# EXTRACTION OF PECTIN FROM POMELO PEELS USING CONVENTIONAL HEATING, ULTRASONIC, MICROWAVE AND SUBCRITICAL WATER TECHNIQUES

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**FACULTY OF ENGINEERING**
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# EXTRACTION OF PECTIN FROM POMELO PEELS USING CONVENTIONAL HEATING, ULTRASONIC, MICROWAVE AND SUBCRITICAL WATER TECHNIQUES

## ABSTRACT

Citrus fruit peels can be converted into value-added products such as pectin and via the bioconversion to manage its disposal problems. Despite the wide utilization of pectin in the food industry, knowledge on pectin extraction process and its chemistry is limited. Therefore, this research was aimed to extract pectin from the peel of pomelo, the largest citrus fruit with its peel accounted for approximately 40% of its fruit weight, via different extraction techniques. The performances of various techniques were evaluated and the pectins obtained were compared via physicochemical properties and structural analyses. Conventional heating extraction (CE) method with citric acid as extraction solvent was first used to extract pectin from pomelo peel in the study. A Box–Behnken design was employed to optimize the yield and the degree of esterification (DE) of pectin. The optimized conditions were determined at pH 1.80, extraction time of 141 min, temperature of 88°C and liquid-solid ratio of 29:1 mL/g with a pectin yield of 39.13% and a DE value of 59.23%. The latter signifies a high methoxyl (HM) pectin was obtained. The extraction pH impacted pectin yield and its DE value greatly and pH of 1 – 2 resulted in diverse pectin functional groups with different structural modifications. The feasibility of non-conventional extraction methods such as ultrasound (UAE), microwave (MAE), ultrasound-microwave (UMAE), and microwave-ultrasound (MUAE) assisted extraction to extract pectin from pomelo peel using citric acid were also studied. All these methods also gave HM pectin with UMAE achieved the best yield (36.33%) followed by MUAE (31.57%), MAE (30.24%) and UAE (13.24%). The optimized conditions of UMAE were determined at pH 1.80, 27.52 min sonication and 6.40 min microwave irradiation at 643.44 W. In UMAE, pH exerted a significant impact on pectin yield while microwave power on DE value. The combined extraction technique i.e. UMAE and MUAE gave better galacturonic acid (GalA) content (> 65%) than that obtained from sole extraction technique (i.e. UAE or MAE). Furthermore, the morphological structures of pectin were affected by the technique and the sequence of the combined extraction methods; i.e. pectin extracted from MUAE closely resembled to that of MAE whereas it was smaller with more regular surface if subjected to ultrasonication before microwave as in UMAE. In addition, pectin was extracted in an acid free and environmental friendly subcritical water extraction (SWE) system. The SWE yielded 19.63% of pectin and a DE of 40.09% at optimized operating condition of 120°C and 30 bar. The extraction yield predominantly influenced by temperature. The absence of acid and a prolonged exposure to pressure under dynamic SWE conditions facilitated the formation of low methoxyl (LM) pectin. Compared with SWE, other extraction techniques incorporated with acid have higher pectin yields and greater DE values. Comparing the physicochemical properties of pectin extracted using different techniques, all of them possessed good quality within acceptable GalA limit (≥ 68%). They have close viscosity around ~0.01 Pa.s, molecular weight at ~$8.20 \times 10^4$ Da. Also, the pectin gels prepared from various extracted pectin exhibited pseudoplastic behavior. The pectin extracted from UMAE was lighter color and smaller in size. On the other hand, pectin extracted via SWE showed less acidity (pH 4.72) and better solubility attributed to its amorphous structure. In term of energy usage and efficiency, SWE consumed the highest energy at 1.0530 kW.h whereas UMAE achieved the highest efficiency at 0.4909 g/h. As a conclusion, UMAE is commendable to extract pectin from pomelo peel due to its operational efficiency.

**Keywords:** Pectin extraction, Physicochemical property, Yield and degree of esterification, Optimization, Citric acid

# PEKTIN PENGEKSTRAKAN DARIPADA KULIT BUAH LIMAU BALI DENGAN MENGGUNAKAN TEKNOLOGI PEMANAS KONVENSIONAL, ULTRASONIK, GELOMBANG MIKRO DAN AIR SUBKRITIKAL

## ABSTRAK

Kulit buah limau bali (KBLB) boleh ditukar kepada produk tambah nilai seperti pektin melalui biokonversi untuk menguruskan masalah pelupusannya. Walaupun penggunaan pektin yang luas dalam industri makanan, pengetahuan mengenai proses pengekstrakan pektin dan kimianya adalah terhad. Oleh itu, kajian ini bertujuan untuk mengekstrak pektin dari KBLB melalui teknik pengekstrakan yang berbeza, buah sitrus yang terbesar dengan kulitnya menyumbang kira-kira 40% berat buahnya. Prestasi teknik pengekstrakan dinilai dan pektin yang diperoleh dibandingkan dengan harta fizikokimia dan analisis struktur. Kaedah pengekstrakan pemanasan konvensional (CE) dengan asid sitrik sebagai pelarut pengekstrakan pertama digunakan untuk mengekstrak pektin dari KBLB dalam kajian. Box-Behnken design digunakan untuk mengoptimumkan hasil dan tahap esterifikasi (DE) pektin. Keadaan yang dioptimumkan ditentukan pada pH 1.80, masa pengekstrakan sebanyak 141 minit, suhu 88°C dan nisbah padu cecair 29:1 mL/g dengan hasil pektin sebanyak 39.13% dan nilai DE sebanyak 59.23%. Ini menandakan tinggi methoxyl (HM) pektin diperolehi. pH mempunyai pengaruh yang besar terhadap hasil pengekstrakan pektin dan nilai DE; pH 1 – 2 menghasilkan kumpulan fungsi pektin yang pelbagai dengan pengubahsuaian struktur yang berlainan. Pengekstrakan pektin dari KBLB dengan menggunakan asid sitrik juga dikaji dengan menggunakan teknologi pengekstrakan bukan konvensional seperti ultrasonik (UAE), gelombang mikro (MAE), ultrasonik-gelombang micro (UMAE), dan gelombang mikro-ultrasonik (MUAE). Semua kaedah ini juga memberikan pektin HM dengan UMAE mencapai hasil terbaik (36.33%) diikuti oleh MUAE (31.57%), MAE (30.24%) dan UAE (13.24%). Keadaan optimum UMAE ditentukan pada pH 1.80, ultrasonik 27.52 minit dan penyinaran gelombang mikro 6.40 minit pada 643.44 W, memberikan hasil pectin sebanyak 36.33% dan nilai DE sebanyak 59.85%. Dalam UMAE, pH memberikan impak yang besar terhadap hasil pektin manakala kuasa gelombang mikro pada nilai DE. Teknik pengekstrakan gabungan seperti UMAE dan MUAE menghasilkan kandungan asid galakturonik (GalA) yang lebih baik (> 65%) daripada pektin yang diperoleh daripada teknik pengekstrakan tunggal (iaitu UAE atau MAE). Tambahan pula, struktur morfologi pektin dipengaruhi oleh teknik dan urutan kaedah pengekstrakan gabungan; iaitu pektin yang diekstrak dari MUAE sangat mirip dengan MAE manakala ia lebih kecil dengan permukaan yang lebih teratur jika tertakluk kepada ultrasonik sebelum gelombang mikro seperti dalam UMAE. Selain itu, pektin diekstraksi dalam sistem pengekstrakan air subkritikal yang asid (SWE) bebas dan mesra alam. SWE menghasilkan 19.63% pektin dan DE sebesar 40.09% pada keadaan yang dioptimalkan 120°C dan 30 bar. Hasil pengekstrakan dipengaruhi oleh suhu. Di bawah keadaan ketiadaan asid dan pendedahan yang berpanjangan kepada tekanan dalam dinamik SWE memudahkan pembentukan pektin rendah methoxyl (LM). Berbanding dengan SWE, teknik pengekstrakan lain yang digabungkan dengan asid mempunyai hasil pectin yang lebih tinggi dan nilai DE yang lebih besar. Membandingkan sifat fizikokimia pectin yang diekstrak menggunakan teknik yang berbeza, semua pektin mempunyai kualiti yang baik dalam had GalA yang boleh diterima (≥ 68%). Mereka mempunyai kelikatan yang dekat ~0.01 Pa.s, berat molekul pada ~8.20 × $10^4$ Da. Gel pektin yang disediakan dari pelbagai pektin yang diekstrak mempamerkan sifat pseudoplastik. Pektin yang diekstrak daripada UMAE adalah warna yang lebih terang dan lebih kecil saiznya. Sebaliknya, pektin yang diekstrak melalui SWE menunjukkan kurang keasidan (pH 4.72) dan mempunyai kelarutan yang lebih baik dikaitkan dengan struktur amorfusnya. Dari segi penggunaan tenaga dan kecekapan, SWE menggunakan tenaga tertinggi pada 1.0530 kW.h sedangkan UMAE mencapai kecekapan tertinggi pada 0.4909 g/h. Kesimpulannya, UMAE adalah terpuji untuk mengekstrak pektin dari KBLB kerana kecekapan operasi.

**Kata kunci:** Pengekstrakan pektin, Harta fizikokimia, Hasil dan tahap esterifikasi, Pengoptimuman, Asid sitrik

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| | | |
|---|---|---|
| $a^*$ | : | Green value |
| $A_{1630}$ | : | Intensity of non-methyl-esterified carboxyl group (cm$^{-1}$) |
| $A_{1745}$ | : | Intensity of methyl-esterified carboxyl group (cm$^{-1}$) |
| $b^*$ | : | Red value |
| $C^*$ | | Chroma |
| $Da$ | | Daltons |
| $H^*_{\mathrm{ab}}$ | | Hue |
| k | | Number of factors |
| $K$ | | Consistency index |
| $L^*$ | | Lightness of color |
| $m$ | | Weight of dried pomelo peel powder (g) |
| $m_0$ | | Weight of dried pectin (g) |
| $m_1$ | | Weight of dry supernatant (g) |
| $m_2$ | | Weight of extracted pectin sample (g) |
| $m_{exp}$ | | Experimental value |
| $m_{pre}$ | | Predicted value |
| $n$ | | Flow behaviour index |
| n | | Number of combinations |
| $P$ | | Power dissipated (kW.h) |
| $Q$ | | Energy required (kW) |
| $t$ | | Extraction time (min or h) |
| v/v | | Volume per volume |
| w/v | | Weight per volume |
| w/w | | Weight per weight |

| | |
|---|---|
| $T_c$ | Critical temperature |
| $\tau$ | Shear stress (Pa) |
| $\dot{\Upsilon}$ | Shear rate (1/s) |
| $\eta$ | Viscosity (Pa.s) |
| ANOVA | Analysis of variance |
| AOAC | Association of official analytical chemists |
| BBD | Box-Behnken design |
| BV | Ball valve |
| CCD | Central composite design |
| CE | Conventional extraction |
| DE | Degree of esterification |
| DES | Deep eutectic solvent |
| D-GalA | D-galacturonic acid |
| EtOH | Ethanol |
| FAO | Food and agriculture organization |
| FT-IR | Fourier-transform infrared spectroscopy |
| GalA | Galacturonic acid |
| HCl | Hydrochloric acid |
| HM | High methoxyl |
| HPSEC | High performance size exclusion chromatography |
| IPA | Isopropyl alcohol |
| IPPA | International pectin producers association |
| LM | Low methoxyl |
| L/S | Liquid to solid ratio |
| MAE | Microwave assisted extraction |
| MALLS | Multiangle laser light-scattering |

| | |
|---|---|
| M | Molarity |
| Mw | Molecular weight |
| MUAE | Microwave-ultrasound assisted extraction |
| N | Normality |
| NV | Needle valve |
| $P_c$ | Critical pressure |
| pH | Power of hydrogen |
| PI | Pressure indicator |
| $R^2$ | Coefficient of determination |
| RI | OptilabreX differential refractometer |
| rpm | Revolution per minute |
| RSD | Relative standard deviation |
| RSM | Response surface methodology |
| S/F | Solvent to feed ratio |
| SEM | Scanning electron microscope |
| SWE | Subcritical water extraction |
| TI | Temperature indicator |
| UAE | Ultrasound assisted extraction |
| UMAE | Ultrasound-microwave assisted extraction |
| XRD | X-ray diffraction |

# LIST OF APPENDICES

# CHAPTER 1: INTRODUCTION

## 1.1 Research Background

The recovery of valuable compounds from food waste is becoming popular in food processing. Large amounts of non-edible wastes such as peels, seeds and bagasse produced by food industries cause pollution which is difficult to be managed and have resulted in economic loss. Researches over the past 20 years discovered that many food wastes could serve as a source of potentially valuable bioactive compounds which have beneficial effects on human health (Ferrentino et al., 2018). Direct waste from fruits such as citrus peels can be the source of raw material for extraction due to the presence of potential bioactive compounds such as pectin, polyphenols, carotenoids and etc. The major challenge for the recovery of these compounds is to determine an effective and eco-friendly extraction method which is capable of achieving maximum extraction yield without compromising the stability of the extracted products (Ferrentino et al., 2018). Profitability and health consideration, consciousness on efficient extraction process and cleanliness in extracted product have invigorated research into the investigation of up-to-date, innovative and environment friendly extraction technologies to replace conventional solvent extraction procedures in the recovery of pectin from various fruit sources. For example, combination of extraction technologies such as ultrasound with microwave to increase the yield of the extract.

Due to the rapid demand of pectin of over 14 million tonnes per year (Wood, 2016) and its wide application in food industry as thickener, emulsifier, stabilizer and gelling agent, many research works have been conducted to extract pectin from various sources using efficient extraction methods (Kanmani et al., 2014; Methacanon et al., 2014; Minjares-Fuentes et al., 2014; Quoc et al., 2015). Therefore, this research focuses on the exploration of conventional and non-conventional techniques with aim to extract pectin from pomelo peels effectively. Research works on process optimization of extraction parameters were conducted for both the conventional and non-conventional extraction methods. The non-conventional method such as sequential ultrasound-microwave assisted extraction method (UMAE) was conducted in batch mode whereas the subcritical water extraction (SWE) was a dynamic system without involve the use of acid. The physicochemical properties of pectin extracted from UMAE and SWE were compared with those using conventional method (CE). Detailed comparison study of the extraction methods and their performances are presented.

## 1.2 Problem Statement

An estimated 8,830 metric tons of pomelo (*Citrus grandis* (L.) Osbeck) fruits are produced in Malaysia annually (Zaini et al., 2014). This results in huge generation of pomelo waste and has triggered disposal and environmental issues. Pectin were extracted from waste materials such as peels of citrus fruit, apple pomace and sugar-beet pulp (Arslan & Kar, 1998). This suggests that the citrus fruit peel (pomelo peel wastes) can be a good source for pectin extraction. Through pectin extraction from the wastes, the disposal problem of pomelo peel wastes can be mitigated.

Pectin is commonly extracted using strong mineral acids. Reasonable extraction yields can be achieved by using mineral acids (Lim et al., 2012) but the pectin extracted is prone to degradation. Another shortcoming of using acids in the extraction is its high acidity that can accelerate corrosion of equipment and leads to water pollution problems. More importantly, pectin is commonly used in the food industry for human consumption and the use of strong mineral acids may not be consumer friendly. Hence, organic acid was applied in place of mineral acids in non-conventional extraction methods (e.g. ultrasound and microwave) aimed to minimize the use of detrimental chemicals during pectin extraction and to reduce the extraction time. In order to search for a greener extraction technique, an acid free subcritical water extraction was attempted to extract pectin from pomelo peels.

Despite that the optimization study of pectin extraction was often conducted, the optimum extraction conditions reported in the literature are mostly applicable to specific source of extraction and without comprehensive design of experiment involved. Also, the optimum conditions reported are limited to certain extraction method and hence there is a lack of understanding on the interaction among the operating parameters. Therefore, this study adopted design of experiment technique based on Response Surface Methodology (RSM) to elucidate the interactive effects of the extraction parameters for various extraction techniques. Considering the physicochemical properties of pectin extracted may vary according to the extraction method employed involving different parameters, pectin extracted from both the conventional and non-conventional extraction methods in the study was thoroughly characterized to classify the type of pectin for its possible industrial application.

## 1.3 Research Objectives

To overcome the problem statements described, objectives of various related studies are formed and they are:

i. To investigate the feasibility of pectin extraction from pomelo peel by using organic acid incorporated conventional extraction and non-conventional extraction methods.
ii. To evaluate the performance of acid free subcritical water system for pectin extraction.
iii. To perform comparison study of various extraction methods in terms of yield, degree of esterification and physiochemical properties.

## 1.4 Scope of Study

The scopes of the study are in accordance with the objectives. They are:

**Scope Objective 1**

1. Investigate the performance of conventional extraction and sequential ultrasound-microwave assisted extraction of pectin from pomelo peel using citric acid.
2. Optimize the operating condition of conventional extraction method (i.e. pH, extraction temperature, extraction time and liquid-solid ratio) and sequential ultrasound-microwave assisted method (i.e. pH, sonication time, microwave power and irradiation time) for pectin extraction using Box-Behnken design.
3. Compare the pectin extraction performance of UAE, MAE, MUAE and UMAE.
4. Determine the effect of low pH ranges on the chemical structure, morphological and gelling properties of the extracted pectin.

**Scope Objective 2**

1. Set up a dynamic acid free subcritical water extraction system for pectin extraction from pomelo peels.
2. Conduct a preliminary test on heating time required to achieve the desired extraction temperature.
3. Investigate the effects of extraction temperature and pressure on the performance of subcritical water extraction on pectin yield.
4. Optimize SWE operating parameters such as temperature and pressure in the extraction of pectin using face-centred central composite design.

**Scope Objective 3**

1. Characterize the physicochemical properties of the pectin extracted from various extraction methods in terms of degree of esterification (DE), galacturonic acid

(GalA), proximate analysis, pH, molecular weight, solubility, colour, gel viscosity, flow behaviour, FT-IR structure, XRD, micrograph and macrograph structures.

## 1.5 Structure of Thesis

This thesis is presented in five (5) chapters and the content of each chapter is described as follows:

### Chapter 1: Introduction

This chapter presents the background related to pectin extraction, problem statement of the research, the objectives to be accomplished, scopes of study, and the overall structure of the thesis.

### Chapter 2: Literature Review

This chapter reviews on the source of pectin, pectin and its applications, extraction method of pectin, non-conventional extraction methods, influencing parameters, experimental design tools for optimization study.

### Chapter 3: Materials and Methods

This chapter details the methods used for pectin extraction and characterization of the extracted pectin according to the flow of the objectives.

### Chapter 4: Results and Discussion

This chapter discusses the results obtained in five sections. The first section discusses the characteristics of pomelo peels powder followed by a discussion on the optimization of pectin extraction using conventional heating method (CE). Third section discusses the performance of ultrasound extraction (UAE), microwave extraction (MAE) and sequential extraction (UMAE & MUAE) based on pectin yield. In the penultimate section, the results and discussion on pectin extracted using subcritical water extraction

(SWE) set up is presented. The final section of this chapter discusses the physicochemical properties of extracted pectin by SWE and also compares the acid free extraction performance between SWE with both conventional extraction (CE) and non-conventional extraction (UMAE) method.

**Chapter 5: Conclusions and Recommendations**

This chapter summarises the results of various extraction methods used for extracting pectin from pomelo peels. The novelties and the implications of the study with recommendations for future works are also included.

# CHAPTER 2: LITERATURE REVIEW

This chapter presents the topics pertaining to pectin and the extraction methods. A brief introduction of pectin, its properties, classifications and potential applications as well as the source of pectin is provided. The conventional and non-conventional extraction methods employed for pectin extraction are also included. Various extraction methods namely conventional heating extraction, ultrasound assisted extraction, microwave assisted extraction and subcritical water extraction are reviewed. The parameters affecting the extraction performance of pectin are also elucidated.

## 2.1 Pectin

Pectin is a complex heteropolysaccharide mainly consists of α-(1,4)-linked D-galacturonic acid as the backbone with different degrees of esterification (DE) (Mohnen, 2008; Yuliarti et al., 2015). The chemical structure of pectin is depicted in Figure 2.1.

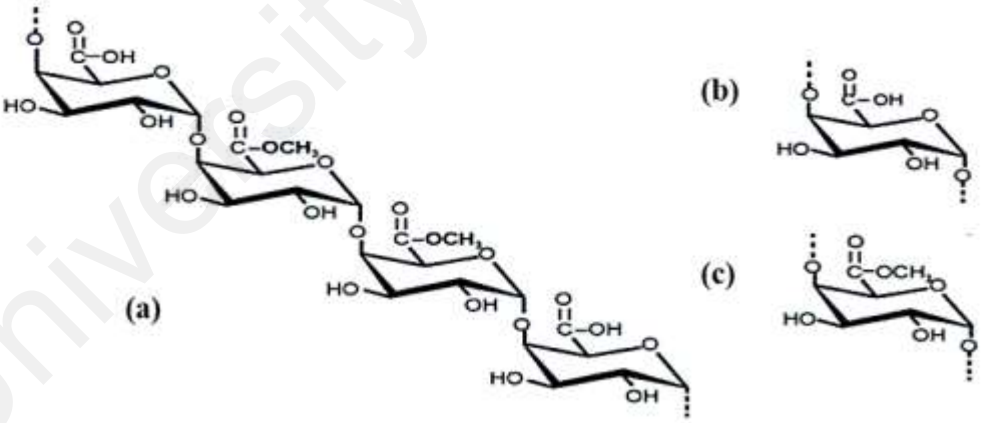


**Figure 2.1: Structure of pectin (a) repeating segment of the pectin molecules and the functional groups (b) carboxyl, (c) ester (Adapted from (Sriamornsak, 2002)).**

### 2.1.1 Properties of Pectin

Pectin is water soluble. Pectin that diluted with water behaves like Newtonian fluid and when it is at a moderate concentration, it exhibits a non-Newtonian (pseudoplastic) characteristic. DE is a property which can be used to determine the class of pectin. The viscosity of a pectin solution will be altered by DE, molecular weight of pectin and pH as well as by the presence of counter ions in the solution (Toha, 2013). The analytical measurement of pectin in term of its “DE” is a useful molecular index to classify it as high methoxyl (HM) or low methoxyl (LM) pectin. The classification of pectin will subsequently determine its application. According to Food and Agriculture Organization (FAO), a galacturonic acid content (GalA) ≥ 65% is considered as the typical minimum level for pectin to be used in food application (IPPA, 2011). Furthermore, the molecular weight of pectin is often an indication of its gelling ability in food industry. Apart from that, proximate analysis, morphological structure, functional group and colour are among the important analytical measures in determining the properties of pectin.

### 2.1.2 Classification of Pectin

Pectin is usually classified according to its degrees of esterification (DE). The degree of esterification can be expressed as the percentage of esterified galacturonic acid units to the total galacturonic acid in the molecule of pectin. DE influences the property of pectin, especially its solubility and the gel forming characteristics (Sundar Raj et al., 2012). Depending on the DE value, pectin can be classified as high methoxyl pectin (HM) with DE ≥ 50 or low methoxyl pectin (LM) with DE < 50 as shown in Figure 2.2. HM and LM pectins form gel under different mechanisms. HM pectin may be used for canning applications. It requires high amount of sugar to gel and it is sensitive to acidity (Pinheiro et al., 2008). LM pectin has been used in food industry to create low-sugar jams because it does not require high sugar levels but calcium to gel. This type of pectin is less sensitive to acidic medium.

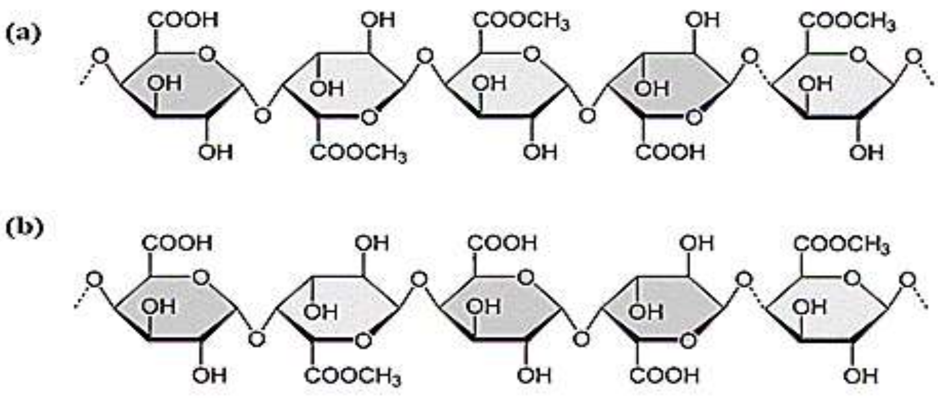


**Figure 2.2: Types of pectin (a) high methoxyl, HM (b) low methoxyl, LM (Adapted from (IPPA, 2011)).**

### 2.1.3 Application of Pectin

Pectin is situated in plant cell walls, a layer between the cells named middle lamella as illustrated in Figure 2.3. In plant, pectin serves as a strengthening or building agent for the plant to bind cells together. It is an important cell wall polysaccharide that allows primary cell wall extension and plant growth. Furthermore, pectin is also responsible to hold soft non-woody parts of the fruit firmly by keeping the walls of adjacent cells joined together (Phillips, 2000). When pectin in the fruit breaks down to simple sugars that are easily to be dissolved, the fruit would become soft and out of shape (Sriamornsak, 2002).

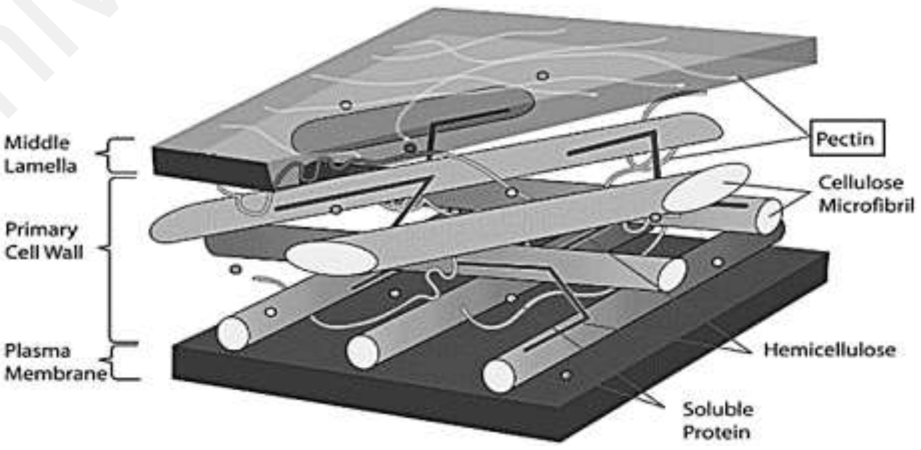


**Figure 2.3: Spatial arrangements of polymers in the cell walls of plants (Adapted from (Ladyofhats, 2007)).**

Pectin is used primarily in food industry as gelling agents, emulsifier, stabilizer and fat substitutes (Liew, Chin, & Yusof, 2015) and it is widely used in the production of jams and jellies, fruit juice, confectionary products, bakery fillings, frozen foods, yogurts, anti-diarrheal agents and in low-calorie foods to replace fat or sugar (Mierczyńska et al., 2014). Due to its unique properties, pectin has gained an increased importance in the pharmaceutical industry and biotechnology industry. For instances; some pectin has been used as biodegradable surfactants and emulsifiers; $Ca^{2+}$ sequestering agent in detergents; rheology modifier; fillers in low calorie food products; edible acidifying agents; and also it can be used in edible packaging (Rhodes, 1995). The application of various types of pectin are presented and summarized in Table 2.1.

**Table 2.1: Applications of HM and LM pectin**

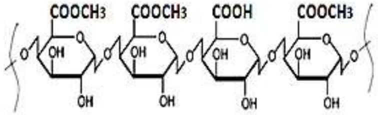
| Types of pectin | Structure | Applications | References |
|---|---|---|---|
| High Methoxyl pectin (HM) |  | -Apply in aerogels for enhancing the bioavailability of low-soluble drugs | Tkalec et al. (2015) |
| | | -Apply in microcapsules for controlled release of kenaf seed oil | Chew et al. (2015) |
| | | -Apply in microcapsules for controlled release of materials | Humblet-Hua et al. (2011) |
| | | -Coating for targeting of drugs to the colon by preventing too fast swelling and solubilization | Semdé et al. (1998) |
| | | -Apply in hydrogel matrix system | Sungthongjeen et al. (1999) |
| | | -Food-grade nano emulsions for containing essential oil | Guerra-Rosas et al. (2016) |
| | | -Apply in biodegradable films intended for food packaging | Piazza et al. (2009) |
| | | -Apply in composite film | L. Liu et al. (2007) |
| | | -Apply in biodegradable nanocomposite films | Lorevice et al. (2016) |
| | | -Reduce egg yolk cholesterol | Garcia Rojas et al. (2007) |

**Table 2.1, continued**

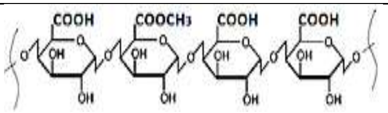
| | | | |
|---|---|---|---|
| | | -Instant jellies applied to bakery products | Hoefier (2012) |
| | | -Ingredient to make flavored candies | Thakur et al. (1997) |
| | | -Apply in edible coatings to inhibit lipid migration in confectionery products | Brake and Fennema (1993) |
| | | -Restore mouth feel for dietetic soft drinks | Hoefier (2012) |
| | | -As a beverage-clouding agent | El-Shamei and El-Zoghbi (1994) |
| Low Methoxyl pectin (LM) |  | -Apply in delivery system to improve encapsulation technology | Chotiko and Sathivel (2016) |
| | | -Apply in biodegradable nanocomposite films | Lorevice et al. (2016) |
| | | -Apply in edible film developed for antioxidant preservation | De'Nobili et al. (2015) |
| | | -Thickener for fruit fillings | Agudelo et al. (2014) |
| | | -Form pectin-calcium gels used in low-sugar products | Thakur et al. (1997) |

**Table 2.1, continued**

| | | |
|---|---|---|
| | -Provide a desired mouthfeel to conserves and produce bakery jams and jellies | Thakur et al. (1997) |
| | -Improve the texture and quality of fruits in ice-creams | Thakur et al. (1997) |
| | -Improve the barbecue sauce final consistency and texture | Hoefier (2012) |
| | -Produce milk gels | Oakenfull and Scott (1998) |

## 2.2 Source of Extraction: Citrus Fruits

### (i) Citrus

Pectin, as previously described is found in most plants, is highly concentrated in citrus fruits and apples. Citrus peels such as orange, lemon, lime and grapefruit are the most common commercial sources for pectin production (Quoc et al., 2015); attributing to its reasonably high pectin content indicated by the conventional extraction of various citrus fruits as shown in Table 2.2.

**Table 2.2: Pectin yield and DE from citrus family sources using conventional heating method**

| Citrus family source | Pectin yield (%) | DE (%) | Reference |
|---|---|---|---|
| Lemon[a] | 36.71 | 33.77 | Kanmani et al. (2014) |
| Lime[b] | 19.80 | 77.00 | Koffi et al. (2013) |
| Orange[a] | 67.30 | 35.85 | Elizabeth Devi et al. (2014) |
| ‘Pera’ sweet orange[b] | 38.21 | 70.21 | Zanella and Taranto (2015) |
| Grapefruit[b] | 21.10 | 68.20 | Arslan and Toğrul (1996) |
| Grapefruit[b] | 19.16 | 75.60 | Bagherian et al. (2011) |
| Pomelo[a] | 27.63 | 55.82 | Methacanon et al. (2014) |

[a] based on optimized results
[b] based on highest but not optimized yield

### (ii) Pomelo

In Malaysia, an estimated annual production of 8,830 metric tons of pomelo fruits was produced for commercial purposes (Zaini et al., 2014). The peels of pomelo (*Citrus grandis* (L.) Osbeck) had been reported to contain natural chemical components, such as cellulose, flavonoids, essential oil, pectin and etc. Pomelo is the largest citrus fruits with its peels accounted for approximately 40% of its fruit weight as illustrated in Figure 2.4, making the peels a potential source for pectin extraction.

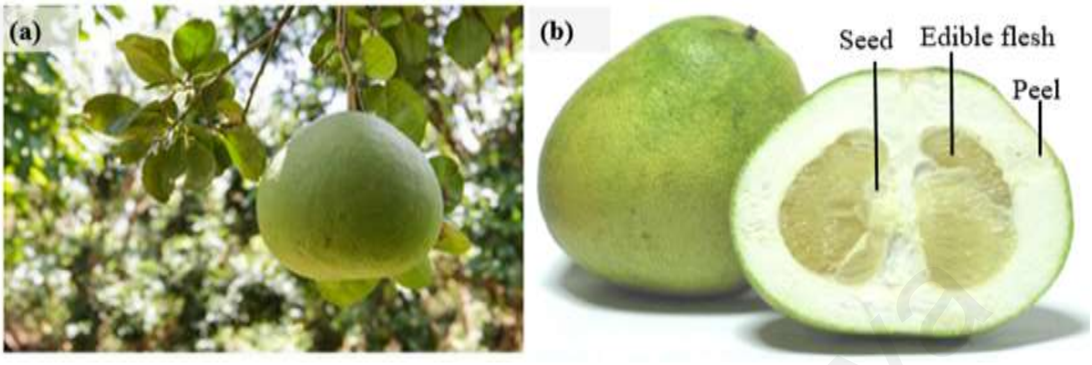


**Figure 2.4: Pomelo (a) fruit and (b) cross sectional diagram of the fruit.**

However, there is limited report on pectin extraction from pomelo peels. One study thus far by Methacanon et al. (2014) who extracted pectin from pomelo peels based on conventional heating method achieved a pectin yield of 27.63%. On the other hand, many different extraction techniques had been employed for the extraction of pectin from other fruit sources. The following section will review the conventional and non-conventional extraction method including their respective extraction mechanisms.

## 2.3 Extraction Techniques

Extraction is a process that separates a substance from a matrix. The process is extremely important to extract desirable product for various applications, i.e. biotechnology, pharmaceutical, food industries and environmental protection. Extraction techniques can be conventional or non-conventional.

The conventional heating extraction (CE) technique is commonly used to extract active compounds from plants. Food industries also employ it to extract and recover valuable active compounds from different plants and waste residues (Chan et al., 2014). However, most of the conventional heating extraction techniques is time consuming (Seixas et al., 2014) and laborious. They consume large amount of solvent and can thermally degrade targeted compounds at high temperature. Thus, the stated problems had been overcome by either applying alternative extraction methods or improving the existing conventional extraction methods such as enzymatic extraction, microwave assisted extraction, ultrasound assisted extraction, subcritical water extraction and etc. For instance, the efficiency of the conventional extraction was improved by incorporating microwaves or/and ultrasounds into the extraction system. The assisted techniques offer advantages and features which are suitable for specific extractions (Chan et al., 2014). Furthermore, subcritical water extraction had also been applied for plant extraction of flavonoids (11.78%), phenolics content (4.96%) and betulinic acid (0.28%)  (Ko et al., 2016; Lee et al., 2018; J. Liu et al., 2015; Pavlić et al., 2016; Tomšik et al., 2017; Zeković et al., 2014). On the other hand, numerous studies have been conducted to obtain pectin using enzymatic extraction (Lim et al., 2012; Yuliarti et al., 2011). However, enzymes are generally very expensive (Perussello et al., 2017) and highly sensitive to various denaturing conditions. The performances of various extraction methods are listed in Table 2.3. Both conventional and non-conventional methods showed very slight difference in their yields. However, the operating condition for the non-conventional extraction method used were milder as compared with that for the conventional method.

**Table 2.3: Performance and optimum condition of pectin extraction using various extraction methods**

| Methods | Substrate | Solvent | DOE | Operating conditions | Performance | References |
|---|---|---|---|---|---|---|
| CE | Grapefruit peel | hydrochloric acid | – | T= 85 °C; t= 90 min; pH= 2.5; S/L ratio= 1 g/25 mL | Y= 21.1%; DE= 68.2% | Arslan and Toğrul (1996) |
| CE | Pomelo peel | nitric acid | CCD | T= 90 °C; t= 180 min; pH= 2.0; S/L ratio= 1 g/30 mL | Y= 27.63%; DE= 55.82% | Methacanon et al. (2014) |
| CE | Orange peel | citric acid | – | T= 80 °C; t= 60 min; pH= 1.5; S/L ratio= 1 g/30 mL | Y= 67.3%; DE= 35.85% | Elizabeth Devi et al. (2014) |
| CE | Lime peel | nitric acid | – | T= 75 °C; t= 90 min; pH= 1.5; S/L ratio= 1 g/25 mL | Y= 19.8%; DE= 77% | Koffi et al. (2013) |
| CE | Lemon peel | citric acid | BBD | T = 65 °C; t = 68 min; pH = 3.5; S/L ratio = 1 g/18 mL | Y= 36.71%; DE= 33.77% | Kanmani et al. (2014) |
| CE | Cacao pod husks | nitric acid | – | T= 100°C; t= 30 min; pH= 1.5 ; S/L ratio= 1 g/25 mL | Y= 9.5% | Vriesmann et al. (2011) |

**Table 2.3, continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| CE | Red dragon fruit peel | citric acid | CCD | T= 73°C; t= 67 min; pH= 2.03 | Y= 26.38% | Muhammad et al. (2014) |
| CE | Sisal waste | – | BBD | T= 85°C, t= 60 min; S/L ratio= 2% | Y= 19.2% | Santos et al. (2013) |
| CE | Lemon by product | citric acid | CCD | T= 84.34°C; t= 3 hr 34 min; pH= 2.8; S/L ratio= 1 g/25 mL | Y= 11.21% | Masmoudi et al. (2008) |
| CE | Sugar beet pulp | hydrochloric acid | CCD | T= 93.7°C; t= 3 hr, pH= 1.21 | Y= 24.45% | Lv et al. (2013) |
| CE | Sugar beet pulp | citric acid | BBD | T= 99°C; t= 166 min; pH= 1.0; S/L ratio= 1 g/20 mL | Y= 23.95% | D.-q. Li et al. (2015) |
| CE | Durian rinds | hydrochloric acid | BBD | T= 86°C; t= 43 min; pH= 2.8; S/L ratio= 1 g/10 mL | Y= 9.3% | Maran (2015) |

**Table 2.3, continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| CE | Sweet potato residues | disodium phosphate | CCD | T= 66°C; t= 3.3 hr; pH= 7.9; S/L ratio= 1 g/20 mL | Y= 10.24% | C. Zhang and Mu (2011) |
| CE | Okra pods | hydrochloric acid | BBD | T= 60°C; t= 64 min; pH= 3.9; S/L ratio= 1 g/42 mL | Y= 2.68% | Y. Chen et al. (2014) |
| CE | *Aloe* leaves | hydrochloric acid | BBD | T= 90°C; t= 120 min; pH= 1.5; S/L ratio= 1 g/20 mL; After coagulation pH= 3.0; T= 50°C | Y= 1.39 g /20 g | Geng et al. (2014) |
| CE | Banana peel | citric acid | CCD | T= 87°C; t= 160 min; pH= 2.0 ; S/L ratio= 1 g/20 mL | Y= 14.23 g/100 g; DM= 53.73 | Oliveira et al. (2016) |
| MAE | Sour orange peel | citric acid | BBD | Power= 700 W; irradiation time= 3 min; pH= 1.50; S/L ratio= 1 g/15 mL | Y= 29.1%, DE=1.7-37.5% | Hosseini et al. (2016) |
| MAE | Orange peel | sulfuric acid | BBD | Power= 422 W; irradiation time= 169 sec; pH= 1.40; S/L ratio= 1 g/16.9 mL | Y= 19.24% | Prakash Maran, Sivakumar, et al. (2013) |

**Table 2.3, continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| MAE | Orange peel | nitric acid | – | Power= 630 W; t= 89 sec;<br>pH= 1.5; S/L ratio= 1 g/20 mL | Y= 13.32% | Kute et al. (2015) |
| MAE | Apple pomace | hydrochloric acid | CCD | Power= 499.4 W; t= 20.8 min;<br>pH= 1.01; S/L ratio= 0.069 | Y= 0.315 g/2 g | S. Wang et al. (2007) |
| MAE | Sugar beet pulp | sulfuric acid | BBD | Power= 152.63 W; t= 3.53 min;<br>pH= 1.57; S/L ratio= 1 g/18.92 mL | Y= 32.4% | Li et al. (2012) |
| MAE | Dragon fruit peel | hydrochloric acid | CCD | t =65 sec; pH= 2.07;<br>S/L ratio= 1 g/66.57 mL | Y= 18.53%;<br>DE= 46.95% | Rahmati et al. (2015) |
| MAE | Papaya peel | unknown | BBD | Power= 512 W; t= 140 sec;<br>pH= 1.80; S/L ratio= 1 g/15 mL | Y= 25.41% | Maran and Prakash (2015) |
| MAE | Pumpkin biomass | hydrochloric acid | BBD | t= 10 min; T= 102.2°C;<br>S/L ratio= 1 g/50 mL | Y= 7.1% | Košťálová et al. (2016) |

**Table 2.3, continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| MAE | Watermelon rinds | hydrochloric acid | BBD | Power= 477 W; t= 128 sec; pH= 1.52; S/L= 1 g/20.3 mL | Y= 25.79% | Prakash Maran et al. (2014) |
| UAE | Grapefruit peel | hydrochloric acid | BBD | T= 66.71°C; sonication time= 27.95 min; Power intensity= 12.56 W/cm$^2$; pH= 1.5 ; S/L ratio= 1 g/50 mL | Y= 27.46% | W. Wang et al. (2015) |
| UAE | Grapefruit peel | hydrochloric acid | BBD | Power intensity= 0.40 W/ml; Duty cycle= 50%, T= 60°C, S/L ratio= 1 g/50 mL | Y= 26.74% | Yuting Xu et al. (2014) |
| UAE | Grape pomace | citric acid | BBD | T=75°C; sonication time= 60 min, pH= 2.0 | Y= 34.70% | Minjares-Fuentes et al. (2014) |
| UAE | Pomegranate peel | unknown | BBD | T= 61.90°C; sonication time= 28.31 min; pH= 1.27 ;<br>S/L ratio= 1 g/17.52 mL | Y= 23.92% | Moorthy et al. (2015) |
| UAE | Sisal waste | – | BBD | T= 50°C; sonication time= 26 min; Power= 61 W; S/L ratio= 1 g/28 mL | Y= 29.32% | Maran and Priya (2015) |

**Table 2.3, continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| UAE+SWE | Sugar beet pulp | water | BBD | T= 120.72°C; t= 30.49 min;<br>Pressure= 10.70 MPa;<br>S/L ratio= 1 g/44.03 mL | Y= 24.63% | H.-m. Chen et al. (2015) |
| UAE+ MAE | Grapefruit | hydrochloric acid | – | sonication time= 30 min;<br>microwave heating= 10 min;<br>microwave power= 0.45 kW;<br>S/L ratio= 1 g/30 mL | Y= 31.88% | Bagherian et al. (2011) |
| UAE+ MAE | Jujube waste | hydrochloric acid | BBD | sonication time= 17.66 min;<br>microwave heating= 52.73 sec;<br>pH= 1.97; S/L ratio= 1 g/10.03 mL | Y= 1.95% | Bai et al. (2015) |
| SWE | Citrus peel | water | – | T=120°C; t= 5 min;<br>S/L ratio= 1 g/30 mL | Y= 21.95% | X. Wang et al. (2014) |

t=time; T= temperature; Y= pectin yield

### 2.3.1 Conventional Heating Extraction Method (CE)

Conventional heating extraction uses solvent such as water or acid to extract targeted component from plant matrices. The extraction of pectin using acid as extraction solvent is a process of acidic hydrolysis of protopectin. Protopectin is the parent substance from which the pectin is derived within the plant matrix. The principal mechanism of the process involves three steps (Figure 2.5): (i) hydrolysis of protopectin occurs in the solid phase of an extractant (extraction agent); (ii) internal diffusion of the dissolved pectin; (iii) external diffusion of dissolved pectin through the liquid boundary layer (Minkov et al., 1996; Ptichkina et al., 2008). Table 2.4 shows some conventional extractions using mineral acid and organic acid to extract pectin. Extraction of pectin using organic acid gave comparable extraction yield as that using mineral acid, and in some cases the extraction yield was even better. In the extraction of pectin using CE, the commonly used raw materials are citrus fruit peel, sugar beet, banana peel, durian rinds and etc. The extraction yields from different sources varied greatly from 2.68 % to 67.30%.

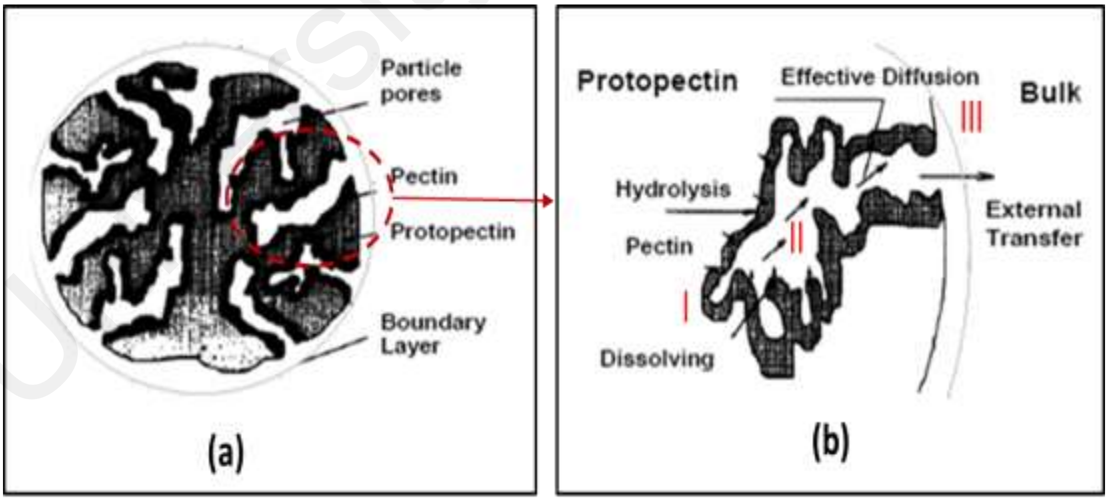


**Figure 2.5: Illustration of (a) a porous particle and (b) the mechanism of pectin hydrolysis and transport of the pectin to the particle surface (adapted from Minkov et al. (1996)).**

**Table 2.4: Different acidic extraction agents used in pectin extraction**

| Source | Extraction agent | Pectin yield | References |
|---|---|---|---|
| Grapefruit peel | hydrochloric acid | 21.10%; | Arslan and Toğrul (1996) |
| Sugar beet pulp | hydrochloric acid | 24.45% | Lv et al. (2013) |
| Durian rinds | hydrochloric acid | 9.30% | Maran (2015) |
| Okra pods | hydrochloric acid | 2.68% | Y. Chen et al. (2014) |
| *Aloe* leaves | hydrochloric acid | 6.95% | Geng et al. (2014) |
| Pomelo peel | nitric acid | 27.63% | Methacanon et al. (2014) |
| Lime peel | nitric acid | 19.80%; | Koffi et al. (2013) |
| Cacao pod husks | nitric acid | 9.50% | Vriesmann et al. (2011) |
| Orange peel | citric acid | 67.30% | Elizabeth Devi et al. (2014) |
| Lemon peel | citric acid | 36.71% | Kanmani et al. (2014) |
| Red dragon fruit peel | citric acid | 26.38% | Muhammad et al. (2014) |
| Lemon by product | citric acid | 11.21% | Masmoudi et al. (2008) |
| Sugar beet pulp | citric acid | 23.95% | D. Li et al. (2015) |
| Banana peel | citric acid | 14.23% | Oliveira et al. (2016) |

### 2.3.2 Non-conventional Extraction

#### 2.3.2.1 Ultrasound (UAE), microwave (MAE) assisted extraction and combined assisted extraction methods

##### i. Ultrasound assisted extraction method (UAE)

Ultrasounds are mechanic waves that required an elastic medium to spread. The difference between sound and ultrasounds is in their wave frequency. Ultrasounds have frequencies above human hearing frequencies (16 Hz to 20 kHz) but below microwaves frequencies (20 kHz to 10 MHz) (F. Chemat et al., 2011). Ultrasound-assisted extraction (UAE) uses acoustic energy and solvent to extract compounds from plant matrices (Reis et al., 2015). This extraction technique requires short extraction time, low solvent cost, high quality of the extraction and it is environment-friendly (Kumar et al., 2017). Hence, pectin had been extracted using ultrasound assisted extraction on different plant materials, such as tomato waste (Grassino et al., 2016), grapefruit peel (Bagherian et al., 2011; Minjares-Fuentes et al., 2014; W. Wang et al., 2015; Yuting Xu et al., 2014), sisal waste (Maran & Priya, 2015), pomegranate peel (Moorthy et al., 2015) and etc.

The mechanism of UAE is based on sonication via induced micro streaming effect to propagate sound waves that create cavitation in the liquid solution (Figure 2.6) (F. Chemat et al., 2011). The subsequent collapse of the cavitated bubbles near the plant material surface would result in an increase in pressure and temperature. These phenomena destroy the cell walls of the plant matrix and release extractive compounds into the solution. The performances of pectin extraction using ultrasound assisted extraction method are presented in Table 2.3 with yield ranges between ~23.92 and 34.70%.

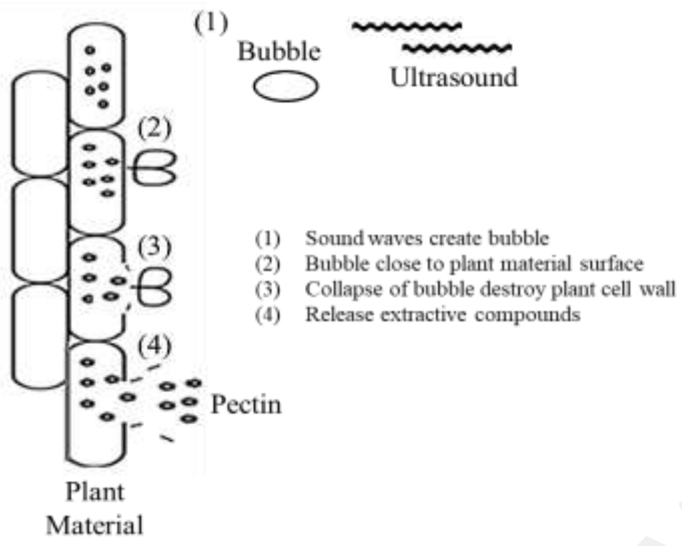


**Figure 2.6: Schematic diagram of ultrasound extraction mechanisms of natural products (modified from F. Chemat et al. (2011)).**

### ii. Microwave assisted extraction method (MAE)

Microwave-assisted extraction is a non-conventional heating method which can directly extract plant constituents from solid matrixes. This method uses microwave energy to heat solvent that comes in contact with a sample in order to partition analytes from the sample matrix into the solvent (S. Wang et al., 2007). It is very effective compared to conventional method as its extraction time usually varies from a few seconds to less an hour. Unlike X-rays and gamma rays, microwaves are non-ionizing radiations and they do not break chemical bonds or cause molecular changes in a compound by removal of electrons (Haynes & Vaclavik, 2018). During extraction, microwave energy heats up the moisture inside the cells and causes it to evaporate which subsequently leads to a tremendous increase in pressure on the cell walls. As a result, plant tissue ruptures and releases the targeted compounds into the surrounding solvent (Figure 2.7) (Dhobi et al., 2009). MAE had been successfully applied to extract pectin from a wide variety of natural resources such as lime, orange peel and pomelo peel (Fishman et al., 2006;

Prakash Maran, Sivakumar, et al., 2013; Quoc et al., 2015). The extraction yield of different resources varied from 7.10% – 32.40% as shown in Table 2.3.

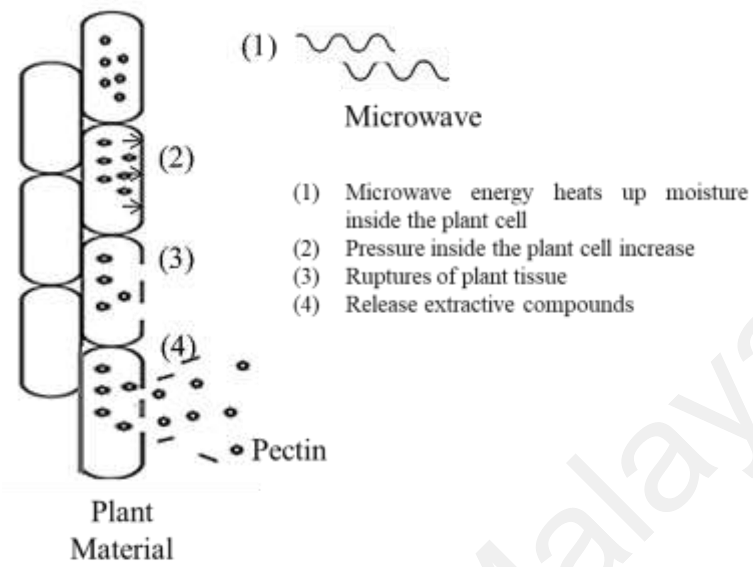


**Figure 2.7: Schematic diagram of microwave extraction mechanisms of natural products (modified from F. Chemat et al. (2011)).**

### iii. Combined assisted extraction method

Different extraction techniques offer different advantages. For example, ultrasound extraction can be operated under non-thermal conditions while microwave radiation extraction requires shorter extraction time compared with other extraction techniques. In view of their advantages, employing a combined ultrasound and microwave assisted extraction could potentially minimize or prevent the degradation of extract. The combined techniques may achieve higher yields and are suitable for the extraction of thermally labile active compounds. The findings obtained by Bagherian et al. (2011) on pectin extraction from grapefruit using ultrasound as a pre-treatment step before microwave extraction (31.88%) showed better results than that only employed MAE (27.81%). In the extraction of anthraquinones from *Heterophyllaeapustulata* Hook f. (Rubiaceae) by Barrera Vázquez et al. (2014) via the combined ultrasound with microwave exhibited highest efficiency among the Soxhlet and the UAE extractions. Meanwhile, the energy consumption for the extraction of *Heterophyllaea pustulata* Hook f. (Rubiaceae) using UMAE and UAE was rather similar at around 1.45×$10^7$ kJ/gAQs while Soxhlet consumed 2.88×$10^7$ $10^7$ kJ/gAQs. (Barrera Vázquez et al., 2014). Despite recent investigations utilizing ultrasound with microwave assisted extraction technique on natural products, there is a lack of comprehensive study on UMAE using citric acid in the extraction of pectin. In view of their (UAE and MAE) respective mechanisms, combining UAE and MAE (Figure 2.8) with suitable operating conditions might accelerate the extraction processes and would probably yield better quality pectin while avoiding possible thermal degradation.

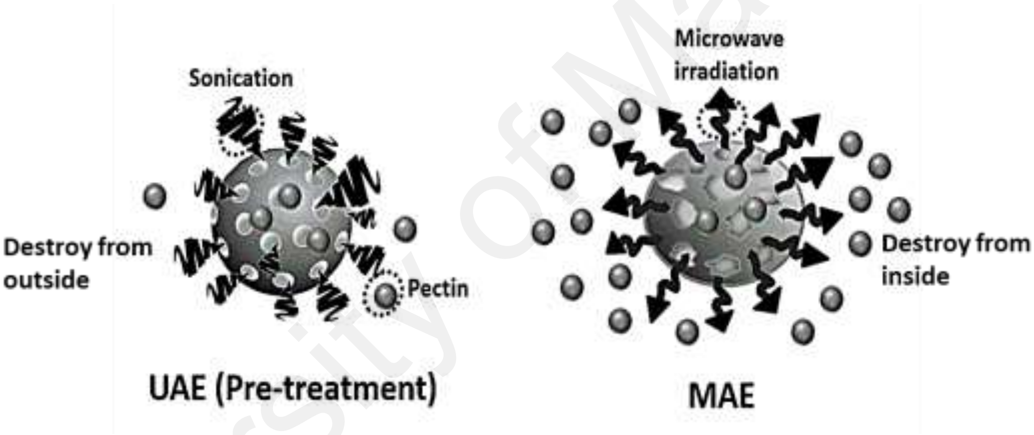


**Figure 2.8: Modified schematic diagram of sequential ultrasound-microwave assisted extraction mechanisms of pectin (Adapted from Liew et al. (2016)).**

#### 2.3.2.2 Subcritical water extraction method (SWE)

Conventional method for extraction of pectin generally requires the use of harsh acid extraction solvent that commensurate with long extraction time. The assisted extraction techniques such as ultrasonic and microwave, also often require the use of acid as extraction agent to facilitate the hydrolysis of protopectin. Acid is corrosive and hazardous; requiring equipment that are resistant to corrosion and necessitating further treatment to the liquid waste generated from the acid extraction process (Maurya et al., 2015; Rojas et al., 2015). As a consequent, the use of acid tends to increase production costs along with increasing wastewater treatment and maintenance costs. Hence, an approach to extracting pectin without acid can potentially reduce the cost of extraction is essential (Zakaria & Kamal, 2016).

One of the alternative methods without involving acid in the extraction of pectin is to use subcritical water approach. Subcritical water extraction (SWE) is a technique that based on the use of an attractant between 100 to 374°C under pressure (Ayala and De Castro 2001); the operating conditions usually are below the water critical pressure and critical temperature ($P_c$= 22MPa (220 bar); $T_c$= 374 °C) (Lancas, 2003) (Figure 2.9). The chief reason being that the pH of water drops as temperature increases (Carr et al., 2011; Shitu et al., 2015) and thus, circumventing the need for acid solvents.

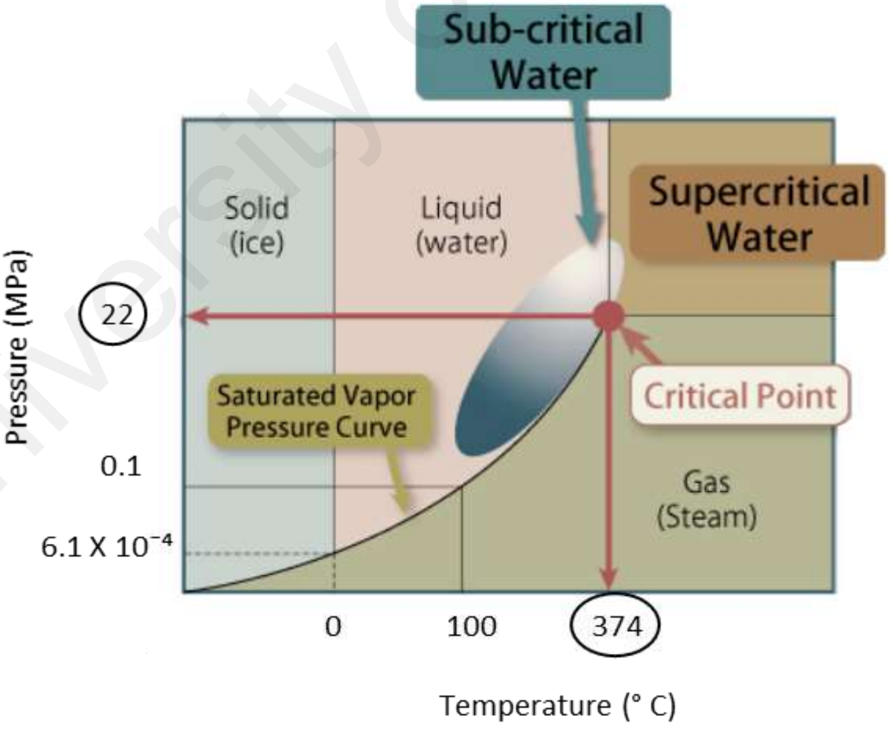


**Figure 2.9: The phase diagram of water representing subcritical region (Adapted from Thiruvenkadam et al. (2015)).**

The use of subcritical water has other added advantages including an increase in the quality and purity of the extract (Ramos et al., 2002). In addition, water is non-toxic, inexpensive, readily available and can easily be disposed (Teoh et al., 2013).

Subcritical water technology had also successfully been used to extract value-added products i.e. pectin enriched materials, polyphenols, hemicelluloses, pectin, essential oil (H.-m. Chen et al., 2015; Duba et al., 2015; Hanim et al., 2012; Hoshino et al., 2009; Ozel et al., 2003; X. Wang et al., 2014; Zeković et al., 2014).

The mass transfer of subcritical water extraction process (Figure 2.10) is generally involved: (1) rapid fluid entry; (2) desorption of solutes from matrix active sites; (3) diffusion of solutes through organic materials; (4) diffusion of solutes through static fluid in porous materials; (5) diffusion of solutes through layer of stagnant fluid outside particles; and (6) elution of solutes by the flowing bulk of fluid (Asl & Khajenoori, 2013).

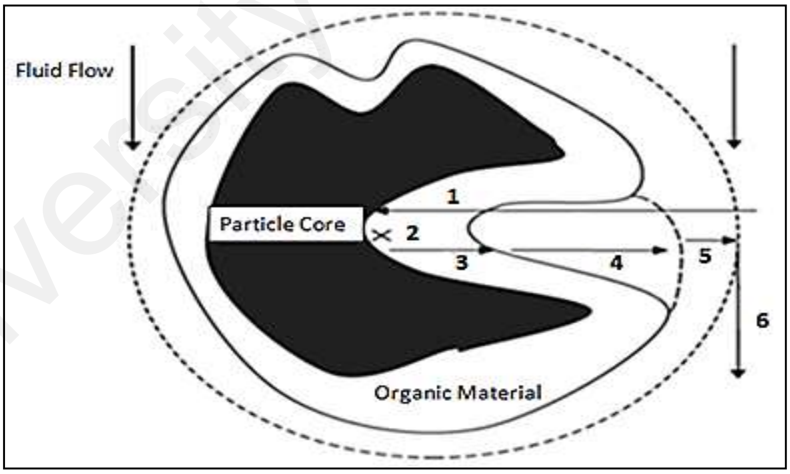


**Figure 2.10: Mechanism of pectin extraction in subcritical water extraction (Adapted from Asl and Khajenoori (2013)).**

The review compiled from the literature shows that different extraction techniques gave different pectin yields. The factors applied in the extraction also have different degree of impact on the performance of the extraction. Hence, some important influencing factors of pectin extraction will be discussed.

## 2.4 Factors of Extraction

Extraction of pectin can be influenced by process parameters such as extraction solvent, pH, temperature, time, liquid to solid ratio and etc (Table 2.3) (Anuar et al., 2013; Borges et al., 2011; Wijngaard & Brunton, 2010). Furthermore, specific factors such as sonication time, microwave power and irradiation time are also needed to be considered in ultrasound and microwave extraction, respectively. As for the subcritical water extraction, it is important to give proper consideration to factors such as temperature and pressure to ensure the extraction can be implemented effectively. The effect of each of the process variables was elucidated in the following sections.

### 2.4.1 Extraction Solvent

The extraction of natural products from plants requires the use of solvent to separate the solute from the plant material. For an extraction to be efficient, the selected solvent must be able to solubilize the target analytes while leaving the sample matrix intact (Mottaleb & Sarker, 2012). In plant or herb extraction that intended for food/drug/medical study, the extraction solvents used should not have toxic effect and interfere strongly with living cells, animals, and human beings. For examples; extraction solvents such as methanol, acetone, chloroform, dichloromethane, etc. have high toxicity to living cells; and extraction solvents of low boiling point that have high risk and safety issues (Mottaleb & Sarker, 2012). The solvents stated should not be used as extraction solvents. Besides that, cost effectiveness and working efficiency of the solvents should be taking into consideration for the selection of extraction solvent.

Solvents that had been used as extraction solvents are water, ethanol, ethyl acetate and hexane. Water can be used to extract product mainly containing metals, ions, high hydrophilic compounds, water soluble proteins/enzymes, glycoproteins, peptides, amino acids, nucleotides, sugars and polysaccharides (Yaqin Xu et al., 2016). On the other hand, ethanol can be used to extract high hydrophilic compounds (i.e. amino acids, nucleotides, sugars and polysaccharides). Furthermore, ethyl acetate has been used to extract medium hydrophobic compounds (i.e. steroids, wax, fatty acids, alkaloids and etc). To extract low or non-polar hydrophobic compounds with extremely high lipophilicity (i.e. low polar neutral compounds, steroids and high carbon fatty acids), hexane is commonly used.

In the extraction of pectin, solvents that commonly used are strong mineral acids (Table 2.4). Strong mineral acids can achieve reasonable extraction yield and the extraction using mineral acids are time efficient (Lim et al., 2012). However, pectin extracted using high acidic solvent could result in pectin degradation, corrosion of equipment and might also impose a negative impression to customers. To address the issues, organic acid has replaced mineral acids in pectin extraction of many fruits wastes (Jamsazzadeh Kermani et al., 2015; Kontogiorgos et al., 2012; Kurita et al., 2008; D.-q. Li et al., 2015; Oliveira et al., 2016; Virk & Sogi, 2004; Vriesmann et al., 2012). Among the organic acids, citric acid is favourable for pectin extraction in terms of its yield and physicochemical properties, lower production cost and environmental friendliness (Canteri-Schemin et al., 2005; Kliemann et al., 2009; Pinheiro et al., 2008; Virk & Sogi, 2004; Vriesmann et al., 2012; B. M. Yapo, 2009a; Beda M. Yapo, 2009b). Thus, citric acid has been employed for conventional heating extraction and ultrasound-microwave assisted extraction of pectin.

### 2.4.2 pH

pH is important for pectin extraction (Kalapathy & Proctor, 2001; Pagán et al., 2001). The pH that required for pectin extraction varies depending on the substrate used for pectin extraction. The commonly applied pH for pectin extraction ranges from 1 to 3 (Adetunji et al., 2017). In the extraction of pectin, acidic pH is necessary to split up the soluble pectin and the cellulose by eliminating the water molecules for the hydrolysis of protopectin (Hamidon & Zaidel, 2017) in which the protopectin is a combined compound of cellulose with pectin molecules (Elizabeth Devi et al., 2014). Although neutral and alkaline conditions can be used for the hydrolysis of pectin, it is not suitable for pectin extraction. This is because pectin can be degraded by β-elimination mechanism which involves the hydroxide ions. Therefore, the β-elimination degradation process takes place more rapidly under neutral and alkaline pH environment (Kravtchenko et al., 1992; Panda, 2011). On the other hand, too strong an acid may also cause hydrolysis degradation (Panda, 2011). Both the acid and alkaline pH dependent mechanisms for pectin degradation are depicted in Figure 2.11.

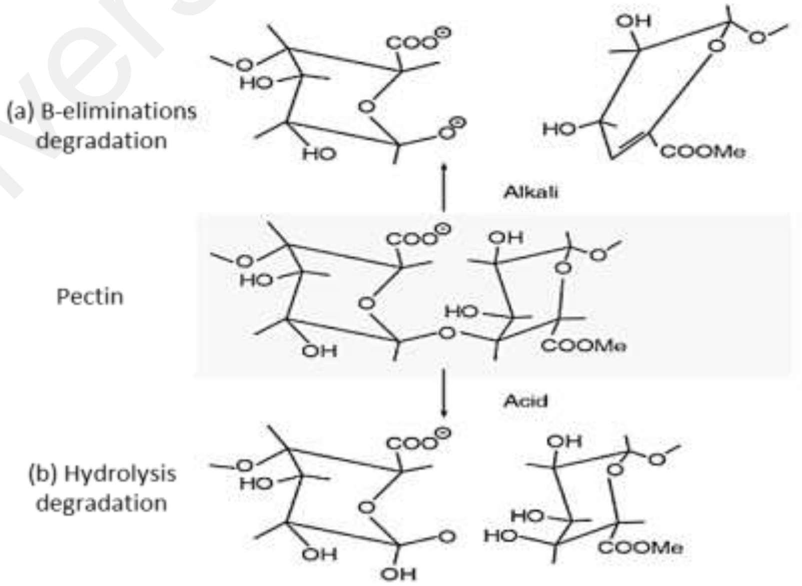


**Figure 2.11: Mechanism of pectin degradation (a) β-eliminations degradation caused by alkali treatment (b) hydrolysis degradation caused by acid treatment (Adapted from (Panda, 2011)).**

The optimum pH for pectin extraction by conventional heating varies for different substrates when different types of solvent are involved. For example, optimum pH for sugar beet pulp was 1 (D. Li et al., 2015) and pomelo peel was 2 (Methacanon et al., 2014) and the extraction yield was 23.95% and 27.63%, respectively. When alkaline medium such as disodium phosphate was used in pectin extraction from sweet potato residues,  the optimum pH was 7.9 and the pectin yield was 10.24% (C. Zhang & Mu, 2011). For extraction using UAE and MAE, the optimum extraction pH was generally in the range between 1.5 and 2 (Table 2.3). This indicates that acid medium is preferred for pectin extraction.

### 2.4.3 Temperature

Temperature is an influencing parameter for pectin extraction. The temperature selected for extracting a specific group of substances is dependent on the targeted compound’s molecular structure, plant matrix characteristics, degradation tendency, and also the extraction time (Meireles, 2008). During extraction, increase in temperature causes an increase in solubility and diffusion coefficients of the targeted compound while decreases the viscosity of the solvent; and this in turn has enhanced mass transfer and penetration of the solvent into the plant matrix (Al-Farsi & Lee, 2008; Hemwimon et al., 2007; Silva et al., 2007; Tan et al., 2013; J. Wang et al., 2008). Also, this accelerates the softening of the plant tissue and weakens the interactions between the targeted compound and other compounds in the plant matrix. Consequently, more targeted compound can be transferred to the solvent  (Shi et al., 2003). On the other hand, elevating the temperature up to a certain level can risk the denaturation of membranes and the degradation of targeted compound by hydrolysis, internal redox reactions and polymerizations, resulting in a decrease in extraction yield (Abad-García et al., 2007).

The temperature for pectin extraction of citrus family sources using conventional heating involving acid solvents normally ranges from 65°C to 90°C (Kanmani et al., 2014; Methacanon et al., 2014). The extraction temperature for Okra pods and Cocao pod husks was 60°C and 100°C, respectively (Y. Chen et al., 2014; Vriesmann et al., 2011). In the case of ultrasound-microwave assisted extraction, the extraction temperature is dependent on the microwave power while the best temperature for subcritical water extraction of pectin was reported to be 120°C for citrus (X. Wang et al., 2014), 150°C for apple pomace (X. Wang et al., 2014) and 120.72°C for sugar beet (H.-m. Chen et al., 2015).

### 2.4.4 Extraction Time

Extraction time is another factor that influences the extraction efficiency. Depending on the extraction methods used, the extraction time required for pectin extraction can vary accordingly. For examples, long extraction time up to 214 min (Masmoudi et al., 2008) was needed for conventional heating extraction. In general, extension of extraction time can improve the extraction yield. This is due to the fact that longer time is needed to soften the plant matrix and to ease the release of the targeted compound. However, too long an extraction time is not cost effective and it is inefficient.

In ultrasound extraction of pectin, sonication time must be adjusted to obtain good recovery of pectin. The extraction efficiency increased with the sonication time until an equilibrium was reached (Capelo-Martinez, 2009). Longer extraction times could reduce the pectin recovery due to degraded compound or decomposition of the solvent caused by ultrasound waves (Melecchi et al., 2006). A sonication time of 26 min (Maran & Priya, 2015) and 60 min (Minjares-Fuentes et al., 2014) were used to extract pectin via ultrasound extraction.

In microwave extraction, the factor to be considered is the irradiation time. The irradiation time for pectin extraction usually ranged from a few seconds (i.e. 65 sec) to a few minutes (i.e. 20.8 min)(Rahmati et al., 2015; S. Wang et al., 2007). In the review of Deo et al. (2015), microwave power and temperature were reported to be interrelated. Furthermore, the extraction yields were low at high microwave power or low microwave power with long irradiation time (20 – 25 min). The lengthy irradiation period causes the loss of chemical structure of the targeted compounds. For the combined extraction method i.e. UMAE and MUAE, a total extraction time of 40 min was needed for grapefruit (Bagherian et al., 2011) and ~19 min for *Jujube* waste (Bai et al., 2015).

There is limited report on extraction time required for subcritical water extraction of pectin. The extraction time for the subcritical water extraction in dynamic mode is subject to the time from first yield recorded until there is no yield obtained. The time required for the subcritical water pectin extraction in dynamic mode from juice processing residue was discovered to be ~140 min (Hoshino et al., 2009).

### 2.4.5 Liquid to Solid Ratio

The liquid to solid (L/S) ratio is always important for pectin extraction. The driving force for mass transfer is the concentration gradient between the solid and the bulk of the liquid, and it is greater when the liquid to solid ratio is higher (Meireles, 2008). From an economical point of view, liquid consumption incurs a direct cost for extraction process. A large liquid to solid ratio implies a high demand on the chemical solvent. It does not only incur higher chemical cost, but also requires more energy and time to heat up the extraction solution during extraction. Also, extra cost is needed for solvent removal after the extraction. On the other hand, low liquid to solid ratio builds the mass transfer barrier as the distribution of active compounds is concentrated in certain regions which limits the movement of the compounds out of plant matrix (Mandal & Mandal, 2010). Thus, the recommended range of liquid to solid ratios for plant extraction such as anthocyanins, phenolic antioxidants and total flavonoid compound is 10:1 – 50:1 mL/g (W. Liu et al., 2010; Pedro et al., 2016; Zuorro & Lavecchia, 2013). The minimum liquid to solid ratio needed for the extraction process is governed by the surface area of the feed (sample solid). The minimum liquid to solid ratio for pectin extraction should be controlled at 15 mL/g to make sure sufficient solvent is available for the extraction of pomelo peel powder at particle size of ~300 μm. Table 2.3 lists the optimum liquid to solid ratios are in the range of 20:1 – 30:1 mL/g for various extractions.

### 2.4.6 Microwave Power

Microwave power required is directly dependent on the quantity of sample and the time of extraction (Deo et al., 2015). Microwave power provides localized heating in the sample, which acts as a driving force for microwave assisted extraction to destroy the plant matrix, so that the solute can diffuse out and be dissolved in the solvent. As previously described, microwave power and temperature are interrelated, high microwave power elevated the temperature of the system and increased the extraction yield in shorter extraction time (S. Chemat et al., 2005; Mandal & Mandal, 2010). However, too high a microwave power may result in poor extraction yield due to pectin degradation. Thus, it is important to select suitable microwave power that compatible with irradiation time to maximize the extraction efficiency. In the MAE of flavonoids from mulberry leaves (Q. Chen, 2011) under different microwave powers (200 – 600 W), the yield of flavonoids increased with increasing microwave power until the highest yield of flavonoids of 1.38% was achieved at 500 W. No decomposition of flavonoids was detected under the selected microwave power (200 – 600 W). Several studies also suggested that microwave power at ~500 W gave the best pectin yield from various extraction sources such as waste *Carcia* *papaya* L. peel (25.41%), *Citrullus lanatus* fruit rinds (25.79%) and apple pomace (15.75%) (Maran & Prakash, 2015; Prakash Maran et al., 2014; S. Wang et al., 2007).

### 2.4.7 Pressure

Pressure plays an important function in subcritical water extraction, as it is the parameter that maintaining water in the liquid state at high temperatures. Pressures of 30 – 100 bar are usually employed to keep the liquid state of water. According to Kronholm et al. (2007), the mass-transfer property of water was enhanced under sufficient pressure. Therefore, it is crucial to evaluate the effect of pressure on the performance of extraction. In the SWE of pectin from juice processing residue (Hoshino et al., 2009) under extraction temperature of 120°C, pectin yield was higher at lower pressure. The pressure did not affect the yield of pectin at higher temperature region and the effect of pressure was not obvious in SWE extraction.

## 2.5 Optimization Techniques and Tools

Optimization process is a technique that can be applied to reduce processing cost and time, while enhancing the process performance such as yield. Apart from the traditional "one-factor-at-a-time" statistical technique and the factorial design/two level factorial, response surface methodology is widely used in optimization study.

In the one-factor-at-a-time approach, only one factor is varied at a time while all other factors are remained constant. This technique is tedious and time-consuming. Besides, the technique requires a large number of experiments which could be expensive. Moreover, it does not establish any equation to describe the relationship between variables and their responses. The results of one factor at a time experiments do not reflect the actual changes in the environment as the technique ignores the interactions between factors (Ma & Ooraikul, 1986). On the other hand, the factorial design has minimum one factor with more than two levels. The two level factorial is designed in such a way whereby all factors have only two levels, i.e. n= $2^k$, (where; k= number of factors, n= number of combinations). Hence, the factorial experiments can get larger very quickly with several levels for each of the factors.

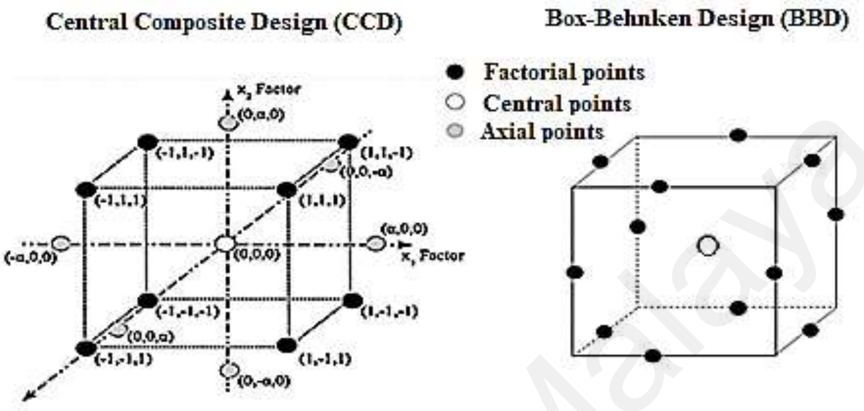


**Figure 2.12: Response surface design: Central Composite Design (CCD) and Box-Behnken Design (BBD) (Modified from Mousavi et al. (2018).**

Response Surface Methodology (RSM) is widely applied in industrial, biological and clinical sciences, social science, food science, and physical and engineering sciences. Unlike the factorial approach, it is an effective statistical and mathematical technique that can determine the interaction between variables (Clark & Williges, 1973). This technique can reduce the number of experimental trials and evaluate multiple parameters and their interactions. Therefore, RSM can be used in modelling and analysis of applications where a response of interest is influenced by several variables, and also it is useful for optimizing the response. Based on the Response Surface Methodology design as shown in Figure 2.12, Central Composite Design (CCD) and Box-Behnken Design (BBD) are two most commonly used design of experiment for optimizing pectin extraction as shown in Table 2.3. A CCD has three groups of design points, factorial points (±1), axial points (±α) and center points (0). A BBD has two groups of design points, factorial points (±1) and center points (0). Both designs used to estimate the coefficients of a quadratic model. BBD require fewer treatment combinations than a CCD in problems involving 3 or 4 factors. The BBD is preferred if many factors are involved. This is because BBD requires fewer numbers of experiments, and it is feasible to provide valuable information on the interactions among experimental parameters within a shorter time frame (Gong et al. 2007). Ferreira et al. (2007) also reported that the BBD was more efficient than the CCD.

# CHAPTER 3: MATERIALS AND METHODOLOGY

This chapter describes the materials used in this study, the overall research flow, the methodologies for the extraction of pectin from pomelo peel powder using conventional heating, ultrasound, microwave, combined ultrasound and microwave, and subcritical water. The design of experiment and the optimization study on pectin extraction and the analytical methods for characterizing of pectin are also included.

## 3.1 Materials and Reagents

Citric acid, ethanol of 70% (v/v) and 95% (v/v) that used for extraction and the chemicals for pectin analysis i.e. acetone, lactic acid, glucose, glycine, hydrochloric acid, sodium nitrate, sodium azide, sucrose, sulphuric acid were purchased from R&M, Malaysia. Standard compounds including D-galacturonic acid (> 98%) and dextran (~95%) were purchased from Sigma-Aldrich, Germany.

## 3.2 Research Flow Chart

Figure 3.1 shows the flow of the overall research methodology and the activities required to achieve the objective set for this study.

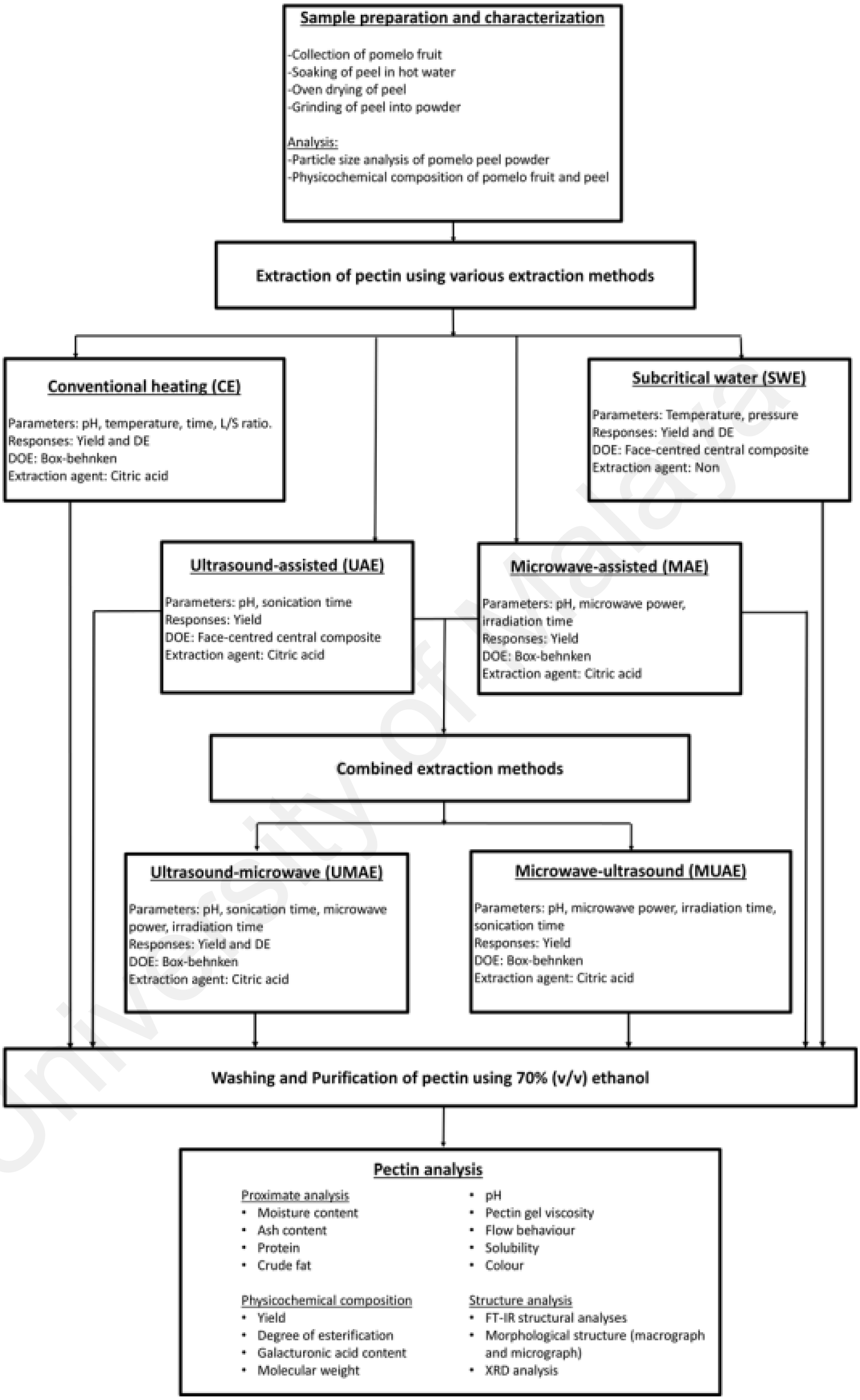


**Figure 3.1: Flow chart of overall research methodology and activities.**

## 3.3 Sample Preparation

Fresh Pomelo (*Citrus grandis* (L.) Osbeck) fruit peel was collected from Go Chin Pomelo Nature Park, Perak, Malaysia. Pomelo peel was soaked in water bath at 90°C for 5 min to inactivate enzymes and dried in a hot air oven (Model 600, Memmert, Germany) at 60°C until a constant weight was attained. The dried peel was grinded and sieved into 250 – 355 µm powder. The peel powder was stored in an air tight container and kept dry prior to use (Figure 3.2).

**Figure 3.2: Pomelo peels and pomelo peel powder.**

The particle size of the pomelo peel powder was measured using the Mastersizer 2000 (Malvern Instruments, United Kingdom) that equipped with a Scirocco 2000 dry dispersion unit, connected to a computer equipped with Malvern software. Particle size distribution of the peel sample was performed to ensure consistency of raw material used in various pectin extraction. The dimension of a pomelo fruit and the physicochemical composition of the pomelo peel powder was determined according to the methods described in section 3.6.

## 3.4 Extraction Methods

The procedures of the extraction techniques for conventional heating extraction, ultrasound, microwave, ultrasound and microwave combined extraction, and the instrumental setup for subcritical water extraction are provided in this section.

### 3.4.1 Conventional Heating Extraction (CE)

Ten (10) g of dried pomelo peel powder was weighed and mixed with distilled water based on the desired liquid-solid ratio. The pH of the mixture solution was adjusted by using citric acid. The mixture with diverse adjusted pH values (1.50 – 2.50) was extracted under different extraction temperature (65 – 90°C) at varying liquid-solid ratio (20:1 – 30:1 mL/g) and extraction time (40 – 180 min). The extraction was performed in triplicates under different operating conditions. During the extraction, the desired temperature was set and controlled using a water bath (TW20, Julabo, Germany).

### 3.4.2 Ultrasound (UAE), Microwave (MAE) Assisted Extraction and Combined Assisted Extraction Methods (UMAE & MUAE)

The procedures of UAE, MAE, UMAE and MUAE are described below and the extraction was conducted in triplicates for each of the mentioned techniques.

#### i. UAE and MAE

The extraction samples were made up of 10 g of pomelo peel powder and 290 mL of distilled water. The pH of the sample mixture was adjusted to 1.7 – 2.3 using citric acid. In the UAE, the sample solution was placed in an ultrasonic bath (Model 3800, Branson, USA) (frequency of ultrasound= 40 kHz; sonication power= 110 W) and sonicated at different sonication time of 12 – 28 min. In the MAE, the sample solution was placed in a microwave oven (ME711K, Samsung, South Korea) and irradiated at different microwave power (350 – 650 W) and irradiation time (4 – 12 min).

#### ii. UMAE and MUAE

In the UMAE, the extraction sample solution with adjusted pH (same as for UAE and MAE) was first placed in an ultrasonic bath at different sonication time (12 − 28 min). After the ultrasound extraction was completed, the pretreated sample was transferred to a microwave oven and further heated under different power (350 − 650 W) and irradiation time (4 − 12 min).

In the MUAE, the pH adjusted extraction sample solution was first placed in a microwave oven and heated under different power (350 − 650 W) at irradiation time (4 − 12 min) before was transferred to an ultrasonic bath to be sonicated at different sonication time (12 − 28 min).

The mixture sample solution after the extraction processes (CE, UAE, MAE, UMAE and MUAE) was centrifuged (Sigma 3-15P, Osterode am Harz, Germany) at 4000 rpm (3130 × g) for 10 min. The supernatant after centrifugation was decanted and then filtered using a filter cloth. The soluble pectin in the filtered supernatant was precipitated and floated onto the surface of the supernatant after 250 mL of 95% (v/v) ethanol was added. The sample was then stored in the dark at room temperature for 24 hours to ensure all the soluble pectin was precipitated. All the precipitated pectin in the sample was filtered and subsequently washed twice using 70% (v/v) ethanol to remove low molecular weight sugars, amino acids, organic acids and inorganic salts (H.-m. Chen et al., 2015). The washed pectin was dried in a hot air oven (Model 600, Memmert, Germany) at 65°C until a constant weight was reached.

### 3.4.3 Subcritical Water Extraction

Subcritical water extraction is a dynamic extractive method which was utilized to extract pectin from pomelo (*Citrus grandis* (L.) Osbeck) peel powder without involving the use of acid in this study. The experimental setup and the schematic diagram of the subcritical water extraction system is shown in Figure 3.3 and Figure 3.4, respectively. Five grams (5 g) of dried pomelo peel powder was placed together with 4 ± 1 mm glass beads (R&M, Selangor, Malaysia) in a 50 mL extraction vessel (31.8 mm width × 95.2 mm length). The two ends of the extraction vessel were fitted with filters of 0.5 μm porosity to prevent the entrainment of the peel powder in the liquid phase. A Yamato Scientific DKL410C oven was used for heating. The temperature indicator of the oven was calibrated with a mercury-type thermometer.

At the start of a dynamic subcritical water extraction (SWE) process, water was delivered into the system via a syringe pump (260D, Teledyne Isco, USA). Valves BV2 to BV5 and valve NV were opened. Once water dripped out at the end of the line, all valves were closed and the oven was switched on. At the onset of heating, valves BV4, BV5 and NV were occasionally opened to reduce the pressure in the system caused by the thermal expansion of water.

Due to the presence of filter stones at both ends of the vessel, a thermocouple could not be inserted into the pressure vessel during extraction. Hence, a preliminary study to measure the heating time required for water in the system to attain the desired temperatures was conducted. The apparatus and experimental setup is given as a supplementary material shown in Appendix B6. The heating time required for water in the system to attain the desired temperature is shown in Appendix B7.

Once the system attained the desired pressure and temperature, it was allowed to equilibrate for 20 minutes prior to the start of the dynamic extraction (Appendix B1 – B5) [Note that the pressure profile is not given in the first 1-20 bar due to the rapid increase in the water pressure and the continuous manual depressurization of the system via valve]. The pressure in the system was maintained by the syringe pump and measured with a pressure transducer (PDCR 4030, Druck, England) coupled with a digital pressure indicator (DPI 280, Druck, England).

After equilibration, extraction commenced. All valves were opened and the flow rate of the solution was maintained at 1 mL/min with the aid of a needle valve (NV). When the extract solution exited the oven, it was allowed to cool by passing the solution through a coil immersed in an ice bath. The extract solution was collected in 20-min fractions. When fifty milliliters (50 mL) of 95% (v/v) ethanol was added into each fraction of the extract collected, the soluble pectin was precipitated and floated onto the surface of the solution. At certain time fraction whereby the precipitation of pectin in the extract solution was no longer observed, the extraction process was considered complete. The samples with precipitated pectin from all time fractions were then stored in the dark at room temperature for 24 hours. The pectin in the sample was filtrated and washed in duplicate using 70% (v/v) ethanol to remove low molecular weight sugars, amino acids, organic acids and inorganic salts (H.-m. Chen et al., 2015) (in section 4.4.4, ethanol or 5% (v/v) hydrochloric acid (HCl) in 60% isopropyl alcohol (IPA) was used as the washing solvent for LM and HM pectin verification). The washed pectin was dried in a hot air oven (Memmert 600, Schwabach, Germany) at 65°C until a constant weight was attained.

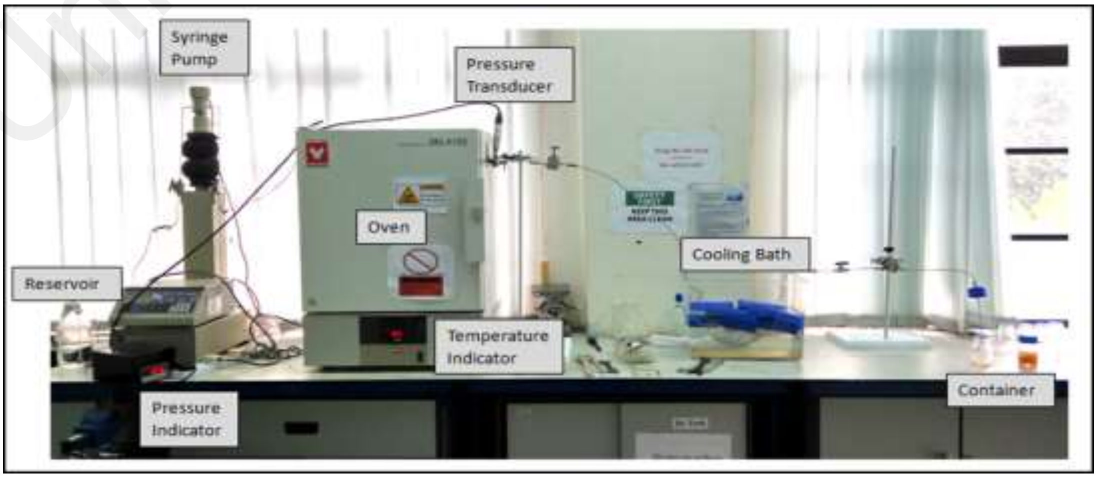


**Figure 3.3: Instrumental setup of dynamic subcritical water system.**

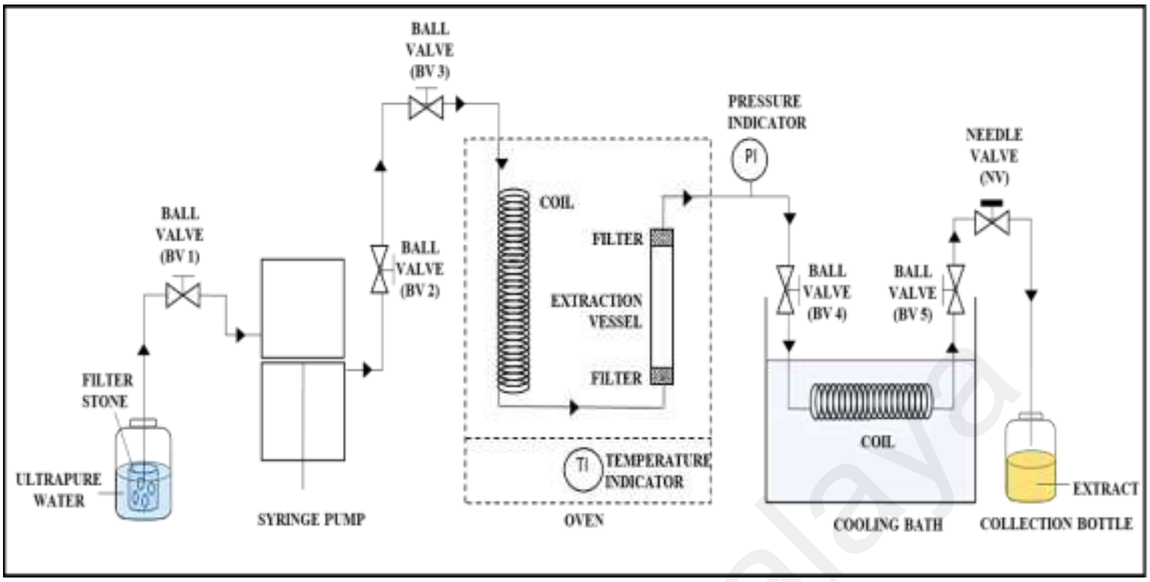


**Figure 3.4: Schematic diagram of the dynamic subcritical water extraction system.**

## 3.5 Design of Experiments

Different design of experiments were applied to various extraction techniques in the study according to the number of influencing variables involved. In all the experiments except those for UAE and SWE, three levels (-1, 0, 1) Box-Behnken Response Surface Methodology design was employed to investigate and optimize the effect of their respective process variables on pectin yield from pomelo peel powder. The coded variables and the levels are tabulated in Table 3.1.

In the Box-Behnken Response Surface Methodology design, a total of experiments ($N$) including 5 center points were designed ($N= 2K(K\text{-}1) + C_0$, where $N$ is the total number of experiments, $K$ is the number of influencing variables and $C_0$ is the number of centre points). In the ultrasound assisted extraction (UAE), two-factor, three-level (−1, 0, 1) face-centred central composite design was used to investigate and optimize the effect of process variables on pectin yield. The variables considered were pH and sonication time.

A total 13 sets of test conditions including 5 replicates of the centre point were conducted for theoretical modelling. Similar to ultrasound assisted extraction, dynamic subcritical extraction of pectin, a two-factor, three-level (−1, 0, 1) face-centred central composite design was used for optimizing the extraction of pectin. The variables considered were extraction pressure and temperature.

The statistical package Design Expert 6.0.6 (State-Ease Inc., USA) was used to construct the experimental design and analyze the experimental data. Experimental data were fitted to a second-order polynomial equation to establish the relationship between independent variables and responses. The generalized form of the equation is:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_{\mathrm{j}} X_{\mathrm{j}} + \sum_{j=1}^{k} \beta_{\mathrm{jj}} X_{\mathrm{j}}^2 + \sum_{i} \sum_{<\mathrm{j}=2}^{k} \beta_{\mathrm{ij}} X_{\mathrm{i}} X_{\mathrm{j}} \qquad (3.1)$$

where $Y$ represents the response variable, $X_{\mathrm{i}}$ and $X_{\mathrm{j}}$ are the independent variables affecting the response, and $\beta_0$, $\beta_{\mathrm{i}}$, $\beta_{\mathrm{ii}}$, and $\beta_{\mathrm{ij}}$ are the regression coefficients for intercept, linear, quadratic and interaction terms. The effects of process variables were analyzed statistically by using an analysis of variance (ANOVA). The adequacy of the model equation for predicting the optimum response values was validated with experimental results. The summary table for the independent variables and the coded/actual levels for different extraction methods are presented in Table 3.1.

**Table 3.1: Independent variables and their coded and actual levels**

| **Variable** | **Unit** | **Coding** | **Level** | | |
|---|---|---|---|---|---|
| | | | -1 | 0 | 1 |
| ***Conventional heating extraction (CE)*** | | | | | |
| RSM: Box-Behnken | | | | | |
| pH | - | $X_1$ | 1.50 | 2.00 | 2.50 |
| Time | min | $X_2$ | 40 | 110 | 180 |
| Temperature | °C | $X_3$ | 65 | 77.5 | 90 |
| L/S ratio | mL/g | $X_4$ | 20:1 | 25:1 | 30:1 |
| ***Ultrasound assisted extraction (UAE)*** | | | | | |
| RSM: Face-centred central composite | | | | | |
| pH | - | $X_1$ | 1.70 | 2.00 | 2.30 |
| Sonication time | min | $X_2$ | 12 | 20 | 28 |
| ***Microwave assisted extraction (MAE)*** | | | | | |
| RSM: Box-Behnken | | | | | |
| pH | - | $X_1$ | 1.70 | 2.00 | 2.30 |
| Microwave power | W | $X_2$ | 350 | 500 | 650 |
| Irradiation time | min | $X_3$ | 4 | 8 | 12 |
| ***Ultrasound-microwave assisted extraction (UMAE)*** | | | | | |
| RSM: Box-Behnken | | | | | |
| pH | - | $X_1$ | 1.70 | 2.00 | 2.30 |
| Sonication time | min | $X_2$ | 12 | 20 | 28 |
| Microwave power | W | $X_3$ | 350 | 500 | 650 |
| Irradiation time | min | $X_4$ | 4 | 8 | 12 |
| ***Microwave-ultrasound assisted extraction (MUAE)*** | | | | | |
| RSM: Box-Behnken | | | | | |
| pH | - | $X_1$ | 1.70 | 2.00 | 2.30 |
| Irradiation time | min | $X_2$ | 4 | 8 | 12 |
| Microwave power | W | $X_3$ | 350 | 500 | 650 |
| Sonication time | min | $X_4$ | 12 | 20 | 28 |
| ***Subcritical water extraction (SWE)*** | | | | | |
| RSM: Face-centred central composite | | | | | |
| Pressure | bar | $X_1$ | 30 | 65 | 100 |
| Temperature | °C | $X_2$ | 90 | 105 | 120 |

## 3.6 Characterization of Pomelo Peel Powder and Pectin

Analytical methods for characterizing of pomelo peel and pectin are presented in sub-sections 3.6.1 − 3.6.12. Evaluation and chemical characterization on the pomelo peel and pectin were performed. The characterization of pomelo peel is to ensure that a consistency of the pomelo peel was used for the extraction; whereas the characterization of the extracted pectin was performed to identify the pectin extracted from different methods for their industrial application.

### 3.6.1 Determination of Pectin Yield

The yield of pectin extracted from pomelo peel powder using various extraction techniques in this study was calculated according to Equation (3.2):

$$Pectin\ yield(\%) = \frac{m_0}{m} \times 100 \tag{3.2}$$

where $m_0$ is the dried pectin weight (g) and $m$ is the dried peel powder weight (g).

### 3.6.2 Physical Characteristics

The composition of pomelo in which its peel powder was used for the study was studied. The physical characteristics such as the length, diameter, weight and the contents of the pomelo peel and pomelo pulp were determined using the average measurement of 20 ripe pomelo fruits. The pH of the peel powder and the pectin extracted was determined with a pH meter (827 pH lab, Metrohm, Switzerland) by mixing 0.5 g of sample with 50 mL of distilled water.

### 3.6.3 Proximate Analyses

Proximate analyses such as moisture, ash, crude fat and crude protein content of the dried pomelo peel and the extracted dried pectin were performed as follows:

### i. Determination of Moisture Content

The moisture content of the dried pomelo peels before extraction and the dried pectin obtained from the extractions were determined using the association of official analytical chemists (AOAC) official method 930.15 (2005). Two gram (2 g) of the sample to be analysed was dried in an oven at 135 ± 2°C for 2 h. The moisture content (%, w/w) of the sample was estimated by Equation (3.3) based on the loss of moisture content on drying (LOD) of the sample.

$$Moisture\ (\%) = \frac{LOD\ wt\ (\mathrm{g})}{Analysis\ sample\ wt\ (\mathrm{g})} \times 100 \tag{3.3}$$

### ii. Determination of Ash Content

The ash content of the dried pomelo peel powder before extraction and the dried pectin obtained from the extractions were determined with AOAC Official method 942.05 (2005). Two gram (2 g) of the sample was weighed in a porcelain crucible and placed in a pre-heated furnace (KL15/11, Thermaconcept, Belgium) at 600°C for 3 hours. The crucible was transferred to an oven and cooled down to 100°C for an hour before it was placed in a desiccator for bringing its temperature down to room temperature. The carbon-free ash was weighed immediately and the average ash content (%, w/w) of the sample was estimated by Equation (3.4).

$$Ash\ (\%) = \frac{Analysis\ sample\ wt\ (\mathrm{g}) - Loss\ on\ ashing\ wt\ (\mathrm{g})}{Analysis\ sample\ wt\ (\mathrm{g})} \times 100 \tag{3.4}$$

### iii. Determination of Crude Fat

The crude fat content of the dried pomelo peel powder before extraction and the dried pectin obtained from the extractions was determined based on the protocol set by Food and Agriculture Organization (FAO). Three gram (3 g) of analysis sample was put into a thimble lined with a circle of filter paper. A thimble with the sample was placed in a 50 mL extraction cup and dried in a mechanical convection oven at 125°C for 0.5 – 1 hours. After drying, the sample containing thimble was then transferred to a Soxtherm extraction unit (Soxtherm 2000, Gerhardt, Germany) and the extraction cup was rinsed several times with ethyl ether. The beaker rinsing solvent was then added to the Soxtherm apparatus to extract the crude fat. Once the extraction was completed, the fat extract was transferred from the extraction flask into a pre-weighed evaporating dish with several rinse of ethyl ether. After that, the evaporating dish was placed in a fume hood to evaporate off the ethyl ether. The dish and contents were dried in a mechanical convection oven at 100°C for 30 minutes before cooled down in a desiccator. The weight of the evaporating dish and the contents were weighted and the crude fat (%, w/w) of the sample was determined by Equation (3.5).

$$Crude\ fat\ (\%) = \frac{W_2 - W_1}{S} \times 100 \tag{3.5}$$

where $W_1$ is the weight of empty evaporating dish (g), $W_2$ is the weight of evaporating dish and the contents after drying (g), and $S$ is the weight of analysis sample (g).

### iv. Determination of Crude Protein

The crude protein content of the dried pomelo peel powder and the dried pectin were determined by the Kjedahl method. Half gram (0.5 g) of each sample was mixed with 12 mL of concentrated sulphuric acid ($H_2SO_4$) in a digestion tube. A Kjeltab Cu 3.5 tablet was added to the sample mixture solution. The digestion tube containing the sample mixture solution was then placed in a digestion block and digested at 420°C until the solution was clear and left to be cooled. The cooled sample mixture solution was diluted with 75 mL $H_2O$. Following that, 25 mL of boric acid and 50 mL of 40% NaOH were added and mixed with the sample mixture solution. After that, the sample mixture solution was distilled and titrated with 0.1 M HCl standardised acid solution and 1% of Boric acid with bromocresol green was used as the indicator solution. Similar procedure was repeated for a reagent blank as control. The total nitrogen determined was multiplied by the conversion factor of 6.25 (Muhammad et al., 2014) to calculate the protein content as expressed in Equation (3.6).

$$Crude\ protein\ (\%) = \%\,N\ \times 6.25 \tag{3.6}$$

### 3.6.4 Determination of Degree of Esterification (DE) of Pectin

The degree of esterification (DE) of pectin was analyzed using FT-IR (Tensor 27, Bruker, USA) spectroscopy. The FT-IR spectra of the samples were recorded from 600 to 4000 cm$^{-1}$ with 32 scans. The measuring resolution was 2 cm$^{-1}$ and the resultant spectra were smoothened to remove noise. DE is a ratio of esterified carboxyl group to the number of total carboxyl groups present which can be calculated using the absorbance intensities at 1630 cm$^{-1}$ and 1745 cm$^{-1}$; corresponding to the non-methyl-esterified carboxyl groups and the methyl-esterified carboxyl groups, respectively. The sum of the two bands absorbance intensities corresponding to the total carboxyl groups was measured by the OPUS software (Opus, USA). The percentage of DE was determined according to Equation (3.7) (Manrique & Lajolo, 2004).

$$DE(\%) = \frac{A_{1745}}{A_{1745}+A_{1630}} \times 100 \tag{3.7}$$

### 3.6.5 Galacturonic Acid (GalA)

The galacturonic acid (GalA) content of the extracted dried pectin was determined according to the colorimetric method (Filisetti-Cozzi & Carpita, 1991; Kliemann et al., 2009). The dried pectin sample was dissolved in distilled water (0.5 mg/mL) under gentle magnetic stirring. A 400 μL mixture (0.5 mg/mL) in a test tube was kept on ice. Then, 40 μL of 4 M sulfamic acid-potassium sulfamate solution (pH 1.6) was added and mixed thoroughly. Following that, an analytical grade (96.4%) $H_2SO_4$ containing 75 mM sodium tetraborate (2.4 mL) was added and the mixture was stirred vigorously by vortex mixing. The stirred mixture was then incubated in a boiling water bath for 20 min and then left to be cooled. After the mixture was cooled down to room temperature at 27°C, 40 μL of 0.15% (w⁄v) m-hydroxydiphenyl in NaOH 0.5% (w⁄v) was added to the mixture and subject to vigorous stirring. The mixture was turned into pink colour in around 5 to 10 min, and stable after about 1 h. Next, the absorbance of the mixture was read using a spectrophotometer (PRIM Light, Secomam, France) at a wavelength of 520 nm. The calibration curve was plotted using D-galacturonic acid (> 98%, Sigma-Aldrich, Germany) as standard (Figure C1). All the analysis of samples was performed in triplicates.

### 3.6.6 Molecular Weight

The molecular weight of the extracted pectin was determined by using High Performance Size Exclusion Chromatography (HPSEC) coupled with a Dawn Heleos Multiangle Laser Light-Scattering (MALLS) detector (Wyatt Technology, USA) and an OptilabreX Differential Refractometer (RI) at 633 nm wavelength at 25°C. The extract was dissolved in distilled water (1.5 mg/mL) and passed through a 0.45 mm membrane filter (Milipore Co., USA). This was followed by manually injecting the extract through a 100 mL loop using a PL aquagel-OH MIXED-H 8 mm column (Agilent Technologies, USA). The mobile phase was 0.1 M sodium nitrite ($NaNO_2$) solution containing 0.5 g/L sodium azide ($NaN_3$) as a bactericide which was carried out at 25°C with a flow rate of 0.6 mL/min (Muhammad et al., 2014). Monodisperse dextrans (~95%, Sigma-Aldrich, Germany) was used as standard and the data were collected by the Astra software (Wyatt Technology, USA).

### 3.6.7 Solubility

The solubility of the extracted pectin was measured using the method described by Jiaxing et al. (2015). A half gram (0.5 g) of sample was mixed with 50 mL distilled water. The mixture solution was stirred evenly and incubated at 40°C for 30 min. The mixture solution was centrifuged at 4200 rpm for 20 min at 25°C. The supernatant was then transferred to a beaker and allowed to evaporate in water bath at 90°C followed by oven drying at 105°C until a constant weight was achieved. The solubility of the extracted pectin was determined using Equation (3.8):

$$Solubility\ (\%) = \frac{m_1}{m_2} \times 100 \tag{3.8}$$

where $m_1$ is the constant weight of dried supernatant (g) and $m_2$ is the weight of sample (g).

### 3.6.8 Colorimetry

The colour of the pectin sample was measured by a colorimeter (WF30, iWAVE, China). The CIELAB coordinates ($L^*$, $a^*$, $b^*$) of the pectin samples were directly obtained from the colorimeter. The $L^*$ value represents lightness, ranging from 0 (black) to +100 (white); $a^*$ value ranges from −100 (green) to +100 (red) and $b^*$ value ranges from −100 (blue) to +100 (yellow). The hue angle ($H^*_{ab}$) and chroma ($C^*$) were calculated according to Equation (3.9) and Equation (3.10), respectively:

$$H^*_{\mathrm{ab}} = \arctan\left(\frac{b^*}{a^*}\right) \tag{3.9}$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \tag{3.10}$$

### 3.6.9 Surface Morphology Analysis

Half gram (0.5 g) of pectin extracted from the pomelo peel powder was mounted onto a specimen stub laid with a double-sided tape. The morphological structure of the pectin samples was observed using a Scanning Electron Microscope (SEM) (Quanta 200 FESEM, FEI, USA) with an accelerating voltage of 10 kV at magnification 500x.

### 3.6.10 X-ray Diffraction Analysis (XRD)

The X-ray diffraction patterns of the extracted pectin were recorded with the aid of an X-Ray Diffractometer (X'Pert$^3$ Powder, PANalytical, Netherlands). Half gram (0.5 g) of the extracted pectin powder was scanned from 5° to 60° diffraction angle (2θ) with Cu Kα radiation at voltage of 40 kV, current of 40 mA, step-scan mode with a step size of 0.02° (2θ) and counting time of 0.2 s/step.

### 3.6.11 Rheological Measurement

The flow behaviours of pectin solutions were investigated using a controlled-stress rheometer (Paar Physica MC 301, Anton Paar, Austria) with a 50 mm parallel plate. The pectin gels for rheological tests were prepared using the procedure described by Jiang et al. (2012) with a slight modification. To form pectin gels, 60% (w/v) of sucrose was added to 3% (w/v) pectin solutions. The pH of the mixture was adjusted to 2 and the mixture was heated and stirred at 80°C for 30 min. The sample mixture was subject to steady-shearing at 25°C, with shear rates ranging from 1 to 500 $s^{-1}$, and a geometry gap of 0.150 mm. The findings were fitted to Ostward–DeWaele equation, $\eta = K\gamma^{n-1}$, where $K$ is the consistency index and $n$ is the flow behaviour index.

### 3.6.12 Energy Consumption

The energy requirement of an extraction process was measured by a Primera-Line Wattage current meter (PM231E, Hugo Brennenstuhl GmbH & Co. KG, Germany) and calculated according to Equation (3.11):

$$Q = P \times t \tag{3.11}$$

where $Q$ is the energy required (kW.h), $P$ is the power dissipated (kW) and $t$ is the extraction time (h).

# CHAPTER 4: RESULTS AND DISCUSSION

This chapter is divided into five main sections discussing the findings obtained for various research objectives. In the first section, discussion on particle size and compositions of pomelo peel powder is presented. The second section discusses the performance of the conventional heating extraction of pectin, followed by another section discusses on ultrasound and microwave assisted extraction as well as the combined assisted extraction method in different sequence. In the fourth section, the suitability of the potential dynamic subcritical water extraction method for the pectin extraction was evaluated and the performance of the technique is thoroughly discussed. The last section of this chapter compares the feasibility of various extraction methods employed and also the physicochemical properties of pectin extracted from different extraction methods.

## 4.1 Particle Size and Compositions of Pomelo Peel Powder

The particle size of raw material is an influential parameter for phytochemical extraction (Yeop et al., 2017). Thus, a preliminary study was conducted in the present work to examine the effect of particle size of pomelo peel powder on pectin yield based on the optimum extraction condition (90°C, pH 2, 180 min and 1:30 g/mL) reported by Methacanon et al. (2014). The result of the study shows that pomelo peel powder with smaller particle size has better pectin yield i.e. the peel powder of less than 1 mm was 2.5% and 7% higher in pectin yield than the other two bigger size particles (Figure 4.1). This is because smaller size pomelo peel powder offers a shorter path for the extraction solvent to pass through the sample (Baldosano et al., 2015; Shi et al., 2005). Therefore, that, pomelo peel powder of < 1 mm (250 – 355 µm) (Y. Chen et al., 2014; Guo et al., 2012; Rahmati et al., 2015) was used throughout the study to ensure the consistency of the sample for all extractions investigated. The particle size distribution of the fruit peel powder was shown in Appendix D1.

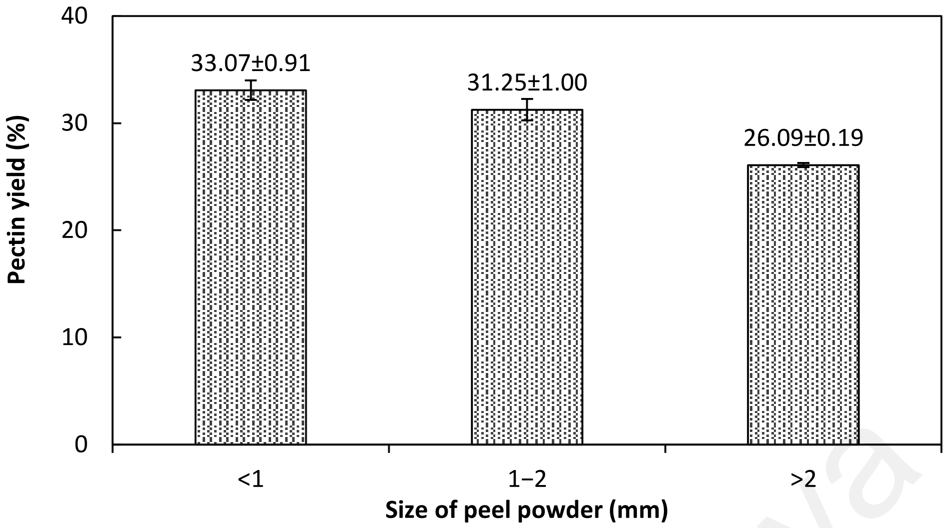


**Figure 4.1: Effect of particle size of pomelo peel powder on pectin yield.**

Besides identifying the suitable size of pomelo peel powder for the extraction, physicochemical compositions of peel powder were determined and are summarized in Table 4.1. An average fruit weight of 1.21 kg and average peel weight of 0.53 kg were obtained from pomelo. The peel powder exhibited a pH value of 4.72.

**Table 4.1: Physicochemical composition of pomelo fruit and its peel powder**

| | |
|---|---|
| **Pomelo fruit** | |
| Length of fruit (cm) | 15.10 ± 0.69 |
| Fruit diameter (cm) | 8.13 ± 0.68 |
| Fruit weight (kg) | 1.21 ± 0.12 |
| Peel weight (kg) | 0.53 ± 0.09 |
| Peel content (%) | 44.29 ± 8.75 |
| Peel thickness (cm) | 1.55 ± 0.27 |
| **Pomelo peel powder** | |
| pH | 4.72 ± 0.03 |
| Moisture (%) | 6.34 ± 0.06 |
| Ash (%) | 4.37 ± 0.05 |
| Protein (%) | 7.04 |
| Fat (%) | 0.25 |

Each value is expressed as mean ± standard deviation of triplicate tests

## 4.2 Conventional Heating Extraction (CE) of Pectin

In the optimization study of pectin extraction from pomelo peel powder using citric acid incorporated conventional heating method, the Box–Behnken design was employed to optimize its yield and the degree of esterification (DE). The effect of types of extraction solvent and diverse sources of citrus family on the yield of pectin and its DE values were compared with the literature reported findings. The effects of pH, temperature, extraction time and liquid-solid ratio were also discussed. As acidity of the extraction medium plays a significant role on the extraction performance, additional discussion was provided on the effect of low range pH on pectin yield through examination of the physicochemical properties of the extracted pectin.

### 4.2.1 Statistical Analysis and Effect of Parameters on Pectin Yield and DE

Two important responses that can reflect accurately on the performance of the extraction process and the specific application of pectin are yield and DE, respectively. The responses can be influenced by certain variables such as pH, temperature, time, liquid-solid ratio, etc. The yield and DE of the pectin obtained in this study using conventional heating method are presented in Table 4.2. It can be seen that the yield of the extracted pectin varies greatly from 4.47 to 39.57% and the values of DE are found between 49.71 and 67.50%. The yield achieved was the lowest under the condition of pH 2.5, extraction time of 110 min, extraction temperature of 65°C and L/S ratio of 25:1; best yield was achieved at pH of 1.5, extraction time of 110 min, extraction temperature of 90°C and L/S ratio of 25:1. This clearly shows that pectin yield is mainly governed by pH and extraction temperature which concurs with most of the study reported in the literature (Vriesmann et al., 2012; Wai et al., 2010) and the ANOVA in Table 4.3.

**Table 4.2: Experimental conditions for conventional heating extraction (CE) of pectin based on BBD and their responses**

| Run | Independent variables | | | | | | | | Responses | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $x_1$ | $(X_1)$ | $x_2$ | $(X_2)$ | $x_3$ | $(X_3)$ | $x_4$ | $(X_4)$ | Yield (%) | DE (%) |
| 1 | 1 | (2.5) | -1 | (40) | 0 | (77.5) | 0 | (25:1) | 4.51 | 54.52 |
| 2 | 1 | (2.5) | 0 | (110) | -1 | (65.0) | 0 | (25:1) | 4.47 | 51.32 |
| 3 | 0 | (2.0) | 0 | (110) | 0 | (77.5) | 0 | (25:1) | 14.57 | 59.70 |
| 4 | 0 | (2.0) | 0 | (110) | 0 | (77.5) | 0 | (25:1) | 12.30 | 58.03 |
| 5 | 0 | (2.0) | -1 | (40) | 0 | (77.5) | 1 | (30:1) | 16.84 | 58.61 |
| 6 | -1 | (1.5) | 1 | (180) | 0 | (77.5) | 0 | (25:1) | 37.70 | 63.13 |
| 7 | 0 | (2.0) | 1 | (180) | 1 | (90.0) | 0 | (25:1) | 31.79 | 58.00 |
| 8 | 0 | (2.0) | 1 | (180) | 0 | (77.5) | 1 | (30:1) | 24.45 | 59.24 |
| 9 | 0 | (2.0) | 1 | (180) | 0 | (77.5) | -1 | (20:1) | 12.18 | 57.81 |
| 10 | 0 | (2.0) | 1 | (180) | -1 | (65.0) | 0 | (25:1) | 12.39 | 58.28 |
| 11 | 1 | (2.5) | 0 | (110) | 0 | (77.5) | 1 | (30:1) | 5.22 | 49.71 |
| 12 | 0 | (2.0) | 0 | (110) | 1 | (90.0) | 1 | (30:1) | 37.50 | 54.22 |
| 13 | -1 | (1.5) | -1 | (40) | 0 | (77.5) | 0 | (25:1) | 18.46 | 57.91 |
| 14 | 1 | (2.5) | 1 | (180) | 0 | (77.5) | 0 | (25:1) | 7.24 | 54.61 |
| 15 | 0 | (2.0) | 0 | (110) | 0 | (77.5) | 0 | (25:1) | 20.61 | 61.01 |
| 16 | -1 | (1.5) | 0 | (110) | 1 | (90.0) | 0 | (25:1) | 39.57 | 61.35 |
| 17 | 1 | (2.5) | 0 | (110) | 0 | (77.5) | -1 | (20:1) | 6.87 | 54.53 |
| 18 | 0 | (2.0) | 0 | (110) | 0 | (77.5) | 0 | (25:1) | 10.88 | 58.90 |
| 19 | -1 | (1.5) | 0 | (110) | 0 | (77.5) | 1 | (30:1) | 36.38 | 55.93 |
| 20 | 0 | (2.0) | -1 | (40) | 1 | (90.0) | 0 | (25:1) | 20.75 | 60.94 |
| 21 | 0 | (2.0) | -1 | (40) | -1 | (65.0) | 0 | (25:1) | 12.73 | 59.77 |
| 22 | 0 | (2.0) | 0 | (110) | 1 | (90.0) | -1 | (20:1) | 24.75 | 63.29 |
| 23 | -1 | (1.5) | 0 | (110) | 0 | (77.5) | -1 | (20:1) | 28.25 | 62.64 |
| 24 | 1 | (2.5) | 0 | (110) | 1 | (90.0) | 0 | (25:1) | 10.21 | 55.00 |
| 25 | -1 | (1.5) | 0 | (110) | -1 | (65.0) | 0 | (25:1) | 35.51 | 59.58 |

**Table 4.2, continued**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 0 | (2.0) | 0 | (110) | -1 | (65.0) | -1 | (20:1) | 10.39 | 62.85 |
| 27 | 0 | (2.0) | 0 | (110) | 0 | (77.5) | 0 | (25:1) | 13.20 | 67.50 |
| 28 | 0 | (2.0) | -1 | (40) | 0 | (77.5) | -1 | (20:1) | 9.54 | 67.34 |
| 29 | 0 | (2.0) | 0 | (110) | -1 | (65.0) | 1 | (30:1) | 18.41 | 64.79 |

The analysis of variance (ANOVA) was applied to examine the statistical significance of the model terms and the findings are tabulated in Table 4.3. According to the *p*-value shown in the table, pH poses the most significant effect on pectin yield, followed by temperature and liquid-solid ratio, whereas time has the least effect. As for the DE, pH poses the most significant effect, followed by liquid-solid ratio and time, and temperature has the least effect. The low *p*-values ($< 0.05$) indicated respectively by pectin yield and DE model, demonstrated that the developed model is significant and well fitted. The lack of fit for both models with high *p*-value ($> 0.05$) further emphasizes that the models could be used to predict the responses. In addition, the $R^2$ value at 0.94 and adj-$R^2$ value at 0.87 of the yield models further signify that the response and independent variables are well correlated. However, the low $R^2$ value at 0.74 and adj-$R^2$ value at 0.47 of the DE models indicated that the response and independent variables are not well correlated to DE; where the non-significant variables in the model resulting in the big differences between $R^2$ value and adj-$R^2$ value.

### Table 4.3: ANOVA for regression model of pectin yield and DE in CE

| Term | Pectin Yield | | | | | DE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **SS** | **DF** | **MS** | ***F*** | ***p*** | **SS** | **DF** | **MS** | ***F*** | ***p*** |
| Model | 3217.04 | 14 | 229.79 | 14.6 | < 0.0001 | 376.25 | 14 | 26.88 | 2.78 | 0.0330 |
| $X_1$-pH | 2063.25 | 1 | 2063.25 | 131.11 | < 0.0001 | 139.06 | 1 | 139.06 | 14.37 | 0.0020 |
| $X_2$-time | 153.51 | 1 | 153.51 | 9.75 | 0.0075 | 5.36 | 1 | 5.36 | 0.55 | 0.4691 |
| $X_3$-temperature | 416.19 | 1 | 416.19 | 26.45 | 0.0001 | 1.20 | 1 | 1.20 | 0.12 | 0.7303 |
| $X_4$-L/S ratio | 182.68 | 1 | 182.68 | 11.61 | 0.0043 | 56.16 | 1 | 56.16 | 5.80 | 0.0304 |
| $X_1^2$ | 48.04 | 1 | 48.04 | 3.05 | 0.1025 | 105.99 | 1 | 105.99 | 10.95 | 0.0052 |
| $X_2^2$ | 1.65 | 1 | 1.65 | 0.11 | 0.7507 | 0.59 | 1 | 0.59 | 0.06 | 0.8089 |
| $X_3^2$ | 213.08 | 1 | 213.08 | 13.54 | 0.0025 | 1.02 | 1 | 1.02 | 0.11 | 0.7498 |
| $X_4^2$ | 33.43 | 1 | 33.43 | 2.12 | 0.1670 | 0.26 | 1 | 0.26 | 0.03 | 0.8716 |
| $X_{12}$ | 68.15 | 1 | 68.15 | 4.33 | 0.0563 | 6.58 | 1 | 6.58 | 0.68 | 0.4235 |
| $X_{13}$ | 0.71 | 1 | 0.71 | 0.05 | 0.8354 | 0.91 | 1 | 0.91 | 0.09 | 0.7634 |
| $X_{14}$ | 23.91 | 1 | 23.91 | 1.52 | 0.2380 | 0.89 | 1 | 0.89 | 0.09 | 0.7658 |
| $X_{23}$ | 32.38 | 1 | 32.38 | 2.06 | 0.1734 | 0.53 | 1 | 0.53 | 0.05 | 0.8191 |
| $X_{24}$ | 6.18 | 1 | 6.18 | 0.39 | 0.5411 | 25.81 | 1 | 25.81 | 2.67 | 0.1248 |
| $X_{34}$ | 5.59 | 1 | 5.59 | 0.36 | 0.5606 | 30.31 | 1 | 30.31 | 3.13 | 0.0986 |
| Residual | 220.31 | 14 | 15.74 | | | 135.51 | 14 | 9.68 | | |
| Lack of Fit | 163.52 | 10 | 16.35 | 1.15 | 0.4845 | 78.34 | 10 | 7.83 | 0.55 | 0.7992 |
| Pure Error | 56.79 | 4 | 14.2 | | | 57.17 | 4 | 14.29 | | |
| Cor Total | 3437.35 | 28 | | | | 511.77 | 28 | | | |
| $R^2$ | 0.94 | | | | | 0.74 | | | | |
| Adj $R^2$ | 0.87 | | | | | 0.47 | | | | |

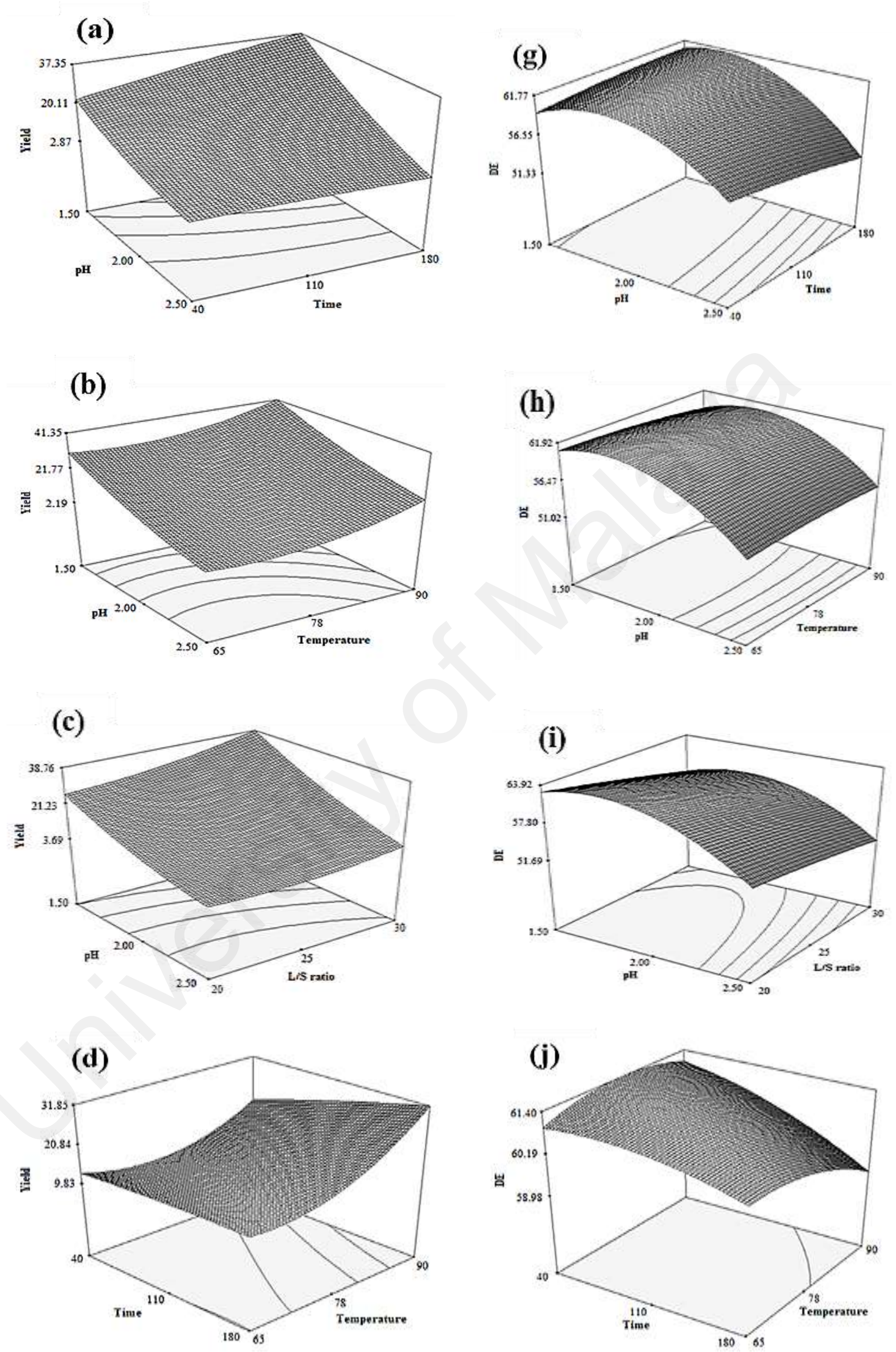


**Figure 4.2: Response surface plots showing the effect of process variable on pectin yield and DE in CE.**

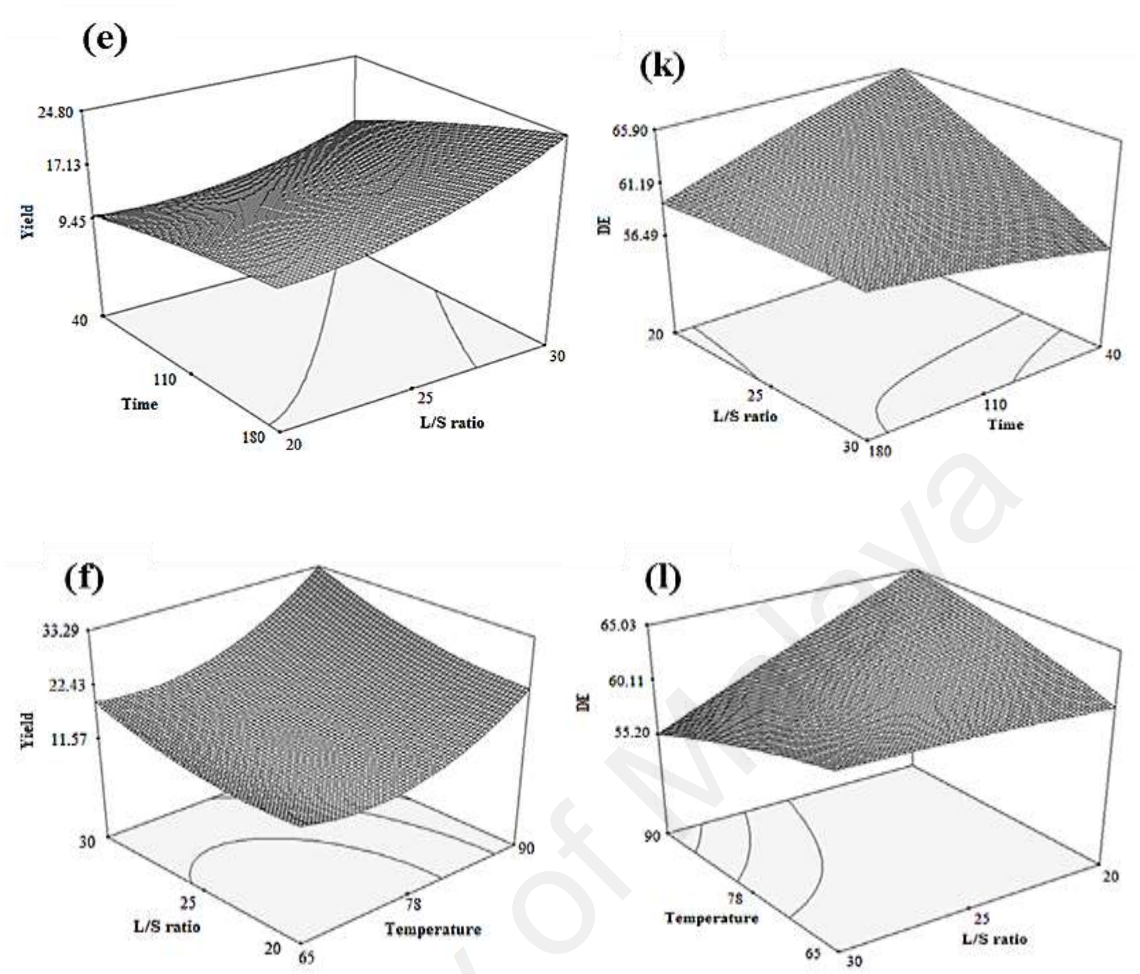


**Figure 4.2, continued.**

The single individual effects and interactive effects of parameters on both pectin yield and DE can be observed in the three-dimensional surface plots as shown in Figure 4.2. The plot depicts a reasonable good pectin yield when low pH was combined with either long extraction time or high temperature or high liquid-solid ratio (Figure 4.2(a), (b) and (c)). This is due to the fact that low pH in the extraction medium establishes a concentration gradient between the extraction medium and the pomelo peel powder (plant matrix), to enable the extraction medium that was in contact with the insoluble pectin to easily induce the hydrolysis of insoluble pectin into soluble pectin (Prakash Maran, Sivakumar, et al., 2013). With longer extraction time, pectin can diffuse across the plant structure to reach the extraction solvent. On the other hand, higher temperature provides more thermal energy required to soften the pomelo peel structure, allowing the targeted compound to diffuse out much easier into the extraction medium which has in turn sped up the extraction process. As shown in Figure 4.2, high liquid-solid ratio of 1:30 g/mL promoted the diffusion process of pectin (the targeted compounds) from pomelo peel powder to citric acid extraction medium which is agreed with the liquid-solid ratio reported by (Radojkovic et al., 2012).

Comparing the interactive effect between operating parameters, the effect between liquid-solid ratio and pH was found to be the most obvious (Figure 4.2(c), (e) and (f)). A combination of the highest liquid-solid ratio with the strongest acid strength resulted in a yield of 38.76% (Figure 4.2(c)) while a combination of the highest liquid-solid ratio with highest temperature only yielded 33.29%. This is followed by a yield of 24.80% when combining the highest liquid-solid ratio with the longest extracting time. Prolonging extraction time is not operational commendable and which can be replaced by adopting a higher operating temperature to speed up the reaction rate (Yu & Sun, 2013). Both the extraction time and the temperature can be made to supplement and complement each other, such that the energy consumption at a higher temperature can be reduced with shorter extraction time. Furthermore, the issue on overheating extracted pectin can also be prevented.

The DE findings listed in Table 4.2 show all the extracted pectin using CE are fairly consistent in DE with an average value of 58.98%. This indicates that the pectin extracted is HM pectin. The highest DE was obtained experimentally at pH 2.0, 77.5°C, with a solid-liquid ratio of 1:25 and an extraction time of 110 min. The response surface plot shown in Figure 4.2(g) – (l) demonstrates that pH exerted the most significant effect on DE with $p < 0.005$, followed by liquid-solid ratio ($p < 0.05$). The effect of extraction time and temperature ($p > 0.5$) were found to be insignificant. Moreover, the DE values obtained are similar to those reported using nitric acid incorporated convention extraction of pectin from pomelo (Methacanon et al., 2014). Table 4.4 shows that the DE values of extracted pectin vary with various citrus sources. Different DE values can classify them into LM or HM pectin. The DE values of extracted pectin obtained from this study using pomelo peel are higher than those extracted from orange (Elizabeth Devi et al., 2014) and lemon (Kanmani et al., 2014) but lower than that extracted from grapefruit using hydrochloric acid (Jiang et al., 2012; L. Liu et al., 2010).

In order to study the effects of the extraction parameters more thoroughly, the extraction conditions of pectin from different citrus family sources were compared as summarized in Table 4.4. It shows that temperature range between 65°C and 90°C was suitable to extract pectin from various citrus sources since thermal degradation of the pectin is unlikely to be triggered at these temperatures. The table also shows no specific trend of parametric effects on pectin extraction for all the citrus sources. This implies that it is essential to investigate the effect of each individual parameter for pectin extraction even though pectin is being extracted from source of materials of same citrus family. It can also be observed from the table that the optimized extraction pH for all the citrus sources was between 1.5 and 2.5, except for lemon, which required a higher extraction pH of 3.5. The disparity in the optimized extraction pH between lemon and other citrus sources may be due to the acidic nature of the fruit itself. Lemon is highly acidic (~pH 2) which might prevent it from creating a substantial concentration gradient between the extraction medium and the plant matrix. This has the effect of reducing the diffusion ability of the pectin into the extraction medium.

**Table 4.4: The effects of different extraction conditions on the characteristics of pectin extracted from various citrus family sources**

| Parameters | | | | Extraction agent | Sources | Responses | | References |
|---|---|---|---|---|---|---|---|---|
| Temperature (°C) | pH | Time (min) | S/L ratio (g/mL) | | | Yield (%) | DE (%) | |
| 65 | 3.5 | 68 | 1:18 | citric acid | Lemon (~pH 2) | 36.71 | 33.77 | Kanmani et al. (2014) |
| 75 | 1.5 | 90 | 1:25 | nitric acid | Lime | 19.80 | 77.00 | Koffi et al. (2013) |
| 80 | 1.5 | 60 | 1:30 | citric acid | Orange (~pH 3) | 67.30 | 35.85 | Elizabeth Devi et al. (2014) |
| | 2.5 | 120 | 1:70 | citric acid | ‘Pera’ sweet orange | 38.21 | 70.21 | Zanella and Taranto (2015) |
| 85 | 2.5 | 90 | 1:25 | hydrochloric acid | Grapefruit | 21.10 | 68.20 | Arslan and Toğrul (1996) |
| 88 | 1.8 | 141 | 1:29 | citric acid | Pomelo | 39.72 | 57.56 | Present study |
| 90 | 1.5 | 90 | 1:30 | hydrochloric acid | Grapefruit | 19.16 | 75.60 | Bagherian et al. (2011) |
| | 2.0 | 180 | 1:30 | nitric acid | Pomelo (~pH 3.3) | 27.63 | 55.82 | Methacanon et al. (2014) |

It is apparent that the effect of pH on extraction performance depends on the inter-relationship between the pH of an extraction solvent and the pH of the source of extraction. The extraction solvent whether it is of mineral acid or organic acid may impact the yield and DE of the pectin.

The optimum pectin yield obtained (39.72%) from the present work is comparatively higher than that obtained from the work of Methacanon et al. (2014) at 27.63% with similar DE value within 1% discrepancy. In both cases, pectin was extracted from pomelo peel powder and nitric acid was used instead of citric acid as the extraction solvent in the latter. Better yield performance in the present work commensurate with the observation that yields obtained from organic acids i.e. citric acid tend to be higher than those obtained through mineral acids i.e. hydrochloric acid. For instance, extractions of pectin from various citrus sources showed that citric acid has better yield performance in the range of 36.71% − 67.30% as compared with mineral acids such as nitric acid (19.80% − 27.63%) and hydrochloric acid (19.16% − 21.10%) (Arslan & Toğrul, 1996; Bagherian et al., 2011; Elizabeth Devi et al., 2014; Kanmani et al., 2014; Koffi et al., 2013; Methacanon et al., 2014; Zanella & Taranto, 2015). Referring to Table 4.4, the optimum amount of extraction solvent required and the optimum extraction temperature for citric acid-based extraction on pomelo were marginally lower as compared to the extraction using nitric acid. However, the extraction time for using citric acid was approximately three-quarter of the time required for using nitric acid as extraction solvent. Hence, if the operating conditions of the present study were apply to a large scale extraction, it may result in substantial energy and material savings.

Furthermore, the optimal pectin yield of the present study (39.72%) is comparable to that extracted from 'Pera' sweet orange (38.21%) by Zanella and Taranto (2015) and to that extracted from lemon (36.71%) by Kanmani et al. (2014). It is however, comparatively lower than the yield extracted from orange (67.30%) by Elizabeth Devi et al. (2014). In all four studies, citric acid was used as extraction solvent. The pectin extracted from pomelo (present work) and that from ‘Pera’ sweet orange both yielded HM pectin with DE value higher than 50.

### 4.2.2 Modelling and Model Validation

The yield and DE of the pectin obtained in this study (Table 4.2) using conventional heating method were optimized and a second order polynomial equation was developed by relating the process variables with the responses in terms of coded factors as shown in Equation (4.1) and Equation (4.2).

$$\text{Pectin yield (\%)} = 14.31 - 13.11X_1 + 3.58X_2 + 5.89X_3 + 3.90X_4 + 2.72X_1^2 - 0.50X_2^2 + 5.73X_3^2 + 2.27X_4^2 - 4.13X_{12} + 0.42X_{13} - 2.45X_{14} + 2.85X_{23} + 1.24X_{24} + 1.18X_{34} \quad (4.1)$$

$$\text{DE (\%)} = 61.03 - 3.40X_1 - 0.67X_2 - 0.32X_3 - 2.16X_4 - 4.04X_1^2 - 0.30X_2^2 - 0.40X_3^2 - 0.20X_4^2 - 1.28X_{12} + 0.48X_{13} + 0.47X_{14} - 0.36X_{23} + 2.54X_{24} - 2.75X_{34} \quad (4.2)$$

The obtained quadratic models can be reduced by taking out all insignificant terms ($p > 0.05$), given by Equation (4.3) and Equation (4.4).

$$\text{Pectin yield (\%)} = 14.31 - 13.11X_1 + 3.58X_2 + 5.89X_3 + 3.90X_4 + 5.73X_3^2 \quad (4.3)$$

$$\text{DE (\%)} = 61.03 - 3.40X_1 - 0.67X_2 - 2.16X_4 - 4.04X_1^2 \quad (4.4)$$

The quadratic models of Equation (4.1) – (4.4) show good correlation between the parameters investigated with pectin yield and degree of esterification. The optimum condition was validated in order to confirm the experimental result was consistent with the predicted result. The optimum pectin yield of 39.72% and DE of 57.56% for full models, and pectin yield of 37.26% and DE of 59.72% for the reduced models were successfully predicted using the models obtained at extraction conditions of pH 1.80, time 141.38 min, at 88 °C with a liquid-solid ratio of 29:1. The models were validated 3 times with pectin yields of 39.88%, 40.54% and 36.98% while the validation results for DE were 58.55%, 60.14% and 58.99%. The average yield of 39.13% and the average DE value of 59.23% are consistent with the predicted values. This suggests that the models are well fitted with the experimental data and their predictability are further confirmed.

### 4.2.3 Effect of pH

The influence of low pH (~2) on pectin extraction was reported in a number of studies (Wai et al., 2010; Xue et al., 2011). The effect of pH on pectin yield and DE within a low pH range were investigated via a series of experiments conducted at pH of 1.00, 1.25, 1.50, 1.75, 2.00 with the other extraction parameters i.e. temperature, time, liquid-solid ratio fixed at their centre points. The effect of pH on yield and DE are illustrated in Figure 4.3. At pH 1.50, the pectin yield was at its maximum and the DE value was considerably high. At pH below 1.50, the DE values were observed to markedly increase as pH decrease. As DE is a ratio of the number of esterified carboxyl group to the number of total carboxyl groups present, lowering pH increases the concentration of the $H^+$ ions which will allow more free-carboxyl groups to be ionized to esterified carboxyl groups. Ionization causes the free carboxyl groups i.e. the non-methyl-esterified carboxyl groups (~1630 $cm^{-1}$) to decrease as more methyl-esterified carboxyl groups (~1745 $cm^{-1}$) are formed. Therefore, lowering the pH results in an increase in DE value as illustrated in the FT-IR spectra in Figure 4.6 (section 4.2.4).

Apart from affecting the DE value, pH has impacted the galacturonic acid (GalA) content of the pectin. The determination of GalA in food is crucial as it affects the chemical and sensorial characteristics of the matrix i.e. pH, total acidity, microbial stability, sweetness, global acceptability and it provides information on the wholesome quality of the food (Manuela M. Moreira et al., 2010). The GalA content for a good quality pectin is recommended by the Food and Agriculture Organization (FAO) to be above 65% (Zanella & Taranto, 2015). In the present study, the GalA contents of pectin extracted at different pH are shown in Figure 4.3. The pectin extracted at pH 2.00, pH 1.75 and pH 1.50 both exceeded the 65% threshold. Pectin extracted at 1.25 gave GalA content lower than 65% but that extracted at pH 1.00 gave an extremely low GalA content which was probably caused by the degradation of acid hydrolysis under high acidity (de Oliveira et al., 2015; Garna et al., 2004).

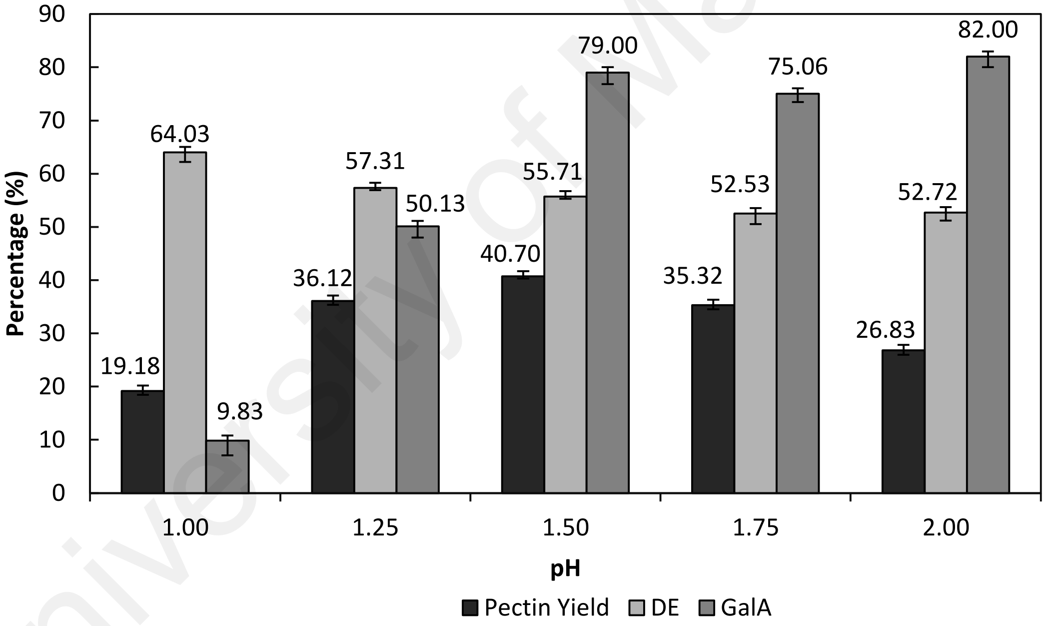


**Figure 4.3: Effect of pH on pectin yield, DE and GalA value.**

It has been reported that the morphological structural of pectin was greatly influenced by the extraction process and pectin extracted with different extraction agent exhibit different morphologies (Jiang et al., 2012). Hence, both macroscopic and microscopic examination of the extracted pectin were performed to gain an insight into the morphological structure of pectin. The findings are shown in Figure 4.4. The macroscopic scanning image on pectin extracted at pH 1.00 was very viscous, which was difficult to be filtered and dried into solid form (Figure 4.4(a)). The pectin extracted at pH 1.50 had a smooth and compact texture (Figure 4.4(b)) whereas the pectin extracted at pH 2.00 showed rough and fragmented surfaces (Figure 4.4(c)).

The micrograph scanning image of pectin extracted at different pH values are shown in Figure 4.4(d) – (f). The pectin extracted at pH 1.50 (Figure 4.4(e)) had smoother and more compact surface structure with fewer fragments than those obtained at pH 1.00 (Figure 4.4(d)) and pH 2.00 (Figure 4.4(f)). Theoretically, particles of smaller size provide greater contact area for mass transfer, which can speed up the dissolution process (Smith, 2015). Thus, pectin extracted at pH 2.00 can be considered as a potential food additive powder.

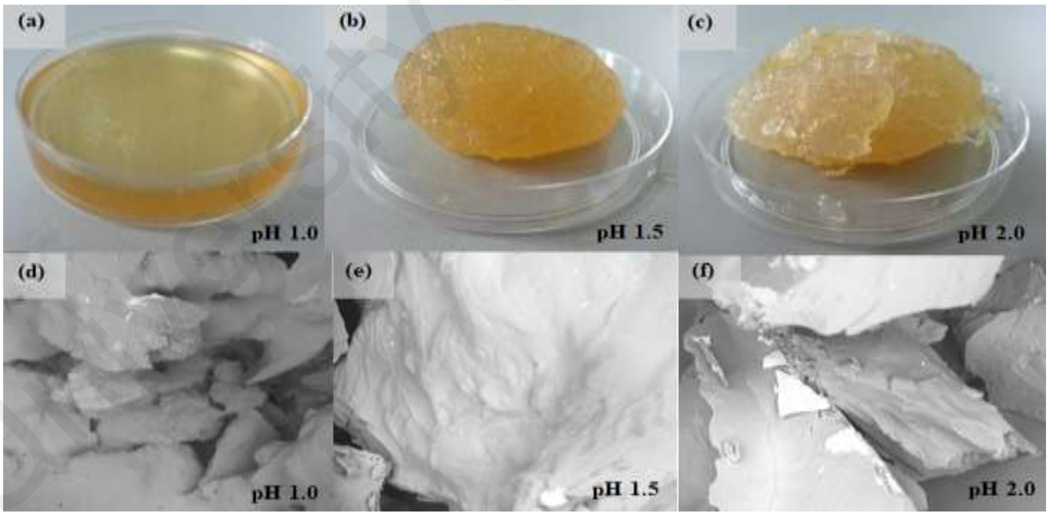


**Figure 4.4: Macrograph of pectin at (a) pH 1.00; (b) pH 1.50 and (c) pH 2.00 and scanning electron micrograph of pectin at (d) pH 1.00; (e) pH 1.50 and (f) pH 2.00; at 500× magnification, 100 µm.**

As previously discussed GalA affects the sensory matrix of food, and thus when pectin is used as food additive, its viscosity can affect the sensory performance of food products.

The rheological analyses of pectin gel samples are presented in Figure 4.5. The calculated *n* values shown in Figure 4.5(a) is ranged from 0.97 to 1.03, whereby the shear rate increased under steady-shear conditions. The flow behaviour of all pectin solutions under steady-shear conditions are shown in Figure 4.5(b). The pectin gel samples exhibited a shear-thinning (pseudoplastic) behaviour. The rheological property of the polysaccharides allows liquid food to be pumped more easily and further imparts a thinner consistency with better mouth feel (Y. Chen et al., 2014; L. Liu et al., 2010). Figure 4.5(b) shows that the viscosity of pectin decreased rapidly with increasing shear rate from 1 to 50 $s^{-1}$ and then stabilized at shear rates of 100 $s^{-1}$. The viscosities of all the samples (~0.01 Pa.s) of this study are similar to those obtained from pomelo pectin extractions using tartaric acid (Quoc et al., 2014) and oxalic acid (Quoc et al., 2015).

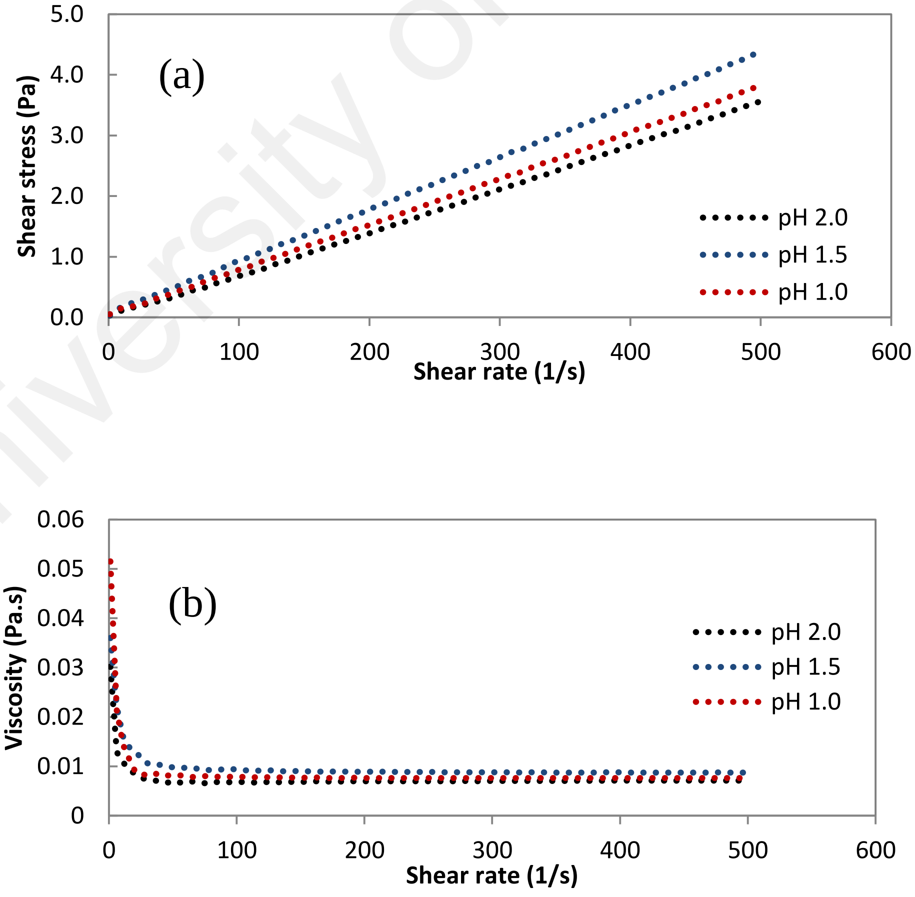


**Figure 4.5: Flow behaviour curves and viscosity curves for pectin gel at different pH.**

### 4.2.4 Functional Groups of Extracted Pectin

The effect of pH on the extracted pectin can be related to its functional groups. FT-IR spectroscopy was performed to identify the major functional groups of pectin extracted at different pH values as illustrated in Figure 4.6. The functional group region between 1600 cm$^{-1}$ and 1800 cm$^{-1}$ is useful for the identification and quantification of pectin (L. Liu et al., 2010). The mixed peaks in this region are caused by vibrations in the ester and carboxylic groups. As shown in Figure 4.6, the absorbance intensity of the methyl-esterified carboxyl groups (~1745 cm$^{-1}$) decreased with an increase in pH from 1 − 2 while the non-methyl-esterified carboxyl groups (~1630 cm$^{-1}$) increased as the pH increased. A clearer peak for non-methyl-esterified carboxyl groups (~1630 cm$^{-1}$) was observed at both pH 2.00 and pH 1.75. As pH decreased from pH 1.50 to 1.00, the non-methyl-esterified carboxyl groups (~1630 cm$^{-1}$) gradually disappeared. This suggests that the degradation of extracted pectin might have taken place and the identity of the pectin became unclear or the non-methyl-esterified carboxyl groups (~1630 cm$^{-1}$) had possibly been transformed to methyl-esterified carboxyl groups (~1745 cm$^{-1}$). This confirms the significant impact of pH on the functional groups of the pectin.

pH does not only affect the methyl-esterified carboxyl groups (~1745 cm$^{-1}$) and the non-methyl-esterified carboxyl groups (~1630 cm$^{-1}$), but also affects the band around 1014 cm$^{-1}$ that indicates the ester group C-O stretching region. It has also been reported that pectin sources were related to the fingerprint pattern of the characteristic region between 950 cm$^{-1}$ to 1200 cm$^{-1}$ (Kamnev et al., 1998). Within the stated region of wavelengths, the characteristic trend at pH 1.00 was different from the trends exhibited at pH 1.25 − 2.00 (Figure 4.6). The DE obtained in the present study, is in agreement with the reported findings of Singthong et al. (2004) whereby the methyl-esterified carboxyl groups increased with DE values and decreased with non-methyl-esterified carboxyl groups. The FT-IR spectra of the present work at 1630 cm$^{-1}$ are found to exhibit similar %Transmittance patterns to the work of Jiang et al. (2012) in which pectin was extracted from *Akebia trifoliate* var using hydrochloric and citric acids. The chemical structure and functional groups of the pectin extracted in this study also resemble those reported by Jiang et al. (2012); further confirming that the polysaccharide extracted from pomelo peel was pectin.

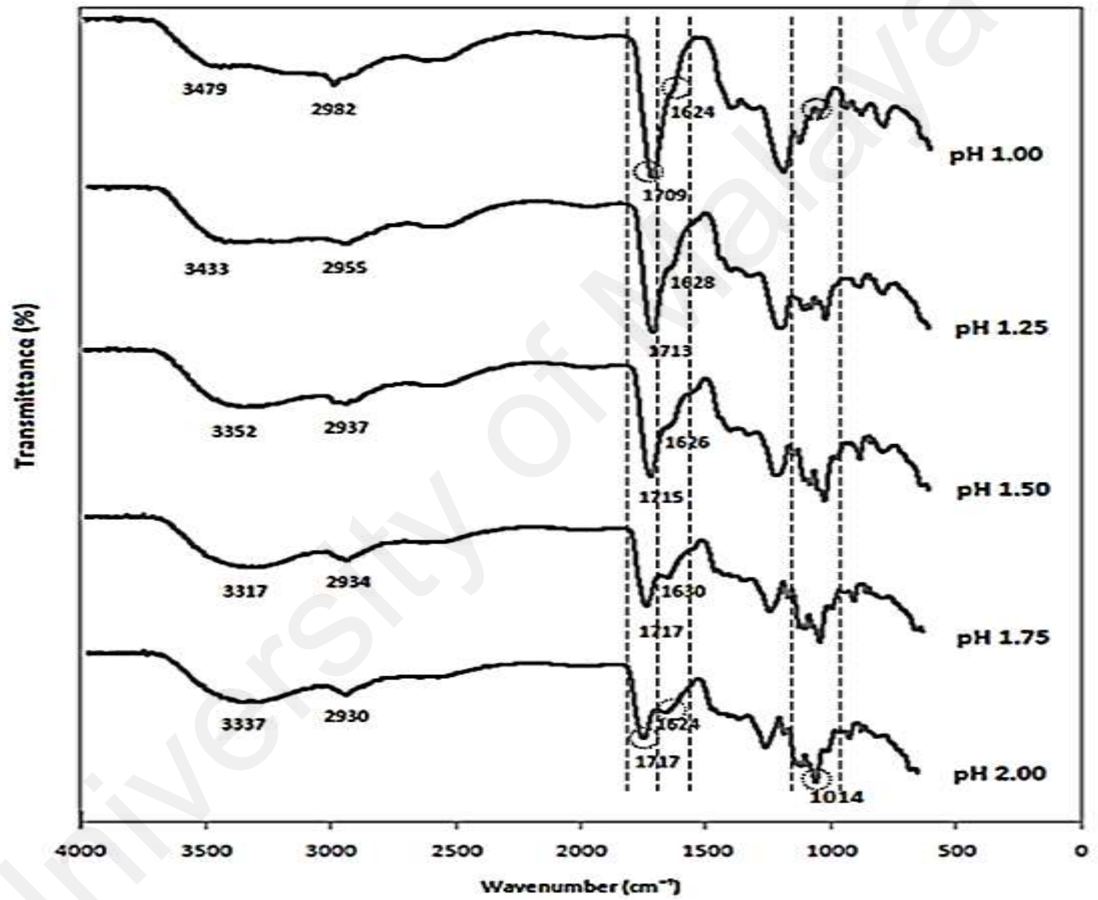


Figure 4.6: FT-IR spectra of pomelo pectin at different pH.

## 4.3 Ultrasound (UAE), Microwave (MAE) Assisted Extraction and Combined Assisted Extraction (UMAE & MUAE) of Pectin

This section discusses the pectin extracted using citric acid incorporated ultrasound-assisted extraction (UAE), microwave assisted extraction (MAE), microwave-ultrasound assisted extraction (MUAE) and ultrasound-microwave assisted extraction (UMAE) methods. The extraction yield of pectin from the stated extraction methods was optimized using response surface methodology (the optimization results for UAE, MAE and MUAE are showed in Appendix A1 – A6). Among the methods used, UMAE (36.33%) gave the best yield followed by MUAE (31.57%), MAE (30.24%) and UAE (13.24%). This section focuses the best performance UMAE and its optimized results will be discussed in more detail. In addition, discussion on the performance of pectin performance for UAE, MAE and MUAE in terms of compositional properties, morphological structure and rheological analyses will be presented.

### 4.3.1 Statistical Analysis and Effect of Parameters on Pectin Yield and DE

The yield and the DE of pectin extracted from pomelo peel using sequential UMAE system are presented in Table 4.5. The yield of the extracted pectin varies greatly from 10.79 to 37.52% and the values of DE were found between 53.14 and 68.78%. The yield was the lowest under the condition of pH 2.3, sonication time of 20 min, microwave power of 350 W and irradiation time of 8 min; the best yield was achieved at pH of 2.0, sonication time of 28 min, microwave power of 650 W and irradiation time of 8 min. This shows that pectin yield obtained via UMAE was affected by all the influencing parameters i.e. pH, sonication time, microwave power and irradiation time as can be seen from the ANOVA in Table 4.6.

Table 4.5: **Experimental conditions for sequential ultrasound-microwave assisted extraction (UMAE) of pectin based on BBD and their responses**

| | Independent variables | | | | | | | | Responses | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Run** | $x_1$ | ($X_1$) | $x_2$ | ($X_2$) | $x_3$ | ($X_3$) | $x_4$ | ($X_4$) | **Yield (%)** | **DE (%)** |
| 1 | 1 | (2.3) | 1 | (28) | 0 | (500) | 0 | (8) | 11.14 | 57.34 |
| 2 | -1 | (1.7) | 0 | (20) | 0 | (500) | 1 | (12) | 25.87 | 55.13 |
| 3 | 0 | (2.0) | -1 | (12) | 1 | (650) | 0 | (8) | 29.28 | 58.94 |
| 4 | -1 | (1.7) | 1 | (28) | 0 | (500) | 0 | (8) | 29.56 | 59.16 |
| 5 | 1 | (2.3) | 0 | (20) | -1 | (350) | 0 | (8) | 10.79 | 54.07 |
| 6 | 0 | (2.0) | 0 | (20) | -1 | (350) | -1 | (4) | 15.71 | 53.82 |
| 7 | 0 | (2.0) | 0 | (20) | 0 | (500) | 0 | (8) | 23.57 | 53.14 |
| 8 | 1 | (2.3) | 0 | (20) | 1 | (650) | 0 | (8) | 14.63 | 57.54 |
| 9 | 0 | (2.0) | 0 | (20) | 0 | (500) | 0 | (8) | 18.82 | 58.31 |
| 10 | 0 | (2.0) | 0 | (20) | 0 | (500) | 0 | (8) | 23.26 | 59.46 |
| 11 | 0 | (2.0) | -1 | (12) | 0 | (500) | -1 | (4) | 20.96 | 58.23 |
| 12 | 0 | (2.0) | 0 | (20) | 1 | (650) | -1 | (4) | 20.72 | 59.04 |
| 13 | 0 | (2.0) | 0 | (20) | 0 | (500) | 0 | (8) | 22.37 | 59.09 |
| 14 | 1 | (2.3) | 0 | (20) | 0 | (500) | 1 | (12) | 12.79 | 57.66 |
| 15 | 0 | (2.0) | 0 | (20) | 0 | (500) | 0 | (8) | 22.54 | 58.88 |
| 16 | 0 | (2.0) | -1 | (12) | 0 | (500) | 1 | (12) | 26.82 | 59.49 |
| 17 | 0 | (2.0) | -1 | (12) | -1 | (350) | 0 | (8) | 32.55 | 63.86 |
| 18 | 0 | (2.0) | 1 | (28) | -1 | (350) | 0 | (8) | 21.48 | 63.70 |
| 19 | 1 | (2.3) | -1 | (12) | 0 | (500) | 0 | (8) | 17.62 | 62.26 |
| 20 | 1 | (2.3) | 0 | (20) | 0 | (500) | -1 | (4) | 13.95 | 58.45 |
| 21 | -1 | (1.7) | -1 | (12) | 0 | (500) | 0 | (8) | 26.91 | 65.37 |
| 22 | 0 | (2.0) | 1 | (28) | 0 | (500) | 1 | (12) | 30.05 | 61.86 |
| 23 | -1 | (1.7) | 0 | (20) | -1 | (350) | 0 | (8) | 30.43 | 68.78 |
| 24 | 0 | (2.0) | 0 | (20) | -1 | (350) | 1 | (12) | 34.45 | 64.43 |
| 25 | 0 | (2.0) | 1 | (28) | 1 | (650) | 0 | (8) | 37.52 | 58.02 |

**Table 4.5, continued**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 0 | (2.0) | 0 | (20) | 1 | (650) | 1 | (12) | 22.10 | 53.68 |
| 27 | -1 | (1.7) | 0 | (20) | 0 | (500) | -1 | (4) | 27.70 | 54.97 |
| 28 | 0 | (2.0) | 1 | (28) | 0 | (500) | -1 | (4) | 24.35 | 56.15 |
| 29 | -1 | (1.7) | 0 | (20) | 1 | (650) | 0 | (8) | 29.98 | 57.40 |

The results of the analysis of variance (ANOVA) that examined the statistical significance of the model terms are listed in Table 4.6. According to the ANOVA results, the changes in pH ($p < 0.0001$) and irradiation time ($p < 0.05$) exerted greater effects on pectin extraction than the other variables. The low $p$-values (< 0.05) indicated by pectin yield ($p = 0.0008$) and DE ($p = 0.0477$) demonstrated that, the developed model is significant and well fitted. The lack of fit for both models with high $p$-value (> 0.05) further emphasized that the models are suitable to be used to predict the responses. The high value of $R^2$ (0.86 and 0.72) further signify that the response and independent variables are well correlated.

**Table 4.6: ANOVA for regression model of pectin yield and DE in UMAE**

| | Pectin Yield | | | | | DE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Term** | **SS** | **DF** | **MS** | ***F*** | ***p*** | **SS** | **DF** | **MS** | ***F*** | ***p*** |
| Model | 1192.98 | 14 | 85.21 | 6.18 | 0.0008 | 282.77 | 14 | 20.20 | 2.52 | 0.0477 |
| $X_1$-pH | 667.97 | 1 | 667.97 | 48.41 | < 0.0001 | 15.17 | 1 | 15.17 | 1.89 | 0.1909 |
| $X_2$-Sonication | 0.00 | 1 | 0.00 | 0.00 | 0.9976 | 11.84 | 1 | 11.84 | 1.47 | 0.2447 |
| $X_3$-Power | 6.48 | 1 | 6.48 | 0.47 | 0.5042 | 48.16 | 1 | 48.16 | 6.00 | 0.0281 |
| $X_4$-Irradiation | 68.59 | 1 | 68.59 | 4.97 | 0.0427 | 11.19 | 1 | 11.19 | 1.39 | 0.2573 |
| $X_1^2$ | 69.56 | 1 | 69.56 | 5.04 | 0.0414 | 1.55 | 1 | 1.55 | 0.19 | 0.6672 |
| $X_2^2$ | 95.35 | 1 | 95.35 | 6.91 | 0.0198 | 41.19 | 1 | 41.19 | 5.13 | 0.0399 |
| $X_3^2$ | 49.38 | 1 | 49.38 | 3.58 | 0.0794 | 8.32 | 1 | 8.32 | 1.04 | 0.3260 |
| $X_4^2$ | 0.45 | 1 | 0.45 | 0.03 | 0.8599 | 12.97 | 1 | 12.97 | 1.62 | 0.2245 |
| $X_{12}$ | 20.84 | 1 | 20.84 | 1.51 | 0.2393 | 0.42 | 1 | 0.42 | 0.05 | 0.8232 |
| $X_{13}$ | 4.60 | 1 | 4.60 | 0.33 | 0.5728 | 55.13 | 1 | 55.13 | 6.87 | 0.0202 |
| $X_{14}$ | 0.11 | 1 | 0.11 | 0.01 | 0.9294 | 0.23 | 1 | 0.23 | 0.03 | 0.8693 |
| $X_{23}$ | 93.22 | 1 | 93.22 | 6.76 | 0.0210 | 0.14 | 1 | 0.14 | 0.02 | 0.8952 |
| $X_{24}$ | 0.01 | 1 | 0.01 | 0.00 | 0.9831 | 4.95 | 1 | 4.95 | 0.62 | 0.4454 |
| $X_{34}$ | 75.34 | 1 | 75.34 | 5.46 | 0.0348 | 63.76 | 1 | 63.76 | 7.94 | 0.0137 |
| Residual | 193.16 | 14 | 13.80 | | | 112.39 | 14 | 8.03 | | |
| Lack of Fit | 178.63 | 10 | 17.86 | 4.92 | 0.0692 | 84.84 | 10 | 8.48 | 1.23 | 0.4546 |
| Pure Error | 14.53 | 4 | 3.63 | | | 27.56 | 4 | 6.89 | | |
| Cor Total | 1386.14 | 28 | | | | 395.16 | 28 | | | |
| $R^2$ | 0.86 | | | | | 0.72 | | | | |
| Adj $R^2$ | 0.72 | | | | | 0.43 | | | | |

Process variables have different degree of impact on pectin extraction. Some parameters on their own have very little effect on pectin yield and DE, but the interactive effect of certain parameters can affect the responses significantly. The percentage yield of pectin extracted in the sequential extraction system is presented in Table 4.5. Highest pectin yield of 37.52% was obtained when the pomelo peel powder was first sonicated at pH 2.00 for 28 min ensued by the microwave irradiation at 650 W for 8 min. This yield is 5.64% higher than the result reported by Bagherian et al. (2011) from grapefruit peel. Comparing the literature reported condition, the microwave power 450 W employed is lower than that of the present study. However, the reported sonication and irradiation times are 2 min longer. Furthermore, the pectin yield from UMAE of this study was 13.69% higher than the yield of pectin extracted from pomelo peel using sole MAE (Quoc et al., 2015). This suggests that ultrasound pre-treatment has assisted in the extraction of pectin.

The relationship between the responses and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Figure 4.7). A suitable pH is essential to ensure good extraction of pectin and a pH over a narrow acidic range has significant effect. The present study on UMAE that was carried out at different low pH (1.70 – 2.30) shows that the yield of pectin was increased from 8.56% to 32.41% as pH declined from 2.30 to 1.70 (Figure 4.7(a) – (c)). This is because, at lower pH values, more $H^+$ ions are present which increases the hydrolysis of protopectin to yield pectin or pectinic acids. From the surface plot on pH with another parameter, it shows that maximum pectin could be extracted at pH of 1.70 and this finding is in agreement with the results reported (Y.-Y. Zhang et al., 2013). Meanwhile the optimization result based on all four parameters under studied recorded the highest pectin yield at pH 1.80.

This demonstrates negligible fluctuation of pH over a narrow pH range of acidity as far as pectin extraction is concerned. The pH gave most significant effect ($p < 0.5$) with sonication time as compared with other variables as presented in Table 4.6.

In ultra-sonication, micro streaming triggered by the collapse of cavitational bubbles enhances mass transfer. This phenomenon has destructed the cell wall to allow better contact and interactions between solvents and plant materials. However, sonication time did not seem to have obvious influence ($p > 0.05$) on pectin yield in UMAE. In the present work, ultra-sonication resembles pre-treatment in breaking the outer cell wall of plant without involving any heating. Therefore, longer period of sonication such as 28 min is required to facilitate pectin extraction as agreed with the reported UAE from pumpkin by Prakash Maran, Mekala, et al. (2013). On the other hand, greater effect is observed when sonication time has interacted with other parameters. For instance, Figure 4.7 shows that higher pectin yield can be obtained at longest sonication time of 28 min with either of the parameters i.e. pH of 1.7 (32.41%), microwave irradiation time of 12 min (28.12%) or 650 W microwave power (34.26%). The combination of irradiation time with microwave power that extracted the most pectin is as shown in Figure 4.7(d).

Microwave power can affect extraction performance and it must be controlled to obtain desired set of temperature to avoid excessive temperature and overpressure in a closed vessel. As can be seen from Figure 4.7, the yield of pectin increased gradually with the increase of microwave power. At higher microwave power, temperature of the solution increased to promote the diffusion of targeted compound. Thus, the combined effect of microwave power with irradiation time (Figure 4.7(f)) was more significant ($p > 0.05$) than other combined effects shown in Figure 4.7(b) and Figure 4.7(d), as such a lower microwave power needs a longer irradiation time to obtain higher yield and vice versa. This observation concurs with research findings reported on the influence of microwave power and extraction time on MAE (Dragovic-Uzelac et al., 2012). Some researchers prefer the approach of low power with longer extraction duration for extraction as high microwave power reported might reduce purity. However, low power with long exposure might have caused overheating and reduce the nutrient content of the extracted pectin. The present work indicates that 650 W microwave power and 8 min extraction time gave highest yield while no overheating of samples was observed.

The main advantage of MAE compared to conventional method is time saving. Careful selection of irradiation time is crucial as it can take several seconds up to several minutes for significant extraction to take place. Many studies reported that pectin yield was reduced with lengthy microwave heating as pectinic acid by- product was produced instead of pectin (Quoc et al., 2015). Thus, it should be refrained from applying long irradiation time in the extraction process despite longer time allows more reaction to take place as more heat can be used to soften the structure of the carbohydrate polymer (Liew, Chin, Yusof, et al., 2015). On the other hand, short reaction time might be inadequate to severe components such as cellulose, hemicelluloses that link between pectin. According to Figure 4.7(c), 12 min irradiation time combined with pH 1.7 gave 28.26% yield. Also, in the combined effect study of sonication and irradiation time, 28.12% of pectin yield can be obtained in either 12 min or 28 min sonication time. This indicates that the combined effect of sonication and irradiation time is less significant compared to other combined parametric effect, and therefore solely based on the two combined parameters cannot accurately elucidate the interactive effect within the UMAE system. From the response surface plot of DE (Figure 4.7(g) – (l)), the values obtained are fairly consistent within 15% difference ranging from 53.14% to 68.78%. Therefore, the extracted pectin can be classed as HM pectin.

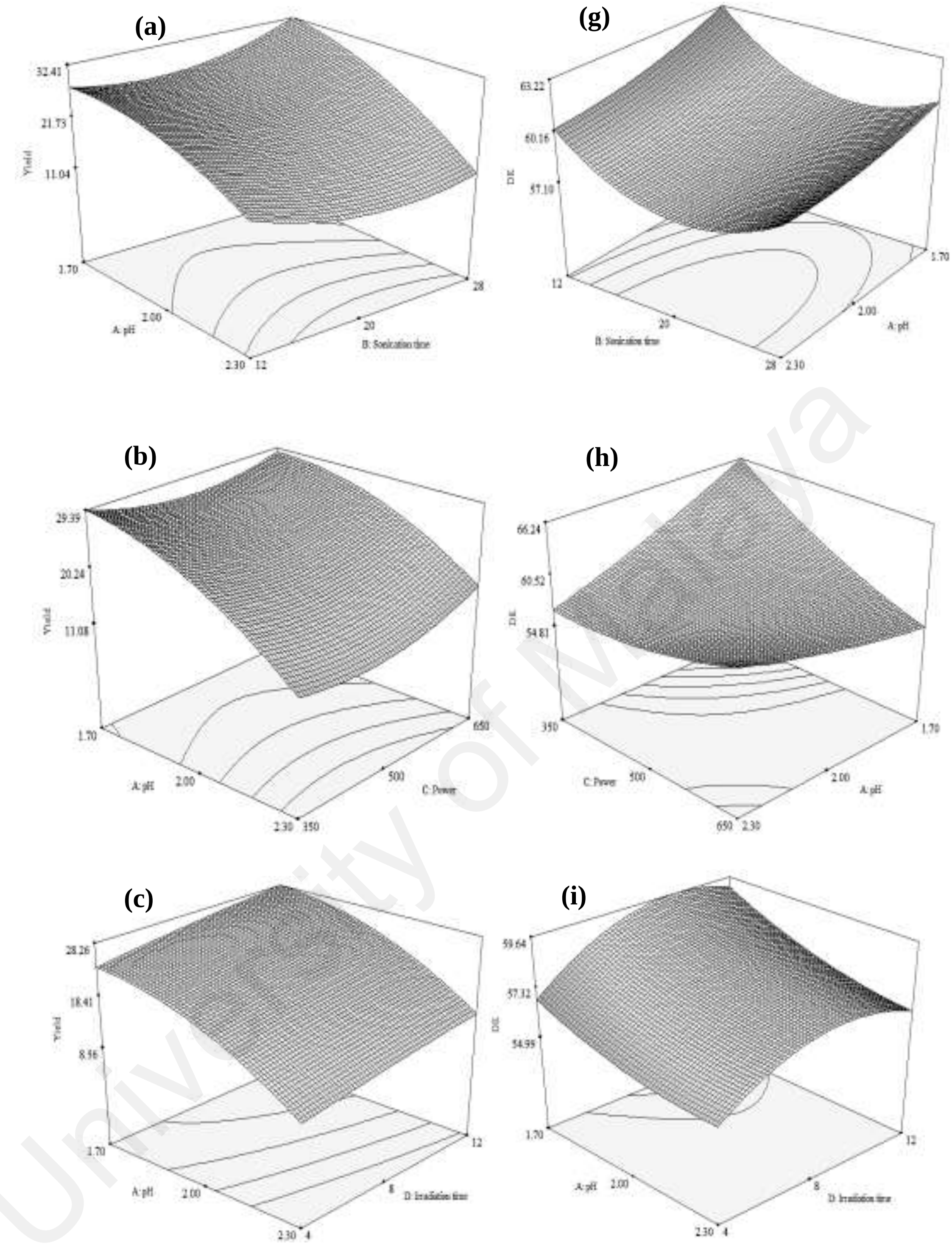


**Figure 4.7: Response surface plots showing the effect of process variable on pectin yield and DE in UMAE.**

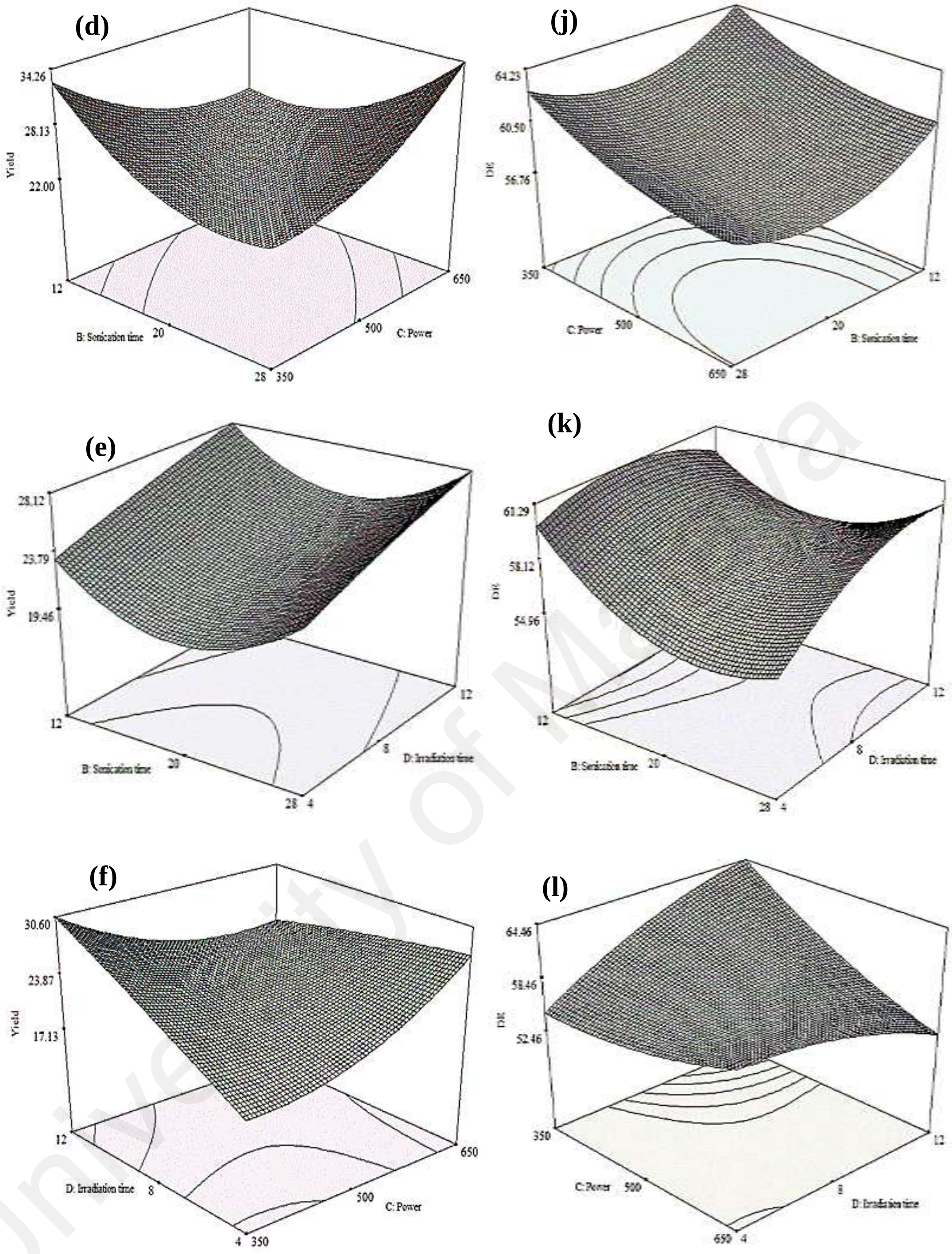


**Figure 4.7, continued.**

### 4.3.2 Modelling and Model Validation

A second order polynomial equation was developed to determine the optimum conditions that maximize the extraction yield and DE of pectin and also to relate the process variables with the responses. Their respective expressions in terms of coded factors are given in Equation (4.5) and Equation (4.6).

$$\text{Pectin yield (\%)} = 22.11 - 7.46X_1 - 0.003X_2 + 0.74X_3 + 2.39X_4 - 3.27X_1^2 + 3.83X_2^2 + 2.76X_3^2 - 0.26X_4^2 - 2.28X_{12} + 1.07X_{13} + 0.17X_{14} + 4.83X_{23} - 0.04X_{24} - 4.34X_{34} \quad (4.5)$$

$$\text{DE (\%)} = 57.78 - 1.12X_1 - 0.99X_2 - 2.00X_3 + 0.97X_4 + 0.49X_1^2 + 2.52X_2^2 + 1.13X_3^2 - 1.41X_4^2 + 0.32X_{12} + 3.71X_{13} - 0.24X_{14} - 0.19X_{23} + 1.11X_{24} - 3.99X_{34} \quad (4.6)$$

Various diagnostic plots such as predicted versus actual plot, normal % probability plot, and residuals plot were constructed to evaluate the adequacy of the model as shown in Figure 4.8. It can be observed that the data points lie close to the straight line showing adequate agreement between the experimental data and the predicted data obtained from the developed models. Furthermore, the figures show that the normal % probability plot of residuals for response are reasonably close to the straight line indicating negligible deviation of variance.

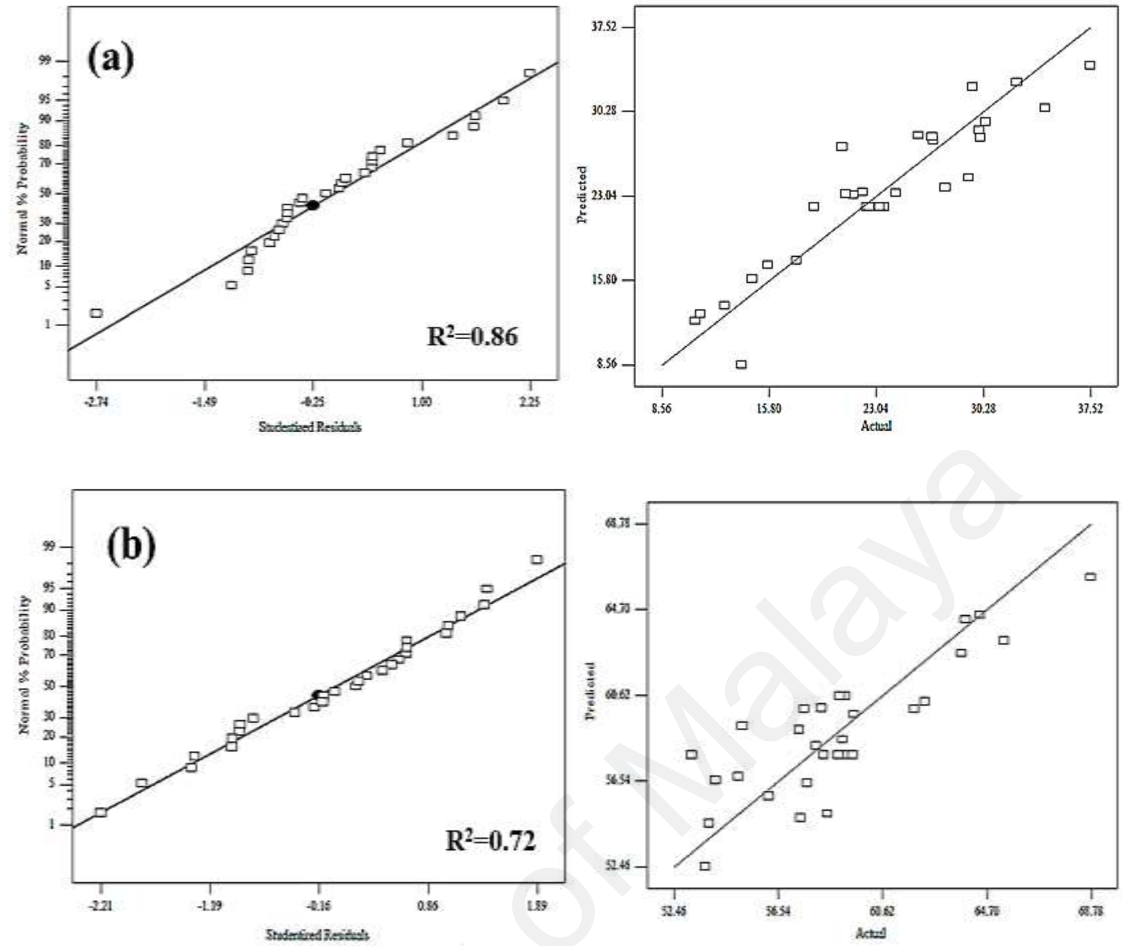


Figure 4.8: Diagnostic plots for the model adequacy of (a) yield and (b) DE in UMAE.

An optimum pectin yield of 38.00% and DE of 56.88% were successfully predicted using the quadratic model obtained at extraction conditions of pH 1.80, 27.52 min sonication time, 643.44 W microwave power and irradiation time of 6.40 min. Models were validated to ensure the predicted result was consistent with the experimental result. The model was validated 3 times with pectin yield of 35.98%, 37.45% and 35.55% while the replicate validation results for DE are 61.15%, 59.70% and 58.70%. The average pectin yield of 36.33% and a DE value of 59.85% are consistent with the predicted values. This confirms that the model is well fitted for the experimental data.

### 4.3.3 Extraction Performance of Pectin Using Different Techniques

The combined ultrasound with microwave extraction has been reported to give better yield than a single extraction technique (Bagherian et al., 2011). This has initiated the comparison study on the effect of single and combined extraction techniques on pectin extraction. Considering the extraction performance between the sequencing orders of the combined extraction might have improved the pectin yield, UMAE was performed and compare with MUAE. As shown in Table 4.7, the highest yield for UMAE was 36.33% and that for MUAE was 30.50%.

UMAE gave better pectin yield is probably due to the effect of ultrasonic waves in the sonication stage of extraction that induces the cracking on the plant tissue to allow the release of pectin. This action provides a larger contact area between pectin (the targeted compounds) with citric acid (the extraction solvent). Subsequently, the auxiliary rapid heating of a microwave ruptured the plant tissue more completely and release more pectin (the targeted compounds) into the surrounding solvent (L. Wang & Weller, 2006).

On reversing the order of the sequential extraction technique, the yield of MUAE was about 5% lower than that achieved by the UMAE. This might be due to overlapping of “micro streaming effect” and “microwave effect” imparted respectively by UAE and MAE in pectin extraction. Having had the microwave irradiation ruptured the plant tissue, further incorporation of ultrasound effect which associated with the collapse of cavitation bubble in ultra-sonication was less effective. Therefore, the enhancement role of ultrasound played in MUAE is not as distinct as in the UMAE. This also implies that microwave effect dominates in the MUAE and thus rupturing by using ultrasound is inefficient in MUAE. This also explains a mere 3% yield difference between the sole MAE and the MUAE.

Comparing UMAE with UAE and MAE, the combined extraction techniques showed a greater potential and encouraging efficiency of 154.95% with a 31.39% improvement on yield (e.g. (($Y_{UMAE} – Y_{UAE}$) / $Y_{UAE}$) x 100%)). Furthermore, when comparing various citrus fruit sources and acids used in sole MAE, the pectin yield obtained from the current study is close to that from grapefruit peel (27.81%) using HCl (Bagherian et al., 2011) and 3.82% higher than that extracted from pomelo peel (23.83%) using tartaric acid (Quoc et al., 2015). The yield difference could be caused by the extraction agents used at different concentration. For safety, environment considerations, low toxic and weak acid such as citric acid is a preferred choice.

**Table 4.7: Extraction yield, DE and GalA from the UAE, MAE, MUAE and UMAE techniques**

| Extraction technique | Yield (%) | DE (%) | GalA (%) | Conditions |
|---|---|---|---|---|
| UAE | 14.25 ±0.49 | 64.40 ±1.41 | 10.46 ±1.07 | pH= 1.8; sonication time= 27.52 min |
| MAE | 27.65 ±0.90 | 64.11 ±4.64 | 48.00 ±1.30 | pH= 1.8; irradiation time= 6.40 min; microwave power= 643.44 W |
| MUAE | 30.50 ±0.56 | 67.01 ±5.29 | 74.92 ±1.27 | pH= 1.8; irradiation time= 6.40 min; microwave power= 643.44 W; sonication time= 27.52 min |
| UMAE | 36.33 ±0.99 | 59.85 ±1.23 | 71.09 ±1.27 | pH= 1.8; sonication time= 27.52 min; irradiation time= 6.40 min; microwave power=643.44 W |

The results represent the average of triplicates ± standard deviation.

### 4.3.4 Morphology of Pectin Extracted from Different Techniques

As mentioned before, pectin morphological structural was greatly influenced by extraction process (Jiang et al., 2012). To study the influence of the extraction methods on pectin more in depth, SEM analysis was applied to observe the morphological changes in the extracted pectin. The structure of the pectin extracted by UAE, MAE, MUAE and UMAE are shown in Figure 4.9. The pectin derived from UAE of pomelo peel powder presents a smooth, loosened and wrinkled surface. The surface of the pectin extracted using MAE was rough and slightly ruptured. This might be attributed to the sudden temperature rise and also the internal pressure build up caused by microwave power during the extraction process (Figure 4.9(b)). When pomelo peel powder was subject to MUAE processes (Figure 4.9(c)), cracked and small fragments were observed on the surface of pectin. However, UMAE method gave pectin a more compact, smoother and flatter surface with less fragments, and also with smaller size of the particle distribution (Figure 4.9(d)). The morphological structures of pectin from both MAE and MUAE are almost similar whereas the pectin structure from UAE is very different from that of UMAE. In the cases of pectin extracted from MUAE and UMAE, the structure differences are apparent. Though pectin from MUAE is more compact, it is the pectin obtained from UMAE that have smaller particle size distribution might increase dissolution rate of pectin which is particularly favourable for food, cosmetic and pharmaceutical industry. Moreover, this combined extraction technique also gave the highest yield among the extraction techniques investigated.

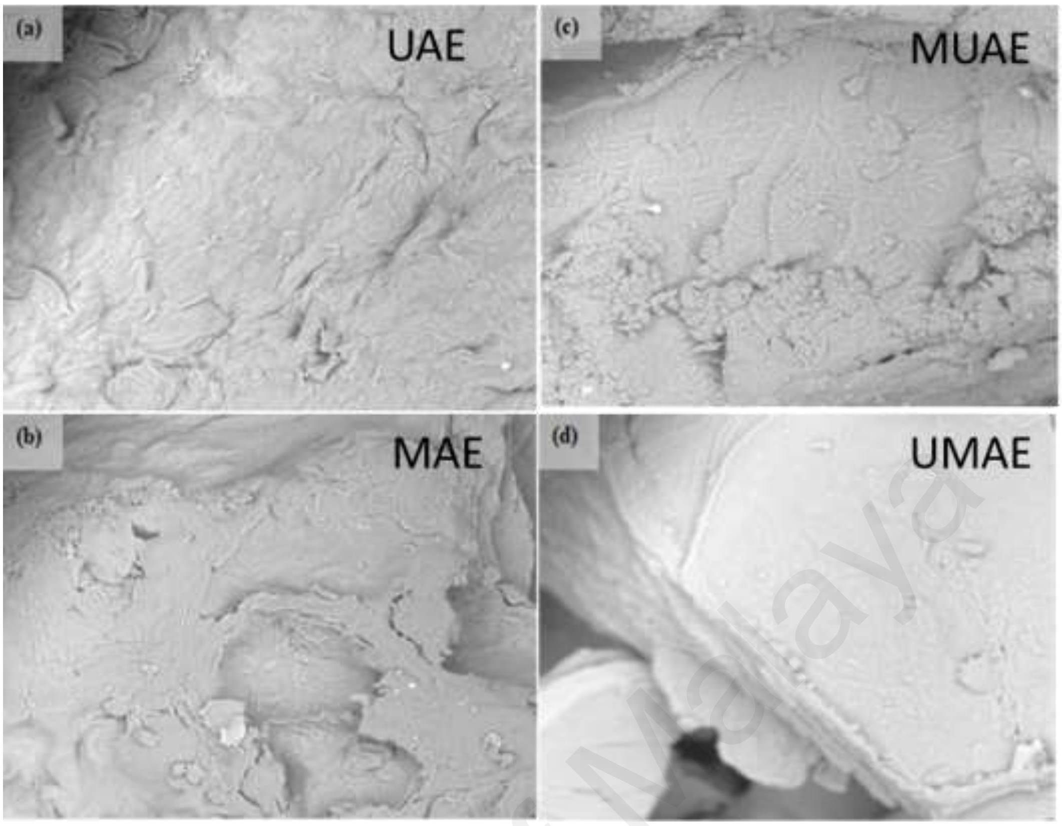


**Figure 4.9: Scanning electron micrograph of pectin at (a) ultrasound assisted; (b) microwave assisted and (c) microwave-ultrasound assisted (d) ultrasound-microwave assisted; at 500× magnification, 100µm.**

### 4.3.5 GalA Content and DE of Pectin Extracted from Different Techniques

The galacturonic acid (GalA) content and the degree of esterification (DE) of pectin are two important parameters that can determine the quality of pectin and contribute to its gelling properties (de Oliveira et al., 2015). Pectin structure consists of mainly esterified D-galacturonic acid resided in an alpha-(1-4) chain. The GalA content reflects the gelling ability of pectin. At high GalA content, the ability to form gel also increases (de Oliveira et al., 2015). Industrial pectins have specification as stipulated by FAO and EU, which should meet a minimum of 65% GalA content (Willats et al., 2006). As shown in Table 4.7, the GalA content of MUAE (74.92%) and UMAE (71.09%) both have met the quality standard. This evidences that MUAE and UMAE provide better gelling ability compared with UAE and MAE. This is probably due to the more dominant effect of combined technique extraction over single technique extraction. The combined technique extraction is able to release pectin compound more completely than the single technique extraction from deeper plant matrix that might preserve a better GalA content.

The degree of esterification (DE) represents the carboxyl groups of the main chain of galacturonic acid that are esterified with methyl or acetyl groups. DE is responsible for certain gel formation in various commercial applications. Depending on the DE value, pectins are classified as high methoxyl pectin (HM) with DE ≥ 50 or low methoxyl pectin (LM) with DE < 50. In this work, the DE obtained from various extraction techniques varied slightly (59.85% − 67.01%) as shown in Table 4.7 indicating that they are the HM pectin which often used for jam and jellies making.

### 4.3.6 Viscosity of Pectin Extracted from Different Techniques

Viscosity is an important factor to be considered for processing and sensory assessment of products. Literature has reported that pectin viscosity was significantly affected by extraction methods (Yoo et al., 2012). Hence, the rheological property and the viscosity of pectin extracted from UAE, MAE, UMAE and MUAE were evaluated. The rheological analyses of pectin gel samples are presented in Figure 4.10. In Figure 4.10(a), the flow behaviour of pectin solutions under steady-shear conditions was characterized by a plot of stress versus shear rate. The viscosity curves of the pectin dispersions are shown in Figure 4.10(b). The figures clearly show that all the pectin gels samples are pseudoplastic materials exhibiting shear-thinning behaviour ($n < 1$), whereby the viscosity decreases with increasing shear rate under steady-shear conditions. The shear-thinning region of the samples at different techniques was fitted with Ostward–DeWaele equation, $\eta = K\gamma^{n-1}$, where $K$ is the consistency index and $n$ is the flow behaviour index. The $K$ and $n$ values of the pectin are shown in Table 4.8. The table shows that the viscosities obtained from different techniques are close to each other. This finding is in agreement with that reported by Quoc et al. (2014) and L. P. T. Quoc et al. (2015), who performed pectin extraction from pomelo peel using MAE.

**Table 4.8: The rheological properties of pectin gel prepared from pectin extracted by various extraction techniques**

| Techniques | Consistency index (*K*) | Flow behaviour index (*n*) | Viscosity (Pa.s) |
|---|---|---|---|
| UAE | 0.0125 | 0.9272 | 0.0125 |
| MAE | 0.0159 | 0.8748 | 0.0159 |
| MUAE | 0.0101 | 0.9461 | 0.0101 |
| UMAE | 0.0147 | 0.9297 | 0.0147 |

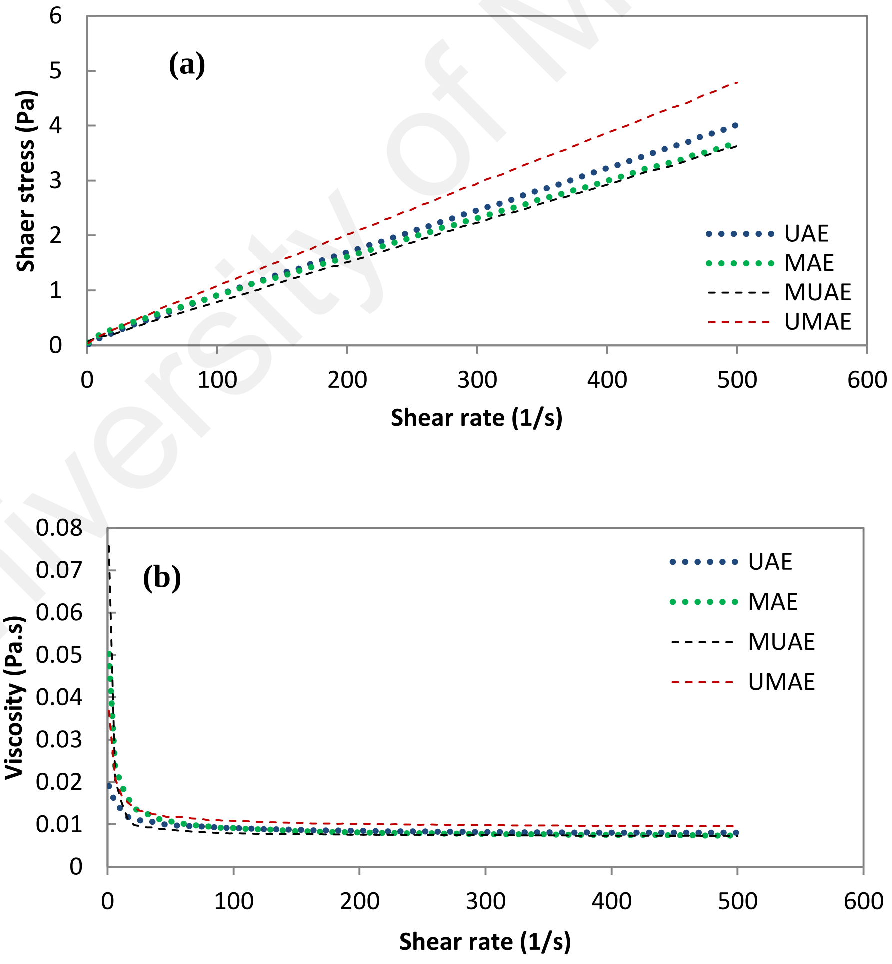


**Figure 4.10: (a) Flow behaviour curves and (b) viscosity curves for the pectin gel prepared from the pectin extracted by various extraction techniques.**

## 4.4 Subcritical Water Extraction (SWE) of Pectin

The performances of various methods in the extractions of pectin thus far suggest reasonable pectin yields which have concurred with literature reported findings as previously discussed. However, the extraction processes incorporated acids to facilitate the performance would incur operational cost and equipment coat attributing to the corrosiveness of the acidic extraction agents. Hence, pectin extracted from pomelo peels using subcritical water in a dynamic mode without the involvement of acids was attempted. An optimization study of process parameters, based on temperature and pressure, was also conducted to recover a maximum yield of the extracted pectin. This section discusses the performance of the acid free SWE and the effects of temperature and pressure as well as the physicochemical properties of the pectin obtained.

### 4.4.1 Process Optimization

The extraction of pectin by acid free SWE was optimized based on two main factors namely temperature and pressure. The optimum yield of the extracted pectin at various temperatures and pressures are presented in Table 4.9. It can be seen that the yield of the extracted pectin varies greatly from 3.7 to 20.4%. The yield of pectin extracted in this study using dynamic SWE (20.4%) is comparable with those extracted using batch SWE (21.95%) from citrus peel (X. Wang et al., 2014).

**Table 4.9: Face-centred central composite design matrix and pectin extracted using subcritical water extraction (SWE) method**

| Run | Temperature (°C) | Pressure (bar) | Pectin Yield (%) |
|---|---|---|---|
| 1 | 105 | 100 | 13.1 |
| 2 | 90 | 65 | 5.8 |
| 3 | 105 | 65 | 11.4 |
| 4 | 90 | 30 | 4.3 |
| 5 | 120 | 30 | 20.4 |
| 6 | 105 | 65 | 10.9 |
| 7 | 105 | 30 | 9.2 |
| 8 | 105 | 65 | 9.7 |
| 9 | 90 | 100 | 3.7 |
| 10 | 120 | 65 | 16.6 |
| 11 | 105 | 65 | 10.3 |
| 12 | 105 | 65 | 10.8 |
| 13 | 120 | 100 | 18.7 |

Analysis of variance (ANOVA) was employed to determine the significant level of the main parameters and the interaction effects between the parameters. The ANOVA results listed in Table 4.10 show a low *p*-value (0.0003) of the regression model; demonstrating that the developed model is significant and well fitted. The lack of fit for the quadratic model with a *p*-value at 0.0146 which is insignificant further emphasizes that the model can be used to predict the responses.

**Table 4.10: ANOVA for regression model of pectin yield in SWE**

| | Quadratic | | | | |
|---|---|---|---|---|---|
| | **SS** | **DF** | **MS** | ***F*** | ***p*** |
| Model | 297.95 | 5 | 59.59 | 23.46 | 0.0003 |
| $X_1$-pressure | 0.42 | 1 | 0.42 | 0.17 | 0.6960 |
| $X_2$- | 294.28 | 1 | 294.28 | 115.85 | <0.0001 |
| $X_1^2$ | 0.83 | 1 | 0.83 | 0.33 | 0.5844 |
| $X_2^2$ | 0.98 | 1 | 0.98 | 0.38 | 0.5547 |
| $X_{12}$ | 0.32 | 1 | 0.32 | 0.13 | 0.7334 |
| Residual | 17.78 | 7 | 2.54 | | |
| Lack of Fit | 16.19 | 3 | 5.40 | 13.54 | 0.0146 |
| Pure Error | 1.59 | 4 | 0.40 | | |
| Cor Total | 315.73 | 12 | | | |
| $R^2$ | 0.94 | | | | |
| Adj$R^2$ | 0.90 | | | | |

A second order polynomial equation was first suggested to determine the optimum conditions that maximizing the extraction yield of pectin. The quadratic model is given in Equation (4.7).

$$\text{Pectin yield (\%)} = 10.63 + 0.27X_1 + 7.00X_2 + 0.55X_1^2 + 0.59X_2^2 - 0.28X_{12} \qquad (4.7)$$

Value of $R^2$ (0.94) and an adj-$R^2$ above 0.90 signify good correlation between the response and the independent variables. Based on the quadratic model, an optimum pectin yield of 18.8% and a DE of 40.5% were predicted at extraction temperature and pressure at 120°C and 30 bar.

The predicted optimum extraction condition was validated in triplicate with an average pectin yield of 19.6 ± 0.9% and a DE value of 40.1 ± 2.1%; the results substantiated the goodness of fitting of the quadratic model to the experimental data. Furthermore, 0.8% difference between the predicted optimum yield and the validated experimental value is well within the relative standard deviation (RSD) of the center point (RSD center point= 7.3%). The relationship between the responses and the experimental variables are illustrated graphically in a three-dimensional response surface plot as shown in Figure 4.11.

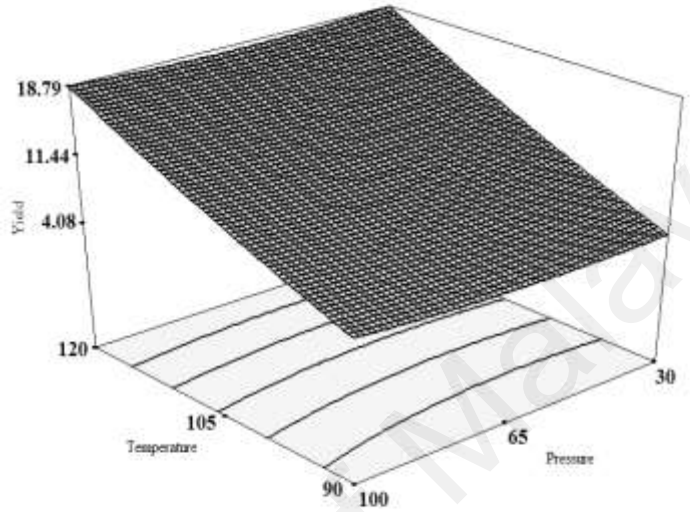


Figure 4.11: Response surface plots showing the effect of process variable on pectin yield in SWE.

The results obtained from ANOVA in Table 4.10 shows that the effect of temperature on pectin yield is significant ($p < 0.0001$). The linear and quadratic terms of the pressure are insignificant within the range considered in the present study. Furthermore, the quadratic term of temperature and the interaction between temperature and pressure are insignificant. The obtained quadratic model can be reduced to a linear equation by taking out all insignificant terms with $p > 0.05$, giving Equation (4.8).

$$\text{Pectin yield (\%)} = 10.63 + 7.00X_2 \tag{4.8}$$

The dominant influence of temperature over pressure on the yield of subcritical water extraction is consistent with the findings obtained by Thiruvenkadam et al. (2015) and Zeković et al. (2014).

### 4.4.2 Temperature Effect

The results of the present study (Table 4.9) show that the yields of pectin obtained are ranged from 3.7 – 20.4%. It can be seen that the yield of pectin was mainly dependent on the extraction temperature. The yield of pectin increased as the extraction temperature increased. The yield obtained at 105°C was 9.2 – 13.1% and the yield obtained at 120°C (i.e. 16.6 – 20.4%) was at least three times higher than the yields obtained at 90°C (3.7 – 5.8%). Extraction temperature affects the physicochemical properties of water such as viscosity, surface tension and dielectric constant (Hata et al., 2008). It also affects the sublimation pressures of solutes, thereby affecting the solubility of a solute in a solvent (Teoh et al., 2013) and its subsequent extractability. As water temperature increases, the strength of the hydrogen bonds in water reduces. The weakened hydrogen bond leads to a decrease in the dielectric constant value followed by a reduction in water polarity; subsequently, reducing the energy needed for division in solute-matrix interactions and increasing extraction efficiency (Ho et al., 2007). Hence, an increase in temperature generally commensurate with an increase in extraction yield.

The maximum temperature used in the present study is 120°C as pectin floatation was not observed at 130°C (Figure 4.12(a)). One possible explanation for the observation is the degradation of pectin due to high temperature and/or prolonged exposure to subcritical conditions. The degradation of pectin can be observed from the appearance of the extract. For example, the colour of the extract turned dark brown at 130°C as shown in Figure 4.12. The dark brown extract is of contrast to a typical non-degraded light brown extract.

For the extract obtained at and below 120°C, the odor was found to be mild fruity. Whereas, burning smell of the extract was detected at 130°C. Similar observations (in terms of solution color and odor) were made in a number of studies (Asl & Khajenoori, 2013; Zeković et al., 2014). Also, a number of authors have found that an increase in temperature to 120°C in SWE increased the yield of the extract (H.-m. Chen et al., 2015; X. Wang et al., 2014). However, as the extraction temperature continued to increase, a decline in extraction yields was observed; pointing to a possible degradation of the extract above 120°C.

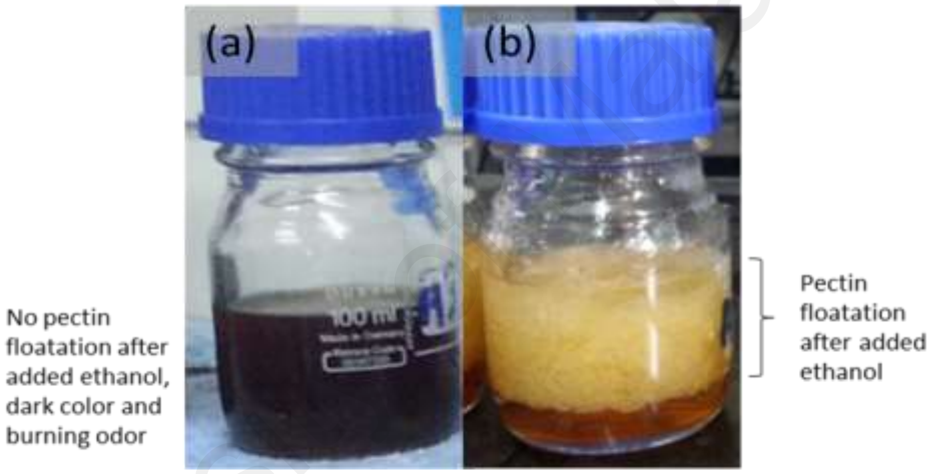


**Figure 4.12: Products extracted at 120°C and 130°C using SWE: (a) Thermal degraded extract at 130°C and (b) pectin extract obtained at 120°C.**

### 4.4.3 Pressure Effect

Like extraction temperature, pressure can also influence the pectin yield. According to the ANOVA result presented in Table 4.10, the effect of pressure on pectin yield is insignificant (*p* > 0.05) and the interaction between extraction temperature and pressure did not significantly affect extarction yields of pectin. Nevertheless, Figure 4.11 shows that no matter at high pressure of 100 bar or low pressure at 30 bar, it is preferred to have high temperature of 120°C in order to obtain a better pectin yield. The insignificant influence of pressure on SWE yield was found to be in agreement with various published works (J. Liu et al., 2015; Zeković et al., 2014). For each level of temperature investigated in the present study, no distinct trend was observed between pressure changes and yield. Hence, in optimizing the SWE of pectin, it is desirable that the extraction pressure is kept low provided that the liquid state of the solvent is maintained. A lower extraction pressure will reduce exhaustion on equipment use and thus, reduces equipment and operating costs.

### 4.4.4 The Effects of SWE Conditions on DE Values

Apart from affecting the yields of pectin, the condition of a specific extraction technique affects the DE of pectin. The values of DE obtained in the present study ranged between 29.7% and 45.5%, as shown in Table 4.11. All DE values obtained are found to be lower than 50%; indicating that the extracted pectin is a type of LM pectin. This class of pectin is often used to produce low-sugar or sugar-free jellies.

**Table 4.11: DE of pectin extracted using SWE method**

| Run | Temperature (°C) | Pressure (bar) | DE (%) |
|---|---|---|---|
| 1 | 105 | 100 | 36.7 |
| 2 | 90 | 65 | 37.3 |
| 3 | 105 | 65 | 45.5 |
| 4 | 90 | 30 | 29.7 |
| 5 | 120 | 30 | 41.1 |
| 6 | 105 | 65 | 43.0 |
| 7 | 105 | 30 | 38.2 |
| 8 | 105 | 65 | 42.6 |
| 9 | 90 | 100 | 33.4 |
| 10 | 120 | 65 | 42.2 |
| 11 | 105 | 65 | 43.3 |
| 12 | 105 | 65 | 43.0 |
| 13 | 120 | 100 | 40.1 |

The subcritical water extraction of pomelo peel powder of this study produces LM pectin whereas conventional heating, ultrasound and microwave-assisted extraction methods are found to produce high methoxyl (HM) pectin (Liew et al., 2016; Methacanon et al., 2014). One possible explanation for the marked difference is the absence of acid in the present work. For the conventional heating, ultrasound and microwave methods, the use of acid was essential to the extraction process. When acid was added during the extraction process, non-methyl-esterified carboxyl groups in pectin were converted to methyl-esterified carboxyl groups; thus, increasing DE value and, thereby, producing HM pectin. The HM pectin produced can then be converted to LM pectin through the de-esterification of HM pectin using acids, alkali and enzyme (Renard & Thibault, 1996; Zhao et al., 2015).

In order to test the hypothesis that a lack of acid hindered the production of HM pectin, the precipitates extracted from dynamic SWE at 30 bar and 120 °C, were washed with two different solutions; one with ethanol and the other with 5% (v/v) hydrochloric acid (HCl) in 60% isopropyl alcohol (IPA). The DE of pectin was found to increase from 40.1% (LM grade) with ethanol washing to 61.1% (HM grade) with acid washing. The corresponding FT-IR analyses for both washing conditions are shown in Figure 4.13. When acid washing was applied, the corresponding FT-IR spectrum showed an increase in the intensity of the peak at around 1745 cm$^{-1}$ and a decrease in the intensity of the peak at around 1630 cm$^{-1}$, relative to the FT-IR spectrum with ethanol washing. The DE value is generally reflected in proportion to the 1745 cm$^{-1}$ peak. Hence, the FT-IR spectra lead to an inference that the washing of the precipitated pectin extract with acid increases the DE value. In addition, the outcome of the present dynamic SWE with acid washing can be juxtaposed with the batch SWE conducted by Wang et al. (2014). Pectin was extracted from citrus peel by the authors in a 5-min batch SWE. The authors subsequently washed the precipitates with HCl in IPA; resulting in HM pectin being produced. Hence, the use of acid can aid in the production of HM pectin and a lack thereof, lowers the DE values.

Another possible explanation for the ensuing production of the LM pectin under dynamic SWE is a prolonged exposure to pressure. In a high hydrostatic pressure assisted enzymatic treatment of HM pectin to LM pectin, Zhao et al. (2015) found that the value of DE decreased as they increased the pressure holding time in the de-esterification process. In the present study, the total extraction time for one SWE generally took up to 140 min for completion. Hence, a prolonged exposure to pressure may aid in the conversion of pectin to the LM type. Therefore, the production of LM pectin in the present study, as oppose to other studies, can be due to a direct influence of prolonged exposure to high pressure and the absence of acid in the process.

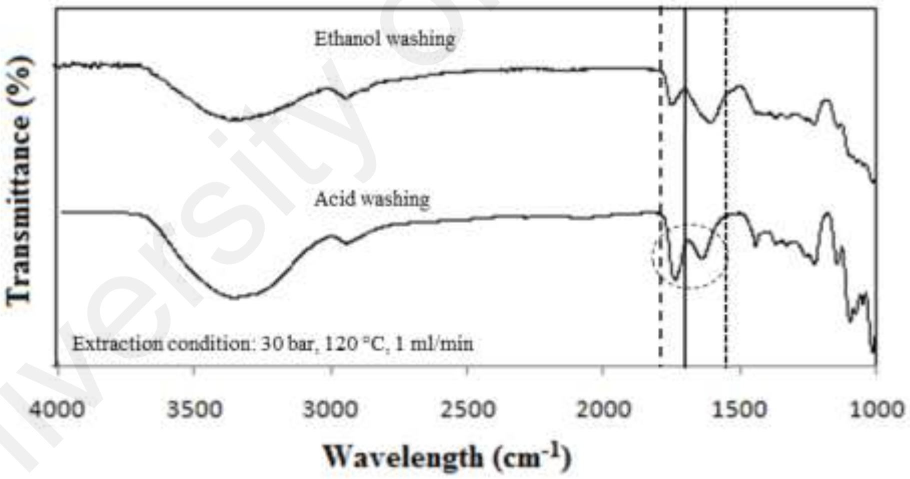


Figure 4.13: FT-IR spectra of pomelo pectin using different washing solvent.

Pectin extracted via CE, UAE, MAE, UMAE and MUAE involved the use of acid, except the acid free SWE, will incur additional equipment cost and this has prompted the investigation of various extraction methods without using acid. The results of various extraction methods with or without involved acid are presented in Figure 4.14 and Table

4.12. Results clearly showed that those extraction methods involved acid (13.24 – 39.13%) gave better yield of pectin compared to those without involved acid (3.18–19.21%). For the extraction without involved acid, UAE, MAE, UMAE and MUAE gave similar yield ~3.5% while CE has slightly higher extraction yield (5.34%). This might due to the longer heating time in CE method that offering a better pectin yield. Besides, extraction methods without involved acid also showed lower DE than those involved acid.

**Table 4.12: Pectin yield and DE obtained from various extraction methods with or without acid**

| | With acid | Pectin yield (%) | DE (%) | Without acid | Pectin yield (%) | DE (%) |
|---|---|---|---|---|---|---|
| CE | pH=1.80, t=141.38 min, T= 88°C, L/S=29:1 | 39.13±1.89 | 59.23 | t= 141.38 min, T= 88°C, L/S= 29:1 | 5.34±0.24 | 36.65 |
| UAE | pH=1.79, sonication time=28 min | 13.24±0.44 | 57.91 | Sonication time= 28 min | 3.18±0.13 | 33.14 |
| MAE | pH=1.74, irradiation time=11.97 min, microwave power=649.94 W | 30.24±0.97 | 59.47 | Irradiation time= 11.97 min, microwave power= 649.94 W | 3.43±0.17 | 50.51 |
| MUAE | pH=1.73, irradiation time=10.11 min, microwave power=649.90 W, sonication time=17.72 min | 31.57±0.77 | 61.15 | Irradiation time= 10.11 min, microwave power= 649.90 W, sonication time= 17.72 min | 3.50±0.12 | 41.70 |
| UMAE | pH=1.80, sonication time=27.52 min, microwave power=643.44 W, irradiation time=6.40 min | 36.33±1.00 | 59.85 | Sonication time= 27.52 min, microwave power= 643.44 W, irradiation time= 6.40 min | 3.68±0.53 | 39.37 |
| SWE | - | n/d | n/d | T= 120°C, pressure= 30 bar | 19.6±0.9 | 40.1 |

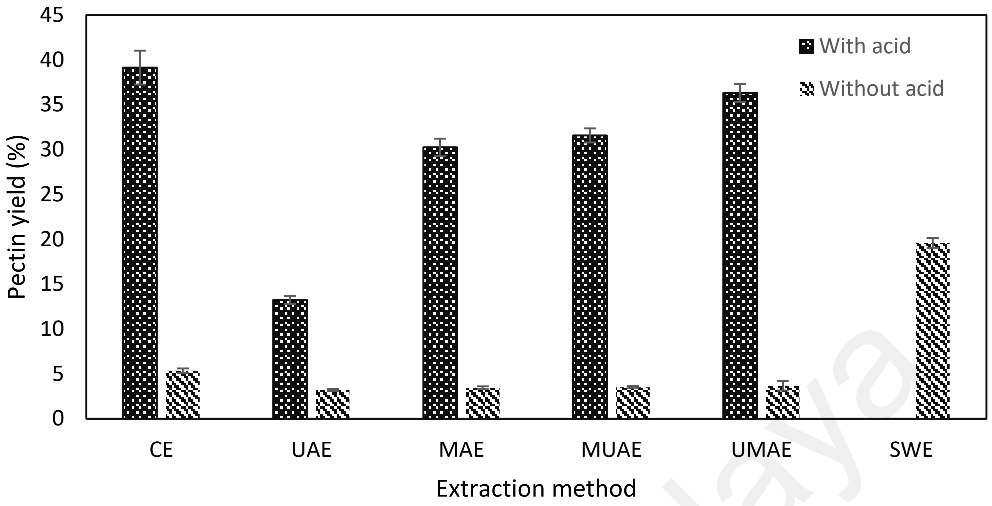


**Figure 4.14: Pectin yield from various extraction methods with or without acid.**

## 4.5 Physicochemical Properties of Pectin Extracted using CE, UMAE and SWE

The yield and pectin quality can be influenced by extraction methods (Jiang et al., 2012; Sari et al., 2018). Considering the possible influence of extraction method on the properties of pectin, a series of analyses were performed on the pectin extracted from CE, UMAE and SWE. The analyses associated with the investigation on pectin yield, degree of esterification, galacturonic content, molecular weight of the extracted pectin, solubility, flow behaviour, colorimetry, morphological study, structural analyses, proximate analyses, process efficiency and energy consumption. The physicochemical composition of pectin extracted using CE, UMAE and SWE are shown in Table 4.13.

Pectin is commonly used as food additive. The pH of food additive would influence the taste of final product. In general, a neutral pH food additive is preferred to be used in food industry. Furthermore, easily soluble food additive which can speed up the dissolution time are also in favor. In this study, the pectin extracted from CE, UMAE, and SWE exhibited pH value of 2.11, 2.47 and 4.51, respectively. The extracted pectin from

SWE showed less acidity due to the reason of water was used as the extraction agent. In terms of solubility, pectin extracted from SWE has a slightly better solubility as comparing with CE and UMAE.

The extraction methods used to extract pectin can influence the molecular weight of pectin (Hui & Sherkat, 2005); thereby, affecting its gelling and rheological properties (Lim et al., 2012). The molecular weight of the subcritical water extracted pomelo pectin ($6.26\times10^4$ Da) was slightly lower than that of the CE technique ($9.05\times10^4$ Da) and UMAE technique ($9.30\times10^4$ Da). It is also lower than those conventionally extracted from mango ($2.45\times10^5$ Da) and lime ($1.23\times10^5$ Da) (Koubala et al., 2008) but higher than that from Akebia *trifoliata* var. australis peel ($4.54\times10^4$ Da) and sunflower pectin ($4.80\times10^4$ Da) (Jiang et al., 2012). In term of viscosity, pectin extracted from different methods have very close viscosity at ~0.01 Pa.s and exhibit pseudoplastic behaviour. The pseudoplastic behaviour of pectin can be rationalized in terms of polymer entanglements, where (under shear) the rate of disentanglement exceeds the rate at which the new entanglements form and this leads to a reduction in the cross-link density and, as a consequence, the viscosity decreased (Manoj et al., 1998).

Lighter coloured pectin is generally preferable for industrial use. The pectin extracted from UMAE is of light brown colour with the lightness, $L^*$ higher than the pectin extracted using CE and SWE. One possible reason for the lighter colour in UMAE pectin is a shorter exposure to heat treatment compared to a CE and SWE. UMAE achieved the highest extraction efficiency of 0.4909 g/h while the SWE only obtained an efficiency of 0.0480 g/h. In terms of energy usage, SWE required 1.053 kW.h which is higher than those obtained by CE and UMAE respectively at 0.8299 kW.h and 0.2269 kW.h. It can also be observed that the energy required to get 1g of pectin is higher using SWE (5.4815 kW.h/g) than CE (2.0894 kW.h/g) and UMAE (0.6245 kW.h/g). Higher energy consumption was attributed to the energy required to heat up the extraction oven in CE and SWE. For the latter which is in dynamic mode whereby pumping was needed throughout the extraction, energy demand is comparatively higher.

**Table 4.13: Physicochemical property of pectin extracted from CE, UMAE and SWE**

| | CE[a] | UMAE[b] | SWE[c] |
|---|---|---|---|
| Moisture (%) | 14.60±0.31 | 18.69±0.36 | 16.30±0.34 |
| Ash (%) | 1.28±0.12 | 1.10±0.31 | 2.96±0.11 |
| Protein (%) | 2.11 | 2.47 | 4.51 |
| Fat (%) | 0.02 | 0.08 | n.d. |
| pH | 2.07±0.01 | 2.23±0.02 | 4.72±0.02 |
| Molecular weight, Mw (Da) | $9.05\times10^4$ | $9.30\times10^4$ | $6.26\times10^4$ |
| Solubility (%) | 76.27±0.51 | 71.79±0.89 | 79.80±0.50 |
| Type of pectin | HM | HM | LM |
| Gel viscosity (Pa.s) | 0.0103 | 0.0108 | 0.0127 |
| Color, CieLab coordinates | | | |
| $L^*$ | 16.49±0.14 | 19.57±0.37 | 17.28±0.07 |
| $a^*$ | 7.12±0.04 | 7.41±0.20 | 6.67±0.11 |
| $b^*$ | 15.55±0.22 | 14.72±0.21 | 17.07±0.80 |
| $H^*_{ab}$ | 65.38±0.38 | 63.28±0.46 | 68.62±1.15 |
| $C^*$ | 17.10±0.19 | 16.48±0.25 | 18.59±1.13 |
| Efficiency (g/h) | 0.1576 | 0.4909 | 0.0480 |
| Energy usage (kW.h) | 0.8299 | 0.2269 | 1.0530 |
| Energy require to produce 1g pectin (kW.h/g) | 2.0894 | 0.6245 | 5.4815 |

[a] Conditions: pH 1.80, time 141.38 min, at 88°C with 29:1 liquid-solid ratio.
[b] Conditions: pH 1.80, 27.52 min sonication time, 643.44 W microwave power and irradiation time of 6.40 min.
[c] Conditions: 120°C and 30 bar.

According to Figure 4.15, it shows that pectin yield, DE and GalA content are significantly affected by the extraction methods. The pectin extracted by CE (39.13%) is significantly greater than those extracted by UMAE (36.33%) and SWE (19.63%).

Similar DE values of the pectin extracted from CE (59.23%) and UMAE (59.85%) indicating that the pectin is a type of HM pectin (DE ≥ 50%). However, it is the LM pectin that was obtained from SWE (40.09%). The most probable reason is that in SWE there was no involvement of acid for the extraction of pectin as discussed in section 4.4.4. On the other hand, the results of GalA content obtained by different extraction methods indicate that the extracted pectin from CE (68.54%), UMAE (73.93%) and SWE (76.62%) are of good quality within the acceptable limit (GalA ≥ 65%) stipulated by the Food and Agriculture Organization (FAO) specification for pectin.

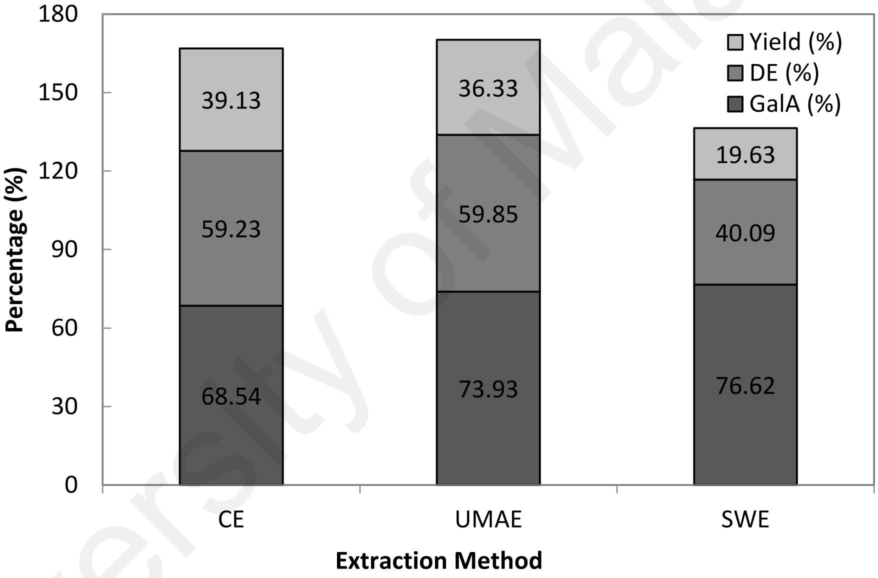


**Figure 4.15: Comparison of pectin yield extraction using different extraction methods.**

FT-IR structure analysis can be used to prove identity of pectin. Figure 4.16 presents the FT-IR spectra of the extracts extracted using CE, UMAE and SWE. It was found that the FT-IR pattern of the extracts obtained from the three extraction methods is similar to the work of Jiang et al. (2012) and thus the extracts can be confirmed are pectin. In the figure, the FT-IR spectra of pectin extracted using CE and UMAE methods are very similar to each other, with a higher band at 1745 cm$^{-1}$ and a lower band at 1630 cm$^{-1}$. It is different from that extracted using SWE with opposite trend due to acid free extraction via SWE as previously discussed in section 4.4.4.

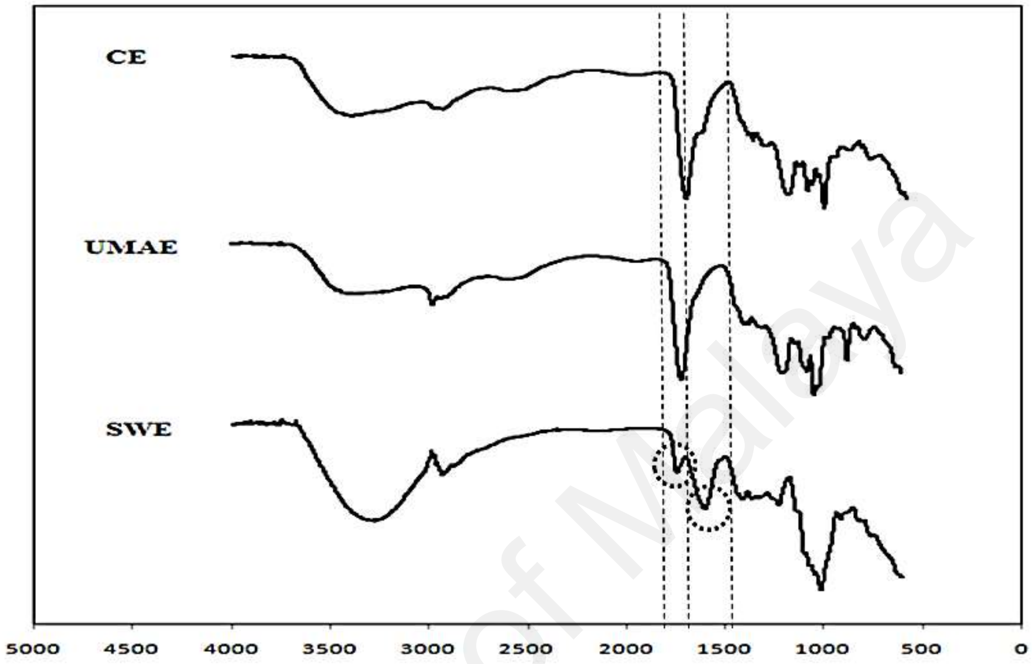


**Figure 4.16: FT-IR spectra of pectin extracted using different extraction methods.**

While FT-IR is for pectin identification, macrograph and micrograph are indicators that can be used in the food industry to determine the quality of pectin. The colour of pectin can be observed in a macrograph, and the morphological structural of the pectin is always presented using a micrograph. Figure 4.17(a) and Figure 4.17(b) show the macrographs of the wet and dried pectin powders extracted from CE, UMAE and SWE, respectively. The fine brownish powder liked pectin were obtained from CE and UMAE. However, the dried pectin extracted from SWE appeared to have large yellowish brown shiny crystals. The surface morphologies of pectin extracted using CE, UMAE and SWE methods are illustrated in Figure 4.17(c). The micrographs of pectin extracted using CE and SWE have similar structures which consist of smooth and compact surface with less fragments compared to the pectin extracted from UMAE. This signifies that more exposed surface plane for pectin extracted from UMAE with smaller size of particle and thus pectin extracted using UMAE is favourable for industrial application.

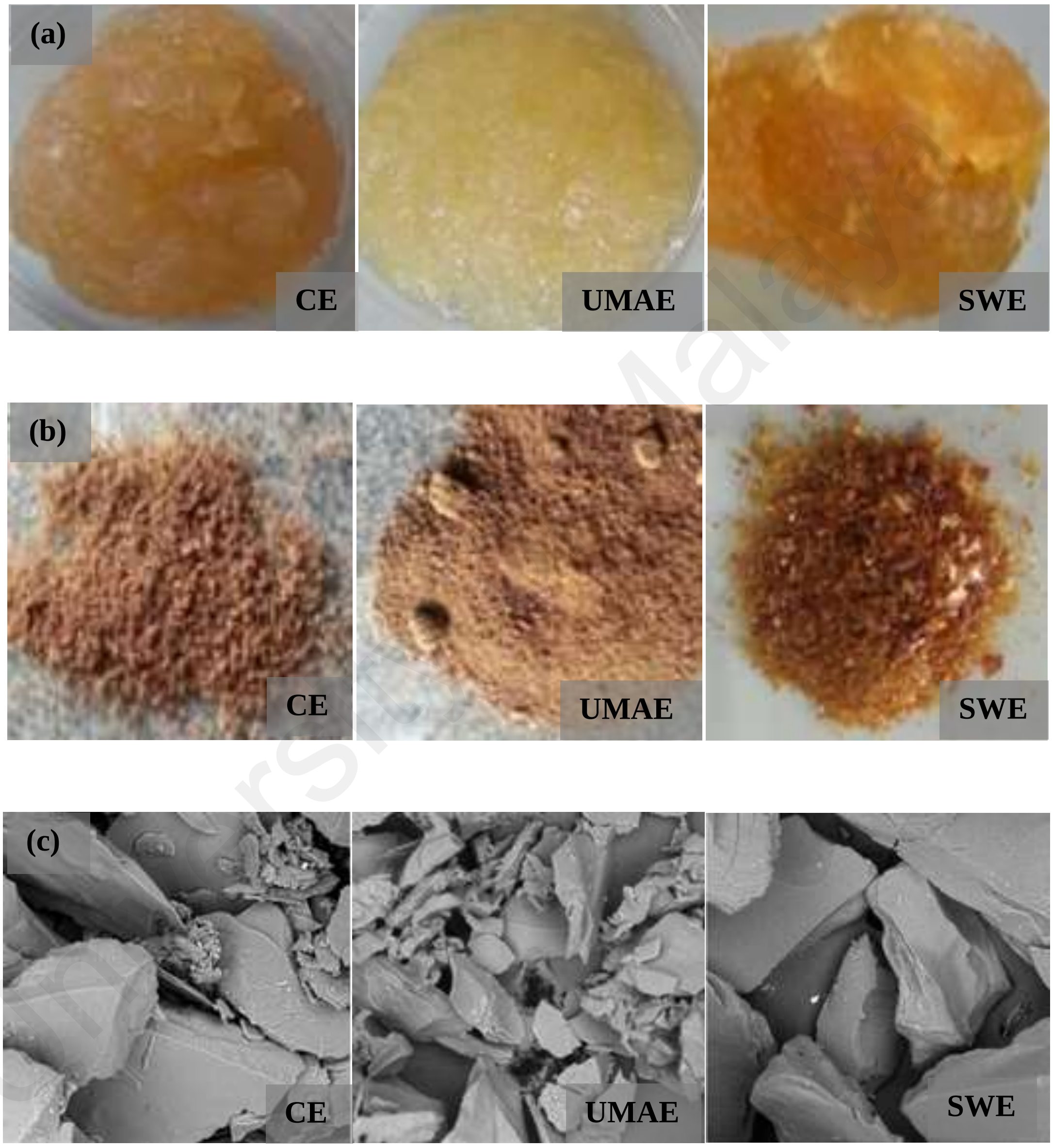


**Figure 4.17: Photographs of (a) wet pectin, (b) dried pectin and (c) scanning electron micrograph of pectin from CE, UMAE and SWE; at 500× magnification, 100µm.**

X-ray diffraction (XRD) analysis was used to examine the pectin structure (amorphous or crystalline) obtained and the XRD patterns of pectin observed at the range of 5° to 60° (2θ) are shown in Figure 4.18. The XRD pattern displayed for CE appeared to have similar illustration as that for UMAE; denoting both of them are typical crystalline polymers. Sharp and great intensities of characteristics peaks can be observed for pectin extracted from both CE and UMAE which appeared at $2\theta = 18.5°$, 26.3°, 31.5° and 36.3°. Comparatively, pectin obtained from CE has greater peak intensity than that of UMAE. On the other hand, there is no significant characteristic peaks showed by the XRD pattern for SWE. This has deduced that the pectin structure resulted from SWE is typically an amorphous polymer.

A well-ordered polymer is considered crystalline, the structure of crystalline is strong and rigid. Conversely, amorphous polymers have flexible and elastic structure (CPNikadee, 2017). Hence, the amorphous polymer can dissolve faster than the crystalline polymer because of less intermolecular forces involved. This explains the amorphous pectin extracted from SWE has better solubility. Moreover, XRD analysis has also proven that different extraction methods would attribute to the difference in the pectin X-ray diffraction patterns.

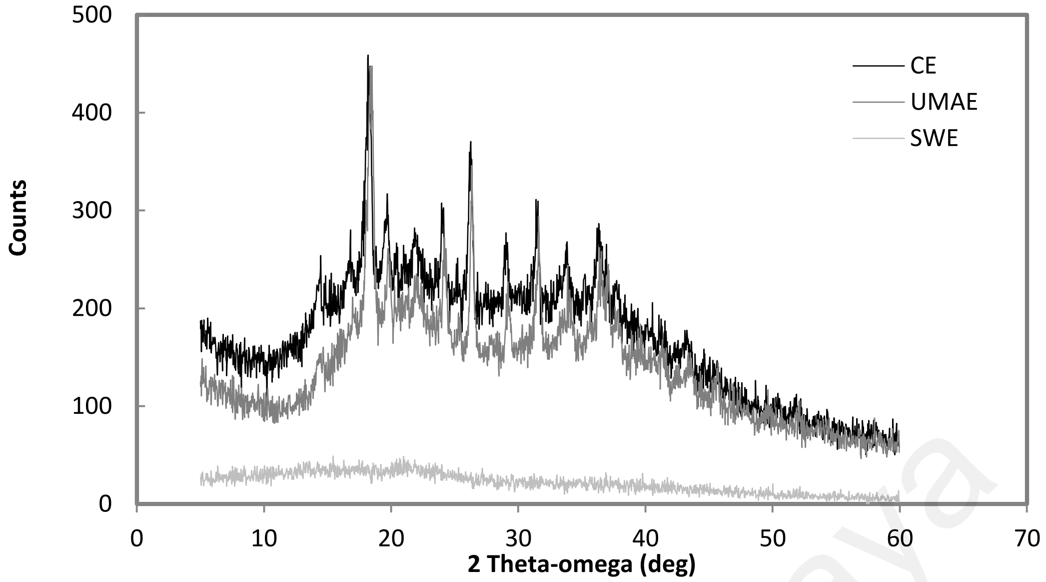


**Figure 4.18: X-ray diffraction patterns of pectin from CE, UMAE and SWE.**

The performances of pomelo pectin extractions using CE, UMAE and SWE were thoroughly compared and are tabulated in Table 4.14. Taking into account of the quantity and quality of the extracted pectin, extraction agent, extraction efficiency, operating cost, capital cost, energy requirement and the complexity of the operation, the comparison results collectively suggest that ultrasound-microwave assisted extraction (UMAE) of pectin is preferable to conventional heating extraction (CE) and subcritical water extraction (SWE).

**Table 4.14: Summary of pectin extraction using CE, UMAE and SWE**

| Extraction technique | CE | UMAE | SWE |
|---|---|---|---|
| Brief description | Pomelo peel powder treating with citric acid in a water bath. | Pomelo peel powder treating with citric acid in ultrasonic bath following with a microwave. | Pomelo peel powder treating with subcritical water without acid involved |
| Feature | Water bath heating | Ultrasound radiation and microwave heating | Pressure, Oven heating |
| Extraction mechanism | Diffusion | Ultrasonic: Provides stirring and structure effects on plant cells through the cavitation phenomenon.<br>Microwave energy: Localized heating of microwave builds internal pressure to rupture plant cells. | Diffusion |
| Capital cost | Low | Low | High |
| Operating cost | Moderate | Low | Low |
| Energy requirement | Moderate | Low | High |

**Table 4.14, continued**

| | | | |
|---|---|---|---|
| Extraction time | Long | Short | Moderate |
| Efficiency rating | Moderate | High | Low |
| Solvent | Acid | Acid | Water |
| Mode | Batch | Batch | Dynamic |
| Advantages | Easy to handle; high extraction yield | Easy to use; fast; high extraction yield, no heating up time | Cheap solvent (water); environment-friendly system |
| Drawbacks and limitations | Long extraction time; use acid as solvent | Sequential extraction; use acid as solvent | Low extraction yield; possible thermal degradation of the sample and powder blockage; long equilibrium time; clean up step for the extraction line is needed; expensive and complicated setup; not easy to handle; high pressure and dangerous. |

# CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

This chapter summarizes the research findings of various extraction methods according to the objectives set. The novelties of the present study and the recommendations for future work are also provided.

## 5.1 Conclusion

Pectin was successfully extracted from pomelo (*Citrus grandis* (L.) Osbeck) peels using different extraction methods. The yields obtained are comparable to those extracted from other citrus fruit families signified that pomelo peel is a good source of pectin.

Pectin extracted using CE incorporated with citric acid achieved the highest pectin yield (39.13%) amongst the methods studied. The optimum extraction conditions for CE were pH 1.80 and 88ºC, with an extraction time of 141 min at a liquid-solid ratio of 29:1. From the assessment of ultrasound assisted, microwave assisted and combined assisted extraction, UMAE gave the highest pectin yield of 36.33% and the optimum conditions were determined at pH of 1.80, sonication of 27.52 min, microwave power of 643.44 W and irradiation times of 6.40 min. In CE, pH posed the greatest impact on both pectin yield and DE value. By varying extraction pH between 1 and 2, pectin extraction performance, chemical structure, as well as its morphological and gelling properties were significantly affected. Low extraction pH of 1.0 gave the least GalA content in pectin. The extraction pH of 2.00 greatly modified the morphological structure of pectin and also indicated clearly the functional groups of pectin. Pectin gels prepared from pectins extracted at pH 1 – 2 exhibited a pseudoplastic behaviour with viscosity ~0.01 Pa.s. Among the parameters investigated, pH has the most significant impact on pectin yield whereas DE is significantly affected by microwave power in UMAE. Both the CE and the UMAE extracted HM pectin with similar DE values of 59.23% and 59.85%. In view of its shorter extraction time and a mere 2.8% difference in yield compare with CE, UMAE is a good alternative technique for pectin extraction.

Further comparison with acid free subcritical water extraction that achieved the yield of 19.63% at its optimum condition of 30 bar and 120°C shows that extraction involving acid can obtain a higher pectin yield. In SWE, temperature plays a more significant role than pressure. SWE extracted LM pectin as its DE is low at 40.09%. It was attributed to acid free extraction system and a prolonged exposure to pressure under dynamic SWE conditions. This signifies that LM pectin can therefore be extracted from pomelo peel powder in a one-step extraction process instead of the conventional two-step extraction–de-esterification process.

The physicochemical properties of pectin extracted from CE, UMAE and SWE were performed orderly. Good quality of GalA (≥ 68%) was characterized from pectin extracted via CE, UMAE and SWE with molecular weight ~$8.20 \times 10^4$ Da. Besides that, all the pectin gels obtained have viscosity around 0.01 Pa.s exhibiting pseudoplastic behavior. From acid free SWE, the pectin extracted is less acidic and more amorphous with better solubility whereas those extracted from UMAE are lighter in color and with smaller particle size. Comparing among the extraction techniques, UMAE required the shortest extraction time of 34 min to give a satisfactory pectin yield of 36.33% and the highest efficiency at 0.4909 g/h; SWE required the highest energy usage at 1.0530 kW.h despite it is a more environmental friendly acid free extraction system.

Based on the comparison findings in terms of extraction performance, operational feasibility, energy requirement, UMAE is preferred to CE and SWE for pectin extraction from pomelo peel powder.

## 5.2 Novelties and Implications of Study

Several novelties and implications of this research are highlighted below:

i. Comprehensive characterization of pectin extracted from pomelo peels using conventional heating and non-conventional extraction methods. The physicochemical properties of the extracted pectin provide useful information pertaining to the structure and properties of pectin for various industrial applications.

ii. The optimization of pectin extraction from pomelo peel powder using citric acid incorporated CE, UMAE and acid free dynamic SWE have never been reported. Moreover, single and interactive effects of the parameters employed in pectin extraction were thoroughly studied to facilitate the maximum recovery of pectin from the extraction source.

iii. HM pectin was recovered from extraction techniques incorporated with citric acid whereas LM pectin can be obtained in a one-step acid free SWE extraction process. This SWE extraction system is encouraged to be used in place of the conventional two-step extraction–de-esterification process. In addition, acid free SWE can contribute to the movement of the pectin industry into the natural realm by eco-friendly processing technology without the use of harmful chemical.

## 5.3 Recommendations

This research study has provided several insights into pectin extraction using different extraction methods. Some potential areas related to the pectin extraction for possible future studies are recommended as follows:

i. Further investigation on the use of biodegradable green extraction agent such as deep eutectic solvent and enzymes on the extraction of pectin to minimize environmental impact imparted by acids.
ii. Exploration on non-conventional extraction techniques that have not been thoroughly investigated for pectin extraction such as negative pressure cavitation extraction and enzyme assisted extraction. If it is proven feasible, they can replace the existing less eco-friendly extraction techniques.
iii. Kinetic model study on pectin extraction is recommended to enable scale up of the extraction process of the present work.
iv. Investigation on the storage and purification of the extracted pectin as the shelf life and purity of the extracted pectin are crucial for possible pectin commercialization.
v. Further study on the manipulation of DE value via different extraction techniques to produce either LM pectin or HM pectin using acids, alkali, ammonia in alcohol, concentrated aqueous ammonia and pectin methyl esterase.

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# LIST OF PUBLICATIONS AND PAPERS PRESENTED

## Technical Papers

1. Liew, S. Q., Ngoh, G. C., Yusoff, R., & Teoh, W. H. (2016). Sequential ultrasound-microwave assisted acid extraction (UMAE) of pectin from pomelo peels. *International Journal of Biological Macromolecules*, 93, 426–435. (ISI cited publication, Tier 1 in the category of Biology and Biochemistry with impact factor of 3.671).

2. Liew, S. Q., Ngoh, G. C., Yusoff, R., & Teoh, W. H. (2018). Acid and Deep Eutectic Solvent (DES) extraction of pectin from pomelo (*Citrus grandis* (L.) Osbeck) peels. *Biocatalysis and Agricultural Biotechnology*, 13, 1–11. (Tier 2 in the category of Biotechnology with impact factor of 1.96).

3. Liew, S. Q., Teoh, W. H., Tan, C. K., Ngoh, G. C., & Yusoff, R. (2018). Subcritical water extraction of low methoxyl pectin from pomelo (*Citrus grandis* (L.) Osbeck) peels. *International Journal of Biological Macromolecules*, 116, 128–135. (ISI cited publication, Tier 1 in the category of Biology and Biochemistry with impact factor of 3.671).

## Conference Papers (Proceedings)

1. Liew, S. Q., Ngoh, G. C., Yusoff, R., & Teoh, W. H. (2017). Microwave Assisted Extraction (MAE) and Microwave-ultrasound Assisted Extraction (MUAE) of Pectin from Pomelo Peels. *International Journal of Food Engineering*, 3(2), 117–121. (ISI cited publication, Tier 4 in the category of Food Science and Technology with impact factor of 0.685). *Proceedings of the 8th International Conference on Food Engineering and Biotechnology-ICFEB 2017*, International University of Malaya-Wales, Kuala Lumpur, Malaysia, 24th–26th April.

# APPENDIX

## Appendix A: Optimization of ultrasound (UAE), microwave (MAE) and microwave-ultrasound assisted (MUAE) extraction.

This subsection includes the optimization results for the individuals UAE method, MAE method and MUAE method. The ANOVA results and optimum pectin extraction conditions for each method are also included.

**Table A1: Design matrix of BBD and pectin extraction yield obtained from UAE**

| Run | Independent variables | | | | Responses |
|---|---|---|---|---|---|
| | $x_1$ | ($X_1$) | $x_2$ | ($X_2$) | Yield (%) |
| 1 | 1 | (2.3) | -1 | (12) | 7.39 |
| 2 | 1 | (2.3) | 1 | (28) | 8.68 |
| 3 | 0 | (2.0) | 0 | (20) | 10.74 |
| 4 | -1 | (1.7) | -1 | (12) | 14.85 |
| 5 | 0 | (2.0) | 0 | (20) | 8.13 |
| 6 | 0 | (2.0) | 0 | (20) | 9.35 |
| 7 | 0 | (2.0) | 0 | (20) | 9.68 |
| 8 | 1 | (2.3) | 0 | (20) | 7.59 |
| 9 | 0 | (2.0) | 1 | (28) | 14.88 |
| 10 | -1 | (1.7) | 0 | (20) | 10.64 |
| 11 | 0 | (2.0) | 0 | (20) | 10.83 |
| 12 | 0 | (2.0) | -1 | (12) | 12.89 |
| 13 | -1 | (1.7) | 1 | (28) | 14.20 |

## Table A2: ANOVA for regression model of pectin yield obtained from UAE

| **Source** | **Sum of square** | **DF** | **Mean square** | ***F*** | ***p*** |
|---|---|---|---|---|---|
| Model | 73.40 | 5 | 14.68 | 8.22 | 0.0076 |
| $X_1$-pH | 42.83 | 1 | 42.83 | 23.99 | 0.0018 |
| $X_2$-sonication time | 1.15 | 1 | 1.15 | 0.65 | 0.4481 |
| $X_1^2$ | 6.93 | 1 | 6.93 | 3.88 | 0.0895 |
| $X_2^2$ | 28.04 | 1 | 28.04 | 15.70 | 0.0054 |
| $X_{12}$ | 0.94 | 1 | 0.94 | 0.53 | 0.4914 |
| Residual | 12.50 | 7 | 1.79 | | |
| Lack of Fit | 7.56 | 3 | 2.52 | 2.04 | 0.2505 |
| Pure Error | 4.94 | 4 | 1.23 | | |
| Cor Total | 85.90 | 12 | | | |
| R-Squared | 0.85 | | | | |
| Adj R-Squared | 0.75 | | | | |

## Table A3: Validation of optimum UAE conditions

| | **Ultrasound assisted extraction (UAE)** |
|---|---|
| Optimum conditions | pH= 1.79, sonication time= 28 min |
| Equation Models | $Y = 10.02 - 2.67X_1 + 0.44X_2 - 1.58X_1^2 + 3.19X_2^2 + 0.49X_{12}$ |
| Predicted yield (%) | 14.4 |
| Experimental yield (%) | 13.24±0.44 |
| Percentage error (%) | 8.72 |

## Table A4: Design matrix of BBD and pectin extraction yield obtained from MAE and MUAE

| | Microwave assisted extraction (MAE) | | | | | | | Microwave-ultrasound assisted extraction (MUAE) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Run | Independent variables | | | | | | Responses | Independent variables | | | | | | | | Responses |
| | $x_1$ | ($X_1$) | $x_2$ | ($X_2$) | $x_3$ | ($X_3$) | Yield (%) | $x_1$ | ($X_1$) | $x_2$ | ($X_2$) | $x_3$ | ($X_3$) | $x_4$ | ($X_4$) | Yield (%) |
| 1 | 0 | (2.0) | -1 | (350) | 1 | (12) | 14.03 | 0 | (2.0) | 1 | (12) | 1 | (650) | 0 | (20) | 23.28 |
| 2 | 1 | (2.3) | 1 | (650) | 0 | (8) | 13.83 | 1 | (2.3) | -1 | (4) | 0 | (500) | 0 | (20) | 11.90 |
| 3 | 1 | (2.3) | -1 | (350) | 0 | (8) | 10.48 | 1 | (2.3) | 0 | (8) | 0 | (500) | -1 | (12) | 13.46 |
| 4 | 1 | (2.3) | 0 | (500) | 1 | (12) | 13.39 | 0 | (2.0) | 1 | (12) | 0 | (500) | -1 | (12) | 20.96 |
| 5 | 0 | (2.0) | 1 | (650) | -1 | (4) | 19.24 | -1 | (1.7) | 0 | (8) | 0 | (500) | -1 | (12) | 24.14 |
| 6 | 0 | (2.0) | 0 | (500) | 0 | (8) | 15.67 | 1 | (2.3) | 0 | (8) | 0 | (500) | 1 | (28) | 13.02 |
| 7 | -1 | (1.7) | 0 | (500) | -1 | (4) | 21.12 | 0 | (2.0) | 0 | (8) | 0 | (500) | 0 | (20) | 21.91 |
| 8 | 0 | (2.0) | 0 | (500) | 0 | (8) | 15.11 | 0 | (2.0) | 0 | (8) | 0 | (500) | 0 | (20) | 22.05 |
| 9 | 0 | (2.0) | 0 | (500) | 0 | (8) | 13.78 | 1 | (2.3) | 1 | (12) | 0 | (500) | 0 | (20) | 14.62 |
| 10 | 0 | (2.0) | 1 | (650) | 1 | (12) | 20.22 | 0 | (2.0) | 0 | (8) | -1 | (350) | 1 | (28) | 15.17 |
| 11 | -1 | (1.7) | -1 | (350) | 0 | (8) | 14.32 | -1 | (1.7) | 0 | (8) | 1 | (650) | 0 | (20) | 30.24 |
| 12 | -1 | (1.7) | 0 | (500) | 1 | (12) | 24.78 | 0 | (2.0) | -1 | (4) | 0 | (500) | -1 | (12) | 16.65 |
| 13 | 0 | (2.0) | -1 | (350) | -1 | (4) | 11.21 | 0 | (2.0) | -1 | (4) | 0 | (500) | 1 | (28) | 10.59 |
| 14 | 1 | (2.3) | 0 | (500) | -1 | (4) | 12.93 | 1 | (2.3) | 0 | (8) | -1 | (350) | 0 | (20) | 16.65 |
| 15 | -1 | (1.7) | 1 | (650) | 0 | (8) | 29.02 | 0 | (2.0) | 0 | (8) | 0 | (500) | 0 | (20) | 22.21 |
| 16 | 0 | (2.0) | 0 | (500) | 0 | (8) | 14.95 | 0 | (2.0) | -1 | (4) | 1 | (650) | 0 | (20) | 19.45 |
| 17 | 0 | (2.0) | 0 | (500) | 0 | (8) | 15.45 | -1 | (1.7) | 0 | (8) | -1 | (350) | 0 | (20) | 20.18 |
| 18 | | | | | | | | 0 | (2.0) | 1 | (12) | -1 | (350) | 0 | (20) | 16.00 |
| 19 | | | | | | | | -1 | (1.7) | 0 | (8) | 0 | (500) | 1 | (28) | 25.26 |
| 20 | | | | | | | | 1 | (2.3) | 0 | (8) | 1 | (650) | 0 | (20) | 15.59 |
| 21 | | | | | | | | 0 | (2.0) | 0 | (8) | 1 | (650) | 1 | (28) | 21.19 |
| 22 | | | | | | | | -1 | (1.7) | -1 | (4) | 0 | (500) | 0 | (20) | 16.41 |

**Table A4, continued**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 23 | 0 | (2.0) | 1 | (12) | 0 | (500) | 1 | (28) | 19.98 |
| 24 | 0 | (2.0) | 0 | (8) | 0 | (500) | 0 | (20) | 19.33 |
| 25 | -1 | (1.7) | 1 | (12) | 0 | (500) | 0 | (20) | 23.60 |
| 26 | 0 | (2.0) | 0 | (8) | 1 | (650) | -1 | (12) | 23.00 |
| 27 | 0 | (2.0) | 0 | (8) | -1 | (350) | -1 | (12) | 13.73 |
| 28 | 0 | (2.0) | -1 | (4) | -1 | (350) | 0 | (20) | 14.91 |
| 29 | 0 | (2.0) | 0 | (8) | 0 | (500) | 0 | (20) | 21.22 |

## Table A5: ANOVA for regression model of pectin yield obtained from MAE and MUAE

| **Microwave assisted extraction (MAE)** | | | | | | **Microwave-ultrasound assisted extraction (MUAE)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source** | **SS** | **DF** | **MS** | ***F*** | ***p*** | **Source** | **SS** | **DF** | **MS** | ***F*** | ***p*** |
| Model | 382.054 | 9 | 42.45 | 73.58 | < 0.0001 | Model | 545.833 | 14 | 38.99 | 11.01 | < 0.0001 |
| $X_1$-pH | 186.342 | 1 | 186.34 | 323.00 | < 0.0001 | $X_1$-pH | 248.339 | 1 | 248.34 | 70.12 | < 0.0001 |
| $X_2$-microwave | 130.169 | 1 | 130.17 | 225.64 | < 0.0001 | $X_2$-irradiation | 67.830 | 1 | 67.83 | 19.15 | 0.0006 |
| $X_3$-irradiation time | 7.841 | 1 | 7.84 | 13.59 | 0.0078 | $X_3$-microwave | 108.661 | 1 | 108.66 | 30.68 | < 0.0001 |
| $X_1^2$ | 15.204 | 1 | 15.20 | 26.35 | 0.0013 | $X_4$-sonication | 3.774 | 1 | 3.77 | 1.07 | 0.3194 |
| $X_2^2$ | 0.002 | 1 | 0.00 | 0.00 | 0.9579 | $X_1^2$ | 4.918 | 1 | 4.92 | 1.39 | 0.2583 |
| $X_3^2$ | 5.693 | 1 | 5.69 | 9.87 | 0.0164 | $X_2^2$ | 56.861 | 1 | 56.86 | 16.06 | 0.0013 |
| $X_{12}$ | 32.206 | 1 | 32.21 | 55.83 | 0.0001 | $X_3^2$ | 0.710 | 1 | 0.71 | 0.20 | 0.6613 |
| $X_{13}$ | 2.560 | 1 | 2.56 | 4.44 | 0.0732 | $X_4^2$ | 22.459 | 1 | 22.46 | 6.34 | 0.0246 |
| $X_{23}$ | 0.846 | 1 | 0.85 | 1.47 | 0.2651 | $X_{12}$ | 4.995 | 1 | 5.00 | 1.41 | 0.2547 |
| Residual | 4.038 | 7 | 0.58 | | | $X_{13}$ | 30.914 | 1 | 30.91 | 8.73 | 0.0105 |
| Lack of Fit | 1.884 | 3 | 0.63 | 1.17 | 0.4263 | $X_{14}$ | 0.608 | 1 | 0.61 | 0.17 | 0.6848 |
| Pure Error | 2.154 | 4 | 0.54 | | | $X_{23}$ | 1.877 | 1 | 1.88 | 0.53 | 0.4786 |
| Cor Total | 386.092 | 16 | | | | $X_{24}$ | 6.452 | 1 | 6.45 | 1.82 | 0.1985 |
| $R^2$ | 0.990 | | | | | $X_{34}$ | 2.641 | 1 | 2.64 | 0.75 | 0.4024 |
| Adj $R^2$ | 0.976 | | | | | Residual | 49.583 | 14 | 3.54 | | |
| | | | | | | Lack of Fit | 43.942 | 10 | 4.39 | 3.12 | 0.1423 |
| | | | | | | Pure Error | 5.640 | 4 | 1.41 | | |
| | | | | | | Cor Total | 595.416 | 28 | | | |
| | | | | | | $R^2$ | 0.917 | | | | |
| | | | | | | Adj $R^2$ | 0.833 | | | | |

**Table A6: Validation of optimum MAE and MUAE conditions**

| | **Microwave assisted extraction (MAE)** | **Microwave-ultrasound assisted extraction (MUAE)** |
|---|---|---|
| Optimum conditions | pH= 1.74, microwave power= 649.94 W, irradiation time= 11.97 min | pH= 1.73, irradiation time= 10.11 min, microwave power= 649.90 W, sonication time= 17.72 min |
| Equation Models | $Y = 14.99 - 4.83X_1 + 4.03X_2 + 0.99X_3 + 1.90X_1^2 + 0.02X_2^2 + 1.16X_3^2 - 2.84X_{12} - 0.80X_{13} - 0.46X_{23}$ | $Y = 21.34 - 4.55X_1 + 2.38X_2 + 3.01X_3 - 0.56X_4 - 0.87X_1^2 - 2.96X_2^2 - 0.33X_3^2 - 1.86X_4^2 - 1.12X_{12} - 2.78X_{13} - 0.39X_{14} + 0.69X_{23} + 1.27X_{24} - 0.81X_{34}$ |
| Predicted yield (%) | 29.37 | 31.11 |
| Experimental yield (%) | 30.24±0.97 | 31.57±0.77 |
| Percentage error (%) | 2.88 | 1.47 |

## Appendix B: Preliminary test prior subject to subcritical water extraction (SWE).

### (a) Pressure Profile at Different Temperatures

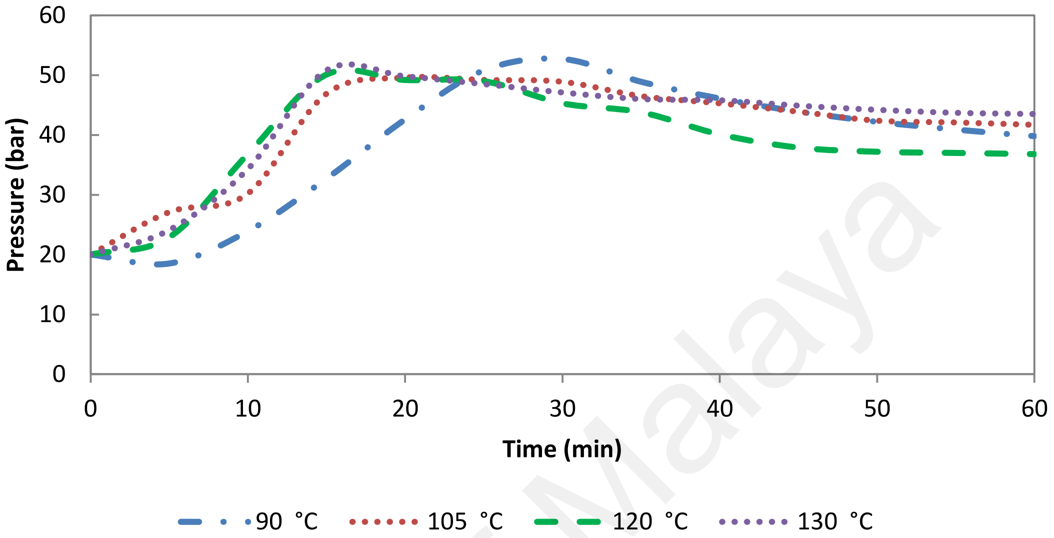

Figure B1: Pressure profile at various temperature of SWE.

## (b) Temperature and Pressure Profile

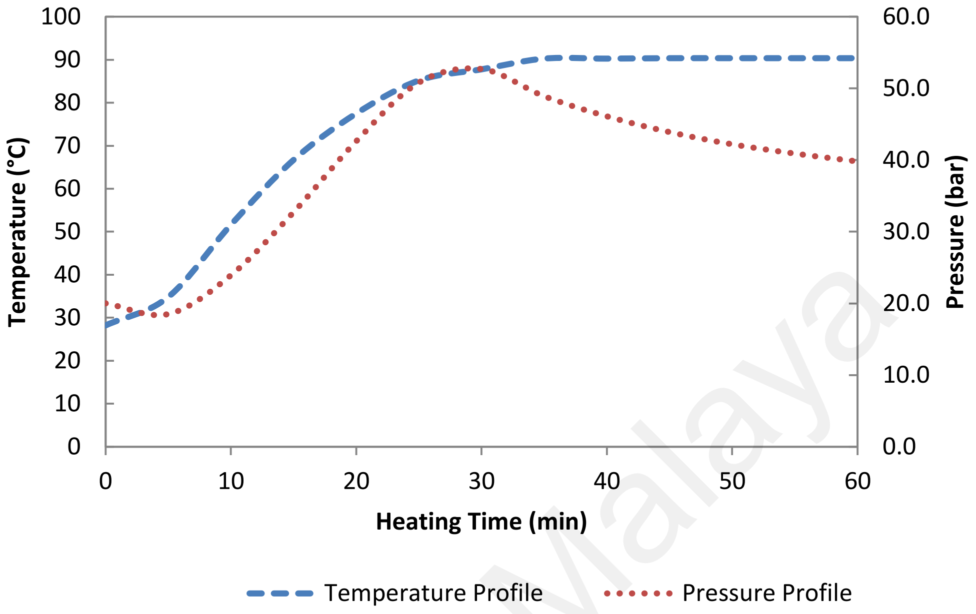

**Figure B2: Pressure and temperature profiles of subcritical water in an equilibrium vessel at 90°C.**

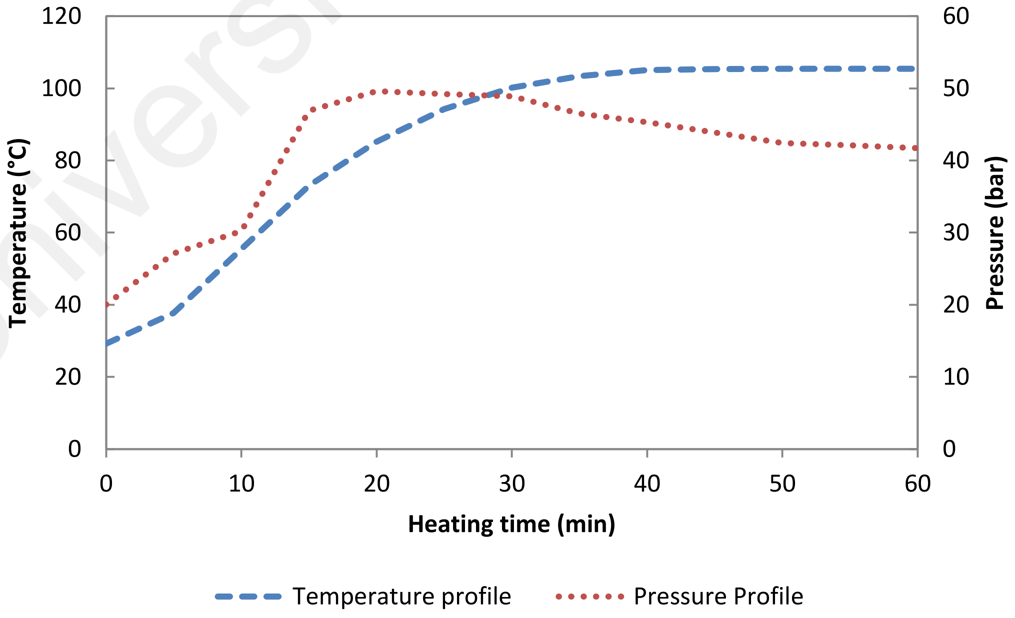

**Figure B3: Pressure and temperature profiles of subcritical water in an equilibrium vessel at 105°C.**

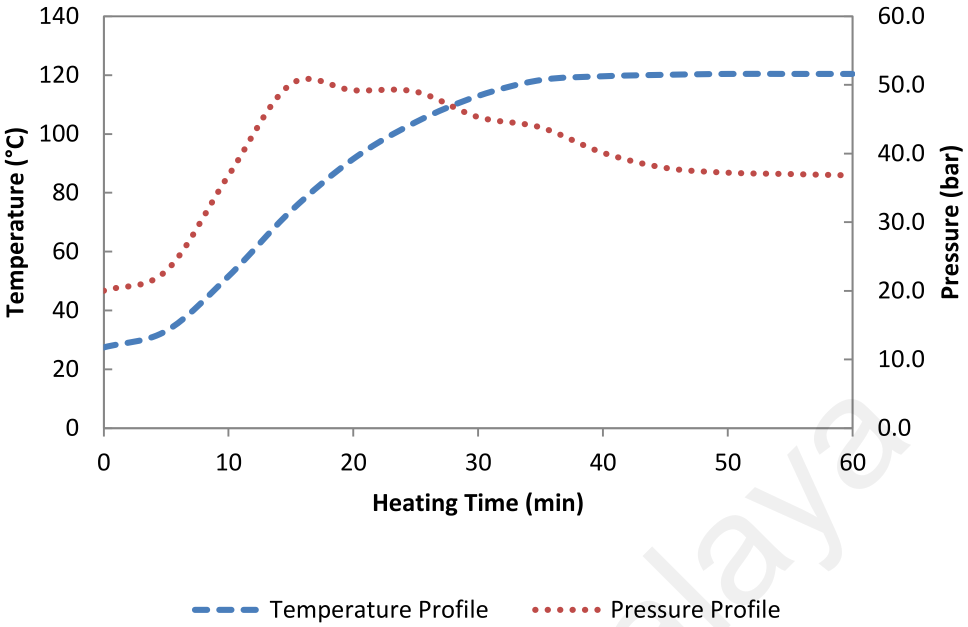

**Figure B4: Pressure and temperature profiles of subcritical water in an equilibrium vessel at 120°C.**

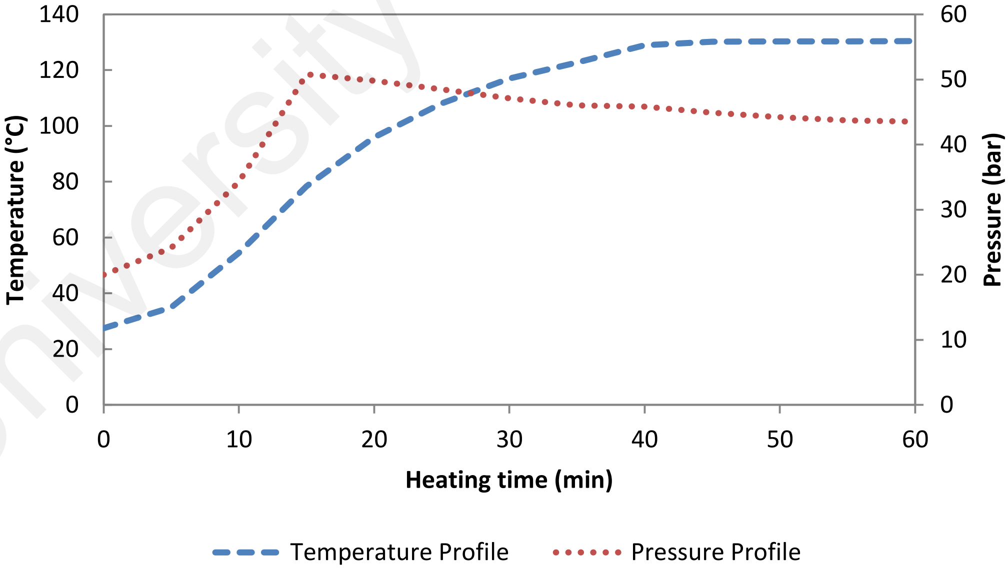

**Figure B5: Pressure and temperature profiles of subcritical water in an equilibrium vessel at 130°C.**

## (c) Instrumental Setup Schematic Diagram

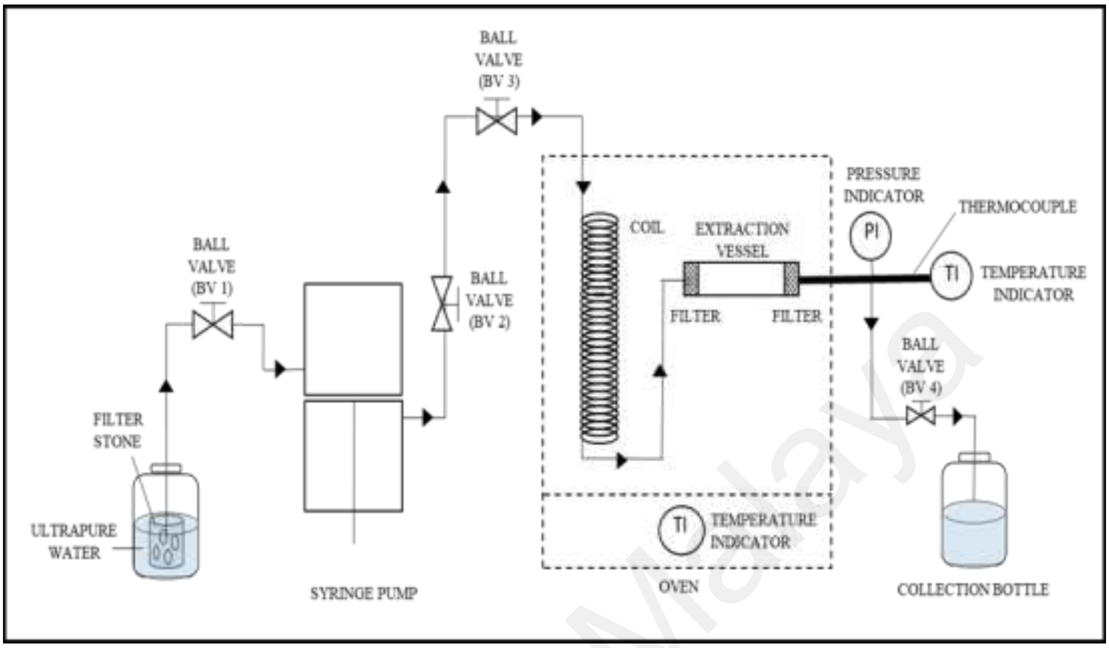

Figure B6: Schematic diagram for the determination of heating time.

### (d) Heating and Equilibration Time

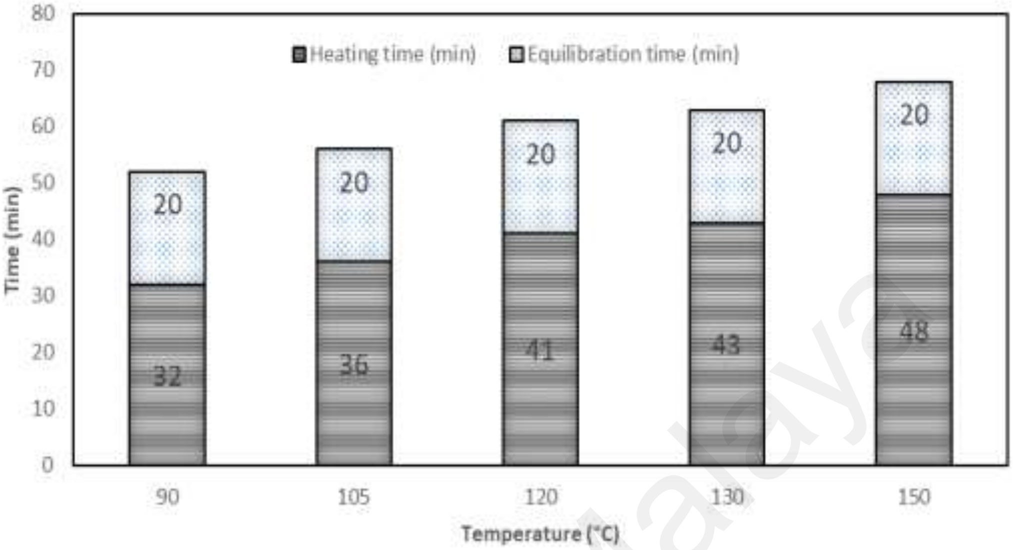

**Figure B7: Heating and equilibration time for the extraction of pectin at various temperatures.**

## Appendix C: Calibration curve generated for galacturonic acid content.

The following Figure C1 is a five-point calibration curve generated to determine the amount of galacturonic acid for the extracted pectin. The calibration curve for galacturonic acid was obtained based on the 520 nm peak absorbance with $R^2 = 0.97$.

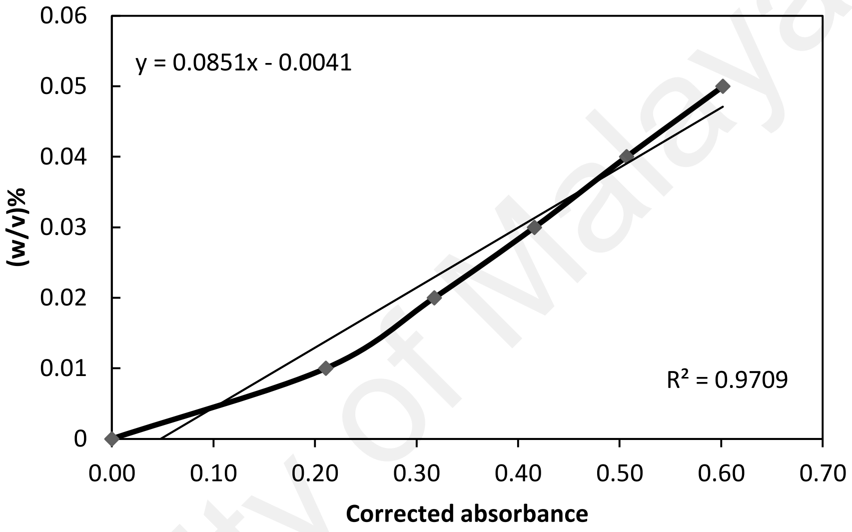

**Figure C1: Calibration curves of galacturonic acid (GalA) content.**

## Appendix D: Particle size analysis of pomelo peel powder.

An averaged fruit peel powder particle size distribution graph was plotted with particle size (µm) axis versus volume (%) as shown in Figure D1. The particle size distribution of the powder was fallen in the range between 250 – 355 µm.

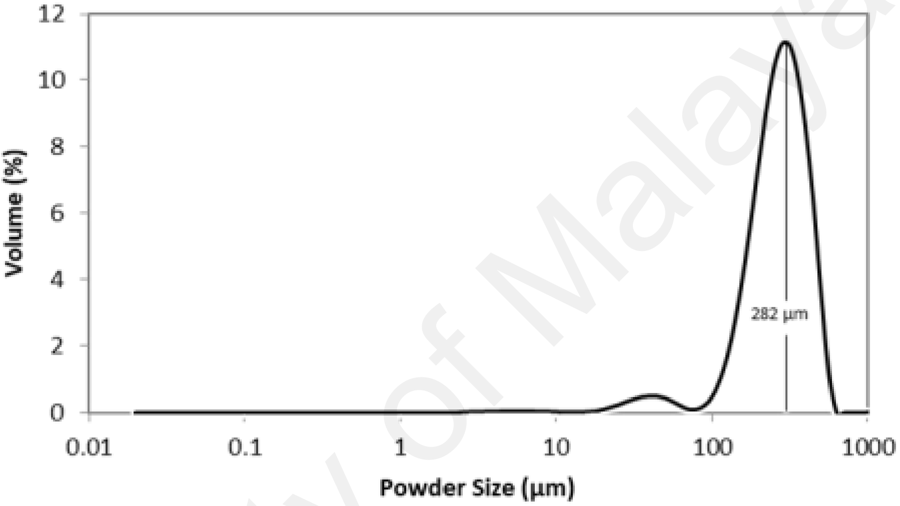

**Figure D1: Particle size distribution of pomelo peel powder.**