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By

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ACIDIC AND ENZYMETIC EXTRACTION OF PECTIN FROM PEELS OF SOURSOP, PASSION FRUIT AND DRAGON FRUIT

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Chairperson : Associate Professor Ir. Chin Nyuk Ling, PhD

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Pectin has been intensively used as natural gelling agent and stabilizer to alter the rheological properties of food ingredients in most food processing industries today in order to achieve desired textural quality. Three tropical fruit peels, the yellow passion fruit, red dragon fruit, and soursop were selected for optimising the pectin extraction using citric acid by varying pH from 2.0 to 4.5 and extraction time from 30 to 120 minutes at 70 xC.

The extracted pectin was measured in term of pectin yield and the degree of esterification (DE). Peel of yellow passion fruit gave the highest pectin yield of 14.60% and DE of 67.31%, followed by the red dragon fruit with pectin yield of 13.04% and DE of 57.81% and lastly the soursop with 5.87% and 47.65% respectively. The optimised pectin yield and DE obtained from the response surface methodology using the central composite design were within 13.40 ± 0.8% and 51.71 ± 3.83% at extraction pH of 2.37 and extraction time of about 61 ± 3 minutes for both the yellow passion fruit and dragon fruit. The pH of extraction solvent had more significant effect on pectin yield ($p < 0.05$) with high pectin yield produced at pH 2.0.

With high amount of pectin and DE obtained from the yellow passion fruit peels, further pectin extraction method using enzyme was studied as to compare with the acidic extraction method. The celluclast® 1.5L enzyme was used and concentration was varied from 0.49-2.84% (w/w). The extraction temperature studied was from 35 to 85 xC and extraction time was from 30 to 120 minutes. The extracted pectin yield and DE were compared with those extracted using citric acid concentration from 0.05-0.21% (w/w). The enzymatic extraction gave higher pectin yield of 9.17% with DE of 86.96% compared to acidic extraction at 7.71% with 78.57% respectively. The acidic and enzymatic extraction methods of pectin from yellow passion fruit peels gave similar optimised yields of about 7.14 ± 0.02%. However, optimised DE of the enzymatic extraction method was higher at 85.45% compared to the acidic extraction method at 71.02%. The optimised enzyme concentration was 1.67% (w/w) at 102 minutes at 61.11 xC. Pectin yield was most significantly affected by extraction time

(p<0.05) while DE was most affected by enzyme concentration (p<0.05). Similarly, for acidic extraction, citric acid concentration had most significant effect on DE (p<0.5) while temperature had more significant effect on pectin yield (p<0.05). The optimised citric acid concentration was 0.19% (w/w) at 75 ◦C for 102 minutes.

Morphology of pectin obtained using the scanning electron microscopy (SEM) suggested that the wet passion fruit pectin obtained from the acid extraction gave a smooth, compact and little wrinkle structure while the enzymatic extraction was with more wrinkle. The pectin obtained from acidic and enyzmatic extraction respectively WRWHVWIRULW¶VDELOLWWRIRUPJHOEDGGLQJZDWHUFDOFLXP ing water, calcium chloride, sugar. Combination of water, sugar and calcium gave the highest pectin gel viscosity with sugar giving much influence on the viscosity of pectin gel. The pectin gels exhibited shear thinning or pseudoplastic behavior with n<1.

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

PENGEKSTRAKAN PEKTIN DARIPADA BUAH KULIT DURIAN BELANDA, BUAH NAGA MERAH DAN MARKISA KUNING DENGAN MENGGUNAKAN ASID DAN ENZIM

Oleh

LIEW SHAN QIN

Januari 2015

Pengerusi : Prof. Madya Ir. Chin Nyuk Ling, PhD

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Pektin digunakan secara intensif sebagai agen penggelan semula jadi dan penstabil untuk mengubah sifat-sifat reologi bahan-bahan makanan di kebanyakan industri pemprosesan makanan hari ini untuk mencapai kualiti tekstur dikehendaki. Tiga jenis kulit buah-buahan tropika, iaitu buah markisa kuning, buah naga merah, dan durian belanda telah dipilih untuk pengekstrakan dioptimumkan pektin menggunakan pengekstrakan asid sitrik dengan mengubah pH 2.0 hingga 4.5 dan pengekstrakan masa 30 hingga 120 minit pada 70 °C.

Pektin yang diekstrak diukur dari segi hasil pektin dan tahap pengesteran (DE). Kulit buah-buahan markisa kuning memberikan hasil pektin tertinggi 14.60% dan DE 67.31%, diikuti dengan buah naga merah dengan hasil pektin 13.04% dan DE 57.81% dan akhir sekali durian belanda dengan masing-masing 5.87% dan 47.65%. Keadaan optimum untuk hasil pektin dan DE diperolehi daripada kaedah gerak balas permukaan menggunakan reka bentuk komposit pusat ialah 13.40 ± 0.8% dan 51.71 ± 3.83% pada pH pengekstrakan 2.37 dan masa pengekstrakan 61 ± 3 minit untuk buah markisa kuning dan buah naga merah. pH pengekstrakan pelarut mempunyai kesan yang lebih ketara ke atas hasil pektin ($p<0.05$) dan pH 2.0 memberikan hasil pektin yang tinggi.

Dengan jumlah yang tinggi dari segi hasil pektin dan DE diperolehi daripada kulit buah markisa kuning, kaedah pengekstrakan pektin dengan meggunakan enzim telah dikaji untuk membandingkan dengan kaedah pengekstrakan yang berasid. Celluclast® 1.5L enzim digunakan dan diubah kepekatannya 0.49-2.84% (w/w). Suhu pengekstrakan dikaji adalah 35-85 °C dan masa pengekstrakan adalah antara 30 hingga 120 minit. Hasil pektin dan DE yang diperoleh daripada pengekstakan enzim dibandingkan dengan kaedah pengekstrakan berasid dengan menggunakan asid sitrik kepekatan 0.05-0.21% (w/w). Pengekstrakan enzim memberikan hasil pektin lebih tinggi 9.17% dengan DE sebanyak 86.96% berbanding dengan pengekstrakan berasid pada 7.71% dengan 78.57% masing-masing. Kaedah pengekstrakan berasid dan

enzim memberikan hasil optimum pektin yang hampir sama kira-kira 7.14 ± 0.02%. Walau bagaimanapun, kaedah pengekstrakan enzim memberikan DE yang lebih tinggi pada 85.45% berbanding kaedah pengekstrakan berasid di 71.02%. Keadaan pengoptimuman pektin pengekstakan enzim adalah kepekatan enzim 1.67% (w/w) di 102 minit pada 61.11 xC. Masa pengekstrakan ($p<0.05$) memberikan kesan yang paling ketara kepada hasil pektin manakala kepekatan enzim lebih ketara memberi kesan kepada DE ($p<0.05$). Bagi pengekstrakan berasid, kepekatan asid sitrik memberi kesan ketara ke atas DE ($p<0.5$) manakala suhu lebih ketara memberi kesan kepada hasil pektin ($p<0.05$). Keadaan pengoptimuman pektin pengekstakan acid adalah kepekatan acid 0.19% (w/w) di 102 minit pada 75 xC.

Morfologi pektin diperolehi dengan menggunakan mikroskop imbasan elektron (SEM) mencadangkan bahawa nano struktur basah pektin buah markisa kuning yang diperolehi daripada pengekstrakan asid memberikan struktur kedut, licin dan padat manakala struktur pektin daripada pengekstrakan enzim terdapat lebih banyak kedut. Pektin yang diperolehi daripada pengekstrakan berasid dan enyzmatic digunakan untuk penggelan lanjut dan diuji dengan merangkak syarat bentuk gel dengan menambah air (kawalan), kalsium klorida dan gula. Kombinasi air, gula dan kalsium memberikan kelikatan yang tertinggi dan gula memberi pengaruh lebih ketara pada kelikatan gel pektin. Gel pektin memberikan kelakuan pseudoplastik dengan $n < 1$.

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I certify that a Thesis Examination Committee has met on 29 January 2015 to conduct the final examination of Liew Shan Qin on his thesis entitled "Acidic and Enzymetic Extraction of Pectin from Peels of Soursop, Passion Fruit and Dragon Fruit" 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 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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LIST OF SYMBOLS / ABBREVIATIONS

AIS	Alcohol insoluble solid
ANOVA	Analysis of variance
BBD	Box-behnken design
CCD	Central composite design
CPE	Combined physical enzymatic
DE	Degree of esterification
GalA	Galacturonic acid
Gal	Galactose
HCl	Hydrochloric acid
HM	High methoxyl
LM	Low methoxyl
MAE	Microwave-assisted extraction
NaOH	Sodium hydroxide
PE	Percentage error
Rha	Rhamnose
RSM	Response surface methodology
SI	System International
TPA	Texture profile analysis
UAE	Ultrasonic-assisted extraction
Da	Daltons
Ft	Final titration volume, mL
It	Initial titration volume, mL
M	Molarity
M_w	Molecular weight
N	Normality
2	Shear stress
È	Shear rate
k	Consistency index
n	Power law index
m_{exp}	Experimental value
m_{pre}	Predicted value
rpm	Revolution per minute
w/w	Weight per weight

CHAPTER 1

INTRODUCTION

1.1 Background

Malaysia is a fruit enthusiasts' paradise. The tropical climate creates a luxuriant plant life and produces a wide and remarkable diversity of edible and succulent fruit. The fruit industry in Malaysia has also shown a steady increase in production. In 1990, the total fruit production in Peninsular Malaysia was about 1.05 million tons, while in 1994 the total production increased to about 1.47 million tons (Sarif *et al.*, 1996).

Processing of fruits produces solid residue wastes of peel, seeds, stones. In some fruits, the percent of discarded portions could be very high, for example, 20% from banana, 30-50% from mango and orange, and 40-50% from pineapple (Yeoh *et al.*, 2008). The increase of these fruit wastes is a challenge for waste management. Proper management of fruit wastes is necessary because the maintenance of clean environment is vital in fruit industries. The wastes from food industries are normally disposed as organic fertilizer or as animal feed. Recovery of bioactive compound or useful material from the fruit waste is an alternative method to minimize fruit wastes. Thus, pectin extraction from fruit peels is one method to reduce and fully utilize fruits waste.

Pectin is a carbohydrate polymer which contains at least 65% of galacturonic acid units where the acid groups may either be free or in the form of a simple salt, i.e. sodium, potassium, calcium or ammonium or naturally esterified with methanol (Espitia *et al.*, 2014; Kertesz, 1951) contained in the primary cell walls of terrestrial plants. Pectin has been intensively used as natural gelling agent and stabilizer to alter the rheological properties of food ingredients in most food processing industries today in order to achieve desired textural quality. It is also being used as edible food packaging (Bierhalz *et al.*, 2012; Penhasi and Meidan, 2014) and film (Espitia *et al.*, 2014) due to its antimicrobial property, simple and cytocompatible gelling mechanism. Pectin also has been recently exploited for different biomedical applications including drug delivery, gene delivery, wound healing and tissue engineering (Munarin *et al.*, 2012).

With many ways of pectin usage and its health benefits, the world demand for pectin has increased and pectin production is urgently needed in the market (Yeoh *et al.*, 2008). Pectin production has been extensively studied and this included recovery from by-products from food processing industries such as fruit wastes.

Pectin has been discovered from various kinds of fruits. Yellow passion fruit (*Passiflora edulis var.* flavicarpa), red dragon fruit (*Hylocereus* polyrhizus) and soursop (*Annona* muricata *L.*) are consumed widely in Southeast Asia as juice. Besides the juicy pulp, pectin has been extracted from peels of passion fruit (Pinheiro *et al.*, 2008; Seixas *et al.*, 2014) and dragon fruit (Muhammad *et al.*, 2014; Tang *et al.*, 2011) in recent effort of waste recoveries.

Extraction is the most important processes in the pectin production. Pectin extracted in a hot diluted with strong mineral acid solution is the most commonly employed method. However, strong acids are corrosive, and may be a potential threat to health. Moreover, the liquid waste generated from the industrial processes might lead to a burden to the environment and a high cost might incur in treating the strong acidic waste (Lúcia *et al*., 2013). Most probably for this reason, extraction of pectin from fruit peel using weak organic acid such as citric acid has been intensively conducted in recent studies (Fuentes *et al*., 2014; Kulkarni *et al*., 2010; Pinheiro *et al*., 2008).

Besides the conventional acid extraction (Pinheiro *et al*., 2008), the wide applications of pectin in food and drug industries have also led to various explorations to obtain pectin from various methods and sources, such as using subcritical water from apple pomace and citrus peel (Wang *et al*., 2014), microwave-induced heating from passion fruit peels (Seixas *et al*., 2014), and high hydrostatic pressure (Guo *et al*., 2014) from pomelo peels. However, due to recent awareness on of food safety considerations and environment impact, enzymatic extraction has emerged as a popular method to obtain pectin. Pectin is extracted using various different enzymes from varying sources. The enzymes used to extract pectin from sources were cellulose and Ǔ-amylose from pumpkin (Cui and Chang, 2014), cellulose from butternut (Fissore *et al*., 2013), Viscozyme® L from *Yuza* (Lim *et al*., 2012) and apple pomace (Min *et al*., 2011), celluclast and alcalase from rapeseed cake (Jeong *et al*., 2014), Ǔ-amylose and neutrase from banana peel (Qiu *et al*., 2010).

Different pectin sources, extraction methods, and processing parameters have been observed affecting the pectin yields (Yeoh *et al.*, 2008; Bahherian *et al.*, 2011; Pranati *et al.*, 2011; Fissure *et al*., 2009). Therefore, it is important to investigate an optimum extraction factors and techniques to produce a good pectin yield. The pectin yield and DE are usually affected by extraction factors of pH, concentration, time and temperature.

1.2 Aims and Objectives

The general objectives of this research were to investigate the extraction process of pectin from tropical fruit peels of passion fruit, red dragon fruit and soursop.

The specific objectives are:

i. To optimise and compare pectin extracted from different sources, i.e. passion fruit peel, dragon fruit peel and passion fruit peel by varying pH and time to obtain highest pectin yield and DE using acidic extraction.
ii. To compare enzymatic extraction with acidic extraction from passion fruit peel by varying extractor concentration, time and temperature.
iii. To investigate the pectin viscosity and surface morphology.

1.3 Scope of Work and Thesis Outlines

This is a research of pectin extraction from fruit peels. Chapter 1 states the research background and problems statements which inspire hypotheses and objectives for pectin extraction. Chapter 2 is literature reviews on soursop, dragon fruit and passion fruits. Detail background, structure, types and application of pectin are also presented. Types of extraction method, factors that affect the extraction process, gelling mechanism of pectin, statistical analysis tool are summarized.

Chapter 3 describes the materials, methods, procedures, equipments, experimental designs and statistical analysis used in experimental works. The research work was performed in two parts. In the first part of this research, research covered on using citric acid extraction solvent on pectin yield from fruit peels of soursop, dragon fruit and passion fruit. The second part of the research was on the optimization and comparison of acidic and enzymatic pectin extraction of passion fruit peel. Viscosity analysis of pectin gel was also obtained.

Chapter 4 presents results optimization of pectin extraction from the three different fruit peels. Comparison and recommendation were made based on pectin yield and DE obtained.

Chapter 5 discusses results of the optimization of pectin from yellow passion fruit peel extracted using acidic and enzymatic extraction methods. Morphological investigation on extracted pectin gel using scanning electron microscopy (SEM) was presented.

Lastly, Chapter 6 summarizes the main findings and recommendations are suggested for future research.

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