UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF WATER SOLUBLE POLYSACCHARIDES FROM BAMBOO SHOOT (*Gigantochloa levis* Blanco Merr) AND THEIR PREBIOTIC POTENTIAL

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By

AIDA FIRDAUS BT MUHAMMAD NURUL AZMI

Thesis submitted the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the requirements for the degree of Doctor of Philosophy

September 2012

Specially dedicated to Abahmak, my siblings, my hubby & my lovely son, wafi…

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chair : Assoc. Prof. Shuhaimi bin Mustafa, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Health benefits of bamboo shoots are often neglected as limited study was carried out to reveals its medicinal properties. Increasing demands in functional foods like prebiotics have driven many researches to isolate these functional ingredients from natural sources. Therefore this research was conducted as to explore the prebiotic potential of bamboo shoots from *Gigantochloa levis*. Characterization on its compositions, molecular weight, solubility, digestibility, intrinsic viscosity, rheological properties, thermal stability and morphological properties were also covered. Hot water extraction (90 ^{0}C) of water soluble polysaccharides extracted from the shoots of *Gigantochloa levis* (which was named as Bamboo Shoots Crude Polysaccharides, BSCP) was found to be 3.27 ± 0.18 % of dry basis, with molecular weight of about 7.49 kDa, while other purified sub-fractions (F1 to F5) were 1550.96, 1471.63, 1685.78, 1691.61 and 1551.67 Da respectively.

BSCP was composed of glucose, galactose and arabinose as the major sugar components with the molar ratio of 10 : 5 : 4 (50.51 ± 1.16, 25.11 ± 0.31 and 19.81 ± 0.52 % molar respectively). The percentage of xylose, mannose, rhamnose and fucose in crude BSCP were however found to be relatively low; 2.05 ± 0.03, 1.77 ± 0.25, 0.62 ± 0.53 and 0.14 ± 0.02 % molar respectively. It also posses good solubility in water (99.81 ± 0.01 % solubility at 5 % (w/v) of BSCP) and its non-digestibility towards artificial human gastric juices was proven to be significantly higher (> 99 %) as to compare with the commercial fructooligosaccharides (FOS) (98 %). This might be due to the presence of (1→3),(1→4)-β-glucan in BSCP as elucidated through Fourier Transform Infra Red (FTIR) spectrum as well as Nuclear Magnetic Resonance (NMR). Fingerprint of BSCP gathered through structural analysis using FTIR showed that BSCP contains polysaccharide with traces of phenolic and uronic acids presence.

Intrinsic viscosity of BSCP was 6.89 dl/g, with a rod-like conformation presumed in the dilute domain through the measurement of power law model. The onset of molecular entanglements or the critical concentration, C^* for BSCP was 0.88 g/dl, while the relative chain stiffness for BSCP was found to be 0.3086, which was about ten times greater than pectin and alginate. This value showed a more flexible chain of BSCP polymer, which was not aligned to the conformation of BSCP presumed through power law model measurement. This is because rod-like polymer tends to have a stiff chain. Atomic Force Microscopy (AFM) analysis conducted for morphological studies of BSCP was however elucidated the conformation of BSCP to be globular rather than rod-like polymer.

Rheological test conducted had confirmed the weak gel properties of BSCP. It was found to be a heat-setting gel, where gel is formed upon heating of a solution. Thermal analysis using Differential Scanning Calorimetry (DSC) also proves the weakness of BSCP gelling properties, with a very small enthalpy value obtained (0.03 to 0.37 mJ).

In vitro fermentation conducted using BSCP as a carbon source gave a positive indicator as a potential prebiotic. BSCP was found to potently support the growth of *B. animalis, B. longum* and *L. acidophilus.* On top of that, BSCP was able to suppress the growth of *Salmonella* sp. pH of Trypticase Peptone Yeast (TPY) medium supplemented with BSCP and FOS were seen to decrease as an indication of bacterial growth. Lactic acid and SCFA produced by the probiotic strains supplemented with BSCP were also found to be higher as compared to FOS, even though there was no significant trend noted. Results obtained throughout this research showed that BSCP meets most of prebiotic characteristics, which is highly resistance to the artificial gastric juice, able to be fermented by the intestinal microbiota like bifidobacterium and lactobacilli while suppressing the growth of pathogens as well as producing SCFA which later could enhance the host health indirectly.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCIRIAN POLISAKARIDA LARUT AIR DARIPADA REBUNG BULUH (*Gigantochloa levis* Blanco Merr) DAN POTENSI PREBIOTIKNYA

Oleh

AIDA FIRDAUS BT MUHAMMAD NURUL AZMI

September 2012

Pengerusi : Prof. Madya Shuhaimi bin Mustafa, PhD

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Khasiat kesihatan rebung buluh sering dipinggirkan ekoran kekurangan penyelidikan yang dijalankan untuk membongkarkan khasiat perubatannya. Permintaan tinggi terhadap makanan berfungsi seperti prebiotik telah mendorong ramai penyelidik untuk mengekstrak makanan berfungsi ini daripada sumber alam. Oleh sebab itu, penyelidikan ini telah dijalankan untuk mengkaji potensi prebiotik daripada rebung buluh yang boleh dimakan iaitu *Gigantochloa levis*. Pengekstrakkan polisakarida larut air daripada rebung *Gigantochloa levis* (yang dipanggil Polisakarida Kasar Rebung Buluh, BSCP) dengan menggunakan air panas (90 ^{0}C) memberikan sebanyak 3.27 ± 0.18 % hasil dengan berat molekular sebanyak 7.49 kDa, sementara sub-fraksi yang telah ditulenkan (F1 hingga F5) mempunyai berat molekular sebanyak 1550.96, 1471.63, 1685.78, 1691.61 and 1551.67 Da masing-masing.

BSCP mengandungi glukosa, galaktosa dan arabinosa sebagai gula utama dengan nisbah molar sebanyak 10 : 5 : 4 (50.51 ± 1.16, 25.11 ± 0.31 dan 19.81 ± 0.52 % molar masing-masing). Manakala peratusan bagi xylosa, mannosa, rhamnosa dan fukosa di dalam BSCP didapati agak rendah iaitu sebanyak; 2.05 ± 0.03, 1.77 ± 0.25, 0.62 ± 0.53 dan 0.14 ± 0.02 % molar masing-masing. Ia mempunyai kadar keterlarutan yang tinggi di dalam air (99.81 ± 0.01 % keterlarutan pada 5 % (w/v) BSCP) serta menunjukkan kadar ketidakhadaham yang tinggi terhadap jus gastrik tiruan (> 99%) berbanding fruktooligosakarida (FOS) (98%). Ini mungkin disebabkan oleh kehadiran (1→3),(1→4)-β-glucan yang ditemui melalui spektra Fourier Fransform Infra Red (FTIR) dan juga Nuclear Magnetic Resonance (NMR). Spektra FTIR juga menunjukkan BSCP mengandungi polisakarida beserta sedikit kandungan phenolik dan asid uronik.

Kelikatan intrinsik bagi BSCP ialah 6.89 dl/g, dengan konformasi yang diandaikan berbentuk rod pada tahap kelikatan paling cair menerusi pengukuran "power law model". Permulaan simpulan molekul atau dikenali sebagai konsentrasi kritikal, C^* bagi BSCP adalah 0.88 g/dl, dengan kekuatan rantaian relatif sebanyak 0.3086, iaitu sepuluh kali ganda berbanding pektin dan alginate. Nilai ini menunjukkan rantaian BSCP yang lebih fleksibel, di mana ianya tidak selari dengan konformasi BSCP yang diandaikan menerusi pengukuran "power law model". Ini kerana molekul berbentuk rod cenderung untuk bersifat lebih kaku. Analisa menggunakan Atomic Force Microscopy (AFM) untuk mengkaji keadaan morfologi BSCP walau bagaimanapun menunjukkan konformasi BSCP berbentuk globul, berbanding bentuk polimer berbentuk rod.

Kajian rheologi menunjukkan bahawa BSCP mempunyai kebolehan membentuk gel yang lemah. Ia merupakan perekat gel panas, di mana gel terbentuk apabila larutan dipanaskan. Analisa terma menggunakan Differential Scanning Calorimetry (DSC) juga membuktikan kelemahan ciri gel BSCP, dengan nilai entalpi yang terlalu kecil (0.03 hingga 0.37 mJ).

Fermentasi secara *in vitro* menggunakan BSCP sebagai sumber karbon menunjukkan potensi yang positif sebagai prebiotik. BSCP didapati mampu untuk menyokong pertumbuhan *B. animalis, B. longum* dan *L. acidophilus.* Disamping itu juga, BSCP mampu untuk membendung pertumbuhan *Salmonella* sp . pH bagi media Trypticase Peptone Yeast (TPY) yang mengandungi BSCP didapati menurun sebagai tanda pertumbuhan bakteria. Laktik asid dan asid lemak rantaian pendek (SCFA) yang dihasilkan oleh bakteria yang diberi medium mengandungi BSCP menunjukkan kuantiti yang tinggi berbanding FOS, walaupun tiada corak yang siknifikan diperhatikan.

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I certify that an Examination Committee has met on 03 SEPTEMBER 2012 to conduct the final examination of AIDA FIRDAUS BT MUHAMMAD NURUL AZMI on her DOCTOR OF PHILOSOPHY thesis entitled **"Characterization of Water Soluble Polysaccharides from Edible Bamboo Shoot (*Gigantochloa levis*) and Its Prebiotic Potential"** in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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AIDA FIRDAUS BT MUHAMMAD NURUL AZMI

Date:_____

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