UNIVERSITI PUTRA MALAYSIA

Pullulanase Type 1-Assisted Modification of Sago (*Metroxylon sagu*) Starch

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BY

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Pullulanase Type 1-Assisted Modification of Sago (*Metroxylon sagu*) Starch

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Chairman : Professor Hasanah Mohd Ghazali, PhD

Faculty : Food Science and Technology

Starch is a versatile food ingredient and is widely used in numerous food and industrials applications. Whether in its native form, or modified through chemical substitution or enzyme modification and/ or physical modification, starch is used as a texturizer, gelling agent, thickener, adhesive, and moisture-retainer. Starches also provide an essential carbohydrate energy source and are obtained from roots, tubers, palms, fruits and cereals, all of which possess unique starch chemistries that impact their properties and function as food ingredients. Starch is a mixture of two polysaccharides, the linear molecule of amylose, which consists of polymers of glucose, and amylopectin, a highly branched molecule. Most starches contain between 15%-30% amylose. Since the major characteristics of starches such as viscosity, shear resistance, gelatinization temperature, solubility, gel stability and textures are affected by the amylose to amylopectin ratios, a wider range of amylose content could extend their applications.

In the present study, sago starch was used as a starting material to produce a starch with higher linear chain molecules (amylose) content due to the fact that it is an indigenous and is the main carbohydrate source in Malaysia. Physico-chemical properties were characterized including their amylose content. Result showed that the native sago starch contained of 24.9% amylose. Scanning electron microscopic (SEM) analysis of sago starch granules were predominantly oval, round or bean-shaped and the size is ranging from 15 to 60 μm. The enthalpies of gelatinization (ΔH_G) and gelatinization transition temperature range ($T_c - T_o$) of 6% (w/v) sago starch suspensions based on differential scanning calorimetric analysis were 19.16 ± 0.79 J/g and 73.9 ± 0.10 °C, respectively. The gelatinization temperature for sago starch obtained by Brabender Viscograph was 72.4°C. Gelatinization temperatures are associated with the loss of birefringence characteristic of starch. Rheological analysis demonstrated that sago starch showed high swelling power and solubility.

Pullulanase Type 1 (EC.3.2.1.41) was used to generate more amylose chains (linear long-chain dextrin) from sago starch which contains 24.9% amylose. Starch suspensions of 2.0– 15.0% (w/v) sago starch were heated at 100°C for 45 minutes, which after cooling, the gelatinized sago starch were hydrolyzed with 0.1–10.0% (v/dry weight starch) pullulanase (Promozyme 400L, Novozymes A/S, Denmark) for 0–24 hours. The amylose contents of the hydrolysates after spray-drying (180°C), were then compared with the initial amylose content. The surface morphology of the starch granules was observed with a Scanning Electron Microscope (SEM). The effects of gelatinization and non-gelatinization, time of reaction, pretreatment with different strengths of hydrochloric acid prior to enzyme hydrolysis, and starch and enzyme concentrations were also studied. Raw sago starch was found to be resistant

to the action of pullulanase, but caused an increase in the amylose (linear long-chain dextrin) content of that sago starch from an initial concentration of 24.9% to 30.0 – 33.2% following gelatinization. The addition of pullulanase to gelatinized sago starch suspensions resulted in a more runny solution, being more runny with longer reaction time. The effect of acid pretreatment on starch, in place of gelatinization of starch, on the ability of pullulanase to hydrolyze the amylopectin of sago starch was examined. Sago starch was pretreated with various concentrations of HCl (0.5– 6.0 M) at 20°C and 40°C for durations of 1–24 hours. Acid pretreatment of the sago starch did not cause greater improvement in the accessibility and susceptibility of pullulanase as the amylose content following pullulanase action did not change significantly. Shrinkage on the surface of the starch granules was observed with SEM. The best condition to maximize the amount of amylose was 5.0% (w/v) sago starch and 2.0% (v/w) enzyme and 12 hours reaction time.

A high-performance size-exclusion chromatograph (HPSEC) equipped with a refractive index (RI) detector and a two-separation columns set up was used to determine and investigate the molecular characteristics of native, fractionated amylose and amylopectin from native sago, and enzyme-catalyzed debranched sago starch. Native sago starch showed a bimodal molecular weight distribution, which indicates fractions that corresponded to amylopectin and shorter linear dextrin chain molecules. The weight average molecular weight (M_w) for the fractionated amylose and amylopectin from sago starch were 8.414×10^5 g/mol and 1.359×10^7 g/mol, respectively. The enzyme-modified starch [5.0% sago starch treated with 0.5-5.0% (v/w) pullulanase for 0.5-24 hours] showed a high molecular weight peak corresponding to that of partially debranched-amylopectin (M_w= 2.875×10^5 – 7.410

$\times$ 10^6 g/mol) and long-chain amylose and a lower molecular weight peak corresponding to the shorter chain amylose molecules, possibly generated from amylopectin by pullulanase, indicating increased concentration of amylose content. The peak area of short chain amylose increased with increasing time of debranching and also enzyme concentration. The M_w for the short chain amylose was between $2.978 \times 10^3 - 4.520 \times 10^3$ g/mol, equivalent to degrees of polymerization (DP_n) between 18 – 23 or number-average molecular weight (M_n) of $2.872 \times 10^3 - 3.713 \times 10^3$ g/mol. The M_w for amylose of the debranched sago starch is smaller as compared to the native sago starch.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Kedoktoran

Pengubahsuaian Kanji Sagu (*Metroxylon sagu*) Secara Bantuan Enzim Pullulanase Jenis 1

Oleh

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Kanji merupakan ramuan makanan yang versatile dengan pelbagai aplikasi industri. Sama ada di dalam bentuk yang asal, atau diubahsuai melalui penggantian secara kimia, atau pengubahsuaian enzim dan/ atau pengubahsuaian fizikal, kanji digunakan sebagai bahan tekstur, ejen pembentukan jel, bahan pemekat, bahan pelekat dan penekalan kelembapan. Kanji juga merupakan sumber tenaga karbohidrat penting yang diperolehi dari akar, batang, pohon, buah dan bijian, yang kesemuanya mempunyai sifat kimia kanji yang unik yang dapat mempengaruhi sifat dan fungsi sebagai ramuan makanan. Kanji terdiri daripada dua campuran polisakarida, molekul amilosa yang linear, polimer glukosa, dan amilopektin, molekul yang bercabang. Kebanyakan kanji mempunyai 15%-30% kandungan amilosa. Memandangkan cirri-ciri utama bagi kanji seperti kelikatan, suhu pengelatinan, kelarutan, kestabilan jel dan tekstur adalah dipengaruhi oleh nisbah amilosa dan amilopektin, maka, julat kandungan amilosa yang lebih lebar dapat memperluaskan aplikasi mereka.

Dalam kajian ini, kanji sagu digunakan sebagai bahan permulaan untuk menghasilkan kanji yang mempunyai molekul rantai linear (amilosa) yang lebih tinggi disebabkan ia merupakan kanji tempatan yang utama di Malaysia. Sifat-sifat fisiko-kimia kanji sagu ditentukan termasuk kandungan amilosa. Kanji sagu didapati mengandungi 24.9% amilosa. Analisis mikroskopi imbasan electron (SEM) menunjukkan bentuk granul kanji sagu adalah kebanyakannya bujur, bulat, atau bentuk semacam kacang dan saiznya adalah di antara 15 hingga 60 μm. Entalpi haba untuk proses gelatinasi (ΔH_G) dan kadar perubahan suhu untuk pengelatinan ($T_c - T_o$) bagi 6% (b/i) ampaian kanji sagu dengan menggunakan alat 'differential scanning calorimetry' adalah masing- masing 19.16 ± 0.79 J/g dan 73.9 ± 0.10 °C. Suhu pengelatinan bagi kanji sagu adalah 72.4°C diperolehi dengan menggunakan Brabender Viscograph. Suhu pengelatinan adalah berkait dengan kehilangan sifat 'birefringence' kanji. Analisis reologi mendemonstrasikan kanji sagu mempunyai keupayaan sifat pengembangan dan kelarutan yang tinggi.

Pullulanase jenis 1 (EC.3.2.1.41), sejenis enzim untuk nyah-cabang amilopektin, telah digunakan untuk menghasilkan lebih banyak amilosa (dekstrin rantai-linear panjang) daripada kanji sagu asal di mana kandungan amilosa ialah 24.9%. Ampaian kanji sagu daripada 2.0 – 15.0% (b/i) dipanaskan pada suhu 100°C untuk 45 minit, kanji sagu yang telah melalui proses penggelatinan ditindak balas dengan 0.1–10.0% (i/ berat kering kanji) enzim pullulanase (Promozyme 400L, Novozymes A/S, Denmark) untuk 0 – 24 jam. Kandungan amilosa bagi hidrolisat selepas pengeringan semburan (100°C) dibandingkan dengan kandungan amilosa asal. Morfologi permukaan butir-butir kanji diperhatikan dengan menggunakan mikroskop imbasan elektron (SEM). Kesan pengelatinan, masa tindak balas, pra-rawatan kanji mentah

dengan asid hidroklorik sebelum tindak balas enzim, kepekatan kanji dan kepekatan pullulanase telah dikaji. Kanji sagu mentah rintang terhadap tindakan pullulanase seperti yang dijangkakan. Walaubagaimanapun, tindak balas dengan pullulanase selepas proses pengelatinan menambahkan kandungan amilosa kepada 30.0–33.2%. Tambahan enzim pullulanase kepada suspensi kanji sagu yang sudah melalui proses pengelatinan adalah lebih cair selepas masa tindakan tertentu. Kesan pra-rawatan dengan asid juga diperhatikan. Kanji sagu dipra-rawat dengan pelbagai kepekatan asid hidroklorik, HCL (0.5-6.0M) pada suhu 20°C dan 40°C untuk jangka masa selama 1-24 jam. Pra-rawatan dengan asid ke atas kanji mentah didapati tidak memudahkan pullulanase untuk nyah-cabang amilopektin kerana kandungan amilosa tidak bertukar secara ketara selepas bertindak dengan pullulanase. Analisa SEM menunjukkan tindak balas dengan pullulanase menyebabkan pengecutan berlaku ke atas granul kanji. Keadaan optimum untuk memaksimumkan kandungan amilosa ialah 5.0% (b/i) kanji sagu, 2.0% pullulanase (i/b) kanji sagu dan masa tindak balas 12 jam.

'High-performance size-exclusion chromatograph' (HPSEC) yang dilengkapi dengan detector indeks biasan (RI) dan dua turus pemisah untuk menentukan ciri molekular bagi kanji sagu mentah, amilosa dan amilopektin yang telah difraksinasikan daripada kanji sagu mentah dan yang sudah dinyah-cabang dengan enzim. Kanji sagu mentah memberikan satu taburan berat molekul secara 'bimodal', menunjukkan pecahan yang berkaitan dengan molekul amilopektin dan rantai linear dekstrin yang lebih pendek. Purata berat molekul (M_w) bagi pecahan amilosa dan amilopektin daripada kanji sagu adalah masing- masing 8.414×10^5 g/mol dan 1.359×10^7 g/mol. Kanji yang diubahsuai dengan enzim [5.0% kanji sagu dirawat dengan 0.5-5.0% (i/b)

pullulanase untuk 0.5-24 jam] menunjukkan puncak berat molekul yang lebih tinggi, dan selaras dengan amilopektin yang telah sebahagian dinyah-cabangkan (M_w= 2.875×10^5–7.410×10^6 g/mol) manakala puncak berat molekul yang lebih rendah selaras dengan amilosa rantai pendek, berkemungkinan dihasilkan daripada amilopektin dengan mengunakan pullulanase, menunjukkan penambahan kepekatan dalam kandungan amilosa. Keluasan puncak bagi amilosa rantai pendek semakin bertambah sekadar dengan masa nyah-cabang dan juga kepekatan enzim. M_w bagi amilosa rantai pendek adalah dalam lingkungan 2.978×10^3- 4.520×10^3 g/mol, sekadar dengan tahap polimerisasi (DP_n), iaitu antara 18-23 atau purata nombor berat molekul (M_n) di antara 2.872×10^3- 3.713×10^3 g/mol. M_w bagi amilosa kanji sagu yang sudah dinyah-cabang adalah lebih kecil berbanding dengan kanji sagu mentah.

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I certify that a Thesis Examination Committee has met on 03 July 2009 to conduct the final examination of Wong Chen Wai on her thesis entitled "Pullulanase Type 1-Assisted Modification of Sago (*Metroxylon sagu*) Starch" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently for any other degree at UPM or other institutions.

WONG CHEN WAI

Date: 19 July 2009

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