UNIVERSITI PUTRA MALAYSIA

EXTRACTION AND CHARACTERIZATION OF SOLUBLE NON-DIGESTIBLE POLYSACCHARIDES FROM COCONUT KERNEL CAKE AND PALM KERNEL CAKE AS POTENTIAL SOURCE OF PREBIOTICS

BASHIRAT BELLO

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By

BASHIRAT BELLO

Thesis Submitted to the School of Graduate Studies Universiti Putra Malaysia, in Fulfillment of the Requirements for the degree of Master of Science

June 2017

DEDICATION

This project is dedicated to my family, teachers and the humanity at large, especially to my parents for their unconditional love and infinite supports given to me in my life journey.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

EXTRACTION AND CHARACTERIZATION OF SOLUBLE NON-DIGESTIBLE POLYSACCHARIDES FROM COCONUT KERNEL CAKE AND PALM KERNEL CAKE AND AS POTENTIAL SOURCE OF PREBIOTICS

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Chairman : Professor Shuhaimi Mustafa, PhD
Faculty : Biotechnology and Biomolecular Sciences

The health benefits associated with consumption of prebiotic and probiotics have made it more preferable than conventional food products. Extraction of prebiotics from local plant materials are associated with low yield; therefore, the aim of this research is to study the effects of solvents on extraction yield and biochemical composition of soluble polysaccharides from palm kernel cake (PKC) and coconut kernel cake (CKC) that could have prebiotics potential. The specific objectives are, (i) To extract and characterized soluble polysaccharides from palm kernel cake and coconut kernel cake (ii) To determined the non-digestibility of the extracted soluble polysaccharides on artificial human gastric juice and alpha amylase and, (iii) To evaluate the prebiotics potential on *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus rhamnosus* ATCC 53103 *in-vitro*. Towards achieving these objectives, three different solvents namely water, citric acid and NaOH were employed to extract soluble polysaccharide from PKC and CKC. The chemical properties of the extracted soluble polysaccharides which include total carbohydrates, protein content, solubility rate, monosaccharides composition, structural information and thermal properties were determined. The extracted soluble polysaccharides were further subjected to digestibility test using artificial human gastric juice and alpha amylase. Their prebiotic potential was determined on *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus rhamnosus* ATCC 53103 as probiotics strains *in-vitro*.

It was observed that, there were significant differences (P<0.05) among the crude polysaccharides extracted with the three different solvents. In both PKC and CKC, NaOH had the highest percentage polysaccharides yield (8.73 and 2.90%), whereas the lowest percentage yield of soluble polysaccharide obtained was recorded using hot water in both PKC and CKC. The soluble polysaccharides obtained from PKC and CKC, (PKCSP) and (CKCSP) respectively, were found to contain a considerable percentage composition of total carbohydrates 57.11%, 56.94% and 50.95% for water, citric acid and NaOH in PKC samples. In CKC, the total percentage carbohydrate composition for water, citric acid and NaOH were 55.26%, 42.56% and 43.46% respectively, with traces of proteins ranging from 0.39 to 0.72% in all the soluble polysaccharides. The extracted crude polysaccharides from both PKC and CKC were found to be highly soluble with an average solubility rate of 98%. There was no significant difference (P>0.05) between the solubility rate of PKCSP and CKCSP in all solvents extracts.The polysaccharides of both PKCSP and CKCSP composed of mannose, glucose, galactose, arabinose with traces of rhamnose and xylose as revealed by GC-FID. The highest monosaccharaides contents were glucose in CKCSP (78%) and mannose in PKCSP (64%) in water and citric acid as extracts respectively. FTIR spectroscopy revealed that the polysaccharides extracts were linked together by β and α-glycosidic bonds with absorption bands at around 1072 cm^{-1} and 888 cm^{-1}, with traces of protein in water extracts at around 1534 cm^{-1}.Thermal analysis revealed by differential scanning calorimeter (DSC) showed the main degradation temperature of both polysaccharides at around 120 °C to 130 °C.

PKCSP and CKCSP were found to be highly resistance (>96%) when subjected to artificial human gastric juice. There was no significant differences (P>0.05) of the percentage hydrolysis observed from non-digestibility tests on artificial human gastric juice among the tested polysaccharides extracts. The prebiotics potentials of the polysaccharides on probiotics *in-vitro* revealed an increase in growth of *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus rhamnosus* ATCC 53103 with decrease in the pH of the medium, indicating that short chain fatty acid (SCFA) were produced at the end of the incubation period when compared with the fructo-oligosaccharides (FOS) control. It can be concluded that soluble polysaccharides from PKC and CKC were highly resistance to artificial human gastric juice and were able to increase the growth of the tested probiotics *in-vitro*. It can be concluded that, the highest yield of soluble polysaccharides was obtained when NaOH was used as an extracting solvent. But this extract exhibited lower percentage of monosaccharides composition as compared to water and citric acid extracts in both PKC and CKC samples. In general, results from this study demonstrated that soluble polysaccharides obtained from PKC and CKC could be used as potential prebiotics.

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

PENGEKSTRAKAN DAN PENCIRIAN KETIDAKHADAMAN KOLISAKARIDA TERLARUT DARIPADA DEDAK ISORONG SAWIT (PKC) DAN SEDAK ISIRONG KELAPA (CKC) SEBAGAI SUMBER BERPOTENSI PREBIOTIK

Oleh

BASHIRAT BELLO

Jun 2017

Pengerusi : Profesor Shuhaimi Mustafa, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Faedah kepada kesihatan melalui penggunaan prebiotik dan probiotik telah menyebabkania dipilih berbanding produk-produk makanan konvesional. Pengekstrakan prebiotik daripada tumbuhan tempatan dikaitkan dengan hasil pengekstrakan yang rendah; Oleh itu, tujuan penyelidikan ini adalah untuk mengkaji kesan pelarut pada hasil pengeluaran dan komposisi biokimia polisakarida larut dari kek isirung sawit (PKC) dan kek isirung kelapa (CKC) yang boleh mempunyai potensi prebiotik. Objektif-objektif spesifik adalah, (i) Mengekstrak dan mencirikan polisakarida larut dari kek isirung kelapa sawit dan kek isirung kelapa (ii) Untuk menentukan ketidakbolehtelapan polisakarida larut dalam jus gastrik buatan manusia dan pada alpha amylase dan, (iii) Untuk menilai potensi prebiotik pada Lactobacillus plantarum ATCC 8014 dan Lactobacillus rhamnosus ATCC 53103 secara in-vitro. Bagi mencapai matlamat ini, tiga pelarut berbeza iaitu air, asid sitrik dan NaOH digunakan untuk mengeluarkan polisakarida larut dari PKC dan CKC. Sifat kimia polisakarida terlarut yang mengandungi jumlah karbohidrat, kandungan protein, kadar kelarutan, komposisi monosakarida, maklumat struktur dan sifat terma telah dikenalpasti. Polisakarida terlarut yang telah diekstrak seterusnya dicernakan menggunakan jus gastrik manusia buatan. Potensi prebiotik telah ditentukan secara in-vitro ke atas strain-strain probiotik *Lactobacillus plantarum* ATCC 8014 dan *Lactobacillus rhamnosus* ATCC 53103.Terdapat perbezaan yang signifikan ($P<0.05$) terhadap kandungan polisakkarida mentah yang diekstrak menggunakan tiga pelarut berbeza.

Di dalam kedua-dua PKC dan CKC, NaOH mempunyai peratusan polisakkarida tertinggi (8.73 dan 2.90 %), di mana peratusan terendah polisakkarida terlarut adalah di dalam air panas bagi kedua-dua PKC dan CKC. Polisakkarida terlarut yang diperolehi daripada PKC dan CKC, PKCSP serta CKCSP masing-masing mengandungi jumlah karbohidrat sebanyak 57.11%, 56.94% dan 50.95% bagi air,

asid sitrik dan NaOH di dalam sampel PKC. Di dalam CKC, jumlah peratusan karbohidrat di dalam air, asid sitrik dan NaOH adalah masing-masing 55.26%, 42.56% dan 43.46% dengan kandungan protin surih antara 0.39 hingga 0.72 % di dalam polisakarida terlarut Polisakarida mentah yang diekstrak daripada PKC dan CKC didapati sangat larut dengan kadar keterlarutan purata sebanyak 98 %. Tiada perbezaan signifikan (P>0.05) antara kadar keterlarutan PKCSP dan CKCSP di dalam semua ekstrak pelarut.Polisakkarida daripada kedua-dua PKCSP dan CKCSP terdiri daripada mannosa, glukosa, galaktosa dan arabinosa dengan kehadiran surih rahmnosa dan xilosa seperti yang diperolehi daripada analisis GCFID. Kandungan monosakarida tertinggi adalah glukosa di dalam CKCSP (78 %) dan mannosa di dalam PKCSP (64 %) yang diekstrak menggunakan air dan asid sitrik. Spectroscopi FTIR menunjukkan polisakarida yang menunjukkan ikatan β dan α-glycosidic bonds pada ikatan penyerapan 1072 cm^{-1} dan 888 cm^{-1}, dengan kehadiran surih protin di dalam ektrak air pada 1534 cm^{-1}. Analasis termal oleh pengimbasan pembezaan kalorimeter (DSC) menunjukkan suhu degradasi utama bagi kedua-dua polisakarida adalah antara 120 °C hingga 130 °C.

PKCSP dan CKCSP didapati tahan (>96%) kepada jus gastrik manusia buatan. Tiada perbezaan signifikan (P>0.05) pada peratusan hidrolisis dilihat daripada ujian ketidakhadaman pada jus gastrik manusia buatan di antara extrak-estrak polisakkarida yang dikaji. Potensi prebiotic polisakkarida ke atas probiotik in-vitro menunjukkan peningkatan pertumbuhan *Lactobacillus plantarum* ATCC 8014 dan *Lactobacillus rhamnosus* ATCC 53103 dengan penurunan nilai pH medium, menunjukkan bahawa rantaian kecil asid lemak (SCFA) terhasil di penghujung masa pengeraman apabila dibandingkan dengan frukto-oligosakkarida (FOS) kawalan. Ianya boleh disimpulkan bahawa polisakkarida terlarut daripada PKC dan CKC mempunyai ketahanan yang tinggi kepada jus gastrik manusia buatan dan boleh meningkatkan pertumbuhan probiotik yang diuji secara in-vitro. Dapat disimpulan bahawa, hasil pengekstrakan tertinggi bagi polisakarida terlarut diperoleh apabila NaOH digunakan sebagai pelarut. Tetapi, ekstrak ini menunjukkan peratusan yang rendah bagi komposisi monosakkarida apabila dibandingkan dengan air dan asid sitrik daripada kedua-dua sample PKC dan CKC. Secara umumnya, keputusan kajian ini menunjukkan polisakarida terlarut yang diperoleh daripada PKC dan CKC boleh digunakan sebagai prebiotik yang berpotensi.

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I certify that a Thesis Examination Committee has met on 21 June 2017 to conduct the final examination of Bashirat Bello on his thesis entitled "Extraction and Characterization of Soluble Non-Digestible Polysaccharides from Coconut Kernel Cake and Palm Kernel Cake as Potential Source of Prebiotics" 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 Master of Science.

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

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LIST OF ABBREVIATIONS

%	percentage
µL	microliter
AOAC	Association of Official Analytical Chemist
ATCC 53103	*Lactobacillus rhamnosus*
ATCC 8014	*Lactobacillus plantarum*
$CaCl_2$	Calcium chloride
CKC	coconut kennel cake
CKCSP	coconut kennel cake soluble polysaccharide
$CKCSP_{CA}$	Coconut kernel cake soluble polysaccharide, citric acid extract
$CKCSP_{N}$	Coconut kernel cake soluble polysaccharide, NaOH extract
$CKCSP_{W}$	Coconut kernel cake soluble polysaccharide, water extract
CEM	Conventional extraction method
DEAE	Diethylaminoethyl
DNS	Dinitrosalicylic acid
DSC	deferential scanning calorimeter
FID	Flame ionization detector
FOS	fructose oligosaccharide
FTIR	Fourier Transform infrared
g	gram
GC	Gas chromatography
h	hours
H_2O	water

HCl	Hydrochloric acid
J/g	Enthalpy change
KCl	Potassium chloride
LAB	Lactic acid bacteria
MAE	Microwave Assisted Extraction
mg	milligram
$MgCl_2$	Magnisium Chloride
mins	minutes
mL	milliliter
mM	millimolar
MRS	Man Rogosa Sharp
Na2HPO4	Di-sodium hydrogen phosphate
NaCl	Sodium chloride
NaCl	Sodium chloride salt
NaOH	sodium hydroxide
N	Normality
nm	nano meter
NMR	Nuclear Magnetic Resonance
PBS	phosphate buffer saline
PKC	palm kennel cake
$PKCP_N$	palm kennel cake NaHO extraction
PKCSP	palm kennel cake soluble polysaccharide
$PKCSP_{CA}$	palm kennel cake citric acid extraction
$PKCSP_N$	Palm kernel cake soluble polysaccharide, NaOH extract
$PKCSP_W$	palm kennel cake water extraction

rpm	revolution per minute
SCFA	Short chain fatty acids
Te	End temperature
TFA	trifluoroacetic acid
To	Onset temperature
Tp	Peak temperature
UAE	Ultrasonic Assisted Extraction
USA	United State of America
w/v	weight by volume
α	alpha
β	beta

CHAPTER 1

INTRODUCTION

1.1 Background of study

The Knowledge of consumers regarding food products that promotes health and wellbeing is increasing. The development in food technology and nutritional research has led to various invention of novel foods ingredient that reduces the risks towards chronic diseases when consumed. The health benefits related to consumption of prebiotic and probiotics have made it more preferable compared to conventional food products (Ares et al., 2009). Prebiotics are selective non digestible, short chain carbohydrates food substances, that pass through the upper portion of the gastrointestinal tract unchanged and stimulate the growth and activities of the beneficial bacteria (probiotics) that colonized the colon by enhancing their growth performance (Al-Sheraji et al., 2013; Wang, 2009).

Prebiotics can be considered as a food for probiotics that resides predominantly in the large intestine of mammals and other higher animal species (Gibson et al., 2004; Slavin, 2013). Studies have shown that prebiotics have other beneficial effects apart from being acting on probiotics. It was found out that they possess potential chances to reduce colon cancer risk in the human body (Topping and Clifton, 2001; Wollowski et al., 2001) increases gastrointestinal absorption of magnesium and calcium ions in the body (Bornet et al., 2002; Roberfroid, 2000) lipid regulation , serves as antioxidants agents (Bornet et al., 2002; Van Den Ende et al., 2011) immunomodulatory effects (Grethel et al., 2011) relief constipation and suppress diarrhea (Younis et al., 2015) and have potentials to suppress the growth of some pathogenic bacteria in the gastrointestinal tract (Topping et al., 2001; Van Den Ende et al., 2011).

The most important proposed mechanism of action of prebiotics is the fermentation in the colon and enhanced modification in the gut bacterial microflora (Gibson et al., 2004; Slavin, 2013) . It was estimated that about 400 bacterial species resides in the human intestinal microbiota, but the colonic environment is more suitable for bacterial growth due to its slow up time, nutrients availability, and convenient pH (Rycroft et al., 2001). The main gut microbiota members are *Bifidobacteria and Lactobacilli* (Gerritsen et al., 2011). It was established that *Bifidobacteria* constitute about 25% of the total bacteria number present in the gastrointestinal tract (Rodrı´guez et al., 2015), serving as beneficial to the host thereby promoting the health of the individual (Suavarna and Boby, 2005; Wang and Weller, 2006).

Short chain carbohydrates that have potentials for prebiotics applications are widely extracted from different plants and animals sources (de Jesus Raposo et al., 2016). In recent years, different prebiotics gained pharmaceutical relevance in the market such as fructo-oligosaccharides, inulin, galacto-oligosaccharides, polydextrose and lactulose, (Mitchell and Stowell, 2007). Other emerging prebiotics are isomalto-oligosaccharides, xylo-oligosaccharides and lactitol (Patel and Goyal, 2012). Recently, prebiotic properties have been found in some carbohydrates such as resistance starch, disaccharides, oligosaccharides and polysaccharides. Polysaccharides (long chain complex carbohydrate) from plants such as cereal, sweat potato, mushrooms, chicory roots and soybean have gained attention as novel source prebiotics (Al-Sheraji et al., 2013). Non-digestible polysaccharides are carbohydrates that are resistance to digestion in the upper gastro intestinal tract (GIT). Thus arriving the colon intact, where its serves as foods for the intestinal microbiota. Highly soluble non-digestible polysaccharides have been reported to confers more beneficial effects by increasing the population of probiotics bacterial as compared to fructo-oligosaccharides and inulin (commercial prebiotics) (Azmi et al., 2012; Wichienchot et al., 2010).

Polysaccharides extraction from natural sources is usually affected by the extraction techniques used. Other factors which greatly affect polysaccharides yield and structural behaviors are extracting solvent, temperature, ratio of solvent to water and time (Chen et al., 2008; Huang et al., 2010; Yuliarti et al., 2008). Effects of solvents in particular have been reported to have significant effects on extraction yield and chemical composition of polysaccharides (Gannasin et al., 2015). The most commonly used extracting solvents are water, acid and alkaline and enzymes. Therefore, this research focused on investigating the effect of extracting solvents (water, citric acid and NaOH) on yield and biochemical characteristic of PKC and CKC soluble polysaccharides. Furthermore, the prebiotic potential of PKC and CKC soluble polysaccharides under artificial human gastric juice, alpha amylase and proliferation effects on some *Lactobacillus* species were studied.

1.2 Statement of problems

(i) Extraction of prebiotics from plants sources are usually associated with low yield, therefore modification of the conventional extraction methods could increase the yield and enhanced the biochemical composition of the prebiotics.
(ii) The commercially available prebiotics obtained in Malaysia are imported, therefore this research focus on extracting prebiotics from locally available plants materials (palm kernel cake and coconut kernel cake) which are economically cheap and available.

1.3 Research Hypothesis

(i) It was hypothesized that extracting solvents may exert effects on the yield and structural characteristics of polysaccharides from palm kernel cake and coconut kernel cake.
(ii) Soluble polysaccharides from palm kernel cake and coconut kernel cake could have potential to be used as a source of prebiotics.

1.4 Aims and Objectives

The general aim of this research is to extract and characterized soluble non-digestible polysaccharides from PKC and CKC that could have potential prebiotic properties.

The specific objectives are:

(i) To extract and characterize soluble polysaccharides from palm kernel cake and coconut kernel cake using three different solvents.
(ii) To determine the non-digestibility of the extracted soluble polysaccharides on artificial human gastric juice and alpha amylase and,
(iii) To evaluate prebiotics potential of the soluble polysaccharides on *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus rhamnosus* ATCC 53103 *in-vitro*.

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