

PRODUCTION OF MALTO-OLIGOSACCHARIDES FROM CASSAVA CULTIVAR KUNING

Nanik Rahmani, Ade Andriani, Yopi and Sri Hartati

Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI)
Jl. Raya Bogor Km. 46, Cibinong, Bogor 16911, Indonesia
Phone (021) 8754587/Fax (021) 8754588
e-mail: yop_i@yahoo.com

(Diterima 20-02-2015; Disetujui 30-11-2015)

ABSTRACT

Characteristic the physic-chemical of Indonesia cassava starch from four cultivated varieties has been conducted for maltooligosaccharide production. Result of proximate analysis of the extracted starch indicated that the extracted starch was quite pure. The purity of the extracted starch was visually confirmed by microscopic analysis by using SEM micrographs at 2500X magnifications show that the integrity of the granules starch as intact. Based on the amylopectin and amylase content showed that one of cultivated variety of cassava, cultivated variety Kuning contain the amylopectin higher than amylose was compared with the other cultivated variety. The next focus research was analysis potential of starch from cultivated variety Kuning for maltooligosaccharide production by enzymatic hydrolysis by α -amylase from marine bacterium *Brevibacterium* sp. The optimum hydrolysis condition for cultivated variety Kuning was obtained substrate concentration 4.5% (b/v), comparison of substrate: enzyme 1:2, temperature reaction 30°C with reducing sugars concentration of 13.359 ppm. The hydrolysis products of cassava starch cultivated variety Kuning were maltooligosaccharides mixture, yielding maltose, maltotriose, maltotetraose, maltopentaose. This result showed that cassava starch of cultivated varieties Kuning potential for maltooligosaccharides production.

Key words: Tuber, cassava, starch, phisic-chemical, maltooligosaccharides

ABSTRAK

Nanik Rahmani, Ade Andriani, Yopi and Sri Hartati. 2015. Produksi maltooligosakarida dari ubi kayu varietas kuning.

Karakteristik fisiko kimia karbohidrat dari empat varietas kultivar asal Indonesia dilakukan untuk melihat potensinya sebagai bahan baku untuk produksi maltooligosakarida. Analisa proksimat karbohidrat hasil ekstraksi dari keempat varietas kultivar ubi kayu mengindikasikan bahwa karbohidrat yang dihasilkan cukup murni. Kemurnian dari karbohidrat tersebut terlihat setelah dikonfirmasi dengan analisa mikroskopis dengan menggunakan mikroskop electron SEM dengan pembesaran 2500X yang menunjukkan bahwa granulanya utuh. Berdasarkan kadar amilopektin dan amilosa menunjukkan bahwa salah satu varietas kultivar ubi kayu yaitu varietas Kuning mengandung amilopektin lebih tinggi dibandingkan kadar amilosanya jika dibandingkan dengan tiga varietas kultivar lainnya. Fokus penelitian selanjutnya adalah analisa potensi karbohidrat varietas kultivar Kuning tersebut untuk produksi maltooligosakarida dengan hidrolisis enzimatis oleh α -amylase dari *Brevibacterium* sp. Kondisi optimum hidrolisis dari varietas kultivar Kuning diperoleh pada konsentrasi substrat 4.5% (b/v), perbandingan substrat dan enzim 1:2, suhu reaksi 30°C dengan kadar gula reduksi yang diperoleh 13.359 ppm. Produk hidrolisis dari ubi kayu varietas kultivar Kuning adalah berupa campuran maltooligosakarida yang terdiri atas maltose, maltotriose, maltotetraose, maltopentaose. Hasil ini menunjukkan bahwa karbohidrat ubi kayu dari varietas kultivar Kuning mempunyai potensi untuk digunakan sebagai bahan baku untuk produksi maltooligosakarida.

Kata kunci: umbi-umbian, ubi kayu, karbohidrat, fisiko kimia, maltooligosakarida

INTRODUCTION

Tubers become one of the alternative source developments of carbohydrate than rice and maize. One of tuber groups is cassava (*Manihot esculenta*) that most popular especially at the most rural areas in Indonesia either consumed directly or as a main raw material (starch) in various industry. Indonesia is the fourth country which largest producer cassava in the world after Nigeria, Brazil and Thailand in 2010¹. Starch from cassava is the main ingredient in a number of industrial textiles, paper, pharmaceuticals and food.

The utilization and processing of tuber become foodstuffs depends on starch characteristic that contained in each of the tuber. There are many advantages of utilizing cassava for starch production. Cassava offers a relatively cheap source of raw material containing a high concentration of starch (dry matter basis) that can match or better the properties offered by other starches (maize, wheat, sweet potato and rice). Cassava starch is easy to extract using a simple process (when compared to other starches) that can be carried out on a small-scale with limited capital. In addition cassava starch has a high level of purity due to the low levels of proteins and lipids found in cassava roots².

Today people are becoming more health conscious and they are looking for foods with special health-promoting functions. Oligosaccharides are attracting increasing interest as prebiotic functional food ingredients. They can be extracted or obtained by enzymatic hydrolysis from a variety of biomass sources or synthesized from simple oligosaccharides by enzymatic transfer reactions³. Analysis characteristics of physico-chemical of starch from cassava was important to develop processing of foodstuff product and its derivatives that potential for food functional application. Starch from tubers can be used as a material for maltooligosaccharides production. The use of cassava as a material for maltooligosaccharide production is one of the current challenges for increasing both of the value of cassava and human nutrition.

Maltooligosaccharides potential were used for functional food, such as symbiotic health-food products containing both probiotic bifidobacteria and prebiotic maltooligosaccharides^{4,5}. The purpose of this study is to determine the characteristic of physico-chemical of Indonesia cassava starch from four varieties cultivar (FEC 25, Adira I, Kuning and Roti) and analysis their potential for maltooligosaccharide productions.

METODOLOGY

Materials

Samples of cassava which harvested in nine months were provided by Laboratory of Plant Molecular Genetics and Biosynthetic Pathway Alteration, Research Center for Biotechnology-LIPI.

Methods

Starch extraction

The starch was extracted through the stages of the process, such as stripping, washing, grater, extraction, filtration, precipitation, drying, and sieving. Fresh cassava tuber from four varieties cultivar were peeled and washed by using manually to clean tubers from soil and the other dirt. The tuber was shredded using grater machine and then starch extracted by added water with a ratio of material and water was 3.5: 1. Furthermore, the filtering has been done to separate the starch from the residue. Residue obtained from the screening process again extracted 5 times with the same ratio of the water addition and precipitation on night. After precipitation, the supernatant were removed until the only remaining part of the wet starch deposition. Furthermore, drying starch obtained using the sun. Starches continue were crushed with mortar and then continue to the sifting process to obtain a uniform particle size using a filter pore size of 50 meshes. Finally, starch obtained from four varieties cultivar were weighed and subsequently for next analyzed.

Proximate analysis

Moisture, protein, lipid, ash and crude fiber contents of the isolated starches were determined using the approved methods of AOAC⁶.

Physico-chemical analysis

Physico-chemically analysis consist of carbohydrate content (by different), amylose, amylopectin, viscosity, starch digestibility, water and oil absorptions. The amylose content was determined by using the iodine blue complex method using a solution of 0.2% iodine in 2% potassium iodide⁷. The digestibility of starch was performed by in vitro using methods developed by⁸. The principle of this method, the sample is hydrolyzed using α -amylase enzyme into simple units such as maltose. The amount of maltose hydrolysis enzyme was measured using spectrophotometric method.

Scanning electron microscopy (SEM) analysis

Granula of the isolated starch was additionally checked by scanning electron microscopy (SEM)⁹.

Optimization of hydrolysis condition starch Kuning variety cultivar for maltooligosaccharide production

Enzymatic hydrolysis was carried out under various conditions, such as diverse substrate concentrations (w/v) 1.5, 3, 4.5, 6 and 7.5%, comparison of substrate (mL) with enzyme (mL) ratio (v/v) 1:10 (1 mL substrate : 23 U amylase), 1:5 (1 mL substrate : 11.5 U amylase), 1:2 (1 mL substrate : 4.6 U amylase), and 1:1 (1 mL substrate : 2.3 U amylase) and temperature reaction (30, 40 and 50°C). Amylase enzyme was used in this analysis from marine bacterium, *Brevibacterium* sp. with enzyme activity 2.3 U/mL. Reactions were carried out in 100 mL Erlenmeyer flasks containing 20 mL of reaction mixtures in a rotary shaker (Stuart orbital incubator S1500, Staffordshire, United Kingdom) at room temperature. Samples were taken at regular intervals (after 1, 2, 4, 6, and 8h), reactions were stopped by heating the samples in boiling water.

Reducing sugar analysis

Product hydrolysis was analyzed by calculating the reducing sugars. Reducing sugars were determined by the DNS method¹⁰.

Thin Layer chromatography (TLC) analysis

TLC analysis of maltooligosaccharides products was carried out by the ascending method (three time development) on silica gel 60F254 plates (Merck Art20-20 cm, Darmstadt, Germany). All samples were applied

in equal quantities (4 µL) and then resolved by two runs with a solvent mixture of n-Butanol/aceticacid/water (12:6:6, by volume). Spots were visualized by spraying the sugar color (0.5 g α-diphenylamine, 25 mL acetone, 2.5 mL phosphate acid, 0.5 ml aniline) and subsequent heating at 100°C for 15 min.

RESULT AND DISCUSSIONS

Strach recovery from four varieties cultivar cassava

First step was extraction of starch from four varieties cultivar cassava. Fresh tuber and result of starch powder extraction from four varieties cultivar were presented in Figure 1. From appearance characteristics, varieties Kuning, FEC 25 and Adira I have yellow color most likely due to the high β-carotene content with the most highest was Kuning varieties and Roti varieties has white color.

The total weight rendement of cassava starch obtained from extraction of fresh tubers from four varieties cultivar was presented at Figure 2. The total amount of starch, expressed as a percent of dry matter. The yield of starch from four varieties cultivar cassava was ± 20 % on a grain dry matter basis. The relatively low yield could be attributed to starch losses occurred during the repeated washing steps during the starch extraction process.

Microscopic properties

Starch exists as starch granules. Starch granules from four varieties cultivar were evaluated by using scanning electron micrographs presented in Figure 3. There are not differences results of SEM analysis from



Figure 1. Photo of fresh cassava tubers, cassava tuber after peeled and starch powder from four varieties (Kuning, FEC 25, Adira I and Roti)

Gambar 1. Foto ubi kayu segar, ubi kayu setelah dikupas dari kulitnya dan tepung karbohidrat dari empat varietas kultivar (Kuning, FEC 25, Adira I dan Roti)

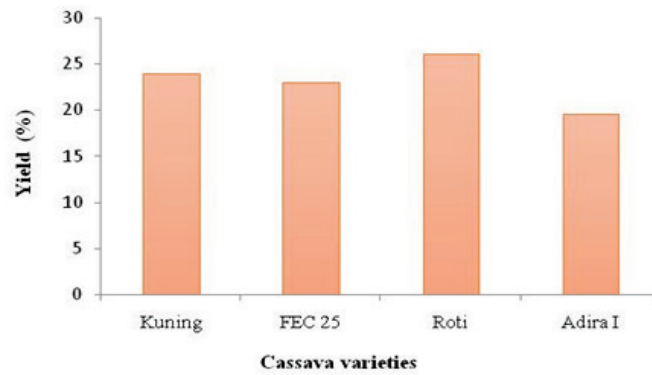


Figure 2. Yield of cassava starch from four varieties cultivar
 Gambar 2. Berat rendemen karbohidrat dari empat varietas kultivar ubi kayu

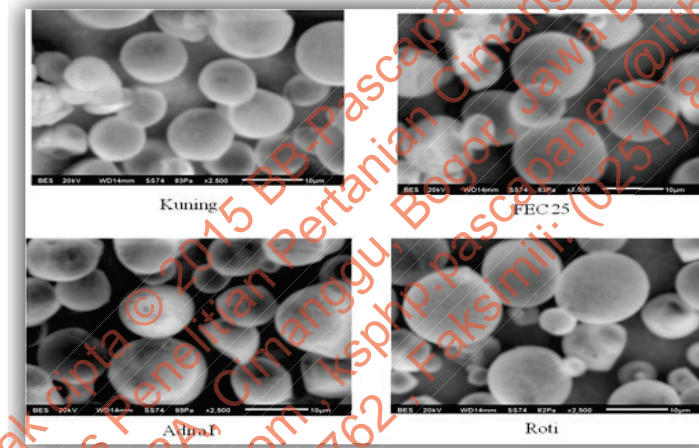


Figure 3. Microscopic analysis of starch from four varieties cultivar cassava: Kuning, FEC 25, Adira I and Roti by using scanning electron microscopy (SEM) with magnification 2500X
 Gambar 3. Analisa mikroskopis karbohidrat dari empat varietas kultivar ubi kayu: Kuning, FEC 25, Adira I and Roti dengan menggunakan scanning electron microscopy (SEM) pembesaran 2500X

four varieties cultivar cassava. The purity of the extracted starch was visually confirmed by SEM micrographs at 2500X magnifications with scale 10 μ m show that the integrity of the granule starches as intact and the granule size of starches from four varieties cultivar small oval shaped (Figure 3).

Proximate analysis

Proximate analysis of extracted cassava starch were presented in Table 1. The raw starch from four varieties cultivar employed in this study (Kuning, Adira I, FEC 25 and Roti) differs in proximate analysis properties each other with respect to water content, ash content, protein content, fat content and crude fiber. Based on proximate analysis showed that protein and fat content were law, these values indicated that the extracted starch was quite pure, so we can used this starch for next analysis.

Physic-chemical analysis

Physic-chemical composition of extracted cassava starch were presented in Table 2.

Based on Table 2 showed that cassava starch from all varieties cultivar have similar content of carbohydrate total around 80%. The percent amylose content of the four varieties cultivar extracted starch varied from 13.0 to 16.4%, where is amylopectin content varied from 28.54 to 47.30%. Three varieties cultivar, such as FEC 25, Adira I and Roti contain amylopectin (30%) lower than amylose and Kuning variety cultivar has amylopectin higher (47%) than amylose. Fenema OR¹¹ described that cassava starch is a good raw material for maltooligosaccharide production because it has a high content of amylopectin. The amylopectin content of the cassava starch was lower than amylose and high starch digestibility, which confirmed that these starch easier

Production of Malto-Oligosaccharides from Cassava Cultivar Kuning
(Nanik Rahmani et al)

Table 1. Proximate components of starch from four varieties cultivar cassava
Tabel 1. Komponen proksimat karbohidrat dari empat varietas kultivar ubi kayu

Proximate components Komponen proksimat (%)	Cassava varieties cultivar/ Varietas kultivar ubi kayu			
	Kuning	FEC 25	Roti	Adira
Kadar air/ Moisture	15.56	11.39	11.5	11.29
Kadar abu/ Ash content	0.25	0.24	0.22	0.17
Protein kasar/ Crude protein	0.48	0.42	0.65	1.18
Lemak kasar/ Crude lipid	0.37	0.5	0.76	1.05
Serat kasar/ Crude fiber	0.09	0.27	0.13	1.22

n = triple

Table 2. Physic-chemical characteristics of starch from four varieties cultivar cassava
Tabel 2. Karakteristik fisiko kimia karbohidrat dari empat varietas kultivar ubi kayu

Physic-chemical characteristics of starch	Cassava varieties cultivar/ Varietas kultivar ubi kayu			
	Kuning	FEC 25	Roti	Adira I
β -caroten / β -karoten (ppm)	7.2	5.9	8.5	-
Carbohydrate content / Kadar pati (by different)	75.7	85	85.5	85.0
Amylopectin / Amilopektin (%)	47.3	30.9	28.5	30.5
Amylose /Amilosa (%)	28.4	54.1	57.1	54.5
Viscosity /Viskositas (Cp)	16.4	14.4	14.8	13.0
Starch digestibility / Daya cerna karbohidrat (%)	97.5	83.4	64.7	88.2
Water absorption / Penyerapan air (g/g)	0.6	1.3	0.8	0.9
Oil absorption / Penyerapan minyak (g/g)	1.1	1.1	1.2	1.2

n = triple

to hydrolysis by α -amylase. While cassava starch from Kuning variety cultivar contain amylopectin higher than amylose showed that this variety same with the characteristic of black potato starch from Indonesia contain amylopectin higher than amylose, 67.69%¹².

Starches from all cassava variety cultivar were potential to be used for produce maltooligosaccharide. It showed by value of high digestibility assay (65-100%). Based on the amylopectin content show that Kuning variety can be used for maltooligosaccharde production linier type, different with the other variety cultivar. So, in this research we focus to analysis optimization hydrolysis condition of starch cassava Kuning variety cultivar for maltooligosaccharide production.

Optimization hydrolysis condition of starch variety cultivar Kuning for maltooligosaccharide production

The parameter analysis for optimation hydrolysis condition consist of substrate concentration, comparison of enzyme and substrate and temperature of hydrolysis. The enzymatic hydrolysis of starch by α -amylase leads to the formation of smaller maltosaccharide fragments. Every starch polysaccharide molecule possesses exactly one reducing end which can reduce dinitrosalicylic

acid; therefore, the measurement of reducing sugars is a useful method to determine the molar concentration of starch molecules in solution¹³. So, we used measurement the reducing sugar as a parameter to decide the optimum condition for each parameter analysis. First step, we decide the optimum substrate concentration. Values of reducing sugars on some substrate concentration (1.5, 3, 4.5, 6 and 7.0 %) were shown at Figure 4. The value reducing sugar optimum at substrate concentration 4.5% and the lowest reducing sugar at substrate concentration 1.5%.

Next step was to decide the comparison between substrate and enzyme amylase concentration used in the hydrolysis. We used substrate concentration 4.5% from the previously result. Comparison between substrate and enzyme concentration were made in v/v in hydrolysis reaction. The volume of substrate were made fix (1 mL), whereas enzyme concentration were made in variance 1 mL, 2 mL, 5 mL, 10 mL with each activity 2.3 U, 4.6 U, 11.5 U and 23 U. Yield of reducing sugar produced by enzymatic hydrolysis on variance comparison substrate and enzyme were shown at Figure 5. Based on this figure shown that optimum comparisons between substrate and enzyme was 1:2 with the highest value of reducing sugar.

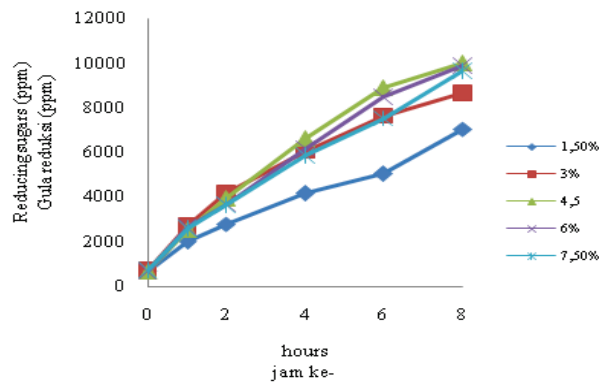


Figure 4. Yield of reducing sugar (ppm) through enzymatic hydrolysis on variance substrate concentration of starch Kuning variety cultivar, n = 3 kali ulangan

Gambar 4. Kadar gula reduksi (ppm) yang dihasilkan melalui hidrolisis enzimatis pada berbagai konsentrasi substrat karbohidrat varietas kultivar Kuning, n = triple

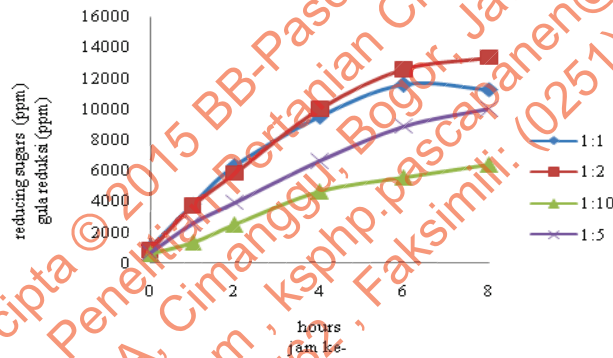


Figure 5. Yield of reducing sugar (ppm) through enzymatic hydrolysis on substrate enzyme comparison optimization of starch variety cultivar Kuning, n = 3 kali ulangan

Gambar 5. Kadar gula reduksi yang dihasilkan melalui hidrolisis pada berbagai variasi perbandingan substrate dan enzim varietas kultivar Kuning, n = triple

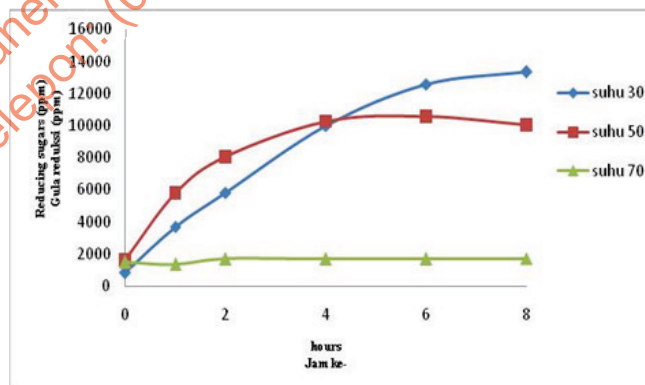


Figure 6. Yield of reducing sugar (ppm) through enzymatic hydrolysis on variance of temperature hydrolysis of starch variety kultivar Kuning, n = 3 kali ulangan

Gambar 6. Kadar gula reduksi yang dihasilkan pada berbagai suhu hidrolisis dari varietas kultivar Kuning, n = triple

The final step was to decide the temperature optimum for hydrolysis substrate by α -amylase from *Brevibacterium* sp to produce maltooligosaccharide from variety cultivar Kuning. We used substrate concentration 4.5% and comparison substrate and enzyme 1:2 (v/v) in three variance temperature hydrolysis, such as 30, 50 and 70°C. The values of reducing sugars from three variance temperature hydrolysis were shown at Figure 6. From this data showed that temperature 50°C was optimum for production maltooligosaccharide with the highest value of reducing sugars from variety cultivar Kuning.

Based on the value of reducing sugars were shown at Figure 4, 5, and 6, respectively showed that optimum hydrolysis condition for maltooligosaccharide production from Kuning variety cultivar were substrate concentration 4.5% (b/v), comparison of substrate: enzyme 1:2, and temperature hydrolysis 30°C with the reducing sugars 13.359 ppm.

Duedahl-Olesen et al¹⁴ described that chromatographic methods using different separation techniques have been applied to determination of the starch hydrolysis products formed by the maltooligosaccharide forming amylases. Thin-layer chromatography (TLC) is a low cost method and still commonly used as an analytical tool for the detection of starch hydrolysis products because of its simplicity and relatively high sensitivity^{15,16,17,18,19,20,21,22}. So, we used the TLC method to decide the type of maltooligosaccharide were formed by Kuning variety cultivar. Many amylases producing specific maltooligosaccharide. Enzyme α -amylase from *Brevibacterium* sp can be degraded of cassava starch of Kuning variety cultivar to produce various maltooligosaccharides type, such as maltose, maltotriose, maltotetraose and maltopentaose based on the TLC result (Figure 7).

Based on the TLC data on Figure 7 showed that Kuning variety cultivar can produce variance maltooligosaccharide from 0 hour until 8 hour reaction, with the dominant product was maltotriose, maltotetraose and maltopentaose. This result was similar to²³ described that potato starch hydrolysis by thermophilic α -amylase from *B. megaterium* VUMB109 produced higher quantity of maltopentaose and maltotriose²⁴ also reported amylase from *Pseudomonas stutzeri* AS22 produce high maltotetraose (G4).

Result analysis from variety Kuning was very interested, because cassava starch from Kuning variety can produce mainly maltotriose, maltotetraose and maltopentaose that have properties are compatible for use in the food industry²⁶. Many report the application of maltooligosaccharide, such as maltotriose, maltotetraose and maltopentaose. Specific interest was many kind applications for maltotetraose by²⁵. Maltotetraose is being tested for its use as a food additive to improve the texture or to reduce the sweetness of foods without affecting their inherent taste and flavor^{27, 28}. It's also can be used in baking due to its high moisture retention power which serves to prevent retrogradation of starch ingredient^{29, 30} was also described that maltotetraose has a prebiotic effect by ingestion of maltotetraose syrup could improve intestinal flora and suppress the formation of putrefactive products. In particular, maltopentaose which has five glucopyranoses is highly demanded as a high value-added material in the medical field since the pure maltopentaose can be used as a diagnostic reagent to examine the activity of α -amylase in serum^{18,31,32,33}. Based on the result of maltooligosaccharide type from Kuning varieties show that variety Kuning have challenge used as a material for maltooligosaccharides and used for food industry application.

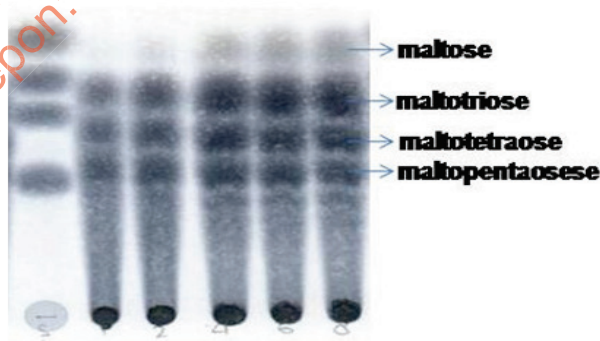


Figure 7. Chromatogram of TLC for enzymatic hydrolysis produce Kuning variety cultivar. S was standard mix (maltose, maltotriose, maltotetraose and maltopentaose); 0, 2, 4, 6 and 8 showed time of enzymatic hydrolysis (hours)

Gambar 6. Kromatogram TLC hasil hidrolisis pada kondisi optimum varietas kultivar Kuning. S adalah standar campuran yang terdiri atas maltose, maltotriose, maltotetraose and maltopentaose; 0, 2, 4, 6, and 8 menunjukkan waktu hydrolysis enzimatis dalam jam

CONCLUSIONS

Based on the phisic-chemical characterization showed that starch from all of cultivated varieties casaava can be used for maltooligosaccharides production, especially for Kuning variety has ability to produce variance maltooligosaccharide. The optimum hydrolysis condition for maltooligosachharide production from cultivated variety Kuning were substrate concentration 4,5% (b/v), comparison of substrate: enzyme 1:2, and temperature hydrolysis 30°C with the reducing sugars 13.359 ppm. The dominant product hydrolysis was maltotriose, maltotetraose and maltopentaose. These maltooligosaccharides was important for many kind application in industry.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of DIPA PN UMBI 2011-2012 granted to Puslit Biologi-LIPI. We also thanks to Dicky Gustiawanto for cassava carbohydrate extraction.

REFERENCES

1. Angelucci F. Analysis of incentives and disincentives for cassava in Ghana. Technical notes series, MAFAP, FAO, Rome. © FAO 2013.
2. FAO Corporate document repository. 1999. Global cassava market study: cassava starch.
3. Rastall RA. Functional Oligosaccharides: Application and Manufacture. *Annu. Rev. Food Scr. Technol.* 2010. 1:305–39. doi: 10.1146/annurev.food.080708.100746
4. Crittenden RG, Playne MJ. Production, properties and applications of food grade oligosaccharides. *Trends FoodSci. Technol.* 1996; 7:353-361.
5. Barreateau H, Delattre C, Michaud P. Production of oligosaccharides as promising new food additive generation. *Food Technol. Biotechnol.* 2006; 44:323-333.
6. AOAC. Official Methods of Analysis. Arlington, VA: Association of Official Analytical Chemists, Inc. Washington D.C. 1984; 14th Edn.
7. Sowbhagya CM, Bhattacharya KR. Simplified determination of amylose in milled rice. *Starch.* 1979; 31:159-163.
8. Muchtadi D. 1992. Metode Kimia, Biokimia, dan Biologi dalam Evaluasi Nilai Gizi Pangan Olahan. Bogor (ID): Pusat Antar Universitas Pangan dan Gizi. Institut Pertanian Bogor.
9. Tharanathan RN and Ramadas Bhat U. Scanning electron microscopy of chemically and enzymatically treated black gram (*Phaseolus mungo*) and ragi (*Eleusine coracana*) starch granules. *Starch.* 1988; 40:378-382.
10. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *J Anal Chem.* 1959; 31:426-428.
11. Fennema OR (Ed.). *Food Chemistry*, third ed. Marcel Dekker, Inc., New York, USA. 1996.
12. Rahmani N, Rohanah, Sukarno, Ade andriani, Yopi. Production of maltooligosaccharides from black potato (*Coleus tuberosus*) starch by α -amylase from a marine bacterium (*Brevibacterium* sp.). *Microbiology Indonesia.* 2013; 7(3):129-136.
13. Hiromi K, Ogawa K, Nakanishi N, Ono S. A kinetic method for the determination of number-average molecular weight of linear high polymer by using an exo-enzyme. *J. Biochem.* 1966; 69:439-449.
14. Lene Duedahl-Olesen, Lars Haastrup Pedersen, Kim Lambertsen Larsen. Suitability and limitations of methods for characterisation of activity of maltooligosaccharide-forming amylases. *Carbohydrate Research.* 2000; 329:109-119.
15. Robyt JF, RJ Ackerman. Isolation, purification, and characterization of a maltotetraose-producing amylase from *Pseudomonas stutzeri*. *Arch. Biochem. Biophys.* 1971; 145(1):105-114.
16. Kainuma K, S Kobayashi, T Ito, S Suzuki. Isolation and action pattern of maltohexaose-producing amylase from *Aerobacter* (Enterobacter) aeroges. *FEBS Lett.* 1972; 26: 281-285.
17. Takasaki Y. An amylase producing maltotriose from *Bacillus subtilis*. *Agric. Biol. Chem.* 1985; 49: 1091-1097.
18. Saito N. A Thermophilic Extracellular alpha-Amylase from *Bacillus licheniformis* Archives. *Biochem. Biophys.* 1973; 155:290-298.
19. Taniguchi H, CM Jae, N Yoshigi, Y Maruyama. Purification of *Bacillus circulans* F-2 amylase & its general properties. *Agric. Biol. Chem.* 1983; 47:511-519
20. Hayashi T, Teruhiko Akiba, Koki Horikoshi. Production and Purification of New Maltohexaose-forming from Alkalophilic *Bacillus* sp. H-167. *Agric. Biol. Chem.* 1988a; 52 (2):443-448.
21. Hayashi T, T Akiba, K Horikoshi. Properties of new alkaline maltohexaose forming amylases. *Appl. Microbiol. Biotechnol.* 1988b; 28: 281-285.
22. Okemoto H, S Kobayashi, M Monma, H Hashimoto, K Hara, K Kainuma. 1986. Isolation and cultivation of a novel microorganism producing a maltopentaose-forming enzyme. *Appl. Microbiol. Biotechnol.* 1986; 25:137-142.
23. Jana M, Chiraujit MB, Saptadip SB, Bikos RPB. Syed Sirajul IC, Prodeep KDM, Keshab CM. Salt-independent thermophilic α -amylase from *Bacillus megaterium* VUMB109: An efficacy testing for preparation of maltooligosaccharide. *Industrial crops & Products.* 2013; 41:386-391. Doi:10.1016/j.indcrop.2012-04.048.

Production of Malto-Oligosaccharides from Cassava Cultivar Kuning
(Nanik Rahmani et al)

24. Maalej H, Hanen Ben Ayed, Olfa Ghorbel-Bellaaj, Moncef Nasri, and Noomen Hmidet. Production and biochemical characterization of a high maltotetraose (G4) producing amylase from *Pseudomonas stutzeri* AS22. BioMed Research International. 2014.
25. Trupti K, Sharma, Vaibhav A, Bhadane, Lalitha S, Kumar, Meenakshi V, Rele, Gajanan Bhawar and Imran Rahman. Optimization of the production of a maltooligosaccharides producing amylase from the alkalophilic *Streptomyces lonarensis* strain NCL 716 using SVR Modeling. Starch. 2013; 65:179-185. DOI 10.1002/star.201200094.
26. Byoung-Cheol Min, Sang-Hyeon Yoon, Jeong-Weon Kim, Yin-Won Lee, Young-Bae Kim, Kwan Hwa Park. Cloning of Novel Maltooligosaccharide-Producing Amylases as Antistaling Agents for Bread. J. Agric. Food Chem. 1998; 46:779-782.
27. Rivero MU, A. Santamaria-Orleans. 2001. Oligosaccharides: application in infant food. Early Human Development. 2001; 65(2):S43-S52.
28. Aiyer PV. Amylases and their applications. African Journal of Biotechnology. 2005; 4(13): 1525-1529.
29. Palacios HR, PB Schwarz, BL D'Appolonia. Effect of α -amylases from different sources on the retrogradation and recrystallization of concentrated wheat starch gels, relationship to bread staling. Journal of Agricultural and Food Chemistry. 2004; 52(19): 5978-5986.
30. Malabendu J, M Chiranjit, S Saptadip. Salt-independent thermophilic α -amylases from *Bacillus megaterium* VUMB109; an efficacy testing for preparation of maltooligosaccharides. Industrial Crops and Products. 2013; 41:386-390.
31. Pankratz T J. Process for producing soluble amylose. U. S. Patent 4 1977; 029,516.
32. Masao K, H Kanchin, M Takashi. Enzymatic manufacture of a-maltopentaose in waterhydrophobic solvent systems. Japan Kokai Tokkyo Koho JP 9180093 A2. 1991.
33. Takashi O, J Akihisa, H Kozo, H Hitoshi, K Shoichi. Manufacture of maltopentaose syrups with c-amylase. Japan Kokai Tokkyo Koho. JP 9245794 A2.1992.

Hak cipta © 2015 BB-Pascapanen
Kampus Penelitian Pertanian Cimanggu, Jawa Barat, Indonesia
Jl. Tentara Pelajar no 12A, Cimanggu, Bogor, Jawa Barat, Indonesia
Email: bb_pascapanen@yahoo.com, ksphp.pascapanen@lib.umpertanian.go.id
Telepon: (0251) 8321762, Faksimili: (0251) 8350920