Characterization of Insoluble Fiber in Cassava Peel and Its Hydrolyzate Potential as a Prebiotic for Lactobacillus Plantarum

Ilham Marvie[1,2], Azis Boing Sitanggang[2] and Slamet Budijanto[2]
[1]*Food Technology Study Program, Institute Technology of Sumatera, South Lampung, Indonesia*
[2]*Department of Food Science and Technology, IPB University, West Java, Indonesia*

Keywords: Cassava Peel, Cellobiose, Cellulase, Cellulose, Prebiotic.

Abstract: The cassava peel is an uncommon material for consumption as food for Indonesians because its use is widely used in non-food industries. Exploration of the use of cassava peel in the food industry in this case as a functional food ingredient such as prebiotics has the potential to be carried out. This study aims to determine the characteristics of the insoluble fiber content of the cassava peel and testing its hydrolysate potential as a prebiotic in growing *Lactobacillus plantarum*. The varieties of the cassava plant used in this study were Ratim (RTM 22) and Ulujami (UJ 17). The method used in this study is the characterization of insoluble fiber. As well as testing the prebiotic potency by determining the prebiotic activity score by doing *Lactobacillus plantarum*. The results of this study indicate that the characteristics of insoluble fiber in the cassava peel are hemicellulose content is more dominant than cellulose and lignin in the cassava peel. The hydrolysate of cellulose from cassava peel showed its potential as a prebiotic in growing *Lactobacillus plantarum*. RTM 22 varieties had a higher prebiotic activity score than UJ 17. The prebiotic activity scores of RTM 22 and UJ 17 were 1.70 and 1.48, respectively.

1 INTRODUCTION

The Cassava peel is an uncommon material for consumption as food for Indonesian people, its use is more widely used as industrial raw material (Aripin *et al.*, 2013), animal feed (Oluwanike and Adeneye, 2014), and biodegradable packaging (Widiarto *et al.*, 2017). The use of the cortex of cassava peel as a traditional Sumedang food ingredient called kadedemes has inspired the development of cassava peel as a food ingredient. Meanwhile, the cassava peel has a dry weight that reaches 13% of the total weight of cassava peel (Aripin *et al.*, 2013). The availability of raw material for cassava is also predicted to continue to increase in line with the increase in the national consumption of cassava reaching 12.7 million tons in 2020 (BPS, 2013). Therefore, exploration of utilization in the food industry in this case as functional food ingredients such as prebiotics has the potential to be carried out.

Cassava peel has a crude fiber content of up to 12.2% (Idugboe, Nwokoro and Imasuen, 2017). Insoluble fiber is a part of crude fiber (Idris *et al.*, 2020). Cellulose under natural conditions is coated by a matrix of hemicellulose and lignin (Elechi et al. 2016). The presence of lignin and hemicellulose content is thought to affect the hydrolysis of cellulose (Surendran *et al.*, 2018). Therefore, it is necessary to characterize the content and insoluble in the skin of the cassava peel. The process to reduce the hemicellulose and lignin content is also needed to obtain the dominant cellulose content. Cellulose is composed of D-anhydro glucopyranose in β-1,4-glycoside or β-glucose bonds (Elechi *et al.*, 2016). The hydrolysate from the hydrolysis of cellulose with cellulase enzymes can produce cellobiose as an intermediate product (Razie *et al.*, 2011).

Previous research has been carried out by knowing the ability of cellobiose as a stimulus in the growth of *Lactobacillus acidophilus NCFM* (van Zanten *et al.*, 2012). In-vivo test results have also been carried out and do not give unwanted side effects to humans (Van Zanten *et al.*, 2014). Testing the ability to grow other *Lactobacillus* strains such as *Lactobacillus plantarum* also needs to be carried out on products from the hydrolysis of cassava peel to add information on the ability of cellobiose as a prebiotic. This study aims to determine the characteristics of the insoluble fiber content of the skin of cassava peel. and testing its hydrolysate potential as a prebiotic in

Marvie, I., Sitanggang, A. and Budijanto, S.
Characterization of Insoluble Fiber in Cassava Peel and Its Hydrolyzate Potential as a Prebiotic for Lactobacillus Plantarum.
DOI: 10.5220/0010507300003108
In *Proceedings of the 6th Food Ingredient Asia Conference (6th FiAC 2020) - Food Science, Nutrition and Health*, pages 31-37
ISBN: 978-989-758-540-1

growing *Lactobacillus plantarum*. This research is expected to expand the exploitation of cassava peel as raw material for functional food as a prebiotic and increase its added value in the food industry.

2 METHOD

2.1 Material

The main material used in this study were cassava peel (Manihot utilissima Sp) varieties of Ratim (RTM 22) and Ulujami (UJ 17) from the experimental garden of the Department of Agronomy and Horticulture, Bogor Agricultural University, Cigombong, Sukabumi Regency, West Java. The enzymes used were complex cellulase enzymes (Wathringthon, Murni). The bacteria used were *Lactobacillus plantarum* and *Escherichia coli* obtained from SEAFAST IPB. Nylon-66 membrane pore size 0.20 µm and 0.45 µm with a diameter of 25mm (Himedia). The media used for bacterial growth are de Man Rogosa Sharpe (Merck) and M9 (Merck).

2.2 Preparation of Cassava Peel Material

The process of preparing the cassava peel raw material consists of sorting, washing, reducing the size, and peeling the tuber skin. Samples were dried using a cabinet drier at 50°C for 24 hours and crushed using a disc mill to form flour with a size of 40 mesh. The raw material is again dried in the cabinet drier for 24 hours until the moisture content is <10% (Tasaso, 2015). The cooking of the cassava peel is done by heating 50 g of cassava root flour in 1 L of water and 10% NaOH using an autoclave at a temperature of 130°C, a pressure of 190 kPa for 60 minutes. Separation of the extraction results between the solid residue and the black concentrated solution using a filter cloth and washed with distilled water until the solid residue reaches a pH of 7.0. The residue was dried at 70°C for 24 hours. Bleaching process by adding 30% H_2O_2 at 70°C for 3 hours. Cassava peel is separated again to get the residue and rinsed using distilled water. The bleaching residue is dried again at a temperature of 70°C (Tasaso, 2015).

2.3 Characterization of the Insoluble Fiber Content

Characterization of cassava peel flour was carried out using fiber content analysis consisting of Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), cellulose, lignin, and hemicellulose levels (van Soest, 1963). All tests were carried out in two duplicate replications.

2.3.1 Neutral Detergent Fiber (NDF)

NDF solution consisted of distilled water 1 L, Sodium Sulfate 30 g, EDTA 18.81 g; Sodium Borate 10 H_2O 6.81 g, 4.5 g anhydrous di-Na-HPO4 and 10 ml pure 2-ethoxy ethanol. a sample of 0.5 g (A) was put into a 250 mL beaker. The sample was then added with NDF solution and filtered with the help of a vacuum pump, rinsed alternately with hot water and acetone. The filter results were dried in an oven at 105°C until stable, after that they were put in a desiccator for one hour, then weighed (B). The measurement results are reduced by the weight of the dry glass filter before use (C)

$$\text{NDF Content} = ((\text{B-C}))/\text{A}) \times 100\% \quad (1)$$

2.3.2 Acid Detergent Fiber (ADF)

A sample of 1 g (A) was put into a beaker and ml of ADF solution was added. The ADF solution consisted of 1 L of 1 N H_2SO_4 and 20 g of CTAB (cethyle trimethyl ammonium bromide). The sample to which the solution was added was heated for one hour on the back cooler. Filtering is done with the help of a vacuum pump using a glass filter. Washing is carried out alternately with acetone and hot water. The filter results were dried in an oven at 105°C until stable, after that they were put in a desiccator for one hour, then weighed (D). The measurement results are reduced by the weight of the dry glass filter before use (C)

$$\text{ADF Content} = ((\text{D-C})/\text{A}) \times 100\% \quad (2)$$

2.3.3 Cellulose, Hemicellulose, and Lignin Content

ADF residue (E) which is in the glass filter is placed on a tray of water about 1 cm high. Then added H_2SO_4 as high as ¾ part of the glass filter and left for 3 hours while stirring. Filtering with a glass filter is assisted by a vacuum pump. Washing is done with acetone and hot water. Do the drying and put the filter results into the oven. After that, it is put back into the desiccator to cool down and weigh (F). Furthermore, the glass filter is in the furnace at 450°C for 3-4 hours, then put again into the desiccator to cool down and weigh (G)

$$Cellulose\ Content = ((F - G)/E)\ x\ 100\% \quad (3)$$

To find out the hemicellulose and lignin content, you can use the equation below using the results of the ADF and NDF tests.

$$Hemicellulose\ Content = NDF\ Content - ADF\ Content \quad (4)$$

$$Lignin\ Content = ADF\ Content - Cellulose\ Content \quad (5)$$

2.4 Probiotic Growth Testing on Cassava Peel Hydrolysates

The lactic acid bacteria used as probiotics are *Lactobacillus plantarum*. Before use, *Lactobacillus plantarum* was refreshed on MRS Broth for 24 hours at 30ºC. Growth was carried out on MRS Base media with the addition of cellobiose as a substitute for glucose for carbohydrate sources. The composition for making MRS Base is peptone 10 g/L, meat extract 8 g/L, yeast extract 4 g/L, sodium acetate 5 g/L, magnesium sulphate 0.2 g/L, manganese sulphate 0.05 g / l , dipotassium sulphate 0.05 g/L, polysorbate 80 1 g/L and prepared with a pH of 6.2±0.2 at 25ºC (De Man, Ragosa and Sharpe, 1960). Inoculation of 0.1 mL of *Lactobacillus plantarum* on MRS added 0.1 mL of cellobiose. *Lactobacillus plantarum* was also grown on MRS with 0.1 glucose added at a concentration of 250 mg/mL to compare with the growth on MRS with cellobiose. The cellobiose to be added to the MRS must first be filtered on a membrane with a sterile 0.25 µm pore size to avoid bacterial contamination. The growth of *Lactobacillus plantarum* can be observed by growing it on MRS agar. Measurements were carried out on *Lactobacillus plantarum* which had been incubated for 0, 24 and 48 hours at 30ºC (Herawati *et al.*, 2019). as much as 1 mL of *Lactobacillus plantarum* was inoculated on MRS agar and incubated for 48 hours. The growth of *Lactobacillus plantarum* is indicated by the presence of white colonies.

The prebiotic activity score is a comparison between the ability of prebiotics to grow probiotics and inhibit the growth of *Escherichia coli* against growth in glucose during 24 hours of incubation. *Lactobacillus plantarum* was grown on MRS media added by prebiotics and *Escherichia coli* was growth on M9 media added by prebiotics (Moongngarm, Trachoo and Sirigungwan, 2011). The prebiotic activity score can be found using the equation below.

$$Prebiotic\ Activity\ Score = \frac{\log\frac{cfu}{ml}\ probiotic\ \&\ prebiotic\ 24\ hours - \log\frac{cfu}{ml}\ probiotic\ \&\ prebiotic\ 0\ hours}{\log\frac{\text{cfu}}{\text{mL}} probiotic\ \&\ glucose\ 24\ hours - \log\frac{cfu}{ml}\ probiotic\ \&\ glucose\ 0\ hours} - \frac{\log\frac{cfu}{ml}\ E\ coli\ \&\ prebiotic\ 24\ hours - \log\frac{cfu}{ml}\ E\ coli\ \&\ prebiotic\ 0\ hour}{\log\frac{cfu}{ml}\ E\ coli\ \&\ glucose\ 24\ hours - \log\frac{cfu}{ml}\ E\ coli\ \&\ glucose\ 0\ hour}$$

In this test, the composition of the addition of cellobiose and glucose to M9 media followed the concentration on MRS. Before using *Escherichia coli*, its condition can be freshened by growing it on Triptic Soy Broth (TSB) / Brain Heart Infusion Broth (BHIB) and M9 media for 24 consecutive hours respectively. *Escherichia coli* grown on M9 was focused on Triptic Soy Agar (TSA) to count the number of colonies that grew during 24 hours of incubation at 35oC. The number of colonies that grew on TSA and MRS agar media was converted to log cfu/mL and then entered in equation (1)

2.5 Data Analysis

Data presentation was carried out using the Microsoft Excel 2016 program and the Minitab 18 program. Analysis of variance (ANOVA) between samples was carried out using Tukey's honest real difference (HSD) test at the 5% level ($p < 0.05$).

3 RESULT AND DISCUSSION

3.1 Characterization of Cassava Peel

The skin of the Ratim variety of cassava peel (RTM 22) has physical characteristics in the form of red colour on the inner peel (cortex) and has a bland taste. While the Ulujami variety (UJ 17) has white peel with a bitter taste. Based on its structure, the cassava peel consists of two layers, namely the periderm and cortex (Mohd-asharuddin *et al.*, 2017). The cortex layer used as the raw material in this study has a slippery texture, is flexible, is lighter in colour than the periderm. According to Idris *et al.*, (2020) the cortex layer has a higher crude fiber content than the tuber content (flesh), while the cyanide compound content is not significantly different at 0.01 mg/Kg dry basis. The raw material preparation process can be carried out to clean cassava peels from soil impurities and reduce the cyanide acid content found in cassava peels (Falade and Akingbala, 2010).

Based on Table 1, the NDF content of cassava peel of UJ 17 variety was 13.35% and RTM 22 variety was 48.64%. Meanwhile, the ADF content of the cassava peel of UJ 17 variety was 6.36% and RTM 22 variety was 7.63%. Neutral Detergent Fiber (NDF) is an insoluble fiber content in neutral detergents consisting of cellulose and lignin, while Acid Detergent Fiber (ADF) is an insoluble fiber content in acidic detergents consisting of lignin, cellulose, and hemicellulose. The difference between ADF and NDF indicates the amount of hemicellulose

content (Oluwanike and Adeneye, 2014). The total content of cellulose, hemicellulose, and lignin which does not reach 100% both before cooking and after blanching indicates other compounds that do not include insoluble fiber such as starch, protein, fat, and so on. The NDF and ADF content of the two varieties of cassava bark had a significant difference. After cooking with NaOH and blanching with H_2O_2, the NDF and ADF content were not significantly different. The NDF content of cassava peel of UJ 17 variety increased to 68.51% and RTM 22 variety to 67.60%, while the ADF content of cassava peel of UJ 17 variety increased to 60.83% while RTM 22 variety increased to 60.27%.

Table 1: The cassava peel insoluble fiber content.

Insoluble Fiber	Before Cooking		After Bleaching	
	UJ17	RTM 22	UJ17	RTM 22
NDF (%)	13,35 ±0,55[b]	48,64 ±0,09[a]	68,51 ±0,07[a]	67,60 ±0,64[a]
ADF (%)	6,36 ±0,10[b]	7,63 ±0,07[a]	60,83 ±1,21[a]	60,27 ±0,45[a]
Cellulose (%)	4,99 ±0,09[a]	5,74 ±0,05[a]	55,50 ±2,20[a]	55,79 ±0,44[a]
Hemicellulose (%)	6,97 ±0,45[b]	41,02 ±0,02[a]	7,68 ±1,14[a]	7,32 ±0,19[a]
Lignin (%)	1,39 ±0,01[a]	1,88 ±0,02[a]	5,34 ±0,99[a]	4,49 ±0,01[a]

Note: Dry base with 8.98% moisture content. Tests were carried out in 2 replications. a / b: Tukey's real difference test (ANOVA) and honest real difference test (HSD) at the 5% level

Hemicellulose content in cassava peel flour of varieties UJ 17 and RT 22 was 6.97% and 41.02%, respectively. This significant difference shows that different varieties can indicate different characteristics of crude fiber. The lignin content of varieties UJ 17 and RTM 22 were 1.39% and 1.88%, respectively. A study by Barati, Latif and Müller, (2019) states that cassava contains 30.4% hemicellulose. The lignin content in cassava skin reaches 7.50% (Aripin *et al.*, 2013). After the cooking and bleaching process, the hemicellulose content changed to 7.32%, while the lignin content became 4.49%. Changes in hemicellulose and lignin content are caused by dissolving insoluble non-fiber compounds during the cooking process with NaOH and bleaching with H_2O_2. The process of dissolving lignin and hemicellulose also occurs during cooking with NaOH at high temperatures. The reaction between NaOH and lignin in hot conditions results in the formation of a thick black and sticky solution that can be separated from the solvent. The bleaching process carried out after extraction through the addition of H_2O_2 aims to remove the remaining lignin by oxidizing the chromophore molecules in the lignin so that it becomes polar and water-soluble. This process is important because naturally lignin is water-insoluble and binds to hemicellulose and lignin (Allen *et al.*, 2016).

The cellulose content of the RTM 22 variety of cassava peel after cooking with NaOH and blanching with H_2O_2 showed an increase from 5.74% to 55.70%. The increase in cellulose content in the cassava peel varieties UJ 17 from 4.99% to 55.50% The increase in cellulose content is due to the nature of cellulose which has good resistance to alkaline and heat compounds up to 280ºC used in the extraction process (Suryanto, 2015). Meanwhile, according to Widiarto *et al.*, (2017) the use of acids in extraction makes cellulose hydrolysed into a simpler form. In addition to the extraction method used in this study, the alternative use of 4% NaOH and 4% NaOCl in extraction resulted in 40.5% cellulose, 11.7% lignin, and 21.4% hemicellulose (Widiarto *et al.*, 2019). Therefore, the extraction method using 11% NaOH and 30% H_2O_2 has a better result to increase the percentage of cellulose. In this bleaching process, it is also possible to decrease cellulose in the amorphous form and increase the percentage of cellulose in the crystalline form (Leite, Zanon and Menegalli, 2017).

3.2 Probiotic Growth Testing on Cassava Peel Hydrolysates

The hydrolysate of the cassava peel was obtained based on the results of the hydrolysis of the cassava peel flour which had been cooked with NaOH and blanched with H2O2. Hydrolysis was carried out using cellulase enzymes with a concentration of 4.03 U/mL on UJ 17 and RTM 22 varieties. The enzyme concentration was obtained from the results of preliminary research using a concentration of 18.91 U/mL and 22.06 U/mL with the results of the first 1 hour of hydrolysis. has reached the degree of polymerization 1,00. Hydrolysis using a substrate of 200 mg, 0.1 mL, 200 mL of citrate buffer pH 4.8, and 10% sodium azide as antimicrobial. The cellulase enzyme activity used was 115 U/mL (Worthington, 2020). Hydrolysis was performed using a shaker incubator at 37ºC with an agitation speed of 150 rpm for 24 hours (Selig, Weiss and Ji, 2008). Hydrolysate is obtained when the hydrolysis process has been going on for 12 hours. The results of hydrolysis of cassava peels for 12 hours were used as a carbohydrate source substitute in MRS media to

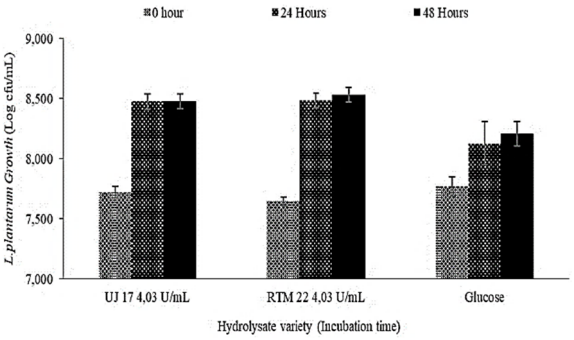

Figure 1: *Lactobacillus plantarum* growth on cassava peel hydrolysate and MRS.

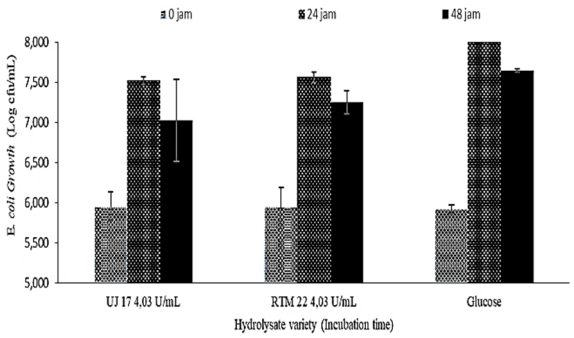

Figure 2: *Escherichia coli* growth on cassava peel hydrolysate and M9.

growth *Lactobacillus plantarum* and as a carbohydrate source substitute in M9 medium to grow *Escherichia coli*. Based on the preliminary research that has been carried out, the hydrolysate has a degree of polymerization below 2.00 which indicates that the content of cellobiose and cello-oligosaccharide has been dominant.

The growth of *Lactobacillus plantarum* growth with UJ 17 hydrolysate at 0 hour was 7,719 log cfu/mL and increased significantly at 24 hours of growth with 8,475 cfu/mL, but after 48 hours there was no significant difference, namely 8,479 cfu/mL. The growth of *Lactobacillus plantarum* on RTM 22 variety hydrolysate was at 7,643 log cfu/mL at 0 hours and increased significantly to 8,482 log cfu/mL. Growth at 24 hours and 48 hours had no significant difference with 8,535 log cfu/mL. In the two varieties of cassava peel at 24 and 48 hours, the growth was not significantly different. This shows that the microbes have been in a stationary phase because they are still in the same log colony number with growth at 24 hours. (Karnaouri, Matsakas, Bühler, *et al.*, 2019) reported that the growth of *Lactobacillus plantarum* in media added with cellobiose also showed a stationary phase at the incubation time of 48 hours to 72 hours. The amount of cellobiose consumed would correlate with the increase in the amount of lactic acid, acetic acid, and propionic acid formed. The growth of *Lactobacillus plantarum* is suspected not because there are still peptides from the inactivation of the cellulase enzyme by heating at 85oC for five minutes. Apart from the percentage use of the enzyme which is 1% of the total hydrolysis volume, the stratified filtration process with a membrane measuring 0.45µm and 0.20µm is expected to minimize the contamination of macronutrients and microorganisms that bias the growth of *Lactobacillus plantarum*.

The growth of *Escherichia coli* on M9 medium grown on the hydrolysate of cassava peels of UJ 17 variety in 0 hours was 5.953 log cfu/mL and increased significantly to 7.524 log cfu/mL at 24 hours of growth. While the growth of *Escherichia coli* on M9 media added to the hydrolysate of cassava varieties RTM 22 in 0 hours was 5.952 log cfu / mL. The increase occurred in growth within 24 hours to 7.566 log cfu / ml. The growth of *Escherichia coli* at 48 hours experienced a decrease in both the hydrolysates of UJ 17 and RTM 22 varieties, respectively 7,026 log cfu / mL and 7,253 log cfu / mL. The growth of *Escherichia coli* on M9 media with added glucose had a higher growth than in M9 media which was added by hydrolysis of cassava peel. The growth of *Escherichia coli* by two logs shows that the products of cellulose hydrolysis of the three varieties still contain β glucose which is easily digested by *Escherichia coli*, but after 24 hours the incubation of β glucose contained in M9 is reduced and the growth of *Escherichia coli* is lower because cannot digest cellobiose and cello-oligosaccharides found in M9. Growth conditions in the hydrolysate that are expected to occur in cellobiose as a prebiotic in providing the ability to grow *Lactobacillus plantarum* as a probiotic and not a source of carbohydrates for *Escherichia c*oli in the human digestive tract. Cellobiose which is thought to be dominant in the product of cellulase enzyme hydrolysis has the ability to grow *Lactobacillus plantarum* which is better with an optical density (OD) value of 600 more than 5 compared to other Lactobacillus strains and Bifidobacterial strains as probiotics, *Lactobacillus plantarum* has low growth. on media with added glucose for 24 hours (Karnaouri, Matsakas, Krikigianni, *et al.*, 2019).

The ability of hydrolysis products needs to be measured as a prebiotic potency using prebiotic activity score analysis. The hydrolysate of RTM 22 cassava peel with 2.88 U / mL cellulase had a higher prebiotic activity score than the UJ 17 variety. The difference in prebiotic activity scores between

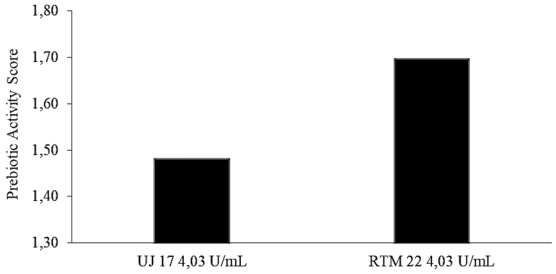

Figure 3: Prebiotic Activity Score on cassava peel hydrolysate.

cassava varieties was thought to be due to differences in the amount of cellobiose and β glucose found in hydrolysis products. The high percentage of cellobiose content will increase the growth of *Lactobacillus plantarum*, while the higher percentage of β glucose content will increase the growth of *Escherichia coli*. The percentage of growth of the two bacteria affects the prebiotic activity score. The difference in the content of cellulose, lignin, and hemicellulose contained in the substrate was thought to affect, however, the cellulose extraction process made the composition of the lignocellulose compounds not significantly different between cassava varieties. The prebiotic activity scores of the two-cassava peel hydrolysates of UJ 17 and RTM 22 varieties were 1.48 and 1.70, respectively. The prebiotic activity score was higher than the score on inulin from the hydrolysis of red fruit grown on *Lactobacillus casei* of 0.88 (Murtiningrum *et al.*, 2019). The prebiotic activity score was also higher than the prebiotic activity score of gallactooligosaccharide (GOS) grown on *Lactobacillus plantarum 12006* (Huebner, Wehling and Hutkins, 2007). The prebiotic activity score on the RTM 22 hydrolysate was also higher than the growth of *Lactobacillus acidophilus* on fructooligosaccharides (FOS), but lower than the growth of Lactobacillus acidophilus on inulin (Moongngarm, Trachoo and Sirigungwan, 2011).

4 CONCLUSION

The characteristics of insoluble fiber in the cassava peel consist of cellulose, hemicellulose, and lignin. Hemicellulose content is more dominant than cellulose and lignin in the cassava peel. The hemicellulose content of UJ 17 and RTM 22 varieties had a significant difference. After cooking with NaOH and blanching with H_2O_2, cellulose content became dominant compared to hemicellulose and lignin. This process causes changes in the characteristics of insoluble fiber, such as the dissolution of non-soluble fiber compounds and hemicellulose and lignin. The cassava peel varieties UJ 17 and RTM 22 contained no significant difference in cellulose. The hydrolysate of cellulose from the cassava peel showed its potential as a prebiotic in growing *Lactobacillus plantarum*. The hydrolysates from cassava peels of RTM 22 varieties had a higher prebiotic activity score than UJ 17. The prebiotic activity scores of the cassava peel hydrolysates of RTM 22 and UJ 17 varieties were 1.70 and 1.48, respectively.

ACKNOWLEDGEMENTS

Thank you to the Directorate of Higher Education, Ministry of Education and Culture of the Republic of Indonesia for funding this research through the master's thesis research scheme. Thanks to Prof. Dr. Ir. Lilis Nuraida, M.Sc. who has allowed the use of *lactobacillus plantarum 026* culture that is owned and thanks to Dr. Ir. Nurul Khumaida, M.Sc. which has permitted the use of cassava peel of a Ratim variety (RTM 22) dan Ulujami variety (UJ 17) that were owned for this study.

REFERENCES

Allen, S. A. *et al.* (2016) 'Lignocelluloses : An Economical and Ecological Resource for Bio-Ethanol Production – A Review', *International Journal of Natural Resource Ecology and Management*, 1(3), pp. 128–144. doi: 10.11648/j.ijnrem.20160103.18.

Aripin, M. *et al.* (2013) 'Cassava Peels for Alternative Fibre in Pulp and Paper Industry : Chemical Properties and Morphology Characterization', *International Journal of Integrated engineering*, 5(1), pp. 30–33.

Barati, Z., Latif, S. and Müller, J. (2019) 'Enzymatic hydrolysis of cassava peels as potential pre-treatment for peeling of cassava tubers', *Biocatalysis and Agricultural Biotechnology*. Elsevier Ltd, 20 (December 2018), p. 101247. doi: 10.1016/j.bcab.2019.101247.

BPS, B. P. S. (2013) *Pengeluaran dan Konsumsi Penduduk Indonesia*, *Survey Sosial Ekonomi Nasional*.

Elechi, O. O. *et al.* (2016) 'Acid Hydrolysis Of Cassava Peel', *International Journal of Scientific & Technology Research*, 5(01), pp. 184–187.

Falade, K. O. and Akingbala, J. O. (2010) 'Utilization of Cassava for Food', *Food Review International*, 9129(27), pp. 51–83. doi: 10.1080/87559129.2010.518296.

Herawati, E. R. N. *et al.* (2019) 'Oligosaccharides Profile and Prebiotic Potential of Gembolo Tuber (Dioscorea

bulbifera) Oligosaccharides Profile', in *IOP Conf. Series: Earth and Enviromental Science*. IOP Publishing. doi: 10.1088/1755-1315/251/1/012048.

Huebner, J., Wehling, R. L. and Hutkins, R. W. (2007) 'Functional activity of commercial prebiotics', *International Dairy Journal*, 17(7), pp. 770–775. doi: 10.1016/j.idairyj.2006.10.006.

Idris, S. *et al.* (2020) 'Physicochemical composition of different parts of cassava (Manihot esculenta crantz) plant', *Food Research*, 4, pp. 78–84. doi: 10.26656/fr.2017.4(S1).S33.

Idugboe, Nwokoro, O. and Imasuen, S. (2017) 'Chemical Composition of Cassava Peels Collected from Four Locations (Koko , Warri , Okada and Benin City), Brewers` Spent Yeast and Three Grades of "Caspeyeast", *International Journal of Science and Research*, 6(4), pp. 2015–2018. doi: 10.21275/ART 20172389.

Karnaouri, A., Matsakas, L., Bühler, S., *et al.* (2019) 'catalysts Tailoring Celluclast ® Cocktail ' s Performance towards the Production of Prebiotic Cello-Oligosaccharides from Waste Forest Biomass', *Catalysts*, 9, p. 897.

Karnaouri, A., Matsakas, L., Krikigianni, E., *et al.* (2019) 'Valorization of waste forest biomass toward the production of cello-oligosaccharides with potential prebiotic activity by utilizing customized enzyme cocktails', *Biotechnology for Biofuels*. BioMed Central, 12(1), pp. 1–19. doi: 10.1186/s13068-019-1628-z.

Leite, A. L. M. P., Zanon, C. D. and Menegalli, F. C. (2017) 'Isolation and characterization of cellulose nanofibers from cassava root bagasse and peelings', *Carbohydrate Polymers*. Elsevier Ltd., 157, pp. 962–970. doi: 10.1016/j.carbpol.2016.10.048.

De Man, J. ., Ragosa, M. and Sharpe, M. E. (1960) 'A Medium For Cultivation of Lactobacilli', *J Appl Bact*, 1, pp. 130–135.

Mohd-asharuddin, S. *et al.* (2017) 'A Chemical and Morphological Study of Cassava Peel : A Potential Waste as Coagulant Aid', in *MATEC Web of Conferences*. EDP Sciences, pp. 1–8. doi: 10.1051/ matecconf/201710306012.

Moongngarm, A., Trachoo, N. and Sirigungwan, N. (2011) 'Low Molecular Weight Carbohydrates, prebiotic content, and prebiotic activity of selected food plants in Thailand', *Advance Journal of Food Science and Technology*, 3(4), pp. 269–274.

Murtiningrum *et al.* (2019) 'Identification of Inulin Profile From Red Fruit (Pandanus Conoideus L) Pedicel Extract Using LC-MS And Its In Vitro Prebiotic Activity Test', *International Journal of Advance Reasearch*, 7(11), pp. 344–351. doi: 10.21474/IJAR01/ 10014.

Oluwanike, A. and Adeneye, M. A. (2014) 'Evaluation of chemical composition and nutritive potential of oil palm slurry fermented with cassava peel as feed for livestock', *African Journal of Agricultural Research*, 9(26), pp. 2062–2067. doi: 10.5897/AJAR2013.7565.

Razie, F. *et al.* (2011) 'Aktivitas Enzim Selulase Mikroba Yang Diisolasi Dari Jerami Padi Di Persawahan Pasang Surut di Kalimantan Selatan', *J. Tanah Linkungan*, 13(2), pp. 43–48.

Selig, M., Weiss, N. and Ji, Y. (2008) *Enzymatic Saccharification of Lignocellulosic Biomass Laboratory Analytical Procedure*. Colorado.

van Soest, P. J. (1963) 'Use of Detergents in the Analysis of Fibrous Feeds. II. A Rapid Method for the Determination of Fiber and Lignin', *Journal of AOAC*, 46, pp. 829–835.

Surendran, A. *et al.* (2018) 'Inhibition and Kinetic Studies of Cellulosa and Hemicellulose Degrading Enzymes of Ganoderma Boniense by NaturallyOccurring Phenolic Compounds', *Journal of Applied Microbiology*, 124(6), pp. 1544–1555. doi: 10.1111/jam.13717.

Suryanto, H. (2015) 'Thermal degradation of mendong fiber', in *The 6th Green Techonlogy*. Malang.

Tasaso, P. (2015) 'ptimiz tio of Re ctio o ditio s for Sy thesis of rboxymethy e u ose from i P m Fro ds', *International Journal of Chemical Engineering and Applications*, 6(2), pp. 3–6. doi: 10.7763/IJCEA.2015.V6.460.

Widiarto, S. *et al.* (2017) 'Preparation and Characterization of Cellulose and Nanocellulose from Agro-industrial Waste - Cassava Peel', in *IOP Conference Series: Materials Science and engineering*. IOP Publishing, p. 176. doi: 10.1088/1742-6596/755/1/011001.

Widiarto, S. *et al.* (2019) 'Cellulose Nanofibers Preparation from Cassava Peelsvia Mechanical Disruption', *Fibers*, 7(44), pp. 1–11. doi: 10.3390/fib7050044.

Worthington (2020) *Worthington Enzyme Manual Cellulase*, *Worthington Biochemical Corporation*. Available at: http://www.worthington-biochem.com/ CEL/default.html.

van Zanten, G. C. *et al.* (2012) 'The Effect of Selected Synbiotics on Microbial Composition and Short-Chain Fatty Acid Production in a Model System of the Human Colon', *PloS ONE*, 7(10). doi: 10.1371/journal.pone.0 047212.

Van Zanten, G. *et al.* (2014) 'Synbiotic Lactobacillus acidophilus NCFM and cellobiose does nt affect humat gut bacterial diversity but increases abudance f lactobacili, bifidobacteria and branched-chain fatty acids: a randomizes, double-blinded cross-over trial.' doi: 10.1111/1574-6941.12397.