food's resistance to damage caused by microorganisms (Hilman et al., 2018). In addition, Lestari et al. (2017), A lower water content can extend the product's shelf life, while a higher water content can cause the product to be easily damaged due to chemical reactions or microorganisms.

pH

As shown in Table 3, the pH of stone banana hump inulin is 6,31. El-Kholy et al. (2020), The pH value of standard inulin is between 5 – 7, while dahlia tuber inulin has a pH of 6 – 7 (Petkova et al., 2018). Therefore, banana weevil inulin is still in this value range.

The pH measurement aims to determine the suitability of the degree of acidity of inulin for digestive conditions. Matching the pH value of inulin with the types of microbes that grow in the intestine is necessary so that inulin can be utilized by microbes during the fermentation process. Inulin is fermented by microbes into short chain fatty acids which are the prebitoic properties of inulin (Hartati et al., 2023). In addition, the pH value of inulin is also an important parameter because inulin is often added to various food products to increase its functional value (Hilman et al., 2018).

Solubility

Solubility states the ability of a material to dissolve in a certain solvent, for example water. The higher the solubility value, the easier it was to dissolve. Inulin is a hydrophilic compound that is slightly difficult to dissolve in cold water or ethanol and is easily soluble in hot water (Wan et al., 2020). As shown in Table 3, the solubility of banana tuber inulin was 21,70%, whereas that of dahlia tuber inulin was 97,97%. The solubility of banana tuber inulin was lower than that of the dahlia tuber inulin.

There are several factors that influence the solubility of inulin, including the degree of polymerization, temperature, type of solvent, particle size, and water content (Hartati et al., 2023; Yudhistira et al., 2020). Inulin with a high degree of polymerization has a small solubility value (Teferra, 2021). According to Mohammadi et al. (2023), short-chain inulin (DP <10) has a higher solubility than long-chain inulin (DP >23). The greater the degree of polymerization, the greater the molecular weight, which makes it difficult for water to enter the inulin particles, resulting in low inulin solubility (Yudhistira et al., 2020). The solubility of inulin is influenced by temperature, where the higher the temperature, the higher the solubility. At a temperature of 10^{o}C, the solubility of inulin reaches 6% and 35% at a temperature of 90^{o}C (Ahmed & Rashid, 2017).

a_w

The a_w value is the amount of water in food that can be used by microbes for growth or enzyme activity (Ulfah et al., 2018). Water activity is one of the important factors that influences food damage due to microbial growth, chemical and biochemical reactions. According to Ramadhani *et al*. (2017) in (Ardiansyah et al., 2019b), The higher the a_w value of food, the easier it is for microorganisms to grow.

The a_w inulin value of the rock banana weevil is 0,432. This value is still higher than a_w inulin extracted from dahlia tubers, namely 0,333 (Petkova et al., 2018). The a_w value of banana hump inulin is still within safe limits because microorganisms can only grow at a_w >0,6 (Rubel et al., 2017).

Color

Based on Table 2, banana weevil inulin has a value of L* = 66,76 ± 0,46; a* = 8,28 ± 0,56; and b* = 15,90±0,49. The decrease in brightness value in banana hump inulin flour was caused by the browning reaction in the process of making banana hump inulin. The enzymatic browning reaction occurs due to the activity of the enzyme polyphenol oxidase. The reaction between the polyphenol oxidase enzyme and oxygen that occurs during the process of making banana hump flour can oxidize phenolic compounds to produce a brown pigment, namely melanin. Apart from that, the Maillard reaction can also occur due to the heating process. The reaction between amino acids and reducing sugars contained in the banana weevil forms melanoidin, resulting in a brown color in the inulin of the banana weevil (Priyani et al., 2019).

Table 2. Color test results

	L*	a*	b*	ΔE
IBW	66,76±0,46	8,28±0,56	15,9±0,49	33,11
IS	96,43±0,04	-0,86±0,01	4,38±0,06	

Information: L lightness is scaled from 0 – 100 with 0 – 50 for dark colors, 51 – 100 for light colors; a* redness for green to red on a scale of -80 – 80, green on a scale of -80 – 0, red on a scale of 0 – 80; b* yellowness is blue to yellow, blue is on a scale of -70 – 0, yellow is on a scale of 0 – 70.*

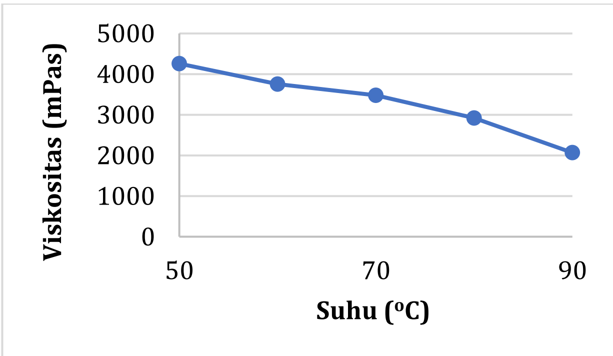
The measurement of the b* value is the assessment of the blue – yellow color on the IBW. The b* IBW value was 15,90, which indicates that the banana weevil tends to have a yellow color. A yellow color is produced by the browning reaction during the extraction and drying of IBW. The measurement of the a* value is an assessment of the green – red color of the stone banana weevil. The a* value of 8,28 shows that banana weevil inulin tends to have a red color.

Picture 1. Stone Banana Weevil Inulin

The color difference between IBW and commercial inulin is expressed as ΔE. If the ΔE value is 3 – 6, then the total difference is in the medium category, whereas ΔE <2 indicates that the total color difference is not visible. A value of ΔE>6 indicates that the total color difference between the sample and standard is real and can be seen with the eye (Prabaningrum et al., 2022). Based on Table 4, the ΔE value of stone banana hump inulin is 33,11 (ΔE>6), indicating that the color difference between IBW and commercial inulin is very large and can be seen by the human eye.

Viskosity

IBW viscosity was measured using a rapid visco analyzer (RVA). The results of testing the viscosity of banana weevil inulin can be seen in the graph



Picture 2. Banana Weevil Inulin Viscosity Graph

Based on the graph, the viscosity of banana weevil inulin increased as the temperature decreased. This is in accordance with a study conducted by Hilman et al. (2018)

Inulin produced from gembili tubers has a viscosity that increases as temperature decreases. The viscosity of banana hump inulin is still much greater than that of standard chicory inulin. According to Shoaib et al. (2016), a 30% chicory inulin solution has a viscosity of 100 mPas at a temperature of 10 °C. Another study by Hilman et al. (2018) showed that the viscosity of inulin from gembili at a temperature of 9 °C is 30 mPas. The high viscosity value of banana hump inulin is probably caused by the high starch content in banana hump inulin of 46,01%. When the banana hump inulin solution is heated, the starch contained in it undergoes a gelatinization process so that it becomes thick, causing the viscosity value of the banana hump inulin to increase.

Sensori Analysis

The results of the sensory analysis of the banana hump inulin are presented in Figure 3.

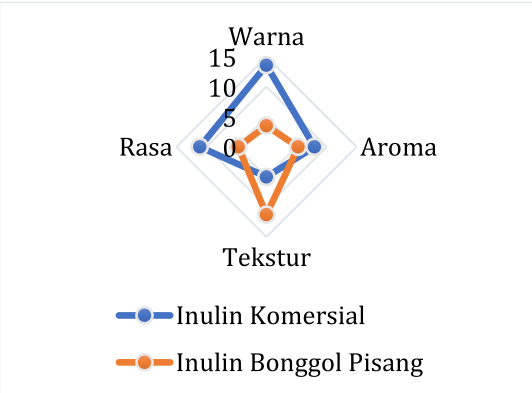


Figure 3. Results of descriptive sensory test of Batu Banana Weevil Inulin

The results of IBW sensory analysis produced a quality that was still below that of commercial inulin in terms of color, taste, and aroma. If the line on the graph is closer to 0 (center), then the value becomes smaller, which indicates a weak parameter/quality of the product being tested. Based on Figure 3, the value of the sensory analysis of the color of banana hump inulin was much smaller than that of commercial inulin. This shows that the color of IBP is darker than that of the commercial inulin. Making IBP does not involve a bansing process or the addition of chemicals such as metabisulfite, ascorbic acid, or other reducing agents to provide a bright color as a result of enzymatic browning activity. Phenolase is an enzyme present in plant tissue. If it reacts with oxygen, enzymatic oxidation can occur, causing browning of the substrate(Mandasari et al., 2015).

The values of the sensory analysis results for IBW aroma and taste are also much smaller than those of commercial inulin. Stone banana hump inulin, which has a small taste value, indicates that it has a bitter taste. Batu banana hump flour contains 11,616 g/100 g g tannin, which is a class of flavor compounds that can cause an astringent taste (Sumanti, 2017). The low aroma value of IBW indicates that inulin has a stronger aroma than commercial inulin. The results of the sensory analysis showed that the texture value of rock banana hump inulin was greater than that of commercial inulin, indicating that it had a smoother texture. The degree of inulin fineness was influenced by the particle size. The IBW particle size was 100 mesh, which resulted in a smooth texture.

Conclusion

The characterization results show that IBW is a group of low-fructose short-chain inulins that can be used for functional food as a source of prebiotics with a low glycemic index; therefore, consumption of IBW according to needs can stimulate the growth of probiotic bacteria in the intestine to produce bacteriorin, which functions as a stimulus for the immune system. Further research can be done to diversify the formulation of prebiotic and diet food products, so that we can develop products to prevent stunting and diabetes.

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