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Physical Characteristics and Functional Carbohydrates of Three Varieties of Batu Banana (*Musa Balbisiana Colla*) in Central Java and Special Region of Yogyakarta

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ABSTRACT

Indonesia is rich in various types of bananas. Wild banana (Batu Bananas) is a banana type that has no economic value, because it has so many seeds. There are three varieties of batu banana in Central Java and the Special Region of Yogyakarta Indonesia, namely *M. balbisiana* var *balbisiana* (Klutuk Batu), *M. balbisiana* var *brachycarpa* (Klutuk Sukun) and *M. balbisiana* var. *liukuensis* (Klutuk Wulung). Raw bananas are source of complex carbohydrates, including dietary fiber (DF), resistant starch (RS) and fructooligosaccharides which are very beneficial for health. The aim of this research was to identify the physical properties and evaluate the complex carbohydrate content of batu banana flesh and seeds. This research is an experimental study with a completely randomized design. The resistant starch test uses the multienzyme method (AOAC, 1995), the dietary fiber test uses the multienzyme method (AOAC, 1995), while the fructan test (FOS and inulin) uses AOAC method 999.03. The results of the analysis of resistant starch and total fiber were highest in klutuk batu seeds at 50.07% and 56.42%, consisting of 53.76% insoluble fiber and 2.66% soluble fiber. The highest fructan and FOS content was obtained from klutuk batu seeds at 6.16% and 6.11%, while the highest inulin content was found in klutuk wulung seeds at 0.51%.

Keywords: Stone Banana, Functional Carbohydrates

Introduction

Indonesia is a tropical country with abundant banana production, because the climate is very suitable for banana growth. Bananas can grow in the lowlands and highlands, up to an altitude of 1,600 m above sea level, with an optimum temperature of 27-38°C, pH 4.5-7.5 and optimal rainfall between 2000-2500 mm/year. The Central Statistics Agency East Java Province (2023) noted that banana production in Indonesia reached 8.74 million tonnes in 2021, an increase of 6.85% or 0.56 million tonnes compared to 2020 of 8.18 million tonnes.

There are more than 200 types of local banana varieties in Indonesia. These varieties are divided into three groups, namely 1) bananas that can be consumed directly (*banana*), for example ambon and cavendish, 2) bananas that should be cooked before consuming (*plantain*), for example kepok, tanduk, raja nangka, and siam, 3) *wild bananas*, for example batu banana, in Javanese called Pisang Klutuk (Poerba, 2016).

In general, raw bananas are a source of complex carbohydrates such as resistant starch, dietary fiber and fructans. Menezes et al. (2011) reported that banana carbohydrates contain resistant starch 48.99 g/100 g, total dietary fiber 56.24 g/100 g, and fructan (0.05 g/100 g) in dry weight (db). Bamigbade et al. (2022) stated that in general fresh bananas contain fructans in the form of fructooligosaccharides (FOS) and inulin 0.3-0.7%. Bananas are one of the main sources of FOS among the four other types of fruit that are sources of FOS (apples, grapes, tomatoes and pumpkin). Bananas have 1-ketose (GF2) value 125 and nystose (GF3) 129, the highest among other FOS source materials, only the fructofuranosyl nystose (GF4) value is in the middle range, in the amount of 97 (Jovanovic-malinovska et al., 2014).

Batu bananas are generally not specially cultivated, the fruit with seeds has almost no economic value. Batu banana cultivation generally only uses the leaves as wrappers, the flowers as vegetables, and the young fruit as a salad mixture, while the old or ripe fruit is not used and is wasted. Several researchers have studied the potential of complex carbohydrates on various types of fresh bananas and batu banana seeds, but there has been no research regarding the potential of raw old batu bananas. Yuli (2006) reported that whole, ripe batu bananas contain 15.90% carbohydrates and 6.90% fiber, while batu banana seed flour contains 69.3% carbohydrates and 4.31% fiber (Sharma et al., 2021). Batu bananas seed flour has the advantage that its fiber content is twice as high as bananas in general, so batu bananas have the potential to be a source of complex carbohydrates.

Sotto & Rabara (2017) stated that the morphological diversity of *Musa balbisiana* is relatively small compared to other types of banana, character diversity is more abundant in flower/fruit characters, shape and color of banana fruit which is influenced by growing environmental conditions. The banana variety in Central Java and Special Region of

Yogyakarta (DIY) consists of three varieties, namely *M. balbisiana var balbisiana* (Klutuk Batu), *M. balbisiana var brachycarpa* (Klutuk Sukun) with accession number LIPI-063 and *M. balbisiana var. liukuensis* (Klutuk Wulung) with accession number LIPI-064 (Poerba, 2016). Many studies have been conducted on various types of banana consumption, including research by Welli et al. (2019) and by Horigome et al. (1991) regarding Cavendish bananas, research by Kusumawardani et al. (2019) regarding Uter bananas and research by Agustin et al. (2019) regarding Berlin bananas, but not much research has been carried out on batu bananas. So far, research on stone bananas has been carried out mostly on the flowers and leaves, as well as in the form of fruit flour as a substitute for processed food products. Research on complex carbohydrate content in batu bananas in the form of resistant starch, dietary fiber and fructans (FOS and inulin) are still very limited. Therefore, research is still needed to explore the potential complex carbohydrate content of batu bananas and their health benefits.

Bananas are climacteric fruits that are easily damaged, due to their high water content due to increased metabolic processes during ripening. The shelf life of bananas is relatively short so processing banana is needed to extend its shelf life, by processing it into flour (Rosalina et al., 2018). According to Putri et al. (2015); Wulandari et al. (2023), the type of banana that is suitable for making flour is an old but not yet ripe banana which has a high starch content of around 73.4% and a low sugar content of less than 18%. Bananas are at stage 1 which shows the entire surface of the banana is green, or stage 2 which shows changes in 1 or 2 bananas in the bunch are slightly yellow. Banana flour that is old but not yet ripe contains more complex carbohydrates than ripe bananas.

Research methods

Research Materials

The materials used in this research were batu banana (*Musa balbisinia Colla*) from 3 variants, namely klutuk batu (*M. balbisiana var balbisiana*), klutuk sukun (*M. balbisiana var brachycarpa*) and klutuk wulung (*M. balbisiana var. liukuensis*) from the flesh and seeds. Klutuk Wulung was obtained from the Malangan banana germplasm plantation, Jalan Lingkar Selatan, Giwangan, Umbulharjo, Yogyakarta, while Klutuk Batu and Klutuk Sukun were obtained from Ungaran, Semarang Regency, Central Java. Sample analysis was repeated three times and analysis was carried out in triplicate.

Food fiber analysis materials: 85% ethanol, α amylase enzyme, protease and amyloglucosidase, 2-N-Murpholino ethanesulfonic, Tris (hydroxymethyl) aminomethane, 0.561 N HCL. Resistant starch analysis materials: 100 mM sodium maleate buffer, pH 6.0; 1.2 M sodium acetate buffer, pH 3.8; 100 mM sodium acetate buffer pH 4.5; 2M KOH

solution; IMS was dissolved in 50% (v/v) 95%/99% ethanol; amyloglucosidase stock 3300 units/mL in 50% glycerol; AMG Solution 300 U/mL; pancreatic α -amylase suspension 30 U/mL; GOPOD-aminoantipyrine buffer mixture. Inulin analysis materials: fructose standard solution, 0.05 N KOH, 0.05 N HCL, inulinase enzyme, acetate buffer, amyloglucosidase, 20 ppm glucoheptose internal standard. FOS analysis materials: carbohydrate standards glucose, fructose, sucrose, lactose, 1-kestose and nistose with an average polymerization degree of 22, deionized water, vacuum filtered through a 0.2 m membrane.

Flour Making

The process of making flour begins by steaming batu banana at 90°C for 20 minutes, peeling the skin, and separating the flesh and seeds. Drying of batu banana chips is carried out at temperature of \pm 65°C for 8 hours, and for batu banana seeds 60°C for 6 hours. Drying is done until the texture is hard and brittle (moisture content \pm 8-10%). The flouring process is carried out by grinding and sifting 80 mesh, packing the batu banana flour into closed plastic bags and storing it at room temperature (Pragati et al., 2014).

Analysis of Resistant Starch (multienzymatic method) AOAC Official Method 2002.02

A total of 0.5 g of batu banana flour was added to 25 ml of 0.1 M phosphate buffer solution pH 7, stirred until a suspension was formed then 0.1 ml of alpha amylase enzyme was added, covered with aluminum foil and incubated in a waterbath with a shaker at temperature 100°C for 15 minutes, stirring occasionally. The sample was cooled, added 20 ml of distilled water, 5 ml of 1 N HCL and 1 ml of 1% pepsin enzyme, covered and incubated in a water bath at 40°C for 1 hour. Remove from the incubator and add 5 ml distilled water, 1 N NaOH and 0.1 ml beta amylase enzyme. The Erlenmeyer was closed and incubated again in a water bath with a shaker at 40°C for 1 hour. Continue filtering using filter paper, then the dregs are analyzed for starch content.

Analysis of total dietary fiber (soluble & insoluble) multienzymatic method AOAC 991.43)

A total of 0.5 g of batu banana flour was added to 25 ml of 0.1 M phosphate buffer solution pH 7, then stirred until a suspension was formed. Afterwards 0.1 ml of alpha amylase enzyme was added, covered with aluminum foil and incubated in waterbath at 100°C for 15 minutes, and stirred occasionally. The sample was cooled then 20 ml of distilled water and 5 ml of 1 N HCL were added. Then 1 ml of 1% pepsin enzyme was added, covered and incubated in a water bath at 40°C for 1 hour. Remove and add 5 ml distilled water, 5 ml 1 N NaOH and 0.1 ml beta amylase enzyme. The Erlenmeyer was closed and incubated again in a shaking waterbath at 40°C for 1 hour. Filter using constant-known-weight filter paper. The sample was washed, the filtrate was then filtered using ash-free filter paper and washed with 2 ml of ethanol. Total Dietary Fiber is calculated as the sum of insoluble dietary fiber and

soluble dietary fiber.

FOS analysis (HPLC method) Petkova et al. (2014)

The analysis process begins with making a standard solution: fructose, glucose, sucrose, lactose, 1-ketose, nystose with polymerization degree of 22 with an initial concentration of 10 mg/mL. Working standard solution 0.05; 0.1; 0.5; 1.0; 2.5; 5.0, 10 mg/mL prepared from standard stock solution. The operating conditions of the device are with a flow rate and temperature of 1 mL/minute and a temperature of 85°C. Extract 1 g of sample using 30 ml of boiled deionized water for 10 minutes. The protein was precipitated by adding 5 ml of Carrez I reagent (K₄ Fe (CN)₆).

Results

Batu Banana Character

Batu bananas (*Musa balbisiana* Cola) or known in Javanese as klutuk bananas, have pseudo stems and can grow up to ± 3 m high. At the top of the pseudo stem, there are many leaves with a leaf sheath length of $\pm 1-2$ m. Batu bananas grow well in hot areas up to an altitude of ± 2.200 m above sea level. Batu banana leaves tear easily, the flowers emerge from the tip of the stem near the leaves, the color of the flowers is very dark red like a heart, while the male flower part is white. The fruit is in the form of a bunch, after the fruit is ripe the color will change from green to yellow, apart from that there is also a change in the nutritional composition. The Purwodadi Botanical Gardens National Research and Innovation Agency (2020) stated that ripe batu bananas taste sweet but have many seeds, each fruit contains ± 50 small black seeds resembling *Ceiba pentandra* seeds. The distribution of stone bananas is almost even throughout Indonesia, specifically in Central Java and the Special Region of Yogyakarta there are three varieties of batu bananas, namely *M. balbisiana* var *balbisiana* (klutuk batu), *M. balbisiana* var *brachycarpa* (klutuk sukun) with access numbers LIPI-063 and *M. balbisiana* var. *liukiensis* (klutuk wulung) with accession number LIPI-064 (Poerba, 2016).

These three types of stone bananas can be easily recognized based on the shape of the fruit (Figures 1 and 2). The Klutuk Batu plant has smaller fruit, the color is light green, the fruit is ± 15 cm long and ± 3.5 cm in diameter. The fruit of the Klutuk Sukun type is dark green, large in size but longer than the Klutuk Wulung, with a length of ± 20 cm and a diameter of ± 4.5 cm, while the Klutuk Wulung is dark green with a purple tinge, shorter, and fatter than Klutuk Sukun with a length of ± 17 cm and a diameter of ± 5 cm.



Figure 1. Display of three varieties of batu bananas

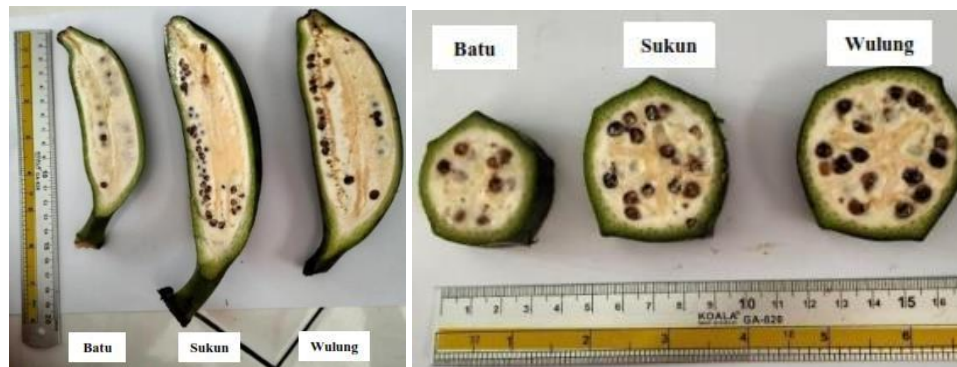


Figure 2. Longitudinal and transverse views of three batu bananas

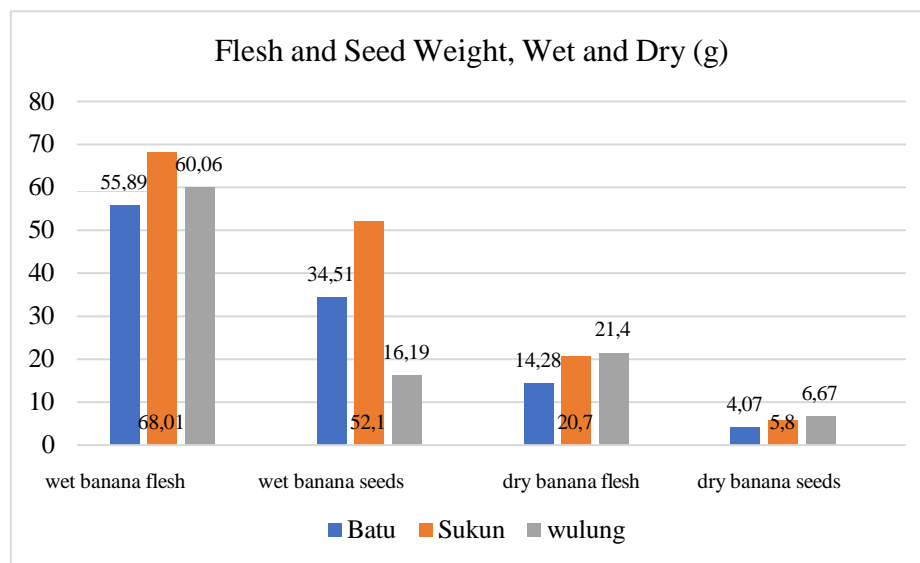


Figure 3. Average banana weight three batu bananas in top, middle & bottom bunch

Evaluation of banana weight shows that Klutuk Sukun and Klutuk Wulung bananas in the top bunch have the highest average weight compared to the middle and bottom, amounting to ± 158 g and ± 148 g, while Klutuk Batu bananas in the middle bunch have a weight per fruit highest (± 142 g) compared to the top and bottom. Complete data on the average weight of top, middle and bottom banana bunches according to the three batu banana varieties is in Figure 3.

This research was carried out on the weight and percentage of the flesh and seeds of the three varieties of batu banana in wet and dry conditions. The results of the weight analysis of

the three varieties of batu banana (Figure 4), show that the order of weight of the wet flesh part from the heaviest is Klutuk Sukun, Klutuk Wulung, and Klutuk Batu, while the order of weight of the wet seed part from the heaviest is klutuk sukun, klutuk batu and klutuk wulung. Drying of fruit flesh is carried out at a temperature of 65°C for 8 hours, in contrast to seeds at a temperature of 60°C for 6 hours. The weight reduction in klutuk sukun flesh was greater than klutuk wulung flesh, this was due to the water content in klutuk sukun flesh being 69.59% db which was greater than klutuk wulung flesh at 64.72% db. Figure 5 shows that the highest water content is found in klutuk batu flesh 74.45% db, while the lowest water content is found in klutuk wulung flesh 63.68% db. As for seeds, the water content of klutuk batu seeds and klutuk wulung seeds is almost the same, namely 84.64% and 84.90% respectively.

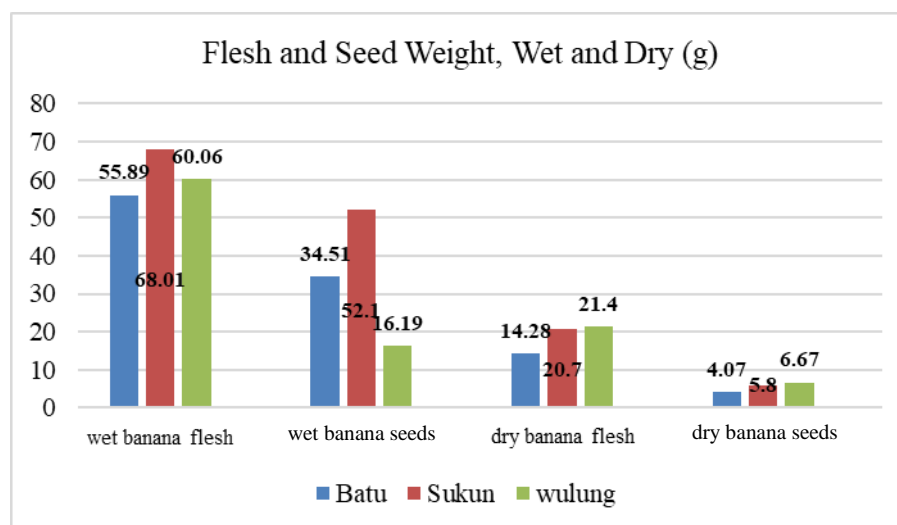


Figure 4. Weight of flesh and seeds of 3 batu bananas

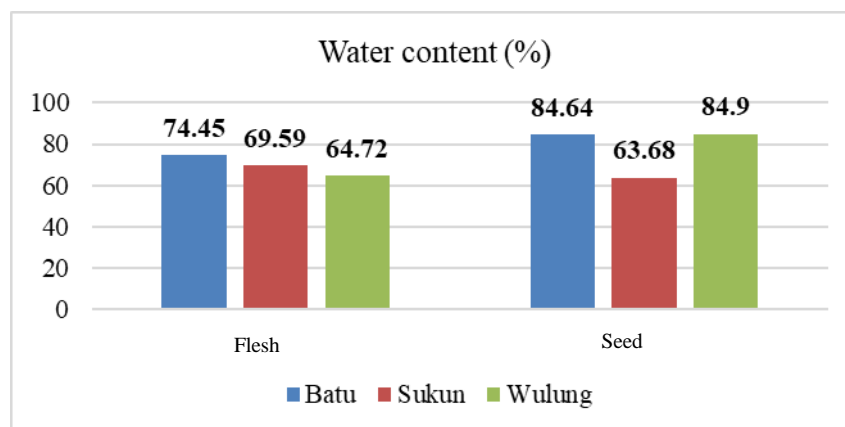


Figure 5. Water content of the flesh and seeds of 3 batu bananas

Figure 6 shows that the highest percentage of klutuk batu seeds and flesh reached 27.47%, after drying it decreased to 23.59%. This is because the water content of klutuk batu seeds is very high, reaching 84.64%. The percentage of klutuk sukun flesh experienced the smallest decrease, from 79.44% to 65.77%, due to the water content of klutuk sukun flesh being only 69.59%.

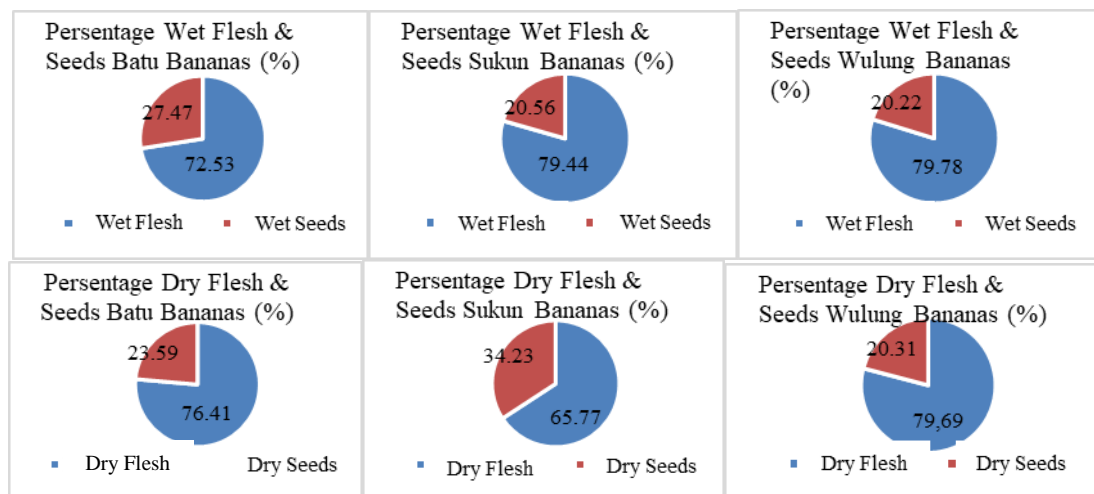


Figure 6. Percentage of flesh and seeds of three varieties of wet & dry batu bananas

Banana Complex Carbohydrates

Complex carbohydrates are a type of carbohydrate that has benefits for the body's health. Batu bananas contain resistant starch, total fiber in the form of insoluble fiber and soluble fiber, as well as fructans in the form of FOS and inulin. Yuli (2006) conducted an analysis of batu banana fiber, comparing it with Raja and Siam bananas, the results showed that the fiber content of batu bananas was higher (6.90%) than Raja (4.08%) and Siam bananas (4.75). Research by Sharma et al. (2021) analyzing banana seed flour obtained 69.3% carbohydrates and 4.31% fiber. The fiber content of batu banana seeds is twice as high as batu banana flour in general, $\pm 2\%$.

In this study, the content of resistant starch, total fiber, including soluble and insoluble fiber and fructans in the form of FOS and inulin, was analyzed. Resistant starch is a type of complex carbohydrate that cannot be broken down into a simpler form (glucose) and cannot be digested by the small intestine. The digestive process for resistant starch is similar to that of insoluble fiber. This substance only passes through the small intestine and does not undergo breakdown or change in any form. The resistant starch content of batu banana flour was highest in klutuk wulung flesh at 31.35% and klutuk batu seeds at 50.07% (Figure 7).

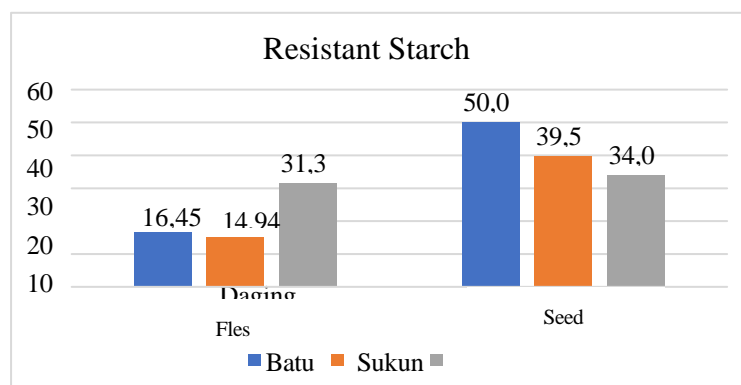


Figure 7. Resistant starch content of flesh and seeds of three varieties of batu bananas flour

Dietary fiber is the part of plants that can be consumed and is composed of carbohydrates that are resistant to digestion and absorption in the human small intestine, but undergo partial or complete fermentation in the large intestine. Dietary fiber is a part of food that cannot be hydrolyzed by digestive enzymes. Dietary fiber consists of soluble fiber which will form a gel when it interacts with water or other body fluids in the digestive tract, while insoluble fiber will absorb water to form feces.

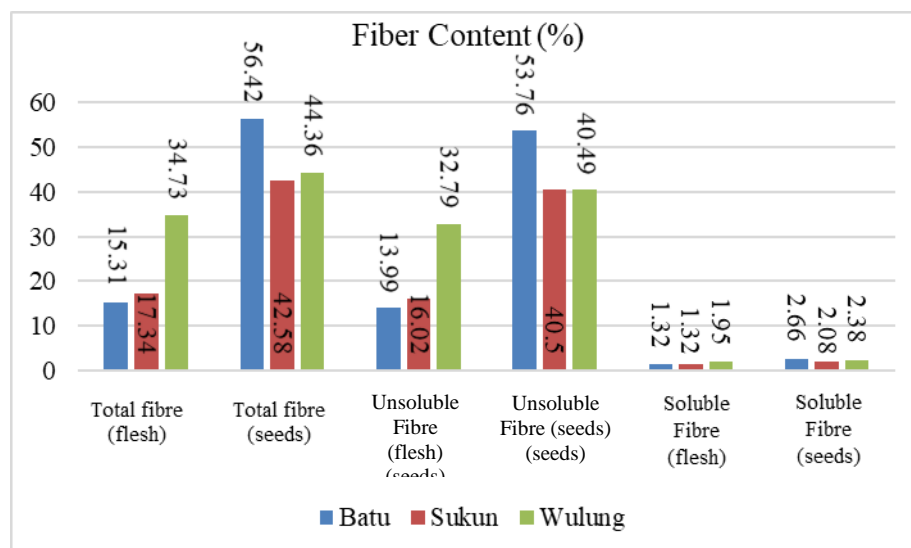


Figure 8. Fiber content of flesh and seeds of three varieties of banana flour

The fiber content of the three batu banana variants in this study was calculated based on total fiber, insoluble fiber and soluble fiber. Figure 8 is data from fiber analysis, it can be seen that the highest total fiber, insoluble fiber and soluble fiber content is found in klutuk batu seeds reaching 56.42%, 53.76% and 2, 66%.

Fructans are fructose polymers that have three to several hundred fructose units. Fructans are very water soluble, generally consisting of inulin and fructooligosaccharides (FOS), which are food ingredients that are classified as prebiotic, because they cannot be digested by the host, but can be selectively fermented by some colonic microflora. Inulin and FOS are water soluble, cannot be digested by digestive enzymes, but can be fermented by colonic microflora to become Short Chain Fatty Acids (SCFA) and lactic acid which are very beneficial for health.

Figure 9 is data from the analysis of FOS and inulin. The fructan content of the three batu banana varieties in this study was calculated based on the total content of inulin and FOS. The highest fructan values were found in klutuk batu seeds and flesh at 6.16% and 3.4%. Meanwhile, the highest inulin content is found in klutuk wulung seeds, namely 0.51%. The highest FOS content is found in klutuk batu seeds, namely 6.11%.

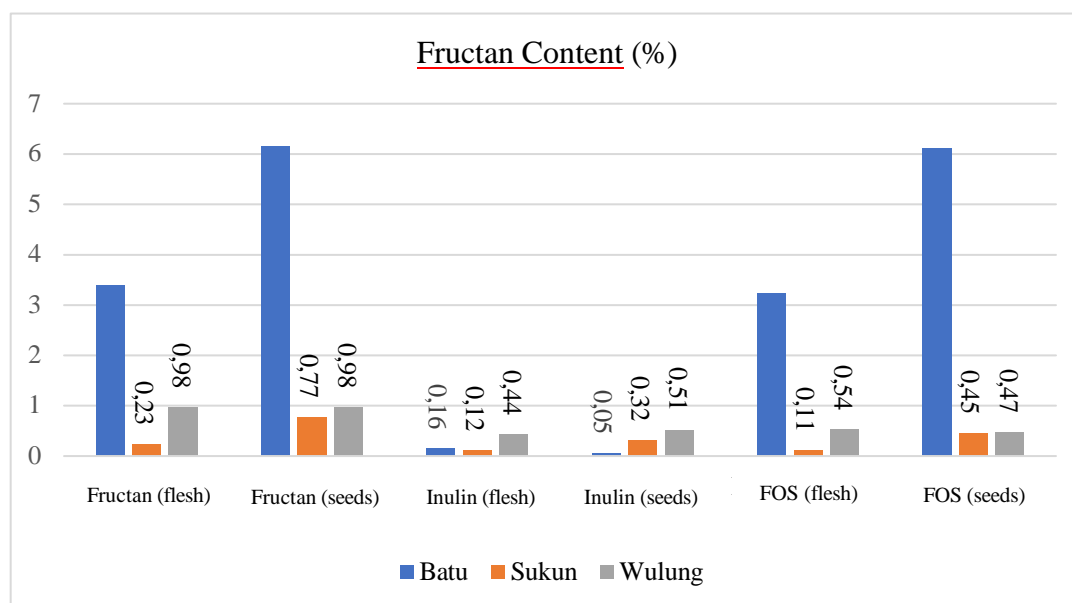


Figure 9. Fructan content of flesh and seeds of three varieties of batu bananas flour

Discussion

Batu bananas grow abundantly in Indonesia, but the fruit is underutilized so much of it is wasted. Batu bananas are included in the *Musa Branchycarpa* group, namely bananas with seeds which are generally eaten when they are still raw. The varieties of batu bananas (*Musa balbisiana Colla*) in Indonesia are very diverse. The characterization of bananas in Indonesia has been carried out by Indonesian Institute of Sciences (LIPI), inventorying and obtaining the diversity of bananas in Indonesia based on their varieties. According to LIPI, there are three varieties of batu bananas in Central Java and the Special Region of Yogyakarta with the names *M. balbisiana var balbisiana* (klutuk batu), *M. balbisiana var brachycarpa* (klutuk sukun) with accession number LIPI-063 and *M. balbisiana var. liukiensis* (klutuk wulung) with accession number LIPI-064. The three batu banana varieties have different percentages of flesh and seeds which have an impact on differences in complex carbohydrate content (resistant starch, dietary fiber and fructan).

The batu banana flour in this study was made from old but still unripe batu bananas, characterized by the presence of 1 or 2 yellowed bananas in one bunch. Ripe bananas are related to the degradation of chlorophyll and the formation of carotenoid pigments, so that mature bananas have a yellower banana color. The characteristics of banana skin color changes provide information about the banana ripening process in seven stages. The stone bananas used in this study were grade 1, full green in color, indicating the entire surface of the banana was still green or grade 2, light green, there was a change in the color of the fruit skin to yellow.

The results of the analysis of resistant starch and fiber in batu banana flour from the three variants show that the resistant starch and fiber content in the seeds is higher than in the

flesh. The range of resistant starch of seed content 34.05-50.07% db, which is higher than the flesh, which is 16.45-31.35% db. The range total fiber of seed content 42.58-56.42% db, higher than the flesh 15.31-34.73% db. The range insoluble fiber of seed content 40.49-53.76% db, higher than the flesh 13.99-32.79% db, while the range soluble fiber of seed content 2.08-2.66%, higher than the flesh 1.32-1.95% db. An interesting thing is shown by klutuk batu, the fructan content of the seed reaches 6.16% db and the flesh is 3.4% db, much higher compared to klutuk sukun and klutuk wulung which have a fructan content of less than 1%. The FOS content of klutuk batu, both flesh and seeds, is higher than the inulin content. Research by Menezes et al. (2011), results of analysis of raw banana flour *Musa acuminata*, var. Nanicão in dry weight (db), the resistant starch content is 48.99%, total fiber 56.24%, and fructan (7.2%), starch content 27.78%, while the dissolved sugar is very low at 1.81%. According to Bamigbade et al. (2022), bananas contain fructooligocharide (FOS) compounds of 0.3-0.7% and inulin of 0.3-0.7%.

Batu banana seeds have advantages because their resistant starch and dietary fiber content is higher than the flesh of the fruit. Research by Yuli (2006) analyzed the flesh and seeds of batu bananas, comparing them with the flesh of Raja and Siam bananas. The results showed that the carbohydrate content of batu bananas was lower than that of Raja and Siam bananas, but the fiber content of batu bananas was higher than Raja and Siam bananas. Research by Sharma et al. (2021), analyzing banana seed flour, obtained 69.3% carbohydrates and 4.31% fiber. The fiber content of batu bananas is twice as high as banana flour in general, only approximately by 2%.

Conclusion

The results of the research show that banana flour contains quite high levels of complex carbohydrates. Klutuk batu seeds contain higher levels of resistant starch, dietary fiber and fructan than the flesh. Klutuk batu seeds contain higher levels of resistant starch, dietary fiber and fructan than klutuk sukun and klutuk wulung. Klutuk Batu seeds are superior in fructan content, especially FOS, while klutuk wulung seeds are superior in soluble and insoluble fiber content.

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