

**Faculty of Engineering and Science**

**Microwave-Assisted Hydrodistillation: Garlic Essential Oil  
Recovery**

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**This thesis is presented for the Degree of  
Master of Philosophy (Chemical Engineering)  
of  
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## **Declaration**

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material, which has been accepted for the award of any other degree or diploma in any university.

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## ABSTRACT

In recent decades, the usage of phytochemicals generated from natural plants for food, beverage, drug and pharmaceuticals have increased steadily in response to increasing demand and advancements in extraction and analytical technologies. Amongst the numerous natural plants, the use of garlic (*Allium sativum*) is widespread owing to its desirable flavour and bioactive components. Garlic essential oils possess several interesting pharmacological which include antimicrobial, anti-inflammatory and antioxidant properties. One of the key challenges in extracting garlic essential oil is to maintain the molecular and functional characteristics of the extract, which comprises of a mixture of more than 20 heat sensitive organosulfur compounds. In conventional extraction techniques, garlic essential oil is often exposed to a long extraction durations under high temperature conditions. Reducing the extraction time is a strategy to maintain the structural integrity of the extract; however, this can compromise the extraction yield. To address the aforementioned challenge, this study proposed microwave-assisted technology to reduce the extraction period whilst releasing sufficient energy for oil enhanced extraction. Thus, rapid heat transfer via the microwave exposure is crucial to improving the extraction rate.

The present work investigated the effectiveness and characteristics of a microwave-assisted hydrodistillation (MAHD) process for recovering garlic essential oil. A modified microwave oven was used to carry out the process. Both solvent free and solvent-assisted (MAHD) processes were investigated under the influences of four operating parameters: microwave power, water to ethanol ratio, solid to total liquid ratio and extraction period. The antimicrobial properties of the extracted essential oils were analysed. Experimental results showed that the MAHD extraction is better than the SFME. The optimum conditions for the MAHD extraction process with water-ethanol extracting medium occurred at a ratio of ethanol to water of 1:1.5 which reduced the extraction time by about 50% relative to that of the MAHD alone (without extracting liquid medium). This corresponds to an optimal yield of 0.07% (g essential oil / 100 g of fresh garlic). Moreover, analyses showed that the microwave did not destroy the functional group responsible for the antimicrobial action during the extraction process. This was proven through the disc diffusion method, MIC and MBC analyses. The results showed that the extracted oil is effective against the target bacteria: *E. coli* and *S. Aureus*.

A rigorous fundamental model was developed to characterize the MAHD process. Three constitutive equations were proposed to represent: (1) microwave power conversion into heat, (2) overall mass transfer coefficient, and (3) Vapour-Liquid Equilibrium (VLE) coefficient of the garlic oil in ethanol-water mixture. By incorporating the three constitutive equations into the MAHD model, a good prediction of garlic essential oil recovery was achieved within  $\pm 5\%$  of experimental yield values. Based on the MAHD model, the solubility of garlic oil in the ethanol was estimated to be about 80-100 times as high as that in water at room temperature. The fundamental MAHD model can also be applied to theoretically studying MAHD extraction processes for extraction of other plant.

**Keywords:** Garlic essential oil, Microwave technology, Natural plant extraction, Antimicrobial properties

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## NOMENCLATURE

### Abbreviation

3-VDT	3-Vinyl-[4H]-1,2-Dithiin
BBD	Box Behnken Design
CCD	Central Composite Design
DATS	Diallyl Trisulphide
DATTS	Diallyl Tetrasulphide
DDS	Diallyl Disulphide
DMS	Dimethyl Disulphide
DMTS	Dimethyl Trisulphide
DS	Diallyl Sulfide
<i>E Coli</i>	<i>Escherichia Coli</i>
EVS	Ethyl Vinyl Sulphide
Exp	Experiment
FTIR	Fourier Transform Infrared Spectroscopy
GC-FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography – Mass Spectrometry
HCl	Hydrochloric Acid
HD	Hydrodistillation
H <sub>2</sub> S	Hydrogen Sulfide
HIPEF	High Intensity Pulsed Electric Fields
KBr	Potassium Bromide
LDPE	Low Density Polyethylene
MADS	Cis or trans Methyl Allyl Disulphide
MAE	Microwave-Assisted Extraction
MAHD	Microwave-Assisted Hydrodistillation
MATS	Methyl Allyl Trisulphide
MBC	Minimum Bactericidal Concentration
MHG	Microwave Hydro Diffusion Gravity
MIC	Minimum Inhibitory Concentration

NAOH	Sodium Hydroxide
NRTL	Non-Random-Two-Liquid
OFAT	One-Factor-at-A-Time
PMAE	Pressurized Microwave-Assisted Extraction
PSFME	Pressurized Solvent Free Microwave Extraction
RMS	Root Mean Square
<i>S Aureus</i>	<i>Staphylococcus Aureus</i>
SCCO <sub>2</sub>	Supercritical Carbon Dioxide
SDE	Simultaneous Distillation Extraction
SFE	Supercritical Fluid Extraction
SFME	Solvent Free Microwave Extraction
Simul	Simulation
S/N	Signal-to-Noise
UAE	Ultrasonic-Assisted Extraction
VMAE	Vacuum Microwave-Assisted Extraction
VMHD	Vacuum Microwave Hydro Distillation
VLE	Vapour-Liquid Equilibrium

### **Greek letters/ Physics**

$\tan \delta$	Loss Tangent	dimensionless
$\epsilon'$	Dielectric constant	$F/m$
$\epsilon''$	Dielectric Loss	$F/m$
$m_e$	Oil extracted at time	$g \text{ oil}/g \text{ matrix feed}$
$k$	Cellular mass transfer coefficient	$\text{min}^{-1}$
$m_{s,t}$	Quantity of unextracted oil at time	$g \text{ oil}/g \text{ matrix feed}$
$m_{s,t}^*$	Quantity of unextracted oil at time	$g \text{ oil}/g \text{ matrix feed}$
$M$	The mass of oil contained in the column	$g$
$y_0$	The input essential oil fraction by weight	$g$
$y(t)$	Steam essential oil fraction by mass at the outer column at a time	$g$
$G_v$	Mass flow rate of steam	$gm^{-1}$
$K$	Overall mass transfer coefficient	$g \text{ oilmin}^{-1}m^{-2}$

$\Omega$	Surface area available for mass transfer	$m^2$
$x$	Difference between an average composition of seeds	dimensionless
$x^*$	Oil fraction assumed to be in equilibrium composition with steam	dimensionless
$k$	Second-order extraction rate constant	$Lg^{-1}min^{-1}$
$C_s$	Extraction capacity	$gL^{-1}$
$C_t$	Concentration of sandalwood oil at any time	$gL^{-1}$
$h$	Initial extraction rate	$Lg^{-1}min^{-1}$
$\rho_f$	Solvent density	$g/mL$
$\rho_s$	Solid density	$g/mL$
$\varepsilon$	Bed void fraction	dimensionless
$U$	Superficial fluid velocity	$ms^{-1}$
$Y$	Solute mass fraction in fluid phase	$kg/kg$
$x$	Solute mass fraction in solid phase	$kg/kg$
$J$	Flux of solute	$kg/m^3s$
$Z$	Axial co-ordinate	dimensionless
$x_0$	Initial mass fraction of solute in solid flux	$kg/kg$
$k_f$	Fluid phase mass transfer coefficient	$m/s$
$k_s$	Solid phase mass transfer coefficient	$m/s$
$a_0$	Specific surface area per unit volume of extraction bed	$m^2/m^3$
$Y^*$	Equilibrium fluid phase mass transfer	$kg/kg$
$x_k$	Easily accessible solute in solid phase	$kg/kg$
$M(t)$	Mass of extract	$kg$
$\dot{m}$	Solvent flow rate	$kg/s$
$H$	Extraction bed length	$m$
$M(\infty)$	Maximum value for the extract obtained	$kg$
$L$	Thickness of the plate	$m$
$D$	Effective diffusion coefficient	$m^2/s$
$c_A$	Oil concentration at the boundaries	$g/mL$
$m(t)$	Mass flow as a function of time	$g$
$\lambda$	Core dimension	$m$

$x_i$	Mean solute concentration in the core	<i>g oil/kg matrix</i>
$k$	Mass transfer rate	<i>m/s</i>
$x_w$	Mean solute concentration in boiling water	<i>g oil/ kg water</i>
$k_w$	Mass transfer rate of boiling water	dimensionless
$q_p$	Average concentration of essential oil in the plant material	<i>mg/g</i>
$x$	Distance along the direction of diffusion	<i>m</i>
$D_{\text{eff}}$	Effective diffusion coefficient	<i>h<sup>-1</sup></i>
$q$	Average yield at time	<i>g</i>
$q_0$	Initial average concentration	<i>mg/g</i>
$A$	Constant area	<i>m<sup>2</sup></i>
$K$	Kinetic constant including the effective diffusion coefficient	<i>h<sup>-1</sup></i>
$q_w$	Essential oil yield	<i>mg/g</i>
$q$	Essential oil yield	<i>mg/g</i>
$b$	Washing coefficient	<i>l</i>
$k$	Diffusion coefficient	<i>h<sup>-1</sup></i>
$q$	Function of a specific quantity solvent	<i>g solvent/g solute</i>
$q_m$	Extraction of the solute within the particles	<i>g solvent/g solute</i>
$q_n$	Fully extraction of accessible solute	<i>g solvent/g solute</i>
$\dot{q}$	Solvent specific mass flow rate	<i>kg/s</i>
$Z$	Mass transfer parameters in solid phase	dimensionless
$W$	Mass transfer parameters in liquid phase	dimensionless
$z_w$	Boundary coordinated between slow and fast extraction	dimensionless
$x_0$	Initial total concentration of the solute in bed	<i>g solvent/g solute</i>
$x_k$	Initial content of the barely accessible solute	<i>g solute/g solute</i>
$\rho_s$	Density of solid phase	<i>kg/m<sup>3</sup></i>
$\rho_f$	Density of fluid phases	<i>kg/m<sup>3</sup></i>
$\varepsilon$	Void fraction of the bed	<i>m<sup>3</sup>/m<sup>3</sup></i>
$a$	Specific interfacial area of the solid particles	<i>m<sup>2</sup>/m<sup>3</sup></i>
$y_r$	Extract solubility in SC-CO <sub>2</sub>	<i>g solute/g solvent</i>
$t$	Extraction time	<i>s</i>

$k_s$	Solid phase mass transfer coefficient	$m/s$
$k_f$	Fluid phase mass transfer coefficients	$m/s$
$x_u$	Untreated particles	$g \text{ solute} / g \text{ solute}$
$\Gamma$	Initial ratio of the essential oil in fluid to solute	dimensionless
$\Theta_e$	External mass transfer resistance	dimensionless
$\Theta_i$	Internal mass transfer resistance	dimensionless
$u_t$	Fraction of the essential oil extracted at time	dimensionless
$k$	1 <sup>st</sup> order rate constant	$min^{-1}$
$k_1$	1 <sup>st</sup> order rate constant describing the fast release fraction	$min^{-1}$
$F$	Fast release fraction	dimensionless
$k_2$	1 <sup>st</sup> order constant describing the slowly released fraction	$min^{-1}$
$1 - F$	Slow released fraction	dimensionless
$q_p$	Average concentration of essential oil in the particles at time	$g/mL$
$k$	Process rate constant	$mL \text{ min}^{-1}$
$f$	Washing coefficient	dimensionless
$k_1$	Coefficient for fast washing extraction	$min^{-1}$
$k_2$	Coefficient for slow diffusion extraction	$min^{-1}$
$\zeta$	Zeta Potential	$mV$
$u_e$	Electrophoretic mobility	dimensionless
$\eta$	Solvent viscosity	$kg/ms$
$f(\kappa a)$	Henry's function	dimensionless
$X \in R^n$	Vectors of state variables	dimensionless
$U \in R^k$	Vectors of the input variables	dimensionless
$f \in R^n$	Nonlinear function	dimensionless
$g$	Nonlinear function	dimensionless
$M_s$	Total mass of garlic	$g$
$x_s$	Solid mass fraction of oil	$g/g$
$k_{ex}$	Overall mass transfer coefficient from solid to liquid	$g/mL \cdot min$
$x_l$	Bulk liquid mass fraction of oil	dimensionless
$V_s$	Volume of the garlic	$mL$
$\rho_s$	Garlic density	$g/mL$

$\rho_l$	Average liquid density	$g/mL$
$F_v$	Rate of liquid evaporation	$g/mL$
$y_l$	Vapor mass fraction of oil	dimensionless
$K_l$	VLE constant of oil in the ethanol-water solvent	dimensionless
$y_w$	Mass fractions of water in vapor phase	%
$y_e$	Mass fractions of ethanol in vapor phase	%
$x_w$	Mass fractions of water in liquid phase	$m/v$
$x_e$	Mass fractions of ethanol in liquid phase	$m/v$
$\gamma_i$	Activity coefficient of the $i$ component	dimensionless
$P_i^s$	Vapor pressure of the $i$ component	$mmHg$
$P$	Total pressure built in the vessel	$mmHg$
$\bar{C}_p$	Average specific heat of combined liquid-solid	$J/g^\circ C$
$T$	Liquid temperature	$^\circ C$
$T_{bp}$	Bubble point of the liquid	$^\circ C$
$\varepsilon$	Thermal effectiveness factor of microwave power conversion	dimensionless
$Q_{MW}$	Microwave power	$J/min$
$\lambda_s$	Average latent heat of vaporization	$J/g$
$\eta$	Liquid evaporation	dimensionless
$\rho_w$	Density of water	$g/mL$
$\rho_e$	Density of ethanol	$g/mL$
$\lambda_w$	Latent heat of evaporation of pure water	$J/g$
$\lambda_e$	Latent heat of evaporation of pure ethanol	$J/g$
$\bar{C}_{pw}$	Specific heat capacities water	$J/g^\circ C$
$\bar{C}_{pe}$	Specific heat capacities ethanol	$J/g^\circ C$
$\bar{C}_{ps}$	Specific heat capacities garlic	$J/g^\circ C$
$\beta_1$	Equation parameter	dimensionless
$\beta_2$	Equation parameter	dimensionless
$\beta_3$	Equation parameter	dimensionless
$\tan\delta_w$	Loss tangents for water	dimensionless
$\tan\delta_e$	Loss tangents for ethanol	dimensionless



$V_{l0}$	Initial total liquid volume	mL
$\rho_{l0}$	Average liquid density	$g/mL$
$\lambda_{s0}$	Average latent heat of vaporization	$J/g$
$\bar{C}_{p0}$	Average liquid specific heat	$J/g\ ^\circ C$
$\bar{C}_{ps0}$	Garlic specific heat	$J/g\ ^\circ C$
$a_{e/w}$	Relative solubility of oil in ethanol to its solubility in water	dimensionless
$S_o$	Average solubility of oil	$g/mL$
$k_1$	Kinetic parameter	$\frac{g}{mLmin} / 1/^\circ C$
$k_2$	Kinetic parameters	$K$
$a_1$	Constant	dimensionless
$f_d$	Delay function	dimensionless
$T_{cric}$	Critical temperature	$^\circ C$
$m$	Power value	dimensionless
$S_{ow}$	Solubility of garlic oil in pure water	$g/mL$
$a_{e/w}$	Relative solubility	dimensionless
$a_2$	Relative solubility at reference temperature	dimensionless
$b_w$	Slops of oil solubility against temperature in water	$1/^\circ C$
$b_e$	Slops of oil solubility against temperature in ethanol	$g/mL.\ ^\circ C$
$k_{ex}$	Extraction kinetics	dimensionless
$T_{mp}$	Melting point of ethanol-water mixture	$^\circ C$
$c_1$	Positive constant	dimensionless
$c_2$	Positive constant	dimensionless
$c_3$	Positive constant	dimensionless
$f_e$	Fraction to be accounted for the impact of ethanol on volatility	dimensionless
$x_{s0}$	Initial oil mass fraction in the solid	$m/v$
$x_s(t)$	Oil mass fraction in the solid	$m/v$
$x_l(t)$	Oil concentration in the liquid	$m/v$
$V_l(t)$	Liquid volume at time $t$	$mL$
$P_1$	Optimization problem	dimensionless
$m_t$	Numbers of samples for temperature	dimensionless

$m_v$	Numbers of samples for liquid volume	dimensionless
$T_{ex}$	Experimental temperature	$^{\circ}C$
$V_{ex}$	Experimental liquid volume	$mL$
$\bar{T}$	Average liquid temperature	$^{\circ}C$
$\bar{V}_l$	Average liquid volume	$mL$
$X \in R^6$	Vector of state variables	dimensionless
$\phi_p$	Vector physical properties	dimensionless
$\bar{Z}$	Initial values for $Z$	dimensionless
$f \in R^6$	Vector of nonlinear functions of arguments	dimensionless
$B_{min}$	Lower limits for the fitting parameter	dimensionless
$B_{max}$	Upper limits for the fitting parameter	dimensionless
$T_0$	Initial temperature	$^{\circ}C$
$T_{max}$	Expected maximum temperature	$^{\circ}C$
$\bar{T}$	Average temperature	$^{\circ}C$
$P_2$	Optimization problem	dimensionless
$m_y$	Number of experimental samples for yield	dimensionless
$Yd_{ex}$	Experimental yield	$g/g$
$Z_{min}$	Lower limits for the fitting parameter	dimensionless
$Z_{max}$	Upper limits for the fitting parameter	dimensionless

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# Chapter 1

## INTRODUCTION

### 1.1 Research background

Humankind has explored and used natural plants for their benefits to treat various diseases since the times immemorial. Although different types of medicinal plants have been known and used for centuries, most of the medicinal values and benefits of many species of plants have remained unknown. The progress of phyto-therapeutic researches in recent years has provided a growing pool of evidence where bioactives in natural plants, such as garlic (*A. Sativum*) and turmeric possess various medicinal values. This progress has led to the booming of nutraceutical and related industries. Nevertheless, the nutraceutical properties of garlic remain under emphasized until recent years despite the fact that garlic has been widely utilized as a culinary herb. In a number of articles, it has been reported that garlic possesses antibacterial, antifungal, antiprotozoal and antiviral properties (ur Rehmana and Munirb 2015); hypoglycemic and antiplatelet characteristics (Cavagnaro and Galmarini 2007); anti-parasitic and anti-oxidation properties (Helou and Harris 2007). The medicinal content of garlic essential oil would provide new opportunities to develop high-value products in industries, which is at least in part due to the garlic essential oil antimicrobial activities.

Extraction of essential oil from garlic has gained growing interest among researchers for the development of products in pharmaceutical and food industries. Traditionally, the steam distillation (Sowbhagya, Purnima *et al.* 2009), hydro-distillation (Sylvestre, Legault *et al.* 2005), and Soxhlet extraction (Luque de Castro, Jiménez-Carmona *et al.* 1999) have been used for extracting garlic essential oil. It should be noted that, these conventional methods have low efficiency because they require high temperatures and a large quantity of solvent. High temperature often degrade the extracted oil (Poojary, Barba *et al.* 2016). Furthermore, the conventional methods exhibit slow extraction rates and low extraction yields as well as energy intensive. Hence, the conventional extraction methods often have low economic feasibility in extracting various natural plants of interest. The ability of a given extraction process to achieve the specified quality of extracts is crucial in the new development of sustainable and scalable extraction technologies. These unconventional extraction technologies include ultrasonic-assisted

extraction (UAE) (Kimbaris, Siatis *et al.* 2006), supercritical fluid extraction (SFE) (del Valle, Glatzel *et al.* 2012), and microwave-assisted extraction (MAE) (Luque de Castro, Jiménez-Carmona *et al.* 1999). Credible studies found that the microwave heating technology has a potential of rendering more effective heating than the direct heating by flame or electrical heater. For a microwave technology, its high selectivity for certain compounds together with the ability to produce uniform heating across the whole volume of extraction medium, have been shown to produce higher extraction yield and improved extraction quantity (Gude, Patil *et al.* 2013).

So far, the extraction issues related to garlic in particular and medicinal plants in general remain an open area of research. One of the related gaps is the question of how to design an effective new extraction technique and to enhance the extraction yield and quality of extracted oil. Obviously, an effective extraction method shall demonstrate high selectivity for targeted bioactives, in addition of being able to achieve high yield. In the light of these issues, the present study seeks to find an improved procedure and conditions for extracting garlic essential oil via the microwave technology coupled with solvent extraction. Besides that, in the recent studies, most of the models developed are often oversimplified, which have poor predictive capability and are not suitable for the applications in a large-scale process development and synthesis. At present, there are no reports on the details of the extraction kinetic behaviors, thermal effectiveness of microwave and vapor-liquid equilibrium (VLE) of garlic essential oil; these properties are essential in the development of the microwave-assisted extraction process.

## **1.2 Research questions**

The microwave technology is one of the latest green technology options capable of extracting essential oils from natural plants. Reports of several experimental studies on the microwave-assisted extraction (MAE) to extract essential oils are available, but there have been very limited studies reporting its use to extract garlic essential oil. Recently, growing interests to extract and purify garlic essential oil have been motivated by important properties exhibited by the garlic oil, for examples, antioxidant, anti-cancer and anti-microbial activities. These properties are desirable in the nutraceutical product development. Despite growing research interests in garlic oil, some important data and basic knowledge of its extraction by the microwave technology have remained incomplete, and as a result, this lack of knowledge prevents its commercial-scale process development. The present research study aims to improve understanding of this new process. In

order to achieve this goal, this study will address several important questions. These research questions include but not limited to:

- 1) How several important parameters (microwave power, ethanol to water ratio, liquid volume and mass of feedstock) affect the yield of garlic oil extracted?
- 2) How the microwave power affects the antimicrobial properties of the garlic oil extracted?
- 3) What are the chemical components in the garlic oil extracted?
- 4) Can the microwave hydrodistillation combined with solvent improve the extraction performance compared to that of the solvent free microwave extraction (SFME)?
- 5) To what extent the microwave radiation will degrade the garlic essential oil components, which are responsible for the antimicrobial action?

In this study, the SFME is selected for a comparative study with the MAHD process in order to extract garlic oil. The influences of microwave power, loading feedstock, extraction time, solvent types and solvent ratio on the SFME and MAHD performances will be analysed and optimized. The new data and knowledge obtained by studying the influences of these parameters could help in the process scale-up and optimization of the SFME/MAHD process.

A potential solvent for coupling with MAHD process is ethanol, which has a high microwave absorbing potential (loss tangent of 0.941). For this reason of high loss tangent, ethanol could facilitate the diffusion of targeted compounds from the plant matrix to the bulk of extracting liquid phase. In addition, an increase of solubility of essential oil in the solvent could further enhance the extraction rate. For a mixture of water-ethanol, the presence of water, which has a moderate microwave absorbing potential (lower than that of ethanol), may overcome the degradation of heat sensitive compounds – water acts as quenching effect to avoid over heating of certain heat sensitive compounds. In other words, water could avoid overheating due to high microwave absorbing potential of ethanol. Hence, the combination of these polar solvents could also enhance the extraction process while simultaneously can avoid the degradation of heat sensitive components in the garlic essential oil.

So far, very little investigation has been carried out to study the potential degradation effect of microwave radiation on the essential oil components, which contribute to certain properties, e.g., its antimicrobial activities. The mechanism of microwave heating is through the vibration of polar molecules when induced by the electromagnetic waves. As these polar molecules (solvent and some bioactives) are inside the garlic matrix, the microwave radiation will cause infusion of



direct heating inside the matrix, thus leading to increased diffusion of the bioactives out of the matrix to the bulk solvent. While this is a desirable effect of the microwave radiation, the potential negative effect is that, an excessive radiation may alter the structure of some highly polar compounds, i.e., excessive vibrations of the molecules may lead to localized heating which then destroy the properties of the given compounds. However, if the compounds are mildly polarized molecules, which could be expected of some molecules like allicin in garlic essential oil, the microwave radiation may not cause significant damage to such molecules; hence these compounds are expected to retain most their desirable properties. It is very important though in this study to ascertain whether the use of MAHD, will lead to a significant degradation of the garlic oil antimicrobial properties. More specifically, will the antimicrobial properties of the targeted compound damaged by the microwave irradiation.

The answers several questions above, it is important to conduct sufficient experimental study of both SFME and MAHD processes. For the latter, the extraction performances in the presence of several different solvent or/and co-solvents will be investigated.

The following hypotheses serve as guides in the experimental and theoretical (simulation) study in this project:

- 1 **Hypothesis H1:** The MAHD combined with water-ethanol solvent extraction can achieve a high extraction yield while significantly shorten the extraction period, compared with the SFME or MAHD without ethanol co-solvent. The reason for this improved performance is due to the microwave radiation promoting direct and uniform heating within the plant matrix. In addition, ethanol co-solvent improves solubility of oil in water-ethanol mixture. The combination of these two factors will mediate the diffusion of oil from the solid phase (matrix) to the bulk liquid phase, hence increasing the overall rate of extraction.
- 2 **Hypothesis H2:** Microwave does not destroy the functional groups such as alkanes, amides, C=S stretch, S=O stretch and alkenes, which are responsible for the antimicrobial action in the garlic oil. This is because of the transparency of the compounds to microwave irradiation, i.e., due to weakly polarized molecule.

### 1.3 Objectives

The overall goal of this project is to establish in depth understanding of conditions affecting microwave-assisted extraction technology, as applied to extracting garlic oil. The specific objectives of this project summarized are as follows:

- a. To evaluate the performances solvent free microwave extraction (SFME) and microwave-assisted hydrodistillation extraction (MAHD) for garlic essential oil extraction.
- b. To optimize process conditions for the MAHD combined with water-ethanol solvent in extracting garlic oil.
- c. To analyze antimicrobial properties of the extracted garlic oil by MAHD.
- d. To develop a fundamental model for the MAHD process combined with water-ethanol solvent, to enable evaluation of the microwave power and ethanol co-solvent on the kinetics of extraction.

### 1.4 Novelty, Contributions and Significance

To best of our knowledge, present studies are very limited about the uses of green extraction techniques for garlic oil extraction. In this respect, the novelty of the present study lies in the effort to gain new insights into the MAHD process by combining both experimental and modelling studies, in order to elucidate the mechanism and kinetic behaviours governing the garlic oil extraction under different sets of conditions. Until now, the mechanism and kinetics behaviours of garlic oil extraction in the MAHD process has remained poorly understood. For an example, does the solubility affect the extraction kinetics? Assuming the solubility does affect the extraction kinetics, then to what extent the solubility affect the overall extraction rate?

Note that, it is always desirable that any extraction method adopted should preserve the antimicrobial or other interesting properties contained in the essential oil. Some studies on the applications of the SFME and MAHD techniques showed that these methods are effective for extracting high quality oils in natural plants and herbs. Advantageously, these green extraction methods require shorter extraction time than that of the traditional extraction methods (Filly, Fernandez *et al.* 2014). Unfortunately, a drawback of these green methods is that the yield of

extraction is often lower than in the conventional methods. The question now becomes, can this drawback be overcome by combining the MAHD with water-ethanol solvent? Please note that, there has been no study reported in the open literature showing that the use of microwave will not destroy the antimicrobial properties of the garlic oil. Hence, by taking the above mentioned gaps into consideration, the proposed study attempts to compare for the first time, the performances of extraction via the SFME and MAHD with water-ethanol solvent techniques. To gain detailed insights into the kinetics behaviour, a rigorous fundamental model of the batch MAHD with water-ethanol solvent was constructed. This model is useful to elucidate the current lack of understanding on the role of solvent in altering the overall extraction kinetics in the presence of microwave irradiation.

One of the findings in this study shows that the addition of ethanol as co-solvent helps improve the diffusion of the garlic oil from the plant matrix to the bulk liquid phase. This improvement was made possible due to the better solubility of garlic oil in the water-ethanol mixture compared to that in pure water (in traditional hydrodistillation). This finding agreed with the model prediction, which showed that the solubility of garlic oil in ethanol is about 80 - 100 times as high as the oil solubility in pure water at room temperature. Specifically, in conjunction with the MAHD modelling, the study proposed three important contributions as listed below:

- i. Novel thermal effectiveness equation which enables the calculation on the fractional conversion of microwave energy into thermal energy.
- ii. Novel equation describing the overall extraction rate of essential oil in the presence of water-ethanol mixture, in which solubility is postulated to have a critical role affecting the kinetics.
- iii. Novel empirical equation showing the Vapour-Liquid Equilibrium (VLE) constant of the garlic oil is a function of water-ethanol mixture and liquid temperature.

The present study provides a reasonable ground to conclude that, the MAHD combined with water-ethanol solvent could be a sustainable technology because the process:

- i. Requiring relatively less energy and solvent consumptions than in the conventional processes.
- ii. Giving a higher yield than the conventional processes.

- iii. Leading to non-destruction of interesting properties of the extracted oil, which are derived from are relatively weak polarized molecules.

Significantly, the obtained new data and model in this study should be able at least in assisting understanding of the mechanism and kinetics behaviour governing this MAHD process – useful for process optimization and scale-up. In short, the new knowledge built through the present research should enable engineers to understand the phenomenon of microwave-assisted extraction, which in turn enables the design of scale-up equipment for commercial applications.

## **1.5 Thesis structure**

This thesis is divided into 7 chapters. Chapter 1 introduces the project background, defines the project scope, its significance and research objectives. Chapter 2 presents the importance of phytochemicals in natural plants, especially in garlic. Factors influencing the extraction of an essential oil, the extraction mechanism and the recent development of microwave-assisted extraction technology are also reported. Research methodology for the microwave-assisted extraction technologies and characterization of garlic essential oil are described in Chapter 3. Results obtained from the experiments are discussed in Chapter 4. Chapter 5 illustrates the methodology for kinetic modelling of MAHD and Chapter 6 presents and discusses the simulation results. The comparison of experimental and modelling are deliberated in Chapter 6 as well. Lastly, conclusions and recommendations are presented in Chapter 7. Figure 1.1 illustrates the overview of the thesis structure and chapter interactions.

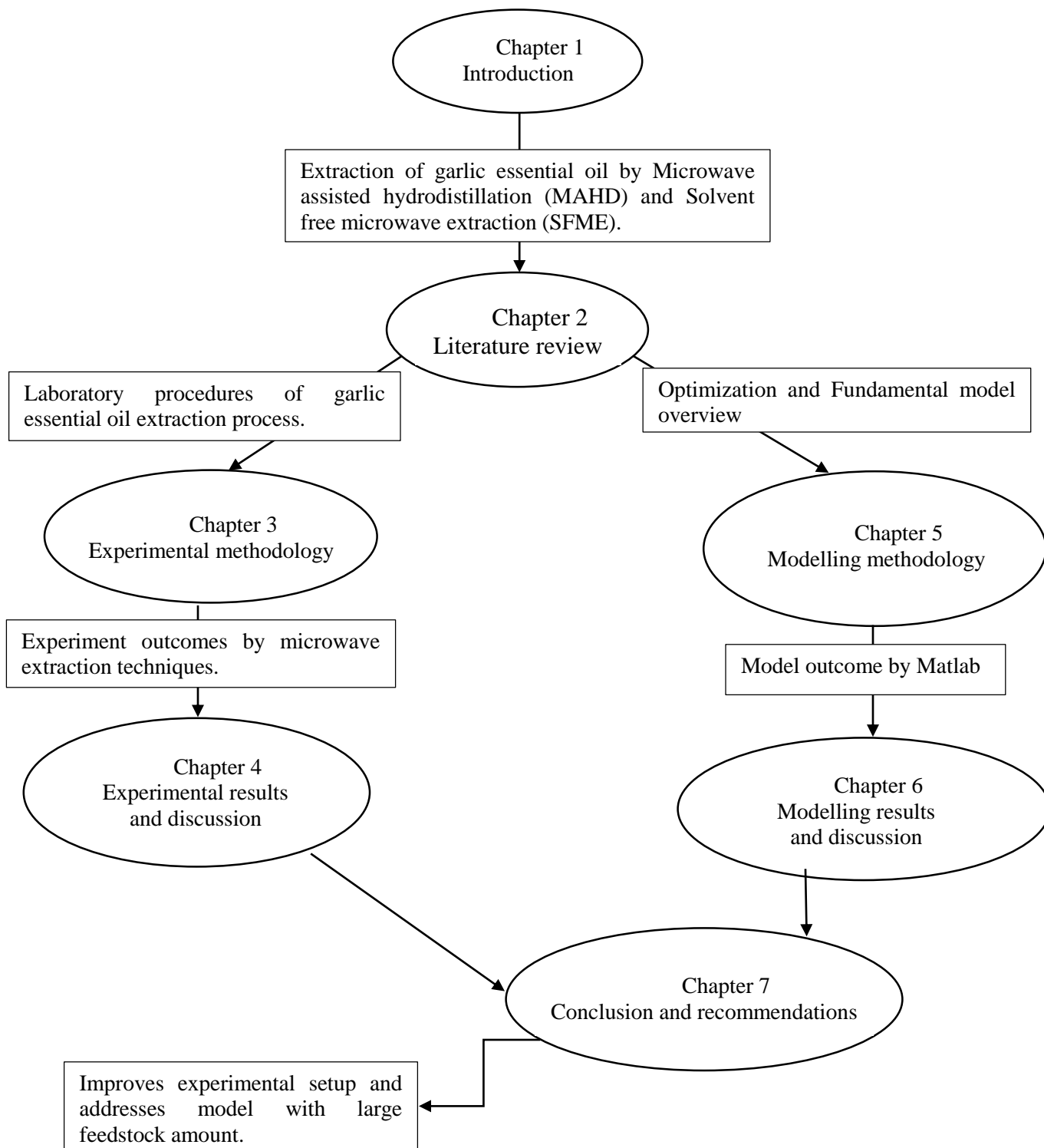


Figure 1.1: Thesis structure overview.

## Chapter 2

### LITERATURE REVIEW

#### 2.1 Garlic

Garlic, or scientifically known *Allium sativum*, is commonly used as spice and seasoning in culinary for centuries. It is also reported as a multi-versatile herb and possesses good therapeutic effects to humans (Li, Chen *et al.* 2010). Garlic has also been studied and reported to contain 58.6 %, of water, 6.4 % of protein and 33 % carbohydrates (Milner and Wildman 2001). These major constituents of garlic are tabulated in Table 2.1.

Table 2.1: Components in Garlic (Milner and Wildman 2001).

Component	Amount/100 g
Water, g	58.6
Protein, g	6.4
Lipid, g	0.5
Carbohydrates, g	33.1
Fiber, total dietary, g	2.1
Calcium, mg	181.0
Magnesium, mg	25.0
Phosphorus, mg	153.0
Potassium, mg	401.0
Selenium, µg	14.2
Vitamin C, mg	31.2

A number of bioactive volatile organosulfur compounds can be found in garlic where one of the interesting compounds is allicin which has been reported to possess therapeutic properties (Sowbhagya, Purnima *et al.* 2009). Allicin is released when the tissues are broken down. This happens when fresh garlic is crushed or chopped to produce alliinase enzyme which converts alliin into allicin as shown in Figure 2.1.

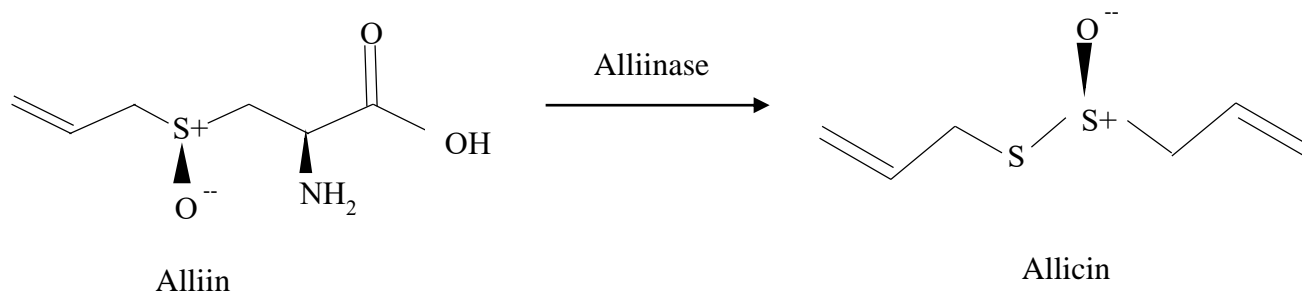


Figure 2.1: Formation of Allicin (Sowbhagya, Purnima *et al.* 2009).

Alliin is a phytochemical that easily degrades to form organosulfur compounds, such as vinyl diithin, allyl sulfide and ajoenes (Cavagnaro and Galmarini 2007). Vinyl diithin has been discovered to have the ability to lower cholesterol risk and help prevent adipocytes development (Dethier, Hanon *et al.* 2013). Meanwhile, allyl sulfide and ajoene have been reported to show anticancer (Wang, Pao *et al.* 2012) and antiplatelet properties (Omar and Al-Wabel 2010).

The health benefits of garlic have been intensively investigated. Among the health benefits reported in the literature of garlic are its inhibition ability of tumor growth, limitation of chemical carcinogenesis, and pathogenic bacteria inhibitions by gram-positive or gram-negative bacteria (Milner 2006). Epidemiologic studies showed that garlic has the potential of lowering the risk of various disease infections and cancer cell development (Bayan, Koulivand *et al.* 2014). Previous studies on the bioactivity of garlic have also confirmed that garlic contains active compounds that demonstrate anti-oxidation, antibacterial, antifungal, antiviral, anti-parasitic, antiprotozoal and hypoglycemic properties (Anwar *et al.* 2009). According to Harris, Cottrell *et al.* (2001), garlic has been used as an antibacterial agent for preventing trauma infection and food spoilage in India, and to combat pulmonary infection in Ireland and lessen intestinal infection in soldiers in Balkan. Antiprotozoals properties are also found in allicin, ajoenes, and other organosulfide compounds (Harris, Cottrell *et al.* 2001).

Note that, Garlic has shown capable of inhibiting the hosts of protozoa, such as *Opalina ranarum* and *Entamoeba histolytica* (ur Rehmana and Munirb 2015), and prevent the growth of various different kinds of fungi, such as *Aspergillus*, *Torulopsis*, *Candida* and etc. The oxygen scavenging molecules present in garlic lead to cell damage by inhibiting the uptake of oxygen (ur Rehmana and Munirb 2015). Recently, anticancer properties from garlic have also been investigated in which the uptake of garlic has led to the shrinkage of the developed cancer cells (Mousa and Mousa 2005).

The medicinal properties of the garlic have resulted in substantial research interests in the development of garlic essential oil related products. Moreover, it has been reported that the garlic essential oil is 200 times as effective as antimicrobial agent compared to powdered garlic and 900 times as potent as a fresh garlic (Razavi Rohani et al. 2011). Due to the extremely high effectiveness of the garlic oil compared to the fresh garlic, this has motivated some industries to extract garlic oil and formulating it into various forms of high-end products. Helou and Harris (2007) reported that the yield and quality of the chemical compositions of the extracted garlic essential oil are dependent on the extraction methods adopted.

## **2.2 Garlic as the essential oil extract**

Garlic has been used in different diet-based therapies to cure many types of diseases, as garlic possesses antimicrobial properties. Hence, food industry has shown a growing demand for garlic oil because of its broad biological and antimicrobial activity spectrum (antibacterial, antifungal, antiviral, insect repellents and etc.). According to Benkeblia (2004), different concentrations of onions and garlic were tested for their antimicrobial activity against two bacteria *Staphylococcus aureus*, *Salmomella Enteritidis*, and three fungi *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum*. As a result, allium extracted from garlic was shown to exhibit higher inhibitory activity against all tested bacteria such as *F. oxysporum*, *A. niger*, and *P. cyclopium*. Amongst them, *F. oxysporum* showed the lowest sensitivity toward garlic essential oil whereas, *A. niger* and *P. cyclopium* showed higher inhibition at low concentrations. Thus, it can be concluded that the essential oil extracted from *Allium* species has proven to possess strong antimicrobial activity.

Furthermore, because of its strong antimicrobial properties, the application of garlic has widely developed especially in the packaging, food and pharmaceutical industries. Seydim and



Sarikus (2006) explored the usages of essential oil by incorporating it into edible films in food packaging industry in order to replace chemically synthesized preservatives. Note that, the antimicrobial activities of the natural plants have received considerable acceptance in the packaging industry due to the increasing demand from consumers. A comparative study of the effectiveness of oregano, rosemary, and garlic oil incorporated antimicrobial film was conducted by Seydim and Sarikus (2006). Among the studied natural plants, garlic antimicrobial film has shown the highest antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Lactobacillus plantarum*.

### **2.3 Existing extraction method for garlic essential oil**

Various extraction techniques on garlic have been used to extract essential oil and these methods are developed and discussed in terms of qualitative and quantitative by several researchers. In Table 2.2, different garlic essential oil extraction methods are summarized and compared.

Table 2.2: Garlic essential oil extraction methods. (Kimbaris, Siatis *et al.* 2006), (Sowbhagya, Purnima *et al.* 2009), (Li, Chen *et al.* 2010), (Yang, Han *et al.* 2012).

Extraction method	Simultaneous distillation extraction (SDE)	Enzyme assisted hydrodistillation extraction	Ultrasonic-assisted extraction (UAE)	Supercritical fluid extraction (SFE)	Microwave-assisted extraction (MAE)
Extraction time	2 h	3 h	0.5 h	2.17 h	1.5 h
Feedstock	100 g	200 g	100 g	50 g	40 g
Solvent use	110 mL	10000 mL	50 mL	-	160 mL
Yield	0.24 %	0.57 %	0.17 %	0.81 %	0.478 %
Purification	Evaporated under gentle nitrogen blow down	Dried over anhydrous sodium sulfate	Transfer to separation funnel with 20 mL of NaCl	-	Filtered and separated by rotary evaporator with a vapor pressure, - 0.71 MPa.
Operating conditions	$\pm 100$ °C	$\pm 100$ °C	Power: 35 kHz 25 °C	Pressure: 10 MPa 35 °C	70 W 30 °C

Different extraction techniques of garlic essential oil have been employed by various researchers such as hydrodistillation (Lucchesi, Chemat *et al.* 2004), soxhlet extraction (Bimakr, Rahman *et al.* 2011), steam distillation method (Sahraoui, Vian *et al.* 2008) etc. Based on literature review, conventional methods for the phytochemical extraction exist with some shortcomings such as requiring long extraction time, large solvent amounts, and low efficiency. Conventional methods are the most common method for the extraction of essential oils from plants, however, due to these shortcomings, MWHD, UAE and SFE have been developed (Kimbaris, Siatis *et al.* 2006), (Sowbhagya, Purnima *et al.* 2009), (Li, Chen *et al.* 2010), (Yang, Han *et al.* 2012).

Kimbaris, Siatis *et al.* (2006) reported that both conventional and recent extraction methods of garlic have a similar extraction yield, however, MWHD and UAE gave a better quality of the extracted essential oil. It is because in the conventional method, garlic was subjected to 2 to 3hr of thermal treatment at boiling temperature. This has led to the degradation of the chemical compound during the extraction process. Even though Kimbaris, Siatis *et al.* (2006) showed that USE diminished the danger of thermal degradation of organosulfur compounds, the lowest extraction yield was reported. Nevertheless, heat is still a crucial factor for garlic essential oil extraction. After the extraction, the garlic essential oil is made concentrated by a gentle nitrogen blowdown stream.

Sowbhagya, Purnima *et al.* (2009) carried out a study of conventional method, hydrodistillation (HD) and steam distillation method (SD) with oil yield of 0.31 and 0.28 % dwb. In order to increase the oil yield, enzyme pretreatment was introduced to the conventional extraction method where there was an obvious increase in the range of 0.45-0.57% compared with 0.31% of HD. Hence, the higher yield can be comprehended as the enzymes having the ability to break down the cell wall and ease the escape of the essential oil from the garlic (Sowbhagya, Purnima *et al.* 2009). The trapped garlic essential oil was removed and dried over anhydrous sodium sulfate for the yield analysis.

The solvent extraction (SE) and supercritical fluid extraction (SFE) of garlic essential oil were evaluated (Li, Chen *et al.* 2010). SFE is a promising method for sensitive heat compounds from plant material. It can perform at low temperatures but it requires a high capital cost. According to Li, Chen *et al.* (2010), compared with SFE (0.81%), the yield essential oil by SE (0.78%) was lower but the organosulfur compounds in garlic essential oil were quantitatively

similar. In order to determine the yield of garlic essential oil for SE, the water layer was extracted with another equal volume of solvent and the mixture was concentrated using rotary vacuum evaporator.

Yang, Han *et al.* (2012) compared and studied on the extraction of garlic essential oil by microwave-assisted extraction and conventional extraction method. According to Yang, Han *et al.* (2012), factors affecting the microwave yield are solvent quantity used, microwave processing time, extraction temperature, extraction time, and evaporator bath temperature. Hence, the microwave extraction went under 10s periodical microwave processing with the power of 70W and the oven was stopped for another 90s to prevent garlic from overheating. A long reaction time of 1.5hr was found to be able to complete the extraction with a yield of 0.478%. Similarly, conventional extraction method took longer extraction time but with a lower yield, 0.186%.

#### **2.4 Phytochemicals in garlic**

It has been identified by Dethier, Hanon *et al.* (2013) that the antimicrobial properties are due to the primary organosulfur compounds such as diallyl sulfide group, vinyl diithin, and methyl allyl sulfides. The organosulfur compound- vinyl diithin is typically present in crushed garlic, therefore extraction has to be carried out using the damaged tissue. Besides, vinyl diithins has also demonstrated its capability in lowering cholesterol level. It was found that 486 mg of vinyl diithins was obtained from the maceration of 20g of garlic at 37°C in 6h. Microwave-assisted extraction reported by Dethier, Hanon *et al.* (2013) showed a significant increase in the amount of extracted vinyl diithins. Results obtained from these studies showed that garlic is a promising prospect in nutraceutical industry for the development of health-promoting products. The major components expected in the essential oil through SFME extraction by GC-MS are 2-vinyl-[4H]-1, 3-dithiin and 3-vinyl-[4H]-1, 2-diithiin whereas minor amount of diallyl disulphide, diallyl trisulfide, methyl allyl trisulfide, methyl allyl disulphide, and diallyl sulphide may be present according to Kimbaris, Siatis *et al.* (2006). As reported by Helou and Harris (2007), different extraction techniques may result in the conversion of different sulphur-containing compounds. Table 2.3 shows the component yields when different extraction methods are employed whereas Figure 2.2 shows the typical chromatogram of garlic oil compounds from Sowbhagya, Purnima *et al.* (2009).

Table 2.3: The compounds in Garlic Oil (Kimbaris, Siatis *et al.* 2006).

No.	Compounds	Diethyl ether			Hexane			Ethyl Acetate			
		Extraction methods	SDE	MWHD	USE	SDE	MWHD	USE	SDE	MWHD	USE
1	Dimethyl disulphide (DMS)		2.2	2.1	-	2.4	2.3	-	1.9	0.7	-
2	Ethyl vinyl sulphide (EVS)		-	-	3.2	-	-	4.2		-	9.7
3	1,2-dithacyclopentane		0.4	0.4	0.2	0.4	0.4		0.3	0.2	
4	Diallyl sulfide (DS)		2.3	0.9	0.3	2.2	0.8	0.4	1.9	0.1	0.4
5	2-vinyl thiophene		-	-	-	-	-	-	-	0.2	-
6	3-methylthio propanal		0.3	-	-	0.2	-	0.1	0.4	0.2	-
7	2-ethyl thiophene		-	-	-	-	-	0.1	-	-	-
8	Cis or trans Methyl allyl disulphide (MADS)		9.1	9.0	0.6	11.2	7.1	0.5	8.5	4.3	1.4
9	Methyl propyl disulphide		0.1	0.1	-	0.2	-	-	0.1	0.2	-
10	Cis or trans Methyl allyl disulphide (MADS)		0.2	0.1	-	0.1	-	0.1	0.2	0.2	-
11	Unknown		2.4	1.8	0.7	1.4	1.3	0.9	1.6	1.5	2.0
12	Dimethyl trisulfide (DMTS)		2.0	2.7	-	2.7	2.0	-	2.3	2.0	-
13	Allyl 2,3-epoxypropylsulfide		-	0.1	0.1	-	-	0.1	-	-	-
14	Diallyl disulfide (DDS)		28.4	17.6	8.2	25.5	17.8	7.3	23.1	6.8	6.0
15	C <sub>6</sub> H <sub>10</sub> S <sub>2</sub>		0.5	0.3	0.1	0.5	0.3	0.2	0.4	0.5	-
16	C <sub>6</sub> H <sub>10</sub> S <sub>2</sub>		1.2	1.4	1.5	1.2	1.0	1.4	0.9	1.1	3.7
17	Methyl butyl trisulfide		-	-	-	-	-	-	-	0.1	-
18	Methyl allyl trisulfide (MATS)		16.3	14.1	0.1	17.4	12.9	0.2	17.5	14.9	0.2
19	3-Vinyl-[4H]-1,2-dithiin (3-VDT)		4.0	15.9	32.7	4.5	15.4	26.3	4.5	14.5	25.7

Table 2.3: The compounds in Garlic Oil. (Kimbaris, Siatis *et al.* 2006). Continue (...)

No.	Compounds	Diethyl ether			Hexane			Ethyl Acetate			
		Extraction methods	SDE	MWHD	USE	SDE	MWHD	USE	SDE	MWHD	USE
22	Diallyl trisulfide (DATS)		20.4	9.1	0.2	18.2	9.5	1.3	22.1	14.1	0.7
23	3,5-diethyl 1,2,4-trithiolane		0.2	0.2	-	-	0.2	0.2	0.2	0.2	0.5
24	Pentadecene		-	-	-	-	-	-	-	-	0.6
25	Heptadecene		-	-	-	-	-	-	-	-	0.5
26	1,2,3,4-tetrathia-5-methyl cyclohexane		-	-	-	-	-	0.4	-	-	-
27	3-(2-thia-4-pentenyl)-1-thacyclohex-5-ene		-	-	-	-	-	0.2	-	-	0.3
28	Diallyl tetrasulfide (DATTS)		0.7	0.1	-	0.5	0.3	0.1	0.6	0.4	-
29	3-(2,3,4-trithia-5-heptenyl)-1-thia-cyclohex-5-ene		0.1	0.2	0.1	-	-	0.4	-	0.2	-
30	2-(1-thia-2-cyclohexen-6-yl)-1,3-dithia-cyclohex-5-ene		0.3	2.1	6.4	0.3	2.1	2.3	0.4	2.7	-
31	Octadecanol		-	-	-	-	-	0.5	-	-	9.7

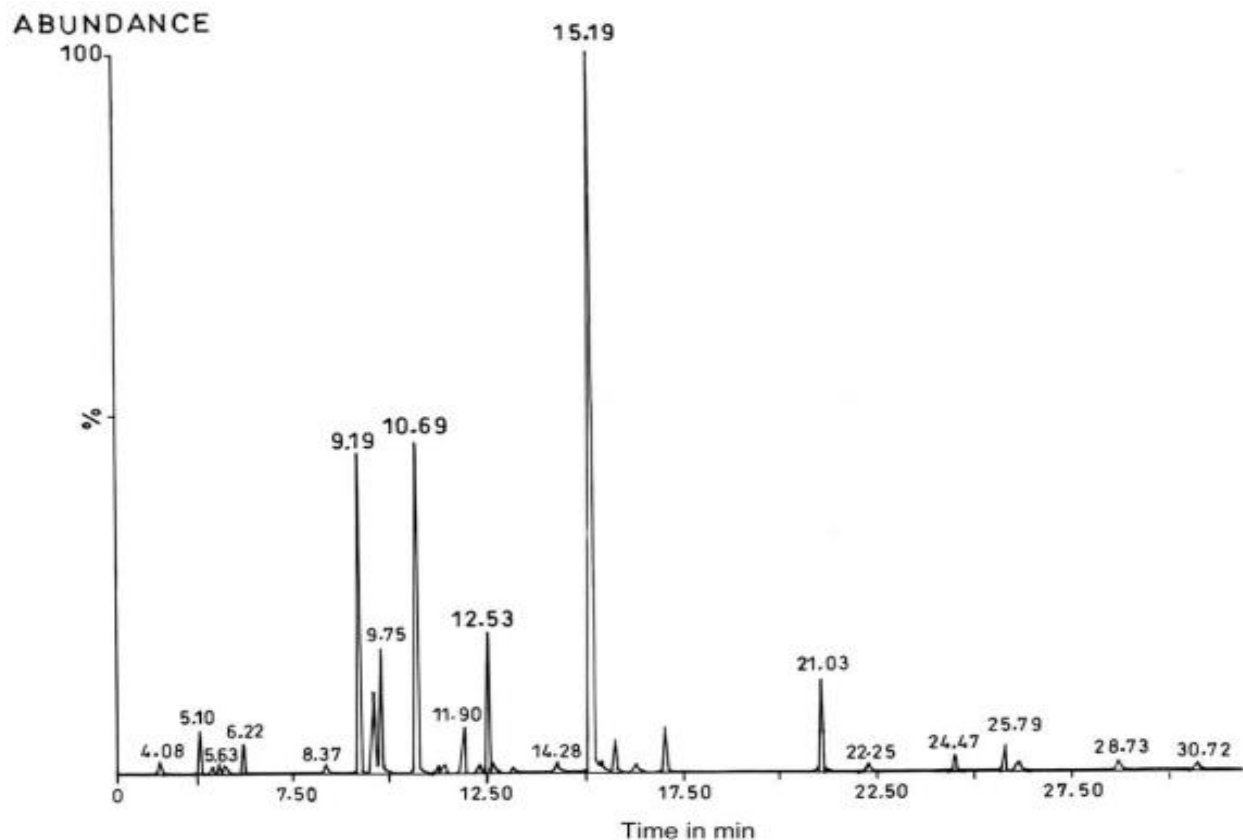


Figure 2.2: The chromatogram of garlic essential oil (Sowbhagya, Purnima *et al.* 2009).

#### 2.4.1 Stability of phytochemicals

Natural plants and herbs contain essential bioactive compounds for health maintenance and disease prevention. However, Asbahani, Miladi *et al.* (2015) stated that essential oils are unstable and fragile volatile compounds which could easily be degraded by oxidation, volatilization, heating, light. These compounds are known as phytochemicals. These phytochemicals are vulnerable to harsh environmental conditions whereby bioavailability of the phytochemicals is greatly affected which lead to a shorter shelf-life (Talcott, Percival *et al.* 2003). According to Dandawate, Subramaniam *et al.* (2016), phytochemicals such as curcumin showed promising effects that are anticancer in the laboratory experiments, however, its clinical use is limited due to the low metabolic stability. Hence, studies have been conducted on phytochemicals analogue and their formation by thermal and non-thermal processing technologies for activity improvement. Thermal processing is the most common method in the food industry. However, excessive heating leads to severe degradation of bioactive compounds as shown in Table 2.4. Given the fact that

thermal processing leads to severe conditions, this might cause several chemical and physical changes that impair the organoleptic properties and result in loss of bioactive compounds. Therefore, non-thermal extraction processes are needed, therefore developed, such as high hydrostatic pressure processing, high intensity pulsed electric fields (HIPEF), dense-phase carbon dioxide, ultrasound and etc (Rawson, Patras *et al.* 2011). These technologies are used to improve the functionalities of the food. In addition to process technology, factors such as pH and temperature have great influence on the stability of the bioactive compounds (Harbourne, Marete *et al.* 2013). According to the literature review, extraction temperature is one of the important parameters for the stability of the functional food. For instance, the extraction of phenolic compounds in feverfew increased with the increase in temperature (Marete, Jacquier *et al.* 2009). Furthermore, storage temperature and pH have been examined as they influence the stability and bioactive content of a given quality with storage (Rawson, Patras *et al.* 2011).



Table 2.4: Effect of thermal processing on bioactive content (Rawson, Patras *et al.* 2011).

Fruit	Type	Conditions	Phytochemicals affected
Acai ( <i>Euterpe olerace</i> and <i>Euterpe precatoria</i> )	Puree	Processing temperature: 80°C	Anthocyanin
Cupuacu	Nectar	Thermally treated at 60 - 99°C	Ascorbic acid Dehydroascorbic acid
Papaya	Cubes	Blanching at 70, 85 and 100 °C. Drying at 65 °C for 5-6 hr	Carotenoid Anthocyanins Ascorbic acid
Guava	Fruit slices	Drying at 30, 40 and 50 °C.	Ascorbic acid

## 2.5 Antimicrobial properties of essential oil

Antimicrobial susceptibility tests are used to detect and determine the sensitivity of bacteria against particular antibiotic. These techniques are very important for clinical microbiology laboratory and pharmaceutical development in order to comprehend the bacterial and assure the susceptibility of antimicrobial agents in inhibiting bacterial. The testing methods have been developed and carried out, these include dilution method (Jiang 2011), rapid automated instrument, antimicrobial gradient method (Reller, Weinstein *et al.* 2009), diffusion method (Dayao, Kienzle *et al.* 2014), and bioautographic method (Valgas, Souza. *et al.* 2007). Dorman and Deans (2000) conducted an antimicrobial susceptibility test with 9 gram-positive and 16 gram-negative bacteria against volatile oil of *Myristica fragrans*, *Origanum vulgare*, *Pelargonium graveolens*, *Piper nigrum*, *Syzygium aromaticum* and *Thymus vulgaris*. These bacteria strains showed a different degree of sensitivity towards the tested plant volatile oil. Antimicrobial properties of *Achillea millefolium* essential oil was studied by Candan, Unlu *et al.* (2003) using *S. aureus*, *S. pneumoniae*, *M. catarrhalis*, *B. cereus*, *A. Iwoffii*, *E. aerogenes*, and *E. coli* through disc diffusion method. From the in vitro study, it showed that oil of *Achillea millefolium* possesses low antimicrobial activity. Sung, Sin *et al.* (2014) conducted an antimicrobial test on a packaging film formulated from low density polyethylene (LDPE) with the incorporation of garlic oil using serial dilution method. It was concluded that the formulated packaging film is effective towards inhibition of food pathogens.

According to Andrews (2001), minimum inhibitory concentrations (MICs) were conducted in the laboratory to confirm resistance and often act as a research tool to determine the in vitro activity of new antimicrobial agents. MICs are used to determine the lowest concentration of an antimicrobial that will inhibit the growth of microorganisms after an overnight incubation. Hence, lower MIC denotes that the material is an effective antimicrobial agent. MIC has been widely performed due to its advantages such as its ease to be conducted, automated, highly reproducible, rapid turnaround of results (Varzakas and Tzia). MICs can be performed on plates of solid growth medium (agar) or broth dilution method (Qi, Li *et al.* 2014a). However, there are several disadvantages where MIC value can differ with minor changes in methodology which results in large variation in the result. For instance, prolonged incubation may increase the apparent MIC, while smaller inoculum concentrations may decrease the apparent MIC and MIC may change with storage conditions (Varzakas and Tzia). Shu and Lai (2001) studied the minimum inhibitory concentration of oregano essential oil and two of its principle components, thymol and carvacrol against *Pseudomonas aeruginosa* and *Staphylococcus aureus* by broth dilution method. In order to obtain the inhibition data, wide ranges of concentrations were tested for each of the compounds. The mathematical analysis was also carried out and it was suggested that mixture of carvacrol and thymol gave an additive effect where it is proven that thymol and carvacrol exhibit the antimicrobial action.

## **2.6 Microwave technology in the extraction of essential oil**

In the early period, due to the advancement of separation technologies using microwave techniques, microwave have been developed and widely used for the extraction of natural plants and herbs. The extensive usage of these techniques has led to the improvement of the extraction method as reported in Table 2.5.

Table 2.5: Microwave-based technologies for essential oil extraction from plants.

Method	Advantages	Disadvantages
Solvent Free Microwave Extraction (SFME)	Carried out at atmospheric pressure without solvent or water added (Li, Wang <i>et al.</i> 2012).	Highly dependent on the moisture and apparent density of the plant material (Navarrete, Mato <i>et al.</i> 2012)
	Effective heating, environmentally friendly, fast energy transfer, and low handling costs (Li, Wang <i>et al.</i> 2012)	
	Suitable for organic compounds with high polar moments (Filly, Fernandez <i>et al.</i> 2014)	Designed for aromatic herbs rich in high volatile oil (Leonelli, Veronesi <i>et al.</i> 2012)
	Short extraction time with low concentrations of volatile compounds (Asbahani <i>et al.</i> 2015)	
Microwave Hydro Diffusion Gravity (MHG)	Suitable for the extraction of pigments, aromatic components, and antioxidants (Li, Fabiano-Tixier <i>et al.</i> 2013)	Highly dependent on the moisture and apparent density of the plant material (Navarrete, Mato <i>et al.</i> 2012)
	Low environmental impact, time saving, and power saving (Asbahani, Miladi <i>et al.</i> 2015)	Only suitable for the extraction of non-volatile compounds which is not suitable for this project (Michel, Destandau <i>et al.</i> 2011)
	Does not require solvent or water (Li <i>et al.</i> 2013)	
	The extraction mechanisms rely on gravity (Li, Fabiano-Tixier <i>et al.</i> 2013)	
Vacuum Microwave-assisted Extraction (VMAE)	Operates under vacuum (Wang, Xiao <i>et al.</i> 2008)	Lesser yield compared to MAE (Pasquet <i>et al.</i> 2011)
	Extraction of oxygen-sensitive and thermosensitive compounds (Xiao <i>et al.</i> 2009)	Complex and expensive infrastructure (Wang, Xiao <i>et al.</i> 2008)
Vacuum Microwave Hydro Distillation (VMHD)	Extraction of high quality plant material under vacuum (Li <i>et al.</i> 2013)	Requires solvent (Li <i>et al.</i> 2013)

In 1940's, microwave was first used as a kitchen appliance that heats and cooks' food. The first commercial microwave oven was invented in 1954 and was named as Radarange. This marks the beginning of the use of microwave oven. Since that, microwave is seen as a tool that provides rapid, high efficiency of heating (Jones, Lelyveld *et al.* 2002). Microwave has also drawn attention as a new heating source in lab scale that was started in the late 1970's. It is applied to the acid digestions (Abu-Samra, Morris *et al.* 1975). The first microwave-assisted extraction publication was reported by Ganzler and co-worker (Kaufmann and Christen 2002).

Kimbaris *et al.* (2006) studied and compared the effects of various extraction techniques for the extraction of garlic essential oil using simultaneous distillation extraction (SDE), microwave assisted hydro-distillation extraction (MAHD), and ultrasound-assisted extraction (UAE). The extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). Similar oil extraction was obtained from different extraction methods, however, significant differences were observed in the compositions of the extracts. The essential oil obtained from SDE showed more R allyl-substituted polysulfides and less vinyl dithiin compounds compared to essential oil obtained from MAHD and UAE. Benavides, Squadrito *et al.* (2007) reported that human red blood cells can convert the allyl-substituted polysulfides into endogenous cardioprotective vascular cell signaling molecule named hydrogen sulfide (H<sub>2</sub>S) while vinyl dithiins have been investigated as a preventative for cardiovascular disease and as antioxidants (Singh and Singh 2008). Therefore, it is crucial to obtain extracts with a good proportion of the allyl-substitute polysulfides and vinyl dithiins. Among the techniques, SDE operates at high temperature for an extended period, which leads to the degradation of garlic essential oil compounds into subunits of sulfur compounds. It is observed in MAHD where a certain degree of active compounds has degraded. UAE, on the other hand, showed a significant reduction in the degradation of the bioactive compounds. Nevertheless, the scalability of UAE for commercial applications remains a challenge. Table 2.6 summarizes the performance comparison of several different advanced extraction methods.

Table 2.6: Performance comparison of different extraction technologies (Chemat *et al.*, 2011).

Extraction method	Ultrasonic-assisted extraction (UAE)	Microwave-assisted extraction (MAE)	Supercritical fluid extraction (SFE)
Extraction time	10–60 min	3-30 min	10–60 min
Sample size	1–30 g	1–10 g	1–5 g
Solvent use	50–200 mL	10-40 mL	2-5 mL (solid) 30-60 mL (liquid)
Capital	Low	Moderate	High
Advantages	Simple manipulation	Rapid	Rapid
	Reduce thermal degradation	Easy to handle	Low solvent consumption
	Low energy consumption	Moderate solvent consumption	Concentration of the extract
	Increase mass and heat transfer	Reduce waste products	No filtration necessary
	Time saving		Possible high selectivity
Disadvantages	Large solvent consumption	Extraction solvent must absorb microwave energy	Many parameters to optimize
	Filtration step required	Filtration step required	Supercritical fluid lack of polarity to extract polar molecules
	Required thin plant material skin May lead to the formation of free radicals	Required moisture contents in plant materials.	Expensive equipment and maintenance cost
	laborious as it requires high surface area from size reduction to improve sonication (Ashokkumar 2015).		

Michel et al (2011) measured the extraction performance of the antioxidants from *H. rhamnoides L. berries* using pressurized microwave assisted extraction (PMAE) and pressurized solvent free microwave extraction (PSFME) techniques. From the results, both techniques gave similar extraction performance, however, PSFME is preferable because the usage of organic solvents can be eliminated and is regarded as a sustainable process (Michel, Destandau *et al.* 2011). Qi, Li *et al.* (2014b) investigated the extraction performance of pigeon pea leaves on solvent-free microwave extraction (SFME). The optimization was carried out by employing central composite design (CCD). The optimum extracts obtained was 0.330 (% w/w) when SFME was conducted at 660 W and humidity 68% for 44 min. The results further confirmed that SFME serves as a superb extraction technique for natural plants and herbs because of the excellent extraction efficiency yet maintaining the rich contents of targeted phytochemicals for the antimicrobial activity. Similarly, Abdelhadi, Meullemiestre *et al.* (2015) has performed extraction of essential oil from *Hypericum perforatum L.* and evaluated the extraction performance in solvent free microwave extraction (SFME) and hydrodistillation (HD) techniques. Results showed that 0.365 %(w/w) of extracts were extracted in 33 min which was about 45 times higher than the extracts extracted using HD technique and it was also 7-fold shorter in time. Moreover, SFME extract is found to contain a higher proportion of valuable health-promoting oxygenated compounds (Golmakani and Moayyedi 2016). It can be concluded that microwave-assisted extraction techniques give higher extraction yield and this may be attributed to the synergy combination effects of mass and heat transfer acting on the plant biomass. Therefore, SFME and MAE techniques are investigated for the extraction of garlic essential oil in this project.

## **2.7 Factors influencing the microwave-assisted extraction of essential oils**

In order to optimize the extraction process in natural product using microwave, it is essential to understand the factors that will influence the process. Pan, Niu *et al.* (2003) reported that the solvent to matrix ratio, microwave power, type of solvent, and the extraction time are the influencing factors in microwave-assisted extraction. Similarly, Setyaningsih, Saputro *et al.* (2015) later in the studies of phenolic compounds extraction of rice grains has reported that the extraction time, microwave power and ratio of solid to liquid are important and significant factors for the microwave-assisted extraction. Hence, this section discusses the effects of different factors in influencing the microwave-assisted extraction of essential oils.

### 2.7.1 Effect of moisture content

Heating mechanisms of the microwave are dependent on the bound water presence in the plant material. This is because the bound water absorbs the microwave energy and hence resulting in the increase of internal temperature of plant cells. The internal heating causes the expansion of plant cells which leads to rupture of the cell wall. The cells' disruption then facilitates the mass transfer of solvent thus promoting the extraction efficiency. Therefore, the moisture content of the plants greatly affects the extraction yield (Zhang, Yang *et al.* 2011). According to Kala, Mehta *et al.* (2016), the extraction of dried matrix can be improved after being pre-treated with water before subjecting it to microwave-assisted extraction. Thostenson and Chou (1999) investigated the physics during microwave-assisted extraction and observed that dipole rotation of water molecules occurred within the plant material when microwave is used. Thus, this leads to heat generation from the plant's interface to the surrounding (Mandal, Mohan *et al.* 2007). Kala, Mehta *et al.* (2016) further explained that the rotation of molecules generate heat and the heating of moisture inside the plant cell resulted in the generation of pressure within the plant cell which leads to disruption of plant cells, hence, facilitates the movement of bioactive phytochemicals from the plant cells.

The effects of moisture on the extraction efficiency of polycyclic aromatic hydrocarbons from soil has been studied by Shu and Lai (2001). The finding showed that the moisture content in the soil enhances the extraction in a microwave heating process. Likewise, Budzinski, Letellier *et al.* (1999) confirmed that the water has a great influence on the microwave extraction and the interaction between water and microwave has attributed to the increase in the recoveries. It is because water are polar molecules which can be partially aligned by electric fields which shown in Figure 2.3 and leads to increase the chances of collision between water and other molecules. The molecules collision increase hence leads to microwave heating (Chaplin 2018b). The influence of water content of the matrix on the extraction efficiency has been tested from 0% to 100% (w/w) of water. Based on the result, the total amount of extract increases with increasing quantity of water from 0% to 30%. From the study, a further increase of water amount has slightly decreased the recoveries. When large amount of water is involved, it led to the problem of miscibility of the extract solvent. Water may act as a barrier which prevents the transfer of extract from the matrix to solvent. Hence it is shown that the amount of water is an important factor in the microwave-

assisted extraction. The results obtained by the microwave-assisted extraction with 30% of water is maximal to allow optimum recovery which is 90%.

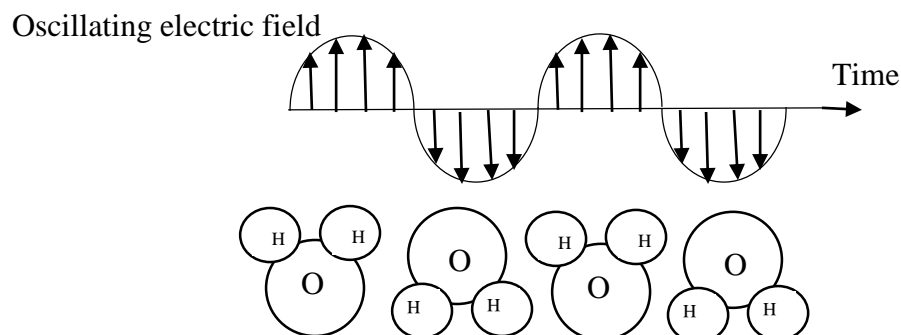


Figure 2.3: Water with oscillation of the electric field (Mishra and Sharma 2016)

### 2.7.2 Effect of solvent selection and solvent to matrix ratio

One of the factors that affect the extraction process is the selection of solvent as it facilitates the extraction of phytochemical through the plant cell. Solvent characteristics are such as the solubility of the targeted extract with the solvent, solvent penetration, solvent and extract interaction, the dielectric properties and the mass transfer kinetics (Varzakas and Tzia 2014). The solvent is required to penetrate the solid cells by diffusion and dissolving the solute. Subsequently, the solution carries the solute then diffuses out to the surface. These characteristics are closely tied with the solvent properties (Veggi, Martinez *et al.* 2012). Furthermore, dielectric properties are a measurement of the ability of a material to absorb microwave energy. Generally, the extraction efficiency of microwave energy is dependent on the dielectric properties of solvents. The ability of solvent to absorb microwave energy and convert it into heat energy at a given temperature and frequency can be defined as loss tangent,  $\tan \delta$  as described in Equation (2.1)

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \quad (2.1)$$

where  $\varepsilon''$  is the dielectric loss and  $\varepsilon'$  is the dielectric constant.

Hence solvent with high loss tangent has higher microwave absorption capacity and consequently leads to better extraction (Mandal, Mohan *et al.* 2007). Loss tangent of some common solvents are tabulated in Table 2.7. Generally, solvent can be classified as high ( $\tan \delta > 0.5$ ), medium ( $\tan \delta 0.1-0.5$ ), and low ( $\tan \delta < 0.1$ ) microwave absorbing potential. High



microwave absorbing potential solvents such as ethanol and methanol are usually used for the extraction of antioxidant compounds especially phenolics. Veggi, Martinez *et al.* (2012) discovered that an aqueous combination of polar solvents could enhance the extraction process. It was reported that better heat dissipation happens in water-mixed solvent because the changes of solvent polarity in the polar solvents increases the heating rate and hence enables the movement of target compounds. Moreover, the penetration depth is inversely proportionate to the loss tangent value. The high loss tangent value is normally characterized as low penetration depth because microwave energy has only absorbed by the outer layer of the matrix due to solvent that has high reaction towards microwave energy (Kappe, Stadler *et al.* 2012).

Besides the selection of solvent, solvent to matrix ratio is also an important factor for the extraction process. Wang, Li *et al.* (2008) has reported that in the conventional extraction method, a larger solvent volume tends to dissolve the bioactive compounds effectively and lead to higher extraction yield. Likewise, an increase in the mass of peanut skins to solvent ratio from 1.5: 37.5 (g/mL) to 3.5: 37.5 (g/mL) caused a reduction in extraction yield of total phenolics. Gao, Huang *et al.* (2007) stated that the yield of scutellarin in *Erigeron breviscaous* decreased with the increasing ratio of solid to liquid from 2.5: 100 (g/mL) to 15.0: 100 (g/mL) by microwave assisted extraction. This occurs when microwave usage is applied to mixtures that contain excessive solvent. In addition, low extraction yield may due to the decrease of the extractable surface area of target molecules although it seems that more solvent could enhance the extraction efficiency. Besides that, excessive solvent leads to poor microwave heating because the solvent absorbs the microwave energy, hence it requires extra power to ensure complete extraction process. Furthermore, the microwave energy will dissolve in the bulk solvent which will affect the internal heating of the matrix (Kala, Mehta *et al.* 2016). Low amount solvent will limit the movement of the compounds out from the matrix (Mandal and Mandal 2010). Hence, optimum ratio is preferable to ensure homogeneous and effective heating.

Table 2.7: Loss tangent ( $\tan \delta$ ) of different solvents (2.45 GHz, 20°C) (Kappe, Stadler *et al.* 2012).

Solvent	$\tan \delta$
Ethylene glycol	1.350
Ethanol	0.941
Methanol	0.659
Acetic acid	0.174

Water	0.123
Ethyl Acetate	0.059
Hexane	0.020

### 2.7.3 Effect of extraction temperature and microwave power

In microwave extraction, the microwave power and the extraction temperature are correlated and both show significant impact on the extraction process. Wang and Weller (2006) found that as the microwave power radiated increases, temperature of mixture will rise while pressure builds up rapidly within the extractant, hence, shorten the extraction time.

Generally, elevated temperature increases the extraction efficiencies. However, high temperature may lead to the degradation of heat-sensitive bioactive phytochemicals from the plant materials (Upadhyay, Ramalakshmi *et al.* 2012). Chen, Xie *et al.* (2007) investigated the effect of temperature on extraction efficiency for the MAE extraction of total triterpenoid saponins from *Ganoderma atrum*. The extractions were conducted with 95% ethanol for 20 min at five different temperatures, respectively 60, 70, 78, 100, 120°C. Generally, the extraction yield increased with temperature elevation, however, optimum yield point was found at 78°C, thus, indicating a further increase of temperature that resulted in a decrease of yield. Likewise, Xiao, Han *et al.* (2008) has concluded that extraction temperature has clear influence on the extraction yield and also the extraction quality. The concentration of extract increased with the increasing temperature from 90 °C to 110 °C. Further increase of temperature decreased the yield and the extract was found to be scorched. Scorching of the extract is a result of degradation and hence affects the extract quality.

It is noticeable that the microwave power also influences the energy transfer efficiency onto the plant material. Generally, increase of microwave power indirectly increases the extraction temperature. In other words, more electromagnetic energy is transferred to the plant material and improved the extraction efficiency. Electromagnetic energy is being transferred to plant material to provide a localized heating effect that lowers the viscosity and surface tension of the solvent and plant materials. Lowering the surface tension has shown to facilitate the extraction process (Leonelli, Veronesi *et al.* 2012). However, Chemat, Ait-Amar *et al.* (2005) reported that there is an optimum extraction efficiency whereby any further increase of microwave power did not increase the extraction yield. Nevertheless, high microwave power may result in poor extract

quality due to degradation of thermally sensitive compounds. Likewise, Chen, Xie *et al.* (2007) has investigated the effect of temperature in the extraction of triterpenoids saponins. The optimum extract (1.218%) of triterpenoids saponins was found to be at 78°C. The extraction percentage increases gradually with the increased of temperature until 78°C. This might be due to the improved diffusivity of solvent into the core of plant matrix with the elevated temperature which indirectly enhances the yield percentage. Therefore, it can be concluded that the selection of microwave power plays an important role in the MAE. Behaviour and the physiochemical characteristics of the targeted extracts should be first identified to ensure the quality and the efficiency during MAE.

#### 2.7.4 Effect of extraction time

Generally, longer extraction time improves the yield of extractions in the microwave. Pan, Liu *et al.* (2000) shown that the yield of glycyrrhizic acid extracted from licorice root increased with the increasing extraction time. Effect of extraction time is studied by Xiao, Han *et al.* (2008) with different extraction time of 5 min, 10 min, 15 min, 20 min, 25 min and 30 min were carried out. The yield increased with the increasing extraction time and reached to a maximum at 25 min. In addition, Hemwimon, Pavasant *et al.* (2007) also supported the finding as the recovery of the extract was found to increase with increasing MAE time.

However, extraction is significantly shortened in MAE. It was reported that 5 min in MAE is comparable with the extraction that takes place at room temperature for 20-24 hours (Pan, Liu *et al.* 2000). Moreover, Pan, Niu *et al.* (2003) reported that the extraction time is shortened when extraction processes are conducted using microwave technology. Shorter extraction time decreases the probability of thermal degradation and oxidation of the extracts. In a study conducted by Kimbaris, Siatis *et al.* (2006) on the extraction methods for sensitive aroma compounds on garlic, it was discovered that the extraction time for ultrasound-assisted extraction and microwave-assisted hydrodistillation extraction was significantly shortened by one-fourth compared to the simultaneous distillation solvent extraction technique. Furthermore, Sowbhagya, Purnima *et al.* (2009) has reported that the extraction of garlic volatile oil using hydrodistillation and steam distillation is time-consuming and also leads to energy wastage. Besides that, the long exposure time may affect the quality of the extracts. Contrary, Pan, Niu *et al.* (2002) reported that the tanshinones extracts decrease with the increase of extraction time. It is because tanshinones

decomposes easily when exposed to a high temperature for a long period of time. Therefore, 2 min was found to be the optimal extraction time for the extraction of tanshinones. Therefore, the study of extraction time in microwave-assisted extraction is crucial to ensure the quality as well as the quality of the extract.

## **2.8 Transport phenomena in oil extraction**

The term transport phenomenon is used to describe processes in which mass, momentum, and heat move about in matter combined with basic principles (Bird, Stewart *et al.* 2007). Mass transport describes the transport of various chemical species in a system whereas momentum transport discusses the transport momentum in fluids. Besides that, heat transfer describes the transport of heat/energy in a system (Leal 2007). Transport phenomena takes place in any system and can be studied at 3 different levels from molecular, microscopic and macroscopic level (Bird, Stewart *et al.* 2007). At the molecular level, the kinetic molecular theory offers a description of the microscopic properties of molecules and their intermolecular forces, which depends on the temperature of the substances (Nguyen 2011). At the microscopic level, the transport equations are formed for a small control volume with appropriate assumptions and boundary conditions which lead to more details and accurate description of the system. Meanwhile, macroscopic level forms the transport equations which may help in understanding the overall performance of systems (Bird, Stewart *et al.* 2007). In this report, the microscopic level of heat and mass transport in the microwave will be further discussed.

### 2.8.1 Heat Transfer

Microwave heating is a multiphysics phenomenon that involves electromagnetic waves, channel heat energy directly to the matrix. This induces polar molecules in the matrix to vibrate, leading to heat generation and rise in temperature inside the matrix. Thus, microwave heat is produced more quickly within the cells from the outside. Heat transfer occurs when thermal energy is transferred in response to a temperature difference. Microwave energy is a form of radiation which transfers the energy and heat by the force fields of electromagnetic waves to the matrix (Pitchai 2011). Furthermore, the heat generated inside the matrix creates an outward flux which leads the diffusion of moisture from the matrix's interface to the surrounding (Torki-Harchegani, Ghanbarian *et al.* 2016). This phenomenon has indirectly increased the extraction rate and decreases the extraction time.

For instance, peppermint leaves consist of high moisture contents which caused friction against the bipolar rotation. This results in heat being generated inside the leaves and creates an outward flux of rapidly escaping vapor and forces the moisture and extract to diffuse outside. (Torki-Harchegani, Ghanbarian *et al.* 2016). Golmakani and Moayyedi (2015) found that MAHD and SFME (15 min) required less time for essential oil extraction from citrus lemon peel compared to HD (120 min). Also, Kusuma and Mahfud (2016b) presented that microwave-assisted extraction method (MAHD for 66 min and SFME for 45 min) offered shorter extraction time compared to conventional method (HD for 417 min). This may be due to the translational and vibrational energy transfer which leads to heat transfer that is involved during heating processes. The energy received via the electromagnetic wave by the cell ruptures the membrane wall of the cell which in turn eases the diffusion and mass transfer of the phytochemicals from the matrix for improve extraction efficiency. Conventional heating method is non-uniform and non-homogenous as compared to microwave method which is more targeted, uniform and homogenous for a given area to volume ratio. Furthermore, Lucchesi, Chemat *et al.* (2004) has reported that the extraction of essential oil from aromatic herbs by SFME for 30 min was superior in terms of saving energy and extraction time compared to conventional HD for 4.5 hr.

Conventional thermal processes are only limited to two general ways of heating such as convection and conduction (Kusuma and Mahfud 2016b). The conventional methods apply heat

to the surface of the product and rely on the thermal conductivity to move the heat to the center by conduction and convection as shown in Figure 2.4(a). The heat transfer by conduction is a slow process which indirectly limits the extraction rate (Vanmeurs, Waxman *et al.* 1987). In the case of HD shown in Figure 2.4 (a), heat transfer occurs from the inside to outside is due to conduction and convection happening around the water. Owing to the poor extraction by conventional method, microwave heating is studied and discovered. Microwave provides a local internal heating with high frequency field passing through the matrix and allows vibrational frequencies of chemical bonds as shown in Figure 2.6. As shown in Figure 2.6 (b), the extraction mechanism of essential oil by MAHD is partly due to the internal heating of solute under microwave radiation from inside to the outside of plant matrix and also mostly due to the heat transfer from the outside to the inside of plant matrix. In SFME, heat transfer mostly occurred from the inside to the outside from the plant matrix via steam and partly occurred from the outside to the inside of the plant matrix as shown in Figure 2.6 (c). This phenomenon promotes uniform heating to ensure optimize product quality. Microwave-assisted extraction offers a rapid transfer of energy to the water and matrix homogeneously and efficiently in order to rupture the cells by internal superheating which eases the diffusion of chemicals from the matrix to improve the essential oil extraction (Golmakani and Moayyedi 2015).

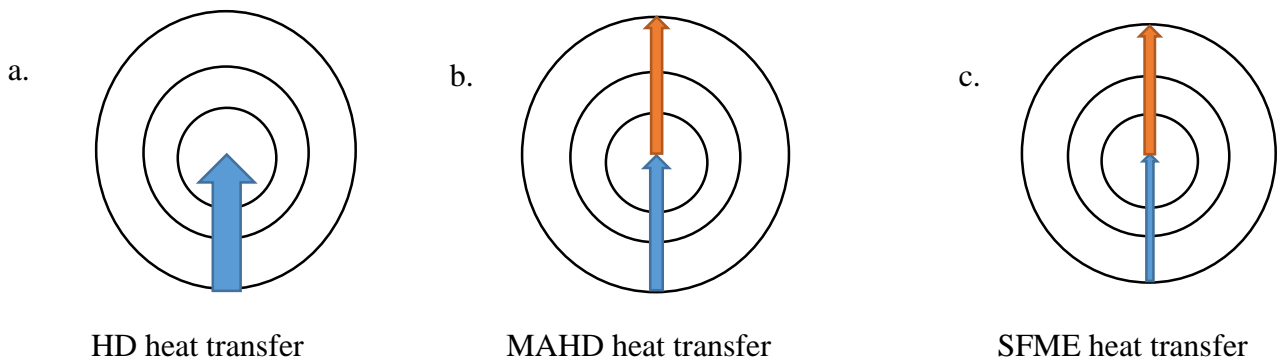


Figure 2.4: A schematic diagram of heat transfer during (a) HD, (b) MAHD and (c) SFME (Golmakani and Moayyedi 2015).

## 2.8.2 Mass transfer (Osmotic Effect)

Mass transfer occurs due to a difference in chemical potential of solvent and solute during an extraction process. For conventional conditions, osmosis and osmotic potential are used to explain mass transfer where the temperature is equal inside and outside of the matrix. Yet, osmosis is not effective when temperature gradients exist due to the selective heating in the microwave. Selective heating takes place with the higher  $\tan \delta$  material which optimizes product quality and quantity. As the chemical potential decreases with the increase of matrix's temperature (Phase Stability and Chemical Potential 2004), temperature gradients exist between the matrix and the solvent which leads to water and solute transfer through diffusion. Furthermore, the energy of radiated microwave increases the temperature of the process and extracts start to evaporate (Torki-Harchegani, Ghanbarian *et al.* 2016).

Bousbia, Abert Vian *et al.* (2009) presented that the higher rate of yield by microwave is due to a synergy combination of the two transfer phenomena (heat and mass transfer) acting in the same direction as shown in Figure 2.5. In Figure 2.6, it showed the schematic diagram of the synergy combination of heat and mass transfer acting in the same direction (from inside to the outside) for MAHD and SFME. Moreover, the rupture of cell wall caused by microwave facilitates the mass transfer of compounds. Kusuma and Mahfud (2016b) carried out a study of essential oil extracted by MAHD and HD and analyzed by GC-MS. 19 compounds were detected in MAHD, while only 14 compounds were detected in HD.

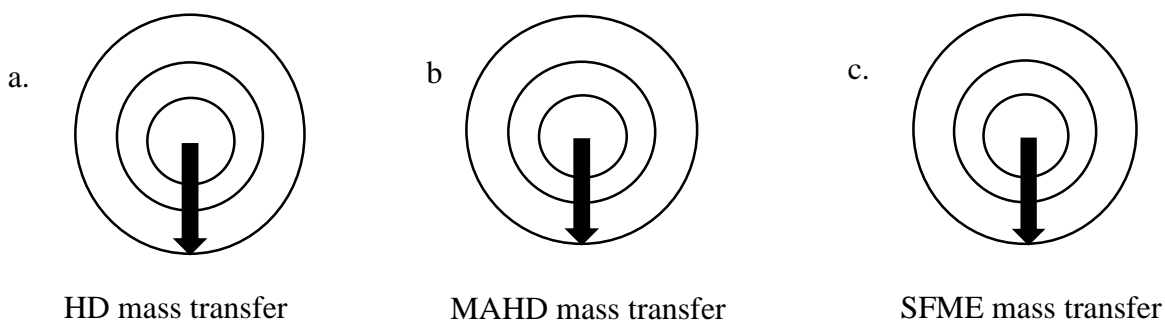


Figure 2.5: A schematic diagram of mass transfer during (a) HD, (b) MAHD and (c) SFME (Golmakani and Moayyedi 2015).

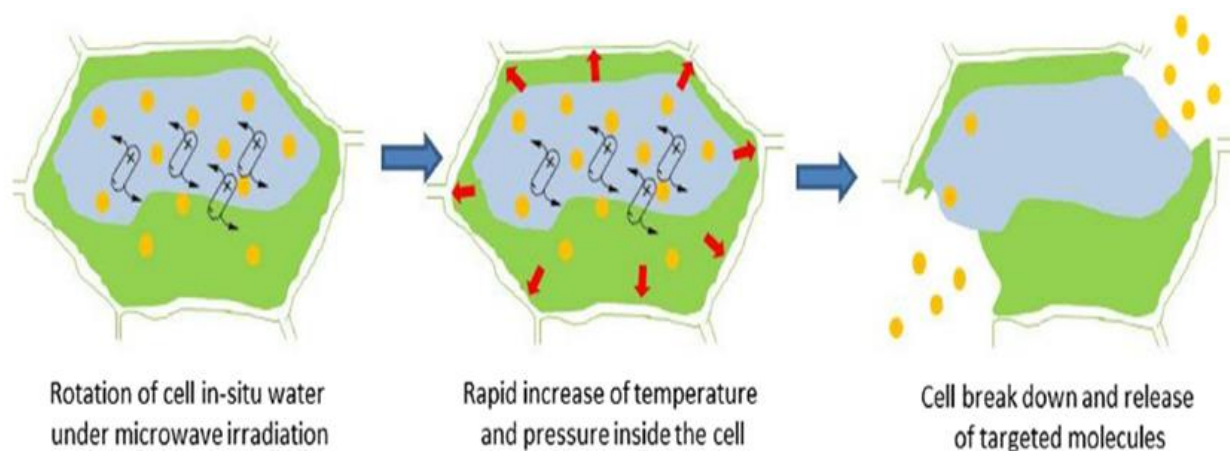


Figure 2.6: Movement of cell under microwave (Li, Fabiano-Tixier *et al.* 2013).

## 2.9 Purification of extracts

Purification is necessary for every extraction process. It is essential to separate the impurities and obtain targeted compounds which improve the functional properties of the extract (Amid and Mirhosseini 2012). The method of the purification of organic compounds depends on the properties of the compounds and types of impurities involved. There are various types of purification method such as sublimation, crystallization, distillation, differential extraction, chromatography, centrifugation, evaporation etc. Hence, the discussion will be focused on the purification of essential oils from the natural plants.

Perez, Fernández *et al.* (2013) evaluated the effects of pectinase oil extracted through *n*-hexane solvent extraction from different genotypes of sunflower. The extracted essential oil recovered by rotary evaporator and the residual hexane is removed under a nitrogen stream. This is because sunflower essential oil (0.93 g/mL) has a higher density than the solvent (0.659 g/mL). On the other hand, the microwave-assisted aqueous enzymatic extraction (MAAEE) of oil from pumpkin seeds was also investigated by Qi, Li *et al.* (2014a). Centrifugation was used for the separation of oil and emulsion before the recovery of oil from de-emulsification process. Therefore, properties of the extracts and solvents are the considerations in the selection of appropriate separation technique. Furthermore, Aziz, Hassan *et al.* (2017) performed studies on the extraction of essential oils from zingiberaceae Famili by using SFME, MAE, and HD. The essential oil was collected at 0°C due to the different physical properties of the solvent and extracted essential oil. It showed that altering the temperature could be an alternative for the recovery of essential oil.



## 2.10 Optimization studies of microwave-assisted extraction

Optimization studies are crucial to improve the performance of a process. Several multivariate approaches have been used as optimization designs, such as L<sub>9</sub> Orthogonal array design, L<sub>16</sub> array design, Box-Behnken experimental design, central composite design and etc (Mohan, Khanam *et al.* 2013). Besides that, researchers have adopted the univariate approach or chemo metric tools such as factorial design, fractional factorial design, taguchi design and various other models. In the optimization of MAE, extraction time, microwave power and temperature are known to be the most influencing parameters (Kala, Mehta *et al.* 2016). These parameters and their operational range are tabulated in Table 2.8 according to the source of the extract.

Table 2.8: MAE parameters and their maximum and minimum operational range (Kala, Mehta *et al.* 2016).

Plant part	Parameter	Range used
Leaves	Power	17.86-900 W
	Time	30 sec-5 hr
	Temperature	45-200 °C
Fruits	Power	90-1000 W
	Time	31 sec-67.11 min
	Temperature	35-300 °C
Roots	Power	300-600 W
	Time	31 sec-10 min
	Temperature	50-100 °C
Stem	Power	140-531 W
	Time	40 sec-5 min
	Temperature	60-100 °C

Isolation of essential oil from lavender using microwave energy is conducted by Farhat, Ginies *et al.* (2009). This study was operated using microwave steam diffusion (MSD) at the optimum condition for the extraction of essential oil from lavender. The optimization carried out by using five-level with the main parameters, steam mass flow rate (3-60 g min<sup>-1</sup>) and microwave power (50-400 W). From the experiment, the extraction rate increased with steam flow rates. The study shown that at low steam flow rate, the extraction of totality of essential oil took longer time.

Microwave power at 200 W has shown to be able to minimize losses of volatile compounds. The optimal conditions to obtain high yield at short extraction time are with a steam flow rate of 25 g min<sup>-1</sup> at 200 W.

Process optimization of essential oil extracted from leaves of lemongrass through microwave and hydrodistillation were investigated and compared by Desai and Parikh (2015). The extraction process was optimized using Taguchi method. Three factors were studied at four levels: volume of water (200-500 mL), rehydration time (30-180 min), extraction time (15-45 min). Extraction time was identified in ANOVA analysis to be the most significant factor affecting the extraction process. The total of signal-to-noise (S/N) ratio for each factor was calculated to find out the optimum conditions. Based on the results, an increased in the volume water and rehydration time was observed to produce a lower yield. Besides that, with an increased on extraction time, the yield was observed to be increased. The maximum total of S/N ratio among the four levels of each factor is 200 mL volume of water, 30 min of rehydration time, and 45 min of extraction time.

Tigrine-Kordjani, Meklati *et al.* (2012) examined the SFME of essential oil from *S. chinensis*. Optimization was carried out using Response Surface Method (RSM) by employing central composite design (CCD) to determine the importance of major factors in affecting the extraction process. It was concluded that microwave power has the greatest effect on the extraction process followed by extraction time and moisture content. In order to determine the upper and lower limit for further optimization process, a single factor of SFME was carried out for moisture of raw material (40-60%), extraction time (25-35 min) and microwave power (230-540 W). Additionally, Tigrine-Kordjani, Meklati *et al.* (2012) reported that the effect of interactions in this model was smaller than the effect of a single factor which also indicated that interactions between moisture of raw material, extraction time and microwave power were not significant. The optimum conditions for extraction of essential oil from *S. chinensis* by SFME were 68% moisture content of *S. chinensis* fruits extracted at 385 W for 30 min with an error about 1.8%. The extraction yield of essential oil obtained from the optimization was 11 mL/kg.

Furthermore, Li, Chen *et al.* (2010) studied and optimized the solvent extraction of garlic essential oil. A four-factor, three-coded level of Box-Behnken design (BBD) was applied to analyze and to determine interaction of the major factors in optimization studies. Extraction temperature, extraction time, ratio of solvent to feedstock, and number of extraction time were

shown to be the independent variables studied to predict the response value. The factorial design had led to 27 sets of experiments.

Based on RSM regression, the predicted maximum extraction yield was 0.78% at 45°C for 42 min with the ratio of solvent to feedstock of 4, and number of extraction of 4 on the production of essential oil. Moreover, the statistical analysis revealed that extraction temperature and the ratio of solvent to raw material have shown the most apparent effect on the yield of garlic essential oil.

The MAE conditions such as irradiation time, temperature, particle size for extraction of andrographolide from *Andrographis paniculata* with water and methanol were studied and optimized by using L<sub>9</sub> orthogonal array design (Mohan, Khanam *et al.* 2013). The optimization process was completed with 9 runs of experiments. After several runs of experiments, the optimum parameters with water were with mesh size #12 for 4 min at 420 W under 80°C whereas for methanol, the mesh size was #44 for 1 min at 420 W under 50°C. According to Bezerra, Santelli *et al.* (2008), optimization in analytical chemistry was carried out by “one-factor-at-a-time” method on an experimental response. This is conducted when one factor is varied while others are kept at constant. However, this technique is tedious and does not study the interaction effects among the parameters (Bezerra, Santelli *et al.* 2008).

## 2.11 Kinetics of essential oil extraction

### 2.11.1 First order reaction

The molecular mechanism of essential oil extraction from microalgae can be described in 3 stages. Stage 1 describes the initial extraction of essential oil located on the surface of the plant material. Stage 2 relates to the internal diffusion of essential oil from the inner components of the plant material while stage 3 describes the equilibrium extraction phase (Tigrine-Kordjani, Meklati *et al.* 2012). In order to study the transport phenomenon of the oil mass from one location (intracellular point) to the extracellular phase, a 1st order kinetic model was proposed. According to Özkal, Salgın *et al.* (2005), the mass transfer behavior of microalgae by supercritical carbon dioxide (SCCO<sub>2</sub>) is described in Equation (2.2) to (2.3)

$$\frac{dm_e}{dt} = k(m_{s,t} - m_{s,t}^*) \quad (2.2)$$

where  $m_e$  is the oil extracted in grams (g oil/g garlic feed) at time  $t$  (min);  $k$  is the garlic cellular mass transfer coefficient in  $\text{min}^{-1}$ ;  $m_{s,t}$  is the quantity of unextracted oil at time  $t$ ;  $m_{s,t}^*$  is the quantity of unextracted oil at time  $t$  at equilibrium between the concentration of garlic oil at the intracellular and extracellular phases. However,  $m_{s,t}^*$  is assumed to be neglected, because pure solvent continuously fed to the extractor and  $m_{s,t}$  is equal to the difference between initial oil content and extracted oil at time,  $t$ .

$$\frac{dm_e}{dt} = k(m_{s,t}) = k(m_{s,0} - m_e) \quad (2.3)$$

Integrating Equation (2.3) gives Equation (2.4) which can be established in a linear form with  $k$  as the slope of the straight line through the origin of the plot of  $\ln\left(\frac{m_{s,0}}{m_{s,0}-m_e}\right)$  against  $t$ . Equation (2.4) will be used to study the transport phenomenon of the oil mass.

$$\ln\left(\frac{m_{s,0}}{m_{s,0} - m_e}\right) = kt \quad (2.4)$$

In addition, Farhat, Ginies *et al.* (2009) also explained the rate of extraction of essential oil from lavender by microwave steam diffusion as 1<sup>st</sup> order kinetics. The overall oil mass balance over the column boundaries gives:

$$G_v y_0 - G_v y(t) = \frac{dm}{dt} \quad (2.5)$$

where  $m$  is the mass of oil contained in the column,  $y_0$  is the input essential oil fraction by weight,  $y(t)$  is the steam essential oil fraction by mass in the outlet of the column at a time  $t$ ,  $G_v$  is the mass flow rate of steam ( $\text{g m}^{-1}$ ) and in Equation (2.6), rate of extraction which oil is transferred to steam can be explained in terms of an overall mass transfer coefficient  $K$  and the difference between an average composition of seeds,  $x$ , and an equilibrium composition,  $x^*$ .

$$\frac{dm}{dt} = K\Omega(x - x^*) \quad (2.6)$$

where  $K$  is an overall mass transfer coefficient ( $\text{g oil min}^{-1} \text{m}^{-2}$ ),  $\Omega$ , is the surface area available for mass transfer ( $\text{m}^2$ ),  $x^*$  is an oil fraction in the flowers assumed to be in equilibrium with steam.

### 2.11.2 Second order reaction

Second-order mechanism model has been applied to the essential oil extraction from sandalwood by microwave-assisted hydrodistillation (Kusuma and Mahfud 2016a). It is because the extraction occurs in two simultaneous processes. The extraction increases rapidly with the time at the beginning and then decreases slowly with the time. The rate of dissolution for the essential oil contained in the solid into solution can be described by Equation (2.7).

$$\frac{dc_t}{dt} = k(C_s - C_t)^2 \quad (2.7)$$

where  $k$  is the second-order extraction rate constant ( $\text{L g}^{-1} \text{min}^{-1}$ ),  $C_s$  the extraction capacity (concentration of essential oil at saturation in  $\text{g L}^{-1}$ ) and  $C_t$  is the concentration of sandalwood oil at any time  $t$  (min).

As the initial and boundary conditions are to be assumed at  $t = 0$  to  $t$  and  $C_t = 0$  to  $C_t$ , the integrated rate law is obtained:

$$C_t = \frac{C_s^2 kt}{1 + C_s kt} \quad (2.8)$$

Equation (2.8) can be transformed into a linear form shown in Equation (2.9) and the extraction rate can be written as Equation (2.10):

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s} \quad (2.9)$$

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{kC_s^2}\right) + \left(\frac{t}{C_s}\right)} \quad (2.10)$$

The initial extraction rate,  $h$ , can be expressed, as  $\frac{C_t}{t}$  when  $t \approx 0$ :

$$h = kC_s^2 \quad (2.11)$$

Moreover, the concentration of essential oil at any time can be defined as:

$$C_t = \frac{t}{\left(\frac{1}{h}\right) + \left(\frac{t}{C_s}\right)} \quad (2.12)$$

where the second-order extraction rate constant,  $k$  can be determined experimentally from the slope and intercept by plotting  $\frac{t}{C_t}$  versus  $t$ .

### 2.11.3 Modelling extraction units

#### I Steam distillation

*Baccharis* (Compositae-Asteraceae) is an American large genus plant which is widely used as traditional medicine. Hence, Xavier, Vargas *et al.* (2011) has investigated the mathematical modelling for the extraction of essential oil from *Baccharis* sap by steam distillation. The new

model has been modified to fit two extraction processes in steam extraction process: phase equilibrium and internal diffusion in the particles. However, there are a few assumptions that have been made where the extracted oil is assumed to be a pseudo component with constant properties throughout the process. The mass balance per unit volume for the solute in the fluid phase and solid phase are shown in Equation 2.13 and 2.14.

$$\rho_f \varepsilon \frac{\delta Y}{\delta t} + \rho_f u \frac{\delta Y}{\delta z} = J(x, Y) \quad (2.13)$$

$$- \rho_s (1 - \varepsilon) \frac{\delta x}{\delta t} = J(x, Y) \quad (2.14)$$

where  $\rho_f$  is solvent density,  $\rho_s$  is solid density,  $\varepsilon$  is bed void fraction,  $u$  is superficial fluid velocity,  $Y$  is mass fraction in fluid phase,  $x$  is mass fraction in solid phase,  $J$  is flux of solute,  $t$  is extraction time,  $z$  is axial co-ordinate and  $x_0$  is the initial mass fraction of solute in solid flux.

The flux for fluid and solid phase was

$$J(x, Y) = k_f a_0 \rho_f (Y^* - Y) \quad \text{for } x > x_k \quad (2.15)$$

And

$$J(x, Y) = k_s a_0 \rho_s x \quad \text{for } x \leq x_k \quad (2.16)$$

where  $k_f$  is fluid phase mass transfer coefficient,  $k_s$  is solid phase mass transfer coefficient,  $a_0$  is specific surface area per unit volume of extraction bed,  $Y^*$  is an equilibrium fluid phase mass transfer and  $x_k$  is an easily accessible solute in solid phase.

The phase equilibrium denotes the rapid extraction of essential oil from the broken cell to the surrounding. In this extraction step, external mass transfer resistance is negligible where the fluid was in equilibrium phase with solid phase throughout the extractor. Moreover, the second parts are controlled by the essential oil's slow diffusion rate from the inner cells. This period corresponds to the diffusion from intact cells by combining Equation 2.14 and Equation 2.16:

$$- \rho_s (1 - \varepsilon) \frac{\delta x}{\delta t} = k_s a_0 \rho_s x \quad (2.17)$$

The solution for Equation 2.18 is

$$x = x_0 e^{-K_2 t} \quad (2.18)$$

where

$$K_2 = \frac{k_s a_0}{(1 - \varepsilon)} \quad (2.19)$$

For fluid phase, the equation is rewritten as

$$\rho_f \varepsilon \frac{\partial Y}{\partial t} + \rho_f u \frac{\partial Y}{\partial z} = K_2 \rho_s (1 - \varepsilon) x_0 e^{-K_2 t} \quad (2.20)$$

The extraction curve is calculated as follows:

$$M(t) = \int_0^t \dot{m} Y(z = H, t) dt$$

where  $M(t)$  is the mass of extract,  $\dot{m}$  is solvent flow rate and  $H$  is extraction bed length. The result is presented in terms of maximum value for the extract obtained,  $M(\infty)$ , for the two periods in the extraction process:  $\frac{K_1 t}{M(\infty)}$  for first period and  $1 - e^{-K_2 t}$  for second period.

where  $K_1$  is defined by:

$$K_1 = \dot{m} Y^* \quad (2.21)$$

Based on Xavier, Vargas *et al.* (2011), the new model is well fitted for the steam distillation extraction method as it is able to stimulate extraction curves and identify them by means of the determining of the parameters as mass transfer coefficient and equilibrium constant.



According to Cassel and Vargas (2006), steam distillation process is based on Fick's law model in a steady state of one-dimensional geometry. The process assumed that the essential oil is homogeneous with constant properties in the beginning of the extraction process. At the boundaries, the steam carried out the oil, hence, the oil concentration at the boundaries is small ( $c_A = 0$ ). The mathematical formulation is expressed as Equation 2.22 and with a few initial and boundary conditions.

$$\frac{\partial^2 c_A}{\partial x^2} = \frac{1}{D} \frac{\partial c_A}{\partial t} \quad 0 \leq x \leq L \quad (2.22)$$

where L is the thickness of the plate and D is the effective diffusion coefficient.

However, a separation variable technique is used and the following solution is shown:

$$c_A(x, t) = \sum_{n=1}^{\infty} \frac{4c_{A_0}}{n\pi} \text{sen}(\beta_n x) e^{-D\beta_n^2 t} \quad (2.23)$$

The mass flux at the boundary with the normal surface area presented the mass flow as a function of time.

$$m(t) = \frac{4c_{A_0}DA}{L} \sum_{n=1}^{\infty} e^{-D\beta_n^2 t} \quad (2.24)$$

The mass of essential oil is

$$m(t) = \frac{8mA_0DA}{\pi^2} \sum_{m=0}^{\infty} \frac{\left(1 - e^{-\frac{(2m+1)^2 \pi^2 Dt}{L^2}}\right)}{(2m+1)^2} \quad (2.25)$$

And the degree extraction

$$e(t) = \frac{m(t)}{m(\infty)} = \frac{\sum_{m=0}^{\infty} \frac{\left(1 - e^{-\frac{(2m+1)^2 \pi^2 Dt}{L^2}}\right)}{(2m+1)^2}}{\sum_{m=0}^{\infty} \frac{1}{(2m+1)^2}} \quad (2.26)$$

## II Hydrodistillation

A mathematical model for hydrodistillation of essential oils from coriander seeds has been developed by Sovová and Aleksovski (2006). This model is carried out by investigating the outbreak and intact cells where the essential oil is rapidly extracted from the broken cell wall and diffusion takes place through the intact cells to the plant surface. However, the controlling step is the diffusion rate of solute from the intact cell. In order to develop the model, assumptions have been considered:

1. Essential oils as single pseudo-component with constant properties throughout the process.
2. Plants particles are uniformed in size, shape, and oil content.
3. The particle in the extractor where fraction  $r$  of essential oil is in broken cells and fraction  $1-r$  is closed in intact cells.
4. Boiling water with plant particles and steam bubbles act as a well-mixed fluidized bed.
5. The amount water in the vapor phase in the extractor, condenser, separator, and connecting tubing is very small which is negligible in the mass balance.
6. Mass transfer resistances in broken cells, boiling water and vapor phase are assumed to be negligible and thus equilibrium between the phases is still established.
7. If intact cells are equal to the concentration of untreated particles, the initial concentration in the cells is composed.
8.  $x_s$  is assumed to be constant when the essential oil concentration in the separator flows back the extractor.

In this case, the equilibrium between the region of broken cell, boiling water and vapor phase is assumed to form quasi-rapidly, hence, the limiting process is the slow diffusion rate of essential oil from the inner particle core to the broken cells. Moreover, the mass transfer rate from the intact cell is determined as quasi-steady with the mass flux.

By introducing core dimension,  $\lambda = \text{core volume/ core surface}$ , the mass transfer rate from particle core with mass flux is known:

$$-\frac{dx_i}{dt} = \frac{k}{\lambda} \left( x_i - \frac{x_w}{k_w} \right) \quad (2.27)$$

where  $x_i$  is the mean solute concentration in the core,  $k$  is the mass transfer rate,  $x_w$  is the mean solute concentration in boiling water and  $k_w$  is the mass transfer rate of boiling water.

Likewise, Pornpunyapat, Chetpattananondh *et al.* (2011) conducted research on the mathematical modelling for extraction of essential oil from *Aquilaria crassna* via hydrodistillation. Agarwood is known in many industries for its application in medicine, perfume, and cosmetics due to its high therapeutic potential and properties. In this work, the mechanisms of hydrodistillation consisted of two processes: washing stage and diffusion stage and the diffusion stage is the controlling step. Moreover, the mass transport of essential oil within the plant particles is an unsteady state. Hence, Fick's second law of one-dimensional unsteady state diffusion is applied as it is a batch hydrodistillation with no chemical reaction.

$$\frac{\partial q_p}{\partial t} = D_{eff} \frac{\partial^2 q_p}{\partial x^2} \quad (2.28)$$

where  $q_p$  is the average concentration of essential oil in the plant material at time  $t$ ,  $x$  is the distance along the direction of diffusion, and  $D_{eff}$  is the effective diffusion coefficient. Few assumptions have been made in order to solve Equation 2.29.

1. The plant particles are isotropic, uniform in size, shape with constant properties throughout the process.
2. The essential oil is assumed as single pseudo-component.
3. The essential oil is distributed around the surface of the broken cells and some are allocated in the inner part of plant particles.
4. The effective diffusion coefficient,  $D_{eff}$  is constant.
5. Due to the washing stage, the oil concentration on the surface of plant particles is always equal to zero.
6. There is no mass transport resistance of essential oil around the surface of plant particles.

By taking account of these assumptions, Equation 2.29 was developed:

$$\frac{q_0 - q}{q_0} = Ae^{-kt} \quad (2.29)$$

where  $q$  is the average yield at time  $t$ ,  $q_0$  is the initial average concentration,  $A$  is a constant, and  $k$  is a kinetic constant including the effective diffusion coefficient.

As mentioned earlier, hydrodistillation is involved in two stages, washing stage and diffusion stage. In the washing stage, the essential oil around the surface of the plant particles is washed during initial step at approximately  $t = 0$ . It can also be determined that the oil yield rapidly increases at the beginning of the process which brings to Equation 2.30:

$$t = 0 : q = q_w$$

$$\frac{q}{q_0} = \frac{q_w}{q_0} = b \quad (2.30)$$

where at  $t=0$ ,  $q_w$  is the essential oil yield and  $b$  is the relative amount of essential oil extracted.

In the diffusion stage, the essential oil diffused from the inner part to the surface of the plant particles. This is described in a slow increase rate of oil yield during the extraction process. Hence, the basic kinetic equation for agarwood oil extraction is derived by combining Equation 2.29 and 2.30.

$$\frac{q_0 - q}{q_0} = (1 - b)e^{-kt} \quad (2.31)$$

In this proposed equation,  $b$  is named as washing coefficient and  $k$  is diffusion coefficient. The linearized form can be derived from Equation 2.31.

### III Supercritical fluid extraction

Experimental and modelling of essential oil extraction by using supercritical fluid extraction (SFE) with carbon dioxide were conducted (Sodeifian, Ghorbandoost *et al.* 2016), (Perrut, Clavier *et al.* 1997). Sodeifian, Ghorbandoost *et al.* (2016) performed a mathematical modelling for extraction of essential oil from *Pistacia khinjuk* by supercritical carbon dioxide. This model has been divided into three stages: washing stage, diffusion stage and internal diffusion stage. In this model, the essential oil is assumed to be trapped in the intact cells. During the first period, the extraction happens at a constant rate. The essential oil covering the external surface of the plant particles is easily accessible and extracted. However, it is controlled by the resistance of

the solvent film surrounded by the plant particles. Next, the second stage involved in the diffusion mechanisms is from the inlet to the outlet of the intact particles where the essential oil is located in the interior sections of these particles. The extraction rate is determined by the diffusion and convection rates at the same time. Finally, the essential oil becomes inaccessible as it is located in the deeper part of the plant particles. This stage is only influenced by the internal mass transfer resistance.

Assumptions:

1. Model is assumed that the flow regime is a plug and axial dispersion is negligible.
2. Plant particle is equal in size and initial oil content.
3. Bed porosity, pressure, and temperature are constant.

The mass balance equations for the solid and fluid phase during the three different stages are shown in Equation 2.32. The following expressions describe the specific amount of extract solute (g solute/g solute-free feed) as a function of a specific quantity solvent  $q$  (g solvent/g solute-free feed).

$$\begin{aligned}
 e &= qy_r [1 - \exp(-Z)] & q < q_m \\
 e &= y_r [q - q_m \exp(Z_w - Z)] & q_m \leq q < q_n \\
 e &= x_0 - \left(\frac{y_r}{W}\right) \ln \left( 1 + \left[ \exp\left(\frac{Wx_0}{y_r}\right) - 1 \right] \exp[W(q_m - q)] \frac{x_k}{x_0} \right) \\
 q_n &\leq q & (2.32)
 \end{aligned}$$

where

$$q_m = \frac{(x_0 - x_k)}{y_r Z} \quad (2.33)$$

$$q_n = q_m + \frac{1}{W} \ln \left[ \frac{x_k + (x_0 - x_k) \exp\left(\frac{Wx_0}{y_r}\right)}{x_0} \right] \quad (2.34)$$

$$\frac{Z_w}{Z} = \frac{y_r}{Wx_0} \ln \left( \frac{x_0 \exp[W(q - q_m) - x_k]}{x_0 - x_k} \right) \quad (2.35)$$

$$Z = \frac{k_f a \rho_f}{q(1 - \varepsilon) \rho_s} \quad (2.36)$$

$$W = \frac{k_s a}{q(1 - \varepsilon)} \quad (2.37)$$

$q_m$  and  $q_n$  are  $q$  values when extraction of the solute within the particles begins and when the easily accessible solute is fully extracted respectively.  $\dot{q}$  is the specific mass flow rate of the solvent.  $Z$  and  $W$  are dimensionless mass transfer parameters in solid and solvent phases being proportionate to the mass transfer coefficients of two phases.  $z_w$  is the dimensionless boundary coordinated between the slow and fast extraction regions while  $x_0$  is the initial total concentration of the solute in the bed (g solute/g solute free feed).  $x_k$  is the initial content of the barely accessible solute inside the particles (g solute/g solute free feed).  $\rho_s$  and  $\rho_f$  are the densities of solid and fluid phases ( $\text{kg}/\text{m}^3$ ) respectively.  $\varepsilon$  is the void fraction of the bed ( $\text{m}^3/\text{m}^3$ ),  $a$  is the specific interfacial area of the solid particles ( $\text{m}^2/\text{m}^3$ ),  $y_r$  is the extract solubility in SC- $\text{CO}_2$  (g solute/ g solvent),  $t$  is extraction time (s),  $k_s$  and  $k_f$  are the solid and fluid phases mass transfer coefficients (m/s), respectively. The model is an effective approach to fit the experimental data.

New modelling of the extraction of volatile oil from natural plants by supercritical  $\text{CO}_2$  was carried out (Sovová and Aleksovski 2006). Sovová and Aleksovski (2006) proposed a model based on the concept of broken and intact cells where this model is involved in two processes:

phase equilibrium and diffusion in the plant particles. In the first stage, this model takes different types of phase equilibrium and solvent flow pattern into account. This model is based on the broken and intact cells which provide an extension of the group. The model equations are simplified and converted into a dimensionless form which enables to solve numerically and used to analyze model properties. In order to build the model, few assumptions have been made.

1. Homogeneous essential oil distributed in the plant particles.
2. Both broken cell near the surface and intact cell in the core are present in the particles.
3. Grinding efficiency,  $r$  is the volumetric fraction of broken cells.
4. The essential oil near the broken cells directly diffuses to fluid phase, while the essential oil in the intact cells diffuses from the inside to the broken cells and then to the fluid phase.
5. The fluid phase mass transfer coefficient is used to characterize the mass transfer from broken cells to the solvent which is by few orders of magnitude larger than solid phase mass transfer relay to the diffusion from intact cells to broken cells.
6. The reduction in the solid mass during the extraction is not influenced by the extraction bed characteristics, void fraction, and surface area.
7. The fluid density does not affect when the essential oil dissolves in the solvent.

The mass balance per unit volume of extraction bed equation below is for plug flow. Equation 2.38 is for the essential oil in the fluid phase, Equation 2.39 is for solid phase with broken cells and Equation 2.40 is for solid phase with intact cells.

$$\rho_f \varepsilon \left( \frac{\partial y}{\partial t} + U \frac{\partial y}{\partial h} \right) = j_f \quad (2.38)$$

$$r \rho_s (1 - \varepsilon) \frac{\partial x_1}{\partial t} = j_s - j_f \quad (2.39)$$

$$(1 - r) \rho_s (1 - \varepsilon) \frac{\partial x_2}{\partial t} = -j_s \quad (2.40)$$

With initial and boundary conditions as follows:

When  $t=0$ ,  $y = y_0$ ;  $x_1 = x_{1,0}$ ;  $x_2 = x_{2,0}$ ; when  $h=0$ ,  $Y = 0$

The extraction curve is calculated by

$$E = \dot{Q} \int_0^t y_{h=H} dt \quad (2.41)$$

Phase equilibrium of fluid phase and the solid phase with broken cells is known by the discontinuous equilibrium function.

$$y^*(x_1) = y_s \quad \mathbf{for} \quad x_1 > x_t;$$

$$y^*(x_1) = Kx_1 \quad \mathbf{for} \quad x_1 > x_t; Kx_t > y_s \quad (2.42)$$

The equilibrium between the fluid and solid with broken cells is assumed to be initiated before the extraction starts and it happens during the extractor pressurization, pressure, and temperature stabilization. On the other hand, the essential oil concentration in the intact cells is assumed to be the same and is equal to the untreated particles,  $x_u$ . Hence, the initial mass balance for the essential oil in the broken cells and in the solvent is given:

$$x_u - x_{1,0} = \frac{\rho_f \varepsilon}{\rho_s (1 - \varepsilon)} \frac{Y}{r} y_0 \quad \mathbf{for} \quad t = 0 \quad (2.43)$$

By combining Equation 2.42 and 2.43, it divides the initial concentrations into three regions with respect to the discontinuity on the equilibrium curve.

The flux mass transfer from the broken cells to the solvent is shown in Equation 2.44 where the discontinuity in the equilibrium relationship has been considered.

$$j_f = k_f a_0 \rho_f (y^* - y) \quad \mathbf{for}$$

$$x_1 \neq x_t \quad \mathbf{or} \quad x_1 = x_t, y < Kx_t, j_f = 0 \quad (2.44)$$

After all the essential oil has been dissolved in the solvent, the flux is interrupted. The dissolved essential oil is then washed out, resulting in a decrease of fluid phase concentration in the extraction bed until it fell below equilibrium concentration of interacting solute,  $Kx_t$  and the flux is renewed. Only in the beginning of the extraction when all essential oil interacts with the matrix, the flux from broken cells is not interrupted. The flux from intact cells to broken cells is described by the equation



$$j_s = k_s a_s \rho_s (x_2 - x_1) \quad (2.45)$$

Finally, the dimensionless equation is introduced, the models are all transformed into dimensionless form using the dimensionless variables

$$z = \frac{h}{H}; \tau = \frac{Ut}{H} = \frac{t}{t_r}; Y = \frac{y}{y_0};$$

$$X_1 = \frac{x_1}{x_{1,0}}; X_2 = \frac{x_2}{x_{1,0}}; \phi = \frac{E}{Nc_u} \quad (2.46)$$

The initial ratio of the essential oil in the fluid phase to the solute in broken cells,  $\Gamma$  the external mass transfer resistance,  $\Theta_e$ , and the internal mass transfer resistance,  $\Theta_i$  which are the three dimensionless model parameters.

$$\Gamma = \frac{\rho_f \varepsilon y_0}{\rho_s (1 - \varepsilon) r x_{1,0}} = \frac{\gamma y_0}{r x_{1,0}};$$

$$\Theta_e = \frac{\varepsilon}{k_f a_0 t_r}; \Theta_i = \frac{1 - \varepsilon}{k_s a_s t_r} \quad (2.47)$$

#### IV Microwave-assisted extraction

Desai and Parikh (2015) studied the kinetic behavior of extraction of essential oil from the leaves of lemongrass by microwave radiation and a data fit for kinetic desorption models was carried out. The kinetic desorption models were applied to study the rate of extraction of MAE with respect to the yield and composition. Both HD and MAE technique modelling were performed with similar concepts of rate process, with MAHD having a different mode of energy supplied. The kinetic models used and the rate of the desorption steps in the process can be further described as a diffusion-based hot-ball model (one site) and two-site model. The one-site kinetic desorption model and two-site kinetic desorption model can be presented by Equation (2.48) and (2.49):

$$u_t = 1 - e^{-kt} \quad (2.48)$$

where  $u_t$  is the fraction of the essential oil extracted at time  $t$ , and  $k$  is the 1<sup>st</sup> order rate constant in  $\text{min}^{-1}$ .

$$u_t = 1 - [Fe^{-k_1t}] - [(1-F)e^{-k_2t}] \quad (2.49)$$

where,  $k_1$  is the 1<sup>st</sup> order rate constant in  $\text{min}^{-1}$  describing the fast release fraction,  $F$ , and  $k_2$  is the 1<sup>st</sup> order constant in  $\text{min}^{-1}$  describing the slowly released fraction,  $1 - F$ .

The rate constant was determined by using the MATLAB program (Mathworks, Natick, MA, U.S.A.) to minimize the error between the experimental and predicted value. The kinetic model has shown to be fit for MAE and HD. MAE showed higher rate constants ( $0.0776 \text{ min}^{-1}$  for the one-site model and  $0.0644$  and  $0.1563 \text{ min}^{-1}$  for the two-site model) where HD obtained lower rate constants ( $0.0339 \text{ min}^{-1}$  for the one-site model and  $0.0300$  and  $0.0469 \text{ min}^{-1}$  for the two-site model). This might be due to the long extraction time of conventional heating occurred during the HD extraction process. This has also proven that the volumetric and uniform heating involved in MAE has led to a fast extraction time of essential oil, hence providing a higher rate.

Kinetic modelling of microwave-assisted hydrodistillation of juniper berry essential oil was also investigated ((Pavićević, Marković *et al.* 2015). This model is developed for batch distillation vessel where the plant particles are immersed in the solvent. During the microwave extraction process, the solvent evaporates with essential oil and carries the essential oil vapor from the distillation vessel into the condenser. Pavićević, Marković *et al.* (2015) has proven that the mechanisms for both microwave-assisted hydrodistillation (MAHD) and hydrodistillation (HD) are the same which include fast (washing) and slow (diffusion) distillation of essential oil. In order to develop the model, few assumptions are made:

1. Plant matrix is isotropic and uniform in size, shape, and initial essential oil content.
2. The essential oil is a pseudo-single component.
3. The amount of essential oil that can be extracted is equal to the essential oil distilled off until an equilibrium is achieved.
4. A part of essential oil is located around the surface of the plant particles,  $f$ , and the rest are homogeneously located inside the particle core,  $(1-f)$ .
5. Constant effective coefficient diffusion.
6. No existence of mass transfer resistance of essential oil from the surface of plant particles.
7. The extracted solvent mixture and essential oil are immiscible.

8. The extraction process occurs simultaneously in the washing and diffusion stage. Both of the stages follow the first order kinetics law with respect to the essential oil in the plant particles as shown in Equation 2.50.
9. The amount of the essential oil collected and divided from the total feedstock is equal to the essential oil yield from the plant matrix in the distillation vessel with a time delay.

$$-\frac{q_p}{q_t} = kq_p \quad (2.50)$$

where  $q_p$  is the average concentration of essential oil in the plant particles at time  $t$ , and  $k$  is the process rate constant.

For  $t = 0$ ,  $q_p = q_\infty$ , by integrating Equation (2.50) gives the following Equation (2.51):

$$\frac{q_{p,i}}{q_\infty} = e^{-k_i t} \quad (2.51)$$

where  $k_i$  is the rate constant and  $i$  is the 1 and 2 for washing and diffusion.

By considering assumption 8, Equation 2.52 is derived where the amount of essential oil remains in the plant matrix until time  $t$  is given by

$$\frac{q_p}{q_\infty} = fe^{-k_1 t} + (1-f)e^{-k_2 t} \quad (2.52)$$

Hence, the amount of essential oil at time  $t$ , with  $q = q_\infty - q_p$ :

$$\frac{q_p}{q_\infty} = 1 - fe^{-k_1 t} - (1-f)e^{-k_2 t} \quad (2.53)$$

Assumption 9 showed that Equation 2.53 defines the changes of essential oil yield with the extraction process. According to this model,  $f$  represents the essential oil in broken cell while the others are time constant, homologous to the values of the rate constant for washing and diffusion. By assuming instantaneous washing followed by diffusion ( $k_1$  to  $\infty$ ) and diffusion with no washing ( $k_1$  to  $\infty$  and  $f = 0$ ), Equation (2.53) can be further derived into:

$$\frac{q}{q_{\infty}} = 1 - (1 - f)e^{-k_2 t} \quad (2.54)$$

$$\frac{q}{q_{\infty}} = 1 - e^{-k_2 t} \quad (2.55)$$

However, Equation 2.54 is the same as the kinetic expression for conventional HD where  $f$  is the washing coefficient,  $k_2$  is the coefficient for slow diffusion extraction. The model has been verified that it is valid for the extraction of essential oil by MAHD. Additionally, Equation 2.55 is based on the assumption of pseudo first order kinetics with respect to the essential oil remaining in the plant matrix. This model has proven to fit the kinetics of HD and MAHD.

Table 2.9 showed that most of the kinetic models involve two stages: washing and diffusion stages. HD and MAHD are able to fit with the same kinetic model. The differences between them are their heat source where HD uses the conventional heating mechanisms while MAHD uses microwave irradiation. Likewise, both HD and SD have the same heat source while SFE does not require heat for the extraction process which makes kinetic model for SFE different. In the SFE process, the temperature, pressure, particles size and flow rate can be easily affected by the mass transfer coefficient. These models have proven that the diffusion stage is the controlling step of essential oil extraction. Furthermore, the assumptions made are similar.

Table 2.9: Comparison of modelling extraction method.

Extraction methods	Approaches	Authors
Steam distillation (SD)	<ul style="list-style-type: none"> <li>• Involves two phases: phase equilibrium and diffusion.</li> <li>• Involves mass balance of essential oil in fluid and solid phase.</li> <li>• Controlling process: diffusion phase in the plant cell.</li> </ul>	(Xavier, Vargas <i>et al.</i> 2011)
	<ul style="list-style-type: none"> <li>• Based on two phases: rapid extraction and slow diffusion.</li> <li>• Controlling process: diffusion step in the plant cell.</li> <li>• Applies Fick's law in steady state for 1D.</li> </ul>	(Cassel and Vargas 2006)
Hydrodistillation (HD)	<ul style="list-style-type: none"> <li>• The equilibrium between broken cells, boiling point, and vapor phase enables forming quasi-immediately.</li> <li>• Controlling process: diffusion phase in the plant cell.</li> </ul>	(Sovová and Aleksovski 2006)
	<ul style="list-style-type: none"> <li>• Involve 2 stages: Washing stage and diffusion stage.</li> <li>• The diffusion stage is the limiting step.</li> <li>• When distillation temperature increases, both mass transfer kinetic coefficients increase.</li> <li>• Applied Fick's second law in unsteady state diffusion.</li> </ul>	(Pornpunyapat, Chetpattananondh <i>et al.</i> 2011)
Supercritical fluid extraction (SFE)	<ul style="list-style-type: none"> <li>• Involves three stages: rapid extraction, diffusion, and internal diffusion.</li> </ul>	(Sodeifian, Ghorbandoost <i>et al.</i> 2016)

Table 2.6: Comparison of modelling extraction method. Continue (...)

Extraction methods	Approaches	Authors
Supercritical fluid extraction (SFE)	<ul style="list-style-type: none"> <li>• Based on the concept of broken and intact cells: phase equilibrium and diffusion.</li> </ul>	(Sovová and Aleksovski 2006)
	<ul style="list-style-type: none"> <li>• Controlling stage: diffusion in the plant particles.</li> </ul>	
	<ul style="list-style-type: none"> <li>• Types of phase equilibrium and solvent flow patterns are taken into considerations.</li> </ul>	
	<ul style="list-style-type: none"> <li>• Able to simulate various type of extraction curves.</li> <li>• Characterized with mutually comparable parameters as mass transfer coefficients and equilibrium constant.</li> </ul>	
Microwave-assisted extraction (MAE)	<ul style="list-style-type: none"> <li>• Modelling is assumed as rate process.</li> </ul>	(Desai and Parikh 2015)
	<ul style="list-style-type: none"> <li>• Limiting process: intraparticle diffusion.</li> </ul>	
	<ul style="list-style-type: none"> <li>• Uses kinetic models which describe the rates of desorption step as diffusion-based hot-ball model (one-site) and two-site model.</li> </ul>	
	<ul style="list-style-type: none"> <li>• Includes two phases: washing and diffusion phase.</li> </ul>	(Pavićević, Marković <i>et al.</i> 2015)
	<ul style="list-style-type: none"> <li>• Applies similar kinetic model as HD.</li> </ul>	
	<ul style="list-style-type: none"> <li>• Controlling stage: slow diffusion</li> </ul>	

In short, based on extensive literature study, the following current research gaps have been identified:

- There has been no study reported on the Solvent-Free Microwave Extraction (SFME) of garlic essential oil.
- There has been no study reported on Microwave-Assisted Hydrodistillation (MAHD) combined with water-ethanol solvent for garlic essential oil extraction. It remains unclear what is the impact of the microwave radiation on the quality of antimicrobial properties of the oil recovered. Also, currently the available data on the thermal stability of garlic essential oil extracted via the microwave-assisted technology remains insufficient.
- No fundamental model with predictive capability has been developed for the MAHD. The existing research methodologies lack of reliable models for the MAHD represents a major obstacle to system engineering studies, such as in the process scale-up, process control development and optimization.

## Chapter 3

### RESEARCH METHODOLOGY

#### 3.1 Microwave-assisted extraction

A customized microwave system (Panasonic NN-SM332M) was used in this study. The garlic was obtained from the local market. It was used for the extraction of garlic essential oil. Garlic was crushed or chopped into small pieces for further process. Absolute ethanol (99.9%) from Merck was used in the experimental work. A feedstock to solvent ratio was 1:1.5 in a total volume of 150 mL of solvent and water at the ratio of 1:2 (w/v) is placed in the system. The microwave power was set and varied at different volumes: low (200 W), mid (400 W), mid-high value (600 W) and high (800 W). The extraction duration was varied in order to investigate the extraction equilibrium region. The condenser was connected to the low temperature bath circulator (Protech, Model 630D). Refrigerated water, 10 °C was circulated from low temperature bath circulator into the condenser to condense the vapor flowing from the microwave oven to the receiving flask. In order to minimize the microwave leakage to surrounding environment, a mesh cage was used while an experiment was being conducted. The microwave direction of flow was shown in Figure 3.1. The receiving flask was soaked in a cold water bath to ensure the condensed vapor will not vaporize in the receiving flask. Condensed vapor containing both steam (water-ethanol) and essential oil was collected and subjected to refrigerated centrifugation (Hettich 320 R) at 4 °C, 4000 rpm for 20 min. The collected essential oil was weighed and stored at 4 °C for further analysis.

##### 3.1.1 Solvent selection

The solvent was selected based on the literature review such as ethanol, water and ethyl acetate (Yang, Han *et al.* 2012) and (Kimbaris, Siatis *et al.* 2006). The plant essential oil extracted obtained by ethanol was shown to be the highest in terms of total antioxidant activity, flavonoid content and phenolic content (Do, Angkawijaya *et al.* 2014). Ethanol has been known as a good solvent as it is environmental friendly, and does not pose health and safety concerns. According to the Wang and Weller (2006), the chosen solvent for microwave should have large capacity in absorbing microwave energy. In this case, solvents such as ethanol and water are sufficiently polar for absorbing the microwave energy. Solvents with low dielectric constant, such as hexane and toluene are not considered as potential solvents in microwave technology. In order to determine



the best solvents for garlic essential oil extraction, several solvents with different dielectric constant were experimentally studied: (1) pure ethanol, (2) ethanol-water mixture, (3) pure ethyl acetate, (4) ethyl acetate-water mixture, and (5) distilled water. Previously, the addition of water has been proven to improve the heating effect of microwave in the extraction process.

Table 3.1: Experiments conditions for SFME and MAHD.

Experiments	SFME	MAHD
Parameters		
Feedstock	300-600	100-300
Microwave power	200-800	200-800
Extraction time	30-120	30
Solvent to water ratio	-	1:0.5-1:2

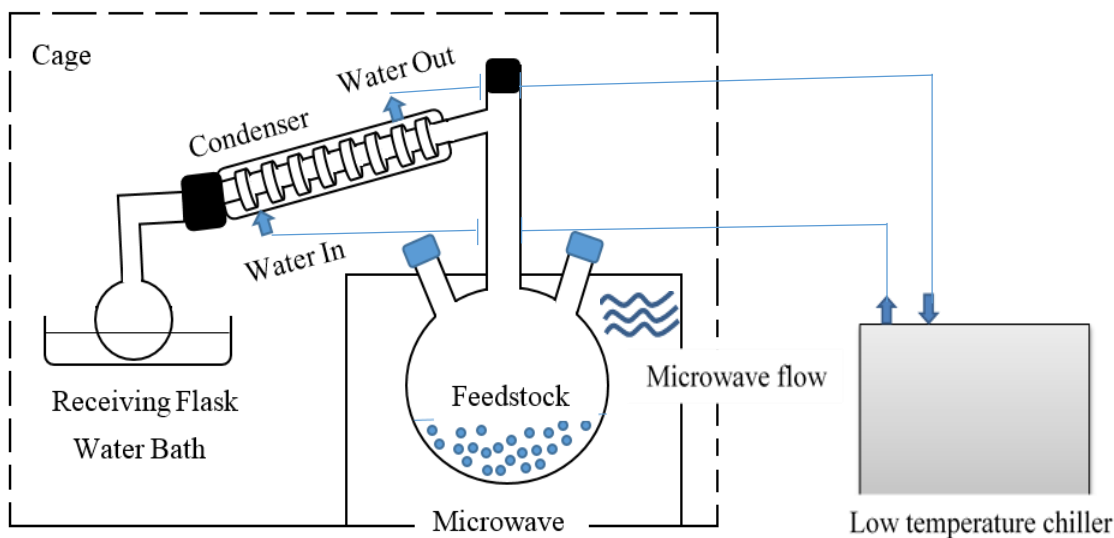


Figure 3.1: The schematic experimental setup.

### 3.2 Statistical experimental design

One-factor-at-a-time (OFAT) method is employed to investigate the influencing operating conditions in the microwave-assisted extraction process.

### 3.2.1 OFAT method

For each process parameter, an optimization was conducted according to the following Table 3.2. The optimization of each experiment requires the identification of the lower and upper bounds of each parameter. Each component composition remains the same according to section 3.1.

Table 3.2: Process parameter.

Set	1	2	3	4
Parameter				
Microwave power, W	200	400	600	800
Extraction time, min	30	60	90	120
Feedstock, g	50	100	150	200

## 3.3 Characterization of garlic essential oil

### 3.3.1 Functional group analysis

Fourier Transform Infrared spectroscopy (FTIR) (Thermo Scientific Nicolet iS1) is a technique to measure infrared frequencies. FTIR was performed by the interference of the incoming radiation between two beams to produce a signal called interferogram. The infrared frequencies from the source produced the signal of their functional group characteristics. For the analysis, the sample was mixed with potassium bromide (KBr) in order to measure the infrared spectrum up to low-wavenumber region ( $250\text{cm}^{-1}$ ). Approximately 10-100 microliter sample is well mixed into 250mg of KBr. The vacuum pelletized sample was then scanned from higher to lower wavenumber to identify the functional groups present in the sample.

### 3.3.2 Gas Chromatography analysis (GC)

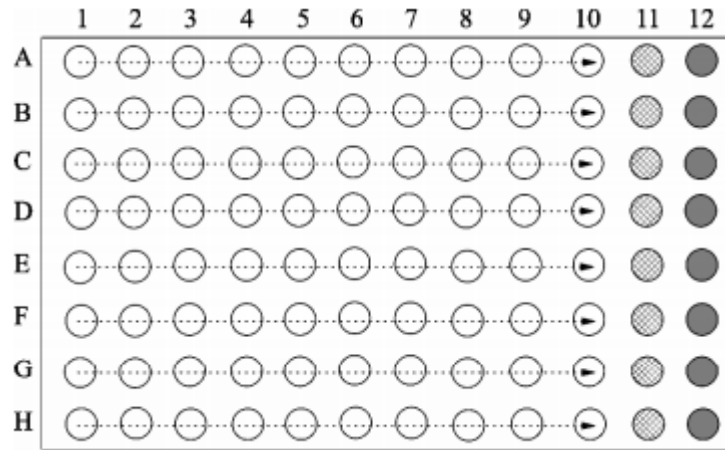
Gas chromatography - flame ionization detector (GC-FID) was carried out using Agilent technologies 6890 n gas chromatograph. A polar HP-5MS column ( $30\text{ m} \times 0.25\text{ mm}$ ,  $0.25\text{ }\mu\text{m}$  film thickness) was used. The injector and FID temperatures were fixed at  $250\text{ }^\circ\text{C}$ . Column temperature was initially kept at  $50\text{ }^\circ\text{C}$  for 3 min, then gradually raised to  $250\text{ }^\circ\text{C}$  at the rate of  $3\text{ }^\circ\text{C}/\text{min}$ . Helium

(HiQ Helium 5.0 zero, purity 99.999% MIN) which acts as the carrier gas is applied at a flowrate of 1.0 mL/min. The essential oil was diluted 50 times in ethanol and filtered. 2 $\mu$ L was injected.

### 3.3.3 Antimicrobial testing

Disc diffusion method was carried out to determine the antimicrobial properties of the garlic essential oil according to the method reported by Ghori (2012). All medium and apparatus were sterilized at 121 °C for 15 min. Müller Hinton agar was prepared to culture the stock bacteria. The inoculum was then prepared by inoculating 1% of culture (*Escherichia coli* or *Staphylococcus aureus*) into Müller Hinton broth and it was then incubated for 24 h. The culture was then harvested by centrifugation at 4000 rpm for 10 min. Cell pellets are later spread onto pre-prepared Müller Hinton agar. 6mm sterile paper discs that soaked with 10  $\mu$ L of garlic essential oil was placed on the growth medium and the Petri plate is incubated at 30 °C for 24 h. The antimicrobial properties of the garlic essential oil were measured by the zones of microbial growth inhibition.

The minimum inhibitory concentrations (MICs) and minimum bacterial concentration (MBC) of extracted garlic essential oil were examined by using microdilution methods as reported by Luo, Yan *et al.* (2014). All microwells were first loaded with 100  $\mu$ L of Müller Hinton broth and 10  $\mu$ L of the tested culture. 10  $\mu$ L of garlic essential oils with known concentrations and phytochemical compositions that had been extracted from different parameters are placed into the first row (row A) of the microwells in triplicates. Rows 11 and 12 were set as positive (without extract) and negative control (without bacteria and extract). 1/3 microdilution was then performed from row A to row H (Referring to Figure 3.2). The plates were inoculated at 37°C for 18 h. After the incubation, the microplate was removed and scanned using UV-Vis spectrometer (Perkin Elmer Lambda Bio) at 600 nm. The lowest concentration of the extract that was found to inhibit the growth of the microorganism was identified as minimum inhibition concentration (MIC).



- ◑ Positive control: 100% growth of test bacteria
- Negative control: 0% growth of test bacteria

Figure 3.2: The experimental scheme of the modified broth microdilution assay.

### 3.3.4 Stability test

The stability test was carried out in dynamic light scattering method using Malvern Zetasizer Nano series (Malvern Instruments ZS). It was used to determine the size and zeta potential of the sample. Sample will spontaneously acquire charge by a molecule or particle called zeta potential which depends on the surface charge, concentrations and types of ions in the medium. Zeta potential of the sample is the measurement of its velocity while particles are moving due to electrophoresis. The speed of the moving particles is proportionate to the field strength and its zeta potential. In the analysis test, the sample was crushed and soaked in ethanol while the pH was varied between 3 to 9 for the stability testing. The data was collected by the zeta sizer software (v.7.11). Data were expressed as means  $\pm$  standard deviation ( $n = 6$ ). Zeta potential is described by Henry equation as shown in Equation 3.1 ('Zetasizer Nano Series User Manual' 2004). All measurements were carried out in triplicate at 25 °C after 2 min of equilibration. The pH of the solution was adjusted by sodium hydroxide (NaOH) and hydrochloric acid (HCl) in 2 molar. The Henry equation is:

$$u_e = \frac{2\varepsilon' \zeta f(\kappa a)}{3\eta} \quad (3.1)$$

where  $\zeta$  is the zeta potential (mV),  $u_e$  is the electrophoretic mobility ( $\text{m}^2/\text{Vs}$ ),  $\varepsilon'$  is the dielectric constant (F/m),  $f(\kappa a)$  is the Henry's function and  $\eta$  is the solvent viscosity. In this case, zeta potential are made in aqueous media hence  $f(\kappa a)$  in this case is 1.5, and is referred to as the Smoluchowski approximation.

Microwave-assisted extraction techniques (SFME and MAHD) have been carried out to extract garlic essential oil. The extracted garlic essential oil has been further characterized by using FTIR and GC-FID. In order to test the antimicrobial properties of the extract, disc diffusion method and minimum inhibitory concentration (MICs) were conducted. Lastly, the stability of garlic essential oil with different pH value was tested. Note that the methods used to achieve objective 1 and 2 have been concisely described in this Chapter 3.

## Chapter 4

### RESULTS AND DISCUSSIONS

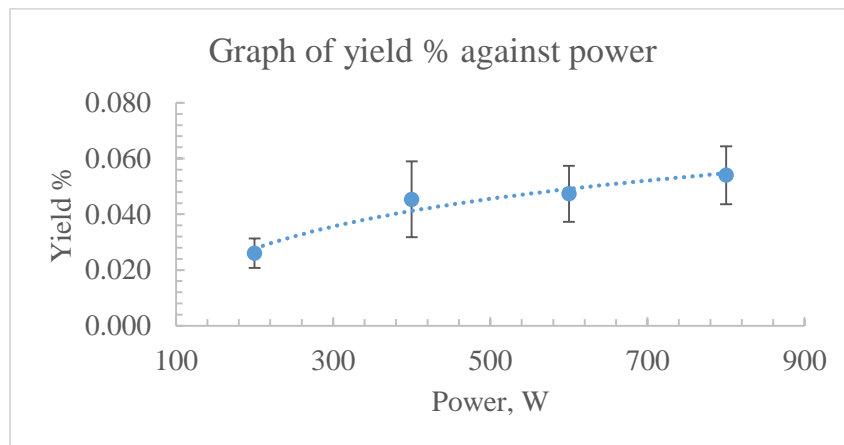
#### 4.1 Microwave-assisted extraction of garlic essential oil

The study of garlic essential oil yield from the microwave-assisted extraction is obtained based on various operating conditions: different microwave powers, solvent composition, solvent-to-feed ratios, extraction temperature, and extraction times. The results of the extraction are reported in the following section.

##### 4.1.1 Solvent Free Microwave Extraction

###### 4.1.1a Effects of Microwave power

Figure 4.1 illustrates the influence of microwave power on the extraction yield. It is found that the extraction yield increases with increasing microwave power. However, as described in Section 2.5.3, the microwave power and temperature are mutually dependent where high microwave power results in higher rate of temperature rise. Higher rate of temperature rise leads to higher extraction yield but may degrade the extractive quality (Mandal, Mohan *et al.* 2007). Consequently, a set of low or moderate microwave power is preferable.



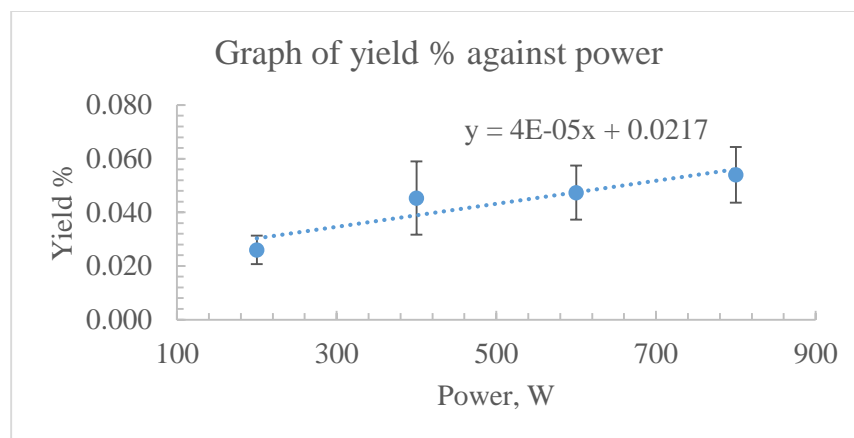


Figure 4.1: Effect of microwave power on the solvent free microwave-assisted extraction (extraction duration of 60 min and feedstock mass of 500 g).

It was reported by Machado, Hasson *et al.* (1999) that the extraction yield is highly dependent on the amount of heat and energy transfer to the plant materials by ionic conduction and dipole rotation. In other words, more electromagnetic energy will induce the extraction efficiency when the microwave power is increased from 200 W to 800 W. Besides that, the increase of temperature indirectly increases the extraction ability of the solvent as the viscosity and surface tension of extracting solvent are reduced (Machado, Hasson *et al.* 1999). Less solvent viscosity provides lower mass transfer resistance and a reduced surface tension will allow solvent cavities to form more easily. Hence, both viscosity and reduction of surface tension of solvent will lead to better contact of extract and solvent, thus enhancing the extraction rate (Richter, Jones *et al.* 1996). Regardless of the degradation, a high temperature of the extraction indeed increases the ability of the solvent to solubilize solutes, boost plant material wetting, and hence enhances the solvent penetration power (Mandal, Mohan *et al.* 2007). Therefore, the yield of the extract increases proportionately with the increase of microwave power as shown in Figure 4.1. In Figure 4.1, the data has used to fit into the first order reaction to obtain the rate constant,  $4 \times 10^{-5} s^{-1}$ . The value of the rate constant from the experiments can be used in the model for realistic modeling.

Microwave irradiation provides localized internal superheating in the plant material to increase extraction rate. Theoretically, a high microwave power enhances extraction yield in a shorter extraction time as it increases the extraction kinetic. However, Mandal, Mohan *et al.* (2007) reported that the use of high extraction temperature or a high microwave power can result in the

rapid breakdown of cell walls leading to release of impurities which lowers the quality of the extractant. In addition, high microwave power may lead to serious degradation of thermally sensitive compounds (Kormin, Ahmed *et al.* 2010). On the other hand, low microwave power shall result in slower temperature rise and gradual breakdown of the cell walls which is beneficial to solvent selectivity. However, Kimbaris, Siatis *et al.* (2006) discovered that conventional extraction method requires 4 times longer extraction time than microwave-assisted extraction for a similar extraction yield. Hence, the selection of a suitable microwave power for extraction is critical. The highest yield of 0.060 % was obtained after 60 min of extraction when the power input was 800 W. However, 400 W has been selected for further optimization of garlic essential oil shown in Figure 4.2(a) because a higher microwave power has been found to result in a severe thermal degradation of the extract as shown in Figure 4.2(b).



Figure 4.2(a): Garlic essential oil (b) Scorched Garlic essential oil.

#### 4.1.1b Effects of feedstock

One of the most important factors that affect MAE process is the amount of feedstock. A feedstock has various biological and chemical characteristics which are related to the heating potential of the material involved. The feedstock might be heated selectively by the microwave and has different microwave transparency. Moreover, the moisture content of the feedstock might affect the extraction efficiency. Therefore, it is important to study the potential effects of feedstock amount on the microwave-assisted extraction yield (Rynk 1998).

Figure 4.3 shows the effect of feedstock on SFME of garlic essential oil. The effect of feedstock was experimented to investigate the influence of extract at constant environment. It was observed that the yield of extract increased gradually as the amount of 500 g of garlic feedstock increased the optimum yield by 0.05 %. Further increase in the feedstock amount did not show further increase in the extraction yield. A decreasing trend was observed at 600 g of feedstock



loading. This may due to the low heat and mass transfer rates in the presence of a large amount of feedstock.

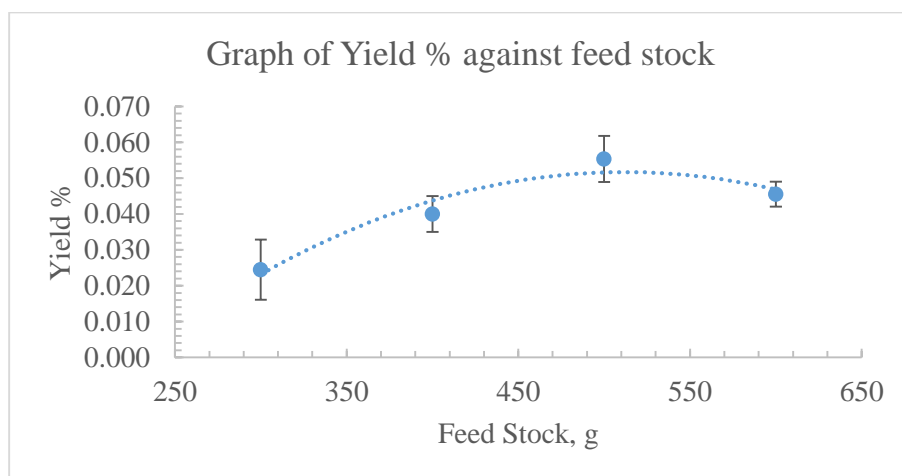


Figure 4.3: Effect of feedstock on SFME of garlic essential oil (conditions: extraction duration of 60 min and microwave power of 400 W).

Besides that, as the feedstock increases, the heat distribution among the molecules is reduced (Kapadiya and Desai). As microwave power is fixed, there is a slower heat distribution when the feedstock increases which in return reduces the extract yield. This study is important in terms of determining an optimum amount of feedstock which should be compatible with the size extraction flask. Moreover, it is necessary to obtain representative sample homogeneity and maximum extract volume with the lowest feedstock amount (Michel, Destandau *et al.* 2011). Based on the experiment, the maximum extraction yield obtained corresponded to a feedstock of 500g (yield = 0.060 %), followed by 600g (yield = 0.046 %), 400g (yield = 0.040%) and 300g (yield = 0.024 %) when the extraction was conducted for 60 min under microwave power of 400 W. However, the larger sample will lead to slow heating and long extraction time compare to small amount. Hence, 500g feedstock has been chosen for further optimization.

#### 4.1.1c Effects of reaction time

Sowbhagya, Purnima *et al.* (2009) reported that a conventional extraction method requires an extraction period that is 3 times as long as that of the microwave-assisted extraction method for a given extraction yield. It is vital to study the optimization of extraction time because the use of different plant parts and type of extraction techniques will affect the extraction time (Mandal,

Mohan *et al.* 2007). Moreover, a prolonged extraction time may lead to undesirable degradation of thermally sensitive compounds contained in the garlic. In many cases, the extraction yield tends to increase as the extraction time is prolonged (Mandal, Mohan *et al.* 2007).

As can be seen in Figure 4.4, the maximum yield of 0.060% was obtained when the extraction process was conducted for 120 min. The extraction yield was gradually increased from 30 min to 60 min, 90 min and finally to 120 min. However, the increment in extraction yield was found to be very small as the extraction period was increased from 60 min to 120 min. As well as, a short extraction time of 30 min gave quite a low yield, i.e., 0.028% as shown in Figure 4.4. Hence, a short extraction time (60 min) is preferable in order to minimize the operational cost and product degradation. Therefore, an extraction period of 60 min has been chosen for further optimization study.

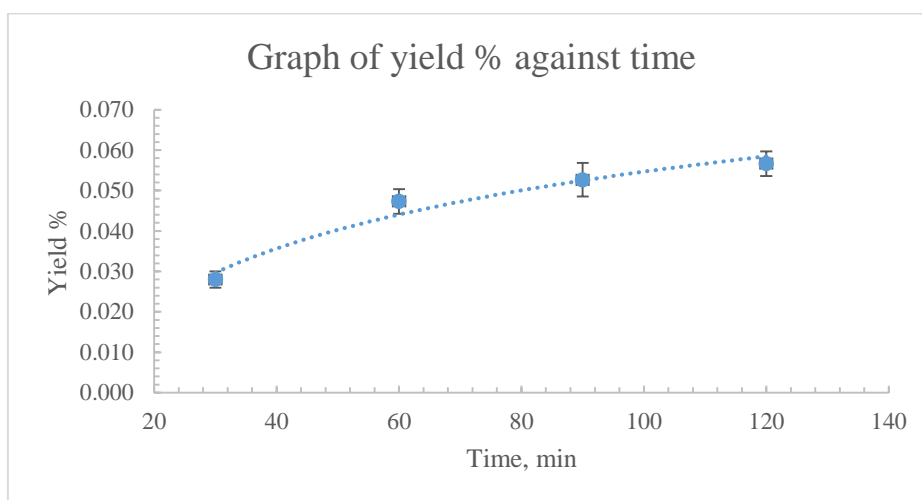


Figure 4.4: Effect of reaction time on SFME of garlic essential oil (conditions: feedstock mass of 500g and microwave power of 400 W).

#### 4.1.2 Microwave-assisted extraction hydrodistillation

##### 4.1.2a Effects of feedstocks

According to Leonelli, Veronesi *et al.* (2012), use of SFME is preferable for extraction of natural plants which are rich in volatile oil. Since the oil yield extracted by SFME is quite low, an enhanced method, i.e., MAHD is introduced. This section aims to study the extraction efficiency between solvent free microwave extraction and microwave-assisted hydrodistillation by optimizing the solvent to feed ratio for the MAE.

Hence, the effect of feedstock loading on the MAE performance with ethanol solvent has been carried out and the results are shown in Figure 4.5. Figure 4.5 shows that the yield increases linearly with the loading amount of feedstock in the MAE process. It shows an increase of yield of 1.8 times (0.05% to 0.09%) for feedstock range from 100 g to 300 g with ethanol-water ratio 1:2. Note that, 0.09% extraction yield was obtained when the extraction was carried out with 300g of garlic feedstock. At a high amount of loading feedstock, the solvent has a higher chance to dissolve the targeted molecules, thus enhancing the extraction yield. However, large amounts of solvent will require more energy and longer time of extraction which leads to feedstock wastage. Furthermore, it is also because of the location of the microwave electron. This is due to the constraints of project which the extraction flask cannot be rotated during the extraction process. Hence, more volume of feedstock involve will easily cause electron hardly penetrate through the feedstock. However, (Yang, Han *et al.* 2012) has obtained higher yield of garlic oil yield (0.478%) by microwave-assisted process. This maybe due to the extraction process underwent periodical microwave processing of 90 s in a microwave oven of 70W for 1.5 h. Besides that, a plastic film was also used to cover the beaker to prevent evaporation loss of garlic oil yield. Owing to different extraction approached, different amount of garlic oil yield was obtained.

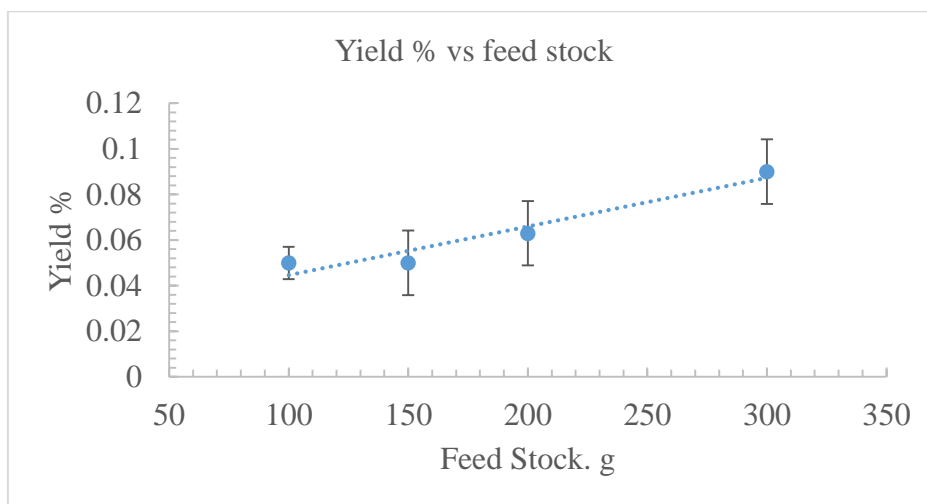


Figure 4.5: Effect of feedstock on MAE (Conditions: time: 60 min, Microwave Power: 400 W, Solvent: Ethanol; Solvent to water ratio: 1:2 (m/V)).

The volume of solvent should be studied because an adequate volume of solvent could act as a barrier to prevent overheating which can lead to charring (Kapadiya and Desai). Adjusting the solvent to water ratio is preferred: Kimbaris, Siatis *et al.* (2006) showed that the entire feedstock

material must be completely covered by the solvent. In order to enhance the extraction process, the feedstock needs to be fully immersed in the solvent. Besides that, with the aid of co-solvent, the extraction process requires a lower amount of feedstock to produce maximum yield (0.060%) compared to solvent free microwave extraction. Therefore, from the economical viewpoint, 100 g of feedstock with the extraction yield of 0.059% has been selected for further optimization.

#### 4.1.2b Selection of solvent

Solvent selection is an important factor in microwave process. Different solvent interacts differently with the microwave depending on the solvent polarity, solubility with respect to a given compound, solvent penetration, molecular interaction between the solvent and a compound, dielectric constant and mass transfer within the process (Chen, Song *et al.* 2008) (Spigno and De Faveri 2009). From Table 4.1, it shows that solvent molecules with lower value of relative polarity have lower chances to dissolve with polar solvents. It is also important that the selected solvent for extraction has a high selectivity towards the targeted components. As the electromagnetic waves are generated during microwave heating, the dielectric properties of a given solvent should be considered as mentioned in Chapter 3 (section 3.1.1). A solvent with higher dielectric constant and dielectric loss has a higher capacity to absorb microwave energy and hence promoting extraction process (Vasu, Palaniyappan *et al.* 2010). Moreover, the selected solvent for extraction should also interact well with microwave radiation as to allow substantial temperature rise in the medium, in which the extraction is taking place (Leonelli, Veronesi *et al.* 2012).

In this work, ethanol ( $\tan \delta = 0.941$ ), water ( $\tan \delta = 0.123$ ) and ethyl acetate ( $\tan \delta = 0.059$ ) were screened for their suitability as co-solvents for extraction based on the procedure in Kimbaris, Siatis *et al.* (2006). Based on Figure 4.6, the extraction with ethanol as solvent gave the lowest yield. The microwave absorbing properties (high  $\tan \delta$ ) of the polar solvent which leads to a rapid and excessive heating but at the same time it has a low power of penetration depth. Generally, high dielectric properties of the solvent lead to high extraction temperature hence pose low values of penetration depth (Wang and Weller 2006). Hence, microwave energy may only break the outer cell wall without breaking the entire cell structure (Veggi, Martinez *et al.* 2012). From the results, it has showed that the microwave has significant effect on the dielectric constant of the extraction solvent.

Table 4.1: Solubility and relative polarity of solvents (Murov 2010)

Solvent	Solubility in H <sub>2</sub> O	Relative polarity
Water	M	1.000
Ethanol	M	0.654
Ethyl Acetate	8.7	0.228

M: Miscible  
 The value for relative polarity are normalized from measurements of solvent shifts of absorption spectra

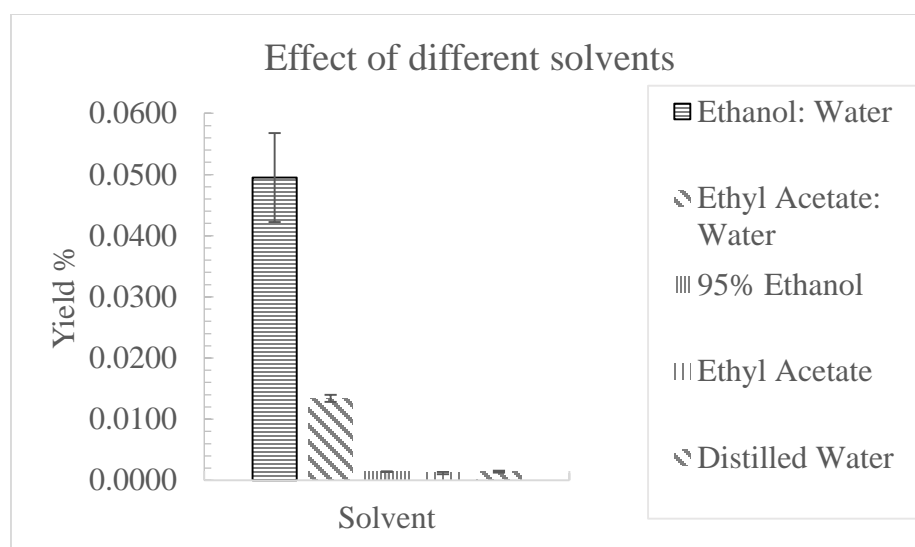


Figure 4.6: Effects of different solvents on MAE.

Note that, a low yield is expected to be obtained when distilled water and ethyl acetate (EA) are used. In a case of high water proportion in a mixture of solvents, a low extraction yield is expected. This could be due to the lack of polarity of the mixture that does not favor the extraction process (Leonelli, Veronesi *et al.* 2012). Another explanation is due to the lack of solubility of the target components in the solvent, hence leading to a low extraction yield. Poor extraction efficiency was also found when using EA as a solvent because of its weak polarity, i.e., it is almost a microwave transparent solvent as it has a very low loss  $\tan \delta$ . Hence, this is one of the factors leading to its poor extraction efficiency in this study (Kappe, Stadler *et al.* 2012).

From Figure 4.6, it can be seen that water-ethanol mixtures give the highest yield (non-optimized) yield of up to 0.050 % at 400 W of microwave power input within an extraction period

of 30 min, with ethanol to water ratio of 1:1.5, total liquid volume of 125 mL and loading feedstock of 100 g. Both water and ethanol are polar molecules due to the OH group. Note that oxygen is partially negative charged while hydrogen is partially positive charged, which lead to polar molecules. When two polar groups meet, this will result in the formation of hydrogen bonding as shown in Figure 4.7. It is because the OH group causes differences in electronegativity (dipoles  $\delta +$  and  $\delta -$ ) (Hydrogen Bonding 2018). However, Farías-Campomanes, Horita *et al.* (2014) have mentioned that allicin is a low polarity molecule which is usually extracted by using polar solvents, such as water or ethanol. Hence, allicin and polar solvents are believed to form hydrogen bond between the hydrogen and oxygen atoms which indirectly releases essential oil from the plant material. This is because water/ethanol mixture creates more bonding site for partially negatives charges for allicin to form a hydrogen bond with the partially positive charges from water and ethanol. Hence, better extraction yield and composition quality of the garlic essential oil can be obtained. In addition, use of fresh batches of solvent is preferable to increase the mass transfer and extraction kinetics which is caused by the saturation of solvent with solute (Routray and Orsat 2012). Indeed, current studies have shown that certain amount of water (60 %) in the solvent could improve the cell diffusion which indirectly leads to better or even heating that promotes higher mass transfer rate.

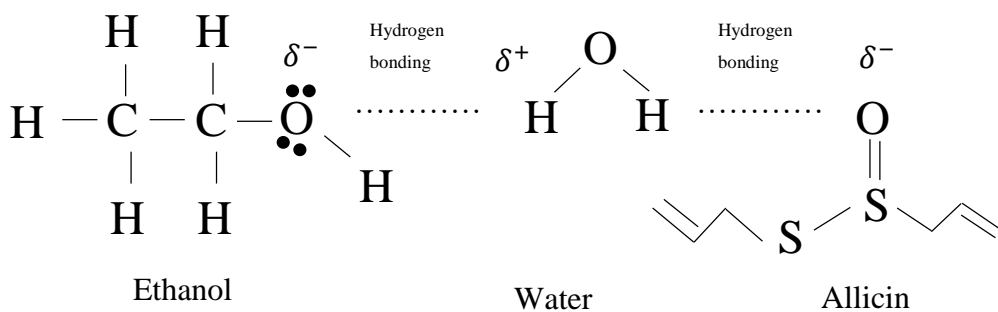


Figure 4.7: Hydrogen bonding between Ethanol, water, and allicin.

#### 4.1.2c Effect of water to ethanol ratio

Figure 4.8 shows the yield versus ethanol to water ratio where the ratio of 1: 1: 1.5 gives a maximum yield of 0.070%.

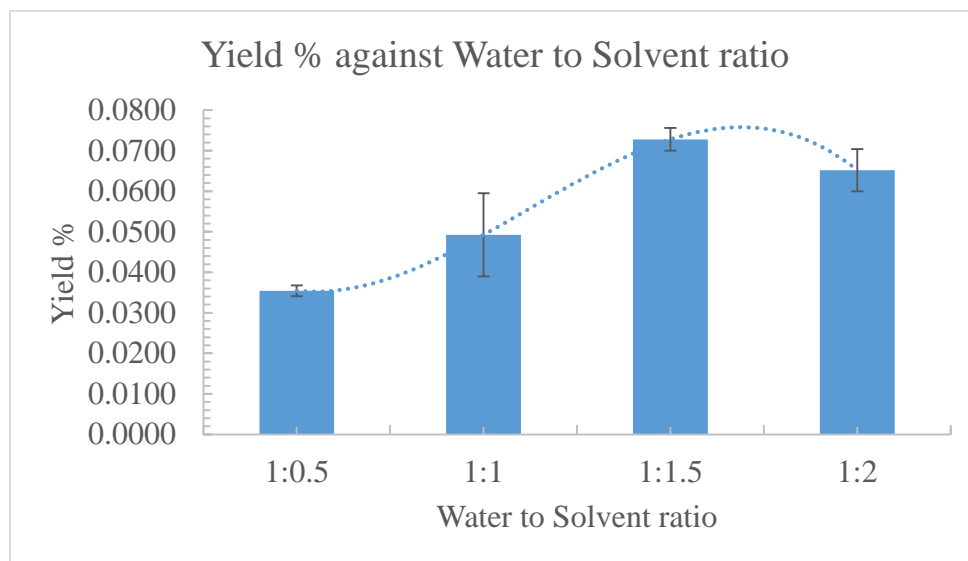


Figure 4.8: Effect of water to ethanol ratio on MAE (Conditions: time: 30 min, Microwave Power: 400 W, Feedstock: 100 g)

Among different solvents: ethanol, water, ethyl acetate, and water/ethyl acetate mixtures, water/ethanol mixture was shown to be the most suitable solvent mixture for optimizing the yield of bioactive constituents' extraction in garlic. It is known that ethanol and water have high dielectric properties which promote the heating rate in the microwave. According to (Routray and Orsat 2012), the solvent combination is favored for prolonged process, the dilution of water boost the heating capacity to prevent degradation of the target compounds. In order to optimize the extract yield, different solvent mixture ratios have been carried out namely (v:v): 1:0.5, 1: 1, 1:1.5, and 1:2 (Farías-Campomanes, Horita *et al.* 2014). Water to solvent ration 1:1.5 gave the highest yield (0.0728 %) followed by 1:2 (0.0652 %), 1:1 (0.0493 %) and 1:0.5 (0.0355 %). Ethanol and water are both polar solvents which assist in microwave absorption (Dent, Dragovic-Uzelac *et al.* 2013). The solvent properties can be varied by mixing different solute in order to enhance the capability of solvent selectivity for different compounds. This is due to the strong attractive forces between solute and solvent which enable overcome the attractive forces holding solute together (Chaplin 2018a). From the experiments, it is observed that the addition of water to solvent



improves the extraction efficiency. It provides a better heating effect and a higher diffusion rate of the transportation of water and oil molecules from the plant cells. As the amount of water increased from 25 mL, 50 mL, 75 mL, and 100 mL, at 100 mL, the yield initially increased but started to drop when the amount of water is more than 100 mL. However high molar fractions of water showed less spontaneity of the solution (Dangui, Santos *et al.* 2018). This suggests that a high water content might lead to hydrolysis effect resulting in the reduction of extraction yield because the high polarity can create an unfavorable effect on the extraction environment (Kappe, Stadler *et al.* 2012). Hydrolysis effect is the separation of water molecules into oxygen and hydrogen atom by electricity which will lead to high polarity solvent. Furthermore, a high amount of water will increase the amount of heat required to boil the water which leads to incomplete extraction process for a given time period.

#### **4.2 Optimization of garlic essential oil by MAHD**

Figure 4.9 shows the graph corresponding to various feedstock (50 g – 200 g) under different microwave power (200 W – 800 W). At 50 g, the maximum yield (0.06%) was obtained at 400 W for 25 min followed by 600 W for 10 min (0.033%), 800 W for 10 min (0.032 %) and 200 W for 60 min (0.026%). At 100 g, the maximum yield was produced at 400 W for 35 min (0.07%), subsequently at 600 W for 15 min (0.052 %), 800 W for 10 min (0.05 %) and 200 W for 135 min (0.043 %). At 150 g, the maximum yield percentage was attained when the extraction process was carried out at 400 W for 60 min, 600 W for 30 min (0.05 %), 800 W for 30 min (0.049 %) and 200 W for 200 min (0.03 %). At 200 g, the maximum yield (0.061 %) was obtained at 600 W for 35 min followed by 400 W for 70 min (0.051 %), 800 W for 30 min (0.04 %) and 200 W for 300 min (0.036 %).

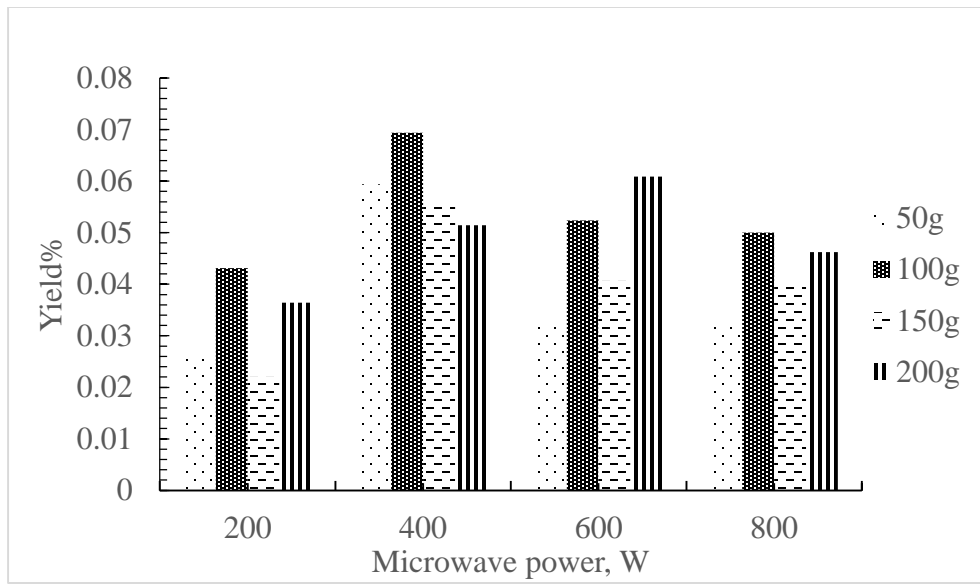


Figure 4.9: Comparison of yield with various feedstock and microwave power.

### 4.3 Effect of microwave power with respect to time and various feedstock amount

From Figure 4.10, the extraction time decreases with the rise of microwave power. The experiment was carried out with maximum time that can be taken by the garlic to extract its essential oil before the plant material experienced charring. From the graph, the maximum extraction time to extract 50 g of garlic is 60 min under 200 W followed by 400 W for 25 min and 600 W and 800 W for 10 min. Furthermore, the graph also showed the maximum extraction time to extract 100 g, 150 g, and 200 g of garlic. The optimum extraction time to extract 100 g of garlic is 135 min for 200 W, 35 min for 400 W, 15 min for 600 W and 10 min for 800 W. Moreover, the maximum time to extract 150 g of garlic is 200 min for 200 W, 60 min for 400 W, 30 min for 600 W and 30 min for 800 W. Likewise, 200 g of garlic takes 300 min for 200 W, 70 min for 400 W, 35 min for 600 W and 30 min for 800 W to obtain the maximum extraction time.

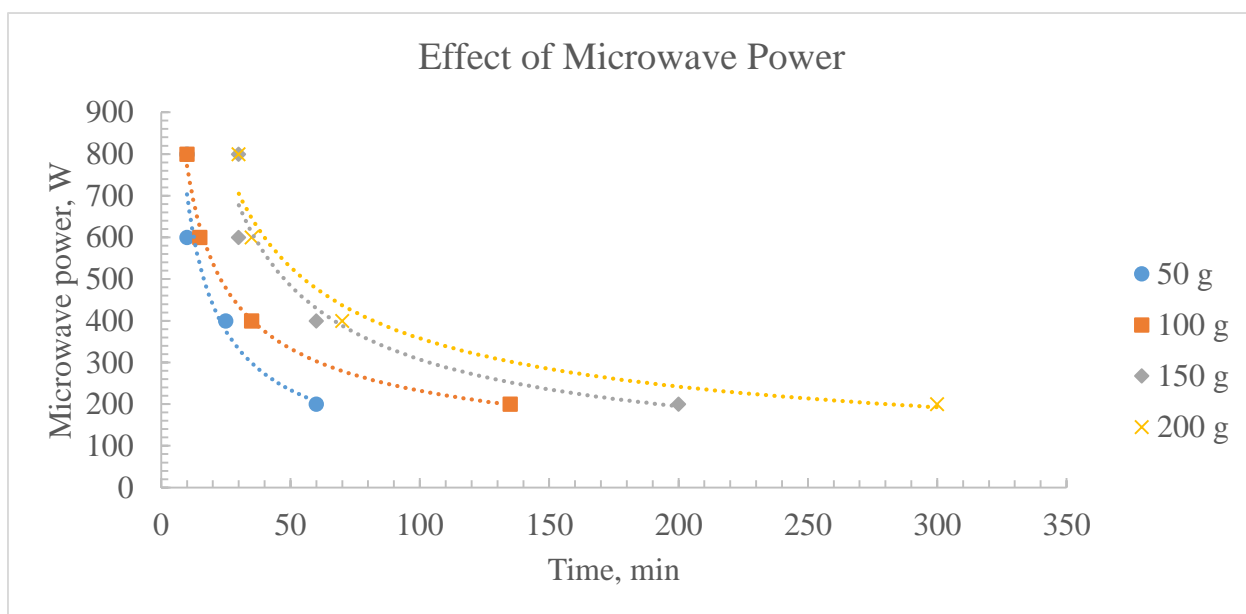


Figure 4.10: Optimum extraction time at each microwave power.

Notwithstanding that microwave-assisted extraction technology possesses superior performance, this technology has been so far only widely developed in lab scale. This might be due to the difficulty to scale up and it is hard to find out the optimum operation conditions with different plants and parts (Chan, Yeoh *et al.* 2016). Figure 4.10 was carried out by using different powers with various amount of feedstock to investigate its optimum extraction time. Through this method, the maximum extraction time of different feedstock to extract garlic essential oil can be

found. However, prolonged time will lead to charring of the garlic. Although higher microwave power required shorter extraction time, the sudden temperature rise in the plant cells can lead to degradation and loss of the target molecules (Mandal, Mohan *et al.* 2007). This might result in a low extraction oil yield. From Figure 4.10, it is showed that 200 W required long extraction up to few hours to extract about the same yield as the moderate or high microwave power which is not environmentally friendly and economically practically. Also, the graph shows that the extraction time increases with the amount of feedstock from 50 g to 200 g. As the feedstock increased, the solvent required longer time to react with the target compounds in the reaction (distillation) flask.

#### 4.4 Comparison plots of yields between SFME and MAHD

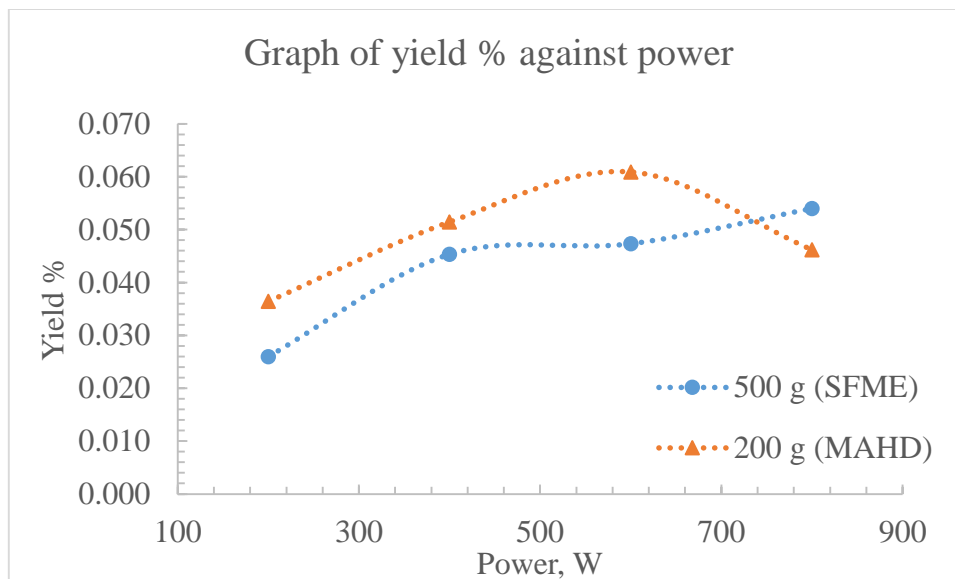


Figure 4.11: Comparing feedstock between SFME and MAHD at 400W (SFME Conditions: (time: 60 min and feedstock mass of 500 g; MAHD Conditions: time: 70 min, Microwave Solvent: Ethanol; Solvent to water ratio: 1:1.5 (m/V)).

Figure 4.11 showed SFME and MAHD are compared at 500g and 200g. From the graph, the yield % increased gradually with the microwave power from 200 W to 600 W and decreased at 800 W. At 800 W, the charring occurred easily due to sudden high temperature exert to feedstock. Hence, resulted in low yield %. On the other hand, MAHD showed yield % increased with the microwave power from 200 W to 800W. This might due to the aid of solvent during the extraction process. Hence, it can be concluded that solvent helps to improve the extraction of target compounds with higher microwave absorbing potential (loss tangent value).

#### 4.5 Kinetic temperature profile

According to Chen, Xie *et al.* (2007), extraction process at elevated temperature and pressure will enhance the extraction rate due to the desorption of target compounds from active sites. Furthermore, at higher temperature, extraction solvent has higher capability to dissolve the target compounds. This is because the increase in temperature decreases the surface tension and solvent viscosity which indirectly enhances plant matrix penetration and wetting. Hence, temperature is one of the influential parameters in microwave heating. The microwave allows short

extraction time by direct heating on the plant matrix. This aids in minimizing the temperature gradient and hence speed up the extraction rate (Spar Eskilsson and Björklund 2000).

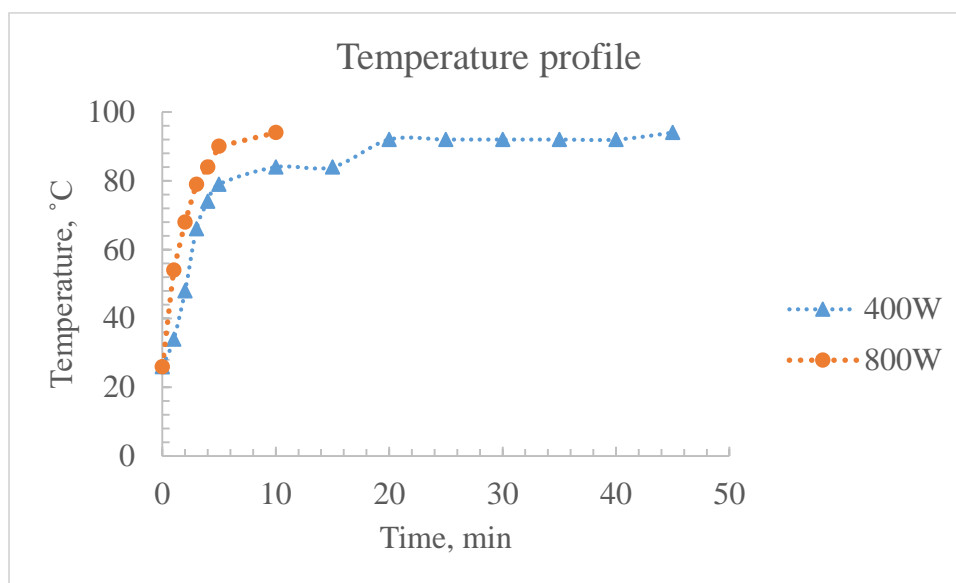


Figure 4.12: The temperature profile for feedstock 100 g under microwave power 400 W and 800 W (Solvent to water ratio: 1:2 (m/V)).

Figure 4.12 shows the temperature profile of MAHD extraction for 400 W and 800 W. In this experiment, both microwave power was observed and compared with two parts. The first part shows the fast temperature increasing until 92 °C at which the process simultaneously underwent distillation of the first drop of essential oil. In the second part, the temperature became constant which was nearly at the boiling point of water around 92 °C and 94 °C in the ethanol-water solvent at atmospheric pressure until the end of the experiment. From *Figure 4.12*, MAHD can raise the temperature quickly to the desired temperature where it can be easily obtained using 800 W within 10 min and 20 min for 400 W with the ethanol-water solvent. The rapid increase of temperature promotes the extraction rate using microwave. Hence, it can be inferred that mass and heat transfers under microwave are synergistic combined which acts in the same direction, from inside to outside, as explained in section 2.8.1 and 2.8.2. This could also explain the long extraction time of conventional extraction method where mass transfer occurred from inside to outside whilst the heat transfer occurred from the outside to inside. The same direction of mass and heat transfer in microwave facilitated the diffusion of essential oil by a rapid increase of temperature. Figure 4.12 proves that microwave power and temperature are interrelated where higher microwave power

leads to higher temperature rate of increase until reaching a plateau at about 92 - 94 °C. Generally, the extraction efficiency increases with the elevated temperature. However, this does not confirm to thermosensitive compounds because high temperature will easily lead to solute degradation. At a microwave power of 800 W, the temperature increases rapidly and leads to a shorter extraction time compared to 400 W. However, the instant rise of temperature expands the cell walls, followed by subsequent rapid rupture of the walls, thus resulting in unfavorable extraction environment for the target compounds. The reduced purity of the extract is shown in Table 4.2. Based on the set of experiment, although 800 W gave the shortest extraction time, but the yield obtained was lower with the value 0.05 % compared to 400 W with the value of 0.07 % with 100 g feedstock. In addition, plant matrix under high microwave power will easily lead to product degradation under a prolonged period of microwave exposure.

#### 4.6 Volume profile

Figure 4.13 shows the liquid volume profile in the distillation flask. The profiles were obtained under various amounts of feedstock at 400 W, respectively: 100 g, 125 g, 150 g, 175 g, and 200 g. The feedstock to solvent to water ratio used in this experiment was 2:1:2. In the experiment, the initial volume of the solvent increased as the feedstock amount increased. This is because the feedstock has to be entirely immersed in the solvent in order to enhance the extraction efficiency.

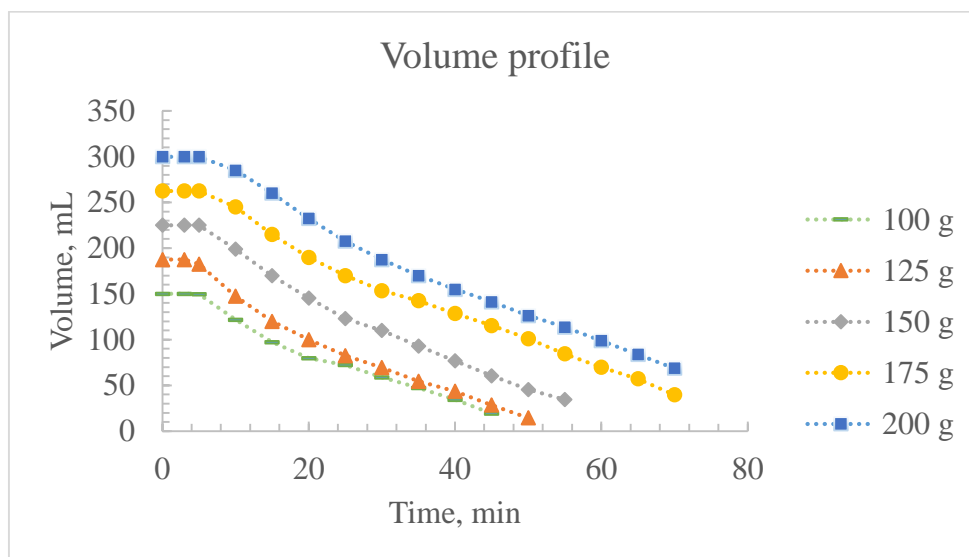


Figure 4.13: The volume profile for feedstock 100 g, 125 g, 150 g, 175 g and 200 g (Microwave power: 400 W; Solvent to water ratio: 1:2 (m/V)).

In the first 5 min, the microwave required time to heat up the ethanol-water solvent. In the second part of the heating process, the solvent started to boil and evaporated into the receiving flask which can be observed to occur substantially when the ethanol-water solvent started to boil at around 74°C. From the Figure 4.13, the volume of solvent in the extractor decreased linearly with the increase of time after 5 min.



## 4.7 Characterization

The result of GC-FID is shown in the APPENDIX.

### 4.5.1 Functional group analysis of garlic essential oil

According to (Motteshard), volatile oil contents in garlic are normally 0.1-0.36% which are mostly responsible for the pharmaceutical properties in garlic. Garlic has been reported to contain roughly about 33 organosulfur compounds such as alliin, allicin, ajoene, allyl propyl disulfide, diallyl trisulfide, sallylcysteine, vinyl dithiins, S-allylmercaptocystein, enzymes (allinase, peroxidases, myrosinase, and others), 17 amino acids (arginine and others), and minerals (selenium, germanium, tellurium and other trace minerals) (Omar and Al-Wabel 2010). Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to identify the presence of the organosulfur compounds. Previously, Jirovetz, Jäger *et al.* (1992) studied and identified the Egyptian garlic essential oil by GC-FTIR which showed the presence of only eight organosulfur compounds and 26 minor components. According to Jirovetz, Jäger *et al.* (1992), diallyl trisulphide is the main volatile compound present in the Egyptian garlic essential oil followed by diallyl tetrasulfide, diallyl disulfide, diallyl sulfide and methyl allyl trisulphide as the minor components. In a different study, Rastogi and Arunachalam (2011) characterized the compounds present in an aqueous garlic extract by using FTIR where the main peaks found are similar to the results obtained in the current study. According to the peaks that had been discovered by (Rastogi and Arunachalam 2011), functional groups that exist in the present extract are O-H stretching of hydroxyl group (3323), asymmetric stretching of C-H bonds (2930), -C=O stretch (1638), -O-H bend of carboxylates (1400), SO<sub>2</sub> absorption of sulfones (1129), C-N stretching vibrations of primary amines (1027),  $\gamma$ -C-H (934), N-H bend of primary amines (819), and C-H bend of alkynes (603).

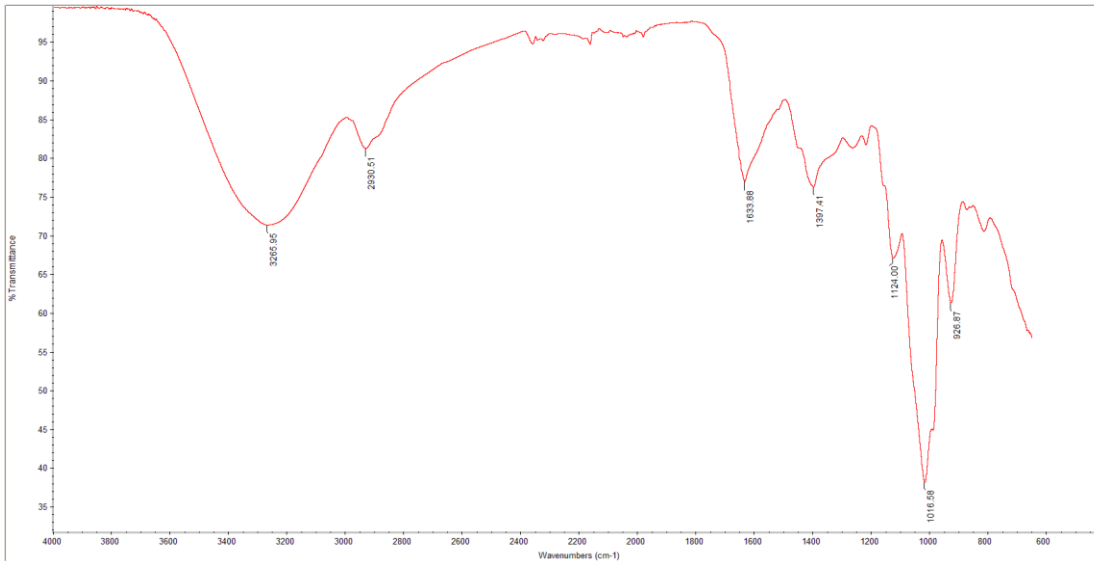


Figure 4.14: Analysis of garlic essential oil by FTIR.

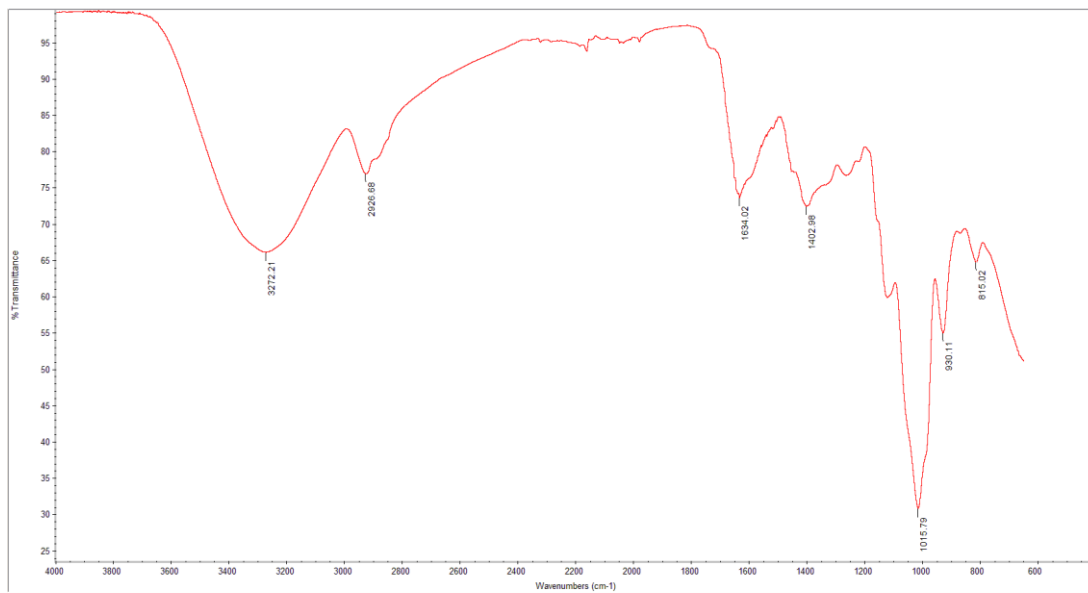


Figure 4.15: Analysis of garlic by FTIR.

Table 4.2: Compounds detected by FTIR.

Wavenumbers (cm <sup>-1</sup> )	Functional Groups	Compounds
2930.51	Alkanes	Allyl propyl disulfide
1633.88	Amides	Sallylcysteine,
1124.00	C=S stretch	Vinyl dithiin
1016.58	S=O stretch	Aliin Thiosulfinate Ajoene
926.87	Alkenes	diallyl trisulfide, diallyl disulfide, Methyl allyl trisulfide Allyl methyl Allyl Mercaptan

An analysis of garlic essential oil has been carried out by using FTIR as shown in Figure 4.14 where the presence of functional groups is tabulated in Table 4.1. The organosulfur compounds which have been reported to possess medicinal properties are confirmed to be present in the extract of garlic essential oil as shown in Figure 4.14, which is in agreement with the literature review (Jirovetz, Jäger *et al.* 1992). The compounds with double bonds are all categorized under alkenes groups such as diallyl trisulfide, diallyl disulfide, methyl allyl trisulfide, and allyl methyl. Figure 4.16 shows the structure of diallyl trisulfide with a C double bond which is recognized to be under alkenes functional group. Hence, the sulfur compounds which possess medicinal properties are confirmed to be present in the extracted garlic essential oil due to the presence of alkanes, alkenes, S=O stretch, C=S stretch, and amides functional group which is supported by the literature review (Rastogi and Arunachalam 2011).

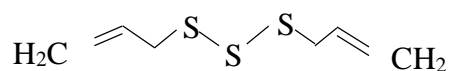


Figure 4.16: Structure of Diallyl Trisulfide.

#### 4.5.2 Assessment of the antimicrobial activity of garlic essential oil

##### 4.5.2a Disc diffusion method

Optimal garlic essential oil conditions and their antimicrobial activity was evaluated using disc diffusion method. According to Seydim and Sarikus (2006), garlic essential oil exhibits inhibitory zones on *S. aureus*, *S. enteritidis*, *L. monocytogenes*, *E.coli* and *L. plantarum*. Results in Table 4.3 clearly shows that garlic essential oil exhibits antimicrobial activity against gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria.

Table 4.3: Antimicrobial activity of garlic compound standards against *E. coli* and *S. aureus*.

Bacteria	Control	Standard (DAD & DAT)
Inhibition Zone (cm)		
<i>E. coli</i>	NIL	1.1
<i>S. aureus</i>	NIL	1.2

NIL: No antimicrobial activity

Table 4.4: Antimicrobial activity of different parameters of garlic essential oil against *S. aureus* and *E. coli*.

		Garlic Essential oil				
		Control	200 W	400 W	600 W	800 W
Inhibition Zone (cm) $\pm$ SD						
<i>S. aureus</i>	NIL	1.3 $\pm$ 0.14	1.5 $\pm$ 0.14	1.3 $\pm$ 0.14	1.2 $\pm$ 0.071	
<i>E. coli</i>	NIL	1.2 $\pm$ 0.071	1.35 $\pm$ 0.071	1.4 $\pm$ 0.14	1.2 $\pm$ 0.071	

NIL: No antimicrobial activity

From Table 4.4, 400 W and 600 W gave the highest antimicrobial activity as judged from the diameter of inhibition zone. It is suggested that the antimicrobial properties of garlic essential oil are due to its content of different sulfides. The investigation indicated that the numbers of disulfide bonds (S – S) in the garlic essential oil are responsible for the antimicrobial activity (El-Sayed, Chizzola *et al.* 2017).

Figure 4.17 shows the disc diffusion method which was used to test the antibacterial properties of the extracted garlic essential oil. The inhibition zone was measured and tabulated as in Table 4.3 and Table 4.4.

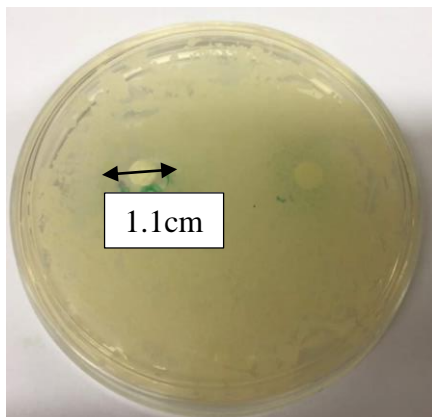


Figure 4.17: Antimicrobial properties by disc diffusion method.

#### 4.5.2b Minimum inhibitory concentration (MICs) and Minimum bacterial concentration (MBC)

The potential use of essential oils as natural antimicrobial agents is less exploited compared to their usage as flavoring and as antioxidant products. Minimum inhibitory concentration (MICs) is defined as the lowest concentration of an antimicrobial that will inhibit the growth of microorganisms (Andrews 2001) whereas MBC is the minimum concentration of antibacterial agent that is required to kill the bacteria. The antibacterial activity of the extracted garlic essential oil against *S. aureus* and *E. coli* are confirmed by this method. A lower MIC value indicated the effective concentration of essential oil that inhibits the visible growth of bacteria after overnight incubation.

From the table above, garlic essential oil has shown antibacterial properties against *E. coli* and *S. aureus*. Table 4.5 and Table 4.6 present the minimum inhibitory concentration (MIC) of *E. coli* and *S. Aureus* on 96 well plate. Indeed, at lower concentrations of garlic essential oil, the media became turbid due to the growth of bacteria. The tables showed that different microwave power results in different concentration of garlic essential oil.

From Table 4.5, the minimum inhibitory concentration of garlic essential oil against *E. coli* was 0.0095 g/mL. Under microwave power, 600 W and 400 W with a concentration of 0.0114 g/mL and 0.00951 g/mL showed better inhibition of *E. coli*, followed by 800 W (0.0130 g/mL) and 200 W (0.00401 g/mL). However, from the MIC value, minimum bacterial concentration (MBC) value can be determined. The minimum concentration of garlic essential oil to kill *E. coli* was at 0.00334 g/mL for 200 W, 0.00792 g/mL for 400 W, 0.00978 g/mL for 600 W and 0.00976

g/mL for 800 W. From the result, 200 W gave the lowest MIC in which this might be due to the degradation of antimicrobial compounds. The matrix was exposed under high temperature for a long time which led to minimum antimicrobial compounds in 200 W.

Moreover, the MIC of 400 W of microwave power was 0.0015 g/mL, followed by 800 W (0.0024 g/mL), 200 W (0.00358 g/mL) and 600 W (0.00378 g/mL). This has indicated that garlic essential oil at 400 W has a minimum concentration of 0.0015 g/mL which was shown by the inhibition of *S. aureus*. The MIC value at 400 W gave the lowest value which might be due to the presence of other organosulfur compounds in the extracted garlic essential oil. In other cases, the concentration of garlic essential oil which gave lesser than 0.0026 g/mL was shown in the MBC value for *S. aureus*.

Table 4.5: MICs and MBC of *E. coli*.

Microwave power (W)	200		400			600			800		Blank	
Concentration (g/mL)	0.2005		0.4757			0.6848			0.3907			
96 well plate	1	2	3	4	5	6	7	8	9	10	11	12
A	0.128	0.226		0.172	0.173		0.290	0.387		0.386	0.267	0.411
B	0.372	0.573		0.315	0.396		0.403	0.961		0.251	0.391	0.488
C	0.450	0.437		0.031	0.373		0.372	0.370		0.272	0.268	0.615
D	0.195	0.27		0.363	0.400		0.205	0.288		0.290	0.366	0.438
E	0.246	0.331		0.399	0.384		0.318	0.309		0.448	0.359	0.648
F	0.263	0.278		0.353	0.315		0.385	0.391		0.540	0.358	0.570
G	0.682	0.754		0.033	0.379		0.193	0.360		0.483	0.491	0.422
H	0.608	0.547		0.627	0.438		0.535	0.516		0.974	0.436	0.549

Table 4.6: MIC and MBC of *S. Aureus*.

Microwave power (W)	200		400			600			800		Blank	
Concentration (g/mL)	0.1076		0.0463			0.0756			0.0477			
96 well plate	1	2	3	4	5	6	7	8	9	10	11	12
A	0.021	0.226		0.203	0.068		0.150	0.079		0.400	0.254	0.441
B	0.142	0.241		0.112	0.304		0.274	0.261		0.120	0.125	0.846
C	0.173	0.143		0.138	0.379		0.285	0.174		0.362	0.696	0.673
D	0.322	0.307		0.191	0.384		0.531	0.584		0.553	1.249	0.438
E	0.765	0.631		0.557	0.804		0.460	1.042		0.569	0.910	0.487
F	0.546	0.610		0.585	0.636		0.911	0.548		0.520	1.263	0.531
G	0.587	0.530		0.620	0.822		0.465	1.051		0.835	0.673	0.854
H	0.754	0.636		0.623	0.676		0.451	0.730		1.124	0.656	0.629

### 4.5.3 Zeta potential

The sample used to test for garlic zeta potential was 100 g garlic with ethanol to water ratio 1:2. Notice in Figure 4.18 that with the increment of pH value, the zeta potential of the particles switched from a positive value to a negative value with the isoelectric points lying between pH 5 and 7. From Figure 4.18, the graph showed the sample was negatively stable under basic pH and positively stable under acidic pH. This might due to the existence of oxygen and hydrogen atoms in the solution (Júnior and Baldo 2014). Figure 4.19 (A) shows the molecule in dispersion state, while (B) shows the particle under normal environment and (C) shows when external force is applied to the particles. It is important for the particles to stay in dispersion state (A) instead of aggregation state so that the targeted molecules could be extracted easily.

The stability of garlic extract is tested over a wide pH range between 3 and 9 by using Malvern Zetasizer Nano series. It helps to measure the magnitude of the electrostatic or charges between particles since this is known to affect stability. Note that small particles tend to have greater stability under a high negative or positive zeta potential value due to more influential electrostatic repulsions that inhibit the aggregation of the particles. On the other hand, when the zeta potential value is close to zero, the repulsion force is smaller than the attractive force which breaks the dispersion hence promoting coagulation and flocculation of particles (Sathishkumar, Sneha *et al.* 2009).

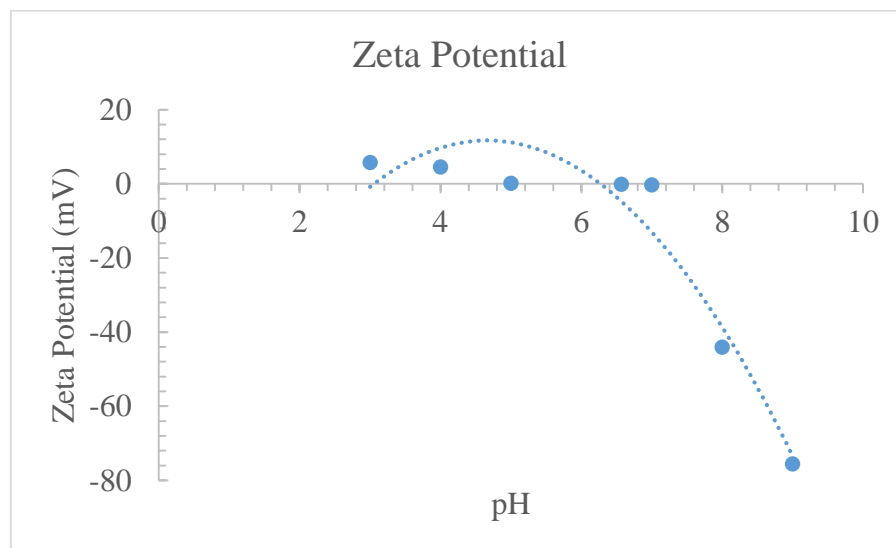


Figure 4.18: Zeta Potential of garlic extract at various pH.



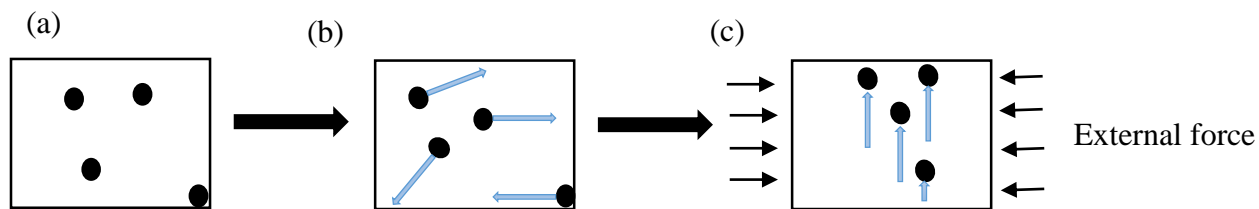


Figure 4.19: Illustration of dispersion states of particles.

At a higher pH values, the colloidal suspension showed good stability. Indeed, this result showed that pH value plays a critical role in controlling the size of the colloidal suspensions. Hence, for garlic essential oil extraction, higher pH (>7) is preferable. Besides that, according to Zeta Potential 1999), the magnitude of zeta potential is necessary to be kept above 25 mV (positive or negative) in order to maintain the dispersion state (B) of molecules. Besides, it has also been studied that smaller particles are easier to be removed from the surrounding particles as shown in Figure 4.19. Furthermore, under high temperature, smaller particles tend to have higher kinetic energy, therefore promoting better chemical reaction as shown in Figure 4.19(C) (Chan, Yeoh *et al.* 2016). Hence, it can be anticipated that when the suspension is in good stability, an increase in the kinetic energy would result in an enhanced extraction rate.

The extraction of garlic essential oil by SFME and MAHD was carried out. Due to the low yield obtained by SFME, MAHD has been introduced. The maximum yield obtained by SFME and MAHD were 0.060 % from 500 g of garlic and 0.059 % from 100 g of garlic respectively. It was shown that, with the smaller amount of feedstock, MAHD produced a comparable oil yields with SFME. However, the efficiency of the MAHD depends in part, on the distribution of microwave irradiation coverage, length of extraction period and microwave power. For a constant microwave power, with a larger feedstock will require a larger volume of liquid, which means that large surface area for receiving the microwave irradiation. Furthermore, with larger volume and feedstock for a given microwave power, the extraction time will tend to be longer. These effects in combination tend to produce larger extraction yield. FTIR was conducted where the detected functional groups are consistent with the literature. Furthermore, zeta potential has been studied, that at higher pH, particles are smaller in size which eases the extraction yield.

#### 4.6 Summary

The summaries of this chapter are as follows:

- The experimental methodology shown in Chapter 3 for the SFME and MAHD has been applied to the extraction of garlic essential oil. The experiments were conducted by One-factor-at-a-time (OFAT) method to study the influencing factors in the microwave-assisted extraction process (SFME and MAHD). The MAHD showed superior performance over the SFME in terms of extraction yield.
- The optimization was carried out by investigating the maximum extraction time at each microwave power before charring occurred to the garlic matrix.
- The profiles of temperature and volume are shown in this chapter and two distinct phases can be observed in the profiles.
- The characterization of the extracted garlic essential oil has been carried out via FTIR, GC-FID, Disc diffusion method, MIC, MBC, and zeta potential. Such characterization is important in order to ensure the quality of the extract by the proposed method. The results showed that the extracted garlic essential oil contains the targeted compounds which provide antimicrobial properties against bacteria: *E. Coli* and *S. Aureus*.
- The stability of the garlic essential oil was also tested through zeta potential. With the increment of the pH value, the zeta potential of the particles changed from positive value to negative value where the isoelectric points were between pH 5 to 7. Hence, higher pH showed better stability in the dispersion phase.

## Chapter 5

### MODELLING METHODOLOGY

#### 5.1 Modelling approach

##### 5.1.1 General modelling description

The proposed fundamental model is given as follows

$$\frac{dX}{dt} = f(X, U, \Theta) \quad (5.1)$$

$$Y_i = h(X) \quad (5.2)$$

where the notations  $X \in R^n$  is the vectors of state variables,  $U \in R^k$  is the vectors of the input variables,  $f \in R^n$  and  $g$  are nonlinear functions of their arguments. Model (5.1) represents the fundamental mass-energy balance equations of the extraction system while (5.2) describes the extracted yield as an implicit function of the state variables. In (5.2), the extracted yield can be predicted by first solving the mass-energy balance system to obtain the values of the state variables involved. However, theoretically, this can only be materialized when the fundamental system, i.e. (5.1), is complemented with a set of reliable constitutive equations. Through extensive simulation and experimental studies, three important constitutive relations are proposed to complement the fundamental model (5.1) so that (5.2) can provide better prediction of the experimental yield. The features of these three empirical relations are summarized as follows:

- i. Thermal effectiveness factor given in Equation 5.21 which describes the microwave power conversion into heat as a function of three interaction terms: (1) microwave interaction with pure water/ethanol molecules, (2) microwave interaction with combined water and ethanol molecules, and (3) microwave interaction with combined water and garlic material.
- ii. Overall mass transfer coefficient given in Equation 5.24, represents the essential oil mass transfer kinetic from plant material to bulk liquid. This coefficient takes into account explicitly the effects of: (1) essential oil's solubility in water-ethanol mixture, (2) liquid temperature, and (3) ethanol mass fraction in the liquid on the overall mass transfer rate.

- iii. Vapour-Liquid Equilibrium (VLE) coefficient of the essential oil represented by Equation 5.31 which is assumed to be a nonlinear function of temperature, ethanol and water mass fractions in the liquid.

#### 5.1.2 Model assumptions

1. Garlic essential oil is assumed as a single pseudo-component.
2. Uniform temperature distribution throughout the vessel - liquid and solid have analogous temperature.
3. Both solid and liquid phases have uniform concentration profiles.
4. At any operating temperature, Vapor-Liquid Equilibrium (VLE) occurs in the vessel.
5. Non-ideal mixing between water and ethanol as well as between garlic and ethanol-water solvent.
6. Stable total pressure around 1.1-1.2 atms in the vessel.
7. Oil mass fraction is small in liquid hence does not drastically influence the regular properties of ethanol-water solvent.
8. Microwave irradiation leads to the delayed break down of the cell walls. Significant mass transfer of essential oil to bulk liquid only occurs after the cell walls are broken.

### 5.1.3 Mass balance equations

In line with the assumptions 1-3, the rate of change of garlic essential oil mass fraction can be written as

$$M_s \left( \frac{dx_s}{dt} \right) = -k_{ex} (x_s - x_l) V_s \quad (5.3)$$

where  $M_s$  is the total mass of garlic [g],  $x_s$  is the solid mass fraction of oil [g/g],  $k_{ex}$  is the overall mass transfer coefficient from solid to liquid [g/mL.min] and  $x_l$  is the bulk liquid mass fraction of oil. However, the  $V_s$  can be calculated by

$$V_s = M_s / \rho_s$$

where  $\rho_s$  is the garlic density [g/mL].

Moreover, in view of Equation 5.3, the rate of change of garlic essential oil mass fraction in the bulk liquid can be expressed by

$$\rho_l \left( \frac{dV_l x_l}{dt} \right) = k_{ex} (x_s - x_l) V_s - F_v y_l \quad (5.4)$$

where  $\rho_l$  is the average liquid density [g/mL],  $F_v$  is the rate of liquid evaporation [g/min] and  $y_l$  is the vapor mass fraction of oil in equilibrium with the mass fraction of oil in the liquid solvent.

Due to assumption 4 which mentions the VLE condition that is applied in the vessel throughout the operating temperature, the VLE relationship between the vapor and liquid mass fractions of oil are represented in terms of K values can therefore be written as

$$y_l = K_1 x_l \quad (5.5)$$

where  $K_1$  is the VLE constant of oil in the ethanol-water solvent.

Owing to the evaporation occurring in the vessel, the rate of change of liquid volume is written as follows

$$\rho_l \left( \frac{dV_l}{dt} \right) = -F_v \quad (5.6)$$

The mass balance for the oil in the liquid can be simplified by substituting Equation 5.5 and 5.6 into Equation 5.4, thus leading to

$$\rho_l V_l \left( \frac{dx_l}{dt} \right) = k_{ex} (x_s - x_l) V_s + x_l F_v (1 - K_l) \quad (5.7)$$

Moreover, the mass balance for water and ethanol in liquid phase due to evaporation can be expressed as

$$\rho_l \left( \frac{dV_l x_w}{dt} \right) = -F_v y_w$$

$$\rho_l \left( \frac{dV_l x_e}{dt} \right) = -F_v y_e$$

where the mass fractions of water and ethanol  $y_w$  and  $y_e$  in the vapor phase are interrelated to  $x_w$  and  $x_e$ , respectively in the liquid phase.

$$y_w = \gamma_w \left( \frac{P_w^s}{P} \right) x_w \quad (5.8)$$

$$y_e = \gamma_e \left( \frac{P_e^s}{P} \right) x_e \quad (5.9)$$

where  $\gamma_i$  is the activity coefficient of the  $i$  component,  $P_i^s$  is the vapor pressure of the  $i$  component [mmHg], and  $P$  is the total pressure built in the vessel; subscripts 'w' and 'e' represent water and ethanol.

Non-Random Two-Liquid (NRTL) model has been used to calculate the activity coefficients of a compound  $i$  with its mole fraction,  $x_i$  in the liquid phase where activity coefficient is used to consider the deviation from ideal liquid behavior in a mixture of chemical substances (Gebreyohannes, Neely *et al.* 2014). In this case, for binary mixture of water and ethanol, the following functions are shown in Appendix Equation (A.1).

From the saturated vapor pressure and activities coefficients, the mass balance for water and ethanol can be developed from Equation 5.8 and 5.9 into

$$\rho_l V_l \left( \frac{dx_w}{dt} \right) = F_v x_w \left[ 1 - \gamma_w \left( \frac{P_w^s}{P} \right) \right] \quad (5.10)$$

$$\rho_l V_l \left( \frac{dx_e}{dt} \right) = F_v x_e \left[ 1 - \gamma_e \left( \frac{P_e^s}{P} \right) \right] \quad (5.11)$$

#### 5.1.4 Thermal energy balance equation

The energy balance of a total mass of liquid and solid can be expressed as

$$\frac{d(\bar{C}_p M_T (T - T_{bp}))}{dt} = \varepsilon Q_{MW} - F_v \lambda_s$$

where  $\bar{C}_p$  is the average specific heat of combined liquid-solid [ $J/g \text{ } ^\circ C$ ],  $T$  is the liquid temperature [ $^\circ C$ ],  $T_{bp}$  is the bubble point of the liquid [ $^\circ C$ ],  $\varepsilon$  is the thermal effectiveness factor of microwave power conversion into heat,  $Q_{MW}$  is the microwave power [ $J/min$ ],  $\lambda_s$  is the average latent heat of vaporization [ $J/g$ ] respectively.

However, the total mass of liquid and solid can be calculated with

$$M_T = \rho_l V_l + M_s \quad (5.12)$$

Through Equation 5.14, energy balance can be expressed as

$$M_T \bar{C}_p \frac{dT}{dt} + (T - T_{bp}) \bar{C}_p \rho_l \frac{dV_l}{dt} = \varepsilon Q_{MW} - F_v \lambda_s \quad (5.13)$$

However, evaporation takes place in the vessel, therefore, the amount of vapor flow is calculated via

$$F_v = \varepsilon \eta Q_{MW} / \lambda_s \quad (5.14)$$

where  $\eta$  is used for liquid evaporation that represents the fraction of microwave power converted into heat. By substituting Equation 5.14 into Equation 5.13, a new equation is formed that leads to

$$M_T \bar{C}_p \frac{dT}{dt} = \varepsilon Q_{MW} \left( 1 - \eta + \frac{\eta \bar{C}_p}{\lambda_s} (T - T_{bp}) \right) \quad (5.15)$$

In terms of the general model (5.2), the corresponding mass and energy balance of MAHD model established in the above has a total of six state variables,  $X = [x_l, x_s, V_l, x_w, x_e, T]^T$ . Matlab *ode45 solver* (fourth-order Runge-Kutta method) is used to solve the ordinary differential equations (ODEs) numerically with time step size of 0.1 min.

#### 5.1.5 Average properties calculations

Recalling back to assumption 7, it is applied for all calculations related to the average properties. The average liquid density of ethanol-water mixture is calculated via

$$\rho_l = x_w \rho_w + x_e \rho_e \quad (5.16)$$

Where  $\rho_w$  is the density of water and  $\rho_e$  is the density of ethanol.

Next, the average latent heat of evaporation is established in Equation 5.17.

$$\lambda_s = x_w \lambda_w + x_e \lambda_e \quad (5.17)$$

Here,  $\lambda_w$  is the latent heat of evaporation of pure water while  $\lambda_e$  is the latent heat of evaporation of pure ethanol.

Furthermore, Equation 5.18 is used as approximation to calculate the average specific heat capacity of the liquid-solid:

$$\bar{C}_p = \frac{(x_w C_{pw} + x_e C_{pe}) \rho_l V_l + M_s C_{ps}}{M_T} \quad (5.18)$$



where  $\bar{C}_{pw}$ ,  $\bar{C}_{pe}$ ,  $\bar{C}_{ps}$  are the specific heat capacities of water, ethanol and garlic respectively [J/g °C].

By adopting the data in (Kramkowski, Kaminski *et al.* 2001), the specific heat capacity of garlic can be expressed as a function of liquid temperature [ °C]:

$$C_{ps} = 1.5101 + 0.036 T - 7 \times 10^{-5} T^2$$

### 5.1.6 Microwave thermal conversion efficiency

The thermal effectiveness factor of microwave power conversion into heat is proposed to be a function of lost tangents, garlic mass and component mass fractions as shown below

$$\varepsilon = \beta_1 \left[ \sqrt{(x_w \tan \delta_w)^2 + (x_e \tan \delta_e)^2} + \beta_2 \left( \frac{x_e \tan \delta_e}{x_w \tan \delta_w} \right) \right] + x_w \tan \delta_w \left( \frac{M_s}{\rho_w V_l} \right)^{\beta_3} \quad (5.19)$$

where  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the equation parameters while  $\tan \delta_w$  and  $\tan \delta_e$  are the loss tangents for water and ethanol respectively. From Equation 5.19, it presumes that the heat conversion from microwave power relies on several factors such as:

1. Root mean square (RMS) of individual microwave conversion into heat by molecules of pure water and ethanol interacting with microwave.
2. Ethanol-water mixed molecules interacting with microwave.
3. Water-solid interactions interacting with microwave.

During the early transient phase in MAHD process, the first and second conversion terms are believed to be dominant while the third term is relatively negligible. Yet, in the end of the MAHD process where all the liquid nearly dries up, the third term is expected to become important. This is because the third term only involves water and garlic interacting with the microwave. At the end of MAHD process, all ethanol should have been evaporated which leaves only water and solid (garlic plant).

In addition, the microwave power converted into thermal energy is partially used to vaporize the liquid below its bubble point temperature during the early MAHD process. Hence, from this

thermal fraction used for vaporization, a power law expression has been assumed in terms of liquid temperature and bubble point of the mixture given as follows:

$$\eta = \left( \frac{T}{T_{bp}} \right)^n, \quad 2 < n < 10 \quad (5.20)$$

In the beginning of the process, the thermal fraction in Equation 5.20 nearly equals to zero. When all thermal energy used for liquid vaporization and liquid temperature reaches its bubble point, the thermal fraction should be equal to 1. In this study, an estimation model is proposed to define power  $n$  by assuming that it is a function of ratio of the total latent heat to the total sensible heat at the beginning of the process. The equation is given as follows:

$$n = \left( \frac{V_{l0} \rho_{l0} \lambda_{s0}}{V_{l0} \rho_{l0} \bar{C}_{p0} + M_s C_{ps0}} \right)^{1/3} \quad (5.21)$$

where  $V_{l0}$  is the initial total liquid volume,  $\rho_{l0}$  is the average liquid density,  $\lambda_{s0}$  is the average latent heat of vaporization,  $\bar{C}_{p0}$  is the average liquid specific heat and  $\bar{C}_{ps0}$  is the garlic specific heat and all are determined at the condition at  $t = 0$ .

### 5.1.7 Estimation of overall mass transfer coefficient

The overall mass transfer can be further developed into the following Arrhenius-like expression

$$k_{ex} = k_1 f_d \left[ 1 + a_1 \alpha_{e/w} \left( \frac{x_e}{x_w} \right) \right] \left( \frac{S_o - \rho_l x_l}{S_o} \right) e^{-k_2/(T+273.15)} \quad (5.22)$$

where  $\alpha_{e/w}$  is the relative solubility of garlic oil in ethanol to its solubility in water,  $S_o$  is the average solubility of oil in a given ethanol-water mixture [g/mL],  $k_1$  and  $k_2$  are the kinetic parameters to be found with the unit  $\left[ \frac{g}{mLmin} \right]$ , and [K], respectively, and  $a_1$  is the constant describing that the given solvent can either increase or decrease the kinetics. In order to take into account the delay in the cell rupture which is described under the assumption 8, the delay function  $f_d$  is incorporated into Equation 5.22 and is shown as follows:

$$f_d = \left( \frac{T_{crit}}{T_{max}} \right)^m \quad (5.23)$$

where  $T_{crit}$  is the critical temperature. However, it has been defined that the extraction kinetic becomes more significant when the temperature reaches its critical value ( $T_{crit}$ ). The onset of significant extraction is assumed to occur when  $f_d \approx 0.001$ . In this study, the approximate  $T_{crit}$  is 85°C and the  $T_{max}$  is obtained based on the experimental studies (92 – 94°C). The power  $m$  is varied based on the amount of total mass of liquid and feedstock. Theoretically, under a fixed microwave power, the value of  $m$  will increase with the amount of feedstock. Therefore, the value of  $m$  is estimated first from the experimental observation, i.e., corresponding to the time taken for the first drop of oil to appear in the receiving flask.

Besides that, the data on garlic solubility,  $S_o$  in ethanol-water mixture is essential in Equation 5.22 which can be expressed in the form of

$$S_o = S_{ow} \alpha_{e/w} \quad (5.24)$$

where  $S_{ow}$  is the solubility of garlic oil in pure water measured at the reference temperature and  $\alpha_{e/w}$  is the relative solubility. The relative solubility is assumed to follow a function given in Equation 5.22 by considering the temperature's dependence on oil solubility as follows

$$\alpha_{e/w} = x_w \left[ 1 + b_w (T - T_{ref}) \right] + a_2 x_e \left[ 1 + b_e (T - T_{ref}) \right] \quad (5.25)$$

where  $a_2$  is the relative solubility at reference temperature,  $T_{ref}$  at 25°C,  $b_w$  and  $b_e$  are the slope of oil solubility against temperature in water and ethanol respectively.

The first order of Taylor's series can be used to estimate the exponential term in Equation 5.22, hence the following equation is obtained

$$k_{ex} \cong k_1 f_d \left[ 1 + a_2 \alpha_{e/w} \left( \frac{x_e}{x_w} \right) \right] \left( \frac{S_o - \rho_l x_l}{S_o} \right) \left( 1 - \frac{k_2}{T + 273.15} \right) \quad (5.26)$$

Additionally, the following effects on extraction rate can be captured by the model of overall mass transfer in Equation 5.22 and 5.25:

- i. Effect of inhibition of oil concentration in the bulk liquid on the extraction rate. For instance, when the bulk oil concentration approaches its solubility limit in the given liquid, the extraction kinetic slows down. Hence,  $\rho_l x_l \rightarrow S_o$  and the extraction kinetics,  $k_{ex} \rightarrow 0$ .
- ii. In a given solvent, the relative solubility relies on garlic essential oil extraction. For example, the ethanol-water mixture. If the relative solubility of ethanol is greater than 1, this means that the addition of ethanol as co-solvent aids in the rate of extraction. However, in hydrodistillation, ethanol mass fraction in the water decreases rapidly owing to the high volatility of ethanol than water. The ethanol mass fraction in water will continue decreasing until  $x_e \rightarrow 0$  while that of water will continue increasing until  $x_w \rightarrow 1$ . Sooner or later, ethanol will evaporate from the liquid and the extraction rate will be reduced.
- iii. Extraction rate relying on the temperature is as shown in Equation 5.25. The equation predicts that the extraction rate will become zero when liquid temperature reaches the melting point of the solvent. This means that the lower the liquid temperature, the lower the extraction rate will be.

Generally, when the solvent temperature is close to the melting point, the essential oil extraction rate will be expected to come close to zero. Thus, when there is still liquid remaining in the vessel, essential oil extraction can still be carried out from the solid plant. From the theoretical viewpoint,  $k_2$  can be expressed as

$$k_2 = 273.15 + T_{mp} \quad (5.27)$$

$$T_{mp} = -149.94 x_e^3 + 131.31 x_e^2 - 92.076 x_e \quad (5.28)$$

$T_{mp}$  is the melting point [°C] of ethanol- water mixture, Note that the melting point data can be found in (Flick 1998) which can be fitted into the third order polynomial equation as shown in Equation 5.28. In this work, Equation 5.24, which is the original kinetics expression is used in the MAHD model simulation.

#### 5.1.8 Determination of bubble point

The bubble point of the ethanol-water-oil can be calculated by the following equality

$$\frac{\gamma_w P_w^s x_w + \gamma_e P_e^s x_e}{P} + K_l x_l = 1 \quad (5.29)$$

Since Equation 5.29 is a nonlinear equation, *fzero function* can be used to solve the temperature which is available in the Matlab Optimization Toolbox or by trial-and-error method to find the liquid temperature that will correspond to the equality.

### 5.1.9 Garlic essential oil VLE coefficient

Based on the literature review, garlic essential oil contains almost 30 different kinds of organosulfur compounds which are quite polar in nature. Due to this reason, a significant influence between essential oil, water and ethanol is expected with non-ideal liquid behavior. As there is no information of garlic essential oil in VLE data, therefore, in order to represent essential oil VLE coefficient in the ethanol-water mixture, an empirical equation is proposed as follows

$$K_l = \frac{y_l}{x_l} = c_1 [1 - c_2(1 - f_e)x_e^2 + c_3 f_e x_w^2] \quad (5.30)$$

$$f_e = \left(1 - \frac{x_e}{x_{e0}}\right)^4$$

where  $c_1$ ,  $c_2$ ,  $c_3$  are positive constants. Equation 5.30 shows that an increase in ethanol mass fraction will decrease the VLE coefficient in a liquid mixture. This statement is established based on the general observations that have been made by various researchers where many plant oils and flavors have the tendency to stay longer in solvent compared to water. This suggests that between oil and ethanol molecules, there is a negative deviation from Raoult's Law, hence the bond between ethanol and oil molecules is stronger than the bonds between two pure ethanol or oil molecules. Oil and ethanol molecules are expected to have negative deviation while positive deviation is likely to occur between oil and water molecules. Garlic essential oil in pure water is expected to have positive deviation because of the garlic essential oil's poor solubility. Both assumptions on these deviations are considered in the empirical equation which is shown in Equation 5.30. The  $f_e \in [0, 1]$  shown in Equation 5.30 represents a fraction to be accounted for the impact of ethanol on volatility, for instance,  $f_e$  will be less than 1 that occurs at the end of the process when most of the ethanol would have been evaporated. The equation can realistically account for the effect of ethanol on volatility with the aid of  $f_e$ . Hence, it should be nearly to zero towards the end of the process when most of the ethanol has been evaporated. Due to the presence of a significant amount of ethanol in mixture, the equation can predict that negative deviation will be more dominant. In contrast, towards the end of the process when ethanol evaporates, the equation predicts that the positive deviation will be dominant instead.

If positive deviation occurs, this denotes that the maximum liquid temperature of the mixture in the end of the process should be lower than the boiling point of pure water. On the other hand, if the deviation does not exist, at the end of the MAHD process, the maximum liquid temperature before drying up should be around the boiling point of pure water.

#### 5.1.10 Prediction of extraction yield

By using the MAHD model, the following implicit equation can be used to predict the yield  $Yd(t)$  of essential oil extracted at any time,  $t$

$$Yd(t) = \frac{M_s(x_{s0} - x_s(t)) - \rho_l V_l(t)x_l(t)}{M_s} \quad (5.31)$$

where  $x_{s0}$  is the initial oil mass fraction in the solid,  $x_s(t)$  is the oil mass fraction in the solid,  $x_l(t)$  is the oil concentration in the liquid and  $V_l(t)$  is the liquid volume at time  $t$ .

## 5.2 MAHD parameter identification

The fitted model parameters are varied based on the process conditions in order to meet the model predictions of the real experimental work. In this work, there are seven fitting parameters for the MAHD model:  $\beta_1, \beta_2, \beta_3, b_w, k_1, c_1$  and  $m$ . Some fitting model parameters values are well tabulated in Table 6.2. To obtain the values for these parameters, the following two sequential steps are applied. First let define  $B = [\beta_1, \beta_2, \beta_3]$  and  $Z = [b_w, k_1, c_1, m]$ .

### 5.2.1 Temperature and volumetric profile identification

In the first step, optimization problem  $P_1$  is used to identify the values of  $\beta_1, \beta_2, \beta_3$ , minimizing the deviations of MAHD model predictions for the experimental data of temperature and liquid volume.

$$P_1 : \min_B (J_1) = \frac{1}{m_t} \sum_{i=1}^{m_t} \left( \frac{T_{ex,i} - T_i}{\bar{T}} \right)^2 + \frac{1}{m_v} \sum_{i=1}^{m_v} \left( \frac{V_{ex,i} - V_{l,i}}{\bar{V}_l} \right)^2$$

s.t.:

$$B_{\min} \leq B \leq B_{\max}$$

$$\frac{dX}{dt} = f(X, \Phi_p, B, \bar{Z})$$

where  $m_t$  and  $m_v$  are the numbers of samples for temperature and liquid volume respectively,  $T_{ex}$  is the experimental temperature,  $V_{ex}$  is the experimental liquid volume,  $\bar{T}$  is the average liquid temperature,  $\bar{V}_l$  is the average liquid volume,  $X \in R^6$  is the vector of state variables,  $\phi_p$  is the vector physical properties,  $\bar{Z}$  is the initial values for  $Z$  and  $f \in R^6$  is the vector of nonlinear functions of their arguments. The notations  $B_{\min}$  and  $B_{\max}$  are the lower and upper limits for the fitting parameters.

The solution to problem  $P_1$  obtained where the minimum value of the cost function  $J_1 = J_{1,\min}$  corresponds to the optimal values,  $B^* = [\beta_1^*, \beta_2^*, \beta_3^*]$ .



The average temperature is calculated as a sum of  $T_0$  and the expected maximum temperature  $T_{max}$  is divided by two. Meanwhile, the average liquid volume is given by half of the initial volume.

$$\bar{T} = (T_0 + T_{max})/2$$

$$\bar{V}_l = V_{l0}/2$$

### 5.2.2 Yield profile identification

Next, the value for  $Z$  is solved by another optimization problem  $P_2$  where it minimizes the deviation of MAHD model prediction for yield from the experimental yield.

$$P_2 : \min_Z (J_2) = \frac{1}{m_y} \sum_{i=1}^{m_y} \left( \frac{Yd_i - Yd_{ex,i}}{\bar{Yd}} \right)^2$$

s.t.:

$$Z_{min} \leq Z \leq Z_{max}$$

$$\frac{dX}{dt} = f(X, \Phi_p, B^*, Z)$$

where  $m_y$  is the number of experimental samples for yield,  $Yd_{ex}$  is the experimental yield,  $Z_{min}$  and  $Z_{max}$  are the lower and upper limits for the fitting parameters.

By solving  $P_2$ , the minimum value of cost function is  $J_2 = J_{2,min}$  which corresponds to the optimal values of  $Z^*$ . Average value of yield  $\bar{Yd}$  is used to normalize the cost function via

$$\bar{Yd} = Yd_{ex,final} / 2 \quad (5.32)$$

The optimization problems can be solved sequentially by Matlab Optimization Toolbox by using *fmincon* function.

As the oil mass fraction is very small, the model identification can be solved in two sequential steps showed in 5.2.1 and 5.2.2. This results in the oil mass fraction to have insignificant effect on temperature profile compared to water and ethanol components. For this reason, the three parameters ( $B$  identified independently of the other parameters  $Z$ ); the former are called thermal

balance parameters while latter the mass balance parameters. In other words, mass balance parameters only affect the oil mass fraction profiles without changing the liquid temperature and volume profiles. Once the temperature and volume profiles are fixed in 5.2.1, it will not be affected by changes in values of Z because the amount of the garlic essential oil is very small compared to that of garlic and water-ethanol components.

The constant parameters for the MAHD model is shown in **Error! Reference source not found.** and Table 5.2 shows the fitting parameters. The ranges of the fitting parameters have been determined via the experimental process conditions in order to fit the model predictions.

Table 5.1: Constant model parameters

Notations	Values
$C_{pw}$ [J/(g·C)]	4.1840
$C_{pe}$ [J/(g·C)]	2.4435
$\lambda_w$ [J/g]	2258
$\lambda_e$ [J/g]	838.3
$\rho_w$ [g/mL]	1.000
$\rho_e$ [g/mL]	0.789
$\rho_s$ [g/mL]	0.574
$\tan\delta_w$	0.123
$\tan\delta_e$	0.941
$S_{ow}$ [g/mL]**	$9.1 \times 10^{-5}$
$a_1$ **	0.02
$a_2$ **	81.4
$b_e$ [g/mL·°C]**	0.1
$c_2$ **	0.08
$c_3$ **	$3.9 \times 10^{-4}$

\*\*data collected from model

((Thermodynamics: Heat and Enthalpy 2018), (Latent Heat 2018), (Pure Water Density Standard 2018), (Density of Ethanol 2018), (Density of Garlic 2018), (Kappe, Stadler *et al.* 2012), )

Table 5.2: Fitting parameters

Fitting Parameters	Ranges
$\beta_1$	0.07 – 0.16
$\beta_2$	0.85 – 0.96
$\beta_3$	0.10 – 0.60
$b_w$ [1/°C]	0.1 – 5.0
$k_1$ [1/°C]	$1.7 – 11 \times 10^{-2}$
$c_1$	1.52 – 30.02

### 5.3 Summary

The summaries of this chapter are as follows:

- In this chapter, a fundamental modelling of MAHD with ethanol-water mixture has been developed. The modelling approach has been presented in this chapter, i.e., mass and thermal energy balance equations and the steps to identify the MAHD parameters.
- In order to simulate the constructed fundamental model, and to obtain good yield prediction, three important constitutive relations are proposed: (1) thermal effectiveness factor as a function of three interaction terms, (2) overall mass transfer coefficient which considers the relationship between essential oil, ethanol-water mixture, and liquid temperature and (3) Vapour-Liquid Equilibrium of the essential oil in ethanol-water mixture. Note that the existing models reported in the literature only take into account a limited number of state variables, which are often oversimplified and hence unable to give a full description of the given extraction process.
- The developed MAHD model can be used to predict the extraction yield in addition to accounting for six state variables, which are liquid temperature, liquid volume, mass fraction of oil in the solid and liquid phases, mass fraction of water and mass fraction of ethanol.
- **Error! Reference source not found.** shows the constant model parameter for the MAHD model while Table 5.2 shows the fitting parameters. The ranges of the fitting parameters have been determined via the experimental process conditions in order to fit the model predictions.
- The development of a fundamental and dynamic MAHD model is a challenging task because it involves a non-ideal phase change, delayed plant cell rupture, unknown thermal effectiveness of microwave conversion into heat, unknown solubility effect on mass transfer and time-varying properties. Hence, extensive simulation and experimental studies need to be employed to enhance the model reliability. In the present study, the model shall provide new insights into the extraction kinetics in the MAHD system as well as describing the effects of solvent, garlic essential oil and microwave power on the extraction kinetics.
- In the next chapter, the developed model will be validated against some experimental data.



## Chapter 6

### MODELLING RESULTS AND DISCUSSION

#### 6.1 Experimental design

In order to identify the model parameters, a series of simulation runs are first conducted. Table 6.1 shows the conditions used in the experimental study where the collected data includes a series of liquid temperature, liquid volume and mass of garlic oil taken over time.

Table 6.1: Experimental run for MAHD modelling and identification

Experiment	Garlic: Ethanol: Water	Liquid volume (mL)	Mass garlic (g)
1	100g: 50mL: 100mL	150	100
2	125g: 62.5mL: 125mL	187.5	125
3	150g: 75mL: 150mL	225	150
4	100g: 50mL: 75mL	125	100

A total of four experimental runs are carried as shown in Table 6.1. In all of the experimental runs, the microwave power is fixed at 400 W while the garlic to initial total liquid ratio is kept at 1:1.5 to ensure that the garlic is completely immersed in the solvent.

## 6.2 Model fitting parameter values

The parameter identification technique described in Chapter 5 is applied to determining the optimal fitting parameter values. Table 6.2 shows the fitting parameter values corresponding to the different experimental runs (1-4). The values of  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ,  $b_w$ ,  $c_1$ , and  $k_1$  are obtained via the identification technique in Chapter 5, section 5.2.

Table 6.2: Fitting parameter values for different experimental runs

Exp	$\beta_1$	$\beta_2$	$\beta_3$	$b_w$	$c_1$	$k_1(10^{-2})$	$m$
1	0.124	0.825	0.190	0.191	5.738	2.09	4.5
2	0.144	0.957	0.190	0.364	16.30	1.70	35.0
3	0.160	0.825	0.570	2.327	30.02	1.70	45.5
4	0.0781	0.825	0.190	4.940	1.52	11.0	7.5

However, another fitting parameter  $m$  is estimated from the experimental observation for time taken by the first drop of oil appearance in the receiving flask. Take note that the value of  $\beta_1$  increases with garlic feedstock for the fixed ethanol to water ratio 0.5:1.0 and 1.0 g: 1.5 mL initial ratio of feedstock to solvent ratio. This is likely to occur because as the solid and liquid mass increases, a bigger surface area (view factor) is exposed to the microwave irradiation. Additionally,  $\beta_3$  demonstrates the interaction between microwave and garlic inside the distillation flask. As the mass increases, the value of  $\beta_3$  also tends to increase. In the experimental run 3 (150 g garlic), it shows that a larger value of  $\beta_3$  is obtained when a larger mass of garlic is used, e.g., compared with the experimental run 1 or 4 (100 g garlic). This is confirmed by Equation 5.21, i.e. from the microwave thermal conversion efficiency, the increase of  $\beta_3$  with garlic mass proves that the effectiveness of microwave conversion into heat is related to the feedstock amount. A further notice is that  $\beta_2$  does not vary when the feedstock amount increases from 100 g to 150 g and total liquid volume increases from 150 mL to 225 mL. This is again expected from Equation 5.21 which indicates that  $\beta_2$  describes the contribution of ethanol towards the microwave thermal efficiency. As the ratio of ethanol to water is kept constant, this parameter should in theory be considered constant. Moreover, both ethanol and water are polar molecules, hence the microwave irradiation on ethanol-water mixture in the vessel shows not much dependency on the amount of initial liquid or mass of garlic used.

Owing to the essential oil's ability to dissolve in the ethanol-water mixture, this will cause parameter  $b_w$  to tend to increase with the feedstock amount. Garlic essential oil contains different kinds of organosulfur compounds. Therefore, it can be explained that the overall solubility of garlic essential oil in water is highly nonlinear. When the amount of feedstock increases from 100 g to 150 g, the microwave requires longer time to boil up the mixture, hence, longer delay time is observed for the extraction process. This delay time is represented by the parameter  $m$ , which is the power of the delay function (Equation 5.25) during the extraction process. An increase in  $m$  is observed as the feedstock amount increases. The delay time of the oil extraction in the distillation flask depends on two factors:

1. Delay due to the time taken to achieve the bubble point at a given composition of the liquid during the early stage of the process.
2. Delay due to time taken for cell rupture by microwave radiation.

However, the second delay type is expected to be dominant for large feedstock amount under a fixed microwave power input as longer time is required for cell ruptures. Conversely, for a small amount of feedstock (e.g, 100 g or less), it is believed that the first delay type is more significant, i.e. the time taken to reach the bubble point. Besides that, the microwave power also plays an important role in the delay. A higher microwave power used is supposed to lead to a shorter delay time because of the shortened time taken to reach the bubble point or/and more energy to break the cell walls, similar to how parameter  $m$ ,  $k_1$  value varies with the experimental conditions. The  $k_1$  value increases with smaller amount of feedstock (e.g., 100 g or less). It is shown that the parameter is significantly influenced by the ethanol to water ratio. From Table 6.2 at lower ethanol to water ratio, i.e. 1:1.5 (Exp 4), the  $k_1$  value is significantly large compared to that at a higher ratio, i.e. in Exp 1. This might relate to the relative solubility of essential oil in water and ethanol. From Table 6.2, it proves that with more ethanol is added, the higher it is the solubility of garlic essential oil in the water-ethanol solvent. This indirectly implies that the  $k_1$  value increases with the ethanol mass fraction the water-ethanol solvent.



## 6.3 Model prediction

### 6.3.1 Predicted temperature profiles

Figure 6.1 displays the predicted temperature profiles as compared with the experimental temperature profiles. Simul 1 indicates the predicted temperature profile corresponds to Exp 1, i.e. to the experimental data from the experiment run 1 (see Table 6.1). From Figure 6.1, all the predicted temperature results match very closely the experimental temperature profiles under all experimental runs shown in Table 6.1.

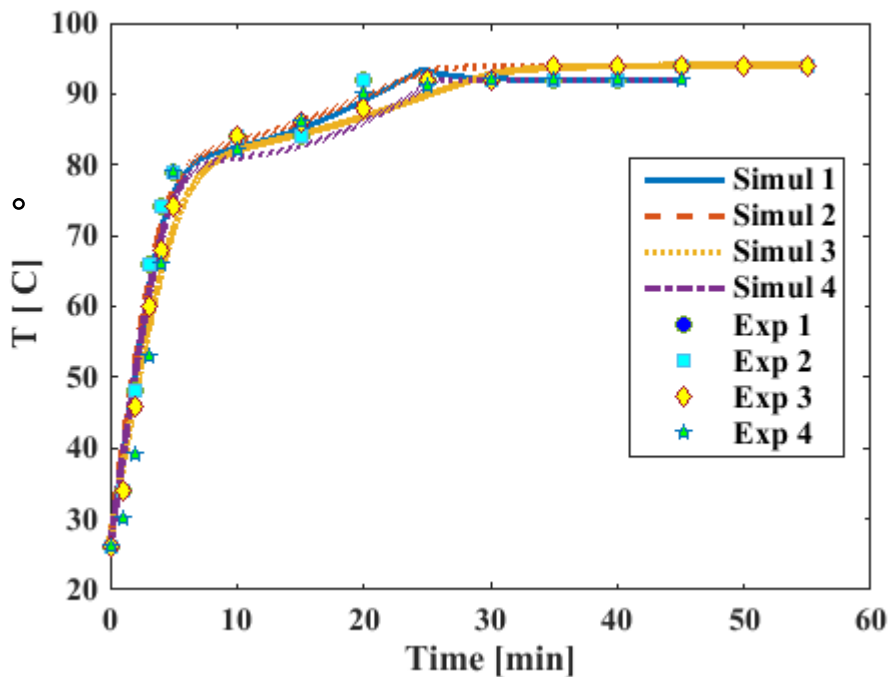


Figure 6.1: Model prediction of temperature with 4 different amount feed stocks used.

### 6.3.2 Predicted liquid volume profiles

Figure 6.2 compares the predicted liquid volume profiles with the experimental volume profiles. According to Figure 6.2, under the different experimental conditions, the model can closely fit the experimental liquid volume data. It is interesting to point out that all experimental runs and the rate of evaporation seems to be quite similar, the difference is mainly in terms of the delayed time involved prior to fully reaching evaporation. The predicted volume profile also includes the estimation on the delayed time in the liquid volume change for all the experiment runs.

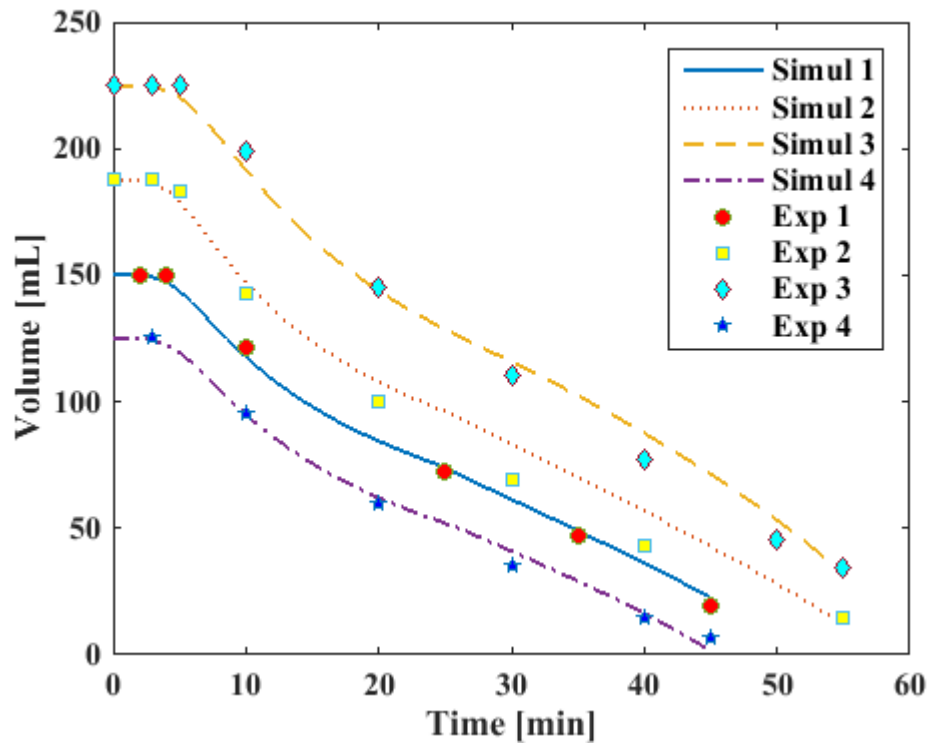


Figure 6.2: Model predictions of volume in liquid.

### 6.3.3 Simulated profiles of oil mass fractions

Figure 6.4 and Figure 6.3 demonstrate the simulated profiles of oil mass fractions in solid and bulk liquid phases respectively. From Figure 6.3, by comparing the Simul-Exp 1 (ethanol: water initial ratio = 0.5) and Simul-Exp 4 (ethanol: water initial ratio = 0.67) in which both have the same amount of feedstock, a higher ratio of ethanol to water results in a rapid drop of the garlic oil fraction in solid phase. This is owing to the high concentration of ethanol that can dissolve more of garlic essential oil from the solid phase, hence, lead to a shorter extraction time. Due to the higher extraction rate in Simul-Exp 4, it can be seen that there is almost an instant decrease of oil mass fraction in the solid phase, whereas in the liquid phase, the oil mass fractions increase rapidly. Based on the figures, it can be observed that a larger feedstock amount results in longer extraction delay time. Although large amount of feedstock (125 g or more) requires longer delay time compared to a smaller amount of feedstock, once the delay time reaches the oil mass fraction in the solid phase, it diffuses more rapidly into the fluid phase. Based on Figure 6.4, the lower ethanol to water ratio gives lower profiles (Simul-Exp 1-3) of oil mass fraction in the bulk liquid compared to that of the higher ethanol to water ratio (Simul-Exp 4). In addition, the evaporation rates are similar for all experiment runs. Hence, the addition of more ethanol in the extracting liquid will enhance the mass transfer of oil from the solid to liquid phase. The increase in the mass transfer rate proves that there is a better solubility of garlic essential oil in ethanol than in water. This can be further confirmed by **Error! Reference source not found.** where the solubility of garlic essential oil in ethanol is about 81 times as high as its solubility in water. This prediction is based on the simulation using the MAHD model against several experimental data. The solubility of garlic essential oil in pure water is estimated to be about  $9.1 \times 10^{-5}$  g/mL.

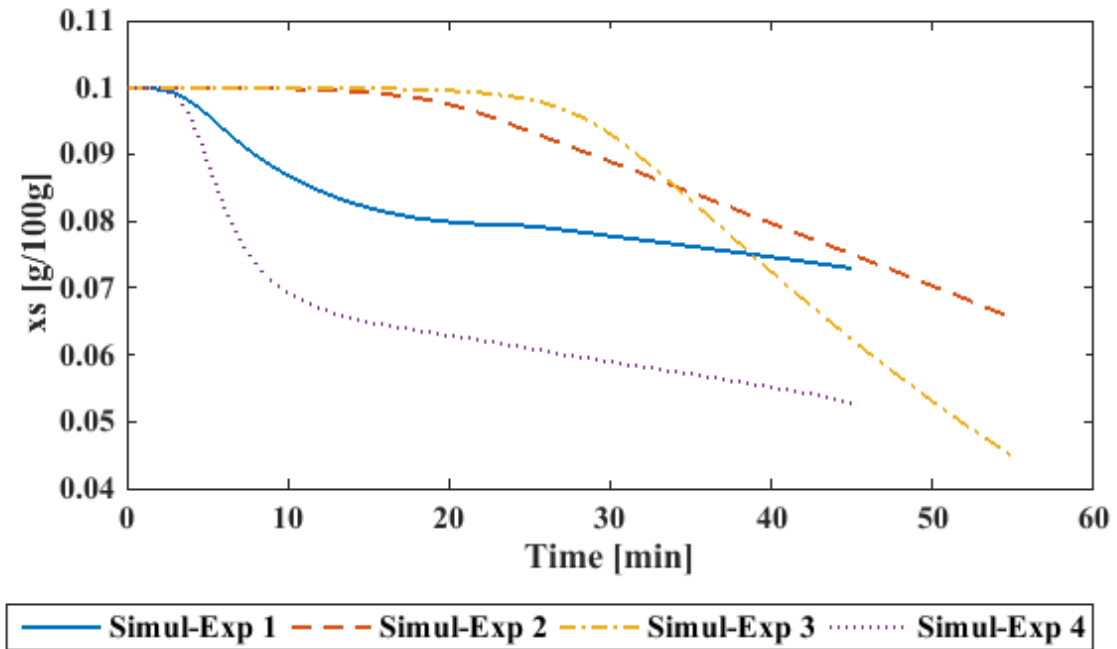


Figure 6.3: Plot of mass fraction of oil in solid phase.

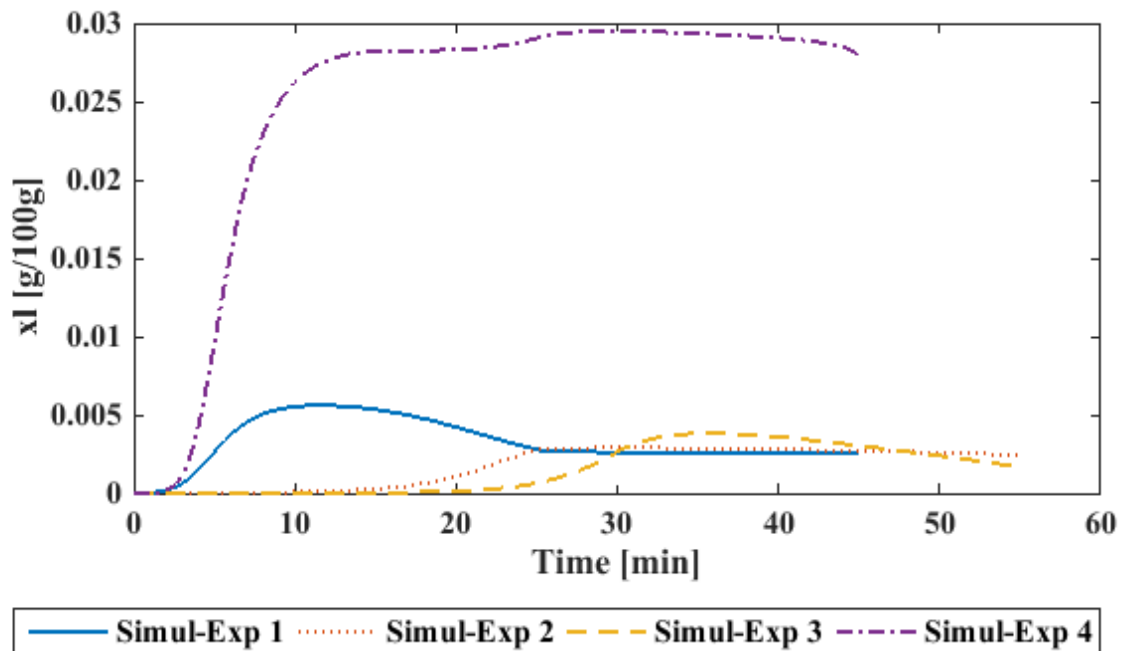


Figure 6.4: Plot of mass fractions of oil in liquid phase.

### 6.3.4 Extracted oil yield profiles

The model is also used to predict the extracted oil yield. Note that the simulated MAHD model can be used for further optimization of the process and economic evaluation if it is able to predict the extracted oil yield reasonably well. Figure 6.5 displays the graphs of the predicted experimental yields corresponding to Experiment runs 1 to 4 in Table 6.1. From the graphs, the model can closely predict the experimental yields at intermediate time and final extraction time. The final extraction time is chosen to be the time taken before the liquid in the distillation flask completely dries up.

Figure 6.5 clearly indicates that the extraction delay time increases as the feedstock amount increases. This can be shown by comparing between Simul 1 (100 g) and Simul 3 (150 g).

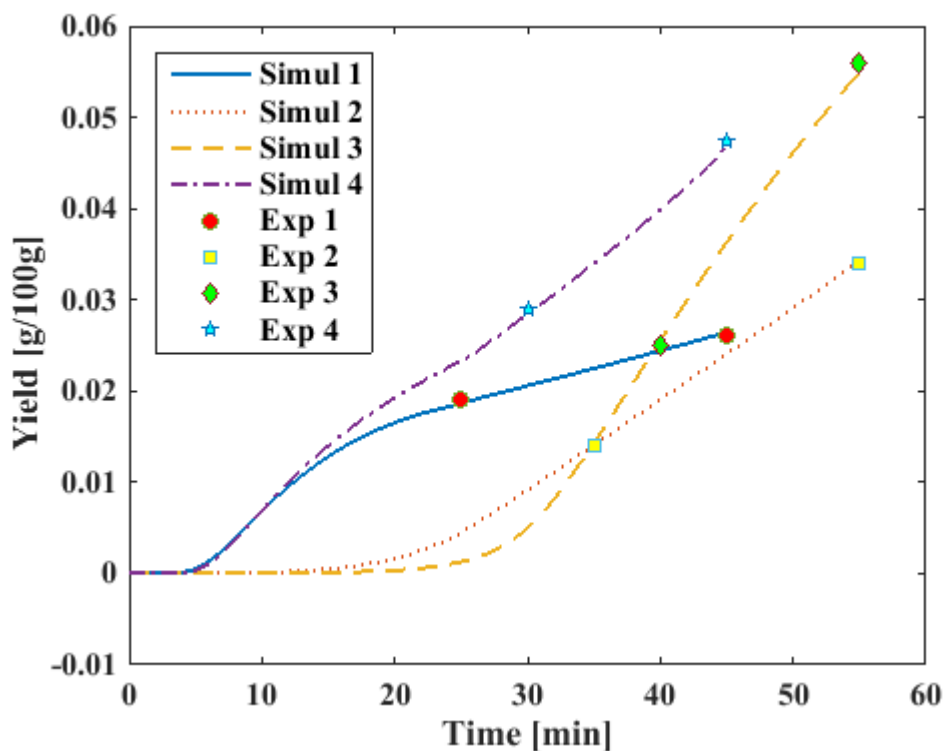


Figure 6.5: Plots of comparison of predicted and experimental yield values between experiments 1,2,3 and 4.

For a fixed microwave power at 400 W with the increase in feedstock amount, the extraction process requires a longer time for the cell to rupture; cell ruptures promote enhanced mass transfer of the garlic essential oil from the solid to fluid phase. Hence, the delay for the first

oil drop to appear in the receiving flask increases with the feedstock amount. Furthermore, by evaluating Simul 1 and Simul 4, it can be noticed that the ethanol to water ratio does not have significant effect on the delay time compared to the feedstock amount. It is worth mentioning that the developed MAHD model can fit the experimental data reasonably well where the prediction accuracy is within  $\pm 5\%$  of error margin from the experimental values.

Table 6.3 tabulates the comparison between the calculated yield by the model versus experimentally obtained yield. It can be seen that ethanol to water ratio plays a crucial role on the final extraction yield whereby the larger the ratio, the higher the extraction yield. This again proves that garlic essential oil has better solubility in ethanol than in pure water. The observation shows that solubility does significantly influence the mass transfer of garlic essential oil. In addition, Table 6.3 displays that  $t_m$  and  $t_f$  slowly increase with the amount of feedstock. In other words, an increase of feedstock has the tendency to increase the extraction period as well as the extraction yield under fixed ethanol to water ratio. From Table 6.3, the errors in model prediction of the values of experimental yields are within 2% under varying garlic feedstock amounts, total liquid volumes and ethanol to water ratios.

Table 6.3: Comparison between model prediction and experimental data for yield

Experiment	Yield at $t = t_m$ mins			Yield at $t = t_f$ mins		
	Model (g/100g)	Data (g/100g)	Error (%)	Model (g/100g)	Data (g/100g)	Error (%)
1 ( $t_m = 25, t_f = 45$ )	0.0187	0.019	-1.8244	0.0263	0.026	1.7819
2 ( $t_m = 35, t_f = 55$ )	0.0141	0.014	0.6165	0.0343	0.034	0.9576
3 ( $t_m = 40, t_f = 55$ )	0.0255	0.025	1.9649	0.0549	0.056	-1.9347
4 ( $t_m = 30, t_f = 45$ )	0.0284	0.029	-1.9785	0.0469	0.0474	1.1709

Moreover, the MAHD model can well predict the extraction yield at intermediate time,  $t_m$  and final time,  $t_f$  of the extraction process. Under a fixed microwave power, the extraction of oil yield becomes slower with the feedstock mass and total volume increases due to the delay time as mentioned above. Therefore, it can be assumed that the essential oil transferred from the solid

matrix to the bulk liquid occurs after the cell ruptures have taken place by the microwave irradiation.

#### 6.4 Summary

This chapter can be summarized as follows:

- The developed fundamental model showed in previous Chapter 5 has been proven effective, in the sense that it can be applied to the real MAHD extraction process in Chapter 6. The results obtained via Matlab simulation are presented and discussed in this chapter. The validations of the model include the model fitting parameter values, model predictions of temperature and liquid volume profiles, simulated profiles of garlic essential oil mass fractions and extracted yield profiles.
- The fitting parameter values, i.e.  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ,  $b_w$ ,  $c_1$ ,  $k_1$  and  $m$  for different experimental runs are well tabulated. Note that  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  explain the interaction between ethanol-water mixture, garlic solid and microwave irradiation.  $b_w$  represents the slope of solubility in water in which it tends to increase with the feedstock amount. However, the solubility of essential oil involves various kinds of organosulfur compounds, hence the solubility of garlic essential oil in water is expected to be highly nonlinear. The delay time, as indicated by the parameter  $m$ , increases with the feedstock amount. At small amount of feedstock, delay time involved is due to the time taken to reach the bubble point whereas for a large amount of feedstock, the delay is dominated by time taken for the cell rupture. The  $k_1$  values increase with the ethanol concentration, whose presence also increases the solubility of garlic essential oil. The model can give prediction of the experimental data under varying conditions of different amount of feedstock and ethanol to water ratio with accuracy within 5 % of error margin.
- The fundamental model can be used to perform system engineering study such as process simulation, optimization and process control.
- This fundamental model allows implicit prediction of the extracted oil yield. Majority of the existing models are explicit types in terms of describing the extracted yield. These kind of models lack prediction capability due to (1) insufficient number of state variables used, and (2) unrealistic assumption, as an example, the solubility has no effect on the extraction kinetics but this has been proven wrong in the present study.

## Chapter 7

### CONCLUSIONS AND RECOMMENDATIONS

#### 7.1 Conclusions

From the current study, it can be concluded that the garlic essential oil extracted by mean of microwave-assisted technology has been proven to possess antimicrobial properties. Therefore, the garlic oil extracted has an appealing potential to be applied in industries such as food, medicines and others.

The main findings from this project can be highlighted as follows.

Firstly, the garlic essential oil yield extracted via the MAHD method was higher than that via the SFME, which is 0.0728 %, with experimental conditions given as follows for the MAHD,

- Microwave power = 400 W
- Extraction time = 30 minutes
- Feedstock amount = 100 g
- Water to Ethanol ratio:1:1.5

and 0.060 % for SFME with conditions given by

- Microwave power = 400 W
- Extraction time = 60 minutes
- Feedstock amount = 500 g

The results show that with the incorporation of microwave technology in the conventional extraction process (hydrodistillation), higher yield and good quality garlic oil can be produced. Note that, the optimization of garlic essential oil in the MAHD process has been carried out by investigating the optimum extraction time at several microwave powers inputs. From a series of experimental studies conducted, the optimized parameters for the MAHD process have been determined as given above. The MAHD technique gave a higher yield and required shorter period of extraction than the SFME, which supports the hypothesis that the addition of water-ethanol as solvent mediates the diffusion of oil from the solid phase to the bulk liquid phase. Hence, this combined effect of microwave heating and solvent increases the overall extraction rate. On the



other hand, in the SFME process, the predominant mode of extraction is similar to the drying process where oil in water or water-ethanol mixture diffuses to the bulk gaseous phase from the plant matrix. This diffusion rate was lower than the one in which the extraction was mediated by water-ethanol mixture. In other words, an increased solubility of oil in ethanol-water mixture helps the diffusion of oil from the plant matrix to the bulk liquid phase – leading to higher extraction rate.

Secondly, the characterization study has confirmed that garlic essential oil extracted via the MAHD/SFME possesses good antimicrobial properties – comparable with the oil extracted via conventional method as reported in the literature, e.g. Soxhlet extraction. Several analyses of the garlic essential oil were carried out through FTIR, disc diffusion method, MIC and MBC measurements. All of the useful recorded functional groups were confirmed present in the extracted garlic essential oil. Both gram-positive (*S. Aureus*) and gram-negative bacteria (*E. coli*) showed inhibited growth against garlic essential oil. The lowest concentrations of garlic essential oil that inhibited the visible growth of gram-positive (*S. aureus*) and gram-negative (*E.coli*) bacteria were 0.0015 g/mL and 0.0095 g/mL respectively. Meanwhile, the MBC value can also be determined from the 96 well plates, which was 0.00334 g/mL for *E.coli* and 0.0026 g/mL for *S. aureus*. This result supported the hypothesis that microwave radiation has little downgrading effect on the organosulfur compounds since they are slightly polarized molecules. Hence they are quite transparent to the microwave radiation.

Thirdly, the kinetic behavior of the microwave-assisted hydrodistillation (MAHD) extraction has been modelled using empirical equations, which hypothesized that solubility plays a key role in influencing the overall extraction rate, together with mass fractions of water-ethanol and temperature. A rigorous fundamental mass-energy balance model of the MAHD with mixed ethanol-water solvent has been developed. Based on the model, the garlic essential oil has been shown to have a higher solubility in ethanol, which is about 80 to 100 times as high as compared to its solubility in water (approx..  $9.1 \times 10^{-5}$  g/mL) at room temperature. The model can satisfactorily predict the extracted oil yield under a series of experimental conditions with error margin within  $\pm 5$  %. Obviously, the developed model has a satisfactory prediction capability, which means that it could be used in process simulation, control and optimization studies of the microwave-assisted extraction system. The model-based simulation using this fundamental model

again supports the hypothesis that ethanol mediates the diffusion of oil from the plant matrix to bulk liquid because of the improved oil solubility in the ethanol-water mixture.

Alongside with the development of fundamental model of the MAHD, three empirical constitutive equations have been proposed as follows:

- i. A new equation describing the thermal effectiveness factor of microwave power conversion into heat was developed. This equation proposes that the conversion of the microwave irradiation into heat depends on the loss tangents of water and ethanol, and their mass fractions and ratio of garlic to liquid volume in the extracting/distilling flask. This equation has never been reported in the open literature.
- ii. A new overall extraction kinetics equation was proposed. This empirical equation suggests that the rate of extraction is affected by the solubility of oil in the water-ethanol mixture. The experimental-simulation result has confirmed that as more ethanol is introduced in the liquid mixture, the rate of extraction increases dramatically leading to a reduced extraction period.
- iii. An equation representing the vapor-liquid equilibrium constant of the oil in water-ethanol mixture was also developed. This is the first time that an attempt has been made to model the equilibrium constant of essential oil in water-ethanol mixture. The empirical equation postulates that the constant is a quadratic function of the water and ethanol mass fractions in the distilling flask.

Note that, the fundamental model must be supplemented with the aforementioned three empirical equations in order for the model to be able to accurately predict the temperature, liquid and yield profiles. The fact that this model can predict the experimental profiles within 5 % error margin confirms the validity of the three empirical equations proposed. Another striking feature of the proposed fundamental model is its ability in capturing the delay in the process, e.g., delay in the oil appearance in the receiving flask and delay in significant evaporation process.

Overall, based on the experimental and modelling studies, one can conclude that this research project has achieved all of its intended objectives. Noteworthy, the results of the study seemingly support all of the hypotheses and answer the main research questions. This research project is established on a novel fundamental model of the MAHD, which can be applied in process scale-up, process control development and optimization.

## 7.2 Recommendations

Some recommendations are listed for possible future works. During the extraction process, stirring is suggested in order to prevent hotspot. Hotspot can occur due to the local behavior of the electromagnetic radiation effect on the solid-liquid mixture in the distillation flask. These waves tend to bounce around in the distillation flask, which can either cancel out each other or double the radiation intensity. Hence, stirring the mixture or turning the plate around is needed throughout the extraction process for improved homogeneous heating. Moreover, the moisture content of the feedstock might affect the extraction efficiency. Hence, it should be further studied on the effect of moisture content against the oil yield.

Furthermore, the receiving flask should have been soaked completely in a very cold liquid bath, preferably close to 0 °C. This is because the garlic essential oil consists of many organosulfur compounds, some of which are very volatile, thus they tend to evaporate under room temperature. Therefore, to prevent the evaporation of essential oil in the receiving flask, it is important to keep the flask in very a low temperature bath. Furthermore, the modelling in this study can still be further refined to include experiments with larger feedstock amount, larger microwave power input, and different solvent to water ratio to assess the effect of scaling-up. It is recommended that these parameters should be used for scale-up study. Besides that, scale up and technoeconomic analysis can be carried out in the future extension of the current work.

## Chapter 8

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**Chapter 9**  
**APPENDIX**

The calculation of the garlic oil yield %:

$$\text{Extraction yield (\%)} = \left( \frac{\text{Mass of extracted essential oil}}{\text{Mass of per 100g biomass}} \right) \times 100$$

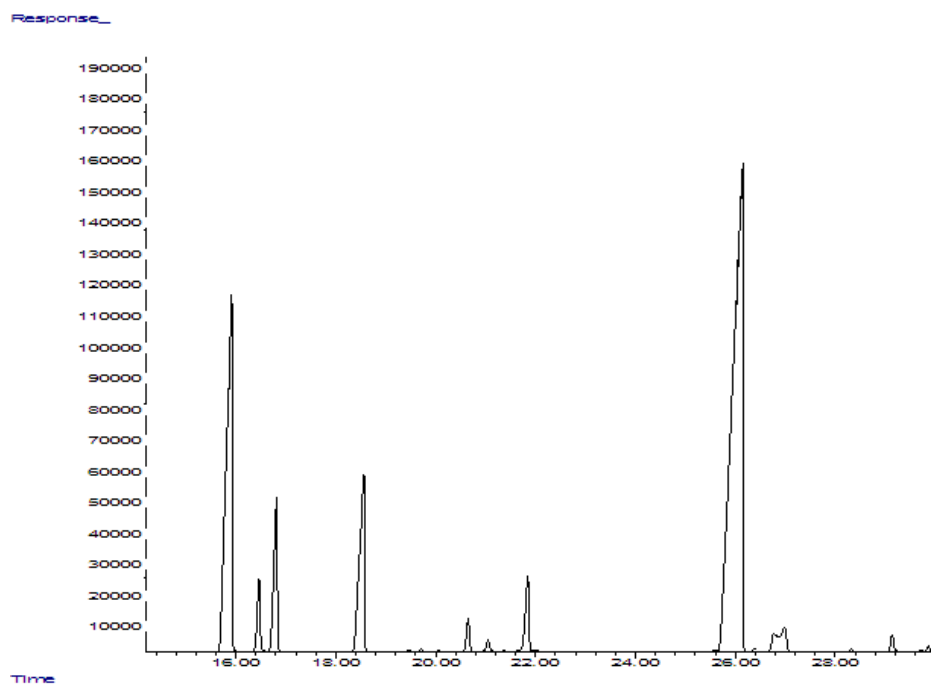


Figure A.1: GC-FID of garlic essential oil with microwave power 200 Wh

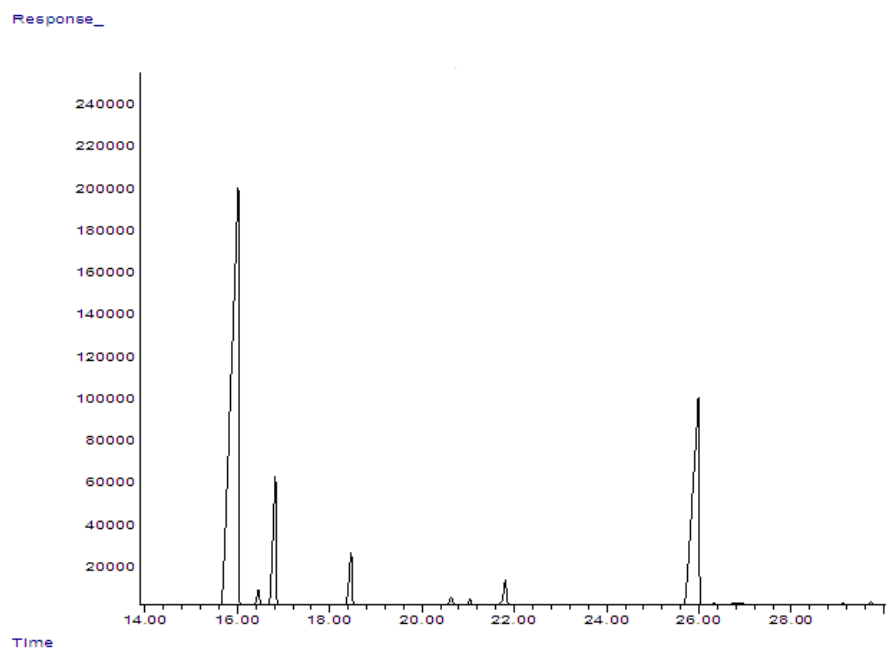


Figure A.2: GC-FID of garlic essential oil with microwave power 400 Wh

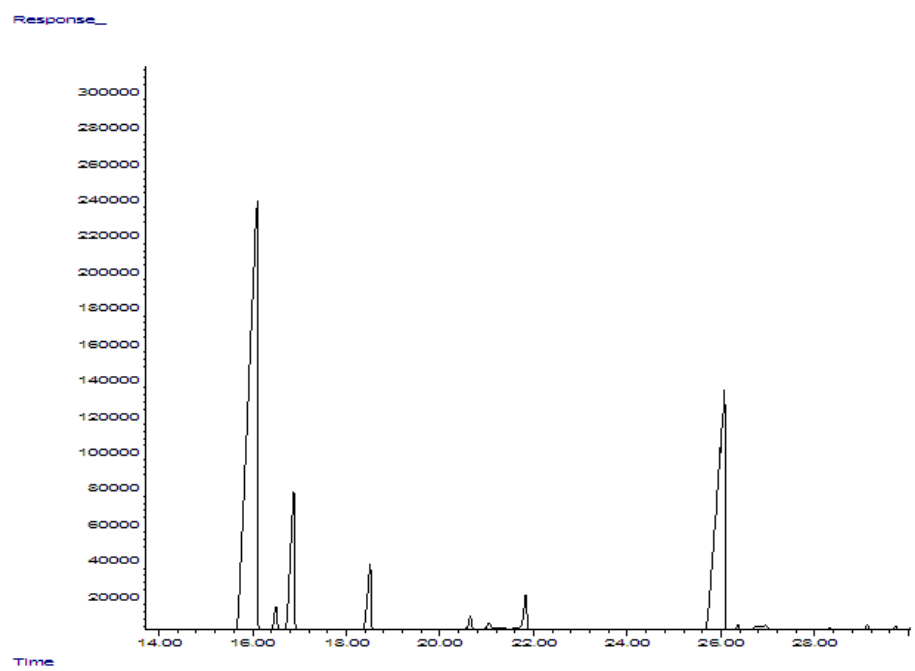


Figure A.3: GC-FID of garlic essential oil with microwave power 600 Wh

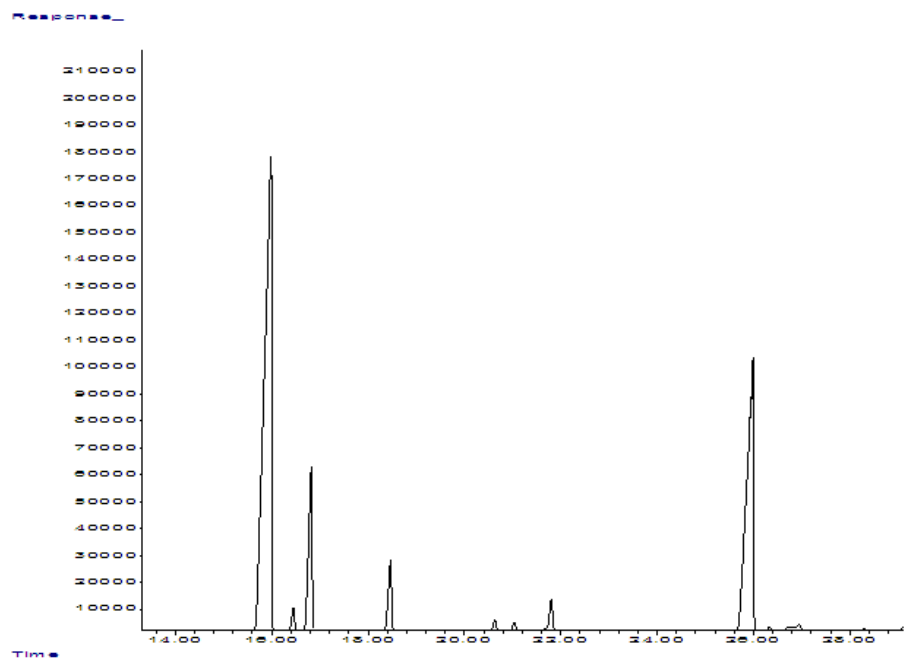


Figure A.4: GC-FID of garlic essential oil with microwave power 800 Wh

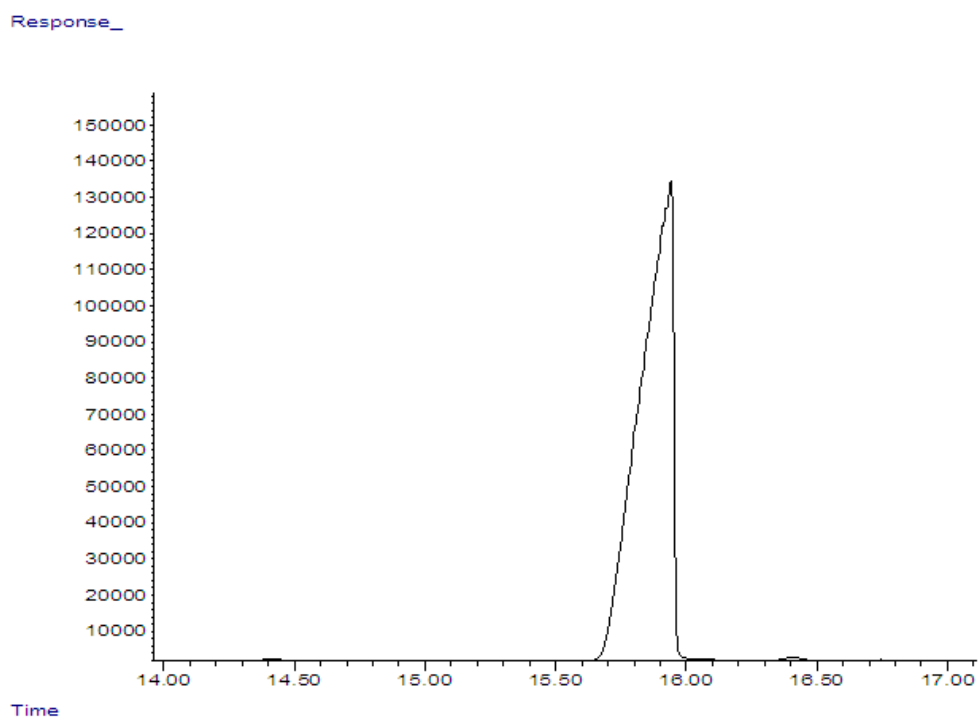


Figure A.5: GC-FID of garlic compound standard (DAD)



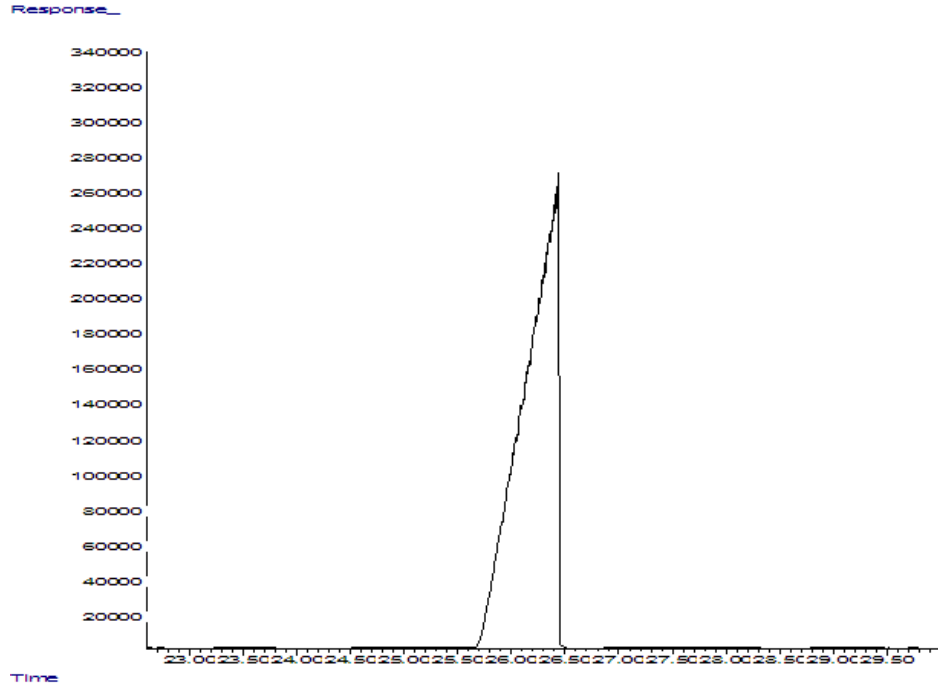


Figure A.6: GC-FID of garlic compound standard (DAT)

Non-Random Two Liquid (NRTL) model which used to calculate the activity coefficients:

$$\ln \gamma_w = x_e^2 \left[ \tau_{ew} \left( \frac{G_{ew}}{x_w + x_e G_{ew}} \right)^2 + \frac{\tau_{we} G_{we}}{(x_e + x_w G_{we})^2} \right] \quad (\text{A.1})$$

$$\ln \gamma_e = x_w^2 \left[ \tau_{we} \left( \frac{G_{we}}{x_e + x_w G_{we}} \right)^2 + \frac{\tau_{ew} G_{ew}}{(x_w + x_e G_{ew})^2} \right] \quad (\text{A.2})$$

Whereas for the NRTL model, parameters  $a_{we}$ ,  $a_{ew}$ ,  $b_{we}$ ,  $b_{ew}$ ,  $c_{we}$ ,  $c_{ew}$  are given as follows:

$$\ln G_{we} = -c_{we} \tau_{we}$$

$$\ln G_{ew} = -c_{ew} \tau_{ew}$$

$$\tau_{we} = a_{we} + b_{we} / (T + 273.15)$$

$$\tau_{ew} = a_{ew} + b_{ew} / (T + 273.15)$$

The saturated vapor pressure for  $i$  component is defined by the Antoine equation:

$$P_i^s = A_i - \frac{B_i}{C_i + T}$$

Table A.1 and Table A.2 shows the NRTL parameters and Antoine's coefficients for the for ethanol-water mixture respectively.

Table A.1: NRTL parameters for ethanol-water mixture

Parameters	Water-Ethanol	Ethanol-Water
$\alpha_{ij}$	3.458	-0.801
$b_{ij}$	-586.1	246.2
$c_{ij}$	0	0.3

Table A.2: Antoine coefficients for ethanol-water mixture

Parameters	Water	Ethanol	
		( $T < 80^\circ C$ )	( $T > 80^\circ C$ )
A	8.07131	8.20417	7.68117
B	1730.63	1642.89	1332.04
C	233.426	230.300	199.200