STUDY ON THE EXTRACTION OF HIGH METHOXYL PECTIN FROM DRAGON FRUIT (*Hylocereus polyrhizus*) PEELS AND ITS ADSORPTION PERFORMANCE AGAINTS CHOLESTEROL

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SUPERVISOR'S DECLARATION

We hereby declare that We have checked this thesis, and, in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Pektin adalah serat makanan terlarut bernilai tinggi yang memberi manfaat kepada kesihatan. Keperluan untuk mengekstrak pektin secara maksimum dengan ciri-ciri tertentu adalah cabaran utama. Ciri pektin adalah kata kunci untuk aplikasinya. Tujuan kajian ini adalah untuk mengkaji pengekstrakan polisakarida hidrofobik yang dikenali sebagai pektin bermethoxyl tinggi yang diekstrak dari *Hylocereus polyrhizus* peels yang berpotensi sebagai agen hipolipidemik melalui eksperimen penyerapan vitro pada model molekul (kolesterol). Dalam kajian ini, gabungan rawatan fizikal dan kimia telah digunakan didalam proses pengekstrakan. Ultrasonik ringan oleh mandian sonicator dan pengadukan oleh kipas dengan asid sitrik sebagai pelarut pengekstrakan digunakan dalam proses pengekstrakan untuk mencapai hasil tinggi pektin metoksil tinggi. Satu kajian mengenai parameter-parameter pengekstrakan (kadar agitasi, suhu, masa, pH dan nisbah pepejal cecair (LSR)) dilakukan oleh one-factor-at-one-time (OFAT) bagi membantu pemilihan julat yang bersesuaian untuk proses pengoptimalan seterusnya. Analisi kinetik bagi proses ekstraksi telah dilakukan selepas kajian OFAT bagi memberikan idea untuk proses meramal dan pengoptimuman selanjutnya terhadap hasil pektin. Analisis kinetik oleh model Panchev menunjukkan bahawa kadar pengekstrakan didapati paling tinggi pada LSR 10 v/w dengan nilai y_{max} 30.85%. Energi pengaktifan yang diperoleh untuk ekstraksi dan degradasi pektin adalah masing-masing 4.532 kJ / mol dan 28.054 kJ / mol. Pemeriksaan awal menggunakan 2^5 reka bentuk faktorial penuh menunjukkan bahawa suhu, masa, pH dan LSR adalah faktor penting dalam memperoleh hasil yang tinggi bagi pektin methoxyl tinggi. Semua faktor penting kemudian dioptimumkan menggunakan metodologi permukaan tindak balas (RSM) untuk mencapai hasil yang tinggi bagi pektin methoxyl tinggi. Nilai hasil pektin dan darjah esterifikasi (DE) selepas pengoptimuman adalah masing-masing 30.11% dan 55.00% berbanding dengan hasil pektin dan DE sebelum pengptimuman iaitu masing-masing 21.52% dan 56.1%. Pectin bermethoxyl tinggi yang diekstrak dari *Hylocereus polyrhizus* peels (HPP) telah diuji sebagai agen yang berpotensi menurunankan kolesterol serta dibanding dengan pektin bermethoxyl tinggi dari citrus (CP). Kemuadianya, penyerapan kolesterol diukur secara kuantitatif oleh kromatografi cecair prestasi tinggi (HPLC). Didapati bahawa pektin bermethoxyl tinggi dari *Hylocereus polyrhizus* peels mempunyai kecendurangan menyerap kolesterol yang mematuhi model isotherm Freundlich dan model pseudo-first order. Sifat hidrofobisiti dan struktur permukaan HPP dianalisis menggunakan sudut sentuhan dan mikroskop elektron pengimbasan pelepasan medan (FESEM) untuk memahami permukaan HMPHPP yang membantu proses penjerapan. Kajian ini membuktikan bahawa pektin bermethoxyl tinggi yang diekstrak dari kulit *Hylocereus polyrhizus* mempunyai pertalian terhadap kolesterol yang membantu penjerapan kolesterol.

ABSTRACT

Pectin is a high value soluble dietary fiber which is beneficial to health. Requirement to manufacture the high yield of pectin with certain characteristic is a major challenge. Pectin characteristic is the keywords to its application. The purpose of this study is to investigate the extraction of hydrophobic polysaccharide which is known as high methoxyl pectin extracted from *Hylocereus polyrhizus* peels as potential hypolipidemic agents by in vitro sorption experiments on model molecules (cholesterol). In this study, combination of physical and chemical treatment has been applied in the extraction process. Mild ultrasonic by sonicator bath and agitation by propeller with citric acid as an extraction solvent was employed in the extraction process to achieve the high yield of high methoxyl pectin. A study on the extraction parameters (agitation rate, temperature, time, pH and liquid-solid ratio (LSR)) was performed by one-factor-at-a-time (OFAT) to facilitate the appropriate range for further optimization process. The extraction process kinetics has been done after OFAT study which would give the idea for process forecasting and optimization of the pectin yield. The kinetic analysis by Panchev's model shows that the extraction rate was found highest at LSR 10 v/w with y_{max} 30.85 %. The calculated activation energy for pectin dissolution and degradation was found to be 4.532 kJ/mol and 28.054 kJ/mol, respectively. The initial screening using 2^5 full factorial design indicated that temperature, time, pH and LSR were significant factors in the extraction of high yield of high methoxyl pectin. All the significant factors were then optimized using response surface methodology (RSM) to achieve the high yield of high methoxyl pectin. The value of pectin yield and degree of esterification (DE) after optimization were 30.11 % and 55.00 %, respectively as compared to the initial pectin yield and DE of 21.52 % and 56.1 %, respectively. High methoxyl pectin extracted from *Hylocereus polyrhizus* peels (HMPHPP) were tested as potential cholesterol-lowering agent and compared with the commercial high methoxyl citrus pectin (HMPCP). The cholesterol sorption was measured quantitatively by high performance liquid chromatography (HPLC). It was found that high methoxyl pectin from *Hylocereus polyrhizus* peels has the affinity towards cholesterol which followed the Freundlich isotherm and pseudo-first order model. Hydrophobicity and surface structure of the HPP was characterized by contact angle analysis and field emission scanning electron microscopy (FESEM) in order understand HMPHPP surface that help the adsorption process. This study proves that high methoxyl pectin extracted from *Hylocereus polyrhizus* peels has affinity towards cholesterol which help the adsorption of cholesterol.

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LIST OF SYMBOLS

mg	milligram
g	Gram
kg	Kilogram
mL	miliLitre
L	Litre
^{o}C	Degree Celsius
mg/g	Milligram per gram
mg/L	Milligram per litre
g/L	gram per litre
mL/min	Mililitre per minute
mg/min	milligram per minute
kJ/mol	Kilo joule per mol
v/w	Volume per weight
k_1	Rate constant of pectin dissolution/rate constant of pseudo-first order adsorption (min^{-1})
k_2	Rate constant of pectin degradation/rate constant of pseudo-first order adsorption (min^{-1})
z(t)	Quantity of protopectin in the plant tissue (%)
y(t)	Quantity of dissolved pectin (%)
q(t)	Quantity of degraded pectin (%)
p(t)	Total dissolved pectin can be obtained (%)
P_E	Percentage of the extractable pectin to the raw material (%)
Ry(t)	Reaction rate of pectin dissolution (%/min)
Rq(t)	Reaction rate of pectin degradation (%/min)
Rp(t)	Reaction rate of total pectin obtained (%/min)

t	time (min)
Ea	Activation energy (kJ/mol)
R	Gas constant (8.314 J/mol.K)
T	Temperature (^{o}C)
$\Delta H^{\#}$	Enthalpy of activation (kJ/mol)
$\Delta S^{\#}$	Entropy of activation (J/mol)
ΔH^{o}	Enthalpy change (kJ/mol)
ΔS^{o}	Entropy change (J/mol)
k_b	Boltzamann's constant (1.38064852 $m^2kgs^{-2}K^{-1}$)
h	Plank's constant (6.62607004 x 10^{-34} m^2kg/s)
q	Adsorption capacity of the Langmuir isotherm (mg/g)
k_L	Langmuir constant (l/g)
K_f	Adsorption capacity of Freundlich isotherm (mg/g)
q_{LF}	Adsorption capacity of Sips isotherm (mg/g)
k_{LF}	Sips constant (l/g)
R^2	Coefficient of determination

LIST OF ABBREVIATION

HMPHPP	High methoxyl pectin extracted from *Hylocereus polyrhozus* peels
HMPCP	High methoxyl of standard citrus pectin commercial
DE	Degree of esterification
HPP	*Hylocereus polyrhizus* peels
HMP	High methoxyl pectin
LMP	Low methoxyl pectin
GalA	Galacturonic acid
PMP	1-phenyl-3-methyl-5-pyrazolone
LSR	Liquid to solid ratio
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infra-Red
OFAT	One Factor at a Time
RSM	Response surface methodology
FFD	Full factorial design
CCD	Centered composite design
ANOVA	Analysis of Variance
SSE	Sum of the square error
RMSE	Residual root mean square error

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