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Saccharum officinarum L. BY-PRODUCTS (PITH AND RIND) AS POTENTIAL SOURCE OF PREBIOTIC IN VITRO

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By

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Saccharum officinarum L. BY-PRODUCTS (PITH AND RIND) AS POTENTIAL SOURCE OF PREBIOTIC _IN VITRO_

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Agricultural residues are normally present in massive amounts, and their costs of disposal are very high. Notwithstanding their rich nutritional values, the wastes remain under-utilized commercially due to inadequate study. Sugarcane (*Saccharum officinarum* L.) by-products; rind (SR) and pith (SP) promote well-being, probably as natural ingredients for functional and medical food industry. The aims of the research were to evaluate the health benefits and functional properties of Sugarcane by-products (SB) through the estimation of their chemical compositions, antioxidant capacities (DPPH and FRAP assays) and phytochemical compounds (phenolic and flavonoids) as well as to evaluate the prebiotic properties of enzymatically produced xylooligosaccharide (XOS) including; non-digestibility experiment using α-amylase and artificial gastric acid juice and selective fermentation using pure cultures namely: *Lactobacillus casei* Shirota (LcS), *Bifidobacterium animals* subsp. *Lactis* and *Escherichia coli*. Both SR and SP were found to have high total dietary fiber contents (58.91% to 69.10% DW, respectively), with high proportion of insoluble dietary fiber. The available carbohydrate content (AC) in SR (31.72% ± 0.47 DW) was significantly (p<0.05) higher compared to SP (23.07% ± 0.45 DW). The high content of dietary fiber and available carbohydrate are characteristics that made SB as fermentable potential food. The result of antioxidant activities revealed that SR had significantly higher radical scavenging activity (62 % ± 1.06) than SP (51.32% ± 1.18) using the DPPH assay and higher reducing power activity (6.98 ± 0.30 mg $FeSO_4$/100g DW), than SP (2.77 ± 0.32 mg $FeSO_4$/100g DW) using the FRAP assay. In addition, SB showed high total phenolic contents (SR= 469 ± 3.25; SP= 273 ± 4.27 mg GA/100g), and total flavonoids contents (SR= 269 ± 16.80; SP= 169 ± 4.36 mg QE /100g), being significantly higher in SR (p<0.05). This study showed that SB contained XOS known as prebiotic agent. In the present study, XOS mixture was successfully produced from SR and SP (XOS-SR and XOS-SP) respectively. XOS-SR was composed of 1.78 ± 1.01 and 1.62 ± 0.02 mg/mL xylobiose and xylotriose respectively, while XOS-SP was composed of 1.75 ± 0.05, 1.04 ± 0.06 and 0.19 ± 0.02 mg/mL xylobiose, xylotetrose and xylotriose, respectively. Based on the non-digestibility test, around 84 to 88.24% of XOS consumed would reach the colon and then be utilized by the probiotic as small amounts of the saccharides were hydrolyzed by α-amylase (8.29 to 12.87%) and by gastric juice (3.47 to 4.07%). The fermentation test demonstrated that XOS derived from SB is a potential prebiotic ingredient due to the evidence that probiotic bacteria (LcS and *Bifidobacterium*) increased significantly ($p<0.05$), while *E. coli* growth was suppressed *in vitro* condition. In addition, the probiotic bacteria supplemented with XOS produced high amount of SCFAs, known as fermentation end products during the fermentation, indicating the ability of tested bacteria to utilize XOS-SR and XOS-SP, where acetic acid was the predominant end product, followed by propionic acid and lactic acid. In conclusion, sugarcane by-products are potential ingredients for development of functional foods.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

Saccharum officinarum L. (EMPULUR DAN KULIT) SEBAGAI SUMBER BERPOTENSI PREBIOTIK _IN VITRO_

Oleh

DINA ABDUL GHANI ZIDAN

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Residu buah-buahan dan produk akhir mereka biasanya boleh diperoleh dalam kuantiti yang banyak, dan kos pelupusan mereka adalah sangat tinggi. Walaubagaimana pun nilai pemakanan mereka kaya, sisa mereka tidak dieksploitasi secara komersial sepenuhnya disebabkan kekurangan penyelidikan. Produk akhir tebu (*Saccharum officinarum* L.) iaitu, kulit (SR) dan empulur (SP) memberikan ciri penggalakan kesihatan, berpotensi sebagai bahan semula jadi baharu bagi industri industri makanan fungsian dan kesihatan. Objektif kajian ini adalah untuk menilai manfaat kesihatan dan ciri fungsian produk akhir tebu melalui penganggaran komposisi kimia, kapasiti antioksidan (ujian DPPH dan FRAP) dan sebatian fitokimia mereka (fenolik dan flavonoid) di samping untuk menilai ciri prebiotik hasil enzim *Xylooligosaccharide* (XOS) termasuk; eksperimen ketidakcernaan menggunakan α-amylase dan jus gastrik tiruan dan penapaian selektif menggunakan kultur tulen iaitu, *Lactobacillus casei* Shirota (LcS), *Bifidobacterium animals* subsp. *Lactis* dan *Escherichia coli*. Kedua-dua SR dan SP didapati mempunyai kandungan jumlah serat makanan yang tinggi (masing-masing 58.91% hingga 69.10% DW), dengan perkadaran serat tidak larut yang tinggi. Kandungan karbohidrat tersedia (AC) di dalam SR (31.72% ± 0.47 DW) adalah tinggi secara signifikan (p<0.05) daripada SP (23.07% ± 0.45 DW). Kandungan serat makanan yang tinggi dan karbohidrat tersedia merupakan ciri yang menjadikan produk akhir tebu sebagai makanan berpotensi untuk penapaian. Dapatan aktiviti antioksidan menunjukkan bahawa SR mempunyai aktiviti radikal hapus sisa yang secara signifikan lebih tinggi (62% ± 1.06) daripada SP (51.32% ± 1.18) menggunakan ujian DPPH. Demikian juga, SR menunjukkan aktiviti pengurangan kuasa yang secara signifikan lebih tinggi (6.98 ± 0.30 mg $FeSO_4$/100g DW), daripada SP (2.77 ± 0.32 $FeSO_4$/100g DW) menggunakan ujian FRAP. Tambahan pula, SB menunjukkan kandungan fenolik total yang tinggi (SR= 469 ± 3.25; SP= 273 ±

4.27 GA mg/100g), dan kandungan flavonoid total (SR= 269 ± 16.80; SP= 169 ± 4.36 QE mg/100g), adalah secara signifikan lebih tinggi dalam SR ($p<0.05$). Kajian ini menunjukkan bahawa Produk akhir tebu mengandungi XOS, dikenali sebagai agen prebiotik. Untuk kajian ini, campuran XOS telah berjaya dihasilkan daripada SR dan SP (XOS-SR dan XOS-SP). XOS-SR terdiri daripada masing-masing, 1.78 ± 1.01 dan 1.62 ± 0.02 mg/mL xylobiosa dan xylotriosa, manakala XOS-SP terdiri daripada, masing-masing, 1.75 ± 0.05, 1.04 ± 0.06 dan 0.19 ± 0.02 mg/mL xylobiosa, xylotetrosa dan xylotriosa. Berdasarkan eksperimen ketidakcernaan, sekitar 84 hingga 88.24% XOS yang dimakan akan sampai ke kolon dan kemudian digunakan oleh populasi probiotik disebabkan jumlah kecil sakrida telah dicerna oleh *α-amylase* (8.29 hingga 12.87%) dan jus gastrik (3.47 hingga 4.07%). Ujian fermentasi memperlihatkan bahawa XOS yang diperoleh daripada SB merupakan bahan berpotensi prebiotik disebabkan bukti yang menyatakan bahawa bakteria probiotik (LcS dan *Bifidobacterium*) meningkat secara signifikan ($p<0.05$), manakala pertumbuhan *E. coli* telah disekat *in vitro*. Tambahan pula, bakteria probiotik yang digabung dengan XOS menghasilkan jumlah SCFA yang tinggi ketika fermentasi, memperlihatkan kebolehan bakteria yang diuji untuk menggunakan XOS-SR dan XOS-SP, di mana asid asetik merupakan produk akhir yang dominan dihasilkan, diikuti oleh asid propionik dan asid laktik. Kesimpulannya, produk akhir tebu merupakan bahan yang berpotensi untuk pembangunan makanan fungsian.

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

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

%	Percentage
µL	Microliter
AC	Available carbohydrate
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
B.	*Bifidobacterium*
BHT	Butylated Hydroxytoluene
CFU	Colony Forming Unit
DP	Degree of Polymerization
DPPH	2,2- Diphenyl-1-Picrylhydrazyl
DW	Dry weight
E.	*Escherichia*
FAO	Food and Agriculture Organization
FOS	Fructooligosaccharide
FRAP	Ferric Reducing Antioxidant Power
g	Gram
h	Hour
IDF	Insoluble Dietary Fiber
kg	Kilogram
L	Liter
L.	*Lactobacillus*
LAB	Lactic Acid Bacteria
LcS	*Lactobacillus casei* Shirota
Log	Logarithm
M	Molar
mg	milligram
min	Minute
mL	Milliliter
mM	Millimolar
MRS	De Man Rogosa Sharpe

MW	Molecular Weight
nd	Not detected
RCM	Reinforced Clostridial Medium
rpm	Route Per Minute
SB	S. *officinarum* L. By-products
SCFAs	Short Chain Fatty Acids
SD	Standard Deviation
SDF	Soluble Dietary Fiber
SP	S. *officinarum* L. Pith
SR	S. *officinarum* L. Rind
ssp.	Sub-species
TDF	Total Dietary Fiber
TFC	Total Flavonoids Content
TPC	Total Phenolic Content
v/v	volume/ volume
w/v	weight/ volume
WHO	World Health Organization
XOS	Xylooligosaccharide
XOS-SP	Xylooligosaccharide from S. *officinarum* L. Pith
XOS-SR	Xylooligosaccharide from S. *officinarum* L. Rind
α-	Alpha-
β-	Beta-

CHAPTER 1

INTRODUCTION

1.1 Background

Sugarcane (*Saccharum* spp.) is one of the most crucial crops in tropical and subtropical areas with immense industrial applications especially in sugar and alcohol industries (Henry & Kole, 2010). Over 1000 million tons of sugarcane are harvested annually and the level of its production excelled among the major grain food crops, i.e. wheat, rice and maize, which have annual production of around 600 million tons (Cordeiro *et al*., 2007). The harvesting and processing of sugarcane to its different products produces massive amount of bagasse residues including rind (outer layer) and pith (inner layer) of plant stalk (Lee & Mariatti, 2008). The bagasse residues are rich in polysaccharides (Jayapal *et al*., 2013; Leang & Saw, 2011; Wang *et al*., 2019c) and oligosaccharides (Reddy & Krishnan, 2016; Bian *et al*., 2013) which are known as prebiotic ingredients. Besides, other beneficial nutrients such as phytochemical compounds (Zhao *et al*., 2015; Feng *et al*., 2015) which may be involved in scavenging of free radicals are also present. Therefore, they are ideal functional food ingredients that improve human health.

Over the last two decades, consumers adopted food products that are well known to promote wellness and health (Cencic & Chingwaru, 2010). As recorded by Institute of Medicine's Food and Nutrition Board, "functional foods" are food products that can enhance health beyond its basic nutritional requirements at the same time alleviate risks toward chronic disease (Al-Sheraji *et al.*, 2013). It is commonly known that prebiotic is one among the most commonly type of functional foods (Al-Sheraji *et al*., 2013). Dietary prebiotic has been identified as ''a selectively fermented ingredient which cause particular alterations in the constitution and/or activity of the gut microbiota, therefore according benefit(s) upon host well-being'' (Gibson *et al*., 2017). Thus, the prebiotic compounds should not undergo hydrolysis nor absorption within the upper section of the gut; and reach the colon to particularly stimulate the development of colonic microbiota (Rastall & Gibson, 2015). The use of prebiotics is not just limited to the regulation of gastrointestinal tract, but also could be used as therapeutic agents against various infections such as reducing the risk of colon carcinogenesis (Laparra & Sanz, 2010), and obesity (Cani *et al*., 2009), enhancing mineral absorption (Whisner *et al*., 2013), improving lipid metabolism and glucose homeostasis (Beserra *et al*., 2015) and modification of inflammatory conditions like Irritable Bowel Syndrome (IBS) (Silk *et al*., 2009).

Nevertheless, large scale study on prebiotics has promoted the desire to explore other possibility and inexpensive origins of prebiotics production in order to meet the market and consumers' requirements (Singdevsachan *et al*., 2016). Xylooligosaccharide (XOS) is sugar polymers with a great prebiotic potential produced from the hydrolysis of xylan and consist of xylose units linked by β-(1,4) bonds, with degree of polymerization (DP) counting from 2 to 10. They are termed according to their number of xylose in their structure such as xylobiose, xylotriose, xylotetraose and so on (Aachary & Prapulla, 2011). XOS as prebiotic has comparative advantages over fructooligosaccharide (FOS), inulin and galactooligosaccharide (GOS) with respect to their high resistance against heat and acidity. In fact, the recommended daily dose of XOS is only 0.7g, compared to FOS of 3g indicating that XOS can exert prebiotic effect at a lower dosage than FOS (Singh *et al*., 2015). Agricultural wastes are among the most widely used sources for XOS production due to their high content of plant cell wall xylan as their utilization can relieve the environmental damage associated with the disposal of waste. Recently, different agricultural materials have been investigated for their XOS content as well as their prebiotic functions such as corn cobs (Samanta *et al*., 2012b), wheat bran (Immerzeel *et al*., 2014) and corn stover (Buruiana *et al*., 2017). Nevertheless, there is insufficient information on prebiotic function of *S. officinarum* L. by-products (rind and pith).

1.2 Problem Statement

While most of the recent studies are focusing mainly on biological activity of fruit or their products, a handful of research investigated residues and by-products of fruit or food processing. Agricultural waste obtained from the food industry is plentiful in Malaysia, while its utilization is still limited. A great amount of untreated wastes can contaminate water, air and land by volatilization, leaching and dusting. On the other hand, various studies demonstrated that these wastes are rich in insoluble and soluble dietary fiber, polyphenol compound, oligosaccharides and others components which can be exploited for human consumption. (Leang & Saw, 2011; Kong *et al*., 2015).

In year 2017, the global generation of sugarcane was calculated to be about 1.9 billion, that was produced on nearly 27.2 million hectares (FAOSTAT, 2017). The production of sugarcane in Malaysia was estimated at 28038 and 29990 tons in 2016 and 2017, respectively. Typically, over 90 countries produce sugarcane and of 100 tons of sugarcane processing in a factory, 30–34 tons of bagasse are obtained (Yadav & Solomon, 2006). Sugarcane remnants are burnt in sugar mills for energy production or stockpiled on site posing environmental issues without recognizing its potential beneficial for a variety of purposes, including for human dietary purpose (Attard *et al*., 2015; Mandelli *et al*., 2014). In Malaysia, there is lack of scientific information of sugarcane by-products in relation to their functional and health properties, and largely uncollected, which require more investigation to find other alternative uses in commercial food formulations. Therefore, to put an end to wasting resources, harnessing these residues for modern means of functional compounds for food application might be a commendable alternative for the health and economic sector.

1.3 Significance of Study

Several organizations (Food and Drug Administration and European Food Safety Authority) have recognized the proven health benefits of agricultural waste obtained from food industry. Therefore, the alternative uses of *S. officinarum* L. wastes in the formulation of different food products would be vital, both from the view of commercial application and food science investigation. Due to unavailability of studies or investigation about the functional and health properties of the *S. officinarum* L. wastes, the present study will offer the methodological information to researchers and provide information regarding the ideal and effective use for each material based on its properties.

From the view of industrial application, the food factories would gain from the conversion of wastes into commercial products either as raw material for intermediate foods ingredients, or as ingredients of new products. Besides, using such inexpensive material as functional ingredients in food products can facilitate the product's availability in affordable and cheaper price to large number of customers. Based on that, *S. officinarum* L. wastes are ideal source of a variety of phytochemical compounds, and indigestible poly- or oligosaccharides which have exhibited different health benefits (Takara *et al*., 2007; Kadam *et al*., 2008; Reddy & Krishnan, 2016). Therefore, this alternative use of *S. officinarum* L. waste could not only reduce the amount of agricultural waste contaminating the environment, but also provide a variety of benefits for the human health and food industry.

1.4 Objectives

1.4.1 General Objective

To determine the ability of Xylooligosaccharide derived from *Saccharum officinarum* L. by-products (pith and rind) as a potential prebiotic to promote the growth of selected probiotics.

1.4.2 Specific Objectives

a. To estimate the chemical composition, antioxidant activity, total phenolic, and total flavonoids content of *Saccharum officinarum* L. by-products.

b. To determine the resistance of xylooligosaccharide derived from *Saccharum officinarum* L. by-products against human hydrolysis using α- amylase and artificial human gastric juice *in vitro*.

c. To determine the effect of xylooligosaccharide derived from *Saccharum officinarum* L. by-products on two probiotic strains namely, *Lactobacillus casei* Shirota *and Bifidobacterium animals* subsp. *Lactis*.

d. To quantify the primary end products of fermentation of xylooligosaccharide derived from *Saccharum officinarum* L. by-products particularly lactic, acetic and propionic acids.

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