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PRODUCTION OF MALTOOLIGOSACCHARIDES FROM HUTAN JATI VARIETY CULTIVAR TACCA (*Tacca leontopetaloides*) STARCH

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ABSTRACT

This research aimed to extract and characterize the physicochemical properties of starch from Tacca tuber, to determine the optimum conditions for enzymatic hydrolysis to produce maltooligosaccharides, and to analyze the character of these maltooligosaccharides. The analysis was conducted by calculating the amount of reducing sugar, total sugar, and the degree of polymerization, and by using the TLC (Thin Layer Chromatography) and HPLC (High-Performance Liquid Chromatography) analyses. The Hutan Jati variety cultivar of 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-amylase. The optimum conditions for the enzymatic hydrolysis of Hutan Jati variety cultivar Tacca starch for the production of maltooligosaccharides were obtained at a substrate concentration of 3% (w/v) and a ratio of 1:5 enzyme and substrate at 6 hours incubation time. From 250 mL of fresh hydrolysate, some 34.49 grams of powder maltooligosaccharide were produced. The TLC and HPLC results showed a similar yield of both the liquid and powder maltooligosaccharides with maltose, maltotriose, and maltotetraose as the main products. Considering its physicochemical characteristics and the product of its maltooligosaccharides, the starch from the tuber of Hutan Jati variety cultivar Tacca possessed strong potential for the future production of maltooligosaccharides particularly, maltotriose and maltotetraose, in food industries.

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

INTRODUCTION

Recently, a great deal of interest is focused on the use of prebiotic oligosaccharides as functional food ingredients to manipulate the composition of colonic microflora for the improvement of the host's health. Prebiotic oligosaccharides stimulate the growth and the colonization of probiotic bacteria, those non-pathogenic organisms which are beneficial to human health when ingested (Rastall & Maitin 2002).

Oligosaccharides can be extracted by enzymatic hydrolysis from a variety of biomass sources or synthesized from simple oligosaccharides by enzymatic transfer reactions (Rastall 2010). The volume and diversity of oligosaccharide products are increasing rapidly with more than 12 classes of food-grade oligosaccharides currently in commercial production around the world, including malto-oligosaccharides (Crittenden & Playne 1996). This is significant particularly, in the discovery and development of novel oligosaccharide sources.

Previous studies (Rahmani *et al.* 2013; Rahmani *et al.* 2015; Rahmani *et al.* 2016) conducted on enzymatic hydrolysis of black potatoes (*Coleus tuberosus*) and cassava (*Manihot esculenta*) starches used the crude *Brevibacterium* sp. α-amylase to produce maltooligosaccharides. Other attempts to hydrolyze other starch sources in Indonesia included *Tacca* spp. Tacca (*Tacca leontopetaloides*) is one minor carbohydrate sources which can be found in the coastal and

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high salinity areas of Indonesia, including the coastal areas of South Garut (where it is locally known as Jalawure), Talaud Islands district, Nanusa District, North Sulawesi (locally known as Anuwun). However, Tacca tuber cannot be consumed directly as the tubers contain a bitter taste of Taccaline and little is known of its optimal utilization, due to limited scientific information, particularly, on its physicochemical and functional properties. Tacca starch as a source of maltooligosaccharides is a potential alternative source of prebiotics which may lead to the development of a new food ingredient, that may promote the economic growth of Tacca starch.

The main objectives of this study are to (1) extract and analyze the physicochemical characteristics of the starch from Tacca tuber, (2) determine the optimum conditions for the enzymatic hydrolysis of Tacca tuber starch for the production of maltooligosaccharides, and to (3) characterize the maltooligosaccharides. By understanding the potential of Tacca tuber starch for maltooligosaccharides production and its characterization, various functional properties of the hydrolyzed starch solution from Tacca tuber can be further developed.

MATERIALS AND METHODS

Samples

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

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

Extraction of Tacca Starch

The Tacca starch was produced through different processing stages; stripping, washing, gratering, extracting, filtrating, precipitating, drying, and sieving. Fresh Tacca tubers were peeled and washed manually to remove the soil and other dirt. The tubers were shredded using a grater machine, and the starch was extracted by adding water in a ratio of 3.5: 1 material and water. Furthermore, the filtering was done to separate the starch from the residue. Starch extraction from the residue was continued for five times with the same addition of water. The solution containing the residue and water underwent precipitation overnight. After precipitation, the supernatant was removed until the only remaining part was the wet starch deposit. Furthermore, the starches were sun-dried then crushed with a mortar and then sifted to obtain a uniform particle size using a filter pore size of 50 meshes. Finally, the starch obtained from the three variety cultivars were separately weighed and subsequently analyzed physicochemically.

Physico Chemical Analysis

The 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 extraction of crude α-amylase 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, Indonesia. This strain is already registered at the Biotechnology Culture Collection (BTCC) with the accession number of B-822.

Crude Extract of α-amylase Enzyme Production

The production of the crude extract of α-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. The media were sterilized at 121°C for 15 minutes. The fermentation was performed for

four days at 150 rpm, 30 °C (Stuart orbital incubator S1500, Staffordshire, United Kingdom). The crude extract of α-amylase enzyme as the culture supernatant was obtained by centrifugation (6764×g, 15 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).

Crude Extracts of α-amylase Assay

The crude extract of α-amylase was assayed 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 °C for 30 min based on Bernfeld (1955). The reaction was stopped by immersing the test tubes in boiling water for 20 min and by subsequent cooling on ice. The 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 Starch

Enzymatic hydrolysis was carried out under various conditions, such as the 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 of 1, 2, 4, 6, or 8 hours. Reactions were carried out at room temperature in 100 mL Erlenmeyer flasks containing 20 mL of reaction mixtures in a rotary shaker (Stuart orbital incubator S1500, Staffordshire, United Kingdom). The samples were taken at regular intervals (after 1, 2, 4, 6, and 8 h) and the reactions were stopped by heating the samples in boiling water.

Production of Maltooligosaccharide

Maltooligosaccharides were produced at the optimum enzymatic hydrolysis condition using a 2L Erlenmeyer flask and shaker at 150 rpm and 30 °C. The reactions were stopped when heated at 90 °C for 10 min and centrifuged at 6764 x g and 4 °C for 15 min. 1 L of malto-oligosaccharides was stored as the liquid maltooligosaccharides, and 250 mL malto-oligosaccharides were made into powder by freeze-drying at a pressure of 10 μHg and a temperature of -50 °C.

Characterization of Maltooligosaccharides

Both the liquid and the powder maltooligosaccharides 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, as described by Dubois *et al.* (1956). The amount of 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 the ascending method (three-time development) on silica gel 60 F_{254} plates (Merck Art, 20 - 20 cm, Darmstadt, Germany). All samples were prepared in equal quantities of 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 °C for 15 min. HPLC was performed using the AGILENT system (Agilent technology 1290 Infinity, USA), the column used was ZorbaxSIL column (silica) coated with 3-aminopropilsilen and at the mobile phase was acetonitrile and distilled water at 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

Various factors affected the conversion of starch into maltooligosaccharides through enzymatic hydrolysis, of which the substrate and enzyme are the key factors for a continuous enzymatic process (Pan *et al.* 2017). Maltooligosaccharide production from indigenous starch, such as black potatoes and cassava starches using the crude extract of *Brevibacterium* sp. α-amylase, has been successful (Rahmani *et al.* 2013; Rahmani *et al.* 2015; Rahmani *et al.* 2016). This study explored the potential of the novel starch for the production of maltooligosaccharide. Three Tacca (*Tacca*

leontopetaloides) variety cultivars, namely; Hutan Jati, Pulau Katang and Gunung Batur were selected for analysis of their physicochemical characteristics.

Physico-chemical Characteristics of Tacca Starch

Starch powders were extracted from three fresh tuber Tacca variety cultivars, namely Hutan Jati, Pulau Katang, and Gunung Batur (Fig. 1).

The total weight yield of Tacca starch was obtained from the fresh tubers extracts of three Tacca varieties cultivar (Table 1). The starch yield was calculated as the ratio of the dry weight of starch to the weight of fresh tuber. The starch yield of Hutan Jati variety cultivar has the highest rendement (± 28.44%) while the rendement of starch from Gunung Batur and Pulau Katang variety cultivars were 2% lower than Hutan Jati variety cultivar.

Results of the proximate analyses of extracted Tacca starch showed that the properties of the raw starch from the three variety cultivars, Hutan Jati, Pulau Katang and Gunung Batur differ from each other concerning water content, ash content, protein content, fat content and crude fiber content (Table 2). Starch digestibility is a level of convenience of the enzyme to hydrolyze the starch to produce the simple sugar or monomer of the sugar. Hydrolysis solution was reacted with DNS solution until it forms a reddish-orange color whose density is directly proportional to the level of maltose in the solution. The content of maltose in each starch was decided based on maltose standard curve. Based on the results, starches from Hutan Jati and Gunung Batur variety cultivars have digestibility capacity of 50% greater than that of Pulau Katang indicating that this starch from Pulau Katang variety cultivar has less amylose and produces lower simple sugar hydrolyzed by the enzymes. The starches from the Hutan Jati and Gunung Batur variety cultivars have high starch digestibility confirming that these starches are easier to hydrolyze using the crude α-amylase. In addition, starch from Hutan Jati and Gunung Batur variety cultivars have high starch digestibility indicating that these variety cultivars had similar characteristics with that of the black potato and cassava Kuning starch variety cultivars from Indonesia (67.69% and 97.5%, respectively) (Rahmani *et al.* 2013, 2016). Based on these physicochemical analysis (percentage of starch digestibility), Hutan Jati variety cultivar Tacca starch was selected for the next analysis.

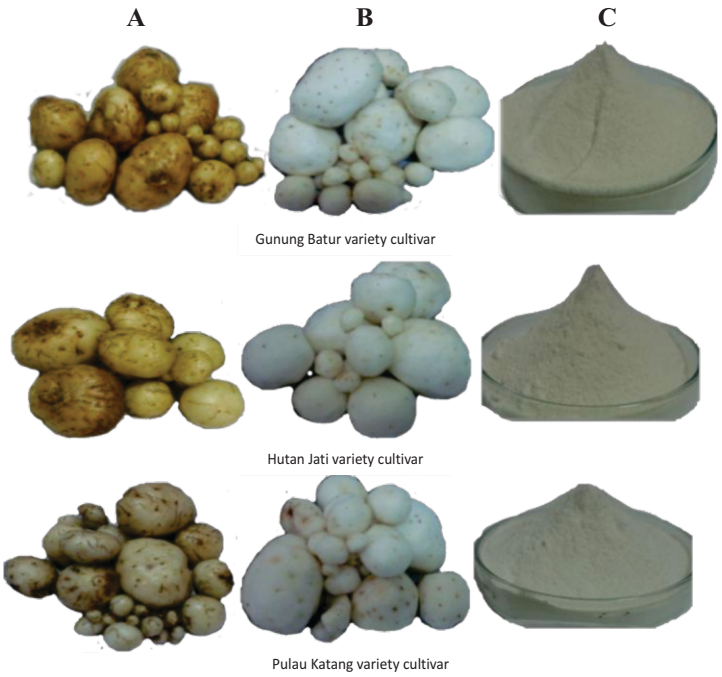

Figure 1 (A) Fresh Tacca tuber, (B) Fresh peeled Tacca tuber, (C) Starch powder

Tabel 1 Percentage of starch yield from three tacca variety cultivars

Varieties Cultivar of Tacca	Weight of Fresh Tacca (g)	Weight of Tacca Starch (g)	Yield (%)
Gunung Batur	1832	442	24.12
Pulau Katang	1337	394	26.10
Hutan Jati	995	283	28.44

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Optimum Conditions for Maltooligosaccharides Production through Enzymatic Hydrolysis of Tacca Starch from Hutan Jati Variety Cultivar

Hutan Jati variety cultivar Tacca starch have digestibility capacity higher than the other varieties, indicating its potential for maltooligosaccharides production (Rahmani *et al.* 2015). To determine the optimum conditions for the enzymatic hydrolysis of Tacca starch from Hutan Jati variety cultivar using the crude extract *Brevibacterium* sp. α-amylase, the analyses were performed on various substrate concentrations, the ratio of enzyme and substrate until 8 hours hydrolysis times. The end products of hydrolysis were measured by calculating the amounts of reducing sugar and total sugar, degree of polymerization (DP) and TLC. TLC is still the simplest chromatographic technique currently used to analyze maltooligosaccharides (Bal *et al.* 2016; Pan *et al.* 2017). By analyzing the starch hydrolysis reaction during the different reaction times, the product component present during each stage can be preliminarily determined (Murakami *et al.* 2008; Maalej *et al.* 2014).

Table 2 Proximate analysis of the starch from three Tacca variety cultivars

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	

Note: *Ukpabi *et al.* 2009

The 4.5% substrate concentration produced larger than 7.5% of reducing sugars (Fig. 2A). The highest reducing sugar was produced at the 8th hour with 4.5% substrate concentration of 2470.81 µg mL^{-1} while the smallest reducing sugar concentration was at the 1st hour with 3% substrate concentration of 1760.46 µg mL-1.

The highest total sugar (amount of sugar contained in the starch) was measured at 7.5% substrate concentration, particularly at the 2nd hour (60304.69 µg mL^{-1}) (Fig. 2B). To identify the hydrolyzed products of Hutan Jati variety Tacca starch, the chromatographic methods using different separation techniques were applied. These methods show different advantages and limitations in identifying the starch hydrolysis products (Olesen *et al.* 2000). The thin-layer chromatography (TLC), a low-cost method, is still commonly used as an analytical tool for the detection of starch hydrolysis products because of its simplicity and relatively high sensitivity (Hayashi *et al.* 1988).

The 4.5% substrate concentration produces the highest reducing sugar concentration, but the TLC data (Fig. 3B, 3C) showed that the maltooligosaccharide in the 4.5% substrate concentration could not be separated very well compared with that of the 3% substrate concentration. Hence, the 3% substrate concentration was selected for the next step of the analysis.

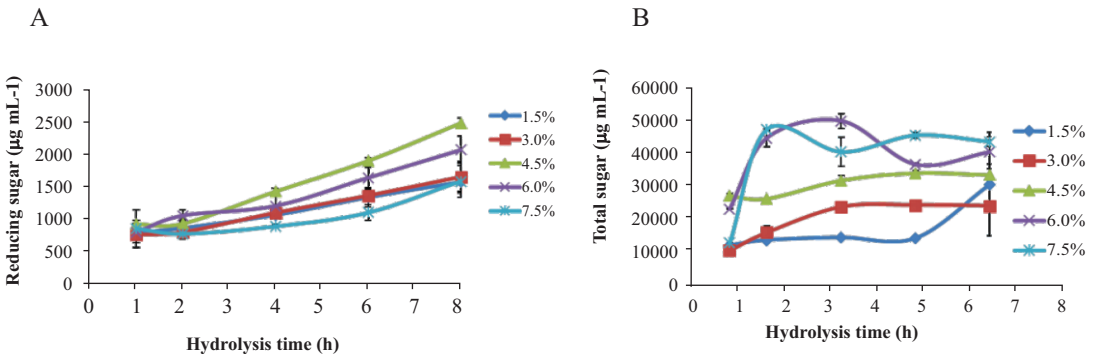

Figure 2 (A) Concentrations of the reducing sugar (µg mL^{-1}) and (B) total sugar (µg mL^{-1}) of the Hutan Jati variety Tacca starch hydrolyzed using the crude extract *Brevibacterium* sp. α-amylase with five variations of Tacca starch concentrations at 1.5%; 3%, 4.5%, 6.0%; 7.5% in 50 mM sodium phosphate buffer, pH 6.6, ratio of enzyme and starch at 1:1 (v/v), and enzyme concentration at 17.8 U/mL and 30 °C

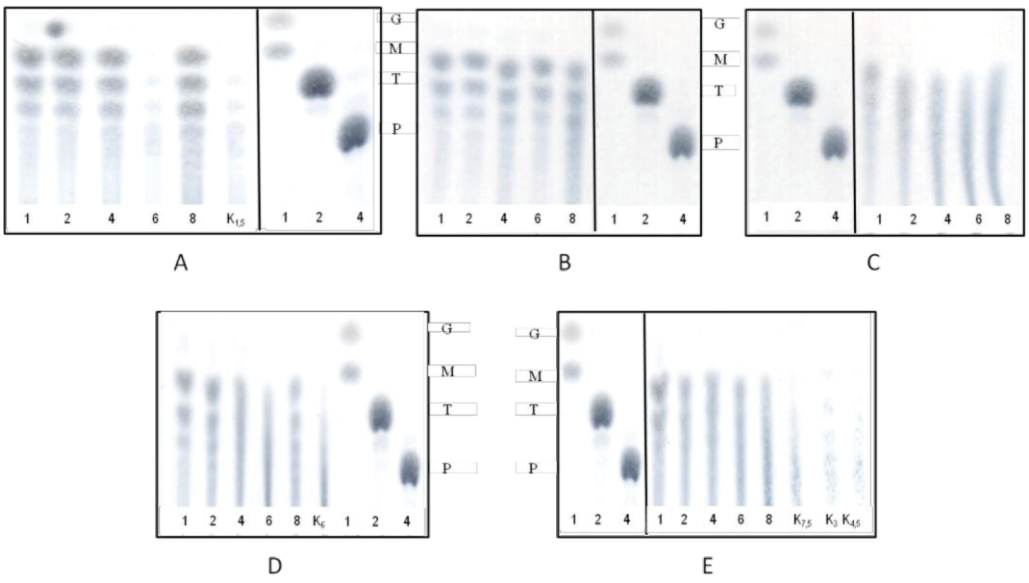

Figure 3 TLC analysis of the Hutan Jati variety cultivar Tacca starch hydrolyzed by the crude extract *Brevibacterium* sp. α-amylase enzyme at various substrate concentrations (A) 1.5%, (B) 3%, (C) 4:5%, (D) 6%, and (E) 7.5%. Standard G: glucose, M: Maltose, T: maltotriose, P: maltopentaose.

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

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

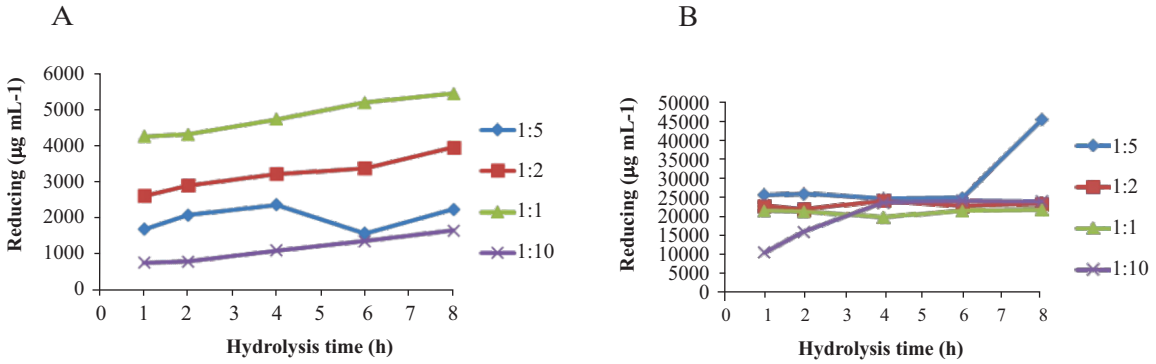

Figure 4 (A) Reducing sugar (µg mL^{-1}) and (B) total sugar (µg mL^{-1}) concentrations of the Hutan Jati variety Tacca starch hydrolyzed using the crude extract *Brevibacterium* sp. α-amylase at 3% substrate concentration, ratios of enzyme and starch at 1:1, 1:2, 1:5 and 1:10 (v/v) and enzyme concentration of 17.8 UmL^{-1}, at 30 ^{o}C in phosphate buffer and pH 6.6

The enzymatic hydrolysis was continued at 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 of the enzyme and substrate increased in line with the length of hydrolysis time. The reducing sugar at 1:5 ratio decreased at the 6th hour and increased again at the 7th and 8th hours (Fig. 4A).

The total sugar concentration is fairly constant at 1:2 and 1:1 ratio of enzyme and substrate, not significantly increased nor decreased (Fig. 4B). The total sugar concentration at 1:10 ratio of enzyme and substrate at the 1st hour is 10445.32 µg mL^{-1} then increased at the 2nd hour (value in µg mL-1) and remained constant until the 8th hour (value in µg mL-1). In contrast, the total sugar concentration value at 1:5 ratio of enzymes and substrate was constant at the beginning until the 6th hour and increased significantly at the 8th hour from 24846.35 to 45463.54 µg mL^{-1}. The degree of polymerization of the Tacca starch variety cultivar at various ratios of the enzyme and substrate were in the range of 4-22 (Table 4).

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

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

Based on the chromatographic analysis of the various ratios of enzyme and substrate (Fig.5), the TLC pattern of the ratio of enzyme and substrates at 1:5 (Fig 5C) and at 1:10 (Fig 5D) were better than the ratio of enzyme and substrates at 1:1 (Fig 5A) and at 1:2 (Fig. 5B). The retention factor (Rf) of the TLC showed that the resulting oligosaccharides consisted of maltose, maltotriose, and maltotetraose, expected that the Rf value was in between maltotriose and maltopentosa. The comparison between the 1:5 ratio and the 1:10 ratio of enzyme and substrate showed that the 1:5 ratio produced stronger chromatogram intensity than the 1:10 in the 6th hour until the 8th hour. Hence, the 1:5 ratio was selected as the optimum ratio of enzyme and substrate and, the optimum hydrolysis time was six hours.

Optimum Conditions for the Enzymatic Hydrolysis of Maltooligosaccharides from the Starch of Hutan Jati Variety Cultivar Tacca

The optimum conditions for the maltooligosaccharide production through enzymatic hydrolysis was obtained at 3% substrate concentration, 1:5 ratio of enzyme and substrate and at a hydrolysis time of six hours. The total volume of hydrolyzed maltooligosaccharide was 1.8 L consisting of 0.3L enzyme and 1.5L substrate with a total activity of 534 U. One (1) L was stored as a liquid maltooligosaccharide (Fig. 6A) and 250 mL maltooligosaccharide was powered by freeze-dry method at a pressure of 10 μHg and a temperature of -50°C resulting in 34.4903 grams of powdered maltooligosaccharide (Fig. 6B).

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

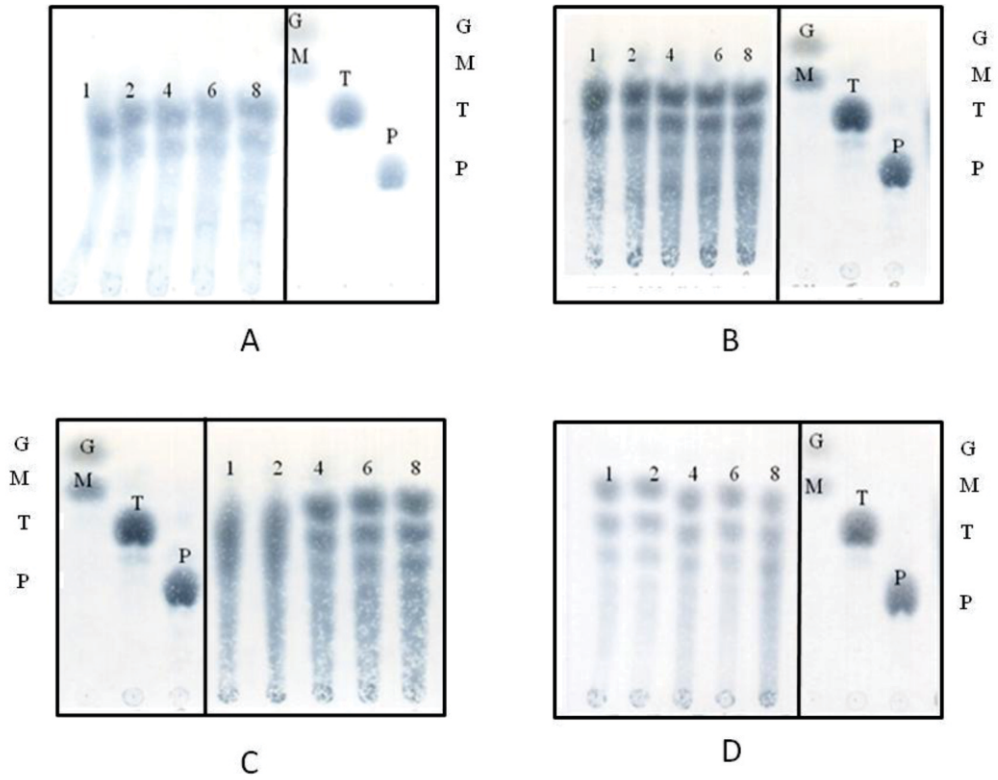

Figure 5 TLC analysis of the Hutan Jati variety cultivar Tacca starch hydrolyzed by the crude extract *Brevibacterium* sp. α-amylase enzyme at various ratios of enzyme and substrate (A) 1:1, (B) 1:2, (C) 1:5, and (D) 1:10. Standard G: glucose, M: Maltose, T: maltotriose, P: maltopentaose.

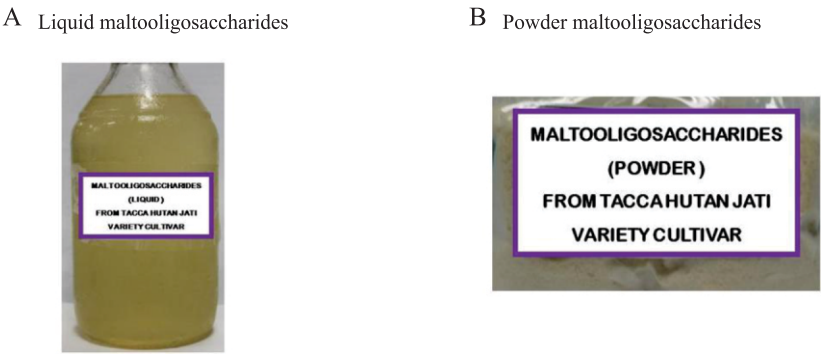

Figure 6 Liquid (A) and powdered tacca maltooligosaccharide (B)

Table 5 Amount of reducing sugar, total sugar and degrees of polymerization of maltooligosaccharides at optimum enzymatic conditions

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

Table 6 TLC analysis of the retention factor (Rf) spot of the standard, liquid and the powdered maltooligosaccharides

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	.

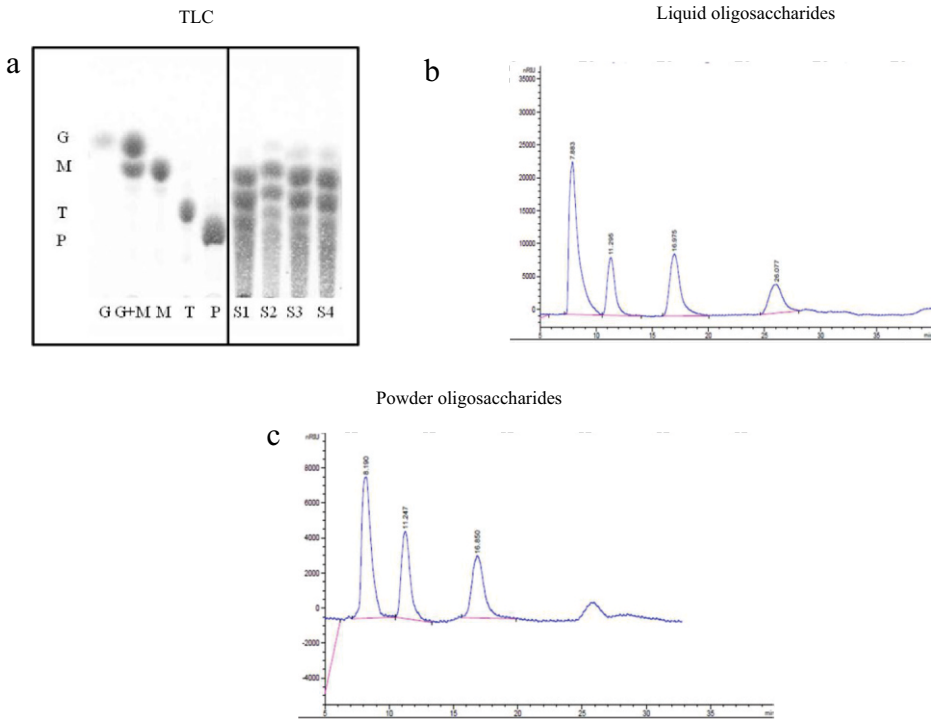

Figure 7 TLC analysis of the Hutan Jati variety cultivar Tacca starch hydrolyzed using the crude extract *Brevibacterium* sp. α-amylase enzyme at the optimum conditions (S1 = liquid maltooligosaccharide, S2 = 1% powdered maltooligosaccharide, S3 = 2% powdered maltooligosaccharide, S4 = 3% powdered maltooligosaccharide, Standard G = glucose, M = Maltose, T = maltotetraose, P = maltopentaose) (a), HPLC chromatograms of liquid maltooligosaccharide (b), HPLC chromatograms of maltooligosaccharide powder (c)

Table 7 HPLC analysis of the retention time of the standard, liquid and the powdered maltooligosaccharides

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

The maltooligosaccharides produced from the Hutan Jati variety cultivar Tacca starch were similar to those of Hwang *et al.* (2013) in which the starch hydrolysis using α-amylase produced maltotriose and maltotetraose as the main products. Generally, starch yield hydrolysis using α-amylase had produced various maltooligosaccharides, such as a higher quantity of maltotriose (Yang & Liu 2004; Aiyer 2005; Yang & Liu 2007; Kashiwagi *et al.* 2014; Kanpiengjai *et al.* 2015), and maltopentaose (Jana *et al.* 2013), and also an evidence of maltotetraose and maltohexaose that were dominated by amylase (Murakami *et al.* 2008; Maalej *et al.* 2014; Li *et al.* 2015). Based on the hydrolysis of the maltose, maltotriose, and maltotetraose produced from the starch of Hutan Jati variety cultivar Tacca, the α-amylase from the *Brevibacterium* sp. used in this study has the action of an endo-type enzyme (Olesen *et al.* 2000).

CONCLUSION

The optimum enzymatic hydrolysis conditions for maltooligosaccharides production from the starch of Hutan Jati variety cultivar Tacca (*Tacca leontopetaloides*) were obtained at substrate concentration (w/v) of 3% and a 1:5 ratio of enzyme-substrate at six hours hydrolysis time. The tuber starch from Hutan Jati variety cultivar Tacca possesses distinct potential characteristics for future applications as a source of maltooligosaccharide, particularly, maltotriose and maltotetraose in the operational food industry.

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