EXTRACTION OF PECTIN FROM SOYBEAN HULLS USING PRESSURIZED INTERMITTENT MICROWAVE ASSISTED METHOD

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ABSTRACT

Thai soybean hulls (*Glycine max* (L.) Merr. cv. 'Chiang Mai 60') as processing biomass can be used as an inexpensive source of pectin and cellulose for the food industry. The objective of this research was to investigate the optimum conditions for extracting pectin from soybean hulls under pressurized intermittent microwave-assisted extraction (PIMAE) with several conditions including pH from 1.5 to 2.5, pulse ratio from 1.0 to 1.51 and extraction time from 5 to 15 minutes. Box-Behnken design for response surface methodology was utilized to study and optimize the effects of processing variables. Results showed that the optimum extraction condition was pH 1.5, pulse ratio of 1 and ~12 minutes of extraction time. Alcohol precipitations using ethanol (10.5% of pectin yield) or propan-2-ol (11.7% of pectin yield) had no significant effect on yield. Moisture content (~10%) and galacturonic acid content (~50-56%) of the pectin varied. Morphology of pectin from both ethanol and propan-2-ol precipitation gave amorphous shapes. The colour of pectin from ethanol precipitation was brown and from propan-2-ol precipitation white, which was significantly different ($p < 0.05$).

Pectin extract from soybean hulls as either flakes (SHF) or flakes ground into powder (SHP) gave highest pectin yield at pH 2, pulse ratio 1.0 and extraction time 15 min as 6.42% from SHF and pH 1.5, pulse ratio 1.0 and extraction time 10 min as 12.09% from SHP. Highest percentage of Degree of esterification was recorded at pH 2.5, pulse ratio 1.25 and extraction time of 5 min, as 80.81% for SHF and 80.59% for SHP. Under optimal conditions, experimental yield correlated with predicted yields.

Pectin and cellulose were extracted from the hulls using acid and alkaline digestion in PIMAE under different conditions of pH 2, pulse ratio of 1 and extraction time of 30 minutes and compared with water bath overhead stirrer extraction (WBOSE) under several

conditions, including pH of 2 at 90°C for two hours. Pectin and cellulose yields were measured, and physicochemical properties were characterized by determining moisture content (~8%). Pectin obtained from propan-2-ol precipitation was white colour, and both celluloses gave yellow colour ($p < 0.05$). SEM morphological analysis confirmed the presence of pectin and cellulose. Water absorption index (WAI) and water solubility index (WSI) values were 5.03 and 5.14% for soybean hull powder, 3.84 and 2.37% for cellulose extract from PIMAE and 3.75 and 2.51% for cellulose extract from WBOSE. Fourier transform infrared spectroscopy (FTIR) confirmed the presence of pectin and showed that soybean hulls pectin under PIMAE and WBOSE compared with high methoxyl and low methoxyl pectin commercial grade. The structure of the molecules included υ(OH), υ(C=H)COOH from methyl of esterified carboxyl group, υ(CH), υ_{as} (COO^-), υ_s (COO^-), δ(CH) and δ(OH)COOH. Finger print nuclear magnetic resonance spectroscopy (NMR) confirmed the presence of pectin and showed that the atoms and molecules included β-glycosidical, α-glycosidical, 1, 4-linked-α-d-Glc*p* residues, α-terminal-d-Glc*p* and α-l-rhamnopyranose residues.

Keyword: soybean hulls, pectin, extraction, pressurized intermittent microwave-assisted extraction.

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LAMNAMBHORN SOBMOR

2020

CONTENTS

CONTENTS (Cont'd)

CONTENTS (Cont'd)

LIST OF TABLES (Cont'd)

LIST OF TABLES (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF ABBREVIATIONS

AGA	Apiogalacturonan
AIS	Alcohol-insoluble solids
ANOVA	One-way analysis of variance
BBD	Box-Behnken Design
Ca^{2+}	Calcium ion
Cu^{2+}	Cupric ion
DA	Degree of amidation
DE	Degree of esterification
DM	Degree of methyl esterification (methylation))
EDS	Energy-dispersive X-ray spectroscopy
FTIR	Fourier transform infrared spectroscopy
Gal	Galactose
GalA	Galactopyranosyluronic acid
HG	Homogalacturonan
HM	High methyl ester
LM	Low methyl ester
MAE	Microwave-assisted extraction
M_w	Molecular weigh
NMR	Nuclear magnetic resonance spectroscopy
PIMAE	Pressurized intermittent microwave-assisted extraction
RGI	Rhamnogalacturonan I
RGII	Rhamnogalacturonan II
Rha	Rhamnose
RMSEP	Root mean square error of prediction
RPM	Rounds per minute
SEM	Scanning Electron Microscope
SHF	Soybean hull flakes
SHP	Soybean hulls powder
SDF	Soybean hulls dietary fibre

LIST OF ABBREVIATIONS (cont'd)

SFA	Soybean hull flakes acid digestion
SFB	Soybean hull flakes alkaline digestion
WAI	Water absorption index
WBOSE	Water bath overhead stirrer extraction
WSI	Water solubility index
XGA	Xylogalacturonan
Zn	Zinc

NOMENCLATURE

α	Alpha
~	Around or about
β	Beta
τ_{on}	Turn on time (sec) of the microwave field
τ_{off}	Turn off time (sec) of the microwave field
${}^{a}\upsilon$	Stretching
δ	Scissoring
γ	Wagging or out-of-plane deformation
s	Symmetric
as	Antisymmetric
μ	Micro

CHAPTER 1

INTRODUCTION

Food processing is one of the largest industries in the world. It has been predicted that there would be increasing trends in the future to reduce food waste by-products by the recovery and utilization of food residues, along with the need to reduce greenhouse gases. The utilization of high volumes of wasted food by-products in the food industry is attracting increasing scientific research at the national and international levels (FAO, 2011a).

Soybean (*Glycine max*) is considered a major agricultural resource throughout the world. Soybean originated in Eastern Asia, probably in the north and central regions of China. It was reported that cultivated varieties were introduced into Korea and later into Japan some 2000 years ago (FAO, 1992). The leading soybean-producing countries are in North America, South America and Asia (FAO, 2002). Soybean sauce is a popular condiment around the world, including in Thailand. It is an important product for both internal consumption and export. It can be classified into 3 categories depending on the process, including fermented soy sauce, chemical soy sauce, and semi-chemical soy sauce. Soybean oil is a vegetable oil obtained from the seeds of the soybean and is the most popular cooking oil. In addition, soy milk is a health drink that has been popular for a long time due to having complete nutrition. The international market can also be promoted with soy products if the quality can be maintained to international standards. However, the growing demand for soy products has increased waste from factories.

Soybean hulls are a by-product released during the initial de-hulling during processing into products including cooking oil and soy sauce. Soybean hulls, which are the seed coat of soybeans, make up approximately 8% of the total mass (Blasi et al., 2000). Soybean hulls are the fibrous layer coating of soybeans. At present, hulls are utilized as a fibre source for cattle (Alemdar and Sain, 2008), but they could potentially be used as a functional food ingredient due to the high content of dietary fibre, including hemicellulose (50%), cellulose (20%) and pectin (30%) (Gnanasambandam and Proctor, 1999).

Pressurized intermittent microwave-assisted extraction (PIMAE) is a technology that integrates two extraction methods: pressurized and microwave extraction. Microwave-assisted extraction is being progressively used for the extraction of pectin. Pressurization can help extraction by exposure to temperatures above the boiling point of the solvent. The use of microwaves can interact with water molecules inside cells to increase the temperature and

force the breakdown of cell walls to discharge pectin (Mandal et al., 2006; Yang et al., 2019). Mao et al. (2019) compared three differences in extraction techniques between conventional solvent extraction, microwave-assisted extraction and microwave-assisted hydrothermal extraction under pressure (130 °C) for extracting pectin from sugar beet pulp. They found that microwave-assisted extraction had both the highest rhamnogalacturonan-I (RG-I) yield at 25.3% and purity at 260.2 mg/g alcohol-insoluble solids (AIS), followed by microwave-assisted hydrothermal extraction under pressure with 7.5% for RG-I yield and 166.7 mg/g AIS for purity. Fishman et al. (2006) extracted lime pectin using microwave-assisted extraction under pressure. Heating times ranged from 1 to 10 min. at 630 W of power. The temperature at that pressure was ~ 140 °C. Extraction time of heating was 3 min. They found that higher yield (~18%), molar mass, radius of gyration, intrinsic viscosity and hydrated radius, all decreased during extraction. In addition, they also studied pectin extracted from the orange albedo using microwaves under pressure (Fishman et al., 1999). The results confirmed that pressurized microwave extraction is an operative method to increase the yield and properties of pectin.

The purpose of this research, therefore, consisted of 3 parts: the first part was process optimization on the yield of pectin extraction from soybean hulls and precipitation methods on yield and physicochemical of pectin extraction from soybean hulls. The second was to study the effects of particle sizes on the yield and degree of esterification of pectin extracted from soybean hulls. The third was a study process extraction and separation of pectin and cellulose from soybean hulls, comparing pressurized intermittent microwave-assisted extraction and water bath overhead stirrer extraction, in order to determine their physicochemical properties.

1.1 Objectives

The specific objectives were:

1. To study the optimization of processing soybean hulls powder on pectin yield using pressurized intermittent microwave-assisted extraction and correctly optimum conditions to compare different alcohol precipitate.

2. To compare the use of soybean hull flakes and soybean hulls powder as basic material on pectin yield and degree of esterification of soybean pectin

3. To compare pressurized intermittent microwave-assisted extraction and water bath overhead stirrer extraction on the determination of physicochemical properties

1.2 Scope of research

The first step in this research concerns the study of the effects of pressurized intermittent microwave-assisted extraction on pectin yield and physicochemical properties of soybean hulls. The experiments on the precipitation were designed to compare two different alcohols, ethanol and propan-2-ol. The powder from soybean hulls was extracted using three levels of pH (1.5, 2 and 2.5), three pulse ratios (1, 1.25 and 1.51) and three extraction times (5, 10 and 15 min). The pectin of two alcohol precipitation methods was dried to ~7% moisture content. The pectin samples from optimal conditions were kept at room temperature for analysis, after which their colour and microstructure were determined using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS) and galacturonic acid.

Only one of the second, propan-2-ol was selected to study the effect of particles size on pectin yield and degree of esterification (DE). The hulls of two particles types, flakes and powder, was used for the determination. Response Surface Methodology, coupled with Box-Behnken Design (BBD) was employed in the investigation. The optimal conditions were selected from this study for extraction.

Extraction and separation of pectin and dietary fibre from hulls were determined. For this, pressurized intermittent microwave-assisted extraction (PIMAE) and water bath overhead stirrer extraction (WBOSE) were used to treat with acid and alkaline to test digestion. The suspension was placed in an overhead stirrer and stainless-steel Impeller (300 rpm) at 90°C for 2 hr. Then, the hulls were dried to ~7% moisture content, and crude pectin was grounded. The dietary fibre powder and crude pectin powder were kept at room temperature for analysis. Yield, moisture content, colour, SEM, water absorption index (WAI), water solubility index (WSI), Fourier transform infrared (FT-IR), and nuclear magnetic resonance (NMR) spectroscopy are the techniques that were used in the analyses.

CHAPTER 2

LITERATURE REVIEWS

2.1 Soybean

2.1.1 Definition and characteristic of soybean

Soybean (*Glycine max* (L.) Merr. cv. 'Chiang Mai 60'*)* is a genetic modification from *Sor Jor 4* and *Williams*. *Sor Jor 4* has rust resistance characteristics. *Williams* has strong stems and the highest yield. The prominent characteristics of cv. 'Chiang Mai 60' include rust resistance, high productivity (~623 kg/ acre), low rate of fertiliser requirements and seeds containing oil (~20%) and protein (~44%) (Office of Agricultural Economics of Thailand, 2017).

A description of fully mature soybean plants includes ~60 cm in height, with leaves that are dark green, wide and thick. White flowers are produced at the age of ~25 days and harvested at the age of ~97 days. The seed pods are dark brown and difficult to crack. 100 seeds weight ~16 g. Soybean is cultivated in Thailand throughout the country, though the most popular area is North Thailand (~44,227 acres), followed by the Northeast (~15,534 acres) and Middle North (~47 acres) of Thailand (Office of Agricultural Economics of Thailand, 2018).

The harvesting of soybean is usually soybean plants dry in the field. Agricultural Engineering from the Department of Agriculture studied drying seeds in a hot air oven with 250 kg of soybeans at a time. To reduce moisture from 34.1-39.9% to 15-16.8% in 6 hours, it costs ~1 baht/kg of dried soybeans. To reduce the problem of production and quality loss when it rains, threshing is commonly used by machines to ensure soybean seed quality. Using this method seed or grain drying, desiccation damage using an oven at 45 °C could be used to reduce moisture to ~10%. Seed processing is carried out by firstly air screen cleaning and grading with a gravity grading. Optimum recommended conditions for storage are 22 °C and relative humidity ~60-65% (Office of Agricultural Economics of Thailand, 2017).

2.1.2 Nutrition and structure of soybean hulls

The nutritional value of soybean hulls is quite high (Blasi et al., 2000). Soybean hulls are available wherever soybeans are produced. It is estimated that soybean hulls represent about 5-8% of soybean seed weight (Blasi et al., 2000). The hulls contain fibre that animals can digest because it contains 50% hemicellulose, 20% cellulose and 30% pectin, and high fibre diets are

suitable for use in animal feed (Liu et al., 2016; Lyu et al., 2018). The nutrition values of soybean hulls are shown in Table 2.1.

Table 2.1 Nutrients in soybean hulls

Nutrients	Soybean Hulls
Total digestible nutrients, %	77.0
Neutral detergent fibre, %	74.0
Acid detergent fibre, %	47.0
Crude fibre, %	35.0
Crude protein, %	10.4
Ash, %	5.0
Ether extract, %	2.5
Potassium, %	1.7
Calcium, %	0.6
Phosphorus, %	0.2
Sulfhur, %	0.1
Sodium, %	0.01
Magnesium, %	-
Net Energy for lactation (Mcal/lb)	0.8
Net Energy for maintenance (Mcal/lb)	0.8
Net Energy for gain (Mcal/lb)	0.5
Iron, ppm	324.0
Zinc, ppm	24.0
Copper, ppm	18.0
Manganese, ppm	11.0
Cobalt, ppm	0.1
Iodine, ppm	-
Iodine, ppm	-

On a DM basis, soybean hulls contain 46% cellulose, 18% hemicellulose, and 2% lignin.
Source: Blasi et al. (2000)

In Thailand, the utilization of hulls is used for livestock feed including goats, sheep, horses, beef and dairy cattle, poultry, pigs, ostriches, and rabbits, as well as in aquaculture. In 2018, the Thai Ministry of Agriculture and Cooperatives estimated that Thailand's soybean industry needed 2.93 million tons of soybeans, but only 1.30% was available from domestic production. The remainder was imported from South America, North America and Canada.

2.2 Dietary Fibre

2.2.1 Definition and characteristics of dietary fibre

The British Nutrition Foundation (2018) defines dietary fibre as a group of substances in plant foods that cannot be completely broken down by human digestive enzymes. These include waxes, lignin, and polysaccharides such as cellulose and pectin. Originally, it was thought that dietary fibre was completely indigestible and did not provide any energy. It is now known that some fibre can be fermented in the large intestine by gut bacteria, producing short-chain fatty acids and gases.

Dietary fibre is a general term denoting chemicals contained in the cell walls of plants including vegetables, beans and peas, oats, fruits, and grains that are not digested in the digestive system, therefore not providing energy. They consist of non-starch polysaccharides and other components such as cellulose, resistant starch, resistant dextrins, inulin, lignins, chitins, pectins, beta-glucans, and oligosaccharides (National Research Council, 1989; Loix et al., 2017).

Types of dietary fibre can be divided into 2 types, which are:

Soluble dietary fibre is dissolved in water and then absorbs water with the body, causing increased viscosity. These substances are not digested by the body, but the bacteria that live in the large intestine can be digested, such as resistant starch (maltodextrin and inulin), Oligosaccharide (fructo-oligosaccharide), heteropolysaccharide (pectin, glucomannan), unabsorbed sugar (sugar alcohol) and gum (guar gum, xanthan gum, gum Arabic, etc.) (National Research Council, 1989). Delayed gastric emptying, regulated blood glucose levels, lower serum, and cholesterol levels are due mainly to the effects of increasing viscosity of gut content, and colonic fermentation are shown in Table 2.2.

Insoluble dietary fibre is a kind of food fibre that is insoluble in water. However, it will swell in water like a sponge, not giving viscosity, and causing the increased volume of water in the stomach. It causes a feeling of fullness as these dietary fibres and bacteria in the large intestine cannot be digested. It helps increase faecal texture. It can reduce constipation and reduce the risk of colon cancer through the regulation of cellulose, hemicellulose, and lignin are shown in Table 2.3. (National Research Council, 1989).

Table 2.2 Nutrients and sources of soluble dietary fibre

Nutrient	Source
Arabinoxylan	Wheat bran (Wang et al., 2019) (Anderson and Simsek, 2019), cereal grains (Wang et al., 2020), barley bar (Guo et al., 2019), rich red apple (Ali et al., 2020)
Fructans	Banana and garlic (Shalini et al., 2017), onion bulbs (Ohanenye et al., 2019), artichoke (Luo et al., 2018), cereal grains (Verspreet et al., 2017)
Inulin	In diverse plants, artichoke (Gao et al., 2010; Khuenpet et al., 2017; Rubel et al., 2014)
Polyuronide	Cell wall of pears (Dong et al., 2018), papaya (Karakurt and Huber, 2003), apples (Vanoli et al., 2009), tomato (Hobson et al., 1987)
Pectin	Pomace of citrus (Talekar et al., 2020), pear (Tang et al., 2020), soy hulls (Kim et al., 2015), carrot (Neckebroeck et al., 2020),
Alginates	In algae (Faidi et al., 2019; Park et al., 2019)
Agar	Seaweed (Ferreira et al., 2019)
Raffinose	Chickpea (Gangola et al., 2016), rice (Li et al., 2018)

Source: Applied from Li and Komarek (2017)

Table 2.3 Nutrients and sources of insoluble dietary fibre

Nutrient	Source
Cellulose	In general plants, apple pomace (Melikoğlu et al., 2019), quinoa stalks (Gil-Ramirez et al., 2018), pineapple (Sumesh et al., 2020)
Chitin	In fungi (Liao and Huang, 2019), crustaceans (Hong et al., 2018; Xin et al., 2019)
Hemicellulose	Sugar beet pulp (Sun and Hughes, 1998), sugarcane (You et al., 2019), wheat straw (Sun and Tomkinson, 2002)
Arabinoxylan	Psyllium, rye (Imjongjairak et al., 2015)
Lignin	Corn (Yang et al., 2018; Zhang et al., 2019), sugarcane (Arni, 2018)

Source: Applied from Li and Komarek (2017)

2.3 Biopolymers

Biopolymers are produced from natural sources. They have been used for many years for food (e.g. thickening and gelling agents), pharmaceutical (e.g. drug delivery and wound covered), and biomedical applications (e.g. polynucleotides and polypeptides). Colloids are classified into two groups: hydrophobic and hydrophilic. Hydrophilic colloids are thermodynamically stable and mixed with the solvent easily, while hydrophobic colloids are antagonistic. Hydrocolloids are hydrophilic that have a high molecular weight. They are utilized as ingredients in food formulation for improving the gelling effect (e.g. agar, alginate, carrageenan, gelatin, gellan gum, and pectin), increasing food consistency (e.g. cellulose derivatives, starch, and gum etc.), and controlling the microstructure (e.g. flavour, texture, and shelf life) (Alba, 2015).

2.4 Pectin

2.4.1 Description, characteristic of pectin

Pectin (beta-D-galacto-hexopyranuronic acid) is a type of soluble dietary fibre found in the intracellular layer of plant cells. It is a heteropolysaccharide with a complex structure. The main structure is composed of D-galactonic acid molecules that are connected from 200-1000 molecules (~10% of total). Joined in chains by α-1, 4 glycosidic linkages. There is a medium charged sugar, such as rhamnose, arabinose, galactose, and other sugars, such as D-glucuronic acid, L-fucose, D-glucose, D-mannose, and D-xylose are sometimes found in side chains (Alba, 2015). The molecular weight of pectin ranges between 50 - 150 kDa. Pectin structure is shown in Figure 2.1. There are two different types of pectin:

2.4.1.1 High methoxyl pectins are characterised by >50% degree of esterification galacturonic acid residues Methyl groups >8.16%, the gel can be formed by 65-70% sugar and adjusting the pH 2.8-3.2. There are two different groups of high methoxyl pectins.

2.4.1.1.1 Rapid-set pectin is pectin with a >70% degree of esterification. Setting gel with sugar and acid in the pH range 3.0-3.4.

2.4.1.1.2 Slow-set pectin is pectin with a 50-70% degree of esterification. Setting gel with sugar and acid in the pH range 2.8-3.2 at a low temperature more than rapid-set pectin.

2.4.1.2 Low methoxyl pectins are characterised by a <50% degree of esterification galacturonic acid residues Methyl groups <8.16%, It has the ability to form gels in the presence

of divalent cations, such as Ca^{2+}, Zn or Cu^{2+}, and low sugar. Ca^{2+} will bind to the carboxylic group between structures. It creates a structure called an egg-box model (Alba, 2015).

2.4.2 The structural classes of pectin

Pectic polysaccharides can be classified into five groups: homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), xylogalacturonan (XGA), and apiogalacturonan (AGA).

2.4.2.1 Homogalacturonan

Homogalacturonan is found in plant cell walls (~65% of pectin). It is the most polymers in pectins. HG is comprised of long chains of linear 1,4-α linked -D-GalpA residues and a few of the carboxyl groups are not fully methyl-esterified at C-6 and/or acetyl-esterified at O-2 and/or O-3 of GalpA contingents of plants egg.

2.4.2.2 Rhamnogalacturonan I

Rhamnogalacturonan I (RG-I) is found in plant cell walls (~20-35% of pectin). Its backbone is composed of the repeating disaccharide consisting of two types: galactonic acid and rhamnose $[\alpha\text{-}(1,2)\text{-D-GalpA-}\alpha\text{-}(1,4)\text{-L-Rhap}]_n$ and has side chains consisting of *α*-(1,5)-L arabinans and *β*-(1,4)-D galactans, arabinogalactans I (AG-I), arabinogalactans II (AG-II) and galacto-arabinans. AG-I is composed of a 1,4 linked β-D-Galp backbone and α-L-Araf are attached to the O-3 position of galactosyl residues. AG-II is composed of 1,3-linked *β*-D-Gal*p* backbone, containing short side chains of *α*-L-Ara*f*-(1→6)-[*β*-D-Gal*p*-(1→6)]$_n$ is shown in Figure 2.2, where galactosyl residues of the sidechains can be substituted with *α*-L-Araf-(1→3) residues. The heterogeneous structures of RG-I arabinan and galactan side-chains are different depending on the pectin source (e.g. apple, sugar beet, soybean, persimmon, and potato).

2.4.2.3 Rhamnogalacturonan II

Rhamnogalacturonan II (RG-II) is a scarce pectic component in cell walls (0.5 - 8% in dicotyledon and <1% in monocotyledon). RGs-II has been detected in the cell walls of edible plants including apple, kiwi, carrot, tomato, and radish. RG-II is not the same as or related to RG-I because it does not have a rhamnogalacturonan backbone. The RG-II structure is highly complex with over 20 different linkages and 12 different types of sugars including 2-keto-3-deoxy-Dmanno octulosonic acid (Kdo), 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha), apiose, 2-O-methyl xylose, 2-O-methyl fucose and aceric acid (Alba, 2015).

2.4.2.4 Xylogalacturonan

Xylogalacturonan (XGA) can be found in the cell walls of soybeans, apples, peas, watermelons, and marine seagrasses (~20-35% of pectin). It has α-(1→4)-linked D-galacturonic acid substituted by β-D-xylose at the O-3 position.

2.4.2.5 Apiogalacturonan

Apiogalacturonan is found in the cell walls of aquatic plants such as duckweeds (*Lemnaceae*) and marine seagrasses (*Zosteraceae*) with D-apiose residues 2,3-linked to homogalacturonan.

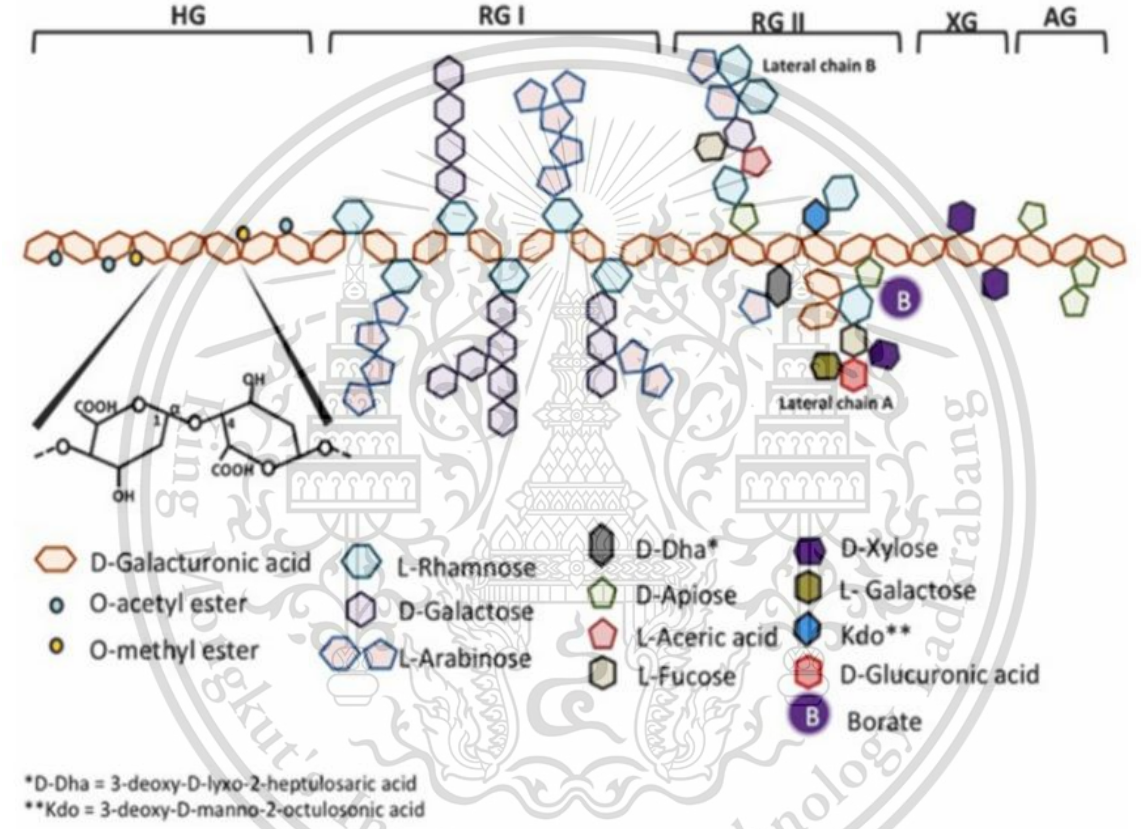


Figure 2.1 Structure of pectin; homogalacturonan (HG), rhamnogalacturonan (RG**),** Xylogalacturonan (XG), arabinogalactan (AG)

Source: Leclere et al. (2013)

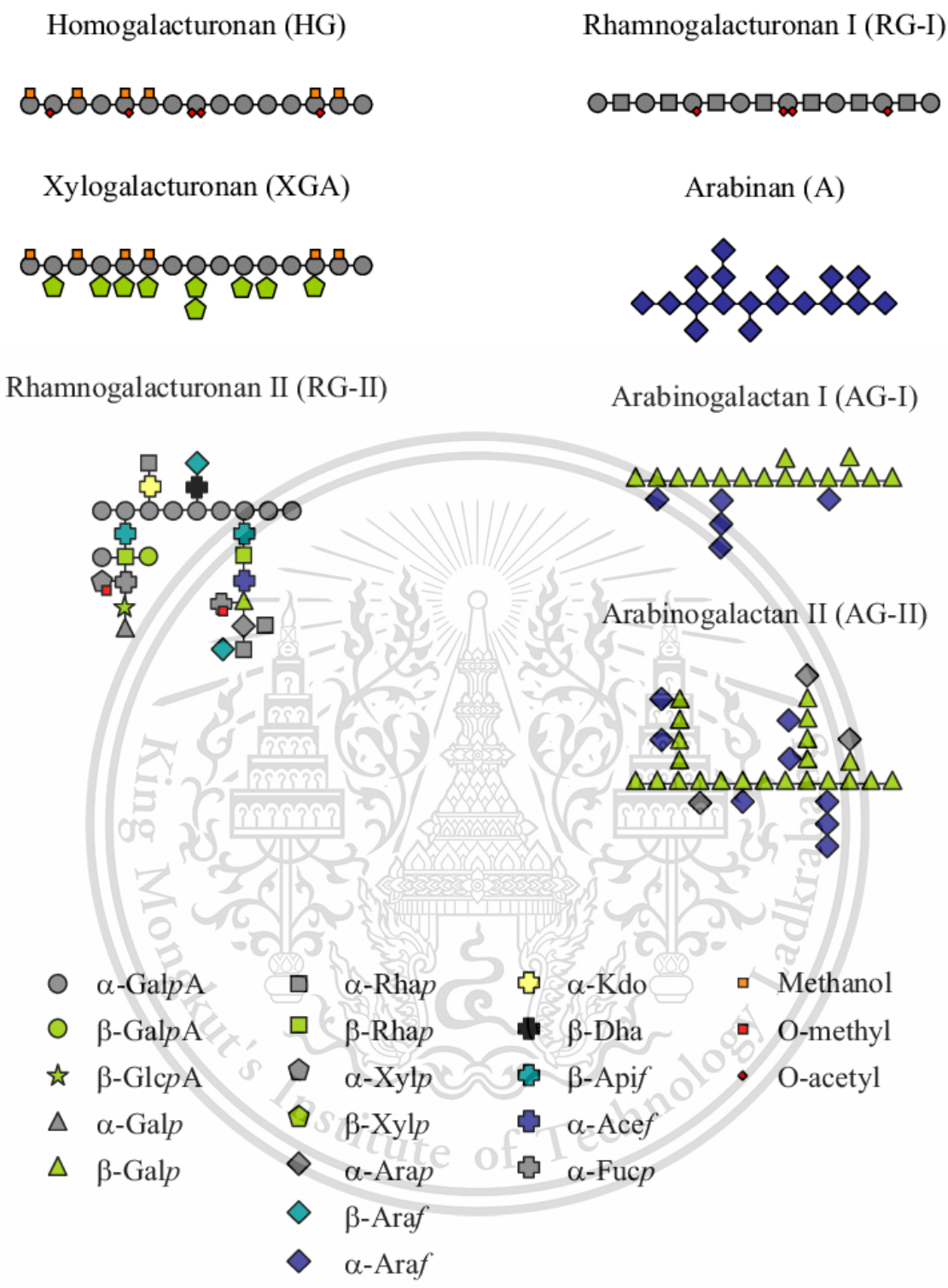


Figure 2.2 Structures of pectic structural domains, HG and HG-derived domains are shown in the left; RG-I and attached neutral sugars side chains are shown in the right column

Source: Bonnin et al. (2013)

2.5 Isolation of Pectin

Conventional methods for the extraction of pectin that can be used in acid or alkaline digestion, cold or hot aqueous buffers, and chelating agents, e.g., potassium oxalate will be conducted for several hours. Thus, the production of environmentally-friendly pectin will be recommended and explored to satisfy the demand for more efficient use. The techniques will include the isolation of pectin with microwave- and ultrasound-assisted isolation enzymes, e.g., polymethylgalacturonases and polygalacturonases, and electromagnetic induction heating. The present production method for pectin uses high temperature acid hydrolysis (70-90°C) with HCl, HNO_3 or H_2SO_4 (HG will be extracted with mild agents; e.g., water or K-oxalate), and RG-I will be extracted with stronger agents, e.g., HCl, NaOH at a pH between 1.5 and 2.5; the extraction time will depend on the raw materials. The pH of the extraction in pectin at a high pH will normally result in pectin with a low degree of methyl-esterification (DM). In contrast, a low pH will result in a high DM. Thus, the functional properties will be different (Alba, 2015). This is because acid hydrolysis will cause depolymerisation. Subsequently, the pectin extract will be separated from the material by filtration and centrifugation to remove the plant tissue residue. The residue will be normally used as livestock feed, which will be adjusted to a pH of 3-4 for degradation of the polymer. It will then be concentrated by vacuum evaporators. The precipitation of pectin will be carried out in alcohol, e.g. ethanol and propa-2-ol, for the removal of contaminants, e.g. sugars, polyphenols, and pigments. After the precipitated pectin is dried, it will be ground into a powder.

2.6 Soybean hulls pectin

Soybean hulls pectin is interesting because it is an inexpensive commercial source of pectin. It is also attractive as a hydrocolloid for various food applications, such as the setting of jams and jellies, form gels, and emulsion stabilisers. It can also be used in certain medicines such as colon-biodegradable drug delivery systems, adsorbent, bulk-forming agents, and coating material for tablets, pellets, and microparticles. SHP principally contains galactose, xylose, galacturonic acid, arabinose, glucose, and rhamnose (Table 2.4).

Table 2.4 Composition of soybean hulls pectin, commercial soybean hulls pectin, and citrus pectin

Composition	Soybean Hulls Pectin	Commercial Soybean Hulls Pectin	Citrus Pectin
Galacturonate (% db)	33	18.5	85.8
Esterified galacturonate (% db)	18.1	0	73.7
Neutral sugar composition (% db)			
Rhamnose + Fucose	8	8	25.1
Arabinose	24.2	26.3	15.6
Xylose	2.7	2.5	1.8
Mannose	0	0	0
Galactose	49.8	59.5	49.5
Glucose	15.3	3.7	8

Source: Yamaguchi et al. (1996)

2.7 Cellulose

2.7.1 Description and characteristics of Cellulose

Cellulose (hexopyranosyl-(1->4)-hexopyranose) is a biopolymer composing the cell walls of plant tissue. It is a linear polymer of β-(1 → 4)-D-glucopyranose consisting of several thousand glucose molecules that are linked end to end is shown in Figure 2.3. The degree of polymerisation (DP) between 200 and 12,000 depends on the origin of the cellulose (Merci et al., 2015). From reported results, cellulose has four phases, namely I, II, III and IV. Cellulose I can be transformed into other polymorphs and has the highest native crystalline form. Cellulose I contains two phases: Cellulose I_α will have a one-chain triclinic structure, while Cellulose I_β will have a two-chain monoclinic structure. Both forms will exhibit differences in the hydrogen bonds, which will be important for application in papermaking, food additives, textiles, and pharmaceutical industries (Liu' and Li, 2017).

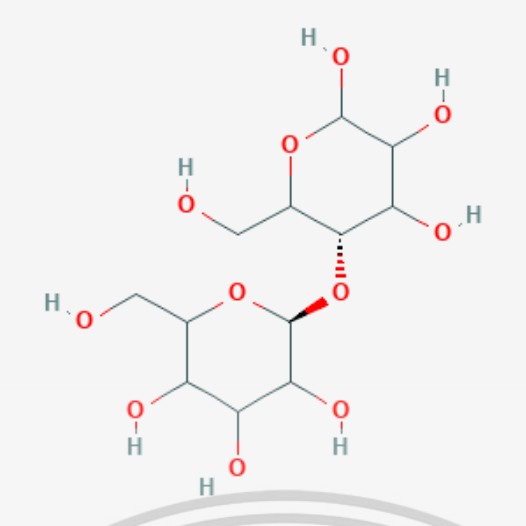


Figure 2.3 2D-chemical structure of cellulose

Source: National Centre for Biotechnology Information (2020)

2.8 Microwave-assisted extraction mechanisms and fundamental

In the late 1980s, microwave-assisted extraction (MAE) began being used in natural products and prominent technological developments. Now, the advancement of MAE technology has gained popularity, e.g., pressurized microwave-assisted extraction (PMAE) and solvent-free microwave-assisted extraction (SFMAE) (Delazar et al., 2012). MAE is an extraction technique that can coordinate the microwave electromagnetic field and conventional solvent extraction, which will be based on the interaction between the materials' or solvents' dipolar molecules at high frequencies. Furthermore, the induced ordered kinetic energy will be transformed into disordered kinetic energy due to the rotation of ions in materials with ionised solvent molecules in the electromagnetic field that will result in the production of heat energy. High temperature and the forced breakdown of cell walls will then cause a discharge of important compounds. Simultaneously, the dielectric constants of solvents; such as, ethanol (2.0, g′) and propanol (1.7, g′) will be less than that of water (2.33, g′), and the tan δ value of ethanol (2,500, $\times 10^{-4}$), methanol (6,400, $\times 10^{-4}$), and propanol (6,700, $\times 10^{-4}$) will be higher than that of water (1,570, $\times 10^{-4}$). Hence, solvents will be more appropriate for usage than water for converting microwave energy into heat. MAE also has advantages, e.g., shorter extraction time, higher yield, less solvent, greater extraction efficiency, and lower cost over the conventional method.

In addition, MAE has been utilized to extract polysaccharides; such as, pectin from black carrot pomace (BCP) (Sucheta et al., 2020), potato pulp (Yang et al., 2019), apple pomace and lemon peel (Zarei et al., 2017), orange peel (Su et al., 2019), etc. is shown in Table 2.5. Other

materials that utilise MAE include phytochemicals extract, e.g., phenolic compounds from fenugreek seeds (Akbari et al., 2019), white and red grape skin (Kwiatkowski et al., 2020), lime peel (Rodsamran and Sothornvit, 2019), as well as essential oil extract from *O. vulgare L. spp. Hirtum* (Drinić et al., 2020).

Table 2.5 The effects of microwave heating on pectin extraction

Source	Microwave Conditions				Effects of Microwaving on Yield and Quality
	Power (W)	Temp. (°C)	Time (min.)	Ratios (g/mL)	
Apple pomace	560	NS	2	1:10	High MW (2.64 kDa), yield (23%); galacturonic acid content (90.60%) (Dranca et al., 2020).
Lime peel	630	40	3	1:25	High MW (310-515 kDa); radius of gyration (Rg) decreased with the heating time (Fishman et al., 2006).
Orange peel	900	NS	10	01:12.5	High MW (6.4kDa); yield (18%); galacturonic acid content (66%) (Kratchanova et al., 2004).
Lime peel	700	NS	3 &5	1:20 & 1:40	Methoxyl content 8.74-10.51%; galacturonic acid content 79.29-95.93%; yield (16%) (Olalere et al., 2018).
Carrot pomace	900	110	5	1:100	MW processing higher yield of pectin without impacting its functionality (Sucheta et al., 2020).

NS - Not stated

2.9 The Principle of Scanning Electron Microscope

A scanning electron microscope (SEM) is an advanced instrument that can be used to detect the surface phenomena of solid materials. SEM uses high-energy electrons shot through solid materials. The signals emitted can then be analyzed, and the results will offer information about the morphology, including the shape and size. The topography will show the surface smoothness or roughness, while the composition will display the elements and compounds.

The crystallographic information will show the arrangement of the atoms, such as in a particular material (Akhtar et al., 2019).

The effect of the microwave treatment on the surface of pectin will depend on various factors, such as microwave conditions, including the ratio, power, time and temperature. Sommano et al. (2018) compared conventional and microwave-assisted extraction on the morphology of pectin from mango peel. The conventional conditions will have a pH of 1.5 at 85°C for 1.5 hours, whereas microwave-assisted extraction will have power ranging between 500 W for three minutes to 900 W for one minute. Sommano et al. (2018) studied the SEM images of the surfaces of commercial citrus pectin, conventionally extracted pectin, pectin extracted by MAE at 500 W, and pectin extracted by MAE at 900 W. The pectin particles of commercial pectin display different shapes. However, the pectin particles that are extracted using the MAE 500 W and 900 W will be crystallised in shapes and with more spongy surfaces compared to pectin that is conducted by the conventional method. Therefore, the extraction method and raw materials produce different pectin morphologies. Pectin with more spongy structures will ordinarily have better solubility than those that will have a firm structure and less spongy structures.

Flauzino Neto et al. (2013) studied the extraction of cellulose from soybean hulls. To undertake this process, hydrolysis was performed at 40°C for 30 or 40 minutes using H_2SO_4, which has 48% cellulose. The results show that the hulls fibre comprised a bone shape with microsize.

2.10 Principles of Fourier transform infra-red Spectroscopy

In 1957, Perkin-Elmer produced the first commercial infrared microscope capable of recording an infrared spectrum. Fourier transform infrared spectroscopy **(**FTIR) is a technique that uses an infrared (IR) spectrum for the absorption or emission of a solid, liquid or gas. FTIR consists of six main parts: 1) Radiation sources that will generally be used in the near-IR (780 nm~2.5 μm) and mid-IR (2.5 μm~25 μm) range for measurements; 2) sample compartment; 3) interferometer that, in practice, will consist of a set of intensities that will measure the discrete values of retardation. The difference between successive retardation values will therefore be constant. Thus, a discrete Fourier transform (FT) is needed, and a fast Fourier transform (FFT) algorithm will be used. As a result, 4,000 cm^1-1300 cm^1 absorbance bands will indicate the functional groups, but 1,300 cm^1-400 cm^1 absorbance bands will be unique to each molecule, similar to a fingerprint (Ismail et al., 1997). 4) Detectors will generally use a pyroelectric form that can respond to changes in temperature as the intensity of IR radiation falling on them will

vary; 5) amplifier and analogue-to-digital convertor (ADC), where the signal will be amplified and converted to a digital signal; 6) a computer, where the signal will be transferred in which FT will be carried out. Table 2.6 presents information concerning the infrared wave ranges of molecular systems. By-products will mostly use this technique for the analysis of the thermochemical process and will provide detailed functional group absorptions.

Flauzino Neto et al. (2013) studied the effect of acid hydrolysis treatment at 40°C for 30 minutes on the functional group of cellulose. Flauzino Neto et al. (2013) displays the FTIR spectra of three samples comprising soybean hulls (SH), soybean hulls extract (TSH), and the cellulose of soybean hulls (WSH_{30}). The acetyl and uronic ester groups (peak at 1,742 cm^{-1}) from soybean hulls will decrease in the spectrum of the soybean hulls extract because the hemicelluloses and lignin will be significantly removed and almost disappear in the spectra of the cellulose because of the acid hydrolysis treatment. The C=C stretch of the aromatic ring in the lignin (peak at 1,630 cm^{-1}) in the soybean hulls and soybean hulls extract will come from the water. The peak at 1,640 cm^{-1} will be assumed to be the absorbed water. Thus, the C=C vibration (peak at 1,520 cm^{-1}) in the cellulose will indicate the removal of the corresponding groups from the lignin. The S=O vibration (peak at 1,205 cm^{-1}) in cellulose is a small peak due to the esterification reaction.

Table 2.6 Types of bonds and typical wave number values during the FTIR process

Types of Bonds	Wave Number (cm^{-1})	Remarks
C–H	3000–2850	alkanes stretch
	1450–1375	alkanes: -CH_3 bend
	1465	alkanes - CH_2 – bend
	3100–3000	alkenes stretch
	1000–650	alkenes out-of-plane bend
	3150–3050	aromatic stretch
	900–690	aromatic out-of-plane bend
	3300	alkyne stretch
	2900–2800	Aldehyde
C=C	1680–1600	Alkene
	1600–1475	Aromatic
C≡O	2250–2100	Alkyne
C=O	1740–1720	Aldehyde
	1725–1705	Ketone
	1725–1700	carboxylic acid
	1750–1730	Ester
	1680–1630	Amide
	1810–760	Anhydride
	1800	acid chloride
		alcohol, ethers, esters, carboxylic
C–O	1300–1000	acids, anhydrides
O–H	3650–3600	free alcohols, phenols
	3400–3200	H-bonded alcohols, phenols
	3400–2400	carboxylic acid
		primary, secondary amines, and
N–H	3500–3100	amides: stretch
		primary, secondary amines, and
	1640–1550	amides: bend
C–N	1350–1000	Amines

Source: Ong et al. (2020)

2.11 The Principle of nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) techniques can detect single atoms and molecules in solution and solid-state samples. The technique is non-destructive and will simultaneously allow structure elucidation and quantification, which will provide information about the chemical structure and dynamics of organic molecules. NMR spectroscopy will use the lowest irradiation energy for excitation. In the past, pectin would be used in food and pharmaceuticals as a gelling agent, thickener, dietary fibre, bulking agent, emulsion stabiliser, foam, encapsulate, emulsifier, adhesive and binder, flocculant, swelling agent, film/coat former, or syneresis inhibitor protective colloid and suspending agent. Thus, NMR techniques can identity the quantity and different functional groups as well as detect minor components and impurities (Cheng and Neiss, 2012).

2.12 Response Surface Methodology

RSM is used to combine mathematical and statistical techniques useful for model design and problem analysis. Interesting results will depend on multiple variables. For the results, the relationship between the results and independent variables will be mostly unknown. Therefore, the first step is finding suitable estimators to be used as representatives showing the actual relationship between y and the set of independent variables. Generally, polynomial functions with low power under independent variables will be used (Chitarree, 2012). If the models of replication show a linear relationship with the independent variables, the function obtained to estimate the relationship will be a first-order polynomial equation.

$$Y = \beta_0 + \sum_{i=1}^{a} \beta_i X_i + \sum_{i=1}^{a} \beta_{ij} X_i X_j + \sum_{i=1}^{a} \beta_{ii} {X_i}^2$$

If arc relates in the system, we will use polynomial functions with a higher power, e.g., second-order polynomial equation.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} {X_i}^2 + \sum_{i>j}^{k} \sum_{j}^{k} \beta_{ij} X_i X_j$$

2.13 Related researches

2.13.1 Pectin product from soybean hulls

Porfiri and Wagner (2018) investigated the extraction of polysaccharide-protein fractions from soybean hulls by using three homogenisation treatments consisting of magnetic stirring (for 10 minutes), a high-speed blender (25,000 rpm for five minutes), and a high-speed blender followed by a high-pressure homogeniser (first valves for 800 bars and second valves for 80 bars). The hulls were ground to a powder (<500 mm). For the first fractions, the hulls powder was dispersed in HCl (0.1M) of 1:15 extract for the three treatments. Then, incubation was carried out in a thermostatic bath at 90°C for 45 minutes and then cooled to room temperature. Subsequently, it was centrifuged (7,000×g for 15 minutes at 4°C), and then received precipitation with 2-propanol, followed by drying at 40°C. For the second fractions, the hulls powder was extracted with NaOH at a pH of 9 (1:10), after which it was treated and incubated using a high-speed blender for two hours at 30°C, and finally centrifuged and precipitated. Supernatants that were obtained from the alkaline (S1) and acid (S2) extractions were precipitated in 2-propanol and then dried, as described for HSF-1, which will generate HSF-2 and HSF-3. Based on the results, HSF-1 from soybean hulls yielded a higher magnetic stirring (5.4%), high-speed blender (10.0%), and high-pressure homogeniser (12%). From using a high-speed blender (R = 3.0%) and high-pressure homogeniser (R = 7.4%), the homogenisation methods exhibited no significance. Consequently, the FTIR showed that the galactomannan proteins and arabinogalactan proteins were solubilised in alkaline or acid. As well, HSF-1, HSF-2, and HSF-3 showed the isoelectric point (PI) as 3.40 (HSF-2), 2.90 (HSF-1) and 2.5 (HSF-3), respectively. Additionally, round the PI, higher viscoelastic parameters were found, while higher polysaccharide ratios offered higher elasticity at a lower pH. Moreover, the turbidimetry and particle size showed that the soy proteins were aggregated at a lower pH. However, this tendency is reduced as the quantity of polysaccharide is increased.

Liu et al. (2016) investigated the effects of various extraction temperature levels (110-180°C) and various extraction times (10-150 minutes) on polysaccharides from soybean hulls powder (40 mesh) using hot-compressed water. From the results, a middling temperature (130-150°C) and short treatment time were shown to be appropriate for the preparation of polysaccharides-A. Arabinose in polysaccharides that are extracted by hot-compressed water would also be suitable (36-47%). Furthermore, the NMR showed that polysaccharides that were extracted at 150°C could be composed of a-L-arabinofuranosyl units, 4-O-Me-a-D-GlcpA units, and a-D-galactose units that would be attached to the substituted sugars. The discovered

polysaccharides were a-L-arabinofuranosyl units, 4-O-methyl-glucuronic acid units, and a-D-galactose units that attached to the substituted units. The raw soybean hulls displayed the crystallinity index (CrI) to have a low CrI (20%), while the hot-compressed water treatment showed an increase in the CrI (22-29%).

Gnanasambandam and Proctor (1999) determined pectin from soybean hulls powder by using a rotary evaporator (90°C for 40 minutes). They extracted the hulls powder by comparing two different treatments. Firstly, three different solvents were used with 0.1 N HNO3, 0.5% Sodium hexametaphosphate (SHMP) and 0.05 N NaOH at a ratio of 1:20 to obtain the sequential extraction. This was followed by cooling at room temperature and centrifuging at 2700×g for 15 minutes, after which the pectin was precipitated three times with 2-Propanol and freeze-dried. The hulls powder was extracted continuously with 0.1% (v/v) mannanase enzyme in 50°C at a pH of 5.5 for one hour. Secondly, the acid extraction was extracted with HNO3 (conditions that are similar to the sequential extraction) with the sediment being extracted twice in HNO3. Then, the repeated methods of precipitation, drying, and enzyme were similar to the sequential extraction, with the results showing their galacturonic acid content (GalA), which is the highest in enzyme repeated acid extraction (~88%) yield ~15%, and methoxyl content (3.9-7.5%). Therefore, it can be concluded that the GalA and methoxyl contents of soybean hulls pectin will be similar to apple (GalA of ~61%; methoxyl content of 4.4%) and citrus pectin (GalA of ~76%; methoxyl content of 4.6%).

Monsoor and Proctor (2001) extracted pectin from soybean hulls powder. Various ratios were assessed for the effect of extracting solvent on the hulls (1:10, 1:15, 1:20, and 1:25, respectively) and different pH (2, 4, 6, 8, and 10, respectively) at 90°C for one hour in a water bath. Following this, the extracts were cooled to room temperature and centrifuged (2,700 × g for 15 minutes). The supernatants were then precipitated with isopropanol, then stirred for one hour and centrifuged. The spray-dried pectin powder was preserved between 200-220°C with the results showing that the extracted pectin at different hull/solvent ratios at 1:15-1:25 was similar and the yield was ~13–16%. Additionally, the galacturonic acid content at ~66-69% was similar to that of citrus pectin (70%). Moreover, the solvent ratio did not significantly affect the degree of esterification. Thus, the FTIR spectroscopy of soybean hulls pectin will reveal similar analytical grades as commercial pectin with the independent solubility of the solvent ratios and pH. Rheology will also show that the viscosity of the pectin solution will increase as the ratio of the solvent decreases.

2.13.2 Cellulose product from soybean hulls

Robles Barros et al. (2020) determined cellulose from soybean hulls using pulping and bleaching processes. The pulping process was optimized for NaOH solutions (1%, 2%, and 2.5% (w/v)) at 90°C for two hours. The bleaching process applied a bleaching solution (VS) of 55-65 mL/g at 85-95°C and VS of 70-75 mL/g at 95°C. The pulping process was optimized with 1% of NaOH and bleaching process applying VS = 75 mL at 95°C/four hours. The results showed that the hulls contained cellulose (40.62%) and had low lignin content (<6%). As a result, the SEM, 1H NMR, XRD, FTIR and TGA/DTG analysis demonstrated that it is possible to synthesise CMC (DS = 1.45) by acetylating bleached pulp with 2.1 g of chloroacetic acid for 192 minutes at 63°C.

Camiscia et al. (2018) compared two different treatments of the traditional alkaline-bleaching pathway and simple pre-alkaline treatment at low temperatures. To pre-treat the SBH samples, the ratio of the hull/solution was 1:10 with a pH of ~14 adjusted by 5% (w/v) NaOH at 20-60°C and the incubation time was 0-48 hours. In the second step, the alkaline pre-treatment will have the residual lignin content by using 2% (w/v) NaOH at 30-90°C with the presence of (4% v/v) H_2O_2. The results showed that cellulose could be easily obtained with just an alkaline pretreatment of 5% (w/v) NaOH within 40 hours at 50°C and be free of any lignin content. The attachment of different functional groups such as -COOH and $(CH_3)_3N+$ will change the physico-chemical properties of the obtained cellulose, which show the major crystalline structures that will consequently modify the swelling capacity and its ability to adsorb model proteins.

Ferrer et al. (2016) studied the physical, thermal, chemical and rheological characterisation of cellulosic microfibrils and microparticles produced from soybean hull. A proprietary extraction method was used by simple alkaline, acid, and mechanical treatments to isolate the cellulosic microfibrils (SMF) and brick-like microparticles (SMP). As such, SMF and SMP were compared with micro and nanofibrillated cellulose (MNFC). The results showed that the SMF, SMP, and MNFC had crystallinity (~> 10%) and good thermal stability (maximum degradation at 361°C and 355°C).

Yang et al. (2014) extracted dietary fibre from soybean hulls and compared two different treatments, with and without soybean hulls powder control (SBH-C). The hulls powder comprised acid that was digested to a pH of 1.5–2.0 by HCl at 60°C for two hours. This was followed by rinsing with water to neutralise the pH and the centrifugation of 5,000g

for 10 minutes. The dewatered hulls were digested by alkaline to a pH of 10-11 by KOH at 60°C for two hours. Similarly, the alkaline digested hulls were rinsed and then centrifuged. After the acid-alkaline digestion, the hulls were autoclaved at 121°C with 15 lbs. of pressure for 10 minutes. Prior to this step, the hulls were dehydrated using a hot-air oven and milled into a dietary fibre powder (SHB-T). The results showed that the colour of the lightness was significantly improved ($p < 0.05$), and the yields of the soluble (2.6%), insoluble IDF (~86%), and TDF (~88%) were increased. WAI of the SBH-C (~4%) and SBH-T (~3.5%), although WSI of SBH-C (~15%) and SBH-T (~3%). Significant variance in WAI, or WSI, between SHB-C and SHB-T was found.

2.13.3 Extraction techniques

Sucheta et al. (2020) studied the kinetics, characterisation and process economics of pectin from black carrot pomace using three different extraction methods: intermittent microwave (110°C for five minutes), ultrasound (70°C for 30 minutes) and conventional heating (110°C for 90 minutes) at a pH of 2.5. The result for the highest yield was shown by conventional heating (0.22 kg/kg pomace), anthocyanins (1,213 mg/l), phenolics (1,832 mg/l), antioxidant activity (180 μM/mL), DE 50%, GalA 47.7%, and particle size (1,373.3 nm). This was followed by microwave extraction, resulting in pectin with the highest kinetic modelling, water holding capacity (0.7 g/g) and higher ultrasound.

Dranca et al. (2020) studied seven different extraction techniques: (microwave (pH 2.2 at 560 W for two minutes), enzyme, ultrasound (pH of 1.8 at 20 kHz for 30 minutes), enzyme with cellulase and celluclast, ultrasound with heating treatment, enzyme with ultrasound treatment and conventional citric acid extraction (pH of 1.9 at 90°C for 148 minutes) to extract pectin from apple pomace. The highest pectin yield was similar to the microwave extraction (23.32%) and conventional (23.26%) method. Pectin from the microwave and ultrasound extraction had a higher GalA, Eq.W, MC, DE, and Mw, whereas pectin from the enzymatic extraction had a lower degree of esterification.

Su et al. (2019) compared three different extraction techniques between microwaves, surfactant and microwave-assisted extraction, and conventional solvent extraction of pectin from orange peel. The results showed that the optimal conditions comprised a pH of 1.2 at seven minutes, and 21.5 v/w LSR. Pectin from surfactant and microwave-assisted extraction was an efficient method for the extraction of pectin and provided a higher pectin

yield (32.8%), galacturonic acid content (78.1%), degree of esterification (69.8%), Mw (286.3 kDa), and lower energy costs.

Swamy and Muthukumarappan (2017) compared two differences in the continuous and intermittent microwave extraction techniques for extracting pectin from banana peel. The continuous process parameters had an extraction power (300-900 W), extraction time of 100-300 seconds, and pH of 1-3. The intermittent process used a pulse ratio (0.5-1), extraction power and pH as similar continuous parameters. The dried banana peel was ground into powder (2 g) and then added to distilled water (20cc) and adjusted with HCl to a pH of 1-3. A Box–Behnken design was utilized for the experiments in this research. The results indicated that the optimized continuous method was 900 W, and the time taken was 100 seconds with a pH of 3.00 with the highest pectin yield (~2.2%). On the other hand, the intermittent process displayed a microwave power of 900 W, pulse ratio of 0.5, and pH of 3.00 that yielded the highest pectin content (~2.6%). R^2 for the continuous method (0.990) and intermittent method (0.994) indicated a high coefficient for the determination values. Optimized solutions for the continuous and intermittent methods were 2.2 ± 0.06% and 2.6 ± 0.03%, respectively, which indicated that the validation experiments were optional for correlation between the experimental and predicted pectin values.

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw materials

Thai soybean hulls (*Glycine max* (L.) Merr. cv. 'Chiang Mai 60'), as a by-product, will be obtained from a local soybean processing company, Kim Kin Pattana Co., Ltd. (Samut Sakhon, Thailand, 13.54714 N, 100.27434 E).

3.2 Chemicals

3.2.1 Methylene blue indicator, $C_{16}H_{18}ClN_3S$ (PA, Carlo Erba, France)

3.2.2 Boric acid, H_3BO_3 (PA, Carlo Erba, France)

3.2.3 Copper sulphate, Cu_2SO_4 (PA, Carlo Erba, France)

3.2.4 Carbazol, $C_{12}H_9N$ (PA, Sigma Aldrich Corp., USA)

3.2.5 Galacturonic acid, $C_6H_{10}O_7 \cdot H_2O$ (PA, Sigma Aldrich Corp., USA)

3.2.6 Gallic acid monohydrate (PA, Sigma Aldrich Corp., USA)

3.2.7 Methyl red indicator, $C_{15}H_{15}N_3O_2$ (ACS, Reag. Ph Eur, Merck, Singapore)

3.2.8 Folin-Ciocalteu reagent (PA, Sigma Aldrich Corp., USA)

3.2.9 Potassium sulphate, K_2SO_4 (PA, Carlo Erba, France)

3.2.10 Phenolphyhalein indicator (PA, Carlo Erba, France)

3.2.11 Deionised water (ELGA Labwater, United Kingdom)

3.2.12 95% ethanol (Merck KGaA, Darmstadt, Germany)

3.2.13 Sodium hydroxide, NaOH (PA, Merck KGaA, Darmstadt, Germany)

3.2.14 Hydrochloric acid, HCl (PA, RCI Labscan, Thailand)

3.2.15 Sulphuric acid, H_2SO_4 (PA, RCI Labscan Co., Ltd., Thailand)

3.2.16 Bromocresol green indicator, $C_{21}H_{14}Br_4O_5S$ (PA, Sigma Aldrich Corp., USA)

3.2.17 Hexane, C_6H_{14} (PA, Macron, Thailand)

3.2.18 Acetone, C_3H_6O (PA, Carlo Erba, France)

3.2.19 Antifoaming agent (PA, Sigma Aldrich Corp., USA)

3.2.20 Isopropa-2-ol (PA, RCI Labscan, Thailand)

3.3 Instruments and equipment

3.3.1 Aluminium foil (Diamond, 37.5 SQ.FT, Thailand)

3.3.2 Centrifuge (Hettich, REF 1401, Germany)

3.3.3 Chroma-meter (CR-400 Konica Minolta, Japan)

3.3.4 Electric stainless grinding machine (Multipurpose Disintregrator Li Xiang-010A)

3.3.5 Microwave pressure cooker (Prestige 57050-N, India)

3.3.6 Tray dryer (Progress electronic Ltd., Thailand)

3.3.7 Fourier Transform Infrared Spectrophotometer (FTIR) (Nicolet 6700, Thermo Scientific, Germany)

3.3.8 Hot air dryer (Binder, FD56, Germany)

3.3.9 Stainless steel mixing bowl (Zebra, Thailand)

3.3.10 Microwave oven (Samsung ME711K/XST)

3.3.11 Water bath (WNB 14, Memmert, Germany)

3.3.12 Vortex (G50E, Scientific industries, USA)

3.3.13 Rotary evaporator (Daihan, WEV-1001V, China)

3.3.14 UV-visible spectrophotometer (UV-1601, Shimadzu, Australia)

3.3.15 Temperature Data logger (Model DS1922E, LINGTEC Instruments Sdn. Bhd., Malaysia)

3.3.16 Glass bowl

3.3.17 Muslin cloth

3.3.18 Beaker (250, 600, 1,000 mL.)

3.3.19 pH meter (Metter Toledo, Switzerland)

3.3.20 Microwave oven (Samsung, ME711K, Japan)

3.3.21 Scanning Electron Microscope (SEM) (Quanta 250, FEI, USA)

3.3.22 Analytical balance (Santorius, Entris224i- 1S, Germany)

3.3.23 Precision Balance (Ohuaus, ARC 120, USA)

3.4 Methodology

3.4.1 Optimization of extraction conditions for producing pectin from soybean hulls by using pressurized intermittent microwave-assisted extraction and compare the effects of precipitation by either ethanol or propan-2-ol on pectin yield and its physicochemical properties

Soybean hulls (SBH) were washed twice in tap water to fully clean. Then, they were dried in a hot air oven (Binder, FD56, Germany) at 60 ºC for 6 h to the moisture content 8% (AOAC, 2000). The dried soybean hulls were called “soybean hull flakes (SHF)”. After that, SHF were ground and screened for particle size by passing through 50 mesh sieves, resulting

in soybean hulls powder (SHP). SHF and SHP were kept in vacuum polyethylene film bags and stored at 4^{o}C until required for analysis. Preparation steps for soybean hull flakes and powder are presented in Figure 3.1. Soybean hulls, soybean hull flakes and soybean hulls powder samples are illustrated in Figure 3.2.

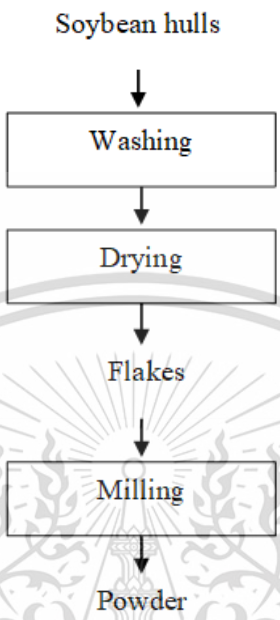


Figure 3.1 Preparation of soybean hulls

(A) Soybean hulls (B) Soybean hull flakes (C) Soybean hulls powder

Figure 3.2 Appearance of soybean hulls (SH), soybean hull flakes (SHF) and soybean hulls powder (SHP)

3.4.1.1 Proximate analysis of soybean hulls powder

Ash, protein, fat, total carbohydrate and fibre were analysed according to the method of AOAC (2000).

3.4.1.2 Key minerals using scanning electron microscopy – Energy-dispersive X-ray spectroscopy (SEM-EDS)

Minerals of soybean hulls samples were analysed, including C, N, P, K Ca, Na, Mg, K, Fe, and Mn. The microstructure of soybean powder samples was examined using a scanning electron microscope (SEM) (FEI, QUANTA-250, USA). Each powder sample was coated with gold in a sputter coater (Cressington sputter coater, 108auto, UK) before being scanned and photographed at 3,000×, equipped with an EDS Spectrometer.

3.4.1.3 Extraction of pectin from soybean hulls powder by pressurized intermittent microwave-assisted extraction and compare precipitation methods by either ethanol or propan-2-ol on pectin yield from soybean hulls and its physicochemical properties

10 g (dry basis) of soybean hulls powder (SHP) was mixed with deionized water at a ratio of 1:15 (w/v) in a 1 L glass bowl. Then, pectin solution samples were adjusted for pH by adding HCL 0.5 mol L^{-1} to pH level at 1.5, 2 or 2.5. Each sample was placed in a microwave pressure cooker and tested at three pulse ratios (1, 1.25 or 1.51) at three extraction times of 5, 10 and 15 minutes. After that, the pectin solution sample was filtered using a muslin cloth, followed by being centrifuged at 6000 rpm for 20 minutes. The supernatant was evaporated by a rotary evaporator until its volume reduced to 50% of the original volume. Extracted pectin was precipitated with an equal volume of 95% (v/v) ethanol (Figure 3.3) and was kept at 4°C for 48 h for extracted pectin turn to the coagulum. Pectin samples were dried in a hot air oven at 60°C until the moisture content was 7%. Extracted pectin was precipitated with an equal volume of 95% (v/v) ethanol or propan-2-ol at the ratio of extracted pectin to ethanol or propan-2-ol (1:2). The pectin solution of two solvents was kept at 4°C for 48 hours until they turned to coagulum, as shown in Figure 3.4 A. Coagulum shown in Figure 3.4 B from the soybean hulls from ethanol or propan-2-ol precipitation was dried in a hot air oven at 60°C until the moisture content was 8%, called crude pectin. The crude pectin samples from ethanol and propan-2-ol precipitation are shown in Figures 3.4 C and E, respectively. They were ground and screened for particle size to pass through 50 mesh sieves, as shown in Figure 3.4 E, and were kept in vacuum polyethylene film bags and then stored at 4^{o}C until required for analysis.

Pulse ratios were calculated following the equation of Soysal et al. (2009), as presented in Eq. 1.

$$\text{pulse ratio} = \frac{(\tau_{on}+\tau_{off})}{\tau_{on}} \quad \text{(Eq. 1)}$$

Where τ_{on} is the turn-on time (sec) of the microwave field, and τ_{off} is the turn-off time (sec) of the microwave field.

One cycle time was set to be $\tau_{on} + \tau_{off}$. When the pulse ratio = 1, it means that τ_{on} = 60 sec and τo_{ff} = 0 sec. (timing of power on during utilized in the PIMAE). When the pulse ratio = 1.25, it means that τ_{on} = 24 sec and τ_{off} = 6 sec. When the pulse ratio = 1.51, it means that τ_{on} = 20 sec and τ_{off} =10 sec.

3.4.1.4 Experimental design of Optimization condition for extracting pectin from soybean hulls powder

Response Surface Methodology coupled with Box-Behnken Design (BBD) was applied to optimize the extracting conditions for pectin from soybean hulls powder to produce pectin with the highest yield, using the statistical software Design Expert 7.0 (Stat-Easy Inc., Minneapolis, USA). The factors of this study were pH (X_1), pulse ratio (X_2) and extraction time (X_3). Each independent factor was coded at three levels: -1, 0, 1 (Table 3.1). The design scheme consisted of 12 treatments with five replications of centre points. Analysis of variance was employed to analyze the data and indicate the significance level at p<0.05. Experiment data were fitted to a second-order polynomial model (Eq. 2) in order to obtain a regression coefficient.

$$\text{Yield or DE (\%)} = \beta_0 + \sum_{i=1}^{a} \beta_i X_i + \sum_{i=1}^{a} \beta_{ij} X_i X_j + \sum_{i=1}^{a} \beta_{ii} {X_i}^2 \quad \text{(Eq. 2)}$$

Where β_0 was a constant coefficient, β_i, β_{ii} and β_{ij} were the coefficients of the linear, quadratic and interactive terms, respectively and X_i and X_j were coded variables.

Table 3.1 Processing condition variables and their ranges

Independent variables	Symbol	Coded levels		
		-1	**0**	**1**
pH	X_1	1.50	2	2.5
pulse ratio	X_2	1	1.25	1.51
Extraction time (min)	X_3	5	10	15

The highest pectin yield from soybean hulls powder in this study was selected for further study.

3.4.1.5 Analysis of physicochemical properties

3.4.1.5.1 Pectin yield (%)

Pectin yield (%) of each extraction condition was analysed according to the equation of Swamy and Muthukumarappan (2017), as shown in Eq 3.

$$\text{Yield (\%)} = \frac{weight\ of\ dried\ pectin\ (g)}{weight\ of\ dried\ soybean\ hulls\ powder\ (g)} \times 100 \quad \text{(Eq. 3)}$$

3.4.1.5.2 Moisture content

The moisture content was analysed, according to AOAC (2000)

3.4.1.5.3 Colour determination

Crude pectin samples were observed for colour in CIE Lab coordinates system (L*, a* and b*), which was tested directly by using a Minolta CR-400 colorimeter. The L* value represents lightness in a range from 0 (black) to +100 (white). The a* value represents a range from -100 (green) to +100 (red) and the b* value represents a range from -100 (blue) to +100 (yellow). The hue angle (H^*_{ab}) and chroma (C*) were also calculated by equations (Eq. 4) and (Eq. 5), respectively:

$$H^* = 180^O - \arctan^{b^*/a^*} \quad \text{(Eq. 4)}$$

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad \text{(Eq. 5)}$$

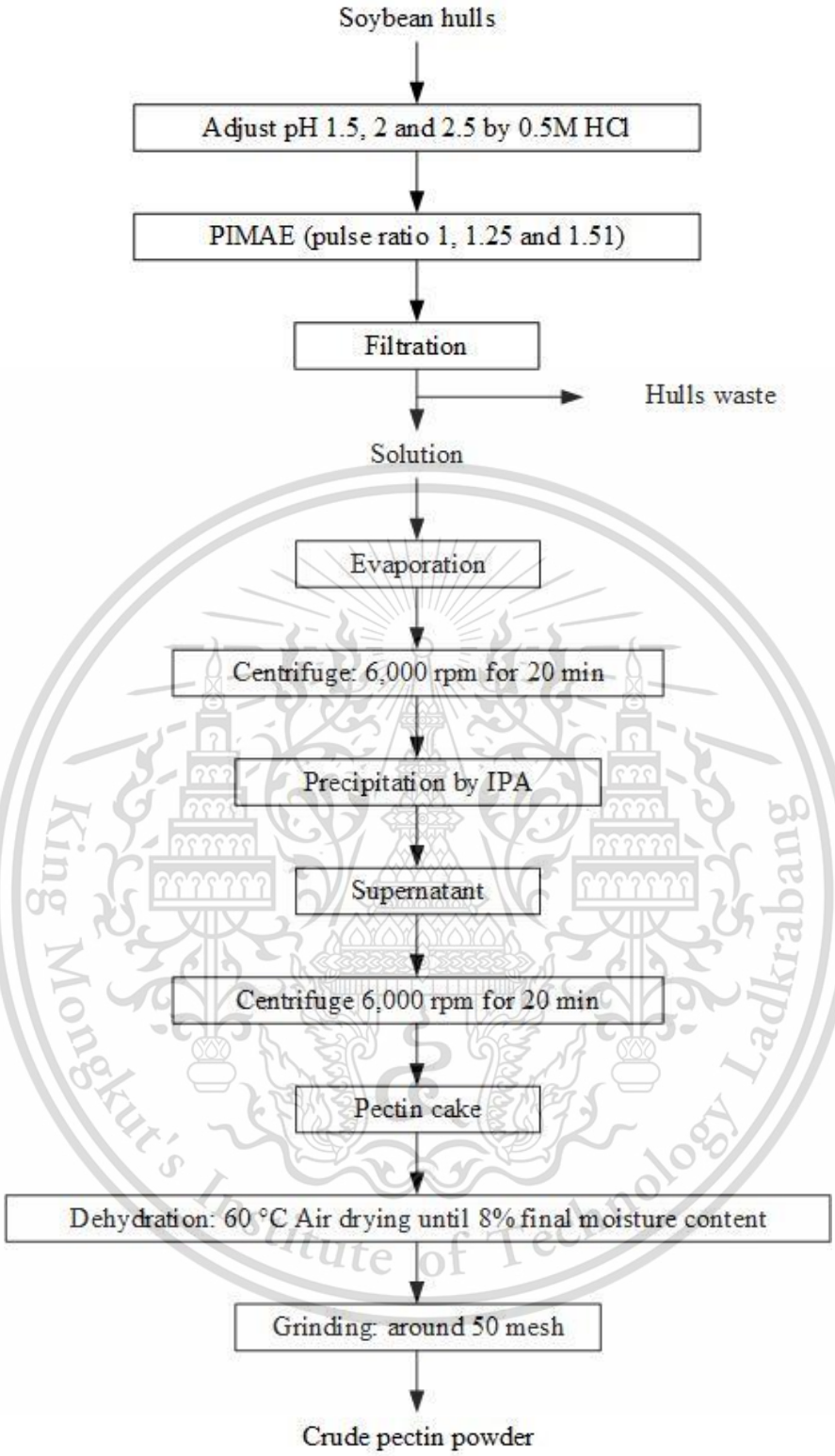
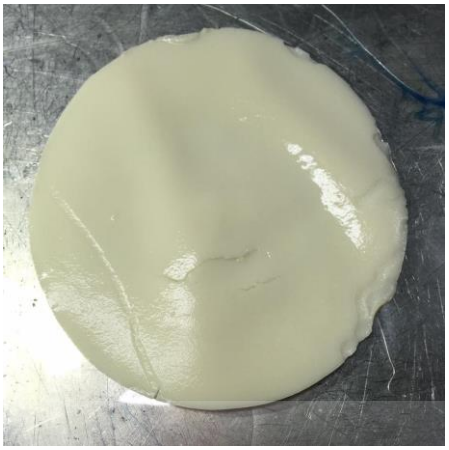
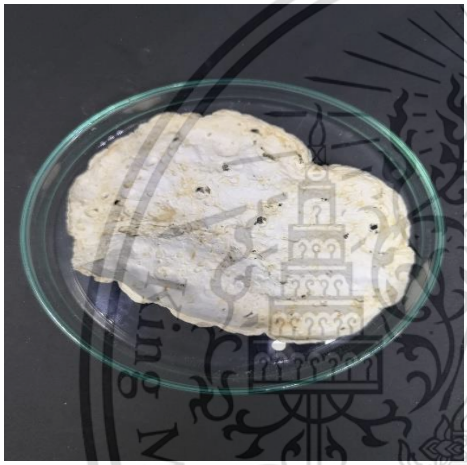


Figure 3.3 Flow diagram of pectin extraction from soybean hulls powder using pressurized intermittent microwave-assisted extraction

(A) Coagulum from soybean hulls extract in alcohol precipitation

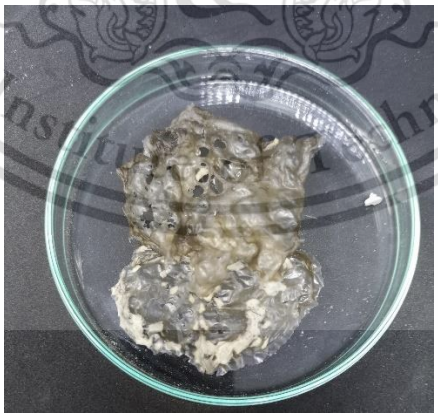


(B) Coagulum cake



(C) Crude pectin from propan-2-ol precipitation

(D) Crude pectin powder from propan-2-ol precipitation



(E) Crude pectin from ethanol precipitation

Figure 3.4 Appearance of coagulum and crude pectin coagulum from soybean hulls

3.4.1.5.4 Determination of galacturonic acid

Galacturonic acid was measured according to Rangana (1977). Dried pectin powder (100 mg) was dissolved in 100 mL of 0.05 N NaOH. Then, it stood for 30 minutes to de-esterify the pectin. 2 mL of this solution was pipetted into a volumetric flask and the volume was made up to 100 mL with DI water. After that, 2mL of de-esterified pectin solution was pipetted into a test tube and 1mL of 0.1% carbazole reagent was added. A white precipitate was formed. Then, 12mL of conc. H_2SO_4 was added with continuous stirring. The test tube was closed with a plastic cap and allowed to stand for 25 minutes to change the colour. 95% ethyl alcohol was used as a blank. Galacturonic acid content of the samples was measured by spectrophotometer (Shimadzu, UV-1601, Australia) at absorbance 525 nm. The sample was measured in triplicate and compared to the D-galacturonic acid standard curve (R^2 = 0.99 shown in Appendix A). Galacturonic acid content was calculated following equation (Eq. 6):

$$\text{GalA (\%)} = \frac{\mu\text{g of GalA in the aliquot x dilution x 100}}{\text{mL taken for estimation x wt.of soybean hulls pectin x 1,000,000}} \quad \text{(Eq. 6)}$$

3.4.1.6 Statistical analysis

Statistical analysis of this study was performed using SPSS version 16.0. The physicochemical properties of extracted pectin using ethanal and propan-2-ol precipitation were analysed using Independent t-test at a significance level $p<0.05$. The highest pectin yield of this study was selected for further study.

3.4.2 Optimization of soybean hull flakes and powder on pectin using pressurized intermittent microwave-assisted extraction

This study selected propa-2-ol for precipitation. Soybean hull flakes or powder was mixed with deionised water at a ratio of 1:20 (w/v). The Optimization of pectin is described in Section 3.4.1.3.

3.4.2.1 Pectin yield (%)

The analysis of pectin yield is described in Section 3.4.1.5.1.

3.4.2.2 Analysis of degree of esterification

The degree of esterification (DE) of pectin samples was determined by the titration method (Singthong et al., 2004) with slight modifications. First, dried pectin powder (500 mg) was placed in a 250 mL flask, to which 2 mL isopropyl alcohol and 100 mL deionised water were added. After dissolving the samples, five drops of phenolphthalein reagent were added and the solution titrated against NaOH (0.5 M). The end-point was recorded as the first titre or V_1. Subsequently, 10 mL of NaOH (0.5 M) was added for hydrolysis, after which the solution was stirred for 15 minutes. Subsequently, 10 mL of HCl (0.5 M) was added then the solution was stirred vigorously until the pink colour disappeared completely. After adding 5 drops of phenolphthalein reagent, the excess HCl was titrated against NaOH (0.5 M) until a pale pink colour was obtained. The volume of NaOH that was required was recorded as the second titre, or V_2.

The DE of pectin was calculated as the following equation (Eq. 7):

$$\text{DE } (\%) = \frac{V_2 \text{ (mL)}}{V_1(mL)+V_2(mL)} \times 100 \qquad \text{(Eq. 7)}$$

Where V_1 is the volume of the 1st NaOH titration, V_2 is the volume of the 2nd NaOH titration.

3.4.2.3 Statistical analysis

Statistical analysis of this study was repeatedly performed using Response Surface Methodology coupled with BBD, as described in Section 3.4.1, whereas extraction yield and degree of esterification of pectin were selected as response variables. The pectin extraction yield and degree of esterification were calculated from equation (Eq. 7). The highest pectin yield of this study was selected for further study.

3.4.3 Extraction and separation of pectin and cellulose from soybean hulls compared to pressurized intermittent microwave-assisted extraction (PIMAE) and water bath overhead stirrer extraction (WBOSE)

3.4.3.1. Pressurized intermittent microwave-assisted extraction

3.4.3.1.1 Acid digestion procedure

PIMAE (performed in three replicates) was conducted in order to compare the results obtained by the conventional extraction. The extraction was a soybean hull/solvent (DI water) ratio of 1:20 (g/mL) put into a glass bowl (size 1 L). HCl (0.5 mol/l) was added to bring the pH to 2.0, and then the glass bowl was placed into a microwave pressure cooker, in which the suspension was an extraction of the pulse ratio of 1 for 30 minutes.

3.4.3.1.2 Pectin purification procedure

After acid digestion, the pH was adjusted to 7 by NaOH (0.5 mol/l). After extraction, the supernatant was concentrated with a rotary evaporator and collect the centrifugation (6,000 rpm for 20 minutes). Precipitation was carried out by adding filtrate to the IPA at a ratio of 1:2 (filtrate: IPA, v/v). The mixture was stirred for 10 minutes at room temperature followed by storing at 4°C for 48 hours. Subsequently, the solid was dried at 60°C to obtain the crude pectin.

3.4.3.1.3 Alkaline digestion procedure

The hulls were digested by alkaline at a pH of 10 with the addition of NaOH (0.5 mol/l). The suspension was extracted at a pulse ratio of 1 for 30 minutes. The pH was adjusted to 7 by NaOH (0.5 mol/l) and by HCl (0.5 mol/l). After extraction, the acid digestion supernatant and pectin purification procedure appeared to be similar. The extraction and separation composition from the soybean hulls is shown in Figure 3.5.

3.4.3.1.4 Separation and dehydration procedure

After the acid-alkaline digestion, the hulls were washed with DI water thrice times. Then, the hulls were dehydrated by hot air at a temperature of 60°C for eight hours in a dehydrator to achieve the final moisture content in the range of 8-10. The dried hulls and crude pectin were ground in sizes ranging between 150- 250 μm as dietary fibre powder and crude pectin powder for analysis.

3.4.3.2 Water bath overhead stirrer extraction (WBOSE)

The extraction was performed by using soybean hull/solvent (DI water) with a ratio fixed at 1:20 (g/mL); HCl (0.5 mol/l) was added to bring the pH up to 2.0. The suspension was an overhead stirrer model HT-50 DX and stainless steel impeller (PL020)) (WNB 14, Memmert, Germany) (300 rpm) at 90°C for two hours in a water bath. The other steps are detailed in Sections 3.4.4.1.1 - 3.4.4.1.4.

3.4.3.3 Statistical analysis

This study was performed using SPSS version 16.0. The physico-chemical properties of the extracted pectin using PIMAE and WBOSE were analysed using an independent T-test at a significance level of $p<0.05$. Pectin and cellulose in this study were selected for further study.

3.4.3.4 Physico-chemical properties

3.4.3.4.1 Analysis of yield and moisture content.

The analysis of the yield of process and moisture content is calculated by using the following equation (Eq. 8) and described in 3.4.2.2.2, respectively.

$$\text{Yield (\%)} = \frac{weight\ (g)\ of\ dry\ sample}{weight\ (g)\ of\ initial\ sample} \times 100 \qquad \text{(Eq. 8)}$$

3.4.3.4.2 Colour determination

The determination of the colour is described in Section 3.4.2.2.3.

3.4.3.4.3 Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

The determination of the SEM is described in Section 3.4.1.2.

3.4.3.4.4 Water absorption index (WAI) and water solubility index (WSI) measurement

The WAI and WSI assay were carried out according to Yang et al. (2014). This was conducted by adding 2.5 g of soybean hulls powder or dietary soybean hulls powder into centrifuge tubes that were weighed separately with individual weights. Subsequently, 30 mL of distilled water was added to each tube and mixed well. The tubes were closed with a lid and incubated in a water bath at 25°C for 20 minutes. Then the tubes were centrifuged (5,000 rpm) at room temperature (25°C) for 10 minutes. Next, the supernatant was decanted from the tube without the sample powder. Finally, the tube and residues were weighed with the WAI being calculated by using the following equation (Eq. 9):

$$\text{WAI (\%)} = \frac{\text{Weight of the centrifuge tube and sample} - \text{Centrifuge tube (g)}}{\text{Weight of the sample powder (g)}} \times 100 \qquad \text{(Eq. 9)}$$

For preparing the WSI assay according to the WAI, the tubes were shaken for five minutes using a shaker mixer before being centrifuged,. The supernatant was poured into a glass petri dish of known weight. The sample was dehydrated at 40°C for 12 hours in a hot-air oven, and the dish was kept in desiccators to maintain the balance. Equation 10 shows the calculation of the WSI.

$$\text{WSI (\%)} = \frac{\text{Weight of the glass petri dish and lid and dried sample} - \text{Weight of the glass petri dish and lid (g)}}{\text{Weight of the sample (g)}} \times 100 \quad \text{(Eq.10)}$$

3.4.3.4.5 Fourier transform infrared spectroscopy

The sample powder was observed using the Fourier transform infrared (FTIR) spectra. The infrared spectra of the samples were observed using a Nicolet

6700, Thermo Scientific (Madison, Germany). The sample powder was transferred into the FTIR system within a wave number range of 4,000-400 cm^{-1} with 36 scans at 4 cm^{-1}.

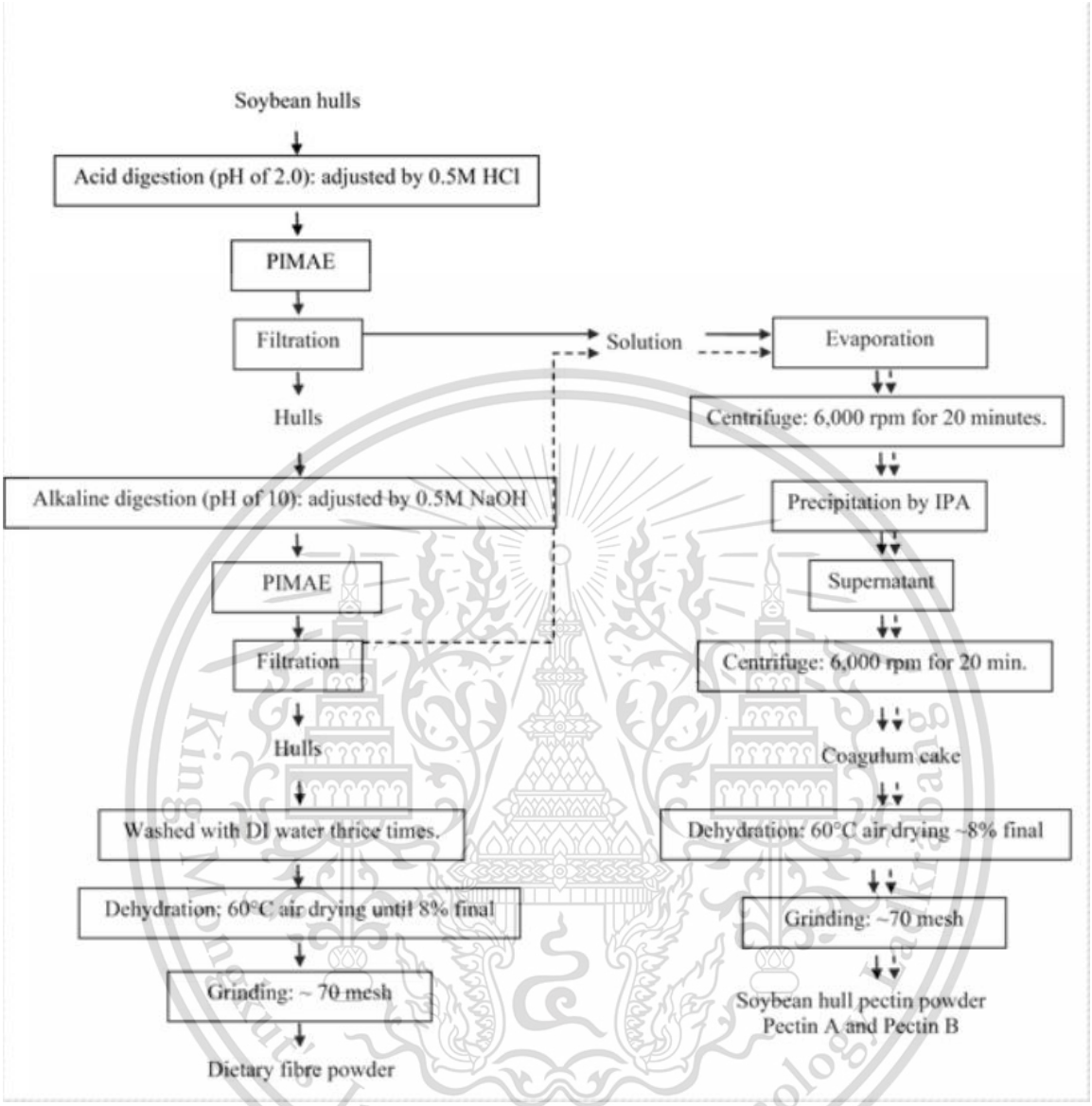


Figure 3.5 Diagram of extraction and separation composition from soybean hulls using pressurized microwave-assisted extraction.

3.4.3.4.6 Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectroscopy was performed with a Bruker Avance III HD 400 MHz spectrometer and magnet as Bruker UltraShield 400 MHz/54mm bore. 15 mg of soybean hulls pectin and commercial pectin were dissolved in 0.5 mL of D_2O as a solvent for the sample that was contained in a 5mm double resonance tube at 60ºC at a frequency of 400.13 MHz for the ^{1}H and using Topspin 3.5pl7 software for analysis.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Optimization of extraction conditions for producing pectin from soybean hulls by using pressurized intermittent microwave-assisted extraction and compare the effects of precipitation by either ethanol or propan-2-ol on pectin yield and its physicochemical properties

4.1.1 Proximate analysis of soybean hulls powder

The chemical composition of soybean hulls powder (*Glycine max* (L.) Merr. cv. 'Chiang Mai 60') used in the experiment and shown in Table. 4.1 was close to Liu et al. (2016), Porfiri and Wagner (2018) and Charudul, (2019). Results gave ~6.5% of moisture content, ~6% of ash, and 0.7% of fat, ~ 11% of crude protein, ~ 40% of crude fibre and ~ 36% of carbohydrate.

The moisture content from the experiment was used to calculate the dry weight of raw material for extraction of pectin from soybean hulls in section 4.2.

Table 4.1 Proximate analysis of soybean hulls powder

Proximate analysis	(Weight% dry basis)
Moisture content	6.47±0.27
Ash	5.98±1.73
Fat	0.71±0.18
Protein	10.97±0.31
Fibre	40.23±0.54
Carbohydrate	35.64±0.72

Data are means ± SD from three replicates.

4.1.2 Scanning electron microscopy

Microstructures of soybean hulls (SH), soybean hull flakes (SHF) and soybean hulls powder (SHP) from SEM are shown in Figure 4.1 A-C. Figure 4.1 A shows the smooth surface of the soybean hulls. After preparation, Figure 4.1 B shows that the palisade layer thickness reduced, characteristic of the cellulose mesh structure. After grinding into powder, Figure 4.1 C shows the small particle size of soybean hulls powder.

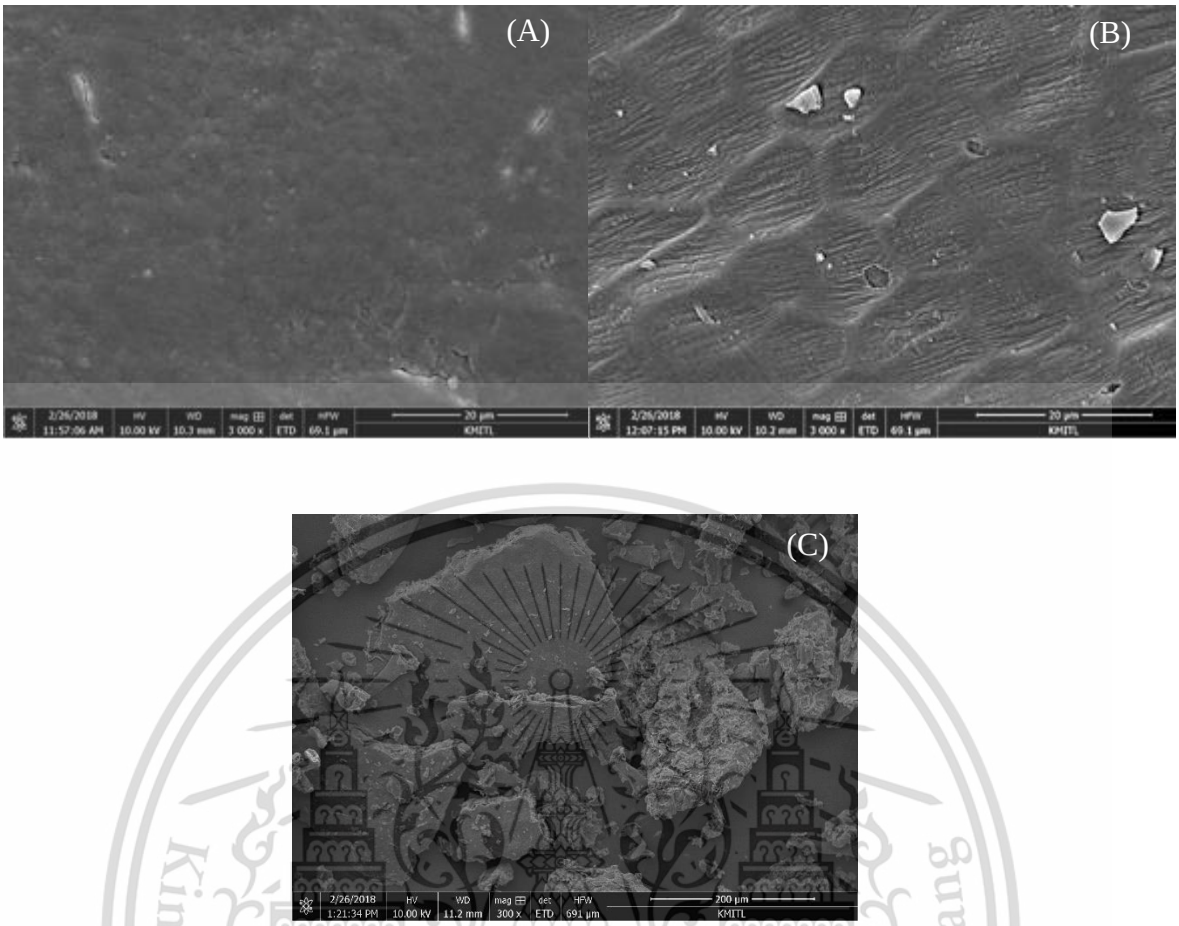


Figure 4.1 Scanning electron microscope images of soybean hulls (A), soybean hull flakes (B) and soybean hulls powder (C)

4.1.3 Key mineral identification using scanning electron microscopy-energy-dispersive X-ray spectroscopy

EDS measures the elemental composition of a sample (Starnes, 2009). There are three main components as primary, secondary and macronutrients. Figure 4.2 shows that C was the most common, followed by O, followed by small amounts of Na and Ca. Soybean hulls (A) were higher in C, O and K than soybean hull flakes (SHF) and soybean hulls powder (SHP). Table 4.2 shows that primary macronutrients included C (26.7-27.2%), O (71.8-72.5%), P (<0.2%) and K (0.2-0.3%). Secondary macronutrients included Ca (up to 0.4%) and S (<0.2%), while micronutrients included Br (up to 0.4%) Si (up to 0.5%) and Na (up to 0.4%).

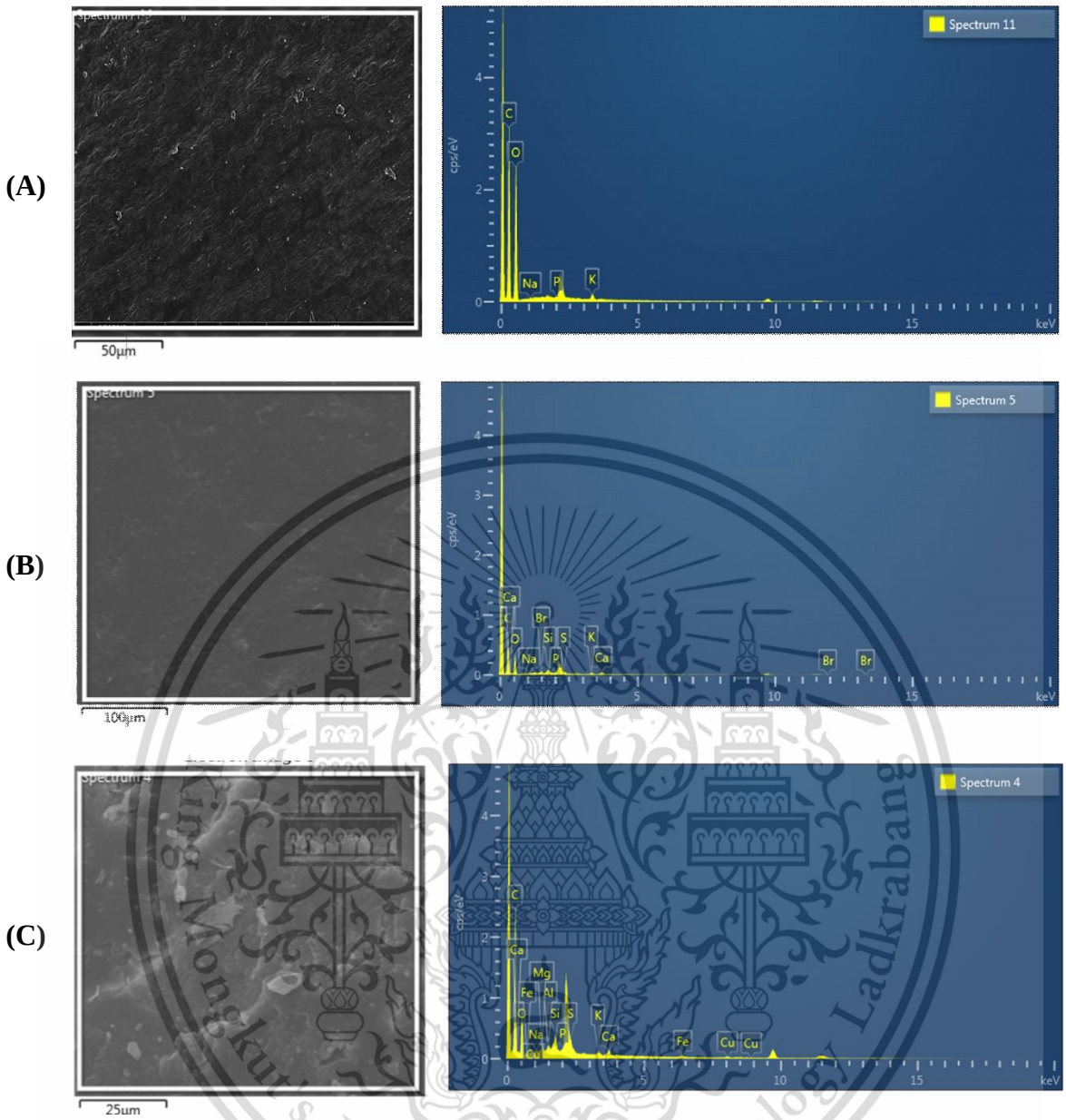


Figure 4.2 Energy dispersive X-ray spectroscopy images of soybean hulls (A), soybean hull flakes (B) and soybean hulls powder (C)

Table 4.2 Element composition using energy dispersive X-ray spectroscopy

Samples	Weight (%)								
	C	O	P	K	Ca	S	Br	Si	Na
Soybean hulls	27.2	72.5	<0.2	0.3	<0.2	-	<0.2	<0.2	<0.2
Soybean hulls flakes	26.7	71.8	<0.2	0.2	0.4	<0.2	0.4	0.5	<0.2
Soybean hulls powder	26.4	71.2	<0.2	0.2	0.3	<0.2	0.4	0.4	<0.2

4.1.4 Extraction from soybean hulls powder using pressurized intermittent microwave-assisted extraction

4.1.4.1 Data analysis and mathematical model assessment

When comparing the suitability of the model (ANOVA) with the linear model, the interactive model (2FI) and the quadratic model, the mathematical model for pectin extraction from soybean hulls by intermittent pressurized microwave-assisted extraction suited the quadratic model. The consideration was based on an R-squared value higher than those of other models (0.9326), as shown in Table 4.3. The quadratic model could also describe factors affecting pectin yield better than the other equation models.

Table 4.3 Comparison of the suitability of mathematical models

Source	Std. dev.	R-Squared
Linear	1.48	0.4884
Interactive	1.1	0.7835
Quadratic	0.73	0.9326

The results of ANOVA are presented in Appendix B. The model was significantly suitable ($p \leq 0.01$). Non significance was found in lack of fit ($p > 0.05$). R-squared was equal to 0.9326, Adj R-squared at 0.8458 showed model precision to predict the experimental results. Coefficient of variation (C.V.) was used to confirm model precision. The C.V. of the model was 9.3%, with adequate precision of 10.71 showing precision based on signal to noise ratio. If the ratio was greater than 4 the model was accepted (Maran, 2015; Senit et al., 2019) and could be used for prediction. Therefore, repetition the quadratic model was suitable to predict pectin yield from soybean hulls by pressurized intermittent microwave-

assisted extraction. This model was used to create a regression equation for predicting the relationship between the variables in the extraction and the expected yield, as shown by Equation 4.1

$$\text{Yield} = -63.63923+1.01266X_1 +25.95661X_2+61.75588X_3 +0.22600X_1X_2 -0.28431X_1 X_3-15.01961X_2X_3- -0.038630X_1^2-2.4930X_2^2-10.39216X_3^2 \quad \text{(Eq.4.1)}$$

Where, X_i is a coded independent factor (X_1= pH, X_2= pulse ratio and X_3= extraction time).

4.1.4.2 Experimental design and analysis

Table 4.4 shows a Box-Behnken design with three individual parameters and a total of 17 runs for optimizing. The values of the responses at different experimental combinations for the variables show that the actual pectin yield of soybean hull powder ranged between 4.8 and 10.9% and predicted values at 4.6 to 10.6% were obtained by solving models of Eq. 4.1. The predicted results were similar to the actual results with a residual error of not more than ± 1. The highest yields of both were from pH 1.5, pulse ratio 1 and extraction time 10 min.

Table 4.4 BBD matrix together with actual, predicted value and residual error on pectin yield of soybean hulls powder

Run	Independent variables			Pectin yield of soybean hulls powder (%)		
	X_1	X_2	X_3	Actual	Predicted	Residual error
1	1.5 (-1)	1.25(0)	15 (1)	8.7	8.7	0.0
2	2 (0)	1(-1)	15 (1)	9.1	9.3	-0.2
3	2 (0)	1.25(0)	10 (0)	9.7	8.9	0.8
4	2.5 (0)	1(-1)	10 (0)	6.7	6.1	0.6
5	2 (0)	1.25(0)	10 (0)	8.4	8.9	-0.5
6	2 (0)	1.25(0)	10 (0)	8.6	8.9	-0.3
7	2 (0)	1.51(1)	5 (-1)	4.8	4.6	0.2
8	1.5 (-1)	1.51(1)	10 (0)	4.8	5.3	-0.5
9	2.5 (0)	1.25(0)	5 (-1)	4.9	4.8	0.1
10	2.5 (0)	1.25(0)	15 (1)	8.9	9.3	-0.4
11	2 (0)	1.25(0)	10 (0)	8.5	8.9	-0.4
12	1.5 (-1)	1.25(0)	5 (-1)	6.9	6.5	0.4
13	1.5 (-1)	1(-1)	10 (0)	10.9	10.6	0.3
14	2 (0)	1.25(0)	10 (0)	9.5	8.9	0.6
15	2 (0)	1.51(1)	15 (1)	9.3	8.6	0.7
16	2 (0)	1(-1)	5 (-1)	6.0	6.7	-0.7
17	2.5 (0)	1.51(1)	10 (0)	8.2	8.6	-0.4

4.1.4.3 Model adequacy

Figure 4.3 presented the internally studentized residues versus normal % probability plot (A) and predicted versus the actual value of pectin extraction (B). Data were evaluated and the experimental data presented a good fit within the limit of the developed model, indicating its accuracy (Maran et al., 2015; Shivamathi et al., 2019).

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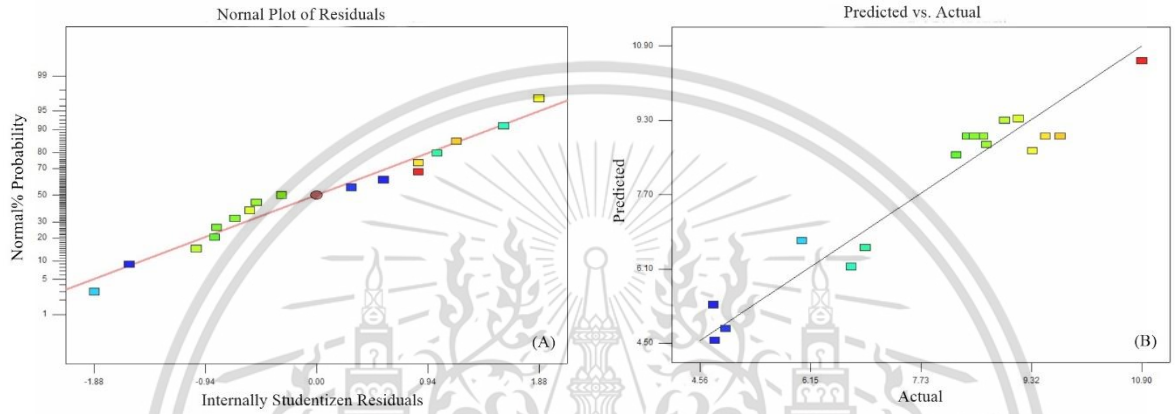
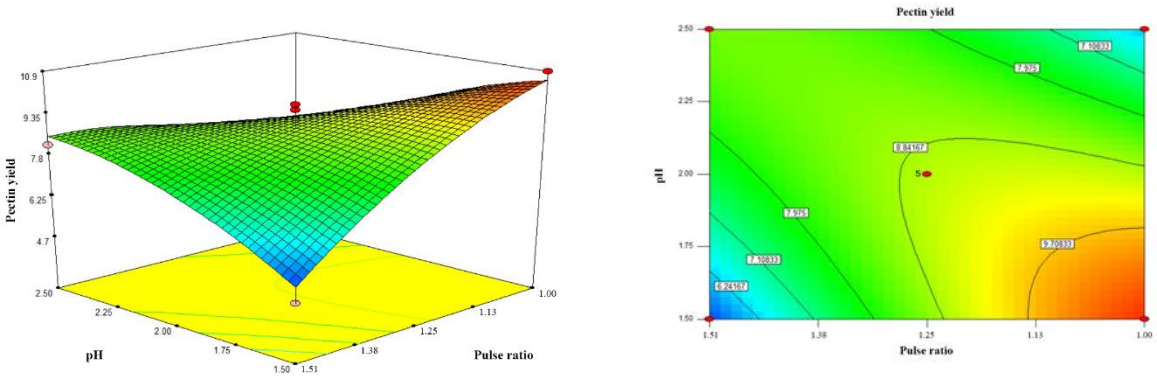


Figure 4.3 Diagnostic plots of internally studentized residuals vs. Normal % probability (A) and Predicted vs. Actual yield of soybean hulls pectin (B)

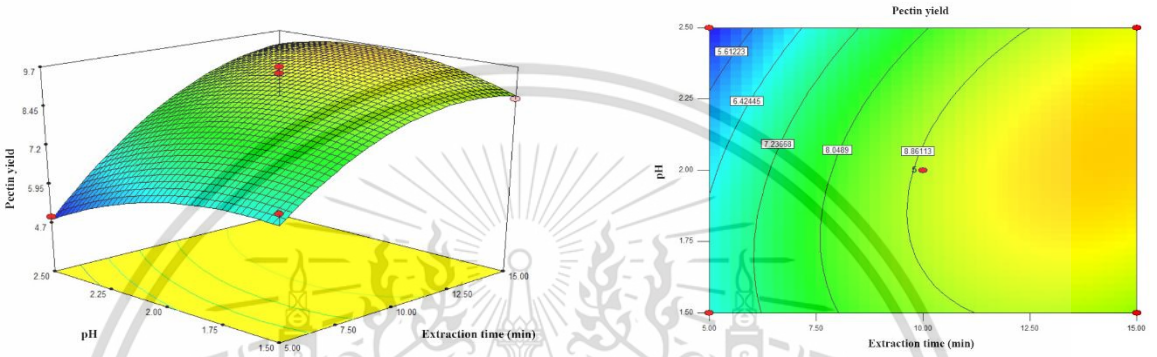
The quadratic model (Eq. 4.1), it was used to create 3D response surface methodology (RSM) and 2D contour plot to describe the relationships of the factors affecting the pectin yield by ANOVA (Appendix B1). The factors affecting pectin yield from soybean hulls by pressurized intermittent microwave-assisted extraction included pH, pulse ratio, pH vs extraction time and pulse ratio vs pulse ratio with significant difference ($p \leq 0.05$). However, extraction time did not affect pectin yield ($p > 0.05$).

According to Figure 4.4 (A) for pH levels used for extraction, low pH showed high acidity. Experimental results of pH 1.5 showed the maximum pectin extraction. This reduced at pH level of 1.5 because low pH levels used for extraction caused assembly of pectins in materials, resulting in lower dissolution (Faravash and Ashtiani, 2008; Prakash Maran et al., 2014). Figure 4.4 (B) showed that extraction duration increased pectin yield because longer duration increased interaction of the solvents on materials (Wong et al., 2010). Figure 4.4 (C) showed that higher extraction time and lower pulse ratio affected higher pectin yield because higher temperature increased heat transfer from the solvents on the material (Pasandide et al., 2017; Zhongdong et al., 2006).

(A)



(B)



(C)

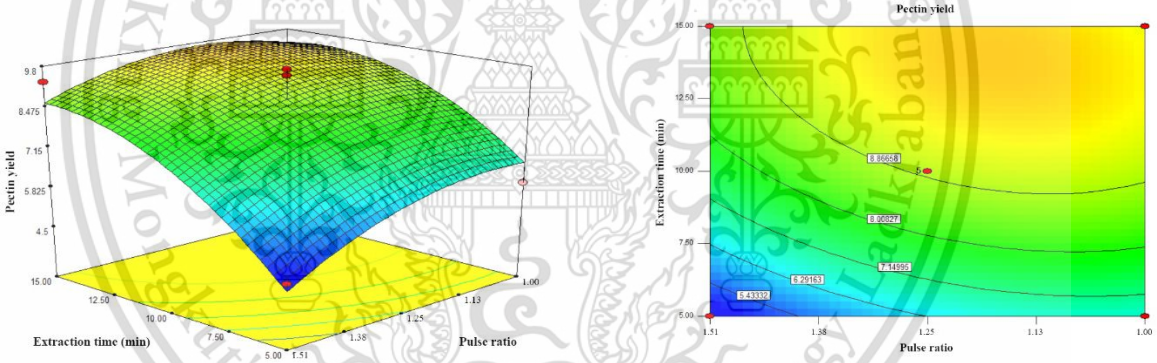


Figure 4.4 3D response surfaces and 2D contour plots for soybean hulls pectin yield pH vs pulse ratio (A), pH vs extraction time (B) and yield: pulse ratio vs extraction time (C)

Validation results at optimum parameters for targeting the maximum pectin yield are shown in Table 4.5, based on a suitable condition for pectin extraction from soybean hulls powder using pressurized intermittent microwave-assisted extraction. Maximum pectin yield was obtained at pH 1.5, pulse ratio 1 and extraction time ~12 min. The pectin amount obtained was 10.5%. This condition was used for the extraction, and experiments were conducted in triplicate to confirm pectin yield. The pectin values from the experiment were 10.5 ± 0.20%. Values from the prediction and from the actual experiment were similar. Therefore, the suitable

condition for extraction by pressurized intermittent microwave-assisted extraction was used in actual pectin extraction.

Table 4.5 Constraints and validation results at optimum parameters for targeting the maximum pectin yield

Parameters	Goal	Lower limit	Upper limit	Optimized condition	Actual value
pH	In range	1.5	2.5	1.5	1.5
pulse ratio	In range	1	1.51	1	1
Extraction time (min)	In range	5	15	11.9	12
Pectin yield	Maximize	4.75	10.9	10.72	10.5±0.2

Data are means ± SD from three replicates.

4.1.5 Comparison of pectin precipitation by ethanol and propan-2-ol on pectin yield and physicochemical of soybean pectin from soybean hulls

4.1.5.1 Pectin yield

The optimum conditions from Table 4.5 were extraction pH 1.5, pulse ratio 1 and extraction time 12 minutes. These conditions provided a pectin yield of 10.72%. This section compares the predicted and actual pectin yields from ethanol as 10.5 ± 0.02 and propan-2-ol as 11.7 ± 0.01 from different alcohol precipitations. Result of this precipitation showed that propa-2-ol was suitable for use in further studies. Physicochemical properties of the extracted pectin are shown in Table 4.6.

Table 4.6 Measured yields vs prediction at optimum conditions: pH 1.5, pulse ratio 1, extraction time 12 minutes

Treatment	Yield (%)
Predicted	10.7^{b}
Ethanol precipitation	10.5 ± 0.2^{b}
Propan-2-ol precipitation	11.7 ± 0.1^{a}

Data are means ± SD from three replicates. Within each column with different superscripts are significantly different ($p<0.05$).

4.1.5.2 Moisture content

Table 4.7 shows that moisture contents of pectins from precipitation by ethanol and propan-2-ol were not significantly different ($p \leq 0.05$). Moisture contents of pectins from soybean hulls precipitated by both types of alcohol were ~10%. The moisture contents of pectins from soybean hulls were similar to those of pectins from soybeans (9.29-11.11%), jackfruit peels (9.98%) and green jelly leaf (*Cyclea barbata* Miers) (9.40-10.05%) (Charudul, 2019; Koh et al., 2014; Yuliarti et al., 2017). The moisture contents of pectins from soybean hulls precipitated by both types of alcohol conformed to the standards of FDA and joint FAO/WHO expert committees on food additives (JECFA). These stipulated that moisture contents of pectins for food production must be below12% (JECFA, 2009) to prevent bacterial growth and pectin degeneration due to decomposition by pectinase enzymes (Ismail et al., 2012).

4.1.5.3 Determination of galacturonic acid (%)

Galacturonic acid is a key structure of pectins, linked by a glycosidic bond at α-1,4. A galacturonic acid amount is used to refer to the pectin amount in a structure and pectin purity (Liang et al., 2012). According to Table 4.7, the galacturonic acid amount of pectins from soybean hulls precipitated by ethanol (56.4±0.9%) and that of pectins from soybean hulls precipitated by propan-2-ol (50.8±1.2%) were significantly different ($p \leq 0.05$). Extracted pectins from soybean hulls contained lower galacturonic acid than commercial pectins, in which galacturonic acid is fixed at 65% up. Pectins are pure and can be used in the food industry. The galacturonic acid amount from soybean hulls was less pure than commercial pectins because soybean hulls contained high protein and fibres, resulting in high temperatures of the extraction process. Other components in the soybean hulls structure were decomposed into smaller molecules, e.g., protein, cellulose and hemicellulose; and then precipitated down with pectins in the precipitation step by alcohol (Wang et al., 2014). The galacturonic acid amount of extracted pectins from soybean hulls by pressurized intermittent microwave-assisted extraction was similar to that of extracted pectins from soybean hulls by the traditional extraction method. The amounts were 54.98-72%, amounts of autoclave extraction were 57.40±0.04% and amounts of microwave pressure cooker extraction were 69.10±1.68% (Charudul, 2019; Gnanasambandam and Proctor, 1999; Kalapathy and Proctor, 2001). Extracted pectins obtained conformed to the regulations of FDA as greater than or equal to 35%.

4.1.5.4 Determination of colour

From the results in Table 4.7, pectins from soybean hulls precipitated by ethanol and pectins from soybean hulls precipitated by propan-2-ol were significantly different ($p \leq 0.05$). According to the experiment, pectins precipitated by ethanol and pectins from soybean hulls precipitated by propan-2-ol had L* or brightness of 54.8±0.8 and 94.2±0.4. The brightness values of both types of pectins were very different. Specifically, pectins from soybean hulls precipitated by propan-2-ol were brighter than those precipitated by ethanol.

Hue showed values of the samples based on degrees whereby 0° referred to the red zone, 90° referred to the yellow zone, 180° referred to the green zone and 270° referred to the blue zone. When the values of hue fell into any degrees of the colour zones, the colours of the measured samples were acknowledged. Pectins precipitated by ethanol had a similar hue of 63.7±0.9, with orange-brown tone. By contrast, pectins from soybean hulls precipitated by propan-2-ol had a hue of -9.5±0.7 with a white tone.

Chroma implied intensity. Results from the experiment showed that values of intensity of pectins precipitated by ethanol were 16.9±0.3, whereas those of pectins from soybean hulls precipitated by propan-2-ol were 5.3±0.2. Pectins precipitated by ethanol had the maximum intensity. The colours of extracted pectins from soybean hulls were orange-brown, similar to those of pectins from soybean hulls by microwave pressure cooker extraction (Charudul, 2019) and from jackfruit peels (Koh et al., 2014).

Table 4.7 Physicochemical properties of soybean hulls pectin

Treatment	Alcohol precipitation	
	Ethanol	Propan-2-ol
Moisture (%)^{ns}	10.2±0.7	10.4±0.6
GalA (%)	56.4±0.9^{a}	50.8±1.2^{b}
Color		
L*	54.8±0.8^{b}	94.2± 0.4^{a}
Hue	63.7±0.9^{a}	-9.5±0.7^{b}
Chroma	16.9±0.3^{a}	5.3±0.2^{b}

Data are means ± SD from three replicates. ^{ns} Moisture contents were not significantly different ($p > 0.05$). Different superscripts within each row are significantly different ($p \leq 0.05$).

4.1.5.5 Scanning electron microscopy

Figure 4.5 shows morphological characteristics by microstructural analysis from SEM micrographs, with pectins from soybean hulls precipitated by ethanol (Figure 4.5A) and pectins from soybean hulls precipitated by propan-2-ol (Figure 4.5B). The surface had a smooth structure, similar to pectin from apple pomace (Dranca et al., 2020), dragon fruit peels (Rahmati et al., 2019) and pistachio green hull with ethanol (Kazemi et al., 2019). Pectin from propan-2-ol precipitation had a rougher structure, similar to pectin from mangosteen rind (Wathoni et al., 2019), fig skin (Gharibzahedi et al., 2019) and eggplant calyces (Kazemi et al., 2019). The pectin also had a surface with relatively smaller particles (5000×) compared to pectin particles precipitated by ethanol (1000×).

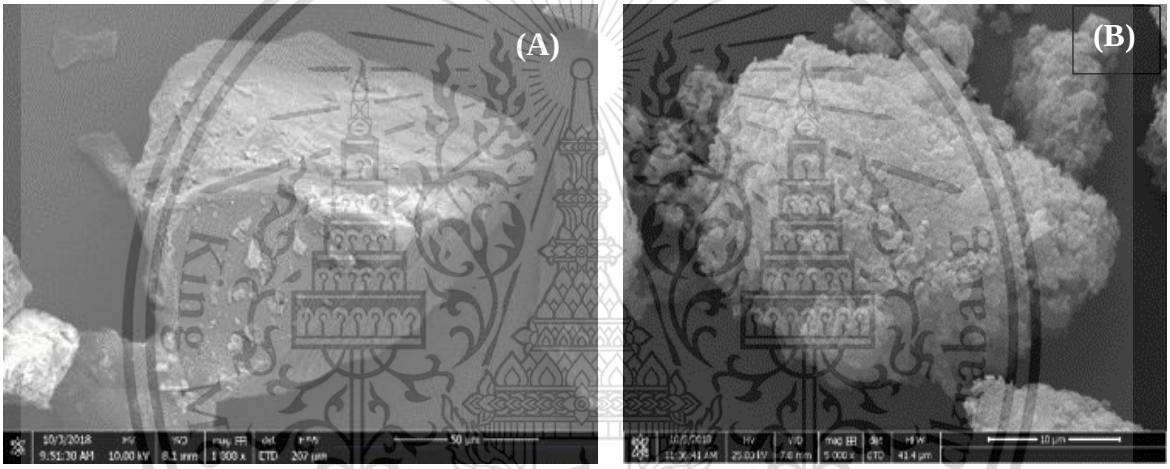


Figure 4.5 Scanning electron microscope images of pectin from soybean hulls precipitated by ethanol (A) and pectin from soybean hulls precipitated by propa-2-ol (B)

Pectin extract using propan-2-ol precipitation under the optimal conditions was lighter and whiter in colour. This could improve its acceptability. Propan-2-ol from the experiment was used to precipitate pectin extract from soybean hulls in section 4.2.

4.2 Optimization of soybean hull flakes and powder on pectin using pressurized intermittent microwave-assisted extraction

This study selected alcohol precipitation of propa-2-ol from the previous section. Soybean hull flakes or powder were mixed with deionised water at a ratio of 1:20 (w/v).

4.2.1 Data analysis and mathematical model assessment

According to Table 4.8, analysis by comparing the suitability of the model (ANOVA) with the linear model, interactive model (2FI) and quadratic model, showed that the mathematical model for pectin extraction and degree of esterification (DE) from soybean hull flakes (SHF) and powder (SHP) using pressurized intermittent microwave-assisted extraction suited the quadratic model. The consideration was based on the R-squared value of all of the treatments higher than those of other models (0.9734-0.9895). The quadratic model could also describe the factors affecting pectin yield better than the other equation models.

Table 4.8 Comparison of the suitability of mathematical models

Source	STD. dev.			R-Squared		
	Linear	Interactive	Quadratic	Linear	Interactive	Quadratic
Pectin of SHF	0.72	0.59	0.27	0.8576	0.9265	0.9895
DE of SHF pectin	1.68	1.74	0.72	0.7321	0.7795	0.9734
Pectin of SHP	0.51	0.54	0.25	0.8805	0.8946	0.9847
DE of SFP pectin	2.04	1.82	0.77	0.703	0.8184	0.9774

According to Table 4.9, the model of all treatments was significantly suitable ($p \leq 0.05$). R-squared at greater than 0.97. Adj R-squared of SHF pectin = 0.98, DE of SHF pectin = 0.97, SHP pectin = 0.98 and DE of SHP pectin = 0.98, showing model precision to predict the experimental results. Coefficient of variation (C.V.) was used to confirm model precision. C.V. of the model of SHF pectin = 6.32%, DE of SHF pectin = 0.98%, SHP pectin = 2.43% and DE of SHP pectin = 1.05%, while adequate precision of the model of SHF pectin = 29.47, DE of SHF = 18.25, SHP pectin = 23.59 and DE of SHP pectin = 27.90 showing precision based on signal to noise ratio. If the ratio was greater than 4 then the model was accepted (Maran et al., 2015; Senit et al., 2019) and could be used for prediction. Therefore, the quadratic model was suitable to predict the pectin yield and degree of esterification (DE) from soybean hull flakes (SHF) and powder (SHP) by pressurized intermittent microwave-assisted

extraction. This model was used to create a regression equation to predict the relationship between the variables in the extraction and the expected yield, as shown in Equations 4.2-4.5. The results of ANOVA and non-significance showed lack of fit ($p > 0.05$) and are presented in Appendix B 2-5.

4.2.2 Experimental design and analysis

Table 4.10 shows the pectin yield. Replicated central point of soybean hull flakes from actual ranged between 0.5 to 6.4% and 4.2 to 4.7% respectively, and predicted 0.4 to 6.4% obtained by solving models of (Eq. 4.2). The highest yields of both were from pH 2, pulse ratio 1 and extraction time 15 min. DE and replicated central point of SHF actual pectin ranged between 70.5 and 80.8% and 70.5 to 72.6%, while predicted values of 70.1 to 80.1% were obtained by solving models of (Eq. 4.3). The highest DE of both were from pH 2.5, pulse ratio 1.25 and extraction time of 5 min. The predicted results of pectin yield from SHF and DE of SHF pectin were similar to the actual results with a residual error of not more than ± 0.3.

Pectin yields of SHP and DE of SHP pectin are shown in Table 4.11. Pectin yield and replicated central point of SHP from actual ranged between 7.8 and 12.1% and from 10.3 to 10.9% respectively, while predicted values of 7.7 to 12.2% were obtained by solving models of (Eq. 4.4). The highest yields of both were from pH 2, pulse ratio 1 and extraction time 15 min. DE and replicated central point of SHP pectin from actual ranged between 70.5 and 80.8% and 70.4 to 72.4% respectively, while predicted values of 70.1 to 80.1% were obtained by solving models of (Eq. 4.5). The highest DE of both were from pH 2.5, pulse ratio 1.25 and extraction time of 5 min. The predicted results of pectin yield from SHF and DE of SHF pectin were similar to the actual results, with a residual error of pectin yield from SHF and DE of SHF at not more than ± 0.5 and ±1.2 respectively. Predicted results were similar to the actual results.

Therefore, particle size affected pectin yields. Yields increased with decreasing particle size, suggesting that decrease in particle size developed extraction of pectin by grinding and breaking the cell walls, thus allowing compounds to easily pass out of the cells. Similar results were previously reported for the extraction of pectin from sugar beet pulp, where different particle sizes were achieved by traditional heating methods (Huang et al., 2018). In addition, pectin extraction using the instantaneous controlled pressure drop process from the expanded vegetal matrix affected extraction. This was previously shown for orange peel (Prakash Maran et al., 2014; Rezzoug et al., 2008) and supported the current results.

4.2.3 Model adequacy

Appendix C presents the internally studentized residues versus normal % probability plot (a) and predicted versus the actual value of pectin extraction (b) of pectin yield and DE from SHF and SHP pectins. All the experimental data presented a good fit within the limits and showed the effects of linear and quadratic terms that depicted the developed model by indicating its accuracy.

The quadratic model in Equations 4.2-4.5 was used to create 3D response surface methodology (RSM) and 2D contour plots. These are presented in Figures 4.6-4.9 and describe the relationships between the factors affecting pectin yield by ANOVA as presented in Appendix B2-B5. The analysis determined the factors affecting pectin yield and degree of esterification from soybean hull flakes and powder by pressurized intermittent microwave-assisted extraction.

The model results of pectin yield from SHF were highly significant ($p < 0.0001$). The main interaction effects on pH (X_1), pulse ratio (X_2) and extraction time (X_3) showed that pH vs extraction time was significant ($p \leq 0.05$), confirming that the model was significant; however, pH vs pulse ratio and pulse ratio vs extraction time did not affect pectin extraction yield ($p > 0.05$). The results of ANOVA showed that for the DE from SHF pectin, the model was significant at $p \leq 0.0001$ (Appendix B3). The main effects of pH (X_1), pulse ratio (X_2) and extraction time (X_3) were significantly different ($p \leq 0.05$). Interactions of pH vs pulse ratio (X_1X_2), pH vs extraction time (X_1X_3) and pulse ratio vs extraction time (X_2X_3) did not affect pectin yield ($p > 0.05$) on the DE of SHF pectin.

The results of the model pectin yield from SHP showed that the main effects of pH (X_1), pulse ratio (X_2) and extraction time (X_3) were highly significant ($p < 0.0001$). Interactions between the main effects gave significance at $p > 0.05$. The results of ANOVA showed that for DE from SHP pectin, the model was significant at $p \leq 0.0001$ (Appendix B5). The main effects and interactions for pH vs pulse ratio (X_1X_2) and pH vs extraction time (X_1X_3) were significantly different ($p \leq 0.05$). However, pulse ratio vs extraction time (X_2X_3) did not affect pectin yield ($p > 0.05$) on the DE of SHP pectin.

The proposed regression model also gave satisfactory results for SHF pectin yield, DE of SHF pectin, SHP pectin yield and DE of SHP pectin with high values of R^2 (0.99, 0.97, 0.98 and 0.98) and adj-R^2 (0.98, 0.95, 0.96 and 0.95) respectively. The selected model showed that the actual relationships between the response and independent variables had good correlations.

The lack of fit for the f-value of SHF pectin yield, DE of SHF pectin, SHP pectin yield and DE of SHP pectin at 2.82, 0.13, 0.68 and 0.75 respectively indicated that they were not statistically significant ($p > 0.05$) relative to the pure error. This was 17.08, 93.45, 60.67 and 57.56% respectively and the results could have occurred due to noise.

Table 4.9 Reduced response model and statistical parameters obtained from ANOVA for central composite design

Response	Regression model		Model *p* value	C.V. (%)	Adjusted R^2	Adequate precision
Pectin yield of SHF (%)	$= +16.315-4.32669X_1-10.90096X_2+0.099867X_3-1.78431X_1X_2+ 0.3270X_1X_3+0.23333X_2X_3+0.5590X_1^2+3.45636X_2^2-0.032410X_3^2$	(Eq.4.2)	< 0.0001	6.32	0.98	29.47
DE of SHF (%)	$= +80.032-5.63818X_1 -0.87814X_2-0.85191X_3 -5.05882X_1 X_3 -0.2860X_1X_3 -0.65294X_1^2 +4.5530X_2^2 +8.31603+0.08223X_3^2$	(Eq. 4.3)	0.0001	0.98	0.97	18.25
Pectin yield of SHP (%)	$= +1.25\ (0)60.34271X_1+11.79154X_2+0.93433X_3+0.76471X_1X_2 +0.049X_1X_3-0.21373X_2X_3-0.6830X_1^2-5.70165X_2^2-0.024830X_3^2$	(Eq.4.4)	< 0.0001	2.43	0.98	23.59
DE of SHP (%)	$=+131.585-23.06028X^1-70.64179X^2 +0.2284X^3+11.96078X^1X^2 -0.6090X^1X^3-0.61569X_2X_3+4.9330X_1^2+22.92580X_2^2+0.062330X_3^2$	(Eq.4.5)	< 0.0001	1.05	0.95	27.90

Where, X_i is a coded independent factor (X_1 = pH, X_2 = pulse ratio and X_3 = extraction time).

Table 4.10 BBD matrix with actual and predicted values and residual error on pectin yield and degree of esterification of soybean hull flakes

Run	Independent variables			Pectin yield of soybean hull flakes (%)			Degree of esterification (%)		
	X_1	X_2	X_3	Actual	Predicted	Residual error	Actual	Predicted	Residual error
1	2.5 (1)	1 (-1)	10 (0)	5.6	5.4	0.2	75.2	75.2	0.0
2	2.5 (1)	1.25 (0)	15 (1)	5.8	6.0	-0.2	73.3	73.2	0.1
3	1.5 (-1)	1.51 (1)	10 (0)	4.5	4.7	-0.2	73.6	73.6	0.0
4	2 (0)	1.25 (0)	10 (0)	4.5	4.4	0.1	72.4	72.0	0.4
5	2 (0)	1.25 (0)	10 (0)	4.7	4.4	0.3	70.5	72.0	-1.6
6	2 (0)	1 (-1)	5 (-1)	2.7	3.0	-0.3	75.8	76.0	-0.2
7	1.5 (-1)	1.25 (0)	5 (-1)	3.3	3.1	0.2	75.7	75.8	-0.1
8	2 (0)	1.25 (0)	10 (0)	4.4	4.4	0.0	72.2	72.0	0.2
9	2 (0)	1.25 (0)	10 (0)	4.3	4.4	-0.1	72.6	72.0	0.6
10	1.5 (-1)	1.25 (0)	15 (1)	5.4	5.5	-0.1	71.1	71.3	-0.2
11	2.5 (1)	1.51 (1)	10 (0)	3.1	3.1	0.0	75.3	75.6	-0.3
12	2 (0)	1 (-1)	15 (1)	6.4	6.4	0.0	71.5	71.6	-0.1
13	2 (0)	1.25 (0)	10 (0)	4.2	4.4	-0.2	72.6	72.0	0.5
14	2 (0)	1.51 (1)	5 (-1)	0.6	0.7	-0.1	79.5	79.3	0.1
15	2 (0)	1.51 (1)	15 (1)	5.5	5.2	0.3	71.9	71.7	0.2
16	2.5 (1)	1.25 (0)	5 (-1)	0.5	0.4	0.1	80.8	80.6	0.2
17	1.5 (-1)	1 (-1)	10 (0)	6.0	6.0	0.0	70.8	70.6	0.3

Table 4.11 BBD matrix with actual and predicted values and residual error on pectin yield and degree of esterification of soybean hulls powder

Run	Independent variables			Pectin yield of soybean hull powder (%)			Degree of esterification (%)		
	X_1	X_2	X_3	Actual	Predicted	Residual error	Actual	Predicted	Residual error
1	2.5 (1)	1 (-1)	10 (0)	10.1	10.1	0.0	73.8	74.0	-0.3
2	2.5 (1)	1.25 (0)	15 (1)	10.7	10.6	0.1	72.9	72.7	0.3
3	1.5 (-1)	1.51 (1)	10 (0)	10.1	10.1	0.0	71.1	70.8	0.3
4	2 (0)	1.25 (0)	10 (0)	10.9	10.8	0.1	72.4	71.2	1.2
5	2 (0)	1.25 (0)	10 (0)	10.9	10.8	0.1	71.4	71.2	0.2
6	2 (0)	1 (-1)	5 (-1)	8.8	8.9	-0.1	75.5	74.9	0.6
7	1.5 (-1)	1.25 (0)	5 (-1)	9.5	9.6	-0.1	72.1	72.3	-0.2
8	2 (0)	1.25 (0)	10 (0)	10.8	10.8	0.1	71.4	71.2	0.2
9	2 (0)	1.25 (0)	10 (0)	10.9	10.8	0.1	70.5	71.2	-0.7
10	1.5 (-1)	1.25 (0)	15 (1)	11.9	12.0	-0.1	70.5	70.2	0.3
11	2.5 (1)	1.51 (1)	10 (0)	8.5	8.7	-0.2	79.8	79.5	0.4
12	2 (0)	1 (-1)	15 (1)	12.1	12.2	-0.1	71.3	71.3	0.0
13	2 (0)	1.25 (0)	10 (0)	10.3	10.8	-0.5	70.4	71.2	-0.8
14	2 (0)	1.51 (1)	5 (-1)	8.0	7.9	0.1	78.8	78.9	0.0
15	2 (0)	1.51 (1)	15 (1)	10.1	10.0	0.1	71.5	72.1	-0.6
16	2.5 (1)	1.25 (0)	5 (-1)	7.8	7.7	0.1	80.6	80.9	-0.3
17	1.5 (-1)	1 (-1)	10 (0)	12.1	11.9	0.2	71.1	71.5	-0.4

According to Figures 4.6 (A-B) and 4.8 (A-B), the effects of pH levels used for extraction on the yield of SHF and SHP show that pH 1.5 obtained the highest yield, while yield decreased slightly with a pH increase of 2 to 2.5. Hamidon and Zaidel (2017) showed that disruption of hydrogen bonds and ester linkages between pectin and the cell walls was triggered by low pH and the rate of diffusion of the pectin increased. Moreover, the molecular weight of pectin was reduced by low pH that caused partial extraction from the tissues of the plant (Prakash et al., 2014).

The effect of pulse ratio in Figure 4.6 A, C and Figure 4.8 A, C showed that a pulse ratio equal to 1 obtained the highest yield, while yield decreased slightly with pulse ratio increase from 1.25 to 1.51. The increased pulse ratio was able to facilitate the dissociation of the pectin into the solvent as previously reported due to the higher on/off ratios resulting from the relatively long 'on' periods that caused the temperature to suddenly rise and the internal pressure within the plant cells to increase. This caused swelling and, consequently, the walls of the cells broke open (Swamy and Muthukumarappan, 2017; Prakash et al., 2014).

Yield of pectin at extraction time of 15 min (Figure 4.6 B, C and Figure 4.8 B, C) was highest and decreased as extraction time decreased. Prakash et al. (2013) showed that as the absorption of microwave energy increased, dissolution was promoted and yield steadily increased due to thermal accumulation within the extraction solution, resulting from increased extraction time.

The DE of SHF and SHP pectin is shown in Figures 4.7 and 4.9. Higher levels of DE at 80% were obtained for pH 2.5, with higher pulse ratio and reduced extraction time. The de-esterification of galacturonic acid chains was the likely cause of reduction in the DE of pectin at lower pH, pulse ratio and short extraction time. These findings concurred with previous research by Pasandide et al. (2017) and Hosseini et al. (2019). The DE of SHF and SHP was between 70 and 80%; therefore, both pectins can be considered to be high methoxyl pectin (HMP).

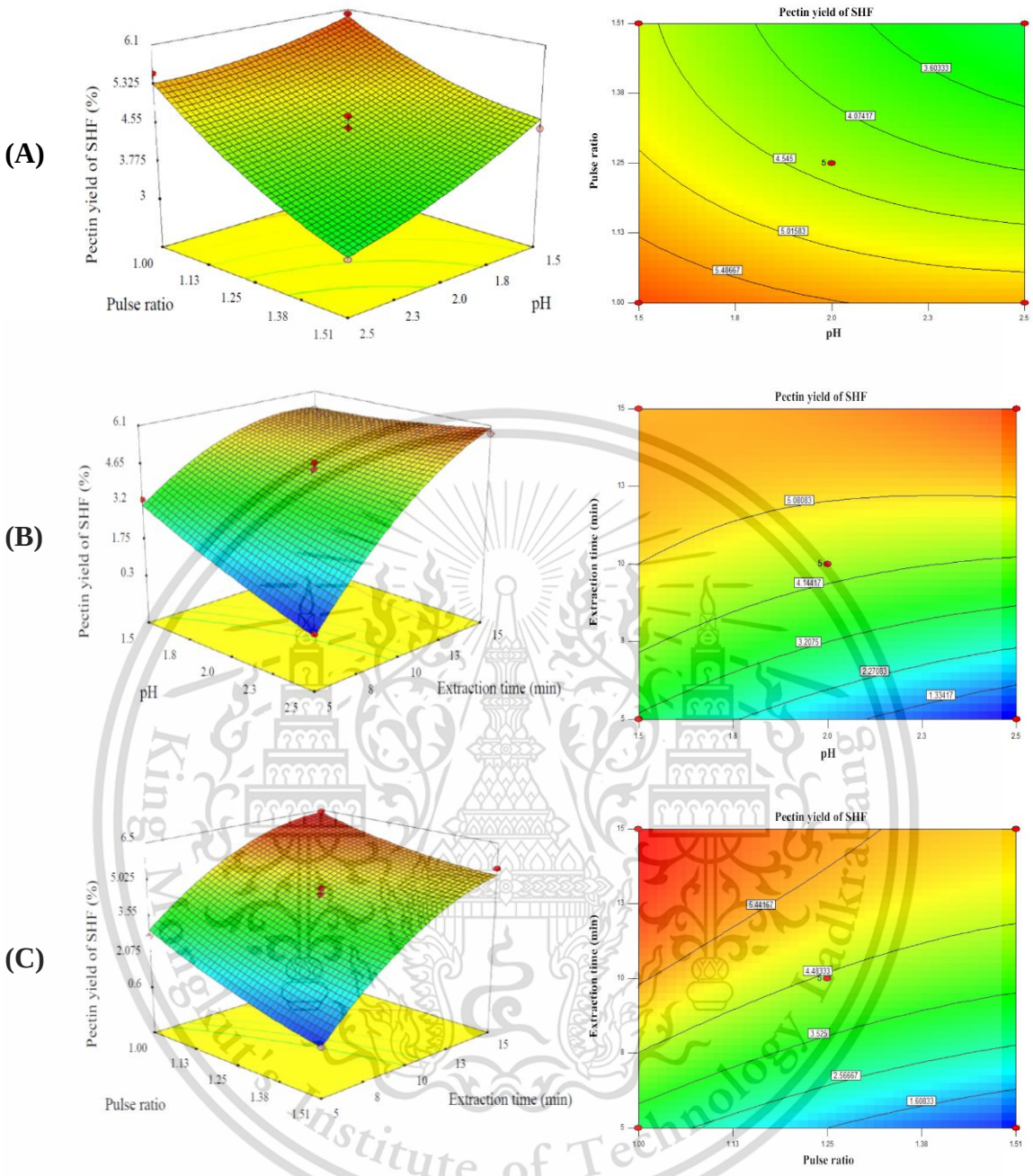


Figure 4.6 3D response surfaces and 2D contour plots for soybean hulls pectin yield from SHF, with yield: pH vs pulse ratio (A), yield: pH vs extraction time (B) and yield: pulse ratio vs extraction time (C)

(A)

(B)

(C)

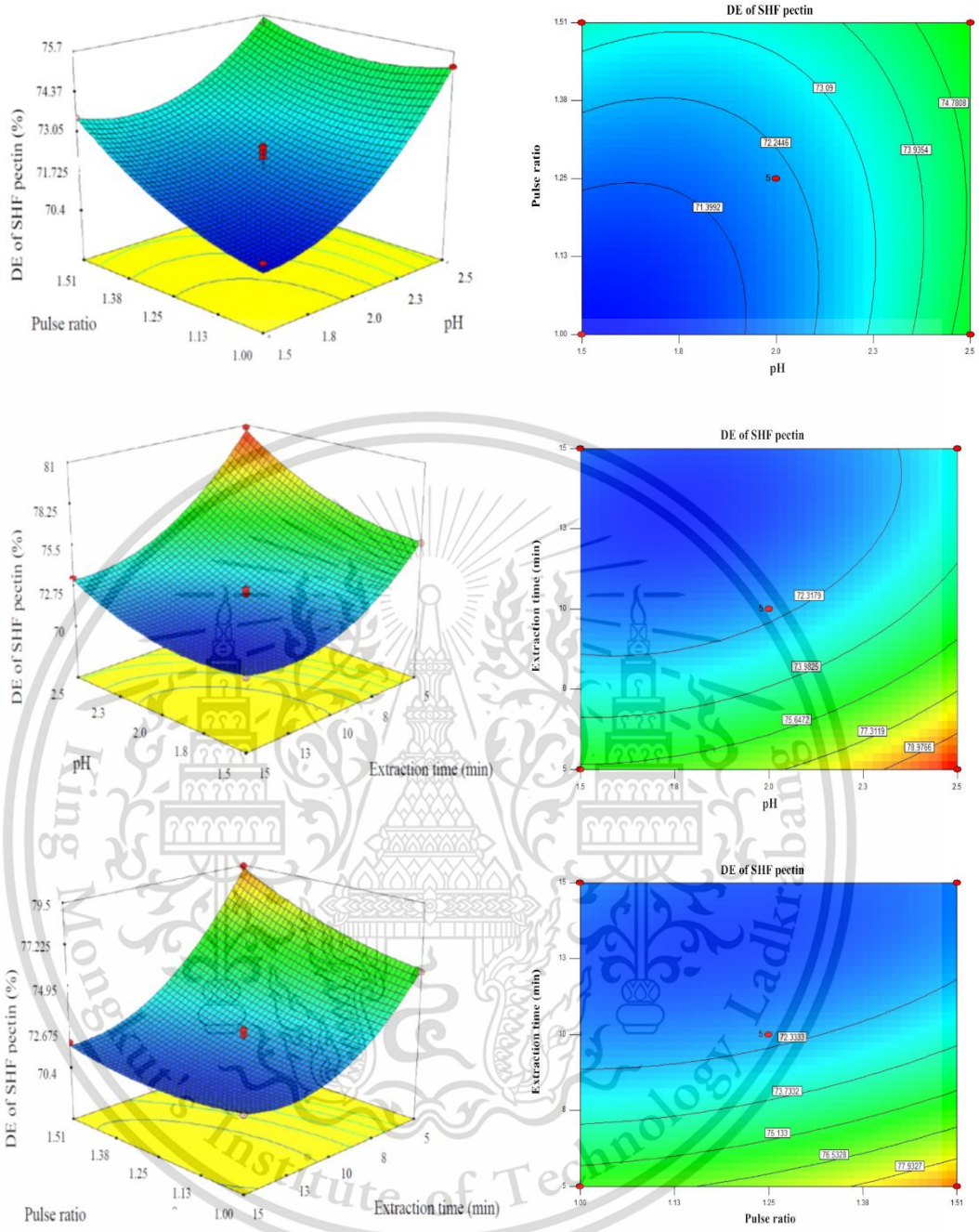


Figure 4.7 3D response surfaces and 2D contour plots for DE from SHF pectin, with DE: pH vs pulse ratio (A), yield: pH vs extraction time (B) and yield: pulse ratio vs extraction time (C)

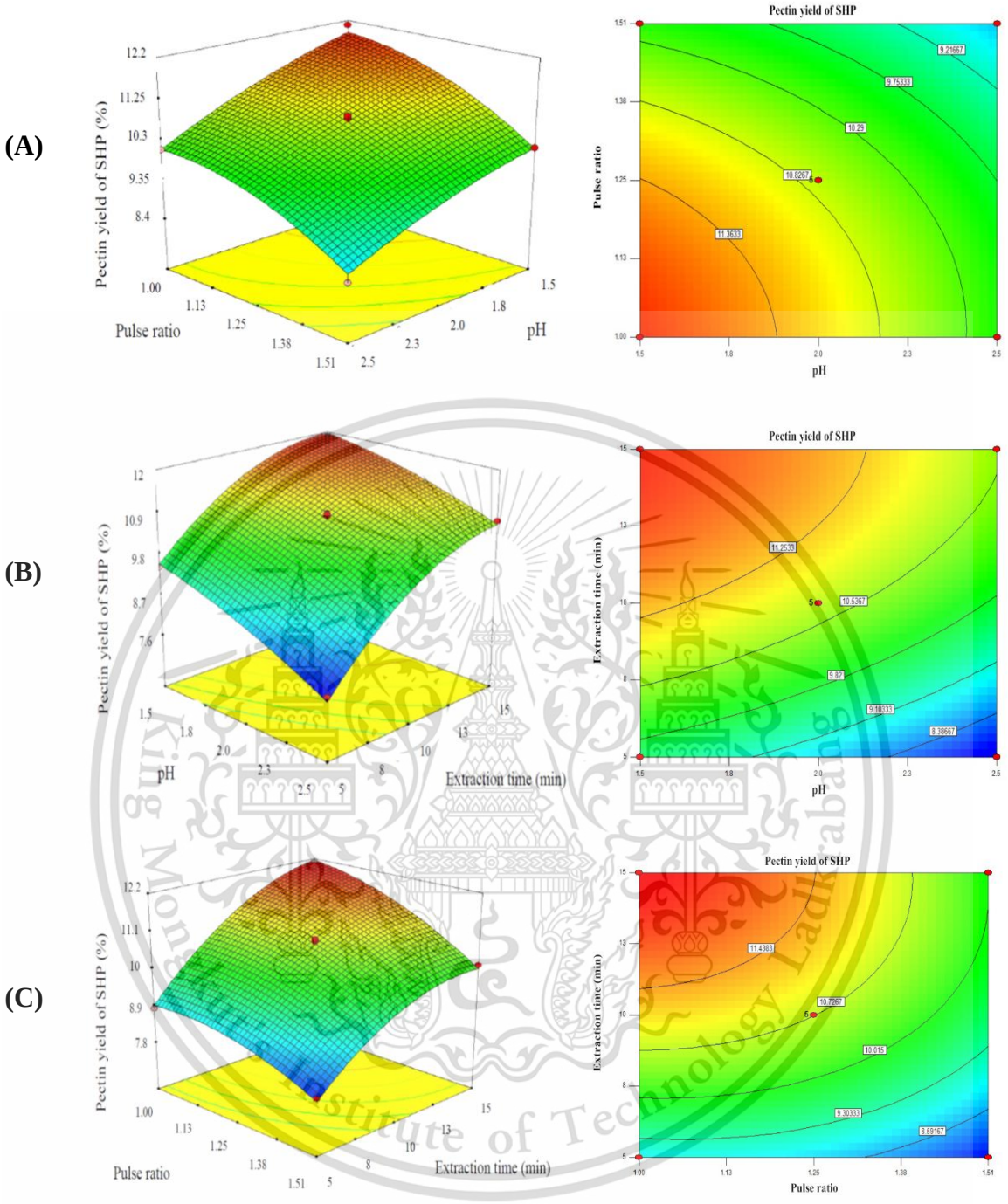


Figure 4.8 3D response surfaces and 2D contour plots for soybean hulls pectin yield from SHP, with yield: pH vs pulse ratio (A), yield: pH vs extraction time (B) and yield: pulse ratio vs extraction time (C)

(A)

(B)

(C)

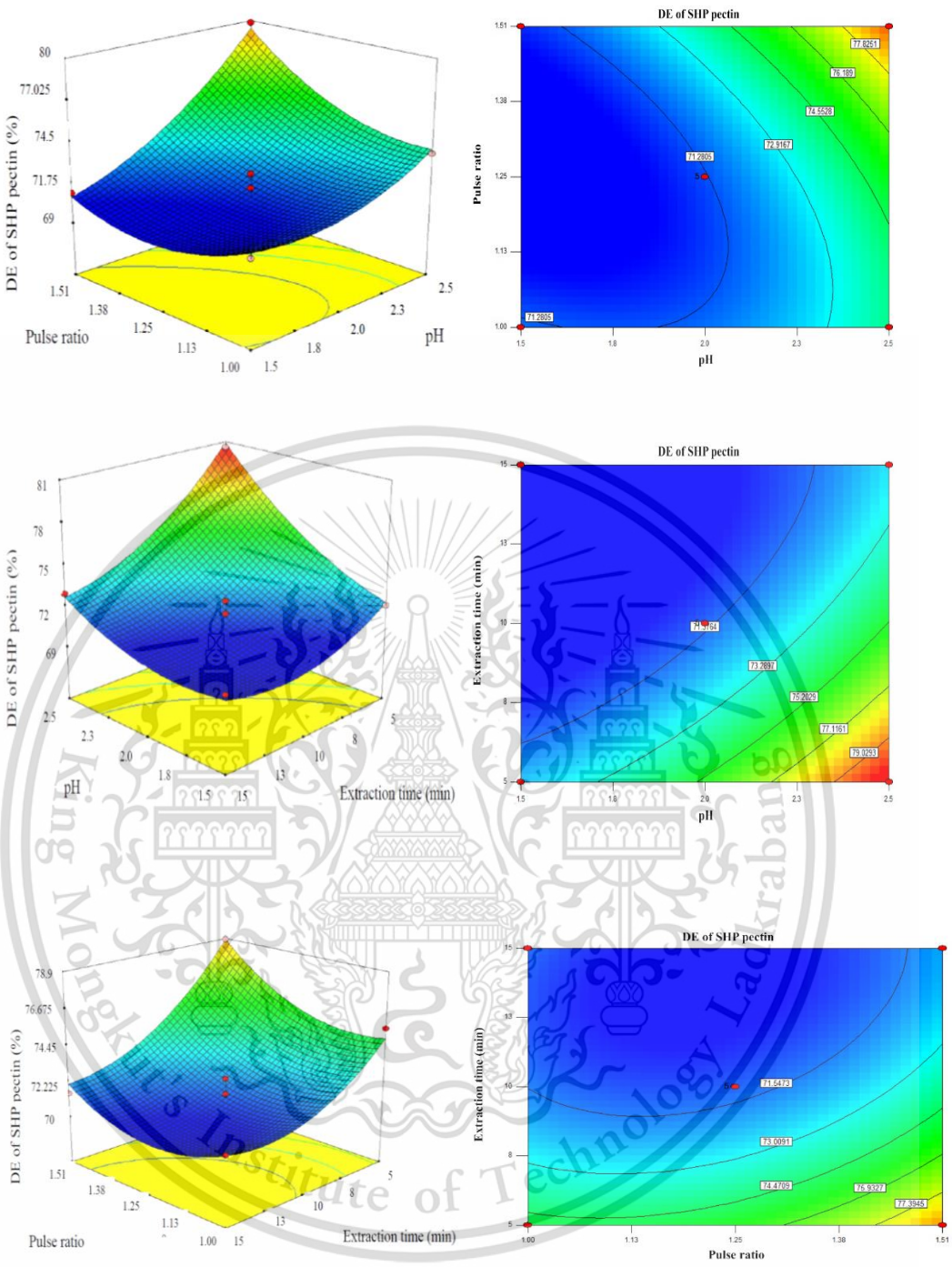


Figure 4.9 3D response surfaces and 2D contour plots for DE from SHP pectin, with DE: pH vs pulse ratio (A), yield: pH vs extraction time (B) and yield: pulse ratio vs extraction time (C)

From the validation results, optimum parameters for targeting the maximum pectin yield are shown in Table 4.12. These parameters are based on a suitable condition for pectin extraction from SHF and SHP using PIMAE to obtain the maximum pectin at pH 2, pulse ratio 1 and extraction time 15 min. The pectin amount obtained was 6.4% for SHF and 12.1% for SHP. This condition was used for the extraction and experiments were conducted in triplicate to confirm the pectin yield. The pectin values from the experiment were 6.3 ± 0.24% for SHF and 12.2 ± 0.36% for SHP. The values from the prediction and the actual experiment were similar. Therefore, the suitable condition for extraction by PIMAE was used for pectin extraction. It was also reported by Eskilsson and Björklund (2000) that there is usually a major influence on the efficiency of MAE resulting from plant particle size distribution and that 100 μm to 2 mm is the usual range of particle size for the extracted material. The extraction can be enhanced by fine powder because the diffusion of chemicals out of the plant matrix is often the step that limits the extraction. Improved contact between the solvent and the plant matrix is provided by the larger surface area of a fine powder, such as finely ground cocoa powder. The MAE of cocaine is facilitated by small particles (Wang and Weller, 2006; Brachet et al., 2002).

Table 4.12 Constraints and validation results at optimum parameters for targeting the maximum pectin yield

Parameter	Goal	Lower limit	Upper limit	Optimized condition	Actual value
pH	In range	1.5	2.5	2	2
Pulse ratio	In range	1	1.51	1	1
Extraction time (min)	In range	5	15	15	15
Pectin yield of SHF (%)	Maximise	0.5	6.4	6.4	6.3±0.24
Pectin yield of SHP (%)	Maximise	7.8	12.1	12.1	12.2±0.36

Data are means ± SD from three replicates.

4.3 Extraction and separation of pectin and cellulose from soybean hulls compared to pressurized intermittent microwave-assisted extraction (PIMAE) and water bath overhead stirrer extraction (WBOSE)

4.3.1 Analysis of yield and moisture content

Table 4.13 shows the percentage yield, moisture and colour content obtained from soybean hulls by acid-alkaline digestion compared to pressurized intermittent microwave-assisted extraction (PIMAE) and water bath overhead stirrer extraction (WBOSE) at each step, i.e. weight loss before and after preparation (9.1%). The pectin yield after acid extraction was 9.9±0.63% for PIMAE and 11.6±0.29% for WBOSE. The pectin from after alkaline extraction was 4.2±0.52% for PIMAE and 3.8±0.86% for WBOSE. The yield before processing until the final output as cellulose extract was 76.8±0.91% for PIMAE and 79.8±1.09% for WBOSE. The moisture content ranged from 6.4 to 7.4%, and was not statistically significant ($p > 0.05$).

4.3.2 Colour determination

L* a* b* measurements from the Minolta device were converted to L*, hue and chroma values. Table 4.13 shows that the highest L* values or brightness were given by soybean hull flake pectins from acid digestion by pressurized intermittent microwave-assisted extraction (SFA-PIMAE) at 94.9±0.20, and 92.4±0.67 for WBOSE. Lower values for both types of pectin from alkaline extraction were 80.4±1.25 for PIMAE and 70.7±0.38 for WBOSE. Cellulose powder was 71.1±0.09 for PIMAE and 73.2±0.21 for WBOSE as significantly different ($p < 0.05$). The L*value measurement of soybean hulls (63.3±2.44) was similar to that previously reported by Slavin et al. (2009) in which L* in soybean hulls ranged from 58.3-63.7 and Yang et al. (2014).

The hue values relate to different colours such as red, blue, green and yellow. When the values of hue equal to any degrees of the colour zones, the colours of the measured samples were accepted. All of the treatments ranged between similar hues of 61.9 to 87.50.9 with a yellow colour, similar to Charudul (2019). By contrast, both types of SFA had hue of -8.6±1.94 for PIMAE and -7.5±1.24 for WBOSE showing white colour and these were significantly different ($p < 0.05$).

The chroma values were used to compare differences in colour intensity in the same shade. It was found that the sample colour intensity ranged from 13.3 to 21.2, i.e. orange to brown tones. This was similar to those of pectins from soybean hulls by microwave pressure cooker extraction (Charudul, 2019) and from jackfruit peels (Koh et

al., 2014). By contrast, both types of SFA had chroma of 5.2±0.02 for PIMAE and 7.1±0.60 for WBOSE with white tone and were significantly different ($p < 0.05$).

Table 4.13 Yield, moisture and colour content obtained from soybean hulls (% dry matter)

Sample	%Yield	Moisture content [NS]	Colour [a]		
			L*	Hue	Chroma
Soybean hulls	100±0.00[a]	6.4±0.36	63.3±2.44[f]	75.8±2.95[b]	21.2±2.61[a]
Soybean hull flakes	90.9±0.98[b]	7.4±0.78	74.2±0.39[d]	81.4±0.37[a]	19.1±0.51[b]
SFA-PIMAE	9.9±0.63[f]	6.5±0.21	94.9±0.20[a]	-8.6±1.94[d]	5.2±0.02[e]
SFB-PIMAE	4.2±0.52[g]	6.4±0.18	80.4±1.25[c]	81.3±2.96[a]	14.1±1.26[c]
SDF-PIMAE	76.8±0.91[d]	6.4±1.22	71.1±0.09[e]	76.6±0.06[b]	17.3±0.35[b]
SFA-WBOSE	11.6±0.29[e]	7.1±0.69	92.4±0.67[b]	-7.5±1.24[d]	7.1±0.60[d]
SFB-WBOSE	3.8±0.86[g]	7.5±0.21	70.7±0.38[e]	67.9±0.77[c]	13.3±0.23[c]
SDF-WBOSE	79.8±1.09[c]	7.4±0.20	73.2±0.21[d]	78.9±0.47[ab]	19.2±0.19[b]

All values are means of three determinations ± standard deviation

[ns] Moisture contents were not significantly different ($p < 0.05$) using Duncan's multiple range test

Different letters in the same column indicate significant differences by Duncan's multiple range test ($p \leq 0.05$).

4.3.3 Thermodynamic measurement

Figure 4.10 shows the temperature profile versus extraction time for acid and alkaline extraction. Up to 12 minutes, the temperature continuously increased until the boiling point at 100°C. After that, temperature rose slowly to 109°C until complete at 30 minutes. The use of a pressure cooker with a microwave caused the temperature to rise faster than using a microwave only. This was consistent with Akhtar et al. (2014) who compared microwave-assisted alkali and acid pretreatment and conventional pretreatments to extract dietary fibre from empty oil palm fruit bunches: their conditions were 800W microwave power for 70 min and 90°C.

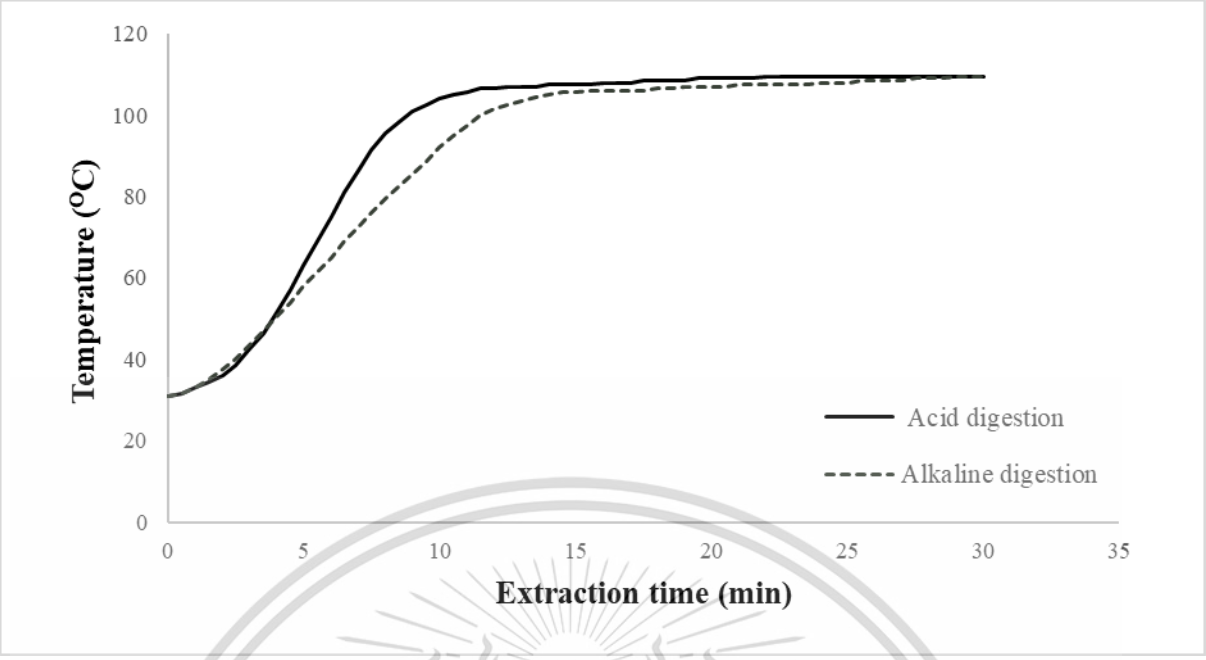


Figure 4.10 Temperature-time profiles for acid digestion (solid line) and alkaline digestion (dashed line) at pH 2.0 and pulse ratio of 1 for 30 minutes

4.3.4 Scanning electron microscopy

Microstructure from Scanning electron microscopy (SEM) images is shown in Figure 4.11 A-F. The surface of the pectin from SFA-PIMAE and WBOSE precipitation by propan-2-ol had a rougher structure. After alkaline extraction, pectin from SFA-PIMAE and WBOSE skin was smooth as in Figure 4.11 (B, E), corresponding to the previous experiment. In Figure 4.11 (C, F), the cellulose from PIMAE and WBOSE was seen as skeletons, consistent with previous reports (Camiscia et al., 2018; Soysal et al., 2009).

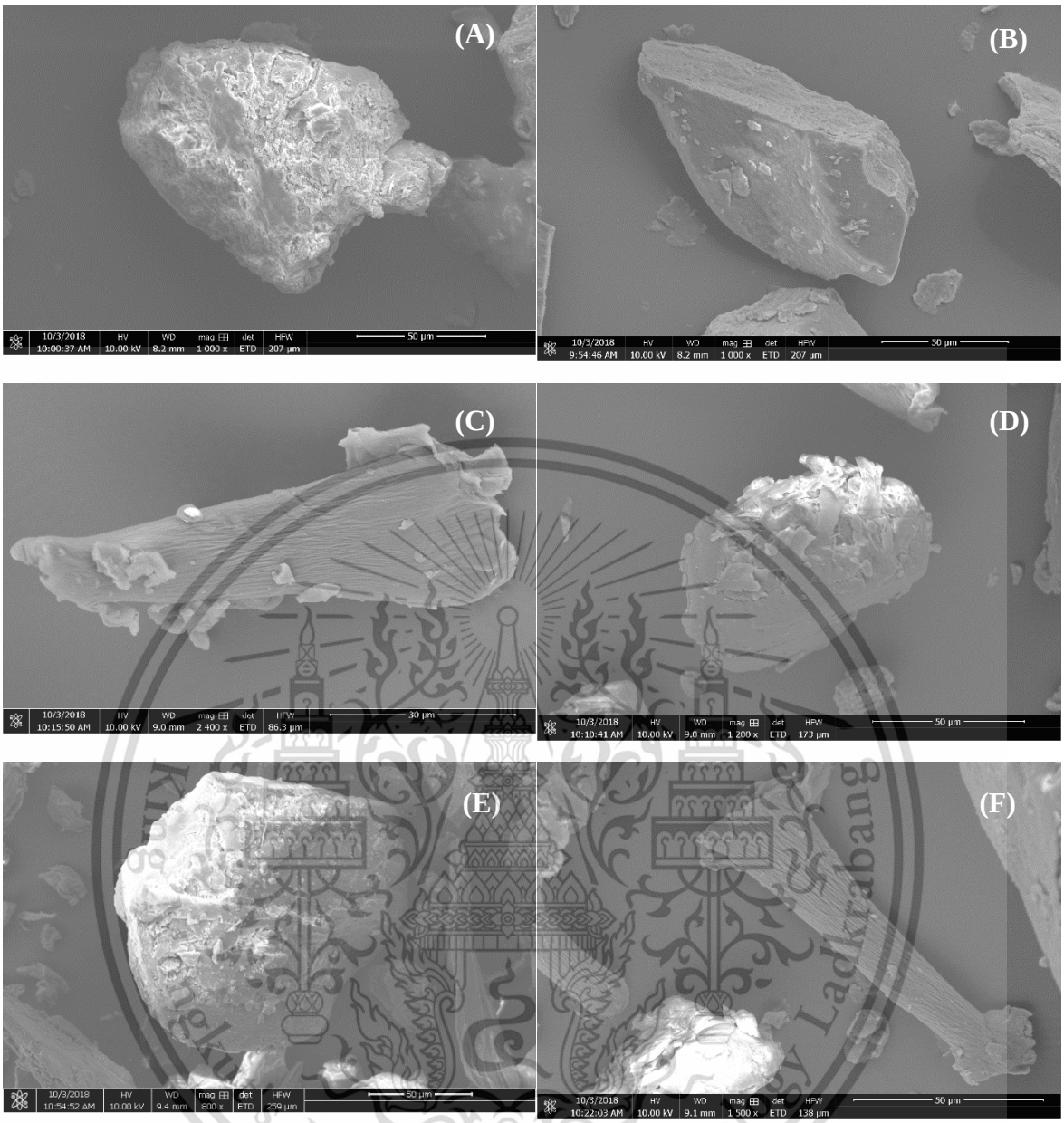


Figure 4.11 Scanning electron microscope images of pectin and cellulose from soybean hull flakes precipitated by propan-2-ol of SFA-PIMAE (A), SFB-PIMAE (B), SDF-PIMAE (C), SFA-WBOSE (D), SFB-WBOSE (E), SDF-WBOSE (F)

4.3.5 Water absorption index and water solubility index

Determination of water absorption index (WAI) and water solubility index (WSI) of cellulose extracts from soybean hull flakes. Experiments were conducted to determine the water holding capacity value and results were used to determine the amount of water-soluble portion from the samples of fibre extracts from soybean hull flakes. In addition, the ability to seize and absorb water was assessed. The ability to inflate the fibre varied with the

elasticity of the fibre surface (Chaplin, 2003). Results from this experiment are shown in Figure 4.12. WAI and WSI values were 5.03 and 5.14 for soybean hulls powder, 3.84 and 2.37 for cellulose extract from PIMAE and 3.75 and 2.51 for cellulose extract from WBOSE. These were similar to the research by Jun et al. (2014). Water absorption and water solubility of fibre extracts from soybean hulls were 3.52 and 3.00% respectively.

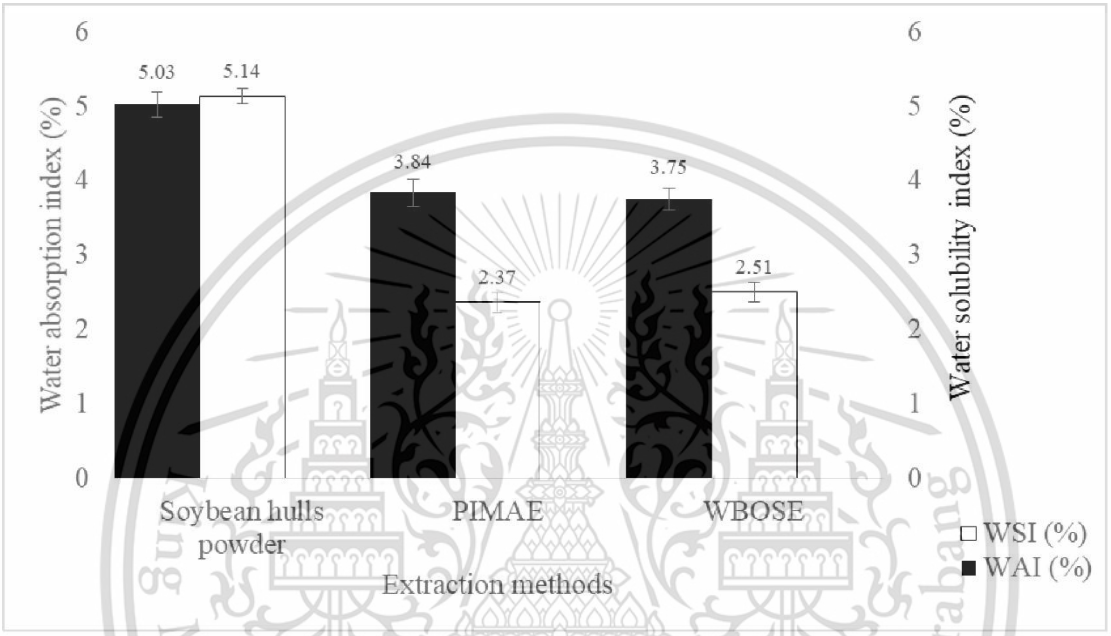


Figure 4.12 Water absorption index (WAI) and water solubility index (WSI) of soybean hulls dietary fibre

4.3.6 Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FTIR) spectra of soybean hulls pectin obtained under PIMAE and WBOSE extraction conditions are presented in Figure 4.13. The functional groups are presented in Table 4.14. The results were comparable for six treatments (SFA-PIMAE, SFB-PIMAE, SFA-WBOSE, SFA-WBOSE, HM pectin commercial grade and LM pectin commercial grade). Pectin peaks were presented at 4000-400 cm^{-1}. The large area in the range of 3300-3500 cm^{-1} was attributed to OH stretching. Only the LM pectin commercial-grade did not show a peak in the range of 2850-3000 cm^{-1}, attributed to CH stretching. This range consisted of CH, CH_2 and CH_3 stretching and bending (Hosseini et al., 2016; Tian et al., 2011). Moreover, peaks in the range of 1732.55 cm and 1224.78 cm^{-1} indicated the existence of COOH from methyl groups of the esterified carboxyl group. Stretching antisymmetric and symmetric COOH and scissoring of CH and OHCOOH

peaks were due to the galacturonic acid chain. Glycosidic linkages between sugar units were presented in peaks between 1000 and 1102 cm^{-1} (Hosseini et al., 2016; Kozarski et al., 2011). Normally, “finger print” presented absorption patterns in the range of 800 to 1200 cm^{-1} are unique to a compound (Chen et al., 2014).

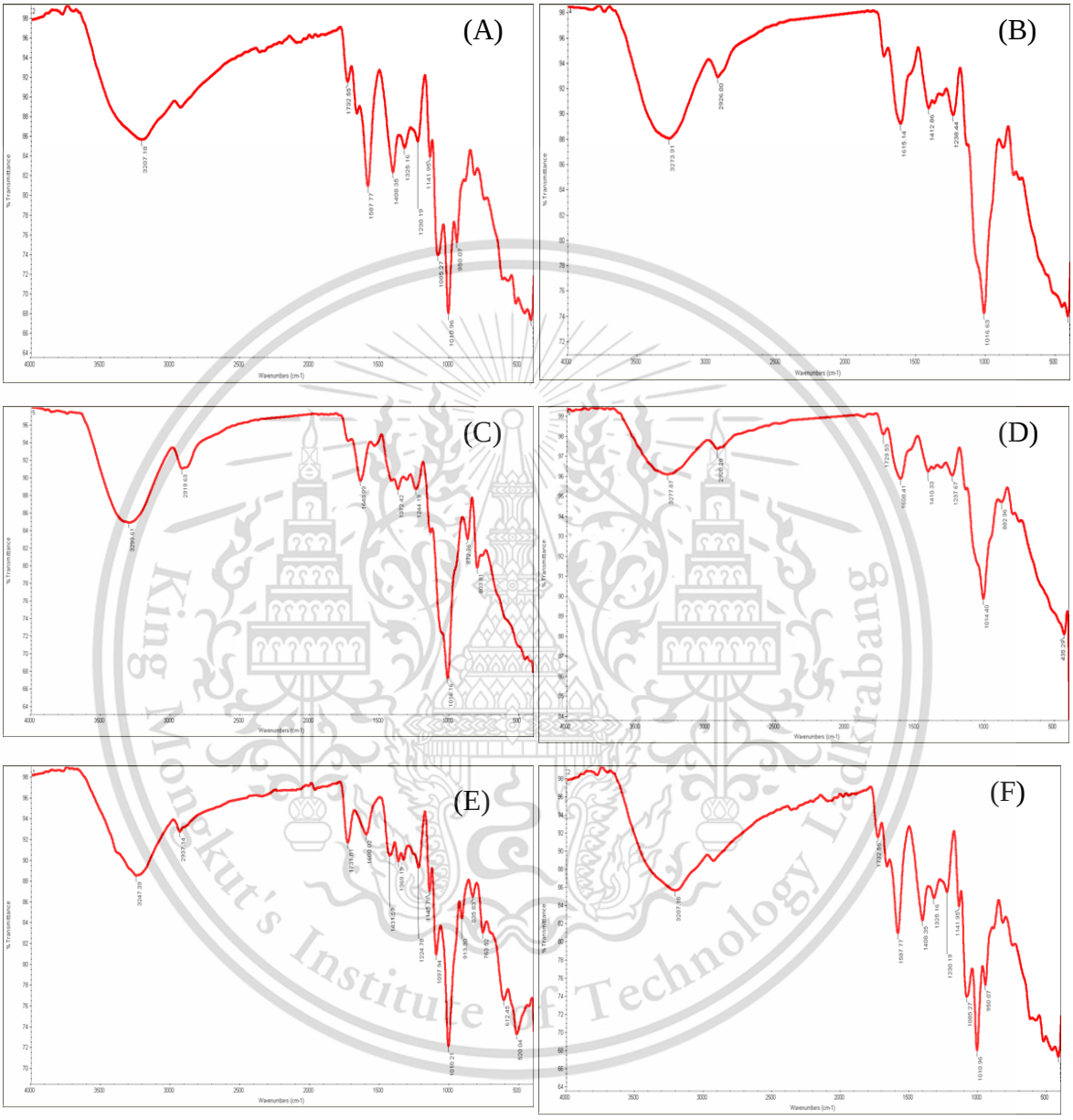


Figure 4.13 FTIR spectra of SFA-PIMAE (A); SFB-PIMAE (B); SFA-WBOSE (C); SFB-WBOSE (D); HM pectin commercial grade (E) and LM pectin commercial grade (F)

Table 4.14 Assignments of FTIR wavenumbers in the range 4000-400 cm^{-1} of soybean hulls and commercial pectin

Pectin peaks (cm^{-1})						Assignments[a]	
PIMAE		WBOSE		Commercial pectin			
Acid	Alkaline	Acid	Alkaline	HM	LM		
3341.85	3273.91	3299.61	3277.87	3247.39	3207.18	υ (OH)	
2926.61	2926	2919.63	2920.28	2937.14	-	υ (CH)	
1727.32	-	-	1729.53	1731.81	1732.55	υ (C=H)COOH from methyl of esterified carboxyl group	
1629.42	1615.14	1640.99	1608.41	1600.02	1587.77	υ_{as} (COO^-)	
-	1412.86	-	1410.33	1431.59	1408.35	υ_s (COO^-)	
-	-	1372.42	-	1369.19	1325.16	δ (CH)	
1241.22	1238.44	1244.19	1237.67	1224.78	1230.31	δ $(OH)_{COOH}$	
-	-	-	-	1145.7	1141.95	υ (COC)	Finger print
-	-	-	-	1097.94	1085.27	υ (CO) + δ(OH)	
1024.03	1016.63	1016.16	1014.4	-	1010.96	υ(CC)(CO)	
-	-	-	-	-	950.07	δ(CCH), δ (COH)	
-	-	-	-	913.3	-		
875.58	-	872.76	882.96	-	-	(C6–C5–O5–C1–O1)	
-	-	-	-	835.83	-	γ (C-OH)	
807.73		803.91	-	-	-	γ (C-OH)	
-	-	-	-	763.52	-		
-	-	-	-	612.45	-	υ (CH)	
525.23	-	-		520.04	-		
-	-	-	435.29	-	-	T(C–O–C) def.	

[a]υ, stretching; δ, scissoring; γ, wagging or out-of-plane deformation; *s*, symmetric; *as*, antisymmetric

4.3.6 Nuclear magnetic resonance (NMR) spectroscopy

The soybean hulls pectin under PIMAE and WBOSE extraction conditions were compared with HM and LM pectin commercial grade. Functionalisation was evaluated by ^{1}H NMR as presented in Figure 4.14. Generally, pectin shows a signal range of polysaccharides at δ 3.0-5.3, *β*-glycosidical at *δ* 4.0-5.0 and *α*-glycosidical at *δ* 5.0-5.6 ppm (Wang et al., 2017; Xu et al., 2018; Yang et al., 2018). Our results showed peaks at *δ* 5.38, 5.27 and 5.1 ppm indicating anomeric protons of 1, 4-linked-*α*-D-Glc*p* residues, *α*-terminal-D-Glc*p* and *α*-l-rhamnopyranose residues respectively according to previous studies (Liu et al., 2017; Yang et al., 2018). The broad peak at *δ* 4.70 ppm was assigned to the D_2O solvent (Jiang et al., 2020; Yang et al., 2018). The peaks at *δ* 4.5-3.2 ppm were attributed to the H1-H5 of 1, 6-linked-*α*-D-Glc*p* residues, Ara 2-O- and 3-O-di-substituted Xyl*p* residues. The weak peaks at *δ* 1.8-2.0 ppm showed soybean hulls pectin that was significantly different from HM and LM pectin commercial grade (Li et al., 2016). The weak peaks at *δ* 1.1-1.2 ppm were from Rha*p* residues of methyl rhamnoses.

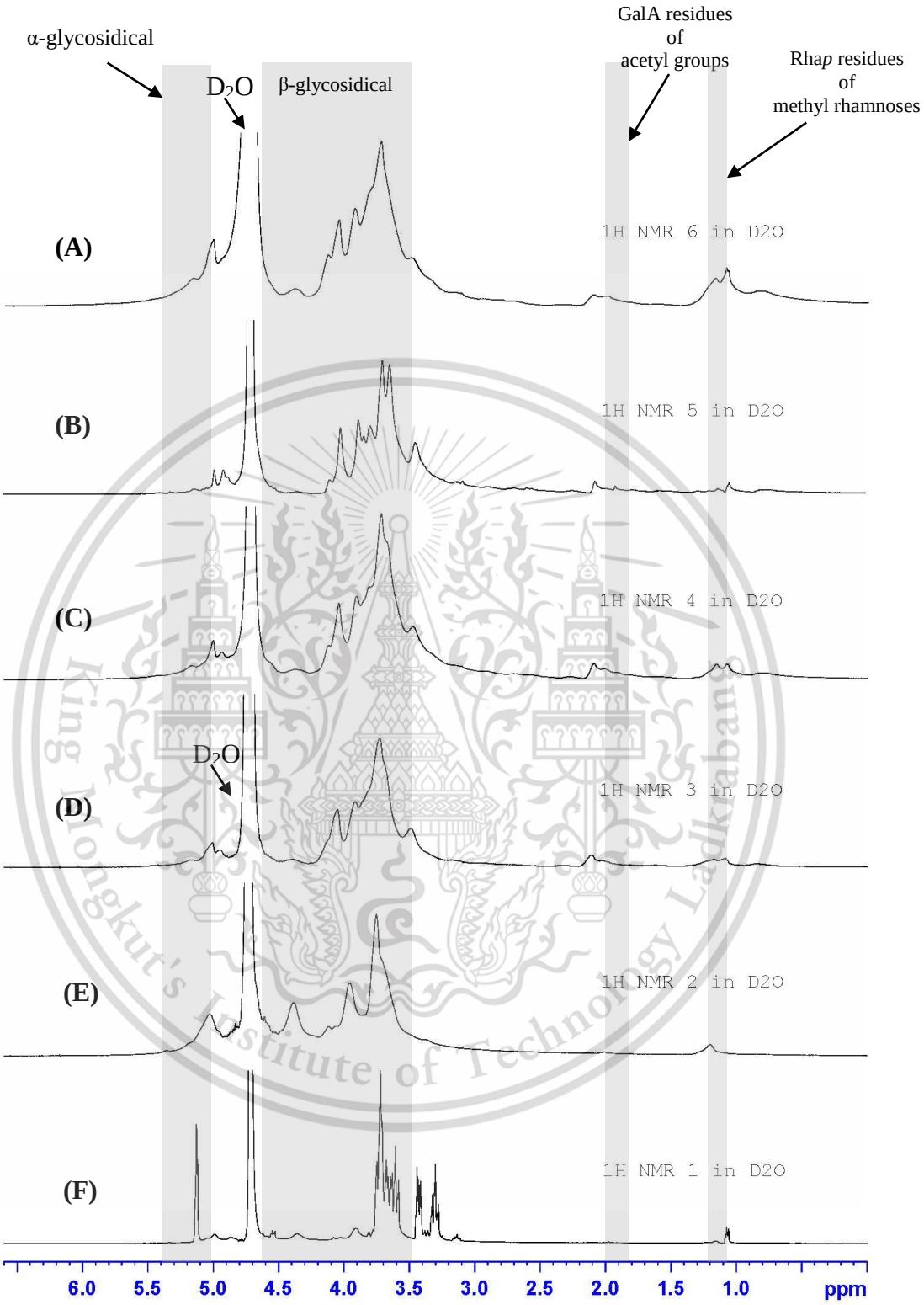


Figure 4.14 H^1 NMR spectra of pectin. H1 NMR spectra of pectin. SFA-PIMAE(A), SFB-PIMAE(B), SFA-WBOSE (C), SFB-WBOSE(D), HM pectin commercial grade (E), and LM pectin commercial grade(F)

CHAPTER 5

CONCLUSIONS

The pressurized intermittent microwave-assisted extraction (PIMAE) process was shown to be effective in extracting pectin from soybean hulls powder. Box-Behnken response surface experimental design was successful for three factors at three levels. The extraction conditions (pH, pulse ratio and extraction time) all significantly ($p < 0.05$) affected the yield of pectin extracted from soybean hulls. Using ethanol resulted in a pectin yield of ~11% at the optimal conditions (pH 1.5, pulse ratio of 1 and ~12 minutes of extraction time) while using propan-2-ol precipitation gave yield of ~12%. However, galacturonic acid content of all extractions, except for pectin extracted at the optimal conditions, was less than the 65% limit established by the FCC purity specification for pectin. Pectin extract using propan-2-ol precipitation under the optimal conditions was lighter and whiter in colour, which could improve its acceptability.

PIMAE was optimized for the extraction of pectin from soybean hulls. For maximum extraction yield and degree of esterification of pectin from soybean hulls, comparison of soybean hull flakes and powder indicated that grinding enhanced pectin extract yield because of improved solvent access to the pectin molecules. On the basis of extraction level, soybean hull pectin can be classified as high methoxyl type with degree of esterification higher than 50%. The PIMAE conditions that resulted in the highest yield were pH 1.5, pulse ratio 1 and extraction time 10 min, giving pectin yields of up to 12.09%. The yield of pectin extracted was shown to be dependent on the extraction technique, pH, temperature and extraction time.

Comparisons of PIMAE and WBOSE on pectin yield (total ~15%) and cellulose yield (~80%) showed similar results. PIMAE reduced extraction time by 3 times less than WBOSE. For morphological properties of pectin and cellulose from soybean hulls, PIMAE generated the highest yield of pectin and cellulose close to WBOSE. Pectin, cellulose and lightness (L* value) were highest in the powder. Chroma showed white and orange-brown tones, respectively. Hue showed that the samples had a white and yellow colour, respectively. SEM morphological analysis showed a surface of pectin and cellulose structure. FTIR confirmed the structure of the sample molecules. NMR indicated the presence of atoms and molecules of pectin.

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Appendixes

Appendix A

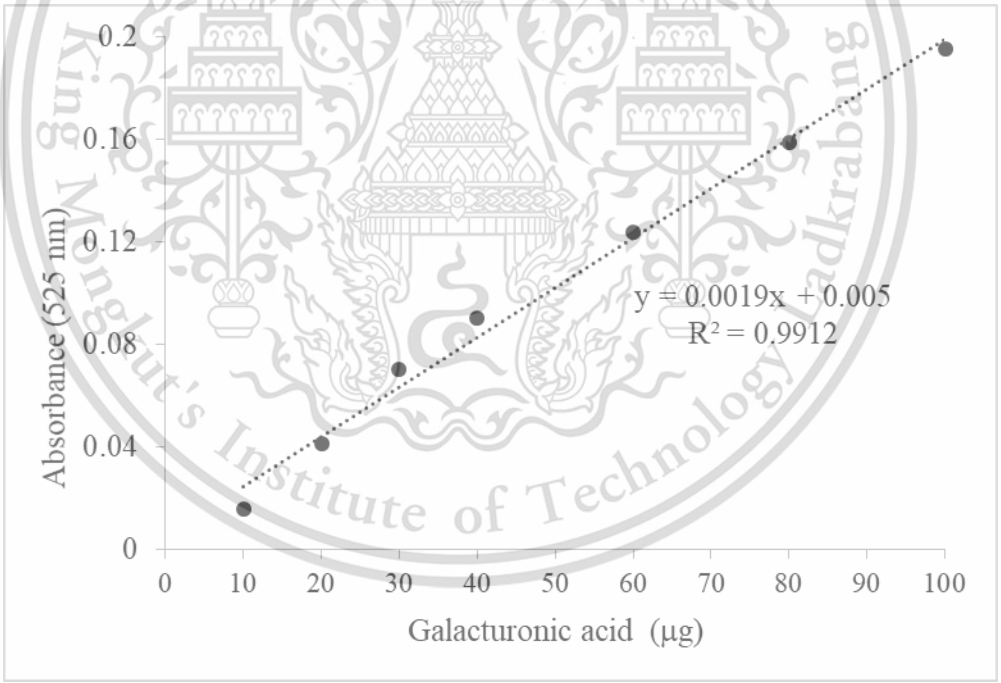
Standard curve of galacturonic acid

Appendix A: Standard curve of galacturonic acid assay and calculation

The stock galacturonic acid solution was prepared by dissolving 120.5 mg in 10 mL of 0.05 N NaOH. 1mL of stock galacturonic acid solution diluted in DI water and completed to volume 100 mL was kept in the dark place by wrapping with aluminium foil. 1, 2, 3, 4, 6, 8, and 10 mL of stock galacturonic acid dilution was placed in volumetric flasks, which were wrapped by aluminium foil and arranged in triplicate. Then, DI water was added to each volumetric flask to bring the total volume to 10 mL. 2mL of these solutions were added to each tube dissolving with 1 ml of 0.1% Carbazole reagent, shaken well and added 12 mL of H_2SO_4 conc. then shaken well again and allowed to react for 30 minutes.

After that, the absorbance was read at 525 nm. DI water was used as blank. The standard curve was plotted as follows:

Where y: Absorbance



x: Mass of Galacturonic acid (µg)

Appendix B

Analysis of variance

Appendix B1: **Analysis of variance for a yield of soybean hulls pectin by ethanol precipitate from SHP**

Source	Sum of squares	DF	Mean square	F-Value	P-Value
Model	52.05	9	5.78	10.75	0.0025**
X_1-pH	4.05	1	4.05	7.53	0.0288*
X_2-Pulse ratio	22.48	1	22.48	41.80	0.0003**
X_3-Extraction time	0.73	1	0.73	1.36	0.2815
X_1X_2	0.53	1	0.53	0.98	0.3558
X_1X_3	14.67	1	14.67	27.28	0.0012**
X_2X_3	1.28	1	1.26	2.37	0.1672
X_1^2	1.92	1	1.92	3.58	0.1005
X_2^2	3.93	1	3.93	7.30	0.0305*
X_3^2	1.64	1	1.64	3.04	0.1247
Residual	3.76	7	0.54		
Lack of Fit	2.22	3	0.74	1.91	0.2693
Pure Error	1.55	4	0.39		
Cor Total	52.05	16	5.78	10.75	0.0025**
R-Squared	0.93				
Adj R-Squared	0.85				
Std. Dev.	0.73				
C.V. %	9.3				
Adeq Precision	10.71				

Where, * is a code $p \leq 0.05$, ** is a code $p \leq 0.01$

Appendix B2: Analysis of variance for a yield of soybean hulls pectin by propa-2-ol precipitate from SHF

Source	Sum of squares	DF	Mean square	F-Value	P-Value
Model	46.43	9	5.16	73.16	< 0.0001**
X_1-pH	2.25	1	2.25	31.87	0.0008**
X_2-Pulse ratio	6.23	1	6.23	88.37	< 0.0001**
X_3-Extraction time	31.76	1	31.76	450.48	< 0.0001**
X_1X_2	0.21	1	0.21	2.94	0.1303
X_1X_3	2.67	1	2.67	37.92	0.0005**
X_2X_3	0.35	1	0.35	5.02	0.06
X_1^2	0.08	1	0.08	1.17	0.316
X_2^2	0.21	1	0.21	3.02	0.126
X_3^2	2.76	1	2.76	39.21	0.0004**
Residual	0.49	7	0.07		
Lack of Fit	0.34	3	0.11	2.82	0.1708
Pure Error	0.16	4	0.04		
Cor Total	46.92	16			
R-Squared	0.99				
Adj R-Squared	0.98				
Std. Dev.	0.27				
C.V. %	6.32				
Adeq Precision	29.47				

Where, * is a code $p \leq 0.05$, ** is a code $p \leq 0.01$

Appendix B3: Analysis of variance on DE of soybean hulls pectin by propa-2-ol precipitate from SHF

Source	Sum of squares	DF	Mean square	F-Value	P-Value
Model	133.20	9	14.80	28.48	0.0001**
X_1-pH	22.65	1	22.65	43.58	0.0003**
X_2-Pulse ratio	5.83	1	5.83	11.22	0.0123*
X_3-Extraction time	71.70	1	71.70	137.99	< 0.0001**
X_1X_2	1.66	1	1.66	3.20	0.1166
X_1X_3	2.04	1	2.04	3.94	0.0877
X_2X_3	2.77	1	2.77	5.34	0.0542*
X_1^2	5.46	1	5.46	10.50	0.0142*
X_2^2	1.23	1	1.23	2.37	0.1676
X_3^2	17.79	1	17.79	34.25	0.0006**
Residual	3.64	7	0.52		
Lack of Fit	0.33	3	0.11	0.13	0.9345
Pure Error	3.30	4	0.83		
Cor Total	136.83	16			
R-Squared	0.97				
Adj R-Squared	0.94				
Std. Dev.	072				
C.V. %	0.98				
Adeq Precision	18.45				

Where, * is a code $p \leq 0.05$, ** is a code $p \leq 0.01$

Appendix B4: Analysis of variance for a yield of soybean hulls pectin by propa-2-ol precipitate from SHP

Source	Sum of squares	DF	Mean square	F-Value	P-Value
Model	27.60	9	3.07	50.00	< 0.0001**
X_1-pH	5.28	1	5.28	86.11	< 0.0001**
X_2-Pulse ratio	5.09	1	5.09	82.96	< 0.0001**
X_3-Extraction time	14.31	1	14.31	233.34	< 0.0001**
X_1X_2	0.04	1	0.04	0.62	0.4569
X_1X_3	0.06	1	0.06	0.98	0.3555
X_2X_3	0.30	1	0.30	4.84	0.0637
X_1^2	0.12	1	0.12	2.00	0.2
X_2^2	0.58	1	0.58	9.44	0.018*
X_3^2	1.62	1	1.62	26.45	0.0013**
Residual	0.43	7	0.06		
Lack of Fit	0.15	3	0.05	0.68	0.6067
Pure Error	0.28	4	0.07		
Cor Total	28.03	16			
R-Squared	0.98				
Adj R-Squared	0.97				
Std. Dev.	0.25				
C.V. %	2.43				
Adeq Precision	23.586				

Where, * is a code $p \leq 0.05$, ** is a code $p \leq 0.01$

Appendix B5: Analysis of variance on DE of soybean hulls pectin by propa-2-ol precipitate from SHP

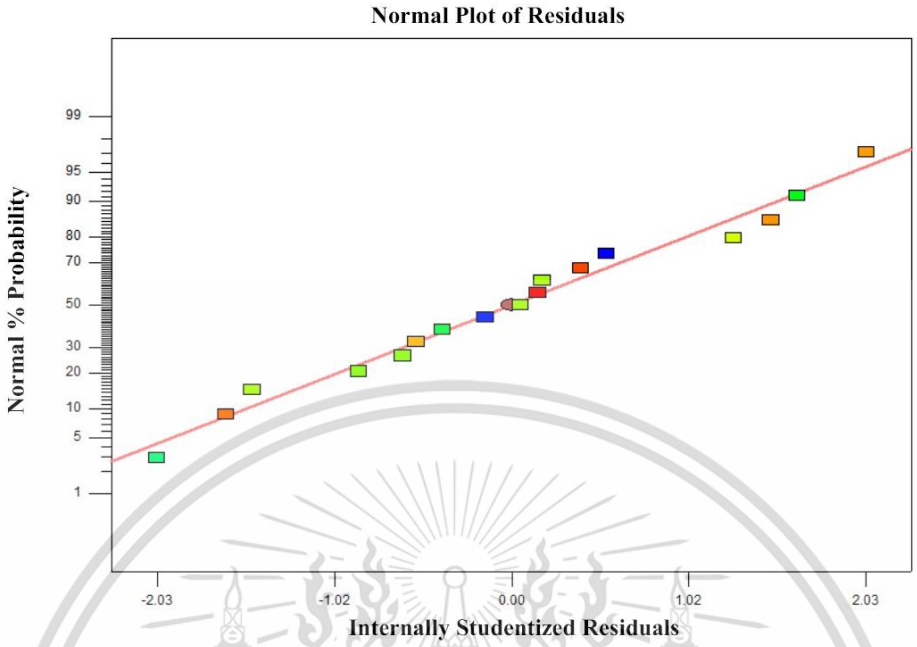
Source	Sum of squares	DF	Mean square	F-Value	P-Value
Model	178.26	9	19.81	33.64	< 0.0001
X_1-pH	62.55	1	62.55	106.24	< 0.0001
X_2-Pulse ratio	11.33	1	11.33	19.24	0.0032
X_3-Extraction time	54.34	1	54.34	92.29	< 0.0001
X_1X_2	9.30	1	9.30	15.80	0.0054
X_1X_3	9.27	1	9.27	15.75	0.0054
X_2X_3	2.46	1	2.46	4.19	0.0800
X_1^2	6.40	1	6.40	10.88	0.0132
X_2^2	9.36	1	9.36	15.89	0.0053
X_3^2	10.22	1	10.22	17.36	0.0042
Residual	4.12	7	0.59		
Lack of Fit	1.49	3	0.50	0.75	0.5756
Pure Error	2.63	4	0.66		
Cor Total	182.38	16			
R-Squared	0.98				
Adj R-Squared	0.95				
Std. Dev.	0.77				
C.V. %	1.05				
Adeq Precision	27.90				

Where, * is a code $p \leq 0.05$, ** is a code $p \leq 0.01$

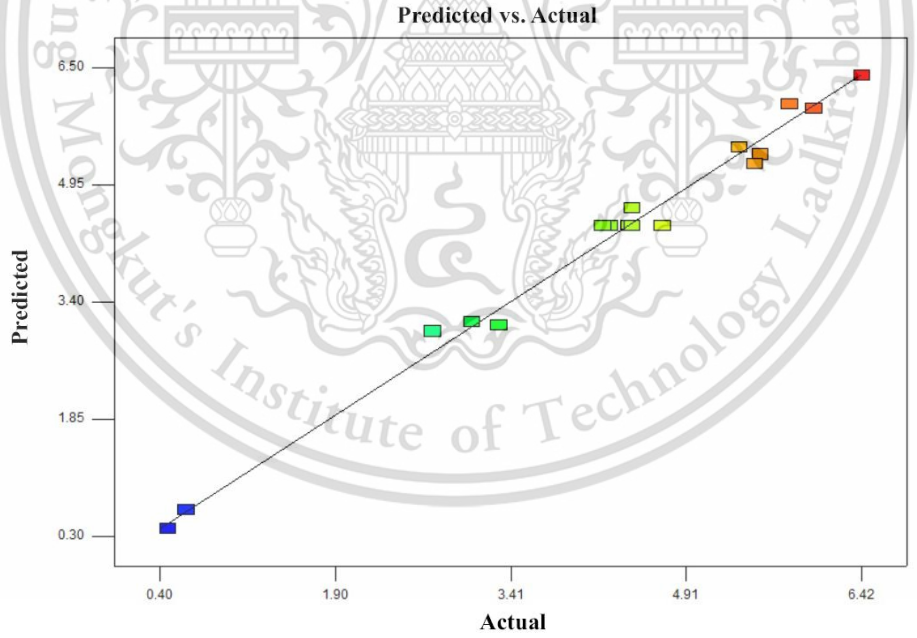
Appendix C

Diagnostic plots

Appendix C1: Diagnostic plots of pectin yield from SHF

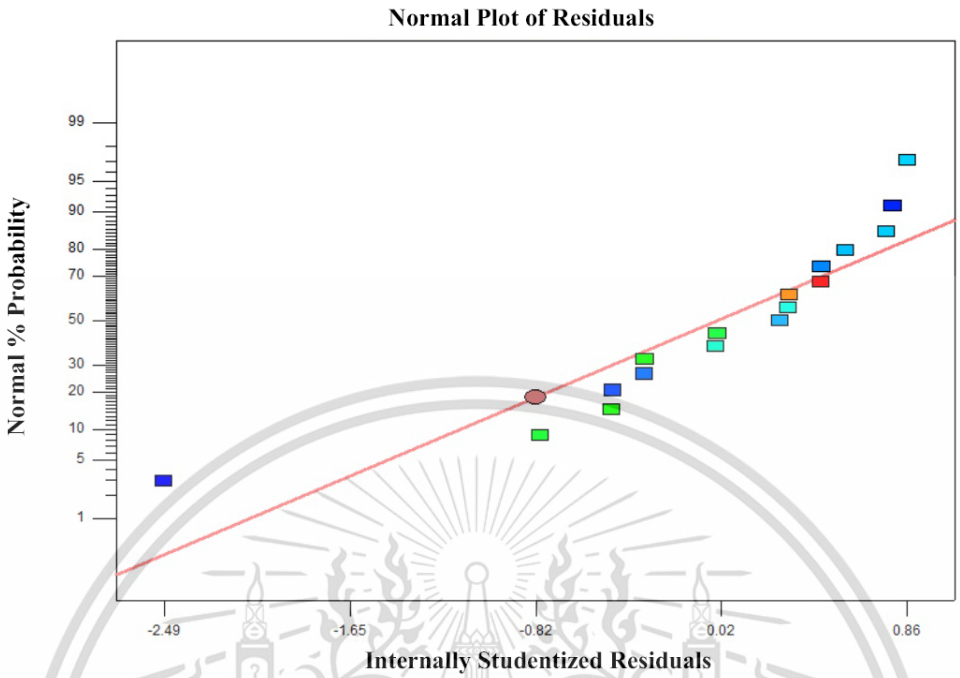


(A) Diagnostic plots of internally studentized residuals vs. Normal % probability of pectin yield from SHF

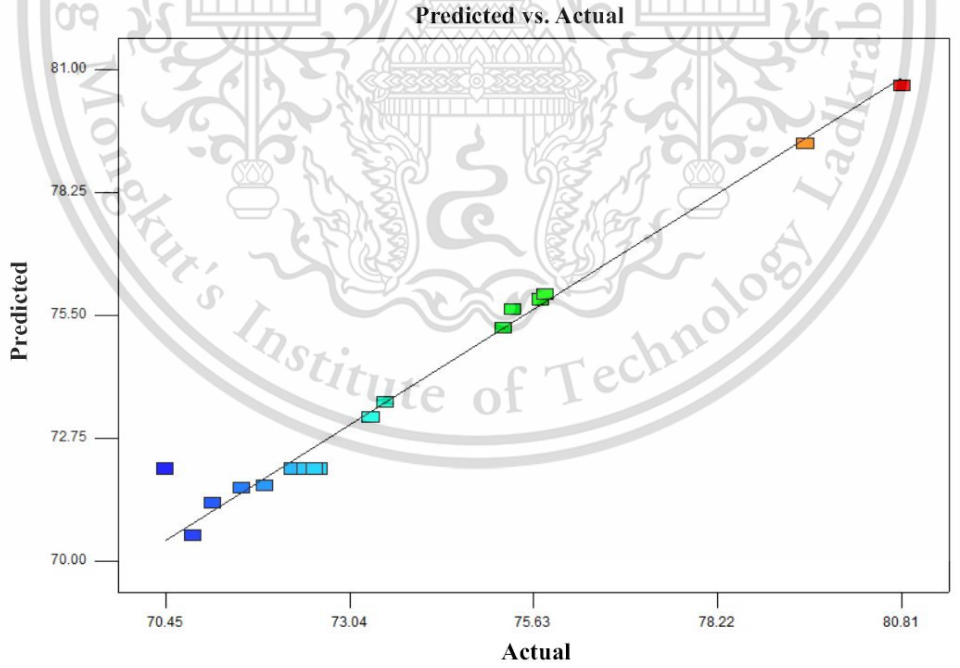


(B) Predicted vs. Actual yield of pectin yield from SHF

Appendix C2: Diagnostic plots of DE from SHF pectin

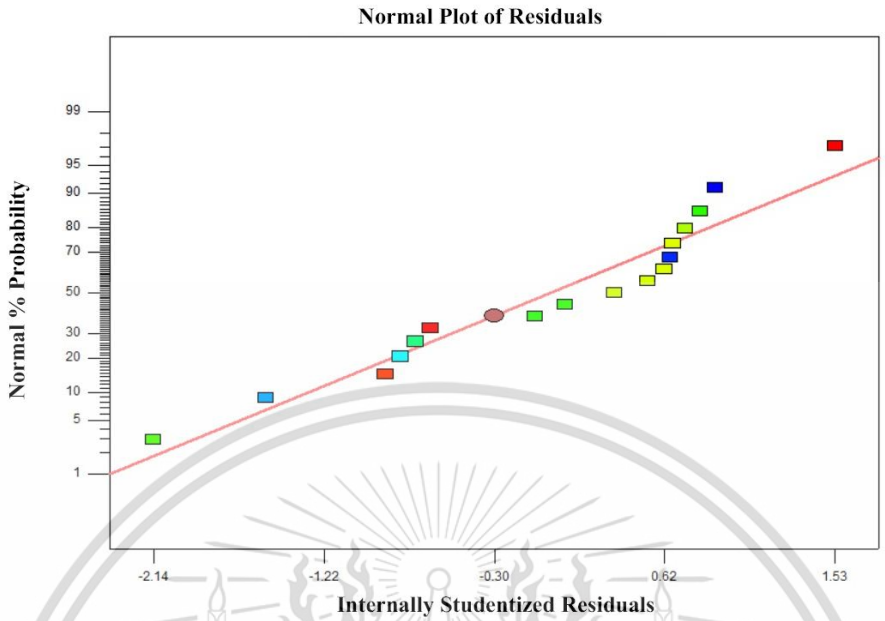


(A) Diagnostic plots of internally studentized residuals vs. Normal % probability of DE from SHF pectin

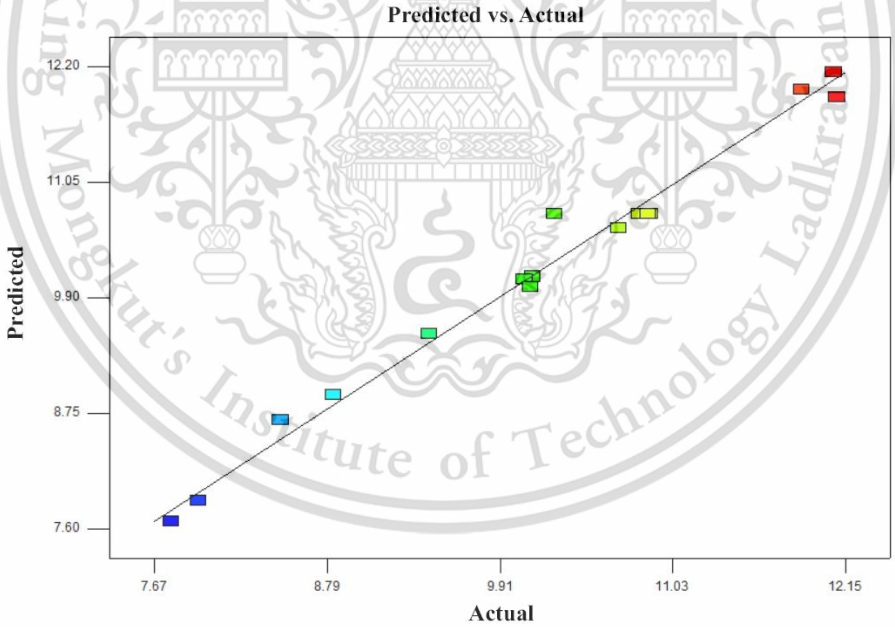


(B) Predicted vs. Actual of DE from SHF pectin

Appendix C3: Diagnostic plots of pectin yield from SHP

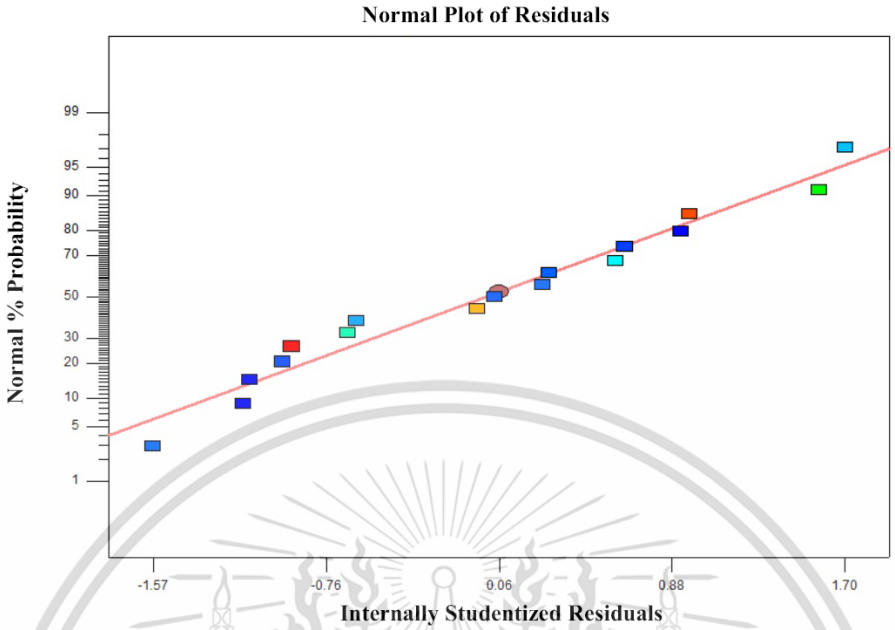


(A) Diagnostic plots of internally studentized residuals vs. Normal % probability of pectin yield from SHP

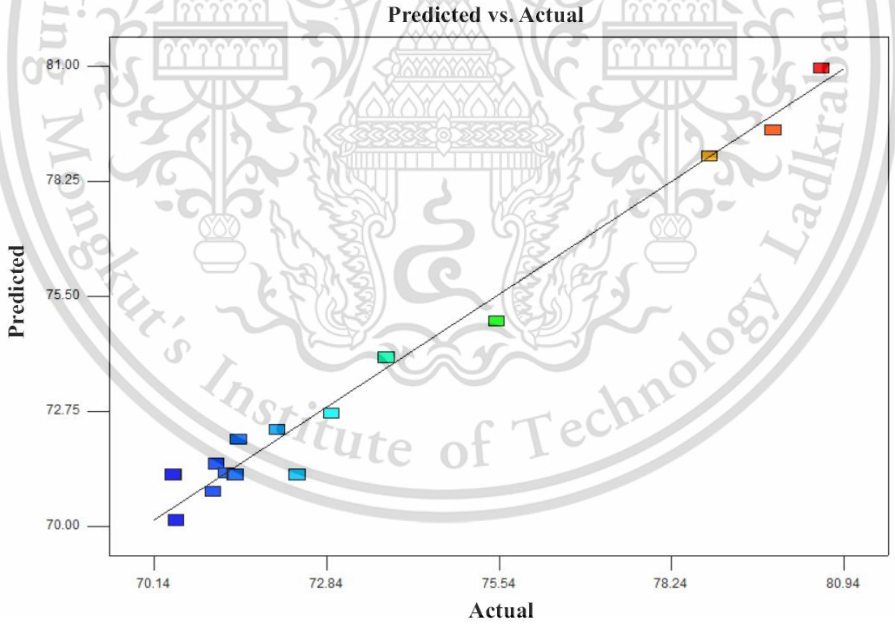


(B) Predicted vs. Actual of pectin yield from SHP

Appendix C4: Diagnostic plots of DE from SHP pectin



(A) Diagnostic plots of internally studentized residuals vs. Normal % probability of DE from SHP pectin



(B) Predicted vs. Actual of DE from SHP pectin

Appendix D

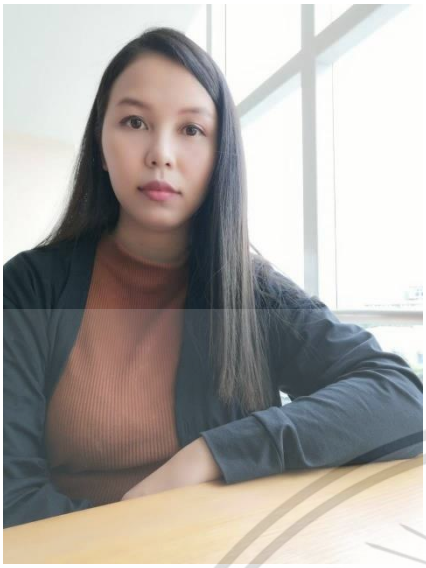
The soybean from the dehulling industry process

Appendix D: The soybean from the industry process.

(A) Whole soybean (B) Cracked soybeans

(C) Dehulled soybean grits (D) Soybean hulls

AUTHOR BIOGRAPHY



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