MICROBIOLOGY INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol 7, No 3, September 2013, p 129-136

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.7.3.6

Production of Maltooligosaccharides from Black Potato (*Coleus tuberosus*) Starch by α-amylase from a Marine Bacterium (*Brevibacterium* sp.)

NANIK RAHMANI[1]*, ROHANAH[2], SUKARNO[2], ADE ANDRIANI[1], AND YOPI[1]

[1]*Biocatalyst and Fermentation Laboratory, Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, Indonesia;*
[2]*Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Institut Pertanian Bogor, Kampus Darmaga, Bogor 16820, Indonesia*

High quality maltooligosaccharides were produced from indigenous Indonesian black potato starch by making use of an amylase from *Brevibacterium* sp. Optimal production was achieved at 2.5% (w/v) substrate concentration, an enzyme-substrate ratio of 1:5 (w/v) and hydrolysis time of 4 h. Under such conditions the yield of reducing sugars was 14 240 ppm with a polymerization degree of 16. Thin layer chromatography (TLC) revealed the formation of glucose, maltose, and maltotriose with Rf values of 0.60, 0.52, and 0.37, respectively. HPLC analysis of freeze-dried samples disclosed Rf values of 0.60, 0.50, 0.37, and 0.12. Maltooligosaccharide profile analysis both using TLC and HPLC showed that the enzymatically hydrolyzed samples contained glucose, maltose, and maltotriose. Thus, black potato starch can be randomly converted into simple sugars and maltooligosaccharides applying by amylolytic enzymes from the marine microbe *Brevibacterium* sp.

Key words: black potatoes, *Brevibacterium* sp., maltooligosaccharides

Maltooligosakarida dengan kualitas baik diproduksi dari pati kentang hitam asli Indonesia menggunakan enzim amylase dari *Brevibacterium* sp. Kondisi terbaik hidrolisis pati kentang hitam telah diperoleh yaitu pada konsentrasi substrat pati kentang hitam 2.5% (b/v), perbandingan enzim-substrat 1:5 (b/v) serta waktu hidrolisis 4 jam. Gula pereduksi yang dihasilkan pada kondisi tersebut sebesar 14 240 ppm dengan derajat polimerisasi 16. Hasil analisis maltooligosakarida menggunakan kromatografi lapis tipis (KLT) menunjukkan jenis maltooligosakarida yang terbentuk adalah glukosa, maltosa, dan maltotriosa dengan nilai *Rf* berturut-turut 0.60, 0.52, dan 0.37. *Spot* kromatogram HPLC sampel hasil *freeze-drying* memiliki nilai *Rf* 0.60, 0.50, 0.37, dan 0.12. Analisis profil maltooligosakarida dengan KLT dan HPLC menunjukkan bahwa hidrolisat mengandung glukosa, maltosa, dan maltotriosa. Munculnya gula-gula tersebut menunjukkan bahwa pati kentang hitam dapat terdegradasi menjadi gula-gula sederhana dan maltooligosakarida secara acak dengan menggunakan enzim amilase dari mikroba laut *Brevibacterium* sp.

Kata kunci: *Brevibacterium* sp., kentang hitam, maltooligosakarida

Starch is the most abundant storage polysaccharide in cereal and legume grains, many roots, and in tubers (vander Maarel *et al.* 2002; Belitz *et al.* 2004); the polysaccharide consists of the two glucose polymers amylose and amylopectin. The former is a linear α-(1-4) linked glucose chain with a plant-specific degree of polymerization of 200-6000; the latter consists of short linear α-(1-4) linked chains with α-(1-6) linked side chains (vander Maarel *et al*. 2002). Starch is hydrolyzed into smaller oligossaccharides by α-amylase, wich is one of the most important commercial enzyme processes (Souza and Pérolade 2010).

Oligosaccharides (OS) have been commercialised since the 1980s as low-calorie bulking agents. The functional food concept was first introduced in Japan. In 1991, several oligosaccharides were classified as "foods for specified health use" (FOSHU) in Japan. Recent research findings across the globe, have led to the inclusion of non-digestible oligosaccharides (NDOs) under functional food. Currently, there exist a 27 billion USD market for functional foods and experts forecast its 8.5-20% growth. The global market for functional foods was valued at 73.5 billion USD in 2005, whereas by 2013, it is expected to reach a value of 90.5 billion USD (Patel and Goyal 2011).

In general, various kinds of oligosaccharides can be produced from starch as the raw material, such as maltooligosaccharides (maltose, maltotriose, maltotetraose, maltopentaose, and maltoheptaose), isomaltooligosaccharides (isomaltose, panose, and isomaltotriose), cyclodextrins (CDs) (α-CD, β-CD, γ-CD, HP- β-CD, and branched CDs), maltitol, gentiooligosaccharides, trehalose, and nigerose. Maltooligosaccharides are by definition glucose oligomeres consisting of 2 to 10 glucopyranosyl residues linked via α-1-4 bonds

*Corresponding author; Phone/Fax: 021-8765066/021-8765062, Email: rahmani_btk@yahoo.com

(Nakakui 2005).

Maltooligosaccharides are produced from starch commercially by the action of debranching enzymes such as the pullulanase (EC 3.2.1.41) or isoamylase (EC 3.2.1.68) and controlled hydrolysis by various α-amylases (EC 3.2.1.1). Alpha-amylases are enzymes that catalyses the hydrolysis of internal-1,4-glycosidic linkages in starch in low molecular weight products, such glucose, maltose, and maltotriose units (Souza and Pérolade 2010). Depending on the organism from which they are produced the latter display diverse reaction specificities facilitating the production of syrups rich in maltooligosaccharides of different chain lengths (Crittenden and Playne 1996).

In recent years there is a steadily growing interest to use maltooligosaccharides in the food industries: as biopreservatives, functional food, and as important components in a great number of other nutritional products (Barreteau *et al*. 2006). They have been used as sweeteners, anti-hygroscopic or truncating agents or humectants (Lee *et al*. 2003). Various kinds of maltooligosaccharides-containing syrups (maltose ~ maltopentaose) having low sweetness impart resistance to retrogradation of starch gels and prevent sucrose crystallization; their rather low browning tendency is due to the improved heat stability. Accordingly, they are useful for enhancing intrinsic properties of various foods, and can be applied as powdering materials, dry milk saccharides, in liquid diets, and for increasing viscosity in refreshing drinks (Nakakuki 2005). Maltooligosaccharides produced by α-amylases can reduce retrogradation which is of practical importance for bakery products (Smits *et al*. 2003).

Amylases producing specific maltooligosaccharides have been reported from a number of microorganisms, such as *Bacillus circulans* and *B. subtilis*. The maltopentaose-generating amylase of *B. licheniformis* is used for the production of maltotriose, maltotetraose, and maltopentaose syrups. Research reagarding maltooligosaccharides produced by the amylase from a marine bacterium is relatively scarce. In this research maltooligosaccharides were produced by *Brevibacterium* sp. isolated from Pari Island. Previously, we have demonstrated that the bacterium is capable of secreting a starch hydrolizing enzyme withan activity of 2.5 U mL^{-1} which can potentially be used for maltooligosaccharides production (Rahmani *et al*. 2011).

The most popular tubers in Indonesia comprise cassava and sweet potatoes but others, such as the black potatoes are locally planted as well. However, Indonesian black potatoes are only rarely exploited and they are cultivated exclusively in rather small scale in Java, Bali, and the Madura Islands (Heyne 1987). The use of indigenous Indonesian black potato tubers as processed foodstuff is still rather limited due to the fact that enzymatic treatment of black potato starch is not thoroughly explored in Indonesia. In this study we determined the optimal conditions for the enzymatic hydrolysis of starch from *Coleus tuberosus* by enzymes from *Brevibacterium* sp. and analyze the profiles of the generated maltooligosaccharide by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Extraction of Black Potato Starch and Physico-chemical Analysis. Black potato tubers used in this study were provided by the Cell and Tissue Culture Laboratory, Research Center for Biology, LIPI, Cibinong, Bogor (Fig 1A). The used variety 3.2 resulted from a tissue culture of a plant from the Sangian area, East Java. The starch was extracted through the stages of the process, such as stripping, washing, grater, extraction, filtration, precipitation, drying, and sieving (Fig 1B). Fresh black potato tuber were peeled and washed by using manually to clean tubers from soil and the other dirt. The tuber be 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 was 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 mesh. Finally, starch obtained from the varieties 3.2 were weighed and subsequently analyzed physico-chemically: moisture, protein, lipid, and ash contents of the isolated samples were determined using approved methods (AOAC 1984). 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.

Scanning Electron Microscopy (SEM) Analysis. The purity of the isolated starch was additionally checked by scanning electron microscopy (SEM) according to the method described by Tharanathan and Ramadas Bhat (1988).

Microorganism. 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.

Crude Enzyme Production. Production of the 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 min. Fermentation was performed for 4 d at 150 rpm, 30 °C (Stuart orbital incubator S1500, Staffordshire, United Kingdom). The crude amylase enzyme preparation was obtained as the culture supernatant by centrifugation (6 764 ×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 Amylase Assay. The 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 pH 6.6 (0.02 M) at 30 °C 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 maltooligosaccharides per min under the above conditions.

Enzymatic Hydrolysis Conditions of Black Potato Starch Varieties 3.2. Enzymatic hydrolysis was carried out under various conditions, such as diverse substrate concentrations (w/v) 1, 2.5, and 5%, enzyme-substrate ratio (v/v) 1:10, 1:5, 1:2, and 1:1, and the reaction time (hours from 1, 2, 4, 6, and 8). 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 8 h); reactions were stopped by heating the samples in boiling water.

Chemical Analysis of Maltooligosaccharides. Product hydrolysis was analyzed by calculating the total sugar content, reducing sugars and the degree of polymerization by TLC and HPLC. Analysis of the total sugar content was performed by applying the phenol-sulfuric acid method with modifications described by Dubois *et al.* (1956). Reducing sugars were determined by the DNS method (Miller 1959). The degree of polymerization was calculated based on the ratio between total and reducing sugar. Thin layer chromatography (TLC) of maltooligosaccharides products was carried out by the ascending method (three time development) on silica gel $60F_{254}$ plates (Merck Art20-20cm, Darmstadt, Germany). All samples were applied in equal quantities (1 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, and 0.5 mL aniline) and subsequent heating at 100 °C for 15 min. Maltooligosaccharide products were freeze dry and analyzed by high performance liquid chromatography (HPLC) (Lee *et al*. 2003; Kandra *et al*. 2002) using the AGILENT system (Agilent technology 1290 Infinity, United State). The column used was Zorbax SIL column (silica) coated with 3-amino propilsilen 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^{-1} and a sample volume of 20 µL. The effluent from the column was monitored with a Refractive Index Detector (RID).

RESULTS

Optimization of Enzymatic Hydrolysis Conditions of Black Potato Starch Varieties 3.2. The yield of starch from black potato was 18.73 % on a grain dry matter basis. The relatively low yield could be attributed to losses occurring during the repeated washing needed for the starch extraction process. The moisture content of the isolated starch was 10.13%. The lipid and protein contents of the starch were 0.81% and 0.51%, respectively, indicating that the isolated starch was quite pure (Table 1). Indeed, the purity of the isolated starch was confirmed by SEM micrographs at 2500X magnifications showing integrity of the starch granules (Fig 2). The amylopectin content of the black potato starch was 67.69%, which agrees with the observed maltooligosaccharide production.

Maltooligosacharides Formation by Enzymatic Hydrolysis. Production of maltooligosaccharides was carried out by making use of the amylase from *Brevibacterium* sp. that was isolated from Pari Island. Optimization of hydrolysis conditions for maltoligosaccharides from starch is a promising method. To determine the most suitable conditions, enzyme reactions were carried out using the same amount of enzyme (2.5 U mL^{-1}) in various substrate concentrations ranging from 1, 2.5 to 5% as starch concentrations

A B

Fig 1 (A) Black potato tubers of the tissue culture *Coleus tuberosus* variety 3.2 and (B) the respective starch powder.

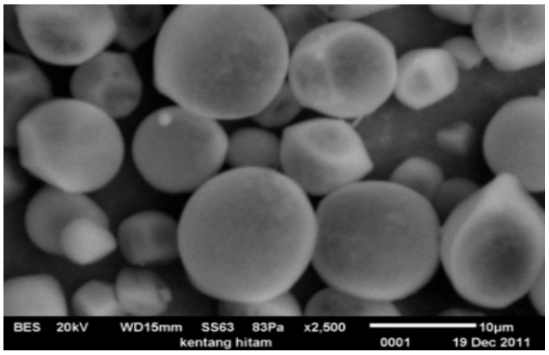

Fig 2 Scanning electron micrographs of the starch granules of variety 3.2 at 2500 times magnifications.

Table 1 Yield and chemical composition of isolated black potato starch varieties 3.2

Yield (%)	Moisture	Protein	Lipid	Ash	Total carbohydrates (%)	Viscosity (Cp)	Amylose content (%)	Amylopectin content (%)
18.73	10.34	0.81	0.50	0.44	83.87	14.2	32.31	67.69

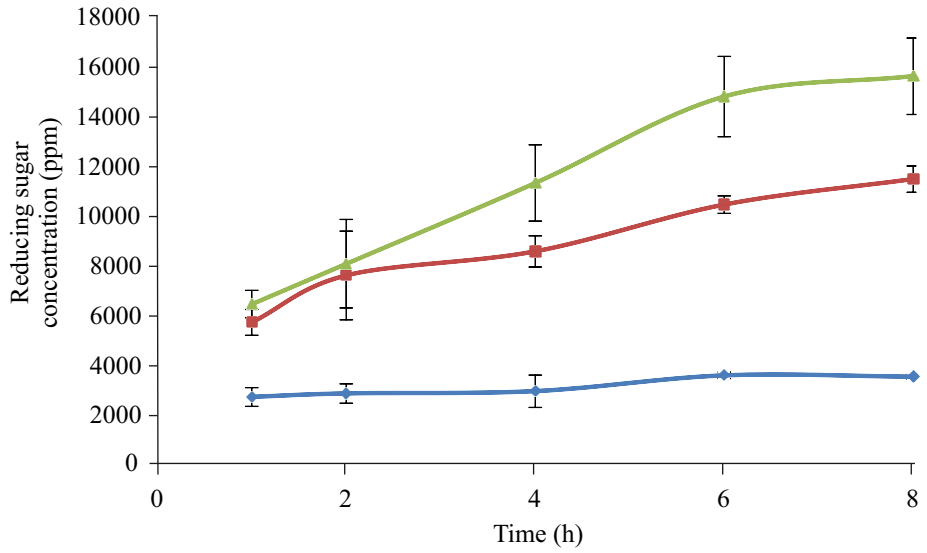

Fig 3 Analysis of reducing sugar content of black potato starch varieties 3.2 were hydrolyzed by amylase enzyme of *Brevibacterium* sp. 10 mL reaction volume consisting of starch and enzyme substrate with a ratio of 1:1 with enzyme activity 2.5 U mL^{-1}. The reaction consists of 3 variations of black potato starch concentration were 1% (◆), 2.5% (■), and 5% (▲) in phosphate buffer pH 6.6, the volume of 1 mL enzyme (2.5 U mL^{-1}), 30 °C.

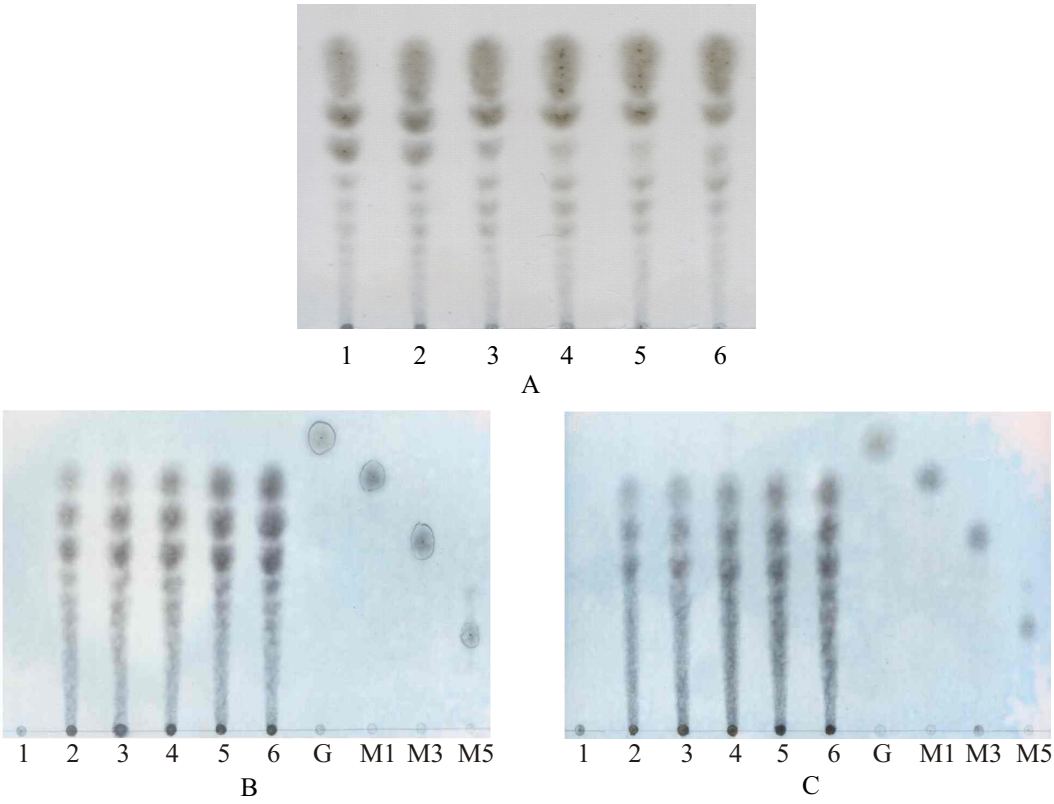

Fig 4 Thin Layer Chromatography analysis of the black potato (*Coleus tuberosus*) starch hydrolyzed by *Brevibacterium* sp. amylase on the substrate concentration 1% (A), 2.5% (B), and 5% (C). Lane 1, control; lane 2, 1 H; lane 3, 2 H; lane 3, 4 H; lane 4, 6 H, and lane 5, 8 H. Lane 7 (G),standard glucose; lane 8 (M1) standard maltose, lane 9 (M3) standard maltotriose, and lane 10 (M5) standard maltopentaose.

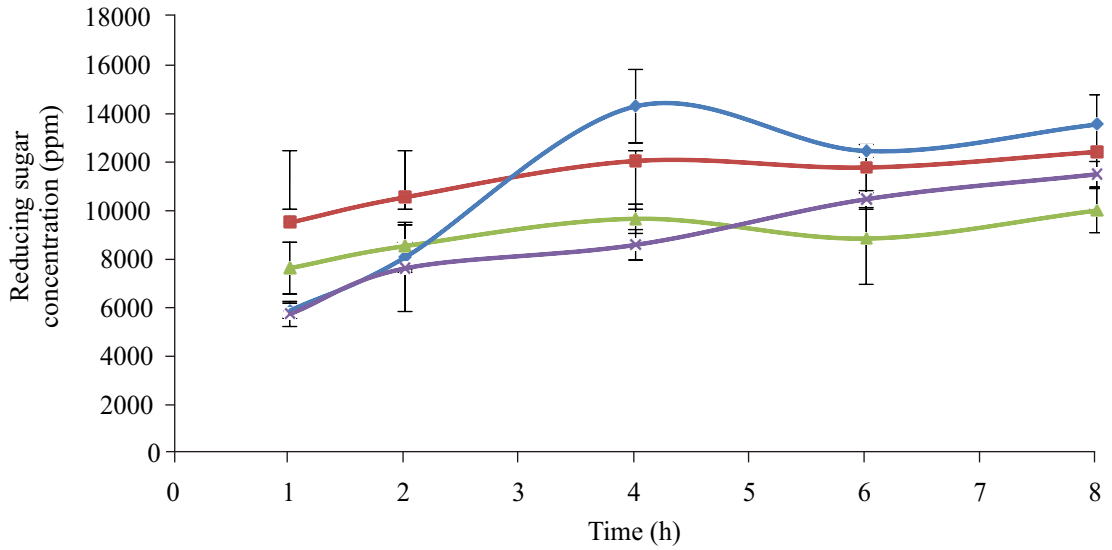

Fig 5 The results of reducing sugar analysis on a variety of enzyme-substrate ratio (v/v) 1:5 (◆) , 1:2 (■), 1:1 (▲), and 1:10 (✕) at a concentration of 2.5% substrate, phosphate buffer pH 6.6, the volume of 1mL enzyme (2.5U mL^{-1}), 30 °C.

exceeding 5% resulted in jelly like solutions. The formation of reducing sugars were assayed to monitor the hydrolysis of starch by the amylase; with respect to the starch concentration different amounts of reducing sugar were produced (Fig 3). The reducing sugar concentration increased from 1 to 5%, for example after 8 h at the substrate concentration of 1, 2.5, and 5%, reducing sugars were 490, 11 435, and 15 570 ppm, respectively. Though the amount of reducing sugars were highest at 5% starch, determination the of the optimal enzyme substrate ratio by TLC was performed at 2.5% starch (Fig 4) as maltooligosaccharides were

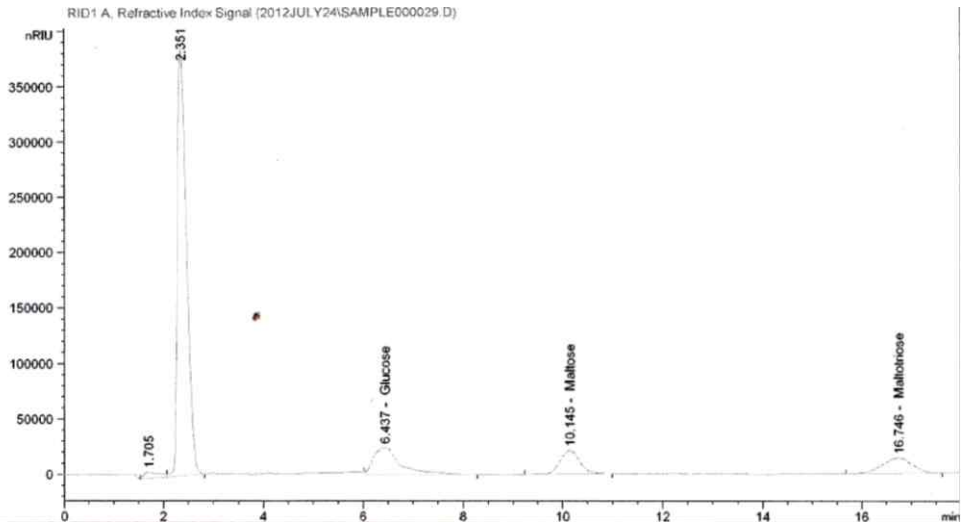

Fig 6 Elution profile of maltooligosaccharides (freeze dry) from hydrolysis the black potato (*Coleus tuberosus*) starch by amylase from *Brevibacterium* sp. Chromatographic conditions:column (ZorbaxSIL(silica) coated with 3-aminopropilsilen); eluent (75:25 acetonitrile:water); flow rate (1.4 mL min^{-1}); detector (Refractive Index/RID).

Table 2 Degree of polymerization (DP) analysis with a variety of enzyme-substrate ratios (v/v) (1:5, 1:2, and 1:1) at a concentration of 2.5% substrate, phosphate buffer pH 6.6, the volume of 1 mL enzyme solution (2.5 U mL^{-1}), 30 °C

Enzyme: substrate ratio	Hydrolysis time (hours to)	DP
1:5	1	22
	2	16
	4	9
	6	10
	8	9
1:2	1	18
	2	17
	4	15
	6	15
	8	14
1:1	1	14
	2	13
	4	11
	6	12
	8	11

readily produced at this concentration and the spots at 2.5% resulted in an increasingly clear separation compared to 5% (Fig 4). There are three kinds of maltooligosaccharides produced: maltose, maltotriose, and a maltooligosaccharides mix, which is dominated by maltose and maltotriose (Fig 6).

DISCUSSION

Generally α-amylase on starch hydrolysis yielded higher quantity of maltotriose (Aiyer 2005; Yang and Liu 2004), but there was also evidence of maltotetraose product dominating amylase (Murakami *et al.* 2008) and Jana *et al.* (2013) described that potato starch hydrolysis by thermophilic α-amylase from *B. megaterium* VUMB109 produced higher quantity of maltopentaose than maltotriose. Patel and Arum (2011) described that thin layer chromatography (TLC) can reveal the degree of polymerization of oligosaccharides. From the products separated by TLC it can be inferred

that the hydrolysis is due to an endo-type in which random starch hydrolysis produced oligosaccharides with DP≥2.

The substrate:enzyme 1:10 has the trend to gradually increase the amount of reducing sugars (Fig 5). At the substrate:enzyme ratio of 1:1; 1:2, and 1:5 the reducing sugar production increased up to 4 h and then decreased for 6 h, subsequently it raised slowly and constantly up-to 8 h but with different degree of polymerization (Table 2). The utilization of very high enzyme dosages caused suboptimal hydrolysis, as low amounts of reducing sugars were produced, as for the ratios of 1:1 and 1:2. The enzyme substrate ratio of 1:5 was chosen as the most effective for further analysis. Similarly, the time for maltooligosaccharides production was determined from the results obtained by investigating enzyme substrate ratios (Fig 5). Hence, a reaction time 4 h were chosen because in this time (substrate concentration 2.5%; enzyme substrate 1:5 ratio) production of reducing sugars were at 14 240 ppm.

Maltooligosaccharides Profile Analysis by HPLC. Patel and Arum (2011) described that to understand the relations between physicochemical properties and the functionality of oligosaccharides, it is important to characterize their structure. Structural analysis requires determination of monosaccharides, their sequence, type of linkages, branching, and anomeric configuration. The oligosaccharides can be isolated using high performance liquid chromatography (HPLC) (Patel and Arum 2011). For the determination of oligosaccharide profiles using HPLC, polar degassed solvents (75% acetonitrile and 25% distilled water) were used according to Eliasson (2006). Separation techniques involved liquid-liquid partition chromatography with retention mechanisms followed by normal-phase with a polar stationary phase and a nonpolar mobile phase. The polarity of the mobile phaseis increased by mixing acetonitrile and water in a ratio of 75:25. The mixture solvent polarity index 6.9 is more nonpolar than distilled water (10.2) and more polar than acetonitrile (5.8).

Oligosaccharides were freeze dried to increase their concentration. Freeze drying is also an ideal final step in the recovery of the product in solid form (Zhu *et al*. 2006). The chromatogram of hydrolyzed samples (Fig 6) displays 5 peaks with different retention times. There were four peaks displaying a retention time similar to the standard. The first peak (retention of 2 351) is characteristic of water. Retention time of the peak of 6 437 corresponds to glucose, maltose was 10 145, and maltotriose 16 746. HPLC analysis of samples resulted in simple sugars such as the glucose monosaccharide, disaccharides such as maltose, and oligosaccharides such as maltotriose. The type of both, simple- and oligosaccharides determined in both, HPLC and TLC analyses are similar. Jana *et al.* (2013) describe that the potato starch which is normally resistant to enzymatic hydrolysis. But, the results of degradation black potato starch, Indegeus Indonesia into simple sugars and oligosaccharides can efficiently be performed using amylolytic enzymes from the marine microbe *Brevibacterium* sp. The end products of α-amylase action are oligosaccharides with varying length with an-configuration and-limit dextrins (Vander *et al*. 2002), which constitute a mixture of maltose, maltotriose, and branched oligosaccharides of 6-8 glucose units that contain both-1,4 and 1,6 linkages (Souza and Pérolade 2010). This research suitable with other marine bacteria amylase. Starch hydrolysis by *Chromohalobacter* sp. TVSP 101 amylase formed maltotetraose, maltotriose, maltose, and glucose as end products (Prakash *et al.* 2009). Chakraborty *et al.* 2011 has been reported that major starch hydrolysis by Halophilic *Saccharopolyspora* sp. A9 were glucose, maltose, and maltotriose as major products. Kumar and Khare (2012) have reported total 72 % soluble starch hydrolysis was achieved in 4 hours by *Marinobacter* sp. EMB8 amylase with the major products were maltotetraose, maltotriose, and maltose by TLC and HPLC analysis.

REFERENCES

Aiyer PV. 2005. Amylases and their applications. Afr J Biotechnol. 4:1525-1529.

AOAC. 1984. Official Methods of Analysis, 14th Edn. Arlington, VA: Association of Official Analytical Chemists.Inc. Washington D.C.

Barreteau H, Cédric D, Philippe M. 2006.Production of oligosaccharides as promising new food additive generation. Food Technol Biotechnol. 44(3):323-333.

Belitz, HD, Grosch W, Schieberle P. 2004. Food Chemistry, 3rd Edn. Heidelberg: Springer. doi:10.1007/978-3-662-07279-0.

Bernfeld P. 1955. Amylase α- and β. Meth Enzymol. 1:149-158.

Crittenden RG and PlayneMJ.1996. Production, properties and applications of food-grade oligosaccharides. Trends in Food Science and Technology. 7(11):353-361. doi:10.1016/S0924-2244(96)10038-8.

Dubois, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugar and related substance. J Anal Chem. 28(3): 350-356.

Eliasson AC, Gudmundsson M. 2006. Starch: physicochemical and functional aspect. *In*: Eliasson AC (ed). Carbohydrates In Food Second Edition. Boca Raton: CRC Press. p 391-469.

Hakraborty S, Khopade A, Biao R, Jian W, Liu X, Mahadik K, Chopade B, Zhang L, Kokare C. 2011. Characterization and stability studies on surfactant, detergent and oxidant stable a-amylase from marine haloalkaliphilic *Saccharopolyspora* sp. A9. J Mol Catal B: Enzym. 68:52-58.

Heyne K. 1987. Tumbuhan Berguna Indonesia [Indonesian Useful Plant]. Jakarta: Yayasan Wana Jaya.

Jana M, Chiranjit MB, Saptadip SB, Bikas RPB, Syed Sirajul IC, Pradeep KDM, Keshab CM. 2013. Salt-independent thermophilic α-amylase from *Bacillus megaterium* VUMB109: An efficacy testing for preparation of maltooligosaccharides. Industrial Crops and Products 41:386-391. doi:10.1016/j.indcrop.2012.04.048.

Kandra L, Gyemant G, Liptak A. 2002. Action pattern of α-amylases on modified maltooligosaccharides. J Biol. 57(11):171-180.

Kumar S and SK Khare. 2012. Purification and characterization of maltooligosaccharide-forming α-amylase from moderately halophilic *Marinobacter* sp. EMB8. Biores Technol. 116:247-251. doi:10.1016/j.biortech.2011.11.109.

Lee JW, Tae OK, Il SM. 2003. Chromatographic separation of maltopentaose from maltooligosaccharides. Biotechnol Bioproc Eng. 8(1):47-53. doi:10.1007/BF02932898.

Lee JW, Kwon TO, Moon IS. 2003. Adsorption of monosaccharides, disaccharides, and maltooligosaccharides on activated carbon for separation of maltopentaose. Biotechnol Bioproc Eng. 8(1):47-53.

Miller GL. 1959.Use of dinitrosalicylic acid reagent for determination of reducing sugar. J Anal Chem. 31(3): 426-428. doi:10.1021/ac60147a030.

Murakami S, Nagasaki K, Nishimoto H, Shigematu R, Umesaki J, Takenaka S, Kaulpiboon J, Prousoontorn M, Limpaseni T, Pongsawasdi P, Aoki K, 2008. Purification and characterization of five alkaline, thermotolerant, and maltotetraose-producing α-amylases from *Bacillus halodurans* MS-2-5, and production of recombinant enzymes in *Escherichia coli*. Enzyme Microb Tech. 43(4-5):321-328. doi:10.1016/j.enzmictec.2008.05.006.

Nakakuki T. 2002. Present status and future of functional oligosaccharides development in Japan. J Pure Appl Chem. 74(7):1245-1251. doi:10.1351/pac200274071245.

Nakakuki T. 2005. Present status and future prospect of fungtional oligosaccharides development in Japan. J. Appl Glicosci. 52(3):267-271. doi:10.5458/jag.52.267.

Okada, M, Nakakuki T. In: Schenck, FW (ed). 1992. Starch hydrolysis products. New York: VHC Publishers. p 335-366.

Patel S, Arun G. 2011. Functional oligosaccharides: production, properties and applications. World J Microbiol Biotechnol. 27(5):1119-1128. doi:10.1007/s11274-010-0558-5.

Prakash B, Vidyasagar M, Madhukumar MS, Muralikrishna G, Sreeramulu K. 2009. Production, purification, and characterization of two extremely halotolerant, thermostable, and alkali-stable A-amylases from *Chromohalobacter* sp. TVSP 101. Process Biochem. 44(2):210-215. doi:10.1016/j.procbio.2008.10.013.

Rahmani N, Yopi, Andriani A, Prima A. 2011. Production and characterization of amylase enzyme from marine bacteria. Proceedings of the International Seminar on Chemistry for a better future, 24-25 November 2011, Jatinangor.

Smits ALM, Kruiskamp PH, Van soest JJG, Vligenthart JFG. 2003. The influence of various small plastisers and malto-oligosaccharides on the retrogradation of (partly) gelatinished starch. Carbohyd Polym. 51(4):417-424. doi:10.1016/S0144-8617(02)00206-0.

Souza PM, Pérolade OM. 2010. Application of microbial α-amylase in industry: a Review. Braz J Microbiol. 41(4):850-861.

Sowbhagya CM, Bhattacharya KR. 1979.Simplified determination of amylose in milled rice. Starch. 31(5):159-163. doi:10.1002/star.19790310506.

Tharanathan RN, Ramadas BU. 1988. Scanning electron microscopy of chemically and enzymatically treated black gram (*Phaseolusmungo*) *andragi* (*Eleusinecoracana*) starch granules. Starch 40(10):378-382. doi:10.1002/star.19880401004.

Vander Maarel MJEC, van der Veen B, Uitdehaag JCM, Leemhuis HL, Dijkhuizen L. 2002. Properties and applications of starch-converting enzymes of the a-amylase family. J Biotechnol. 94(2):137-155. doi:10.1016/S0168-1656(01)00407-2.

Yang CH, Liu WH. 2004. Purification and properties of a maltotriose-producing α-amylase from *Thermobifida fusca*. Enzyme Microb Tech. 35(2-3):254-260. doi: 10.1016/j.enzmictec.2004.05.004.