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Extraction of starch and enzymatic production of high amylose starch from sweetpotato (*Ipomea batatas*) var. Telong

[Pengekstrakan kanji dan penghasilan secara berenzim kanji beramilosa tinggi daripada ubi keledek (*Ipomea batatas*) var. Telong]

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Keywords: glucans, hydrolysis, amylopectin, pullulanase, extraction

Abstract

Starch from sweetpotato var. Telong was extracted at different ratios of sweetpotato and water. A 1:4 ratio (sweetpotato to water) was found to be optimum with 61% of the starch extracted. Further extraction (three times) of the residue at the same ratio of sweetpotato to water resulted in 98% of the total starch extracted. A high amylose starch was produced by debranching the amylopectin of the sweetpotato starch using 0.5% (v/dry weight) pullulanase (Promozyme D2) at 60 °C for 24 h. The effects of pH, temperature, substrate concentration and reaction time on the production of high amylose starch were studied. The optimum conditions for the production of high amylose starch were at pH 5.0, 5.0% (w/v) starch concentration and incubated at 60 °C for 8 h. The amylose content increased from 21 – 84% after 8 h of incubation. The surface morphology of the starch granules observed with a scanning electron microscope (SEM) showed shrinkage on the surface of the starch granules.

Introduction

Starch granules are mainly composed of two macromolecular polymers of α- D-glucose, amylose and amylopectin (Banks and Greenwood 1975). Amylose is a linear glucan with α,1 – 4 glycosidic linkages and limited branching that produces few reducing end groups. Its molecular weight is between 10^5 and 10^6 g/mol (Roger et al. 1996). Chain lengths of amylose are commonly in excess of 6000 D-glucopyranose units. Conversely, amylopectin is a highly branched glucan, which consists of 1 – 4 linked α-D-Glucose with 1 – 6 α-linked branches. Its molecular weight is greater than 108 g/mol (Roger et al. 1999) with an average of 17 – 26 D-glucosyl units separating the α- (1 – 6) branched points (Kennedy et al. 1983).

All starches are made up of these two glucans. The ratio of amylose to amylopectin varies with the starch source. For example corn starch has approximately 28% amylose; genetically modified high amylose corn starch contained about 70% amylose while genetically modified waxy corn starch contained 90 – 100% amylopectin (Kennedy et al. 1983; Cowburn 1989).

Starch accumulates as a complex granular structure with size ranging from 1 – 60 μm. The granules were shown to be made of stacks of amorphous and semi-crystalline growth rings (120 – 400 nm

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thick). The semi-crystalline shells are composed of alternating crystalline and amorphous lamellae repeating in 9 – 10 nm and superimposed to the architecture of amylopectin (French 1984). Starches from different sources differ in overall structure through size distribution of the granules, shape, amylose and lipid content, distribution of chain length in amylopectin and crystalline structure (crystallinity, polymorphic type, crystal size) (Fasihuddin et al. 1999).

High amylose starch is defined as a starch that is composed of at least about 40% amylose (Zallie et al. 1994) and has the ability to form a strong gel and film. These particular properties are exploited in producing jelly gum candies (Lacourse and Zallie 1988; Chiu and Zallie 1989) and as coating for deep fried foods (Van Patten and Freck 1973; Zallie et al. 1994). High amylose starches form hydrogen bonded, insoluble aggregates which are suited for use as a source of dietary fibre (Whistler 1984). It is also used in the production of a range of water soluble or biodegradable plastics (Nawrath et al. 1995). High amylose starch also has a positive effect on insulin production and helps to reduce cholesterol levels after a few weeks in humans (Behall et al. 1988). Amylose was also known to influence both nutritional and technological properties such as susceptibility to enzymatic hydrolysis, gelling and pasting behaviour (Whistler 1984).

High amylose starch is obtained mostly from special hybrids of corn, pea and barley (Banks et al. 1974; Chiu and Zallie 1989). It is well known that high amylose starches are more difficult to isolate from these hybrids as compared to isolating native starches from sources such as corn, rice, tapioca or sago (Chiu and Zallie 1989). Enzyme hydrolysis of native starches by debranching enzymes, such as pullulanase, can produce high amylose content of starch. A linear long chain dextrin from sago starch has been produced using pullulanase enzyme (Wong et al. 2007).

The main objective of this study was to produce high amylose starch from a locally grown sweetpotato variety i.e. Telong. This variety was chosen because it contained substantial amount of starch at 27.4% with amylose to amylopectin ratio of 21:79 (Tan 2000). The tubers are suitable for processing into sweetpotato flour, from which a range of food products can be made such as traditional cakes, extruded products and bakery products.

In this study, starch was extracted from Telong sweetpotato at different ratios of sweetpotato and water. The starch was hydrolyzed into high amylose starch by debranching enzyme (pullulanase) at different pH, temperature, substrate concentration and reaction times. The starch was heat gelatinized prior to hydrolysis to render the starch more accessible to the enzyme. The amylose content and the surface morphology of the starch were determined before and after the hydrolysis.

Materials and methods

Sweetpotato (Telong) was supplied by MARDI (Telong station, Bachok Kelantan). The debranching enzyme, pullulanase (Promozyme D2) with an activity of 1350 NPUN/g was obtained from Novozyme, Denmark and was produced by *Bacillus subtilis*. One Pullulanase Unit Novo (PUN) is defined as the amount of enzyme, which, under standard conditions, hydrolyzes pullulan, liberating reducing carbohydrate with reducing power equivalent to 1 μmole glucose per minute. Amylose and amylopectin standards from potatoes were purchased from sigma-Aldrich, United Kingdom.

Starch extraction from sweetpotato was based on Khatijah and Patimah (1997). The skin was peeled-off and the sweetpotato was cut into small pieces. Water was added at a ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 (sweetpotato to water) in the presence of 0.02% sodium disulphite to prevent enzymatic browning. The suspensions were ground in a mass collider to form slurries.

The starch was extracted by filtering the starch solution from the fibrous part and washed several times to remove the impurities. The starch was then dried in a blowing oven at 45 – 50 °C and ground to 150 μm in size.

The moisture, ash, crude protein and crude fat contents of sweetpotato and sweetpotato starch were determined by moisture analyser, oven method (Pearson 1976), kjeldahl method (AOAC 1990) and soxhlet method (AOAC 1990) respectively. Starch content was determined by the amyloglucosidase/α-amylase method (McCleary et al. 1997) with an assay kit from Megazyme International Ltd. (Ireland). Amylose content of sweetpotato starch was determined by a modified Concanavalin A (Con A) method developed by Yun and Matheson (1990), with an assay kit from Megazyme International Ltd. (Ireland). Three replicates were used for the determination of each constituent.

Differential scanning calorimetry (DSC) was performed to measure the gelatinization temperature of starch. The measurement was performed using a Perkin Elmer DSC (Perkin Elmer corp., Norwalk, Connecticut). Melting point and enthalpies of indium were used for temperature and heat capacity calibration. High pressure steel pan with gold-plated copper seals was used to study the thermal behaviour of the samples. The experiment was carried out in a range of temperatures between 30 – 150 °C with a heating rate of 10 °C/min. Three mg (dry weight) of samples were weighed into stainless steel pans and three times of water was added. The pans were sealed and allowed to equilibrate overnight at ambient temperature. An empty pan served as a reference.

The granular structure of sweetpotato starch was observed using a scanning electron microscope (SEM). Dried starch samples were evenly distributed on SEM specimen stubs with double adhesive tape and coated with a 10 nm gold layer. SEM analysis was conducted using FDI Quanta 400 scanning electron microscope (Netherland).

A 5% starch suspension (100 g starch in 2 litres water) was heated with constant stirring (to prevent sedimentation of the granules) in a water bath at 95 °C until the starch was gelatinized. The suspension was kept at 95 °C for 30 min before being cooled to 40 °C and poured into a 2 litre bioreactor tank (B-Braun Biotech International Biostat®, version 1.0) at 200 rpm. The pH was adjusted to 4.0, 4.5, 5.0, 5.5 and 6.0 with 2 N HCl or 2 N NaOH. The temperature was set at 60 °C. Then, 0.5% (v/w) pullulanase was added once the desired temperature was achieved. One ml of solution was removed every 1, 2, 4, 8, 12, 18 and 24 h to determine the amylose content. The experiment was accomplished in triplicate.

Amylose content was determined according to Morrison and Laignelet (1983). About one ml of dimethyl sulphoxide (DMSO) was added to one ml of 5% starch solution in 16 x 120 mm glass test tubes and incubated in a water bath at 85 °C for 15 min. The solution was cooled to room temperature, vortexed and diluted to 25 ml with distilled water. One ml of the sample was diluted with distilled water and made up to 50 ml in a volumetric flask and iodine solution (5 ml) was added to the sample solution. Iodine solution was prepared by mixing iodine and potassium iodide in distilled water to get a concentration of iodine (I_2) and potassium iodide (KI) of 0.0025 M and 0.0065 M respectively. The solution was then analysed using a spectrophometer (Varian, Cary 50 Probe Spectrophometer) at 635 nm wavelength. A calibration curve of amylose standard (0 – 100% amylose) was constructed to determine the amylose content in the samples.

The effect of temperature was examined by hydrolyzing 5% sweetpotato starch at pH 5.0, 0.5% enzyme concentration and temperatures between 50 and 65 °C. The method described earlier (in effect of pH)

was used to monitor the changes in amylose content of sweetpotato starch as a function of time up to 24 h of incubation.

The effect of substrate concentration was examined by hydrolyzing the starch at pH 5.0, temperature 60 °C, 0.5% enzyme concentration and substrate concentration ranging from 3 – 6%, and incubated for up to 24 h. The method described earlier (in effect of pH) was used to monitor the changes in amylose content of sweetpotato starch.

Statistical analysis

All experiments were performed in three replicates. Statistical significance of differences among means of experimental results was evaluated by analysis of variance (ANOVA) followed by paired wise comparison of means using least significant difference (LSD). The computerized program of SAS system version 6.12 for Elementary Statistical Analysis was used.

Results and discussion

Optimisation of starch extraction

The amount of starch extracted at different ratios of sweetpotato and water is shown in *Figure 1*. The results showed that as the ratio of water increased, more starches were recovered from the sweetpotato. At 1:4 and 1:5 ratios of sweetpotato and water, there was no significant difference in the amount of starch extracted. The amount of starch recovered was about 61%. Further extraction of the residues carried out at the same ratio of sweetpotato to water for another 3 times resulted in a total of 98% starch extracted.

Chemical composition

The sweetpotato starch contained 81.9% starch as compared to only 27.4% in the sweetpotato tubers, while the amylose content was only 21% (*Table 1*). These values were comparable to Gendut and Bukit Naga sweetpotato starch as reported by Khatijah and Patimah (1997). However, the amylose content of Telong sweetpotato starch was lower compared to Gendut and Bukit Naga varieties. Khatijah and Patimah (1998) reported that the amylose content of Gendut and Bukit Naga starches were 34.5% and 34.3% respectively.

The gelatinization temperature of Telong sweetpotato starch was 83.5 °C ± 0.10. The value was higher than Bukit Naga (78.8 °C) and Gendut (78.0 °C) varieties (Khatijah and Patimah 1998). The mechanism of water absorption and gelatinization of sweetpotato starch has been studied by Valetudie et al. (1995). They reported that the gelatinization temperature

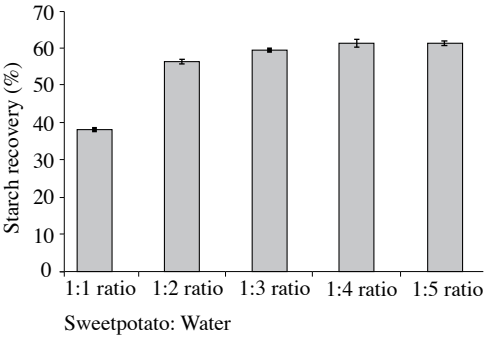

Figure 1. Recovery of starch (%) extracted from Telong sweetpotato tubers at different ratios of sweetpotato tubers and water

Table 1. Chemical composition of Telong sweetpotato and its starch

Chemical composition	Sweetpotato tubers	Sweetpotato starch
Moisture (%)	64.99 ± 1.69	7.3 ± 0.24
Crude Protein (%)	1.60 ± 0.09	0.08 ± 0.00
Crude Fat (%)	0.09 ± 0.01	0.01 ± 0.00
Ash (%)	1.01 ± 0.09	0.05 ± 0.02
Starch content (%)	27.4 ± 0.12	81.9 ± 0.13
Amylose content (%)	n.d.	21.0 ± 0.21
Gelatinisation temperature (°C)	n.d.	83.5 ± 0.10

Means of three replicates; n.d. = Not determined

of sweetpotato starch was 72.7 °C. Perez-Sira and Gonzalez-Parada (1997) reported that gelatinization temperature of sweetpotato starch was 70.5 °C by using Brabendar amylograph.

Production of high amylose starch

The effect of pH on the production of high amylose starch is shown in *Figure 2*. Addition of pullulanase in heated-gelatinized starch suspension resulted in a more runny solution after 24 h of reaction. The starch solution was initially very viscous with the initial amylose content of 21.0%.

The amylose content of the gelatinized sweetpotato starch after hydrolysis by pullulanase was an indicator of the increased amylose content in the reaction. In the first hour, the hydrolysis proceeded at a rapid rate, resulted in a sharp increase of amylose content, which indicated that the enzyme effectively hydrolysed the α-1,6-linkages on the amylopectin molecule (*Figure 2*). As the incubation time is increased, the rate of hydrolysis was subsequently decreased and after 8 h of hydrolysis, there was no significant difference of amylose content (84%) in the reaction mixture. Previous studies reported that hydrolysis of barley starch and sago starch showed no increase in amylose content after 12 h of hydrolysis with pullulanase (Marianna et al. 1993; Wong et al. 2007).

It was noted that the optimum pH for the hydrolysis of sweetpotato starch to produce high amylose starch was at pH 5. In all cases, the amylose content was highest at pH 5. Below and above this pH resulted in lower amount of amylose.

The effect of temperature on the production of high amylose starch is shown in *Figure 3*. The hydolysis was carried out at pH 5.0, 5% (w/v) substrate concentration, 0.5% (v/dry wt) enzyme (pullulanase) concentration and four different temperatures i.e 50, 55, 60 and 65 °C for 24 h. Results showed that the optimum temperatures for the conversion of amylopectin to amylose was at 60 °C. A lower amount of amylose

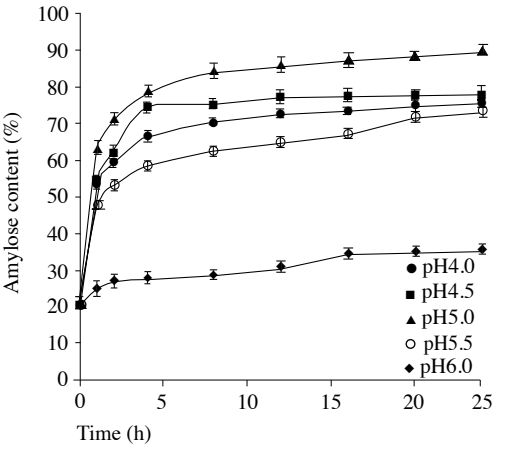

Figure 2. Amylose content of 5.0% gelatinized Telong sweetpotato starch treated with 0.5% pullulanase at different pH

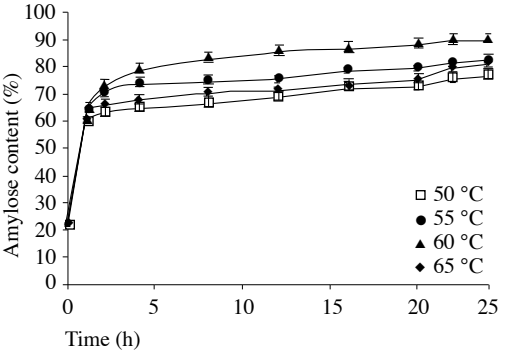

Figure 3. Amylose content of 5.0% gelatinized Telong sweetpotato starch treated with 0.5% pullulanase at different temperatures

was produced at temperature higher or lower than 60 °C.

The effect of substrate concentration on the production of high amylose starch was carried out at 60 °C, pH 5.0, 0.5% enzyme (pullulanase) concentration and four different substrate concentrations (3, 4, 5 and 6%) for 24 h (*Figure 4*). Results showed that the optimum concentration for the conversion of amylopectin to amylose was 5.0% (w/v) substrate concentration. Increasing the concentration of sweetpotato starch resulted in decrease of amylose content. It was probably due to the highly viscous nature of 6.0% gelatinized starch solution compared to 5% starch

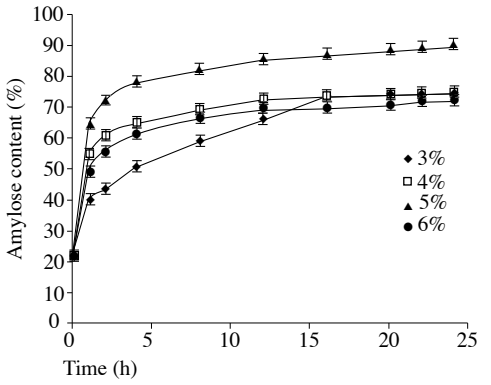

Figure 4. Amylose content of gelatinized Telong sweetpotato starch treated with 0.5% pullulanase, pH 5.0 and temperature 60 °C at different percentages of substrate concentration

concentration (Wong et al. 2007). Lower amylose content was also observed at starch concentrations below than 5%. Wong et al. (2007) also reported that the optimum substrate concentration for the production of high amylose sago starch was 5.0% using 2.0% enzyme concentration but at lower enzyme activity.

Granule structure

Granule size of native sweetpotato (*Figure 5a*) was between 5 – 30 μm and the granule morphology was sphere and irregular polygonal. The gelatinized sweetpotato starch with the addition of pullulanase is shown in *Figure 5b*. It showed some shrinkage of the starch granules and they had lost their structural integrity and no longer firm. Gelatinization disrupted the starch granules, destroying the crystallites and the granule became susceptible to pullulanase attack (Reeve 1992). However, no digestion channel for pullulanase was observed on the starch granules of the gelatinized sweetpotato starch treated with pullulanase although the amylose content of the starch had clearly increased due to hydrolysis of amylopectin.

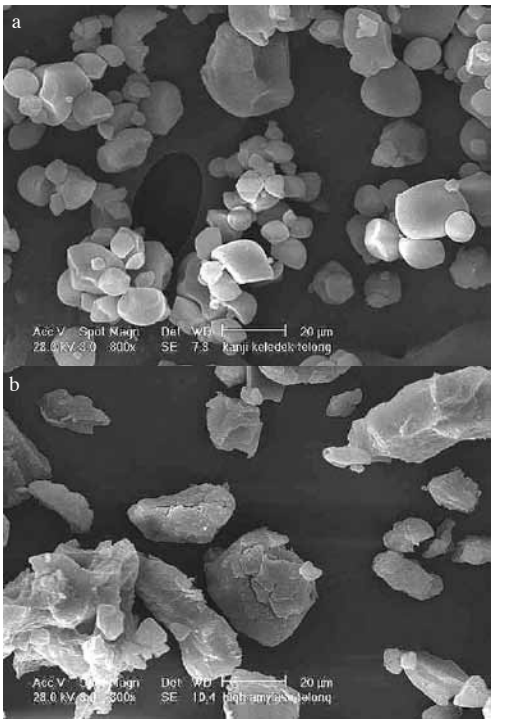

Figure 5. SEM micrographs of (a) Native Telong sweet potato starch, 800x; (b) High amylose Telong sweet potato starch, 800x

Conclusion

Starch was extracted from Telong sweetpotato. A 1:4 ratio of sweetpotato to water was found to be optimum for starch extraction. Further extraction (3 times) of the residue, produced a total starch extraction of 98%. High amylose sweetpotato starch was produced by debranching the amylopectin using pullulanase enzyme. The starch was gelatinized at 95 °C prior to the addition of pullulanase to make it accessible for the action of the enzyme. The best conditions for the production of high amylose starch were at pH 5.0, 5.0% (w/v) starch concentration and incubation at 60 °C for 8 h.

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References

AOAC (1990). *Official methods of analysis,* 15th Edition. Washington DC: Association of Official Analytical Chemists

Banks, W. and Greenwood, C.T. (1975). *Starch and its components,* p. 51 – 66. Edinburgh: Edinburgh University Press

Banks, W., Greenwood, C.T. and Muir, D.D. (1974). Studies on starches of high amylose content. *Starch/Stärke* 26(9): 289 – 328

Behall, K., Schonfield, D.J. and Canary, J. (1988). Effect of starch structure on glucose and insulin response in adults. *Am. J. Clinical Nutr.* 12: 212 – 214

Chiu, C.W. and Zallie, J.P. (1989). *Method for manufacture of jelly gum confections*. US Patent 4886678

Cowburn, P. (1989). The role function of starches in microwaveable formulation. In: *Gum and stabilizers for food industries 5*, (Philips, G.O., Wedlock, D.J. and Williams, P.A., eds.), p. 79 – 88. Oxford: IRL Press

Fasihuddin, A., Williams, P.A., Doublier, J.L., Durand, S. and Buleon, A. (1999). Physico-chemical characterization of sago starch. *Carbohydrate polymers* 38: 361 – 370

French, D. (1984). Organisation of starch granules. In: *Starch, chemistry and technology,* (Whistle, R.L., Bemiller, J.N. and Paschall, E.F., eds.), p. 183 – 247. New York: Academic Press

Kennedy, J.F., Griffiths, A.J. and Atkins, D.P. (1983). The application of hydrocolloids: recent development, future trends. In: *Gum and stabilizers for food industries 2*, (Philips, G.O., Wedlock, D.J. and Williams, P.A., eds.), p. 430 – 431. Oxford: Pergamon Press

Khatijah, I. and Patimah, H. (1997). Physico-chemical properties of cross-linked tuber starches. *J. Trop. Agric. and Fd. Sc.* 25(2): 179 – 185

—— (1998). Physico-chemical properties local native starches. *J. Trop. Agric. and Fd. Sc.* 26(1): 99 – 104

Lacourse, N.L. and Zallie, J.P. (1988). *Starch based jelly gum confections*. US Patent 4726957

Marianna, L., Arja, L., Tapani, S. Karin, A. and Kaisa, P. (1993). Modification of barley starch by α-amylase and pullulanase. *Carbohydrate Polymers* 21: 151 – 152

McCleary, B.V., Gibson, T.S. and Mugford, D.C. (1997). Measurement of total starch in cereal products by amyloglucosidase – α-amylase method: Colaborative study. *J. AOCC Int.* 80: 571 – 579

Morrison, W.R. and Laignelet, B. (1983). An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *J. Cereal Sci.* 1: 9 – 20

Nawrath, C., Poirier, Y. and Somerville, C. (1995). Plant polymers for biodegradable plastics: cellulose, starch and polyhydroxyalkanoates. *Molecular Breeding* 1: 9 – 20

Pearson, D. (1976). *The chemical analysis of food*. London: Longman Group

Perez-Sira, E. and Gonzalez-Parada, Z. (1997). Functional properties of cassava starch (*Manihot esculenta* Crantz) modified by physical methods. *Starch/Starke* 49(2): 49 – 53

Reeve, A. (1992). Starch hydrolysis processes and equipment. In: *Starch hydrolysis product worldwide technology, production and application*, (Schenck, F.W. and Hebeda, R.E., eds.), p. 79 – 120. New York: VCH Publishers

Roger, P., Bello-Perez, L.A. and Colonna, P. (1999). Contribution of amylose and amylopectin to the light scattering behaviour of starches in aqueous solution. *Polymer* 40: 6897 – 6909

Roger, P., Tran, V., Lesec, J. and Colonna, P. (1996). Isolation and characterization of single chain amylose. *J. Cereal Sci.* 24: 247 – 262

Tan, S.L. (2000). New sweetpotato flour varieties: Telong and Jalomas. Retrieved from http://agromedia.mardi.gov.my/magritech/tech_detail_fdcrop.php?id=327

Valetudie, J.C., Colonna, P., Bouchet, B. and Gallant, D.J. (1995). Gelatinization of sweetpotato, tania and yam starches. *Starch/Starke* 47(8): 298 – 306

Van Patten, E.M. and Freck, J.A. (1973). *Methods of coating food products with ungelatinised unmodified high amylose starch prior to deep fat frying*. US Patent 3571286

Whistler, R.L. (1984). History and future expectation of starch uses. In: *Starch chemistry and technology,* (Whistler, R.L., BeMiller, J.N. and Paschall, E.F., eds.), p. 1 – 9. New York: Academic Press

Wong, C.W., Muhammad, S.K.S., Dzulkifly, M.H., Saari, N. and Ghazali, H.M. (2007). Enzymatic production of linear long chain dextrin from sago (*Metroxylan sagu*) starch. *Fd. Chem.* 100: 774 – 780

Yun, S.H., and Matheson, N.K. (1990). Estimation of amylose content of starches after precipitation of amylopectin by concanavalin A. *Starch/Starke* 42: 302 – 305

Zallie, J.P., Eden, J., Kasica, J., Chiu, C.W., Zwiercan, G. and Plutchok, G. (1994). *Methods of making foods containing soluble high amylose starch*. US Patent 5281432

Abstrak

Kanji daripada ubi keledek Telong telah diekstrak dengan nisbah yang berbeza antara ubi keledek dengan air. Nisbah sebanyak 1:4 (ubi keledek:air) adalah paling optimum dengan jumlah kanji yang diperoleh sebanyak 61%. Pengekstrakan tambahan sebanyak tiga kali lagi terhadap hampas ubi keledek pada nisbah 1:4 (ubi keledek:air) menghasilkan jumlah keseluruhan kanji yang diekstrak sebanyak 98%. Kanji beramilosa tinggi telah dihasilkan dengan memutuskan cabang amilopektin kanji ubi keledek (Telong) menggunakan 0.5% (isi padu/berat kering) enzim pululanase (Promozyme D2) pada suhu 60 °C selama 24 jam. Kesan pH, suhu, kepekatan substrat dan tempoh tindak balas terhadap penghasilan kanji beramilosa tinggi telah dikaji. Kesan pH, suhu, kepekatan substrat dan masa tindak balas telah dikaji. Keadaan yang paling optimum bagi penghasilan kanji beramilosa tinggi adalah pada pH 5.0, kepekatan substrat 5.0% dan pengeraman pada suhu 60 °C selama 8 jam. Kandungan amilosa meningkat daripada 21% kepada 84% selepas pengeraman selama 8 jam. Morfologi permukaan granul kanji dilihat melalui mikroskop pengimbas elektron (SEM) menunjukkan pengecutan pada permukaan granul kanji.

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