OPTIMIZATION AND MODELING OF MICROWAVE-ASSISTED EXTRACTION OF ACTIVE COMPOUNDS FROM COCOA LEAVES

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FACULTY OF ENGINEERING

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ABSTRACT

Microwave-assisted extraction (MAE) is a promising technique for extraction of active compounds from plants and it has the potential to be commercialized. However, due to limited significant parameters to describe the MAE process, optimization and modeling of MAE for scaling up are challenging and restricted. To resolve the problem, two intensive energy-related parameters, i.e. absorbed power density (APD) and absorbed energy density (AED) were introduced and they are respectively defined as the amount of microwave power (W/ml) and energy (J/ml) absorbed in the solvent during the extraction. Following that, three methods namely APD predictive method, AED modeling method and combined APD-AED optimization method were developed to model and optimize MAE at various extraction scales. The methods developed in this work are based on the extraction of anti diabetic compounds, i.e. isoquercitrin (0.13-3.51 mg/g), epicatechin (0.23-2.91 mg/g) and rutin (0.30-7.07 mg/g) from cocoa (*Theobroma cacao* L.) leaves. Prior to the evaluation of the developed methods, the optimization and modeling of MAE were performed conventionally using response surface methodology (RSM) and Patricelli model, respectively. The optimum MAE conditions were determined to be 85% (v/v) aqueous ethanol at 50 ml/g (2g), 156 W, and 18 min, and its performance was similar to that obtained in Soxhlet extraction but with lesser solvent (50 ml/g vs. 100 ml/g) and shorter extraction time (18 min vs. 6 hr). From the modeling study, the washing step of MAE is strongly affected by the size of sample while the diffusion step is influenced by both the solvent to feed ratio (S/F) and microwave power (P).

The findings obtained from the proposed methods suggest that the APD predictive method is able to predict the optimum extraction time for large scale MAE between

100-300 ml under various microwave power based on the correlation established between the optimum extraction time region and the APD of the extraction system. By conducting MAE at the predicted optimum extraction time region, more than 85% of equilibrium extraction yields can be achieved and the prediction is valid at solvent to feed ratio varying from 20 to 80 ml/g. Besides that, AED modeling method enables the prediction of overall extraction profiles of MAE. By adapting suitable extraction model i.e. film theory model at AED basis, a predictive model can be developed. The AED extraction model is accurate in capturing the experimental extraction profile of MAE at various microwave power (200-600 W) and solvent loading (100-300 ml) with R-square value > 0.87. In addition, APD-AED optimization method standardizes the optimization of MAE based on its extraction mechanisms. According to this method, the optimization can be performed using sequential single factor experiments based on APD and AED and the result obtained was similar to those obtained from the optimization using RSM. Most important, the intensive optimum MAE conditions (S/F = 50 ml/g, APD = 0.3 W/ml, AED = 300 J/ml) determined from this method can be used to determine the optimum operating parameters (S/F, Power, Time) of MAE at varying extraction scale (100-300 ml).

ABSTRAK

Pengekstrakan terbantu gelombang mikro (MAE) adalah satu teknik pengekstrakan tumbuhan yang bagus dan ia mempunyai potensi untuk dikomersialkan. Walau bagaimanapun, oleh kerana pengoptimuman dan pemodelan MAE untuk skala pengekstrakan yang besar adalah susah dan terhad disebabkan terhadnya parameter yang penting untuk memperihal proses MAE. Untuk menyelesaikan masalah ini, dua intensif parameter yang berkaitan dengan tenaga, iaitu ketumpatan kuasa terserap (APD) dan ketumpatan tenaga terserap (AED) telah diperkenalkan dan mereka masing-masing ditakrifkan sebagai jumlah kuasa (W/ml) dan tenaga (J/ml) gelombang mikro yang diserap dalam pelarut semasa pengekstrakan. Berikutan itu, tiga kaedah iaitu kaedah ramalan APD, kaedah pemodelan AED dan kaedah pengoptimuman APD-AED telah dibangunkan untuk pemodelan dan pengoptimuman MAE di pelbagai skala pengekstrakan. Kaedah yang dibangunkan dalam kerja ini adalah berdasarkan kepada pengekstrakan kompaun anti diabetes, iaitu isoquercitrin (0.13-3.51 mg/g), epicatechin (0.23-2.91 mg/g) dan rutin (0.30-7.07 mg/g) dari daun koko (*Theobroma cacao* L.). Sebelum penilaian kaedah tersebut, pengoptimuman dan pemodelan MAE telah dijalankan secara konvensional dengan masing-masing menggunakan kaedah gerak balas permukaan (RSM) dan model Patricelli. Keadaan optima bagi MAE adalah 85% (v/v) etanol berair pada 50 ml/g (2g), 156 W dan 18 min, dan prestasinya adalah sama dengan yang diperolehi dalam pengekstrakan Soxhlet tetapi dengan pelarut yang lebih sedikit (50 ml/g vs 100 ml/g) dan masa pengekstrakan yang lebih pendek (18 min vs 6 jam). Daripada kajian pemodelan MAE, kinetik pembasuhan MAE sangat dipengaruhi oleh saiz sampel manakala kinetik resapan MAE dipengaruhi oleh nisbah pelarut kepada sampel (S/F) dan kuasa gelombang mikro (P).

Hasil kajian yang diperolehi daripada kaedah yang dicadangkan menunjukkan bahawa kaedah ramalan APD mampu meramal masa pengekstrakan optima bagi skala besar MAE di antara 100-300 ml pada pelbagai kuasa gelombang mikro berdasarkan korelasi yang ditubuhkan antara rantau masa pengekstrakan optima dan APD daripada sistem pengekstrakan. Dengan menjalankan MAE di rantau masa pengekstrakan optima tersebut, lebih daripada 85% daripada hasil pengekstrakan dalam keseimbangan boleh dicapai dan ramalan adalah sah pada nisbah pelarut kepada sampel antara 20-80 ml/g. Disamping itu juga, kaedah pemodelan AED membolehkan ramalan profil pengekstrakan. Dengan mengadaptasikan model pengekstrakan yang sesuai, iaitu, filem teori model berdasarkan AED, sebuah model ramalan boleh dibangunkan. Model pengekstrakan AED adalah tepat dalam meramalkan profil ujikaji pengekstrakan MAE di pelbagai kuasa gelombang mikro (200-600 W) dan jumlah pelarut (100-300 ml) dengan nilai 'R-square' > 0.87. Tambahan pula, kaedah pengoptimuman APD-AED menyelaraskan pengoptimuman MAE secara keseluruhan berdasarkan mekanisme pengesktrakan tersebut. Menurut kaedah ini, pengoptimuman boleh dilakukan dengan menggunakan ujikaji berfaktor tunggal secara berturutan berdasarkan APD dan AED dan keputusan pengoptimuman yang diperolehi adalah sama dengan pengoptimuman menggunakan RSM. Yang paling penting, keadaan intensif MAE optima (S/F = 50 ml/g, APD = 0.3 J/ml, AED = 300 J/ml) yang diperolehi dari kaedah ini boleh digunakan untuk menentukan parameter pengekstrakan optima (S/F, kuasa gelombang, masa pengekstrakan) di pelbagai skala pengekstrakan (100-300 ml).

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NOTATIONS

Abbreviations

AED	absorbed energy density
APD	absorbed power density
BBD	box-behnken design
EC	epicatechin
ESI	electrospray ionization
EtOH	ethanol
HPLC	high performance liquid chromatography
HRE	heat reflux extraction
IQ	isoquercitrin
MAE	microwave-assisted extraction
ME	maceration
MS	mass spectrometry
P	nominal microwave power
RC	regenerated cellulose
RSM	response surface methodology
RT	rutin
SEM	scanning electron microscopy
S/F	solvent to feed ratio
SFE	supercritical fluid extraction
SSE	sum square error
UAE	ultrasonic-assisted extraction
UV-DAD	ultraviolet-diode array detector

Symbols

AED_{eq}	microwave energy required to reach equilibrium stage (J/ml)
AED_t	microwave energy absorbed during certain extraction time (J/ml)
APD	microwave power absorbed in solvent (W/ml)
b	coefficient of washing step for film theory
b'	coefficient of washing step for the AED model
b_1	coefficient of washing step for theory of unsteady diffusion
B_i	linear coefficient of model from RSM
B_0	intercept of model from RSM
B_{ii}	quadratic coefficient of model from RSM
B_{ij}	coefficients of the interactive effects of model from RSM
c	concentration of solute in extraction solvent at any time
c_∞	concentration of solute in extraction solvent after infinite time
C	concentration of solute in solid particle
C_i	concentration of solute at the interface of sample particle
C_0	initial concentration of solute in the sample particle
C_p	heat capacity of the extraction solvent ($Jg^{-1}k^{-1}$)
D	diffusion coefficient
D_1	diffusion coefficient during washing stage
D_2	diffusion coefficient during diffusion stage
E	electric field
f	applied frequency
f_1	fraction of solute extracted from washing stage
f_2	fraction of solute extracted from diffusion stage
H_{vap}	heat of vaporization of the extraction solvent (kJ/mol)
k	coefficient of diffusion step for film theory (min^{-1})

k'	coefficient of diffusion step for the AED model (min^{-1})
k_1	coefficient of diffusion step for theory of unsteady diffusion
k_w	coefficient of kinetic during washing step for Patricelli model (min^{-1})
k_d	coefficient of kinetic during diffusion step for Patricelli model (min^{-1})
K	constant in microwave power dissipation equation
L	half of the thickness of solid particle
m_L	initial mass of the extraction solvent (g)
m_v	mass of the vaporized solvent (g)
M	molecular weight (g/mol)
N	mass flux of solute
P_t	amount of solute extracted at extraction time t for Patricelli model (mg/g)
P_e	equilibrium extraction yields for Patricelli model (mg/g)
P_d	amount of solute extracted in diffusion step for Patricelli model (mg/g)
P_{diss}	microwave power dissipation per unit volume
P_w	amount of solute extracted in washing step for Patricelli model (mg/g)
Q	total heat absorbed in the solvent during microwave heating (J)
R	radius of particle
r	radial distance in the diffusion direction
t	extraction time (min)
$t_{80\%}$	time required to achieve 80% of equilibrium extraction yields (min)
$t_{95\%}$	time required to achieve 95% of equilibrium extraction yields (min)
t_e	estimated optimum extraction time
t_H	microwave heating time (min)
t_{opt}	optimum extraction time
V	solvent loading (ml)
x	distance in the diffusion direction

x_i	coded value of an independent variable
X_1	microwave power in the optimization study using RSM (W)
X_2	solvent to feed ratio in the optimization study using RSM (ml/g)
X_3	extraction time in the optimization study using RSM (min)
X_i	actual value of an independent variable
X_0	actual value of an independent variable at centre point
Y	the predicted extraction yield
$Y_{80\%}$	extraction yields at time $t_{80\%}$ (mg/g)
$Y_{95\%}$	extraction yields at time $t_{95\%}$ (mg/g)
Y_s	equilibrium extraction yield (mg/g)

Greek letters

δ	dissipation factor of material
ε''	dielectric loss
ε'	dielectric constant
ρ	density (g/ml)

Chapter 1

INTRODUCTION

Diabetes is one of the major epidemics nowadays as the world population with diabetes is rising each year and is expected to hit 439 million adults by 2030 (Shaw, Sicree, & Zimmet, 2010). This issue is also critical in Malaysia as it is one of the top ten countries with high prevalence of diabetes (Shaw et al., 2010). The awareness of the issue has led to the research on new medications such as natural products from plants in particular, the herbal medicine which can replace the synthetic drug to avoid causing undesirable secondary effects on patients. The active compounds extracted from plants such as quercetin derivatives and catechin compounds exhibit anti diabetic activities which can be used as alternative medicines for the prevention and treatment of diabetes (Akhlaghi & Bandy, 2010; Fang, Gao, & Zhu, 2008; Jaitak et al., 2010; Vessal, Hemmati, & Vasei, 2003). These flavonoids compounds are highly concentrated in the leaves of plants (Sultana & Anwar, 2008) and currently, researchers are sourcing for this bioresource and effective techniques to recover these valuable compounds.

Solvent extraction forms the primary step to extract valuable active compounds from plants. Among the extraction techniques available for plant extraction, one of the most promising techniques is microwave-assisted extraction (MAE) (Mandal, Mohan, & Hemalatha, 2007). Microwave is an electromagnetic wave which can penetrate into certain material to provide volumetric heating through ionic conduction and dipole rotation (Sparr Eskilsson & Björklund, 2000). With the assistance of microwave in the extraction system, the process can be enhanced in terms of yields, extraction time and solvent consumption (Chen, Xie, & Gong, 2007; Zhu, Su, Cai, & Yang, 2006). MAE

has drawn significant research attention as it is potential to replace conventional techniques due to its special heating mechanism, moderate capital cost, operability under atmospheric conditions and moreover it is suitable to extract polar active compounds (Howard, 1995; Li, Li, & Zhang, 2003; Sparr Eskilsson & Björklund, 2000). Despite that the employment of MAE in plant extractions has becoming popular in recent years, many issues pertaining to the optimization and scaling up of the extraction process remain unsolved. For instances, there is no standardized optimization strategy for MAE in plant extractions. Besides that, the optimum extraction condition of MAE is difficult to be reproduced at larger scales and at different microwave setup. Furthermore, scaling up of MAE process is impeded by the lack of understanding of the interactive effects of operating parameters and also due to unsatisfactory modeling performance of the existing MAE kinetic models. These challenging issues should be resolved first before MAE technique for plant extraction can be commercialized.

1.1. Problem statement

Cocoa (*Theobroma cacao* L.) plants are cultivated globally such as in Malaysia for the production of cocoa powders and chocolates. The leaves of the plant are normally disposed of during pruning without further processing to recover their bioactive compounds and this resulted in great wastage. Up to date, only flavonols compounds such as epicatechin have been identified in the leave via conventional extraction technique (Osman & Lam, 2005; Osman, Nasarudin, & Lee, 2004) and this has prompted the exploration of other anti diabetic flavonoids compounds in the leaves by using non conventional extraction technique in this study.

There are various extraction techniques can be employed for the recovery of active compounds from plants. MAE is widely adopted for the extraction of flavonoids compounds from plants. The operating parameters such as solvent to feed ratio, microwave irradiation power and extraction time are crucial in MAE and they are often optimized in plant extraction. However, the optimum extraction conditions in literature are applicable only for specific microwave systems which limit its application. This suggests that different instrumental setup of microwave system would give different extraction performances if applied with the same operating condition. In other words, the reported optimum extraction conditions can only be used as reference for new extraction using similar type of extractor or to reproduce the extraction at different microwave system. Besides, MAE is hardly applied at larger scale. These operational issues could be alleviated by optimizing more significant MAE parameter. The parameters which can be considered are energy related such as energy density, which can be defined as the microwave irradiation power for a given unit of extraction volume. This parameter is more applicable and significant as compared to microwave power level in the optimization of MAE (Alfaro, Belanger, Padilla, & Pare, 2003; Li et al., 2012). Nevertheless, the irradiation power for the microwave heating (power density) does not reflect the actual power absorbed in the extraction system. The energy absorbed in the extraction system, which depends on dielectric constant of the system (Mandal et al., 2007), is crucial as it provides localized heating to disrupt the cells and elute the active compounds (Sparr Eskilsson & Björklund, 2000). Thus, the absorbed microwave power is important to be investigated for MAE system.

Modeling of MAE is essential for the prediction of extraction behavior and for the scaling up purpose. Various extraction models of MAE, e.g. derivation from Fick's law (Gujar, Wagh, & Gaikar, 2010), chemical kinetic equations (Spigno & De Faveri, 2009;

Xiao, Song, Wang, & Li, 2012) and other empirical models (Amarni & Kadi, 2010) have been developed but the predictive capability of such models is restricted to the extraction constants obtained from a specific instrumental setup. Consequently, the employment of such models to predict MAE profile (extraction yield vs. extraction time) using different instrumental setup would result in lack of fit. Moreover, the extraction constants were constrained to certain operating conditions as the acquisition of experimental data for determining the constants of extraction models is time-consuming. Currently, the application of these kinetic models in the MAE system is scarce as the reported extraction constants, e.g. diffusivity, can only be used to indicate the kinetic of the extraction for comparison purpose such as at different operating conditions and extraction techniques (Amarni & Kadi, 2010; Gujar et al., 2010). As mentioned previously, the microwave energy that is absorbed in the extraction system determines the outcome of the MAE, the kinetic models of MAE should therefore include this parameter for more realistic modeling results.

1.2. Objectives

Based on the research problems previously described, the objectives of this study are:

i. To identify and quantify anti-diabetic compounds in cocoa leaves and to optimize the MAE process.
ii. To study the effects of various operating parameters on the kinetic of MAE.
iii. To study the feasibility of the absorbed microwave power as a new operating parameter for MAE.
iv. To develop MAE kinetic model with incorporation of the absorbed microwave energy during extraction.
v. To standardize MAE optimization procedure for plant extraction using the absorbed microwave power and energy.

1.3. Scope of study

In this study, the subject plant is cocoa leaves and the targeted active compounds are the natural occurring flavonoids compounds which are important for prevention and treatment of diabetes. The extraction of these compounds from the leaves were conducted in a closed vessel microwave system (closed MAE). As the amount of active compounds in the leaves is generally influenced by various factors such as geographical locations, weather and soil conditions, this research only investigates the effects of operating conditions to reach equilibrium extraction and their optimum values without considering the effects of different batches of plant sample taken from plantations as the latter only affects the magnitude of the extraction yields. Other than determination of the optimum extraction conditions and kinetic data of the MAE of anti diabetic compounds from cocoa leaves, this study also develops new techniques for optimizing and modeling of MAE in plant extractions. The scopes of this study are:

i. To identify and quantify anti diabetic compounds in cocoa leaves extract.
ii. To determine optimum extraction conditions of MAE.
iii. To investigate the extraction kinetics under the effects of the proposed MAE parameters namely the absorbed microwave power and other parameters such as particle size of sample, solvent to feed ratio, microwave irradiation power and solvent loading.
iv. To develop a predictive method based on absorbed microwave power for determining the suitable extraction conditions for MAE at different solvent loading.
v. To develop a MAE modeling method that incorporates absorbed microwave energy.
vi. To develop a MAE optimization procedure based on absorbed microwave power and energy for plant extraction.

1.4. Structure of dissertation

This thesis has 6 chapters and the content of each chapters are described as follows:

Chapter 1 introduces the background and objectives of this research.

Chapter 2 discusses the potential of plant extracts including cocoa leaves as alternative treatments for diabetes patients. The chapter also reviews the extraction techniques employed in plant extraction especially the MAE technique. Topics covered in MAE include the development of the technique in plant extraction, influencing parameters of MAE, instrumentation setup, advantages and disadvantages relative to other extraction techniques.

Chapter 3 presents the theory, mathematical modeling of MAE, the development of the proposed predictive method and modeling method as mentioned in the scope of study.

Chapter 4 describes the research methodology for the optimization and modeling of MAE.

Chapter 5 encompasses the result and discussion on the optimization of main anti diabetic compounds in cocoa leaves. The comparison of MAE with conventional Soxhlet extraction was also presented. Besides that, the kinetics of MAE under the influence of various parameters including the absorbed microwave power were studied. In addition, the performance of the predictive method and the modeling method were evaluated accordingly. Also, the optimization of the MAE using absorbed microwave power was performed and the feasibility of the procedure was evaluated.

Chapter 6 concludes all the findings of this research and recommends new research area for future studies. The chapter also highlights the novelty contributed by this study.

Chapter 2

LITERATURE REVIEW

This chapter describes the potential applications and therapeutic values of plant extracts; in particular related active compounds for the prevention and treatment of diabetes. Botanical information of the subject plant, i.e. cocoa leaves is reviewed. Various conventional and non conventional extraction techniques are employed for plant extraction. These techniques are reviewed with special emphasis on the MAE technique. The literature review of the MAE techniques includes its advancement, important operating conditions and its performance in comparison with other extraction techniques.

2.1. Plant extracts for diabetes treatment

Plants extraction has been intensively investigated due to the natural occurring therapeutic compounds presence in plants. These active compounds give medicinal effects as the conventional medicines, and provide additional features such as antioxidative effect to protect cell tissues and can be consumed as supplement for daily diet. In diabetes treatment, most of the extracted anti diabetic compounds are flavonoids. Table 2.1 compiles some of the anti diabetic compounds from plants that had been identified and evaluated in medicinal research. Anti diabetic compounds generally can alter glucose metabolism and assist in treating diabetes and its complications.

Table 2.1: Anti diabetic active compounds extracted from plants

Active ingredients	Plants (part)	Therapeutic function	References
anthocyanins	*Vitis vinifera* (fruits)	inhibitory effect for lens opacity	Morimitsu et al., 2002
catechin	*Cassia fistula* (bark), green tea (leaves), *Theobroma cacao* (leaves)	exhibit hypoglycaemic, glucose oxidizing and insulin mimetic activities	Daisy, Balasubramanian, Rajalakshmi, Eliza, & Selvaraj, 2010; Kamiyama et al., 2010; Osman et al., 2004
chlorogenic acid	*Cecropia pachystachya* (leaves), *cecropia obtusifolia* (leaves)	possess hypoglycaemic effect	Aragao et al., 2010; Toledo, Tellez, Sortibran, Andrade-Cetto, & Rodriguez-Arnaiz, 2008
coumarin	*Hemionitis arifolia* (whole plants), *Clausena anisata* (roots)	exhibit hypoglycaemic effect though stimulation of pancreatic β-cells and subsequent secreation of insulin	Ajikumaran Nair, Shylesh, Gopakumar, & Subramoniam, 2006; Ojewole, 2002
dieckol	*Ecklonia cava* (whole plants)	potential inhibitor for α-glucosidase and α-amylase	Lee et al., 2010
gensenoside	*Panax ginseng* (roots)	enhance glucose uptake, anti hyperglycaemic and anti obese activities by improving insulin and leptin sensitivity	Lee et al., 2010; Yang et al., 2010
kaempferol	*Gynura procumbens* (leaves), *Euonymus alatus* (leaves), *Equisetum myriohchaetum* (aerial parts)	promote hypoglycaemic effect	Andrade Cetto, Wiedenfeld, Revilla, & Sergio, 2000; Fang, Gao, & Zhu, 2008; Rosidah et al., 2009
isoorientin	*Gentiana olivieri* (leaves), *Cecropia pachystachya* (leaves), *Cecropia obtusifolia* (aerial parts)	exhibit hypolgycaemic and anti hyperglycaemic acitivity	Aragao et al., 2010; Sezik, Aslan, Yesilada, & Ito, 2005; Toledo et al., 2008
myricetin	*Abelmoschus moschatus* (aerial part)	lower plasma glucose level	Liu, Liou, Lan, Hsu, & Cheng, 2005
quercetin	*Euonymus alatus* (leaves), *Kalanchoe pinnata* (leaves), *Eucommia ulmoides* (leaves)	exhibit hypoglycaemic effect through stimulation of insulin for glucose uptake, regeneration of the pancreatic islets and prevent against dementia associated with vascular and neurodegenerative disorders.	Cruz et al., 2008; Fang, Gao, & Zhu, 2008

2.2. Cocoa (*Theobroma cacao* L.) leaves

Theobroma cacao is also known as cacao tree or cocoa tree, is a small evergreen tree with the height ranging from 4-8 m tall under the family of Sterculiaceae. Its leaves are alternate, entire, unlobed, 10–40 cm long and 5–20 cm broad as shown in Fig. 2.1. Cocoa plant is native to the tropical region of the Americas (Motamayor et al., 2002). It was cultivated globally for the production of cocoa powder and chocolate. These perennial crops give a total cocoa production of 4,311,000 tonnes globally (ICCO, 2012). In Malaysia, the production of grinded cocoa powder achieved 323,653 tonnes in 2008, is among the largest cocoa grinders in the world and ranked fourth in Asia (Applanaidu et al., 2009). Due to mass plantations of cocoa plants worldwide, the leaves, which are normally disposed of during pruning, could be a good source for the recovery of valuable therapeutic active compounds as leaves are generally the favorable storage site for anti diabetic active compounds (Chan, Yusoff, & Ngoh, 2012). The cocoa leaves extract contains catechin-polyphenols, i.e. (-)-epicatechin, epigallocatechin gallate, epigallocatechin and etc (Osman & Lam, 2005; Osman et al., 2004).

Fig. 2.1: Leaves of cocoa (*Theobroma cacao*) plant

2.3. Solvent extraction

Solvent extraction is one of the oldest techniques in plant extraction for the recovering of valuable active compounds from plant matrices. The conventional and non-conventional solvent extraction techniques are described in the following sections.

2.3.1. Conventional extraction techniques

The traditional extraction techniques employed in plant extraction are soaking and maceration. These extraction techniques can be easily carried out by selecting suitable solvent such as ethanol, hexane and acetone. In cases where the presence of chemical solvent is undesired, simple technique such as decoction in water can be used and it is broadly employed in the traditional Chinese medicine practices (Das et al., 2010; Khan et al., 2009; Meddah et al., 2009). The improved maceration technique applies heat and agitation to enhance the external mass transfer mechanism. Examples are percolation using alcohol, acetone, petroleum ether or hexane in the isolation of natural products (Badole & Bodhankar, 2009; Bamuamba, Gammon, Meyers, Dijoux-Franca, & Scott, 2008; Cunha et al., 2008; Nsonde Ntandou et al., 2010; Pandikumar, Babu, & Ignacimuthu, 2009). All these techniques are traditionally employed and they can not serve as a standard method. The most popular and routinely applied extraction technique in analytical research is the Soxhlet extraction (Soxhlet, 1879). The efficiency of Soxhlet extraction is associated with the changing of transfer equilibrium in which the fresh solvent is repeatedly brought into contact with the solid sample due to reflux (Luque de Castro & García-Ayuso, 1998). Up to date, Soxhlet extraction is widely used to extract active compounds from plants (Kurian & Paddikkala, 2010; Lakshmi & Sudhakar, 2010) and it remains as a standard technique for comparison and evaluation of the non-conventional extraction techniques.

2.3.2. Non-conventional extraction techniques

Various non-conventional extraction techniques such as pressurized solvent extraction (PSE), supercritical fluid extraction (SFE), ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) have been employed in plant extractions and some of these techniques have been commercialized. These extraction techniques are more efficient than the conventional techniques in terms of extraction time, solvent consumption and extraction performance. Among the non-conventional techniques, pressurized solvent extraction is normally used for extracting thermally stable active compounds as this technique often operates at elevated temperature (50-200 ^{o}C) and pressure (10-15 MPa) (Kaufmann, Christen, & Veuthey, 2001; Wang & Weller, 2006). The technique allows the extraction solvent stays in liquid form at elevated temperature and forces it into the sample matrix thus it enhances the extraction kinetics of the extraction (Wang & Weller, 2006).

Similar to pressurized solvent extraction, supercritical fluid extraction (SFE) operates at elevated temperature and pressure but at the supercritical state of solvent. In plant extraction, supercritical CO_2 is usually employed as extraction solvent due to its low critical point (31 ^{o}C, 7.3 MPa), non-flammable and non-toxicity. SFE is superior due to high and adjustable dissolving power of supercritical fluid which is able to fractionate the extract and selectively extract the targeted compounds (Wang & Weller, 2006). The limitation is that the technique is only suitable to extract non-polar compounds, e.g. essential oil due to the properties of the solvent used (Wang & Weller, 2006). Nevertheless, appropriate polar modifier can be added in the supercritical fluid to boost the extraction performance for extracting polar compounds such as flavonoids (Hamburger, Baumann, & Adler, 2004).

Other than the use of heat and agitation in solvent extraction, ultrasonic radiation also can be used to enhance the extraction performance. Ultrasonic-assisted extraction (UAE) is usually employed to enhance the extraction of hardly extracted compounds from plant sample. When ultrasound generates the growth of bubbles inside liquids, the cavitation phenomenon occurs where the cavitation bubbles implode asymmetrically near a solid surface to provide stirring and thermal effects for the extraction solvent, and structural effects on solid sample (Leighton, 1998). In some application, UAE does not impose any significant enhancement on the extraction as reported in the extraction of oil from woad (*Isatis tinetoria*) seeds (Mircea, 2001; Romdhane & Gourdon, 2002). This could be due to irregular shape of plant sample that had weakened the reception of ultrasound and hence resulted in poor performance (Cárcel, García-Pérez, Mulet, Rodríguez, & Riera, 2010).

Furthermore, solvent extraction can also be improved by incorporating microwave heating into the system. Unlike conventional heating (convective heating), the localized heating of microwave radiation improves the heating performance of the extraction system and resulted in fast extraction. The details information pertaining to the theory, development, influencing factors, advantages and disadvantages of MAE are discussed hereafter.

2.4. Microwave-assisted extraction (MAE)

Microwave is an electromagnetic wave. It consists of electric field and magnetic field which oscillates perpendicularly to each others in frequency ranged from 0.3 to 300 GHz. Microwave systems used in domestic and industrial applications usually operate at 2.45 GHz and 915 MHz. Microwave can penetrate into certain materials and interacts with the polar components to generate heat. The heating of microwave energy acts

directly on the molecules by ionic conduction and dipole rotation (Sparr Eskilsson & Björklund, 2000) and thus only selective and targeted materials can be heated based on their dielectric constant. The efficiency of the microwave heating depends on the dissipation factor of the material, or called as dielectric loss tangent (tan δ), which measures the ability of the sample to absorb microwave energy and dissipates heat to the surrounding molecules as shown by Eq. (1) (Mandal et al., 2007),

$$\tan\delta = \varepsilon'' / \varepsilon' \quad (1)$$

where ε'' is the dielectric loss which indicates the efficiency of converting microwave energy into heat while ε' is the dielectric constant which measures the ability of the material to absorb microwave energy. The rate of conversion of electrical energy into thermal energy in the material is expressed by Eq. (2) (Chen, Siochi, Ward, & McGrath, 1993),

$$P_{diss} = Kf\varepsilon' E^2 \tan\delta \quad (2)$$

Where P_{diss} is the microwave power dissipation per unit volume, K is a constant, f is the applied frequency, ε' is the material's absolute dielectric constant and E is the electric field strength.

Microwave heating has been adopted in solvent extraction to replace conventional heating due to its good heating performance and bulk heating characteristic. MAE provides homogenous heating for the extraction solvent and plant matrix. MAE is able to rupture plant cells due to the internal superheating when the water inside the cell absorbs microwave. As a result, the rupture cell facilitates the dissolution of active compounds in the solvent (Kaufmann et al., 2001). The technique of MAE has been continuously improved and its development especially in the plant-based extraction is discussed subsequently.

2.5. The development of MAE techniques

MAE systems can be generalized into multi-mode system and focused-mode system (mono-mode). Multi-mode system allows random dispersion of microwave radiation in cavity by a mode stirrer while focused system (mono-mode) allows focused microwave radiation to be on a restricted zone in the cavity. Usually, the multi-mode system is associated with high pressure while the mono-mode system is applied under atmospheric operating pressure. However, mono-mode system can also run at high pressure. In practice, 'Closed System' and 'Open System' are used to refer to the system that operates above atmospheric pressure and under atmospheric pressure, respectively as illustrated in Fig. 2.2 (Dean & Xiong, 2000; Luque-García & Luque de Castro, 2003).

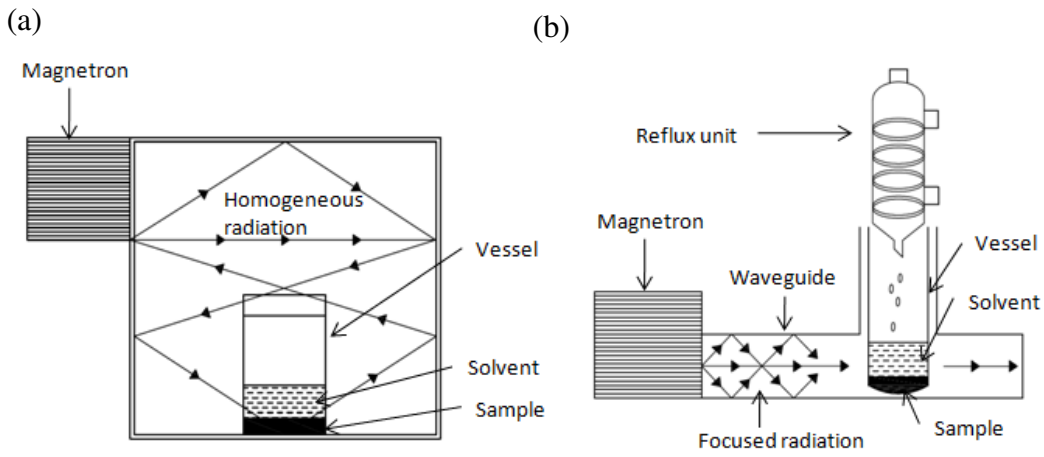


Fig. 2.2: (a) Closed type microwave system and (b) Open type microwave system (adapted from Mandal et al., 2007)

In a closed MAE system, the extractions are carried out in a sealed-vessel with different mode of microwave radiations and under uniform microwave heating. High working pressure and temperature of the system allow fast and efficient extraction. The pressure inside the extraction vessel is controlled in such a way that it would not exceed the working pressure of the vessel while the temperature can be regulated above the normal boiling point of the extraction solvent. Recent advancements in the closed system have

led to the development of high pressure microwave-assisted extraction (HPMAE). The increase in temperature and pressure accelerates microwave-assisted extraction due to the ability of extraction solvent to absorb microwave energy (Wang et al., 2008). Despite the fact that the closed system offers fast and efficient extraction with less solvent consumption, it is susceptible to losses of volatile compounds with limited sample throughput.

Open system is developed to overcome the drawbacks of closed system such as the safety issues and it is considered more suitable for extracting thermolabile compounds. This system has higher sample throughput and more solvent can be added to the system at anytime during the process. Basically, open system operates at milder conditions and it is widely used in the extraction of active compounds and is also used in analytical chemistry. This system operates at atmospheric conditions and only part of the vessel is directly exposed to the propagation of microwave radiation (mono-mode). The upper part of the vessel is connected to a reflux unit to condense any vaporized solvent. Besides that, multi-mode radiation can also be employed in open MAE system with the reflux unit.

The performance of MAE either in a closed or open system can be further enhanced by introducing some modification on the extraction system. For instance, inert gas such as nitrogen can be added in a closed system to prevent oxidation of active compounds during extraction (Casazza, Aliakbarian, Mantegna, Cravotto, & Perego, 2010; Yu et al., 2009). Similar effect can also be achieved by conducting the MAE at vacuum conditions (Pasquet et al., 2011; Xiao, Wang, Wang, Wang, & Li, 2009). Besides that, ultrasonic wave can be incorporated in an MAE system to intensify the mass transfer mechanism (Chen et al., 2010).

2.6. Extraction kinetics of MAE

A typical extraction curve of MAE comprises of two steps, i.e. fast extraction step (washing step) and slow extraction step (diffusion step) as shown in Fig. 2.3 (Franco, Sineiro, Pinelo, & Núñez, 2007; Perez, Carelli, & Crapiste, 2011; So & Macdonald, 1986). The extraction mechanism starts when the solvent molecules penetrate into the plant matrix causes the layer of cytoplasm to be exposed directly to the solvent (Crossley & Aguilera, 2001) which facilitates the dissolution of active compounds in the solvent. At the beginning of the extraction process, the fast extraction step corresponds to a constant rate of extraction (Rakotondramasy-Rabesiaka, Havet, Porte, & Fauduet, 2009). At exceedingly fast rate, the period associated in the extraction step is difficult to be determined (Franco, Sineiro, et al., 2007). During the slow extraction step, active compounds diffuse from the interior of the plants matrices and dissolve in the solvent. The extraction yield during this step is greatly depended on the intact cells that remained after the washing step of the extraction (Crossley & Aguilera, 2001). Conclusively, the characteristics of washing and diffusion steps in the extraction are determined by the proportion of broken and intact cells after sample preparation, e.g. grinding, (So & Macdonald, 1986).

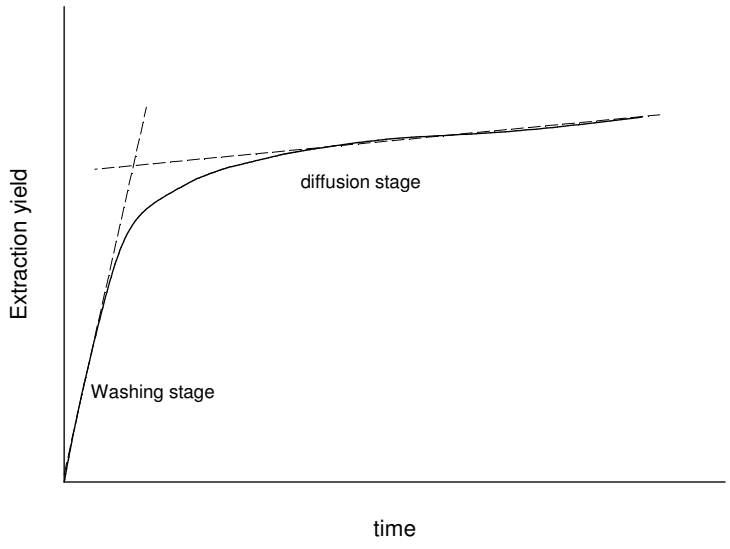


Fig. 2.3: Typical extraction curve of batch type solvent extraction of active compounds from plants

2.7. Factors influencing the performance of MAE

The efficiency of MAE strongly relies on the selected operating conditions and the parameters that affecting the extraction kinetics and the yield. This section discusses the effects of extraction parameters and their interactions on the performance of MAE.

2.7.1. Type of solvent

Extraction solvent and its concentration play important roles in the extraction of active compounds from plants. Different extraction solvents possess different ability to overcome the energy barrier – the activation energy of extraction (Rakotondramasy-Rabesiaka, Havet, Porte, & Fauduet, 2007; Spigno & De Faveri, 2009), to penetrate into the interior of the plant cells. Suitable extraction solvent can enhance the washing step and shorten the extraction time (Rakotondramasy-Rabesiaka et al., 2007). Also, it can improve the diffusivity of solute in the solvent and subsequently maximize the equilibrium extraction yield (Xu, Huang, & He, 2008).

The selection criteria of suitable solvent in MAE extraction process encompasses the solubility of the target analyte, solvent's penetration, the interaction between the solvent with the sample matrix and the solvent's dielectric constant. The solvent for MAE can not be selected based on its performance in the conventional extraction methods as the solvents that work well in conventional techniques might not be suitable for MAE. For example, diethyl ether that has been used extensively in solubilizing steroids from *Saxifragaceae* family is not suitable as MAE solvent (Lu, Yue, Zhang, Li, & Wang, 2007). In general, organic solvents such as ethanol, methanol, acetone, etc are found to be effective in MAE. In particular the ethanol, which is a good microwave absorber suitable for extracting various active compounds from plants (Zhou & Liu, 2006). Aqueous solution of certain organic solvent is often employed as the presence of water

would improve the penetration of solvent into sample matrix and thus enhance heating efficiency (Alfaro et al., 2003). As summary, modifier such as water can be added to the solvent to enhance the extraction performance.

2.7.2. Solvent to feed ratio

Solvent to feed ratio or the ratio of solvent to plant sample is an important parameter which if applied correctly can decrease the mass transfer barrier during the diffusion of active compounds to enhance the extraction yield (Hao, Han, Huang, Xue, & Deng, 2002; Wang et al., 2009; Zhang & Liu, 2008; Zheng et al., 2009). Excessive solvent causes poor microwave heating as the microwave radiation would be completely absorbed by the solvent and additional power is required to ensure complete extraction (Mandal & Mandal, 2010). On the other hand, low amount of solvent in solid builds the mass transfer barrier as the distribution of active compounds is concentrated in certain regions which limit the movement of the compounds out of cell matrix (Mandal & Mandal, 2010). However, extraction which is carried out at low solvent to feed ratio tends to reach equilibrium stage much faster than those carried out at high solvent to feed ratio due to lower equilibrium yield (Stanisavljević, Lazić, & Veljković, 2007). So, an optimum ratio of solvent to solid ratio ensures homogeneous and effective heating. Furthermore, it is worthy to note that the initial extraction rate during washing period is not significantly affected by the solvent to feed ratio (Herodež, Hadolin, Škerget, & Knez, 2003). As a summary, the ratio of solvent to solid depends on the solvent nature which is related to its ability to provide microwave heating to the sample as well as the mobility of extracted compounds in the solvent itself.

2.7.3. Microwave power

Microwave power is critical in MAE as it controls the rate of heating during extraction. While providing localized heating for the extraction system, the microwave power acts as driving force for MAE as it can destroy the plant matrix and allows the active compounds to diffuse out and dissolve in the solvent. In general, increasing the power improves the extraction yield and shortens the extraction time (Chemat, Ait-Amar, Lagha, & Esveld, 2005; Mandal & Mandal, 2010; W. Xiao, Han, & Shi, 2008). The extraction yield also increases proportionally with increasing microwave power up to a limit before the increase becomes insignificant or decline (Chemat et al., 2005; Kwon, Belanger, Pare, & Yaylayan, 2003; Mandal & Mandal, 2010; Xiao et al., 2008). In the stability study of flavonoids during microwave radiation (Biesaga, 2011), increase the microwave power amplifies the degradation as higher microwave heating causes sudden rise in temperature. The elevation in temperature leads to overheating and undesired solvent evaporation resulted in poor yields especially for thermal sensitive extract. As reported in the MAE of flavonoids from *Radix astragali* roots (Xiao et al., 2008), high microwave output of 1000 W decreased the extraction yield if the extraction temperature was higher than 110 ^{o}C due to instability of the flavonoids at those temperatures.

2.7.4. Extraction temperature

Temperature and microwave power are interrelated as high microwave power can elevate the temperature of the extraction system. Increasing the temperature of the solvent causes its solvation power to increase due to a drop in viscosity and surface tension (Mandal et al., 2007). The desired extraction temperature depends on the stability and extraction yield of the desired active compound. In the extraction of phenolic compound from Oolong tea, that phenolic content of the extract increases with

extraction temperature and it decreases when the extraction temperature is increased further beyond its optimum point (Tsubaki, Sakamoto, & Azuma, 2010). Thus, the control of extraction temperature is crucial to maintain the stability and to achieve high extract yield for desired active compound.

2.7.5. Extraction time

The extraction time of MAE controls the exposure of microwave radiation in the extraction system. MAE conducted at optimum extraction time would ensure complete extraction. When extraction time is below its optimum point, incomplete extraction occurs. Similarly, over expose to microwave radiation even though at low temperature or low operating power decreases the extraction yield due to loss of chemical structure of the active compounds (Hao et al., 2002; Wang et al., 2009). To avoid the risk of thermal degradation and oxidation, the extraction time of MAE usually varies from few minutes up to half an hour. If longer extraction time is required, the risk of thermal degradation can be reduced through extraction cycle by feeding fresh solvent to the residue and repeating the extraction step to ensure the completion of the extraction (Chen et al., 2007).

2.7.6. Plant matrix characteristic

Besides the operating parameter discussed previously, the characteristics of the plant sample also have effects on the performance of MAE. The extraction sample is usually dried and powdered prior to the extraction. According to the extraction kinetics, smaller particle sizes increase the diffusivity and enhance the mass transfer mechanism in diffusion step. This creates large surface contact area with the solvent and shorter average diffusion path of active compounds from the solid to the surrounding solvent (Cissé et al., 2012; Herodež et al., 2003; Hojnik, Skerget, & Knez, 2008). As a result,

shorter extraction time is required. In practice, too fine a particle size of sample is not favorable as it would cause difficulty in separating the extract from the residue and incurs additional clean up steps. Nevertheless, the particle size does not affect the initial extraction rate in washing step providing the internal diffusion of active compounds is rate limiting (Herodež et al., 2003). Despite of particle size has effect on extraction process, it depends on the geometry of the extraction sample. For leaves sample that exhibits plate geometry, the effect of the size is not dominant in comparison with the thickness of the leaves as the latter is the relevant dimension for the diffusion of active compounds (Wongkittipong, Prat, Damronglerd, & Gourdon, 2004). The effect of particle size might turn significant only when the particle size of the leaves sample is reduced to below its thickness such as in powder form.

2.7.7. Stirring effect

Generally, stirring affects the mass transfer process. By introducing stirring in MAE, the problem associated with the low solvent to feed ratio on extraction yield can be reduced. The mass transfer barrier created by the concentrated active compounds in a localized region due to insufficient solvent can be minimized. In other words, agitation accelerates the extraction by enhancing the desorption and dissolution of active compounds bound to the sample matrix to give a better extraction yield (Ruan & Li, 2007).

2.8. Comparison between MAE and other extraction techniques

In the extraction of active compounds from plant, MAE is a good and reliable method and it is more efficient as compared to other conventional extraction methods such as Soxhlet extraction (Soxhlet), heat reflux extraction (HRE), ultrasonic-assisted extraction (UAE), maceration (ME) and etc (Dean & Xiong, 2000; Luque-Garcia & de Castro, 2004; Sanchez-Prado, Garcia-Jares, & Llompart, 2010; Sparr Eskilsson & Björklund, 2000). General comparison between MAE and other techniques are shown in Table 2.2 and the comparison on their extraction performance is tabulated in Table 2.3.

Table 2.2: General comparison of various extraction techniques

	Extraction techniques		
	Soxhlet	MAE	SFE
Features	Soxhlet apparatus	microwave heating	supercritical fluid as extraction solvent
Cost	low	medium	high
Effect on extraction kinetic	continuous extraction with fresh solvent changes the transfer equilibrium and enhance the mass transfer	localized heating of microwave builds internal pressure to rupture plant cells and elute the active compounds	high dissolving and penetration power of supercritical solvent
Advantages	high reproducibility and does not require clean up procedure	low solvent consumption, short extraction time, high stability and reproducibility	adjustable solvent power, high selectivity, able to perform fractionation
Drawbacks and limitations	high solvent consumption and long extraction time	low selectivity, additional clean up step is required, poor performance when extracting non polar compounds	expensive setup, require high expertise , poor performance when extracting polar compounds
Applications	standard method in plant extraction	extraction of flavonoids compounds from plants	extraction of essential oil compounds from plants

Table 2.3: Comparison on the performance between MAE and other techniques

References	Extraction	Method	Yield
Chen et al., 2007	*Ganoderma atrum* (triterpenoid saponins)	MAE: 95% ethanol, 25 ml/g, 800 W, 5 min (10 min total), 2 extraction cycles, 78 °C	5.11% [a]
		SFE: CO_2 + ethanol, 30 l/hr (80g sample), 25 MPa, 55 °C	1.52% [a]
		HRE: 95% ethanol, 25 ml/g, 1 hr, 95 °C	2.22% [a]
		UAE: 95% ethanol, 25 ml/g, 15 min, room temperature, ultrasonic bath	1.72% [a]
Yan et al., 2010	*Radix astragali* roots (4 astragalosides (AG))	MAE: 80% ethanol, 25 ml/g, 700 W, 5 min (15 min total), 3 extraction cycles, 70 °C	AG I: 0.788 mg/g AG II: 0.351 mg/g AG III: 0.206 mg/g AG IV: 0.278 mg/g [b]
		Soxhlet: 80% ethanol, 20ml/g, 4 hr, 90 °C	AG I: 0.770 mg/g AG II: 0.347 mg/g AG III: 0.193 mg/g AG IV: 0.242 mg/g [b]
		HRE: 80% ethanol, 20 ml/g, 1 hr, 90 °C	AG I: 0.761 mg/g AG II: 0.352 mg/g AG III: 0.203 mg/g AG IV: 0.257 mg/g [b]
		UAE: 80% ethanol, 20 ml/g, 40 min, ultrasonic bath	AG I: 0.519 mg/g AG II: 0.302 mg/g AG III: 0.19 mg/g AG IV: 0.225 mg/g [b]
		ME: 80% ethanol, 20 ml/g, 12 hr	AG I: 0.411 mg/g AG II: 0.299 mg/g AG III: 0.166 mg/g AG IV: 0.206 mg/g [b]
Li et al., 2010	defatted residue of yellow horn (*triterpene saponins*)	MAE: 40% ethanol, 30 ml/g, 900 W, 7 min, 3 extraction cycles, 50 °C	11.62% [a]
		UAE: 40% ethanol, 30 ml/g, 60 min, 3 extraction cycle, 50 °C, ultrasonic bath	6.78% [a]
		HRE: 40% ethanol, 30 ml/g, 90 min, 3 extraction cycles, 50 °C	10.82% [a]
Zhang, Chen, Xiao, & Yao, 2005	*Macleaya cordata* (Willd) R. Br. Fruits (sanguinarine and chelerythrine)	MAE: 0.1 M HCl, 100 ml/g, 280 W, 5 min	17.10 mg/g (sanguinarine) 7.09 mg/g (chelerythrine) [b]
		UAE: 0.1 M HCl, 100 ml/g, 30 min, ultrasonic bath	10.74 mg/g (sanguinarine) 5.61 mg/g (chelerythrine) [b]
		ME: 0.1 M HCl, 100 ml/g, 30 min, 100 °C	16.87 mg/g (sanguinarine) 7.31 mg/g (chelerythrine) [b]

[a] yield (%) = mass of extracted active compound x 100 / mass of sample; [b] yield (mg/g) = mass of extracted compound / mass of sample . HRE: heat reflux extraction; ME: maceration.

MAE is more efficient as compared to other conventional extraction methods as shown in Table 2.3. Its advantages include high extraction yields, shorter extraction time and low solvent consumption. High efficiency of MAE is attributed to the uniqueness of microwave heating and its interaction with the extraction system which enhances the mass transfer. From the economic aspect, it is feasible as the cost of equipment setup is moderate than the other non conventional extraction methods such as SFE. Moreover, MAE has low risks and no major safety issues as most extractions are generally carried out under atmospheric condition. Besides, MAE is able to rupture the plant cell and accelerate the dissolution of active compounds in the solvent.

The distinct advantages of MAE further confirm its reliability in extraction with high stability and reproducibility (Du, Xiao, Xu, & Li, 2010; ElKhori, Pare, Belanger, & Perez, 2007; Li et al., 2009; Liazid, Guerrero, Cantos, Palma, & Barroso, 2011; Romarís-Hortas, Moreda-Piñeiro, & Bermejo-Barrera, 2009; Sterbová, Matejícek, Vlcek, & Kubán, 2004). In addition, MAE is able to preserve the therapeutic value of the extracted active compounds (Yang et al., 2010). In conclusion, MAE is suitable to be used in analytical chemistry where precision and repeatability of analytical result are valued most

Nonetheless, there are some drawbacks and limitations associated with MAE. MAE incurs additional clean up steps and that might lose the active compounds during the procedure. Besides, non polar solvent, e.g. hexane which is a poor absorbent for microwave radiation, is not suitable to be used in MAE. In other circumstances, application of non polar solvent is essential in MAE as the solubility of the targeted extract is higher as compared to polar solvents. The contradicting role of non polar solvent makes the selection of solvents for MAE difficult. However, many polarity associated problems can be overcome by adding modifiers into non polar solvents to

enhance the microwave absorbing capacity of the solvent (Alfaro et al., 2003; ElKhori et al., 2007). Pretreatment with a polar solvent prior to extraction would also improve the situation (Mandal, Mohan, & Hemalatha, 2008). The disadvantage of MAE is associated with its low selectivity as it is heavily dependent on the solvent nature and the extraction temperature. From the comparison of MAE with SFE, the latter offers higher selectivity than MAE. SFE can also fractionate the extract during extraction process by regulating operating conditions. Unlike MAE, SFE requires expensive setup and severe operating conditions, and is more favorable for extracting non polar compounds (Zougagh, Valcárcel, & Ríos, 2004).

Despite the disadvantages associated with MAE, its advantages are more apparent. The technique is excellent in terms of its extraction efficiency, technique stability and reproducibility and also the ability to retain the functional values of extracted active compounds.

Chapter 3

MATHEMATICAL MODELING

Modeling of MAE process can predict the extraction behaviors under the influences of operating parameters and thus it is useful for scaling up purpose. Modeling of the process can be performed using kinetic model from batch solvent extractions, either analytical equations or empirical models. In general, two extraction steps are involved in MAE; the washing of active compounds from the plant matrix (fast extraction) followed by the diffusion of the compounds from intact plant cells (slow extraction). The fundamental approach to model MAE is based on derivation of Fick's law. Other mathematical approaches such as modified Fick's law and other two-parametric empirical models are also widely adopted. Once the mathematical model is constructed, the parameters of the models can be obtained by fitting with the experimental data. In addition to that, this chapter describes the theory and development of two proposed methods which are useful to predict optimum extraction time and overall extraction curves of MAE, respectively.

3.1. Fick's law

The diffusion step in batch type extraction depends on two extraction mechanisms, i.e. internal diffusion and external diffusion. The internal diffusion of active compounds is driven by the concentration gradient within the solid matrix and the bulk solvent as explained in Fick's law (Coulson, Richardson, Backhurst, & Harker, 1999).

$$N = -D\frac{dC}{dx} \tag{3}$$

where, N is the mass flux of solute, C is the concentration of solute in the solid particle, D is the diffusivity or diffusion coefficient for solute in the solvent, and x is the distance in the direction of the transfer. For external diffusion, the active compounds diffuse from the external surface of solid to the bulk liquid. The determination of rate limiting mechanism in the diffusion step is crucial in kinetic modeling as it determines the suitable mathematical approach for modeling the extraction. To ensure efficient extraction, the external mass transfer resistance has to be minimized so that the rate of extraction is dependent on the internal diffusion of active compounds. Diffusivity in Fick's law (Eq.3) indicates the rate of mass transfer and it is useful for equipment design (Perez et al., 2011). Hence, most of the kinetic modeling in literature engaged in the investigation of the diffusivity or other mass transfer coefficient in solvent extraction.

MAE can be modeled via derivation of Fick's law with their initial and boundary conditions. The mass transfer problem can be solved analytically or numerically depending on the complexity of the equations involved. Several basic assumptions (Crank, 1975) can be used to simplify the mass transfer problem are:

1. Symmetrical and porous sample particles. The geometry of solid particles is assumed to be spherical with radius of R or thin plate with half thickness of L.
2. The solid particle is assumed to be pseudo-homogeneous medium. The concentration of the active compounds in solid particle depends on time and radius, r or thickness, x.
3. Uniform distribution of active compounds in sample matrix.
4. Homogeneous mixing between solvent and plant sample particles. The concentration of solute in the solvent is time dependent.

5. The mass transfer of active compounds from solid is a diffusion phenomenon in which the diffusion coefficient is independent of time.
6. Diffusion of the solute and other compounds are in parallel and no interaction between them.
7. External mass transfer resistance is negligible. The concentration of solute in solvent at the interior of solid particle is equal to the concentration of solute in the bulk solvent.

One of the assumptions of the mass transfer is to treat the external mass transfer resistance as negligible, which is crucial and it depends on the nature of the extraction. This assumption simplifies most of the extraction problems. Considering only the mass balance in spherical solid particle as shown, the kinetic model can be developed as follows:

$$\frac{\partial C}{\partial t} = \nabla(-D\nabla C) \tag{4}$$

where, t is the extraction time. Considering solely the spherical geometry of particles with radius r, the respective initial and boundary conditions can be written as follows:

$$t = 0, \quad C = C_0 \qquad \forall r \tag{5}$$

$$t > 0, \quad C = C_i = 0 \qquad r = R \tag{6}$$

$$t > 0, \quad \frac{\partial C}{\partial r} = 0 \qquad r = 0 \tag{7}$$

where, C_0 is the initial concentration of solute in the sample particle, C_i is the concentration of solute at the interface of sample particle. By assuming negligible external mass transfer resistance, the concentration at the particle interface will become zero as described in Eq. (6). The solution of this ordinary differential equation (ODE)

for both the plate and spherical geometry of sample was respectively given in Eq. (8) and Eq. (9) by Crank (1975).

For spherical:

$$\frac{C-C_0}{C_i-C}=1+\left[\frac{2R}{\pi r}\sum_{n=1}^{\infty}\frac{(-1)^n}{n}\sin\frac{\pi nr}{R}\times\exp\left\{-\frac{Dn^2\pi^2t}{R^2}\right\}\right] \tag{8}$$

For plate:

$$\frac{C-C_0}{C_i-C}=1-\left[\frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\cos\frac{(2n+1)\pi x}{2L}\exp\left\{-\frac{(2n+1)^2\pi^2Dt}{4L^2}\right\}\right] \tag{9}$$

After a short period of time or usually after washing step, only the first term of the series remain significant (Spiro, 1988). The simplified forms are expressed as follows:

For spherical:

$$\ln\left(\frac{c_\infty}{c_\infty-c}\right)=0.498+\frac{9.87Dt}{R^2} \tag{10}$$

For plate:

$$\ln\left(\frac{c_\infty}{c_\infty-c}\right)=0.21+\frac{9.87Dt}{4L^2} \tag{11}$$

where, c_∞ is the concentration of solute in extraction solvent after infinite time. By plotting Eq. (10) or Eq. (11) using experimental extraction curve, two intersecting straight lines can be drawn where the slope of the first line is steeper than the second. The intersection between the lines is the transition point which denotes the point where the extraction changes its phase from washing step to diffusion step (Kandiah & Spiro, 1990; Spiro, Kandiah, & Price, 1989). For better modeling result, Osburn and Katz (1944) suggested to include in the model both the washing and the diffusion steps in parallel, using Eq. (10) or Eq. (11):

For spherical:

$$\frac{c_\infty - c}{c_\infty} = \frac{6}{\pi^2}\left[f_1 \exp\left\{ -\frac{\pi^2 D_1 t}{R^2} \right\} + f_2 \exp\left\{ -\frac{\pi^2 D_2 t}{R^2} \right\} \right] \tag{12}$$

For plate:

$$\frac{c_\infty - c}{c_\infty} = \frac{8}{\pi^2}\left[f_1 \exp\left\{ -\frac{\pi^2 D_1 t}{4L^2} \right\} + f_2 \exp\left\{ -\frac{\pi^2 D_2 t}{4L^2} \right\} \right] \tag{13}$$

where, f_1 and f_2 are fraction of solute extracted from washing and diffusion step with diffusion coefficient of D_1 and D_2, respectively. Both parameters D_2 and f_2 can be determined from the slope and the intersection points of Eq. (10) or Eq. (11). This is because the second exponential term is significant for the second step of the extraction. In early step of extraction, the second exponential term is close to unity thus D_1 and f_1 can be determined.

The models developed based on mass balance in the solid particle can be theoretically obtained by solving the mass transfer in the solid particles. These models are derived fundamentally and they are suitable to be used in scaling up study and equipment design as the parameters involved in the equations are appropriate and theoretically meaningful. For the ease of modeling, models from derivations of Fick's law can be further modified and simplified.

3.2. Modified Fick's law

For more simplified models, film theory and unsteady state diffusion through plant material (Velickovic, Milenovic, Ristic, & Veljkovic, 2006) can be adopted to describe the washing step and diffusion step in the extraction process. These two-parametric

equations are derived from Fick's law and can be expressed in Eq. (14) and (15) respectively:

Film theory:

$$\frac{c}{c_\infty} = 1-(1-b)e^{-kt} \tag{14}$$

Theory of unsteady diffusion through plant material:

$$\frac{C}{C_0} = (1-b_1)e^{-k_1 t} \tag{15}$$

where, b and b_1 denote the coefficients for extraction kinetics in washing step while k and k_1 are the coefficients for diffusion step. To express Eq. (15) on the basis of amount of solute extracted in the extraction solvent, the equation can be modified into Eq. (16).

$$\frac{C_0 - C}{C_0} = (1-b_1)e^{-k_1 t} \tag{16}$$

where, (C_0-C) denotes the amount of solute dissolved in the extraction solvent. The equations based on Fick's law are commonly used in the modeling of solvent extraction in accordance with the fundamental theory for mass transfer. In this study, a new approach based on absorbed microwave energy of the system will be developed from this modified Fick's law model.

3.3. Empirical equations

There are various empirical models based on the kinetic models in Eq. (12) and Eq. (13). The most commonly used empirical models was the model proposed by So and Mcdonald (1986) and Patricelli, Assogna, Casalaina, Emmi and Sodini (1979) in the form as shown in Eq. (17).

$$P_t = P_w\left[1-\exp(-k_w t)\right] + P_d\left[1-\exp(-k_d t)\right] \tag{17}$$

where, P_w and P_d is the amount of solute extracted in the solvent during washing step and diffusion step, respectively. The amount of solute extracted can be expressed per mass of sample used, or expressed in fraction by comparing with equilibrium yield, whereas k_w and k_d are the coefficient of extraction kinetics during washing step and diffusion step, respectively. This empirical equation resembles the model previously shown in Eq. (12) and Eq. (13).

Having elucidated on the mathematical approaches to model MAE process in plant extraction, the following sections will discuss about the development of two proposed methods namely absorbed power density (APD) prediction method and absorbed energy density (AED) modeling method.

3.4. Absorbed power density (APD) prediction method

This method is developed in this study to predict optimum extraction time of MAE based on the absorbed microwave power in the extraction system. The absorbed microwave power is denoted by absorbed power density (APD) in this work. APD is defined as microwave power absorbed per unit volume of solvent (W/ml). Unlike nominal microwave power which indicates the power setting of microwave setup, APD represents the actual microwave power being absorbed in the extraction solvent. A simple procedure to calculate APD experimentally is presented in section 4.4.5.

Hypothetically, the absorbed power provides localized heating to disrupt the cells and elute the active compounds for extraction (Sparr Eskilsson & Björklund, 2000). The amount of energy absorbed in the system, indicated by its temperature profile, was utilized to achieve a certain degree of extraction. Fig. 3.1 shows the extraction curve of MAE with real time temperature profile. This figure was obtained experimentally in this

work and the total extraction yields of isoquercitrin, rutin and (-)-epicatechin is used as a response. Fig. 3.1 indicates that the extraction curve of MAE would exhibit similar characteristics to temperature profile of the solvent during the extraction. Thus, the extraction curve of MAE can be characterized based on the temperature profile, which in turn depends on the amount of power absorbed in the extraction system. Subsequently, the optimum extraction time can be determined based on the absorbed microwave power in the extraction system.

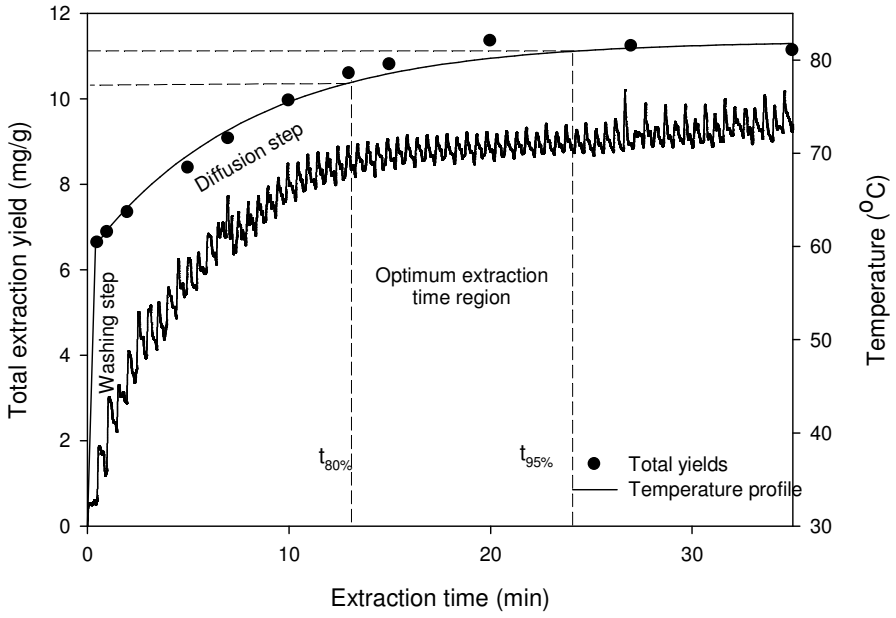


Fig. 3.1: Optimum extraction time region of MAE with real time temperature profile (*MAE conditions: 2 g sample, 100 W, and 100 ml of 85% (v/v) EtOH)*

In this proposed method, the correlation of optimum extraction time region and APD of the extraction system was constructed based on the extraction curves of MAE at various microwave irradiation powers (e.g. 100-600 W). The detail of the experimental can be found in section 4.4.5. From the extraction curve, the optimum extraction time region is defined in this study as the extraction time of MAE that requires to extract 80-95% of total extraction yields during diffusion step in prolonged extraction as shown in Fig. 3.1. Beyond the optimum extraction region, improvement in the yields will not be

substantial and large amount of solvent will be vaporized due to overheating. The extraction times within the optimum region are denoted as $t_{80\%}$ and $t_{95\%}$ respectively. By constructing the correlation, optimum extraction time of MAE at various extraction conditions can be predicted based on their corresponding APD value. The procedure to predict optimum extraction time of MAE based on APD was illustrated in Fig. 3.2.

Procedure of APD predictive method

1	Determine the APD values of MAE at various microwave powers
2	Construct extraction curves of MAE at the investigated range of microwave powers to find the optimum extraction time region i.e. $t_{80\%}$ and $t_{95\%}$.
3	Relate the optimum extraction time region ($t_{80\%}$ and $t_{95\%}$) with their corresponding APD value to form the correlation $t_{80\%} = f(APD)$ $t_{95\%} = f(APD)$
4	Apply the correlation to determine suitable or optimum extraction time of MAE at various extraction conditions based on their respective APD values. Prediction at various conditions: E.g. solvent loading and microwave irradiation power

Fig. 3.2: Proposed procedure of APD prediction method

3.5. Absorbed energy density (AED) modeling method

In this modeling method, the amount of microwave energy absorbed in the extraction solvent forms the basis for modeling of MAE process. As hypothesized, as more microwave energy being absorbed by the extraction system, more active compounds are extracted until it reaches the equilibrium stage. The absorbed energy can be used to indicate the progress of MAE. Taking into account the effect of solvent loading on the absorption of microwave energy, absorbed energy density (AED) is being introduced in this work as the total amount of microwave energy absorbed per unit volume of solvent

used during MAE. Depending on the extraction conditions, AED values of extraction solvent during MAE process can be determined by APD using Eq. (18).

$$AED_t = APD \times t \tag{18}$$

where AED_t is the total amount of microwave energy absorbed per volume of solvent loading during the extraction (J/ml) and t is the extraction time (min).

Fig. 3.3, which was obtained experimentally, depicts the extraction profile of MAE with respect to AED of the extraction system. The plotted extraction profile of MAE in AED basis would exhibit similar trends as those observed in typical dynamic extraction profiles (yield vs time), where two distinct phases are observed, i.e. washing and diffusion steps. Since the prediction of the washing step is challenging as the period of constant extraction rate can hardly be determined (Franco, Sineiro, et al., 2007), only the diffusion step is considered in this work. This is also substantiated by the fact that the equilibrium extraction yield is relatively more significant than the yields associated with the washing step.

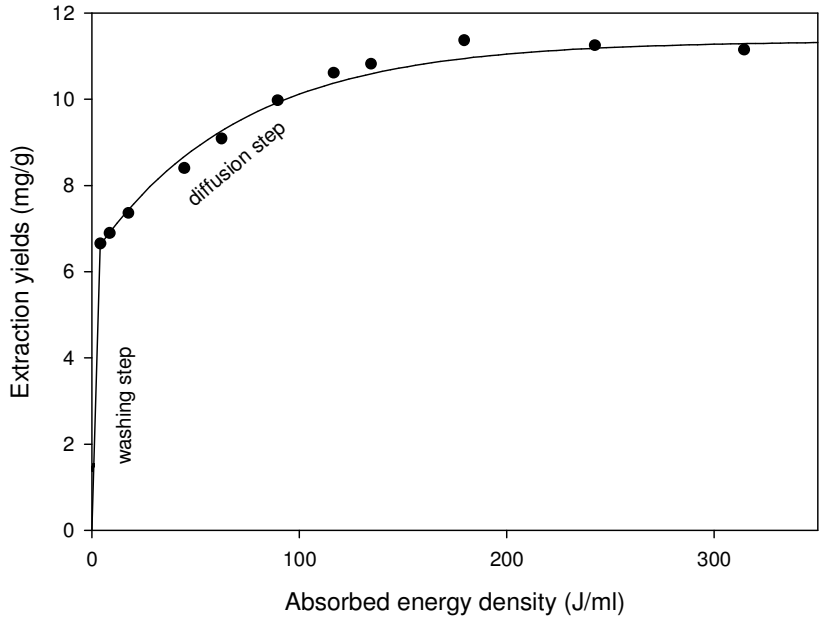


Fig. 3.3: Extraction profile of MAE with respect to AED
(*MAE condition: 2 g sample, 100 W, 100 ml of 85% ETOH, 0.5-35 min, APD of 0.15 W/ml*)

In this modeling method, film theory as in Eq. (14) was adapted using the basis of AED_t. Unlike extraction time, AED_t not only indicates the progress of an extraction but it also gives additional information on the microwave power absorbed in the extraction system. The original film theory and the adapted model are shown in Eq. (19) and Eq. (20).

Original film theory:

$$\frac{Y}{Y_s} = 1 - (1 - b)\exp(-k \cdot t) \tag{19}$$

Adaptation of film theory using AED:

$$\frac{Y}{Y_s} = 1 - (1 - b')\exp(-k' \cdot AED_t) \tag{20}$$

where Y is the extraction yield (mg/g), Y_s is the equilibrium extraction yield (mg/g), b characterizes the washing step, k characterizes the diffusion step (min^{-1}), b' characterizes the washing step in absorbed energy basis and k' characterizes the diffusion step in absorbed energy basis ($(J/ml)^{-1}$).

The washing and diffusion constants (b' and k') are independent of the operating parameters that influence the absorption of microwave energy such as solvent loading, applied microwave power and etc., as the effects of these operating parameters are exerted on the AED_t value due to APD value. The constants are affected by other operating parameters such as solvent to feed ratio, particle sizes and etc., which are not related to the absorption of microwave energy. Once the constant b' and k' are obtained for a particular operating condition, Eq. (20) can be further applied to predict the dynamic extraction profile (yields vs. extraction time) under various applied microwave power and solvent loading. This can be done by substituting Eq. (18) in Eq. (20) to convert the adapted model to extraction time basis as shown in Eq. (21).

$$\frac{Y}{Y_s} = 1 - (1 - b')\exp(-k' \cdot APD \cdot t) \tag{21}$$

Eq. (21) can be used to predict the extraction profiles of MAE (at extraction time basis) at various operating conditions by substituting the APD value at the respective conditions. Comparing the coefficients of the proposed model in Eq. (21) with the original film theory in Eq. (19), the washing coefficients for both models are the same, $b = b'$ while the diffusion coefficients of the original film theory equation, $k = k'$ x APD. The procedure of AED modeling method is presented in Fig. 3.4.

Procedure of AED modeling method

1	Select suitable extraction model $Y = f(t)$
2	Adapt the selected model using AED as the basis $Y = f(AED)$
3	Determine the model parameters by fitting with the extraction profile using AED basis Operating parameters that are dependent on the model parameters E.g. solvent to feed ratio, particle size, extraction solvent.
4	Convert the formulated model to extraction time basis $Y = f(APD, t)$
5	Model the extraction curve by substituting the APD value at specific operating conditions Predict extraction profile of MAE at various conditions: E.g. solvent loading, microwave irradiation power
6	Determine suitable extraction time of MAE based on the modeling result

Fig. 3.4: Proposed procedure for modeling of MAE based on AED

Chapter 4

RESEARCH METHDOLOGY

This chapter describes the methodologies for optimization and modeling of MAE of active compounds from cocoa leaves. The overall flow of the research methodology of this study is presented and ensued by detail descriptions on each experimental procedures and research designs.

4.1. Materials and reagents

The chemicals used for the extractions, mobile phase of HPLC and standard compounds with their sources and supplies are summarized in Table 4.1.

Table 4.1: Chemicals used for experimental studies

Chemicals	Sources and supply
acetonitrile (chromatography grade)	Merck co. (Germany)
ethanol (chromatography grade)	Merck co. (Germany)
denatured alchohol (EtOH)	LGC Scientific co. (Malaysia)
ultrapure water	Milli-Q ultra filtration system
(-)-epicatechin, purity ≥ 98% (HPLC)	Sigma-Aldrich Co. (USA)
(-)-epicatechin gallate, purity ≥ 98% (HPLC)	Sigma-Aldrich Co. (USA)
(-)-epigallocatechin, purity ≥ 95% (HPLC)	Sigma-Aldrich Co. (USA)
(-)-epigallocatechin gallate, purity ≥ 95% (HPLC)	Sigma-Aldrich Co. (USA)
kaempferol, purity ≥ 90% (HPLC)	Sigma-Aldrich Co. (USA)
myricetin, purity ≥ 96% (HPLC)	Sigma-Aldrich Co. (USA)
quercetin, purity ≥ 95% (HPLC)	Sigma-Aldrich Co. (USA)
quercetin-3-β-glucoside, purity ≥ 90% (HPLC)	Sigma-Aldrich Co. (USA)
rutin hydrate, purity ≥ 94% (HPLC)	Sigma-Aldrich Co. (USA)

4.2. Research flow chart

The flow of the overall research methodology with their respective objectives and interrelationship is presented in Fig. 4.1.

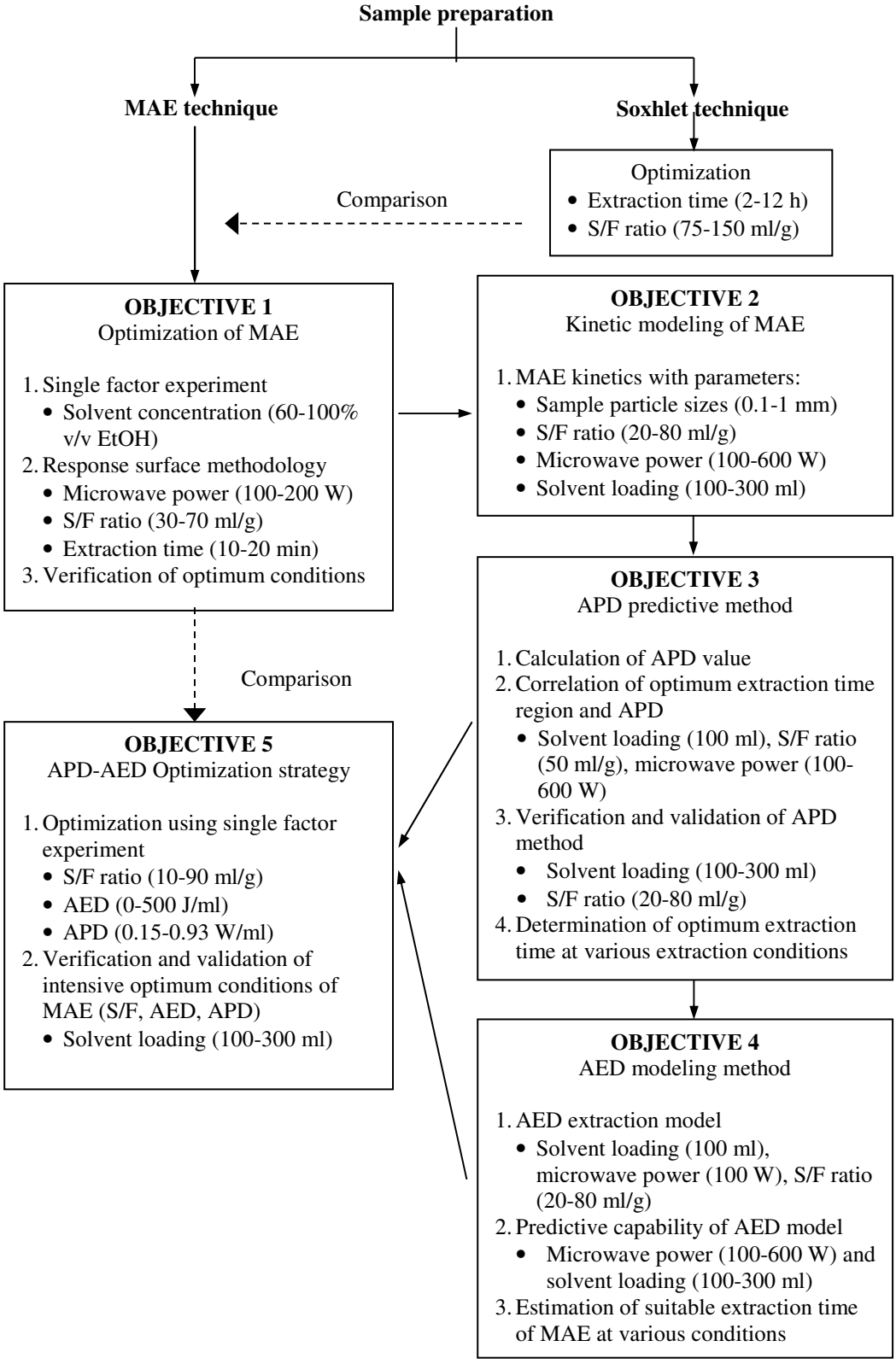


Fig. 4.1: Flow chart of overall research methodology

4.3. Sample preparation

Fresh cocoa leaves were collected during pruning from the local cocoa plantation in Jengka, Pahang, Malaysia (Malaysian Cocoa Board). The collected leaves were washed and then dried in a forced convection oven at 40 ^{o}C for 24 hours. The moisture content of the resulted dried leaves was about 5-6%. The dried leaves were powdered and stored in an air-tight container at 4 ^{o}C until further use for the experiment. Three batches of cocoa leaves were collected from the plantation for specific research works throughout this study as shown in Table 4.2. Each batch of the sample is kept for maximum 3 months to ensure consistency in the extraction result. This is because prolonged storage could decrease the content of active compounds in the leave sample due to degradation (Friedman, Levin, Lee, & Kozukue, 2009).

Table 4.2: Batches of cocoa leaves sample collected for various research purposes

Batch	Research purposes
A	1. Identification of anti diabetic compounds in cocoa leaves 2. Screening of suitable ranges for optimization of MAE 3. Determination of solvent for Soxhlet extraction
B	1. Kinetic modeling to study the effects of MAE parameters 2. Development of APD prediction method 3. Development of AED modeling method
C	1. Optimization of MAE 2. Optimization of Soxhlet extraction 3. Optimization of MAE using APD and AED

4.4. MAE technique

To improve the existing optimization and modeling methodology of MAE in plant extraction, a basic MAE setup, i.e. closed MAE was chosen in this study. The instrumental setup and extraction procedure of MAE technique are described in the following sub-sections.

4.4.1. Instrumental setup of MAE

The microwave system employed for the MAE in this work was a domestic microwave oven (Samsung, model no. MW718) as pictured in Fig. 4.2. The microwave system operates at 2.45 GHz with adjustable nominal power output (100-800 W). Timer is included to set the heating time of the microwave system. The scale of the timer originally in minute is not suitable for MAE time setting which involves time scale of seconds, thus an additional stopwatch was employed for the purpose. The built-in timer in the microwave system functions as switch to turn on or off the microwave heating. A mode stirring turn table is also included in the system as shown in Fig. 4.2 to dissipate the microwave energy during the microwave heating in order to heat up the extraction sample homogeneously. The described system is able to deliver fixed microwave power through pre-determined extraction time and most of the MAE setups employed in plant extraction are based on this heating mode.

Furthermore, the microwave oven was modified and equipped with fiber optic Luxtron I652 thermometer as shown in Fig. 4.2 to measure the temperature of the extraction solvent during microwave heating for APD calculation. Fiber optic temperature sensor probe was inserted into the microwave cavity and the thermometer was connected to a computer through Luxtron TrueTemp^{TM} software for on-line monitoring and recording purpose.

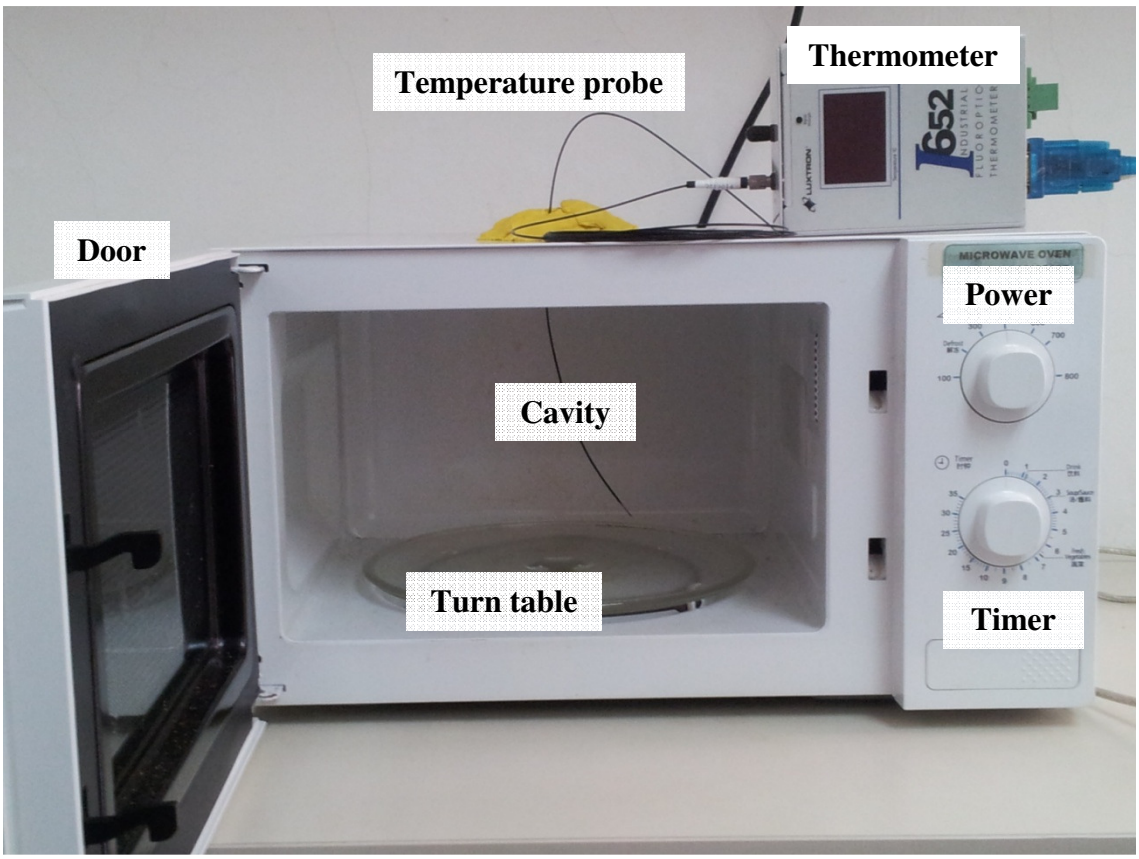


Fig. 4.2: Instrumental setup of MAE

4.4.2. MAE procedure

Cocoa leaves powder (1-6 g) was weighted and mixed with extraction solvent to a desirable predetermined solvent to feed ratio in a 500 ml Duran bottle. The Duran bottle was capped tightly and put in the microwave cavity. After the door of the cavity was closed and the microwave power for the MAE was set, the microwave system was turned on to allow microwave heating of the extraction sample for a predetermined time. Upon completion, the closed bottle was taken out and immersed in a water bath to cool down to room temperature. This helps to condense the evaporated solvent that trapped inside the closed bottle and also to minimize the loss of extraction solvent. The extract in the closed bottle was then filtered by using fine cloth and the volume of the extract was measured to calculate for the extraction yield. Subsequently, the extract obtained was filtered through a 0.2 µm RC (Regenerated Cellulose) syringe filter and

put into an HPLC vial. The extract containing HPLC vial was stored in a refrigerator (4 ^{o}C) before subjected to HPLC analysis.

4.4.3. Optimization of MAE

The main anti diabetic compounds in cocoa leaves extract determined from liquid chromatography – mass spectrometry (LCMS) analysis are the response for the optimization and modeling studies of MAE. Prior to the optimization study, preliminary runs were conducted to investigate the optimum solvent concentration and optimum range of microwave irradiation power. In this study, ethanol (EtOH) was chosen as extraction solvent and the effect of water as modifier (60-100% (v/v) EtOH) was investigated in a single factor experiment. Besides, screening of suitable range of microwave power for the optimization was performed on several power level (100-600 W). The evaluation of microwave power was based on the same input energy such that the final extraction temperature of the extraction system reached 70 ^{o}C.

The RSM with Box-Behnken design (BBD) was employed to optimize the MAE parameters, i.e. microwave power (X_1), solvent to feed ratio (X_2) and extraction time (X_3). In this optimization, the range of microwave power was selected based on the preliminary study whereas the solvent to feed ratio (30-70 ml/g at constant mass of solid) and extraction time (10-20 min) were determined from literature. Each runs of the experiments in BBD were performed at least twice. The total extraction yields of the main anti diabetic compounds are reported as responses in this case. The result from RSM optimization study was fitted into the following second-order polynomial model to predict the optimum point.

$$Y = B_o + \sum_{i=1}^{3} B_i X_i + \sum_{i=1}^{3} B_{ii} {X_i}^2 + \sum_{i>j}^{3} B_{ij} X_i X_j \quad (22)$$

where Y represent the predicted response; X_i was the actual value of an independent variable, B_0 denotes the model intercept; B_i, B_{ii}, and B_{ij} are the coefficients of the linear, quadratic, and interactive effects, respectively. The predicted model was verified by carried out the experiment in triplicate under optimum extraction conditions.

4.4.4. Kinetic modeling of MAE

The extraction kinetics of MAE of flavonoids compounds from cocoa leaves under the influence of particle sizes of sample (0.1-1 mm), solvent to feed ratio (20-80 ml/g), microwave irradiation power (100-600 W) and solvent loading (100-300 ml) were investigated by modeling the extraction curves (yield vs. time) using Patricelli model in Eq. (17).

$$P_t = P_w\left[1-\exp(-k_w t)\right]+P_d\left[1-\exp(-k_d t)\right] \tag{17}$$

The selected model gives information pertaining to the rate of washing steps (k_w) and diffusion steps (k_d) and also the extraction yield associated to each extraction steps (P_w and P_d). The equilibrium extraction yields can be calculated as follows:

$$P_e = P_w + P_d \tag{23}$$

The extraction yields of IQ, EC, RT and their total are the responses for the model. The model parameters were determined by fitting Eq. (17) with MAE curves. The experimental design of the kinetic modeling is tabulated in Table 4.3. Each point of extraction curves was individually constructed using fresh sample through series of extractions at the same operating conditions but with different extraction time.

Table 4.3: Experimental design for kinetic modeling study

No	Particle size of sample (mm)	Mass of sample (g)	Microwave irradiation power (W)	Solvent loading (ml)	Solvent to feed ratio (ml/g)	Extraction time (min)
1	< 0.1	2.00	160	160	80	13
2	0.1- 0.15	2.00	160	160	80	13
3	0.15-0.25	2.00	160	160	80	13
4	0.25-0.6	2.00	160	160	80	13
5	0.6-0.71	2.00	160	160	80	13
6	0.71- 1	2.00	160	160	80	13
7	> 1	2.00	160	160	80	13
8	< 0.25	2.00	100	100	50	30 [a]
9	> 0.25	2.00	100	100	50	35 [a]
10	0.25-0.6	5.00	100	100	20	30 [a]
11	0.25-0.6	1.25	100	100	80	30 [a]
12	0.25-0.6	2.00	100	100	50	35 [a]
13	0.25-0.6	2.00	200	100	50	19 [a]
14	0.25-0.6	2.00	300	100	50	9 [a]
15	0.25-0.6	2.00	450	100	50	6 [a]
16	0.25-0.6	2.00	600	100	50	4 [a]
17	0.25-0.6	3.00	150	150	50	18 [a]
18	0.25-0.6	4.00	200	200	50	35 [a]
19	0.25-0.6	5.00	250	250	50	14 [a]
20	0.25-0.6	6.00	300	300	50	10 [a]

[a] total extraction time of MAE curve.

4.4.5. Development and evaluation of APD predictive method

The APD predictive method is developed to predict the optimum extraction time of MAE. The APD value of extraction system can be calculated using Eq. (24)

$$APD = \frac{Q}{V \cdot t_H} \tag{24}$$

where Q is the total heat absorbed in the solvent during the microwave heating (J), V is the solvent loading (ml) and t_H is the microwave heating time (min). The total heat absorbed, Q can be determined from the temperature profile of solvent during microwave heating using calorimetric method (Incropera, 2007). In this study, two heating cases are considered:

1. When the final heating temperature is less than the boiling point of solvent

$$Q = m_L C_p \Delta T \tag{25}$$

2. When the final heating temperature equals to the boiling point of solvent

$$Q = m_L C_p \Delta T + m_v H_{vap} \tag{26}$$

m_L is the initial mass of the extraction solvent, C_p is the heat capacity of the extraction solvent, ΔT is the differential temperature after microwave treatment, m_v is the mass of the vaporized solvent, H_{vap} is the heat of vaporization of the extraction solvent. Eq. (25) can be used to calculate the absorbed energy required to increase the temperature of the extraction solvent from room temperature to boiling point (≈ 70 ºC) while Eq. (26) includes both the energy required to heat and to vaporize the extraction solvent during boiling. The calculation of APD requires the thermodynamic properties of extraction solvent: heat capacity of solvent components, i.e. ethanol and water from correlation presented by Miller, Shah and Yaws (1976), the equilibrium data of vapor-liquid from Raoult's law (Smith, Van Ness, & Abbott, 1996) and the latent heat of vaporization for the solvent mixture from thermodynamic data presented by Tamir (1982).

In this work, APD values were determined based on microwave heating of blank extraction solvent. To get accurate APD value, the microwave heating was conducted at different heating times (t_H) to include both the heating cases, then the average value of APD was determined. The sample calculation and the APD values at various microwave power and solvent loading are given in Appendix A.

Once the APD values were determined, the APD predictive method can be developed by correlating the optimum extraction time region (defined in chapter 3) with APD. The correlations were constructed based on MAE curves under different microwave irradiation power (100-600 W) at the same solvent loading (100 ml) and solvent to feed ratio (50 ml/g) as detailed in Table 4.3 (no. 12-16). The optimum extraction time regions and its respective extraction yields at these extraction conditions can be determined using Patricelli model such that the extraction yields are between 80-95% of the total extraction yields during the diffusion step. The correlations obtained were then evaluated in terms of their predictive capability of optimum extraction time for larger scale extraction (150-300 ml) as shown in Table 4.3 (no. 17-20). The applicability of the correlations at different solvent to feed ratios (20 ml/g and 80 ml/g) in Table 4.3 (no. 10-11) was also accessed. Furthermore, the optimum extraction time of MAE at various operating conditions as in Table 4.4 were selected within their respective predicted optimum extraction time region and their extraction performances were investigated.

Table 4.4: Random extraction conditions for the evaluation of APD and AED method

No	Particle size of sample (mm)	Mass of sample (g)	Microwave irradiation power (W)	Solvent volume (ml)	Solvent to feed ratio (ml/g)
1	0.25-0.6	3	100	150	50
2	0.25-0.6	3	300	150	50
3	0.25-0.6	4	100	200	50
4	0.25-0.6	4	300	200	50
5	0.25-0.6	5	100	250	50
6	0.25-0.6	5	300	250	50
7	0.25-0.6	6	100	300	50
8	0.25-0.6	6	200	300	50

4.4.6. Development and evaluation of AED modeling method

The modeling of MAE process using AED was performed by using similar extraction curves obtained from the kinetic modeling previously shown in section 4.4.4. In this modeling, the AED adapted film theory in Eq. (20) was employed to model MAE curves under the effect of solvent to feed ratio (20-80 ml/g).

$$\frac{Y}{Y_s} = 1-(1-b')\exp(-k'\cdot AED_t) \tag{20}$$

The constant *b'* and *k'* of the AED-adapted model were determined by fitting Eq. (20) with the experimental extraction curve in Table 4.3 (no. 10-12) using AED basis. The extraction curves of MAE in time basis can be converted to AED basis using Eq. (18).

$$AED_t = APD\times t \tag{18}$$

The constants obtained were then substituted into Eq. (21) to predict extraction curves of MAE at various microwave irradiation powers (100-600 W) and solvent loading (100-300 ml).

$$\frac{Y}{Y_s} = 1 - (1 - b')\exp(-k' \cdot APD \cdot t) \tag{21}$$

The predictive capability of the model was evaluated by comparing with the experimental curves in Table 4.3 (no. 13-20). The predictive model in Eq. (21) was also used to predict the constants of film theory (b and k) and the predicted constants were compared with that obtained from curve fitting using actual film theory in Eq. (19).

$$\frac{Y}{Y_s} = 1 - (1 - b)\exp(-k \cdot t) \tag{19}$$

Furthermore, optimum extraction times of MAE at various extraction conditions in Table 4.4 were estimated from the modeling result and their extraction performance were evaluated.

4.4.7. Optimization of MAE using APD and AED

The procedure to optimize MAE based on APD and AED is generalized in Fig. 4.3. In this optimization strategy, the optimum values of solvent to feed ratio, AED and APD were investigated sequentially using single factor experiments. Each run of the experiments were performed at least twice except for the investigation of the optimum AED. The effect of solvent to feed ratio (10-90 ml/g) was first investigated at an arbitrary extraction condition (100 W, 50 ml, 10 min) and the optimum value was determined. In the subsequent experiment, the effect of AED at extraction condition (100 W, 50 ml, optimum S/F ratio) was studied by conducting the extraction at different extraction time (2-20 min) using fresh sample. The optimum extraction time was converted to AED using Eq. (18) and was adopted for subsequent experiment. Finally, the effect of APD at extraction condition (50 ml, optimum S/F ratio, optimum AED) was investigated by conducting the MAE using various microwave powers (100-300 W). As the extraction had to be conducted at the same AED value, the extraction time

for each microwave power level was calculated based on their APD values using Eq. (18). The optimum solvent to feed ratio, AED and APD are referred as intensive optimum condition of MAE in this study.

Optimization of MAE based on APD and AED

1	Investigate the effect of S/F ratio at any arbitrary condition (nominal power, solvent loading, Time) Use the optimum S/F ratio for subsequent experiment
2	Investigate the effect of AED by changing the extraction time at condition (nominal power, solvent loading, optimum S/F ratio) Change the extraction time into AED basis based on its APD and use the optimum AED value for subsequent experiment
3	Investigate the effect of APD by changing the microwave powers at condition (solvent loading, optimum S/F ratio, optimum AED) Change the microwave power into APD basis and perform the MAE at extraction time determined from the optimum AED value
4	The intensive optimum extraction conditions of MAE are expressed as S/F ratio, AED, APD

Fig. 4.3: The APD-AED optimization procedure of MAE

The intensive optimum condition was validated and compared with the extraction result from the RSM-optimization in section 4.4.3 and they are also verified at larger scale MAE (100-300 ml). The optimum operating parameters of MAE (S/F, power, time) at large scale MAE can be determined from the intensive optimum conditions (S/F, AED, APD). For instance, the optimum microwave power was selected based on the optimum APD and the optimum extraction time was determined based on the optimum AED via Eq. (18).

4.5. Soxhlet extraction technique

Soxhlet extraction was employed as standard technique in this study to evaluate the performance of MAE. Its instrumental setup and extraction procedure are described subsequently.

4.5.1. Instrumental setup of Soxhlet extraction

Soxhlet extractor was constructed by using laboratory apparatus as pictured in Fig. 4.4. The extractor consists of a collecting flask for solvent reservoir, a Soxhlet chamber for sample placement and a condenser for reflux purpose. Extraction thimble was used for sample loading and it was put inside the Soxhlet chamber. The chamber was placed on a collecting flask containing the solvent and was connected to a condenser at its top. A flask heater was used to heat up solvent in the collecting flask and a chiller was employed to circulate cooling water in the condenser. In Soxhlet extraction, the solvent in the collecting flask was evaporated and the vapor was condensed when it travelled along the way reaching the condenser. The Soxhlet chamber was slowly filled up by the condensed solvent and extraction occurred when the solvent contacted with the plant sample. Once the solvent filled in the chamber fully, the solvent containing the active compounds was automatically transferred back to the collecting flask through a siphon side arm. During Soxhlet extraction, the processes of evaporation, condensation, extraction and reflux of solvent are repeated over period of time and the final concentrated extract can be obtained from the collecting flask.

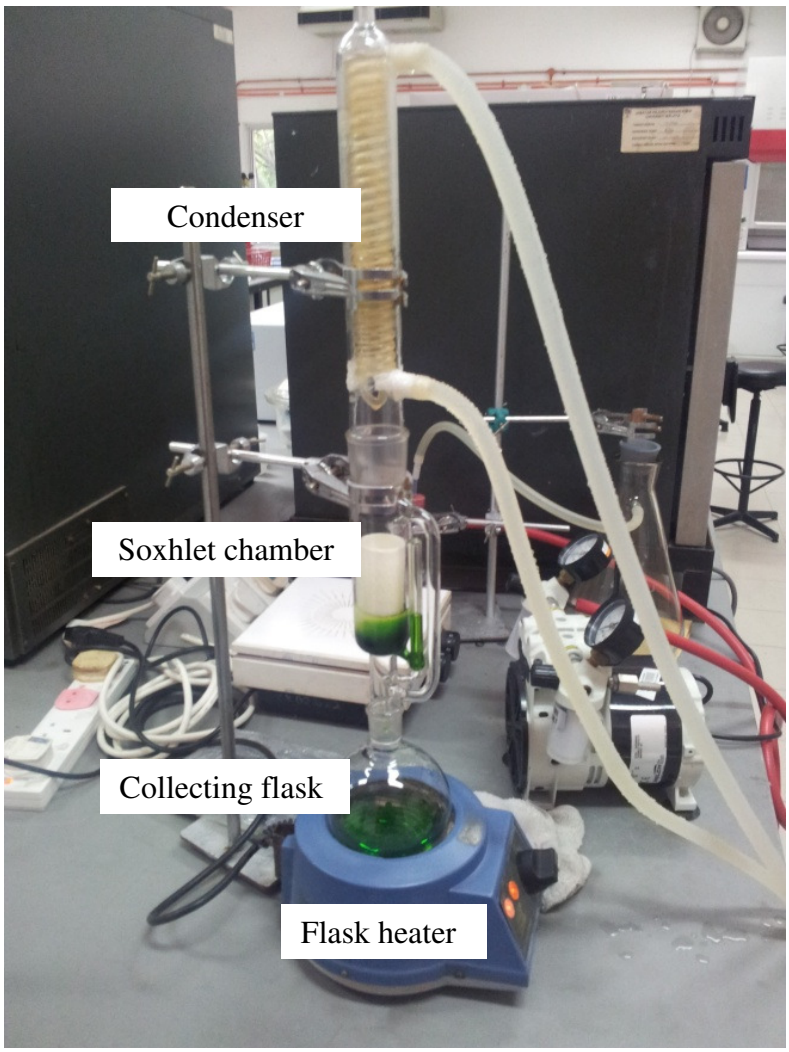


Fig. 4.4: Instrumental setup of Soxhlet extraction

4.5.2. Soxhlet extraction procedure

Two grams (2g) of dried sample was weighted and put into an extraction thimble. Small amount of wool was added to cover the dried sample in the thimble to prevent the sample from spilling out during extraction. The thimble was put in the Soxhlet chamber and a predetermined amount of ethanol was added into the collecting flask. Once the Soxhlet extractor was set up, the chiller connected to the condenser was turned on and the 5 ^{o}C cooling water was filled up the condensing unit. The flask heater was turned on to start the extraction. After extraction, the flask heater was turned off and the extract was cooled down to room temperature then the extract collected was filtered through a 0.2 µm RC (Regenerated Cellulose) syringe filter for HPLC analysis.

4.5.3. Optimization of Soxhlet extraction

Pure EtOH was selected as solvent for Soxhlet extraction due to the solubility of flavonoids compounds in the solvent and the addition of water in ethanol does not enhance the Soxhlet extraction based on preliminary result. There are few operating parameters that can be optimized for Soxhlet extraction. In this study, the extraction time (2-12 hr) and solvent to feed ratio (75-150 ml/g) were investigated using single factor experiment. Each experiment was conducted at least twice. The optimum yields of Soxhlet extraction was used to compare with the optimum extraction yields of MAE (section 4.4.3) and to justify the prediction of optimum extraction time of MAE by APD prediction method (section 4.4.5) and AED modeling method (section 4.4.6).

4.6. Analytical methods

Analytical methods employed in this study are elucidated in the following sub-sections.

4.6.1. HPLC-MS

The identification and quantifications of active compounds in cocoa leaves extract were performed using an Agilent 6500 accurate mass quadropole time-of-flight mass spectrometer coupled with Agilent 1200 Series HPLC system. The mass spectrometer was equipped with electrospray ionization (ESI) source and proprietary Agilent jet stream dual nebulizer. Then HPLC system consists of a vacuum degasser, a thermostatted autosampler, binary pump and a thermostatted column compartment. The separation was performed using Agilent ZORBAX Eclipse Plus C18 column, 5 μm (4.6 mm × 150 mm). The HPLC method proposed by Bonaccorsi, Caristi, Gargiulli and Leuzzi (2008) was modified. In this modified method, , the mobile phase used was linear gradient of acetonitrile in water as follows: 5–20% (0–15 min), 20–30% (15–20 min), 30–50% (20–30 min), 50–100% (30–35 min), 100% (35–40 min), and 100–5%

(40–50 min) at flow rate of 1.0 ml/min. The injection volume of sample was 10 μL and the separations were detected by UV-DAD at wavelength of 280 nm and 350 nm. The calibration curve of HPLC was constructed at the range from 0.01-0.20 mg/ml by dissolving the standards of active compounds into analytical grade ethanol. The extraction yield of active compound can be expressed as follows:

$$\text{Extraction yield} = \frac{\text{Mass of extracted active compound (mg)}}{\text{Mass of sample used (g)}} \quad (27)$$

The mass spectrometer was employed to further confirm the presence of specific active compounds in cocoa leaves extract. Mass spectra were achieved by ESI positive ions mode. Nitrogen was used as both the drying gas and sheath gas with flow rate of 8 L/min and 11 L/min respectively. The electron spray ionize voltage was 3500 fragmentor voltage of 125 V, skimmer voltage of 65 V, nozzle voltage of 1000 V and the collision energy was fixed at 15 V. Continuous full mass spectral data were obtained by scanning from m/z 100 to 800. The recorded full-scan and MS/MS data was processed using Agilent Mass Hunter Workstation Software.

4.6.2. SEM

To investigate the morphological structure of the sample after extraction, the samples after extraction were dried to remove the moisture before subject to SEM analysis. The samples were examined with Field Emission Scanning Electron Microscope (FE-SEM AURIGA, ZEISS) under high vacuum condition and an accelerating voltage of 1 kV at magnification of 500 and1000.

4.6.3. ANOVA

Analysis of variance (ANOVA) in Design Expert 6.0 software (Stat-Ease Inc., USA) was used to determine the regression coefficients of the statistical MAE model constructed in the optimization study (section 4.4.3). The statistical significance of the model parameters were checked by F-test at a probability (P) of 0.001, 0.01 or 0.05. The model accuracy was also evaluated in terms of the R-square value, adjusted R-square value, experimental and standard deviation.

4.6.4. Curve fitting with regression analysis

The curve fitting toolbox (version 2.1) in Matlab (Mathworks Inc., USA) was employed to determine the constants of kinetic models through curve fitting with experimental extraction curves (section 4.4.4 to 4.4.6). The goodness of fit of the fitted model was evaluated based on sum square error (SSE) and adjusted R-square value.

Chapter 5

RESULTS AND DISCUSSION

This chapter presents the optimization study of MAE for the extraction of anti diabetic compounds from cocoa leaves. Optimization of Soxhlet extraction is also included for comparison purpose. Besides, the discussion and analysis of the effects of parameters on the extraction kinetics of MAE are also presented. Subsequently, the focus of this chapter is the evaluation of APD predictive method, AED modeling methods and APD-AED optimization strategy.

5.1. Identification and quantification of anti diabetic compounds in cocoa leaves

The ethanolic extract from MAE of cocoa leaves was analyzed and the results obtained were compared to that of the standard compounds. The detection wavelength and retention time of the standard compounds are tabulated in Table 5.1. The HPLC chromatogram of the cocoa leaves extract and the reference standard are depicted in Fig. 5.1.

Table 5.1: Identification of the selected anti diabetic compounds using HPLC method

Peak no.	Standard compound	Detection wavelength (nm)	Retention time (min)
1	rutin	350	19.31
2	isoquercitrin	350	20.29
3	myricetin	350	23.74
4	quercetin	350	26.77
5	kaempferol	350	29.62
6	(-)-epigallocatechin	280	12.04
7	(-)-epicatechin	280	16.02
8	(-)-epicatechin gallate	280	16.66
9	(-)-epigallocatechin gallate	280	20.81

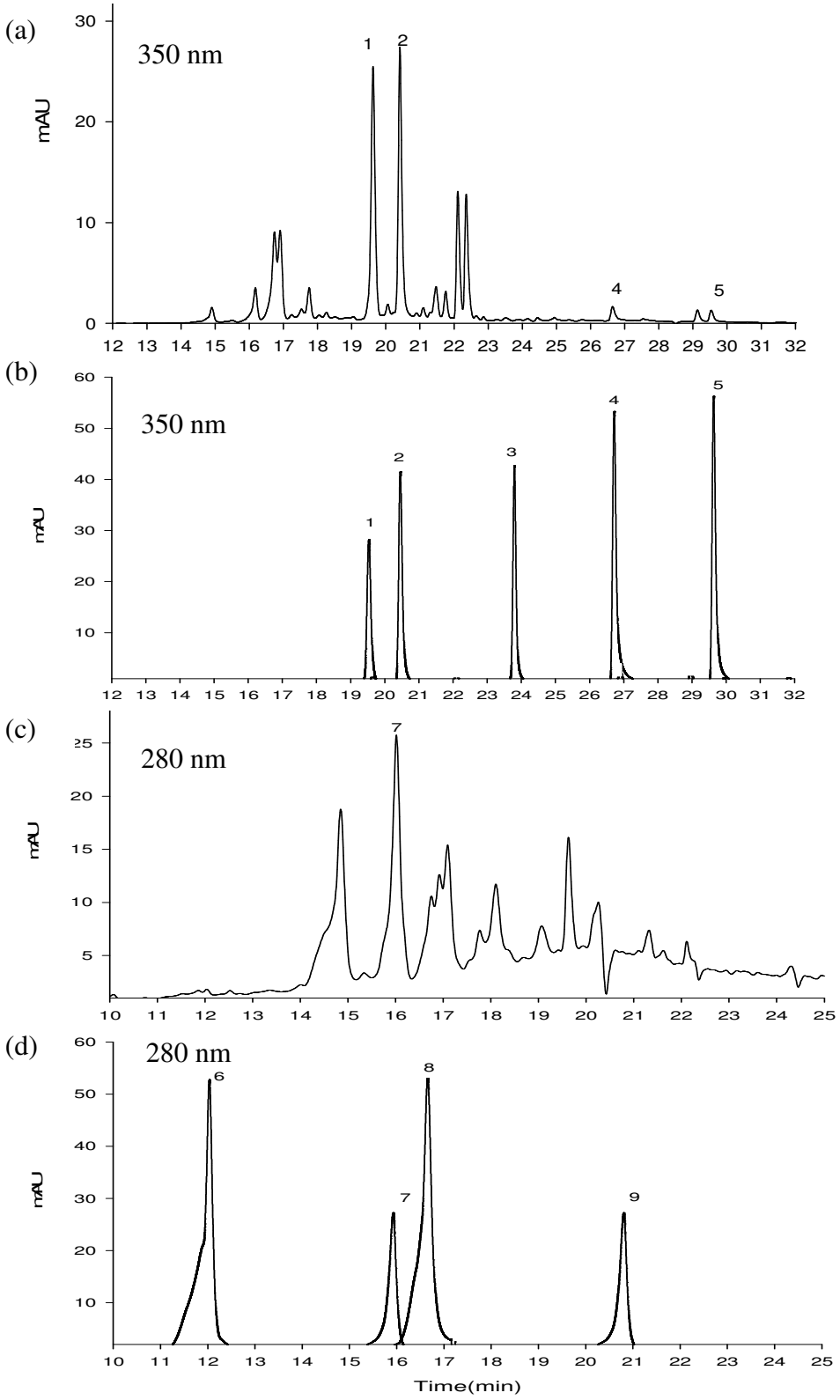
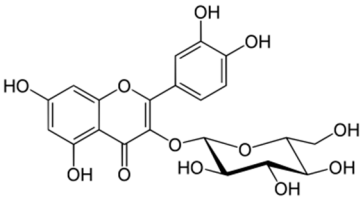
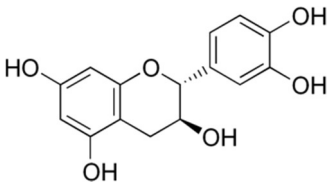
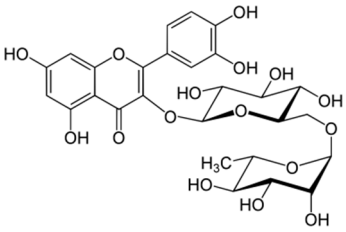


Fig. 5.1: HPLC chromatograms of cocoa leaves extract and standard compounds: (a) cocoa leaves extract detected at 350 nm, (b) standard compounds detected at 350 nm, (c) cocoa leaves extract detected at 280 nm and (d) standard compounds detected at 280 nm

Fig. 5.1 shows that flavonols compounds such as rutin (1), isoquercitrin (2), quercetin (4) and kaempferol (5) are present in cocoa leaves in particular rutin and isoquercitrin are in significant amount. It is interesting to note that quercetin, the aglycone form of rutin and isoquercitrin is negligible in amount indicating that most of the flavonols in cocoa leaves are present in their glucosides forms. On the other hand, (-)-epicatechin is the main catechin compound found in cocoa leaves and other catechin compounds such as (-)-epicatechin gallate and (-)-epigallocatechin gallate are present in little amount. This identification result shows good agreement with the extraction conducted by Osman et al. (2004, 2005). In this work, the key anti diabetic compounds, i.e. isoquercitrin (IQ), rutin (RT) and (-)-epicatechin (EC) were selected based on their substantial amount. The calibration curves of the HPLC analysis for the key compounds are plotted as shown in Appendix B.

The presence of the key compounds in the cocoa leaves extract was further confirmed by using HPLC-MS. The ESI-MS spectra of the cocoa leaves extract and the reference standards are presented in Appendix B. The analysis result in Table 5.2 show that the molecular ions of IQ, EC and RT present in cocoa leaves extract in terms of mass to charge ratio (m/z) are 465.15, 291.08 and 611.16, respectively. The values observed are comparable with those of the reference standards. This confirms the presence of these compounds in cocoa leaves and hence they are used as the subject compounds for the optimization and modeling studies in this research.

Table 5.2: HPLC-MS analysis of key anti diabetic compounds in cocoa leaves extract

Active compound	Molecular structure	Molecular weight, M	Measured m/z of molecular ion $[M+H]^+$ in extract	Measured m/z in molecular ion $[M+H]^+$ in standard
isoquercitrin (IQ) $C_{21}H_{20}O_{12}$		464.38	465.15	465.11
(-)-epicatechin (EC) $C_{15}H_{14}O_6$		290.27	291.08	291.10
rutin (RT) $C_{27}H_{30}O_{16}$		610.52	611.16	611.16

Throughout this research, the amounts of IQ, EC and RT quantified from cocoa leaves were in the ranges of 0.13-3.51 mg/g, 0.23-2.91 mg/g and 0.3-7.07, respectively. The amount of quercetin derivatives (IQ and RT) and catechin compound (EC) in cocoa leaves were compared with those plants that are rich in these flavonoids such as onion and green tea. The quercetin glucosides in onion were in the range of 0.03-2.24 mg/g (Rodríguez Galdón, Rodríguez Rodríguez, & Díaz Romero, 2008; Zill e et al., 2011) whereas the catechin compounds found in green tea was between 0.9-129 mg/g (Perva-Uzunalić et al., 2006). The comparable amount of quercetin derivatives and catechin compounds in cocoa leaves with those in onion and green tea suggesting that cocoa leaves is a potential bioresource of anti diabetic flavonoids compounds.

5.2. Optimization of MAE

There are various significant operating parameters for MAE such as solvent concentration, solvent to feed ratio, microwave irradiation power and extraction time. The operating parameters were first screened followed by the optimization of interactive parameters using RSM. The total extraction yield (IQ, EC and RT) was used as the response in this optimization study.

5.2.1. Determination of optimum solvent concentration

The solvent used in MAE was ethanol (EtOH) due to its non-toxicity, polarity and good microwave absorbing properties (Xiao et al., 2008). The presences of water in the extraction solvent, i.e. 60-100% (v/v) EtOH, acts as positive modifier which can enhances the heating efficiency due to its high dielectric constant and increase the extraction yield as shown in Fig. 5.2. The highest yield of RT and EC were achieved at solvent concentration of 80% (v/v) EtOH whereas for achieving the highest IQ yield, 90% (v/v) EtOH is desired. This is because different active compounds prefer different concentration of aqueous ethanol according to their solubility in the solvent (Wu et al., 2011). When the concentration of water in the EtOH increases above the critical value, the extraction yield decreases as the solubility of the flanovoids in the ethanol decreased (Wu et al., 2011). Since 80-90% (v/v) EtOH (shaded region in Fig. 5.2) represents the region of optimum solvent concentration, a compromise 85% aqueous EtOH was used for the subsequent optimization study.

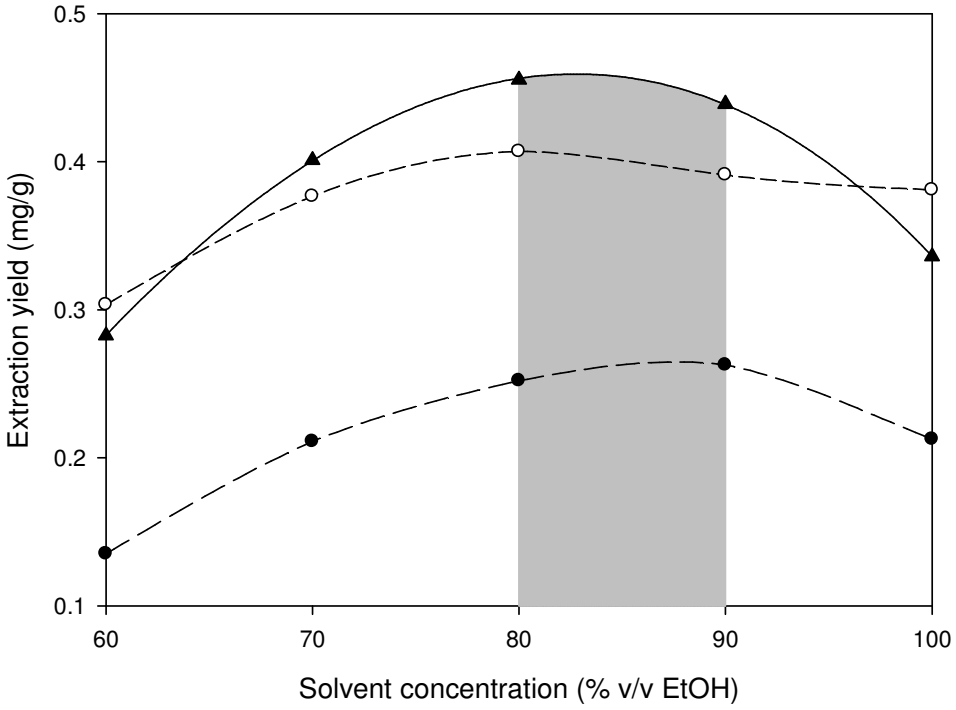


Fig. 5.2: Effect of solvent concentration on IQ, EC and RT yields in MAE (*MAE conditions: 100 W, 5 and 50 ml/g; (●) yield of IQ; (○) yield of EC; (▲) yield of RT*)

5.2.2. Determination of optimum range of microwave power

Microwave heating power is important as it affect the rate of heating in MAE. The effect of heating rate, investigated using microwave power of 100-600 W, was evaluated at the same input energy as illustrated in Fig. 5.3. The evaluation cannot be based on the same extraction time due to the fact that higher microwave power would require shorter extraction time for complete extraction (Chan, Yusoff, Ngoh, & Kung, 2011). The extraction result in Fig. 5.3 demonstrates that MAE at low microwave power (100-200 W) gave higher extraction yields as compared to that at high microwave power (300-600 W). About 10% of total extraction yield was lost at high microwave power when compared to that at low microwave power. Thus, the high microwave power is not recommended for the MAE as high rate of microwave heating could not effectively extract the active compounds from the plants. This also could be due to

thermal degradation at high microwave power (Biesaga, 2011). Based on the findings, microwave power range of 100-200 W was applied in the optimization study.

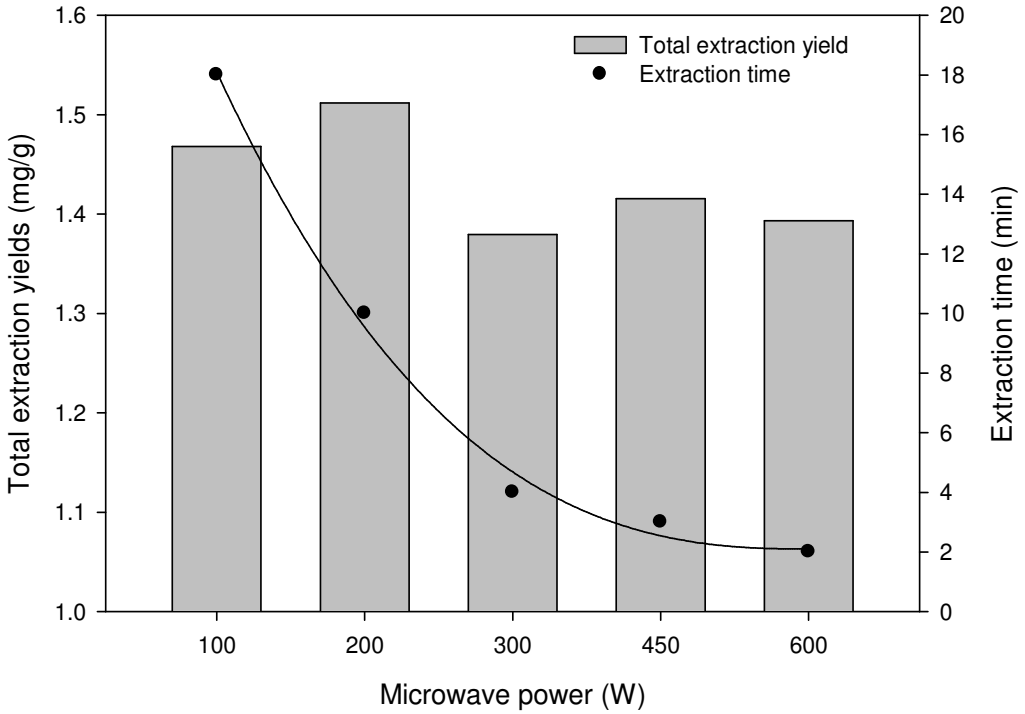


Fig. 5.3: Extraction yields during MAE at different microwave powers (*MAE conditions: 50 ml/g and 100 ml of 85% (v/v) EtOH; The final temperature after extraction for all the operating conditions was 70 ^{o}C*)

5.2.3. Optimization of MAE using RSM

Based on the preliminary results, the microwave irradiation power (100-200 W) together with solvent to feed ratio (30-70 ml/g) and extraction time (10-20 min) determined from the typical ranges of optimum conditions (Chan et al., 2011) were optimized using RSM with BBD in 17 set experiments and the result is tabulated in Table 5.3.

Table 5.3: Box-Behnken design for the optimization of MAE

Standard order	Microwave Power (W)	Solvent to feed ratio (ml/g)	Extraction time (min)	Total extraction yields (mg/g)
1	100	30	15	6.38
2	200	30	15	6.47
3	100	70	15	6.99
4	200	70	15	7.07
5	100	50	10	6.17
6	200	50	10	6.70
7	100	50	20	6.62
8	200	50	20	6.81
9	150	30	10	6.54
10	150	70	10	6.96
11	150	30	20	6.59
12	150	70	20	7.36
13	150	50	15	6.91
14	150	50	15	6.66
15	150	50	15	6.99
16	150	50	15	6.83
17	150	50	15	7.11

The empirical relationship for the total extraction yield with extraction parameters was generated as shown in Eq. (28).

$$Y = 2.76 + 3.50\text{x}10^{-2} X_1 - 1.19\text{x}10^{-2} X_2 + 1.44\text{x}10^{-1} X_3 - 9.20\text{x}10^{-5} X_1^2 + 1.42\text{x}10^{-4} X_2^2 - 3.74\text{x}10^{-3} X_3^2 - 1.77\text{x}10^{-6} X_1X_2 - 3.36\text{x}10^{-4} X_1X_3 + 8.65\text{x}10^{-4} X_2X_3 \quad (28)$$

where X_1 is microwave power (W), X_2 is solvent to feed ratio (ml/g) and X_3 is extraction time (min), respectively. The ANOVA result is presented in Table 5.4. The models are

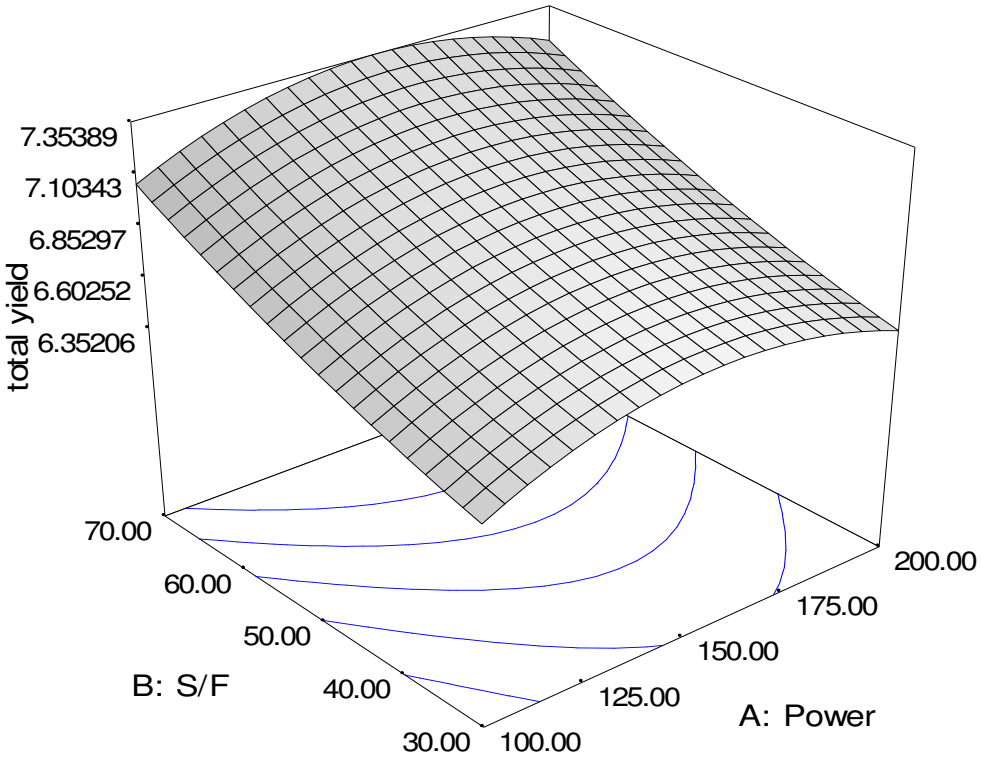
highly significant with insignificant lack of fit indicating its reliability in representing the total extraction yields of MAE at the selected ranges of extraction parameters.

Table 5.4: ANOVA result of optimization of MAE

Transform	None
Model	Quadratic
Sum of squares	1.33
F value	6.51
Prob > F	0.011
Lack of fit	0.7681

Response surface analysis was performed to study the effects of parameters and their interactive effects in the MAE and the response surface curves are plotted in Fig. 5.4. Microwave power has significant effect on the MAE process as it enhances the extraction yields by providing driving force for rupturing the plant matrix to elute the active compounds (Mandal & Mandal, 2010). However, excessive power may have adverse effect on the condition due to the evaporation of solvent which provokes the mass transfer barrier for the active compounds to diffuse out to the solvent (Mandal & Mandal, 2010) and also the thermal degradation. On the other hand, the extraction time shown in Fig. 5.4 (b) allows the extraction to proceed and increases the extraction yield gradually until reaching equilibrium. However, prolong the extraction with low solvent to feed ratio had little effect on the extraction yield as the extraction might have reached equilibrium under the operating condition (Yang et al., 2010).

(a)



(b)

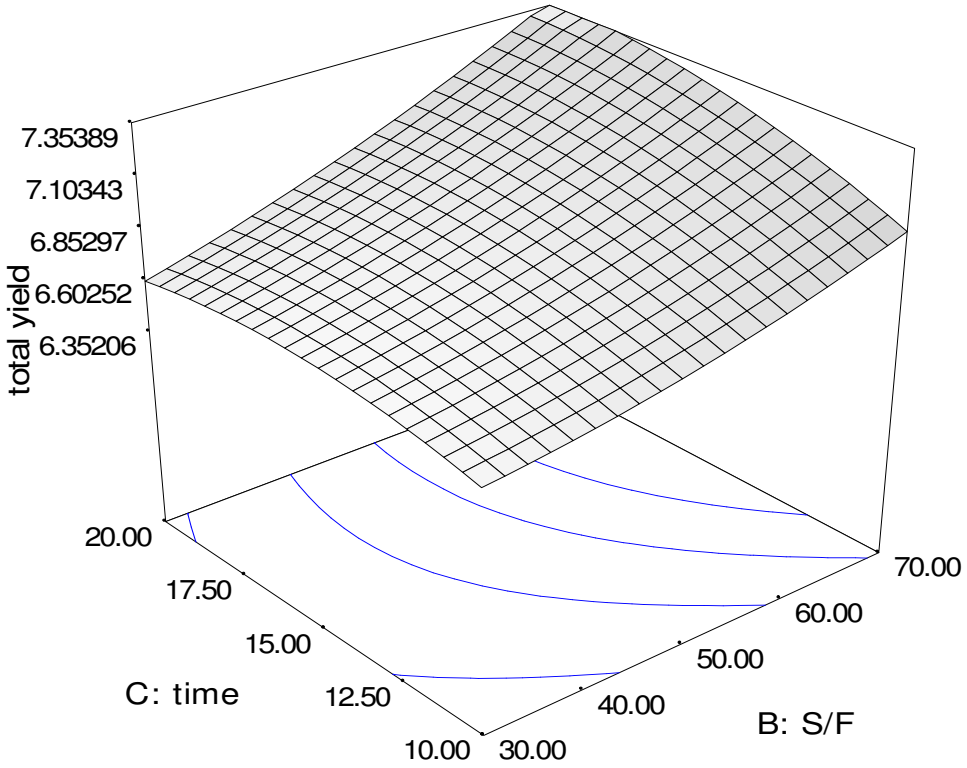


Fig. 5.4: Response surface plots of the (a) effect of solvent to feed ratio and microwave power, and (b) effect of extraction time and solvent to feed ratio on total extraction yield

There are two ways of investigating solvent to feed ratio, either at constant sample mass or constant solvent volume. Increasing solvent to feed ratio at constant sample mass may give poor extraction yield as high solvent volume may give rise to poor mixing and also insufficient heating power for the extraction (Mandal & Mandal, 2010). On the other hand, increasing solvent to feed ratio at constant solvent volume would only enhance the extraction yield of MAE (Spigno & De Faveri, 2009) as illustrated in Fig. 5.4. The response surface curves in Fig. 5.4 are according to that reported in literature without obvious optimum point. Nevertheless, the interaction between microwave power and solvent to feed ratio in Fig. 5.4 (a) shows that for every solvent to feed ratio, there is a specific microwave power for optimum extraction yield. The specific optimum microwave powers are from 161 W to 155 W corresponded with solvent to feed ratios of 30 ml/g to 70 ml/g regardless of its extraction time. The corresponding optimum extraction time can be determined after specifying the solvent to feed ratio and the specific optimum power. Following the procedure, several sets of preferable extraction condition for MAE at various solvent to feed ratios are determined as shown in Table 5.5.

Table 5.5: Preferable extraction conditions of MAE at various solvent to feed ratios

	Solvent to feed ratio (ml/g)				
	30	40	50	60	70
Microwave power (W)	161	159	156	154	155
Extraction time (min)	15.5	16.8	18	19.3	18.8
Total extraction yields (mg/g)	6.67	6.79	6.95	7.14	7.35
Percentage difference (%) [a]	10.2	8.2	5.8	2.9	0.0
Solvent used to extract 1 mg compounds (ml)	4.5	5.9	7.2	8.4	9.5

[a] Percentage difference as compared to the extraction yields at 70ml/g.

The preferable extraction conditions of MAE at various solvent to feed ratios can be used to achieve total extraction yields of 6.67-7.35 mg/g as presented in Table 5.5. It gives operational flexibility in terms of solvent consumption with little compromise of the extraction yields. Comparing between the extraction performance of MAE at 30 ml/g and 70 ml/g in Table 5.5, only 10% difference of total extraction yields between the two cases though the solvent used to extract 1 mg in the case of 70 ml/g is about twice than that in the case of 30 ml/g. Considering both the solvent consumption and the extraction performance of MAE, an intermediate value of the preferable extraction conditions, i.e. 50 ml/g, 156 W and 18 min were selected as optimum extraction conditions of MAE in this study. The optimum condition was verified by carrying out the experiment in triplicate as shown in Table 5.6. The result shows that the experimental extraction yields of IQ, EC and RT were 1.06±0.02 mg/g, 1.32±0.02 mg/g and 4.54±0.03 mg/g, respectively. The total extraction yield obtained is accordance with the predicted yield (6.93 mg/g vs. 6.95 mg/g) thus reflecting the reliability of the extraction model generated using RSM.

Table 5.6: Verification of MAE optimum conditions (50 ml/g, 156 W and 18 min)

Trial.	Extraction yield (mg/g)			
	IQ	EC	RT	Total
1	1.05	1.34	4.55	6.94
2	1.04	1.3	4.51	6.85
3	1.09	1.33	4.57	6.99
Average	1.06 ± 0.02	1.32 ± 0.02	4.54 ± 0.02	6.93 ± 0.06

5.3. Optimization of Soxhlet extraction

Soxhlet extraction was employed as reference extraction technique to evaluate the performance of MAE. The operating parameters such as extraction solvent, extraction time and solvent to feed ratio were investigated for this extraction. In Soxhlet extraction, pure ethanol gave better extraction yields than the aqueous ethanol as shown in Table 5.7 thus it was used for subsequent investigation. The effect of extraction time (2-12 hr) was investigated and the result in Fig. 5.5 illustrates that Soxhlet extraction requires 6 hr to achieve complete extraction and prolong the extraction does not improve the extraction yields. Furthermore, the effect of solvent to feed ratios illustrated in Fig. 5.6 indicates that the parameter has no significant effects on Soxhlet extraction. This can be explained by the amount of solvent used in Soxhlet extraction which does not exert concentration gradient effect on the diffusion of active compounds, in fact, it only acts as reservoir to allow continuous reflux so that the condensed, fresh solvent can be filled in the Soxhlet chamber and extract the active compounds from plant sample. Nonetheless, a minimum amount of 150 ml solvent is required to sustain the reflux operation of Soxhlet extraction. In this study, the optimum conditions of Soxhlet extraction was selected to be pure EtOH in 100 ml/g (2 g sample) and 6 hr extraction time. The optimum extraction yields for batch (B) sample and batch (C) were 11.67 ± 0.41 mg/g and 7.09 ± 0.20 mg/g, respectively.

Table 5.7**:** Effect of aqueous EtOH on Soxhlet extraction.

Soxhlet extraction [a]	IQ yield (mg/g)	EC yield (mg/g)	RT yield (mg/g)	Total yield (mg/g)
2 g sample, EtOH, 200 ml, 6 hr	0.41	0.38	0.56	1.35
2 g sample, 85% EtOH, 200 ml, 6 hr	0.38	0.37	0.55	1.30

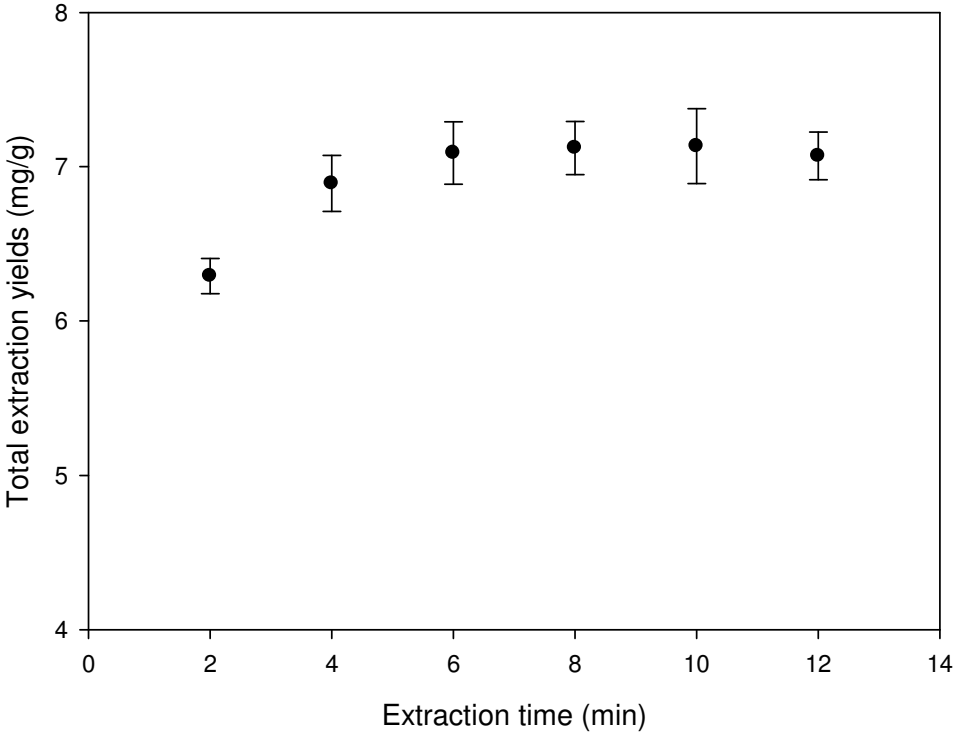


Fig. 5.5: Effect of extraction time on the Soxhlet extraction yield (*Soxhlet conditions: 2 g sample and 200 ml of EtOH*)

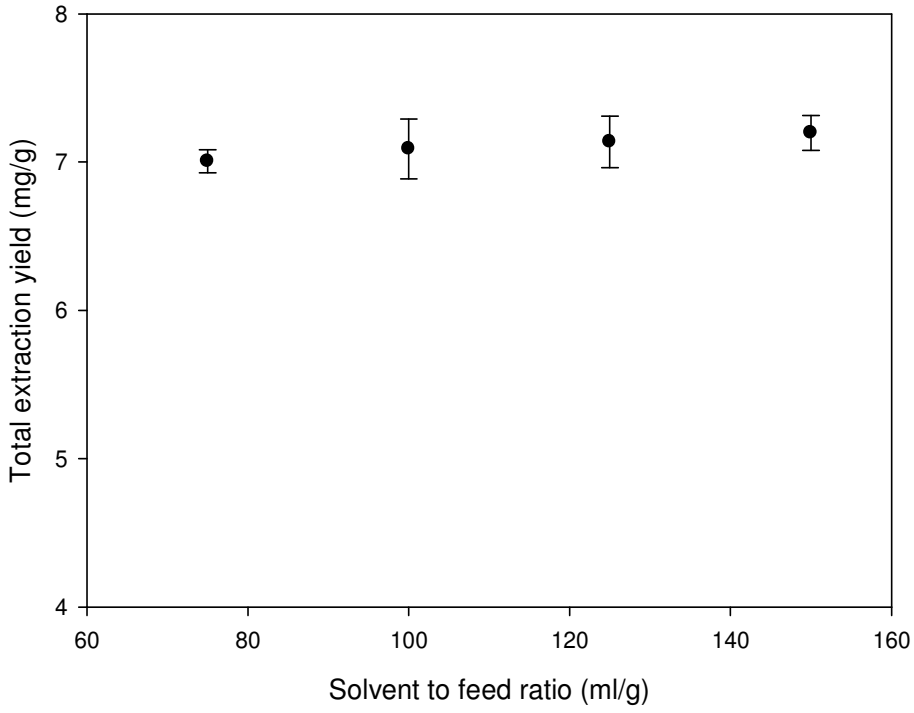


Fig. 5.6: Effect of solvent to feed ratio on the Soxhlet extraction yield (*Soxhlet conditions: 2 g sample and 6 hr*)

5.4. Comparison of MAE and Soxhlet extraction

This section evaluates the extraction performance of MAE using Soxhlet extraction as reference technique. The extraction performances of both techniques at their optimum conditions are compared as shown in Table 5.8. According to the overall performance of both techniques, MAE is more feasible as it gives comparable extraction yields as the Soxhlet extraction (98% of total recovery) but required lesser solvent and shorter extraction time. This is due to the microwave heating that ruptures the plant structure to enhance the extraction more than the conventional heating (Mandal et al., 2007). Besides, the performance of MAE tends to be more consistent and reproducible as compared to Soxhlet extraction based on its smaller standard deviations of total extraction yields (0.06 mg/g vs. 0.20 mg/g) as can be seen from Table 5.8.

Table 5.8: Extraction yields of MAE and Soxhlet extraction

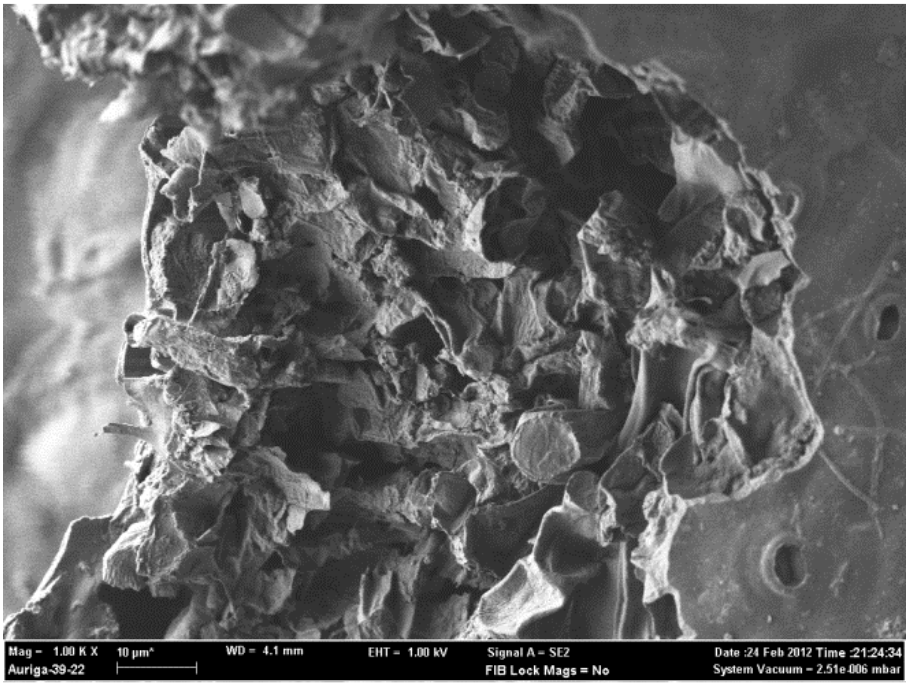
Extraction techniques	MAE	Soxhlet
Optimum conditions	85% EtOH, 50 ml/g, 2 g sample, 156 W and 18 min	100% EtOH, 100 ml/g, 2 g sample and 6 hr
Total extraction yield (mg/g)	6.92 ± 0.06	7.09 ± 0.20
IQ yield (mg/g)	1.06 ± 0.02	1.17 ± 0.09
EC yield (mg/g)	1.32 ± 0.02	1.30 ± 0.13
RT yield (mg/g)	4.54 ± 0.02	4.62 ± 0.01
Total recovery (%) [a]	98	/
Recovery of IQ (%) [a]	91	/
Recovery of EC (%) [a]	102	/
Recovery of RT (%) [a]	98	/

[a] The recovery (%) was calculated based on the optimum extraction yields of Soxhlet extraction.

Table 5.8 also shows that EC and RT can be extracted completely from cocoa leaves using MAE (> 98% recovery) but not for IQ (91% recovery). This can be explained by the thermal stability of these active compounds as reported in literature, whereby RT is more stable than IQ due to its relatively stable sugar moiety when subjected to thermal treatment (Rohn, Buchner, Driemel, & Rauser, 2007). As for catechin compounds e.g. EC, they are stable during MAE up to 100 ^{o}C (Liazid, Palma, Brigui, & Barroso, 2007). Hence, IQ is thermally less stable than RT and EC. In this study, some of the IQ might have degraded during MAE resulted in its yield slightly lower than that of the Soxhlet extraction (1.06 mg/g vs. 1.17 mg/g). This suggests that MAE has greater tendency of thermal degradation than Soxhlet extraction. However, MAE is better than Soxhlet extraction in terms of solvent consumption and extraction time.

The performance of MAE in disrupting of the plant matrix is further confirmed by the structure analysis of microwave-treated samples shown in Fig. 5.7. The figure shows that the plant cells are ruptured and there are pits formed on the surface of the leaves sample attributed to the localized heating of microwave radiation that increased the internal pressure to rupture the cells (Kong et al., 2010). The internal pressure must have been too great that it damaged the surface of the leave and formed channels for rapid dissolution of active compounds into extraction solvent. Comparing the structure of the Soxhlet extracted sample (Fig. 5.8) with the dried sample (Appendix B), both structures were almost the same with shrunk cells. The structural change in the extracted sample explains the positive effects impacted by MAE.

(a)



(b)

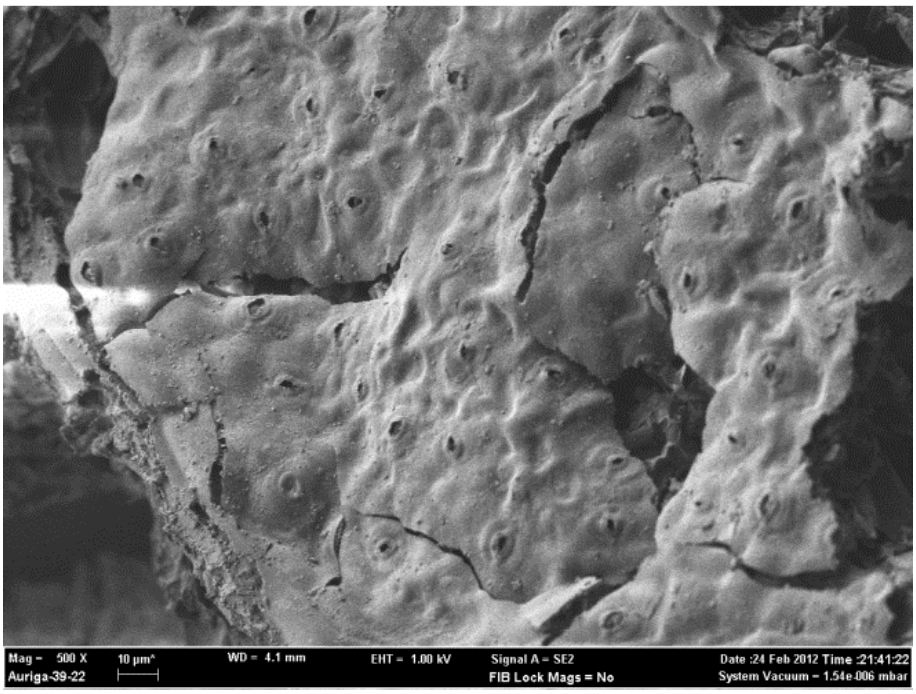
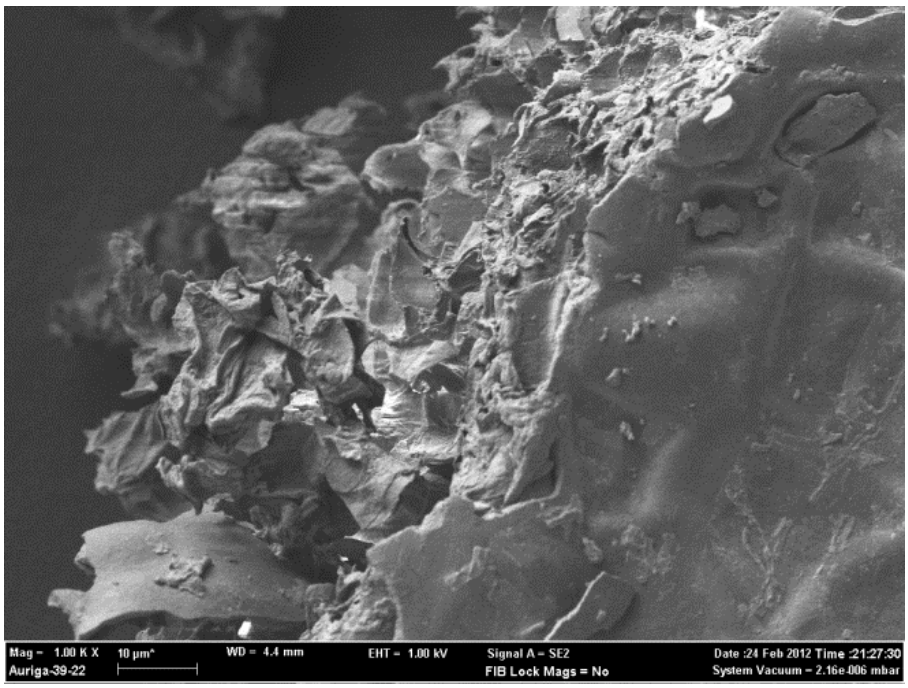


Fig. 5.7: Scanning electron micrographs of microwave-treated sample: (a) plant cells and (b) surface of leave

(a)



(b)

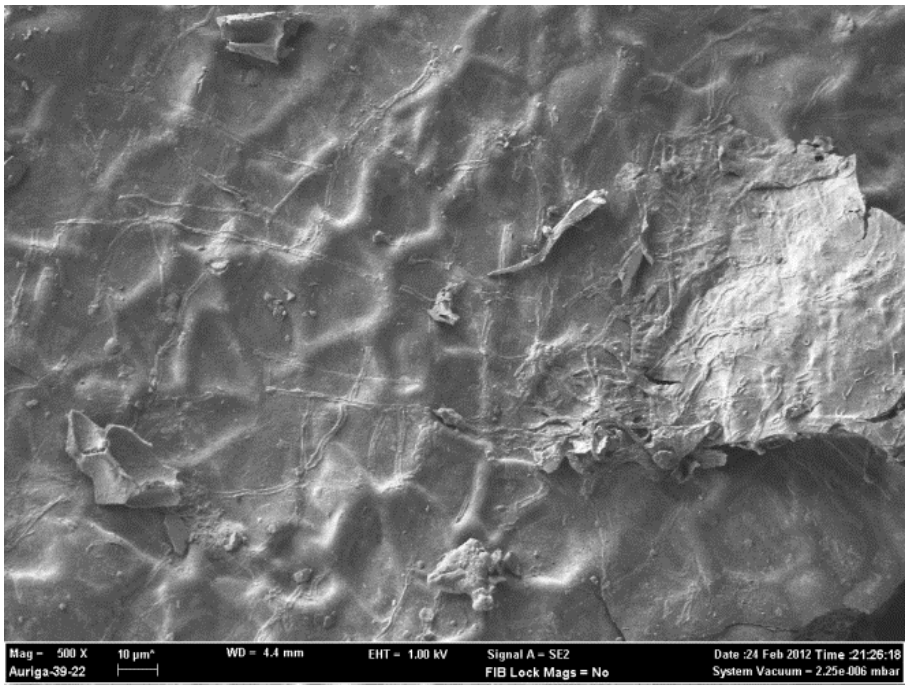


Fig. 5.8: Scanning electron micrographs of sample after Soxhlet extraction: (a) plant cells and (b) surface of leave

5.5. Effects of parameters on MAE kinetics

Having discussed on the optimization of operating parameters for optimum extraction yields, this section investigates the extraction kinetics of MAE under the influences of various factors such as particle size of sample, solvent to feed ratio, microwave power and solvent loading. Patricelli model was employed to determine the extraction rates and extraction yields for both the washing and diffusion steps in MAE. The detail of the experimental data is tabulated in Appendix C. All the extraction curves of MAE obtained in this kinetic study are fitted well with Patricelli model with R-square value greater than 0.97.

5.5.1. Effects of particle size of plant sample

The effect of particle sizes (0.1-1 mm) on the extraction yield of MAE was evaluated in single factor experiments as illustrated in Fig. 5.9. The figure shows that the extraction yields increases when the particle size of sample decreases. Smaller size particles can enhance the surface contact area with the solvent and shorten the diffusion path of active compounds (Cissé et al., 2012; Herodež et al., 2003; Hojnik et al., 2008). As a result, the extraction yields of flavonols (IQ and RT) were significantly improved. This effect is even distinct when the particle size of the sample was reduced below its leaf thickness. As a result, more broken cells were generated and this promotes the washing of active compounds by the solvent. Nevertheless, the effect is insignificant for the extraction of catechin compound (EC) as shown in Fig. 5.9 in which the compound can be extracted easily without having to reduce the sample particle sizes. This suggests that EC has lower mass transfer resistance as compared to IQ and RT. The response of extraction yield of the respective compounds to the influence of particle size indirectly reveals their mass transfer resistance in the extraction. Thus, the mass transfer resistance of active compounds can be expressed in the descending order of RT>IQ>EC.

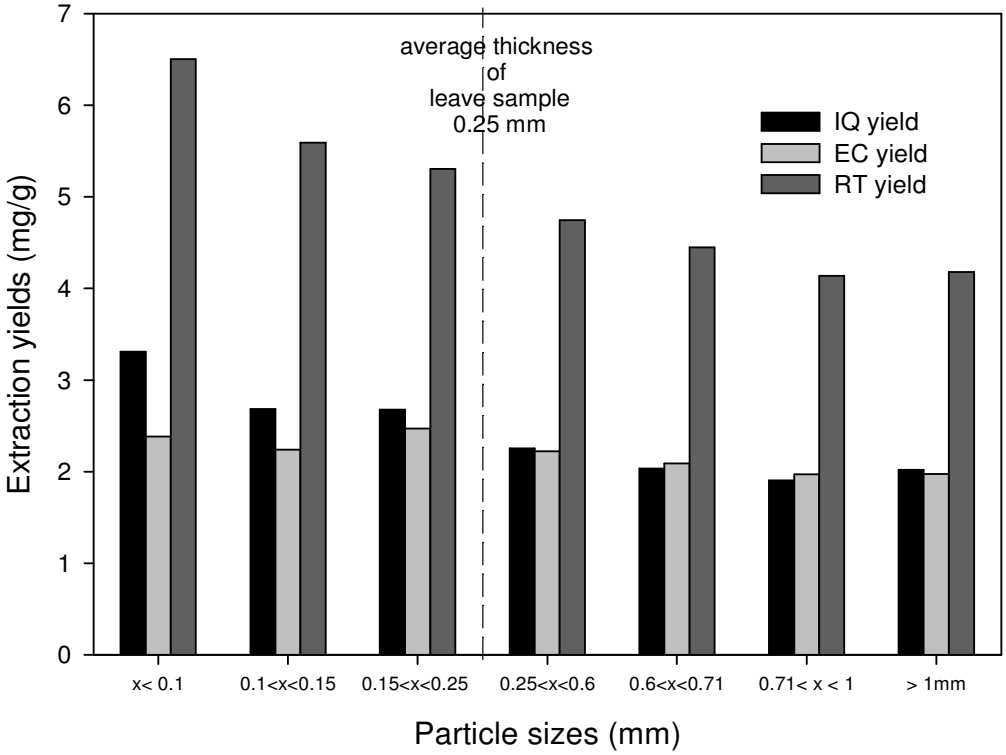


Fig. 5.9: Effect of particle sizes on the extraction yields of IQ, EC and RT (*MAE conditions: 2 g sample, 160 W, 160 ml of 85% (v/v) EtOH and 13 min*)

To explore the effects of particle size on the extraction kinetics of MAE, MAE curves under two ranges of particle sizes, i.e. lower and greater than the average thickness of the plant leaves (0.25 mm) were investigated using Patricelli model. The extraction profiles of MAE are plotted in Fig. 5.10 and the details of the fitted Patricelli model are presented in Table 5.9. It can be seen that additional 15% of equilibrium extraction yields can be achieved when the particle size of sample is reduced below 0.25 mm. This effect is clearly observed in the extraction of IQ and RT but not in the case of EC. This result agrees with the previous results reported that EC can be extracted completely without reducing the sample particle size. The increase of extraction yields could be caused by the washing of active compounds from broken cells at the beginning of the extraction as indicated by high ratio of P_w/P_d shown in Table 5.9.

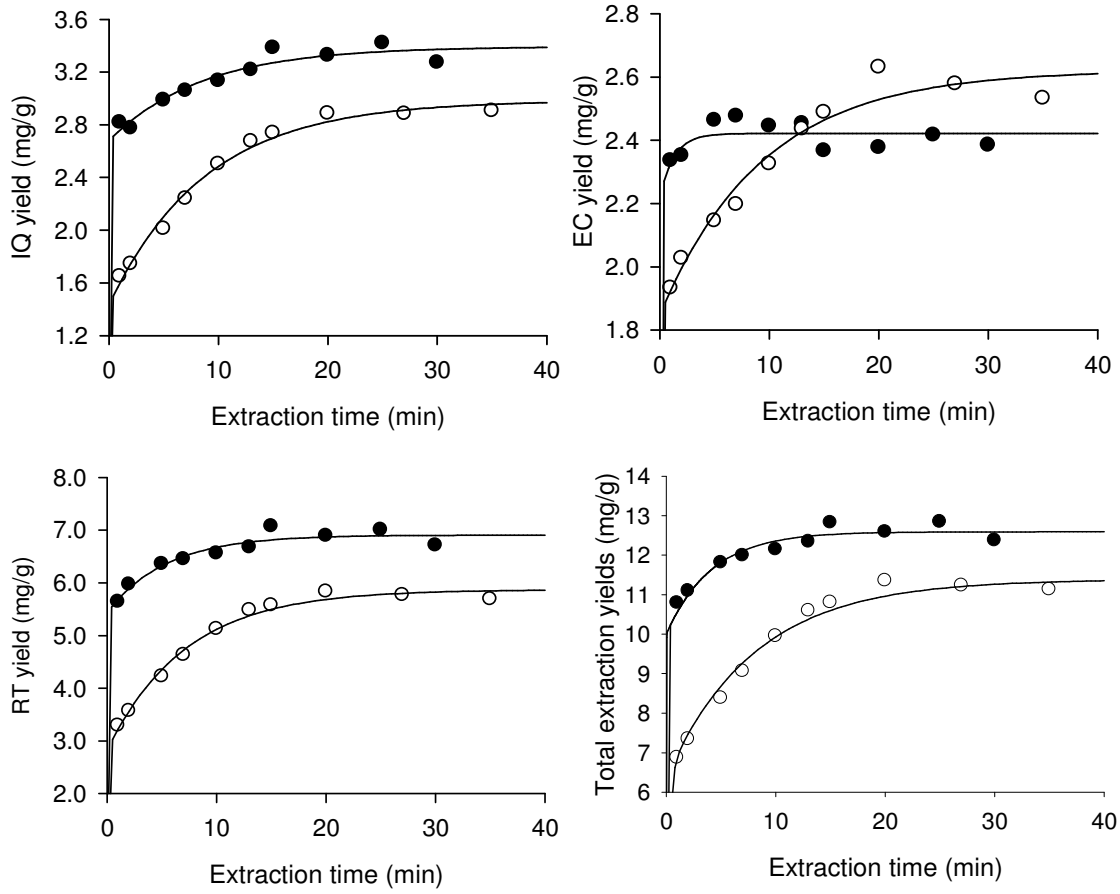


Fig. 5.10: Effect of particle size on the extraction kinetics of MAE (*MAE conditions: 2 g sample, 100 W and 100 ml of 85% (v/v) EtOH*; ● *particle sizes < 0.25 mm;* ○ *particle sizes > 0.25 mm)*

Table 5.9: Coefficient of Patricelli model at varying sample particle size

Compound	Particle sizes (mm)	k_d (min^{-1})	k_w (min^{-1})	P_d (mg/g)	P_w (mg/g)	P_e (mg/g)	P_w/P_d	SSE	Adjusted R-square
IQ	< 0.25	0.12	117.20	0.71	2.68	3.39	3.76	0.03641	0.9961
	> 0.25	0.11	37.13	1.52	1.44	2.96	0.94	0.02413	0.9954
EC	< 0.25	0.75	86.83	0.20	2.23	2.42	11.32	0.01402	0.9962
	> 0.25	0.11	29.30	0.77	1.85	2.62	2.38	0.02164	0.9943
RT	< 0.25	0.18	23.42	1.46	5.45	6.91	3.73	0.1522	0.9946
	> 0.25	0.14	30.38	3.05	2.82	5.87	0.93	0.09578	0.9954
Total	< 0.25	0.24	89.81	2.59	10.00	12.59	3.86	0.3494	0.9968
	> 0.25	0.12	4.16	5.00	6.39	11.39	1.28	0.4323	0.9951

As seen in the Fig. 5.10, the washing step of the MAE is extremely fast that the period associated with this step is difficult to be determined and thus it is impossible to obtain the precise extraction points for the step. Therefore, the coefficient of washing step, k_w obtained from the curve fitting does not represent the real condition as it was determined by minimizing the fitting sum square error. The coefficient k_w in Table 5.9 can only be used to construct the diffusive extraction curves of MAE. With regards to the diffusion step, smaller size of sample improves the extraction rate (indicated by coefficient k_d) as observed in the extraction of EC. Although smaller size of plant sample can enhance the washing of active compounds and the rate of diffusion, it makes the separation of the extract from the residue difficult and incurs additional clean up steps (Chan et al., 2011). Hence, plant sample with particle size of 0.25-0.60 mm was selected for subsequent kinetic studies.

5.5.2. Effect of solvent to feed ratio

The effect of solvent to feed ratio (20, 50 and 80 ml/g) on the extraction kinetics of MAE was evaluated at the same solvent volume as shown in Fig. 5.11. The model parameters determined from the curve fitting are tabulated in Table 5.10. As seen in Fig. 5.11, MAE with high solvent to feed ratio has higher extraction yields as compared to that at low solvent to feed ratio. This could be due to the decrease in the mass transfer barrier during diffusion step (Franco, Pinelo, Sineiro, & Núñez, 2007; Qu, Pan, & Ma, 2010). Once the ratio is increased beyond optimum point, it does not give any significant effect on the equilibrium extraction yields as observed in the extraction of EC. This indicates that EC can be extracted with lesser solvent as compared to the extraction of IQ and RT due to its low mass transfer resistance attributing to its smaller molecular size.

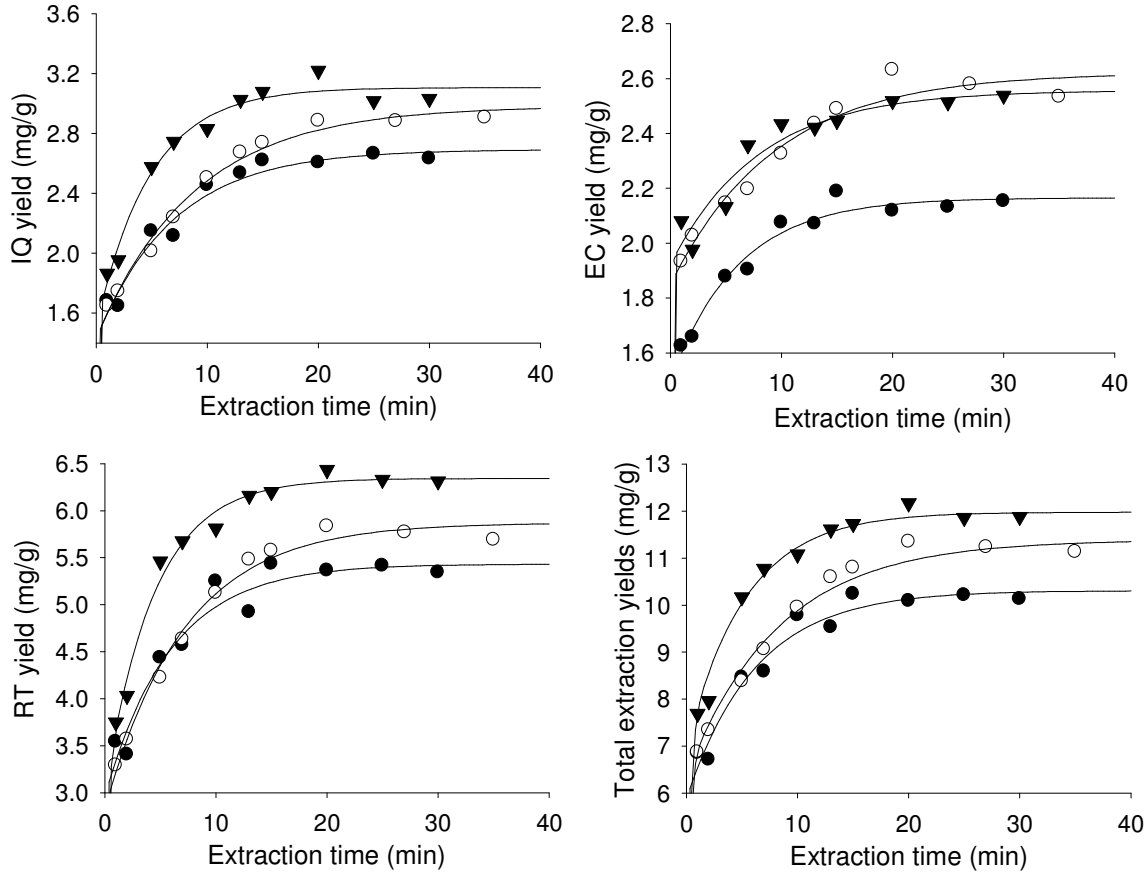


Fig. 5.11: Effect of solvent to feed ratio on the extraction kinetics of MAE
(MAE conditions: sample with particle size of 0.25-0.6 mm, 100 W and 100 ml of 85% (v/v) EtOH; ● *20 ml/g;* ○ *50 ml/g;* ▼*80 ml/g)*

Table 5.10: Coefficient of Patricelli model at varying solvent to feed ratio

compound	Solvent to feed ratio (ml/g)	k_d (min^{-1})	k_w (min^{-1})	P_d (mg/g)	P_w (mg/g)	P_e (mg/g)	P_w/P_d	SSE	Adjusted R-square
IQ	20	0.15	40.80	1.26	1.43	2.69	1.13	0.0479	0.9890
	50	0.11	37.13	1.52	1.44	2.96	0.94	0.0241	0.9954
	80	0.21	41.54	1.61	1.49	3.11	0.93	0.0540	0.9913
EC	20	0.17	33.86	0.67	1.49	2.16	2.23	0.0132	0.9952
	50	0.11	29.30	0.77	1.85	2.62	2.38	0.0216	0.9943
	80	0.12	85.79	0.63	1.92	2.56	3.03	0.0298	0.9921
RT	20	0.17	26.10	2.48	2.96	5.44	1.19	0.2957	0.9837
	50	0.14	30.38	3.05	2.82	5.87	0.93	0.0958	0.9954
	80	5.00	0.23	3.01	3.34	6.34	1.11	0.1335	0.9955
Total	20	0.16	45.39	4.42	5.89	10.30	1.33	0.6568	0.9897
	50	0.12	4.16	5.00	6.39	11.39	1.28	0.4323	0.9951
	80	0.19	3.78	5.00	6.98	11.98	1.40	0.5090	0.9950

In the study on the effect of solvent to feed ratio, the washing step of MAE is unaffected as depicted in Fig. 5.11 and this agrees with the MAE of antioxidants from Balm (*Melissa officinalis* L.) leaves (Herodež et al., 2003). However, the diffusion step of MAE is strongly influenced by solvent to feed ratio. Extraction with high solvent to feed ratio reduces the mass transfer barrier and improves the diffusion of the active compounds in which the coefficient k_d at 80 ml/g are the greatest. However, it is unusual to note that the coefficient k_d of 20 ml/g are higher than that in 50 ml/g. This phenomenon could be due to the saturation of the active compounds in the solvent at 20 ml/g as its equilibrium extraction yields is much lower (Stanisavljević et al., 2007). As a result, shorter extraction time give rise to higher k_d value. Considering the economical and feasibility aspects, subsequent kinetic studies are based on solvent to feed ratio of 50 ml/g.

5.5.3. Effect of microwave irradiation power

In this section, the extraction kinetic of the MAE under the influence of microwave irradiation power (100-600 W) was investigated. The experimental extraction curves were plotted together with the fitted model in Fig. 5.12 and the coefficients of the fitted Patricelli model are tabulated in Table 5.11. The results show that microwave power strongly enhances the diffusion step of MAE. This is because the diffusivity of active compounds increases with temperature (Cissé et al., 2012; Rakotondramasy-Rabesiaka et al., 2007), and the extraction temperature in turn is controlled by the microwave power. About 10 folds increase in the coefficient k_d is observed when the microwave power changes from 100 W to 600 W. Similar findings are also reported in the MAE of oil from olive cake (Amarni & Kadi, 2010). Fig 5.12 shows that the rate of diffusive extraction was improved with the employment of high microwave power. At high microwave power of 300-600 W, though the extraction time of MAE can be shortened

to a few minutes, 10% decrease in the total equilibrium extraction yields was witnessed. Similar observation was also made by Biesaga (2011) on the thermal degradation of active compounds at high microwave power. The highest equilibrium extraction yield can be obtained using low microwave power (100-200 W) as shown in Fig. 5.12 and this confirmed with the reported optimum microwave power of 162 W discussed previously.

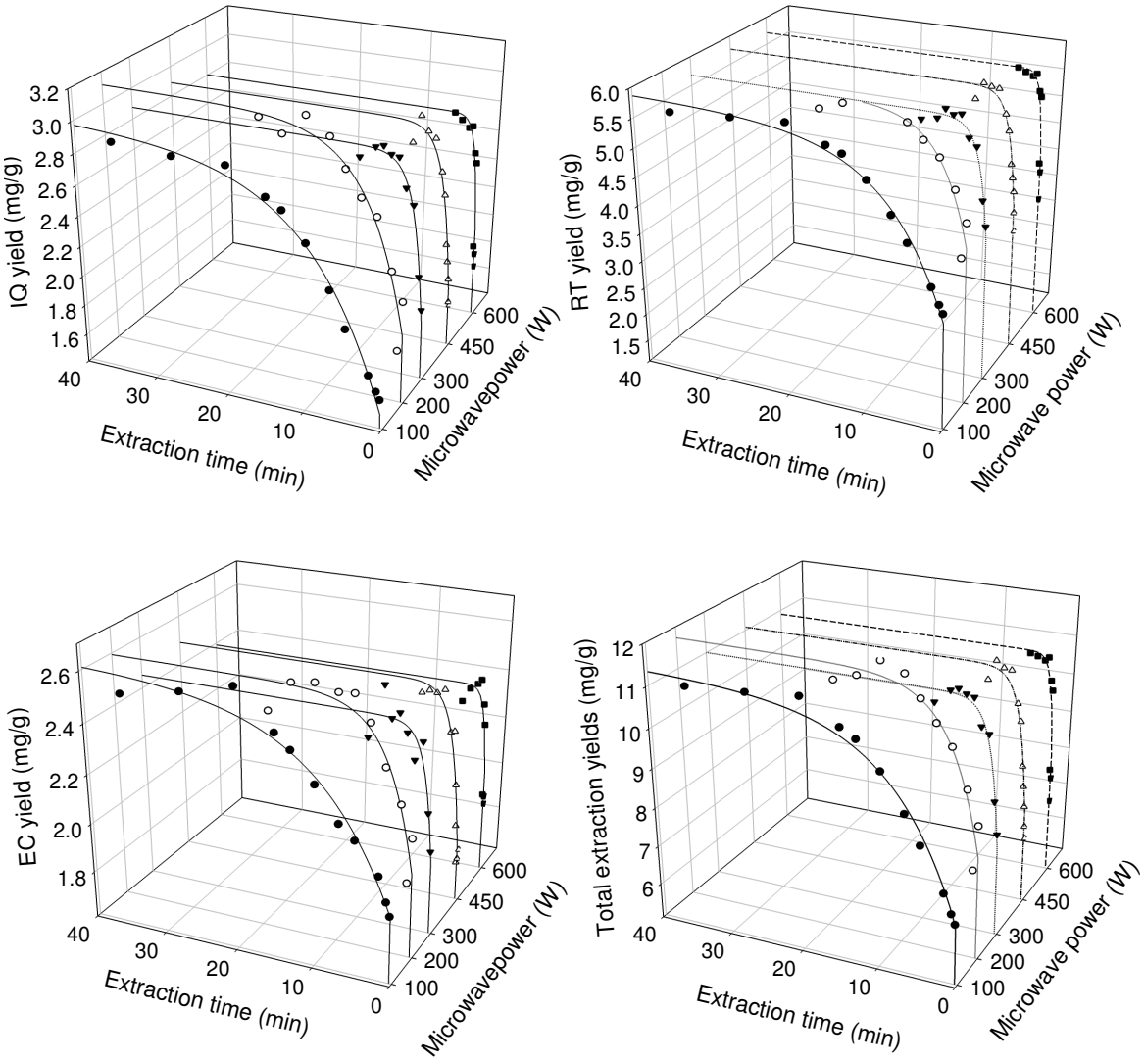


Fig. 5.12: Effect of microwave irradiation power on extraction kinetics of MAE *(MAE conditions: 2 g sample with particle size of 0.25-0.6 mm, 100 ml of 85% (v/v) EtOH and 50 ml/g*; ● *100 W;* ○ *200 W;* ▼ *300 W;* Δ *450 W;* ■ *600 W)*

Table 5.11: Coefficient of Patricelli model at varying microwave power

Compound	Microwave power (W)	k_d (min^{-1})	k_w (min^{-1})	P_d (mg/g)	P_w (mg/g)	P_e (mg/g)	P_w/P_d	SSE	Adjusted R-square
IQ	100	0.11	37.13	1.52	1.44	2.96	0.94	0.0241	0.9954
	200	0.21	68.61	1.37	1.75	3.12	1.28	0.1337	0.9771
	300	0.70	68.35	1.46	1.42	2.88	0.98	0.0177	0.9963
	450	0.72	226.70	1.45	1.44	2.89	0.99	0.0466	0.9911
	600	1.56	173.10	1.45	1.35	2.80	0.93	0.0851	0.9808
EC	100	0.11	29.30	0.77	1.85	2.62	2.38	0.0216	0.9943
	200	0.30	51.76	0.73	1.87	2.59	2.58	0.0578	0.9857
	300	0.82	68.19	0.76	1.69	2.45	2.23	0.0380	0.9890
	450	0.78	180.20	0.89	1.61	2.49	1.82	0.0477	0.9873
	600	1.77	202.20	0.73	1.66	2.39	2.28	0.0414	0.9869
RT	100	0.14	30.38	3.05	2.82	5.87	0.93	0.0958	0.9954
	200	0.29	57.41	2.44	3.61	6.05	1.48	0.6102	0.9730
	300	0.82	70.71	2.79	2.89	5.68	1.04	0.1028	0.9945
	450	0.92	149.00	2.93	2.84	5.77	0.97	0.1264	0.9941
	600	1.98	50.21	3.29	2.43	5.72	0.74	0.3362	0.9824
Total	100	0.12	4.16	5.00	6.39	11.39	1.28	0.4323	0.9951
	200	0.27	88.46	4.50	7.24	11.74	1.61	1.7640	0.9789
	300	0.78	68.28	4.99	6.01	11.00	1.20	0.2896	0.9959
	450	0.81	184.50	5.00	6.09	11.09	1.22	0.4847	0.9945
	600	1.67	23.34	5.00	5.90	10.90	1.18	1.1470	0.9854

Fig. 5.12 shows that the extraction yield during washing step (P_w) was not affected by the microwave power. This is indicated by the similar ratio of P_w/P_d for all the extraction curves. Generally, the influencing effects of microwave power on the extraction kinetics of IQ, EC and RT are of same magnitude. Since the MAE favors low microwave power, thus nominal power density, i.e. nominal microwave power per unit solvent volume of 1 W/ml will be applied in the subsequent study to investigate the effect of solvent loading.

5.5.4. Effect of solvent loading

The effect of solvent loading is important for the scaling up of MAE. In this section, the kinetic of MAE under the effects of solvent loading of 100-300 ml are evaluated at selected solvent to feed ratio of 50 ml/g under nominal power density of 1 W/ml. The experimental curves with the fitted Patricelli model are plotted as shown in Fig. 5.13 and the model parameters are presented in Table 5. 12.

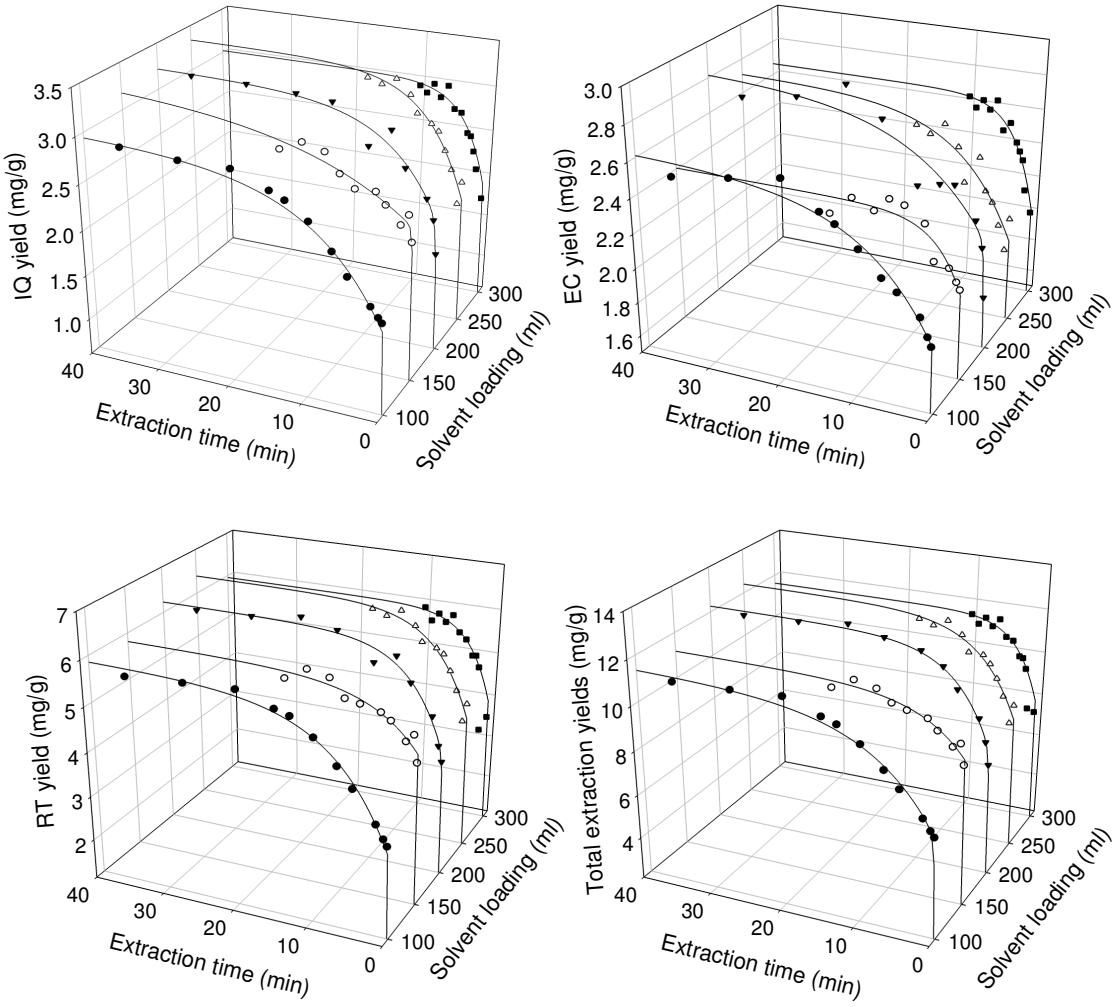


Fig. 5.13: Effect of solvent loading on extraction kinetics of MAE
(MAE conditions: 2 g sample with particle size of 0.25-0.6 mm, solvent of 85% (v/v) EtOH, 50 ml/g and nominal power density of 1 W/ml; ● 100 ml; ○ 150 ml; ▼ 200 ml; ⊿ 250 ml; ■ 300 ml)

Table 5.12: Coefficient of Patricelli model of MAE under the effect of solvent loading

Compound	Solvent loading (W)	k_d (min^{-1})	k_w (min^{-1})	P_d (mg/g)	P_w (mg/g)	P_e (mg/g)	P_w/P_d	SSE	Adjusted R-square
IQ	100	0.11	37.13	1.52	1.44	2.96	0.94	0.0241	0.9954
	150	0.07	5.49	0.99	2.26	3.25	2.28	0.0553	0.9884
	200	0.15	3.38	1.27	1.96	3.22	1.55	0.0764	0.9882
	250	0.19	45.19	1.43	1.91	3.34	1.33	0.0949	0.9851
	300	0.31	14.71	1.25	1.77	3.02	1.42	0.0475	0.9918
EC	100	0.11	29.30	0.77	1.85	2.62	2.40	0.0216	0.9943
	150	0.29	64.55	0.47	1.94	2.41	4.15	0.0339	0.9902
	200	0.10	3.76	0.75	2.08	2.83	2.77	0.0690	0.9847
	250	0.14	149.60	0.78	1.94	2.71	2.50	0.0744	0.9811
	300	0.33	180.20	0.73	1.93	2.67	2.64	0.0405	0.9910
RT	100	0.14	30.38	3.05	2.82	5.87	0.92	0.0958	0.9954
	150	0.16	138.70	1.44	4.38	5.82	3.04	0.2295	0.9878
	200	0.22	5.58	2.60	3.64	6.23	1.40	0.1894	0.9923
	250	0.21	156.80	2.51	3.89	6.40	1.55	0.3038	0.9872
	300	0.31	114.30	2.26	3.69	5.95	1.64	0.1082	0.9951
Total	100	0.12	4.16	5.00	6.39	11.39	1.28	0.4323	0.9951
	150	0.17	80.42	2.70	8.48	11.18	3.15	0.5406	0.9922
	200	0.21	4.72	4.88	7.39	12.26	1.51	0.1808	0.9981
	250	0.19	96.42	4.67	7.74	12.41	1.66	1.1950	0.9864
	300	0.32	166.30	4.26	7.36	11.61	1.73	0.3768	0.9956

Supposedly, MAE conducted at larger scales e.g. 100-300 ml under the same solvent to feed ratio and the same heating power density would give constant rate of extraction and extraction yields. The result obtained shows otherwise as can be seen in Table 5.12. When conducting the MAE at larger scales, the extraction coefficients of diffusion step (k_d) are changed while the extraction yields (P_e) are not significantly affected. Fig. 5.13 demonstrates that at high solvent loading, MAE requires shorter time than at low solvent loading under the same nominal power density. This implies that higher solvent loading tends to speed up microwave heating and the nominal power density can not effectively be used as reference for scaling up of MAE. Thus, the exploration for other extraction parameter i.e. absorbed power density (APD) to account the absorbed power in the MAE system is being carried out which leads to the development of APD predictive method.

5.6. Absorbed power density (APD) predictive method

In this section, APD is introduced and its prediction method is discussed. The method enables the prediction of optimum extraction time for MAE at various extraction conditions. The experimental data used in the development and evaluation of the method can be found in Appendix C.

5.6.1. Introduction to absorbed power density (APD)

APD accounts for the real power to be used for heating up the solvent during extraction. It can be used to indicate the interactive effect between the solvent loading and the nominal microwave power as demonstrated in Fig. 5.14. The figure shows that the absorbed power increases exponentially when the nominal microwave power increases. It also increases with solvent loading especially at low solvent volume. This is because the solvent level was closed to microwave penetration depth of the solution, e.g. 1.99 cm for water and 0.42 cm for ethanol (Horikoshi, Abe, & Serpone, 2009). Under this condition, some of the unabsorbed microwave was reflected and resulted in poor power absorption (Kingston & Jassie, 1988). When the level of the solvent increase farther from the microwave penetration depth of the solvent, the influence of solvent loading on the absorption of microwave power become less significant and the heating will be due to thermal conduction through molecular collision (Kingston & Jassie, 1988). The result showed in Fig. 5.14 explains the inconsistency in extraction result when conducting large scale MAE under the nominal power density of 1 W/ml as previously discussed in section 5.5.4. Since the absorbed power changes in accordance with the changes of solvent loading and nominal microwave power, it can be a significant parameter for MAE. With that, absorbed power density (APD), which is defined as the absorbed microwave power per unit solvent volume, are used as a parameter in the prediction of optimum extraction time for MAE as demonstrated in the subsequent sections.

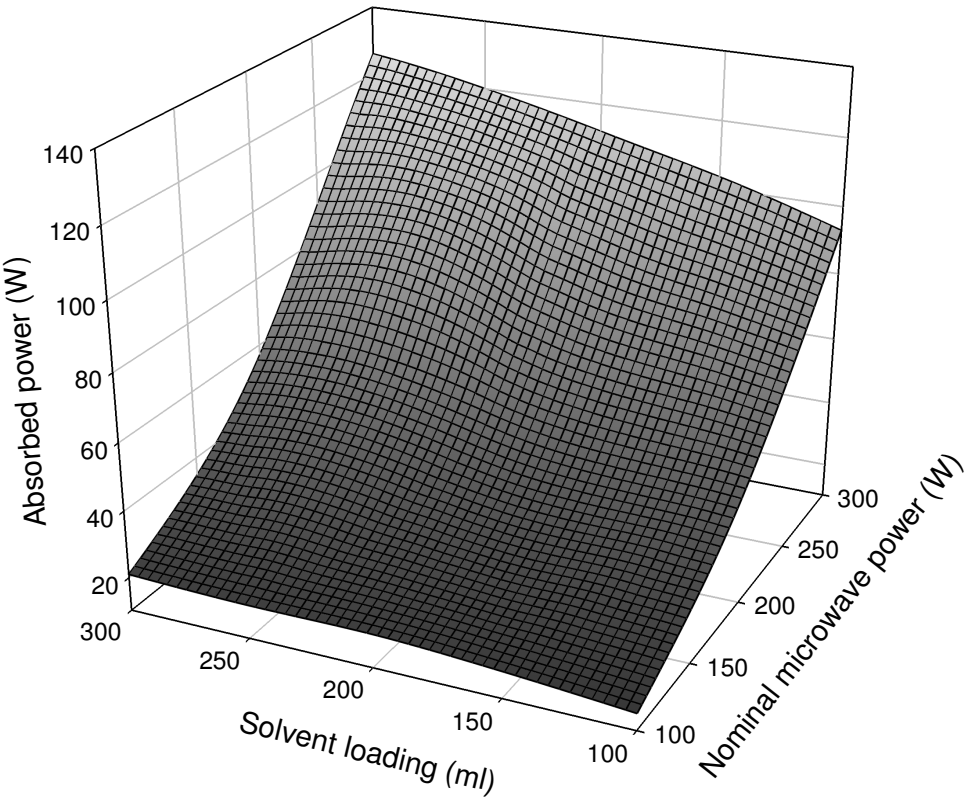


Fig. 5.14: Microwave power absorbed in the solvent under the effect of solvent loading and nominal microwave power

5.6.2. Correlation of optimum extraction time region and APD

The prediction method was established based on the correlation between optimum extraction time region and absorbed power density (APD) of the extraction system. The optimum extraction time region is referring to the required extraction time of MAE to achieve 80-95% of the total extraction yields during diffusion step. Applying Patricelli model previously developed for the MAE, the optimum extraction time region and its respective extraction yields under various microwave irradiation power (100-600 W) and solvent loading of 100 ml were determined as shown in Table 5.13. The optimum extraction time region was then correlated with the respective APD values as shown in Fig. 5.15.

Table 5.13: APD values and optimum extraction time region at different microwave irradiation power

Power (W)	Volume (ml)	Absorbed power density, APD (W/ml)	Optimum extraction time region (min)		Optimum range of total extraction yields (mg/g)	
			$t_{80\%}$	$t_{95\%}$	$Y_{80\%}$	$Y_{95\%}$
100	100	0.15	12.9	23.9	10.36	11.11
200	100	0.43	6.0	11.1	10.84	11.51
300	100	0.93	2.1	3.8	10.00	10.75
450	100	1.35	2.0	3.7	10.10	10.85
600	100	2.24	1.0	1.8	9.90	10.65

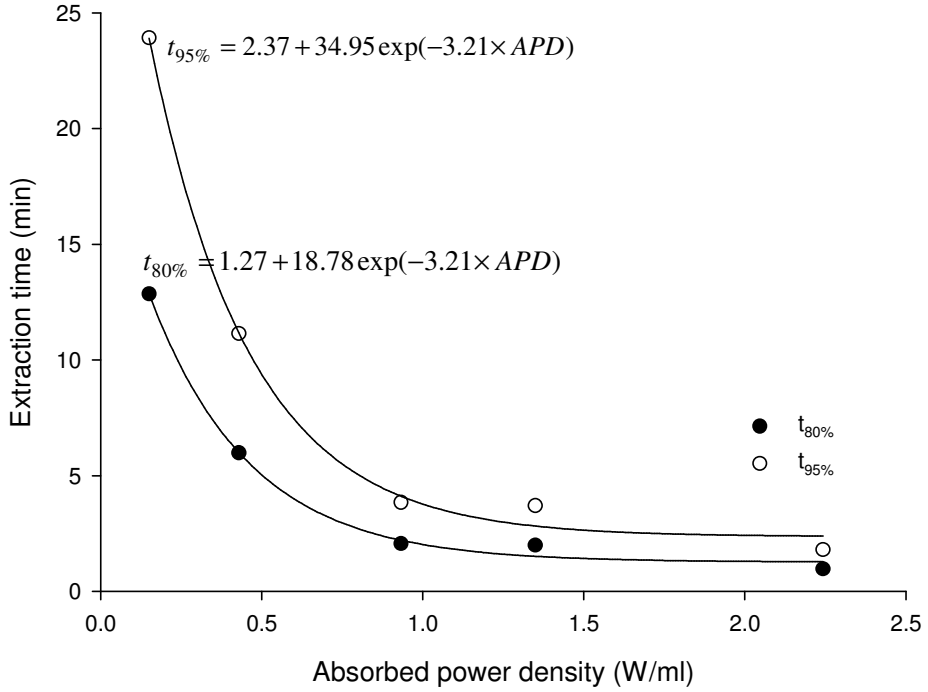


Fig. 5.15: Correlation of optimum extraction time region and APD for MAE (*MAE conditions: 50 ml/g, 100 ml and 100-600 W*)

The established correlations are described in Eq. (29) and Eq. (30).

$$t_{95\%} = 2.37 + 34.95 \exp(-3.21 \times APD) \quad (29)$$

$$t_{80\%} = 1.27 + 18.78 \exp(-3.21 \times APD) \quad (30)$$

where t is extraction time in min and APD is the absorbed power density in W/ml. As shown in Fig. 5.15, the area between the two plots indicates the optimum extraction time region. This region decreases exponentially as the APD increases due to the rapid heating at higher microwave power. High APD resulted in narrow optimum extraction region, thus the prediction of optimum extraction time at this condition is critical as a slight increase above the optimum region will cause significant overheating. The extraction carried out at high APD values reduces the extraction time significantly and also, it affects the equilibrium extraction yields. More than 5% decrease in total yields was observed when the extraction was carried out at high microwave power (>300 W) with corresponding APD value greater than 1.0 W/ml. The correlation of the optimum extraction time region (Eq. (29) and Eq. (30)) was evaluated in terms of their predictive capability for larger scales (150-300 ml) as well as their applicability at different solvent to feed ratios (20, 50, 80 ml/g).

5.6.3. Verification of APD method for large scale MAE

The prediction of optimum extraction time of MAE at large scale MAE (150-300 ml) based on the established correlations are verified in this section. The verification experiments show that the experimental optimum time, t_{opt} of MAE at larger scale extraction falls within the predicted optimum time region by the APD method as tabulated in Table 5.14. In this work, t_{opt} was determined experimentally when 95% of the total extraction yield was achieved during the diffusion step. Table 5.14 shows that the experimental optimum extraction time of MAE decreases from 18 min to 8.3 min

when MAE was conducted at solvent loading of 150 ml to 300 ml respectively under the same solvent to feed ratio (50 ml/g) and nominal power density (1 W/ml). This implies that the increase in solvent loading enhances the absorption of microwave power in the solvent (Kingston & Jassie, 1988). This effect is reflected by the APD values varying from 0.18-0.42 W/ml.

Table 5.14: Predicted and experimental optimum extraction time of MAE at scaled-up conditions

Mass of sample (g) [a]	Solvent loading (ml)	Microwave power (W) [b]	APD (W/ml)	Predicted optimum region		Exp. optimum extraction time, t_{opt} (min)	Exp. yields at t_{opt} (mg/g)
				$t_{80\%}$ (min)	$t_{95\%}$ (min)		
3	150	150	0.18	11.7	21.8	18.0	11.05
4	200	200	0.25	9.8	18.2	14.4	12.02
5	250	250	0.37	7.0	13.1	9.1	11.69
6	300	300	0.42	6.2	11.5	8.3	11.29

[a] Varying mass of sample at each solvent loading to maintain fixed solvent to feed ratio of 50 ml/g. [b] Increasing microwave power at each solvent loading to maintain the same nominal power density of 1 W/ml.

This proposed method is useful in predicting optimum extraction time for larger scale of MAE. A key feature of the method is the advantages of APD over nominal power density as APD can characterize the extraction kinetic of MAE at the diffusion step. Moreover, APD accounts for the real power absorbed in the extraction system whereas nominal power density only serves as an indicator for the power setting of the microwave extractor employed. This strongly suggests that APD is more suitable than nominal microwave power or power density as it reflects the real power used for extraction regardless of the instrumentation setup of the microwave extractor.

5.6.4. Validation of APD method at different solvent to feed ratio

According to the extraction kinetic of MAE, the diffusion step is not only influenced by microwave heating (imposed by the APD value), and it may also be affected by other extraction parameters that have no effect on the absorption of microwave power such as solvent to feed ratio (constant volume). This has prompted the validation study of the APD predictive method for the two extremes of solvent to feed ratios, e.g. 20 and 80 ml/g as plotted in Fig. 5.16. The validation results shows that the experimental optimum extraction time of MAE at 20 ml/g and 80 ml/g were determined to be 16.1 min and 18.5 min respectively which are still bounded by the predicted region (13-24 min) of 50 ml/g. This result confirms the influence of solvent to feed ratio on the prediction is not crucial as the optimum extraction time is in the predicted region.

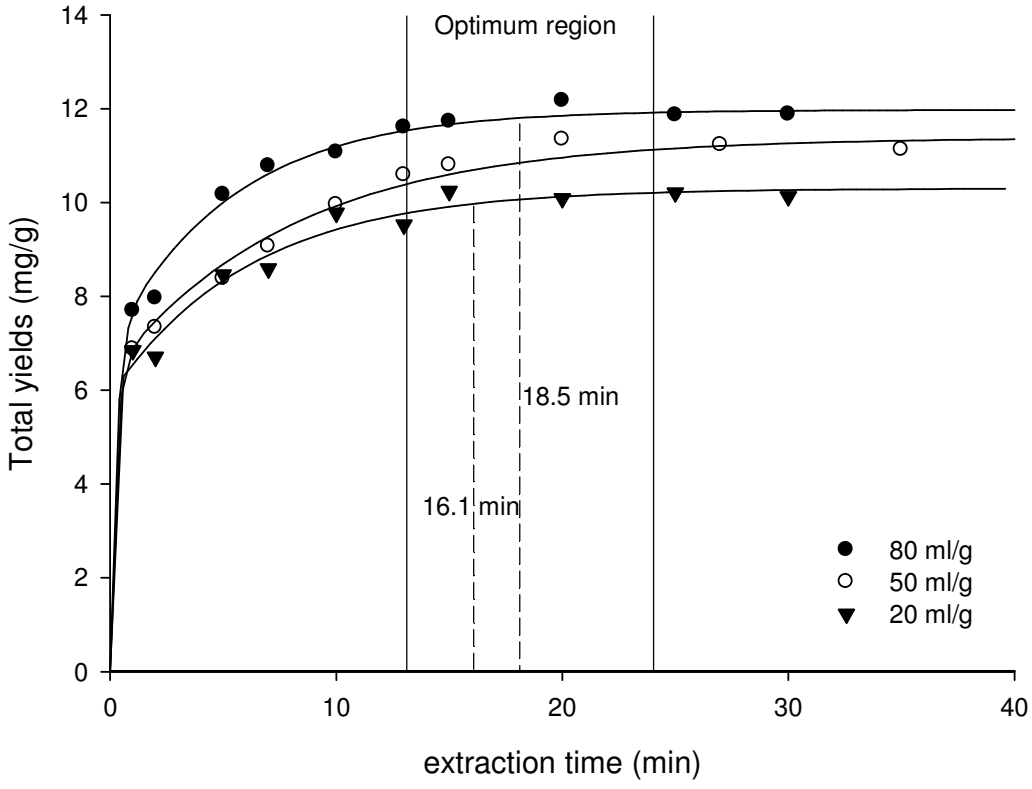


Fig. 5.16: The influence of solvent to feed ratio on the APD prediction method (*MAE condition: 20-80 ml/g, 100 ml and 100 W*)

5.6.5. Determination of optimum extraction time of MAE at various conditions

Having discussed on the verification and validation of the APD predictive method at large scale extraction and at various solvent to feed ratio, this section determines the optimum extraction time of MAE at random extraction conditions based on the established correlations as tabulated in Table. 5.15. The corresponding extraction yields obtained were compared with those from Soxhlet extractions. The result shows that more than 85% of total extraction yields can be recovered if the extraction was carried out in the predicted optimum extraction time region. This signifies that the APD method is capable of predicting optimum extraction time for MAE.

Table 5.15. Recovery of total extraction yields of MAE at the predicted optimum extraction region

MAE conditions	APD (W/ml)	Predicted optimum region		Experimental		
		$t_{80\%}$ (min)	$t_{95\%}$ (min)	Extraction Time (min)	Total extraction Yields (mg/g)	Recovery (%) [a]
3 g, 150 ml, 100 W	0.12	13.9	25.8	18.0	11.28 ± 0.48	97
3 g, 150 ml, 300 W	0.69	3.3	6.1	6.0	10.03 ± 0.35	86
4 g, 200 ml, 100 W	0.10	14.8	27.6	20.0	11.49 ± 0.16	99
4 g, 200 ml, 300 W	0.56	4.4	8.2	7.2	10.96 ± 0.21	94
5 g, 250 ml, 100 W	0.08	15.6	29.1	17.5	10.76 ± 0.80	93
5 g, 250 ml, 300 W	0.48	5.4	10.0	6.5	10.12 ± 0.41	86
6 g, 300 ml, 100 W	0.07	16.3	30.3	18.0	10.55 ± 0.63	91
6 g, 300 ml, 200 W	0.19	11.6	21.5	12.0	10.66 ± 0.17	91
Soxhlet conditions		**Total extraction yields (mg/g)**				
2 g, ETOH, 200 ml, 6 hr		11.67 ± 0.41				

[a] The recovery of total extraction yield (%) was calculated based on Soxhlet extraction (total extraction yields of 11.67 mg/g)

Fig. 5.17 illustrates the selection of optimum extraction time of MAE for the random extraction conditions based on the predicted optimum extraction time region. As can be seen, conducting the extraction close to the upper boundary of the optimum region ensures high extraction yields. The figure also shows that the recovery of extraction yields at high APD values was lower than that conducted at low APD values probably caused by thermal degradation.

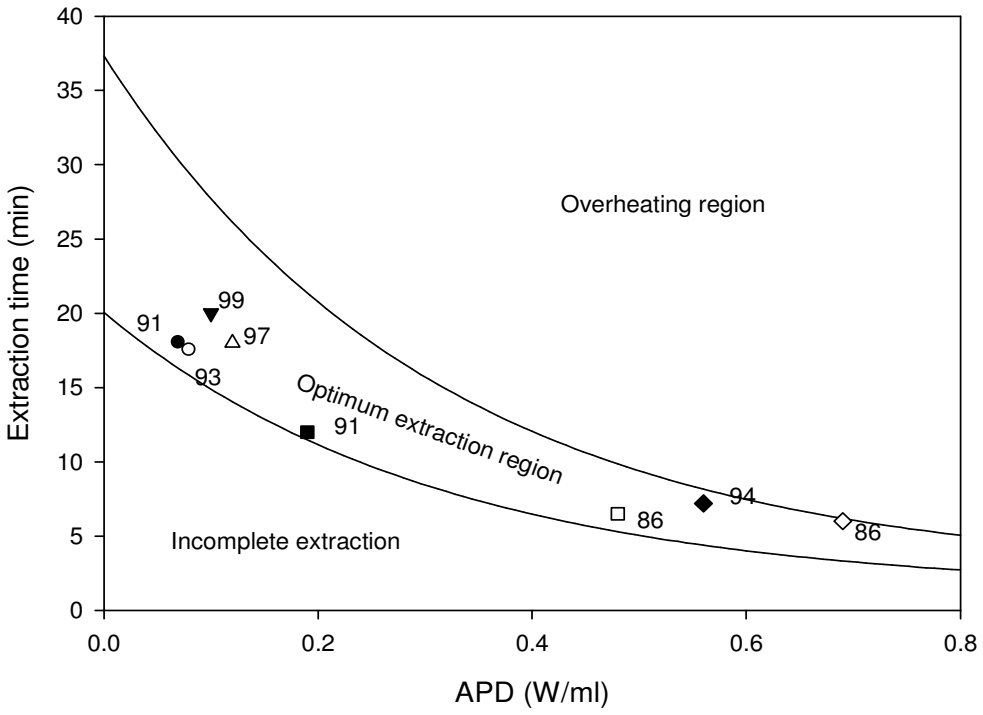


Fig. 5.17: Selection of optimum extraction time of MAE based on APD method (*The values besides the extraction points indicate the percentage recovery of total extraction yields with Soxhlet extraction as reference; MAE conditions: ● 6 g sample, 100 W, 300 ml, 18 min; ○ 5 g sample, 100 W, 250 ml, 17.5 min; ▼ 4 g sample, 100 W, 200 ml, 20 min; ∆ 3 g sample, 100 W, 150 ml, 18 min; ■ 6 g sample, 200 W, 300 ml, 12 min; □ 5 g sample, 300 W, 250 ml, 6.5 min; ♦ 4 g sample, 300 W, 200 ml, 7.2 min; ◇ 3 g sample, 300 W, 150 ml, 6 min*)

The ultimate aim of the APD method is to correlate the optimum extraction time region with the APD of the extraction conditions as plotted in Fig. 5.18. The correlations obtained can subsequently be applied to determine optimum extraction time of MAE at various solvent loading and microwave irradiations power. The operating parameters

mentioned have impact on the rate of microwave heating which indirectly affect the APD value. Nevertheless, the proposed method is not recommended for the prediction of optimum extraction time of MAE at other operating parameters such as solvent to feed ratio and solvent concentration. This is because they can directly affect the kinetic of the washing and diffusion steps rather than the rate of microwave heating during extraction. Overall, APD was found to be a good parameter for extraction time prediction as it characterizes the extraction kinetics of MAE as shown in Fig. 5.18. The APD predictive method provides a means for the establishment of correlation at specific solvent to feed ratio to predict optimum extraction time of MAE with good accuracy and also for larger scale microwave extraction.

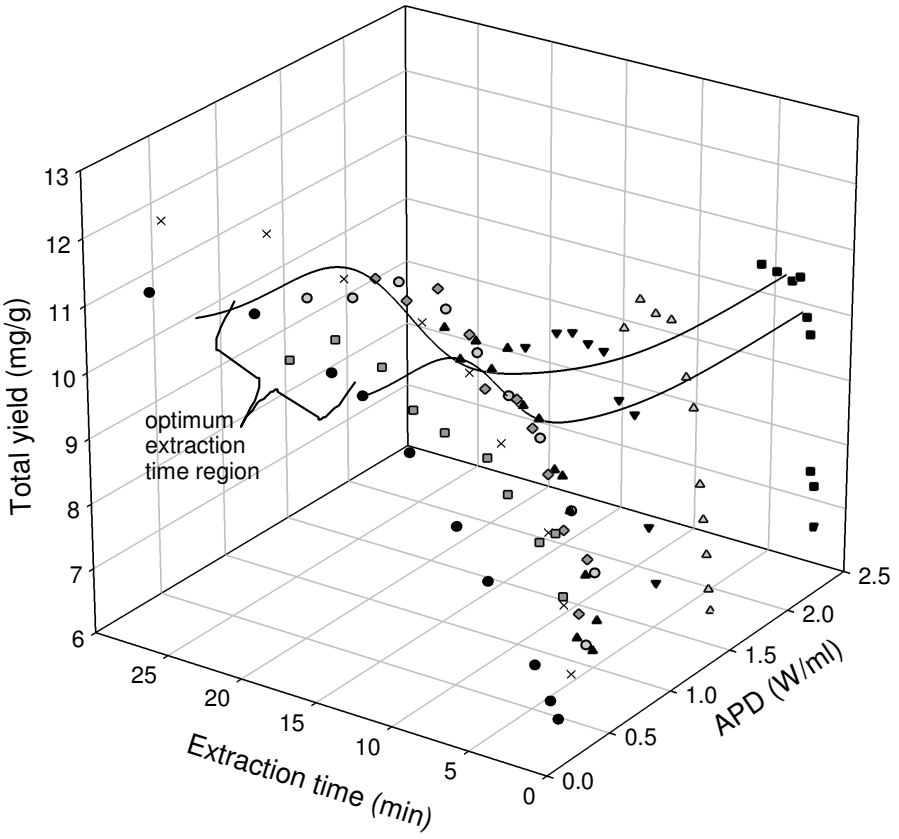


Fig. 5.18: The extraction profiles of MAE under the effect APD
(*— boundaries of optimum extraction time region; MAE conditions: ● 2 g sample, 100 W, 100 ml; ○ 2 g sample, 200 W, 100 ml; ▼ 2 g sample, 300 W, 100 ml; ∆ 2 g sample, 450 W, 100 ml; ■ 2 g sample, 600 W, 200 ml; □ 3 g sample, 150 W, 150ml; ✕ 4 g sample, 200 W, 200 ml; ◇ 5 g sample, 250 W, 250 ml; ▲ 6 g sample, 300 W, 300 ml*)

5.7. Absorbed energy density (AED) modeling method

The capability of APD can be extended by introducing AED, which is the microwave energy absorbed in the solvent during the extraction (J/ml). AED can be calculated by multiplying the APD with the extraction time. The application of AED in the modeling and prediction of overall extraction curves of MAE are demonstrated in this section. By determining the basic coefficients of the model using an extraction curve of MAE, the proposed model can predict extraction curves of MAE at various solvent loading and microwave irradiation power based solely on their APD values. The experimental data used for the investigation are tabulated in Appendix C.

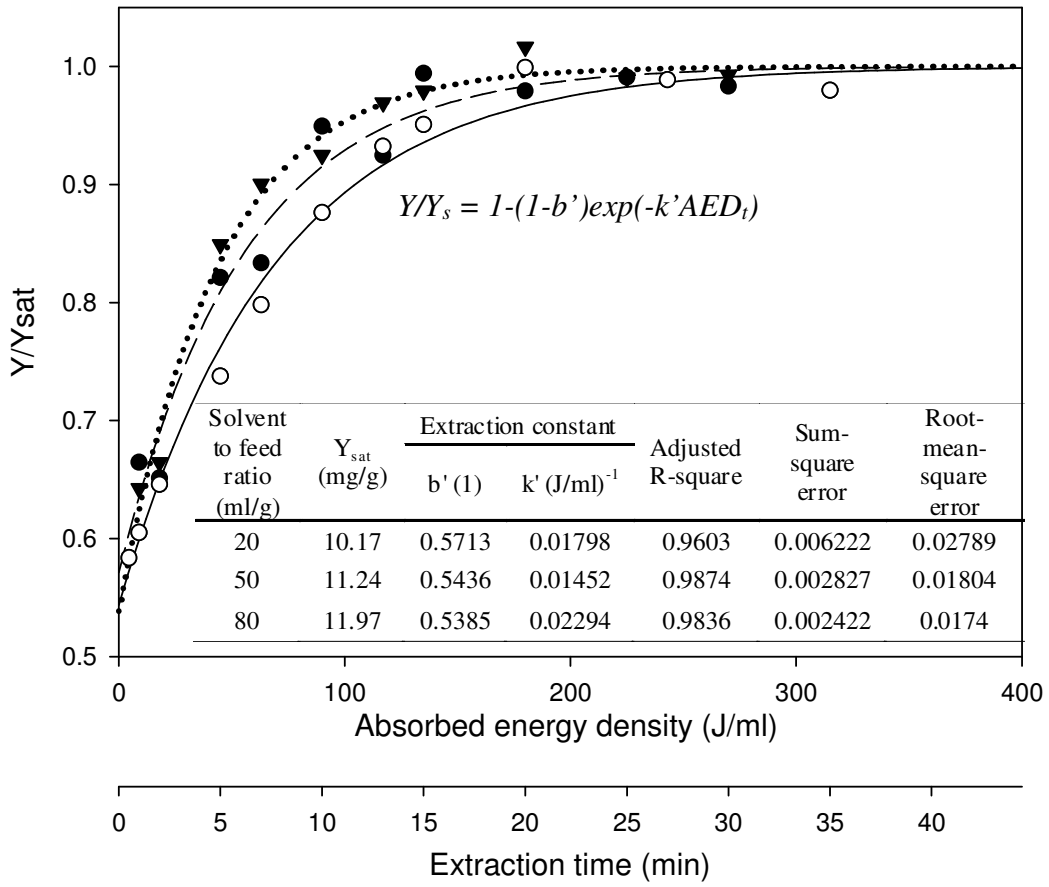
5.7.1. AED extraction model

The modeling of MAE was performed by using AED-adapted film theory in Eq. (20).

$$\frac{Y}{Y_s} = 1 - (1 - b')\exp(-k' \cdot AED_t) \tag{20}$$

The extraction constants (*b'* and *k'*) in Eq. (20) under influence of AED are strongly influenced by other parameters such as sample preparation techniques, e.g. sample drying and grinding (So & Macdonald, 1986), solvent concentration and solvent to feed ratio. In this study, the extraction constants under the effects of solvent to feed ratio (20, 50 and 80 ml/g) were determined by fitting Eq. (20) with the experimental extraction curves as illustrated in Fig. 5.19. The extraction constants, *b'* and *k'* obtained from the non-linear regression indicate that all the extraction curves fitted well with the adapted model (Eq. (20)) as shown in Fig. 5.19. The figure illustrates that MAE at different solvent to feed ratio give different extraction profile and the equilibrium extraction yield increased with solvent to feed ratio as discussed previously. At low solvent to feed ratio, the mass transfer barrier affects the diffusion of active compounds from the plant cell which resulted in poor extraction yield (Franco, Pinelo, et al., 2007;

Qu et al., 2010). In other words, high solvent to feed ratio would enhance the diffusion step (*k'*). In view of the kinetic of the extraction, the equilibrium extraction yield of 20 ml/g solvent to feed ratio was lower than that in 50 ml/g despite that the diffusion constant (*k'*) of the former extraction was higher. This is probably caused by the saturation of extraction solvent at 20 ml/g which has shortened the time for the extractions to reach the equilibrium stage (resulted in higher *k'* value) as previously discussed in section 5.5.2. For evaluation purpose, the proposed model at solvent to feed ratio of 50 ml/g was used in subsequent modeling studies.



Solvent to feed ratio (ml/g)	Y_{sat} (mg/g)	Extraction constant b' (1)	Extraction constant k' $(J/ml)^{-1}$	Adjusted R-square	Sum-square error	Root-mean-square error
20	10.17	0.5713	0.01798	0.9603	0.006222	0.02789
50	11.24	0.5436	0.01452	0.9874	0.002827	0.01804
80	11.97	0.5385	0.02294	0.9836	0.002422	0.0174

Fig. 5.19: Curve fitting of extraction profiles of MAE with AED kinetic model (*MAE conditions: 100 W, 100 ml and APD of 0.15 W/ml; ● 20 ml/g; ○ 50 ml/g; ▼ 80 ml/g; the constant b' and k' were determined with 95% confidence bounds*)

5.7.2. Predictive capability of AED extraction model

The capability of the AED extraction model to predict extraction curves of MAE at various extraction conditions are evaluated in this section. The AED extraction model at solvent to feed ratio of 50 ml/g is expressed in terms of extraction time (s) and APD (W/ml) as shown in Eq. (31).

$$\frac{Y}{Y_s} = 1 - (1 - 0.5436)\exp(-0.01452 * APD * t) \tag{31}$$

Based on APD, Eq. (31) was employed to predict the extraction profile of MAE under various solvent loading (100-300 ml) and nominal microwave power (200-600 W). The prediction results are tabulated in Table 5.16 and the predicted extraction curves together with the experimental extraction curves are depicted in Fig. 5.20 – Fig. 5.25.

Table 5.16: Comparison of the extraction constant (*b* and *k*) obtained from the prediction by the AED extraction model and from the curve fitting by original film theory equation

Extraction conditions	APD (W/ml)	Predicted by the AED extraction model		Curve fitting by original film theory		Percentage difference (%)		Experimental	
		b (1)	*k* (min^{-1})	*b* [a] (1)	*k* [a] (min^{-1})	*b*	*k*	Time required to reach equilibrium (min)	Equilibrium extraction yields, Y_{sat} (mg/g)
2 g, 100 ml, 200 W	0.43	0.5436	0.3746	0.6170	0.2687	11.8	39.4	10	11.64
2 g, 100 ml, 300 W	0.93	0.5436	0.8102	0.5458	0.7812	0.4	3.7	4	10.96
2 g, 100 ml, 600 W	2.24	0.5436	1.9515	0.5032	1.8200	8.0	7.2	2	10.76
4 g, 200 ml, 200 W	0.25	0.5436	0.2178	0.5613	0.2321	3.2	6.2	20	12.22
5 g, 250 ml, 250 W	0.37	0.5436	0.3223	0.5432	0.3127	0.1	3.1	12	11.97
6 g, 300 ml, 300 W	0.42	0.5436	0.3659	0.5717	0.3933	5.0	6.7	10	11.29

[a] values obtained by curve fitting with 95% confidence bounds

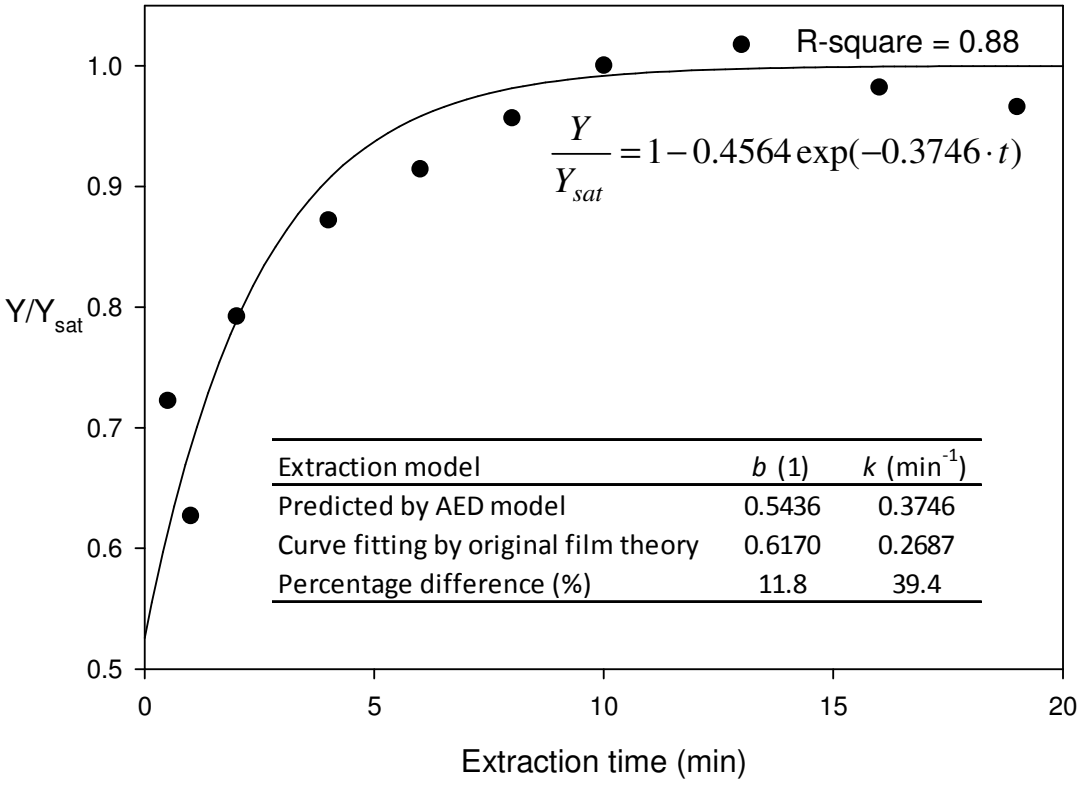


Fig. 5.20: Prediction of extraction curves by using AED extraction model (*MAE conditions: 2 g sample, 100 ml, 200 W and APD of 0.43W/ml)*

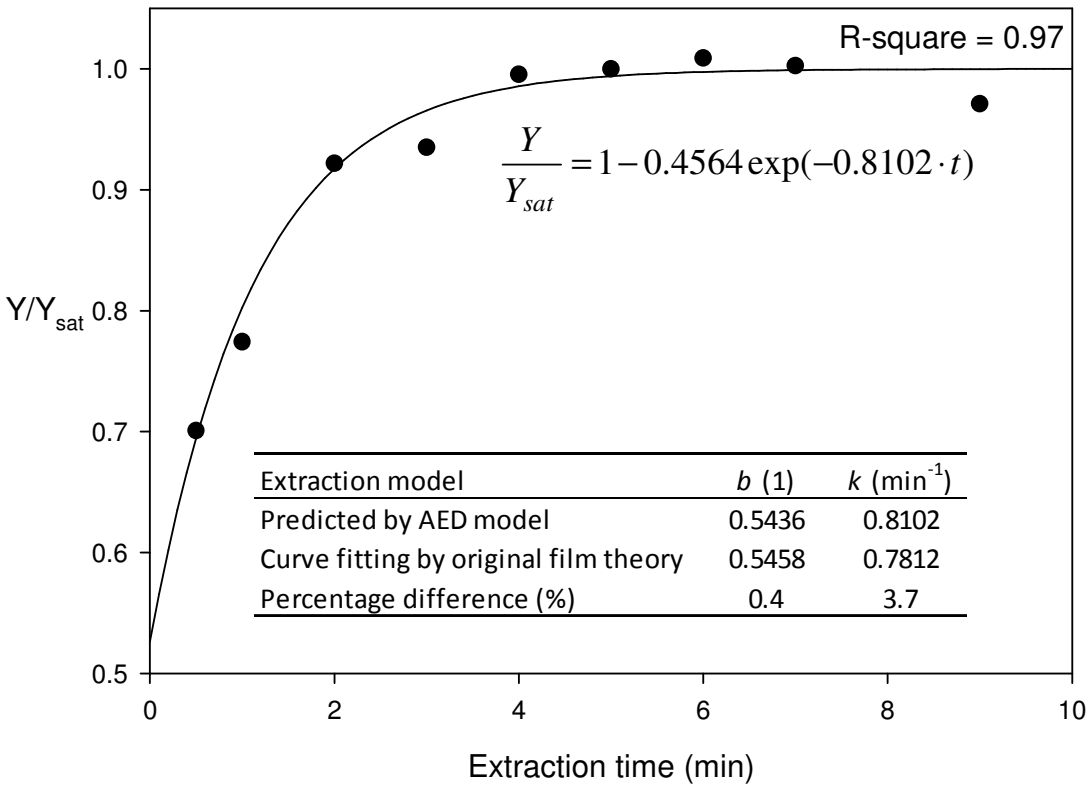
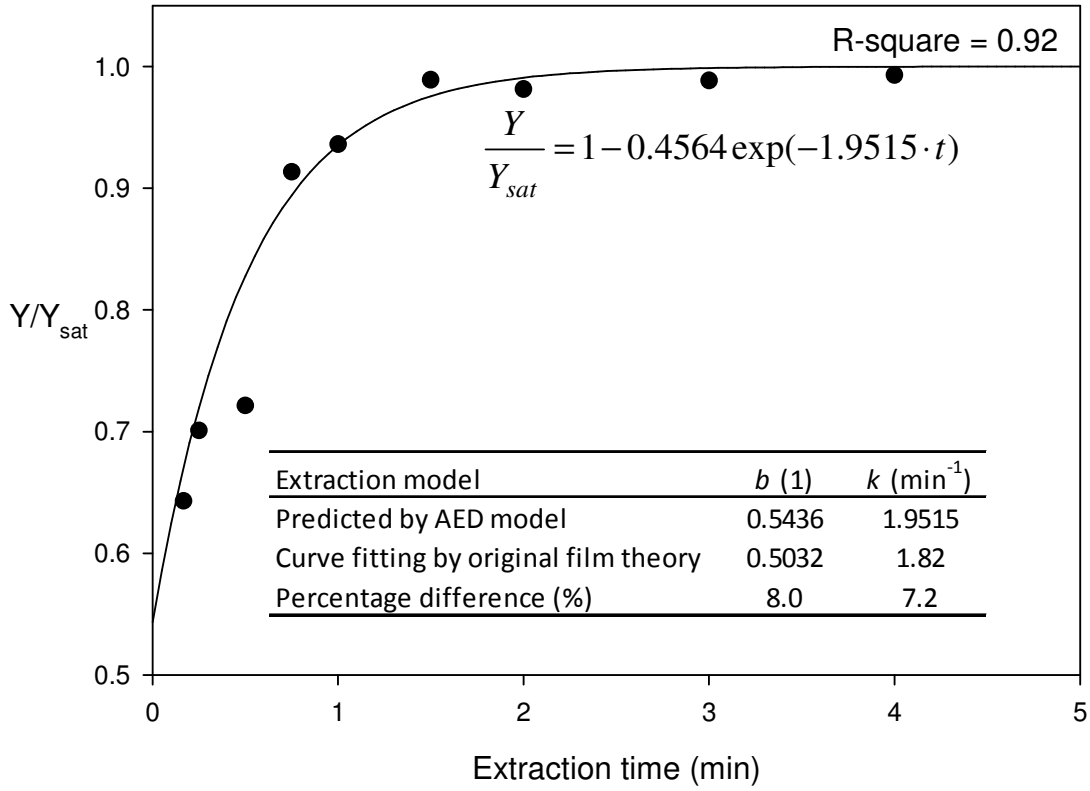
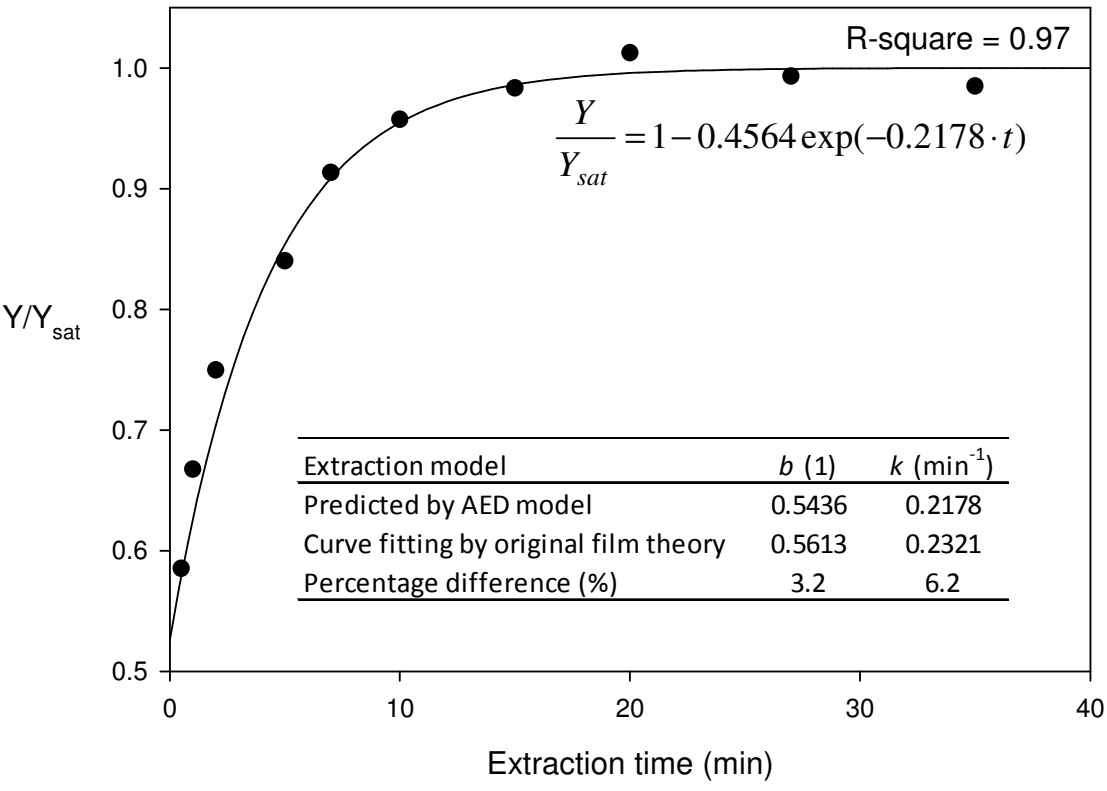


Fig. 5.21: Prediction of extraction curves by using AED extraction model (*MAE condition: 2 g sample, 100ml, 300 W and APD of 0.93W/ml)*



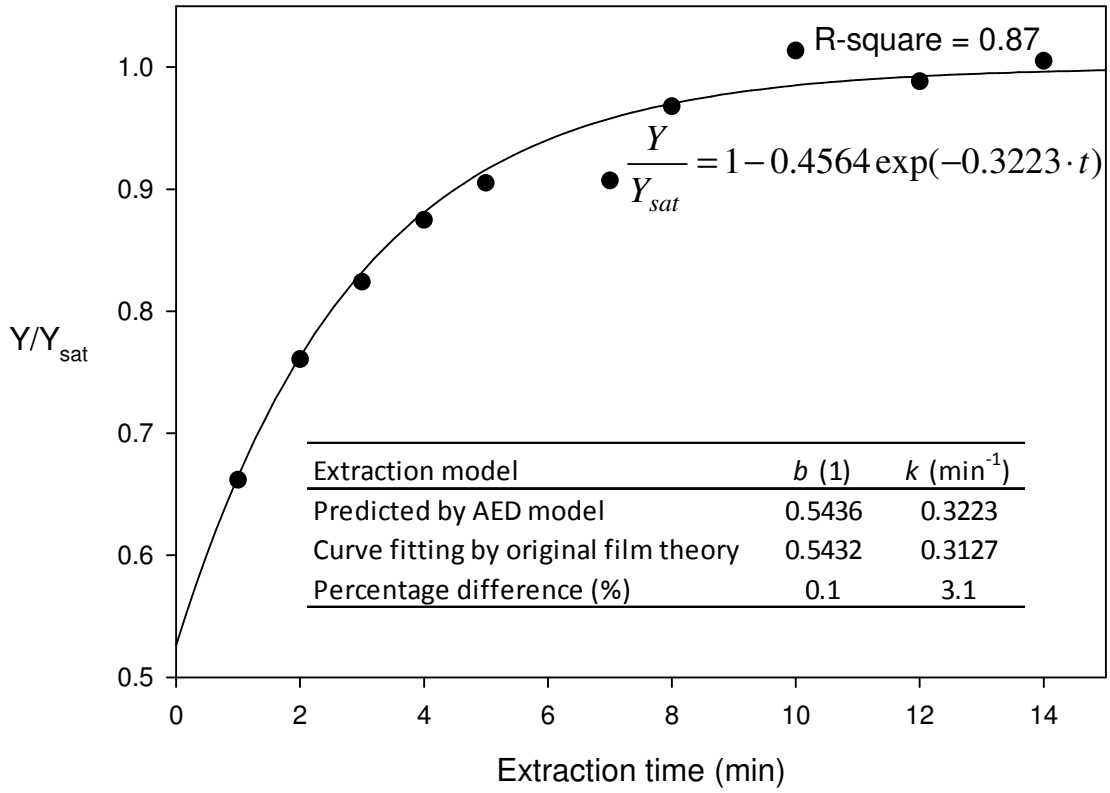
Extraction model	*b* (1)	*k* (min^{-1})
Predicted by AED model	0.5436	1.9515
Curve fitting by original film theory	0.5032	1.82
Percentage difference (%)	8.0	7.2

Fig. 5.22: Prediction of extraction curves by using AED extraction model (*MAE condition: 2 g sample, 100ml, 600 W and APD of 2.24 W/ml)*



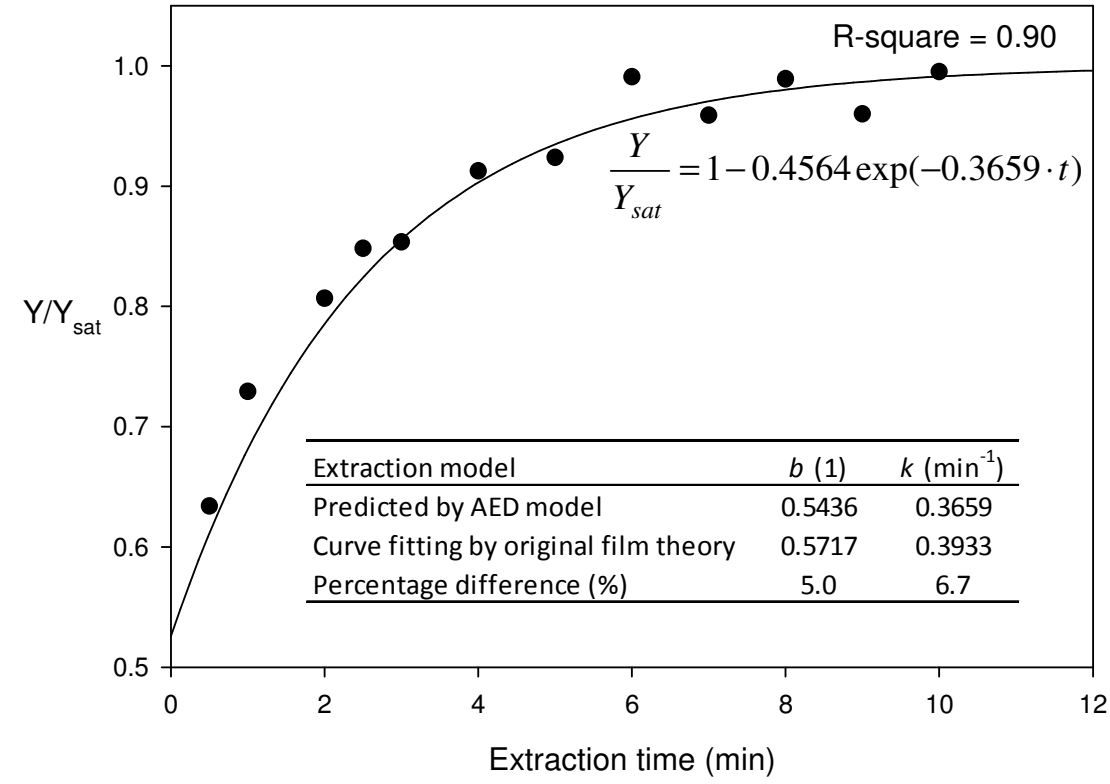
Extraction model	*b* (1)	*k* (min^{-1})
Predicted by AED model	0.5436	0.2178
Curve fitting by original film theory	0.5613	0.2321
Percentage difference (%)	3.2	6.2

Fig. 5.23: Prediction of extraction curves by using AED extraction model *(MAE condition: 4 g sample, 200 ml, 200 W and APD of 0.25W/ml)*



Extraction model	*b* (1)	*k* (min^{-1})
Predicted by AED model	0.5436	0.3223
Curve fitting by original film theory	0.5432	0.3127
Percentage difference (%)	0.1	3.1

Fig. 5.24: Prediction of extraction curves by using AED extraction model (*MAE condition: 5 g sample, 250 ml, 250 W and APD of 0.37W/ml)*



Extraction model	*b* (1)	*k* (min^{-1})
Predicted by AED model	0.5436	0.3659
Curve fitting by original film theory	0.5717	0.3933
Percentage difference (%)	5.0	6.7

Fig. 5.25: Prediction of extraction curves by using AED extraction model (*MAE condition: 6 g sample, 300 ml, 300 W and APD of 0.42W/ml)*

Fig. 5.20 – Fig. 5.25 show that all the predicted extraction curves are capable of capturing the trend of the experimental extraction profiles. This signifies that the diffusion step of MAE can be characterized by APD of the extraction system. In addition, the figures show that the extraction yields of washing step of MAE is about 53% out of the total extraction yield regardless of the operating conditions employed. This suggests that the constant *b'*, which characterizes the washing step of extraction, remain unchanged irrespective of various solvent loading and applied microwave power.

The modeling approach can also be used to predict the coefficients of the extraction model of interest. As the film theory was adapted, the coefficients of the original model (*b* and *k*) can be determined by comparing with the adapted model (Eq. (19) vs. Eq. (21)). The diffusion constant in film theory, *k* is analogous to *k'* x *APD* in Eq. (21) while the coefficient of washing step remain unchanged, $b = b' = 0.5436$. The extraction constants (*b* and *k*) of film theory predicted by using this modeling approach are tabulated in Table 5.16. The comparison of the predicted constants and the constants obtained by fitting with original film theory shows that the proposed method is feasible as the deviations between the two constants were less than 10% in most cases. The only one prediction shows deviations around 40% in Fig. 5.20 could have due to the variation of the amount of active compounds in the plant sample. Nevertheless, the extraction profile of MAE still can be predicted in this case with r-square value of 0.88. This further confirms that the modeling method is reliable in predicting extraction curve of MAE. The APD values used for the prediction are obtained based on the microwave power absorbed in the extraction solvent without considering the effect of microwave heating on the plant material and the interactive effect between the plant material and extraction solvent. When extraction involves sample with high moisture content, the

determination of APD value should take into the account of both the microwave heating on the extraction solvent and plant material as the sample can possibly absorb significant microwave energy.

Table 5.16 also shows that effects of APD on the extraction kinetic and on the equilibrium extraction yields of MAE. It is apparent that k increases with APD of extraction system. This suggests that a high APD value can enhance diffusion thus shorten the extraction time. For instance, the total extraction time required to reach equilibrium extraction yield was reduced by 10 folds from 20 min to 2 min when the APD values increased from 0.25 W/ml to 2.24 W/ml. However, about 10% loss of equilibrium extraction yield was observed when APD was increased to 2.24 W/ml. This implies that thermal degradation of active compounds might have occurred at high APD.

Overall, the presented results showed that the model is capable of modeling the extraction profile of MAE in term of degree of extraction (Y/Y_{sat}). Since there is only a slight 10% decrease of total extraction yield under influence of APD, the model in Eq. (31) can be used to predict extraction yield (mg/g) of MAE under various extraction conditions based on equilibrium extraction yield, Y_{sat}. The equilibrium yield of 11.24 mg/g in Fig. 5.19 (50 ml/g) was used for the prediction as the proposed model was developed from this extraction curve. The predictions of extraction yield of MAE for various extraction conditions under 50 ml/g are shown in Table 5.17. All the predicted yields were close to the experimental values with deviations generally less than 10% demonstrated the model is reliable.

Table 5.17: Experimental and predicted extraction yields of MAE by AED extraction model

MAE conditions	APD (W/ml)	AED (J/ml)	Predicted degree of extraction, Y/Y_{sat}	Predicted extraction yield (mg/g) [a]	Experimental extraction Yields (mg/g)	Percentage deviation of the predicted yield (%)	Recovery (%) [b]
3 g sample, 150 ml, 100 W, 18 min	0.12	130	0.93	10.46	11.28 ± 0.48	7.4	97
3 g sample, 150 ml, 300 W, 6 min	0.69	248	0.99	11.10	10.03 ± 0.35	11.0	86
4 g sample, 200 ml, 100 W, 20 min	0.1	120	0.92	10.34	11.49 ± 0.16	10.1	99
4 g sample, 200 ml, 300 W, 7.2 min	0.56	242	0.99	11.09	10.96 ± 0.21	0.8	94
5 g sample, 250 ml, 100 W, 17.5 min	0.08	84	0.87	9.73	10.76 ± 0.80	10.0	93
5 g sample, 250 ml, 300 W, 6.5 min	0.48	187	0.97	10.90	10.12 ± 0.41	7.9	86
6 g sample, 300 ml, 100 W, 18 min	0.07	76	0.85	9.53	10.55 ± 0.63	10.1	91
6 g sample, 300 ml, 200 W, 12 min	0.19	137	0.94	10.54	10.66 ± 0.17	0.6	91
Soxhlet conditions	Total extraction yields (mg/g)						
2 g sample, ETOH, 200 ml, 6 hr	11.67 ± 0.41						

[a] calculated based on equilibrium extraction yield of MAE at 2 g, 100 ml, 100 W (11.24 mg/g). [b] calculated based on total extraction yields of Soxhlet extraction at 11.67 mg/g)

5.7.3. Estimation of optimum extraction time of MAE based on AED

The effect of microwave energy absorbed in the MAE system on the extraction kinetics is illustrated in the plot of experimental yields at various operating conditions with respect to the amount of absorbed energy in Fig. 5.26. All experimental points are scattered but exhibit the same extraction trend in the figure suggested that the progress of MAE at various microwave power and solvent loading is strongly dependent on AED. However, there is no interactive effect between APD and AED. From the figure, three extraction regions in the diffusion step were identified. The first region describes a constant rate of diffusive extraction where the active compounds diffuse to the extraction solvent steadily. The microwave energy associated with this region was

below 100 J/ml. The diffusion rate decreases as the extraction proceeds to equilibrium. Further increment in the microwave energy beyond 300 J/ml would vaporize large amount of extraction solvent without giving any noticeable increase in extraction yields. Conversely, a decrease in the extraction yields probably due to thermal degradation was observed when the extraction was overheated i.e. AED > 300 J/ml (Fig. 5.26). Thus, the range of energy required to reach equilibrium stage (AED_{eq}) for MAE of anti diabetic compounds from cocoa leaves was suggested to be 100-300 J/ml.

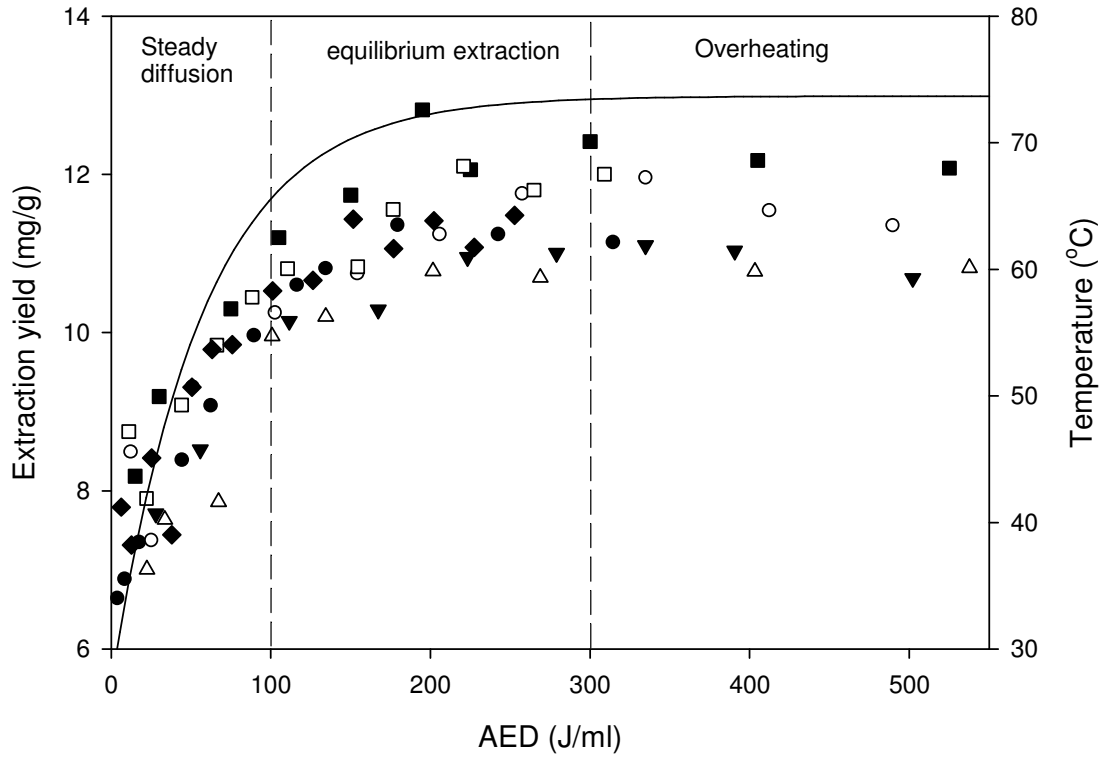


Fig. 5.26: Diffusive extraction stages of MAE at varying AED
(The increase of AED is driven by extraction time; — regressed temperature profile; MAE conditions: ● 2 g sample, 100 W, 100 ml; ○ 2 g sample, 200 W, 100 ml; ▼ 2 g sample, 300 W, 100 ml; ⊿ 2 g sample, 600 W, 100 ml; ■ 4 g sample, 200 W, 200 ml; □ 5 g sample, 250 W, 250 ml; ◆ 6 g sample, 300 W, 300 ml)

Optimum extraction time of MAE can be estimated from AED_{eq}. AED_{eq} denotes the microwave energy required to reach equilibrium stage regardless of the solvent volume and it also indicates the progress of extraction with time. The estimated extraction time (t_e) can be calculated using expression as follows:

$$t_e = \frac{AED_{eq}}{APD} \tag{32}$$

Optimum extraction time of MAE can be estimated with known APD values at designated operating conditions. For evaluation purpose, MAE at solvent to feed ratio of 50 ml/g under various solvent loading and applied microwave power were conducted along the region of AED_{eq} as shown in Table 5.17. The corresponding extraction yields were compared with that obtained from optimized Soxhlet extraction. The comparative study shows that more than 85% of total antioxidant compounds can be recovered and up to 99% recovery can be achieved when MAE was conducted in the AED-equilibrium extraction region. This suggests that the estimation method is feasible to be employed for determining the optimum extraction time of MAE.

5.8. APD-AED optimization method

Having discussed on the significances of APD and AED in the previous modeling studies, this section incorporates the two proposed parameters in the optimization of MAE. This method optimizes MAE operating parameters based on the extraction mechanisms as demonstrated in Fig. 5.27. Basically, MAE comprises of three extraction mechanisms and each one is affected by a group of operating parameters. The first mechanism associates with the penetration of solvent into the plant matrices. Secondly, the polar solvent in the plant cells is heated up by microwave and gradually with the built up internal pressure to rupture the cells. Finally, the active compounds elute from the ruptured cells and dissolve in the solvent. Hypothetically, the rupturing of plant cells

in mechanism 2 is rate limiting as it requires heating energy to proceed. Besides, the first and third mechanisms associate respectively with the penetration of solvent into the plant matrix and the diffusion of compounds from rupture cells are relatively fast. The operating parameters that influence the mechanism 1 and 3 are extraction solvent, solvent to feed ratio (constant volume) and particle size of sample. These parameters can be investigated individually as they do not have interactive effects with each other and they are usually specified prior to the optimization of the mechanism 2.

The rate limiting mechanism of MAE (rupturing of plant cells) is crucial as it determines both the rate of extraction and the yields of the extraction significantly. The operating parameters that affect the mechanism are microwave power and extraction time. They exhibit interactive effect with each other thus usually optimized together with other interactive parameters such as solvent to feed ratio at constant sample mass (Chen et al., 2010; Yang & Zhai, 2010) and also extraction temperature when involved thermal sensitive compounds (Liazid et al., 2011; Wang et al., 2009). As discussed previously on the predictive ability of APD and AED in the modeling studies, APD and AED could be the appropriate alternative to replace the microwave power and extraction time in the optimization of MAE. As independent variables, they can be optimized separately using single factor experiment. In this study, sequential single factor optimization of solvent to feed ratio (constant volume), AED and APD were performed, and based on the optimum AED and APD obtained, the optimum microwave power and the extraction time of MAE at larger scale can be determined accordingly. The details of the optimization result are tabulated in Appendix D.

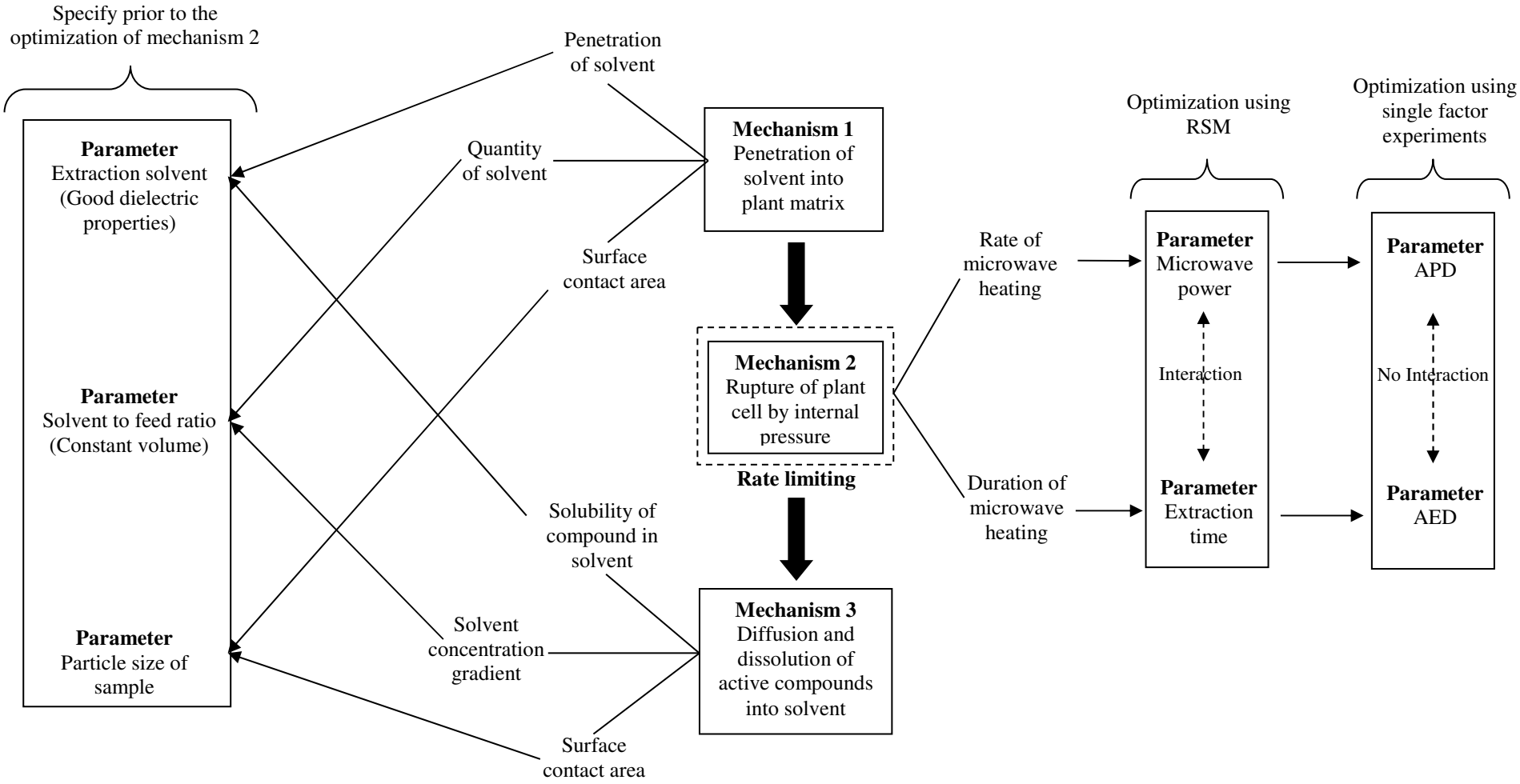


Fig. 5.27: Strategy of optimizing MAE based on its extraction mechanisms

5.8.1. Optimization of solvent to feed ratio

The effect of solvent to feed ratio on the MAE yields at an arbitrary condition (50 ml, 100 W and 10 min) is plotted as shown in Fig. 5.28. The result shows that increasing solvent to feed ratio at constant volume improves the extraction yields of MAE. Once the ratio is increased beyond its optimum value, i.e. 50 ml/g in this case, the increment in the extraction yield will not be substantial (Spigno & De Faveri, 2009). In general, solvent to feed ratio at constant solvent volume does not exhibit interactive effects with APD and AED but only affects the solvent penetration (mechanism 1) and the elution of active compounds into solvent (mechanism 3) as shown in Fig. 5.27. On the other hand, if the solvent to feed ratio is evaluated at constant mass of sample, the change in solvent volume due to different ratio would affect the absorption of microwave energy (Kingston & Jassie, 1988), and might exhibits interaction with APD and AED to affect the microwave heating. Therefore, in this study, solvent to feed ratio at constant volume of 50 ml/g is used for optimization of AED and APD individually.

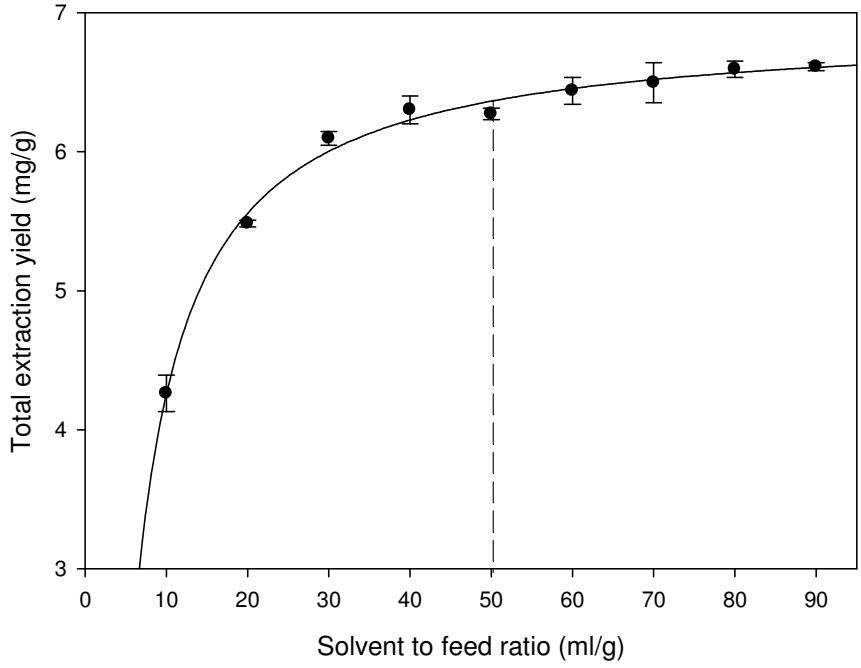


Fig. 5.28: Single factor optimization of MAE at constant volume of solvent to feed ratio (*MAE condition: 50 ml, 100 W, 10 min and APD of 0.43 W/ml)*

5.8.2. Optimization of AED

The effect of AED was investigated by conducting the extraction profile of MAE using AED as a basis as shown in Fig. 5.29. Both the extraction profiles of MAE with varying time and AED exhibit similar trends. The extraction achieves equilibrium yield at 12 min extraction time and 300 J/ml AED, respectively. Despite the similarity in the extraction profiles using two different bases, they have different implications on the optimization of MAE. Unlike extraction time which only addresses the heating time, AED specifies the amount of microwave energy required to achieve certain degree of completion for MAE via rupturing of plant cells. It indicates the progress of MAE to reach equilibrium extraction regardless of microwave irradiation power (or APD) of the extraction system. The optimum AED value obtained, i.e. 300 J/ml, will be used to investigate the optimum APD for the MAE.

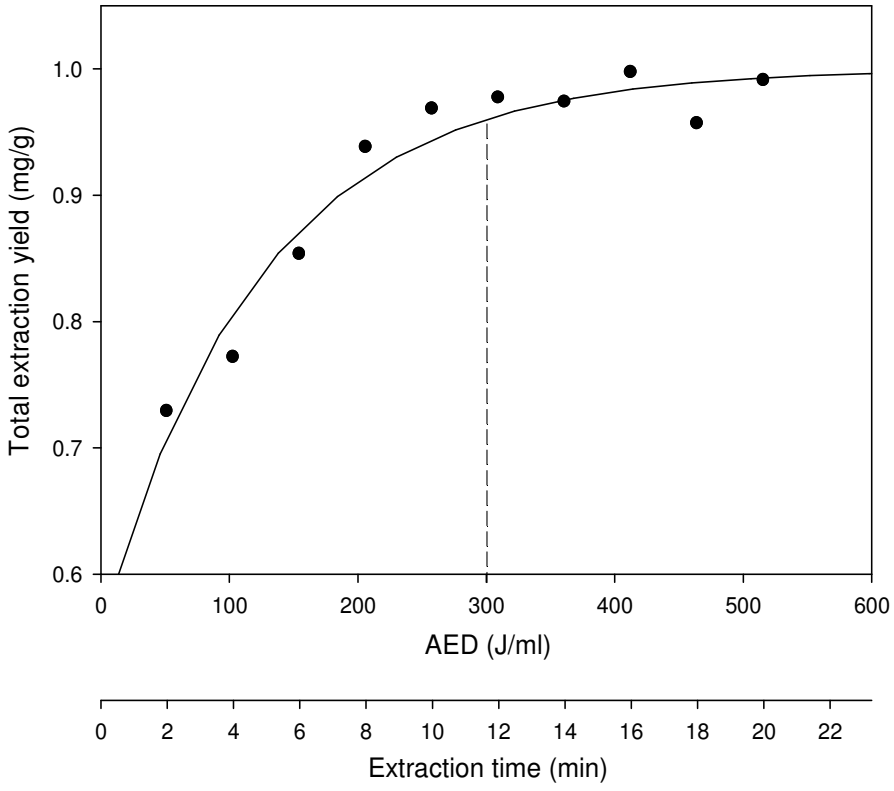


Fig. 5.29: Single factor optimization of MAE at varying AED
(*MAE condition: 50 ml/g, 50 ml, 100 W and APD of 0.43 W/ml)*

5.8.3. Optimization of APD

The effect of APD on the performance of MAE was investigated at the same AED of 300 J/ml by varying microwave power from 100 to 300 W. This is to ensure all extractions had reached the same degree of completion, in this case is the equilibrium extraction. It is not sensible to evaluate the effect of heating power at the same extraction time because the extraction time required for high microwave power differs from that needed for low microwave power to achieve equilibrium extraction. As the nominal microwave power is merely an indication of the power setting of the microwave system employed, the evaluation of heating power in this studies was based on APD as it represents the real heating power in the system. Once APD of a specific microwave power is determined, the corresponding extraction time at AED of 300 J/ml can be calculated using Eq. (33).

$$t\,(\mathrm{s}) = \frac{300\,(\mathrm{J/ml})}{APD\,(\mathrm{W/ml})} \tag{33}$$

The relationship between APD and the extraction time at AED of 300 J/ml is plotted in Fig. 5.30.

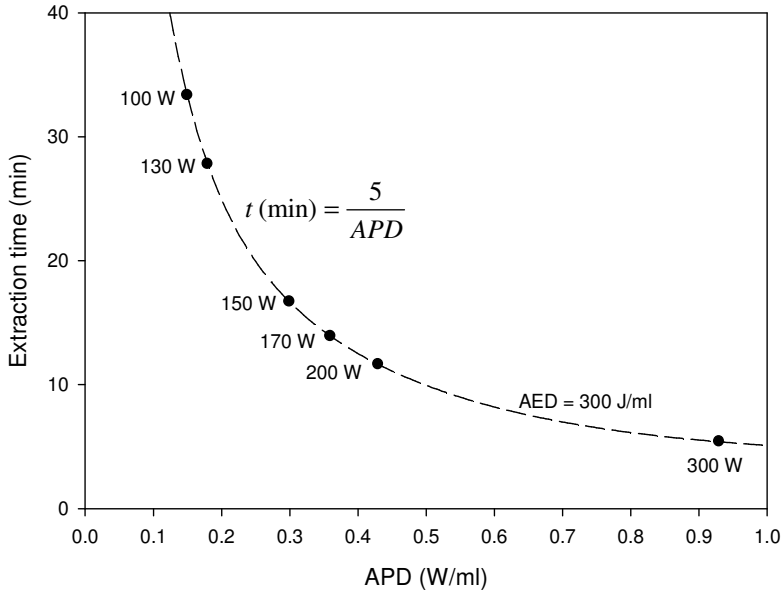


Fig. 5.30: Determination of extraction time of MAE for various microwave power (*Heating condition: solvent loading of 100 ml*)

As seen in the figure, the equilibrium extraction time decreases exponentially with the increase of microwave heating power. The extraction time varies from 30 min to 5 min when the APD of the extraction changes from 0.15 to 0.95 W/ml with the corresponding microwave power of 100-300 W at solvent loading of 100 ml. MAE were conducted in these extraction conditions and the results plotted in Fig. 5.31 suggests that the MAE favors extraction conditions at low APD (< 0.35 W/ml). MAE conducted at APD greater than 0.35 W/ml gives slightly lower yields (< 5% difference) but with shorter extraction time. The decrease in yields at high APD could be caused by the thermal degradation of active compounds. Considering both the performance of MAE and the thermal stability of the active compounds, APD of 0.3 W/ml deemed to be the optimum value for the MAE of this study.

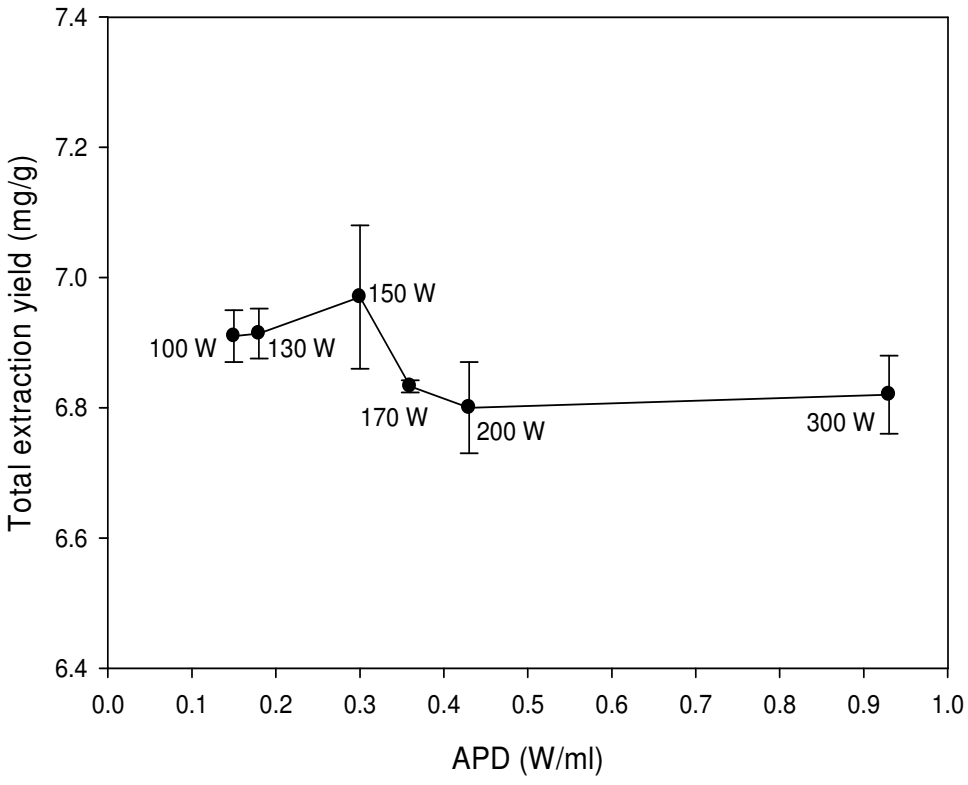


Fig. 5.31: Single factor optimization of MAE for various APD
(*MAE condition: 50 ml/g, 100 ml and AED of 300 J/ml*)

5.8.4. Verification and comparison of optimum extraction conditions of MAE

From the findings of the APD-AED optimization study, the optimum operating conditions of MAE are solvent to feed ratio (constant volume) of 50 ml/g, AED of 300 J/ml and APD of 0.3 W/ml. This optimized condition corresponds to the microwave power of 150 W and the extraction time of 16.7 min for solvent loading of 100 ml. To verify the feasibility of the proposed optimization method, the optimization result obtained was compared to that obtained from the optimization using RSM in section 5.2.3 and also with the conventional Soxhlet extraction in section 5.3. Table 5.18 shows that despite the optimum operating conditions obtained from the two optimization strategies are slightly different in terms of microwave power (150 W vs. 156 W) and extraction time (16.7 min vs. 18 min), they similarly achieve 98% total recoveries of active compounds based on the total extraction yields of Soxhlet extraction.

Table 5.18: Verification of optimum extraction conditions of MAE and comparison with Soxhlet extraction

Extraction technique	MAE		Soxhlet
Optimization strategy	APD-AED	RSM with BBD	Single factor
Optimum condition	85% EtOH, 50 ml/g, 2 g sample, 150 W and 16.7 min	85% EtOH, 50 ml/g, 2 g sample, 156 W and 18 min	100% EtOH, 100 ml/g, 2 g sample and 6 hr
Total yields (mg/g)	6.97 ± 0.11	6.93 ± 0.06	7.09 ± 0.20
IQ yield (mg/g)	1.05 ± 0.06	1.06 ± 0.02	1.17 ± 0.09
EC yield (mg/g)	1.38 ± 0.03	1.32 ± 0.02	1.30 ± 0.13
RT yield (mg/g)	4.49 ± 0.06	4.54 ± 0.02	4.62 ± 0.01
Total recovery (%) [a]	98.3	97.7	/

[a] calculated based on total extraction yield of Soxhlet extraction.

Comparing between the two optimization strategies of MAE, Combined APD-AED optimization method is simpler as it can be performed using a series of single factor experiments whereas in the optimization using RSM, screening of suitable range of parameters is prerequisite for the optimization to achieve reliable result. In brief, the APD-AED incorporated optimization method is proven to be feasible and applicable for MAE of active compounds of cocoa leaves in this study.

5.8.5. Application of intensive optimum MAE condition

The optimum MAE conditions (S/F, APD, AED) obtained from the proposed method can be used to determine the operating parameters (S/F, Power, time) for larger scale of extraction. The optimum operating condition obtained such as 50 ml/g at constant volume, 300 J/ml and 0.3 W/ml can be considered as the intensive optimum MAE conditions as they describe the intrinsic criteria for optimum extraction regardless of the scale of extraction. For instance, solvent to feed ratio is closely related to the concentration gradient effects and has effect on the diffusion and dissolution of active compounds in the solvent as previously described in MAE mechanism (Fig. 5.27). Furthermore, AED and APD both represent respectively the total heating energy and rate of heating required to rupture the plant cells regardless of solvent loading. In this study, the optimum operating condition of MAE (S/F, Power, time) for varying solvent loading (150-300 ml) was determined from the intensive optimum MAE conditions and their extraction performance are evaluated in Table 5.19.

Table 5.19: Validation of intensive optimum MAE condition (50 ml/g, 300 J/ml, 0.3 W/ml) at larger scales extraction

Solvent loading (ml)	100	150	200	250	300
Optimum microwave power (W) [a]	150	200	220	220	260
APD (W/ml)	0.30	0.32	0.34	0.27	0.31
Optimum extraction time (min) [b]	16.7	15.6	14.7	18.5	16.1
Total extraction yield (mg/g)	6.97 ± 0.11	6.82 ± 0.06	7.15 ± 0.19	7.01 ± 0.09	6.97 ± 0.08
IQ yield (mg/g)	1.05 ± 0.06	1.01 ± 0.01	1.04 ± 0.01	1.03 ± 0.01	1.02 ± 0.04
EC yield (mg/g)	1.38 ± 0.03	1.30 ± 0.01	1.47 ± 0.09	1.40 ± 0.04	1.40 ± 0.03
RT yield (mg/g)	4.49 ± 0.06	4.51 ± 0.06	4.64 ± 0.09	4.58 ± 0.05	4.55 ± 0.06
Percentage difference of total extraction yield (%) [c]	/	2.2	2.6	0.6	0

[a] determined based on APD of 0.3 W/ml. [b] determined based on AED of 300 J/ml. [c] determined based on the total extraction yields at solvent loading of 100 ml.

The optimum microwave power and extraction time of MAE at different solvent loading can be determined based on the APD and AED of the intensive optimum conditions respectively. This can be done by adjusting the microwave power of MAE system at each solvent loading so that APD of the system is at about 0.3 W/ml. In cases whereby 0.3 W/ml is difficult to be achieved due to the power setting of the microwave system employed, the best tuning gives the nearest APD value in the range of 0.30 ± 0.04 W/ml for each solvent loading (Table 5.19). The APD values obtained for specific solvent loading was then used to calculate their respective extraction time based on AED of 300 J/ml. Table 5.19 shows that MAE conducted at different solvent loading using the respective determined optimum conditions give similar result (less than 3% discrepancy in the total extraction yields). This proves that the intensive optimum MAE conditions is reliable in determining optimum operating parameters of MAE at different solvent loading. Also, it further stresses the proposed parameters, i.e. APD and AED are significant and reliable in the optimization of MAE for scaling up purpose.

From the findings of this optimization study, it indicates that with different combination of APD and AED, MAE performs differently. By correlating APD with AED, MAE can be classified into nine performance regimes as illustrated in Fig. 5.32. Based on the extraction results, the optimum region of the MAE is confined within APD of 0.25-0.35 W/ml and AED of 250-350 J/ml. MAE conducted outside this optimum region give different characteristics and performances. For instance, MAE conducted below optimum AED values gives incomplete extraction due to inadequate heating time; above the optimum AED, the extraction is risked of thermal degradation due to prolonged extraction and hence resulted in poor equilibrium extraction yields. On the other hand, MAE conducted below optimum APD give poor extraction yield as the heating power is insufficient to rupture all the plant cells and high APD beyond the optimum value subjecting the extraction to high temperature for relatively long time may affect the stability of active compounds such as thermal sensitive compounds.

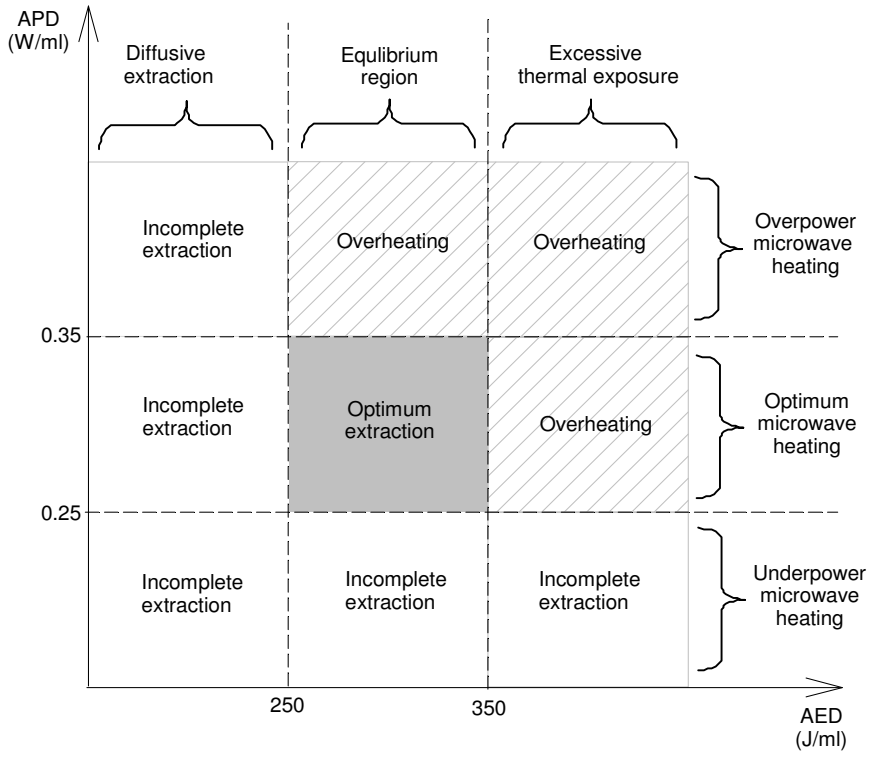


Fig. 5.32: Performance regimes of MAE based on APD and AED

Chapter 6

CONCLUSION AND RECOMMENDATIONS

This chapter summarizes the research findings in accordance with the objectives set in this study. The novelty and contributions of this work as well as the recommendations for future work are also presented.

6.1. Conclusions

The leaf of cocoa (*Theobroma cacao* L.) plant is a potential source of anti diabetic compounds as it contains isoquercitrin (IQ), (-)-epicatechin (EC), rutin (RT), quercetin, kaempferol, (-)-epicatechin gallate and (-)-epigallocatechin gallate. Among all, considerable amounts of IQ (0.13-3.51 mg/g), EC (0.23-2.91 mg/g) and RT (0.3-7.07 mg/g) were found in cocoa leaves. The optimization of MAE performed using RSM gave the optimum extraction conditions for the extraction at 85% aqueous ethanol, solvent to feed ratio of 50 ml/g (2g), microwave power of 156 W and 18 min extraction time. MAE is more efficient than Soxhlet extraction in terms of short extraction time (18 min vs. 6 hr) and less solvent consumption (50 ml/g vs. 100 ml/g). The effectiveness of MAE is due to the ability of microwave heating to rupture the plant cells and subsequently elute the active compounds into solvent.

In view of the MAE kinetics, the washing step of MAE is affected by the size of plant sample used. Reducing the size of sample below the leaf thickness would enhance 15% of extraction yields via washing of active compounds from the disrupted cells. On the other hand, the diffusion step of MAE is influenced by both the solvent to feed ratio and microwave irradiation power. The rate of diffusion step increases with microwave

power while the equilibrium extraction yields increases with the solvent to feed ratio due to concentration gradient effect.

Absorbed power density (APD) parameter was proven to be feasible in the study of MAE. APD addresses the interaction between nominal microwave power and solvent loading. It is potential to replace the nominal microwave power in the investigation of MAE as it indicates the real power used for the extraction regardless of the instrumental setup. Besides, it can characterize the extraction profile of MAE and is useful for scaling up purpose as demonstrated in the APD predictive method for large scale MAE.

The modeling of MAE based on absorbed energy density (AED) is viable as the AED-extraction model exhibits good predictive capability of the MAE profile. The AED kinetic model indicates that the optimum microwave energy required to reach equilibrium extraction was 100-300 J/ml. This implies that AED indicates the progress of MAE toward equilibrium extraction independent of the APD of the extraction system.

The introduced parameters, i.e. APD and AED can be used to replace microwave irradiation power and extraction time in the optimization of MAE. The proposed APD-AED optimization method provides simple means to optimize MAE based on sequential single factor experiments. The intensive optimum conditions obtained from the optimization are solvent to feed ratio of 50 ml/g (constant volume), APD of 0.3 W/ml and AED of 300 J/ml and they are the intrinsic criteria for optimum extraction. Based on these criteria, the optimum operating parameters in MAE such as solvent to feed ratio, microwave power and extraction time similar to those obtained from optimization using RSM can be determined for larger scale extraction.

6.2. Novelty and contributions

The novelty of this study includes the investigation of anti diabetic compounds in cocoa leaves and the development of useful methods in the modeling and optimization of MAE based on the proposed APD and AED parameters. The contributions of this research are listed as follows:

1. Confirm the potential of cocoa leaf as anti diabetic plant

Cocoa leaf is a good bioresource for the recovery of anti diabetic compounds owing to its global availability and its substantial amounts of quercetin derivatives and catechin compounds.

2. Introduce APD and AED as new MAE parameters

The newly introduced parameters, APD and AED describe the real microwave power and energy absorbed in the extraction system respectively regardless of the instrumental setup of microwave system. Due to their intrinsic characteristic in MAE, these parameters can be served as reference conditions for similar extractions by adjusting the microwave power setting of the system employed to match the desired APD value and extending the extraction to reach the desired AED value. Besides, the parameters can be used as comparison basis to evaluate the performance of other MAE system.

3. Devise viable methods to model MAE process at varying extraction scale

The scaling up of MAE is difficult in view of the lack of understanding on the effects of operating parameters and the scarce theoretical models. The methods developed based on APD and AED ensured the modeling of MAE to be performed at varying scale of extraction. The APD prediction method and AED modeling method developed in this study are useful which enables the prediction of optimum extraction time and extraction

curve respectively for large scale MAE with lesser experimental data as compared to others MAE models. In fact, MAE models reported in literature so far do not have predictive capability as they are strongly dependent on the experimental data.

4. Standardize and develop optimization method for MAE

The APD-AED optimization method simplifies and standardizes the optimization of MAE based on its extraction mechanisms. This method is much simpler as compared with the conventional optimization method using RSM whereby the proposed method only involves single factor experiment without the need to screen for suitable range of parameters. Furthermore, the intensive optimum MAE conditions (S/F, APD, AED) obtained from the developed method is useful for scaling up as it can be used to determine the optimum operating parameters (S/F, Power, Time) for various scale of extraction and this is applicable for different microwave extractors.

6.3. Recommendations for future work

To further identify the washing and diffusion steps of MAE individually, the correlation of granulometry and specific surface area on the washing impact and the effect of sample structure on effective diffusivity can be investigated. As the APD and AED methods are well established, applying the methods to other extractions should be attempted to confirm its robustness. Furthermore, the APD and AED parameters introduced in this study can be explored further to develop a complete model for MAE. The temperature factor can also be considered to reduce the thermal degradation of active compounds. Besides, economical analysis of MAE process is important to be investigated. On the other aspects, physicochemical properties of the extract and efficacy of the extracted compounds should be investigated to confirm the quality of the extract.

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APPENDIX A

DETERMINATION OF APD VALUE

The calculation of approximate value of APD for microwave heating of 100 W on 100 ml of 85% (v/v) aqueous ethanol is demonstrated for heating case A (final heating temperature < boiling point of solvent) and case B (final heating temperature = boiling point of solvent). The calculation is conducted by assuming that the solvent mixture is ideal solution and vapor-liquid equilibrium is formed during the vaporization of solvent under microwave heating. The properties of solvent mixture used in the APD calculation are tabulated in Table B.1.

Table A.1: Properties of solvent mixture

Physical properties	EtOH	water
Heat capacity of component, C_p ($Jg^{-1}k^{-1}$)	2.63	4.2
Density of component, ρ (g/ml)	0.789	1.000
Molecular weight of component, M (g/mol)	46	18
Vapor-liquid equilibrium		
Volume fraction of liquid solvent	0.85	0.15
Mass fraction of liquid solvent, x	0.82	0.18
Mass fraction of vaporized solvent, y	0.91	0.09
Density of the solvent mixture at concentration y	0.808	
latent heat of vaporization for the solvent mixture, H_{vap} (kJ/mol)	40.7	

Case A: Final heating temperature < boiling point of solvent

Original solvent volume, V = 100 ml

Microwave heating time, t_H = 5 min

Temperature difference, ΔT = 51 – 29 = 22 °C

$$
\begin{aligned}
Q &= m_L C_p \Delta T \\
&= V_{EtOH}\rho_{EtOH}C_{p.EtOH}\Delta T + V_{water}\rho_{water}C_{p.water}\Delta T \\
&= (85\text{ ml})(0.789\frac{\text{g}}{\text{ml}})(2.63\frac{J}{\text{gK}})(22K) + (15\text{ ml})(1\frac{\text{g}}{\text{ml}})(4.2\frac{J}{\text{gK}})(22K) \\
&= 5266.38\,J
\end{aligned}
$$

$$
\begin{aligned}
APD &= \frac{Q}{V \cdot t_H} \\
&= \frac{(5266.38\,J)}{(100\,ml)(5\text{ min}\times 60\frac{s}{\text{min}})} \\
&= 0.1755\frac{W}{ml}
\end{aligned}
$$

Case B: Final heating temperature = boiling point of solvent

Original solvent volume, V = 100 ml

Microwave heating time, t_H = 27 min

Temperature difference, ΔT = 70 – 29 = 41 °C

Volume of vaporized solvent, V_{vap} = 17 ml

$$
\begin{aligned}
m_L C_p \Delta T &= V_{EtOH}\rho_{EtOH}C_{p.EtOH}\Delta T + V_{water}\rho_{water}C_{p.water}\Delta T \\
&= (85\text{ ml})(0.789\frac{\text{g}}{\text{ml}})(2.63\frac{J}{\text{gK}})(41K) + (15\text{ ml})(1\frac{\text{g}}{\text{ml}})(4.2\frac{J}{\text{gK}})(41K) \\
&= 9814.62\,J
\end{aligned}
$$

$$
\begin{aligned}
m_v H_{vap} &= \left[\left(\frac{\rho_{vap} V_{vap} y_{EtOH}}{M_{EtOH}}\right) + \left(\frac{\rho_{vap} V_{vap} y_{water}}{M_{water}}\right)\right] H_{vap} \\
&= \left[\rho_{vap} V_{vap}\left(\frac{y_{EtOH}}{M_{EtOH}} + \frac{y_{water}}{M_{water}}\right)\right] H_{vap} \\
&= \left[0.808\,\frac{\text{g}}{\text{ml}} \cdot 17\ ml\left(\frac{0.91}{46\ g/mol} + \frac{0.09}{18\ g/mol}\right)\right] 40.7 x 10^3\ \frac{J}{mol} \\
&= 13854.85\ J
\end{aligned}
$$

$$
\begin{aligned}
Q &= m_L C_p \Delta + m_v H_{vap} \\
&= 9814.62\ J + 13851.42\ J \\
&= 23669.47\ J
\end{aligned}
$$

$$
\begin{aligned}
APD &= \frac{Q}{V \cdot t_H} \\
&= \frac{(23669.47\ J)}{(100\ ml)(27\ \text{min} \times 60\ \frac{s}{\text{min}})} \\
&= 0.146\ \frac{W}{ml}
\end{aligned}
$$

Table A.2: APD values at various operating conditions

Solvent loading, V (ml)	Microwave irradiation power, P (W)	Heating time, t_H (min)	Final temperature (°C)	Volume of vaporized solvent (ml)	Amount of energy absorbed, Q (J)	APD (W/ml)	Average APD (W/ml)
50	100	3.00	61.0	0.0	3830	0.43	0.43 ± 0.02
		4.00	67.0	0.0	4548	0.38	
		7.00	71.0	6.0	9910	0.47	
100	100	5.00	51.0	0.0	5266	0.18	0.15 ± 0.02
		13.00	70.0	0.0	10630	0.14	
		27.00	70.2	17.0	23669	0.15	
	130	5.00	53.0	0.0	5745	0.19	0.18 ± 0.02
		10.00	70.0	0.0	9815	0.16	
		15.00	70.5	10.0	17953	0.20	
	150	5.00	68.0	0.0	9336	0.31	0.30 ± 0.03
		10.00	70.0	11.5	19173	0.32	
		15.00	70.0	18.0	24463	0.27	
	170	2.00	48.0	0.0	4548	0.38	0.36 ± 0.03
		5.00	70.0	0.0	9815	0.33	
		7.00	71.2	8.0	16325	0.39	
	200	2.00	49.0	0.0	4788	0.40	0.43 ± 0.03
		8.00	72.5	13.0	20410	0.43	
		16.00	72.0	42.0	44044	0.46	
	300	1.00	50.4	0.0	5123	0.85	0.93 ± 0.06
		5.00	71.3	24.0	29211	0.97	
		7.00	72.0	37.0	39969	0.95	
	450	1.00	62	0	7900	1.32	1.35 ± 0.07
		3.00	72.9	16.8	23506	1.31	
		4.00	74	30	34264	1.43	
	600	0.15	38.3	0.0	2226	2.23	2.24 ± 0.04
		0.75	70.1	0.5	10222	2.27	
		2.00	70.8	20.5	26359	2.20	
150	100	5.00	40.0	0.0	3950	0.09	0.12 ± 0.05
		10.00	58.0	0.0	10413	0.12	
		20.00	70.8	19.0	30207	0.17	
	150	4.00	50.0	0.0	8977	0.25	0.22 ± 0.03
		8.00	67.8	0.0	13932	0.19	
		10.00	70.0	4.3	18251	0.20	
	200	2.00	46.0	0.0	6104	0.34	0.32 ± 0.02
		5.00	68.0	0.0	14004	0.31	
		10.00	70.5	16.0	27743	0.31	

Table A.2, continued: APD values at various operating conditions

Solvent loading, V (ml)	Microwave irradiation power, P (W)	Heating time, t_H (min)	Final temperature (°C)	Volume of vaporised solvent (ml)	Amount of energy absorbed, Q (J)	APD (W/ml)	Average APD .(W/ml)
150	300	1.00	45.0	0.0	5745	0.64	0.69 ± 0.10
		4.00	70.1	10.0	22872	0.64	
		6.00	71.0	35.5	43654	0.81	
200	100	5.00	45.0	0.0	7660	0.13	0.10 ± 0.02
		13.00	58.0	0.0	13884	0.09	
		25.00	72.0	10.0	27779	0.09	
	200	2.00	40.8	0.0	5649	0.24	0.25 ± 0.02
		5.00	63.4	0.0	16469	0.27	
		13.00	70.2	22.5	38048	0.24	
		20.00	72.0	50.5	60868	0.25	
		27.00	73.0	77.0	82384	0.25	
	220	2.0	46.0	0.0	8139	0.34	0.34 ± 0.02
		5.0	70.5	2.0	21257	0.35	
		10.0	71.0	22.0	38012	0.32	
	300	2.00	60.0	0.0	14842	0.62	0.56 ± 0.06
		7.00	70.3	28.0	42449	0.51	
		10.00	71.0	57.0	66084	0.55	
250	100	5.00	43.0	0.0	8378	0.11	0.08 ± 0.03
		15.00	52.0	0.0	13764	0.06	
		25.00	72.0	6.0	29426	0.08	
	220	5.0	65.0	0.0	21544	0.29	0.27 ± 0.02
		10.0	71.0	20.0	40813	0.27	
		15.0	72.0	40.0	57089	0.25	
	250	1.00	39.5	0.0	6284	0.42	0.37 ± 0.07
		5.00	66.0	0.0	22143	0.30	
		7.00	70.0	21.0	41651	0.40	
	300	1.50	50.5	0.0	12867	0.57	0.48 ± 0.08
		8.00	72.5	39.0	56321	0.47	
		12.00	72.0	59.0	72621	0.40	
300	100	5.00	41.5	0.0	8977	0.10	0.07 ± 0.03
		15.00	47.0	0.0	12927	0.05	
		25.00	72.0	4.0	32704	0.07	
	200	5.00	58.0	0.0	20826	0.23	0.19 ± 0.04
		10.00	70.0	6.0	34334	0.19	
		20.00	70.2	30.0	53894	0.15	
	260	2.0	45.0	0.0	11490	0.32	0.31 ± 0.03
		5.0	70.3	0.0	29444	0.33	
		10.0	70.0	25.0	49789	0.28	
	300	1.00	42.0	0.0	9336	0.52	0.42 ± 0.08
		4.00	65.0	0.0	25853	0.36	
		9.00	71.7	41.5	63347	0.39	

APPENDIX B

HPLC, MS AND SEM ANALYSIS

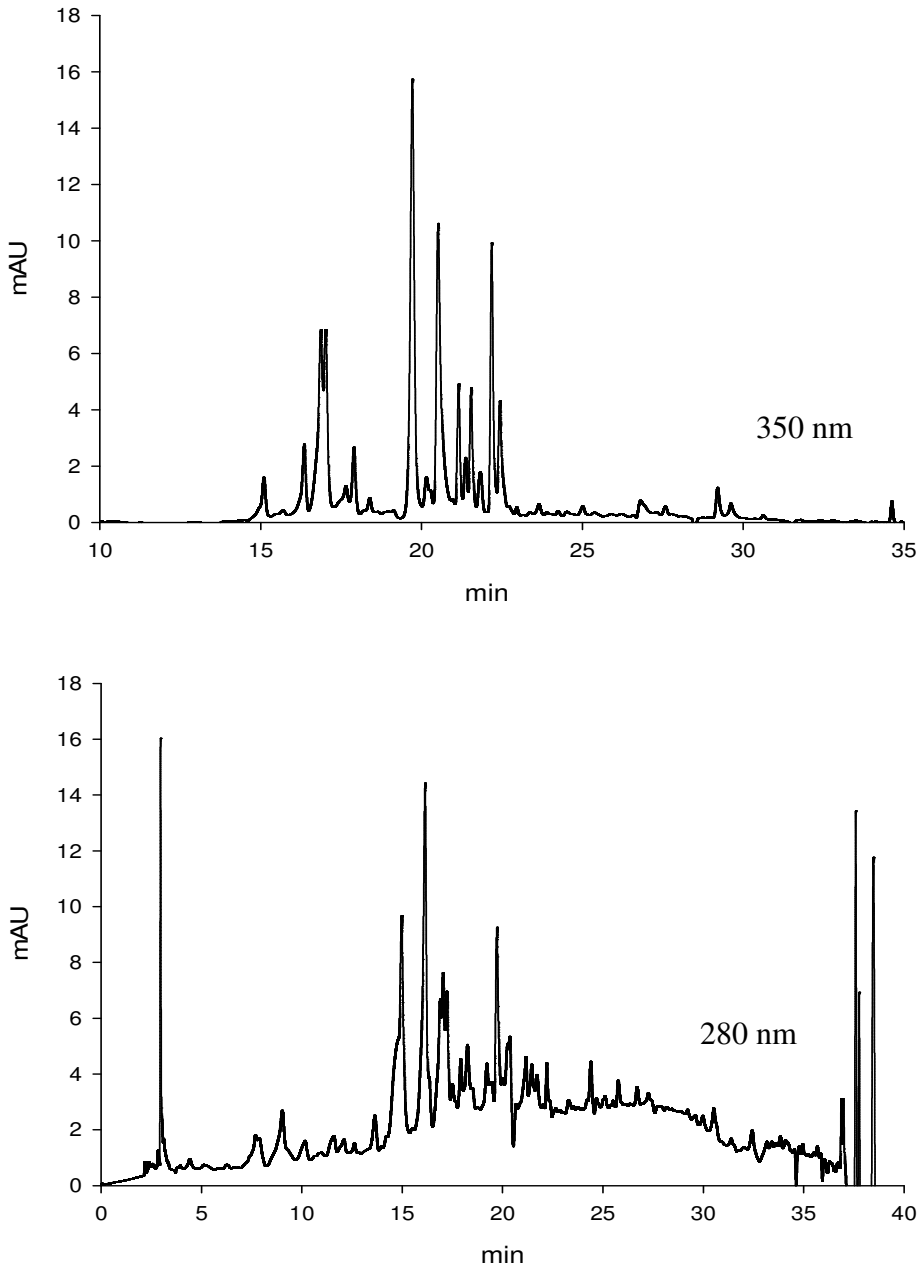


Fig. B.1: Chromatograms of MAE extract from batch (A) cocoa leaves

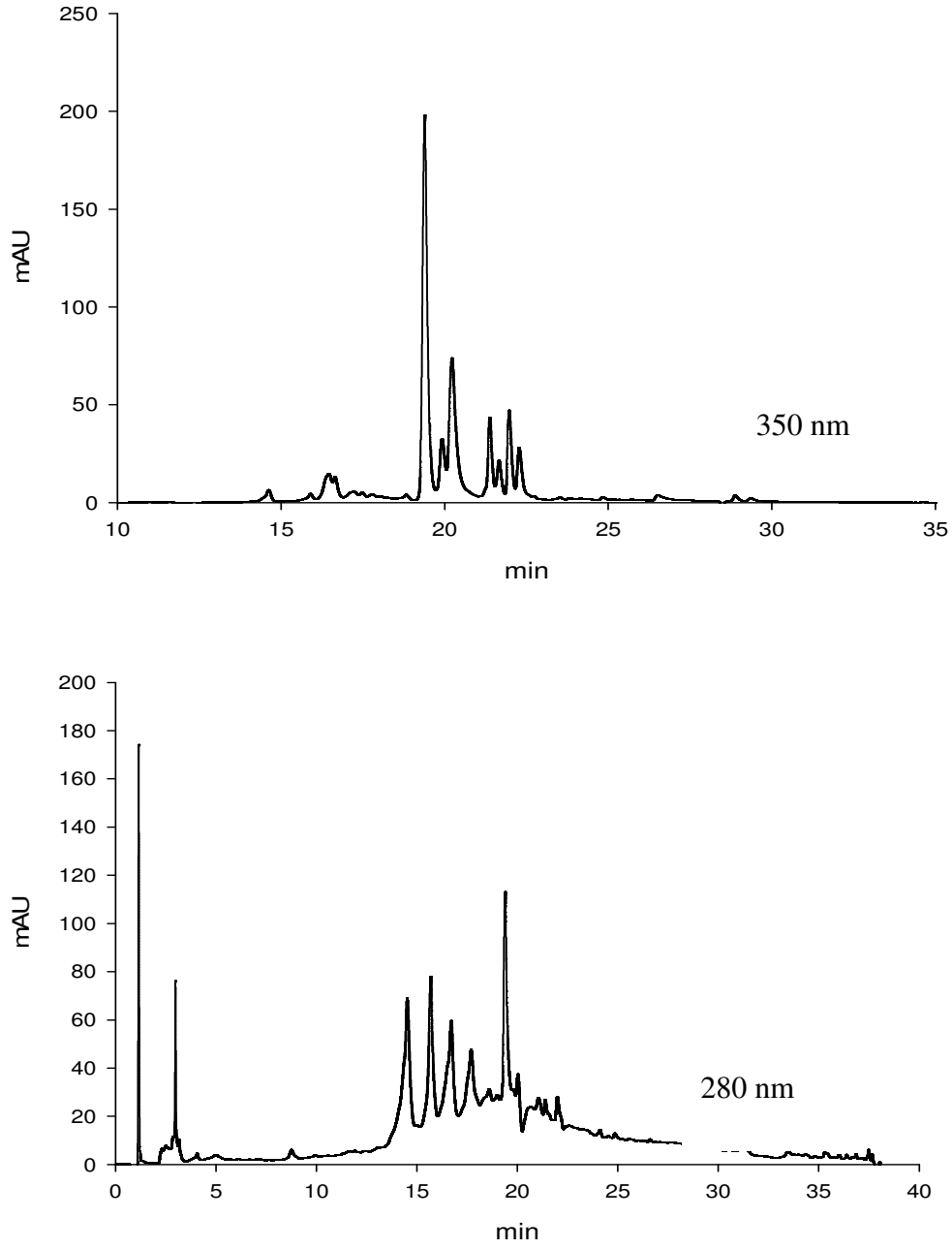


Fig. B.2: Chromatograms of MAE extract from batch (B) cocoa leaves

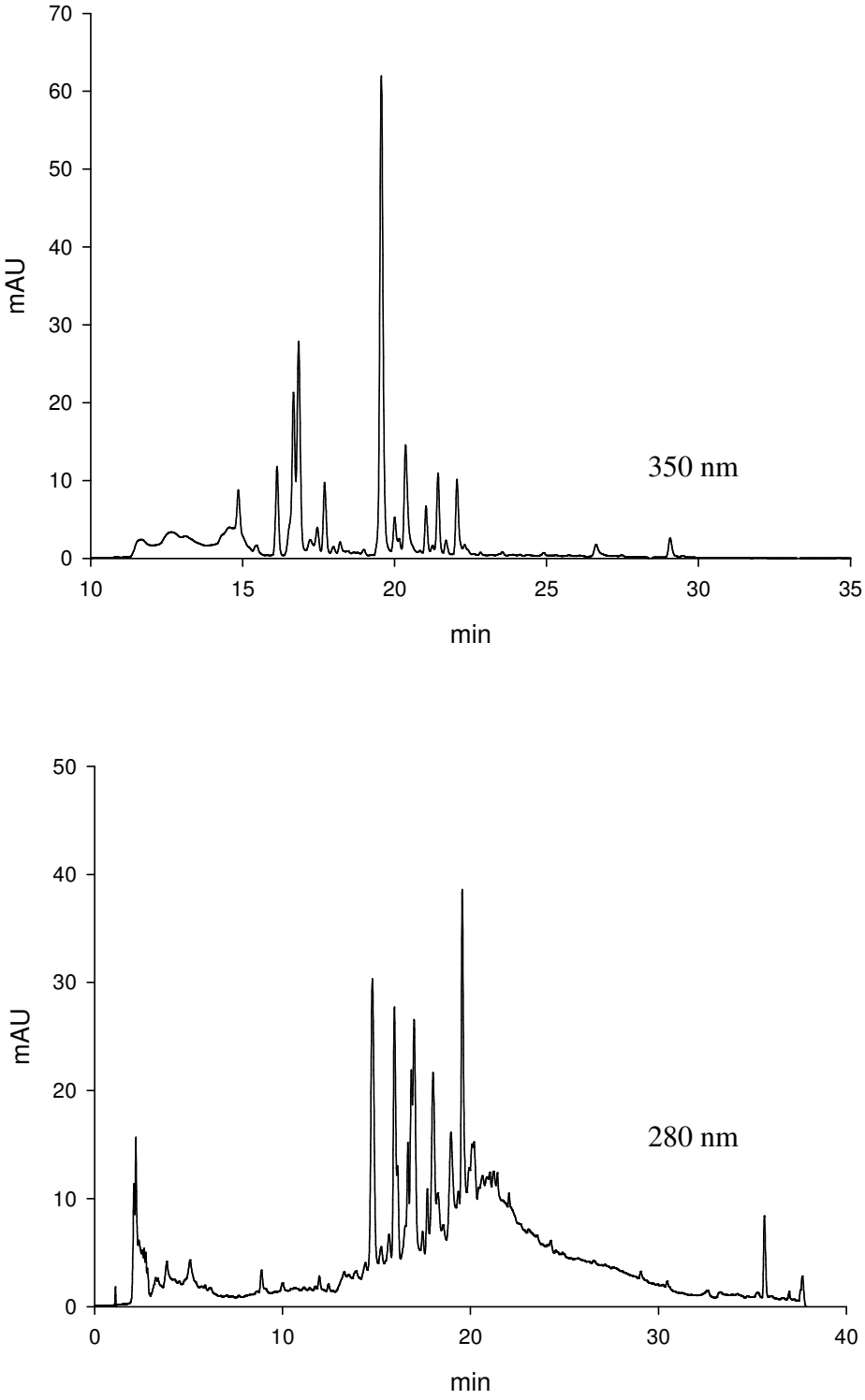


Fig. B.3: Chromatograms of MAE extract from batch (C) cocoa leaves

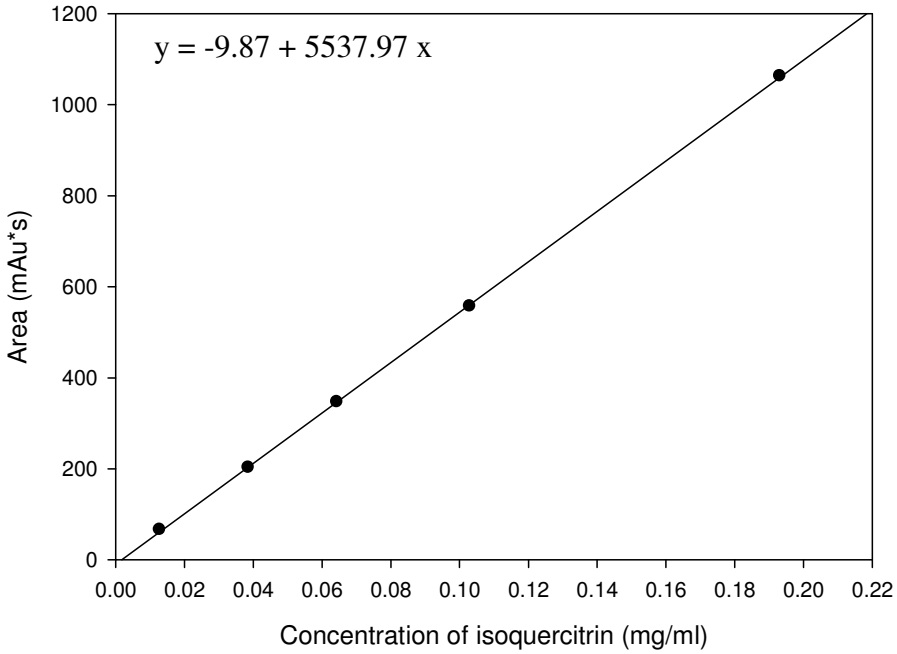


Fig. B.4. Calibration curves of isoquercitrin (IQ)

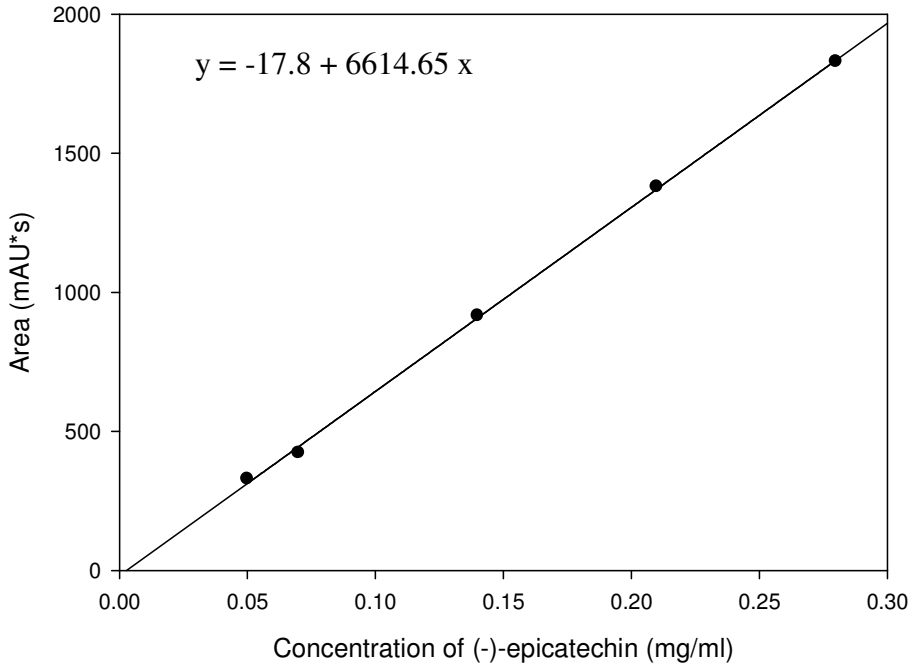


Fig. B.5. Calibration curves of (-)-epicatechin (EC)

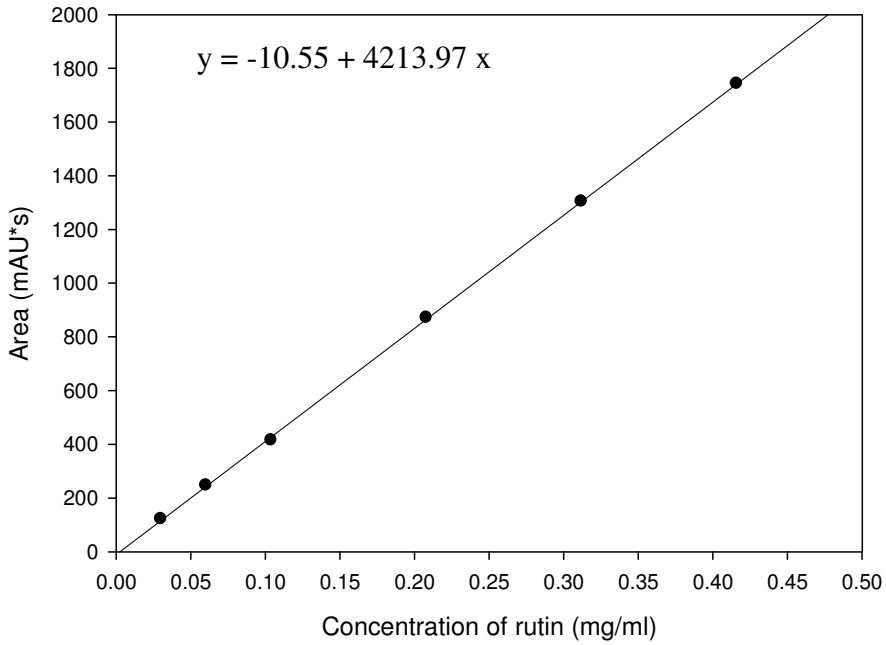


Fig. B.6. Calibration curves of rutin (RT)

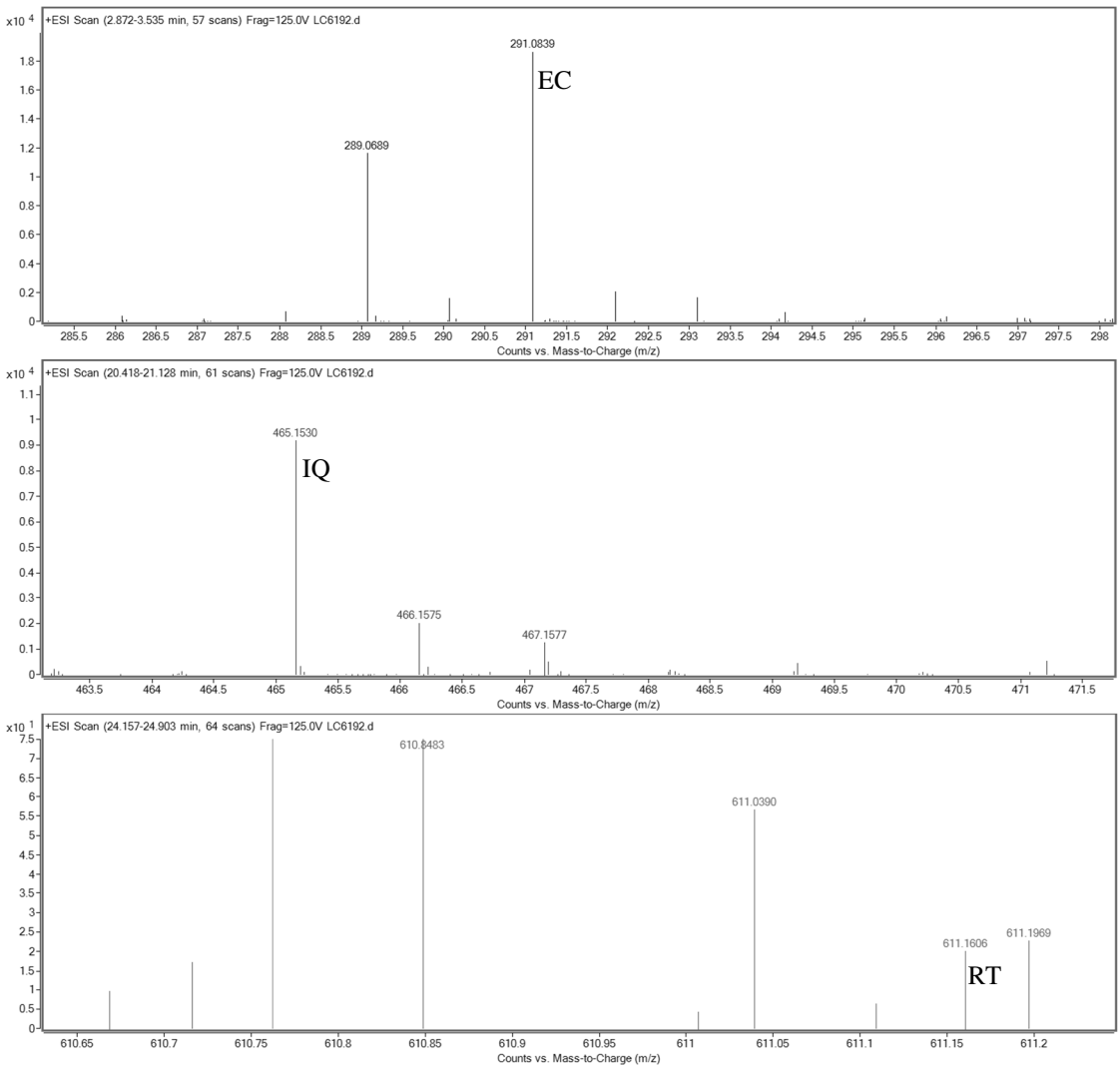


Fig. B.7: MS/MS spectrum of isoquercitrin, (-)-epicatechin and rutin in cocoa leaves extract

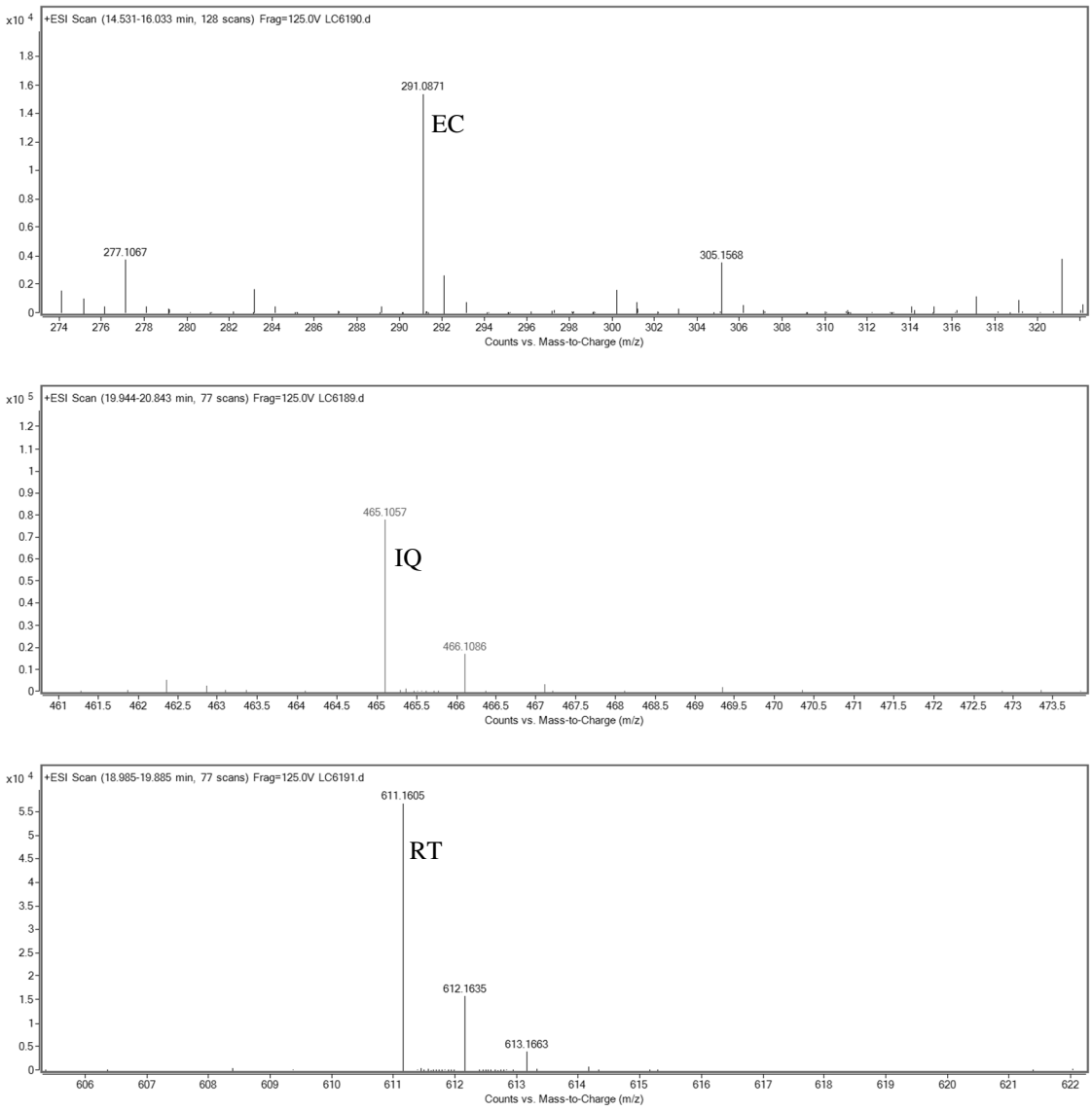
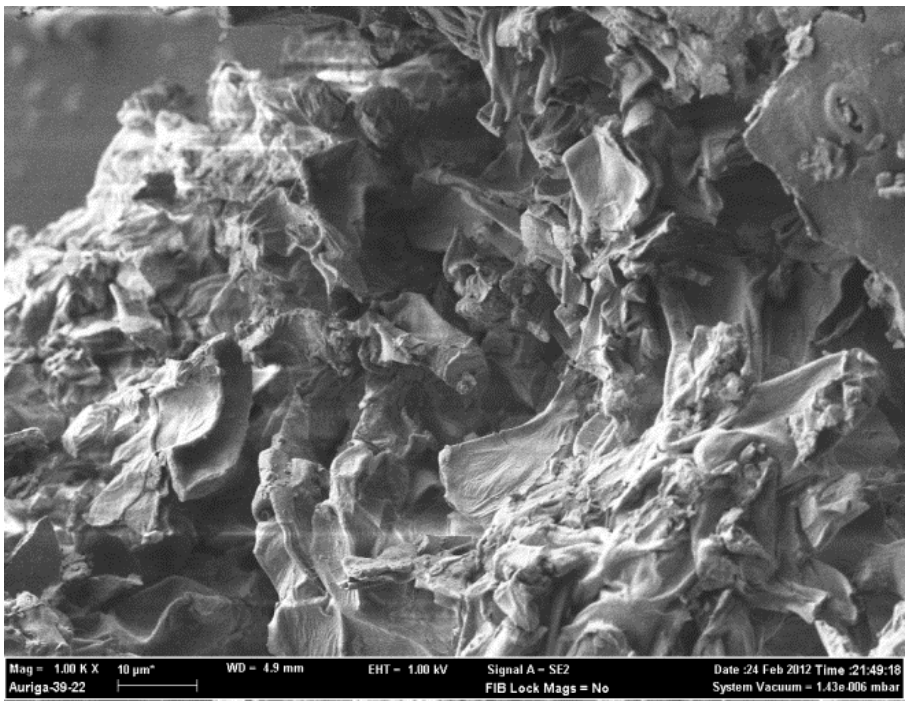


Fig. B.8: MS/MS spectrum of isoquercitrin, (-)-epicatechin and rutin standard compounds

a



b

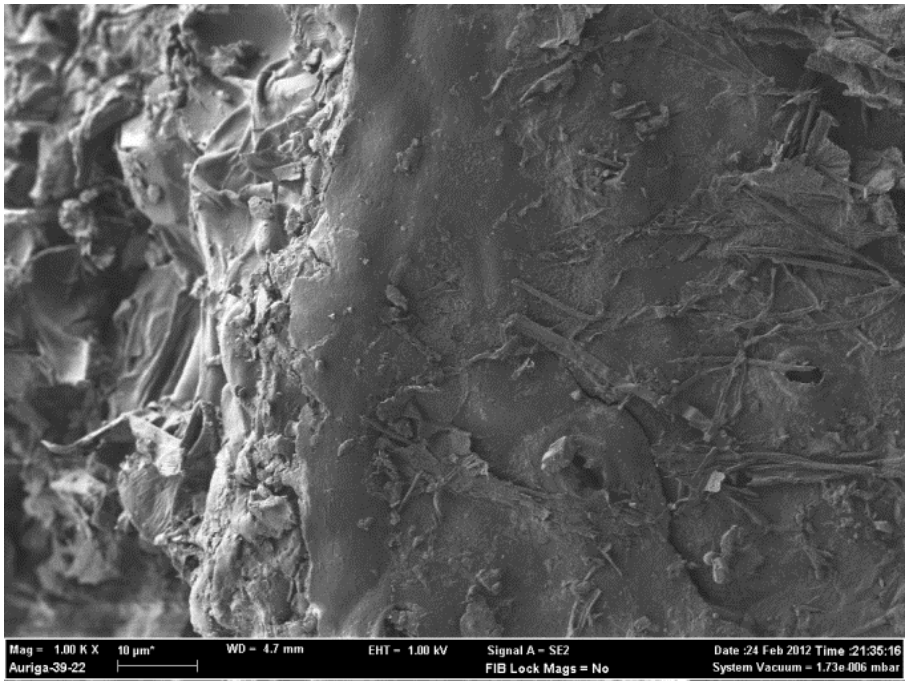


Fig. B.9: Scanning electron micrographs of dried sample
[(*a) plant cells, (b) surface of leave*]

APPENDIX C

EXPERIMENTAL DATA OF KINETIC AND MODELING STUDIES

Table C.1: Extraction data for kinetic and modeling studies

Particle sizes (mm)	Mass of sample (g)	Microwave power (W)	Solvent loading (ml)	Solvent to feed ratio (ml/g)	Extraction time (min)	IQ yield (mg/g)	EC yield (mg/g)	RT yield (mg/g)	Total yield (mg/g)
(a) Effect of particle sizes									
< 0.1					13	3.31	2.38	6.50	12.20
0.1- 0.15					13	2.68	2.24	5.59	10.51
0.15-0.25					13	2.67	2.47	5.30	10.45
0.25-0.6	2	160	160	80	13	2.25	2.22	4.75	9.22
0.6-0.71					13	2.03	2.09	4.45	8.57
0.71- 1					13	1.90	1.97	4.14	8.01
> 1					13	2.02	1.98	4.18	8.17
(b) Effect of particle sizes									
< 0.25	2	100	100	50	1.00	2.82	2.34	5.64	10.79
					2.00	2.77	2.35	5.97	11.09
					5.00	2.99	2.46	6.36	11.81
					7.00	3.06	2.48	6.45	11.99
					10.00	3.13	2.45	6.56	12.14
					13.00	3.22	2.45	6.67	12.34
					15.00	3.38	2.37	7.07	12.82
					20.00	3.33	2.38	6.89	12.59
					25.00	3.42	2.42	7.01	12.84
					30.00	3.27	2.38	6.71	12.37
0.25-0.6	2	100	100	50	0.5	1.6	1.88	3.15	6.63
					1.00	1.65	1.93	3.29	6.87
					2.00	1.75	2.03	3.57	7.34
					5.00	2.01	2.14	4.22	8.38
					7.00	2.24	2.20	4.63	9.06
					10.00	2.50	2.33	5.13	9.95
					13.00	2.67	2.44	5.48	10.59
					15.00	2.74	2.49	5.57	10.80
					20.00	2.89	2.63	5.83	11.35
					27.00	2.88	2.58	5.77	11.23
					35.00	2.91	2.53	5.69	11.13

Table C.1, continued: Extraction data for kinetic and modeling studies

Particle sizes (mm)	Mass of sample (g)	Microwave power (W)	Solvent loading (ml)	Solvent to feed ratio (ml/g)	Extraction time (min)	IQ yield (mg/g)	EC yield (mg/g)	RT yield (mg/g)	Total yield (mg/g)
(c) Effect of solvent to feed ratio									
0.25-0.6	5	100	100	20	1.00	1.68	1.62	3.54	6.85
					2.00	1.65	1.66	3.41	6.71
					5.00	2.15	1.88	4.44	8.46
					7.00	2.11	1.90	4.57	8.59
					10.00	2.45	2.07	5.25	9.78
					13.00	2.54	2.07	4.92	9.53
					15.00	2.62	2.19	5.43	10.24
					20.00	2.61	2.12	5.36	10.09
					25.00	2.66	2.13	5.41	10.21
					30.00	2.63	2.15	5.34	10.13
0.25-0.6	1.25	100	100	80	1.00	1.86	2.08	3.75	7.69
					2.00	1.95	1.98	4.03	7.96
					5.00	2.58	2.13	5.46	10.17
					7.00	2.75	2.36	5.68	10.78
					10.00	2.83	2.43	5.81	11.08
					13.00	3.03	2.42	6.16	11.61
					15.00	3.08	2.45	6.20	11.73
					20.00	3.22	2.52	6.43	12.17
					25.00	3.02	2.51	6.33	11.86
					30.00	3.03	2.54	6.31	11.88
(d) Effect of microwave irradiation power									
0.25-0.6	2	200	100	50	0.50	2.08	2.09	4.31	8.48
					1.00	1.75	1.91	3.69	7.36
					2.00	2.26	2.21	4.82	9.30
					4.00	2.59	2.34	5.31	10.24
					6.00	2.69	2.50	5.55	10.73
					8.00	2.85	2.60	5.79	11.23
					10.00	3.02	2.59	6.13	11.74
					13.00	3.13	2.61	6.21	11.95
					16.00	2.99	2.60	5.94	11.53
					19.00	3.07	2.48	5.79	11.34

Table C.1, continued: Extraction data for kinetic and modeling studies

Particle sizes (mm)	Mass of sample (g)	Microwave power (W)	Solvent loading (ml)	Solvent to feed ratio (ml/g)	Extraction time (min)	IQ yield (mg/g)	EC yield (mg/g)	RT yield (mg/g)	Total yield (mg/g)
0.25-0.6	2	300	100	50	0.50	1.88	1.95	3.89	7.71
					1.00	2.09	2.10	4.32	8.52
					2.00	2.55	2.38	5.22	10.14
					3.00	2.65	2.30	5.34	10.29
					4.00	2.84	2.40	5.72	10.95
					5.00	2.84	2.47	5.69	11.00
					6.00	2.89	2.44	5.77	11.10
					7.00	2.87	2.57	5.59	11.03
					9.00	2.80	2.36	5.53	10.69
0.25-0.6	2	450	100	50	0.50	1.87	1.77	3.99	7.63
					0.17	1.69	1.83	3.28	6.79
					0.33	1.71	1.79	3.61	7.11
					0.75	1.99	1.93	4.23	8.15
					1.00	2.11	2.09	4.47	8.67
					1.50	2.44	2.31	5.05	9.79
					2.00	2.58	2.30	5.33	10.22
					3.00	2.79	2.47	5.75	11.01
					4.00	2.83	2.45	5.76	11.04
					5.00	2.92	2.46	5.81	11.19
					6.00	2.74	2.44	5.51	10.70
0.25-0.6	2	600	100	50	0.17	1.75	1.90	3.36	7.01
					0.25	1.84	1.93	3.87	7.64
					0.50	1.90	1.94	4.03	7.86
					0.75	2.48	2.23	5.24	9.95
					1.00	2.55	2.32	5.34	10.20
					1.50	2.72	2.42	5.64	10.78
					2.00	2.71	2.40	5.59	10.69
					3.00	2.75	2.37	5.64	10.77
					4.00	2.80	2.32	5.70	10.82
(e) Effect of solvent loading at input power density of 1 W/ml									
0.25-0.6	3	150	150	50	0.50	2.13	2.02	4.29	8.43
					1.00	2.41	2.06	4.87	9.33
					2.00	2.29	2.13	4.70	9.13
					4.00	2.47	2.15	5.08	9.70
					5.30	2.59	2.35	5.22	10.15
					8.00	2.58	2.43	5.32	10.34
					10.00	2.71	2.45	5.38	10.54
					12.00	2.91	2.37	5.76	11.04
					15.00	2.97	2.42	5.86	11.25
					18.00	2.86	2.31	5.59	10.76

Table C.1, continued: Extraction data for kinetic and modeling studies

Particle sizes (mm)	Mass of sample (g)	Microwave power (W)	Solvent loading (ml)	Solvent to feed ratio (ml/g)	Extraction time (min)	IQ yield (mg/g)	EC yield (mg/g)	RT yield (mg/g)	Total yield (mg/g)
0.25-0.6	4	200	200	50	0.50	1.69	1.80	3.69	7.18
					1.00	2.06	2.09	4.03	8.18
					2.00	2.28	2.24	4.66	9.19
					5.00	2.57	2.42	5.31	10.30
					7.00	2.95	2.41	5.84	11.20
					10.00	2.74	2.38	5.62	11.74
					15.00	3.15	2.72	6.19	12.06
					20.00	3.18	2.88	6.36	12.41
					27.00	3.20	2.77	6.21	12.18
					35.00	3.20	2.72	6.16	12.08
0.25-0.6	5	250	250	50	0.50	2.21	2.11	4.43	8.75
					1.00	1.96	1.92	4.02	7.90
					2.00	2.33	2.06	4.69	9.08
					3.00	2.53	2.19	5.11	9.84
					4.00	2.73	2.25	5.46	10.45
					5.00	2.80	2.44	5.57	10.81
					7.00	2.89	2.28	5.66	10.83
					8.00	3.08	2.48	5.99	11.56
					10.00	3.23	2.60	6.28	12.10
					12.00	3.15	2.53	6.12	11.80
					14.00	3.20	2.57	6.23	12.00
0.25-0.6	6	300	300	50	0.25	1.82	2.07	3.90	7.79
					0.50	1.75	2.00	3.57	7.32
					1.00	2.16	2.00	4.26	8.42
					1.50	2.08	2.13	3.23	7.44
					2.00	2.28	2.31	4.71	9.31
					2.50	2.45	2.36	4.97	9.79
					3.00	2.48	2.41	4.96	9.85
					4.00	2.70	2.52	5.31	10.53
					5.00	2.73	2.47	5.45	10.66
					6.00	2.98	2.64	5.82	11.43
					7.00	2.84	2.58	5.64	11.06
					8.00	2.98	2.63	5.81	11.41
					9.00	2.87	2.58	5.63	11.08
					10.00	2.94	2.64	5.91	11.48

APPENDIX D

EXPERIMENTAL DATA OF THE OPTIMIZATION USING APD AND AED

Table D.1: Experimental data for the optimization using APD and AED

Solvent to feed ratio (ml/g)	Solvent loading (ml)	Microwave Power (W)	APD (W/ml)	Time (min)	AED (J/ml)	IQ yield (mg/g)	EC yield (mg/g)	RT yield (mg/g)	Total yield (mg/g)
Single factor optimization on solvent to feed ratio									
10	50	100	0.43	10	258	0.67 ±0.02	0.81 ± 0.04	2.79 ± 0.07	4.26 ± 0.13
20						0.85 ± 0.00	1.13 ± 0.01	3.51 ± 0.01	5.48 ± 0.02
30						0.95 ± 0.02	1.25 ± 0.01	3.89 ± 0.04	6.10 ± 0.05
40						0.99 ± 0.01	1.27 ± 0.02	4.04 ± 0.07	6.30 ± 0.10
50						0.92 ± 0.01	1.26 ± 0.02	4.10 ± 0.05	6.27 ± 0.04
60						0.95 ± 0.01	1.29 ± 0.00	4.21 ± 0.08	6.44 ± 0.10
70						0.96 ± 0.02	1.31 ± 0.02	4.23 ± 0.10	6.50 ± 0.14
80						0.98 ± 0.00	1.34 ± 0.00	4.28 ± 0.06	6.59 ± 0.06
90						0.98 ± 0.01	1.34 ± 0.00	4.29 ± 0.03	6.61 ± 0.03
Single factor optimization on AED									
50	50	100	0.43	2	52	0.72	1.08	3.26	5.06
				4	103	0.77	1.14	3.44	5.36
				6	155	0.86	1.20	3.86	5.93
				8	206	0.94	1.30	4.27	6.51
				10	258	0.97	1.35	4.40	6.72
				12	310	0.99	1.30	4.49	6.79
				14	361	0.98	1.35	4.43	6.76
				16	413	1.03	1.38	4.51	6.92
				18	464	0.98	1.36	4.30	6.64
				20	516	1.03	1.35	4.51	6.88
Single factor optimization on APD									
50	100	100	0.15	33.3	300	1.02 ± 0.01	1.43 ± 0.01	4.46 ± 0.02	6.91 ± 0.04
		130	0.18	27.8		1.05 ± 0.06	1.37 ± 0.06	4.50 ± 0.04	6.91 ± 0.04
		150	0.30	16.7		1.05 ± 0.06	1.38 ± 0.03	4.49 ± 0.06	6.97 ± 0.11
		170	0.36	13.9		1.02 ± 0.01	1.33 ± 0.04	4.49 ± 0.02	6.83 ± 0.01
		200	0.43	11.6		1.00 ± 0.00	1.35 ± 0.04	4.44 ± 0.06	6.80 ± 0.07
		300	0.93	5.4		1.03 ± 0.09	1.38 ± 0.03	4.41 ± 0.09	6.82 ± 0.20