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ENZYMATIC HYDROLYSIS OF HUTAN JATI VARIETY CULTIVAR TACCA (*Tacca leontopetaloides*) STARCH BY THE *Brevibacterium* sp. α-AMYLASE AND ITS POTENTIAL FOR PRODUCTION OF MALTOOLIGOSACCHARIDES

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Running title: Enzymatic hydrolysis of tacca starch by *Brevibacterium* sp. α-amylase

ABSTRACT

The aim of research are extraction and physico-chemical characterization, determination of the optimum conditions for enzymatic hydrolysis of starch from Tacca tuber to produce maltooligosaccharides, and analysize of character of the product. The analysis of the product is conducted by calculating the amount of reducing sugar, and total sugar, degree of polymerization, TLC (Thin Layer Chromatography) analysis, as well as HPLC (High Performance Liquid Chromatography) analysis. Hutan Jati variety cultivar Tacca was selected from three Tacca variety cultivars (Hutan Jati, Pulau Katang and Gunung Batur) to produce maltooligosaccharides by enzymatic hydrolysis of crude *Brevibacterium* sp. α-amilase The optimum conditions of enzymatic hydrolysis of Hutan Jati variety cultivar Tacca starch for production of maltooligosaccharides was obtained at a substrate concentration of 3% (w/v), the ratio of enzyme and substrate of 1:5 at 6h incubation time. It was obtained 34.4903 grams of powder maltooligosaccharide from 250 mL of fresh hydrolizate. The results of TLC and HPLC analysis showed similar yield to both of the liquid and the powder maltooligosaccharides with maltose, maltotriose, and maltotetraose as main product. Considering the result of psycho-chemically characteristic and the product of maltooligosaccharides from Hutan Jati variety cultivar Tacca tuber starch, presented in this work, may be considered as a potential, strong candidates for future applications as a source of maltooligosaccharide production, especially maltotriose and maltotetraose in functional food industry.

Keywords: α-amilase, *Brevibacterium* sp., maltooligosaccharides, minor tubers, Tacca (*Tacca leontopetaloides*)

INTRODUCTION

Recently, there is a great deal of interest in the use of prebiotic oligosaccharides as functional food ingredients to manipulate the composition of colonic microflora in order to improve host health. Prebiotic oligosaccharides stimulate the growth and the colonization of probiotic bacteria, non-pathogenic organisms which are beneficial to health when ingested (Rastall and Maitin, 2002).

Oligosaccharides can be extracted or obtained by enzymatic hydrolysis from a variety of biomass sources or synthesized from simple oligosaccharides by enzymatic transfer reactions

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(Rastall, 2010). The volume and diversity of oligosaccharide products are increasing rapidly with more than 12 classes of food-grade oligosaccharides currently in commercial productions around the world, including malto-oligosaccharides (Crittenden and Playne, 1996). It is interesting, especially in the potential for discovering and developing of novel oligosaccharide sources.

An enzymatic hydrolysis of black potatoes (*Coleus tuberosus*) and cassava (*Manihot esculenta*) starches with a crude *Brevibacterium* sp. α-amylase to produce maltooligosaccharides was conducted in our previous investigations (Rahmani *et al*., 2013, Rahmani *et al*., 2015, Rahmani *et al*., 2016). Another attempt was made to develop a hydrolysis for the other starch sources in Indonesia. Tacca (*Tacca leontopetaloides*) is one of minor carbohydrates which can be found in the coastal and high salinity areas in Indonesia, such as in the coastal area of South Garut with the local name of Jalawure, and in the district of Talaud Islands, especially Nanusa District, North Sulawesi under the local name Anuwun. Tacca tuber cannot be consumed directly because the tubers contain a bitter taste of Taccaline. In addition, scientific information for optimal utilization of Tacca tuber is still very limited due to the limited data of basic knowledge on its physicochemical and functional properties. The development ofTacca starch as a source of maltooligosaccharides becomes an alternative source of prebiotics, which it may lead to the development of a new food ingredient, thus it may promote the economic growth of Tacca starch.

The main objectives of the present work are to: (1) extract and analyse the psycho-chemical characteristic of starch from Tacca tuber, (2) determine the optimum conditions for enzymatic hydrolysis of Tacca tuber starch for production of maltooligosaccharides, and (3) characterize maltooligosaccharides. By understanding the potential of Tacca tuber starch for production of maltooligosaccharides and its characterization, various functional properties of hydrolyzed starch solution from Tacca tuber can be further developed.

MATERIALS AND METHODS

Materials

Three cultivar of Tacca tubers consist of Hutan Jati, Pulau Katang and Gunung Batur were obtained from Laboratory of Plant Cell and Tissue Culture, Research Center for Biotechnology, LIPI, Cibinong, Bogor.

Chemicals

Starch was purchased from Merck (Darmstadt, Germany) and Maltose (M1) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Maltobiose (M2), Maltotriose (M3), Maltotetraose (M4), Maltopentaose (M5), and Maltohexaose (M6) were purchased from Megazyme (Wicklow,

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Ireland). All other chemical in the highest commercial grade, were obtained from Merck (Darmstadt, Germany).

The Extraction of Tacca Starch

The starch was extracted through the stages of the process, such as stripping, washing, gratering, extracting, filtrating, precipitating, drying, and sieving. Fresh tacca tubers were peeled and washed manually to clean the tubers from soil and the other dirt. The tubers were shredded using grater machine, and then starch was extracted by adding water with a ratio of material and water was 3.5: 1. Furthermore, the filtering was done to separate the starch from the residue. The residue obtained from the screening process, extracted 5 times with the same ratio of the water addition and precipitation over night. After precipitation, the supernatant was removed until the only remaining part of the wet starch deposition. Furthermore, the dried starches were obtained using the sun. The starches were then crushed with mortar and then continued to the sifting process to obtain a uniform particle size using a filter pore size of 50 meshes. Finally, the starch obtained from the three variety cultivars were weighed and subsequently analyzed physico-chemically.

Physico chemical analysis

Moisture, protein, lipid, and ash contents of the isolated samples were determined using approved methods (AOAC, 1990). The amylose content was determined by the iodine blue complex method of Sowbhagya and Bhattacharya (1979) using a solution of 0.2% iodine in 2% potassium iodide.

Microorganism

The crude extract of α-amylase production was carried out using the *Brevibacterium* sp. from the marine bacterium collection of the Biocatalyst and Fermentation Laboratory, Research Center for Biotechnology, LIPI, Cibinong Bogor. We already registered this strain into Biotechnology Culture Collection (BTCC) with accession number is B-822.

The crude extract of α-amylase enzyme production

The production of the crude extract of αα-amylase was carried out by submerged fermentation. The medium consisted of 38 g L^{-1} Artificial Sea Water (ASW), 2% commercial starch (Merck, Darmstadt, Germany), 1.5% agar, 1 g L^{-1} yeast extract and 5 g L^{-1} peptone, pH 8. Media were sterilized at 121°C for 15 minutes. The fermentation was performed for four days at 150 rpm, 30 ºC (Stuart orbital incubator S1500, Staffordshire, United Kingdom). The crude extract of αα-amylase enzyme preparation was obtained as the culture supernatant by centrifugation (6764×g, 15

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min, 4 °C). Subsequently, the supernatant was analyzed for the enzymatic activity at pH 6.6 in phosphate buffer (0.02 M) at 30 °C (Rahmani *et al*., 2011).

The crude extracts of αα-amylase assay

The crude extract of αα-amylase activity was assayed according to Bernfeld (1955) by incubating 0.5 ml of the enzyme solution with 0.5 ml of a starch solution (0.5% w/v) (Merck, Darmstadt, Germany) prepared in phosphate buffer with 6.6 pH (0.02 M) at 30 ºC for 30 min. The reaction was stopped by immersing the test tubes in boiling water for 20 min and subsequent cooling on ice. Color formation was measured in a spectrophotometer at λ 540 nm (Hitachi, U-3900H, Tokyo Japan). One unit is defined as the production of 1 mM glucose per min under the above conditions.

Enzymatic hydrolysis optimization of Tacca (*Tacca leontopetaloides*) starch

Enzymatic hydrolysis was carried out under various conditions, such as various substrate concentrations (w/v) 1.5, 3, 4.5, 6 and 7.5%, the ratio of enzyme-substrate (v/v) 1:10, 1:5, 1:2, and 1:1, and the reaction time (from 1, 2, 4, 6, and 8 hours). 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.

Production of maltooligosaccharide on optimum enzymatic hydrolysis condition

Production of maltooligosaccharides was conducted using 2 L Erlenmeyer on optimum enzymatic hydrolysis condition according the results of enzymatic hydrolysis optimization at shaker 150 rpm, 30 ºC. The reactions were stopped with heated at 90 ºC for 10 min and centrifuge at 6764 x g at 4 ºC for 15 min. 1L of maltooligosaccharides was stored as the liquid maltooligosaccharides, and 250 mL maltooligosaccharides were continued to be made into powder by freeze drying at a pressure of 10 μHg with a temperature of -50 ° C.

Characterization of maltooligosaccharides

Maltooligosaccharide products (both the liquid and the powder) were analyzed by calculating the total sugar content, the reducing sugar, the degree of polymerization, TLC and HPLC. The analysis of the total sugar content was performed by applying the phenol-sulfuric acid method with modifications described by Dubois *et al* (1956). The Reducing sugar was determined by the DNS method (Miller, 1959). The degree of polymerization was calculated based on the ratio between total sugar and reducing sugar. TLC of maltooligosaccharide products was carried out by

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the ascending method (three-time development) on silica gel60 F_{254} plates (Merck Art, 20-20cm, Darmstadt, Germany). All samples were applied in equal quantities (4 µl) and then resolved by two runs with a solvent mixture of n-Butanol/acetic acid/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 120 ºC for 15 min. HPLC was performed using the AGILENT system (Agilent technology 1290 Infinity, United State), the column used ZorbaxSIL column (silica) coated with 3-aminopropilsilen and the mobile phase was acetonitrile and distilled water in a ratio of 75:25 (v/v). The temperature was kept at 30°C with a flow rate of 1.4 mL/min and a sample volume of 20 µL. The effluent from the column was monitored with a Refractive Index Detector (RID) (Lee *et al*., 2003; Kandra *et al*., 2002).

RESULTS AND DISCUSSION

There are various factors considered in affecting to maltooligosaccharide production by an enzymatic hydrolysis. The substrate and enzyme are key factors impacting the feasibility of a continuous enzymatic process for converting starch into maltooligosaccharides (Pan *et al*., 2017). Production of maltooligosaccharide from indigenous starch, such as black potatoes and cassava starches using the crude extract of *Brevibacterium* sp. α-amilase, has been succeeded in our previous investigations (Rahmani *et al*., 2013, Rahmani *et al*., 2015, Rahmani *et al*., 2016). In this study, we focus on exploring the novel starch, which is potential for the production of maltooligosaccharide. The first step is selected from three Tacca (*Tacca leontopetaloides*) variety cultivars (Hutan Jati, Pulau Katang and Gunung Batur) by physico-chemical characteristic.

Physico-chemically characteristic of Tacca starch

Fresh tuber Tacca and starch powder extraction result inthe three Tacca (*Tacca leontopetaloides*) variety cultivars (Hutan Jati, Pulau Katang and Gunung Batur), which are presented in Fig. 1A, B, C and D, respectively.

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Varieties cultivar of Tacca	Weight of Fresh tacca (g)	Weight of Tacca starch (g)	Rendement (%)
Gunung Batur	1832	442	24.12
Pulau Katang	1337	394	26.10
Hutan Jati	995	283	28.44

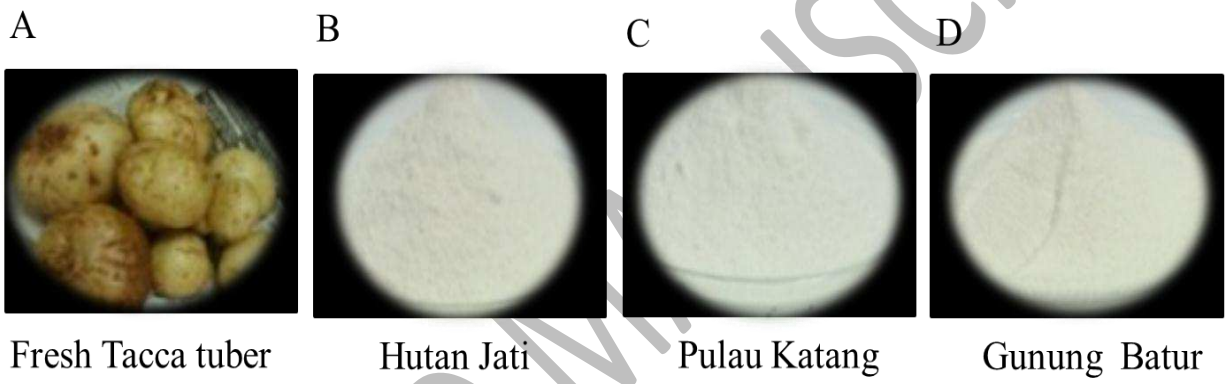

Figure 1 (A) Fresh Tacca tuber, starch from (B) Hutan Jati variety cultivar, (C) Pulau Katang variety cultivar, and (D) Gunung Batur variety cultivar

The total weight rendement of Tacca starch was obtained from extraction of fresh tubers from three varieties cultivar, which was presented in Table 1. The yield of starch was calculated based on the ratio of dry weight of starch to the weight of fresh tuber. The yield of starch from Hutan Jati variety cultivar has the highest rendement, which was ± 28.44%. The rendement of starch from Gunung Batur and Pulau Katang variety cultivars were 2% lower than Hutan Jati variety cultivar.

Tabel 1 Percentage of rendement from three variety cultivars Tacca starch

The proximate analyses of extracted Tacca starch are presented in Table 2. The raw starch from three variety cultivars employed in this study (Hutan Jati, Pulau Katang and Gunung Batur) differs in proximate analysis properties from each other with respect to water content, ash content, protein content, fat content and crude fiber. Starch digestibility is a level of convenience of the starch to hydrolyze by the enzyme to produce the simple sugar or monomor of the sugar. Based on

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the data in Table 2, starches from Hutan Jati and Gunung Batur variety cultivars have digestibility capacity of 50% greater than Pulau Katang. This means that the starch from Pulau Katang variety cultivar has less amylase and produces lower, simple sugar than Hutan Jati and Gunung Batur variety cultivars. On the contrary, the amylopectin content of the Hutan Jati and Gunung Batur variety cultivars starch was higher than amylose and high starch digestibility, confirming that these starch are easier to hydrolysis by the crude of α-amylase while starch from Hutan Jati and Gunung Batur variety cultivars contain amylopectin higher than amylose showed that these variety cultivars similar to the characteristic of black potato and cassava Kuning starch variety cultivars from Indonesia in our previous study (67.69% and 97.5%, respectively) (Rahmani *et al*., 2013, 2016). Based on the data physico-chemically analysis, Hutan Jati variety cultivar Tacca starch was selected for the next analysis.

Table 2 Proxymate analysis from three variety cultivars Tacca starch

Parameters	**Methods**	**Hutan Jati Tacca**	**Gunung Batur**	**Pulau Katang**	**Commerical Tacca starch***
Digestibility (%)		97.23	90.43	52.78	
Water	Gravimetri	14.05	12.67	13.07	
Ash		0.69	0.52	0.34	0.20-1.73
Fat (%)	Soxhlet	0.03	0.30	0.34	0.08-0.30
Protein	Kjeldahl	0.97	1.54	0.77	0.20-1.50
Fiber	Gravimetri	0.27	1.18	0.95	0.00-0.28
Viscocity (c Poise)	Reometri	11.20	12.00	11.60	

***: Ukpabi et al 2009**

Optimizations the enzymatic hydrolysis of Hutan Jati variety cultivar Tacca starch by the crude extract *Brevibacterium* sp. α-amilase for production of maltooligosaccharides

Hutan Jati variety cultivar Tacca starch contains amylopectin higher than amylose compared with the other varieties. Higher ratio of amylopectin to amylase and shorter chain lengths provides a higher production rate in the primary stage (Nakada *et al*., 1990; Woo and McCord, 1993). There are more non-reducing ends in amylopectin starch than in the amylose starch. This increase in non-reducing ends increases the number of sites available for combining with the enzyme (Pan *et al*., 2017).

The first experiment was optimizations the enzymatic hydrolysis of Hutan Jati variety cultivar Tacca starch by the crude extract *Brevibacterium* sp. α-amilase The analyses were performed on various substrate concentrations, the ratio of enzyme and substrate by measurement of reducing sugar, total sugar, degree of polymerization (DP) and TLC. TLC is still the simplest

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chromatographic technique currently used to analyze maltooligosaccharides (Pan *et al*., 2017). By analyzing the starch hydrolysis reaction after it has been underway for different amounts of time, the product component present during each stage can be preliminarily determined (Murakami *et al*., 2008, Maalej *et al*., 2014).

The results of reducing sugar concentrations on various substrate concentrations were presented in Fig. 2A. The 4.5% substrate concentration produces larger reducing sugars than 7.5%. The highest reducing sugar was produced at 8th hour with 4.5% substrate concentration of 2470.81 µg mL^{-1} while the smallest reducing sugar concentration was on the 1st hour with 3% substrate concentration of 1760.46 µg mL-1.

Total sugar describes the amount of sugar contained in the starch with various concentrations. Total sugar on 7.5% substrate concentration showed the highest total sugar when compared with other concentrations, especially at the 2nd hour (60304.69 µg mL^{-1}) (Fig. 2B). The degree of polymerization (DP) produced on various substrate concentration were presented in Table 3.

The 4.5% substrate concentration produce the highest reducing sugar concentration, but the TLC data (data were not shown) showed that maltooligosaccahride in 4.5% substrate concentration cannot be separated very well compared with 3% substrate concentration, so the 3% substrate concentration was selected for the next step analysis.

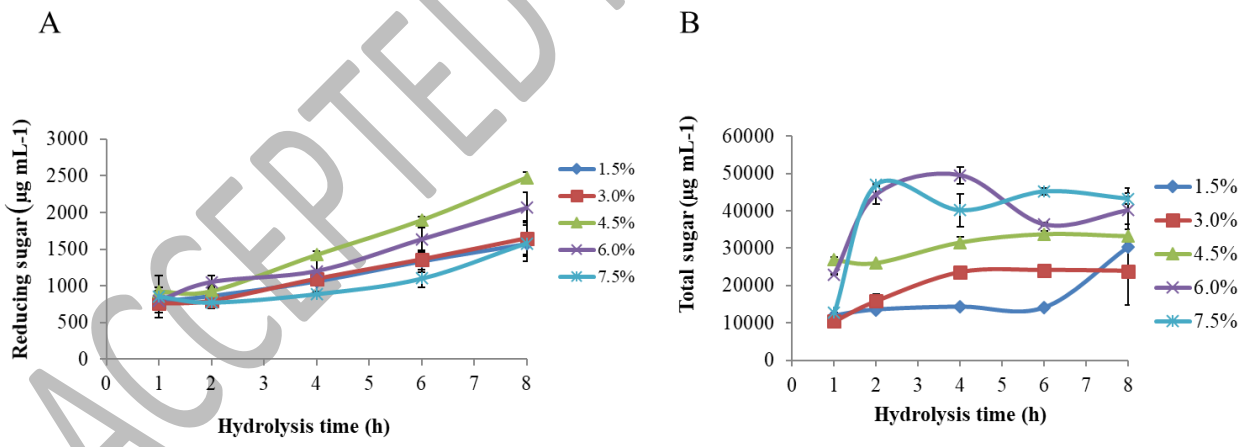

Figure 2 (A) Reducing sugar (µg mL^{-1}) and (B) Total sugar (µg mL^{-1}) concentration of the Hutan Jati variety Tacca starch ~~was~~ hydrolyzed by the crude extract *Brevibacterium* sp. α-amilase The reaction consisting of five variations of Tacca starch concentrations were 1.5%; 3%, 4.5%, 6.0%; 7.5% in 50 mM sodium phosphate buffer pH 6.6, the ratio of enzyme and starch 1:1 (v/v) with enzyme concentration 17.8 U/mL, 30 °C.

Table 3 Degree polymerization (DP) of the hydrolysis product at the ratio of enzyme and substrate 1:1 with the various concentration of substrate and different of hydrolysis time

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Substrate concentration (%)	Degree of polymerization Hydrolysis time (h)				
	1	2	4	6	8
1.5	15	16	14	11	19
3	14	20	22	18	15
4.5	29	28	22	18	13
6	29	42	41	22	19
7.5	19	78	58	53	35

The enzymatic hydrolysis was continued on 3% substrate concentration with various ratios of enzyme and substrate (1:1, 1:2, 1:5 and 1:10, v/v). The reducing sugar concentration from various ratios enzyme and substrate increased with the addition of hydrolysis time. The reducing sugar at 1:5 ratio decreased at 6th hour and increased again at the 7th and 8th hours (Fig. 3A).

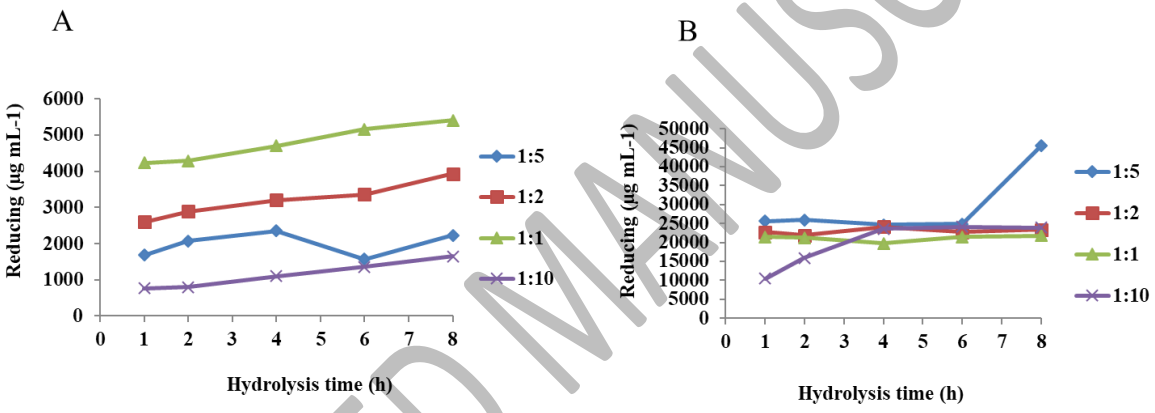

Figure 3 (A) Reducing sugar (µg mL^{-1}) and (B) Total sugar (µg mL^{-1}) concentration of the Hutan Jati variety Tacca starch was hydrolyzed by the crude extract *Brevibacterium* sp. α-amilase The reaction was conducted on 3% substrate concentration, the ratio of enzyme and starch 1:1, 1:2, 1:5 and 1:10 (v/v) with enzyme concentration 17.8 U.mL^{-1}, 30 ^{o}C in phosphate buffer pH 6.6.

The total sugar concentration in the ratio of enzyme and substrate at 1:2 and 1:1 showed in Fig. 3B, is fairly constant, not significantly increased or decreased. The sugar total concentration at 1:10 ratio of enzyme and substrate at 1st hour is 10445.32 µg mL^{-1} then going up at 2nd hour and remaining constant until 8th hour. In contrast, for the 1:5 ratio of enzymes and substrate, the total sugar concentration value is constant at the beginning until 6 hours and increased significantly at the 8th hour from 24846.35 to 45463.54 µg mL^{-1}. The degree polymerization of the hydrolysis result of the variety cultivar Tacca starch at various ratios of both enzyme and substrate shows in the range of 4-22 (Table 4).

Tabel 4 Degree polymerization (DP) of the hydrolysis product at 3% concentration of substrate, the ratio of enzyme and substrate 1:1, 1:2, 1:5 and 1:10 and different of hydrolysis time

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Substrate concentration (%)	Degree of polymerization Hydrolysis time (h)				
	1	**2**	**4**	**6**	**8**
1 : 5	15	13	10	16	20
1 : 2	9	8	8	7	6
1 : 1	5	5	4	4	4
1 : 10	14	20	22	18	15

Chromatographic analysis of the various ratios of enzyme and substrate was presented at Fig.4. The pattern of TLC from the ratio of enzyme and substrates of 1:5 (Fig 4C) and 1:10 (Fig 4D) better than the ratio of enzyme and substrates of 1:1 (Fig 4A) and 1:2 (Fig. 4B). The retention factor (Rf) of the TLC results shows that the formed oligosaccharides consist of maltose, maltotriose, and maltotetraosa,expected that the Rf value was in between maltotriose and maltopentosa. The comparison between the 1:5 ratio of enzyme and substrate and the 1:10 ratio of enzyme and substrate showed that the 1:5 ratio enzyme and substrate produced stronger chromatogram intensity than 1:10 in the 6th hour to the 8th hour. Therefore the 1:5 ratio of enzyme and substrate was selected as the optimum of ratio enzyme and substrate with the optimum hydrolysis time occurred at 6th hour.

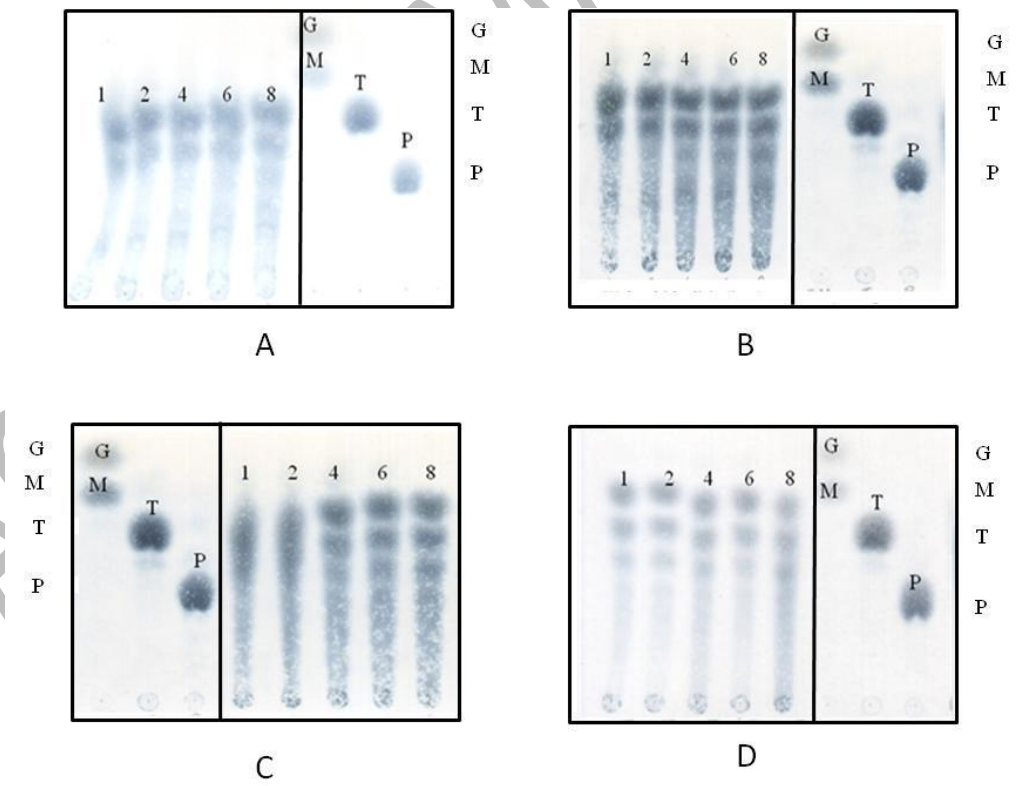

Figure 4 TLC analysis of the Hutan Jati variety cultivar Tacca starch was hydrolyzed by the crude extract *Brevibacterium* sp. α-amilase enzyme on various ratios of enzyme and substrate (A) 1:1, (B) 1:2, (C) 1:5 and (D) 1:10. Standard G: glucose, M: Maltose, T: maltotriose, P: maltopentaose

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Enzymatic hydrolysis of maltooligosaccharides from the Hutan Jati variety cultivar Tacca starch by the crude extract *Brevibacterium* sp. α-amilase on an optimum enzymatic hydrolysis condition and its characterizations

The production of maltooligosaccharide at the optimum condition of enzymatic hydrolysis was obtained at 3% substrate concentration and the 1:5 ratio of enzyme and substrate with the optimum hydrolysis time occurs at 6th hour. The total volume hydrolysis was 1.8 L which consists of 0.3 L enzyme and 1.5 L substrate with a total activity 534 U. One (1) L maltooligosaccharide was stored as a liquid maltooligosaccharide (Fig. 5A) and 250 mL maltooligosaccharide was continued to make powder by freeze dry at a pressure of 10 µHg with a temperature of -50°C. The result of the powder maltooligosaccharide was 34.4903 grams (Fig. 5B).

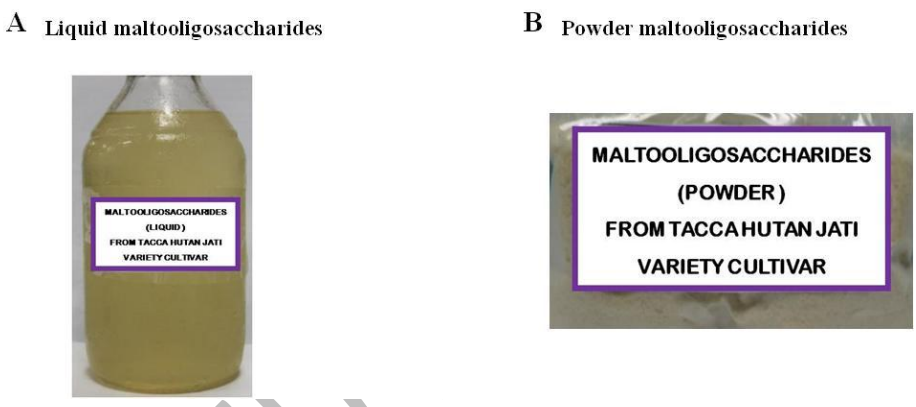

Figure 5 Liquid Tacca Maltooligosaccharide (A) and (B) Powder Form

Reducing sugar concentrations of the liquid maltooligosaccharide and the powder maltooligosaccharide were 6580.46 µg mL^{-1} and 3317.82 µg mL^{-1}, with the degrees of polymerization were 2.7 and 1.3 respectively (Table 5).

Table 5 Reducing sugar, total sugar and degree of polymerization of result hydrolysis on optimum enzymatic hydrolysis condition

Sample	Reducing sugar (µg mL^{-1})	Total sugar (µg mL^{-1})	Degree of polymerization (DP)
Fresh (Liquid)	6580.46	17793.81	2.7
Powder	3317.82	4240.21	1.3

The result of chromatogram of TLC and HPLC from the liquid and the powder (1, 2, 3%) maltooligosaccharides were showed in Table 6 and Table 7, Fig. 6, respectively.

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Table 6 Retention factor (Rf) spot of the standard, the liquid and the powder maltooligosaccharides from TLC analysis

Spot TLC	Retention factor (Rf)				
	Glucose	Maltose	X	Maltotetraosa	Maltopentaosa
Standard	0.486	0.405	-	0.27	0.203
Liquid	-	0.392	0.324	0.243	.
Powder 1%	-	0.419	0.351	0.27	.
Powder 2%	-	0.405	0.324	0.257	.
Powder 3%	-	0.392	0.324	0.27	.

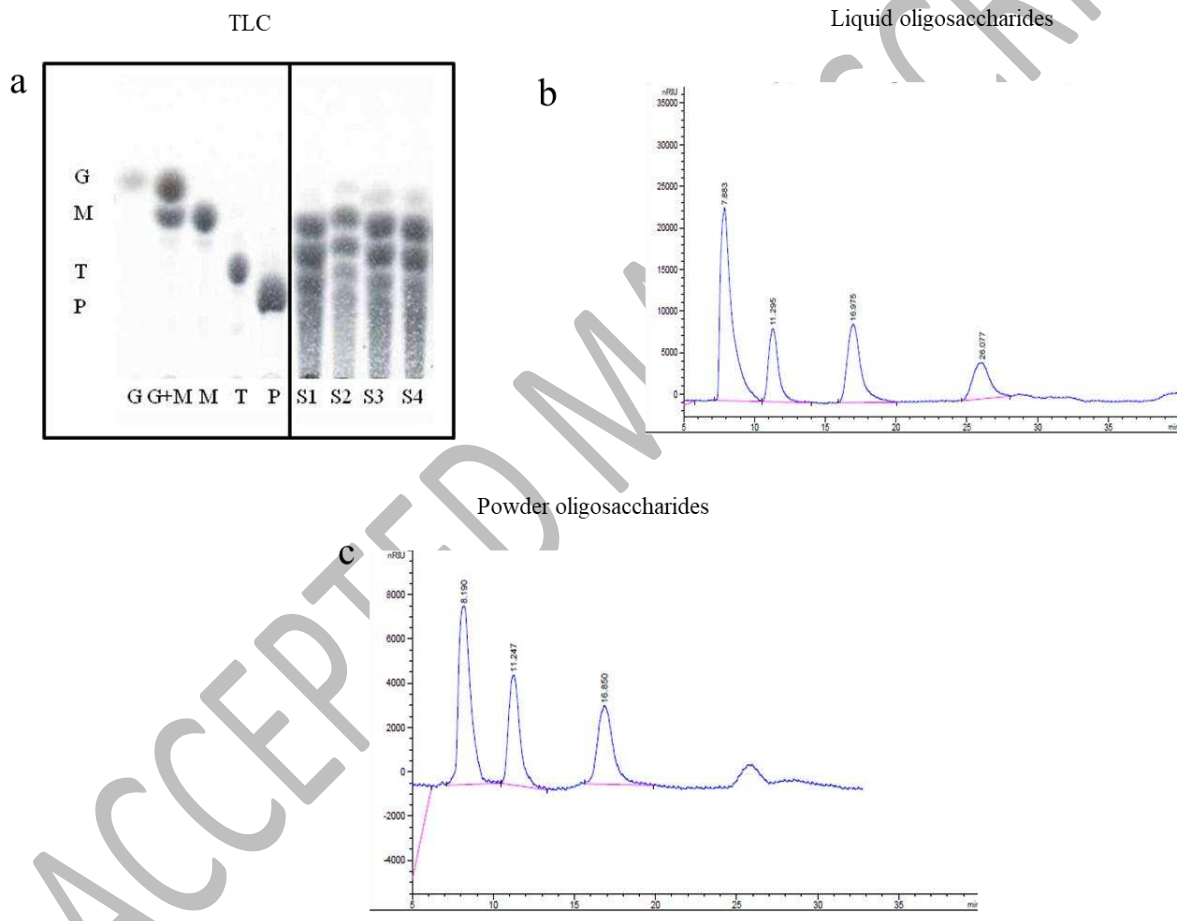

Figure 6 TLC analysis of the Hutan Jati variety cultivar Tacca starch hydrolyzed by the crude extract *Brevibacterium* sp. α-amilase enzyme at the optimum condition. S1 liquid maltooligosaccharide, S2 1% powder maltooligosaccharide, S3 2% powder maltooligosaccharide, and S4 3% powder maltooligosaccharide, Standard G: glucose, M: Maltose, T: maltotetraose, P: maltopentaose (A), HPLC Chromatograms of liquid maltooligosaccharide(B), HPLC chromatograms of powder maltooligosaccharide(C)

Table 7 Retention time of the standard, the liquid maltooligosaccharide and the powder maltooligosaccharides from HPLC analysis

Saccharide	Retention time

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type	Standard	Liquid	Powder (1%)
Glukosa	7.838	7.883	7.838
Maltosa	11.336	11.295	11.247
Maltotriosa	17.05	16.975	16.85
Maltotetraosa	28.823	26.077	28.823

The Rf value of liquid and powder (1, 2, 3%) maltooligosaccharides from TLC analysis contained the same type of the maltooligosaccharide as the optimum conditions such as maltose, maltotetraose, and also maltotriose which was estimated to have a Rf value between maltose and maltotetraose (Fig. 6A and Table 6). HPLC analysis was performed to obtain the information of oligosaccharides type presenting in the sample. The powder maltooligosaccharide (1%) was selected for HPLC analysis because concentration 1% in TLC analysis showed the best separation. Analysis of liquid and powder maltooligosaccharide produced four peaks (Fig. 6B and 6C). Each peak had a retention time similar to the retention time of standard (Table 7).

The result of maltooligosaccharides from the Hutan Jati variety cultivar Tacca starch was similar to Hwang *et al*., (2013) which was a yield of starch hydrolysis by α-amylase produced maltotriose and maltotetraose as main products. Generally, yield of starch hydrolysis by α-amylase produced some various maltooligosaccharides, such as a higher quantity of maltotriose (Yang and Liu, 2004, Aiyer, 2005; Yang *et al*., 2007, Kashiwagi *et al*., 2014, Kanpiengjai *et al*., 2015), maltotriose and maltopentaose by Jana *et al*., (2013), and also an evidence of maltotetraose and maltohexaose product was dominated by amylase, respectively (Murakami *et al*., 2008, Maalej *et al*., 2014) and (Li *et al*., 2014).

CONCLUSION

The optimum conditions of enzymatic hydrolysis of Hutan Jati variety cultivar Tacca starch for production of maltooligosaccharides were obtained at a 3% substrate concentration (w/v), the 1:5 ratio of enzyme-substrate at 6h time hydrolysis. Hutan Jati variety cultivar Tacca tuber starch presented in this work may be considered as a strong potential candidates for future applications as a source of maltooligosaccharide production, especially maltotriose and maltotetraose in a functional food industry.

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