PHYSICOCHEMICAL AND PHYSIOLOGICAL PROPERTIES OF PITAYA [*HYLOCEREUS POLYRHIZUS* (WEBER) BRITTON & ROSE] PEEL PECTIN

By

MAJIDA FADHIL AYFAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

I would like to dedicate this work to:

My beloved father and mother who prayed for my success.

My husband (Ali) and kids (Laya and Maryam) for being my inspiration.

My brothers and sisters for their constant encouragement and support.

My friends and colleagues.

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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Chairperson : Professor Sharifah Kharidah Syed Muhammad, PhD
Faculty : Food Science and Technology

Physicochemical and technological properties, and the cholesterol-lowering potential of high methoxyl pectin extracted from pitaya peel (PP) using citric acid, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) techniques have been reported. However, enzyme-assisted citric acid extraction and its effect on the yield, physicochemical, technological, *in vitro* bile acid binding and hypoglycemic properties, and *in vivo* cholesterolemic and glycemic properties of low and high methoxyl pitaya peel pectin (PPP) have not been investigated. In this study, pectin was extracted from PP using distilled water (DW) and different concentrations (0.5,1.0, 1.5, 2.0 %) of citric acid (CA) with the aid of cellulase (C) enzyme to obtain PPP fractions with different degree of esterification (DE). Pectin yield was determined and physicochemical properties such as proximate composition, DE, galacturonic acid (GalA) content, molecular weight (Mw) and monosaccharide profile of the PPP fractions were then analysed. In addition, technological properties such as water-holding capacity (WHC), oil-holding capacity (OHC), emulsifying activity (EA), emulsion stability (ES) and viscosity of the PPP fractions were also determined. The glycaemic properties of the different PPP fractions were measured using three different in vitro methods: glucose adsorption capacity, glucose diffusion, and amylolysis kinetics. Meanwhile, *in vitro* bile acid-binding capacities (BABC) of the PPP fractions were quantified using high pressure liquid chromatography (HPLC). Low methoxyl and high methoxyl PPP fractions that resulted from cellulase assisted distilled water (CDW) and cellulase assisted 1.5% citric acid (C1.5CA) extractions, respectively, were used for *in vivo* hypoglycemic and hypocholesterolemic activities evaluation using the Sprague Dawley rats. The PPP fraction extracted with C1.5CA had the highest yield (24.63%), GalA content (62.39%), DE (50.20%), WHC (7.53 g water/g dry sample), OHC (2.17 g oil/g dry sample), EA (100%), and ES (100%). Higher viscosity and in vitro normalised BABC were observed for 2% PPP extracted with C1.5CA. Interestingly, the same fraction (PPP extracted with C1.5CA) exhibited the highest glucose diffusion retardation index of 31.24 after 120 min.

The *in vivo* results were consistent with the *in vitro* findings where PPP extracted with C1.5CA demonstrated good hypoglycemic and hypocholesterolemic activities. In conclusion, cellulase assisted 1.5% citric acid (C1.5CA) extraction of pectin from PP resulted in an array of new and improved physicochemical and physiological properties compared to that of commercial pectins and pectin from PP as reported previously. Moreover, PPP extracted with C1.5CA exhibited potent antidiabetic properties. Thus, it could be a suitable alternative food ingredient for the management of Type 2 diabetes mellitus.

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

SIFAT FIZIKOKIMIA DAN FISIOLOGI PEKTIN DARI KULIT PITAYA [*HYLOCEREUS POLYRHIZUS* (WEBER) BRITTON & ROSE]

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Sifat fizikokimia dan teknologi serta potensi penurun kolesterol pektin metoksil tinggi yang diekstrak daripada kulit pitaya (PP) menggunakan asid sitrik, pengekstrakan berbantukan gelombang mikro (MAE) dan teknik pengekstrakan berbantukan ultrasound (UAE) telah dilaporkan. Walau bagaimanapun, pengekstrakan asid sitrik berbantukan enzim dan kesannya terhadap hasil dan sifat fizikokimia, teknologi, pengikatan asid hempedu dan hipoglikemik in vitro serta sifat kolesterolemik dan glisemik in vivo pektin kulit pitaya (PPP) metoksil rendah dan tinggi belum disiasat. Dalam kajian ini, pektin diekstrak dari PP menggunakan air suling (DW) dan kepekatan asid sitrik (CA) yang berbeza (0.5,1.0, 1.5, 2.0%) dengan bantuan enzim selulase (C) untuk memperoleh pecahan PPP dengan tahap esterifikasi (DE) yang berbeza. Hasil pektin ditentukan dan sifat fizikokimia seperti komposisi proksimat, DE, kandungan asid galakturonik (GalA), jisim molekular (Mw) dan profil monosakarida pecahan PPP kemudian dianalisis. Selain itu, sifat teknologikal seperti kapasiti pemerangkapan air (WHC), kapasiti pemerangkapan minyak (OHC), aktiviti pengemulsian (EA), kestabilan emulsi (ES) dan kelikatan pecahan PPP juga ditentukan. Sifat hipoglisemik pecahan PPP yang berbeza ditentukan menggunakan tiga kaedah in vitro yang berbeza iaitu kapasiti penjerapan glukosa, penyebaran glukosa, dan kinetik amilolisis. Sementara itu, kapasiti pengikatan asid hempedu (BABC) in vitro oleh pecahan PPP dikuantifikasi menggunakan teknik kromatografi cecair tekanan tinggi (HPLC). Pecahan PPP bermetoksil rendah dan bermetoksil tinggi yang masing-masing merupakan hasil daripada kaedah pengekstrakan air suling berbantukan selulase (CDW) dan pengekstrakan 1.5% asid sitrik berbantukan selulase (C1.5CA), digunakan untuk penilaian aktiviti hipoglisemik dan hipokolesterolemik in vivo menggunakan tikus Sprague Dawley. Pecahan PPP yang diekstrak dengan C1.5% CA mempunyai jumlah tertinggi bagi hasil pengestrakan (24.63%), kandungan GalA (62.39%), DE (50.20%), WHC (7.53 g air / g sampel kering), OHC (2.17 g minyak / g sampel kering), EA (100%), dan ES (100%). Kelikatan dan BABC dinormalkan in vitro yang lebih tinggi diperhatikan untuk 2% PPP

yang diekstrak dengan C1.5CA. Bukan itu sahaja, pecahan yang sama (PPP yang diekstrak dengan C1.5CA) juga menunjukkan indeks perencatan resapan glukosa tertinggi pada 31.24 selepas 120 minit. Hasil kajian in vivo didapati selaras dengan penemuan in vitro di mana PPP yang diekstrak dengan C1.5CA menunjukkan aktiviti hipoglisemik dan hipokolesterolemik yang baik. Sebagai kesimpulannya, pengekstrakan pektin dari kulit pitaya menggunakan asid sitrik 1.5% dengan bantuan enzim selulase (C1.5CA) menghasilkan pelbagai sifat fizikokimia dan fisiologi yang baru dan lebih baik berbanding sifat pektin komersial dan pektin dari kulit pitaya seperti yang dilaporkan sebelum ini. Selain itu, PPP yang diekstrak dengan C1.5CA mempamerkan sifat antidiabetik yang kuat. Dengan itu ia boleh menjadi ramuan makanan alternatif yang sesuai untuk pengurusan diabetes mellitus jenis 2.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BA	Bile acids
BABC	Bile acid binding capacity
C	Cellulase
C_2H_3N	Acetonitrile
$CHCl_3$	Chloroform
C_3H_6O	Acetone
C_6H_{14}	Hexane
℃	Celsius
CA	Citric acid
CF_3CO_2H	Trifluoroacetic acid
cm	Centimeters
cm^{-1}	Reciprocal centimeter
CVD	Cardiovascular disease
Da	Dalton
DAc	Degree of acetylation
DE	Degree of esterification
DF	Dietary fibre
DM	Degree of methylation
DM	Dry matter
dn/dc	Refractive index increment
DG	diabetic group
DW	Distilled water

EA	Emulsifying activity
ES	Emulsion stability
EU	European union
FT-IR	Fourier transform infrared
G1	Glucose concentration of the original solution
G6	Glucose concentration after 6 h
G	Gram
GAC	Glucose adsorption capacity
g/L	Gram per liter
g/mol	Gram per mol
Gal A	Galacturonic acid
GDRI	Glucose diffusion retardation index
h	Hour
HCl	Hydrochloride acid
HDLC	High-density lipoprotein cholesterol
HFD	High fat diet
HMP	High methoxyl pectin
HMPG	High methoxyl pectin group
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
H_2SO_4	Sulphuric acid
IDF	Insoluble dietary fibre
IPPA	International pectin producers' association

KH_2PO_4	Potassium dihydrogen phosphate
LDL	Low-density lipoprotein
LDLC	Low-density lipoprotein cholesterol
LMP	Low methoxyl pectin
LMPG	Low methoxyl pectin group
LS	Laser light scattering
M	Mol
MAE	Microwave assisted extraction
MC	Moisture content
MeOH	Methanol
MG	Metformin group
mg/dL	Milligrams per deciliter
mmol	Millimole
mmol/L	Millimole per liter
Mn	Number average molecular weight
mm	Millimeter
mg/mL	Milligram per milliliter
min	Minute
mL	Milliliter
mL/g	Milliliter per gram
mL/kg	Milliliter per kilogram
m Pas	Millipascal-second
µL	Microliter
µ/g	Micro per gram

µg/ µL	Microgram per microliter
Mw	Molecular weight
N	Normality (number of equivalents per liter of solution)
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
Na_2HPO_4	Sodium hydrogen phosphate
NaH_2PO_4	Sodium dihydrogen phosphate
NaN_3	Sodium azide
$NaNO_3$	Sodium nitrate
ND	Normal diet
NG	Normal diet group
nm	Nanometer
NaOH	Sodium hydroxide
OHC	Oil holding capacity
pH	Power of hydrogen
PMP	1-phenyl-3-methyl-5-pyrazolone
PP	Pitaya peel
PPP	Pitaya peel pectin
RG	Radius of gyration
RI	Refractive index
rpm	Rotation per minute
s	Seconds
SDF	Soluble dietary fibre
SCFA	Short chain fatty acids

STZ	Streptozotocin
t	Time
UAE	Ultrasound assisted extraction
TC	Total cholesterol
T2D	Type 2 diabetes
TDF	Total dietary fibre
UV	Ultraviolet
VS	Viscometer detector
v/v	Volume per volume
WK1-8	Week 1-8
WHC	Water holding capacity
WHO	World health organization
WIF	Water insoluble fibre
WSF	Water soluble fibre
w: v	Weight to volume
w/v	Weight per volume
Y_d (%)	Percentage of yield on dry matter
ηw	Intrinsic viscosity

CHAPTER 1

INTRODUCTION

Dragon fruit (*Hylocereus polyrhizus*) or red pitaya, a member of Cactaceae, is widely grown in many Asian countries including Malaysia (Muhammad et al., 2014). The fruit pulp is currently exploited for commercial production of pitaya powder as natural red-violet colourant which is rich in betanin (Luu et al., 2021; Ramli et al., 2014; Sonawane, 2017) and development of pitaya beverage and confectionery (Sonawane, 2017). This indirectly results in the accumulation of pitaya peel (PP) as waste material, which may pose as an environmental issue. In turn, PP that consists of high content of dietary fibre (Bakar et al., 2011) can be a promising source of pectin. Muhammad et al. (2014) reported that 26.4% of pitaya peel pectin (PPP) was extracted using 1% of citric acid at the optimum conditions of pH 2 and 70℃ for 70 min.

Pectin is a family of galacturonic acid-rich polysaccharides that contain homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, and xylogalacturonan (Mohnen, 2008). It is usually found in the primary cell walls and middle lamellae of higher plants. These polysaccharides consist of 300-1,000 chains of galacturonic acid units (Yeoh et al., 2008). Pectin is divided into two major groups depending on the degree of esterification (DE): pectin with DE > 50% is known as high methoxyl pectin (HMP) whereas low methoxyl pectin (LMP) has a DE < 50% (Morris et al., 2000). Pectin is widely used in the food, pharmaceutical, cosmetic and polymer industries (Loh, 2016). Specifically, in the food industry, pectin is used as a gelling agent, thickener, texturiser, emulsifier, and stabiliser. Commercial HMPs are extracted from apple pomace and citrus peel using acid extraction method with pectin yield of about 12% and 25%, respectively (Thakur et al., 1997). Meanwhile, commercial LMPs with yield of about 10% to 20% are extracted from sugar beet and sunflower head residues (Miyamoto & Chang, 1992). Many other plant sources have been studied for pectin extraction of higher yield and distinct quality as reviewed by Dranca and Oroian (2018). The composition of pectins varies depending on the plant source and extraction methods and conditions (Sriamornsak, 2007) which in turn affects the pectin properties.

Physicochemical properties of pectin such as monosaccharide composition, molecular weight, viscosity and degree of esterification were reported to influence technological (water-holding capacity, oil-holding capacity, emulsifying activity and emulsion stability) and physiological characteristics of pectin (Belkheiri et al., 2021). Pectin has been shown to reduce blood glucose, total cholesterol and low-density lipoprotein cholesterol (LDLC) levels which is beneficial for human health (Liu et al., 2006). HMP has been shown to reduce the cholesterol in egg yolk and animals (*in vivo*) (Zaid et al., 2019). Viscosity of pectin was reported to influence the hypocholesterolemic and hypoglycemic properties of pectin (Kumar et al., 2012; Logan et al., 2015). One of the main mechanisms for cholesterol-lowering effect is the binding of bile acids in the small intestine by dietary fibre and the excretion of the bound bile acids through faeces (Gunness & Gidley, 2010).

Mild extraction agents (like water and citric acid) and unconventional extraction methods (like microwave- and ultrasound-assisted extraction) have been researched in an effort to create environmentally friendly processes to extract PPP and its physicochemical and technological properties characterized as reported by Ismail et al. (2012), Muhammad et al. (2014), Rahmati et al. (2019), Tang et al. (2011), Thirugnanasambandham et al. (2014), Tongkham et al. (2017), Woo et al. (2010), Zaid et al. (2019a), Zaid et al. (2016), Zaid et al. (2019b), Zaidel et al. (2017). The use of enzyme as a more specific and efficient extraction agent which can deconstruct the plant cell wall and isolating the whole pectin molecule have not been studied. Therefore, in the present study, enzyme-assisted citric acid extraction and its effect on the yield, physicochemical, technological, *in vitro* bile acid binding and hypoglycemic properties, and *in vivo* cholesterolemic and glycemic properties of low and high methoxyl PPP were investigated.

The specific objectives of this research include the followings:

1. To optimise the conditions for cellulase assisted-water and cellulase assisted-citric acid extractions of pectin from pitaya peel.

2. To analyse the physicochemical and technological characteristics of various high methoxyl (HMP) and low methoxyl (LMP) pectins extracted from pitaya peel.

3. To evaluate the *in vitro* hypocholesterolemic and hypoglycemic properties of HPM and LMP from pitaya peel.

4. To assess the *in vivo* hypocholesterolemic and hypoglycemic properties of HMP and LMP from pitaya peel.

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