TEMPERATURE CONTROLLED MICROWAVE CLOSED SYSTEM FOR EXTRACTION OF BIOACTIVE COMPOUND FROM EPIPHYTE'S FERN

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TEMPERATURE CONTROLLED MICROWAVE CLOSED SYSTEM FOR EXTRACTION OF BIOACTIVE COMPOUND FROM EPIPHYTE'S FERN.

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Thesis submitted in fulfillment of the requirements.
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To my beloved parents, brothers, sisters, nieces, nephews, grandchild and friends

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ABSTRACT

In the current work, a temperature controlled microwave closed system (TCMCS) equipped with fluid sealed stirring device was developed to increase the extraction efficiency of bioactive compound from Malaysian palm oil trunk epiphytes fern (MPOTEF). The TCMCS was calibrated with distilled water using the calorimetric method after modification, and the results show power absorbance performance of each microwave power level were much closer to the actual microwave power. In addition, the fluid seal stirring system was found as an important factor to increase uniformity of absorption of electromagnetic energy in the water which reflects to TCMCS efficiency based on % microwave power absorbance and standardized coefficient (*ß*) regression analysis results. The study on the influence of solvents on TCMCS showed that the solubility of the solvent to the antioxidant properties and the ability of the solvent to absorb microwave energy played important role in TCMCS extraction efficiency. TCMAE method combined with statistical design mixtures showed that quaternary mixtures with approximately equal proportions of all four solvents (water, acetone, acetonitrile and ethanol) provided the highest result of antioxidant properties followed by ternary design mixtures and single solvent. But in terms of individual result, water was shown extraordinary results among the single and solvent combination. The application of TCMCS in various extraction parameters (extraction temperature, microwave power and extraction time) on antioxidant properties, physicochemical studies and plant tissue microstructure of *Nephrolepis biserrata* (Sw.) Schott frond (NBF) extract was concluded that, an increase in surface roughness of plant tissue attributed in high antioxidant property's recovery and significant diffusion coefficient through the solid matrix which was observed by physicochemical analysis results. The outcome of standardized coefficient (*ß*) by statistical regression analysis was showing that the extraction temperature is the most important factor as compared to microwave power and extraction time. There are two problems has been overcome in the extraction of plant material using TCMCS: that of releasing the bioactive compounds from the solid matrix with respect to solvent nature under controlled temperature to avoid degradation of sensitive compound and the fluid seal stirring system resulting from uniform heating to avoid supper heating effects.

ABSTRAK

Dalam kajian ini, sistem ekstrasi kawalan suhu terkedap dilengkapi alat pengaduk berlak (TCMCS) dibangunkan untuk meningkat keberkesanan ekstrasi bahan aktif daripada pakis epifit yang terdapat pada pangkal pelepah kelapa sawit (MOFTEF). Selepas proses modifikasi, sistem TCMCS telah dikalibrasi menggunakan kaedah kalorimeter ke atas sampel air. Melalui kaedah ini didapati bahawa TCMCS menunjukkan kecekapan penyerapan radiasi microwave menghampiri kuasa microwave sebenar pada setiap peringkat ujian. Keupayaan sistem pengaduk berlak dianalisa menggunakan kaedah analisis regrasi beta di mana keputusan ujian mendapati alat pengaduk berlak tersebut terbukti membantu meyamaratakan kadar penyerapan radiasi microwave dalam sampel air seterusnya meningkatkan keupayaan TCMCS. Kajian secara statistik melalui "mixture design" untuk kombinasi pelarut secara umumnya membuktikan, kombinasi empat pelarut menghasilkan keputusan yang lebih baik dalam ekstasi antioksidan berbanding kombinasi dua pelarut diikuti satu pelarut. Namun secara keseluruhannya, air sahaja (satu pelarut) telah menunjukkan hasil terbaik berbanding lain-lain keputusan. Melalui keputusan ini apa yang boleh dinyatakan, kebolehan pelarut melarutkan kompoun tertentu serta keupayaan tinggi pelarut menyerap tenaga microwave memainkan peranan utama dalam TCMCS seterusnya meningkatkan keberhasilan ekstasi.Aplikasi TCMCS untuk pelbagai parameter ke atas kompoun tertentu dan kajian fisikal-kimia keatas ekstak *Nephrolepis biserrata* (Sw.) Schott frond (NBF) telah dijalankan. Kesan terhadap stuktur permukaan matrix NBF di bawah pengimbas mikoskopi elektron (SEM), telah membuktikan bahawa kekasaran permukaan ada kaitan dengan peningkatan hasil anti-oksidant dan peningkatan keputusan fisikal-kimia ke atas pelarut terhasil. Melalui analisis regrasi beta, suhu ekstrak memainkan peranan penting dalam parameter ekstasi berbanding masa ekstasi dan kuasa microwave. Melalui sistem ini dua masalah dapat diatasi: pembebasan bahan aktif daripada bahan matrix berlandaskan sifat pelarut di bawah suhu terkawal bagi mengelakkan bahan aktif sensitif suhu terhapus dan alat pengaduk berlak untuk memastikan pemanasan sekata bagi mengelakkan apa yang dikatakan fenomena terlebih bakar.

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LIST OF SYMBOLS

ΔT	final temperature minus the initial temperature
C	concentration
Cp	heat capacity
df	dilution factor
Dp	penetration depth
E_t	efflux time
K	conversion factor for thermochemical sec^{-1} to watt
m	mass of the water sample in gram
M	sample weight
P	apparent power absorbed by the sample in watt
sG	specific gravity
T	relaxion time
V	volume

Greek Symbols

$\tan \delta$	dielectric (loss) tangent
ε'	dielectric constst
ε''	dielectric loss factor
η	viscosity
η_A	dynamic viscosity
η_k	kinematic viscosity
ρ	density

LIST OF ABBREVIATIONS

ALF	*Asplenium longissimum B1*frond
DDF	*Davallia denticulate* (Burm.) Mett frond
GPF	*Gonioplebium percussum* (Cav.) Wagner & Grether frond
MPOTEF	Malaysian palm oil trunks epiphytes ferns
MW	Microwave
NB	*Nephrolepis biserrata* (Sw.) Schott
NBF	*Nephrolepis biserrata* (Sw.) Schott frond
SPF	*Stenochlaena palustis* (Burm.f.) Bedd.frond
TCMAE	Temperature controlled microwave assisted extraction
TCMCS	Temperature controlled microwave closed system
VSF	*Vittaria ensiformis* frond
VLF	*Vittaria elongata* frond

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CHAPTER 1

INTRODUCTION

1.1 MICROWAVE EXTRACTION- AN OVERVIEW

Bioactive compounds extracted from plant materials are of vast importance in the pharmaceutical and the food industries. Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point in qualitative and quantitative analysis of bioactive compounds as medicinal plant constituents. Extraction of plant materials can be done by various processes. Usually, the traditional techniques like soxhlet, maceration, reflux and hydrodistillation, which have been used over decade's forms the first choice for extraction of bioactive compounds. How ever, these techniques also suffer from severe drawbacks such as long extraction time and low efficiency, particularly when trace amount of compounds are present (Mandal et al, 2007). Moreover, many natural products are thermally unstable and could be degraded with increasing temperature during the extraction causing degradation of unsaturated or ester compounds through thermal or hydrolytic effects (Grigonis et al., 2005; Khajeh et al., 2010). The use of large volumes of organic solvent associated with conventional methods are detrimental to environment and their subsequent disposal also becomes an issue of concern. These shortcomings have led to consider the use of new techniques for the extraction of natural substances, which typically use less solvent and energy.

In this respect, a procedure that could obtain most of the effective constituents in a shortest processing time with low production cost and using minimum organic solvent will be an ideal technology. The demand for new extraction techniques has encouraged the development of alternative extraction techniques such as ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and solid phase microextraction (SPME). These techniques have enabled automation, shortened extraction time, reduced organic solvent consumption and improve the reproducibility of compound recovery. However, the use of SFE, ASE or SPME, requires greater financial investment and the presence of water in samples can cause blockage in those techniques (Kerem et al., 1989), while UAE shows lower efficiency compared to the other modern technique.

Microwave-assisted extraction (MAE) has been considered as a potential alternative technique to extract bioactive compounds from medicinal plant for several reasons. By considering economic and practical aspects, microwave-assisted extraction is a strong novel extraction technique for the extraction of bioactive compounds. Microwave extraction has many advantages, such as reducing extraction time, lesser solvent consumption, higher extraction rate, higher purity of final product and cost effective (Kingston and Haswell, 1997; Ganzler, 1986). It has shown higher efficiency compared to conventional methods and easy to operate compared to modern extraction technique. The use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. Many reports on the beneficial effects of MAE with respect to medicinal plants have been published, with significant improvements over conventional extraction methods (Mandal et al., 2007; Kaufmann and Christen, 2002).

Microwave assisted extraction (MAE) is a current technique, has proven to be a promising ideal extraction tool for the extraction of phytochemicals from botanicals (Cui and Mei, 2003). Researchers in many universities are working on novel techniques that could lead to compact, safe, efficient, energy saving, and sustainable extraction processes (Jimenez-Carmona and Luque de Castro, 1999). Few studies have been conducted on the effect of microwave energy on bioactivities and chemicals of medical plants, which will not significantly influence the quality and price of end-product, but also affect the customer desire for the conveniently microwave-derived products. Microwaves have been used increasingly for the fast extraction of a variety bioactive compound such as essential oils from the leaves of rosemary and peppermints (Chen and Spiro, 1994), taxanes from *taxus* biomass (Mattina et al., 1997) and extraction of ergosterol and total fatty acids from fungal hyphae and spores (Young, 1995). Extraction of phenolic compounds from tea leaves has been studied recently (Pan et al., 2003). Phenolic compounds have also been extracted from grape seed (Hong et al., 2001) using microwave assisted extraction (MAE). MAE is increasingly being used as an alternative to traditional extraction method for the removal of phenolics from plant tissues, as it significantly reduces extraction time and solvent consumption while generating higher extraction yields (Pan et al., 2003; Eskilsson and Bjorklund, 2000).

1.2 THEORY OF MICROWAVE ASSISTED EXTRACTION (MAE)

Microwave assisted extraction (MAE) process generally consist of more than one step: solubilization/desorption at the matrix-solvent interface followed by diffusion of the solute into the solvent (Aguilera et al., 2003). In this extraction practice, solvent extraction operating parameter, e.g: extraction temperature, extraction time and microwave power and geometrical structure of the sample may affect the solubilization/desorption and diffusion of the target compounds (Letellier and Budzinski, 1999). Microwave assisted extraction is a process of applying the microwave energy to a liquid-solid system and partition compounds of interest from the solid sample into the surrounding solvents. The special heating mechanism of microwave to materials and the fact different chemical substances absorb microwave to different levels make microwave-assisted extraction an efficient method for extraction and more importantly, make selective extraction of target compounds possible. Since microwave can penetrate into materials, the heating effect happens throughout the material which is called volumetric heating. This special heating effect makes microwave a very efficient method for heating, which is also contributed to the various applications, especially in extraction of bioactive compound from plant material.

During microwave irradiation throughout extraction, direct interaction of microwaves with the free water molecules presents in the glands and vascular systems of plant material, leads to localized heating (Pare et al., 1994) and generate temperature rapidly near or above the boiling point of water. Thus, such systems undergo a dramatic expansion and tremendous increase in internal pressure inside the plant cell due to evaporation of the internal moisture content and consequently, rupture of the plant tissue before releasing of the active compounds into the organic solvent (Mandal et al, 2007). This process is quite different from classical solvent extraction, where the solvent diffuses into the matrix and extracts the components by solubilization (Chemat et al., 2005).

Solvent choice for microwave extraction is dictated by the solubility of the target analyte, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent. To understand how microwave heating can have effects that are different from conventional heating, one must focus on what in the material (solvent) is actually absorbing the microwave energy. Differential absorption of microwaves leads to differential heating and localized thermal in homogeneities that cannot be duplicated by conventional heating (Zhuoyan, 2010). In conventional MAE, microwave power and irradiation, times are two such factors, which influences each other and needs to be given special attention, particularly when working with a closed vessel system. Generally, by increasing the extraction time, the quantity of compound extracted is increased, although there is the risk that degradation may occur. In closed vessel systems, temperature may reach well above the boiling point of the solvent. A combination of low or moderate power with longer exposure may be a wise approach.

1.3 PROBLEM STATEMENT

Various new technologies for efficient and cost-effective extraction of medicinal plants have been discussed. Microwave-assisted extraction (MAE) is highly efficient for obtaining extracts under mild conditions. MAE is particularly important since the active components which are thermally labile can be recovered without any damage. The pressurized microwave assisted extraction (PMAE) is one of the most often used systems, which affords fast extraction with low solvent consumption (Gfrerer and Lankmayr, 2005). Besides that the dynamic microwave assisted extraction (DMAE) has also been found to be an efficient technique. Using a dynamic approach to extraction is generally advantaged, especially with respect to the partitioning of the solute into the extraction media. This can be highly efficient when fresh solvent is continuously introduced into the extraction vessel. The rate constant for desorption does not need to be large in comparison to the rate constant for adsorption for efficient removal of the target solute. A new concept of microwave hydrodiffusion and gravity (MHG) was introduced by Bousbia et al. (2009). The extraction process generated by a combination of microwave heating and gravity working at the atmospheric pressure presents an efficient extraction process, reduce waste, avoids water and solvent consumption and allows substantial energy savings.

The introduction of microwave has met the increasing demand for new extraction techniques, amenable to automation with reduced solvent consumption. In this context, microwave extraction appears as a promising method that still needs to be developed and studied in-depth, but, which will probably be implemented soon for the analytical laboratory industrial, as a consequence of the significant and numerous advantages it shows over alternatives. Nevertheless, conventional techniques continue to dominate this application area. Some procedures have limited application whereas others are more broadly applicable.

The commercial microwave involves the safety issue of increased temperature and pressure leading to burning of the sample and rupture of the container. The potential for commercial microwave heating to be applied in a process is dependent upon the dielectric properties to the target material. Such properties are not fully understood. Not all materials possess the ability to absorb microwave radiation, which, although a limiting factor in some applications, can be advantageous in others (Appleton et al., 2005). Therefore, for a reason of safely and in order to ensure optimum reproducibility, study in this unequal heating of samples in the microwave field must always be taken into consideration. More research is needed to improve the understanding of microwave extraction mechanism, remove technical barriers, improve the design and scale up of the novel extraction systems for their better industrial applications. Accordingly, a primary goal of our novel microwave extraction research is to develop of methods that are more efficient and yield pure and highly representative extracts.

In this research, the extraction system was design with temperature controller and fluid seal stirring system. The temperature of extraction of the microwave system will be controlled by L shape electrically capillary thermometer. The electrically capillary thermometer responsive probe was inserted into a target extracted materials in a microwave oven. The electrically capillary temperature probe is adapted to provide a signal to interrupt the application of microwave power upon reaching a predetermined temperature. So as the operation temperature can be controlled in low temperature on the extraction of thermo sensitive compounds and also can be controlled in unlimited temperature where applicable to heat the extraction solvent above its atmospheric boiling point. Higher temperatures help overcome matrix effects and accelerate desorption and diffusion of the compound molecules. This leads to dramatically reduced extraction times.

Fluid seal stirring system was proposed in the extraction system to increase a heating uniformity of a target matrix. The microwave's penetration depth depends on the dielectric constant and geometry of the target matrix. The loss factor of the compound is also important and is related to the transparency to microwaves and the ability to dissipate the absorbed energy. Since microwaves have low penetration depth (~1.5 cm in water at 2450 MHz), the diameter of the vessel which locating a sample and solvent material should be less than 1.5 cm or the material inside the vessel should be uniformly spread to avoid uneven heating (Pangarkar, 2008). This must be taken in order to account carefully in the design of MAE system.

1.3.1 Significant of New Raw Material Extracted by Microwave

Consumer demand for healthier product has been rapidly increasing, and nearly 77% of consumers would choose a product containing ‘all natural’ ingredients rather than synthetic compounds (Faron, 2010). Thus, the challenge to the food and pharmaceutical industry moving forward is to create products containing more natural ingredients while maintaining sensory quality. Currently, the food industry makes use of a number of synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) (Caillet et al., 2006) to combat the problem of lipid oxidation, and enhance the shelf-life of lipids and lipid-containing foods. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis (Wichi, 1988). Therefore, in order to overcome the problem the researchers are looking forward to the botanical plant source due to increasing consumer demand for products containing natural ingredients.

The interest in the antioxidant properties of the natural phenolics compounds in plant material was growing faster derives from their strong activity and low toxicity compared with those of artifcial phenolics. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or pharmaceutical industry to replace synthetic antioxidant. The use of these naturally occurring antioxidant polyphenol in place of synthetic antioxidants in foods is a potential area worth investigating. Investigations into the chemical and biological activities of ferns during the past two centuries have yielded compounds in the development of modern artificial organic chemistry and the emergence of medicinal chemistry as a major route to the discovery of novel and more effective therapeutic agents. Thus, ferns are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates.

Fern consists of valuable phytochemicals for medical and food application that has been proven by numerous works (Chirstensen, 1997; Siems et al.1996). Research has revealed the potential of fern as sources of various antioxidants (Ding et al, 2001).Studies

Studies Studies on fern also uncovered for their antimicrobial activities (Faleiro et al., 1999), mineral and heavy-metal content, antifungal activities, antiviral, anti-inflammatory, antitussive, antitumor, anti-HIV and anti-aflatoxigenic activity (Johnny et al., 2011; Zhong et al., 2008; Wu, 1999). *Nephrolepisbiserrata* (Sw.) Schott. (NB) is an edible fern, where the leaf and young shoots of NB frondwere eaten as a vegetable. The leaf is traditionally used as a poultice to treat boils, blisters or abscesses and sores (Chirstensen, 1997). Siems et al., (1996), found drimanes in the epicuticular wax of NB frond. Drimane a group of terpeniccompounds which posses a wide variety of biological activities (antiallergic, antiviral, antifungal, antibacterial, antifeedant, insecticidal, cytotoxic, phytotoxic and others). Gupta (1997) found that NB shows positive to Cholinesterase (ChE) activity. Chemical study on NB was indicated the presence of sesquiyitol known as antidiabetic agent in their frond. Previous research shown, NB possesses potent antioxidant activities, which could be described to their phenolic contents and antioxidant activity (Lai and Lim, 2011).

Previous studies on biochemical activities were usually focused on ground fern rather than epiphyte's ferns. Therefore, an evaluation of antioxidant activity, phenolic and flavonoid content between several species of epiphyte's fern is still unknown. *Nephrolepis biserrata* (Sw.) Schott (NB) fern belongs to the family of Davalliacea and is widely distributed in the tropical areas of Malaysia, Indonesia and Australia. NB is a long creeping fern that is commonly found as an epiphyte on the palm oil trunk in Malaysian palm oil plantation. There are 4.8 million hectares of palm oil in Malaysia, which provide significant raw materials for the extraction bioactive compounds purpose. As compare to another well-known medical plant that requires a high cost of farming, epiphyte's fern is a good alternative for an inexpensive source of phytochemical compounds since it’s easily found and growing naturally on the palm oil trunk without cultivation cost. This study can help the pharmaceutical industry to use it as a natural compound for antioxidant, which might be used as an alternative to common medical plants as well as synthetic antioxidants in the market.

Temperature controlled microwave closed system (TCMCS) is an interesting alternative to conventional MAE extraction systems. TCMCS is a novel and ecofriendly system which comprises an effective temperature controlled and fluid seal stirring device to increase's extraction efficiency of bioactive compound from plant material. Temperature controlled microwave assisted extraction (TCMAE) is a competence method, especially in extraction of thermosensitive compound and also in large scale batch. The TCMAE method of active medicinal ingredient from epiphyte's fern is a relatively unexplored area of research. To the best of our knowledge, the effect of extraction method on antioxidant composition of epiphyte's fern, especially *Nephrolepisbiserrata* (Sw.) Schott. fronds (NBF) not been characterized yet and the dissolution properties of solution before and after extraction has not been reported. In the current work, it is proposed to apply a new TCMCS with respect to the different species of epiphyte's ferns. The aim of this thesis has been to present the reader with a new heating concept of microwave technology and overview of the potential application of TCMCS to provide an efficient energy heating in the medical sectors. In respect of epiphyte's fern, this thesis presents the achievements of TCMCS in exploration of new with low cost of medicinal plant sources.

1.4 OBJECTIVES OF THE STUDY

The objectives of this present study are:

1. To develop a temperature controlled microwave closed system (TCMCS) with fluid seal stirring device.
2. To study the TCMCS temperature heating distribution and the fluid seal stirring device efficiency
3. To study the effect of solvent nature in a temperature controlled microwave assisted to extract (TCMAE) method on the antioxidant and physicochemical properties of epiphyte's ferns.
4. To investigate the competency of extraction temperature method in TCMCS extraction procedure.

1.5 SCOPE OF THE STUDY

To accomplish the objective of this study, the scopes of this work focus on:

i. Temperature controlled microwave closed system (TCMCS) was developed, which comprises of L shape electrically capillary temperature controller to run the extraction under controlled temperature and fluid seal stirring device to increase heating uniformity during extraction procedure.

ii. The calorimetric method was used to study the microwave power absorbance in TCMCS at each microwave power level, while standardized coefficient (*ß*) test, regression analysis of R^2 and volume rate of heat generation was exploited to identify the significant of fluid seal stirring device in TCMCS.

iii. The theory behind microwaves heating principles and extraction mechanism of epiphyte's fern extract was investigated under various solvents on antioxidant and physicochemical properties, scanning electron microscopy (SEM) image and also volume rate of heat generation and penetration depth results.

iv. The capability of the extraction parameters, especially extraction temperature on antioxidant and physiochemical properties also microwave heating principle was studied using temperature controlled microwave assisted extraction (TCMAE) of epiphyte's fern water extraction procedure.

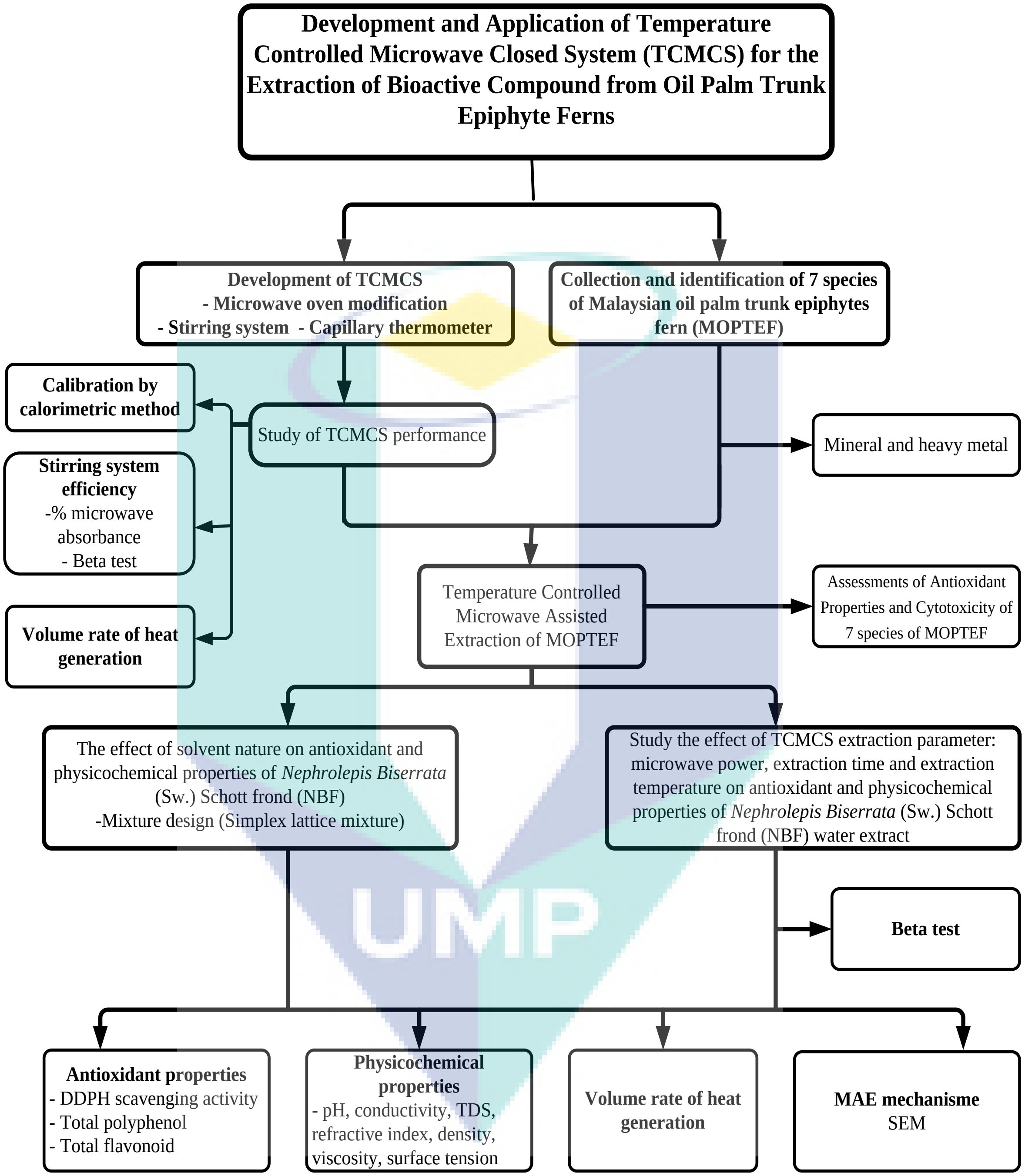

Figure 1.1: Schematic diagram summarizing research methodology

CHAPTER 2

LITERATURE REVIEW

2.1 MICROWAVE TECHNOLOGY

Microwave is an electromagnetic wave of radiant energy. Microwave technology is the outcome of the 1940s electromagnetic energy research on radio frequency was first invented by Sir Henry Tizard during the dismal and desperate early days of World War II (Pound, 1999). Microwave energy for heating has been in commercial use since 1950 (Edgar, 2001). The word microwave defines itself a very short wave. The region of the electromagnetic spectrum of microwave ranging from 300 MHz to 300 GHz falls in the range of infrared radiation and radio frequencies and corresponds on the wavelength of 1cm to 1mm (Camel, 2000; Gould, 1996).

For microwave heating applications, only some discrete frequency bands have been set aside from telecommunication applications for industrial, scientific and medical (then so-called ISM) applications. The ISM bands are defined by the International Telecommunication Union-Radio communication sector in the Radio Regulations dictates that ISM microwave applications operate at specific frequencies. Four frequencies are used for industrial and scientific microwave heating, extraction and drying: 915 ± 25, 2450 ± 13, 5800 ± 75 and 22,125 ± 125 MHz. These frequencies were established for industrial, scientific and medical use by the Federal Communications Commission and confirm to the International Radio Regulations adopted at Geneva in 1959 (Gould, 1996).

Typically, microwave industrial uses the 2450 and 915 MHz band, of which the 2450 MHz band is used for home ovens, and both are used in industrial heating; however, the 915 MHz band should not be used in countries outside Region 2 (Americas, Greenland and some of the eastern Pacific Islands), while outside the United States, frequencies of 433.92, 896 and 2375 MHz are also used. The allowable frequency of microwave for the domestic purpose in Malaysia is 2.45GHz, corresponding to 12.24cm wavelength or 4.9cm depth of penetration in the vacuum (Mujumdar, 1987). At this frequency, the electric field swings the orientation of water molecules 2.45 x 10^9 times every second and the chaos inherent to the system opposes the synchrony of the oscillation with that of the field. It was creating an intense heat that can escalate as quickly as several degrees per second (estimated as 100ºC/s at 4.9 GHz) (Lew, 2002). Thus, within 5 minutes, approximately 43,000 calories are supplied to the microwave cavity for sample heating.

2.2 PRINCIPLE AND MECHANISMS OF MICROWAVE HEATING

Microwave-enhanced chemistry is based on the efficient heating of materials by “microwave dielectric heating” effects. This phenomenon is dependent upon the ability of a specific material or solvent to absorb microwave energy and convert it into heat. The electric component of an electromagnetic field causes heating by two main mechanisms: dipole rotation and ionic conduction (Neas and Collins, 1988). This provides a qualitative understanding of microwave heating mechanism.

2.2.1 Dipole rotation

Dipole rotation refers to the alignment, due to the electric field of the molecules within the sample that have been permanent of induced dipole moments. Dipole rotation is illustrated in Figure 2.1 (a). In the presence of microwave field, the polar molecules of the materials orient themselves with the changing field, like a microscopic magnet, which attempts to align along the field by rotating around its axis. As the electric field of the microwave energy increases, it aligns the polarized molecules. As the field decreases, thermally induced disorder is restored. When the field is removed, thermal agitation returns the molecules to disorder, in relaxation time *t*, and thermal energy is released. At 2450 MHz, the alignment of the molecules followed by their return to disorder occur 4.9×10^9 times per second, and results in very rapid eating. However, the efficacy of heating by dipole rotation depends upon the sample's characteristic dielectric relaxation time, which in turn depends upon temperature and the viscosity of the sample. With increasing temperature, the dipoles try to align more rapidly with the applied field.

Plant materials contain polar molecules that act as a dipole. Water in the material is often the primary component responsible for dipolar rotation. For instance, water molecules are polar molecules with the negative charge centered near the oxygen atom and positive charge nearer the hydrogen atoms and can be said to possess an asymmetric charge center. Other molecules become induced dipoles due to stress causes by the electric field. Dipoles are influence by the rapidly changing polarity of the electric field. When microwaves pass into materials, the electric field tends to pull them into alignment but then, they returned to random orientation as the field decays to zero. The patterns will be continuing with opposite polarity. This build up, and decays of field will occur as much as the electric field reverses 915 or 2450 million times per second depends upon their frequency. The molecules attempting to oscillate at such frequencies generate intermolecular friction, which causes energy conversion from electric field energy to stored potential energy to the material and then to store random kinetic or thermal energy, thus causes the material to heat.

2.2.2 Ionic Conduction

The second mechanism of heating with microwave is through the ionic conduction. Ionic conduction is the conductive (i.e., electrophoretic) migration of dissolved ions in the applied electric field. In the ionic conduction mechanism, ionic species migrate in one direction or the other according to the polarity of the electromagnetic field (Figure 2.1b).

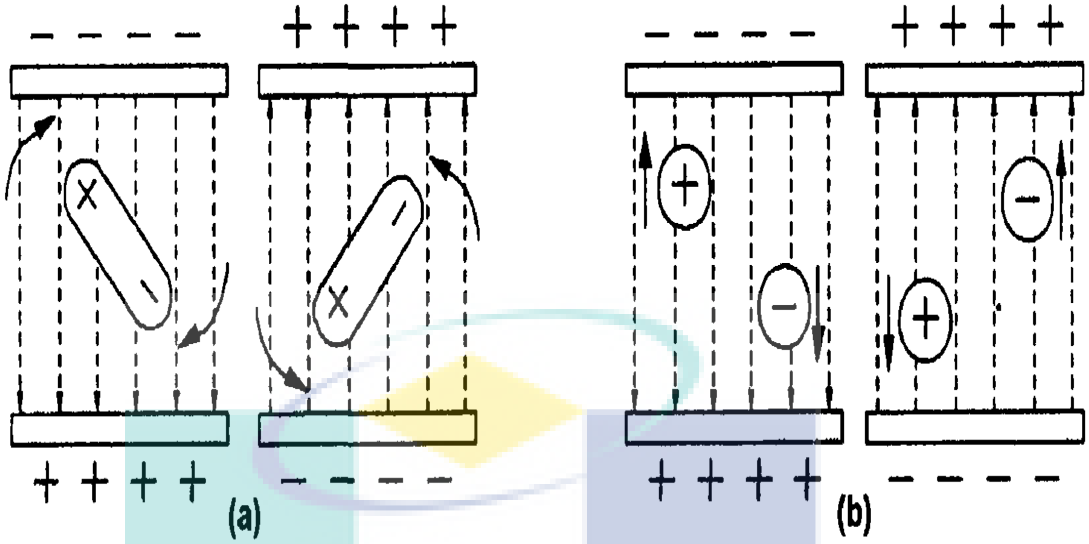

Figure 2.1: Microwave heating mechanism (a) dipolar rotation and (b) ionic conduction

Source: Yam (2006)

Heating is the natural consequence or frequency. It involved in two-step energy conversion: electric field energy is converted to induce ordered kinetic energy, then converted to disordered kinetic energy, which is regarded as heat. Ions, as charged units are accelerated by electric fields. The electric field will cause them to move in the opposite direction of their polarity. As a result, they will collide with unionized water molecules, giving up kinetic energy and causing them to accelerate and collide with other water molecules, and when the polarity changes, the ions accelerate in the adverse pattern.

All ions in a solution contribute to the conduction process, but the fraction of current carried by any given species is determined by its relative concentration and its inherent mobility in the medium. Therefore, the losses due to ionic conduction depend upon the size, charge and conductivity of the dissolved ions and are subject to the effects of ion interaction with the solvent molecules. The parameters affecting ionic conduction are ion concentration ion mobility, and solution temperature. Every ionic solution will have at least two ionic species (e.g. Na^+ and Cl^-ions), and each species will conduct current according to its concentration and mobility. The dissipation factor of an ionic solution will change with temperature because temperature affects ion mobility and concentration.

When microwave energy is introduced into a dielectric material, some part of the energy is transmitted, some part is reflected while some part is absorbed by the material which brings about heating of the material. How well a material heats in a microwave depends on how properly it couples or absorbs the microwave energy and the power level of the microwave. This is controlled in a high degree by the absorption characteristics of the material or product and are more commonly called their dielectric properties.

2.2.3 Dielectric properties

Knowledge of the dielectric properties of the material being heated is of great importance in microwave processing. The ability of a material to interact with microwaves is dependent on the dielectric properties of the material: the dielectric constant (ε') and dielectric loss (ε"). The dielectric constant is a measure of a sample's ability to obstruct the microwave energy as it passes through, and the loss factor measures the sample's ability to dissipate that energy. The word "loss' is used to indicate the amount of input microwave energy that is lost to the sample by being dissipated as heat. The ε' is the measure of the material's ability to absorb microwave energy and the ε" represents the efficiency of converting microwave energy into heat. The basic dielectric relations are as follows (Hoekstra and Delaney, 1974).

$$\varepsilon = \varepsilon' - j\varepsilon'' \qquad (2.1)$$

Where: ε = the complex dielectric constant

ε' = the relative dielectric constant

ε'' = the relative dielectric loss

j = imaginary number (√-1)

$$\varepsilon^{''} = \frac{\sigma}{\omega\varepsilon^{\circ}} \tag{2.2}$$

Where: $\varepsilon^{''}$ = the dielectric constant of free space (8.8 x 10^{-12} Farad/m)
ω = angular frequency (Hz)
σ = electrical conductivity (S/m)

$$\varepsilon'' = \varepsilon' \tan \delta \tag{2.3}$$

Where $\tan \delta$ is the dissipation factor or loss tangent, which represents the ability of the material to absorb microwave energy and pass it on in the form of heat to other molecules. Thus, both ε'' and *tan δ determines* the amount of heat that will be generated when a solvent is subjected to microwave.

When microwave energy penetrates a sample, the energy is absorbed by the sample at a rate dependent upon its dissipation factor. Penetration is considered infinite in materials that are transparent to microwave energy and are considered zero in reflective materials such as metals. The dissipation factor is a finite amount for absorptive samples. Because the energy is quickly absorbed and dissipated as microwaves pass through the sample, the greater the dissipation factor of a sample, the less the penetration of the microwave energy at a given frequency. A useful way to characterize penetration is by the half power depth for a given sample at a given frequency. The half-power depth is defined as that distance from the surface of a sample at which the power density is reduced to one-half that at the surface. The half-power depth varies with the dielectric properties of the sample and approximately with the inverse of the square root of the frequency.The dielectric constant (έ) and dielectric loss (ε̋) of water are given by the following equations 2.4 and 2.5:

$$\acute{E}_{r,w} = 85.215 - 0.33583T \tag{2.4}$$

$$\acute{E}_{r,w} = 320.658T^{-1.0268} \tag{2.5}$$

Which are the least-fit equations to the data given by von Hippel (1954). The temperature, *T* is in ºC. Looyenga's formula for polar solvent's mixture is as follows:

$$Y_{s,m} = \left[Z_{s1} + V_{s2} \left(Z_{s1}^{1/3} + Z_{s1}^{1/3} \right) \right]^{3} \tag{2.6}$$

Where *Y* can be a solvent mixture, where subscription *m* represents a mixture of materials and s represents solvents. The dielectric loss $\left(\varepsilon''\right)$ or tangent loss $\left(\tan\delta_{s,m}\right)$ of mixture of materials was given by Raju (2003):

$$\tan\delta_{s,m} = \frac{\phi_{s,1}\varepsilon'_{s,2}\tan\delta_{s,1} + \phi_{s,2}\varepsilon'_{s,1}\tan\delta_{s,2}}{\phi_{s,1}\varepsilon'_{s,2} + \phi_{s,2}\varepsilon'_{s,1}} \tag{2.7}$$

Where, ϕ is mole fraction. The dissipation factor or dielectric loss tan for the materials in a mxture of two solvents may be approximated by the equation:

$$\tan\delta_{s,m} = \frac{\phi_{s}\varepsilon'_{mix}\tan\delta_{s}}{\phi_{s}\varepsilon'_{mix} + \phi_{mix}\varepsilon'_{s}} \tag{2.8}$$

Usually, most materials are measured in weight grams by so it is often necessary to convert the weight fraction (*w*) to volume fraction (*V*) of the components upon dealing with multicomponent materials (Bicerano, 2002). Thus, the following equation is required for conversion:

$$K_{f}\phi_{m} = V_{n} = \left(\frac{w_{i}}{\rho_{i}} \right) x \sum_{j=1}^{n} \frac{\rho_{j}}{w_{j}} \tag{2.9}$$

Where, ρ_i is the density of each material; w_i is a weight fraction of each material. Subscription n may be a material, a solvent or a mixture of solvents.

2.2.4 Penetration depth

A large penetration depth indicates that radiation poorly is absorbed while short penetration depth means that surface heating predominates. Depth of penetration of microwaves is dependent on dielectric properties of material composition and the frequency, with decreasing frequency being more penetrated (Metaxas and Meredith, 1983). The penetration depth is used to denote the depth at which the power density has decreased to 37 % of its initial value at the surface. Material with higher loss factor ε_r'' show faster microwave energy absorption. The power density will decrease exponentially from the surface to the core region.

As a wave progresses into a dielectric-heating sample, its amplitude diminished owing to absorption of power as heat in the material. In the absence of reflected waves through the material, the field intensity and its associated power flux density fall exponentially with distance from the surface. Because the power absorbed in an element volume of material is proportional to power flux density flowing through it, the power dissipation also falls exponentially from the surface. The rate of decay of the power dissipation is a function of both the relative permittivity, dielectric constant (έ) and loss factor (ε̋). The penetration depth, *Dp* is defined as the depth into material at which the power flux has fallen to 1/e (equal to 0.368) of its surface value and its readily shown to be given by equation 2.10:

$$Dp = \frac{\lambda_0}{2\pi\sqrt{2\varepsilon'}} \frac{1}{\sqrt{\left[\left\{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2\right\}^{0.5} - 1\right]}} \tag{2.10}$$

Where $\varepsilon'' \leq \varepsilon'$, equation (2.10) can be simplified to equation (2.11), with an error up to 10%.

$$Dp = \frac{\lambda_0\sqrt{\varepsilon'}}{2\pi\varepsilon''} \tag{2.11}$$

Where $\lambda_0$is the wavelength of the microwave energy in free space (32.76 cm at 915 MHz and 12.24 cm at 2450 MHz) (Von Hippel, 1954). The penetration depth is a very important parameter for a sample because it gives an immediate first-order indication of the heat distribution within it. Note that *Dp* does not mean that heating at a depth exceeding the penetration depth. The heat dissipates in the layer bounded by the surface and the plane at depth *Dp* is only 63.2% of the total, the balance being dissipated in the material at depth greater than *Dp* (Meredith, 1998). The theoretical examination of the frequency dependence of έ and ε̋ began with the derivation of the Debye equations (Coelho, 1979).

$$\varepsilon'_{d} = \varepsilon'_{\infty} + \frac{\left(\varepsilon'_{\circ} - \varepsilon'_{\infty}\right)}{\left(1+\omega^{2}\tau^{2}\right)} \tag{2.12}$$

$$\varepsilon''_{d} = \frac{\left(\varepsilon'_{\circ} - \varepsilon'_{\infty}\right)\omega\tau}{\left(1+\omega^{2}\tau^{2}\right)} \tag{2.13}$$

Where ε'_{∞} and $\varepsilon'_{\circ}$ are defined as the high frequency and static dielectric constant and ω and τ is the frequency and relaxation times which characterize the rate of build up and decay polarization. The above results apply to both liquids and solids though different models are used to derive it.

2.2.5 Conversion of Microwave Energy into Heat

The thermal properties of materials are also an important factor in the study of microwave heating. As a material is heated by microwaves, the mass heat capacity (*Cp*) of the microwave absorbing material is required to determine the amount of energy absorbed by the material. The mass capacity is the quantity of heat required to raise the temperature of a given mass by 1ºC. The energy absorbed produces a rise in temperature, Δ*T*. If a quantity of energy delivered for a unit of time (*P*) absorbed by a substance (power density) in the microwave cavity may be expressed in the following relation (Kingston and Jessie, 1988). The following equation (2.14) is used to calculate the energy requirements:

$$P_{absorbed} = \frac{\Delta T}{t} \times K \sum C_{p,i}\, m_i \tag{2.14}$$

where:

P = the apparent power absorbed by the sample in watt (W) (joule/sec)

K = the conversion factor for thermochemical sec^{-1} to watt (K=4.184)

C_p = the heat capacity, thermal capacity or specific heat [cal/(g*°C)] of water (heat capacity of water at 25°C is 0.9997)

m = the mass of the water sample in gram (g)

T = the final temperature minus the initial temperature (°C)

t = the time in seconds (s)

The one-dimensional unsteady state heat conduction equation with heat generation may be used to describe microwave heating of material (Taher and Farid, 2001) undergoing to the materials as shown in equation 2.15:

$$\rho_{mix}\, C_{p,mix} \frac{\partial T}{\partial t} = \frac{\partial}{\partial y}\left(\sigma_{mix} \frac{\partial T}{\partial y}\right) + P_{absorbed} \tag{2.15}$$

where

ρ_{mix} = bulk density of dope solution (gm.cm^{-3})

$C_{p,mix}$ = specific heat capacity of mixture of material (cal.g^{-1}.^{0}C^{-1})

$\frac{\partial T}{\partial t}$ = raise of temperature due to microwave radiation (^{0}C.sec^{-1})

σ_{mix} = thermal conductivity of mixture of material (cal.cm^{-1}. ^{0}C^{-1})

y = location of sample

Equation (2.16) comprises of three terms, convective heat transfer, radiation heat due to microwave and conductive heat in the sample respectively. The effect of radiation is very small as well as the convective term, since the sample container (glass) has a low dielectric constant. Therefore, it's in heat generation q_{MW} (cal/sec cm^3) is assumed to be negligible. The volume rate of heat generation with respect to rate of temperature rise (^{0}C.sec^{-1}) would be given by equation 2.16:

:

$$q_{mw} = \rho_{mix} C_{p,mix} \frac{dT}{dt} \tag{2.16}$$

q_{MW} is a constant depending on the electric field and mobility of the materials at fix location.

2.3 MICROWAVE HEATING CHARACTERISTIC IN EXTRACTION PROCESS

The search of natural sources of antioxidant has led many research groups to place their focus on the extraction of new compound from plant materials. The extraction process is one of the most important unit operations in the food and pharmaceutical industry. Compounds obtained from the process may be used as food additives or as nutraceuticals (Moure et al., 2001; Ames, 1983).

Microwave heating is characterized as volumetric heating in which the microwave energy is dissipated as heat to the total volume of mass rather than a surface heating as conventional convective heating. Microwaves pass through most materials uniformly (if the material thickness is relatively smaller than penetration depth). The geometry and the status in which it is presented for microwave extraction can have a profound effect on the recoveries of the compounds. The geometries of the extracted materials are generally in the range of 100 μm – 2 mm. Fine powders can enhance the extraction by providing larger surface area, which provides better contact between the plant matrix, and the solvent, also finer particles will allow improved or much deeper penetration of the microwave.

With microwaves, heat is generated internally within the material. All parts of the material are heated simultaneously and resulting from a uniform heating (if the material is homogeneous), avoiding the large temperature gradients (Schiffmann, 1986). As a result, the thermal gradients and flow of heat are reversed compared to conventional heating, and the microwave heating is volumic and suggesting where microwave technology can offer both technical and financial benefits.

Microwaves selectively heat up the lossy dielectric materials (such as water, etc.) of a matter composed of different dielectric properties, avoids heating of the air, walls or other parts (Schiffmann, 1986). A microwaves couple directly into the core of the material and vaporize the moisture inside (Feng et al., 2002). As a result of the volumetric heating and internal vaporization, the heating time can be reduced substantially (Decareau and Peterson, 1986). This is more dramatically observed for materials having poor thermal diffusivity (Feng, 2000; Stuchly and Stuchly, 1983). Microwave heating can be done at a lower temperatures. This makes possible to eliminate overheating, which are commonly occurred with conventional heating methods (Schiffmann, 1987).

The microwave assisted extraction (MAE) is an interesting alternative to conventional methods, especially in the case of plant material extraction (Ganzler et al., 1986). The use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. The application of microwaves to plant materials was revealed to be more successful in some processes than others as it was shown in Table 2.1. Within specialized areas of application, microwave use has distinct advantages over conventional methods.

Table 2.1: Comparison of MAE and other extraction processes

Extraction technique						
	MAE	**FMASE**	**PLE**	**SFE**	**Soxhlet**	**Sonication**
Brief description	Sample is immersed in a microwave absorbing solvent in a closed vessel and irradiated with microwave energy	Sample is immersed in a microwave absorbing solvent in an open vessel and irradiated with microwave energy	Sample and solvent are heated and pressurized in an extraction vessel when the extraction is finished, the extract is automatically transferred into vial	Sample is loaded in high pressure vessel and extracted with supercritical fluid (most commonly carbon dioxide at pressures of 150-450 bar and temperature of 40-150C). The analytes are collected in a small volume of solvent or onto a solid-phase trap, which is rinsed with solvent in a subsequent step.	Sample is placed in a glass fiber timble and by using a Soxhlet closed the sample is repeatly percoalated with condensed vapours of the solvent	Sample is immersed in solvent in a vessel and placed in an ultrasonic bath
Extraction time	3-30 minutes	10-60 minutes	5-30minutes	10-60minutes	3-48hrs	10-60 minutes
Sample size	1-10g	1-30g	1-30g	1-5g	1-30g	1-30g
Solvent usage	10-40mL	10-150mL	10-100mL	2-5mL (solid trap) 5-20mL (liquid trap)	100-500mL	30-200mL
Investment	Moderate	Moderate	High	High	Low	Low
Advantages	* Fast and multiple extraction * Low solvent volumes * Elevated temperatures	* Fast extraction * Low solvent volume	* Fast extractions * Low solvent volume * Elevated temperatures *No filtration required * Automated	* Fast extractions * Minimal solvent volumes *Elevated temperatures *Relatively selective towards matrix interferences	* No filtration required	* Multiple extractions

Source: Eskilsson and Bjorklund (2000)

2.3.1 Application of Microwave for Extraction of Antioxidant Plant Material

Applications of microwaves in extraction process have been widely investigated due to its unique features in energy transfer. There are many advantages of applying the microwaves technology that has been reported in previous study. Therefore, the applications of microwave to plant material to extract antioxidant and other beneficial compound are detailed in this chapter and these are summarized in Table 2.2

Table 2.2: Application of MAE to plant material extraction

Target Compounds	**Matrix**	**System**	**Extraction condition**	**References**
Azadirachitin	Neem (Azadirachta indica)	Open vessel system, focused	30mL methanol; 150W;30s 0n 30s off; 10minutes	Dai et al. (2001)
Coccaine and Benzoylecgonine	Leaves of Erythroxylum coca var. coca	Open vessel system, focused	100mg sample with 5-30mL methanol; 125W; 30s	Brachet et al. (2002)
Artemisinin	Artemia annua L.	Modified microwave oven (open vessel)	60s power on; water cooling; cycle	Hao et al. (2002)
Piperine	Black pepper (Paper nigrum)	Modified microwave oven	Glass vessel with continuous nitrogen sparging to maintain an inert atmosphere inside the vessel; petroleum ether; 150W;120s	Raman et al. (2002)
Ginger extracts	Ginger (Zingiber officinale)	Open vessel system, focused	5g sample; 1-2mL water or ethanol with 30mL ethanol or hexane; 150-300W; 30-120s	Alfaro et al. (2003)

Table 2.2: Continued

Ginsenosides	Ginseng root	Open vessel system, focused	Ethanol-water; 150W; 15minutes	Shu et al. (2003)
Tea polyphenols and tea caffeine	Green tea leaves	Modified microwave oven (open vessel)	100mL ethanol (0-100% in water); power on (45s) to 90C, then 3s power on for heating and 10s power off for cooling;0.5-8 minutes	Pan et al. (2003)
Berberine	Mahonia bealei (Fort.)	Modified microwave oven (open vessel)	Methanol, ethanol:water (v/v=9:1), ethaol; power on-power off for cycle irradiation	Gao et al. (2004)
Saponins	Chickpea (Cicer arietinum)	Modified microwave oven (closed vessel)	4g sample with 16mL 70% ethanol in water; 300W; 60C; 20minutes	Kerem et al.(2005)
Alizarin, purpurin	Rubiaceae	Closed vessel MARS X system	30% methanol, 120C; 20minutes	Dabiri et al. (2005)
Anthraquinones	Morinda citrifolia	Closed vessel, CEM system	0.1g sample with 10mL 80% ethanol in water, 60C; 30minutes	Hemwimon et al. (2007)
Antihepatotoxic	Actinidia deliciosa	Open vessel system, focused	73% Ethanol; boiling point of solvent; 0.5 hrs;	Bai et al. (2007)
Tetrahydropalmatine; imperatorin; Isoimperatorin	Yuanhu Zhitong prescription	MAE testing system	70% ethanol in water, 500W, 27 minutes	Liao et al. (2008)

Table 2.2: Continued

Flavonoid	Radix Astragali	Closed vessel ETHOS	90% ethanol; 25mL/g material;110°C;25minutes	Xiao et al. (2008)
Phenolic compound	Aromatic plant	Open vessel system, focused	60% methanol and 60% acetone; 20:1 solvent to material; 4 minutes; 750 Watt;	Proestos and Komaitis, (2008)
Glycyrrhizin	Menthazin	Closed vessel MARS system	70°C;13.8minutes;0.25g sample with 2mL solvent methanol: water	Talebpour et al. (2009)
Flavonolignan	Silybum marianum	MAE system	80% ethanol; 25:1 solvent to material; 20minutes; 600W	Dhobi et al. (2009)
Polyphenol	Enteromorpha prolifra	MAE testing HWC-3LA system	40% ethanol;25:1 solvent to material; 25minutes;500W 3 cycle	Luo et al. (2010)
Tyrosine inhibitory activity	Artemisis pallen	Open vessel system, focused	ethanol; 20:1 solvent to material; below 60C;	Kanimozhi et al. (2010)
Tannin	Quercus infectoria	Closed vessel ETHOS system	Methanol 90%;125mL 67C; 30minutes	Asghari et al. (2011)
Phenolic compound	Olive leaves	Modified microwave oven (open vessel)	Ethanol;15minutes boiling point of solvent	Rafiee et al. (2011)
Paclitaxel	Plant cell culture	MAE extraction system (open vessel)	90% MeOH; 1:3 solvent to material; 6 minutes; 40°C)	Lee and Kim (2011)

2.4 LABORATORY MICROWAVE EXTRACTION DESIGN UNIT

The application of the commercial microwave extraction units was performed using two technical/instrumental systems: open vessels (at atmospheric pressure) and closed vessels (under controlled pressure and temperature) (Camel, 2000; Letellier and Budzinski, 1999). Generally, their categorized the open vessels are of the focused/single mode system, and closed vessels are of multi-mode system. Both systems have been shown to have similar efficiencies in extraction of several analytes from plant material (Saim et al., 1997) and each of these systems has advantages and disadvantages. In the open vessel (focused/single mode system) the maximum temperature that can be reached is determined by the boiling point of the solvent or solvent combination used as shown in Figure 2.2(a). The microwave field was focused at “hot spot’, a location where the microwave field strength is high. These cavities require microwave sources that have little variation in the frequency output. Focused/single mode system has had limited (but successful) application in industry because of a limited processing volume over which electric field is useful. Due to the geometric limitations and non-uniformity of the fields, single mode applicators are difficult to scale-up for many industrial applications.

In the closed vessel (muti-mode system), the microwave radiation is allowed to disperse randomly in a cavity, so every zone in the cavity and the sample it contains is evenly irradiated as observed in Figure 2.2(b). In addition, in a closed vessel, the temperature can be elevated by applying correct pressure, similar to the case of the pressurized fluid extraction system (Venkatesh and Raghavan, 2004; Richter et al., 1999). The main parameters to be considered when using the closed systems are: solvent, temperature, pressure, power and extraction time. Figure 2.2 was shown focused mode and multi-mode system.

The home microwave ovens are designing with the multi-mode system where it is usually much larger than one wavelength. Many researchers use home microwave ovens that have been modified to suit their needs as shown in Figure 2.3. The presence of different modes results in multiple hot spots within the microwave cavity. A hotspot is a

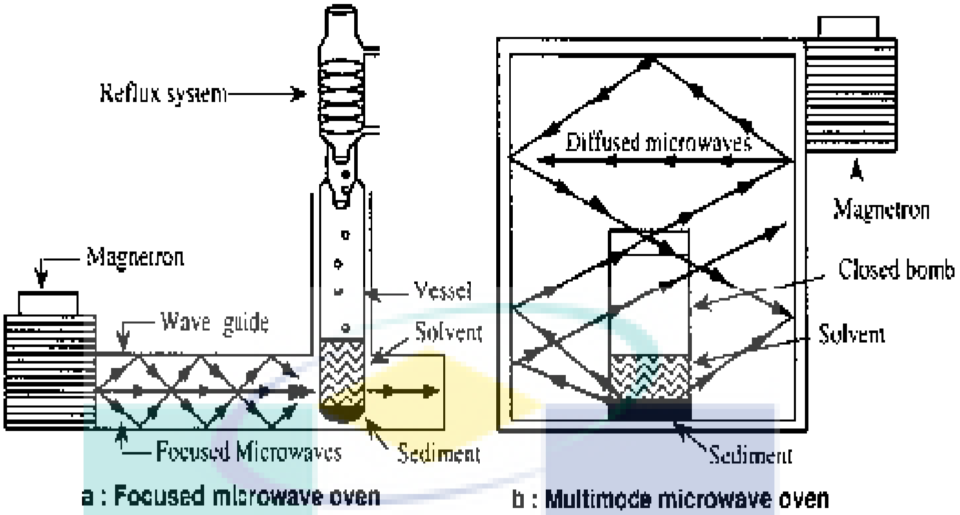

Figure 2.2: a) Focused versus b) Multimode microwave oven

Sources: Mandal et al. (2007)

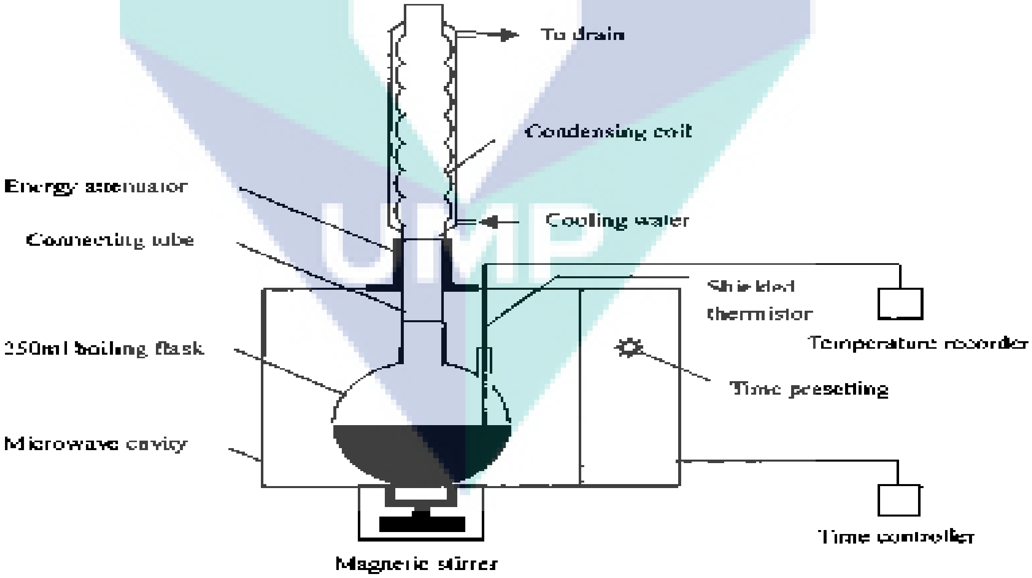

Figure 2.3: Modified microwave ovenfor extraction – reflux system

Sources: Mandal et al. (2007)

type of thermal instability, which arises because of the non-linear dependence of the electromagnetic and thermal properties of the material. It is given operational constraint and controversy of a home microwave oven. Two main factors that cause non-uniform distribution of microwave energy are cavity effects (design limitation, location of feedstock, shape of cavity, hanging parts such as spray gun, mixer or thermometer, etc.) and workload interactions (loss factor, penetration depth and thickness of the workload and particle's features) (Kelen et al., 2006).

Cavity design is an important factor in the control, or the utilization, of this hotspot phenomenon. The formation of standing waves within the microwave cavity results in some regions being exposed to a higher energy than others. This results in an increased rate of heating due to the non-linear dependence. The uniformity of the field increases with the cavity size decrease, thus more uniform heating within a smaller multi-mode oven is the choice. The electric field in a microwave oven can become nonhomogeneous after extended use. Hot spots and cold spots present in the irradiation chamber can be easily visualized using a thin layer of copper sulphate (Ganzler et al., 1986).In a microwave oven, a turnable is equipped to reduce the effect of multiple hot spots by passing the material through areas of high and lower in order to achieve time-average uniformity (Kingston and Haswell, 1997). Mode stirrers and moveable platforms provide some improvements in the field uniformity (Kovach et al., 1998).When the fluid sample is stirred during the extractionprocess, the uneven heat production is equilibrated, and extraction reproducibility is improved (Taib, 2009).

2.4.1 Material Study in the Design of Microwave Extraction System

Microwave heating is a potentially attractive technique as it provides a volumetric heating process at improved heating efficiencies as compared with conventional techniques. If the microwave process is controlled correctly, uniform heating within the material can be obtained. Heat is generated volumetrically within the material rather than from an external source. However, materials differ in their response to microwave heating. Materials possess different optimum frequencies that can be measured and not all materials absorb microwaves. Some materials reflect or appear transparent to microwaves and are thus less responsive to heating.

The interaction of electromagnetic fields with material is important in the design of the microwave extraction system to avoid material's failures such as burning of constructed materials, explosion of extraction system and ineffective extraction process. Materials can be classified into four categories depending on their characteristic when interact with electromagnetic field (Stewart and Amerine, 1982). Conductors such as metals are materials with free electrons. These materials reflect electromagnetic waves without energy absorption and usually are used to contain and directly disposed to microwave. Insulators such as glass are electrically nonconductive materials. Insulators reflect and absorb electromagnetic waves to a negligible extent and primarily transmit them (transparent to the waves). These materials are useful to support or contain materials to be heated by the electromagnetic field. Dielectrics or lossy dielectrics are materials which properties range from conductors to insulators. These materials absorb electromagnetic energy and convert it into heat. The examples of dielectrics are water, oils, wood, sugar and fat. Magnetic compounds such as ferrites are materials interact with the magnetic component of the electromagnetic wave. These materials are often used as shielding or choking devices that prevent leakage of electromagnetic energy.

A material’s ability to couple with (i.e., absorb) microwave energy is determined by its loss factor. Insulating materials do not store microwave energy in the form of heat, as the incident waves travel through the material. Reflecting materials such as metals possess a property such that the incident waves reflect off the material surface. Materials with microwave absorbing properties can be effectively heated at room temperature. However, as the heating mechanisms in microwave systems are strongly temperature-dependent, materials with low microwave conductivities, such as insulators begin to absorb and the even couple more efficiently with microwave radiation when heated above a critical temperature.

Microwaves generate rapid intense volumetric heating of polar substances, which generally results in process energy and time savings (Kingston and Haswel, 1997). In addition, microwave cause reductions in reaction times and, in many cases, higher chemical yields more than 100% for thick, heat sensitive or highly insulating materials. Chemical processes occurring under microwave radiation are believed to be affected by superheating.

The difference between normal (e.g. hotplate) heating and microwave heating is due to the sample heating mechanism. “Normal” heating uses conduction and convection, which are a conventional heating mechanism. Because vessels used in conductive heating are usually poor conductors of heat, it takes time to heat the vessel and transfer who heat to the solution. Also because vaporization at the surface of the liquid occurs, a thermal gradient is established by convection currents, and only a small portion of the fluid is at the temperature of the heat applied to the outside of the vessel. Therefore, when conductively heating, only a small portion of the fluid is above the boiling point temperature of the solution. On the other hand, microwave heating takes place by direct molecular induction. Microwaves heat all of the sample fluid simultaneously without heating the vessel. Therefore, when heating using microwave energy, the solution reaches its boiling point very rapidly.

2.5 DEVELOPMENT OF A MICROWAVE EXTRACTION METHOD

Microwave-assisted extraction (MAE) is a relatively new extraction technique, which utilizes microwave energy to heat the solvent and the sample to increase the mass transfer rate of the solutes from the sample matrix into the solvent. Many reports have been published on the application of microwaves for extracting a bioactive compound from the plants. Aside from material properties and characteristics, operating parameters such as choice of solvent, extraction temperature, extraction time, microwave power and particle size have been found to determine the extent to which a successful treatment is achieved. Control of these parameters is important for the advantages of microwaves to be realised. Without control, non-uniform heating can occur, resulting in the formation of hot spots for example. These areas of high temperature can result in an unexpected heating performance and therefore, reduced overall treatment efficiency and lower effective energy-utilisation

2.5.1 Solvent

i. Solvent nature

The optimal extraction of solvents for microwave extraction cannot always be deduced from those used in conventional procedures. A correct choice of solvent is fundamental for obtaining an optimal extraction. The important natures that must be considered while choosing a solvent are its microwave-absorbing properties, interaction of the solvent with matrix and analyte solubility. The effect of microwave energy is strongly dependent on the nature of the solvent. The solvent should generally be capable of absorbing the microwave energy. Apart from absorbing the energy, the solvent must be able to convert this energy into heat, so the efficiency of the conversion process is dependent on the dielectric factor loss. Dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. The dielectric constant, dipole moment and dissipation factor of some solvents commonly used in microwave extraction are shown in Table 2.3.

Molecules with different dielectric properties, when exposed to microwave radiation, will have a different response to it. Ordinarily, the higher the dielectric constant, the more efficient the molecule absorbs the microwave energy and heated more efficiently. Molecules with very low dielectric constant and loss factors cannot couple with microwave oscillation efficiently. Therefore, will not absorb microwave energy (transparent to microwave energy). Comparison between ethanol and water shows that ethanol has a lower dielectric constant but a higher dielectric loss than water, this indicates that ethanol has lower ability to obstruct the microwave as they pass through, but a higher ability to dissipate the microwave energy into heat. Since dielectric loss and dielectric constant are temperature dependent, thus the dissipation factor (loss parameters of microwave applications) will increase with temperature. Therefore, the heating rate for these polar or nonpolar solvents will increase during microwave heating (Perreux, 2001) probably by limiting the formation of “boiling nuclei” (Baghurst et al., 1992).

Non-polar solvents such as hexane can be heated only if other components in the reaction mixture respond to microwave energy. Non-polar solvents have no dissipation factor value as they are transparent to microwaves. If the reaction mixture contains polar solvent, more concentrated reaction mixtures might be preferable. Under these circumstances, a very high temperature can be achieved. Mingos and Barghust (1991) have claimed that the different rate acceleration can be contributed to the solvent superheating induced by microwave irradiation.

Table 2.3: Dielectric constant, dipole moment and dissipation factor of organic solvents widely used in MAE.

	Dielectric constant, ε' (20°C)	**Dipole movement (25°C)**	**Dissipation factor tan δ**
Acetone	20.7	2.69	0.55
Acetonitrile	37.5	3.12	0.21
Ethanol	24.3	1.96	0.25
Hexane	1.89	<0.1	0.1 x 10^4
Methanol	32.6	2.87	0.64
2-propanol	19.9	-	0.67
Water	78.3	2.3	0.16

Source: Jain et al. (2009); Mandal et al. (2007); Jassie et al. (1997); Zlotorzynski (1995)

It may be worth mentioning that microwave irradiation has been found useful in extraction of specific target substances from plant matrices. It was established that both the efficiency and selectivity of microwave extraction depend significantly on the dielectric constant of the extracting solvent. Most of the time, the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy; however, the extracting selectivity and the ability of the medium to interact with microwaves can be modulated by using mixtures of solvents (Bassirro et al.,1970). For particular purpose, combination of high and low dielectric constant solvent may be used. This allows the extraction of solutes by a cold solvent, thus avoiding any degradation of thermolabile components. In some cases, the matrix itself interacts with microwaves while the surrounding solvent possesses a low dielectric constant and thus remains cold (Jassie et al., 1997). Localised heating leads to the expansion and rupture of cell walls and is followed by the liberation of bioactive compound with the solvent (Garcia-Ayusa et al., 2000).

ii. Effect of solvent to the material ratio

The solvent volume must be sufficient to ensure that the entire sample is immersed, otherwise electrical arcing may be observed (Barnabas et al., 1995). Generally, in conventional extraction techniques a higher volume of solvent will increase the extraction performance, but in MAE a higher solvent volume may give lower yield. One of such type of amount of water in the extracting solvent can penetrate easily into the cells of the plant matrix and facilitate better heating of the plant matrix. This in turn increases the mass transfer of the active constituents into the extracting solvent.

In an industrial extraction process, it is important to maximize extraction yield, but also to minimize the consumption of solvent; that is why the influence of liquid/solid liquid/solid ratio on bioactive compound was considered. In conventional solvent extraction, increasing the liquid/solid ratio (at both constant temperature and time) enhances the solute's recovery since the driving force, that is the gradient concentration, is maximized. In MAE extraction, the same positive effect has been reported by some authors (Xiao et al., 2008; Pan et al., 2003; Hao et al. 2002), but also the opposite effect, that is a reduction in yield with increasing liquid/solid ratio, has also been reported by some authors (Xiao et al., 2008; Wang et al., 2007; Chen and Spiro, 1994). The liquid/solid ratio can influence the solution temperature and the recovery of compound. In fact, for the same extraction duration, if the liquid volume is maintained constant, then the temperature of the mixture will also be almost constant. On the other hand, if the solid mass is maintained constant and the solvent is increased, then the temperature of the mixture will not be constant due to the characteristics of microwave heating, in which the sample heating rate is determined by the power absorption which, on its turn, is determined by the dielectric properties and also by sample size (Barringer et al., 1994).

2.5.2 Extraction temperature

Temperature is one of the most significant parameters influencing extraction. Normally, high temperature usually enhances the extraction, as a result of an increased diffusivity of the solvent into the internal parts of the matrix, as well as an enhanced desorption of the components from the active sites of the matrix (Camel, 2000). In most cases, the extraction temperature is limited by boiling point of the solvent in traditional methods of extraction (e.g. Soxhlet). Closed vessel extraction methods, enable extractions to be performed at higher temperatures than the boiling point of the extracting solvent.

Microwave-assisted extraction consists of heating the extractant (mostly liquid organic solvents) in contact with the sample with microwave energy. The partitioning of the analytes of interest from the sample matrix to the extractant depends on the temperature and the nature of the extractant. Unlike classical heating, microwaves heat the entire sample simultaneously without heating the vessel. Therefore, the solution reaches its boiling point very rapidly, leading to very short extraction times.

Many researchers have been carried out to study the temperature-dependence of recoveries using MAE. They found that there was an optimal temperature in the range of experimental temperature, and the extraction efficiency has linear relationship with the squared difference between the actual and the optimal temperature. Researches by other groups have found that the recovery would be better at a higher temperature. But in most cases, as extractions were done at only two or three temperature points, it is impossible get a model based on the sparse data. In closed systems, increasing the temperature may be detrimental to the extraction, due to the degradation of the selected components. The optimum temperature may depend on the matrix to be extracted. The temperature during microwave heating was difficult to control when the extractions occur at a temperature below the solvent boiling point.

2.4.3 Extraction Time

As in other extraction techniques, time is another parameter whose influence needs to be taken into account. Generally, by increasing the extraction time, the quantity of analyzes extracted is increased, resulted in higher recoveries of compound. However, longer extraction times had a minimal effect on the vitamins E yield (Duvernay et al., 2005). While thermolabile compounds, long extraction times may result in degradation of thermosensitive compounds. Irradiation time is also influenced by the dielectric properties of the solvent. Solvents like water, ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermo labile constituents.

2.5.4 Microwave Power

Microwave power is such factor, which influences each other with extraction time to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. In general, the extraction efficiency was improved by raising microwave power from 100 to 300 W in microwave extraction. During short extraction time, recovery was enhanced with increased microwave power (Mandal et al. 2007). The difference of the components extracted between 100 and 300 watts appeared to be more significant with short extraction time compared to long extraction time. High power with prolonged exposure always involves the risk of thermal degradation. Reports, on the other hand, also exist, which shows that varying of power from 800 watts to 1100 watts had no significant effects on the yield of flavonoids extraction (Camel, 2001). At higher power level settings, the extraction pattern was same whereas purity reduced substantially. Rapid rupture of the cell wall takes place at a higher temperatures when kept at higher power, as a result together with the desired analyzes impurities are also leached out into the solvent. Whereas at low power levels, the cell wall rupture might take place gradually this enables selective microwave extraction.

2.6 ANALYSIS OF PLANT EXTRACTS.

The history of plants being used for the medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several bioactive compounds from these green factories have given birth to some high activity profile drugs. In recent years, considerable attention has been directed towards the identification of natural antioxidants, have shown a growing popularity and faith in the use of herbal medicine worldwide. This may be because of the realization that modern synthetic drugs have failed to provide a “cure all” guarantees to most of the human diseases with often producing undesirable side effects, which at the end the turnout to be more problematic than the actual disease itself. The herbal medicine provides a ray of hope through its cocktail of bioactive compounds, which are believed to act in a synergistic manner, providing excellent healing touch with practically no undesirable side effects, provided its quality is assured off.

The beneficial effects of plant material on health improvement have been partly attributed to their complex mixture of phytochemicals possessing antioxidant activity. Studies have been shown that many plants have chemical components and biological activities that produce finite physiological functions such as antioxidant, antimutagenic and antitumor activities (Kwak et al., 2006) and therefore,could be used to treat various ailments (Omale and Okafor, 2008). The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga et al., 2005; Hill, 1952). Phenolics or polyphenols, including flavonoids are a diverse group of compounds that are present in plants, and are synthesized as secondary metabolites.

“Phenolic compound" is a generic term that refers to a large number of compounds (more than 8, 000) widely dispersed throughout the plant kingdom and characterized by having at least one aromatic ring with one or more hydroxyl groups attached (Cartea et al., 2011). Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway. Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols (Pereira et al., 2007; Crozier et al., 2006). They can be classified based on the number and arrangement of their carbon atoms in flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones and others) and non-flavonoids (phenolic acids, hydroxycinnamates, stilbenes and others) (Crozier et al., 2006), and they are commonly found conjugated to sugars and organic acids. Phenolic has received considerable attention because of, they are known to possess free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action and shows a very good antioxidant activity (Frankel, 1995). Several investigations have shown that many of the plants have antioxidant activities that could be therapeutically beneficial, and it has been mentioned that the antioxidant potential of plants might be due to their phenolic components (Cook and Samman, 1996).

Flavonoids, a group of pholyphenolic compounds with known properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action (Frankel, 1995; Pourmorad et al., 2006). Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties (Miliauskas et al., 2004). The antioxidant ability of flavonoids and phenolic acids is related to the number and position of hydroxyl groups in the molecule; an increase in the number of hydroxyl groups leads to a higher antioxidant activity.

2.6.1 Antioxidant activity

The most commonly used antioxidant methods are those with 2,2-Azinobis (3-ethyl-benzothiazoline- 6-sulfonic acid) - ABTS and with 2,2-diphenyl-1-picryl hydrazyl (DPPH). Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The DPPH free radical (DPPH) does not require any special preparation, while the ABTS radical cation (ABTS) must be generated by enzymes or chemical reactions (Wojdy et al, 2007). In the DPPH free radical method antioxidant efficiency is measured at ambient temperature and thus the risk of thermal degradation of the molecules tested is eliminated (Bondet et al., 1997). DPPH is one of the most widely used methods for reporting the antioxidant potential of plant extracts because it is simple, rapid and relatively inexpensive.

The DPPH antioxidant assay is based on spectroscopic measurement of the loss of color at 517 nm after reaction with the test compounds. This assay is extremely rapid and simple, requiring only a UV-Vis spectrophotometer. DPPH is a commercial oxidizing radical, which can be reduced by antioxidants. DPPH is a stable nitrogen-centered free radical that can be used to estimate antioxidant activity. The DPPH radical has been widely used to test the potential of compounds as free-radicalscavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto et al., 2000 and Soares et al., 1997). It is a free radical donor who accepts an electron or hydrogen to become a stable diamagnetic molecule. In this assay, the violet colour of DPPH was reduced to a pale yellow color due to the abstraction of a hydrogen atoms from an antioxidant compound. The more antioxidants occurred in the extract, the more the DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample (Blois, 1958).

2.6.2 Total Phenolic

Phenolic compounds are very essential for plants due totheir quenching ability because of the presence ofhydroxyl groups (Elmastas et al., 2006). They belong to aclass of antioxidant compounds, which act as free radicalsinhibitors (Ebrahimzadeh et al., 2010). The Folin-ciocalteu assay is the most widely used to estimate the total polyphenol content in a sample, and it was typically characterized by UV-Vis spectrophotometer. A blue color complex of molybdenum blue wasproduced as a result of reaction of phosphomolybdic acidin Folin-Ciocalteu reagent with phenols in an alkaline mediumswhich is then read spectrophotometrically at 760 nm. (Narayanaswamy and Balakrishnan, 2011). The phenolic content is commonly expressed as percent gallic acid equivalents (GAE) per gram of sample. However, reducing substances like sugars and ascorbic acid may also react with the Folin reagent and cause interference and inaccurate estimation, which is one of the main drawbacks of the assay. Phenolics are well established to show antioxidant activity and contribute to human health.

2.6.3 Total Flavonoid

The availability of suitable assay method is an important part of the quality control of plant. Although the active substances of this medical plat are not yet known, flavonoids can be used as chemical's markers of individual flavonoids is, however complex, time consuminutesg and expensive and the measurement of total flavonoid content is an attractive alternative (Soares et al., 2003). The spectrophotometric assay based on aluminutesum chloride complex formation is one of the most commonly analytical procedures applied to flavonoid content determination, which provides a bathochromic displacement and the hyperchromic effect (Mabry et al., 1970).

This procedure include hydrolysis of glycosides, extraction of total flavonoid aglycones with ethyl acetate and complex formation with $AlCl_3$. Although the present in official codes, the method proposed initially by Christ and Müller (1960) presents several analytic limitations that can be configured as a significant source of error. Initially, the technique was developed for analysis of herbal materials containing O-glycoside flavonoids, the ones which, after acid hydrolise and extraction with organic solvent, are quantified starting from the compound formed between the flavonoid aglycones and $AlCl_3$. However, the technique has been indiscriminutesately used for plant material containing C-glycosides flavonoids. How they do not undergo acid hydrolyze, they are discarded in the aqueous phase during the liquid-liquid extraction due to the polarity from glucosyl residue (Petry et al., 2001).

2.7 EPIPHYTES FERN ON THE PALM OIL TRUNK

An epiphyte is a plant that grows upon another plant which it depends for physical support but not for nutrients from their host. Roots may develop primarily for attachment, and specialized structures (for example, cups and scales) may be used to collect or hold moisture. It is non-parasitically where derives its moisture and nutrients from the air and rain and sometimes from debris accumulating around it. Epiphytic plants use photosynthesis for energy and obtain moisture from the air or from dampness (rain and cloud moisture) on the surface of their hosts. Epiphytic plants are sometimes called "air plants" because they do not root in soil.

Ferns belong to a group of non-flowering plants known as Pteridophytes. The pteridophytes constitute the primitive vascular plant group which is found scattered all over the world. Unfortunately, not much consideration has been given towards the utility of pteridophytes yet these possess equal economic importance, including medicinal purpose. In Malaysia, there are 1136 species of pteridophytes (comprising true ferns and fern-alies) have been recorded (Bidin and Jaman, 1999). Fern was commonly used as traditional medicines in the treatment of various ailments or for general healthcare (Baltrushes, 2006; Pigott, 1996; Bidin, 1987). Despite the large number of ferns, knowledge on the epiphyte's fern on antioxidant properties of these botanicals is still scarce. It is well known that many botanicals possess natural antioxidants with high antioxidant activity (Sultana et al., 2007; Hutadilok-Towatana et al., 2006; Juntachote and Berghofer, 2005) and investigations on these were initiated based on their uses in traditional folkloric medicines.

In well-managed palm oil plantations, regular pruning of fronds and weeding to occur and the bases and trunks of oil palms are regularly cleared of weeds, cover crops, creepers and ferns, although some species of epiphytes and climbers still establish. Epiphytes and climbers are plentiful growing on the oil palm's trunk, while herbs, creepers and grasses dominates the ground floor of the palm oil plantations. An epiphytic fern, a group of non-tree plants commonly covered the palm oil trunk. A number of ferns have been identified associated with palm oil epiphytes such as *Davallia denticulate* (Burm.) Mett. and *Nephrolepis biserrata* (Sw.) Schott., *Asplenium longissimum B1*, *Gonioplebium percussum* (Cav.) Wagner & Grether, *Stenochlaena palustris* (Burm.) Bedd, *Vittaria Elongata, Vittaria ensiformis.*

CHAPTER 3

DEVELOPMENT OF TEMPERATURE CONTROL MICROWAVE CLOSED SYSTEM (TCMCS) AND METHODOLOGY.

This chapter presents a development of new temperature control microwave closedapparatus, which is assisted with fluid sealed stirring device for multiple processes. Particularly, this microwave system is designed for carrying out microwave assisted extraction processes. The method includes the steps of placing extract materials in a transparent vessel inside the microwave cavity, which has been sealed by means of two neck lids. The transparent vessels were fabricated in such a way that should contains outside and inside necks at the vessel bottom. Placing the vessel with lids in such a way the lid necks should be outside the microwave cavity, the outside lid's necks can accommodate the fluid sealed stirring device and a condenser attached via Y-type connecter. While the vessel bottom necks were also placed at microwave cavity. The method comprises exposing a plurality of significantly microwave-transparent of vessels containing to extract materials therein to microwave radiation, parallel measuring and controlling the temperature of the extract materials outside vessel using an L-shape capillary temperature sensor embossed at the bottom of the vessel. While measuring the temperature of the materials at outside the cavity, L-shape temperature sensor positioned the externally such way that not only to control the extraction temperature also to control the microwave pulse at any power level at required timing.

3.1 INTRODUCTION

As chemists have long been aware, the application of heat energy is one of the most significant factors in increasing the rate of a wide variety of chemical extraction from plant's materials. Thus, generally familiar devices such as the Bunsen burner, other types of gas burners, hot plates, and other similar devices have historically been used to initiate or accelerate various extraction processes. However, in the modern extraction method, the analysis of elements which are closely related to human health, environment protection, material composition and food safety as well as processing cost attract more and more concern. The term microwave assisted extraction (MAE) refers to the use of electromagnetic radiation within the microwave frequencies to provide the energy required to initiate, drive, or accelerate certain chemical compounds extraction from preferred materials.

Various types of the microwave system for applied chemistry have been suggested as described in Malaysian patent PI20080270, PI 20094768, US Patents 5988877, 5459302, 5601745, 5796080, and 6744024 respectively. Among the patents that reveal apparatus a temperature control microwave digestion and extraction system are U.S. Pat. No. 3859493 which discloses for materials exhibiting a rapid exponential rise in dielectric loss characteristics above a certain critical temperature. U.S. Pat. No. 4081645 discloses a temperature responsive probe is embedded in the food to be cooked, the probe including means for changing its state with variation'stemperature.

U.S. Pat. No. 4553011 discloses microwave heating is controlled by the output value of a temperature sensor. U.S. Pat. No. 4415790 discloses a temperature probe inserted in the product sense temperature and connect to a Wheatstone bridge. U.S. Pat. No. 5595673 discloses a microwave oven comprises microwave radiation sources, a microwave-activable bottom heater and optionally an IR-radiating top heater, which all can be control unit of the oven. U.S. Pat. No. 4217477 discloses a food temperature is sensed by a probe which electrically communicated to a microwave generation control circuit through a plug which is removable engaged in a socket secured by a microwave oven wall.

U.S. Pat No. 6844535 discloses the microwave oven includes a control unit which controls an entire cooking operation of the microwave oven, and a temperature sensing probe connected to the other end of the variable-length wire and a rotating member who winds the variables-length wire therearound.

However, there are several problems with heating material's, particularly organic materials in a microwave, namely the super heated material's problem. For example, water will heat to over the boiling temperature of water at sea level of 100°C without boiling, or going into steam. Therefore, the previous prior art does not mention microwave power and fluid temperature controller. The temperature rise to a microwave process is difficult to measure owing to the sensitive nature of microwaves. The microwave heating is dependent on the dielectric properties of the target material. Unfortunately, such properties are not fully understood, and not all materials possess the ability to absorb microwave radiation.

Certain high cost laboratory microwave use temperature controller to control the temperature inside the cavity. Fiber optic probes are usually employed alternatively, infrared sensor or thermocouple. They have many shortcomings within the probe where, a fiber optics is the best but too much expensive. The infrared sensor was measured without contacted directly to the material inside the reaction vessel. Nevertheless, the temperature reading of infrared sensor was influenced by field of view (target size and distance), type of surface being measured (emissivity considerations), angle of view and temperature range. So most probably the temperature measurement by infrared sensor is not accurate. A thermocouple is the simplest and lowest cost method. It directly reads the temperature when touching the sample. But the shortcoming is, a thermocouple in a microwave field normally can act as an antenna and damage or scramble the temperature meter or “self-heat.". This self-heating can cause errors or uncertainties in the temperature measurement.So in this research, temperature controller so called L shape electrically capillary thermometer with special improvement was studied to solve this gap from previous research.

3.2 DESIGN OF TEMPERATURE CONTROLLED MICROWAVE CLOSED SYSTEM (TCMCS)

3.2.1 Microwave Oven Modification

To accomplish microwave modification several factors must be considered such as the selection of multi mode cavity of the lowest cost commercial microwave oven, which includes the various microwave pulse levels. The MW oven must have a flat roof cavity gap with upper cover and multi mode cavity. Therefore, the selection of multi mode and position of magnetron inside the cavity is very important for microwave modification because of uniform heating (Iqbal, 2009). The microwave oven selected cavity is constructed to distribute a specific frequency (2.45 GHz in most cases) and power inside cavity. A control unit regulates the power value introduced into the cavity in each pulse through an automatic on/off cycle of the magnetron.

In order to extract the epiphyte's fern under microwave irradiation the multi mode cavity of domestic microwave ovens was modified. Figure 3.1 shows the schematic diagram of the modification cavity details of Haier domestic microwave oven model: EA 180M used in this study. The specifications of the Haier domestic microwave ovens are presented in Table 3.1. The magnetron position inside the multi mode cavity was placed according to our experimental requirements. The objective of such size of domestic microwave is to generate as much disarray as possible inside the cavity. The greater the chaos, the higher is the dispersion of radiation, which increases the area that can cause effective heating inside the reaction vessel. Referring Figure 3.1, in order to accommodate the vessel and vessel sealed socket lid inside the microwave cavity, four holes were made into the microwave cavity such that the microwave wavelength must be kept to λ< 10cm Debye. Thus, two holes (2.5cm and 2cm) were drilled inthe roof of microwave cavity for the accommodation of two neck lids, while two holes were made at the bottom of the microwave cavity for the lodging of vessel lids and L-shape capillary temperature controller.

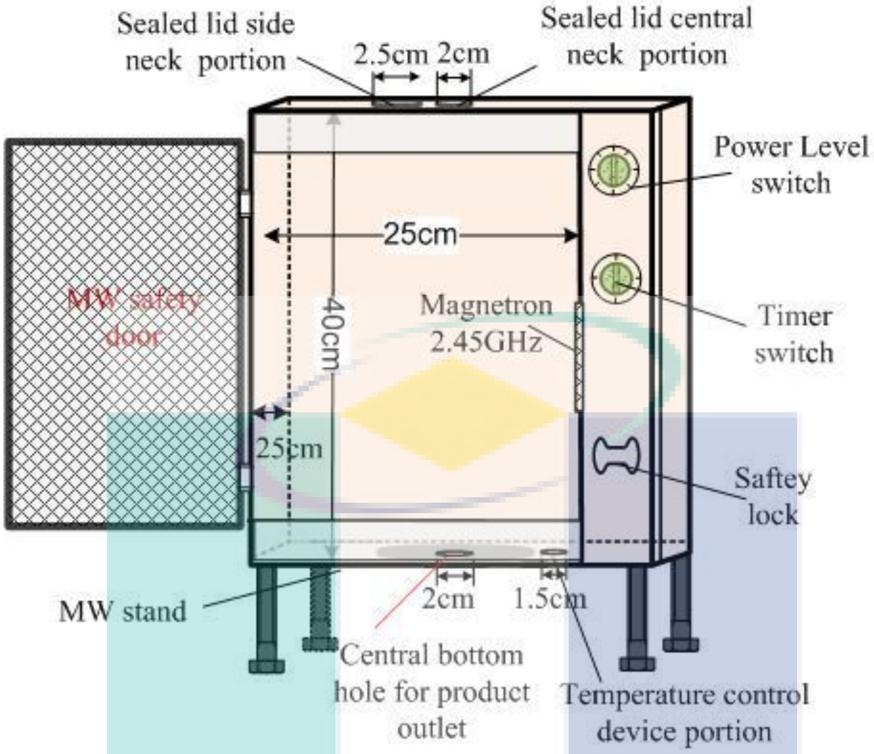

Figure 3.1: Schematic diagram of modified domestic microwave oven cavity

Table 3.1: Specifications of the domestic microwave oven

Brand	Haier
Model	EA-180M
Frequency	2450 MHz.
Power consumption	220~ 240V/ 50Hz ; 1150 Watt
Output power	700 Watt
Pulsed level (%)	20, 40, 60, 80, 100
Power level (watts)	140, 280, 420, 560,700
Cavity dimensions	19 cm (H) * 26 cm (W) * 26 cm (D)
Exterior dimensions	24.7 cm (H) * 29 cm (W) * 40.8 cm (D)
Oven capacity	17 L

3.2.2 Descriptions of Modified TCMCS

It is known in the art that the microwave irradiation is generated through magnetron fixed within the oven and the irradiation is directed to the cavity. The assembled and disassembled closed system improved domestic microwave heating of plant material solution as shown in Figure 3.2. The present study provides a temperature control microwave extraction system assisted with fluid sealed stirring device to accelerate the rate of samples extraction and offers uniform mixing. The apparatus comprises a domestic microwave, a quart glass wider neck vessel, two neck socket lids, Y-type connecter, fluid sealed stirring device made by Teflon, and a L-shape capillary thermometer probe were fabricated by high carbon steel or ceramic but most preferably a quart glass capillary tube. The vessels were fabricated in such a way that its central bottom has 1.85cm neck lid, and a 0.5cm lid embossed besides the wall of the vessel for material temperature sense.

In order to accommodate the vessel and vessel sealed socket lid inside the modified microwave cavity, at first modified reaction vessel and sealed lid necks as illustrated in Figure 3.3 were placed at cavity exterior, the central neck is meant for fluid sealed stirrer, which is then connected to the motor. The stirring blade was positioned inside the vessel by meant of driving shaft. A Y- shape sealed connecter as illustrated in Figure 3.3, which is attached to the perpendicular second neck must be neatly clamped to reflux condenser and the pressure gauge assembly sealing closure. The vessel bottom neck was positioned at the exterior side by means of a central holes of the cavity bottom, while the other vessel neck should be positioned at accurate position on second bottom cavity for the accommodation of the capillary temperature controller.

A quartz glass Y-shape teflon adopter is locked to the vessel sealed lid neck, and a force convection reflux condenser is attached to this portion of the Y-shape openings and a thermocouple to the other. In some instances, it can also act as a hopper for material's addition. The materials to be heated are placed in the vessel during microwave heating and the stirrer is used to ensure homogeneity of the solution. The Y-shape connecter is used as the reflux condenser connecter and is also attached to the thermocouple.

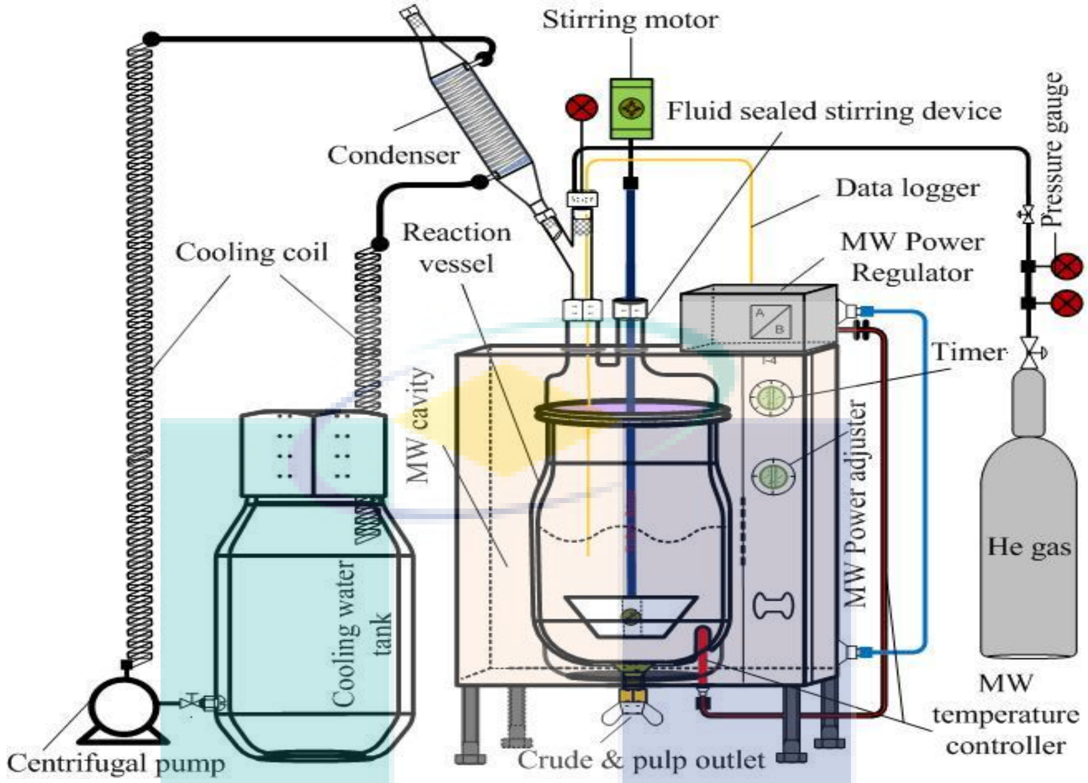

Figure 3.2: Schematic diagram of experimental setup of temperature control microwave closed system

The purpose of the closed system inside the microwave cavity that the system may be pressurized and the temperature of extraction materials may be raised and controlled by means of microwave irradiation. The system must be well enclosed such that it represents an almost closed system. Thus, the solvent's temperature can be raised to their boiling points and their vapor condensed back to the reaction vessel. In this manner, loss of solvents is negligible during the extraction process. The transparent vessel wall is made of microwave permeable material so that the microwave radiation from the source of radiation can be transmitted to the extraction solution in the container through the wall.

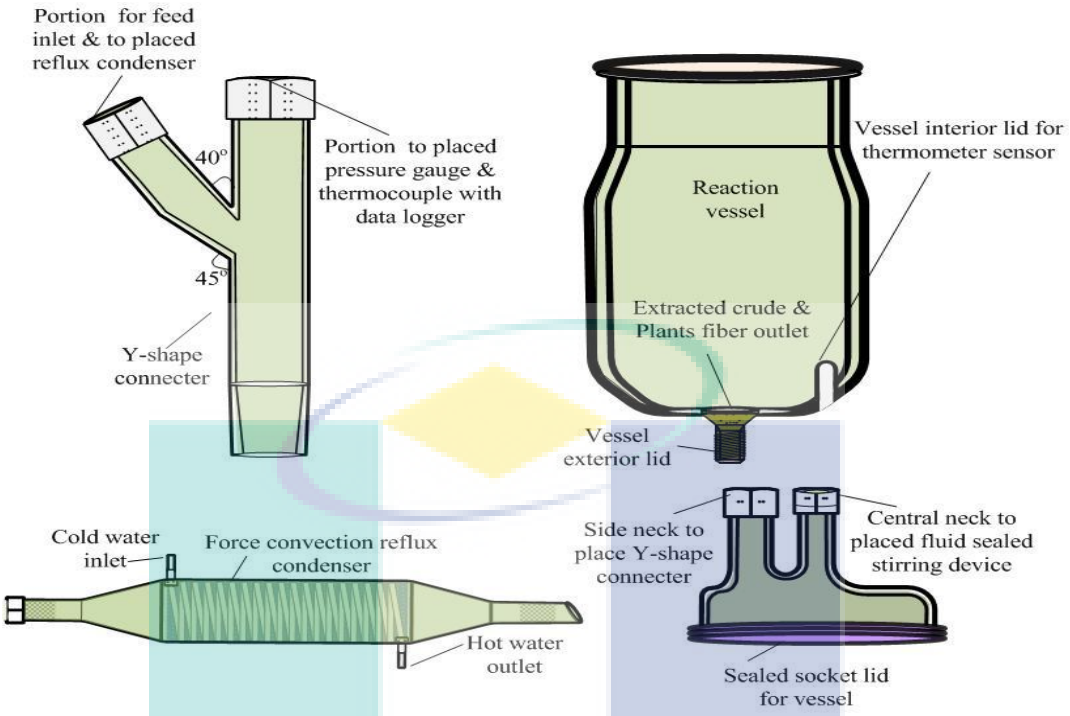

Figure 3.3: Modified microwave closed heating system accessories for extraction

The fluid sealed teflon stirrer rod is placed in the reaction vessel and is rotated by a high speed stirring motor. This means continuous extraction of materials inside the solvent. The stirrer blades rotate unidirectional or oscillate over at least 30-45 degree in a horizontal plane, and a spectacle of the transparent vessel, with a spectacle of materials in therein. In order to control the material's temperature and microwave power the L-shape capillary thermometer were affixed in such a way that the capillary thermometer bulbs were inserted at the bottom of the vessel internal neck by means of inside the microwave cavity. However other side of the capillary a jumper was installed and attached with microwave power controller.

i. Stirring system

In order to assist the temperature controlled microwave closed system with fluid sealed stirring device in this study a Teflon based stirring system were designed according to the desired closed system. Figure 3.4 shows the complete fluid sealed stirring system. The stirring shafts were made of stainless steel rod and coated with teflon. The stirring shafts were inserted inside the tapper form teflon fluid sealed assembly and placed inside the vessel via the central neck of the vessel sealed lid and located in microwave cavity. The stirrer shaft is driven by a high speed motor inside the cavity and to absorb microwave radiations substantially in the horizontal rotary or oscillating between rotary motion. The fluid seal, which is made of teflon material acts as bush or buffer between the neck aperture and the stirrer rod. The high density polyethylene blade is fixed on to the stirring rod or shaft.The fluid seals are placed such that it allows free rotation of the rod but at the same time maintains a substantially fluid-tight chamber. The fluid seals are also adapted such that it can absorb the vibrations caused by the radial and axial motion between the stationary part and the rotatable shaft.

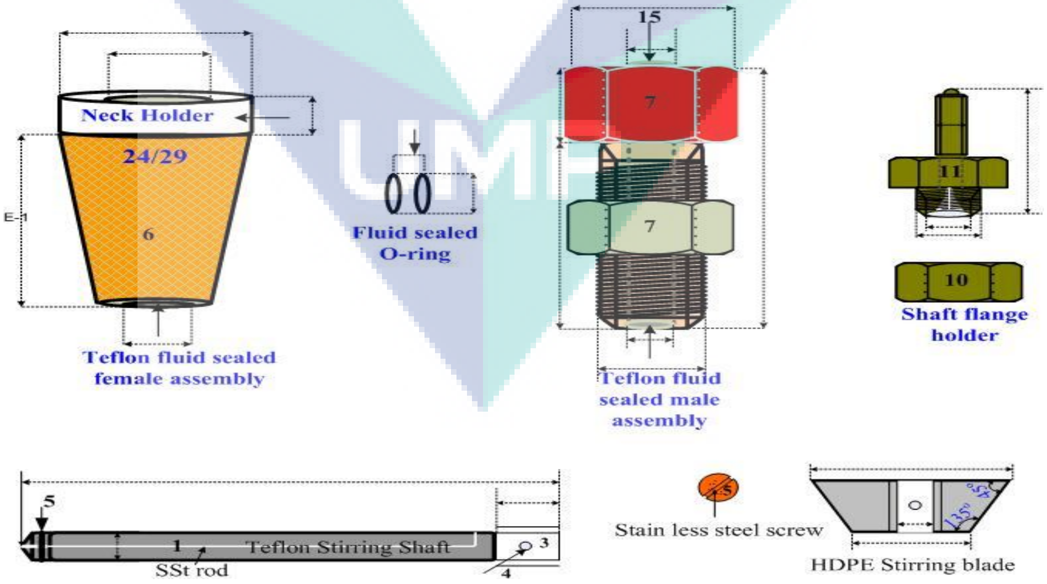

Figure 3.4: Schematic diagram of fluid sealed stirring system for temperature controlled microwave closed system.

ii. Characteristics ofL-shape electricallycapillary thermometer

Certain commercial laboratory microwave also usesthe temperature controller to control the temperature inside the cavity. Most of them use thermocouple, infrared sensor or fiber optic as a temperature probe. They have many shortcomings within the probe which washighlightedin Appendix A. In this study. The L-shape electrically capillary thermometer as shown in Figure 3.5 is attached to the bottom of the reactor in a microwave oven to control a temperature of the material inside the reaction vessel. The temperature responsive probe insertable into materials to be extracts in a microwave oven, and probe is adapted to provide a signal to interrupt the application of microwave power upon reaching a predetermined temperature. Since the microwave temperature is an important factor to get optimum extraction yield, we believe this L-shape electricallycapillary temperature concept is a new innovation as this novel idea never been explored by any researcher. The advantages of this thermometer characteristic also have been highlighted in Appendix A.

Figure 3.5: L-shape electrically capillary thermometer

3.3 MICROWAVE OVEN CALIBRATION

The calibration techniques are necessary to understand and control microwave ovens for microwave-accelerated laboratory procedures (Cheng et al., 2006: Login et al., 1998). All microwave manufacturers perform single setting (100%) full-power output tests as part of their quality assurance program. None perform a multiple calibration procedure to determined linearity of microwave of microwave power output. The reason microwave systems are not calibrated at intermediate to low power level settings by manufacturers relates to how partial level control is accomplished (Bennat et al. 1994).

The advantage of calibration procedure is to avoid assumptions of instrument linearity, and it provides engineering feedback about the contribution to calibration error of each functional extraction process within the instrument. It is necessary to determine the actual output power, which is different from the declared capacity by the manufacturers due to modifications process. Sometimes, this difference is usually because of magnetron filament and heating. Apart from that, variations in power output may also be due to operation of the magnetron for a long period of time or magnetron aging (Cui et al., 2004). When, it was happened, a microwave oven does not convert all electrical energy into microwave and also, not all the microwave energy is totally absorbed during the heating process (Taib, 2009). Therefore, calibration is important to determine the power absorption for each microwave power pulse.

3.3.1 Microwave Power Absorbance of TCMCS Measurements

It is importantto determine the actual microwave power output, which is different from the affirmed capacity by the manufacturers before and after the modification process. In order to measure the output power in a microwave oven, water was selected as standard solvent because of high dielectric constant and dipole moment. In calibrating microwave power output, calorimetrical method, which is to measure the change of temperature of a known mass of water for a known period of time, is applied to determine the power absorption of each pulsed level. The microwave power absorbance, $P_{absorbed}$ in the water was calculated based on the amount of sensible heat required to raise temperature based on equation 2.6. The domestic microwave oven was calibrated before and after modification. The calibration of the domestic microwave oven Haier (EA-180M) before modification was using wide neck vessel closed with glass plate and loaded by 500 g distilled water 28°C ± 2. The multi mode cavity walls reflect the microwaves, which are coming from the magnetron, until it hits the water and is absorbed. Besides that the reflective walls are necessary to prevent leakage of radiation and to increase the efficiency of the oven. There is a rarely perfect match between the frequency used and the resonant frequency of the load, so if the energy is reflected by the walls, absorbance is increased because the energy more often passes through the water and can be partially absorbed on each passage. This can be particularly important if the water volume is dimensionally small. If too much energy is reflected back into the wave guide, the magnetron may be damaged.

To prevent the magnetron from exposure to the excess wall reflected waves, a beaker of water should always be placed inside the cavity, which acts as a dummy load. In this manner, the magnetron and also the operator is protected from the reflected power (Mingos and Baghurst**,** 1991). In order to measure the temperature, the thermocouple probe was inserted inside the microwave cavity and placed inside the reaction vessel (same vessel being used throughout the experiment) at stationary position. The objective of the procedure is to place the sample on a position where the efficiency of the heating is at its maximum. Once the sample is placed in the correct place the heating process starts. Thermocouple type *K* which was equipped to dataloger and connected with computer was used to measure the different temperature of water for every 20 secs until the water reached a boiling point.

Calibration as each power pulse level was carried out in triplicate readings, and the mean value was recorded.In order to determine the power absorption, distilled water was heated in the microwave oven with applying the high mode (700 watts). The similar procedures were repeated for 560 watts, 420 watts, 280 watts and 140 watts. In this research, the temperature different for every 20secs of each pulse was recorded at an atmospheric pressure. The same method was applied in order to investigate the performance of domestic microwave after modification. For the procedure with stirring system, the stirring device was run at 200 rpm at the centre of the reaction vessel loaded with distilled water while 0 rpm for without stirring system.

3.4 MATERIAL AND METHODS

3.4.1 Reagents and solvents

Sodium hydroxide (NaOH), α-tocopherol, butylated hydroxyl-anisole (BHA) were purchased from Sigma-Aldrich. Gallic acid, sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$) was purchased from Benua Sains Sdn. Bhd., 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-cioceltaeu, sodium nitrite ($NaNO_2$), catechin, acetonitrile, acetone and ethanol purchased from Merck Sdn. Bhd. Reagents were of analytical grade.

3.4.2 Apparatus and instruments

Temperature control microwave closed system (TCMCS), thermocouple Type K, Thermocouple data logger (TC-08, Pico Technology), rotary vacuum evaporator (Buchi, Germany), desiccator, freeze drier (Cleanvac 8 BIOTRON, South Korea), cuvette, oven (Memmert, Germany), sonicator (Branson, USA), UV-Vis spectrophotometer (Thermo Scientific model Genesys 10S), cyberscan PCD650 multi meter, digital refractometer (Reichert – r^2i300), micromeritics, AccuPyc II 1340 gas pycnometer, glass pycnometer, Cannon- Fenske Type no. 75 capillary viscometer and surface tension analyzer model DST 60A

3.4.3 Plant Materials

The seven species of epiphytes ferns were collected on palm oil trunk at palm oil plantation in Seri Medan, Batu Pahat, Johor in August 2010, where the soil is combination of clay and peaty soil, pH measured in water (gm/mL) at ratio 10:25 were 5-7, with average temperatures ranging between 25.5 °C (78 °F) and 27.8 °C (82 °F), humidity is between 82 and 86%, annual rainfall is 1778 mm. The species were identified by En. Muhd Ruzi, Faculty of Science and Technology, National University of Malaysia (UKM). The voucher specimens of each species as shown in Table 3.2 have been deposited in the Herbarium of the Rimba Ilmu, University of Malaya for further reference as depicted in Appendix B.

3.4.4 Preparation of TCMAE of Epiphytes Ferns

The microwave assisted extraction's method used here is one of the easiest and quicker than other methods. In this method, the effortless solvent (water, ethanol, acetone, acetonitrile) extracts were used because of its efficient polarity and high dielectric constant properties, which may be more useful for profiling higher amount of extraction of phenolics, flavonoids, and antioxidant's compounds from plant's tissues at a controlled temperatures.

Table 3.2: Collection andIdentification of Malaysian Palm oil Trunk Epiphytes Ferns

Order	**Family**	**Genus**	**Species**	**Common name**		**Specimen no.**	**Voucher no.**
				English	**Malay**		
Polypodiales	Polypodiaceae	*Nephrolepis*	*biserrata*	Giant sword fern	Paku uban, paku larat	FK001	KLU47725
Filicales	Davalliaceae	*Davallia*	*denticulata*	Rabbit's foot fern	Paku tertutup, Sakat laipang	FK002	KLU47726
Polypodiales	Polypodiaceae	*Goniophlebium*	*percussum*	Little foot	Paku lempai	FK003	KLU47727
Blechnales	Blechnaceae	*Stenochlaena*	*palustris*	Miding fern	Paku miding, paku udang	FK004	KLU47728
Polypodiales	Aspleniaceae	*Asplenium*	*longissimum*	Spleenwort	-	FK005	KLU47729
Pteridales	Vittariaceae	*Vittaria*	*Elongate*	Shoestring fern	-	FK006	KLU47730
Pteridales	Vittariaceae	*Vittaria*	*Ensiformis*	Tape fern	-	FK007	KLU47731

In this study, the extraction system was developed by modification of domestic microwave oven (Haier, model EA-180M, Serial number: 6921140329155). It had a rated power output of 700 watts (5.4A, 230-240 V, 50 Hz) with an operation frequency of 2450 MHz. The microwave oven was modified such that the hole length was less than 7 cm to ensure safety and also to accommodate the two necked vessel with fluid sealed stirring device. The details of the vessel and microwave modification were described elsewhere at section 3.2. Each species of epiphytes ferns were initially dried in the microwave oven for about 10 minutes at medium high pulse (420 watts/50°C) to remove moisture until 10-11% moisture content.

Then dried frond of epiphytes ferns were ground by Panasonic MX896TM grinder. They were passed through a stainless steel sieve with pore size of 0.3 mm (Impact Laboratory Test Sieve 850 MIC aperture) before extraction. Subsequently, the plant material (16.5 g) was weighed, approximately but accurately, into a reaction vessel, to which was added 500 mL of solvent placed in the glass vessel setup equipped with glass connecters attached to the reflux condenser and a thermocouple to control the temperature. In order to increase the possibility of interaction between the material inside the reaction vessel and microwaves radiation and to maximize absorption, the plant material and solvent water is placed in reaction vessel which is continuously stirred to ensure uniform extraction. The sample was extracted using TCMCS at specific extraction condition. Then the solution was filtered using Whatman 41 to get supernatant. The supernatant dried by freeze dryer. Sample was stored in a -20°C until further use in antioxidant properties analysis. However for physicochemical properties analysis, supernatant was centrifuge at 9000rpm for 20 minutes to get clear solution before reading process.

3.5 ANTIOXIDANT PROPERTIES ANALYSIS

3.5.1 Determination of total phenolic content (TPC)

Temperature control microwave assisted extraction (TCMAE) water extracts of total phenolic content (TPC) were determined by the Folin-Ciocalteu colometric method (Waterman & Mole, 1994). 0.1 mL of sample extract or blank or gallic acid standard (0-200µg/mL) was added with 2.8mL ultrapure water and 2mL sodium carbonate decahydrate solution (2%). After 4 minutes, 0.1mL of Folin-Ciocalteu reagent (100µL) were added, the reaction mixture was thoroughly shaken and left for 40 minutes in the dark at room temperature. Then, the absorbance was measured at 760 nm using UV-VIS spectrophotometry (Thermo Scientific model Genesys 10S) against the blank prepared for each series of determinations in such way that the sample was replaced by the dilution buffer. The concentration of polyphenols was calculated from the calibration curve using gallic acid and the results were expressed in gallic acid equavalents (GAE mg/g dry weight material). Standard calibration curve was depicted in Appendix C(a). Samples were analyzed in triplicates using equation 3.1:

$$Total\ phenolic\ compound\ (TP) = \frac{C \times V \times df}{M} \tag{3.1}$$

where:

C = gallic acid (µg/mL) (i.e 0-200 µg/mL)

V = volume of plant extract (mL)

M = sample weigh (mg)

df = dilution factor

3.5.2 Determination of Total Flavanoid Content (TFC)

TCMAE water extracts of total flavonoid content of each species was determined according to the method of Marinova et al, (2005) using aluminium chloride colorimetric assay. Briefly, appropriately diluted samples (1 mL) or a standard solution of catechin or blank were transfer into a 10 mL volumetric flask containing water (4 mL) and 0.3mL 5% $NaNO_2$. Reagent blank using water was also prepared. Following 5 minutes, 10% (w/v) $AlCl_3$ solution (0.3 mL) was added with mixing. After 6 minutes, 2mL 1MNaOH was added. The solution was then immediately diluted to a volume of 10 mL with water and was mixed thoroughly. After incubation for 30 minutes at room temperature, the absorbance relative to that of a prepared blank at 435 nm was measured using a UV-Vis spectrophotometer (Thermo Scientific model Genesys 10S). Samples were analyzed in triplicates.The flavonoid was calculated based on the catechin calibration curve with the concentration of 25, 50, 100 µg/mL. Total concentration of flavanols was calculated from the calibration curve using catechinas depicted in Appendix C(b) and the result were expressed in catechin equivalents (mg CE/g dry weight material).

3.5.3 Measurement of DPPH radical scavenging activity (% inhibition, and IC_{50})

The radical scavenging activity of epiphytes species was estimated according to the procedure modified by Braca et al. (2001). An aliquot of 1 mL of epiphytes fern crude extract diluted with 10mL distilled water (1000ppm) was mixed with 4 mL of 0.004% DPPH (dissolved in methanol). The mixture was vigorously shaken and left to stand at room temperature for 30 minutes in a dark room. Absorbance was read at 517 nm by using UV-vis spectrophotometer. Radical scavenging activity was expressed as percent inhibition and was calculated using the following formula:

$$Scavenging\ effect\ (\%) = 1 - \left[\left(\frac{Absorbance\ sample}{Absorbance\ control} \right) \times 100 \right] \tag{3.2}$$

IC_{50} value was determined from the plotted graph of scavenging activity against the concentrations of TCMCS water extracts of each species epiphytes fern samples, which is defined as the concentration of water extract causing 50% inhibition of absorbance. Triplicate measurements at four concentrations were carried, such that a 50% fall in absorbance of the DPPH can be calculated. The IC_{50} value of each extract was measured and compared with the corresponding α-tocopherol and BHA.

3.6 PHYSICOCHEMICAL PROPERTIES ANALYSIS

3.6.1 pH, conductivity and TDS

pH conductivity and TDS was measured by Cyberscan PCD650 Multi meter. The instrument was calibrated before used and the reading was done in triplicate.

3.6.2 Refractive Index

Refractive index was measured using digital refractometer (Reichert – r^2i300). The instrument was calibrated before used and the reading was done in triplicate.

3.6.3 Density

Density was measured in 2 different methods. Electronic pycnometer was used to measure non volatile solvent while volatile solvent was measured by manual pycnometer.

i. Electronic pycnometer

The density of a material is the mass per unit volume. Three replicates of density analysis were performed for each sample. Sample was weighted approximately about 2g and measured by a digital weighing balance with four decimal. The volume of a sample (V, cm^3) was measured using a gas-operated (argon gas) pycnometer (micromeritics, AccuPyc II 1340 gas pycnometer). Measurements were conducted at a temperature of 23°C and a pressure of 101.3 kPa. The mean of five determinations will be used to obtain accurate results.

ii. Manual pycnometer

Density was determined using pycnometer bottle and digital electronic balance with a precision ±1.5 × 10–^{5} g (model AT-201, Mettler Toledo, Switzerland). Prior to measurement the maceration and NBF TCMAE solvent solution, was first empty dry pycnometer bottle weighed, then NBF solvent extract (maceration and extracted) were weights in. Density of the specimens had calculated using the following equation:

$$\rho_s = \frac{A_d}{A_d - B_w} \times \rho_0 \tag{3.3}$$

where ρ_s is density of the solid NBF solvent extract, $A_d$is weight of the solid NBF solvent extract in air; $B_w$is weight of NBF (maceration and extracted crude) in each solvents system and $\rho_0$is density of solvent at given temperature (28^{o}C).

3.6.4 Viscosity

For the measurement of viscosity in controlled settings, ASTM D445 method was applied by Ostwald suspended-level viscometer (Pyrex model number 6942, England) using Cannon- Fenske Type no. 75 capillary viscometer. Capillary viscometer functions by measuring the amount of time a specified quantity of fluid takes to move from its initial position to a final position. It is very important to keep these instruments in a regulated environment with a stable temperature (such as in a water bath) because of viscosity's sensitivity to changes in temperature.

Typically, a suction device holds the bottom of the fluid meniscus at the start position (indicated by the solid horizontal red line), and upon its release the fluid drops an approximate distance of 5 mm (to reach the solid horizontal green line). The time for this drop is recorded so that the acquired data may then be used in Poiseuille’s Law to determine the outcome of kinematic viscosity.

The kinematic viscosity was determined by multiplying the constant of viscometer tube and the measured efflux time, which is the time for a known volume of liquid flowing under gravity to pass through a calibrated glass capillary viscometer tube.

$$\eta_k\,(cSt) = E_t \times K \tag{3.4}$$

Generally speaking, kinematic viscosity (cSt) is related to absolute viscosity (cP) as a function of the fluid's specific gravity (SG) according to the equations (3.5),

$$\eta_A\,(cP) = \eta_k \times SG \tag{3.5}$$

where $\eta_k$is kinematic viscosity in centistoke (cSt), $E_t$is efflux time (second), K is constant of viscometer tube i-e 0.007789, $\eta_A$is dynamic viscosity (cP), and SG is specific gravity of samples, respectively.

3.6.5 Surface Tension

Surface tension analyzer model DST 60A was used with ASTM D971 procedure to measured the surface tension of solvent before and after extraction method. This measurement was equipped with ring and glass sample. The solvent was inserted into glass sample and the ring was adjusted band the analyzer was calibrated at the surface of the sample. Ring was inserted into the sample and the reading of interfacial tension perform was recorded. The ring used in this method must kept under perfectly clean and dust free conditions. It was cleaned by 70% ethanol and burnt with a blue flame from a Bunsen burner after every application.

3.6.6 Statistical and Analysis Data

Standardized coefficients or beta (*ß*) and the mean values of the data from each system were compared using one-way analysis of variance (ANOVA) using SPSS ver. 16.00. *P*-value of less than 0.05 was considered significant. Regression quadratic was used to correlate the relationships between rising temperature and stirring system and regression linear was used to correlate the relationships between antioxidant activities, total phenolic content and total flavonoids content were calculated using Microsoft Excell 2003.

CHAPTER 4

STUDY OF TEMPERATURE CONTROL MICROWAVECLOSED SYSTEM (TCMCS) PERFORMANCE

In this chapter, the performance of temperature control microwave closed system (TCMCS) assisted with fluid sealed stirring device was described. The material, chemical, instrument and method which are involved in the analysis of the research were clarified adequately. A series of experiments was carried out to attain the objectives outlined in Chapter 1. The performance of modified microwave closed system were assessed by calorimetric methods before modification, after modification without stirring system and after modification with stirring system were explained in detailed. Consequently, the different temperature of the particular microwave power level of each condition was studied properly. The % microwave powers absorbance, standardized coefficient (ß) test and regression analysis of R^2 were exploited to identify the competency of fluid seal stirring device in TCMCS. The volume rate of heat generation of different solvents in each microwave pulse was calculated.

4.1 INTRODUCTION

Microwave oven consists of three major components: the sources, the transmission lines and the applicator. The source generates the electromagnetic radiation and transmission lines deliver the electromagnetic energy from source to the applicator. In the applicator, the electromagnetic energy is either absorbed or reflected by the material. The applicator is critical to microwave heating because the microwave energy is transferred to the materials through the applicator. Multi mode applicators are used for designing domestic microwave oven. It is usually much larger than one wavelength. The presence of different modes results in multiple hot spots within the microwave cavity.Non-homogeneous energy distribution in the cavity will create non-uniform heating (Drouzas et al., 1999). Continuous microwave irradiation causes the heating temperature to raise uninterrupted and consequently, results from burning or overheating. Thus, more uniform heating within the smaller multi-mode oven is the choice. Another technique to improve the uniformity is through stirring system. Even microwaves with exceptionally good heat patterns, its still have hot and cool spots were stirring system will minimize the variation. This characteristic has been well discussed in Chapter 3.

Thus, the objective of this chapter is to investigate the performance of temperature controlled microwave controlled system (TCMCS), which is developed by modification of the domestic microwave oven with small cavity, and fluid sealed stirring device The efficiency of TCMCS before and after modification, also with and without stirring system was studied properly to know the mechanism of microwave power distribution of each microwave pulse level and to investigate the rising temperature uniformity, in fact of stirring system. This study very important to define that TCMCS can be performed safely for the extraction of bioactive compound in solvent. Consequently, the volume rate of heat generation in varioussolventin each microwave power level was determined.

4.2 RESULT AND DISCUSSION

4.2.1 Calibration of Microwave Power

Prior to oven modification and after modification, the microwave power absorbance of each microwave power level is conducted. This was carried out to obtain the actual power output since the power absorbance may differ from the value stated by the manufacturer. The microwave power output for each microwave setting was determined calorimetrically. Accurate power measurement is based on a calorimetric method that depends on heating a known amount of water for a certain amount of time. Appendix D was showed the temperature profile of each second for 500 g of distilled water at various MW power levels before modification, and Appendix E was summarized the experimental results of microwave heating, before and after modification. Table 4.1 depicted the power distribution of magnetron for each pulse.

Table 4.2 to Table 4.4 shows the microwave power output for each microwave power setting before and after modification. Table 4.2 shows the maximum output power of the existing microwave oven to be 661.92 watts, which is 94.56% of nominal value of 700watts. It was also observed that, the rates of rising temperature were decreased at when the water almost reached the boiling points. The occurrence is due to the decreasing dielectric properties of water with regards to temperature increase.Moreover, the minimum microwave output power was found to be 72.01% at the low power level which is 100.8watts of nominal value of 140 watts. The heat loss of 140 watts power level due to longer off period as can see in Appendix F where the rest time of 140 watts was 26.63 sec as compared to 280 watts, 420 watts, 560 watts and 700 watts, which show 25.37, 18.55 and 11.58 sec out of 30 sec of the complete cycle respectively.

Table 4.3 and Table 4.4 were revealed that after microwave modification, the results of microwave power absorbance are lower than before modification. The measurement of microwave powers absorbance before oven modification was performed in the reaction vessel closed with glass plate but after modification, it was carried out with the complete set system as shown in Chapter 3 (Figure 3.2-3.5). The high dimension of a complete set of TCMCS was influenced the heating rate of water. In addition, the instrument of the complete system absorbed microwave energy to a certain degree based on the value of the dielectric loss factor depends on the material. In this study, quartz was the use as material of the reaction vessel was categorized as low dielectric loss material but microwave still pass through with little, if any attenuation (Rahmat, 2002).

Overall, the microwave power absorbance shows linear behavior according to ascending microwave power level of microwave before and after modification and with and without stirring system. As observed, the rising temperature of the water increase linearly with the increased of exposure time. Similar finding was reported by Jemaat (2006) who studies the relation between the microwave exposure time and the temperature of the sample at various water-oil ratios.

Table 4.1: Domestic microwave oven power distribution

Power level	**20%**	**40%**	**60%**	**80%**	**100%**
Heat Pulses (watts)	140	280	420	560	700
Radiation time per pulse 60 sec	7	9	23	37	60
Total off time in 60 sec	53	51	37	23	0
Off Time per pulsed (sec)	16	12	8	4	0

Table 4.2: Calibration of microwave oven before modification to heating the water (500g) at 28± 1 °C

MW Power (Watts)	**Time, *t* (sec.)**	**Initial Temp. T_o (°C)**	**Final Temp. T_f (°C)**	**ΔT (°C)**	**P_{absorb} (Watts)**	**Absorbance (%)**
140	200	28.58	38.21	9.64	100.8	72.01
280	200	28.05	50.58	22.22	232.35	82.98
420	200	27.38	63.90	33.47	349.99	83.33
560	200	28.12	74.69	46.57	486.96	86.96
700	200	28.31	91.61	63.30	661.92	94.56

Table 4.3: Calibration of modified microwave without stirring system to heating the water (500g) at 28± 1 °C

MW Power (Watts)	**Time, t (sec.)**	**Initial Temp. T_o (°C)**	**Final Temp. T_f (°C)**	**ΔT (°C)**	**P_{absorb} (Watts)**	**Absorbance (%)**
140	200	28.71	37.75	9.04	94.53	67.52
280	200	28.30	49.71	21.41	223.88	79.96
420	200	28.36	60.65	32.28	337.01	80.36
560	200	28.35	72.07	43.72	457.17	81.64
700	200	28.17	89.76	61.61	644.25	92.04

Table 4.4: Calibration of modified microwave with stirring system to heating the water (500g) at 28 ± 1 °C

MW Power (Watts)	**Time, *t* (sec.)**	**Initial Temp. T_o (°C)**	**Final Temp. T_f (°C)**	**ΔT (°C)**	**$P_{absorb.}$ (Watts)**	**Absorbance (%)**
140	200	28.49	37.59	9.097	95.12	67.95
280	200	29.32	50.96	21.64	226.29	80.82
420	200	28.12	60.78	32.66	341.52	81.13
560	200	28.47	74.04	45.57	476.52	85.09
700	200	27.10	88.91	61.81	646.34	92.33

4.3 STIRRING SYSTEM EFFICIENCY

4.3.1 Percent (%) Absorbance of Microwave Output

Based on the % absorbance readings tabulated in Table 4.2-4.4, Figure 4.1 was plotted to present the relation between stirring system efficiency with microwave output at each level. Upon assembling the setup as shown in Chapter 3 (Figure 3.2), the measurement of % absorbance of microwave output after modification with and without stirring system was performed using Equation 2.6. In all the tests, the output power was noted at low (140 watts) to high pulse (700 watts). The amount of the sample used in this experiment was same as mentioned above with the initial temperature 28°C ± 2. To measure the microwave output after the modification with stirring system, water was continuously stirred at 200rpm and the final temperature was read. % of absorbance was calculated using the same calorimetric method as mentioned above.

The experimental results as illustrated in Figure 4.1 shows the output power of the microwave oven after modification with and without stirring system is to be 92.33% and 92.04% of nominal value of 700 watts respectively. The results revealed that after microwave modification the % of absorbance of TCMCS with and without stirring system are 7.67-32.05% and 7.96-32.48% respectively, lower than before modification, which shows 5.44-27.99% of absorbance and also with the actual microwave power setting given by manufacturer. This is attributed that because of the modification, the microwaves might be having some leakages; however, the microwave leakage detector showed the microwaves in safe mode. Give another reason like surface area of complete system and dielectric properties of each material involved in the complete system like stirrer, reaction vessel and condenser.

As can be observed in Figure 4.1, the result of % absorbance of the microwave oven after modification without stirring system was lower than with stirring system. This is attributed by the design of the domestic microwave oven itself. Domestic microwave ovens are designing with multi-mode system. The presence of different modes results in multiple hot spots within the microwave cavity. A hotspot is a type of thermal instability, which arises because of the non-linear dependence of the electromagnetic and thermal properties of the material (Kelen et al., 2006). In order to get uniform heating of domestic microwave, the materials should be stirred to increase uniformity of the sample where the stirring system tends to even out the temperature caused by uneven heating. Kovach et al. (1998) shows that mode stirrer provides some improvements in the field uniformity within hot and cold spots, which resulted higher % of absorbance where cannot be reached without stirring system.

Based on Figure 4.1, the rising temperature of the water was increased linearly with the increased of exposure time ascending to microwave power level of microwave before and after modification with and without stirring system. The rate of rising temperature for each examination was dependent on the radiation time used in each power level. Overall result shows at 700 watts, the % of absorbance was quite high as compared to other power levels. In this level, the rate of the temperature increase is high because of continuous pulse without rest time. However, according to Bicerano (2002), higher pulse level making it more difficult for dipoles to align in the direction of an applied electric power field on amorphous polymer. But that finding not applicable for water because of it was high in dielectric properties and polarity.

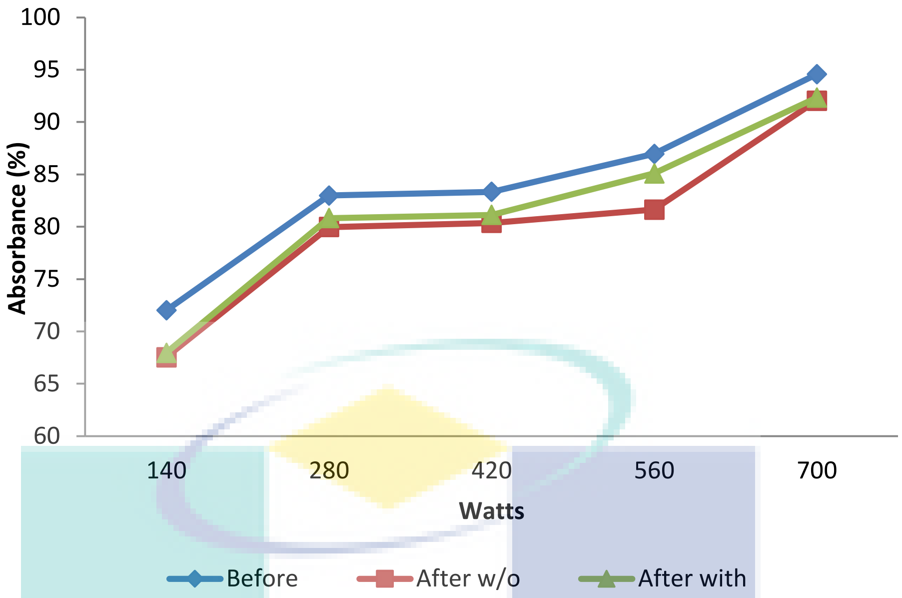

Figure 4.1: % absorbance of microwave output before modification, after modification without stirring system and after modification with stirring system to microwave power level

4.3.2 Standardized Coefficientsor BetaTest

The direction of the relationship between dependent and independent variables can be determined by looking at the regression coefficient associated with dependent variables. There are two kinds of regression coefficients: B (unstandardized) and beta (standardized). The B associated with each variable is given in terms of the units of the variable. The beta uses a standard unit that is the same for all variables in the equation. Beta values are useful to compare two variables that are measured in different units. Values for standardized and unstandardized coefficients can also be derived subsequent to either type of analysis. But in many cases, we need to standardize the coefficients in order to make comparisons. Because a regression carried out on standardized variables produces standardized coefficients.

If the regression coefficient is positive, then there is a positive relationship between variables. If this value is negative, then there is a negative relationship between dependent and independent variables. Thus, the more specifically determine the relationship between dependent and independent variables by looking at the beta coefficient (Beta/*ß*). Standardized coefficients or beta are the estimates resulting from an analysis carried out on independent variables that have been standardized so that their variances are 1. Therefore, standardized coefficients refer to how many standard deviations a dependent variable will change, per standard deviation increase in the predictor variable. In addition, standardized beta coefficient also can be used to compare the strength of the effect of each independent variable on the dependent variable. The independent variable with the largest standardized Beta (independent of the sign) has the strongest effect.

The multiple linear regression analysis findings out of the original (unstandardized) variables produce unstandardized coefficients, which are presented in Table 4.5. Without stirring system, the rising temperature for every 20 sec on distilled water (β=0.230) was significantly associated (p<0.05) with positive correlation. The stirring system (β=0.304) had a positive correlation (p<0.05) with the rising temperature. The result before modification is not significant (p<0.05) and it can be clear observed in Table 4.6. From this result, we can conclude that the stirring system was very important in the microwave process as compared to other factor parameters.

This result was shows that the stirring system is an important factor in the uniformity of the solvent in the microwave system. According to Kovach et al., (1998), mode stirrers and moveable platforms provide some improvements in the field uniformity. The electric field in a microwave oven can become nonhomogeneous after extended use, but by using stirring system the hot spots and cold spots present in the irradiation chamber can be easily homogenized. Therefore, in future experiment, the stirring system was applied in the extraction process because of it believed that during the extraction the uneven heat production is equilibrated, and extraction reproducibility is improved by stirring system.

Table 4.5: Model summary of extraction effect between system and temperature difference increased for every 20 secs.

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	382.362	10.787		35.448	0.000
Before Modification	12.494	13.738	0.107	0.909	0.363
After Modification Without Stirring System	25.197	10.375	0.230	2.429	0.02
After Modification With Stirring System	35.005	14.03	0.304	2.495	0.013
R R-Squared Adj R-Squared		0.219 0.048 0.043	Std error of the estimation		193.655

4.3.3 Heating Mechanism of TCMCS under Different Condition

i. The Influence of Stirring System

Figure 4.2 presents of a heating mechanism of TCMCS before and after modification; with and without stirring system.Temperatures of all conditions were measured across the vertical mid-plane of the reaction vessel. In this study, the TCMCS performance assisted with fluid seal stirring device were investigated at the full microwave power level (700 watts)and the temperature of water was recorded from the microwave oven at 60 sec, 120 sec, 180 sec, 240 sec, 300 sec, 360 sec, 420 sec, 480 sec, 540 sec and 600 sec. The temperature measurements were made using the type-K thermocouple probe connected to a digital meter.

Generally, Figure 4.2 indicates that the temperature increased with respect to increased of exposure time. In the first 240s of microwave radiation, the temperature was increased before become constant when reached the boiling points led by the microwave which is after modification with stirring system, followed by before modification and after modification without stirring system.At 600 seconds microwave radiation, microwave after modification with stirring system achieved the highest temperature of 105^{0}C, while the lowest temperature recorded was 97^{0}C, indicated by after modification without stirring system.In this study, the temperature was reached well above the boiling point of the solvent because the system was categorized as closed vessel.

The temperature elevation is very much dependent on the non-uniform heating due to ‘hotspot’formation phenomenon in microwave cavity (Hill and Marchant, 1996). This thermal uncertainty ascends because of the creation of standing waves within the microwave cavity results in some regions being bare to higher energy than others (Jones et al., 2002). Therefore, stirring system is an important factor in the control, or the utilisation of this hotspot phenomenon especially in liquid form inside the reaction vessel as shown in this study.

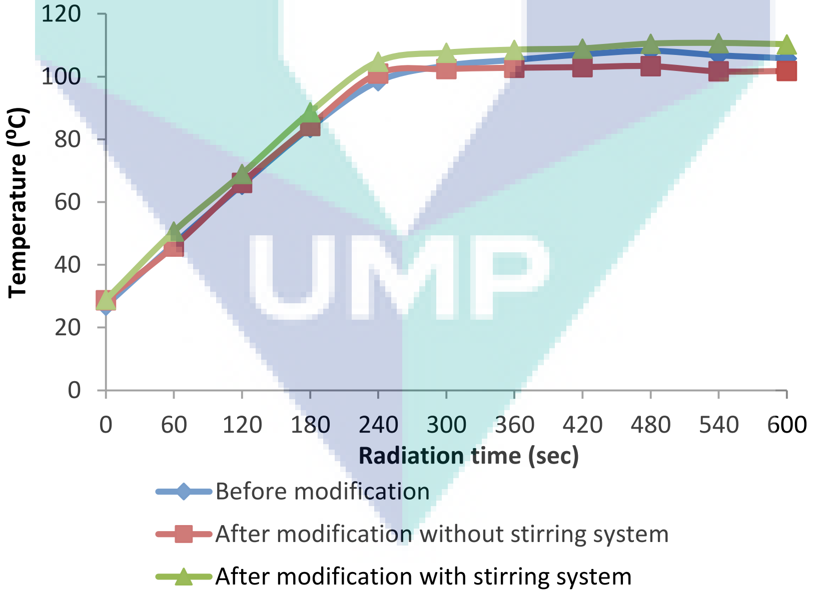

Figure 4.2: Rising temperature of TCMCS before modification, after modification without stirring system and after modification with stirring system to radiation time

Figure 4.3 shows the heating rate of TCMCS before and after modification with/without stirring system.The heating rate was calculated by divided temperature increasing with microwave irradiation time. Results in Figure 4.3 show that the correlation of heating rate was inversely proportional with radiation after 60sec.The trends of heating rate in those three conditions also began constant at 300s. The increasing of heating rate at the first 60 sec shows that microwave having a rapid heating especially for water which is highly polar solvent. Then the heating rate was reduced consistently until reaching a boiling point at 300 sec. From the result, we can conclude that the temperature increase was reflected to declining of dielectric properties of water. Water has a non-symmetric molecule which induces a temporary dipole movement inside the dielectric and generated a heat in the presence of an external electric field.When thetemperature was increases in the matter of radiation time, the dipole rotation, alignment in the microwave field and dielectric of water decreses, which affected the abilty of water to absorb microwave energy.

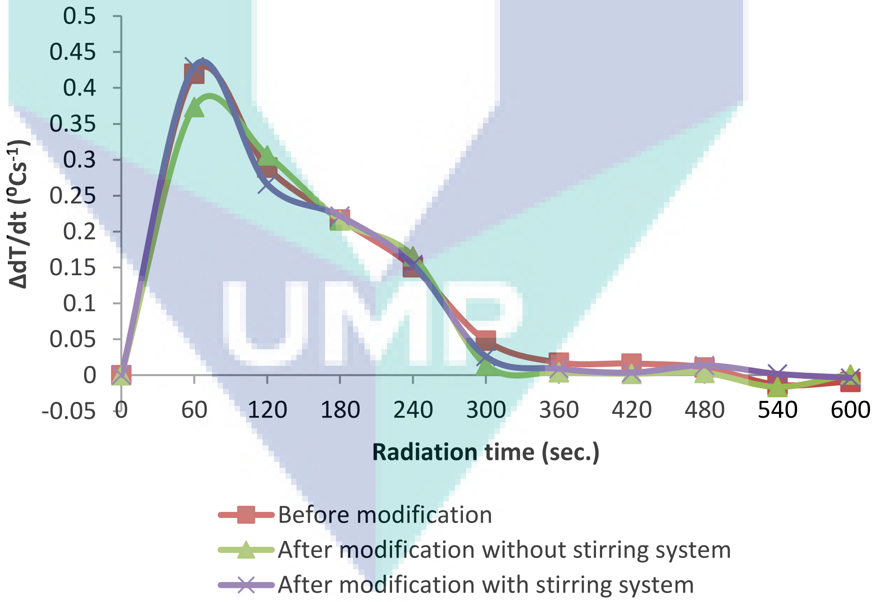

Figure 4.3: Heating rate of TCMCS before modification, after modification without stirring system and after modification with stirring system to radiation time

In order to study the heating mechanism of modified TCMCS with and without stirring device, the relation of microwave power with respect to pulse and rest time on rising temperature of the water during heating at each microwave power level was analyzed respectively. In this study, the estimations of the regression coefficients of all models were done using Microsoft Excel 2003. Linear and quadratic graph was plotted in respect of rising temperature in every 20 sec and the figures with and without stirring system at each power level from 140 watt to 700 watt were illustrated at Appendix G. The results of the regression analysis were tested for R^2 value will give some information about the goodness of fit of a model were summarized at Table 4.6. R^2 was used to statistically measuring of how well a regression line approximates real data points. $R^2$is a descriptive measure between zero and one, indicating how good one term is at predicting another where R^2 of 1.0 indicates that the regression line perfectly fits the data (Himmelblau, 1970). In this topic, the microwave oven before modification was not studied because the rising temperature for every 20 sec are not significant at ($P<0.5$) as shown in Table 4.6.

Table 4.6: Regression coefficient model for with and without stirring system

MW	Linear model (R^2)		Quadratic model (R^2)	
Power	Without	With	Without	With
140	0.03	0.08	0.692	0.704
280	0.437	0.725	0.684	0.874
420	0.675	0.875	0.718	0.886
560	0.665	0.797	0.711	0.893
700	0.628	0.738	0.811	0.901

The TCMCS performance for the water in without stirring and with stirring system under microwave irradiation was shown in Table 4.6 at 140, 280, 420, 560 and 700 watts, respectively. In this study, the quadratic model is the best prediction model which actual data can fit the model as compared to linear model. The quadratic model observed that the interactions between temperatures different of every 20 sec are strongly affecting the length of pulse of microwave power. It was observed from figures in Appendix G, that the temperature difference (ΔT) of water was rising gradually according to irradiation time as per microwave power enhancement, even though without stirring device. But the enhancement of temperature profile is not uniform for the water sample without stirring device as can be observed from the plot. These figures also revealed that during the first 20 secs of radiation, fluctuations occurred for all the pulses, due to many variables such as the alignment of the polar and dipolar molecules oscillation. A physical distribution of the solution shows a significant temperature fluctuation by polar groups. However after this duration, the rates of temperature increase remain almost constant.

Table 4.6 was also shown the quadratic model of with stirring system is a good model as compared to without stirring system. From these result, we found that the rising temperature could not be eliminated by mechanical stirring system since the stirring proved to be efficient method for less viscos to highiest viscos materials to heatup the sample inside microwave cavity.The enhancement of temperature difference profile significantly uniform at presence of stirring device, solvent-free or dry media reactions and for very viscous or biphasic reaction systems where standard magnetic stirring is not effective (Iqbal et al., 2009).

In addition using the stirring device of TCMCS, surprisingly uniformed the temperature gradients on heating water inside a common double-mode microwave with various microwave power. In order to verify the influrecne of stirring system, a similar experiment was performed by heating a sample of N-methylepyrrolidone (NMP) using the microwave system assited with magnetic stirring setup shows insugnificant heating results (Herrero et al., 2008). In this study Herrero and coworker revealed that if competent stirring/agitation cannot be make sured, temperature gradients may develop as a consequence of inherent field inhomogeneities inside a single-mode microwave cavity.

From the result of quadratic model of with stirring system, at microwave power of 700 watt revealed a good model followed by 560 watt, 420 watt, 280 watt and 140 watt. As can be observed in Appendix F, the average of pulse time for 700, 560, 420, 280 and 140 watt is 30 sec, 18.49 sec, 11.49 sec, 4.64 sec and 3.38 sec out of 30 sec of complete cycle respectively. It shows the microwave pulse time length influences the water heating. It is suggested that oflong pulse time period at high power level increase the water reached up the boiling points as compared to short pulse at lower power level.

ii Volume Rate of Heat Generation

The volume rate of heat generation of TCMCS before and after modification also with and without stirring system was calculated based on equation 2.16 as depicted in Appendix H. The heat generation is directly related to the rate of temperature increase, density and heat capacity of the sample. Figure 4.4 illustrates the volume rate of heat generation of water relative to microwave radiation time at 700 Watts of microwave power. In this study, 700 Watts was chosen as consequences of the result of section 4.3.3 were in this power level, its shows a best regression model as compared to other models.

Figure 4.4 illustrates that the volume rate of heat generation was influenced to TCMCS before and after modification also with and without stirring system. In general, the increasing order of volume rate of heat generation is as follows: before modification<after modification without stirring system<after modification with stirring system. This figure depicted the general trend shows the decrease of volume rate of heat generation as a function of temperature, except in the earlry experimental starts (60 sec). At the first 60 sec. of microwave radiation, the volume rate of heat generation increased rapidly. As the exposure time was increased further, the volume rate of heat generation decreased and in certain cases move towarded planteau condition. The incidence is due to the declining dielectric properties of water with regards to temperature increase. The polarity of water decreses with increasing temperature which resulted decreasing of the abilty of water to absorb microwave energy and affected the dipole rotation and alignment in the microwave field. The dielectric properties which is attribute to the solvent polarity is dependent on mobility of the dipoles within the structure (Rahmat, 2002). The dielectric properties which affected the ability of samples to absorb and dissipitate the microwave energy are influenced by heating temperature (Fang and Lai, 1996; Chan and Chen, 2002; Saound, 2004).

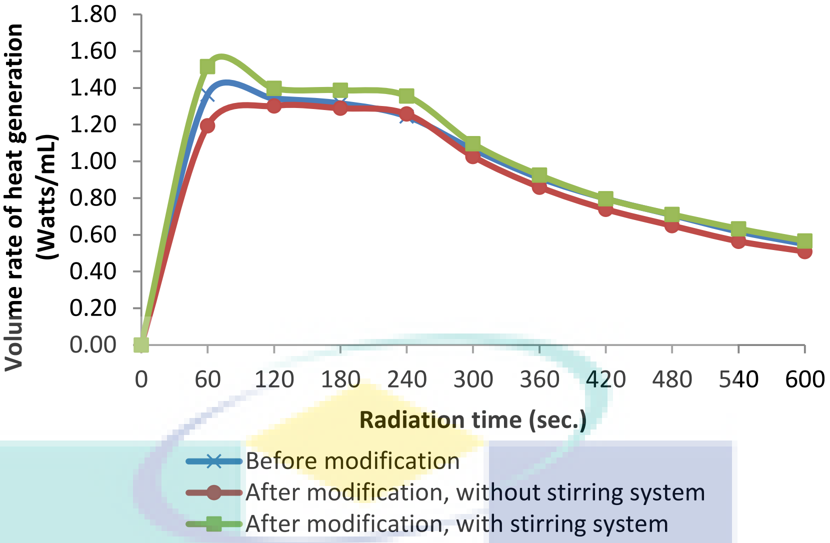

Figure 4.4: Volume rate of heat generation of TCMCS to microwave radiation time

4.4 CONSEQUENCE OF SOLVENT NATURE IN TCMCS

4.4.1 Microwave Power Absorbance

Based on the measurements of rising temperature of every 20 sec for pure polar (aprotic and protic) solvents like acetone, acetonitrile, and ethanol, the microwave power absorbance was calculated and tabulated in Table 4.7. Microwave power absorbed of each solvent in the microwave oven was determined experimentally by measuring rate of the temperature rise after heating known weight of each solvent at every interval time of each cycle pulse. It was observed that the power absorbance by acetonitrile is higher as compared to acetone and ethanoldue to its high dielectric constant and dipolar moment. The microwave powers absorbance by acetonitrile increases with increased microwave power levels, respectively. Almost, a similar phenomenon was observed in acetone and ethanol. In such cases, equalization of thermal energy due to conduction during microwave power-off periods tends to result in a more uniform temperature profile across the sample (Iqbal et al., 2009). But in our study, continuous heating or short power off period showing a good result as can be observed in Table 4.6. The stirring device was believed the influence the uniformity of microwave energy distribution inside the reaction vessel as has been proven from the previous results.

Tables 4.8 showed the experimental results of rising temperature (*ΔT*) during the duty cycle of microwave heating for four solvents. The rises of temperature and microwave power absorbance by the materials are very much related to the duty cycle pulse. At the high pulse, continuous heating occurs and this means that materials are continuously exposed at non stopof radiation time. Consequently, during a very short radiation time, the temperature increments is high.However, at the low and medium low pulse, the temperature increments due to of time per pulse are lower since shorter exposure time to microwave radiation. Similar findings were also reported by Zhu et al. (2003), they has found that the rise of temperature and microwave power absorbed to the materials depend upon duty cycle pulse, and it is establish that at a high pulse level (fixed duty cycle), the duty cycle is constant.

The higher pulse resulted the longer exposure time to microwave. Therefore, it would affect the movement state and reaction activity of the various molecules in the reaction system of solution. However, at low and medium low pulse, fluctuation in cycle duty is observed in each solvent due to its brief exposure time to microwave where the each solvent is irregularly exposed to the irradiation. The same findings were also reported by Huacai et al. (2006).

Overall results showed the greatest temperature rise occurred at medium high (560 watts) and high pulse (700 watts) where the microwave energy absorbed is maximums. This is expected as the sample is dipolar and continuously moving at high (200 rpm) in the closed system inside the cavity. It was observed the continuously high rpm stirring tends to result in a more uniform temperature profile across the sample. Since the closed system is transparent thus microwaves can easily penetrate inside the materials at every direction and also the reflection of microwaves inside the cavity helps maintain the uniform temperature profile

Table 4.7: Performance of TCMCS assisted with stirring system to heating different solvents (500g) at 28 ± 1 °C

MW Power (Watts)	**P_{absorb} (Watts)**				**Absorbance (%)**			
	Water	**Acetone**	**Acetonitrile**	**Ethanol**	**Water**	**Acetone**	**Acetonitrile**	**Ethanol**
140	100.8	79.78	88.1	82.1	72.01	56.98	62.92	58.86
280	232.35	211.15	220.21	217.22	82.98	75.54	78.64	77.75
420	349.99	322.79	335.65	330.93	83.33	76.68	80	78.79
560	486.96	456.55	467.57	462.89	86.96	81.51	83.5	82.66
700	661.92	617.74	631.98	626.97	94.56	88.24	89.28	89.56

Table 4.8: Experimental results of microwave heating at duty cycle pulsed for 500 g of each aprotic and protic solvent

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Polar/dipolar Solvents.	**Low/140 watts**		**Med. low/280watts**		**Medium/420watts**		**Med. high/560 watts**		**High/700watts**	
	ΔT °C	Cycle duty (60 sec.)	ΔT °C	Cycle duty (60 sec)	ΔT °C	Cycle duty (60 sec)	ΔT °C	Cycle duty (60 sec)	ΔT °C	Cycle duty (60 sec)
Water	6.4	0.2	9.1	0.4	16.4	0.6	27.4	0.8	35	1
Acetone	4.3	0.2	6.5	0.4	13.7	0.6	23.3	0.8	30.8	1
Acetonitrile	5.1	0.2	7.7	0.4	15.1	0.6	25.9	0.8	33.2	1
Ethanol	4.8	0.2	6.8	0.4	14.2	0.6	24.7	0.8	31.9	1

4.4.2 Heating Mechanism of TCMCS under Different Solvents

The heating mechanisms of microwave heating and conventional heating are different. In microwave heating, time-temperature profiles within the product are mainly caused by internal heat generation due to absorption of electrical energy from the microwave field, and then heat is transferred by conduction, convection, and evaporation (Mudgett, 1982). Various solvents with dissimilar physical and dielectric properties reveal at different microwave frequencies and exposure time, their polarity, dipole strength, and composition were changed.

i The Influence of Physical and Dielectric Properties of Solvent

In order to investigate the effect of dielectric properties in TCMCS heating mechanism, relation between dielectric properties with rising temperature was studied. In this study, the TCMCS performance assisted with fluid seal stirring device were investigated at the full microwave power level (700 watts) and the temperature of each solvent (water, acetone, acetonitrile, and ethanol) was recorded from the microwave oven at 60 sec, 120 sec, 180 sec, 240 sec, 300 sec, 360 sec, 420 sec, 480 sec, 540 sec and 600 sec.. Temperatures of all solvents were measured across the vertical mid-plane of the reaction vessel. The physical and dielectric behaviors of protic polar solvents; water and ethanol also aprotic polar solvent; acetone and acetonitrile, were obtained from literature as illustrated in Appendix I.

Figure 4.5 shows that the correlation of dielectric constant was inversely proportional with rising temperature. The dielectric constant is the measure of the solvent ability to absorb microwave energy. The interaction of dielectric properties of solvent with electromagnetic radiation in the microwave range results in energy absorbance. If the solvent molecule is not able to absorb microwave energy, there will be no heating. Microwave heating is also referred to as dielectric heating (Oespchuck, 1984). The molecules or atoms comprising the dielectric exhibit a dipole movement. A dipole is essentially two equal and opposite charges separated by a finite distance. This movement generates friction inside the dielectric and the energy is dissipated subsequently as heat

(Kelly and Rowson, 1995). It is observed at Figure 4.5, water shows higher dielectric constant value as compared to other solvent. The interaction of dielectric properties of water with electromagnetic radiation in the microwave range results in energy absorbance. The ability of a water to absorb energy in a microwave cavity is related to the high dielectric properties of the water. This depends on the relaxation times of the molecules in the material, which in turn depends on the nature of the functional groups and the volume of the molecule (Gabriel et al., 1998).

The dielectric constant is one of the most important parameters reflecting solvent polarity. Generally, the dielectric properties of a material are related to temperature, moisture content, density and material geometry (Metaxas and Meredith, 1993). In this study, the lessening of dielectric constant value by rising of temperature can be explained by decreasing of solvent density (Eumatsu & Frank, 1980; Morimoto et al., 2012).

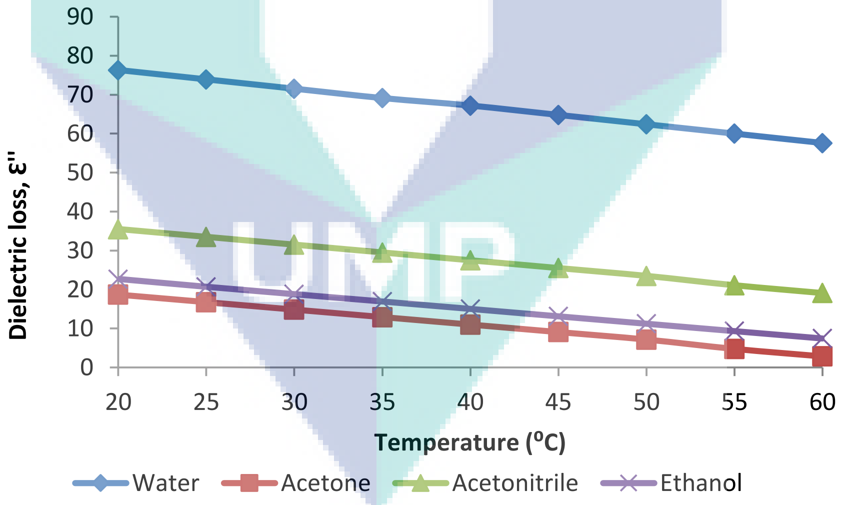


Figure 4.5: Dielectric loss to rising temperature.

In order to investigate the effect of physical properties in TCMCS heating mechanism, the relation between rising temperatures with microwave exposure time was studied. Figure 4.6 was plotted to present the relation between the microwave exposure time and the rising temperature of various solvent (water, acetone, acetonitrile, and ethanol) respectively.As illustrated, the temperature of various solvent increased linearly with increased of exposure time. The rising temperature in various solvent was due to dielectric properties of the solvent. These factors significantly influenced the ability of microwave to interact with the materials and transfer the microwave energy (Chan and Chen, 2002; Islam and Bjorndalen, 2004).

According to Ryynanen (2002), the physical properties such as water volume fraction, viscosity, mass heat capacity and thermal conductivity, was greatly influenced the rising temperature of the emulsion as compared to slight influence on dielectric properties of emulsion. However in this study, it can conclude that physical properties such as boiling points and density of the solvent was affected the temperature increased with time. The lower density of solvent like acetone, ethanol and acetonitrile was increased rapidly within 120 seconds as compared to water which is having higher density. In addition, water which is higly boiling points was taking time to reach that point as compared to other solvent which is reaching constant temperature less than 120 seconds.

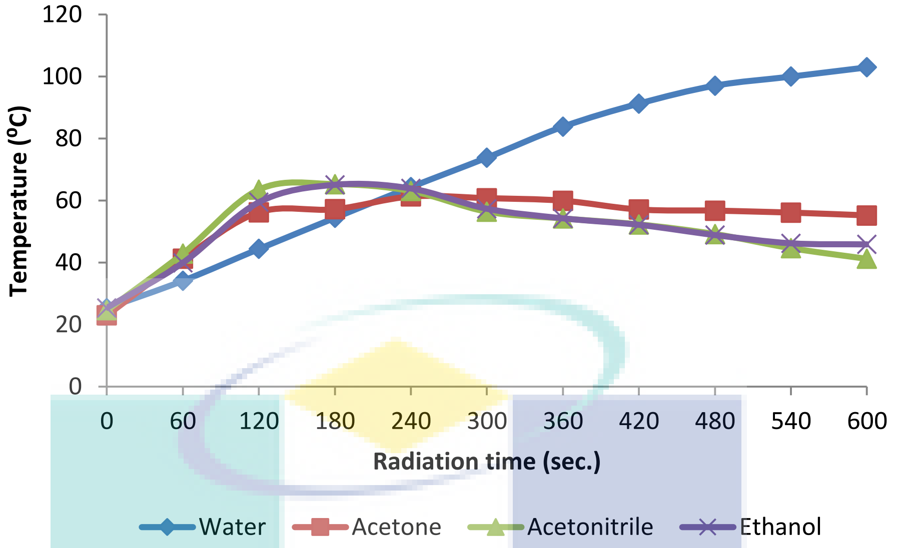


Figure 4.6: Rising temperature increased of TCMCS versus radiation time

In order to investigate the effect of thermal properties in TCMCS heating mechanism, the relation between heating rate with microwave exposure time was studied.Based on the heating rates tabulated in Appendix J, Figure 4.7 was plotted to present the relation between heating rate with microwave exposure time. The heating rate was calculated by divided temperature increasing with microwave irradiation time. As observed, in the first 60 sec of microwave radiation, higher temperature increament was recorded. After that, the temperature decresed drastically led by acetone, ethanol and acetonitrile.

The temperature elevation is very much dependent on the thermal property of the material. When a material is heated by microwave, the mass heat capacity (cp) of the microwave absorbing material determines the amount of energy absorbed by the material. The mass heat capacity is the amount of energy required to raise one degree of temperature of a given mass of material (for example, it tales 4.18 joules of energy to raise the temperature of one gram of water 1^0C, so the heat capacitry of water is 4.18J/g^0C or 75.327J/m.K). From this study, it was found that the highest heat capacity of acetone (124.2 J/m.K) while the lowest was water (75.327 J/m.K).

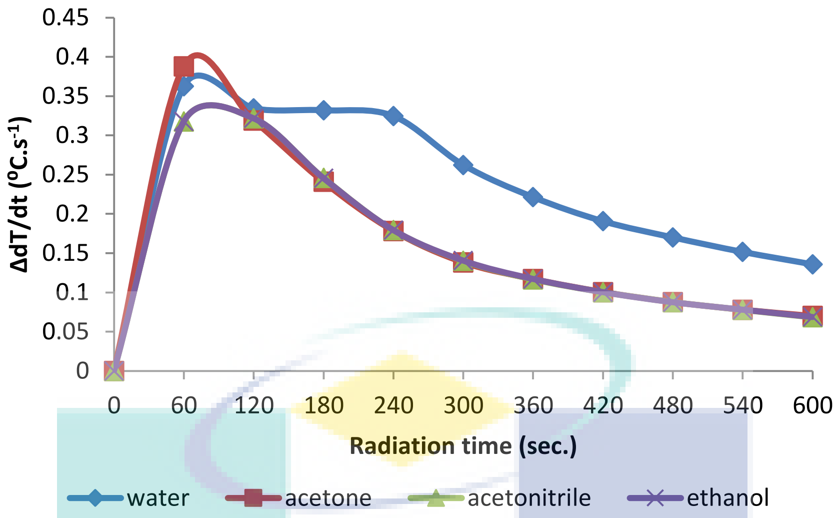


Figure 4.7: Heating rate of TCMCS to radiation time

Overall results was also depicted in Appendix J showed the greatest temperature rise occurred at medium and high power level according to dielectric values of each solvent where the microwave energy absorbed is maximums. It's clearly showing that the continuous or high pulsed microwave power level has enormous influence on rate of the temperature increase inside the closed system with respect to radiation time (sec) for each solvent.This is expected as each solvent is polar and dipolar in nature and continuously moving at high rpm in the closed system inside the cavity. It was observed the continuously high rpm stirring tends to result in a more uniform temperature profile across the sample. Since the reaction vessel in the closed system is transparent thus microwaves can easily penetrate inside the materials at every direction and also the reflection of microwaves inside the cavity helps maintain the uniform temperature profile.

ii. Volume Rate of Heat Generation

In order to investigate the effect of physical and dielectric properties in TCMCS heating mechanism, relation between dielectric constant, dipole moments and heat capacity of protic and aprotic polar solvents with volume heat generation value was studied. In this study, the TCMCS performance assisted with fluid seal stirring device were investigated at the full microwave power level (700 watts). The heat generation is directly related to the rate of temperature increase, density and heat capacity of the solvents. Since the rate of heat generated by the materials inside the microwave cavity is instantly interrelated to the aptitude of the molecules to align itself with the frequency of the applied field. Therefore, the rate of heat generation of solvents can be calculated using equations 2.16.

As illustrated in Figure 4.8, the result of volume of heat generation solvents increased almost linearly with the increased of exposure time. The maximum rate of heat generates at the high-power level were observed in water, which is 341.65 Watts/mL and the minimum is 71.98 Watts/mL of acetone.It is observed that each solvent, exhibits temperature increments according to the dipole and high dielectric constant asset value, which is followed as water> ethanol>acetonitrile>acetone, respectively at all power levels. The difference is the microwave energy absorbed is due to the different dielectric properties of each solvent. Acetone has a lowest dielectric constant of among all selected solvents, thus attributing to low microwave energy absorbed.

As a strongly high dipolar moment, water can efficiently absorb the microwave energy and transform it into thermal energy, leading to the efficient heating of the sample. Apart from absorbing the energy, the solvent also must be able to convert this energy into heat.The capability of a water to convert microwave energy into heat at a given frequency and temperature is determined by the so-called loss tangent ($tan\ \delta$), expressed as the quotient, dielectric loss/dielectric constant ($\varepsilon'' /\varepsilon'$) were describing the ability of molecules to be polarized by the electric field (Herrero et al., 2008). As a conclusion, asolvent with a high $tan\delta$ at the standard operating frequency of a microwave synthesis reactor (2.45 GHz) is required for good absorption and, consequently, for efficient heating.

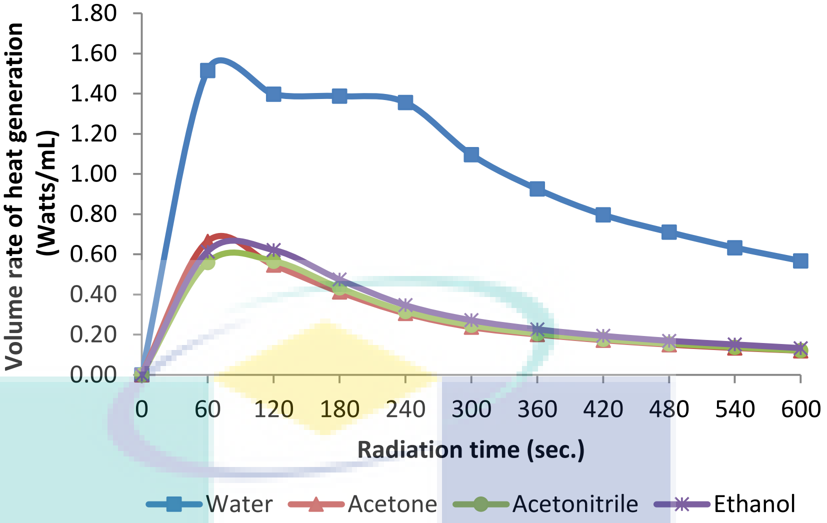


Figure 4.8: Volume rate of heat generation (q_{MW}), of various solvents to radiation time

The result of heat generation between acetonitrile, ethanol and acetone are close each other even clearly showing far value of dielectric properties. The magnitude of the solvent dipole moment is another main factor that correlates with the microwave heating characteristic of protic and aprotic polar solvent. Inspection of dipole moments values suggest that acetone with dipole moments of 2.69 or acetonitrile with dipole moments of 3.44 will rotate easily when exposed to an alternating electric field of microwave energy (Bottcher, 1952) as compared to water (2.3) and ethanol (1.96). This oscillation produced collisions with surrounding molecules, then energy was transferred with subsequent heating (Saoud, 2004). Acetone molecules illustrate a high heat capacity (Appendix I) as compared to be other solvent. This led to higher requirement for energy to be absorbed and consequently, difficult to raise the temperature inside the microwave cavity (Jemaat, 2006). These led to lower volume rate of heat generation of acetone than other solvent. Therefore, certain criteria must be taken into consideration before solvent or combination of solvents is chosen for microwave extraction in the future study.

4.4.3 Heating Mechanism of TCMCS under Different Microwave Power Level

i. The Influence of Microwave Power Level

Figure 4.9 and Figure 4.10 presents of rising temperature and a heating rate of TCMCS in different microwave power level respectively. Temperatures of all conditions were measured across the vertical mid-plane of the reaction vessel. In this study, the temperature of water was recorded from the microwave oven at every ten intervals of time. Each interval of time was kept to 60 sec, 120 sec, 180 sec, 240 sec, 300 sec, 360 sec, 420 sec, 480 sec, 540 sec and 600 sec and temperature measurements were made using the type-K thermocouple probe connected to a digital meter. The rising temperature (Figure 4.9) results show the temperature TCMS at 700 watts increase rapidly as compared to another power level. In general, the increasing order of rising temperature is as follows: 700>560>420>280>140 watts. At 240, 320 and 500 sec.the water temperature reached the boiling points (100^{0}C) in 700, 560 and 420 watts respectively before the temperature rises in those three conditions began to constant and uniform.

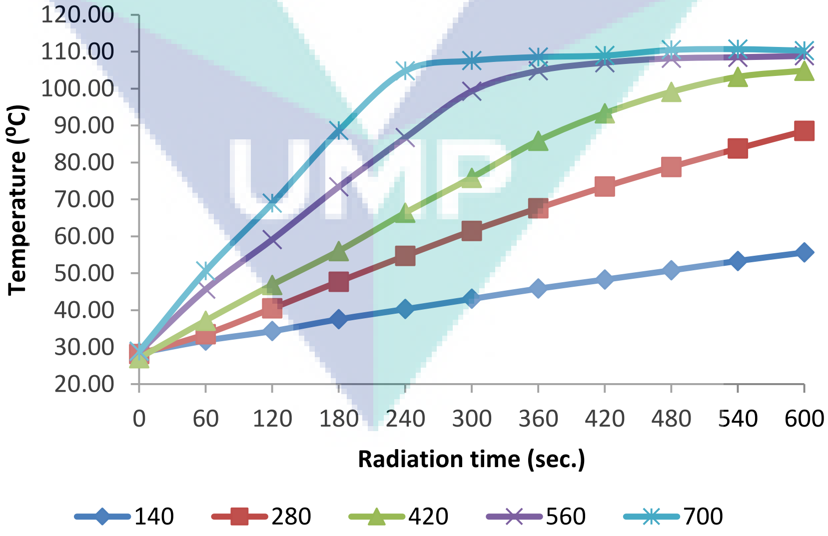


Figure 4.9: Rising temperature of TCMCS in various power levelto radiation time

Based on the heating rate readings tabulated in Appendix J, Figure 4.10 was plotted to present the relation between the heating rate and the microwave exposure time at various microwave power level. The heating rate was calculated by divided temperature increasing with microwave irradiation time. As observed, the temperature of the water increased drastically at first 60 sec. before dropped for 60 sec.with increased of exposure time. On the other hand, the rate of heating rate was related to the watts of microwave. In general, the increasing order of heating rate is as follows: 700>560>420>280>140 watts.

The accelerated heating rate by increasing microwave power can be correlated to the direct effects of microwave energy on ionic conduction and dipole rotation which result in power dissipated in a volumetric fashion inside the water which resulted molecular movement and heating. More electromagnetic energy was transferred to the water when the microwave power increased from 140 to 700 watts and improved the heating rate.

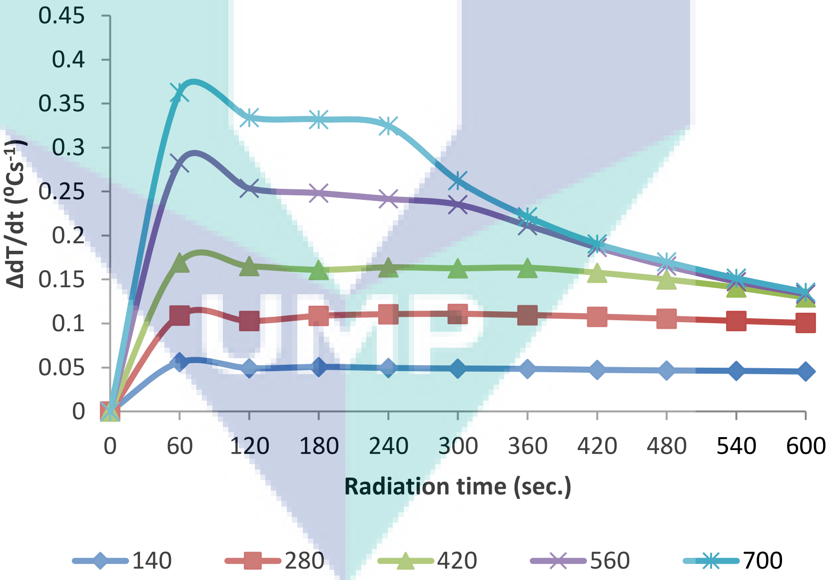


Figure 4.10: Heating rate of TCMCS in different power level to radiation time

ii. Volume Rate of Heat Generation

Based on the result of volume of heat generation reading tabulated in Appendix I, Figure 4.11 was plotted to present the relation between the rates of temperature increment of waterwith the different microwave power level. As observed, temperature increment of water as was shifted from the low, medium low, medium, medium high and high power level. At high power level (700 watts), the rate of temperature increases is high, at about 0.45%, and this indicates that the microwave heating is very rapid at the high pulse. At the initial period, heating rate (*dT/dt*) increased because microwave energy was applied continuously and this resulted in higher average ofq_w is about75.57 Watts/mL. Figure 4.4 also revealed that during the first 60 secs of radiation, fluctuations occurred at all microwave power levels, due to many variables such as the alignment of the protic molecule's oscillation and significant temperature fluctuation by polar groups due to physical distribution of the water molecules. However, after this duration, the rate of temperature increases remains almost constant.

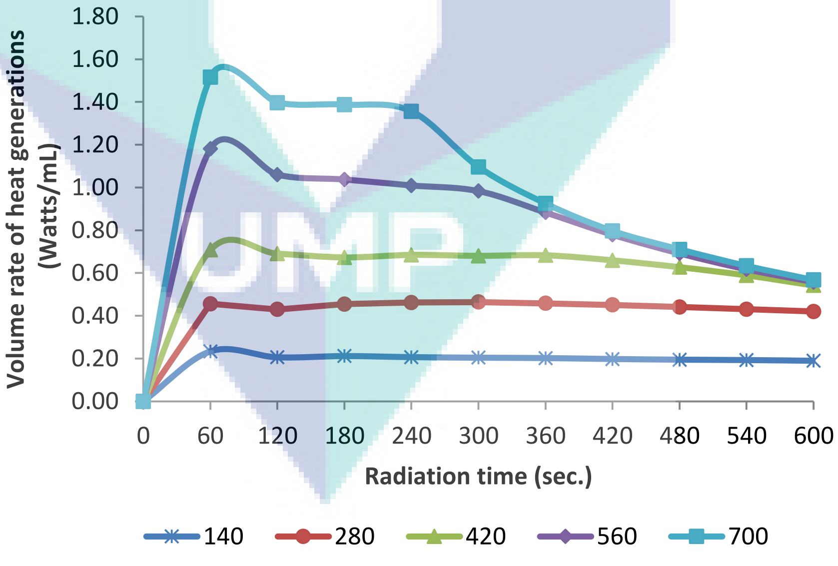


Figure 4.11: Volume rate of heat generation (q_{MW}), of various microwave power levels to radiation time

It is also observed that the acceleration of microwave absorbed energy inside the water during the initial duty cycle is higher at high to low pulse where the molecules of the water become aligned and rapidly absorbed microwave's energy due to short of period time. Bicerano (2002) also reported same manners of amorphous polymer in the liquid state because the microwave energies and thus amplitude of the random thermal motions increase at the high pulse level. Moreover, the increment of temperature in water at high power was much stronger than that at the low power level, thus the increment of temperature reaching at high level smoothly and in a very short time. Figure 4.11 also illustrated, as the exposure time was increased further, the volume rate of heat generation decreased and in certain cases approached plantae condition.

The decreasing of heat generation within radiation time is due to the decreasing dielectric properties of solvents with regards to temperature increase. The polarity of solvent decreses with increasing temperature, which resulted decreasing of the ability of water to absorb microwave energy and affected the dipole rotation and alignment of water molecules in the microwave field. The dielectric property which is attributed to the solvent polarity is dependent on mobility of the dipoles within the structure (Rahmat, 2002). The dielectric properties which are affected the ability of samples to absorb and dissipitate the microwave energy are influenced by heating temperature (Fang and Lai, 1996; Chan and Chen, 2002; Saound, 2004). In general, the ability of material to dissipitate energy changes as the property of the material changes (Jemaat, 2006).

4.5 SUMMARY

Extraction using microwave technology has numerous advantages compared to traditional extraction techniques; however, conventional microwave extraction technology suffers from variable product quality due to uneven processing or heating. In this study, the new concept of temperature controlled microwave closed system (TCMCS) equipped with a fluid seal stirrer was successfully built up and calibrated. After the calibration process, results were revealed the excellent of each microwave power absorbance at all power levels. The power absorbance performances of TCMCS with stirring system were much closed to the actual microwavepower as compared to without stirring system. It was found that the fluid seal stirrer is a very important factor to convert an electromagnetic energy into uniform heat of the fluid. The performance of the TCMCS was clearly shown by the demonstration of several solvents, which being used in this study shows the decreases in super heating effect and dissolution time. The results showed that in presence of stirring device the volume rate of heat generation in high dielectric constant solvents show good performanceas as compared tothe low dielectric constant of solvents.

CHAPTER 5

THE EFFECT OF SOLVENT NATURE ON ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF EPIPHYTE'S FERNS TCMAE

This chapter presents the result on the effect of various solvent having a different polarities on antioxidant properties of Malaysian palm oil trunk epiphyte's ferns (MPOTEF): *Nephrolepisbiserrata* (Sw.) Schott., *Davallia denticulate* (Burm.) Mett. *Asplenium longissimum B1, Gonioplebium percussum* (Cav.) Wagner & Grether, *Stenochlaena palustis* (Burm.f.) Bedd. , *Vittaria elongata,* and *Vittaria ensiformis* which extracted using temperature controlled microwave closed system (TCMCS) with fluid seal stirring device. One must consider in the extraction method of microwave assisted extraction, including solubility, dielectric constant, and the dissipation factor of solvent because solvents with high dielectric constants (e.g. water) can absorb more microwave energy, the polarity of the solvent is very important in microwave extraction. Polar solvents are usually believed better than non-polar ones. Thus, in this study, water and ethanol were chosen to extract 7 species of epiphyte's fern. Then a single and combination solvent of water, ethanol, acetonitrile and acetonewere considered to extract a selected epiphyte's ferns (*Nephrolepis biserrata* (Sw.) Schott). The mixture design was applied to study the relationship of each or mixture of solvent nature on total polyphenol, total flavonoid and DPPH radical scavenging activity (% inhibition and IC_{50}) which is considered as antioxidant properties of extract. The physiochemical analysis like density, refractive index, viscosity and surface tension was observed before extraction (maceration) and after temperature controlled microwave assisted extraction (TCMAE) process to study the effect of solvent nature after each extraction process under various solvent. The effect of microwave irradiation on microstructure changes of *Nephrolepis biserrata* (Sw.) Schott frond's tissues under single or mixture of solvent was observed by scanning electronic microscopy technique.

5.1 INTRODUCTION

Plant's organism utilized for medicinal have a long history of use in therapy throughout the world and still make an important part of traditional medicine. Extraction of several active phytochemical ingredients from new plants gives help to increase the growth and development of medicines (Mandal et al., 2007). Data from various studies indicate that medicinal plants contain a wide variety of natural antioxidants, such as phenolics, and flavonoids, which possess more potent antioxidant activity than common dietary plants. The phenolics and flavonoid compounds extensively existing in plants and are important for their contribution to have numerous biological effects, including antioxidant activity, antitumor, antimutagenic and antibacterial properties.

In order to recover the natural antioxidant's constituents from plants for medicinal purpose, the extraction forms the first basic step. The extraction process is one of the most imperative steps for active component's preparation and the activity evaluation of various plants. Generally, the extraction procedure is chronological and analytically conceded out using aprotic (polar, dipolar) and nonapprotic (nonpolar) solvents to extract polyphenolic compounds in plant samples. Thus, with the intention of recovery of significant yield of phytoconstituents several researchers were used different extraction techniques and solvents for obtaining antioxidant extracts from plant sources. Extraction methods solid-liquid, liquid-liquid include traditional solvent extraction, soxhlet extraction and supercritical fluid extraction, ultrasonic extraction and microwave assisted extraction (MAE). Among all extraction, MAE is a relatively new method used for the extraction of natural products (Pan et al., 2003; Eskilsson and Bjorklund, 2000).

The increasing interest in the powerful biological activity of plant phenolics and flavonoids outlined the necessity of determining their content in botanical plant. Previous studies on microwave assisted solvents extraction were utilized without stirring device and uncontrolled temperature. In addition, the studies of biochemical activities were usually focused on edible plants and ground fern as well most of study mainly focused on plants potential as antimicrobial activities, antifungal activities, antiviral, anti-inflammatory, antitussive, antitumor, anti-HIV and anti-aflatoxigenic activity (Johny et al., 2011; Zhong et al., 2008; Wu, 1999). The evaluation of polyphenolic compound (phenolic and flavonoid content) and antioxidant capacity between several species of epiphyte's fern which are commonly available at abundant resource in Malaysian palm oil plantation is still unknown. Therefore, a newtemperature controlled microwave closed system (TCMCS) with fluid stirring device was used to study the antioxidant properties of Malaysian palm oil trunk epiphyte’s ferns (MPOTEF): *Nephrolepisbiserrata* (Sw.) Schott., *Davallia denticulate* (Burm.) Mett. *Asplenium longissimum B1, Gonioplebium percussum* (Cav.) Wagner & Grether, *Stenochlaena palustis* (Burm.f.) Bedd. , *Vittaria elongata,* and *Vittaria ensiformis.*

Many researchers were found the consequence of microwave energy is strongly dependent on the solvent nature and proportion of the solvent system (mixture solvent). No doubt the heating rate of microwave was related with dielectric constant (ε’) and dielectric loss factor (ε”) of the extraction solvent which is plays an important role in extraction efficiency. Knowledge of the dielectric properties of solvents is essential for proper understanding of the heating pattern during microwave irradiation. The dielectric properties of the solvents provide a quantitative characterization of the interactions between microwave electromagnetic energy and solvents. From these two properties can be defined another solvent dielectric property, called the dissipation factor (δ), which is expressed mathematically in equation 2.3. This property describes the ability of the solvent to absorb microwave energy and dissipitate it in therethe form of heat. Considering the dissipation factor, the higher this factor, the faster the heat will be distributed through the extraction matrix and the quicker the heat will be transferred to the solvent (Escribano-Bailon and Santos-Buelga, 2003).

Solvent that heats up rapidly under microwave radiation typically has a high dielectric constant and dielectric loss constant (Hemwimon et al., 2007). Most of the time, the chosen solvent possesses a high dielectric constant and strongly absorbs microwave energy. However, the extracting selectivity and the ability of the medium to interact with microwaves can be modulated by using mixtures of solvents (Renoe, 1994). In some cases, the matrix itself interacts with microwaves while the surrounding solvent possesses a low dielectric constant and thus remains cold (Jassie et al., 1997). Indeed, microwaves interact selectively with the polar molecules present in vascular tissues. Localized heating leads to the expansion and rupture of cell walls and is followed by the liberation of pytomolecules into the solvent. This latter situation presents some obvious advantages in the case of thermosensitive compounds and has been successfully used for the extraction of phytomolecules from plant material.

The secondary metabolites usually occur in low concentrations, the choice of extraction solvent effects, both the yield (Soares et al., 2009) and the number of potential antioxidant metabolites that can be detected in metabolomic analysis (Almeida et al., 2006; Borges et al., 2007). To obtain complete extraction, the ideal solvent is obviously one that presents maximum selectivity, has the best capacity for extraction in terms of the saturation coefficient of the product in the medium, and is compatible with the properties of the material to be extracted (Wijesekera, 1991).

In the literature, different solvent systems have been used for the extraction of secondary metabolites from plant materials, because their extraction efficacy depends on their chemical nature. For example, ethyl alcohol extracts a large number of flavonoids whereas terpenes can be selectively extracted at neutral pH with ethyl acetate or aliphatic ketones. Aqueous mixtures of ethanol, methanol and acetone have been used for extraction of polyphenols from vegetal material, but no single solvent could extract all the antioxidants with their different polarities (Garcia-Salas, 2010). Methanol has could absorb much of the microwave energy and transform it into heat better than other solvents (Zlotorzynski, 1995) which mean can easily extract polar and non polar compound. But methanol was not tested in this work because it is highly toxic and is not practical for use in food and pharmaceutical processing (Hemwimon et al., 2007).

Mixture designs (Soares et al. 2007; Cornel, 2011) have been shown to be useful in describing the behaviors of solvent mixtures to extract substances from plants (Soares et al., 2009; 2003; 1997). The The literature shows many studies on aqueous mixtures of pure organic solvents, no investigations of these solvent systems were found using statistical mixture designs on NBF. In this work, a simplex centroid mixture design involving water, acetone, acetonitrile and ethanol has been applied to the extraction of NBF. Mixture of design model was assessing the importance of the effects of solvents and their mixtures on their antioxidant properties in terms of total polyphenol contents, total flavonoid content, DPPH radical scavenging activity (% inhibition and IC_{50}). The physicochemical properties of solvent extract in terms of viscosity, refractive index, density, surface tensions are determined with the aim of study the solvent extraction properties of each solvent mixture. The simplex lattice method in the mixture design, provided by the software Design-expert (ver. 6.0.5, Stat-Ease Int. Co., Minutesneapolis, MN, USA), was used to study the relationships between the proportion of different factors and responses.

5.2 ASSESSMENTS OF ANTIOXIDANT PROPERTIES OF MPOTEF

The results in the extraction of medicinal active compounds into water and ethanol (EtOH) of seven Malaysian palm oil trunk epiphyte's fern (MPOTEF) were subjected to evaluate their polyphenol composition by total phenolic content (TPC) and total flavonoid content (TFC) which was estimated by Folin-Ciocalteau test and aluminium chloride colorimetric assay respectively. Then antioxidant capacity was evaluated by their % inhibition and IC_{50} value using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method. The $IC_{50}$was then compared with that of natural antioxidant (α-tocopherol) and synthetic antioxidant (BHA) where it was also determined with parallel experiments.

5.2.1. Total Phenolic Contents (TPC)

Phenolic acids known as kind of multipurpose bioactive agents frequently occur in herbal plant (He, 2000). Phenolic's compounds present in plant material have received considerable attention because of their potential antioxidant activity play an important role in human nutrition as preventive agents against several diseases and protecting the body tissues from oxidative stress. Moreover, the phenolic acid was considered in recent years as potentially protective compound against cancer and heart disease in part because of their essential antioxidant properties (Cartea et. al, 2011). Therefore, in this work, the antioxidant potential of epiphyte's fern extracts is discovered by expressing antioxidant activity in terms of total phenolic content using Folin–Ciocalteu (FC) phenol assay where the method was clearly explained in Chapter 3 (section 3.5). The principle of this method is the reduction ability of the phenol functional group. An oxidation and reduction reaction of the phenolic ion takes place in basic conditions. Reduction of phosphortungstant-phosphormolybdenum complex (Folin-Ciocalteu reagent) by phenolic ions changes the reagent to dark blue (Prior et al., 2005). The colour becomes darker, absorbing at progressively higher wavelength as reduction ability increases with increasing phenolic compounds as in the case with epiphyte’s fern.

The TPC content of perceptive extracts obtained with TCMCS using two different solvents (distilled water, and ethanol) was determined by the Folin-Ciocalteu method. Absorbance was measured at 517nm for water and EtOH, and gallic acid was used as a standard phenolic compound for the calibration curve $y = 0.0005x + 0.0213$ and $y = 0.0005x + 0.0232$ in the range of 25–700 μg/mL with a coefficient of determination (R^2) value of each solvents 0.9998 and 0.9975 respectively, was obtained. TPC of the seven species of epiphyte's fern in water and ethanol extracts was demonstrated in Table 5.1.

In Table 5.1 the result indicates the higher amount of TPC in water extracts as compared to EtOH extract of all epiphyte’s fern species. TPC water extract demonstrated in the range of 175.4 to 639.4 mg GAE/g of dry weight material and 45.60 to 271.60 mg GAE/g in EtOH solvents. SPF (639.4 mg GAE/g dry weight) contained the highest average of TPC in water extract followed by VLF (445.4 mg GAE/g dry weight), VSF (431.4 mg GAE/g dry weigh), DDF (417.4 mg GAE/g dry weight), GPF (289.4 mg GAE/g dry weight), NBF (199.4 mg GAE/g dry weight) and ALF (175.4 mg GAE/g dry weight), respectively. TPC in EtOH extracts indicated SPF (271.60 mg GAE/g dry weight) contained the highest average of TPC followed by VLF >VSF> GPF > NBF > ALF and > DDF, respectively.

Temperature control microwave assisted extraction in closed system and supported by fluid sealed stirring system was reflected the higher amount of TPC in water compared to ethanol solvents extract due to significant solubilisation of phenolics compounds. The higher amount of phenolic compounds in these plants is also attributed the presence of carbonyls and organic acids and these can dissolve in water rapidly as compared to EtOH extracts at presence of microwave irradiation due to their favourable polarity.

Table 5.1: Summary of TCMA water (H_2O), ethanol (EtOH) extraction of total polyphenol, total flavonoid of MPOTEF

	TCMA water extracts		**TCMA EtOH extracts**	
Epiphyte's Ferns	**TPC** (mg GAE/g)	**TFC** (mg CE/g)	**TPC** (mg GAE/g)	**TFC** (mg CE/g)
NBF	199.4	52.71	119.60	152.77
DDF	417.4	12.5	45.60	137.17
ALF	175.4	20.71	95.60	170.77
SPF	639.4	172.71	271.60	174.77
GPF	289.4	61.57	97.60	115.97
VLF	445.4	115.57	125.60	132.37
VSF	431.4	117.57	117.60	178.37

Note**:** *Davallia denticulate* (Burm.) Mett. (DDF), *Nephrolepis biserrata* (Sw.) Schott. (NBF), *Asplenium longissimum B1* (ALF), *Gonioplebium percussum* (Cav.) Wagner & Grether (GPF), *Stenochlaena palustris* (Burm.f.) Bedd (SPF), *Vittaria Elongata* (VLF), *Vittaria ensiformis* (VSF).

In this study, the TPC results also revealed that among all seven epiphytes fern species the TCMCS water extracts of SPF, VLF, VSF, and DDF shows highest level. The high level of phenolics in these four plants might be due to consisting of a hydrogen hydroxyl group ($^{-}$OH) in water because of mutual polarity. However, TCMA water extracts of ALF, and NBF showed the lowest total phenolics (175.4, and 199.4 mg GAE/g dry weight), which is revealed lower contents of organic compounds due to their underprivileged polarities.

It has been reported that the plants had the highest total phenolics are contain gallic acid, caffeic acid, neochlorogenic acid, and ferulic acid, chebulic acid, 1,6-di-O-galloyl-β-D-glucose, punicalagin, 3,4,6-tri-O-galloyl-β-D-glucose, casuarinin, chebulanin, corilagin, neochebulinic acid, terchebulin, ellagic acid, chebulagic acid, chebulinic acid, and 1,2,3,4,6-penta-O-galloyl-β-D-glucosein, which might be responsible for its high antioxidant activity (Juang et. al, 2004). However, it should be also noted that some chemical group of ascorbic acid, organic acids, sugars, aromatic amines can also react with Folin Ciocelteau reagent (Meda et al., 2005). Phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu (FC) reagent (Prasad et al., 2005).

In addition, the higher phenolic content of plants are influenced by a number of intrinsic (genus, species, cultivar) and extrinsic (agronomic, environmental, handling and storage) factors (Tomas- Barberan and Espin, 2001). Few studies have investigated the impact of nitrogen and sulfur application on the total phenolic concentrations and antioxidant activity. Several studies reported that an increase in sulfur fertilization significantly promotes the total phenolic contents and the antioxidant activity in leaf mustard (Li et al., 2008) and ‘friarello’, a local *B. rapa* crop widely grown in Southern Italy (Vallejo et al., 2003). Li et al. (2008) also determined the effect of nitrogen supply on leaf mustard and found that the total phenolic content was considerably decreased by increasing nitrogen fertilization. In this study, higher amount of TPC in SPF, VLF VSF and DDF might be contributed by their fertilizer. Among to the other epiphytes fern, SPF, VLF and VSF are growing at the bottom of palm oil which contacted indirectly to the fertilizer that put on the base of palm oil plant. These studies provide clear evidence that nitrogen and sulfur nutrition can be used to manipulate total phenolic concentrations of epiphytes fern with potential benefits to human health and as a result, it can be concluded that sulphur fertilization may improve the nutritional value of these epiphytes fern.

5.2.2 Total Flavonoid Compounds (TFC)

Flavonoids are polyphenolic compounds that are everywhere in nature and most commonly known it have been implicated in antioxidant activity. Flavonoids as one of the most diverse and widespread groups of natural compounds are probably the most important natural phenolics. The potential of dietary flavonoids has recently created an interest among a scientist for treating many diseases (Terao and Piskula, 1998). These compounds possess a broad spectrum of chemical and biological activities, including radical scavenging properties. The TCMA water extracts of total flavonoid contents of each epiphyte's fern samples were expressed as milligram's catechin equivalents (CE) per gram dry sample. Flavonoid compounds have been implicated in antioxidant activity. Calibration analytical parameter for water and EtOH was calculated and resulted in R^2=0.999, y = 0.003x - 0.007 and R^2=0.999, y = 0.0025x + 8x10^{-05} respectively for catechin. The flavonoids of epiphyte's fern with aluminium to form a complex flavonoid-aluminium that could be measured at 435 nm and the results are summarized in Table 5.1.

Flavonoids compounds usually consist of a glycone or glycosylated forms, are located in the vacuoles within plant cells and are in the polar soluble fraction. Therefore, flavonoids can be easily extracted under TCMA with polar solvents such as water, ethanol and methanol, which is not the case for insoluble lignins and tannins that bind to proteins on cell disruption during the extraction. Using the standard curve of catechin, the flavonoid content of TCMA water extracts epiphytes fern varied from range from 12.5 to 172.71 mg CE/g of dry weight material. TCMA water extracts of SPF (172.71 mg CE/g dry weight) contained the highest average of TFC followed by DDF (88.37 mg CE/g dry weight), VSF (117.57 mg CE/g dry weight), VLF (115.57 mg CE/g dry weight), GPF (61.57 mg CE/g dry weight), NBF (52.71 mg CE/g dry weight), and the lowest flavonoid content was found of ALF (20.71 mg CE/g dry weight).

TFC values of the EtOH extract of each epiphyte's fern and compared with the water extract of TFC are summarized in Table 5.1. The results of ETOH extract obtained in this study shows a little bit higher amount as compared to the water extract. The EtOH extracts of each plant dry weight; VSF (178.34 mg CE/gdry weight) contained the highest average of TFC followed by SPF (174.77 mg CE/g dry weight), ALF (170.77 mg CE/gdry weight), NBF (152.77 mg CE/gdry weight), DDF (137.17 mg CE/gdry weight), VLF (132.37 mg CE/gdry weight), and GPF (115.97 mg CE/gdry weight), respectively. Results revealed the EtOH extract of epiphyte's higher recovery as compared to water extract of each epiphte's fern. It is suggested that the TFC of epiphyte's fern plants has pathways of transformation of different aliphatic, aromatic and polycyclic aromatic compounds and having a tendency to dissolve in EtOH inside the microwave closed system.

Repeatedly, the results obtained in this study revealed that the amount of these phenolic and flavonoids compounds in the TCMA water and EtOH extracts of the epiphyte's fern; SPF, NBF, VLF and VSF, respectively mainly due to the level of essential elements and their redox properties. These oxidation/reductions potential play a significant part in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides due to the chain session of polar compounds under the closed heating system at presence microwave irradiation. In fact, many medicinal plants contain large amounts of antioxidants such as polyphenol. Many of these phytochemicals seize significant antioxidant capacities that are related with subordinate occurrence and inferior mortality rates of several human diseases. The results strongly recommend that phenolics and flavonoids are important components of these plants, and some of its pharmacological effects could be recognized in the presence of these precious constituents. Nevertheless, among all the seven species, the SPF shows the highest phenol, flavonoids contents, because of high antioxidant activity of this plant. Therefore, it is concluded that the water as a universal extraction procedure appropriate for microwave extraction of epiphyte ferns.

5.2.3 Measurements of DPPH Radical Scavenging Activity

i. Scavenging activity (% inhibition)

With the increasing interest in function and diversity of antioxidants, some methods have been developed in order to determine this activity in plant extract. Among chemical methods applied to determine, the antioxidant activity of a compound, DPPH (2,2-diphenyl-1-picrylhydrazyl) is one of the most used methods because it is practical, fast and stable. DPPH is a free-radical compound and has been used to test the free radical scavenging ability of various samples widely in present studies. DPPH is a commercial oxidizing radical, which can be reduced by antioxidants.

The higher value of absorbance (at 517 nm) of the microwave assisted plant extracts reveals the maximum antioxidant activity. The results of scavenging effect of TCMCS water extracts 7 species of epiphyte's fern on DPPH radical are given in Table 5.4, these test samples could reduce the stable free radical DPPH to the yellow-coloured diphenylpicrylhydrazine. Morever, in this assay, it was observed that the violet colour of DPPH was reduced to a pale yellow colour due to the abstraction of a hydrogen atoms from antioxidant compound, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants (Yazdanparast and Ardestani, 2007). The more antioxidants occurred in the extract, the more DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by particular sample as reported by Blois (1958).

The antioxidants present in the sample extract might be different resourceful properties, such as reactive oxygen species scavenging due to presence of microwave influence (quercetin and catechins), inhibition of the production of free radicals and chain-breaking activity might be due to the chain session under microwave irradition, e.g. hydroxycinnamic acid and macro and micronutrients elements chelating. These compounds are usually phenolic compounds, which are efficient hydrogen donors, such as tocopherols, flavonoids, and derivatives of cinnamic acid, phosphatidic and other organic acids, which are reported to be multi functional. One of the mechanisms was also involved in antioxidant activity is the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant chain session of polar compounds under microwave irradiation condition, and this might be the cause of the formation of a nonradical form DPPH-H by the reaction. The propensity of the hydrogen donation is the critical factor that involves free radical scavenging in presence of microwave irradiaition at a particular fixed temperatures. All the species of the epiphyte's fern extract exhibited radical scavenging activity. The strongest effect was observed for the SPF.

Radical scavenging activities of TCMA water and EtOH extract of each epiphyte’s fern were tested using DPPH assay. DPPH is a free radical compound and has been used to test the free radical scavenging ability of various samples widely in present studies. Thus, the results of % inhibition DPPH is shown in Table 5.2, and it was observed that the % inhibition DPPH radical scavenging activity of water extracts of SPF (94.85%) was highest compared to the rest of the other epiphyte’s fern species and it was followed by VSF>VLF>DDF>GPF>NBF>ALF, respectively while α-tocopherol and BHA were found to be 17.79 µg/mL and 9.25 µg/mL. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability as reported by Yazdanparast & Ardestani (2007). Though the DPPH radical scavenging abilities, scavenging capacity of the seven epiphytes fern TCMA water extracts on DPPH the extracts show that the extracts have the proton-donating affinity and could provide as free radical inhibitors or scavengers, acting perhaps as key antioxidants because of their radical scavenging ability due to their OH groups.In addition, the percent of inhibition values for DPPH radical with ethanol extract of each epiphyte’s fern are also summarised in Table 5.2. It was observed that the % DPPH radical scavenging activity of epiphyte’s fern ethanol extracts was lower than water extract. The reasons for these variations are not quite clear, but it could be that some of the target compounds of these plants were purified/isolated in water extract, which had inhibitory roles in the test experiments might be not dissolved properly in EtOH extract. In EtOH extract, SPF shows the highest inhibition value which is 98.65% among all epiphyte’s fern species, and it was followed by VLF (73.01%), GPF (62.6%), VSF (56.49%), ALF (55.79%), DDF (33.57%), and NBF (48.12%), respectively.

Table 5.2: DPPH radical scavenging activity (% inhibitory and IC_{50}) of NBF by TCMCS

	TCMA water extracts		**TCMA EtOH extracts**	
Epiphytes Ferns	**Inhibition**[a] %	**IC_{50}**[b] (μg/mL)	**Inhibition**[a] **(%)**	**IC_{50}**[b] (μg/mL)
NBF	86.67	215.67	48.12	723.27
DDF	92.73	65.14	33.57	897.76
ALF	86.36	235.98	55.79	775.63
SPF	94.85	40.03	98.67	145.43
GPF	91.01	165.55	62.6	861.56
VLF	92.83	82.72	73.01	528.93
VSF	93.74	107.97	56.49	512.89
Standard				
α-tocopherol	-	47.79	-	60.05
BHA	-	9.25	-	13.15

[a] Percent of inhibition and scavenging (μg/mL) as a mean of triplicate experiments.

[b] Values obtained from regression lines with 95% of confidence level. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum inhibition.

ii. Scavenging activity (IC_{50})

The change in colourisation from violet to yellow and subsequent fall in absorbance of the stable radical DPPH was measured at 517 nm at 30 minutes after addition for seven species. Epiphyte's fern extracts, and standards were individually assessed for their possible antioxidative activities by employing DPPH free radical scavenging. The IC_{50} value for sample and standard defined as the concentration of extract causing 50% inhibition of absorbance. Since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value would reflect greater antioxidant activity of the sample..

As shown in Table 5.2, IC_{50} of water extract in a range from 40.03 µg/mL to 235.98 µg/mL. SPF (40.03 µg/mL) contained the lowest number followed by DDF (65.14 µg/mL), VLF (82.72 µg/mL), VSF (107.97 µg/mL), GPF (165.55 µg/mL), NBF (215.67 µg/mL), ALF (235.98 µg/mL). According to the results obtained, IC_{50} values of the synthetic antioxidant BHA was found the more active with an IC_{50} value of 9.25 µg/mLwhile, for the natural antioxidant α-tocopherol also shows more active except for SPF as compared to all epiphyte's fern.

While IC_{50} of each epiphyte's fern species in EtOH was found to be the high value as compared to water extract plants, which show lower antioxidant activity. As shown in Table 5.2, IC_{50} range from 145.43 µg/mL to 897.76 µg/mL. SPF contained the lowest number followed by VSF>VLF>NBF>ALF>GPF>DDF. According to the results obtained, IC_{50} values of the synthetic antioxidant BHA was found the more active with an IC_{50} value of 13.15 µg/mLwhile, for the natural antioxidant α-tocopherol also shows more active (60.05) as compared to all epiphyte's fern.

As shown in Appendix K(a), the results of the present study reveal that the TPC and TFC of water extract showed a significant relationship with antioxidant capacity (R^2= 0.8998 &R^2 = 0.9388) respectively. This good correlation between TPC and TFC with DPPH scavenging activity indicated that the extracts obtained from epiphytes fern has remarkable antioxidant activities. The correlation between antioxidant capacity and TPC of EtOH extract was also good, (R^2 = 0.8868), but the TFC of EtOH extracts (R^2 = 0.7908) shows the moderate correlation with antioxidant. These results suggested that EtOH extracted flavonoid compounds contributed the higher antioxidant capacity (IC_{50}) of the extract as compared to TPC from epiphytes.

Appendix K(b) was depicted the correlation of water extract total phenolic content (TPC) andtotal flavonoid content (TFC). The results obtained was positively correlated with a correlation coefficientsR^2=0.959). Since flavonoids are a group of plant phenolics, this finding suggested that the phenolic compounds find in epiphytes fern may be mainly flavonoids. Appendix K(b) also shows the TPC and TFC of EtOH extracts also have goodcorrelation with the extraction at a fixed temperatures, with the correlation coefficient (R^2) 0.823. Experimental results showed that epiphyte's fern had significant ($p<0.05$) effect on polyphenolic compound (TPC and TFC) and antioxidant activities (DPPH) of crude extract in both solvents. However, it was observed that the ethanols were quite significant solvent for the amount of TFC but lower in TPC and antioxidant activity than water.

Medicinal plants comprise an essential constituent of tropical plants and are extensively scattered in Malaysia. Thus, in order to investigate the potential of important medicinal constituents of Malaysian epiphyte'sferns, particularly in the seven epiphyte's fern species the medicine and biology concerned with the study of drug action analysis were carried out. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds, which can lead to the development of novel and safe medicinal agents. Based on the ethnopharmacological literature, several tropical plant's species of medicinal plants used in traditional medicine in Malaysia were collected. In the present study, aqueous and water extracts of these epiphyte's fern were screened for their cytotoxicity using brine shrimp lethality test.

Brine shrimp lethality bioassay was applied to detect cytotoxic activity of each species. The in vivo lethality in a simple zoological organism, such as the brine shrimp test is simple bioassay for natural product research was considered a useful tool for preliminary assessment of cytoxicity and for pharmacological activities screening in plant extracts (Montanher et al., 2002). Brine shrimp test is simple bioassay for natural product research. The procedure determines lethal concentration of active compounds in brine media. This method is rapid reliable and has been used for over 30 years in toxicological studies. Brine shrimp larvae have been used as a bioassay for a variety of toxic substances. The method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds. A general bioassay that appears capable of detecting a board spectrum of the bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). The technique is easily mastered, costs little, and utilizes small amount of test material.

As shown in Appendix L, all the 7 species of epiphyte's fern ethanol extract used in this study showed significant toxicity against brine shrimp larvae of *Artemia salina* at lower LC_{50} values. The result obtained from the brine shrimp lethality bioassay of epiphytes fern can be used as a guide for the isolation of cytotoxic compounds from the aqueous and ethanol extract of the frond of this plant. These findings provide scientific evidence to support its traditional medicinal uses and indicate a promising potential for the development of an antioxidant, anticancer and antibacterial agent from this plant. The result could serve for further pharmacological and phytochemical research.

However, the data on antioxidant properties and cytotoxicity itself is incomplete without support data on their safety and efficiency when epiphyte’s fern was intentionally explored for pharmaceutical and food industry. Several regulations setting high-quality requirements for medicinal plants and related products on the market are shared at the global level in pharmacopoeias, while legal frameworks exist at the national or regional level (Kosalec et al., 2009). Though there is no standard for herbal materials, which establish a permissible level of metals in such materials. The World Health Organization (WHO) has fixed maximum permissible levels in the dry mass of medical plants only for arsenic, cadmium, and lead is 1.0, 0.3 and 10 mg kg^{-1}, respectively.

Hence, this researches offer a complete data not only on their potential in medical or related product but at the same time provides data on their safety and their essential elements as well. Therefore, the content of some macronutrients elements (Na, K, Mg and Ca), micronutrients elements (Zn, Co, Cu, Cr, Al, Mn, and Fe) and toxic elements (Pb, Cd, As, Hg) in the dry mass of a seven species of palm oil epiphytes was assessed to render their potential in medical, food and safety side and at the same time support the result of antioxidant properties and cytotoxicity in terms of an extraction mechanisms in TCMCS different solvent assisted.

Conducted investigations was depicted in Appendix M which shows that the epiphyte's fern species are particularly rich in Mg, K Ca and Mn, where some of them can be used as beneficial sources for Ca, Mg, K and Mn. Rich in Mg and Mn are belonging to NBF while DDF showing high amount of Ca and Cu. VLF having a good amound of K, Cr and Fe whereas all of epiphyte's fern species poor, especially in Na, Co, and Fe.In conclusion, seven epiphyte's fern, which are widely available in Malaysia, contained good amount of essential macronutrients, essential micronutrients while the amount of toxic elements is under the permissibility limit.

This study can help the pharmaceutical industry to explore a new plant and use it as a natural compound for antioxidant activity, which might be used as an alternative to edible plants as well as synthetic antioxidants. From these findings, epiphyte's fern has been proven as a good alternative and more economical source of phytochemical compound comparing to other medical plants because it's can easily found in the significant amount in palm oil trunk without cultivation cost. The significant amounts of TPC, TFC and antioxidant activity extraction in water and ethanol solvents using TCMCS revealed the potential of seven species for herbal and medicinal compounds as compared to conventional methods. The use of epiphyte's fern due to their capability as a natural antioxidant source appears to be an alternative to synthetic antioxidants. The content of essential and toxic trace elements in seven epiphyte's fern plants, revealed a good potential for medicinal compounds. The mortality of brine shrimp in seven epiphyte's fern plants in EtOH and water are also proven importance of these plants at presence of essential and nonessential elements and since it is environmentally friendly and safe for consumption.

Nephrolepis biserrata (Sw.) Schott (NB) is an epiphyte's fern was grown abundantly on palm oil trunk and also on ground of palm oil plantation. It is popularly known as giant sword fern or paku larat/ paku uban in Malay and is widely used as salad while in folk medicine as a tonic for the treatment of impotence, fatigue, stress and memory deficits. Chemical studies on NB have indicated the presence of sesquiyitol (Sun et al., 2011) known as antidiabetic agent and drimane-type sesquirterpenes (Siems et al, 1996) which recognized as defensive and antifeedants. Studies on NB frond extract shown it has antfungal activity (Johnny et al., 2011). An experimental study on the potential of NB in medical and food industry was clearly discussed in the previous study (Lai and Lim, 2011) using the traditional method. On this research, NB was chosen in the further study because of their huge resources in palm oil plantation as compared to other species, which have been proven in Appendix N. In the next section, the exploration of NBfrond extracts was conducted in single and mixture of the solvent system (water, acetone, acetonitrile and ethanol) using TCMCS with fluid stirring system.

5.3 DESIGN OF EXPERIMENT

Statistical experimental design, also called design of experiment (DoE), is a well established concept for planning and execution of informative experiments (Box et al., 1978) which can be used in many applications. These strategies were originally developed for the model fitting of physical experiments, but can also be applied to numeral experiments. The objective of DoE is the selection of the points where the response should be evaluated. Most of the criteria for optimal design of experiments are associated with the mathematical model of the process. Mixture design is one of the design families instead of fractional factorial and central composite, which commonly used to study the relationship of factors on response.

5.3.1 Mixture Design

Mixture design is usually employed to investigate the relationship between the blend fraction matrix (factors) and the final blend product property matrix (response), given that the raw materials to be blended are already chosen. The response in mixture of experiments primarily depends on the mixing proportions. Ever since Scheffe devised a single-lattice and single-core design in 1958, the mixture design has developed a variety of methods (Muteki et al., 2007, Wang et al., 2006). The characteristics feature of a mixture design is that the sum of mixture factors=100% ($\sum X_k = 1$). That meant these components (X_k), mixture factors, dependently of one, another and their proportions must be somewhere between 0 and 1.

i. Regression Analysis

Regression analysis was used to find a suitable approximation for the true functional relationship between response and the independent variables. In regression analysis, data are collected from an experiment. The data are then used to empirically quantify, through some form of the mathematical model, the relationship that exists between the response variables and its influencing factors (Cornell, 1990). The The behaviors of solvent mixtures to extract substances from plants was studies by Soares, (2009; 2003; 1997) using the mixture model (Cornell, 2011; Soares, 2007). The extracted has been found to depend on the proportions of the solvents in the extracting medium using quadratic or special cubic mixture models as equation 5.1:

$$\hat{y} = \sum_{i} b_i x_i + \sum_{i}\sum_{<j} b_{ij} x_i x_j + \sum_{i}\sum_{<j}\sum_{<k}\sum_{<l} b_{ijkl} x_i x_j x_k x_l \tag{5.1}$$

The first summation contains linear blending terms for all the mixture components. It describes the relative extraction effects of the pure components in the mixture. The second summation describes the binary interactions between solvent pairs in the extraction process. A significant positive term in this summation indicates that a solvent binary mixture extracts an excess of material relative to that extracted using each solvent separately. The last summation contains terms that will be important if quaternary interactions among mixture of solvents are important for the extraction process.

Modeling is based on gravimetric measurements of extracted material by solvent mixtures whose compositions are determined using statistical criteria. The simplex-lattice design used here is a basic one and permits the evaluation of the model mixtures having important binary and ternary interactions. The qualities of the mixture models are judged by analysis of variance (ANOVA) of the regression results.

A. Test for lack of fit

As replicate measurements are available, a test for lack of fit on the regression model can be performed. This test verifies the variation of the data around the fitted model. The non-significant for lack of fit indicates that there is no significant and systematic departure from the staight-line relationship, which has been fitted. The insignificant for lack of fit is achieved if the model fits the data well.

B. Test for residual analysis

Residual analysis consists of the term to judge model adequacy (Mokhtar, 1994). It is used to estimate the difference between the observed and predicted response. In order to check the normality assumption, normal probability plot of the residual was constructed. A normality assumtion is satisfied if the residual plots approximately along a straight line (Myers and Montgomery, 2002).

C. Test of significant based on F value.

The *F* ratio calculated from the data of the experiments explained the mean square of error between the regression. The *F*-test and the corresponding *P*-value are used as a tool to check the significance of each of the coefficients which in turn are necessary so to understand the pattern of the mutual interactions between the best variables. The smaller the magnitude of *P*, the more significant is the corresponding coefficient (Akhnazarova and Kafarov, 1982). In brief, a good model must be significant. Various methods have been developed for model optimization by either adding or deleting regressor one at a time. These methods are generally referred to as stepwise-type procedure that can be classified into three broad categories:

a. Forward selection

The forward selection begins with no variables in the model and then adding variables based on their contribution to prediction. For each of the independent variables, the forward method calculates *F* value that reflects the variable's contribution to the model if it is included. The forward selection process adds the term sequentially to the model until further additions do not improve the fit. At each stage, the term which has the least damaging effect on the model (larger *P* value) is removed (Agresti, 2002).

b. Backward elimination

The backward elimination procedure is the opposite of forward selection (Neter et al., 1996). The backward elimination technique begins by calculating *F* test for a model, including all the independent variables. This is followed by eliminating those variables that do not make a significant contribution to prediction (Hair et al., 2009). Those variables with minimum *F* value which is less than the predetermined limits are dropped.

c. Stepwise methods

This procedure is a combination of the backward elimination and forward selection procedure. It consists of the forward selection where each step is followed by a backward elimination, assessing the significance of all variables already entered in the model.

d. Test for R^2 and adjusted R^2

In general, R^2 is used to decide either a regression model is appropriate or not. It measures the amount of variations around the mean explained by the model. A value of R^2 close to 1 means a perfect fit to the experimental data. A value of $R^2 > 0.75$ indicates the aptness of the model. The R^2 decreases in range from 1 to 0 if the residual value increases (residual is the difference between the observed value and the fitted value). Adding the variables to the model increases R^2, regardless of whether the additional regression model judgment, an adjusted R^2 is preferably used. Adjusted R^2 measures the amount of variations in the dependent variables for which the model accounts. It adjusts the R^2 value based on the number of coefficient in the model. The adding of variables to the model does not always influence the adjusted R^2. If the value of R^2 and adjusted R^2 differ dramatically, there is a good chance that the non-significant terms have been included in the model (Myers and Montgomery, 2002).

5.4 STATISTICAL MODELING OF ANTIOXIDANT PROPERTIES

Experimental level of independent process variables (percentage of water, acetone, acetonitrile and ethanol) studies for dependent variables of antioxidant properties: TPC, TFC and DPPH radical scavenging activity (% inhibition and IC_{50}) was shown in Table 5.3. The temperature controlled assisted extraction (TCMAE) of NBF in each solvent was conducted using a same method as was explained in Chapter 3, section 3.4.4 with little modification. Extraction temperature was kept at 60°C for 4 minutes of extraction times, and microwave power is 420 Watts (Temperature profile of TCMAE at various solvent was depicted at Appendix Q). After extraction, the solution of extract was filtered using Whatman 41. The extracted solution was evaporated using rotary evaporator. Sample was stored in a -20°C until further use in antioxidant property's analysis. However, for physicochemical property's analysis, the extracted solution was a centrifuge at 9000rpm for 20 minutes to get clear solution before reading process.

To fit the response function and experiment data, regression analysis was performed and the model was evaluated by (ANOVA) for dependent variable or response. As mentioned in section 5.3.1(i), a regression analysis was performed to represent the effect of each variable, including their interaction to each other on the response. The result of antioxidant properties in terms of TPC, TFC, DPPH radical scavenging activities (% inhibition and IC_{50}) as per the experimental plan are presented in Table 5.4. The antioxidant property's results in Table 5.4 were input into the Design Expert software for further analysis following the steps outlined previously. Examination of the fit summary output revealed that the quadratic model is statistically significant for the TPC, IC_{50} while the special cubic model is statistically significant for the TFC, and % inhibition. Therefore, both these models were used to represent each of the responses for further analysis.

Table 5.3: Completed design layout (Simplex lattice mixture)

Std. run no.	Run	Factor 1 % Water	Factor 2 % Acetone	Factor 3 % Acetonitrile	Factor 4 % Ethanol
1	20	100	0	0	0
2	11	50	50	0	0
3	18	50	0	50	0
4	6	50	0	0	50
5	12	0	100	0	0
6	10	0	50	50	0
7	13	0	50	0	50
8	4	0	0	100	0
9	5	0	0	50	50
10	9	0	0	0	100
11	19	62.5	12.5	12.5	12.5
12	16	12.5	62.5	12.5	12.5
13	2	12.5	12.5	62.5	12.5
14	3	12.5	12.5	12.5	62.5
15	8	25	25	25	25
16	15	0	100	0	0
17	14	0	0	100	0
18	17	100	0	0	0
19	7	0	0	0	100
20	1	00	50	50	0

Table 5.4: Experimental Results of Antioxidant Properties

Std. run no.	Response A TPC mg GAE/g	Response B TFC mg CE/g	Response C inhibition %	Response D IC_{50} µg/mL
1	91.6	29.97	91.67	318.66
2	73.6	23.4	79.42	592.05
3	79.6	28.83	72.07	482.56
4	59.6	18.26	69.11	799.72
5	11.6	3.97	18.02	15132
6	13.6	6.83	92.65	8926.86
7	13.6	9.11	19.38	6591.64
8	7.60	4.26	11.29	13453
9	21.6	12.83	79.13	7943.51
10	96.1	10.83	75.75	513.21
11	89.6	24.26	79.30	612.60
12	45.6	9.69	54.06	1654.62
13	43.6	9.40	59.98	2081.5
14	49.6	7.40	29.50	2559.76
15	87.6	11.11	63.34	1796.35
16	19.6	3.69	15.10	19145
17	17.6	4.83	12.91	15012
18	13.6	10.54	90.02	521.12
19	105.6	29.69	72.79	317.66
20	99.6	23.11	91.86	623.22

5.4.1 ANOVA Analysis for Antioxidant Properties

In order to ensure a good model, test for significant of regression model, test for significance on individual model coefficients and test for lack of fit needed to be performed. An ANOVA table is commonly used to summarize the tests performed. Table 5.5 shows the ANOVA table for the response quadratic model for TPC.

Table 5.5: ANOVA for Mixture Quadratic Model (Response: TPC)

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	21799.290	9	2422.140	15.10	0.0001	significant
Linear						
Mixture	18601.550	3	6200.520	38.64	< 0.0001	
AB	1749.630	1	1749.630	10.90	0.008	
AC	899.090	1	899.090	5.60	0.0395	
AD	185.570	1	185.570	1.16	0.3074	
BC	61.490	1	61.490	0.38	0.5497	
BD	111.870	1	111.870	0.70	0.4232	
CD	332.350	1	332.350	2.07	0.1806	
Residual	1604.510	10	160.450			
Lack of Fit	1070.510	5	214.100	2.00	0.2318	not significant
Pure Error	534.000	5	106.800			
Cor Total	23403.800	19				

Std. Dev.	12.667	R-Squared	0.931
Mean	47.700	Adj R-Squared	0.870
C.V.	26.555	Pred R-Squared	0.254
PRESS	17455.189	Adeq Precision	9.548

The value of “Prob.>F” for the model is less than 0.05, which indicates that the model is significant. It also indicates that the term in the model has a significant effect on the response. In a similar manner, the main effect of linear mixture, two level interactions of water and acetone (AB) also two level interaction of water and acetonitrile (AC) are significant model terms. Other model terms are said to be not significant. These insignificant terms (not counting those required to support hierarchy) can be removed and the result in an improved model. The lack of fit can also be said to be insignificant. This is desirable, as we want a model that fits.

The backward elimination procedure was selected to automatically reduce the insignificant terms, and the resulting ANOVA table for reduced special cubic model is depicted in Table 5.6. The results indicated that the model is still significant. The main effect is still linear mixture, two level interactions of water and acetone also two level interaction of water and acetonitrile. The main effect of a linear mixture is the most factors associated with TPC. The significant factors were ranked based on the value of *F*-ratio. The larger the magnitude of the F-value and the smaller the *P*-value, the more significant is the corresponding coefficient (Cochran and Cox, 1992). Thus in this study, the ranking is as follows: linear mixture>AB >AC.

R-squared value is first criteria to see the appropriateness of the model from the determination coefficient. It also reveals the total variation of the observed values of activity about its mean (Montgomery, 2001). As the show in Table 5.6, the R^2 value calculated is 0.9, reasonably close to 1, which is acceptable. It implies that about 90% of the variability in the data is explained by the model. The predicted R^2 is in reasonable agreement with the adjusted R^2. The adjusted R^2 value is particularly useful when comparing models with a different number of terms. This comparison is however, done in the background when model reduction is taking place. Adequate precision compares the range of the predicted values at the design points to the average prediction error.Ratio greater than 4 indicate adequate model discrimination. In this case, the value is well above 4.

Table 5.6: ANOVA for Mixture Reduced Quadratic Model (Response: TPC)

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	21093.879	5	4218.776	25.569	< 0.0001	significant
Linear						
Mixture	18601.546	3	6200.515	37.580	< 0.0001	
AB	1702.974	1	1702.974	10.321	0.0063	
AC	952.452	1	952.452	5.773	0.0307	
Residual	2309.921	14	164.994			
Lack of Fit	1775.921	9	197.325	1.848	0.2584	not significant
Pure Error	534.000	5	106.800			
Cor Total	23403.800	19				

Std. Dev.	12.845	R-Squared	0.901
Mean	47.700	Adj R-Squared	0.866
C.V.	26.929	Pred R-Squared	0.686
PRESS	7337.811	Adeq Precision	11.674

The same procedure is applied on the TFC and the resulting ANOVA table for the reduced special cubic model is shown in Table 5.7. Values of "Prob > F" less than 0.0500 indicate model terms are significant. For TFC, the linear mixture, two level interaction of water and acetone, water and acetonitrile, acetonitrile and ethanol, three level interaction of water, acetonitrile and ethanol, also three level interaction of acetone, acetonitrile and ethanol showed principle effect. Other model terms were determined as the secondary effect.

Table 5.7: ANOVA for Mixture Reduced Special Cubic Model (Response: TFC)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	1590.600	12	132.550	701.099	< 0.0001	significant
Linear						
Mixture	1369.843	3	456.614	2415.178	< 0.0001	
AB	50.201	1	50.201	265.530	< 0.0001	
AC	113.498	1	113.498	600.326	< 0.0001	
AD	3.461	1	3.461	18.307	0.0037	
BC	5.407	1	5.407	28.601	0.0011	
BD	1.855	1	1.855	9.811	0.0166	
CD	23.896	1	23.896	126.395	< 0.0001	
ABC	3.047	1	3.047	16.116	0.0051	
ACD	8.220	1	8.220	43.478	0.0003	
BCD	11.171	1	11.171	59.089	0.0001	
Residual	1.323	7	0.189			
Lack of Fit	0.668	2	0.334	2.547	0.1727	not significant
Pure Error	0.656	5	0.131			
Cor Total	1591.923	19				

Std. Dev.	0.435	R-Squared	0.999
Mean	14.101	Adj R-Squared	0.998
C.V.	3.084	Pred R-Squared	0.957
PRESS	68.341	Adeq Precision	73.734

Table 5.8 shows the ANOVA table for the special cubic model for % inhibition. The value of Prob > F for the model is less than 0.05, which indicates that the model have a significant effect on the response. In a similar manner, linear mixture and all of two and three level interaction of factor are also significant. Based on F value, the ranking is a follows: linear mixture>AB>AD>AC>BCD>ABD>ABC>ACD>CD>BD. The R^2 value calculated is 0.99. It implies that 99% of the variability in the data is explained by the model. The predicted R^2 is in reasonable agreement with the adjusted R^2. The adjusted R^2 value is particularly useful when comparing models with different number of terms.

Table 5.9 shows the ANOVA table for the mixture quadratic model IC_{50}. The value of Prob > F for the model is less than 0.05, which indicates that the model have a significant effect on the response. In a similar manner, linear mixture and all of two and three level interaction of factor are also significant. Based on *F* value, the ranking is a follows: linear mixture>AB>AC>BC.The R^2 value calculated is 0.9707 reasonable close to 1, which is acceptable. The predicted R^2 is in reasonable agreement with the adjusted R^2. Adequate precision value is well above 4 which indicate adequate model discrimination.

Table 5.8: ANOVA for Mixture Special Cubic Model (Response: % inhibition)

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	17761.73	13	1366.29	601.29	< 0.0001	significant
Linear Mixture	13975.42	3	4658.47	2050.14	< 0.0001	
AB	469.73	1	469.73	206.72	< 0.0001	
AC	1240.44	1	1240.44	545.90	< 0.0001	
AD	143.18	1	143.18	63.01	0.0002	
BC	5.58	1	5.58	2.45	0.1683	
BD	540.26	1	540.26	237.76	< 0.0001	
CD	925.91	1	925.91	407.48	< 0.0001	
ABC	381.50	1	381.50	167.89	< 0.0001	
ABD	42.94	1	42.94	18.90	0.0048	
ACD	115.50	1	115.50	50.83	0.0004	
BCD	671.65	1	671.65	295.58	< 0.0001	
Residual	13.63	6	2.27			
Lack of Fit	2.00	1	2.00	0.86	0.3959	not significant
Pure Error	11.63	5	2.33			
Cor Total	17775.36	19				

Std. Dev.	1.51	R-Squared	0.9992
Mean	35.87	Adj R-Squared	0.9976
C.V.	4.20	Pred R-Squared	0.9178
PRESS	1460.57	Adeq Precision	64.720

Table 5.9: ANOVA for Mixture Reduced Quadratic model (Response: IC_{50})

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	6.772 x 10^8	6	1.125 x 108	60.80	< 0.0001	significant
Linear Mixture	5.338 x 108	3	1.779 x 108	96.13	<0.0001	
AB	6.899 x 107	1	6.899 x 107	37.52	<0.0001	
AC	5.150 x 107	1	5.150 x 107	28.59	0.0002	
BC	3.944 x 107	1	3.944 x 107	20.78	0.0008	
Residual	1.844 x 107	11	1.8551 x 106			
Lack of Fit	2.23 x 106	6	7.458 x 105	0.21	0.8719	not significant
Pure Error	1.621 x 107	5	3.241 x 106			
Cor Total	6.956 x 108	17				

Std. Dev.	1360.51	R-Squared	0.9707
Mean	5296.69	Adj R-Squared	0.9548
C.V.	25.69	Pred R-Squared	0.9019
PRESS	6.824 x 107	Adeq Precision	19.935

i. Final Equation in Terms of Pseudo Components:

The application of mixture design yielded the following regression equation. The empirical relationship between response and variables in coded unit of TPC, TFC, % inhibition and IC_{50}. The final obtained model to predict the response between the solvent extract in terms of pseudo components the actual factors of mixture components can be expressed as follows:

$$TPC = 98.82A + 16.69B + 17.29C + 20.60\,D + 137.57\,AB + 128.82\,AC$$

$$TFC = 29.81\,A + 4.38\,B + 3.96\,C + 10.67D + 24.48AB + 47.51AC - 8.23AD$$
$$+ 10.37BC + 6.03BD + 21.80CD + 196.98\,ABC - 328.14ACD - 382.54BCD$$

$$\%\ inhibition = 90.88A + 16.59\,B + 12.13\,C + 74.30D - 96.76\,AB - 157.24\,AC$$
$$- 53.42\,AD - 8.18BC - 103.77BD - 135.85\,CD + 2333.04ABC + 786.92\,ABD$$
$$+ 1290.60ACD - 3095.602BCD$$

$$IC_{50} = 615.24\,A + 14793.35\,B + 16184.43C + 234.31D - 27978.81AB$$
$$- 30852.55AC + 26772.64\,BC$$

where A, B, C and D are the coded variables for water, acetone, acetonitrile and ethanol respectively.

5.4.2 Residual Analysis of Antioxidant Properties

Residual analysis consists of terms used to judge model adequacy (Mokhtar, 1994). It is used to estimate the difference between the observed and predicted response. In order to check the normality assumption, a normal probability plot of the residual was constructed and the results are shown in Figure 5.1, 5.2, 5.3 and 5.4. A normality assumption is satisfied if the residual plots approximately along a straight line (Myers and Montgomery, 2001) implying no abnormalities. The S shape will appear in the plot if the model was abnormal, thus showing that the statistical model was not acceptable (Haaland, 1989). Plot of the residual versus the predicted response detection is important for effective model. If the V shape appears in the residual plots, it indicates that some other factors exert might have a significant impact on response variables (Xu and Ting, 2004).

The normal probability plot of the residuals and the plot of the residuals versus the predicted response for TPC, TFC, % inhibition and IC_{50} are shown in Figure 5.1, 5.2, 5.3 and 5.4 respectively. The graph in Figure 5.1 revealed that the residuals generally fall on a straight line implying that errors are distributed normally and thus support adequacy of the least-square fit. Similarly, the plot in Figure 5.2 of the residuals versus the predicted response for TPC revealed that no obvious pattern and unusual structure. It also shows an equal scatter above and below the x-axis. It was found that 20 data experiment's points were located within the ranges. This implies that the model proposed is adequate and there is no reason to suspect any violation of independence or constant variance assumption. A similar trend is also observed for the normal probability plots of residuals and the plot of the residual versus the predicted response for TFC, % inhibition and IC_{50}

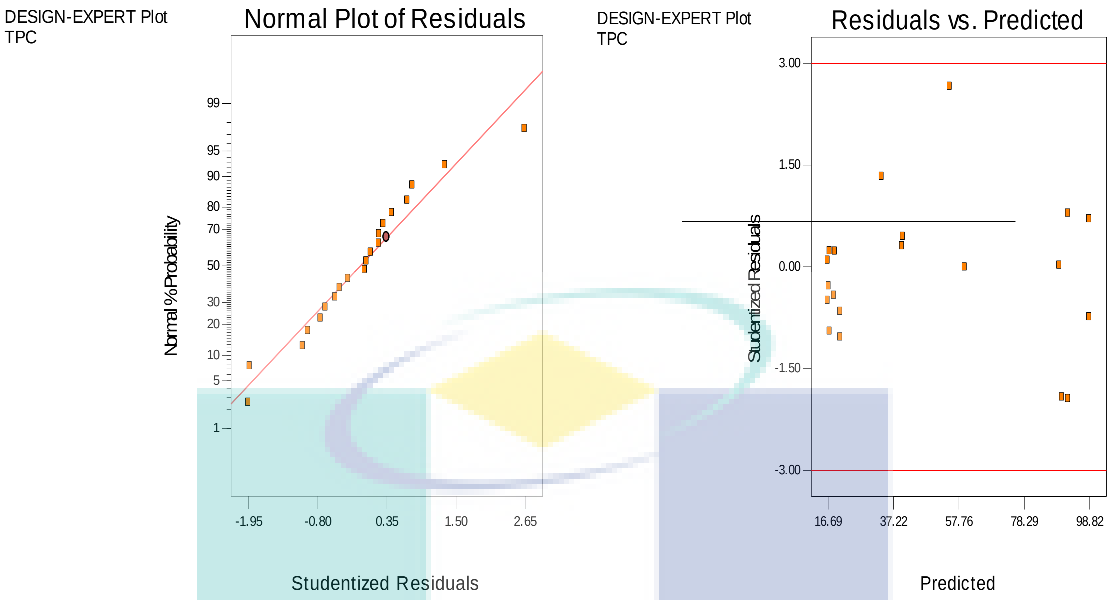


Figure 5.1: Normal probability plot of residual and residual versus. predicted response for TPC

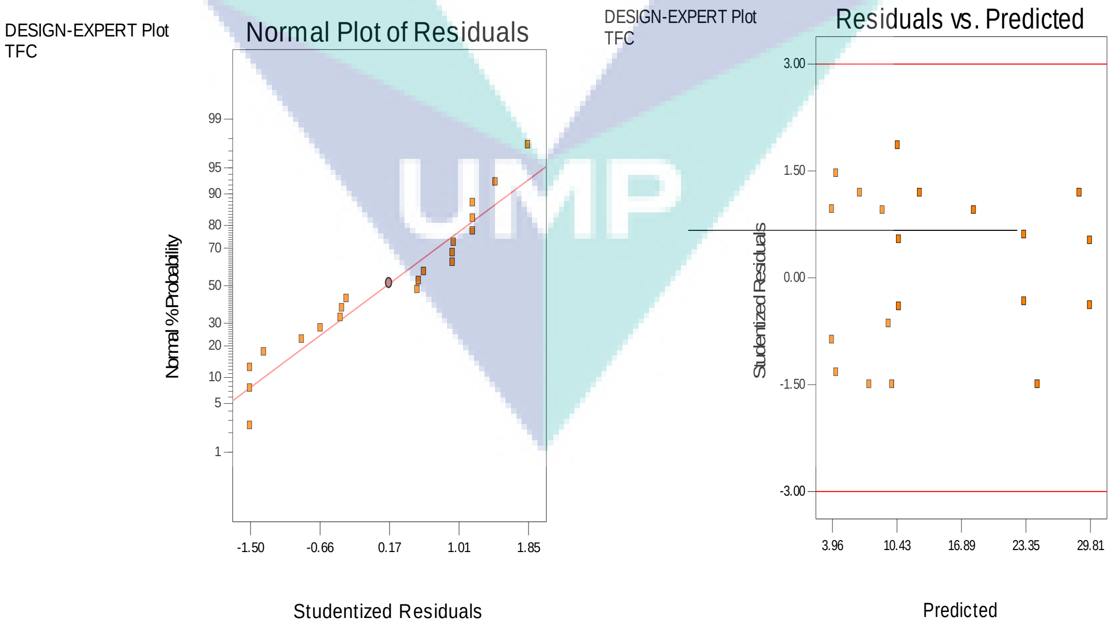


Figure 5.2: Normal probability plot of residual and residual versus. predicted response for TFC

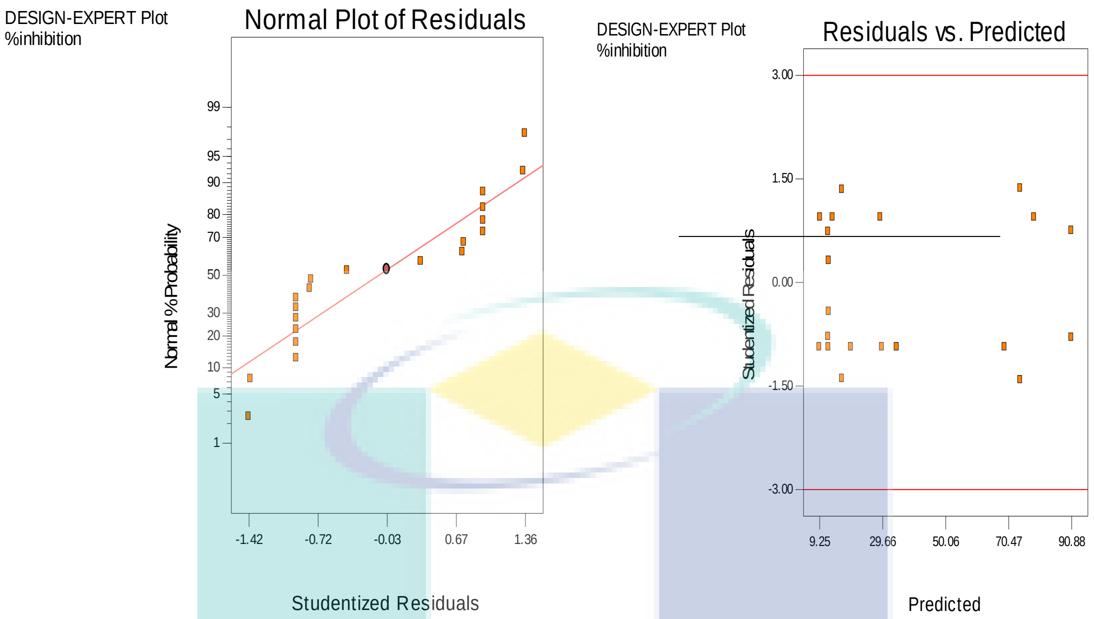


Figure 5.3: Normal probability plot of residual and residual versus. predicted response for % inhibition

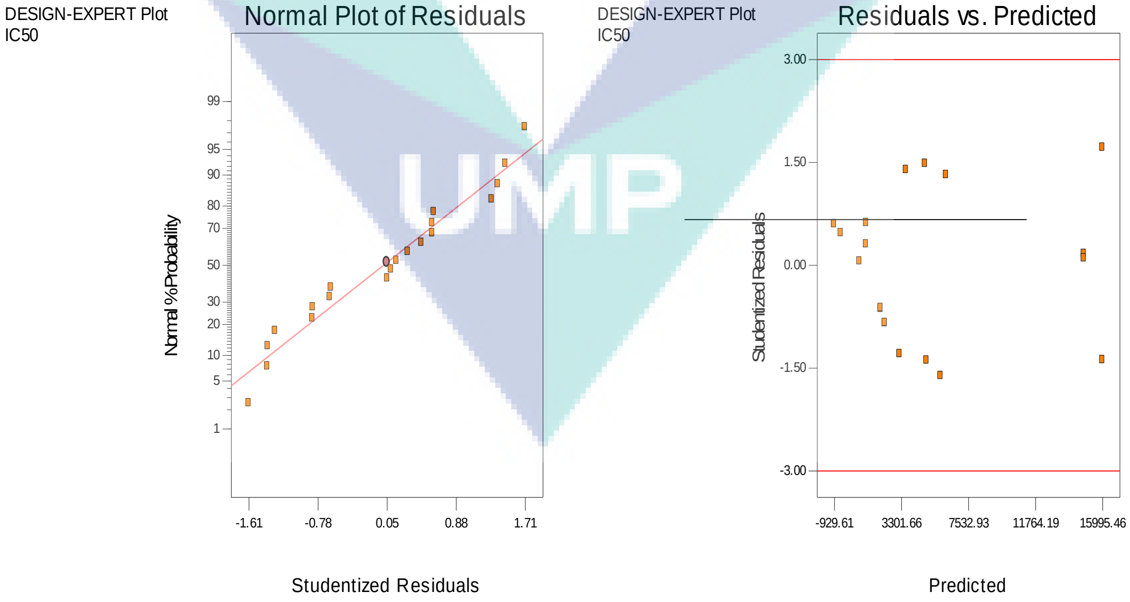
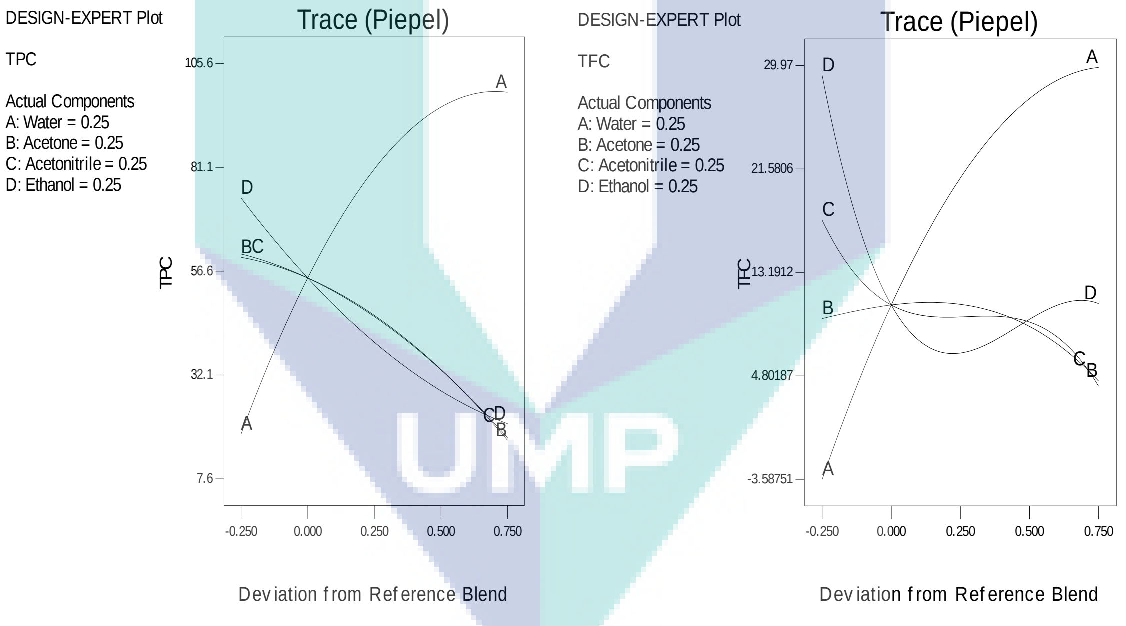


Figure 5.4: Normal probability plot of residual and residual versus. predicted response for IC_{50}

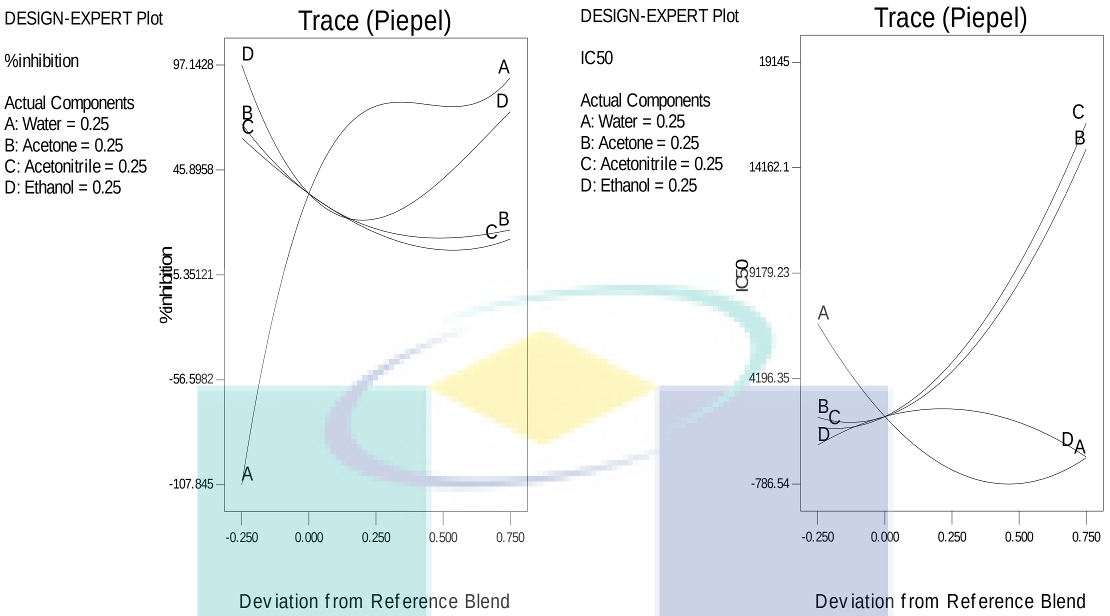
5.4.3 Trace Plots of Antioxidant Properties

On the basis of the mixture model, a trace plot was generated to show the response trend of each component in the mixture Figure 5.5(a-f). The trace plots showed that TPC and TFC were positively affected by water while the addition of acetone, acetonitrile and ethanol could negatively affect of TPC and TFC respectively (Figure 5.5a and 5.5b). This is attributed that due to microwave absorbance, inside water as compare to the rest solvent. As shown in Figure 5.5(c), % inhibition has the positive effect on water and ethanol while the addition of acetone and acetonitrile gave negative effect on % inhibition. Similar trend was found on IC_{50} as shown in Figure 5.5(d).



(a) (b)

Figure 5.5: Effects of setting treatment on antioxidant properties of NBF extract. (a) TPC; (b) TFC: (c) % inhibition (d) IC_{50}: A-A: water; B-B: acetone; C-C: acetonitrile. D-D: ethanol



(c) (d)

Figure 5.5: Continued

5.5 STATISTICAL MODELING OF PHYSICOCHEMICAL PROPERTIES

Experimental level of independent process variables (percentage of water, acetone, acetonitrile and ethanol) studies for dependent variables of physicochemical properties was shown in Table 5.3. The temperature controlled microwave assisted extraction (TCMAE) of NBF in each solvent was conducted using a same method as was explained in section 3.4.4. However for physicochemical property's analysis, maceration solvent before extraction and TCMAE solution was a centrifuge at 9000rpm for 20 minutes to get clear solution before reading process.

To fit the response function and experiment data, regression analysis was performed and the model was evaluated by (ANOVA) for dependent variable or response.As mentioned in section 5.3.1(i), a regression analysis was performed to represent the effect of each variable, including their interaction to each other on the response. The result of physicochemical properties in terms of % different (%Δ) before extraction and TCMAE of viscosity, refractive index, density and surface tension as per the experimental plan are presented in Table 5.10 (The raw data for the for physicochemical properties was tabulated in Appendix N) Then, the physicochemical property's results in Table 5.10 were input into the Design Expert software for further analysis following the steps outlined previously. Examination of the fit summary output revealed that the special cubic model was statistically significant for the viscosity, refractive index, density and surface tension. Therefore, this model was used to represent each of the responses for further analysis.

Table 5.10: Experimental Results of Physicochemical Properties

	Density (%Δ)	**Refractive Index** (%Δ)	**Viscosity** (%Δ)	**Surface Tension** (%Δ)
1	1.005	0.115	1.031	3.735
2	0.168	0.076	0.736	2.027
3	1.058	0.035	0.987	5.569
4	0.052	0.039	0.175	3.572
5	0.603	0.042	0.132	4.452
6	0.058	0.057	0.217	1.849
7	0.301	0.028	0.481	1.178
8	0.233	0.005	0.124	0.235
9	0.435	0.029	0.546	2.242
10	0.326	0.017	0.446	0.365
11	0.677	0.064	0.796	4.681
12	0.578	0.059	0.614	3.322
13	0.631	0.041	0.721	0.501
14	0.515	0.066	0.608	0.321
15	0.781	0.071	0.834	1.416
16	0.613	0.025	0.141	4.552
17	0.201	0.001	0.121	0.331
18	1.003	0.125	1.079	3.735
19	0.316	0.007	0.432	0.365
20	0.056	0.059	0.212	1.941

The result of physicochemical was calculated by the percentage of difference (%Δ) of NBF extract solution before (maceration) and after extraction (TCMAE).

5.5.1 ANOVA Analysis for Density, Refractive Index, Viscosity and Surface Tension Model

In order to ensure a good model, test for significant of regression model, test for significance on individual model coefficients and test for lack of fit needed to be executed. An ANOVA table is commonly used to summarize the tests performed. The backward elimination procedure was selected to automatically reduce the insignificant terms and the resulting ANOVA table for reduced special cubic model for density is depicted in Table 5.11. The value of “Prob.>F” for the model is less than 0.05, which indicates that the model is significant. It also indicates that the term in the model has a significant effect on the response. In a similar manner, the linear mixture and two level interactions of water acetone (AB), water acetonitrile (AC), water ethanol (AD), acetone with acetonitrile (BC), acetone with ethanol (BD) and acetonitrile with ethanol (CD) and three level interactions of water, acetone, with acetonitrile (ABC), water, acetone, with ethanol (ABD), water, acetonitrile with ethanol (ACD) also acetone, acetonitrile and ethanol (BCD) are significant model term. Other model terms are said to be not significant. The lack of fit can also be said to be insignificant. This is desirable, as we want a model that fits.

The linear mixture, two level interactions of water acetone (AB), water acetonitrile (AC), water ethanol (AD), acetone with acetonitrile (BC), acetone with ethanol (BD) and acetonitrile with ethanol (CD) are the most factors associated with density. The significant factors were ranked based on the value of *F*-ratio where the larger the magnitude of the *F*-value the more significant is the corresponding coefficient. Thus in this study, the ranking is as follows: AB>AD>linear mixture>BC>AC>CD>BD>ABD>ABC>BCD.

Table 5.11 also shows the R^2 value calculated is 0.99, reasonably close to 1, which is acceptable. The predicted R^2 is in reasonable agreement with the adjusted R^2. Adequate precision compares the range of the predicted values at the design points to the average prediction error. In this case, the value is well above 4 which indicate adequate model discrimination. In this case, the value is rightly above 4.

Table 5.11: ANOVA for mixture reduced special cubic model (response: Density)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	1.94	13	0.15	947.42	<0.0001	significant
Linear Mixture	0.70	3	0.23	1476.39	<0.0001	
AB	0.33	1	0.33	2066.63	<0.0001	
AC	0.16	1	0.16	1025.86	<0.0001	
AD	0.30	1	0.30	1891.99	<0.0001	
BC	0.17	1	0.17	1068.08	<0.0001	
BD	0.021	1	0.021	134.27	<0.0001	
CD	0.022	1	0.022	142.42	<0.0001	
ABC	1.587×10^{-3}	1	1.587×10^{-3}	10.07	0.0192	
ABD	8.300×10^{-3}	1	8.300×10^{-3}	52.69	0.0003	
ACD	4.443×10^{-4}	1	4.443×10^{-4}	2.82	0.1441	
BCD	0.033	1	0.033	206.79	<0.0001	
Residual	9.451×10^{-4}	6	1.575×10^{-4}			
Lack of Fit	3.291×10^{-4}	1	3.291×10^{-4}	2.67	0.1631	not significant
Pure Error	6.160×10^{-4}	5	1.232×10^{-4}			
Cor Total	1.94	19				

Std. Dev.	0.013	R-Squared	0.9995
Mean	0.048	Adj R-Squared	0.9985
C.V.	2.61	Pred R-Squared	0.8791
PRESS	0.23	Adeq Precision	95.804

Table 5.12 shows the ANOVA table for the response reduced special cubic model for refractive index. The value of “Prob.>F” for the model is less than 0.05, which indicates that the model is significant. It also indicates that the term in the model have a significant effect on the response. In a similar manner, the linear mixture and two level interactions of water acetonitrile (AC), water ethanol (AD), acetone with acetonitrile (BC), acetonitrile with ethanol (CD) and three level interaction of water, acetone and acetonitrile also acetone (ABC), acetonitrile and ethanol (BCD) are significant model term. Other model terms are

said to be not significant. The lack of fit can also be said to be insignificant. This is desirable, as we want a model that fits. The linear mixture is the most factors associated with refractive index. The significant factors were ranked based on the value of *F*-ratio. The larger the magnitude of the *F*-value and the smaller the *P*-value, the more significant is the corresponding coefficient (Cochran and Cox, 1992). Thus in this study, the ranking is as follow: linear mixture> BC>BCD>AC>AD>ABC>CD. Table 6.12 also shows the R^2 value calculated is 0.98, reasonably close to 1, which is acceptable. The predicted R^2 is in reasonable agreement with the adjusted R^2. Adequate precision compares the range of the predicted values at the design points to the average prediction error. In this case, the value is well above 4 which indicate adequate model discrimination.

Table 5.12: ANOVA for mixture reduced special cubic model (response: Refractive index)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	0.021	9	2.278×10^{-3}	49.90	<0.0001	significant
Linear Mixture	0.015	3	4.915×10^{-3}	107.66	<0.0001	
AC	5.368×10^{-4}	1	5.368×10^{-4}	11.76	0.0064	
AD	5.033×10^{-4}	1	5.033×10^{-4}	11.02	0.0077	
BC	2.093×10^{-3}	1	2.093×10^{-3}	45.85	<0.0001	
CD	3.941×10^{-4}	1	3.941×10^{-4}	8.63	0.0148	
ABC	4.038×10^{-4}	1	4.038×10^{-4}	8.85	0.0139	
BCD	1.260×10^{-3}	1	1.260×10^{-3}	27.59	0.0004	
Residual	4.565×10^{-4}	10	4.565×10^{-5}			
Lack of Fit	2.020×10^{-4}	5	4.040×10^{-5}	0.79	0.5970	not significant
Pure Error	2.545×10^{-4}	5	5.090×10^{-5}			
Cor Total	0.021	19				

Std. Dev.	6.756×10^{-3}	R-Squared	0.9782
Mean	0.048	Adj R-Squared	0.9586
C.V.	14.06	Pred R-Squared	0.7987
PRESS	4.219×10^{-3}	Adeq Precision	24.816

The backward elimination procedure was selected to automatically reduce the insignificant terms and the resulting ANOVA table for reduced special cubic model for viscosity is depicted in Table 5.13. The value of “Prob.>F” for the model is less than 0.05, which indicates that the model is significant. It also indicates that the term in the model have a significant effect on the response. In a similar manner, the linear mixture and two level interactions of water acetone (AB), water acetonitrile (AC), water ethanol (AD), acetone with acetonitrile (BC), acetone with ethanol (BD) and acetonitrile with ethanol (CD) and three level interactions of acetone, acetonitrile and ethanol (BCD) are significant model term. Other model terms are said to be not significant. The lack of fit can also be said to be insignificant. This is desirable, as we want a model that fits.

The linear mixture, two level interaction of water acetonitrile, water ethanol, acetone ethanol and acetonitrile ethanol and three level interaction of acetone, acetonitrile and ethanol are the most factors associated with viscosity. The significant factors were ranked based on the value of *F*-ratio. The larger the magnitude of the *F*-value and the smaller the *P*-value, the more significant is the corresponding coefficient (Cochran and Cox, 1992). Thus in this study, the ranking is as follow: linear mixture>AD>AC>BCD>CD>BD>AB>BC.

As show in Table 5.13, the R^2 value calculated is 0.99, reasonably close to 1, which is acceptable. The predicted R^2 is in reasonable agreement with the adjusted R^2. Adequate precision compares the range of the predicted values at the design points to the average prediction error. In this case, the value is well above 4 which indicate adequate model discrimination

Table 5.13: ANOVA for mixture reduced special cubic model (response: Viscosity)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	2.00	9	0.20	499.82	<0.0001	significant
Linear Mixture	1.16	3	0.39	966.31	<0.0001	
AB	0.016	1	0.016	40.01	0.0001	
AC	0.14	1	0.14	340.84	<0.0001	
AD	0.29	1	0.29	713.96	<0.0001	
BC	9.957 x 10^{-3}	1	9.957 x 10^{-3}	24.91	0.0007	
BD	0.029	1	0.029	73.22	<0.0001	
CD	0.058	1	0.058	145.03	<0.0001	
BCD	0.097	1	0.097	242.10	<0.0001	
Residual	2.553 x 10^{-4}	7	3.997 x 10^{-4}			
Lack of Fit	1.517 x 10^{-4}	2	5.725 x 10^{-4}	2.19	0.2064	not significant
Pure Error	2.401 x 10^{-3}	5	2.615 x 10^{-4}			
Cor Total	4.78	16				

Std. Dev.	0.020	R-Squared	0.9982
Mean	0.52	Adj R-Squared	0.9962
C.V.	3.83	Pred R-Squared	0.9839
PRESS	0.032	Adeq Precision	62.517

Table 5.14 shows the ANOVA table for the response special cubic model for surface tension. The value of "Prob.>F" for the model is less than 0.05, which indicates that the model is significant. It also indicates that the term in the model have a significant effect on the response. In a similar manner, the linear mixture and two level interactions of water acetone (AB), water acetonitrile (AC), water acetonitrile (AC), water ethanol (AD), acetone with acetonitrile (BC), acetone with ethanol (BD) and acetonitrile with ethanol (CD) are significant model term. Other model terms are said to be not significant. The lack of fit can also be said to be insignificant. This is desirable, as we want a model that fits.

Table 5.14: ANOVA for mixture special cubic model (response: Surface tension)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	74.84	13	5.76	1569.17	<0.0001	significant
Linear Mixture	34.18	3	11.39	3105.04	<0.0001	
AB	3.49	1	3.49	951.71	<0.0001	
AC	24.87	1	24.87	6777.82	<0.0001	
AD	1.87	1	1.87	510.73	<0.0001	
BC	0.33	1	0.33	89.30	<0.0001	
BD	1.25	1	1.25	341.66	<0.0001	
CD	2.97	1	2.97	809.79	<0.0001	
ABC	0.42	1	0.42	114.23	<0.0001	
ABD	2.84	1	2.84	774.16	<0.0001	
ACD	3.83	1	3.83	1044.52	<0.0001	
BCD	2.15	1	2.15	0.8	<0.0001	
Residual	0.022	6	$3.669\text{x}10^{-3}$			
Lack of Fit	$8.173\text{x}10^{-3}$	1	$8.173\text{x}10^{-3}$	2.95	0.1464	not significant
Pure Error	0.014	5	$2.768\text{x}10^{-3}$			
Cor Total	74.86	19				

Std. Dev.	0.061	R-Squared	0.9997
Mean	2.42	Adj R-Squared	0.9991
C.V.	2.50	Pred R-Squared	0.9222
PRESS	5.86	Adeq Precision	143.889

The linear mixture, two level interaction of water acetone, water ethanol, acetone ethanol and acetonitrile ethanol are the most factors associated with surface tension. The significant factors were ranked based on the value of F-ratio. The larger the magnitude of the *F*-value and the smaller the *P*-value, the more significant is the corresponding coefficient (Cochran and Cox, 1992). Thus in this study, the ranking is as follow: linear mixture>AB>AC>AD>BD>CD>BC.As show in Table 5.14, the R^2 value calculated is 0.99, reasonably close to 1, which is acceptable. The predicted R^2 is in reasonable agreement with the adjusted R^2. Adequate precision compares the range of the predicted values at the design points to the average prediction error. In this case, the value is well above 4 which indicate adequate model discrimination

i. Final Equation in Terms of Pseudo Components:

Using Design Expert software, a quadratic model was fitted to the experimental results. The final obtained model to predict the response between the solvent extract in terms of pseudo components the actual factors of mixture components can be expressed as follows:

$\text{Density} = 1.00A+0.61B+0.22C+0.32D-2.55AB+1.79AC-2.44AD-1.42BC$
$-0.65BD+0.67CD+4.76ABC+10.94ABD-2.53ACD+21.56BCD$

$\text{Refractive index} = 0.12A + 0.034B + 2.024\times10^{-3}C + 0.014D - 0.10AC$
$-0.097AD +0.16BC-0.087CD-2.01ABC+3.56BCD$

$\text{Viscosity} = 1.06A + 0.14B + 0.13C + 0.44D + 0.55AB - 1.60AC$
$- 2.32AD +0.34BC - 0.76BD + 1.07CD+18.59BCD$

$\text{Surface tension} = 3.74A+4.50B+0.28C+0.37D-8.34AB+22.26AC-6.11AD$
$-1.98BC-5.00BD+7.70CD+77.33ABC+202.39ABD-235.09ACD-175.28BCD$

where A, B, C and D are the coded variables for water, acetone, acetonitrile and ethanol respectively.

5.5.2 Residual Analysis of Physicochemical Properties

Residual analysis is used to estimate the difference between the observed and predicted response and was constructed. The normal probability plot of the residuals and the plot of the residuals versus the predicted response for density, refractive index, viscosity and surface tension was constructed and was shown in Figure 5.6, 5.7, 5.8 and 5.9 respectively in order to check the normality assumption, a normal probability plot of the residual The graph in Figure 5.6 for density revealed that the residuals generally fall on a straight line implying that errors are distributed normally and no obvious pattern and unusual structure. It also shows equal scatter above and below x-axis. It was found that 20 data experiments points were located within the ranges. This implies that the model proposed is adequate and there is no reason to suspect any violation of independence or constant variance assumption. A similar trend is also observed for the normal probability plots of residuals and the plot of the residual versus the predicted response for refractive index, viscosity and surface tension.

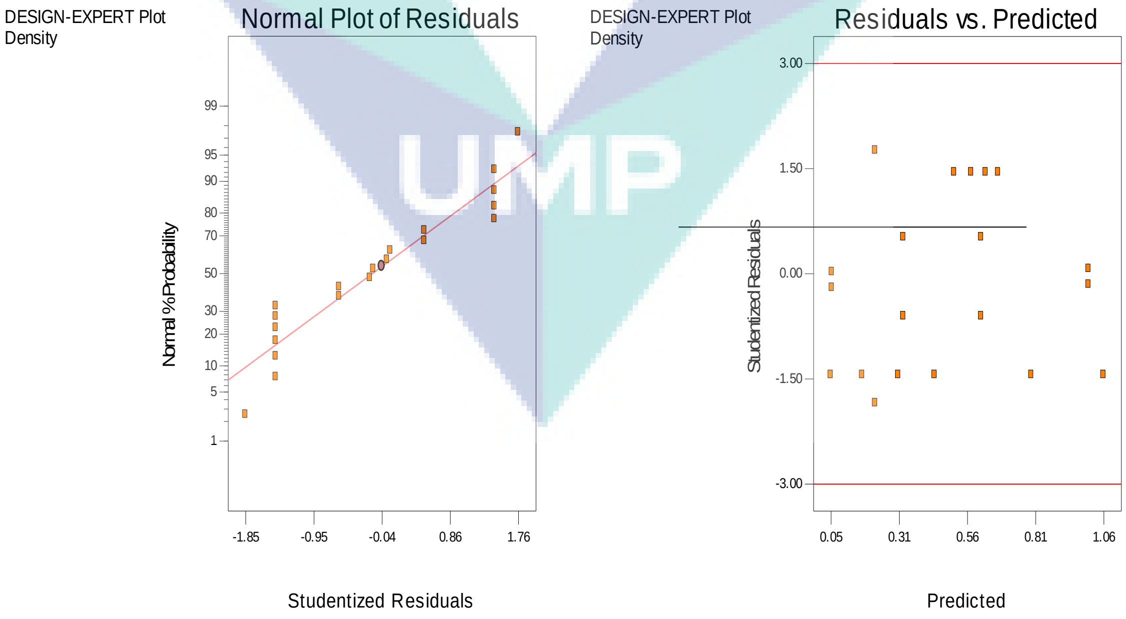


Figure 5.6: Normal probability plot of residual and residual versus. predicted response for density

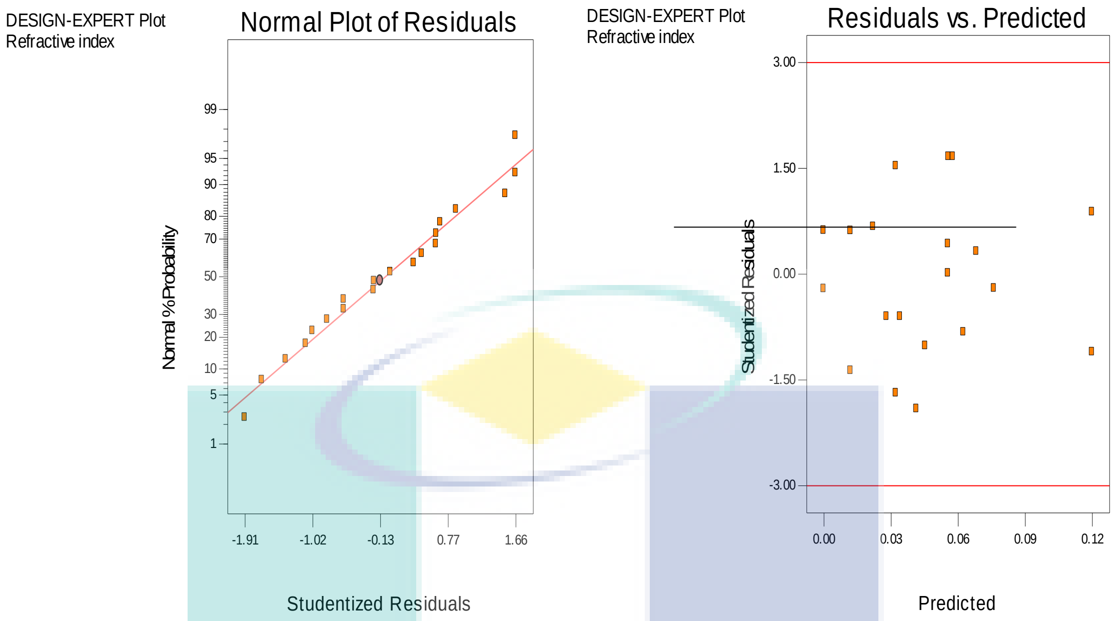


Figure 5.7: Normal probability plot of residual and residual versus predicted response for refractive index

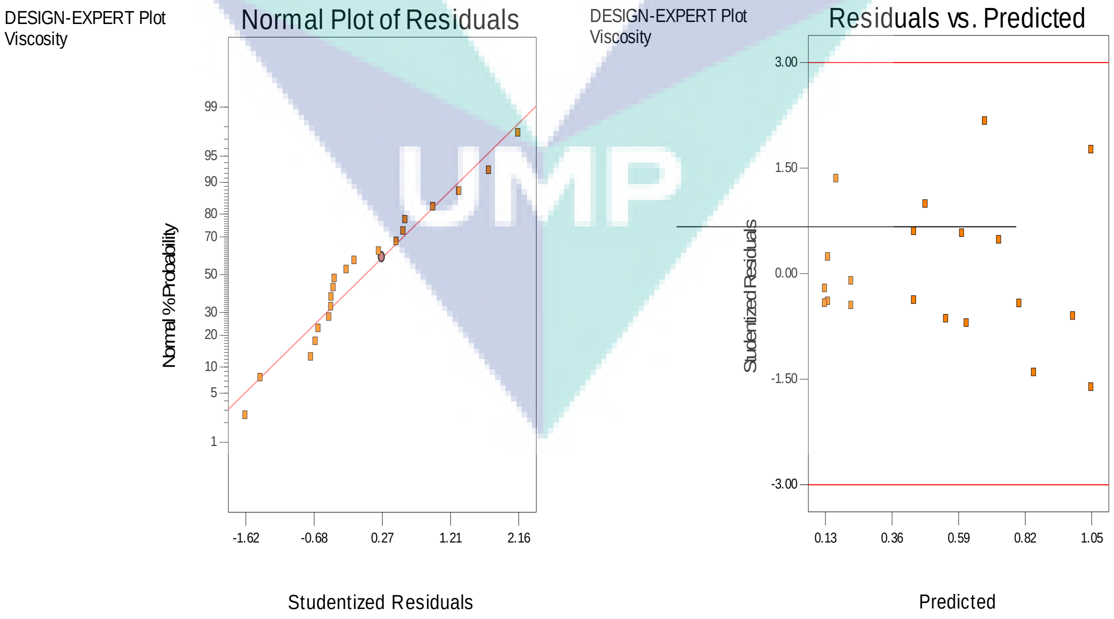


Figure 5.8: Normal probability plot of residual and residual versus. predicted response for viscosity

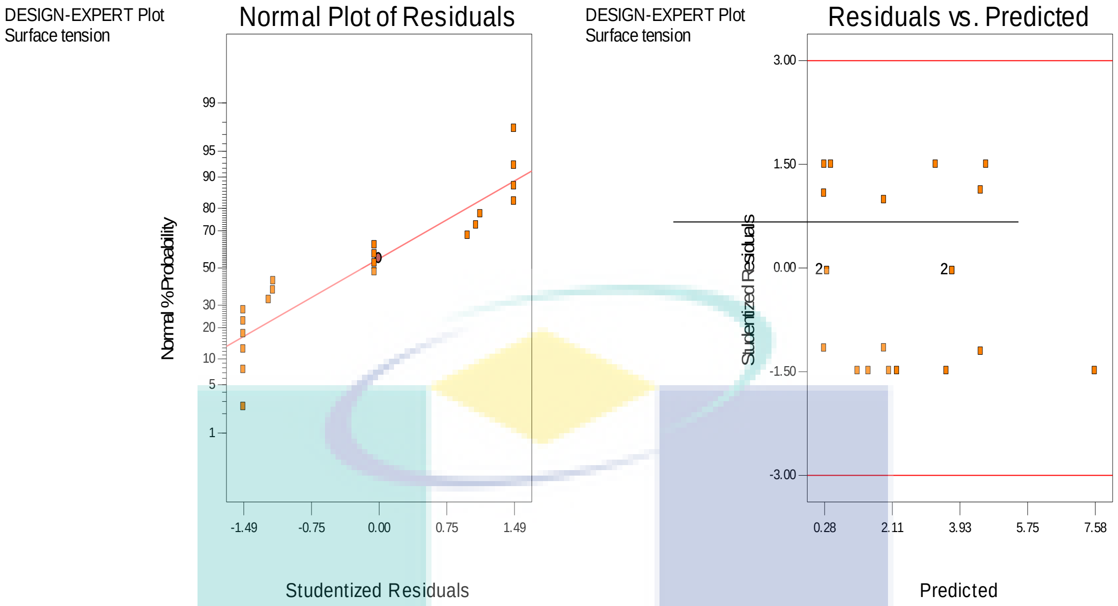
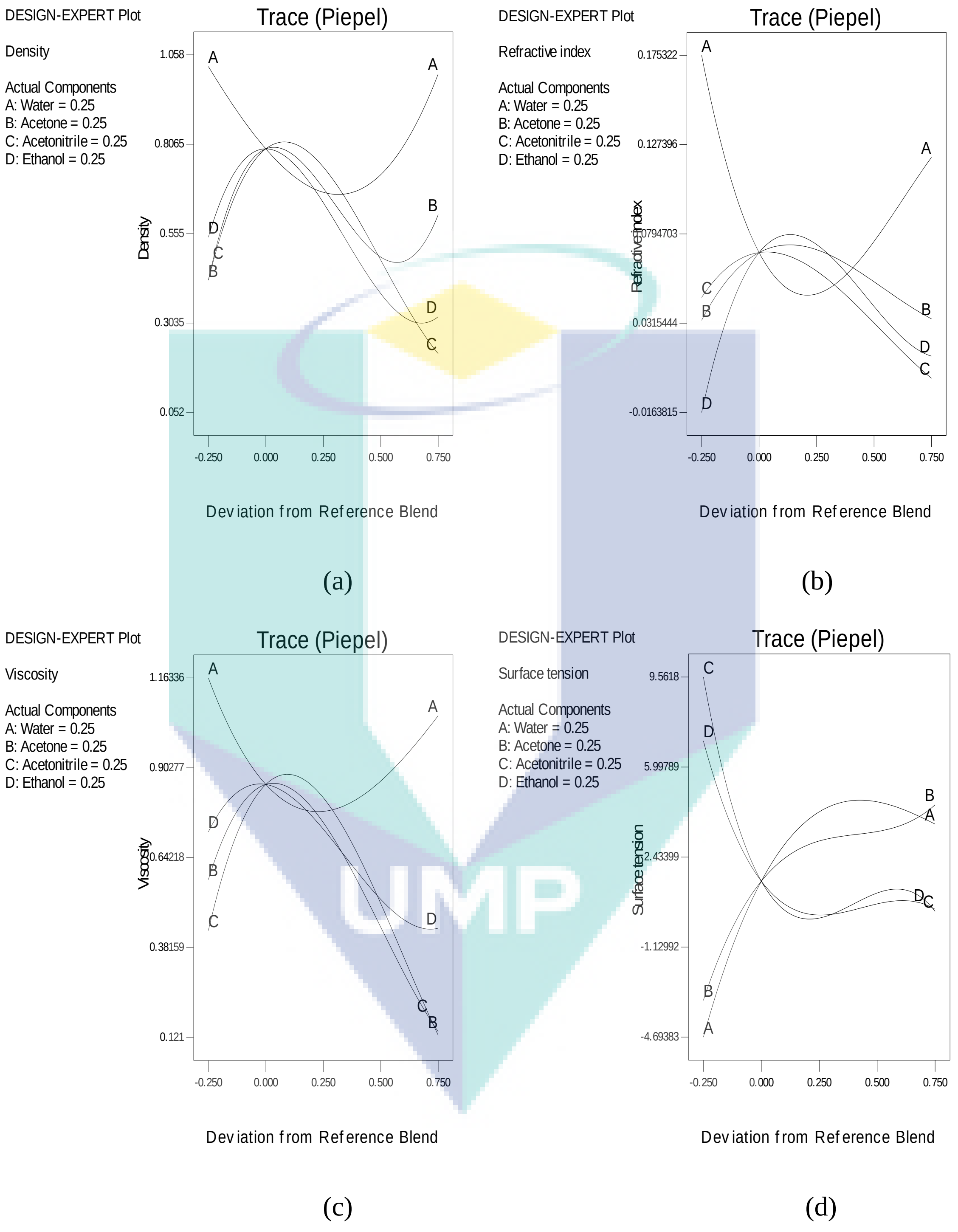


Figure 5.9: Normal probability plot of residual and residual versus. predicted response for surface tension

5.5.3 Trace Plots of Antioxidant Properties Surface

On the basis of the mixture model, a trace plot was generated to show the response trend of each component in the mixture as shown in Figure 5.10(a-d). The trace plots showed that density, refractive index, viscosity and surface tension were positively affected by water while the addition of acetone, acetonitrile and ethanol could negatively affect on viscosity, refractive index and density as depicted in Figure 5.10(a-c). This is attributed that due to higher microwave absorbance of water as compare to the rest solvent. As shown in Figure 5.10(d), surface tension has positive effect on water and acetone and negative effect on acetonitrile and ethanol.



(a) (b)

(c) (d)

Figure 5.10: Effects of setting treatment onphysicochemical properties of NBF extract. (a) density (b) refractive index (c) viscosity (d) surface tension: A-A: water; B-B: acetone; C-C: acetonitrile. D-D: ethanol

5.6 Antioxidant andPhysiochemical Characteristics of Temperature Control Microwave Assisted (TCMA) NBF Extract

5.6.1 Effect of Dielectric Properties of Solvent Nature on Extraction Effeciency

Water, acetone, acetonitrile, ethanol and their combination were used as the solvents for NBF extraction using TCMCS with fluid seal stirring system to examine variations in extraction efficiency according to the solvent used. The dielectric constant (ε’) and dielectric loss factor (ε”) and dissipation factor (δ) of the extraction solvent which plays an important role in extraction efficiency was calculated according to equation 2.3 was tabulated in Table 5.15.

Table 5.15: Dielectric constant and dissipation factors of selected solvent

Solvent	**ε’**[a]	**ε"**[b]	**tan δ**[c]	**tan δ**[d]	**Boiling points (°C)**[c]
Water	78.3	11.67	0.15	0.16	99.8
Acetone	20.7	10.08	0.49	0.55	56.2
Acetonitrile	37.5	7.15	0.19	0.21	81.6
Ethanol	24.3	5.43	0.22	0.25	78.3

[a] Determined at 20°C
[b] At 2450 MHz
[c] Calculation
[d] Zlotorzynski, (1995)

The proper choice of solvent is the key to successful extraction. The ability of material to absorb microwave energy is defined by its dissipation factor tan δ, which is the ratio of the dielectric loss (ε”) to the dielectric constant (ε’) of the material. The former (ε”) is a measure of material’s ability to absorb microwave energy and convert it into heat and other forms of energy. As shown in Table 5.15, the highest recovery for antioxidant properties obtained with water. Water molecules have a high dipole moment, and so absorb microwave energy strongly, leading to efficient heating of the sample. When water which is polar solvent with a relatively high dielectric constant, and loss factor is used; the solvent will be heated by the microwave energy through dipole rotation. The heated solvents will accelerate the process of the desorption of matrix-solvent interface, and the diffusion of the target compounds into solvent (Hawthorneand Miller, 1994). As a consequence, obtaining reproducible results requires control of the matrix water content.

In addition, TCMAE may be subject to interferences from the presence of microwave energy-absorbing mater in the sample that can cause arcing. Furthermore, the organic carbon content of the matrix (Appendix P) is known to hinder the extraction, owing to strong analyte-matrix interactions that are difficult to disrupt. For the same reason, spiked compounds are readily extractable, while native solutes are much harder to extract under the same conditions.In addition, NBF having the huge amount of sequiyitol with the sugar moieties of glucose, fucose, and rhamnose as well as hydroxyl groups attached to the molecular backbones. They were quite soluble in polar solvent, such as water and diluted ethanol, but were insoluble in nonpolar solvents. Conversely, flavonoids are compounds containing hydroxyl groups conferring a high solubility in water.

In this study, acetonitrile extraction was found to give lower amounts of extractable antioxidant properties when compared with another acetone and ethanol solvent. Acetonitrile has the higher dielectric constant of both solvents. However, the dissipation factor is significantly lower than other solvents. Hence, the rate at which solvent absorbs microwave energy is higher than the rate at which the system can dissipate the heat. These phenomena account for the ‘‘superheating’’ effect, which occurs when acetonitrile is used. So, intense heating may have caused degradation of the analyte. This is why it is best to choose a solvent that has a high dielectric constant as well as a high dissipation factor, to facilitate heat distribution through the matrix. However, this theory is not applicable for water in this study even it’s having a higher dielectric constant and lower dissipation factor as compared to ethanol and acetonitrile. “Superheating” phenomena not affected for water because this experiment was run at 60°C which is far from water boiling point (99.98°C) as compared to acetonitrile boiling points (82°C).

i. Volume Rate of Heat Generation

The volume rate of heat generation is directly related to the rate of temperature increases, density and heat capacity of the sample. Temperature profile at different various solvent and their mixture: water, acetone, acetonitrile and ethanolwere depicted in Appendix Q. Figure 5.11 below illustrates the volume rate of heat generation for various solvents at 420 Watts for 300 sec. Results of other solvent, and its combinations are presented in Appendix R.Various solvents with dissimilar physical and dielectric properties reveal at different microwave frequencies and exposure time, their polarity, dipole strength, and composition were changed (Iqbal, 2009). Thus, in order to investigate the effect of physical and dielectric properties in TCMCS heating mechanism, relation between dielectric constant, dipole moments and heat capacity of protic and aprotic polar solvents with volume heat generation value was studied. The physical and dielectric behaviors of protic polar solvents, water and ethanol also aprotic polar solvent, acetone and acetonirile, were obtained from experimental work as observed in Appendix I.

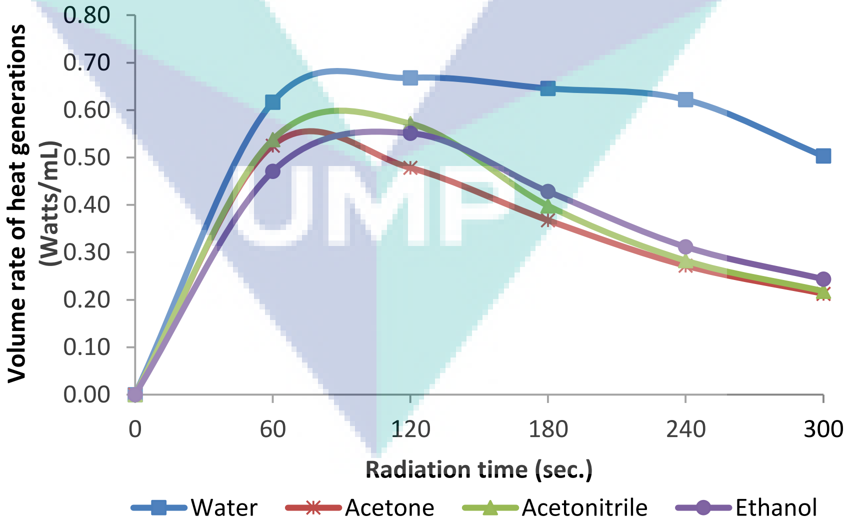


Figure 5.11: Volume rate of heat generation of various solvent vs radiation time

The heating rate of sample by microwave is affected by dielectric constant, loss factor, specific heat capacity, emissivity of the sample and the strength of the applied field and temperature. As illustrated in Figure 5.11, the result of volume of heat generation solvents increased almost linearly with the increased of exposure time. Water shows higher result followed by ethanol, acetonitrile, and acetone. Materials that absorb microwave energy are known as dielectrics, and they are characterized as non-conductive materials. Dielectrics can be heated if the molecules possess an asymmetrical structure where water is the typical case of such a molecule. In the presence of an electricfield, these asymmetric or polar molecules will align themselves according to the electric field.The capability of a water to convert microwave energy into heat at a given frequency and temperature is determined by the so-called loss tangent (*tan δ*), expressed as the quotient, dielectric loss/dielectric constant ($\varepsilon'' / \varepsilon'$) were describing the ability of molecules to be polarized by the electric field (Herrero et al., 2008). As a conclusion, a solvent with a high *tanδ* at the standard operating frequency of a microwave synthesis reactor (2.45 GHz) is required for good absorption and, consequently, for efficient heating.Because of its low dielectric loss factor, ethanol, acetonitrile and acetone are more transparent to microwaves than water, therefore water heats up much more rapidly when exposed to microwaves than other solvents.

The result of heat generation between acetonitrile, ethanol and acetone are close each other even clearly showing far value of dielectric properties. The magnitude of the solvent dipole moment is another main factor that correlates with the microwave heating characteristic of protic and aprotic polar solvent. Inspection of dipole moments values suggest that acetone with dipole moments of 2.69 or acetonitrile with dipole moments of 3.44 will rotate easily when exposed to an alternating electric field of microwave energy (Bottcher, 1952) as compared to water (2.3) and ethanol (1.96). This oscillation produced collisions with surrounding molecules, then energy was transferred with subsequent heating (Saoud, 2004). Acetone molecules illustrate a high heat capacity compared to other solvent (Appendix I). This leads to higher requirement for energy to be absorbed and consequently, difficult to raise the temperature inside the microwave cavity (Jemaat, 2006) which leads to lower volume rate of heat generation of acetone than other solvent.

ii. Penetration depth

Penetration depth is an effective and convenient measure to compare the relative microwave absorbing characteristics of samples and to explain the effect of the dielectric properties and geometry on microwave heating. A large penetration also indicates that radiation is poorly absorbed while a short penetration depth means that surface heating predominates (Saoud, 2004). The penetration depth also affected by microwave frequency (Hoekstra and Delaney, 1974). High frequency has a short wavelength which corresponds to a smaller penetration depth (Rao et al., 1988; Ratanadecho, 2006). In this study, the penetration depth was calculated for TCMAE of water, acetone, acetonitrile and ethanol. Figure 5.12 shows the result of penetration depth are as follows water>ethanol>acetonitrile>acetone. The result was accordance to Palascious et al., (1996) which found that ethanol has the shorter penetration depth than of water, which shows microwave heating rate of ethanol is higher than water.

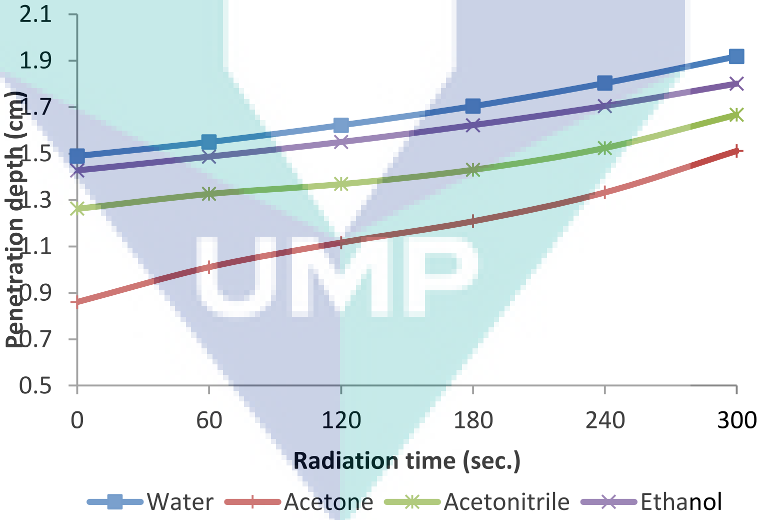


Figure 5.12: Penetration depth of various solvent vs radiation time

5.6.2 Effect of Aqueous Solvent Concentration on Extraction Efficiency

The extraction efficiency was dependence on the plant extracted, as well as the solvent used for the extraction. Polar solvents are usually believed better than non-polar solvents. It has been reported that by addition of water to the solvent, increased yields are obtained (Escribano-Bailon and Santos-Buelga, 2003). The extractable phenolic substances for the microwave extraction method decreased significantly (p<0.05) for all the plants with decreasing polarity of the solvent in the order water, ethanol, acetone and acetonitrile. The principle of heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. Polar molecules (such as polyphenols) and ionic solutions absorb microwave energy strongly because they have a permanent dipole moment. This results in the rapid rise of the temperature and fast completion of the reaction (Gfrerer and Lankmayr, 2005; Venkatesh and Raghavan, 2004; Eskilsson and Bjorklund, 2000).

Effects of water concentration on antioxidant properties of TCMA extraction were carried out at different solvent combination with other conditions kept unchanged (Microwave power, 420 watts; ratio of solid to liquid, 1:25; time, 240s; temperature 70°C). From these results, it is clear that the addition of some amount of water enhances the antioxidant extraction efficiency as shown in our result where 62.5% > 50% > 25% > 12.5% of water proportion in terms of antioxidant properties values.This value was supported by the result of physicochemical properties,which was summarized in Table 5.10. The difference between maximum and minimum values (% Δ) in results of density, refractive index, viscosity and surface tension, before and after TCMAE of NBF, revealed that 100% water showing significant effects on to the solvent extract as compared to 62.5%, 50%, 25% and 12.5%.

High in water content resulted in positive up in extraction of antioxidant properties value. The reason is, with a presence of some amount of water can increase the mass transfer process by increasing the relative polarity of the solvent thus improving its solubilizing capacity and through effective swelling of the plant material, thus increasing the surface area for solute solvent interaction. The interaction of phytochemicals with water also can enhance the dispersion rate of water molecules into the plant matrix due to its favorable dipole moment inside microwave. This interaction rate depends on the hydrogen bonding and heating rate as well as the formation of water clusters. Besides that the water dissolution depends on the presence of hydroxyl groups, nature of monosaccharide, inter sugar linkages (α or β) and the ability to be associate by intermolecular interactions (Amid and Mirhosseini, 2012). A Similar results was also reported previously in microwave-assisted solvent extraction of effective constituents from *Eucommia ulmodies Oliv*. (Li et al., 2004).

i. Volume Rate of Heat Generation

Based on the result of volume of heat generation reading tabulated in Appendix R, Figure 5.13 was plotted to present the relation between the rates of temperature increment of solventwith different aqueous (water) concentration. As observed, temperature increment of water as was shifted from 12.5%, 25%, 50%, 62.5% and 100% aqueous composition. At 100% water, the interaction with microwave is much higher due to its high water composition, which resulted high in temperature increases rate. This study indicates that the microwave heating is very rapid in high composition of waterthus higher interaction with electric fields, therefore, increases in dielectric constant. This result was supported the explanation from Calla et al., (2007) who studied the effect of microwave irradiation with radiation time in soil.

Figure 5.13 also illustrated, the acceleration of microwave absorbed energy inside the water during the initial duty cycle is higher at high to low water concentration because the molecules of the water become aligned and rapidly absorbed microwave's energy due to rising temperature. The results show the increment of temperature in water at 100% was much stronger than that at 12.5% water, thus the increment of temperature reaching at the high amount of water smoothly and in a very short time. However, as the exposure time was increased further, the volume rate of heat generation decreased and in certain cases approached plantaeu condition. The decreasing of heat generation within radiation time is due to the polarity of water decreases with increasing temperature, which resulted decreasing of the ability of water to absorb microwave energy and affected the dipole rotation and alignment in the microwave field. The dielectric property which is attributed to the solvent polarity is dependent on mobility of the dipoles within the structure (Rahmat, 2002). The dielectric properties of solvents are influenced by heating temperature and significantly affected the ability of samples to absorb and dissipitate the microwave energy (Fang and Lai, 1996; Chan and Chen, 2002; Saound, 2004).

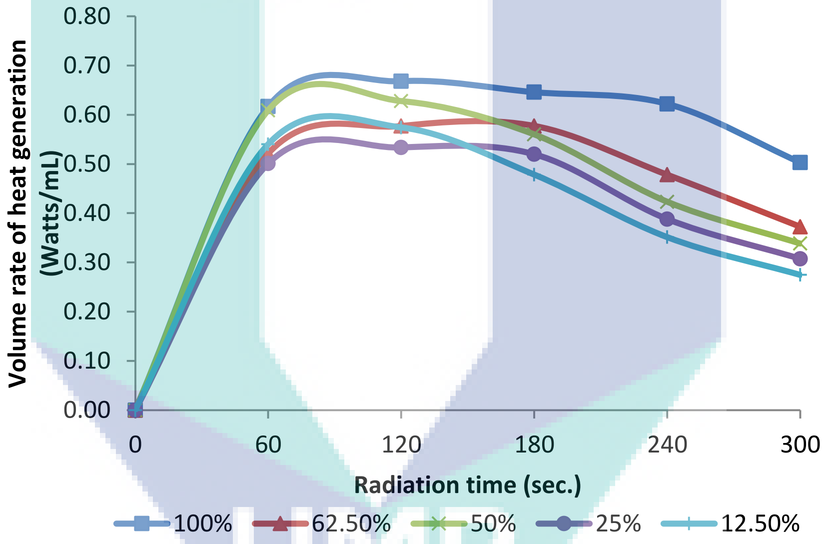


Figure 5.13: Volume rate of heat generation of aqueous solvent concentration vs radiation time

ii. Penetration depth

Penetration depth is an effective and convenient measure to compare the relative microwave absorbing characteristics of samples and to explain the effect of the dielectric properties and geometry on microwave heating. A large penetration also indicates that radiation is poorly absorbed while a short penetration depth means that surface heating predominates (Saoud, 2004). The penetration depth also affected by microwave frequency (Hoekstra and Delaney, 1974). High frequency has a short wavelength which corresponds to a smaller penetration depth (Rao et al., 1988; Ratanadecho, 2006). In this study, the penetration depth was calculated for TCMAE of water, acetone, acetonitrile and ethanol. Based on the result of penetration depth tabulated in Appendix S, Figure 5.14 was plotted. The figure shows the result of penetration depth is as follows water>ethanol>acetonitrile>acetone. The result was accordance to Palascious et al., 1996 which found that ethanol has the shorter penetration depth than of water, which shows microwave heating rate of ethanol is higher than water.

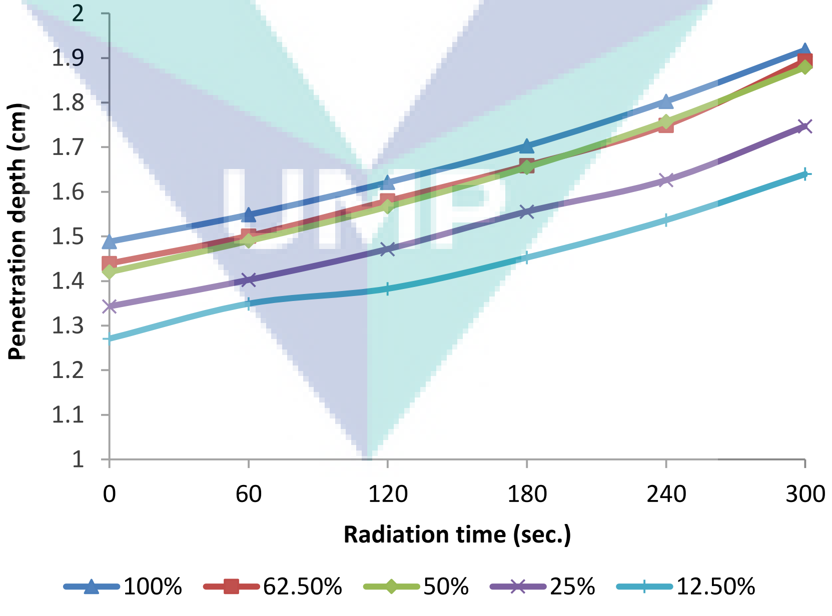


Figure 5.14: Penetration depth of aqueous solvent concentration vs radiation time

5.6.3 Effect of Solvent Composition on Antioxidant and Physicochemical Properties

Table 5.16 shows the effects of the solvent proportion on the average value of antioxidant properties (TPC, TFC, % inhibition and IC_{50}) of NBF extract. It was observed that the TPC, TFC, % inhibition and IC_{50} were greatly influenced by the different solvent proportion.By comparing the average of the TPC, in general one can notice that the both values increased in the following order, pure solvents (34.64 mg GAE/g), binary solvent mixtures (51.62 mg GAE/g) and quaternary solvent mixtures (63.22 mg GAE/g) respectively. The binary solvent mixture shows higher TFC value following by pure quarternary and all the ternary mixtures except pure water. Pure acetone and acetonitrile presented the lowest TFC value whereas maximum values were obtained with the pure water. The experimental results of TPC and TFC were in accordance with previous studies, which reported that binary-solvent system was more useful and favorable in the extraction of phenolic compounds from plant samples as compared to the mono-solvent system (Yang and Zhang, 2008; Kim et al., 2007; Nawaz et al., 2006; Turkmen et al., 2006).

The average of % inhibition, one can be observed that pure acetone, pure acetonitrile and pure ethanol, and acetone–acetonitrile mixture presents lowest % inhibition value. Note that the highest yield values were obtained when extractions were performed with the quaternary solvent mixtures that have maximum % inhibition value, followed by the binary and pure solvent. A lower value of IC_{50} indicates a higher antioxidant activity. It is interesting to notice that higher antioxidant activities were also obtained with the quaternary solvent mixtures. In this study, IC_{50}values also having a same trend with % inhibition.

Table 5.16: Amount of TPC, TFC, % inhibition and IC_{50} in different solvent proportion

	TPC mg GAE/g	TFC mg CE/g	Inhibition %	IC_{50} µg/mL
single solvent	34.64	12.22	29.43	8051.54
binary	51.62	17.48	58.37	3708.48
quaternary	63.22	12.37	69.45	1740.97

Though, as stated by prior research (Amid and Mirhosseini, 2012), a heating process was required to totally dissolve some of the phytochemicals in order to provoke the full dissolution of compound. However, the pulse level or heating rate should not be very high because the dissolution might be decreased due to phytochemicals was degraded because super heating effect and this theory was reflect the result of antioxidant properties in Table 5.4. In this study, the extraction process was fixed in 60°C which near to acetone boiling points. This given the reason why the acetone and acetone binary solvent extract not giving a higher antioxidant value even the colour of dissolution (Appendix T) was darker as compared to other solvent.

Solvent extractions are the most part frequently used procedures to practice extracts from plant materials due to their simplicity of use, effectiveness, and extensive applicability. It is known that the quantitative and qualitative results of extraction depend on the type of solvents with changeable polarities and sample to solvent ratio as well on the chemical composition and physiochemical characteristics of the plant extract. The dissolution of phytochemical is governed by the chemical nature of the plant sample, as well as the polarity of the various solvents used in this study. Thus, the effect of TCMA solvents extraction conditions on the physicochemical properties such as dissolution, density, refractive index, viscosity surface tension and their relation with extraction mechanism of NBF extract were investigated.

Table 5.17: Amount of density, refractive index, viscosity and surface tension in different solvent proportion

	Density (% Δ)	Refractive Index (% Δ)	Viscosity (% Δ)	Surface Tension (% Δ)
single solvent	0.538	0.042	0.438	2.221
binary	0.345	0.044	0.524	2.739
quaternary	0.636	0.060	0.714	2.048

Table 5.17 includes the density, refractive index, viscosity and surface tension of different values for the TCMA NBF solvent extract in various solvents before and after extraction. Values obtained in the present work for the density different (% Δ)of NBF extract in single, binary and quarternary are 0.538, 0.345 and 0.636 respectively.The difference here is likely attributable to an integral linearity in the several solvent systems owing to the use of different dielectric values of solvents. Nevertheless as compared to before TCMAE process in various solvent systems improved the density of NBF extract solution due to the efficient TCMAE closed heating system. That seems to be a promising strategy to achieve density higher than before TCMAE process. Though, the higher density indices of NBF extracted in cooperated with several solvent systems, respectively shows higher density as compared to before extraction of TCMAE of NBF plant. Which is attributed that sometimes higher dissolution of NBF in TCMAE undergo from higher dissolution rate, lower optical loss and higher extraction process.

Refractive index of materials varies with the wavelength.The different colors correspond to light with different wavelengths, and are refracted to differing degrees.Results of refractive index revealed that the water NBF extract before and after TCMAEshows highest different value (0.115) as compared to acetone, acetonitrile, and ethanol as well as binary and quaternary solvents respectively. The results revealed that the change of colour before and after extraction (Appendix T) especially in water extract provided information about relatively higher phytochemical results in the closed heating

TCMCS. This is attributing that the NBF phytochemicals may be fully dissolved in water system due to the presence of soluble compounds (polar) and impurities due its hydrophilic nature.

The % difference (%Δ) of viscosities employed by the various solvents system of NBF plant extract before and after temperature controlled assisted extraction (TCMAE) and results are summarized in Table 5.10. Results revealed that the viscosities of the NBF extract solution in various solvent systems are not large differences. While like density and refractive index, the viscosity of the NBF extract solutions slightly decrease in ethanol, acetonitrile and ethanol except water as can be observed in the Figure 5.5 (c). It was observed that the viscosity of water and ethanol NBF extract solution exhibited higher viscosity as compared to other solvents. It suggested that among four single solvent the water and ethanol has good tendency to dissolve the maximum amount of phytochemical due to their favorable polarities properties. Unlike single solvent system the quaternary solvent plant extract crude exhibitted higher viscosity difference before and after extraction process even as compared to binary solvent. The difference in viscosity is approximately 2 times between these two solvent systems. In general it is attributed that the interactions of each solvent system effect dissolution of phytochemicals according to polarities inside TCMAE.

Surface tension is an important to characterize and determining interfacial tension of each solvent systems. Table 5.17 shows the surface tension % difference values before and after extraction in various solvents system. Results revealed that the difference between maximum and minimum values in single solvents systems, binary and quaternary solvent showing that significant affects on to the plants crude surface tension. In addition higher and lower surface tension values in each solvents intensifies the dissolution of phytochemicals particularly in TCMAE. Results revealed that the acetonitrile and ethanol solvents systems as shown in Table 5.17 including binary and quaternary system drastically reduces the surface tension of the crude in both conditions either in maceration and TCMAE. Some plant phytochemicals compunds are sluggish with respect to their natural frequency. Water and acetone due to their hydrophobic and hydrophilic natures or dual dipolar tendency has an inherent molecular vibration that "stirs up" the polar and nonpolar phytochemical compounds, to break the surface tension of immiscible liquids.

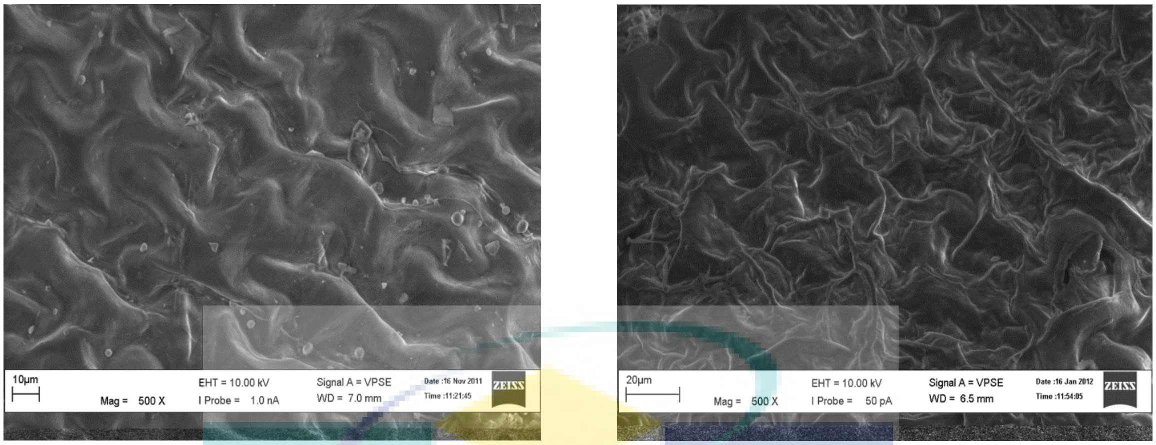
5.6.4 Effect of TCMA Extraction on NBF Microstructure under Various Solvent

Microwave-assisted extraction is a fast extraction process where microwave energy is delivered efficiently to materials through molecular interaction with the electromagnetic field and offers a rapid transfer of energy to the extraction solvent and raw plant materials (Criado et al., 2004; Liu et al., 2004). The direct interaction of microwave with solvent also results in the rupture of the plant cells and release of intracellular products into the solvent quickly (Lay-Keow and Michel, 2003).

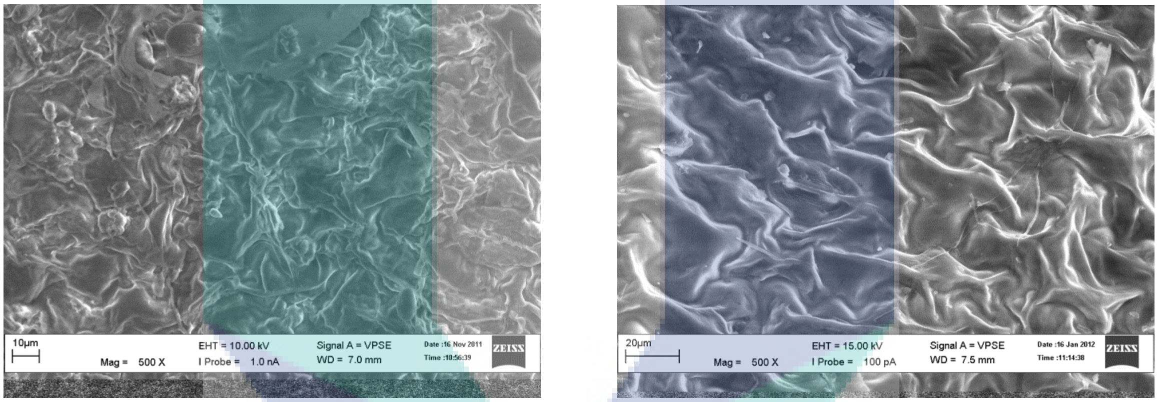
For TCMAE, the choice of extraction solvent takes into account not only its ability to absorb microwave energy but also solubility for target component. The mechanism of the extraction process with the solvent system was performed by scanning electron microscopy. As shown in Figure 5.15, the plant matrix before and after treatment, also with high dielectric properties to low dielectric properties solvent, the correlation was found between the values of antioxidant properties with the change in plant matrix surface. Higher the recovery of antioxidant properties shows greater the damage to the plant matrix surface which allows the migration of the compounds out of the matrix. Microwave heating of the matrix should lead to the destruction of the macrostructure of the matrix, thereby increasing the surface available for the extraction solvent.

SEM image shows that the water extracts had more damage as compared to be other solvent. The presence of the excess amount of water can cause excess thermal stress due to rapid heating of the solution and increase in swelling of plant material. Based on the high dielectric constant of water, the free water content of the sample induces a very rapid and concentrated heating during the microwave extraction process. The resulting pressure increase may result in the rupture of membranes in sample matrix enabling increased the contact surface area between the plant matrix and the solvent and various constituent to be released.

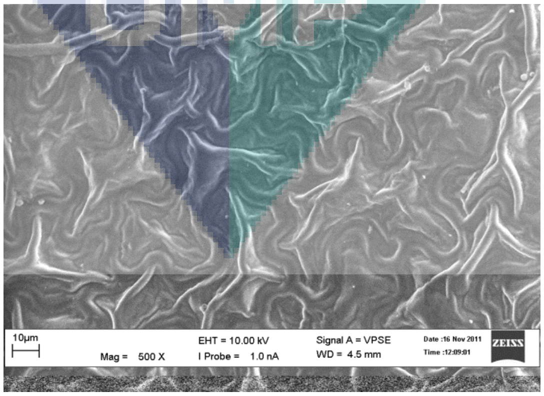
In addition, microwaves interact selectively with the free polar molecules present in the gland and vascular systems; this leads to localized heating, and the temperature increases rapidly near or above the boiling point of solvent. Thus, such systems undergo a dramatic expansion, with subsequent rupture of their walls, allowing the compound to flow towards the solvent. This process is quite different from classical solvent extraction, where the solvent diffuses into the matrix and extracts the components by solubilization. In addition, in TCMAE a wider range of solvents could be used, as the technique should be less dependent on a high solvent affinity.



Before Water



Acetone Acetonitrile



Ethanol

Figure 5.15: The surface of plant matrix at various solvent. Magnification 500x.

5.7 SUMMARY

Mixture design was determined for antioxidant (TPC, TFC, % inhibition and IC_{50}) and physicochemical (density, refractive index, viscosity and surface tension) properties as a function of the extraction solvent compositions. The quadratic model for TPC and IC_{50} also special cubic for TFC and % inhibition showed no significant lack of fit at the 95% confidence level. The difference of density, refractive index, viscosity and surface tension before (maceration) and after extraction (TCMAE) also shows no significant lack of fit at the 95% confidence level on the quadratic model respectively. The highest recovery for TPC, TFC and IC_{50} was obtained with water, and the result was supported by physicochemical properties of the extracted solvent solution. The mechanism of the enhanced extraction by TCMCS was discussed by observing cell destruction of NBF material after TCMAE treatment by scanning electron microscopy. The results showed that the plant materials were significantly destroyed especially for water extract due to the cell rupture after TCMAE treatment. It needs to be noted here, water polar molecules, which can very efficiently absorb microwave energy, plays an important role in the TCMAE process. Thus, water was determined to be appropriate extraction solvent for further study.

CHAPTER 6

THE EFFECT OF EXTRACTION PARAMETER ON ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF NBF TCMAE METHOD

This chapter was discussed the feasibility of employing temperature controlled microwave closed system (TCMCS) as a capable technique to study their efficiency of a microwave extraction parameters; specifically, extraction temperature (50, 60, 70, 80 and 90°C), microwave power (140, 280, 420 560 and 700 watts) and extraction time (0, 2, 4, 6, 8 minutes) to antioxidant and physicochemical properties of *Nephrolepis biserrata* (Sw.) Schottfrond (NBF). The extraction using temperature extraction microwave assisted extraction (TCMAE) method was carried out with water. Generally, absorption of the microwave energy increases with the dielectric constant of the molecule, resulting in power dissipated inside the solvent and plant materials and then generating more effective molecular movement and heating. As a polar solvent, water can efficiently absorb microwave energy and leads to efficient heating. The effect of an extraction parameter on antioxidant properties in terms of total phenolic, total flavonoid and DPPH radical scavenging antioxidant activity (% inhibition and IC_{50}) was determined and result on physicochemical properties of extract in terms of pH, conductivity, total dissolved solid (TDS), density, refractive index, viscosity and surface tension was also studied to support the result on antioxidant properties. The rate of heat generation was calculated. The mechanism of the enhanced extraction before and after extraction with TCMS at various extraction parameters was also discussed by observing cell destruction of NBF material by scanning electron microscopy (SEM).

6.1 INTRODUCTION

Temperature controlled microwave closed system (TCMCS) with fluid stirring device is a relatively provide new extraction technique that delivers microwave energy rapidly to a total volume of the solvent and solid plant matrix. This results in subsequent heating of the solvent and solid matrix, efficiently and homogenously. The principle of heating using microwaves is based on the direct effect of the waves on molecules by ionic conduction and dipole rotation. The plant matrix was immersed in high dielectric constant solvent (water) which is rapidly heated by microwaves, disrupting the cellular structure and releasing the desired components into the surrounding medium.

Evidence has been presented that during the extraction of bioactive compound from plant material; microwave extraction allows the migration of the compounds out of the matrix (Kwon et al., 2003; Wang et al., 1997; Chen and Spiro, 1994; Steinheimer, 1993; Pare et al., 1995; Ganzler et al., 1987). The efficiency of the microwave assisted extraction (MAE) depends on the duration of the process, applied temperature and microwave power. The proper selection of these parameters is strongly dependent on the properties of the sample and target compounds. The amount of the compounds that released into the external liquid was characterized by the degree of cell disintegration, which influenced the efficiency of the extraction process (Chalemchat et al., 2004). For a better understanding of the effect on the microstructure, the application of scanning electron microscopy technique, which microscopically explored the complex changes at the cell level in really biological systems would be helpful. In previous research, the majority of the researchers that related to planting cell structure put the focus on understanding cell wall or cell membrane in relation to the texture and extraction efficiency in plant materials under different extraction parameter. There are a number of parameters that influence the microwave extraction process. Those most commonly studied to include extraction temperature and time, and microwave power.Thus, the objective of this work was to study the effect of an extraction parameters on antioxidant properties and physicochemical properties of *Nephrolepis biserrata* (Sw.) Schott frond (NBF) as a plant matrix in water as a solvent.

6.2 THE EFFECT OF AN EXTRACTION PARAMETERS OF TCMCS ON ANTIOXIDANT PROPERTIES OF NBF EXTRACTS.

6.2.1 Effect of Extraction Temperature

Study the effect of extraction temperature, microwave power and extraction time on antioxidant properties by TCMCS were carried out at different parameter with other conditions kept unchanged (ratio of solid to liquid: 1:25, time: 240 s, microwave power: 420 watts).Water was used in this study due to that is safer in handling as compared to other organic solvents, such as methanol and acetone and more importantly, they are acceptable for human consumption. Temperature profile of water extracts at various power levels was shown in Appendix U. Figure 5.1 and 5.2 shows the effect of extraction temperature on antioxidant properties.

The effect of extraction temperature on antioxidant properties is shown in Figure 5.1 and 5.2, which clearly demonstrates that increasing the temperature of the extract from 50 to 90°C significantly increases the extraction efficiency. This is because the higher temperature causes intermolecular interactions within the solvent to decrease, giving rise to higher molecular motion, and causing the bioactive compound release to surrounding. The increasing temperature may also cause opening cell matrix, and as a result, antioxidant's availability for extraction increases. Moreover, at high temperature, solvent viscosity decreases and the diffusivity increases, thus the efficiency of extraction increases.

The enhancement of product recovery by microwave is generally attributed to its heating effect, which occurs due to the dipole rotation of the solvent in the microwave field. This causes the solvent temperature to rise, which then increases the solubility of the compound of interest. Specifically, solvent heating by microwave occurs when molecules of the polar solvent could not align themselves quickly enough to the high frequency electric field (typically 2450 MHz) of microwave. This discrepancy causes the solvent molecules to dissipate the absorbed energy in the form of heat.

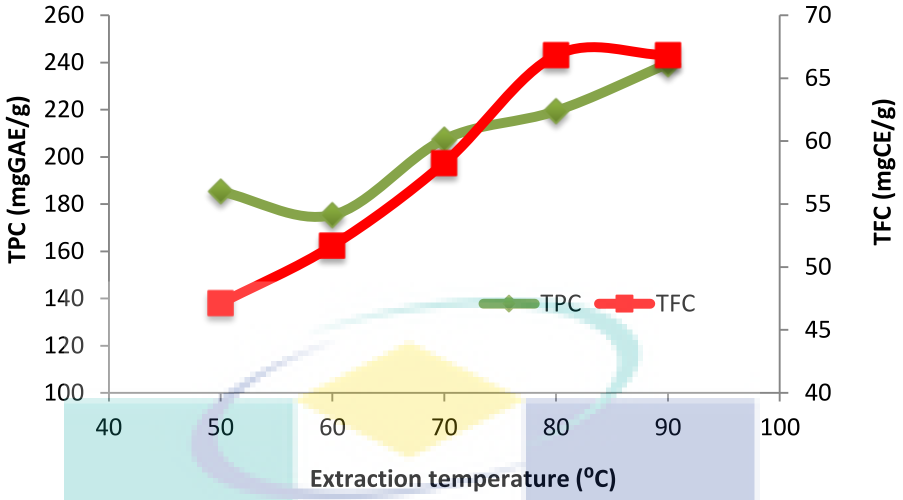


Figure 6.1: Extraction temperature versus TPC and TFC

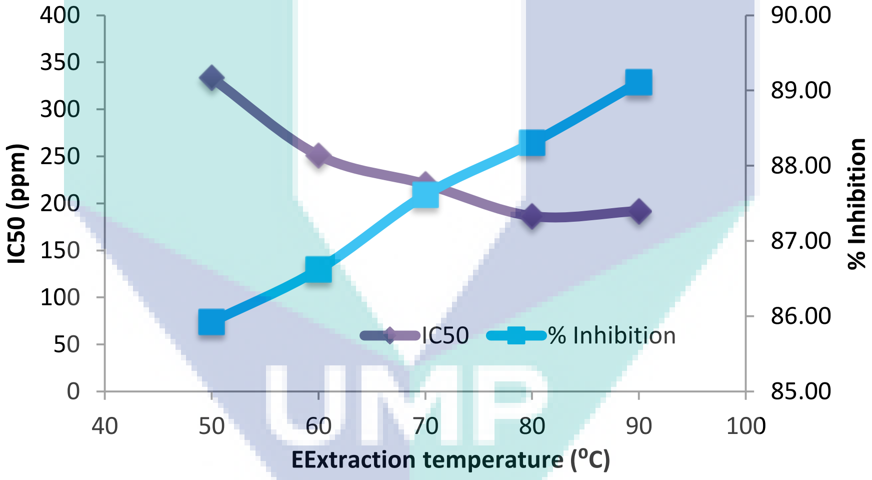


Figure 6.2: Extraction temperature versus IC_{50} and % inhibition

The increment of TPC, TFC and DPPH radical scavenging activities (% inhibition and IC_{50})as a function of temperature were in agreement with previous studies, which reported that the yield of phenolic compounds would be increased with increasing of extraction temperature (Cacace and Mazza, 2006; Pathirana and Shahidi, 2005; Pinelo et al., 2005). Heat could enhance the recovery of phenolic compounds from plant materials by increasing the diffusivity of extraction solvent into plant cells and also enhancing the solubility of phenolic compounds in extraction solvent (Vongsangnak et al., 2004; Cacace and Mazza, 2003).

Generally, when using high temperatures, it is advisable to assess the stability of target compounds.For example, Xiao et al. (2008) evaluated the stability of flavonoids during MAE from *Radix Astragali* found out that extraction performed between 70 and 110ºC did not negatively affect the flavonoid content of the extract, while at temperature above 130ºC degradation of some components occurred (particularly, the degree of degradation increased with increasing molecular polarity for the microwave).

6.2.2 Effect of extraction time

Effects of extraction time on antioxidant properties of TCMAE were carried out at different extraction time with other conditions kept unchanged (ratio of solid to liquid: 1:25, microwave power: 420 watts, temperature: 70°C) . The effect of extraction time on antioxidant properties was shown in Figure 6.3 and 6.4. The duration of microwave radiation of 0, 2, 4, 6, and 8 minutes at 420 watts microwave power on the extraction of antioxidant. According to Mandal et al., (2007), microwave power and extraction time as two factors that influence each other to a great extent. There are two approaches: low or moderate power with long exposure and high power with short exposure, but first is considered to be a wiser choice since high microwave power can reduce purity of the extract. However, this theory was working when the extraction was done in the common domestic microwave oven where the temperature was uncontrolled.

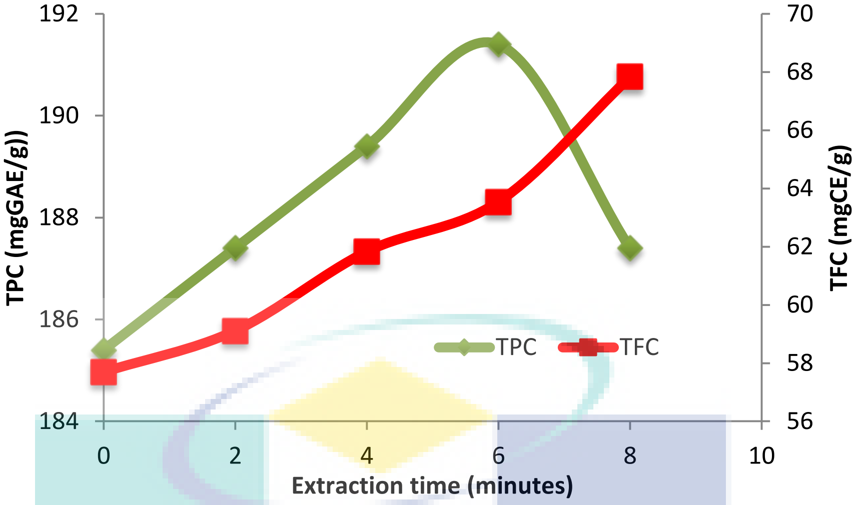


Figure 6.3: Extraction Time versus TPC and TFC

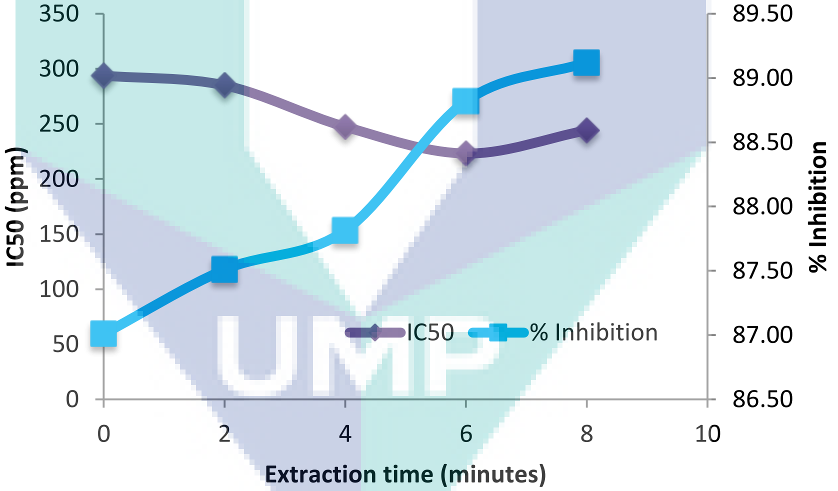


Figure 6.4: Extraction Time versus % inhibition and IC_{50}

In our study, we develop our own microwave with special temperature controller where both of time and microwave power we can separately organize. On the other side, the extraction time counting begins after the temperature reach 70°C. This technique easier to understand the exactly optimum period of time in extraction method without any influenced factor.In this study, DPPH radical scavenging activity (% inhibition and IC_{50})

UMP

was increased as time increased. TCMAE reached the highest antioxidant activity when extraction time was 8 minutes as this is our highest extraction time in our study. However, TPC value was reduced at 8 minutes. Low value of phenolic compounds not necessary accompanies with high % inhibition, as the antioxidant activity of crude extracts can also be influenced by the structure and interaction between extracted phenolic compounds (Huang et al. 2005). Hence, further study should be carried out to identify the predominutesant phenolic compounds in NBF extract, which are extracted at a different temperatures with respect to their antioxidant mechanisms and synergistic effects.

According to literature, long extraction time not significantly effect on yield of artemisnin (Kanimozhi et al., 2010) and *slavia miltiorrhiza* (Pan et al. 2007). The difference of the silybinin yield between 200 to 600 watts appears more significant with short irradiation times compared to long irradiation times. When the extraction solutions were heated long enough (8 minutes), the yields under different microwave power (watts) (Dhobi et al.2009) were similar.

In addition, extraction time varies depending of the extracted analyte, plant material and extraction solvent. Using polar solvents with high dielectric constant (water, ethanol, methanol and acetone) during longer extraction time can result in degradation of analyte, as microwave radiation causes a rapid temperature increase of those solvents. The significance of the extraction time can be presumed to be related to the time required for the desorption process to take place.

6.2.3 Effect of Microwave Power

Effects of microwave power on antioxidant properties of TCMCS were carried out at different microwave power with other conditions kept unchanged (ratio of solid to liquid: 1:25, time: 240 s, temperature: 70°C) . The effect of microwave power on antioxidant properties was revealed in Figure 6.5 and 6.6. As shown in Figure 6.5, it was observed that the total polyphenol and total flavonoid dropped from 140 watts to 280 watts before increased markedly with power increased to 700 watts. The lower activity antioxidant of at 140 watts was the influence by extended extraction time, hence exposure to unfavorable conditions such as light and oxygen where as commonly known that antioxidant very sensitive with both.

The accelerated extraction of TPC and TPC by increasing microwave power from 280 to 700 Watts can be correlated to the direct effects of microwave energy on phytomolecules by ionic conduction and dipole rotation, which result in power dissipated in a volumetric fashion inside the solvent and plant material and then generate molecular movement and heating. More electromagnetic energy was transferred to the extraction system quickly and improved the extraction efficiency when the microwave power increased from 140 to 700 watts. Similarly Figure 6.6 highlights the % inhibition and IC_{50} for the extraction of NBF water extract. In general, the extraction efficiency was improved by raising the microwave power from 140 to 700 watts at 4 minutes of extraction time.

Microwave irradiation caused the explosion and rupture of NBF cells; therefore the target compounds within the cell were rapidly released into the surrounding extraction solvents. As the liquid phase absorbed the microwaves, the kinetic energy of the molecules increased, and consequently, the diffusion rate accelerated and resulted in better extraction efficiency and significantly in antioxidant activity where this finding was accordance to a previous researchers (Xiao et al., 2008).

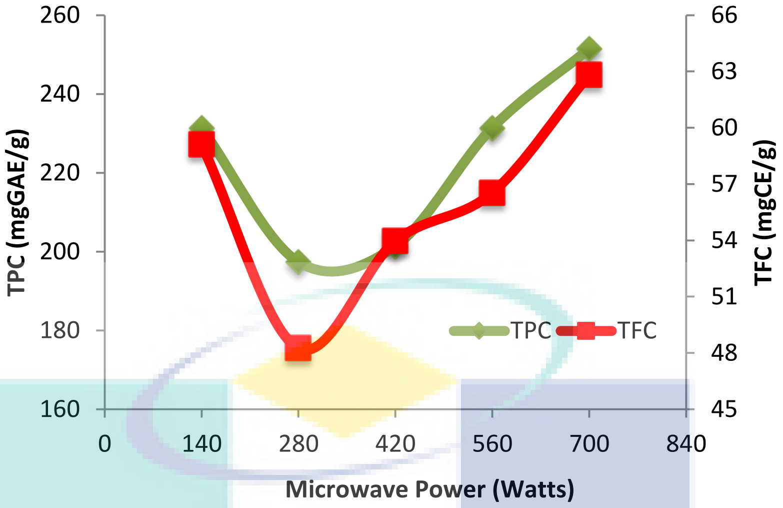


Figure 6.5: Effect of microwave power versus TPC and TFC

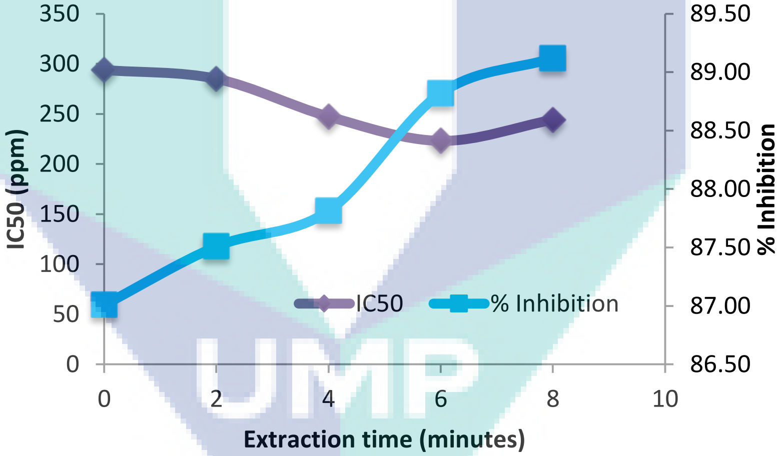


Figure 6.6: Effect of microwave power versus % inhibition and IC_{50}

6.3 THE EFFECT OF AN EXTRACTION PARAMETERSON PHYSICOCHEMICAL PROPERTIES OF TCMA NBF EXTRACTED SOLUTION.

In this study, the effects of various extraction parameters such as extraction temperature, microwave power, and extraction time of NBF TCMA extracted solution on physicochemical properties in terms of pH, conductivity, total dissolve solid, refractive index, density, viscosity and surface tension were investigated. The physicochemical properties of NBF TCMA water extract was revealed in Figure 6.7-6.13.

6.3.1 pH

The pH of extract solution, for the control (before) and after TCMA extracts of NBF are shown in Figure 6.7. The pH value before extraction is 4.96 and increase after extraction in the ranged of 5.34-5.45. Different trend was observed for each different extraction parameter. Extraction temperature (50°C-90°C) influence the reduction ofpH from 5.44 to 5.34, these results indeed support the dissolution of the soluble short-chain fatty acids and carbohydrates in the plant matrix. While microwave power (140 - 700 watts) shows the pH was increased from 5.38 to 5.42. Time of extraction shows the pH was increased from 2 minutes to 6 minutes until was dropped at 8 minutes. Change in pH can be explained by the change in hydrogen ion concentration and their relationship existing between pH and free acid content where it was observed through by the value of antioxidant properties after each treatment.

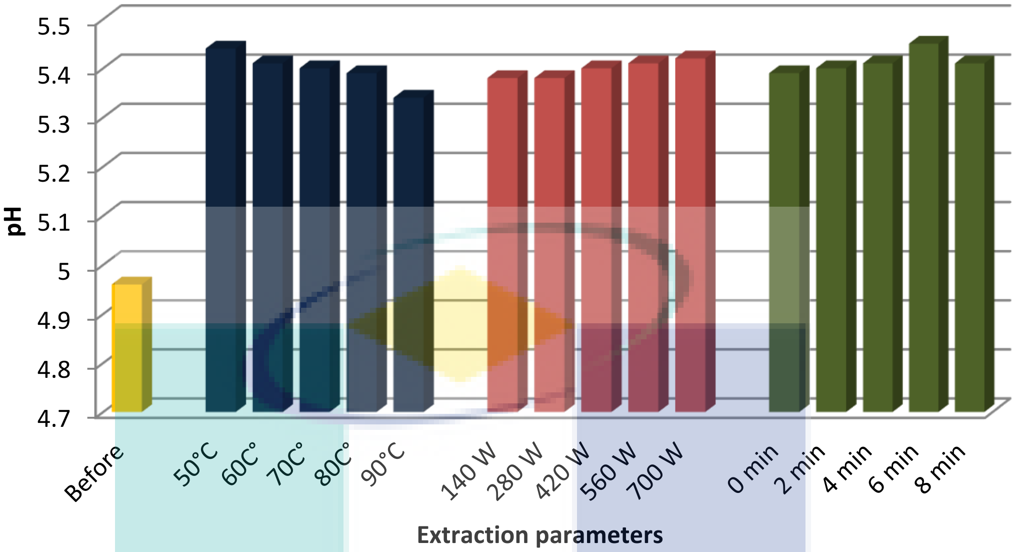


Figure 6.7: Effect of an extraction parameters on pH of extract

6.3.2 Conductivity

Figure 6.8 illustrated the effect of an extraction parameters (temperature, microwave power and extraction time) on conductivity of plant extract crude. The electrical conductivity (µSm^{-1}) values showed different fluctuations within the parameter during the experiment. Results revealed that the conductivity of TCMA NBF water extractssolutionwas increased from 3.71 to 4.06 µSm^{-1}when the extraction temperature parameter changes from 50°C to 90°C. Low conductivity at lower temperature shows lower mineral's dissolution and higher conductivity at higher temperature is attributed the total amount of basic cations and efficient mineral's dissolution of plants inside water. While the conductivity of plant's crude was decreased from 3.98 to 3.82 µSm^{-1}when microwave power increased. It is suggested that the dissolution rate of the soluble polar organic compounds in plant cell are efficiently in dissolve in water. However, the time of extraction influences the increases the conductivity from 3.70 to 3.97µSm^{-1}.

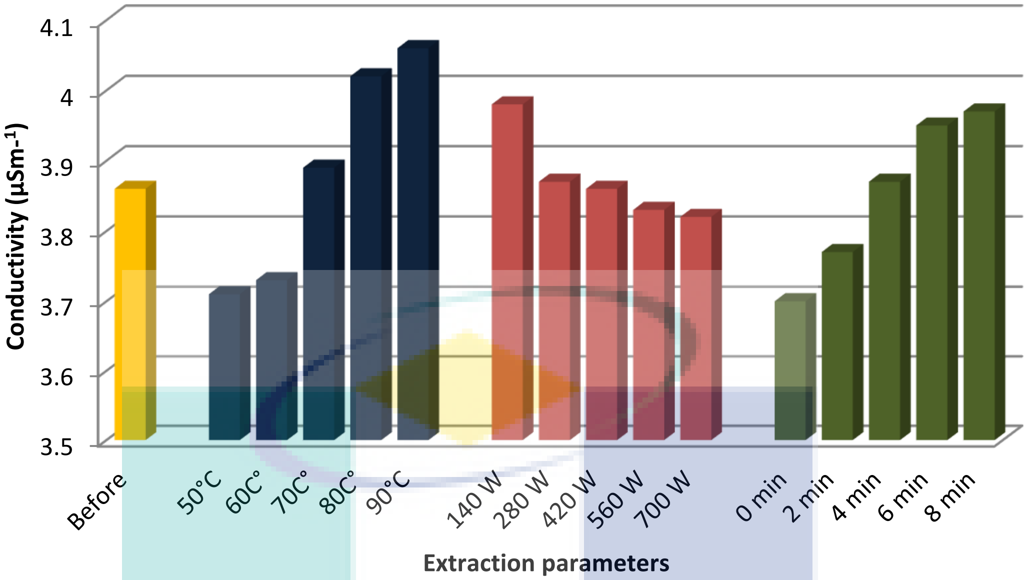


Figure 6.8: Effect of an extraction parameters on conductivity of extract

6.3.3 Total Dissolved Solid

Figure 6.9 shows the effect of an extraction parameters on total dissolved solid of plant extracted crude inside water. An increment in the value of the total dissolved solids was observed in temperature from 50°C to 9°0C and extraction time from 0 to 8 minutesin the extraction solution of TCMA extraction of NBF. This increment may be due to the presence of minerals solid itself in the plant matrix. The value of TDS was reduced according to microwave watt from 140 to 700 watts but increase from 2.357 to 2.5 according to extraction time and 2.33 to 2.567 to extraction temperature respectively. This trend is similar with conductivity results.

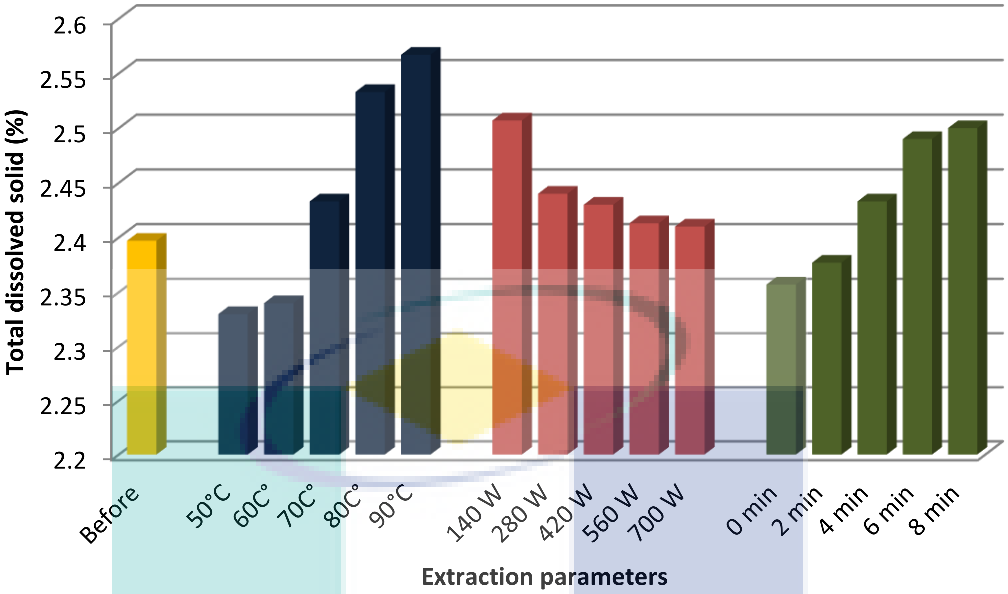


Figure 6.9: The effect of an extraction parameters on total dissolved solid of extract.

6.3.4 Density

Figure 6.10 summarized the effect of an extraction parameters on density of the plant water extracted crude. The density values showed fluctuations in microwave power parameter during the experiment. Results revealed that the density of NBF water extractssolution was increased from 1.00025 to 1.00089 gm.cm^{-1}at 50°C to 80°C. The higher temperature causes intermolecular interactions within the solvent to decrease, giving rise to higher molecular motion, and solvent density to increase. The density increased when the TCMAE temperature was increased, which related to the effect of the boiling points and solubility of antioxidant in water. At 140 watts of it shows 1.0009 gm.cm^{-1}and decrease to 1.0059 at 420 watts before increased to 1.00068 gm.cm^{-1}at 700 watts. The density of extraction solution of TCMAE of NBF also was increased from 1.0039 to 1.00061 gm.cm^{-1}at 0 to 6 minutes before reduce to 1.0051gm.cm^{-1} at 8 minutes. The density increased with prolonging the stirring time. The high density which associated to high solubility of the TCMA NBF extracts could be related to the considerable impact of the continuous stirring on the chemical composition of duringextraction.

The relatively wide range of solubility could be due to the fine particle size (0.3 mm) of crude plant extracted under controlled extraction conditions (closed system). The particle size is a critical factor affecting the solubility. The plant particle size and the status in which it is presented for microwave extraction can have a profound effect on the solubility of the compounds. The larger and coarser particles take longer time to be dissolved due to the slower time required for the penetration of water into the sample matrix. Conversely, the presence of smaller particle leads to shorter time for the penetration of proposed solvent through the sample matrix system inside TCMA extracts, thus inducing better solubility. Fine powders can enhance the extraction by providing larger surface area, which provides best contact between the plant matrix, and the solvent, also finer particles will allow improved or much deeper penetration of the microwave.

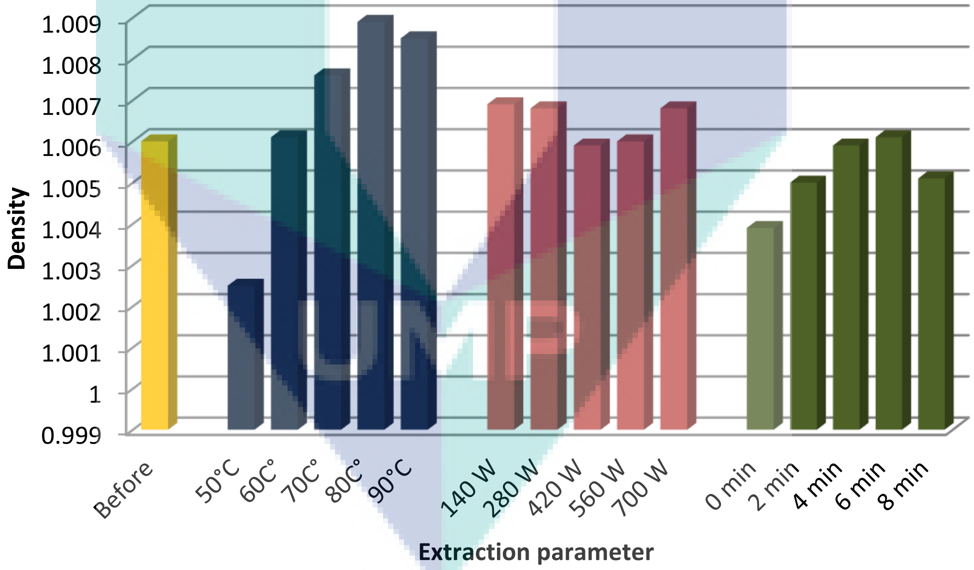


Figure 6.10: The effect of an extraction parameters on density of extract

6.3.5 Refractive Index (RI)

Figure 6.11 summarized the effect of an extraction parameters on the refractive index of plant water extracted crude. The refractive index values showed linearity in all the parameter of the experiment. It was observed that the average value of RI is 1.3335 after extraction was higher than before extraction (1.3332). The higher value of RI was observed at 50°C then it was decreased for every 10°C different until 90°C. At 140, 280 and 420 Watts the RI shows a same value (1.3335) and was dropped to 1.3334 at 420 and 700 Watts. The RI value was decreased from 1.3336 to 1.3334 for every 2 minutes different according to time extraction.

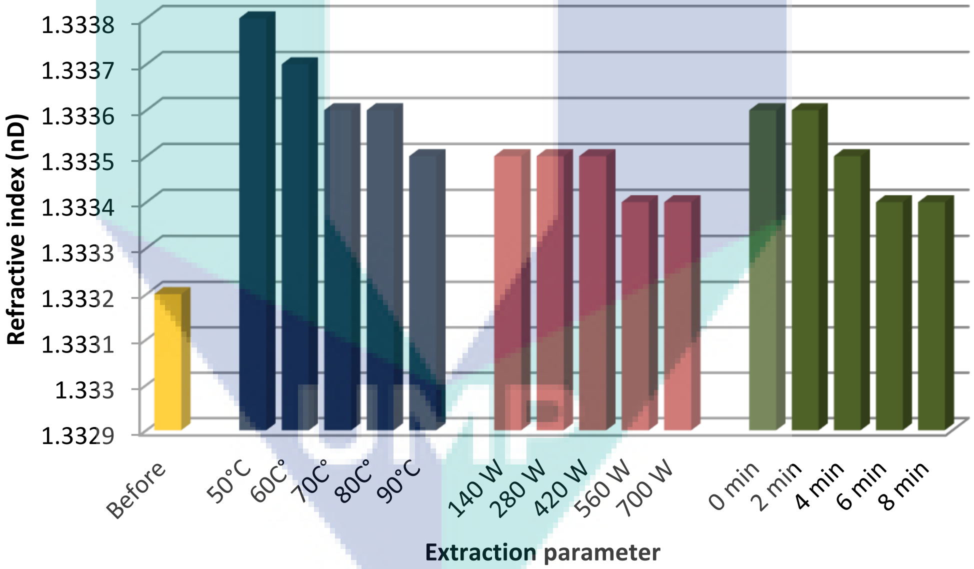


Figure 6.11: The Effect of an extraction parameters on the refractive index of extract

6.3.6 Viscosity

Figure 6.12 shows the effect of an extraction parameters on viscosity of TCMA NBF water extract solution. The viscosity value after extraction was increased in the ranged of 0.8877 to 0.9398 at 50°C to 90°C. Different trend was observed for each different extraction parameter. The viscosity value of microwave power (140 to 700 Watts) shows the insignificant value for each different watts with average are 0.91282 cPs with standard deviation 0.00132. Time of extraction revealed the viscosity was increased from 0.8962 to 0.9164cPs at 2 minutes to 6 minutes until drop to 0.9103cPs at 8 minutes.

A sample's viscosity affects its ability to absorb microwave energy (dissipation factor) because it affects molecular rotation. The higher the viscosity, the lesser is the ability of the molecule to rotate. This locking greatly restricts the molecular mobility and makes it difficult for the molecules to align with the microwave field. Thus, the dielectric dissipation factor is low, which affects the viscosity.

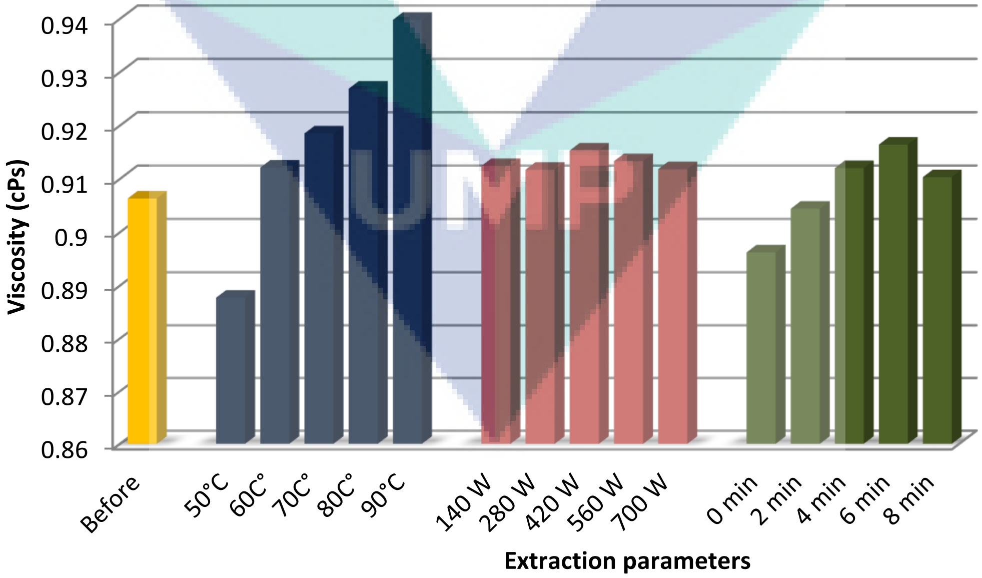


Figure 6.12: The effect of an extraction parameters on viscosity of extract

When the temperature of the water is increased to 50°C from room temperature, the viscosity has decreased. But the higher extracting temperature is profitable for extraction due to the increased solubility, when the temperature rise up from 50 to 90°C. An increase in the temperature also extraction time of the extraction procedure resulted in higher solution viscosity, probably due to dissolution of high molar mass macromolecules.

6.3.7 Surface Tension

Figure 6.13 illustrated the effect of an extraction parameters on surface tension of extract. It was observed that the surface tension of TCMA NBF water extractssolution was decreased from 66.067 to 59.323 when the extraction temperature parameter changes from 50°C to 90°C. At 140 watts, the surface tension revealed 60.804 before drop to 59.121 at 280 watts before increased to 60.091 at 420 watts. Again, the surface tension value was raised up to 59.891 at 700 watts according to microwave power. The time of extraction influences the increasing of surface tension from 58.355 to 61.976 at 0 minutes to 8 minutes.

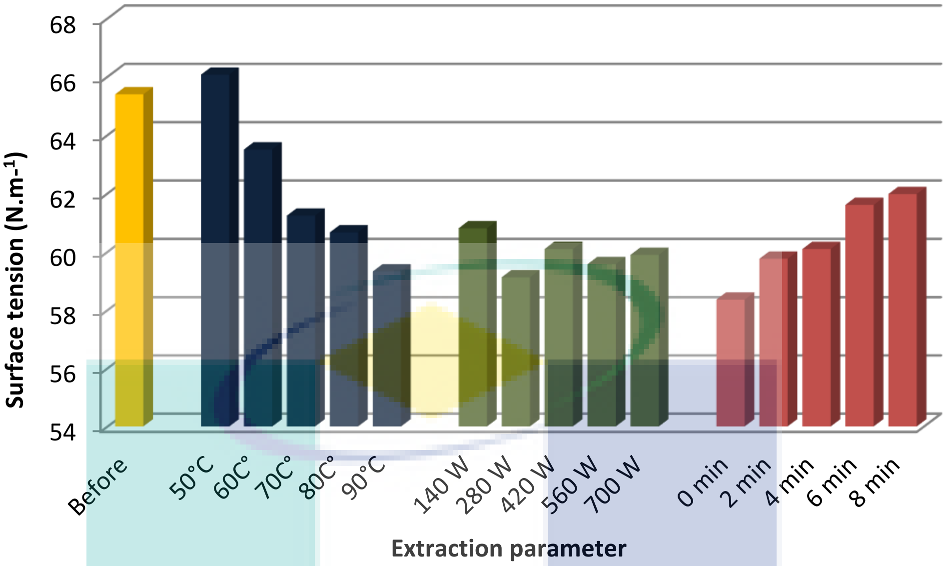


Figure 6.13: The effect of an extraction parameters on surface tension of extract

The use of microwave energy for the extraction of active substances from plant materials results in more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up and increased production. During absorption, the microwaves' energy is converted into kinetic energy, thus enabling the selective heating of the microwave-absorbent parts of the plant material. The volume increased in this way makes cells explode, releasing their content into the liquid phase. When the liquid phase absorbs the microwaves, the kinetic energy of its molecule's increases, and consequently, the diffusion rate increases too (Mandal, 2007). A surface tension is reflected of viscosity of the extract. Juntachote et al. (2006) was reported that elevated extraction temperature would increase the mass transfer of phenolic compounds and also reduce the solvent viscosity and surface tension and hence to promote the extraction of phenolic compounds. In addition, mild heating was also found to have the ability to soften the plant tissues, weaken the cell wall integrity and thus favored the release of bound phenolic compounds (Spigno et al., 2007; Junctachote et al., 2006).

6.4 THE EFFICIENCY OF TEMPERATURE CONTROLLED MICROWAVE CLOSED SYSTEM (TCMCS) PARAMETER ON ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES.

Temperature is an important factor in conventional extraction methods, especially in the case of botanical extractions. But, study on the extraction temperature is quite rare in microwave extraction technique as it is not easy to control the temperature in the microwave oven. Therefore, the efficiency of TCMCS parameters comprises extraction parameter, microwave power and extraction time on antioxidant and physicochemical properties of TCMA of NBF extract was statistically studied in terms of standardized coefficient (β) using SPSS version 16.0.

6.4.1 Standardized coefficients or Beta (β) analysis on Antioxidant Properties

Standardized coefficients or Beta (ß) was intensely elaborate in section 4.3.2. Standardized beta coefficient was used to compare the strength of the effect of each independent variable on the dependent variable where the independent variable with the largest standardized Beta has the strongest effect.

The multiple linear regression analysis finding out the original (unstandardized) variables produce unstandardized coefficients (ß) of TPC whichis presented in Table 6.1. TPC significantly associated (p<0.05) with positive correlation on extraction temperature (β=0.476) and microwave power (ß = 0.221) respectively. The extraction time (β=-0.313) had a negative correlation (p<0.05) with TPC. The ß value shows that extraction temperature having a strongest effect on TPC. Analysis of variance (ANOVA) revealed significant (p< 0.05) difference in the TFC from each parameter. As presented in Table 6.2, extraction temperature (β= 0.711), microwave power (β= 0.171) and extraction time (β= 0.301) was shown positive correlation (p<0.05) in TFC. Based on standardized coefficient (β), the ranking is a follows: temperature> extraction time>microwave power.

As shown in Table 6.3 and 6.4, extraction temperature, microwave power and extraction time was shown strongly positive correlation ($p< 0.01$) in % inhibition but strongly negatively correlation ($p<0.01$) in IC_{50} respectively. Extraction temperature (β = 0587; β = 0.734) showing as strongest effect in % inhibition and IC_{50} and followed by microwave power (β = 0.491; β = 0.351) and extraction time (β = 0.481; β = 0.339) respectively.

Table 6.1: Unstandardized and standardized coefficient value and model summary of TPC

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	106.692	31.432		3.394	0.002
Temperature	1.520	0.395	0.476	3.846	0.000
Microwave	0.052	0.029	0.221	1.79	0.028
Time	-5.000	1.976	-0.313	-2.53	0.015
R		0.611	Std error of the estimation		21.646
R-Squared		0.373			
Adj R-Squared		0.327			

Table 6.2: Unstandardized and standardized coefficient value and model summary of TFC

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	11.672	5.84		1.997	0.050
Temperature	0.546	0.073	0.711	7.428	0.000
Microwave	0.010	0.005	0.171	1.783	0.018
Time	0.003	0.367	0.301	3.148	0.003
R		0.79	Std error of the estimation		4.025
R-Squared		0.625			
Adj R-Squared		0.597			

Table 6.3: Unstandardized and standardized coefficient value and model summary of % inhibition

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	80.121	0.612		130.815	0.000
Temperature	0.068	0.080	0.587	8.765	0.000
Microwave	0.004	0.001	0.491	7.326	0.000
Time	0.276	0.039	0.481	7.181	0.000
R		0.903	Std error of the estimation		4.218
R-Squared		0.816			
Adj R-Squared		0.803			

Table 6.4: Unstandardized and standardized coefficient value and model summary of IC_{50}

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	576.189	27.866		20.677	0.000
Temperature	-3.487	0.350	-0.734	-9.952	0.000
Microwave	-0.122	0.026	-0.351	-4.753	0.000
Time	-8.055	1.752	-0.339	-4.598	0.000
R		0.881	Std error of the estimation		
R-Squared		0.777			19.190
Adj R-Squared		0.761			

6.4.2 Standardized coefficients or Beta (β) analysis on Physicochemical Properties

pH was strongly negative correlation (p <0.01) with extraction temperature (β = 0.688) but strongly positively correlation with extraction time microwave power (β = 0.322) and extraction time (β = 0.314) as clearly observed in Table 6.5. Based on standardized coefficient (β), the ranking is a follows: temperature> microwave power > extraction time. As shown in Table 6.6 and 6.7, conductivity and TDS has significantly associated (p <0.05) with positive correlation on extraction temperature (β = 0.688; β = 0.744) and extraction time (β = 0.500β = 0.451) respectively. The microwave power (β = -0.268; β = -0.253) had a negative correlation (p<0.01) with conductivity and TDS respectively. The ß value shows that extraction temperature having a strongest effect where the ranking is a follows: temperature> extraction time >microwave power.

The density of NBF TCMAE in Table 6.8 was shown negative correlation coefficient on extraction time (β = -0.495). Extraction temperature and microwave power is insignificant. As shown in Table 6.9 refractive index has significantly associated (p <0.05) with negative correlation on extraction temperature (β = 0.503) microwave power (β = -0.228) and extraction time (β = 0.432) respectively. The ß value shows that extraction temperature having a strongest effect where the ranking is a follows: temperature> extraction time >microwave power.

Table 6.10 was shows, viscosity was strongly negative correlation (p <0.01) with extraction temperature (β = 0.707) but not significant (p>0.05) in term of microwave power and extraction time. Table 6.11 revealed, the surface tension was strongly negative correlation (p<0.01) in extraction temperature (β = -0.697) and positive correlation in extraction time (β = 0.707) but insignificant (p>0.05) in microwave power. The ß value shows that extraction temperature having a strongest effect on surface tension. Extraction time and microwave power is not significant. The improvement of extraction at elevated temperature might be due to the surface tension decreased and viscosity of solvent being increased at high temperature, thus accelerating the diffusion of solvent into the matrix, enhancing desorption of the analytes from the matrix, and promoting the high yield as of antioxidant properties as can be seen at Figure 6.1-6.6.

It has been observed, few results was shown insignificant like in density, viscosity and surface tension at certain extraction parameter. In this study, the extraction was performinutesg in temperature range 50°C to 90°C and at low microwave power (140 - 700 watts) only. Maybe this range didn’t giving a good diffusity which reflecting to solvent density, viscosity and surface tension. Nevertheles, the solvent was centrifugre at 9000rpm for 20 minutes before test for density and other physiochemical properties analysis to avoid any unnecessary particle disturbing the result.

Table 6.5: Unstandardized and standardized coefficient value and model summary of pH

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	5.508	0.023		236.216	0.000
Temperature	-0.002	0.000	-0.688	-7.731	0.000
Microwave	7.770×10^{-5}	0.000	0.322	3.617	0.000
Time	0.005	0.001	0.314	3.524	0.001
R		0.822	Std error of the estimation		0.016
R-Squared		0.675			
Adj R-Squared		0.652			

Table 6.6: Unstandardized and standardized coefficient value and model summary of conductivity

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	3.141	0.078		40.301	0.000
Temperature	0.010	0.001	0.688	10.203	0.000
Microwave	0.000	0.000	-0.268	-3.920	0.000
Time	0.036	0.005	0.500	0.500	0.001
R		0.899	Std error of the estimation		0.054
R-Squared		0.808			
Adj R-Squared		0.794			

Table 6.7: Unstandardized and standardized coefficient value and model summary of TDS

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	1.965	0.047		42.033	0.000
Temperature	0.007	0.001	0.744	11.23	0.000
Microwave	0.000	0.000	-0.253	-3.815	0.000
Time	0.020	0.003	0.451	6.806	0.000
R		0.906	Std error of the estimation		0.032
R-Squared		0.820			
Adj R-Squared		0.800			

Table 6.8: Unstandardized and standardized coefficient value and model summary of density

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	1.007	0.005		212.382	0.000
Temperature	2.8×10^{-5}	0.000	0.064	0.47	0.641
Microwave	1.875×10^{-7}	0.000	-0.006	-0.043	0.966
Time	0.001	0.000	-0.495	-3.658	0.001
R		0.499	Std error of the estimation		0.00326
R-Squared		0.249			
Adj R-Squared		0.194			

Table 6.9: Unstandardized and standardized coefficient value and model summary of refractive index

Model	Unstandardized Coefficient		Standardized coefficient	t	Sig.
	B	Std. Error	Beta		
(Constant)	1.334	0.000		1.084×10^4	0.000
Temperature	7×10^{-6}	0.000	-0.503	-4.522	0.000
Microwave	2.326×10^{-7}	0.000	-0.228	-2.051	0.047
Time	3.00×10^{-5}	0.000	-0.432	-3.876	0.000
R		0.701	Std error of the estimation		0.00008
R-Squared		0.492			
Adj R-Squared		0.455			

Table 6.10: Unstandardized and standardized coefficient value and model summary of viscosity

Model	Unstandardized Coefficient		Standardized coefficient	t	Sig.
	B	Std. Error	Beta		
(Constant)	0.829	0.014		59.845	0.000
Temperature	0.001	0.000	0.707	6.475	0.000
Microwave	7.429×10^{-7}	0.000	-0.006	-0.058	0.954
Time	0.001	0.001	0.108	0.988	0.329
R		0.715	Std error of the estimation		0.00953
R-Squared		0.511			
Adj R-Squared		0.476			

Table 6.11: Unstandardized and standardized coefficient value and model summary of surface tension

Model	Unstandardized Coefficient		Standardized coefficient		
	B	Std. Error	Beta	t	Sig.
(Constant)	73.166	2.180		33.559	0.000
Temperature	-0.193	0.027	-0.697	-7.051	0.000
Microwave	-0.001	0.002	-0.069	-0.699	0.489
Time	0.454	0.137	0.328	3.315	0.002
R		0.774	Std error of		
R-Squared		0.599	the estimation		1.50150
Adj R-Squared		0.569			

6.5 EFFECT OF MICROWAVE POWER ON HEATING TEMPERATURE

6.5.1 Volume Rate of Heat Generation

The heat generation is directly related to the rate of temperature increase, density and heat capacity of the solvents. Since the rate of heat generated by the materials inside the microwave cavity is instantly interrelated to the aptitude of the molecules to align itself with the frequency of the applied field. Therefore, the rate of heat generation of solvents in the various microwave power (140, 280, 420, 560 and 700 watt) can be calculated using equations 2.16. Based on the result of volume of heat generation reading tabulated in Appendix V, Figure 6.14 was plotted.

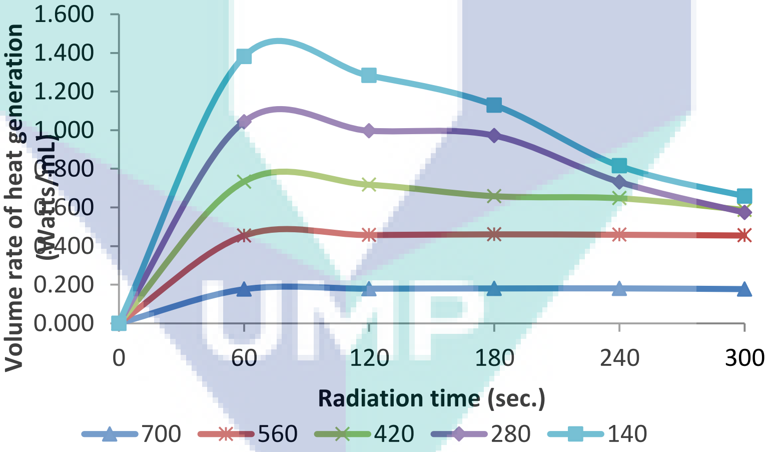


Figure 6.14: Volume rate of heat generation of various microwave power vs radiation time

Temperatures of all microwave power levels were measured across the vertical mid-plane of the reaction vessel. In this study, the temperature of each sample was recorded from the microwave oven at every fiveintervals of time. Each interval of time was kept to 60 sec, 120 sec, 180 sec, 240 sec and300 sec. It’s clearly showing that the continuous or high pulsed microwave power level has enormous influence on rate of the temperature increase inside the closed system with respect to radiation time (sec). Overall results in Figure 6.14 showed the greatest temperature rise occurred at lower to high power level. It is also observed that the acceleration of microwave absorbed energy inside the water during the initial duty cycle is higher at high to low pulse where the molecules of the water become aligned and rapidly absorbed microwave's energy due to short of period time. Bicerano (2002) also reported same manners of amorphous polymer in the liquid state because the microwave energies and thus amplitude of the random thermal motions increase at the high pulse level. Moreover, the increment of temperature in water at high power was much stronger than that at the low power level, thus the increment of temperature reaching at high level smoothly and in a very short time. Figure 6.14 also illustrated, as the exposure time was increased further, the volume rate of heat generation decreased and in certain cases approached plantaeu condition.

The decreasing of heat generation within radiation time is due to the decreasing dielectric properties of solvents with regards to temperature increase. The polarity of solvent decreses with increasing temperature, which resulted decreasing of the abilty of water to absorb microwave energy and affected the dipole rotation and alignment in the microwave field. The dielectric property which is attributed to the solvent polarity is dependent on mobility of the dipoles within the structure (Rahmat, 2002). The dielectric properties which are affected the ability of samples to absorb and dissipitate the microwave energy are influenced by heating temperature (Fang and Lai, 1996; Chan and Chen, 2002; Saound, 2004). In general, the ability of material to dissipitate energy changes in the property of the material changes (Jemaat, 2006).

6.5.2 Penetration Depth

The penetration depth, D_p at the varied microwave power level was investigated within radiation time as shown in Figure 6.15. The figure illustrated that the penetration depth significantly increased at 60sec to 300 sec at all microwave power levels. The penetration depth at high microwave power is higher than at lower microwave power. Elevated value of penetration depth revealed more intense electromagnetic inside the molecules. At higher microwave power, waves can transmit through the water layer rapidly thusfewer amounts of waves loss at the wall of the sample vessel due to the decreasing of dielectric constant. The decreasing in dielectric constant is attributed to the higher temperature within the water layer since the electromagnetic field is more intense (Aris, 2011).

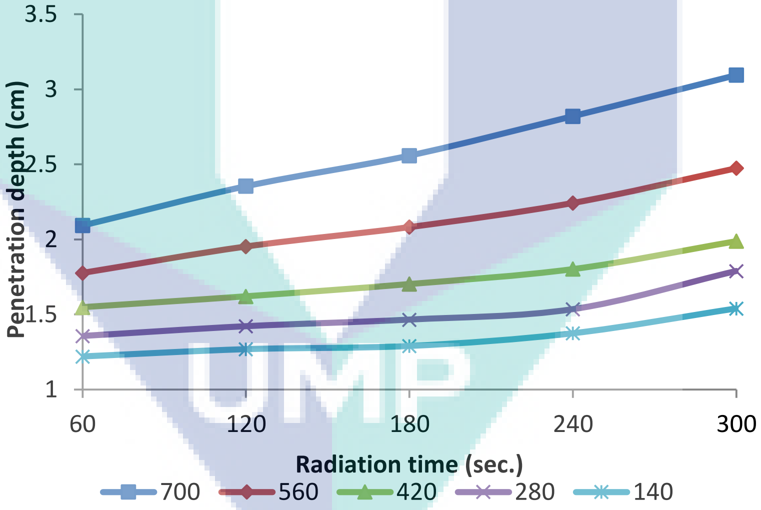


Figure 6.15: Penetration depth of various microwave powers vs radiation time

6.6 EFFECTS OF TCMA EXTRACTION ON NBF MICROSTRUCTURE UNDER VARIOUS PARAMETERS

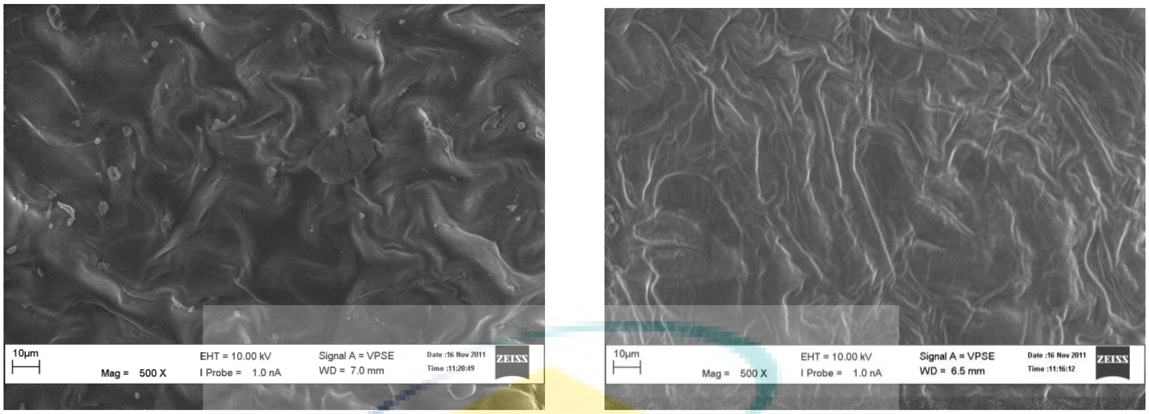
Figure 6.16 – 6.18 showed the micrographs of tissues of NBF before and after temperature controlled microwave assisted extraction (TCMAE) under different extraction parameter. It is observed that different extraction parameters produced distinguishable changes on the microstructure of NBF. As shown in Figure 6.16, as compared to before extraction, microwave treatment affects the structure of the cell due to the sudden temperature rise and the internal pressure increase. The higher temperature attained by the cell wall, during TCMAE, causes dehydration of cellulose and reduces its mechanical strength, which allows the solvent to gain an easy entry inside the cellular channels (Mandal et, al., 2008, Latha, 2007).

During the cell wall rupture process, a rapid exudation of the chemical substance within the cell into the surrounding solvents takes place. This mechanism of TCMAE based on exposing the analytes to the solvent through cell rupture is different from that of heat reflux extraction that depends on a series of permeation and solubilization processes to bring the analytes out of the matrix.

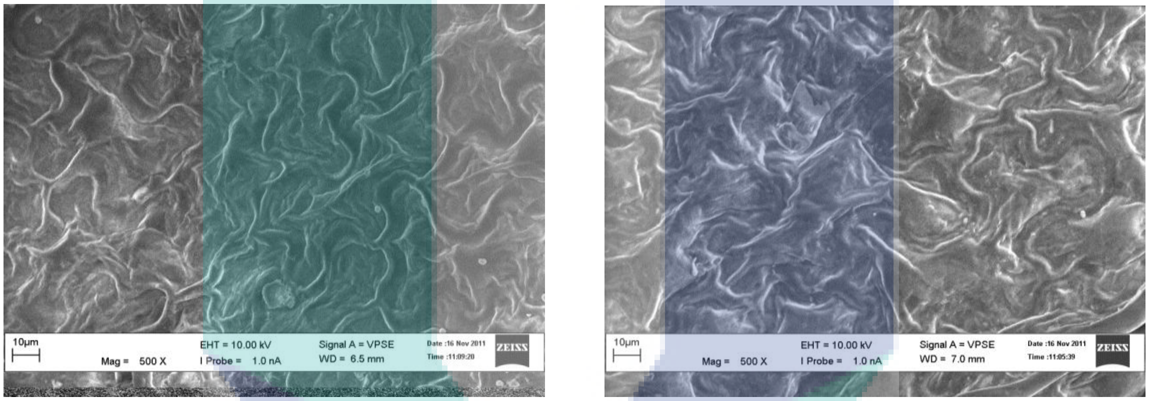
As shown in Figure 6.17 and 6.18, the mechanism of the enhanced microwave power and extraction time by TCMAE was discussed by observing cell destruction of plant material after TCMAE treatment by scanning electron microscopy. The results showed that the plant materials were significantly destroyed due to the cell rupture after TCMAE treatment from 140 – 700 Watts and 0 to 8 minutes respectively. Enhance in extraction time and extraction power, increased the water absorption in plat cell where the microwave's energy is converted into kinetic energy, thus enabling the selective heating of the microwave-absorbent parts of the plant material.

The volume increased in this way makes cells explode, releasing their content into the liquid phase. When the liquid phase absorbs the microwaves, the kinetic energy of its molecule's increases, and consequently, the diffusion rate increases too (Mandalet al., 2007). This mechanism of cell structure was corresponding to antioxidant and physicochemical property'sresults.

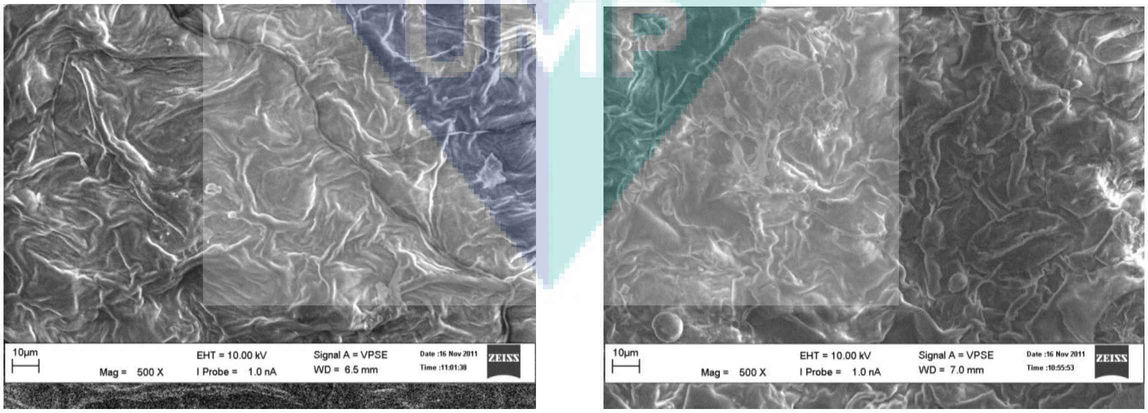
Microwave irradiation caused the explosion and rupture of NBF cells; therefore the target compounds within the cell were rapidly released into the surrounding extraction solvents. As the liquid phase absorbed the microwaves, the kinetic energy of the molecules increased, and consequently, the diffusion rate accelerated. As a result, better extraction efficiency and significantly reduced extraction time for extraction of the effective compounds from NBF matrices were obtained using TCMCS as compared to the conventional method. Explanation was also given to support the effect of microwave power on the MAE of flavonoids from *Radix astragali* (Xiao et al., 2008).



Before 50°C

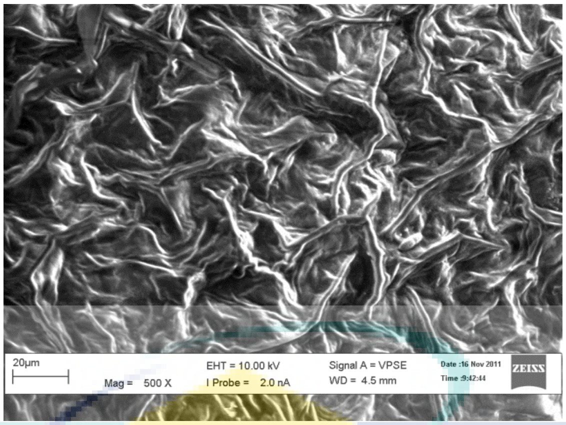


60°C 70ºC

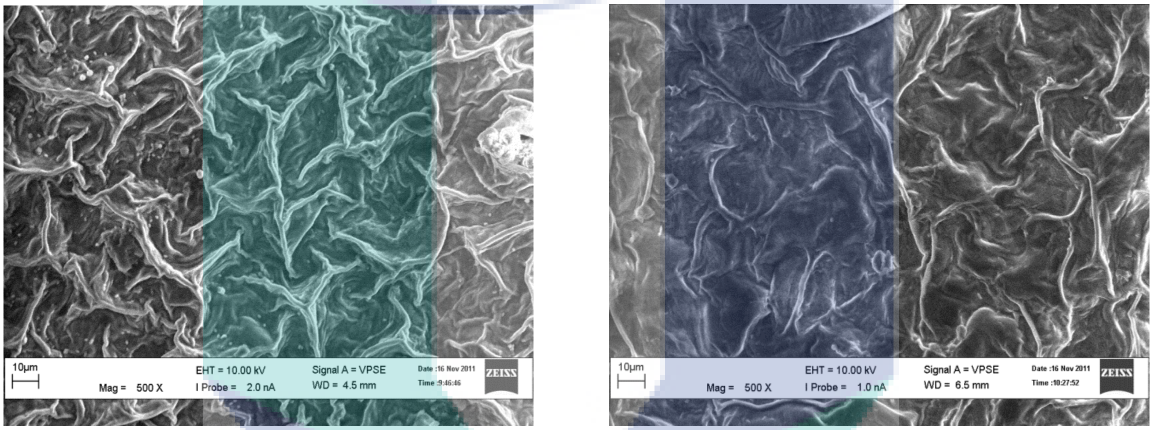


80°C 90°C

Figure 6.16: Effect of extraction temperature on NBF surface structure (Magnification 500x)

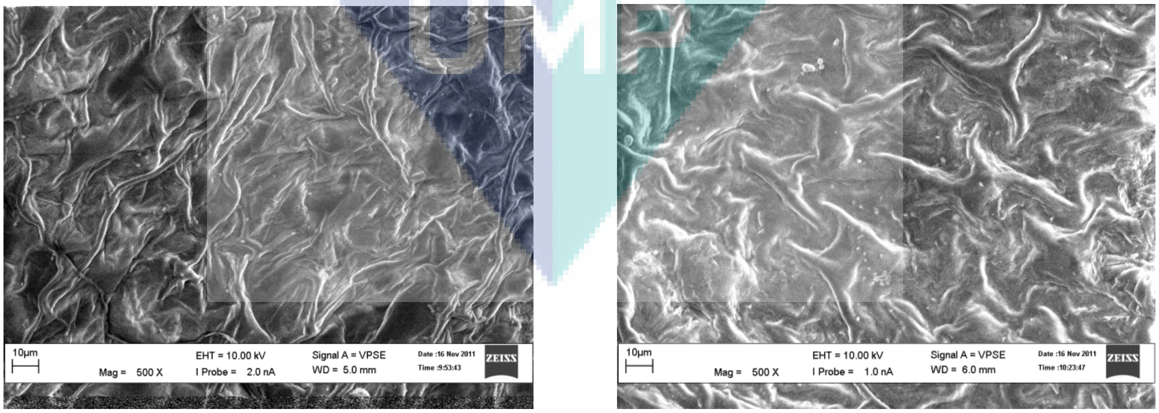


140 watts



280 watts

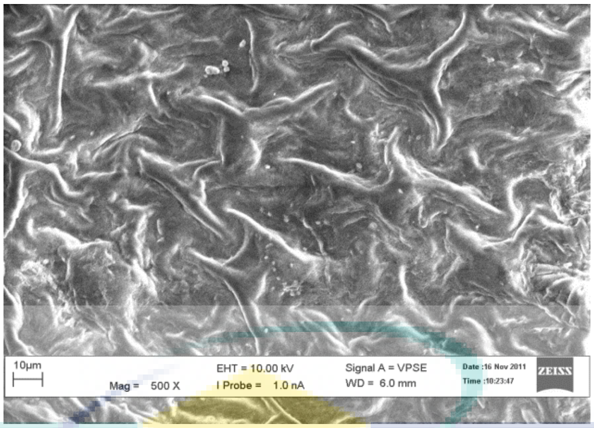
420 watts



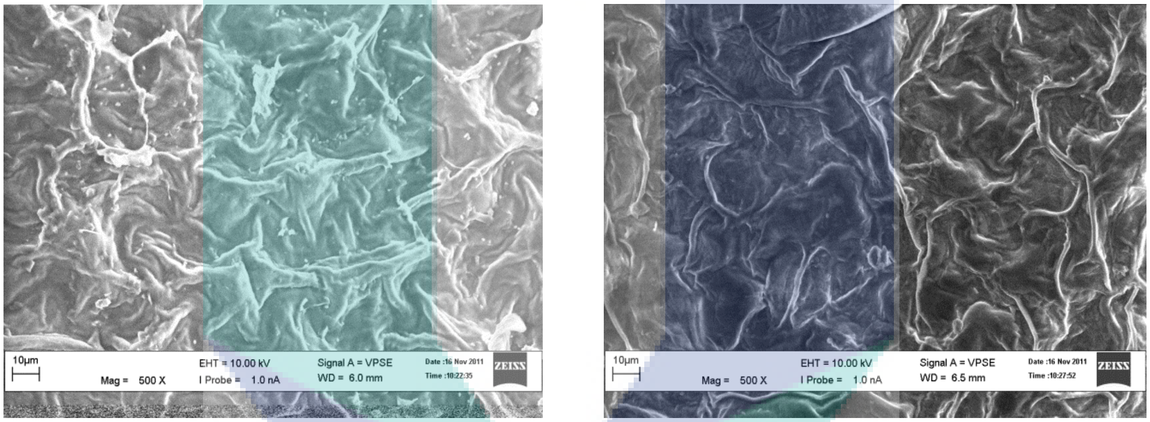
560 watts

700 watts

Figure 6.17: Effect of microwave power on NBF surface structure (Magnification: 500x)

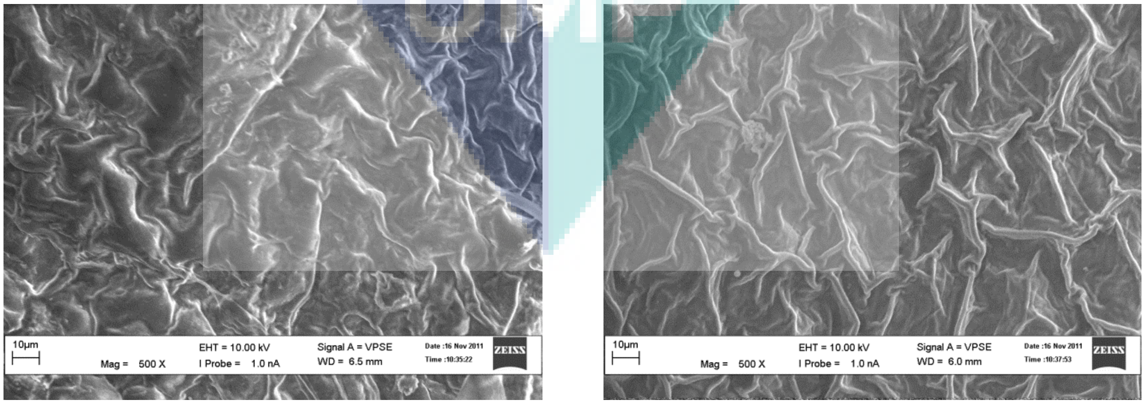


0 minutes



2 minutes

4 minutes



6 minutes

8 minutes

Figure 6.18: Effect of extraction time on NBF surface structure (Magnification: 500x)

6.7 SUMMARY

Our results confirmed that the extraction efficiency of NBF by TCMCS was influenced by the parameters of extraction temperature, microwave power and extraction time. Our findings indicated that extraction parameter was the most significant parameter amongst the others. TCMAE which is specifically based on material temperature controlling concept in the microwave system has been proven as a good technique for the rapid extraction resulting in better extraction recovery. In addition, TCMAE produced distinguishable microstructure changes and rupture the tissues of NBF and caused bioactive compounds of NBF to explode from tissue's cells, thus the target compounds within the cells were rapidly free into the surrounding extraction solutions. This mechanism was leading the releasing of bioactive compound, which has been seen by antioxidant properties and has been support by physiochemical property'sresults.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSION

The conducted study enlightened several aspects of temperature controlled closedsystem (TCMCS). This thesis deals with the temperature controlled microwave assisted extraction (TCMAE) method of antioxidant and physicochemical properties of epiphyte's fern. Conclusions from the results were drawn are listed below:

i. The influence of mechanical stirring device on microwave power distribution was explored. TCMCS shows a highly promising future in extraction on bioactive compound purpose. The hallmark of TCMCS is accelerated dissolution kinetics as a consequence of the rapid and uniform heating processes that occur when a microwave field is applied to a sample. It has gained acceptance as a mild and controllable processing tool.

ii. In order to understand the theory behind microwaves and the heating mechanism, the extraction procedures with various solvents are also stated. The experimental results of statistical design mixtures of water, acetone, acetonitrile and ethanol and their mixture was shown that quaternary mixtures with approximately equal proportions of all four solvents provided the highest result of total polyphenol contents and antioxidant activities of the crude extracts followed by ternary design mixtures and single solvent. The study on the influence of solvents on TCMCS showed that the solubility of the solvent to the antioxidant properties and the ability of the solvent to absorb microwave energy played important role in TCMCS.

iii. The factors which considerably influence the extraction parameters, especially extraction temperatureswere discussed. The principles behind the extraction process are clarified by physicochemicals analysis and extraction mechanism was elucidated by scanning electron microscopy (SEM) image. Our results confirmed that the extraction efficiency of antioxidant properties by TCMAE was influenced by the parameters of microwave power, irradiation time, extraction temperature and it was proven by physicochemical properties and SEM image results. Standardized coefficient or Beta outcome revealed that extraction temperature was the most important factor as compared to other factors.

7.2 RECOMMENDATION

The findings from the study indicate the need for future work to be carried out in this field. Below are some recommendations of future works, which can be carried out for better understanding and improvements of the TCMAE method.

i. A continuous microwave extraction study on TCMCS especially for L-shape electrically capillary thermometer should be carried out to evaluate its feasibility for commercialization.

ii. Modeling of the TCMAE method should be performed to enable the prediction of microwave heating of extraction behavior as function of related parameters in a wider range.

iii. Detailed studies on the role of individual phytochemicals involved in the antioxidant activity of specific species are required for their use as functional foods and in the pharmaceutical industry. So, further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for antioxidant and cytotoxic activities and their mode of actions. More studies should be conducted on extraction, identification and usage of other bioactivities such as anticancer activities and its major constituents. This will provide more scientific evidence on quality, safety and efficacy of epiphyte's fern to support its development as herbal supplements or value-added products.

ii. The initial capital cost of microwave equipment is high in industrial scale, this can be off setted by economic benefits attained in saleable by-products and environmental compatibility. The waste from microwave extraction of the plant material process is shown to produce high cellulose content of pulp that can be further treated and sold as a bioethanol or animal feed. The evidence shows that markets are available for these products. Alternatively, the by-products can be reused in the microwave process itself and provide further energy and cost savings. For example, the bioethanol can be employed as secondary fuels, thereby reducing fossil-fuel consumption, and the pulp can be mixed into the feed material and employed as animal feed. Then, future study of this idea is compulsory.

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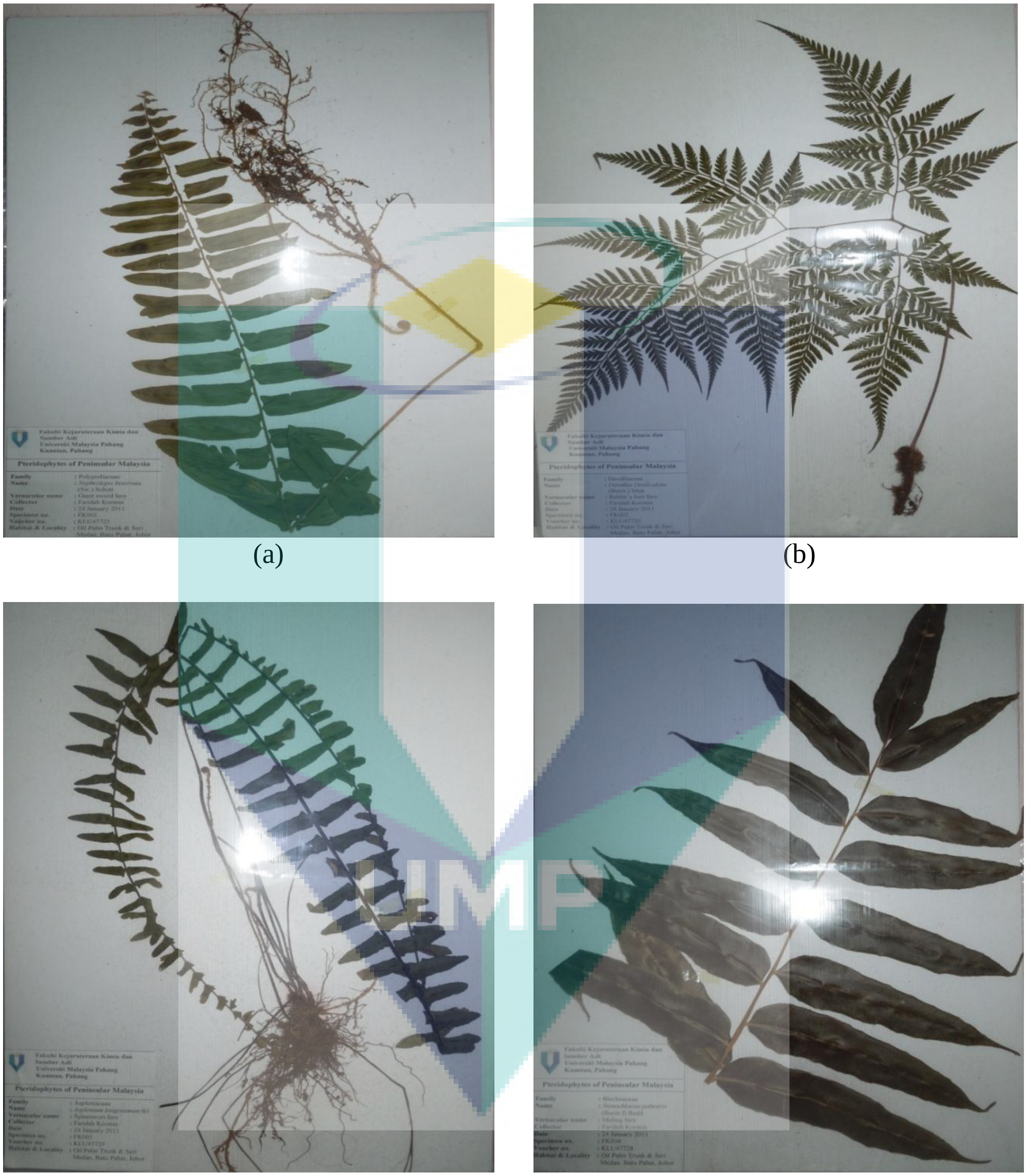
APPENDIX A

Table A: Temperature measurements option

	Thermocouple probe	Infrared sensor	Fiber optic system	L shape electrically microwave thermometer
Cost	Low	Moderate	Very costly	Low
Temperature range	Wide	Limited	Limited	Wide
Recommended for small samples	Discuss applications	Yes	Consult factory	Yes
Ease of use	Moderate	Moderate	Easy	Easy
Accuracy	Medium	Very good	Good	Good
Potential to change to heat pattern	Potentially Yes	No	Minimal, if any	No
Potential for arcing	Yes	No	Minimal, if any	No
Multiple probes	Consult factory	No	Yes	Yes

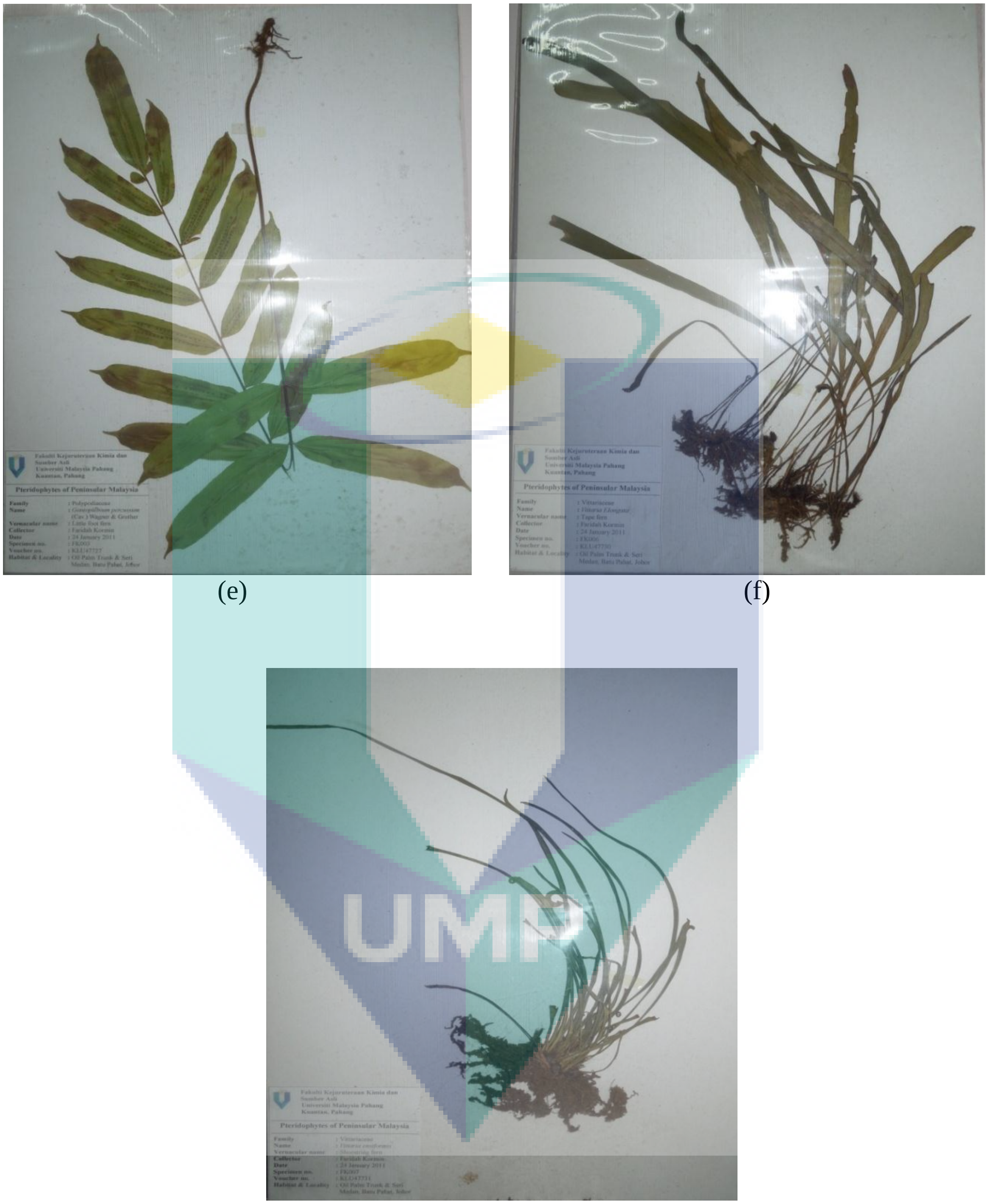
APPENDIX B



(a) (b)

(c) (d)

Figure B: Specimen voucher of epiphytes fern a) *Nephrolepis biserrata* (Sw.) Schott.,b) *Davallia denticulate* (Burm.) Mett. c) *Asplenium longissimum B1*, d) *Stenochlaena palustis*(Burm.f.) Bedd. e) *Gonioplebium percussum* (Cav.) Wagner & Gretherf) *Vittaria elongata*, andg) *Vittaria ensiformis*



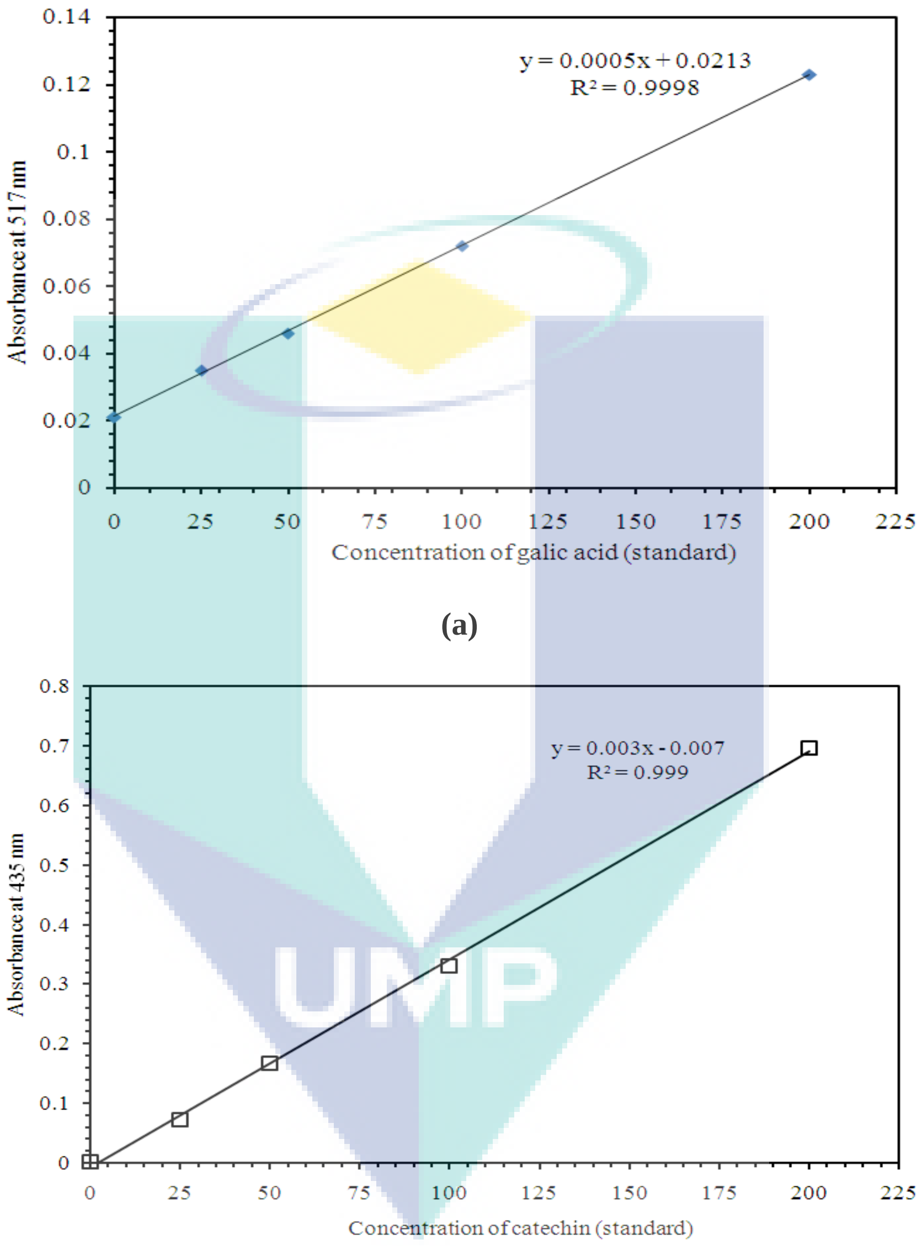
(e)

(f)

(g)

Figure B: Continued

APPENDIX C



(a)

(b)

Figure C: Standard calibration curve of a) Gallic acid and b) Catechin

APPENDIX D

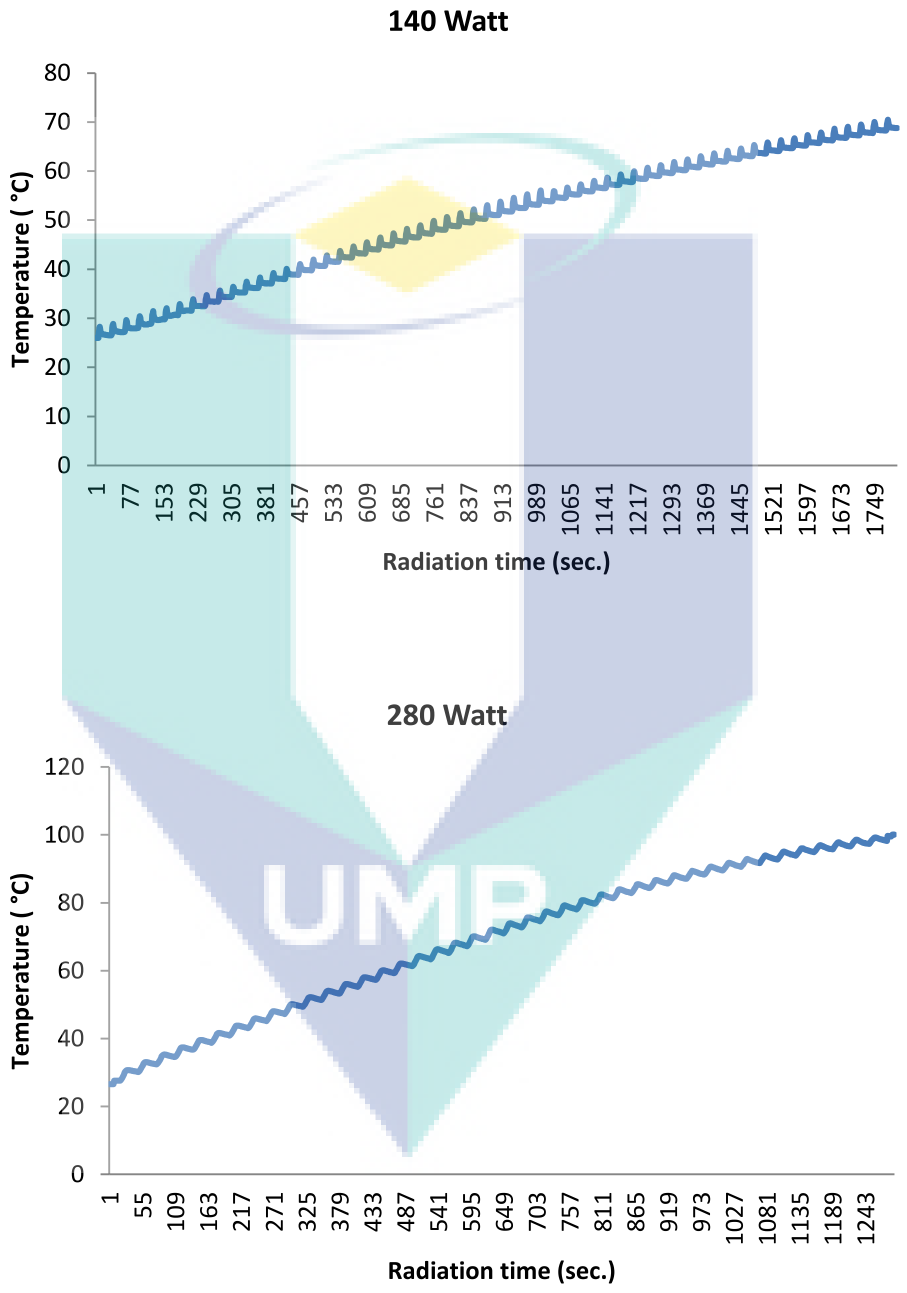


Figure D: Temperature profile for every seconds for each microwave power level

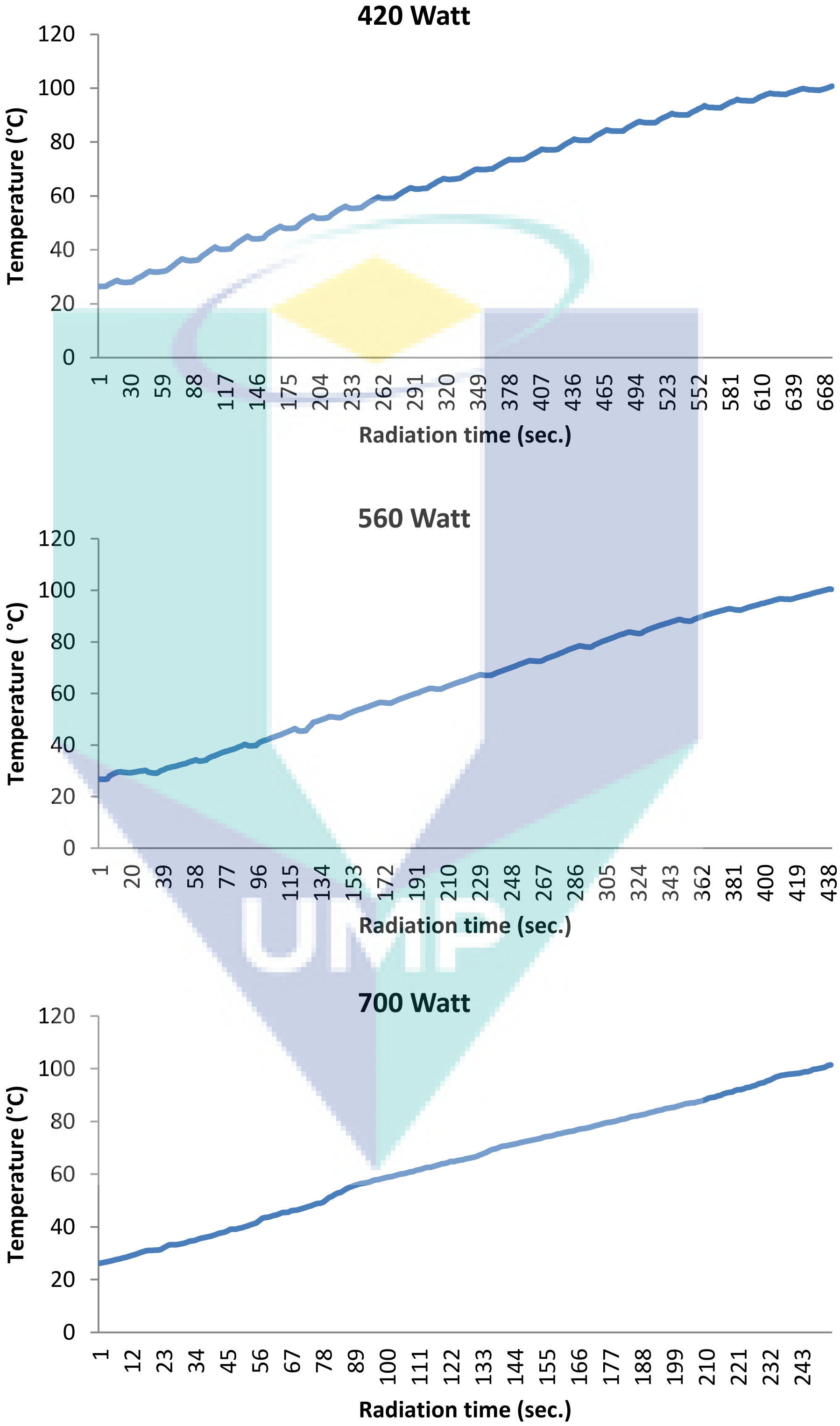


Figure D: Continued

APPENDIX E

TEMPERATURE PROFILE OF MICROWAVE POWER ABSORPTION BEFORE MODIFICATION, AFTER MODIFICATION WITHOUT STIRRING SYSTEM AND AFTER MODIFICATION WITH STIRRING SYSTEM FOR MICROWAVE POWER 140, 280, 420, 560 AND 700 WATTS

	140 Watt Microwave Power																	
	Before Modification						**After Modification Without Stirring System**						**After Modification With Stirring System**					
	1	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**
0	28.42		28.56		28.49		28.73		28.44		28.97		28.46		28.9		28.37	
20	29.36	0.94	29.59	1.03	29.5	1.01	29.61	0.88	29.27	0.83	29.76	0.79	29.72	1.26	30.57	1.67	29.04	0.67
40	30.30	0.94	30.61	1.02	30.68	1.18	30.46	0.85	30.02	0.75	30.66	0.90	30.81	1.09	31.62	1.05	29.98	0.94
60	31.25	0.95	31.48	0.87	31.56	0.88	31.27	0.81	30.84	0.82	31.68	1.02	31.81	1.00	32.6	0.98	30.59	0.61
80	32.33	1.08	32.34	0.86	32.36	0.80	32.08	0.81	31.68	0.84	32.8	1.12	32.75	0.94	33.54	0.94	31.39	0.80
100	33.27	0.94	33.31	0.97	33.17	0.81	32.95	0.87	32.51	0.83	33.98	1.18	33.77	1.02	34.39	0.85	32.23	0.84
120	34.21	0.94	34.22	0.91	34	0.83	33.81	0.86	33.32	0.81	34.08	0.10	34.36	0.59	35.13	0.74	33.44	1.21
140	35.20	0.99	34.91	0.69	34.84	0.84	34.68	0.87	34.12	0.80	36.15	2.07	35.74	1.38	36.42	1.29	34.01	0.57
160	36.07	0.87	35.7	0.79	35.67	0.83	35.54	0.86	35.09	0.97	37.2	1.05	36.61	0.87	37.45	1.03	34.91	0.90
180	37.00	0.93	36.58	0.88	36.53	0.86	36.40	0.86	35.99	0.90	38.29	1.09	37.56	0.95	38.42	0.97	35.81	0.90
200	37.85	0.85	37.54	0.96	37.37	0.84	37.30	0.90	36.89	0.90	39.06	0.77	38.48	0.92	39.42	1	36.74	0.93
220	38.67	0.82	38.44	0.90	38.21	0.84	38.20	0.90	37.79	0.90	39.41	0.35	39.44	0.96	40.39	0.97	37.61	0.87
240	39.57	0.90	39.29	0.85	39.05	0.84	39.10	0.90	38.7	0.91	39.86	0.45	40.31	0.87	41.38	0.99	38.93	1.32
260	40.42	0.85	39.89	0.60	39.87	0.82	40.04	0.94	39.51	0.81	42.43	2.57	41.29	0.98	42.31	0.93	39.4	0.47
280	41.39	0.97	40.82	0.93	40.72	0.85	40.96	0.92	40.39	0.88	43.44	1.01	42.10	0.81	43.3	0.99	40.29	0.89
300	42.25	0.86	41.71	0.89	41.54	0.82	41.85	0.89	41.28	0.89	44.05	0.61	43.08	0.98	44.24	0.94	41.18	0.89
320	43.07	0.82	42.64	0.93	42.35	0.81	42.81	0.96	42.26	0.98	44.42	0.37	43.98	0.90	45.21	0.97	42.07	0.89
340	43.90	0.83	43.43	0.79	43.15	0.80	43.75	0.94	43.18	0.92	45.41	0.99	44.92	0.94	46.14	0.93	42.9	0.83
360	44.72	0.82	44.34	0.91	43.96	0.81	44.70	0.95	44.08	0.90	46	0.59	45.86	0.94	47.09	0.95	44.14	1.24
380	45.49	0.77	45.2	0.86	44.76	0.80	45.67	0.97	44.96	0.88	48.35	2.35	46.63	0.77	48.01	0.91	44.68	0.54
400	46.38	0.89	46.08	0.88	45.52	0.76	46.62	0.95	45.81	0.85	49.3	0.95	47.41	0.78	48.94	0.94	45.56	0.88
420	47.19	0.81	46.85	0.77	46.3	0.78	47.58	0.96	46.92	1.11	50.21	0.91	48.31	0.90	49.82	0.88	46.43	0.87
440	48.01	0.82	47.69	0.84	47.04	0.74	48.58	1.00	47.91	0.99	51.09	0.88	49.13	0.82	50.71	0.89	47.3	0.87
460	48.89	0.88	48.5	0.81	47.79	0.75	49.52	0.94	48.95	1.04	51.94	0.85	49.88	0.75	51.61	0.9	48.13	0.83
480	49.64	0.75	49.32	0.82	48.71	0.92	50.50	0.98	50.07	1.12	52.53	0.59	50.80	0.92	52.51	0.9	48.98	0.85
500	50.24	0.60	50.19	0.87	49.23	0.52	51.45	0.95	51.03	0.96	53.73	1.20	51.72	0.92	53.34	0.83	49.81	0.83
520	50.88	0.64	51.06	0.87	49.94	0.71	52.41	0.96	51.65	0.62	54.59	0.86	52.50	0.78	54.19	0.85	50.61	0.80
540	51.75	0.87	51.66	0.60	50.7	0.76	53.36	0.95	52.48	0.83	55.42	0.83	53.35	0.85	55.02	0.83	51.46	0.85
560	52.49	0.74	52.74	1.08	51.4	0.70	54.32	0.96	53.21	0.73	56.24	0.82	54.13	0.78	55.81	0.79	52.28	0.82
580	53.18	0.69	53.23	0.49	52.1	0.70	55.24	0.92	53.95	0.74	57.05	0.81	54.92	0.79	56.61	0.8	53.05	0.77
600	53.86	0.68	53.81	0.58	52.85	0.75	56.18	0.94	54.87	0.92	57.25	0.20	55.64	0.72	57.41	0.8	53.93	0.88
620	54.56	0.70	54.85	1.04	53.47	0.62	57.11	0.93	55.68	0.81	58.66	1.41	56.43	0.79	58.17	0.76	54.68	0.75
640	55.10	0.54	55.74	0.89	54.15	0.68	58.05	0.94	56.48	0.80	59.42	0.76	57.15	0.72	58.96	0.79	55.51	0.83
660	55.96	0.86	56.3	0.56	54.8	0.65	58.98	0.93	57.2	0.72	60.2	0.78	57.87	0.72	59.69	0.73	56.29	0.78
680	56.55	0.59	57.15	0.85	55.43	0.63	59.95	0.97	58.01	0.80	60.94	0.74	58.64	0.77	60.42	0.73	57.07	0.78
700	57.21	0.66	57.62	0.47	56.09	0.66	60.87	0.92	59.02	1.02	61.66	0.72	59.35	0.71	61.13	0.71	57.78	0.71
720	57.82	0.61	57.93	0.31	56.7	0.61	61.78	0.91	60.09	1.07	62.38	0.72	60.04	0.69	61.87	0.74	58.53	0.75
740	58.37	0.55	58.25	0.32	57.31	0.61	62.68	0.90	61.02	0.93	63.1	0.72	60.77	0.73	62.51	0.64	59.23	0.70
760	59.01	0.64	59.08	0.83	57.9	0.59	63.57	0.89	61.95	0.93	63.78	0.68	61.43	0.66	63.21	0.7	59.95	0.72
780	59.58	0.57	60.04	0.96	58.5	0.60	64.45	0.88	62.91	0.96	64.4	0.62	62.11	0.68	63.85	0.64	60.64	0.69
800	60.16	0.58	60.95	0.91	59.04	0.54	65.33	0.88	63.72	0.81	65.02	0.62	62.75	0.64	64.53	0.68	61.33	0.69
820	60.69	0.53	61.24	0.29	59.61	0.57	66.16	0.83	64.42	0.70	65.67	0.65	63.45	0.70	65.17	0.64	61.97	0.64
840	61.31	0.62	61.51	0.27	60.25	0.64	66.98	0.82	65.01	0.59	66.97	1.30	64.03	0.58	65.84	0.67	62.65	0.68

	280 Watt Microwave Power																	
	Before Modification						**After Modification Without Stirring System**						**After Modification With Stirring System**					
	1	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**
0	28.09		28.95		30.91		28.01		28.01		28.87		28.18		28.42		28.47	
20	29.88	1.79	31	2.05	33.14	2.23	29.11	1.10	29.21	1.20	30.85	1.98	29.31	1.13	30.18	1.76	29.56	1.09
40	31.78	1.90	33.46	2.46	35.27	2.13	30.78	1.67	30.88	1.67	32.67	1.82	31.34	2.03	32.5	2.32	31.46	1.90
60	33.96	2.18	36.29	2.83	37.4	2.13	32.62	1.84	32.72	1.84	34.29	1.62	33.53	2.19	34.7	2.20	33.5	2.04
80	36.16	2.20	38.86	2.57	39.54	2.14	34.61	1.99	34.71	1.99	36.77	2.48	35.80	2.27	36.98	2.28	35.92	2.42
100	38.40	2.24	40.12	1.26	41.67	2.13	36.76	2.15	36.86	2.15	39.04	2.27	38.19	2.39	39.19	2.21	38.34	2.42
120	40.72	2.32	41.78	1.66	43.78	2.11	39.02	2.26	39.11	2.25	40.75	1.71	40.52	2.33	41.12	1.93	40.63	2.29
140	42.79	2.07	44.01	2.23	45.92	2.14	41.17	2.15	41.17	2.06	45.01	4.26	42.94	2.42	44.19	3.07	43.02	2.39
160	44.93	2.14	46.91	2.9	48.03	2.11	43.46	2.29	43.46	2.29	47.77	2.76	45.35	2.41	46.63	2.44	45.42	2.40
180	47.09	2.16	49.4	2.49	50.15	2.12	46.01	2.55	46.01	2.55	50.05	2.28	47.74	2.39	49.14	2.51	47.78	2.36
200	49.27	2.18	51.42	2.02	52.18	2.03	48.49	2.48	48.49	2.48	52.14	2.09	50.06	2.32	51.5	2.36	50.18	2.40
220	51.49	2.22	53.03	1.61	54.24	2.06	50.96	2.47	50.96	2.47	54.05	1.91	52.45	2.39	53.84	2.34	52.57	2.39
240	53.75	2.26	54.82	1.79	56.35	2.11	53.51	2.55	53.51	2.55	55.09	1.04	54.72	2.27	56.15	2.31	54.84	2.27
260	56.04	2.29	57.21	2.39	58.23	1.88	56.06	2.55	56.06	2.55	58.32	3.23	57.03	2.31	58.46	2.31	57.19	2.35
280	58.33	2.29	59.23	2.02	60.19	1.96	58.63	2.57	58.63	2.57	60.36	2.04	59.22	2.19	60.65	2.19	59.47	2.28
300	60.41	2.08	61.24	2.01	62.09	1.90	61.10	2.47	61.10	2.47	62.34	1.98	61.47	2.25	62.93	2.28	61.71	2.24
320	62.45	2.04	63.18	1.94	63.98	1.89	63.59	2.49	63.59	2.49	64.33	1.99	63.52	2.05	64.97	2.04	63.82	2.11
340	64.31	1.86	64.7	1.52	65.97	1.99	66.04	2.45	66.04	2.45	67.21	2.88	65.61	2.09	67.06	2.09	65.83	2.01
360	66.30	1.99	66.64	1.94	67.92	1.95	68.49	2.45	68.49	2.45	69.85	2.64	67.64	2.03	69.09	2.03	67.88	2.05
380	68.28	1.98	67.98	1.34	69.48	1.56	70.85	2.36	70.85	2.36	70.98	1.13	69.63	1.99	71.11	2.02	69.88	2.00
400	70.27	1.99	69.59	1.61	71.19	1.71	73.18	2.33	73.18	2.33	73.29	2.31	71.55	1.92	73.02	1.91	71.79	1.91
420	72.08	1.81	71.63	2.04	72.94	1.75	75.44	2.26	75.44	2.26	75.87	2.58	73.45	1.90	74.91	1.89	73.65	1.86
440	73.86	1.78	73.01	1.38	74.86	1.92	77.73	2.29	77.73	2.29	77.82	1.95	75.27	1.82	76.75	1.84	75.5	1.85
460	75.57	1.71	74.88	1.87	76.87	2.01	79.93	2.20	79.93	2.20	79.74	1.92	77.12	1.85	78.57	1.82	77.34	1.84
480	77.20	1.63	76.95	2.07	78.51	1.64	82.14	2.21	82.14	2.21	81.65	1.91	78.81	1.69	80.29	1.72	79.11	1.77
500	78.84	1.64	78.01	1.06	79.11	0.60	84.25	2.11	84.25	2.11	80.18	-1.47	80.51	1.70	82.07	1.78	80.83	1.72
520	80.51	1.67	79.08	1.07	81.6	2.49	86.22	1.97	86.22	1.97	81.66	1.48	82.19	1.68	83.88	1.81	82.5	1.67
540	82.13	1.62	80.77	1.69	82.08	0.48	88.14	1.92	88.14	1.92	83.04	1.38	83.78	1.59	85.57	1.69	84.19	1.69
560	83.71	1.58	82.7	1.93	83.47	1.39	90.05	1.91	90.05	1.91	84.43	1.39	85.33	1.55	87.18	1.61	85.87	1.68
580	85.16	1.45	84.11	1.41	86.9	3.43	91.85	1.80	91.85	1.80	85.75	1.32	87.00	1.67	88.9	1.72	87.52	1.65
600	86.78	1.62	85.53	1.42	88.23	1.33	93.47	1.62	93.47	1.62	91.07	5.32	88.53	1.53	90.53	1.63	89.01	1.49
620	88.22	1.44	87.49	1.96	89.11	0.88	95.07	1.60	95.07	1.60	88.32	-2.75	90.12	1.59	92.11	1.58	90.53	1.52
640	89.63	1.41	89.48	1.99	89.75	0.64	96.68	1.61	96.68	1.61	89.56	1.24	91.59	1.47	93.68	1.57	92.07	1.54
660	90.81	1.18	91.16	1.68	91.12	1.37	98.27	1.59	98.27	1.59	90.71	1.15	93.12	1.53	95.3	1.62	93.54	1.47
680	92.15	1.34	92.87	1.71	91.95	0.83	99.85	1.58	99.85	1.58	91.83	1.12	94.47	1.35	96.51	1.21	94.98	1.44
700	93.40	1.25	94.98	2.11	93.24	1.29	101.08	1.23	101.08	1.23	92.95	1.12	95.80	1.33	97.94	1.43	96.37	1.39
720	94.67	1.27	97.05	2.07	94.91	1.67	101.48	0.40	101.48	0.40	99.03	6.08	97.03	1.23	99.35	1.41	97.7	1.33
740	95.88	1.21	98.59	1.54	95.89	0.98	101.86	0.38	101.86	0.38	98.04	-0.99	98.25	1.22	100.54	1.19	98.9	1.20
760	97.07	1.19	100.35	1.76	96.79	0.90	102.13	0.27	102.13	0.27	98.71	0.67	99.20	0.95	101.11	0.57	99.92	1.02
780	98.25	1.18	101.06	0.71	97.75	0.96	102.33	0.20	102.33	0.20	99.73	1.02	100.24	1.04	101.34	0.23	100.45	0.53
800	99.20	0.95	102.4	1.34	98.45	0.70	102.52	0.19	102.52	0.19	99.89	0.16	101.19	0.95	101.63	0.29	100.69	0.24
820	100.24	1.04	103.07	0.67	99.99	1.54	102.57	0.05	102.57	0.05	99.59	-0.30	101.90	0.71	102.28	0.65	100.93	0.24
840	101.19	0.95	103.7	0.63	100.83	0.84	102.64	0.07	102.64	0.07	100.35	0.76	102.06	0.16	102.91	0.63	101.23	0.30

	420 Watt Microwave Power																	
	Before Modification						**After Modification Without Stirring System**						**After Modification With Stirring System**					
	1	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**
0	28.35		28.27		27.75		28.01		28.18		28.9		27.02		27.78		27.34	
20	31.92	3.57	32.29	4.02	32.06	4.31	29.13	1.12	30.33	2.15	32.01	3.11	29.97	2.95	31.12	3.34	29.7	2.36
40	34.63	2.71	35.87	3.58	35.66	3.60	31.55	2.42	32.47	2.14	34.03	2.02	33.82	3.85	34.38	3.26	33.01	3.31
60	38.05	2.71	39.09	3.22	38.91	3.25	34.35	2.80	35.03	2.56	36.94	2.91	37.18	3.36	38.25	3.87	36.27	3.26
80	41.50	3.42	42.18	3.09	42.16	3.25	37.25	2.90	37.95	2.92	39.76	2.82	40.45	3.27	41.5	3.25	39.76	3.49
100	45.20	3.45	45.31	3.13	45.36	3.20	40.32	3.07	40.97	3.02	42.18	2.42	43.69	3.24	45.05	3.55	43.24	3.48
120	48.29	3.70	48.02	2.71	48.03	2.67	43.56	3.24	43.96	2.99	45.9	3.72	46.85	3.16	48.44	3.39	46.9	3.66
140	51.59	3.09	51.44	3.42	51.64	3.61	46.99	3.43	46.91	2.95	47.64	1.74	49.83	2.98	51.94	3.5	50.68	3.78
160	54.80	3.30	54.54	3.10	54.71	3.07	50.60	3.61	49.79	2.88	50.68	3.04	52.88	3.05	55.48	3.54	54.22	3.54
180	57.90	3.21	57.66	3.12	57.67	2.96	54.26	3.66	52.77	2.98	52.94	2.26	56.01	3.13	59.04	3.56	57.63	3.41
200	60.97	3.10	60.7	3.04	60.68	3.01	57.95	3.69	55.77	3.00	56.83	3.89	59.06	3.05	62.46	3.42	61.02	3.39
220	63.98	3.07	63.77	3.07	63.57	2.89	61.38	3.43	59.82	4.05	60.74	3.91	62.75	3.69	65.71	3.25	64.25	3.23
240	67.01	3.01	66.76	2.99	66.65	3.08	64.66	3.28	63.97	4.15	64.99	4.25	66.35	3.60	68.38	2.67	66.92	2.67
260	70.17	3.03	69.84	3.08	70.15	3.50	68.34	3.68	66.71	2.74	68.39	3.40	69.91	3.56	71.93	3.55	70.5	3.58
280	73.33	3.16	72.83	2.99	72.86	2.71	71.93	3.59	69.68	2.97	71.98	3.59	73.19	3.28	75.03	3.1	73.45	2.95
300	76.32	3.16	75.83	3.00	76.55	3.69	75.44	3.51	72.55	2.87	75.31	3.33	75.86	2.67	78.06	3.03	76.43	2.98
320	79.01	2.99	78.82	2.99	80.18	3.63	78.86	3.42	76.33	3.78	78.59	3.28	79.49	3.63	80.99	2.93	79.38	2.95
340	81.55	2.69	81.77	2.95	81.73	1.55	82.26	3.40	80.02	3.69	81.81	3.22	83.00	3.51	83.81	2.82	82.32	2.94
360	84.04	2.54	84.37	2.60	83.95	2.22	85.64	3.38	83.28	3.26	84.93	3.12	85.85	2.85	86.97	3.16	86.2	3.88
380	86.47	2.49	87.25	2.88	84.67	0.72	88.71	3.07	82.09	-1.19	87.89	2.96	88.02	2.17	89.36	2.39	88.13	1.93
400	88.78	2.43	89.62	2.37	87.04	2.37	91.67	2.96	84.93	2.84	90.71	2.82	90.49	2.47	92.03	2.67	90.81	2.68
420	91.06	2.31	91.83	2.21	89.33	2.29	94.64	2.97	87.2	2.27	93.48	2.77	93.30	2.81	94.63	2.6	93.42	2.61
440	93.25	2.28	93.95	2.12	91.5	2.17	97.48	2.84	90.06	2.86	96	2.52	94.69	1.39	96.95	2.32	95.89	2.47
460	95.19	2.19	95.84	1.89	93.55	2.05	100.07	2.59	92.49	2.43	98.32	2.32	96.91	2.22	99.23	2.28	98.27	2.38
480	97.11	1.94	97.62	1.78	96.99	3.44	100.85	0.78	97.86	5.37	99.81	1.49	99.08	2.17	100.18	0.95	100.06	1.79
500	98.98	1.92	99.39	1.77	97.48	0.49	101.83	0.98	97.58	-0.28	100.33	0.52	100.37	1.29	100.45	0.27	100.8	0.74
520	100.81	1.87	101.18	1.79	99.29	1.81	102.13	0.30	99.32	1.74	100.57	0.24	102.12	1.75	101.05	0.6	101.27	0.47
540	102.34	1.83	102.29	1.11	100.28	0.99	102.24	0.11	100.35	1.03	100.73	0.16	103.14	1.02	102.12	1.07	102.02	0.75
560	102.89	1.53	102.66	0.37	100.35	0.07	102.35	0.11	101.24	0.89	101.32	0.59	103.91	0.77	103.62	1.5	102.56	0.54
580	103.01	0.55	103.25	0.59	100.87	0.52	102.48	0.13	100.24	-1.00	102.04	0.72	104.29	0.38	103.91	0.29	103.02	0.46
600	104.34	0.12	104.6	1.35	101.36	0.49	102.55	0.07	100.08	-0.16	102.35	0.31	104.82	0.53	104.08	0.17	104.57	1.55
620	105.92	1.33	104.77	0.17	102.02	0.66	102.58	0.03	99.33	-0.75	103.41	1.06	105.05	0.23	103.73	-0.35	104.14	-0.43
640	105.97	1.58	104.87	0.10	102.88	0.86	102.62	0.04	99.41	0.08	103.55	0.14	105.42	0.37	103.79	0.06	104.6	0.46
660	105.21	0.05	105.72	0.85	103.05	0.17	102.65	0.03	99.22	-0.19	103.55	0.00	106.09	0.67	103.87	0.08	104.88	0.28
680	105.05	-0.76	105.64	-0.08	102.41	-0.64	102.71	0.06	99.18	-0.04	103.56	0.01	106.32	0.23	103.95	0.08	105.12	0.24
700	104.07	-0.16	105.32	-0.32	102.33	-0.08	102.81	0.10	99.19	0.01	103.61	0.05	106.83	0.51	104	0.05	105.16	0.04
720	105.22	-0.98	105.63	0.31	102.41	0.08	102.72	-0.09	100.37	1.18	103.22	-0.39	106.84	0.01	104.99	0.99	105.72	0.56
740	105.29	1.15	105.36	-0.27	102.46	0.05	103.68	0.96	99.52	-0.85	103.68	0.46	108.2	1.36	104.16	-0.83	105.67	-0.05
760	105.34	0.07	104.74	-0.62	102.64	0.18	103.29	-0.39	99.27	-0.25	103.29	-0.39	108.83	0.63	104.21	0.05	105.78	0.11
780	105.41	0.05	103.83	-0.91	102.77	0.13	103.12	-0.17	99.25	-0.02	103.12	-0.17	108.43	-0.40	104.35	0.14	105.83	0.05
800	105.64	0.07	103.97	0.14	102.96	0.19	103.97	0.85	100.12	0.87	103.97	0.85	107.36	-1.07	104.38	0.03	105.72	-0.11
820	104.67	0.23	103.9	-0.07	102.99	0.03	105.19	1.22	100.24	0.12	105.19	1.22	107.09	-0.27	104.4	0.02	105.84	0.12
840	104.76	-0.97	103.71	-0.19	103.06	0.07	104.33	-0.86	101.29	1.05	104.33	-0.86	108.2	1.11	104.49	0.09	105.82	-0.02

	560 Watt Microwave Power																	
	Before Modification						**After Modification Without Stirring System**						**After Modification With Stirring System**					
	1	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**
0	28.47		28.17		28.78		28.12		28.02		28.9		28.73		27.94		27.69	
20	33.42	4.95	31.56	3.39	32.58	3.80	31.35	3.23	30.52	2.50	32.09	3.19	34.45	5.72	31.79	3.85	30.17	2.48
40	38.49	5.07	36.45	4.89	37.18	4.60	34.48	3.13	33.37	2.85	35.87	3.78	40.72	6.27	36.69	4.9	35.61	5.44
60	43.51	5.02	41.23	4.78	41.65	4.47	38.30	3.82	37.12	3.75	39.89	4.02	45.68	4.96	41.52	4.83	40.91	5.30
80	48.43	4.92	45.9	4.67	46.11	4.46	42.73	4.43	41.58	4.46	44.06	4.17	50.45	4.77	46.25	4.73	45.96	5.05
100	53.17	4.74	50.73	4.83	50.71	4.60	47.52	4.79	46.45	4.87	48.81	4.75	55.25	4.80	50.91	4.66	50.74	4.78
120	57.83	4.66	55.38	4.65	56.16	5.45	52.59	5.07	51.52	5.07	53.16	4.35	59.16	3.91	56.27	5.36	55.09	4.35
140	62.37	4.54	60.15	4.77	59.69	3.53	57.93	5.34	56.44	4.92	57.2	4.04	64.17	5.01	59.9	3.63	59.92	4.83
160	66.91	4.54	64.79	4.64	64.16	4.47	63.35	5.42	61.27	4.83	61.24	4.04	68.74	4.57	64.29	4.39	64.52	4.60
180	71.37	4.46	69.25	4.46	68.51	4.35	68.68	5.33	65.98	4.71	66.66	5.42	73.41	4.67	68.65	4.36	68.88	4.36
200	75.90	4.53	73.5	4.25	72.73	4.22	73.80	5.12	70.43	4.45	71.98	5.32	78.04	4.63	72.89	4.24	73.13	4.25
220	79.34	3.44	77.52	4.02	76.91	4.18	78.64	4.84	74.89	4.46	77.13	5.15	83.18	5.14	76.92	4.03	77.21	4.08
240	83.09	3.75	80.78	3.26	81.55	4.64	81.93	3.29	80.78	5.89	82.91	5.78	86.71	3.53	82.49	5.57	81.29	4.08
260	86.62	3.53	85.17	4.39	84.9	3.35	87.69	5.76	83.01	2.23	86.83	3.92	92.12	5.41	85.16	2.67	85.31	4.02
280	89.94	3.32	88.6	3.43	88.61	3.71	92.22	4.53	86.88	3.87	90.59	3.76	95.94	3.82	88.9	3.74	89.35	4.04
300	92.95	3.01	91.89	3.29	92.29	3.68	96.64	4.42	90.96	4.08	94.25	3.66	99.31	3.37	92.61	3.71	93.04	3.69
320	95.83	2.88	95.01	3.12	95.86	3.57	100.54	3.90	94.76	3.80	97.61	3.36	102.14	2.83	96.14	3.53	96.3	3.26
340	98.49	2.66	97.75	2.74	99.21	3.35	101.82	1.28	98.85	4.09	99.87	2.26	104.50	2.36	98.7	2.56	98.46	2.16
360	101.53	3.04	99.65	1.90	100.43	1.22	102.14	0.32	99.52	0.67	99.98	0.11	104.81	0.31	100.22	1.52	99.58	1.12
380	102.43	0.90	100.32	0.67	102.02	1.59	102.23	0.09	100.1	0.58	100.01	0.03	105.98	1.17	100.84	0.62	100.69	1.11
400	103.79	1.36	101.45	1.13	102.99	0.97	102.29	0.06	100.25	0.15	100.29	0.28	106.59	0.61	101.88	1.04	101.35	0.66
420	104.55	0.76	102.98	1.53	103.59	0.60	102.31	0.02	100.39	0.14	100.31	0.02	107.02	0.43	102.32	0.44	101.53	0.18
440	104.68	0.13	102.98	0.00	103.71	0.12	102.41	0.10	100.49	0.10	100.53	0.22	107.36	0.34	103.05	0.73	101.72	0.19
460	105.14	0.46	102.83	-0.15	104.18	0.47	102.51	0.10	100.52	0.03	100.68	0.15	107.92	0.56	103.29	0.24	101.83	0.11
480	105.31	0.17	102.86	0.03	104.19	0.01	102.76	0.25	100.58	0.06	100.73	0.05	108.18	0.26	103.67	0.38	103.12	1.29
500	105.62	0.31	102.92	0.06	104.23	0.04	102.66	-0.10	100.73	0.15	100.71	-0.02	108.25	0.07	103.85	0.18	102.54	-0.58
520	106.04	0.42	102.91	-0.01	103.43	-0.80	102.69	0.03	100.71	-0.02	100.82	0.11	108.35	0.10	104.01	0.16	102.74	0.20
540	106.18	0.14	103	0.09	104.52	1.09	102.72	0.03	100.82	0.11	100.87	0.05	108.48	0.13	104.22	0.21	103.24	0.50
560	106.25	0.07	102.99	-0.01	104.99	0.47	102.81	0.09	100.87	0.05	100.77	-0.10	108.93	0.45	104.45	0.23	103.44	0.20
580	105.35	-0.90	103.15	0.16	104.59	-0.40	102.78	-0.03	100.77	-0.10	100.77	0.00	108.49	-0.44	104.54	0.09	103.72	0.28
600	105.78	0.43	103.36	0.21	104.98	0.39	102.89	0.11	100.77	0.00	100.81	0.04	108.84	0.35	104.79	0.25	104.12	0.40
620	104.98	-0.80	103.28	-0.08	103.98	-1.00	101.96	-0.93	100.81	0.04	100.46	-0.35	107.95	-0.89	104.95	0.16	104.06	-0.06
640	104.43	-0.55	103.43	0.15	103.43	-0.55	101.79	-0.17	100.46	-0.35	100.79	0.33	106.36	-1.59	105.21	0.26	103.96	-0.10
660	104.23	-0.20	104.23	0.80	104.23	0.80	101.72	-0.07	100.79	0.33	100.72	-0.07	106.54	0.18	105.54	0.33	104.02	0.06
680	103.43	-0.80	103.43	-0.80	104.03	-0.20	101.78	0.06	100.72	-0.07	100.78	0.06	106.71	0.17	105.71	0.17	104.11	0.09
700	104.52	1.09	104.52	1.09	104.52	0.49	101.91	0.13	100.78	0.06	100.91	0.13	106.09	-0.62	106.09	0.38	104.2	0.09
720	104.99	0.47	104.99	0.47	104.99	0.47	102.12	0.21	100.91	0.13	101.92	1.01	107.45	1.36	106.45	0.36	104.28	0.08
740	104.59	-0.40	104.59	-0.40	104.59	-0.40	101.15	-0.97	100.92	0.01	101.15	-0.77	106.76	-0.69	106.76	0.31	104.39	0.11
760	104.41	-0.18	104.41	-0.18	104.41	-0.18	101.79	0.64	101.15	0.23	101.79	0.64	107	0.24	107	0.24	104.44	0.05
780	103.68	-0.73	103.28	-1.13	103.68	-0.73	102.78	0.99	101.79	0.64	102.78	0.99	107.35	0.35	107.35	0.35	104.64	0.20
800	103.43	-0.25	103.43	0.15	103.43	-0.25	103.7	0.92	102.78	0.99	103.7	0.92	107.51	0.16	107.51	0.16	104.82	0.18
820	103.23	-0.20	104.03	0.60	103.23	-0.20	103.09	-0.61	103.7	0.92	103.09	-0.61	107.82	0.31	107.82	0.31	105.03	0.21
840	102.43	-0.80	102.93	-1.10	103.43	0.20	103.76	0.67	103.09	-0.61	103.76	0.67	108.16	0.34	108.16	0.34	105.87	0.84

	700 Watt																	
	Before Modification						**After Modification Without Stirring System**						**After Modification With Stirring System**					
	1	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**	**1**	**ΔT**	**2**	**ΔT**	**3**	**ΔT**
0	27.01		27.07		27.22		28.73		28.43		28.45		28.89		28.01		28.04	
20	33.01	6.00	32.33	5.26	33.93	6.71	33.88	5.15	33.67	5.24	33.70	5.25	36.62	7.73	34.58	6.57	30.93	2.89
40	39.81	6.80	38.27	5.94	40.15	6.22	39.85	5.97	39.45	5.78	39.33	5.63	43.90	7.28	41.46	6.88	36.93	6.00
60	46.57	6.76	45.51	7.24	46.52	6.37	45.86	6.01	45.78	6.33	45.64	6.31	50.65	6.75	48.53	7.07	44.16	7.23
80	52.96	6.39	52.24	6.73	52.77	6.25	52.74	6.88	52.38	6.60	52.37	6.73	57.72	7.07	55.45	6.92	50.75	6.59
100	59.29	6.33	58.79	6.55	59.17	6.40	59.74	7.00	59.99	7.61	59.91	7.54	63.87	6.15	61.07	5.62	56.94	6.19
120	65.51	6.22	64.98	6.19	65.80	6.63	66.10	6.36	65.57	5.58	65.49	5.58	69.00	5.13	67.89	6.82	63.01	6.07
140	71.91	6.40	71.06	6.08	71.95	6.15	72.69	6.59	71.55	5.98	71.30	5.81	75.85	6.85	73.73	5.84	68.56	5.55
160	78.14	6.23	76.91	5.85	77.95	6.00	78.53	5.84	77.89	6.34	77.94	6.64	82.31	6.46	80.31	6.58	73.96	5.40
180	83.67	5.53	82.72	5.81	83.71	5.76	84.25	5.72	83.77	5.88	83.69	5.75	88.65	6.34	86.54	6.23	79.32	5.36
200	88.95	5.28	88.45	5.73	89.34	5.63	90.13	5.88	89.79	6.02	89.42	5.73	95.03	6.38	93.91	7.37	85.89	6.57
220	93.98	5.03	93.12	4.67	93.81	4.47	95.70	5.57	94.81	5.02	94.99	5.57	101.46	6.43	97.02	3.11	90.48	4.59
240	98.49	4.51	97.19	4.07	98.98	5.17	100.95	5.25	100.08	5.27	100.44	5.45	104.78	3.32	100.29	3.27	96.79	6.31
260	100.31	1.82	100.06	2.87	100.62	1.64	102.05	1.10	101.19	1.11	101.92	1.48	106.68	1.90	100.61	0.32	98.48	1.69
280	102.51	2.20	100.08	0.02	102.18	1.56	102.28	0.23	101.54	0.35	102.11	0.19	107.28	0.60	101.44	0.83	99.82	1.34
300	103.47	0.96	100.76	0.68	103.30	1.12	102.38	0.10	101.89	0.35	102.21	0.10	107.59	0.31	101.26	-0.18	101.63	1.81
320	104.15	0.68	100.94	0.18	104.06	0.76	102.55	0.17	101.94	0.05	102.34	0.13	107.92	0.33	103.28	2.02	102.78	1.15
340	104.67	0.52	101.56	0.62	104.90	0.84	102.68	0.13	101.98	0.04	102.37	0.03	108.43	0.51	103.39	0.11	103.63	0.85
360	105.32	0.65	102.96	1.40	105.73	0.83	102.80	0.12	102.01	0.03	102.45	0.08	108.57	0.14	104.10	0.71	103.98	0.35
380	106.28	0.96	102.98	0.02	105.84	0.11	102.84	0.04	102.14	0.13	102.46	0.01	108.71	0.14	104.47	0.37	104.34	0.36
400	106.79	0.51	103.03	0.05	106.48	0.64	102.91	0.07	102.27	0.13	102.49	0.03	108.85	0.14	104.81	0.34	104.69	0.35
420	107.05	0.26	103.69	0.66	107.11	0.63	103.00	0.09	102.33	0.06	102.51	0.02	108.97	0.12	105.16	0.35	105.00	0.31
440	107.62	0.57	103.77	0.08	107.18	0.07	103.15	0.15	102.48	0.15	102.55	0.04	108.98	0.01	105.44	0.28	105.20	0.20
460	107.89	0.27	104.05	0.28	107.24	0.06	103.29	0.14	102.58	0.10	102.65	0.10	109.05	0.07	105.77	0.33	105.30	0.10
480	108.21	0.32	104.68	0.63	107.60	0.36	103.32	0.03	102.67	0.09	102.87	0.22	110.49	1.44	106.08	0.31	106.00	0.70
500	107.24	-0.97	104.04	-0.64	108.02	0.42	102.73	-0.59	102.90	0.23	102.44	-0.43	109.72	-0.77	106.01	-0.07	106.36	0.36
520	107.37	0.13	104.37	0.33	108.38	0.36	102.74	0.01	102.83	-0.07	102.55	0.11	110.36	0.64	106.05	0.04	106.24	-0.12
540	106.78	-0.59	104.78	0.41	108.16	-0.22	101.65	-1.09	103.76	0.93	101.29	-1.26	110.70	0.34	106.47	0.42	106.13	-0.11
560	105.12	-1.66	105.12	0.34	108.44	0.28	101.60	-0.05	103.21	-0.55	101.28	-0.01	111.13	0.43	106.88	0.41	106.02	-0.11
580	105.49	0.37	105.49	0.37	108.19	-0.25	101.66	0.06	103.40	0.19	101.99	0.71	110.71	-0.42	107.75	0.87	106.22	0.20
600	105.82	0.33	105.82	0.33	108.82	0.63	101.77	0.11	103.54	0.14	101.05	-0.94	110.30	-0.41	108.30	0.55	106.64	0.42
620	106.11	0.29	106.11	0.29	107.41	-1.41	101.98	0.21	102.98	-0.56	100.89	-0.16	108.93	-1.37	108.93	0.63	107.23	0.59
640	106.44	0.33	106.44	0.33	106.44	-0.97	101.05	-0.93	101.05	-1.93	100.05	-0.84	109.48	0.55	109.48	0.55	107.94	0.71
660	106.68	0.24	106.68	0.24	106.68	0.24	101.12	0.07	100.12	-0.93	100.04	-0.01	110.06	0.58	110.06	0.58	108.38	0.44
680	106.89	0.21	106.89	0.21	106.89	0.21	101.24	0.12	101.24	1.12	100.96	0.92	110.48	0.42	110.48	0.42	108.77	0.39
700	107.08	0.19	107.08	0.19	107.08	0.19	102.28	1.04	102.28	1.04	100.82	-0.14	110.54	0.06	110.54	0.06	109.08	0.31
720	107.15	0.07	107.15	0.07	107.15	0.07	102.84	0.56	102.84	0.56	100.65	-0.17	110.75	0.21	110.75	0.21	109.13	0.05
740	107.03	-0.12	107.03	-0.12	106.03	-1.12	104.36	1.52	104.36	1.52	100.79	0.14	110.71	-0.04	110.71	-0.04	109.03	-0.10
760	106.90	-0.13	106.90	-0.13	106.90	0.87	105.44	1.08	105.44	1.08	100.77	-0.02	110.65	-0.06	110.65	-0.06	109.28	0.25
780	106.69	-0.21	106.69	-0.21	106.69	-0.21	104.37	-1.07	105.37	-0.07	100.77	0.00	110.69	0.04	110.69	0.04	109.49	0.21
800	106.37	-0.32	106.37	-0.32	106.37	-0.32	103.41	-0.96	105.41	0.04	100.91	0.14	111.02	0.33	111.02	0.33	109.91	0.42
820	106.06	-0.31	106.06	-0.31	106.06	-0.31	103.79	0.38	104.79	-0.62	100.80	-0.11	111.44	0.42	111.44	0.42	110.60	0.69
840	105.82	-0.24	105.82	-0.24	105.82	-0.24	103.38	-0.41	104.38	-0.41	100.78	-0.02	112.07	0.63	112.07	0.63	110.16	-0.44

APPENDIX F

Table F: Experimental results of microwave heating at for 500 g of distilled water at various MW power level

140 Watt

	Pulse	**Rest time**	**Total heat**
1	3.36	26.7	
2	3.41	26.6	
3	3.35	26.68	
4	3.4	26.54	
	3.38	**26.63**	**30.01**

280 Watt

	Pulse	Rest time	
1	4.7	25.4	
2	4.64	25.37	
3	4.65	25.35	
4	4.57	25.37	
	4.64	**25.37**	**30.01**

420 Watt

	Pulse	Rest Time	
1	11.52	18.56	
2	11.45	18.58	
3	11.46	18.55	
4	11.53	18.5	
	11.49	**18.55**	**30.04**

560 Watt

	Pulse	Rest Time	
1	18.47	11.57	
2	18.48	11.56	
3	18.52	11.58	
4	18.5	11.6	
	18.49	**11.58**	**30.07**

700 Watt
Continuously

APPENDIX G

Different temperature of every 20 seconds before and after modification

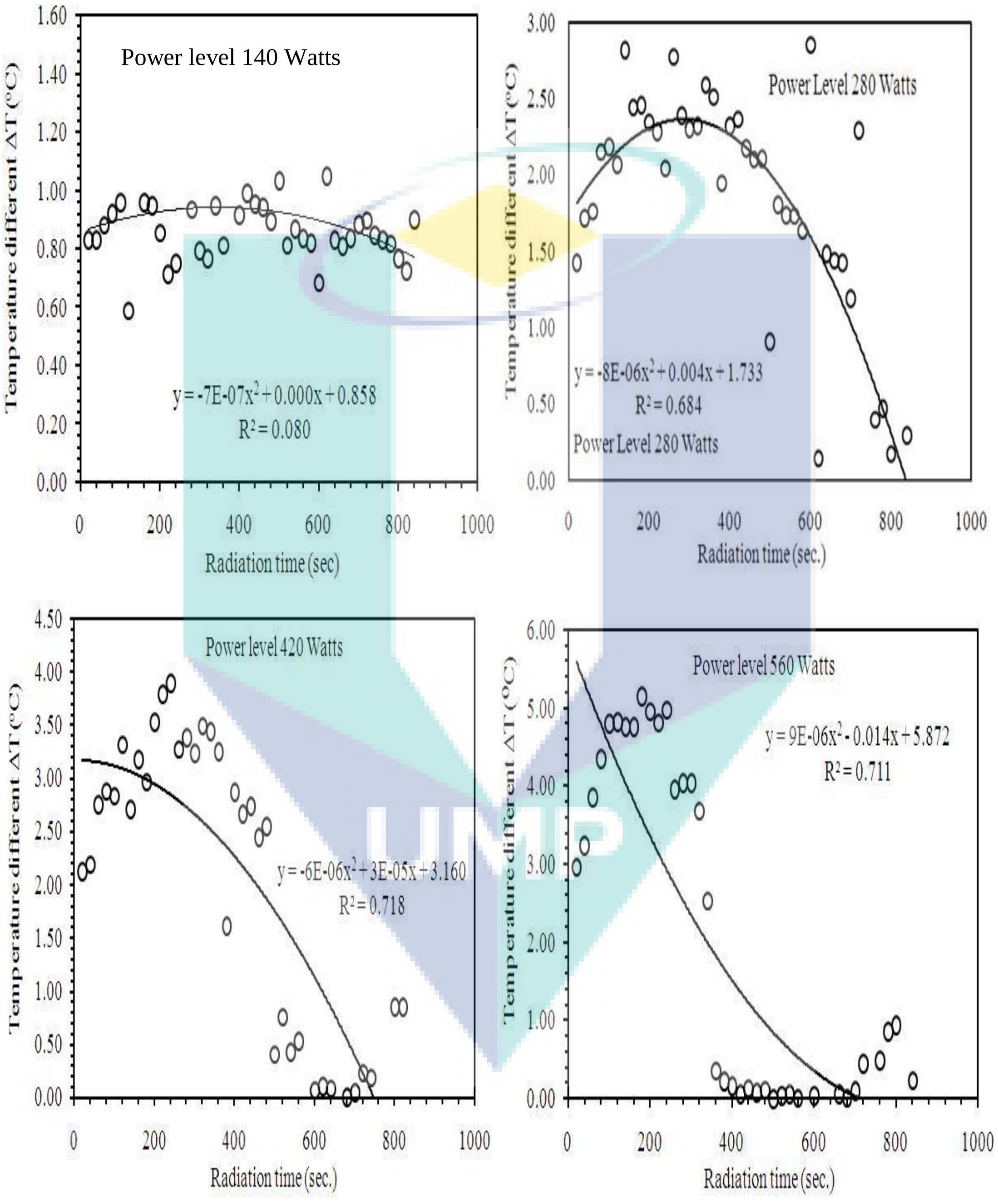


Figure G (a): Without stirring system at 140, 280, 420, 560 and 700 Watts

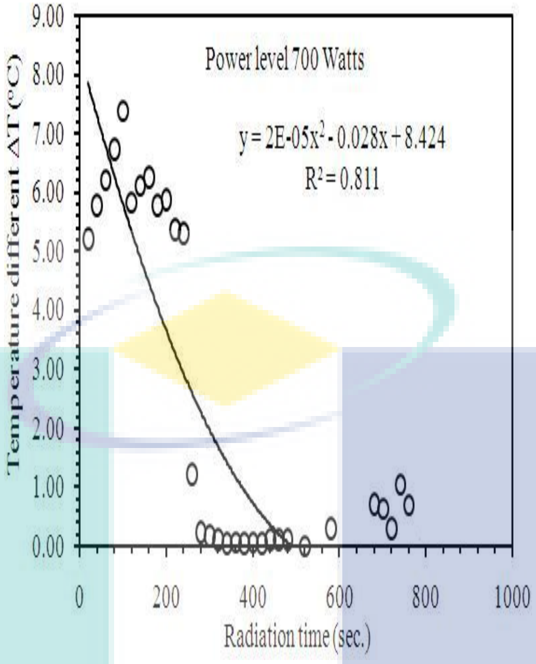


Figure G (a): Continued

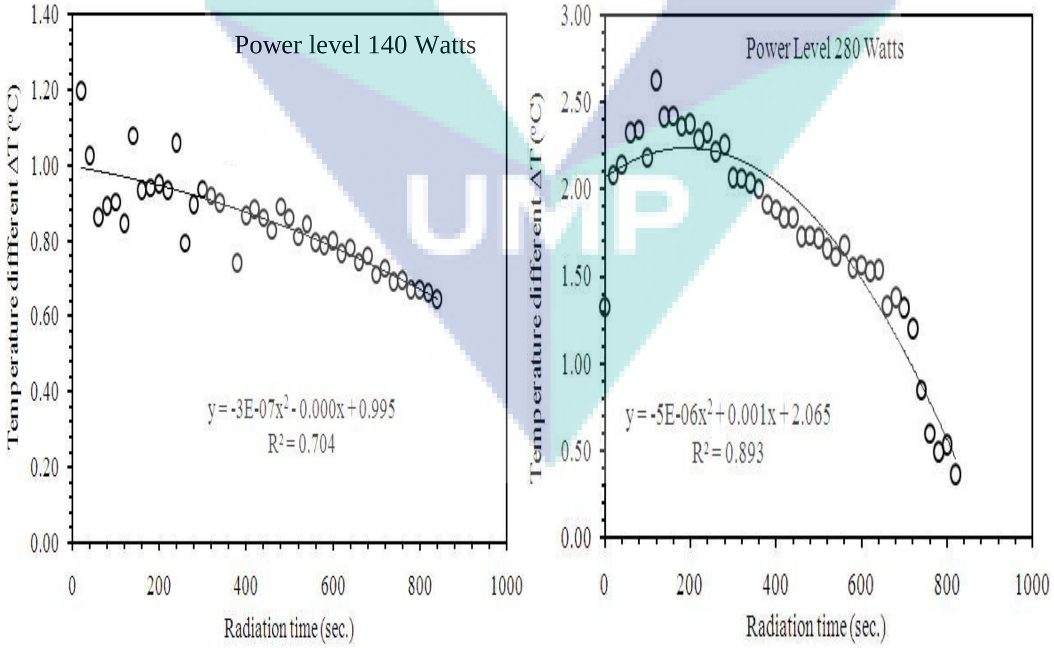


Figure G (b): With stirring system at 140, 280, 420, 560 and 700 Watts

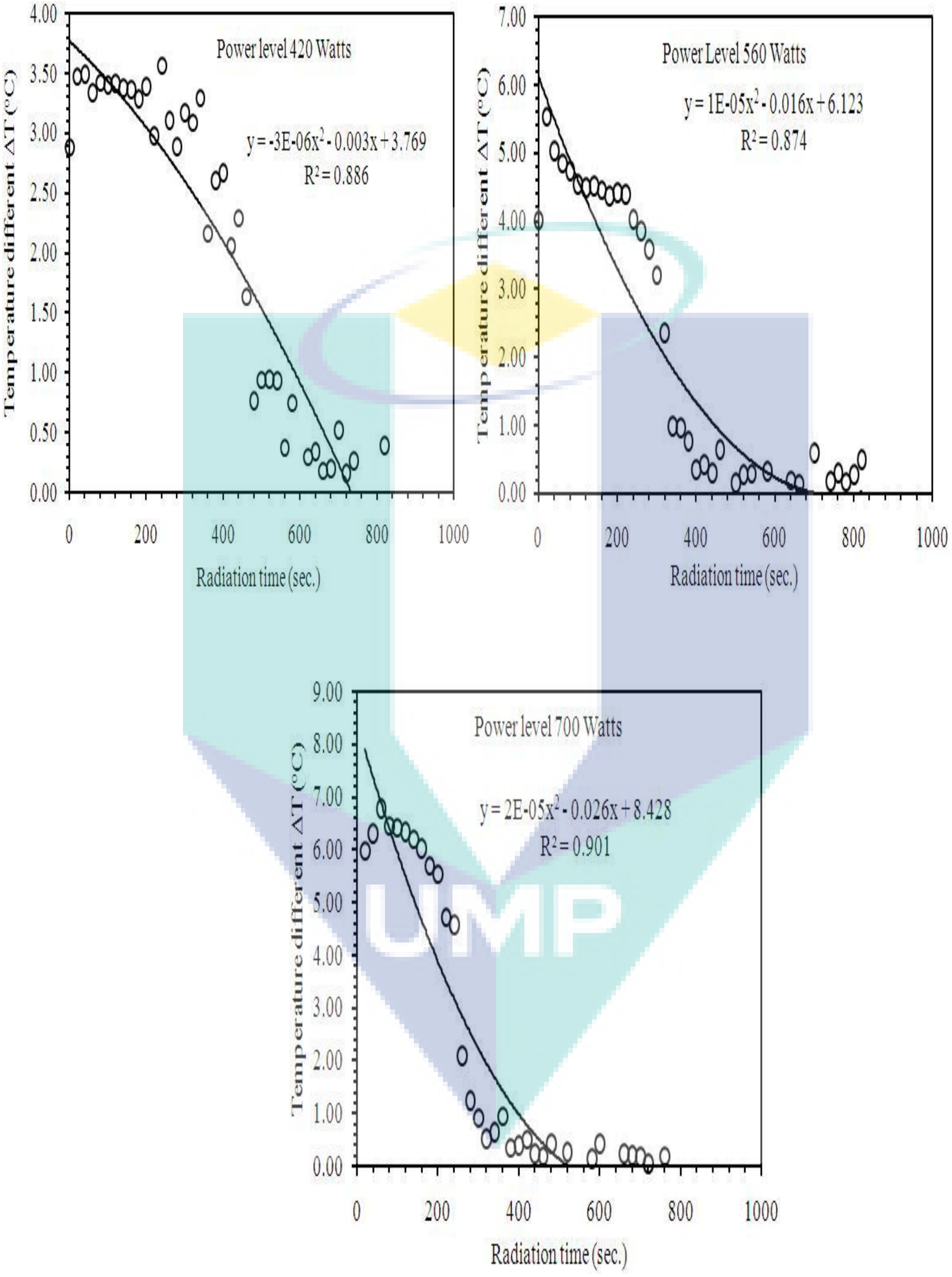


Figure G(b): Continued

APPENDIX H

Sample calculation for rate of temperature increases

a) For sample of 100% water after modification, exposed with 60 seconds of microwave radiation in 420 Watts

$$\frac{dT}{dt_{60}} = \frac{\Delta T}{60} = \frac{8.81}{60} = 0.147^{0}C/s$$

b) For sample 50% water-50% acetone, exposed with 60 secons of microwave radiation in 420 Watts

$$\frac{dT}{dt_{60}} = \frac{\Delta T}{60} = \frac{12.83}{60} = 0.2138^{0}C/s$$

Sample calculation for the volume rate of heat generation

a) For pure water

$$q = \rho C_p\left(\frac{dT}{dt}\right)$$

$C_{p,\ water}$ = 0.9987 *cal/g.°C*

ρ_{water}= 1.0 g/cm^3

$$q_{water,60} = \frac{1.0g}{cm^3}\,x\,\frac{0.9987cal}{g\,^{0}C}\,x\,\frac{0.147^{0}C}{s}\,x\,\frac{1000cm^3}{1L}\,\frac{1L}{1000mL}\,x\,\frac{4.184Joule}{cal} = 0.62Watts/mL$$

b) For 50-50 Water/Ethanol

$C_{p,water} = 0.9987$

$C_{p,acetone} = 0.5181$

$C_{p,acetonitrile} = 0.5372$

$C_{p,ethanol} = 0.5826$

$$C_{p,mix} = C_{p,water}\phi + C_{p,ethanol}(1-\phi)$$

$C_{p,\ 50\text{-}50water/ethanol} = 0.7909\ cal/g.°C$

$\rho_{50\text{-}50water/ethanol} = 0.9371\ \text{g/cm}^3$

$$q_{water/ethanol,60} = \frac{0.9371g}{cm^3}\,x\,\frac{0.7907cal}{g.^0C}\,x\,\frac{0.1792^0C}{s}\,x\,\frac{1000cm^3}{1L}\,x\,\frac{1L}{1000mL}\,x\,\frac{4.184Joule}{cal} = 0.56Watts/mL$$

APPENDIX I

Table I: Physical and dielectric properties of the materials

Material properties	Water	Acetone	Acetonitrile	Ethanol
Purity (%)	100	98.8	99.5	99.9
Molecular weight (g/mol)	18.01	58.08	41.05	46.07
Density (g/cm^3)	0.9987	0.79	0.782-0.783	0.790-0.793
Refractive index (nD)	-	1.35	1.343-1.345	-
Moisture (%)	-	<0.1	<0.1	<0.1
Dielectric constant	78.3	20.7	37.5	24.3
Dipole moment	2.3	2.69	3.12	1.96
Heat capacity (J/m.K)	75.327	124.2	92.36	112.4
Brand	Distilled water	MERCK Technical grade	MERCK Technical grade	MERCK Technical grade

APPENDIX J

The Effect of Various Pulse Output Power Level on Volume Rate of Heat Generation for Various Solvent

Pulse Microwave	**Time (sec.)**	**Water**		**Acetone**		**Acetonitrile**		**Ethanol**	
		$\Delta dT/dt$ **(^{0}C.s^{-1})**	**q_{mw}** **(*Watts. mL^{-1}*)**	**$\Delta dT/dt$** **(^{0}C.s^{-1})**	**q_{mw}** **(*Watts. mL^{-1}*)**	**$\Delta dT/dt$** **(^{0}C.s^{-1})**	**q_{mw}** **(*Watts. mL^{-1}*)**	**$\Delta dT/dt$** **(^{0}C.s^{-1})**	**q_{mw}** **(*Watts .mL^{-1}*)**
Low 20% 140 Watts	60	0.056	0.233	0.281	0.480	0.256	0.451	0.205	0.396
	120	0.049	0.205	0.265	0.454	0.300	0.528	0.265	0.513
	180	0.051	0.211	0.205	0.351	0.210	0.369	0.208	0.402
	240	0.049	0.206	0.151	0.259	0.148	0.261	0.151	0.292
	300	0.049	0.204	0.118	0.203	0.114	0.201	0.118	0.228
	360	0.048	0.202	0.098	0.169	0.096	0.169	0.097	0.187
	420	0.047	0.197	0.085	0.145	0.082	0.144	0.082	0.159
	480	0.047	0.194	0.075	0.128	0.070	0.124	0.072	0.140
	540	0.046	0.193	0.066	0.114	0.062	0.108	0.063	0.122
	600	0.045	0.189	0.059	0.101	0.056	0.098	0.056	0.109
Medium low 40% 280 Watts	60	0.109	0.456	0.301	0.515	0.276	0.485	0.209	0.405
	120	0.103	0.430	0.275	0.472	0.310	0.545	0.268	0.517
	180	0.109	0.454	0.212	0.363	0.216	0.381	0.210	0.405
	240	0.111	0.462	0.157	0.268	0.153	0.269	0.152	0.294
	300	0.111	0.464	0.123	0.210	0.118	0.207	0.119	0.230
	360	0.110	0.458	0.102	0.175	0.099	0.175	0.099	0.192
	420	0.108	0.450	0.088	0.150	0.085	0.149	0.085	0.164
	480	0.105	0.441	0.076	0.131	0.074	0.130	0.073	0.142
	540	0.103	0.430	0.066	0.113	0.065	0.114	0.065	0.126
	600	0.101	0.420	0.061	0.104	0.057	0.100	0.059	0.114

APPENDIX K

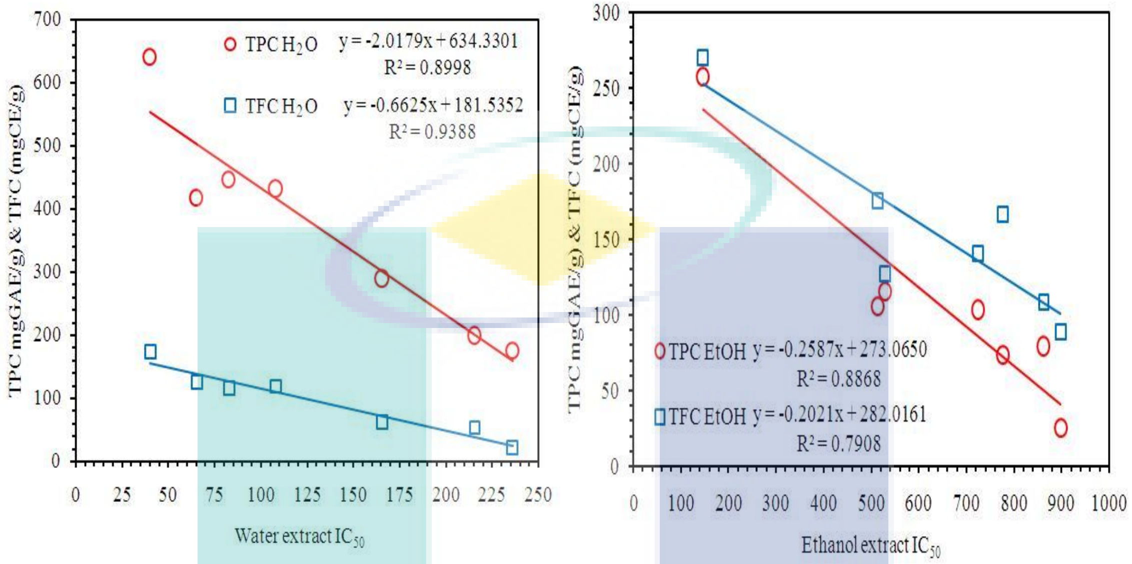


Figure K(a): Relationship between antioxidant capacity (IC_{50}) with total flavonoid (mg CE/g) and total phenolics (mg GAE/g) of water and ethanol extract epiphyte's fern

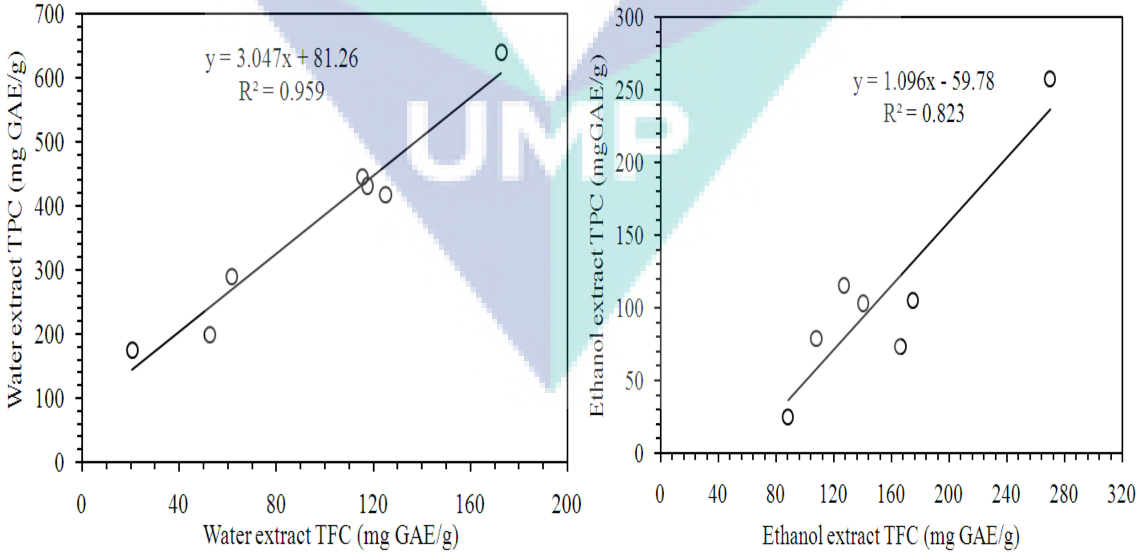


Figure K(b): Relationship between total phenolics (mg GAE/g)with total flavonoid (mgCE/g) of water and ethanolextract epiphyte's fern

APPENDIX L

Assessment of Bioactivity of MPOTEF Plants using Brine Shrimp (Artemia salina) Lethality Assay

Consequently the water and EtOH MPOTEF extracts deliberate in this work showed significant lethality against brine shrimp, which has been successfully used as a simple biological test to guide the fractionation process of plant extracts in order to detect antitumour compounds. A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT).

The lethality of the aqueous and ethanol extracts of epiphytes fern to brine shrimp was determined on brine shrimp of *Artemia salina* after 48 hours of exposure to the test solutions and the the screening results revealed a positive control. The LC_{50} results of the seven plant species evaluated in this screening are summarised in Table J. The table was observed that the brine shrimp lethality bioassay, all the extracts produced concentration dependent increment in percent mortality of brine shrimp nauplii indicating the possible presence of cytotoxic principles in these extracts. All of epiphytes fern ethanol extract tested showed medium result of brine shrimp larvicidal activity according to Meyer et al. (1982), who classified crude extracts and pure substances into toxic (LC_{50} value < 1000 μg/mL) but not for water extract which showing poor activity or non-toxic because all the extract having a value of LC_{50} value more than 1000 μg/mL except for SPF. It is believed that plant extracts with low LC_{50} valuespossibly will have metabolites with cytotoxic, antifungal, insecticidal or pesticide activities which contribute to the toxicity of the plants.

The LC_{50} values of ethanol extract (Figure 7.6) for NBF, DDF, ALF, GPF, SPF, VLF, VSF were 340 µg/mL, 130 µg/mL, 220 µg/mL, 430 µg/mL, 440 µg/mL, 400 µg/mL and 430 µg/mL respectively. However, as compared to other medical plant in literature, this amount was considered medium value. Regarding to this result, medium or poor result of IC_{50} of both extract might be because of the factors like temperature variations during the process which force denatured some of the active compound in the plant and another reason these plants may be active only when fresh.

Although some of these plants had similar secondary metabolites, their toxicity against the brine shrimp larvae was seen to be varying. According to Olwa Odyek (Pers. Com), the brine shrimp test is not enough to disqualify the medicinal values of these plants variation. Quantitative analysis only gives a rough guide about what the extracts contain. He elaborated that some of the compounds are in such tiny amount that cannot be detected by this method. This statement gave a good explanation behind a bad result of toxicity in water extract. In this research, water extract showed LC_{50} value >1000 µg/mL for all species except SPF 825 µg/mL.

Table L: Brine shrimp cytotoxicity of water and ethanolic extract of MPOTEF

Epiphytes ferns	**IC_{50} Water µg/mL**	**IC_{50} EtOH µg/mL**
NBF	> 1000	340
DDF	> 1000	130
ALF	> 1000	220
SPF	825	430
GPF	> 1000	440
VLF	> 1000	400
VSF	> 1000	430

APPENDIX M

Assessment of Elements in Seven MPOTEF Species

Traditional medicine is an integral part of Malaysian culture and has been practiced by various cultural groups long before and the introduction are widely consumed as home remedies. Thus the knowledge of the elemental contents during the extraction of target compound in Malaysian medicinal plants is very important because some of these elements are closely related to human health. Since most of the selected abundant source of MPOTEF plants in this study are non-edible except SPF and NBF and such kind of ferns usually have higher amount of metallic and non-metallic elements. However, the human body needs both metallic and non-metallic elements within positive permitted amounts for growth and good health. Therefore, determination of element compositions in MPOTEF is essential for understanding their nutritive importance and safety permissibility limit. Thus it is very necessary to investigate the macronutrient elements, micronutrients elements and toxic elements of each MPOTEF plants before the potential of the TCMA water and EtOH extraction of TPC, TFC, and antioxidant capacities of each species has been explored. In this present work, the concentrations of macronutrient elements (Na, Mg, K, Ca), micronutrients elements (Co, Al, Cr, Mn, Fe, Ni, Cu, Zn) and toxic elements (As, Cd, Pb, Hg) were determined in seven MPOTEF using ICP-MS method.

i. Macronutrients elements

Macronutrients are a number of minerals needed by the body in large amounts. Figure M(a) show that all seven MPOTEF products contained high concentrations of macronutrients except sodium. Commonly sodium was found from soil, maturation, environment and weather condition. Therefore, the low amount of sodium is because of epiphytes fern not contacted directly to soil and the MPOTEF raw material was picking from palm oil plantation which situated in rural area. MPOTEF showing a high concentrations of Mg were in the range of 160-949.93 ppm, with the highest level values of Mg is belongs to NBF. According to literature, that 15-30% of the total magnesium in plants is associated with chlorophyll molecule. From our observation, compared to another 6 species of MPOTEF, NBF was commonly found on the top part of palm oil tree where easily reach to capture the sunlight for photosynthesis process. Figure M(a) also showed that the potassium (K) content to range from 1871-5019ppm. Plants were absorbed potassium elements from soil for growth and development. The highest value of VEL in potassium concentration (5019ppm) maybe influenced by their growth part is at the bottom of palm oil tree which more close to the soil. DDF was found to have the highest amount o calcium (823.01ppm). Palm oil absorbed calcium from soil to stimulate root and leaf development. Once DDF having a stout and long creeping rhizome compared to the other species, maybe this is a reason the rhizome of DDF penetrate deeply on the palm oil trunk and engrossed the calcium from the host. The present results revealed that these the MPOTEF plants contained large amounts of these three elements (Mg, K and Ca) essential macronutrients. The plenty of Mg, K and Ca in each MPOTEF species in the present study was also in agreement with the previous studies, which indicated that these elements were the most abundant elements in many medicinal plants either edible or non-edible.

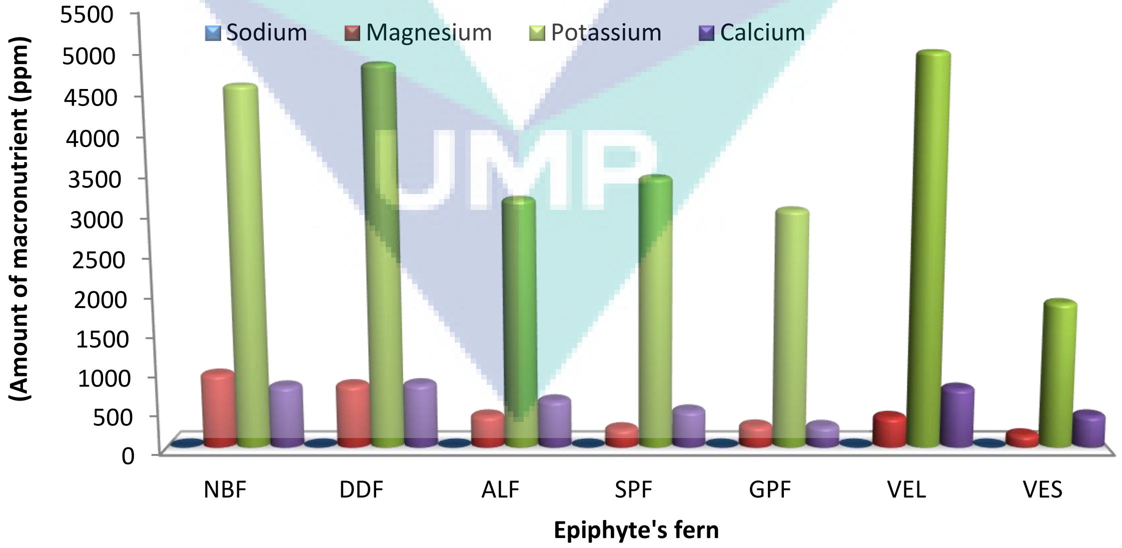


Figure M(a):Concentrations of essential macronutrients elements in seven species of MPOTEF

ii. *Micronutrients elements*

The essential micronutrients are a number of minerals needed by body in small amounts, but could not be produced by human body. Figure M(b) revealed the concentrations of essential micronutrients elements (Al, Cr, Mn, Fe, Cu, Zn) in MPOTEF. Al shows a concentration varied between 22.75 in VLF and 51.56 ppm in SPF. The concentrations of Fe show the lowest value in all MPOTEF plants and varied over wide ranges of 0.09-0.27 ppm. The concentration of Zn varied from 31.4 to 226.97 ppm, respectively and the maximum amount was observed in VSF (226.97 ppm) and lowest in SPF (31.4 ppm). The concentration of Cu was in the range of 9.06-49.53 ppm, with the highest level in DDF. These results were in the same range as those of other plants from the previous studies. For other essential micronutrients element like Co, and Ni, the concentrations of Ni element are varied over a very narrow range among the seven MPOTEF species. The maximum concentration of Ni was observed in VSF (4.5 ppm) and the concentration was observed in VLF (0.89 ppm). However the contents of Co revealed less than 0.01 ppm in all MPOTEF plants. Other elements like Cr, is implicated in sugar metabolism in human to help keep blood sugar levels stable but it was become hazardous to humans, depending on their oxidation states and present at high concentrations.

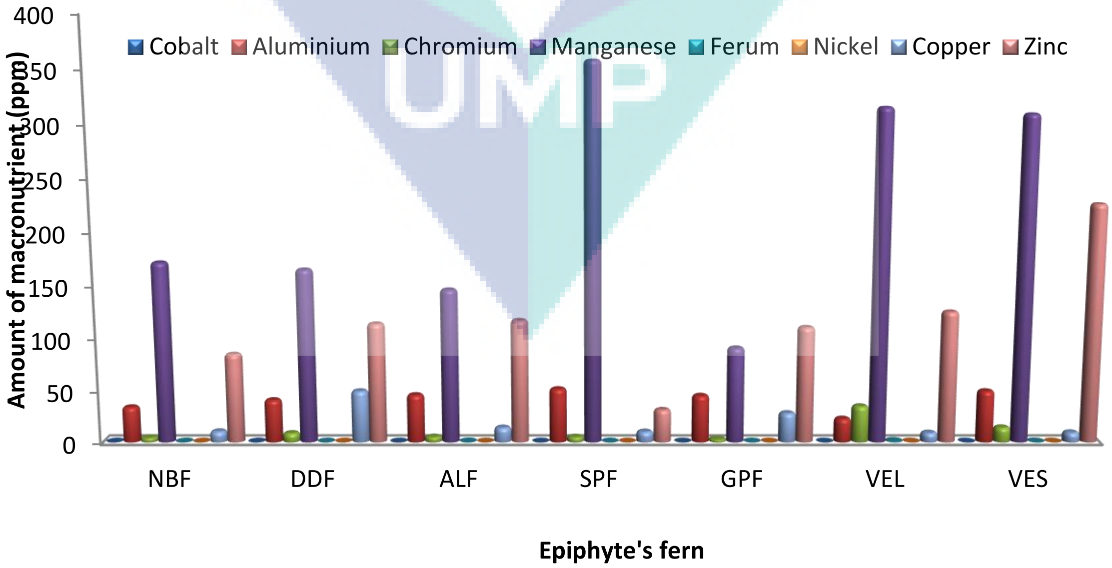
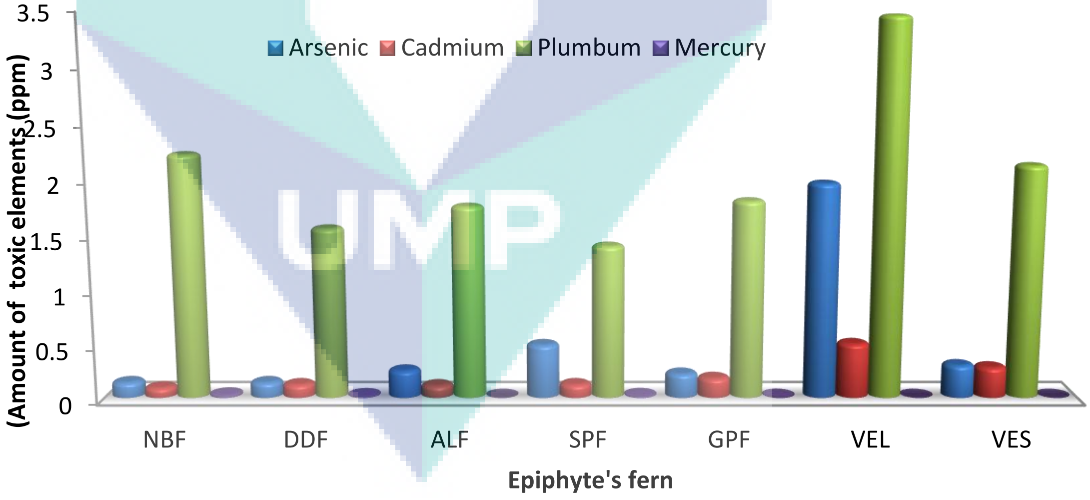


Figure M(b): Concentrations of essential micronutrients elements in seven species of MPOTEF

iii. Toxic elements

Arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) is commonly found in herbal plant. As, Cd, Hg, and Pb, respectively has known as nonessential elements and it was become toxic if consumed exceeded the recommended limit but cadmium and lead are toxic for human biosystem even in low doses. These minerals are detected in plants along with using of inorganic fertilizer and pesticide also because of increasing industrialization and biosphere pollution as well. Figure M(c) demonstrated edible epiphytes fern; NBF and SPF contains As, Cd, Pb and Hg at 0.14, 0.09, 2.23, 0.023 ppm and 0.49, 0.12, 1.40, 0.00148 ppm respectively and its shows both of them is safe in food and pharmaceutical industry application. However the other four of non edible MPOTEF demonstrated low concentration of toxic elements according to the demand of the standard maximum value for medicinal plants except VLF. The high amount arsenic and cadmium in VLF (1.97 and 0.50ppm) maybe influence by pesticide and fertilizerused in palm oil plantation because this plant grows at the lower part of palm oil tree compare to the other.



FigureM(c): Concentrations of toxic elements in seven species of MPOTEF

APPENDIX N

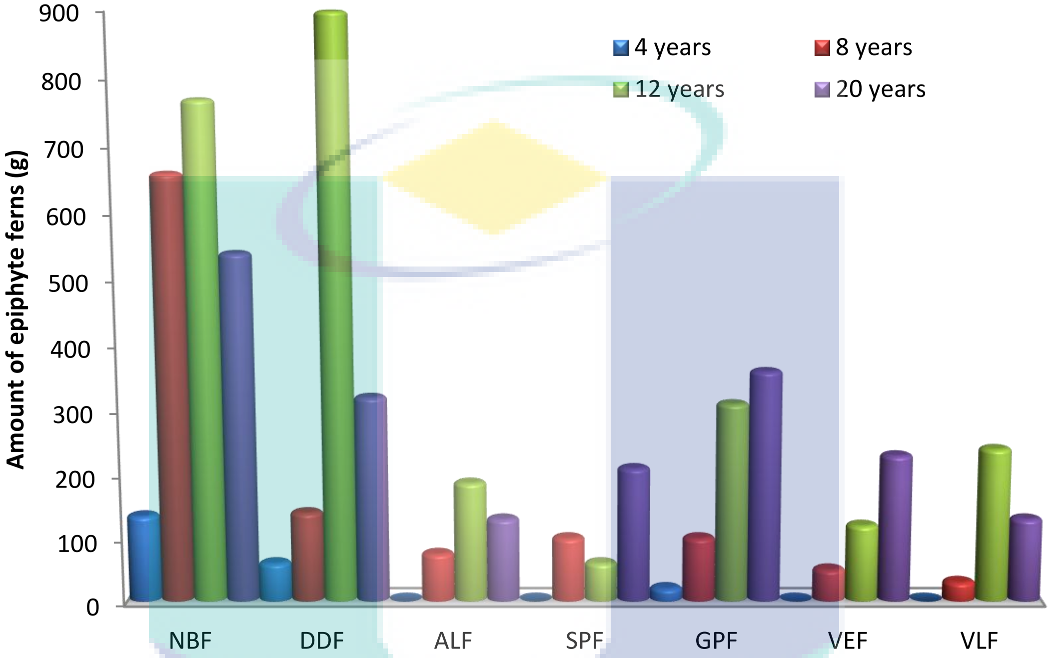


Figure N: Average amount of epiphytes fern on each palm oil tree

Epiphytes ferns were collected from four palm oil plantation with different age (4, 8, 12 and 20 years) of tree which located at Seri Medan, Batu Pahat, Johor where each location is in 30km radius. The average amount of each species in each palm oil tree was determined and the data was depicted in Figure N. Seven species was observed are: *Davallia denticulate* (Burm.) Mett. (DDF), *Nephrolepis biserrata* (Sw.) Schott. (NBF), *Asplenium longissimum B1*(ALF),*Gonioplebium percussum* (Cav.) Wagner & Grether (GPF), *Stenochlaena palustris* (Burm.f.) Bedd (SPF), *Vittaria Elongata* (VLF), *Vittaria ensiformis* (VSF). The result shows NBF was found abundantly in each area as compared to other species.

APPENDIX O

RAW DATA FOR PHSICOCHEMICAL PROPERTIES OF MIXTURE DESIGN

No	**Water (%)**	**Acetone (%)**	**Acetonitrile (%)**	**Ethanol (%)**	**Reading**	**pH**	**Brix (%)**	**TDS**	**Refractive index (nD)**	**Weight of sample (g)**	**Density (g/cm3)**	**Effluent velocity (sec.)**	**Kinematic viscosity (cps)**	**Dynamic Viscosity (cps)**	**Surface tension**
1 **Before**	**100**	**0**	**0**	**0**	1	5.97	0.20	3.65	1.3332	20.2845	1.0050	114.55	0.8922	0.8967	49.096
					2	5.96	0.10	7.30	1.3332	20.2843	1.0050	114.55	0.8922	0.8967	49.262
					3	5.95	0.10	7.30	1.3332	20.284	1.0050	114.80	0.8942	0.8986	47.845
					Mean	**5.96**	**0.13**	**6.08**	**1.3332**	**20.28**	**1.0050**	**114.63**	**0.8929**	**0.8973**	**48.734**
					Std	**0.01**	**0.06**	**2.11**	**0.0000**	**0.00**	**0.0000**	**0.15**	**0.0011**	**0.0011**	**0.775**
1 **After**	**100**	**0**	**0**	**0**	1	5.50	0.30	2.43	1.3334	20.2849	1.0051	114.83	0.8944	0.8990	48.595
					2	5.50	0.30	2.43	1.3334	20.2845	1.0050	114.86	0.8946	0.8992	47.096
					3	5.50	0.30	2.43	1.3334	20.2847	1.0051	114.85	0.8946	0.8991	46.514
					Mean	**5.50**	**0.30**	**2.43**	**1.3334**	**20.28**	**1.0051**	**114.85**	**0.8945**	**0.8991**	**47.402**
					Std	**0.00**	**0.00**	**0.00**	**0.0000**	**0.00**	**0.0000**	**0.02**	**0.0001**	**0.0001**	**1.074**
2 **Before**	**0**	**100**	**0**	**0**	1	6.24	16.40	0.04	1.3574	18.4624	0.7949	52.53	0.4092	0.3252	22.800
					2	6.09	16.80	0.04	1.3577	18.4620	0.7948	52.37	0.4079	0.3242	22.723
					3	6.22	16.90	0.04	1.3579	18.4616	0.7948	52.43	0.4084	0.3246	29.380
					Mean	**6.18**	**16.70**	**0.04**	**1.3577**	**18.46**	**0.7948**	**52.44**	**0.4085**	**0.3247**	**24.968**
					Std	**0.08**	**0.26**	**0.00**	**0.0003**	**0.00**	**0.0000**	**0.08**	**0.0006**	**0.0005**	**3.821**
2 **After**	**0**	**100**	**0**	**0**	1	6.41	16.70	0.04	1.3579	18.4763	0.7965	52.03	0.4053	0.3228	21.564
					2	6.44	17.00	0.04	1.3583	18.4761	0.7965	52.24	0.4069	0.3241	21.257
					3	6.45	17.00	0.04	1.3585	18.4757	0.7964	52.12	0.4060	0.3233	21.257
					Mean	**6.43**	**16.90**	**0.04**	**1.3582**	**18.48**	**0.7965**	**52.13**	**0.4060**	**0.3234**	**21.359**
					Std	**0.02**	**0.17**	**0.00**	**0.0003**	**0.00**	**0.0000**	**0.11**	**0.0008**	**0.0007**	**0.177**
3 **Before**	**0**	**0**	**100**	**0**	1	4.88	6.50	0.11	1.3423	18.372	0.7845	56.38	0.4391	0.3445	27.170
					2	4.90	6.60	0.11	1.3425	18.3719	0.7844	56.43	0.4395	0.3448	27.484
					3	4.96	6.70	0.11	1.3425	18.3718	0.7844	56.49	0.4400	0.3451	27.642
					Mean	**4.91**	**6.60**	**0.11**	**1.3424**	**18.37**	**0.7844**	**56.43**	**0.4396**	**0.3448**	**27.432**
					Std	**0.04**	**0.10**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.06**	**0.0004**	**0.0003**	**0.240**
3 **After**	**0**	**0**	**100**	**0**	1	5.06	6.50	0.11	1.3427	18.3615	0.7832	56.41	0.4394	0.3441	27.878
					2	4.98	6.60	0.11	1.3428	18.3614	0.7832	56.46	0.4398	0.3444	27.642
					3	5.10	6.60	0.11	1.3429	18.361	0.7832	56.39	0.4392	0.3440	27.721
					Mean	**5.05**	**6.57**	**0.11**	**1.3428**	**18.36**	**0.7832**	**56.42**	**0.4395**	**0.3442**	**27.747**
					Std	**0.06**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.04**	**0.0003**	**0.0002**	**0.120**
4 **Before**	**0**	**0**	**0**	**100**	1	5.68	18.00	0.04	1.3607	18.4593	0.7945	176.73	1.3765	1.0937	21.410
					2	5.65	18.10	0.04	1.3608	18.4590	0.7945	176.82	1.3773	1.0942	21.564
					3	5.61	18.10	0.04	1.3608	18.458	0.7944	176.79	1.3770	1.0939	20.718
					Mean	**5.65**	**18.07**	**0.04**	**1.3608**	**18.46**	**0.7945**	**176.78**	**1.3769**	**1.0939**	**21.231**
					Std	**0.04**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0001**	**0.05**	**0.0004**	**0.0003**	**0.451**
4	**0**	**0**	**0**	**100**	1	5.20	18.30	0.04	1.3608	18.474	0.7962	181.20	1.4114	1.1238	21.719
					2	5.26	18.40	0.04	1.3609	18.4740	0.7962	180.89	1.4090	1.1218	20.949
					3	5.28	18.40	0.04	1.3610	18.473	0.7961	180.93	1.4093	1.1219	21.257

After					Mean	**5.25**	**18.37**	**0.04**	**1.3609**	**18.47**	**0.7962**	**181.01**	**1.4099**	**1.1225**	**21.308**
					Std	**0.04**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0001**	**0.17**	**0.0013**	**0.0011**	**0.388**
5 **Before**	**50**	**50**	**0**	**0**	1	5.83	17.50	0.04	1.3596	19.8405	0.9538	179.35	1.3970	1.3325	25.993
					2	5.82	16.90	0.04	1.3596	19.8205	0.9515	179.56	1.3986	1.3308	26.934
					3	5.81	16.70	0.04	1.3596	19.8204	0.9515	179.25	1.3962	1.3285	27.327
					Mean	**5.82**	**17.03**	**0.04**	**1.3596**	**19.83**	**0.9523**	**179.39**	**1.3972**	**1.3306**	**26.751**
					Std	**0.01**	**0.42**	**0.00**	**0.0000**	**0.01**	**0.0013**	**0.16**	**0.0012**	**0.0020**	**0.686**
5 **After**	**50**	**50**	**0**	**0**	1	5.74	16.90	0.04	1.3586	19.8134	0.9507	180.72	1.4076	1.3382	26.071
					2	5.75	16.90	0.04	1.3587	19.8133	0.9507	180.58	1.4065	1.3372	29.459
					3	5.75	16.70	0.04	1.3584	19.8132	0.9507	180.67	1.4072	1.3378	26.384
					Mean	**5.75**	**16.83**	**0.04**	**1.3586**	**19.81**	**0.9507**	**180.66**	**1.4071**	**1.3378**	**27.305**
					Std	**0.01**	**0.12**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.07**	**0.0006**	**0.0005**	**1.872**
6 **Before**	**50**	**0**	**50**	**0**	1	5.84	8.50	0.09	1.3457	19.5292	0.9179	112.79	0.8785	0.8064	33.125
					2	5.84	8.60	0.08	1.3456	19.5293	0.9179	112.74	0.8781	0.8061	33.285
					3	5.84	8.60	0.08	1.3457	19.529	0.9179	112.60	0.8770	0.8050	32.664
					Mean	**5.84**	**8.57**	**0.09**	**1.3457**	**19.53**	**0.9179**	**112.71**	**0.8779**	**0.8058**	**33.025**
					Std	**0.00**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.10**	**0.0008**	**0.0007**	**0.322**
6 **After**	**50**	**0**	**50**	**0**	1	5.62	8.50	0.09	1.3455	19.6144	0.9278	115.16	0.8970	0.8322	27.721
					2	5.62	8.50	0.09	1.3456	19.6142	0.9277	115.23	0.8975	0.8327	27.327
					3	5.62	8.50	0.09	1.3456	19.6141	0.9277	115.30	0.8981	0.8332	26.620
					Mean	**5.62**	**8.50**	**0.09**	**1.3456**	**19.61**	**0.9277**	**115.23**	**0.8975**	**0.8327**	**27.223**
					Std	**0.00**	**0.00**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.07**	**0.0005**	**0.0005**	**0.558**
7 **Before**	**50**	**0**	**0**	**50**	1	5.84	16.80	0.04	1.3586	19.6998	0.9376	312.21	2.4318	2.2801	28.431
					2	5.85	16.80	0.04	1.3584	19.6997	0.9376	311.98	2.4300	2.2784	29.063
					3	5.85	16.80	0.04	1.3584	19.6998	0.9376	312.12	2.4311	2.2794	28.668
					Mean	**5.85**	**16.80**	**0.04**	**1.3585**	**19.70**	**0.9376**	**312.10**	**2.4310**	**2.2793**	**28.721**
					Std	**0.01**	**0.00**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.12**	**0.0009**	**0.0009**	**0.319**
7 **After**	**50**	**0**	**0**	**50**	1	5.74	16.30	0.04	1.3582	19.6956	0.9371	315.57	2.4580	2.3034	27.091
					2	5.74	16.40	0.04	1.3579	19.6955	0.9371	316.51	2.4653	2.3102	28.115
					3	5.73	16.50	0.04	1.3578	19.6955	0.9371	315.76	2.4595	2.3048	27.878
					Mean	**5.74**	**16.40**	**0.04**	**1.3580**	**19.70**	**0.9371**	**315.95**	**2.4609**	**2.3061**	**27.695**
					Std	**0.01**	**0.10**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.50**	**0.0039**	**0.0036**	**0.536**
8 **Before**	**0**	**50**	**50**	**0**	1	5.37	11.8	0.06	1.3504	18.398	0.7875	54.29	0.4229	0.3330	24.742
					2	5.43	11.8	0.06	1.3506	18.4280	0.7909	54.42	0.4239	0.3352	24.431
					3	5.39	11.8	0.06	1.3506	18.4124	0.7891	54.50	0.4245	0.3350	24.664
					Mean	**5.40**	**11.80**	**0.06**	**1.3505**	**18.41**	**0.7892**	**54.40**	**0.4237**	**0.3344**	**24.612**
					Std	**0.03**	**0.00**	**0.00**	**0.0001**	**0.02**	**0.0017**	**0.11**	**0.0008**	**0.0012**	**0.162**
8 **After**	**0**	**50**	**50**	**0**	1	5.39	12	0.06	1.3507	18.4169	0.7896	54.83	0.4271	0.3372	24.820
					2	5.44	11.8	0.06	1.3508	18.4168	0.7896	54.86	0.4273	0.3374	24.119
					3	5.44	11.9	0.06	1.3508	18.4167	0.7896	54.62	0.4254	0.3359	24.197
					Mean	**5.42**	**11.90**	**0.06**	**1.3508**	**18.42**	**0.7896**	**54.77**	**0.4266**	**0.3369**	**24.379**

					Std	**0.03**	**0.10**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.13**	**0.0010**	**0.0008**	**0.384**
9 **Before**	**0**	**50**	**0**	**50**	1	5.52	17.5	0.04	1.3602	18.4613	0.7948	77.85	0.6064	0.4819	22.259
					2	5.61	17.8	0.04	1.3605	18.4607	0.7947	77.91	0.6068	0.4822	21.410
					3	5.61	17.8	0.04	1.3605	18.4603	0.7946	77.97	0.6073	0.4826	21.564
					Mean	**5.58**	**17.70**	**0.04**	**1.3604**	**18.46**	**0.7947**	**77.91**	**0.6068**	**0.4823**	**21.744**
					Std	**0.05**	**0.17**	**0.00**	**0.0002**	**0.00**	**0.0001**	**0.06**	**0.0005**	**0.0003**	**0.452**
9 **After**	**0**	**50**	**0**	**50**	1	5.53	18.2	0.04	1.3602	18.4403	0.7923	79.65	0.6204	0.4916	22.104
					2	5.57	18.3	0.04	1.3605	18.4400	0.7923	79.73	0.6210	0.4920	20.718
					3	5.60	18.4	0.04	1.3608	18.4398	0.7923	79.55	0.6196	0.4909	21.642
					Mean	**5.57**	**18.30**	**0.04**	**1.3605**	**18.44**	**0.7923**	**79.64**	**0.6203**	**0.4915**	**21.488**
					Std	**0.04**	**0.10**	**0.00**	**0.0003**	**0.00**	**0.0000**	**0.09**	**0.0007**	**0.0006**	**0.706**
10 **Before**	**0**	**0**	**50**	**50**	1	6.25	13.1	0.06	1.3522	18.443	0.7926	75.69	0.5895	0.4673	23.808
					2	6.25	13.1	0.06	1.3525	18.4425	0.7926	75.52	0.5882	0.4662	23.420
					3	6.23	13.1	0.06	1.3526	18.4421	0.7925	75.25	0.5861	0.4645	23.964
					Mean	**6.24**	**13.10**	**0.06**	**1.3524**	**18.44**	**0.7926**	**75.49**	**0.5880**	**0.4660**	**23.731**
					Std	**0.01**	0.00	**0.00**	**0.0002**	**0.00**	**0.0001**	**0.22**	**0.0017**	**0.0014**	**0.280**
10 **After**	**0**	**0**	**50**	**50**	1	5.73	13	0.06	1.3527	18.413	0.7892	74.62	0.5812	0.4587	23.886
					2	5.78	13	0.06	1.3528	18.4126	0.7891	74.66	0.5815	0.4589	24.197
					3	5.81	13.2	0.06	1.3529	18.4123	0.7891	74.85	0.5830	0.4601	24.742
					Mean	**5.77**	**13.07**	**0.06**	**1.3528**	**18.41**	**0.7891**	**74.71**	**0.5819**	**0.4592**	**24.275**
					Std	**0.04**	0.12	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.12**	**0.0010**	**0.0007**	**0.433**
11 **Before**	**62.5**	**12.5**	**12.5**	**12.5**	1	5.96	11.80	0.06	1.3501	19.8548	0.9555	178.17	1.3878	1.3260	35.135
					2	5.95	11.50	0.06	1.3503	19.8545	0.9554	177.93	1.3859	1.3241	34.732
					3	5.94	11.50	0.06	1.3501	19.8541	0.9554	178.18	1.3878	1.3259	34.973
					Mean	**5.95**	**11.60**	**0.06**	**1.3502**	**19.85**	**0.9554**	**178.09**	**1.3872**	**1.3254**	**34.947**
					Std	**0.01**	**0.17**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.14**	**0.0011**	**0.0010**	**0.203**
11 **After**	**62.5**	**12.5**	**12.5**	**12.5**	1	5.76	12.00	0.06	1.3510	19.8776	0.9581	181.05	1.4102	1.3511	30.173
					2	5.76	11.90	0.06	1.3511	19.8774	0.9581	180.95	1.4094	1.3503	30.054
					3	5.76	11.90	0.06	1.3510	19.8773	0.9581	181.10	1.4106	1.3514	29.221
					Mean	**5.76**	**11.93**	**0.06**	**1.3510**	**19.88**	**0.9581**	**181.03**	**1.4101**	**1.3510**	**29.816**
					Std	0.00	**0.06**	**0.00**	0.0001	**0.00**	**0.0000**	**0.08**	**0.0006**	**0.0006**	**0.519**
12 **Before**	**12.5**	**62.5**	**12.5**	**12.5**	1	6.14	18.20	0.04	1.3603	18.8361	0.8380	90.93	0.7083	0.5935	24.742
					2	6.17	18.20	0.04	1.3608	18.8357	0.8379	90.73	0.7067	0.5922	25.054
					3	6.18	18.30	0.04	1.3608	18.8347	0.8378	90.81	0.7073	0.5926	26.071
					Mean	**6.16**	**18.23**	**0.04**	**1.3606**	**18.84**	**0.8379**	**90.82**	**0.7074**	**0.5928**	**25.289**
					Std	**0.02**	**0.06**	**0.00**	**0.0003**	**0.00**	**0.0001**	**0.10**	**0.0008**	**0.0007**	**0.695**
12 **After**	**12.5**	**62.5**	**12.5**	**12.5**	1	5.94	18.20	0.04	1.3614	18.8413	0.8386	91.29	0.7111	0.5963	25.601
					2	5.95	18.40	0.04	1.3613	18.8412	0.8386	91.35	0.7115	0.5967	25.289
					3	5.95	18.50	0.04	1.3616	18.841	0.8385	91.30	0.7111	0.5963	25.993
					Mean	**5.95**	**18.37**	**0.04**	**1.3614**	**18.84**	**0.8386**	**91.31**	**0.7112**	**0.5964**	**25.628**
					Std	**0.01**	**0.15**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.03**	**0.0003**	**0.0002**	**0.353**

13 Before	**12.5**	**12.5**	**62.5**	**12.5**	1	5.71	11.70	0.06	1.3511	18.7133	0.8238	76.76	0.5979	0.4925	28.510
					2	5.72	12.10	0.06	1.3512	18.7130	0.8238	76.67	0.5972	0.4919	28.431
					3	5.73	12.10	0.06	1.3513	18.7127	0.8237	76.70	0.5974	0.4921	28.273
					Mean	**5.72**	**11.97**	**0.06**	**1.3512**	**18.71**	**0.8238**	**76.71**	**0.5975**	**0.4922**	**28.405**
					Std	**0.01**	**0.23**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.05**	**0.0004**	**0.0003**	**0.121**
13 After	**12.5**	**12.5**	**62.5**	**12.5**	1	5.71	11.70	0.06	1.3511	18.7133	0.8238	76.76	0.5979	0.4925	28.510
					2	5.72	12.10	0.06	1.3512	18.7130	0.8238	76.67	0.5972	0.4919	28.431
					3	5.73	12.10	0.06	1.3513	18.7127	0.8237	76.70	0.5974	0.4921	28.273
					Mean	**5.72**	**11.97**	**0.06**	**1.3512**	**18.71**	**0.8238**	**76.71**	**0.5975**	**0.4922**	**28.405**
					Std	**0.01**	**0.23**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.05**	**0.0004**	**0.0003**	**0.121**
14 Before	**12.5**	**12.5**	**12.5**	**62.5**	1	6.23	18.90	0.04	1.3617	18.7988	0.8337	157.86	1.2296	1.0251	24.586
					2	6.26	19.10	0.04	1.3618	18.7988	0.8337	156.95	1.2225	1.0192	24.353
					3	6.26	19.20	0.04	1.3620	18.7986	0.8337	157.21	1.2245	1.0208	24.742
					Mean	**6.25**	**19.07**	**0.04**	**1.3618**	**18.80**	**0.8337**	**157.34**	**1.2255**	**1.0217**	**24.560**
					Std	**0.02**	**0.15**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.47**	**0.0037**	**0.0030**	**0.196**
14 After	**12.5**	**12.5**	**12.5**	**62.5**	1	4.89	19.20	0.04	1.3626	18.8	0.8338	160.40	1.2494	1.0417	24.431
					2	4.84	19.10	0.04	1.3628	18.7998	0.8338	160.36	1.2490	1.0414	24.583
					3	4.92	19.20	0.04	1.3628	18.7996	0.8338	160.37	1.2491	1.0415	24.431
					Mean	**4.88**	**19.17**	**0.04**	**1.3627**	**18.80**	**0.8338**	**160.38**	**1.2492**	**1.0416**	**24.482**
					Std	**0.04**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.02**	**0.0002**	**0.0002**	**0.088**
15 Before	**25**	**25**	**25**	**25**	1	6.25	16.50	0.04	1.3584	19.254	0.8862	150.22	1.1701	1.0369	29.697
					2	6.27	16.60	0.04	1.3585	19.2536	0.8861	150.13	1.1694	1.0362	29.221
					3	6.27	16.70	0.04	1.3584	19.2535	0.8861	150.17	1.1697	1.0365	29.459
					Mean	**6.26**	**16.60**	**0.04**	**1.3584**	**19.25**	**0.8861**	**150.17**	**1.1697**	**1.0365**	**29.459**
					Std	**0.01**	**0.10**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.05**	**0.0004**	**0.0003**	**0.238**
15 After	**25**	**25**	**25**	**25**	1	5.88	16.70	0.04	1.3586	19.2834	0.8896	160.20	1.2478	1.1100	30.014
					2	5.88	16.80	0.04	1.3583	19.2830	0.8895	160.58	1.2508	1.1126	30.094
					3	5.88	16.80	0.04	1.3584	19.2828	0.8895	160.43	1.2496	1.1115	29.538
					Mean	**5.88**	**16.77**	**0.04**	**1.3584**	**19.28**	**0.8895**	**160.40**	**1.2494**	**1.1114**	**29.882**
					Std	**0.00**	**0.06**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.19**	**0.0015**	**0.0013**	**0.301**
16 Before	**100**	**0**	**0**	**0**	1	4.91	0.10	7.30	1.3331	20.2845	1.0050	114.97	0.8955	0.9000	49.096
					2	4.93	0.10	7.30	1.3331	20.2843	1.0050	114.80	0.8942	0.8987	49.262
					3	4.94	0.10	7.30	1.3331	20.284	1.0050	114.85	0.8946	0.8990	47.845
					Mean	**4.93**	**0.10**	**7.30**	**1.3331**	**20.28**	**1.0050**	**114.87**	**0.8947**	**0.8992**	**48.734**
					Std	**0.02**	**0.00**	**0.00**	**0.0000**	**0.00**	**0.0000**	**0.09**	**0.0007**	**0.0007**	**0.775**
16 After	**100**	**0**	**0**	**0**	1	5.47	0.20	3.65	1.3333	20.2849	1.0051	114.83	0.8944	0.8990	48.595
					2	5.47	0.20	3.65	1.3333	20.2845	1.0050	114.82	0.8943	0.8988	47.096
					3	5.47	0.20	3.65	1.3333	20.2843	1.0050	114.86	0.8946	0.8991	46.514
					Mean	**5.47**	**0.20**	**3.65**	**1.3333**	**20.28**	**1.0050**	**114.84**	**0.8945**	**0.8990**	**47.402**
					Std	**0.00**	**0.00**	**0.00**	**0.0000**	**0.00**	**0.0000**	**0.02**	**0.0002**	**0.0001**	**1.074**
					1	5.91	15.90	0.05	1.3576	18.4624	0.7949	52.53	0.4092	0.3252	22.800
					2	5.94	16.00	0.05	1.3584	18.4620	0.7948	52.37	0.4079	0.3242	22.723

17 **Before**	**0**	**100**	**0**	**0**	3	5.94	16.20	0.05	1.3585	18.4616	0.7948	52.38	0.4080	0.3243	29.380
					Mean	**5.93**	**16.03**	**0.05**	**1.3582**	**18.46**	**0.7948**	**52.43**	**0.4084**	**0.3246**	**24.968**
					Std	**0.02**	**0.15**	**0.00**	**0.0005**	**0.00**	**0.0000**	**0.09**	**0.0007**	**0.0006**	**3.821**
17 **After**	**0**	**100**	**0**	**0**	1	6.37	16.40	0.05	1.3577	18.4763	0.7965	52.03	0.4053	0.3228	21.564
					2	6.33	16.80	0.05	1.3578	18.4761	0.7965	52.24	0.4069	0.3241	21.257
					3	6.23	16.90	0.05	1.3580	18.4757	0.7964	52.17	0.4064	0.3236	21.257
					Mean	**6.31**	**16.70**	**0.05**	**1.3578**	**18.48**	**0.7965**	**52.15**	**0.4062**	**0.3235**	**21.359**
					Std	**0.07**	**0.26**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.11**	**0.0008**	**0.0007**	**0.177**
18 **Before**	**0**	**0**	**100**	**0**	1	4.81	6.50	0.13	1.3427	18.372	0.7845	56.38	0.4391	0.3445	27.170
					2	4.84	6.60	0.13	1.3428	18.3719	0.7844	56.43	0.4395	0.3448	27.484
					3	4.83	6.60	0.13	1.3428	18.3718	0.7844	56.40	0.4393	0.3446	27.642
					Mean	**4.83**	**6.57**	**0.13**	**1.3428**	**18.37**	**0.7844**	**56.40**	**0.4393**	**0.3446**	**27.432**
					Std	**0.02**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0000**	**0.03**	**0.0002**	**0.0002**	**0.240**
18 **After**	**0**	**0**	**100**	**0**	1	4.84	6.80	0.13	1.3425	18.3615	0.7832	56.41	0.4394	0.3441	27.878
					2	4.97	6.70	0.13	1.3428	18.3614	0.7832	56.46	0.4398	0.3444	27.642
					3	5.19	6.70	0.13	1.3428	18.361	0.7832	56.43	0.4395	0.3442	27.721
					Mean	**5.00**	**6.73**	**0.13**	**1.3427**	**18.36**	**0.7832**	**56.43**	**0.4396**	**0.3443**	**27.747**
					Std	**0.18**	**0.06**	**0.00**	**0.0002**	**0.00**	**0.0000**	**0.03**	**0.0002**	**0.0002**	**0.120**
19 **Before**	**0**	**0**	**0**	**100**	1	4.94	17.90	0.05	1.3605	18.4593	0.7945	176.73	1.3765	1.0937	21.410
					2	5.03	17.90	0.05	1.3605	18.4590	0.7945	176.82	1.3773	1.0942	21.564
					3	5.19	18.00	0.05	1.3606	18.458	0.7944	176.76	1.3768	1.0937	20.718
					Mean	**5.05**	**17.93**	**0.05**	**1.3605**	**18.46**	**0.7945**	**176.77**	**1.3769**	**1.0939**	**21.231**
					Std	**0.13**	**0.06**	**0.00**	**0.0001**	**0.00**	**0.0001**	**0.05**	**0.0004**	**0.0003**	**0.451**
19 **After**	**0**	**0**	**0**	**100**	1	5.21	18.10	0.05	1.3606	18.474	0.7962	181.20	1.4114	1.1238	21.719
					2	5.25	18.10	0.05	1.3606	18.4740	0.7962	180.89	1.4090	1.1218	20.949
					3	5.31	18.10	0.05	1.3607	18.473	0.7961	181.10	1.4106	1.1230	21.257
					Mean	**5.26**	**18.10**	**0.05**	**1.3606**	**18.47**	**0.7962**	**181.06**	**1.4103**	**1.1229**	**21.308**
					Std	**0.05**	**0.00**	**0.00**	**0.0001**	**0.00**	**0.0001**	**0.16**	**0.0012**	**0.0010**	**0.388**
20 **Before**	**50**	**50**	**0**	**0**	1	5.83	17.30	0.05	1.3593	19.8405	0.9538	179.35	1.3970	1.3325	27.406
					2	5.82	17.30	0.05	1.3593	19.8205	0.9515	179.56	1.3986	1.3308	27.327
					3	5.81	17.30	0.05	1.3594	19.8204	0.9515	179.25	1.3962	1.3285	26.934
					Mean	**5.82**	**17.30**	**0.05**	**1.3593**	**19.83**	**0.9523**	**179.39**	**1.3972**	**1.3306**	**27.222**
					Std	**0.01**	**0.00**	**0.00**	**0.0001**	**0.01**	**0.0013**	**0.16**	**0.0012**	**0.0020**	**0.253**
20 **After**	**50**	**50**	**0**	**0**	1	5.74	17.00	0.05	1.3590	19.8134	0.9507	180.72	1.4076	1.3382	26.777
					2	5.75	17.00	0.05	1.3590	19.8133	0.9507	180.58	1.4065	1.3372	26.777
					3	5.75	17.00	0.05	1.3590	19.8132	0.9507	180.67	1.4072	1.3378	27.012
					Mean	**5.75**	**17.00**	**0.05**	**1.3590**	**19.81**	**0.9507**	**180.66**	**1.4071**	**1.3378**	**26.855**
					Std	**0.01**	**0.00**	**0.00**	**0.0000**	**0.00**	**0.0000**	**0.07**	**0.0006**	**0.0005**	**0.136**

APPENDIX P

CERTIFICATE OF ANALYSIS OF CNOS CONTENT IN: NBF EXTRACT-TCMAE (MBF), NBF PLANT MATERIL ON EPIPHYTES FERN (NBF) AND NBF PLANT MATERIAL ON GROUND (NBF GROUND)

APPENDIX Q

TEMPERATURE PROFILE AT DIFFERENT VARIOUS SOLVENT AND THEIR MIXTURE:
WATER, ACETONE, ACETONITRILE AND ETHANOL

50W:50ACN		50W:50ACL		50W:50ETOH		50ACN:50ACL		50ACN:50ETOH	
	ΔT		ΔT		ΔT		ΔT		ΔT
28.71		20.59		30.68		25.45		19.85	
32.98	4.27	24.77	4.18	34.52	3.84	30.39	4.94	24.13	4.28
37.26	4.28	28.92	4.15	37.94	3.42	36.81	6.42	30.12	5.99
41.54	4.28	33.21	4.29	41.43	3.49	44.03	7.22	36.79	6.67
45.83	4.29	37.51	4.30	45.39	3.96	51.39	7.36	43.72	6.93
50.17	4.34	41.74	4.23	49.32	3.93	59.08	7.69	50.75	7.03
54.49	4.32	46.85	5.11	53.23	3.91	62.83	3.75	57.63	6.88
58.41	3.92	51.77	4.92	56.99	3.76	63.62	0.79	61.61	3.98
61.67	3.26	54.97	3.20	60.51	3.52	63.45	-0.17	61.71	0.10
61.04	-0.63	58.08	3.11	60.72	0.21	63.00	-0.45	61.50	-0.21
61.23	0.19	61.44	3.36	60.80	0.08	62.47	-0.53	61.05	-0.45
60.88	-0.35	60.27	-1.17	60.53	-0.27	61.92	-0.55	60.56	-0.49
60.42	-0.46	60.08	-0.19	60.14	-0.39	61.39	-0.53	60.13	-0.43
60.00	-0.42	60.89	0.81	60.23	0.09	60.85	-0.54	61.05	0.92
60.06	0.06	60.08	-0.81	60.18	-0.05			60.94	-0.11
60.15		60.43	0.35	59.97	-0.21				
		60.44	0.01						
		60.44	0.00						

50ACL:50ETOH		62.5W		62.5ACN		62.5ACL		62.5ETOH	
	ΔT		ΔT		ΔT		ΔT		ΔT
19.38		27.56		23.45		19.72		23.98	
26.19	6.81	29.67	2.11	29.50	6.05	22.71	2.99	27.88	3.90
32.42	6.23	33.21	3.54	35.68	6.18	27.64	4.93	33.00	5.12
39.19	6.77	37.00	3.79	41.93	6.25	33.11	5.47	38.49	5.49
46.07	6.88	40.86	3.86	48.26	6.33	38.82	5.71	44.19	5.70
53.18	7.11	44.62	3.76	54.86	6.60	44.44	5.62	49.76	5.57
59.44	6.26	48.45	3.83	60.60	5.74	49.86	5.42	55.28	5.52
63.15	3.71	52.15	3.70	61.13	0.53	55.07	5.21	60.68	5.40
63.99	0.84	55.58	3.43	61.43	0.30	59.32	4.25	62.14	1.46
63.93	-0.06	58.91	3.33	61.23	-0.20	66.65	7.33	62.53	0.39
63.76	-0.17	61.92	3.01	60.91	-0.32	66.41	-0.24	62.32	-0.21
63.47	-0.29	62.34	0.42	60.51	-0.40	66.77	0.36	61.92	-0.40
63.14	-0.33	62.18	-0.16	60.12	-0.39	66.27	-0.50	61.46	-0.46
62.68	-0.46	61.76	-0.42	59.71	-0.41	65.55	-0.72	60.99	-0.47
61.64	-1.04	61.28	-0.48			64.82	-0.73	60.54	-0.45

W:CAN:ACL:ETOH		**100W**		**100ACN**		**100ACL**		**100ETOH**	
	ΔT		**ΔT**		**ΔT**		**ΔT**		**ΔT**
23.04		25.31		23		24.63		25.39	
27.00	3.96	27.79	2.48	25.96	2.96	29.57	4.94	28	2.61
30.76	3.76	30.87	3.08	34.15	8.19	35.97	6.4	33.67	5.67
35.29	4.53	34.12	3.25	41.25	7.1	42.93	6.96	39.94	6.27
40.38	5.09	37.50	3.38	48.81	7.56	50.04	7.11	46.6	6.66
44.73	4.35	40.93	3.43	54.08	5.27	57.19	7.15	53.1	6.5
49.14	4.41	44.40	3.47	56.23	2.15	63.55	6.36	59.48	6.38
53.76	4.62	47.86	3.46	57.72	1.49	65.37	1.82	64.3	4.82
57.87	4.11	51.21	3.35	60.43	2.71	65.63	0.26	65.11	0.81
61.18	3.31	54.43	3.22	61.31	0.88	65.31	-0.32	65.11	0
61.61	0.43	57.59	3.16	61.38	0.07	64.73	-0.58	64.8	-0.31
61.43	-0.18	60.60	3.01	61.13	-0.25	63.94	-0.79	64.39	-0.41
61.02	-0.41	61.58	0.98	60.77	-0.36	63.1	-0.84	63.92	-0.47
60.60	-0.42	61.70	0.12	60.39	-0.38	62.28	-0.82	63.47	-0.45
60.14	-0.46	61.53	-0.17	60.02	-0.37			63.02	-0.45
60.64	0.50	61.25	-0.28	59.96	-0.06				
61.29	0.65	61.25	0.00						
		61.24	-0.01						
		61.23	-0.01						

W: Water, ACN:Acetone, ACL: Acetonitrile, ETOH: Ethanol

APPENDIX R

VOLUME RATE OF HEAT GENERATION RESULTS OF MIXTURE DESIGN

No	Water (%)	Acetone (%)	Acetonitrile (%)	Ethanol (%)	$\Delta T/dt$ (^{0}C.s^{-1})					q_{mw} (*Watts.mL*$^{-1}$)				
					60	**120**	**180**	**240**	**300**	**60**	**120**	**180**	**240**	**300**
1	**100**	**0**	**0**	**0**	0.147	0.159	0.154	0.148	0.120	0.617	0.668	0.646	0.622	0.503
2	**0**	**100**	**0**	**0**	0.304	0.277	0.213	0.157	0.123	0.525	0.478	0.367	0.272	0.213
3	**0**	**0**	**100**	**0**	0.305	0.324	0.226	0.160	0.124	0.538	0.572	0.398	0.283	0.218
4	**0**	**0**	**0**	**100**	0.243	0.284	0.221	0.161	0.125	0.471	0.551	0.428	0.312	0.243
5	**50**	**50**	**0**	**0**	0.214	0.215	0.180	0.132	0.105	0.645	0.648	0.542	0.399	0.316
6	**50**	**0**	**50**	**0**	0.210	0.219	0.208	0.165	0.133	0.627	0.652	0.621	0.490	0.396
7	**50**	**0**	**0**	**50**	0.179	0.188	0.167	0.123	0.098	0.555	0.583	0.517	0.381	0.303
8	**0**	**50**	**50**	**0**	0.310	0.312	0.209	0.150	0.118	0.540	0.543	0.364	0.261	0.206
9	**0**	**50**	**0**	**50**	0.282	0.315	0.231	0.168	0.137	1.030	1.149	0.844	0.612	0.500
10	**0**	**0**	**50**	**50**	0.330	0.334	0.248	0.182	0.141	0.610	0.617	0.458	0.337	0.260

11	**62.5**	**12.5**	**12.5**	**12.5**	0.157	0.174	0.174	0.144	0.112	0.521	0.577	0.577	0.478	0.372
12	**12.5**	**62.5**	**12.5**	**12.5**	0.308	0.310	0.210	0.153	0.121	0.636	0.639	0.433	0.316	0.250
13	**12.5**	**12.5**	**62.5**	**12.5**	0.223	0.251	0.261	0.194	0.150	0.460	0.518	0.538	0.400	0.310
14	**12.5**	**12.5**	**12.5**	**62.5**	0.242	0.261	0.214	0.156	0.122	0.524	0.565	0.464	0.338	0.264
15	**25**	**25**	**25**	**25**	0.204	0.218	0.212	0.158	0.125	0.501	0.534	0.520	0.388	0.307

APPENDIX S

Table S: Estimated values of dielectric properties and penetration depth results of mixture of solvents

No	Water (%)	Acetone (%)	Acetonitrile (%)	Ethanol (%)	Radiation time (sec.)											
					Dielectric constant						**Dielectric loss**					
					0	**60**	**120**	**180**	**240**	**300**	**0**	**60**	**120**	**180**	**240**	**300**
1	**100**	**0**	**0**	**0**	76.870	74.672	72.275	69.877	67.957	66.038	11.670	11.055	10.390	9.725	9.060	8.395
2	**0**	**100**	**0**	**0**	18.900	16.960	15.030	13.120	11.300	9.580	10.020	8.078	6.884	5.942	5.000	4.058
3	**0**	**0**	**100**	**0**	32.500	30.502	28.505	26.507	24.510	22.512	8.950	8.253	7.732	7.135	6.438	5.641
4	**0**	**0**	**0**	**100**	21.700	19.902	18.005	16.128	14.231	12.333	6.470	5.949	5.428	4.907	4.386	3.865
5	**50**	**50**	**0**	**0**	47.885	45.974	44.063	42.151	40.240	38.329	10.845	10.209	9.572	8.936	8.299	7.663
6	**50**	**0**	**50**	**0**	54.685	52.774	50.863	48.942	47.020	45.099	10.310	9.674	9.086	8.498	7.910	7.323
7	**50**	**0**	**0**	**50**	49.285	47.331	45.377	43.456	41.502	39.548	9.070	8.419	7.768	7.117	6.466	5.815
8	**0**	**50**	**50**	**0**	25.700	23.812	22.834	21.856	20.879	19.901	9.485	8.906	8.327	7.676	7.097	6.518
9	**0**	**50**	**0**	**50**	20.300	18.412	16.524	14.635	12.812	10.924	8.245	7.654	7.063	6.472	5.881	5.289
10	**0**	**0**	**50**	**50**	27.100	25.233	23.367	21.500	19.577	17.610	7.710	7.063	6.417	5.770	5.164	4.717
11	**62.5**	**12.5**	**12.5**	**12.5**	57.155	55.331	53.465	51.641	49.817	47.994	10.406	9.817	9.171	8.582	7.993	7.404
12	**12.5**	**62.5**	**12.5**	**12.5**	28.070	26.071	24.072	22.073	20.074	18.296	9.611	9.031	8.451	7.871	7.291	6.711
13	**12.5**	**12.5**	**62.5**	**12.5**	34.971	33.083	31.195	29.307	27.419	25.641	8.746	8.166	7.545	6.935	6.314	5.693
14	**12.5**	**12.5**	**12.5**	**62.5**	29.571	27.793	25.905	24.127	22.349	20.572	7.805	7.194	6.584	5.974	5.353	4.743
15	**25**	**25**	**25**	**25**	38.300	36.424	34.547	32.771	30.874	29.198	9.278	8.675	8.063	7.441	6.920	6.277

No	Water (%)	Acetone (%)	Acetonitrile (%)	Ethanol (%)	Radiation time (sec.)											
					Dissipation factor						**Penetration depth**					
					0	**60**	**120**	**180**	**240**	**300**	**0**	**60**	**120**	**180**	**240**	**300**
1	**100**	**0**	**0**	**0**	0.152	0.148	0.144	0.139	0.133	0.127	1.488	1.549	1.621	1.703	1.803	1.918
2	**0**	**100**	**0**	**0**	0.530	0.476	0.458	0.453	0.442	0.424	0.860	1.010	1.116	1.208	1.332	1.511
3	**0**	**0**	**100**	**0**	0.275	0.271	0.271	0.269	0.263	0.251	1.262	1.326	1.368	1.430	1.524	1.667
4	**0**	**0**	**0**	**100**	0.298	0.299	0.301	0.304	0.308	0.313	1.426	1.486	1.549	1.622	1.704	1.800
5	**50**	**50**	**0**	**0**	0.226	0.222	0.217	0.212	0.206	0.200	1.264	1.316	1.374	1.440	1.514	1.601
6	**50**	**0**	**50**	**0**	0.189	0.183	0.179	0.174	0.168	0.162	1.421	1.488	1.555	1.631	1.717	1.817
7	**50**	**0**	**0**	**50**	0.184	0.178	0.171	0.164	0.156	0.147	1.533	1.619	1.718	1.835	1.974	2.143
8	**0**	**50**	**50**	**0**	0.369	0.374	0.365	0.351	0.340	0.328	1.059	1.086	1.137	1.207	1.276	1.356
9	**0**	**50**	**0**	**50**	0.406	0.416	0.427	0.442	0.459	0.484	1.083	1.111	1.140	1.171	1.206	1.238
10	**0**	**0**	**50**	**50**	0.285	0.280	0.275	0.268	0.264	0.268	1.338	1.409	1.492	1.592	1.698	1.763
11	**62.5**	**12.5**	**12.5**	**12.5**	0.182	0.177	0.172	0.166	0.160	0.154	1.439	1.501	1.580	1.659	1.749	1.854
12	**12.5**	**62.5**	**12.5**	**12.5**	0.342	0.346	0.351	0.357	0.363	0.367	1.092	1.120	1.150	1.183	1.217	1.263
13	**12.5**	**12.5**	**62.5**	**12.5**	0.250	0.247	0.242	0.237	0.230	0.222	1.340	1.395	1.467	1.547	1.643	1.762
14	**12.5**	**12.5**	**12.5**	**62.5**	0.264	0.259	0.254	0.248	0.240	0.231	1.380	1.452	1.532	1.629	1.750	1.895
15	**25**	**25**	**25**	**25**	0.242	0.238	0.233	0.227	0.224	0.215	1.322	1.378	1.444	1.524	1.591	1.705

APPENDIX T

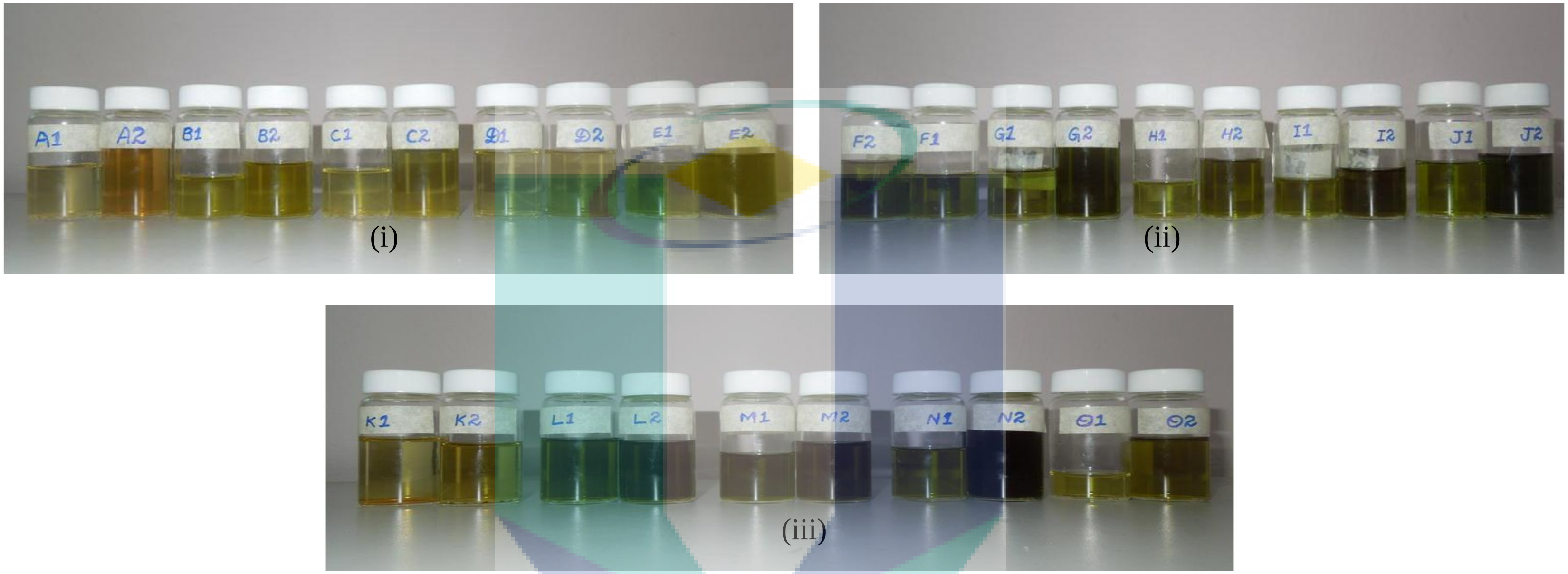


Figure T (i) shows water maceration (A1), water TCMAE (A2), B1 and B2 are maceration and TCMAE in water/acetone binary solvent at ratio 1:1, C1 and C2 are maceration and TCMAE in water/acetonirile binary solvent at ratio 1:1, D1 is maceration and D2 is TCMAE in water/acetonirile binary solvent at ratio 1:1, E1 (maceration) and E2 (TCMAE) in acetone, respectively.

Similarly, Figure T (ii), F1 (maceration) and F2 (TCMAE) in acetone/acetonirile binary solvent, G1 (maceration) and G2 (TCMAE) in acetone/ethanol binary solvent, H1 (maceration) and H2 (TCMAE) in acetonitrile solvent, I1 (maceration) and I2 (TCMAE) in acetonirile/ethanol binary solvent, J1 (maceration) and J2 (TCMAE) in ethanol solvent, respectively

Figure T (iii), summarized the NBF maceration and extraction in quaternary solvent system i-e, K1 (maceration) and K2 (TCMAE) in water/acetone/acetonitrile/ethanol at ratio 0.63/0.13/0.13/0.13, respectively, L1 (maceration) and L2 (TCMAE) in quaternary solvent system at ration 0.13/0.63/0.13/0.13, respectively. Simultaneously the M1, N1, O1, P1, and Q1 (maceration), and M2, N2, O2, P2, and Q2 (TCMAE) in quaternary solvent system at ratio 0.13/0.13/0.63/0.13, and 0.13/0.13/0.13/0.63, respectively.

Figure S: Effects of various solvents system on the dissolution of NBF in maceration and TCMAE condition

APPENDIX U

TEMPERATURE PROFILE AT DIFFERENT VARIOUS EXTRACTION PARAMETER: EXTRACTION TEMPERATURE, MICROWAVE POWER AND EXTRACTION TIME

	TRACTION TEMPERATURE																													
	50°C						60°C						70°C						80°C						90°C					
	1	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**
0	28.1		28.09		28.22		28.01		28.15		28.33		28.17		28.15		28.42		28.68		28.27		28.03		28.85		28.35		28.05	
20	31.69	3.59	31.99	3.9	31.81	3.59	31.52	3.51	32.09	3.94	31.84	3.51	31.45	3.28	32.09	3.94	32.36	3.94	31.84	3.16	32.49	4.22	32.25	4.22	31.76	2.91	32.06	3.71	31.76	3.71
40	35.07	3.38	35.65	3.66	35.19	3.38	34.96	3.44	35.55	3.46	35.28	3.44	34.7	3.25	35.66	3.57	35.93	3.57	34.93	3.09	35.92	3.43	35.68	3.43	35.21	3.45	35.89	3.83	35.59	3.83
60	38.53	3.46	39.36	3.71	38.65	3.46	38.33	3.37	39.08	3.53	38.65	3.37	37.85	3.15	38.79	3.13	39.06	3.13	38.06	3.13	39.28	3.36	39.04	3.36	38.11	2.9	39.68	3.79	39.38	3.79
80	41.93	3.4	42.85	3.49	42.05	3.4	41.7	3.37	42.66	3.58	42.02	3.37	41	3.15	42.21	3.42	42.48	3.42	41.28	3.22	42.78	3.5	42.54	3.5	41.31	3.2	43.32	3.64	43.02	3.64
100	45.43	3.5	46.43	3.58	45.55	3.5	45.08	3.38	46.1	3.44	45.4	3.38	44.17	3.17	45.47	3.26	45.74	3.26	44.52	3.24	46.28	3.5	46.04	3.5	44.52	3.21	47.04	3.72	46.74	3.72
120	48.93	3.5	49.97	3.54	49.05	3.5	48.48	3.4	49.7	3.6	48.8	3.4	47.36	3.19	48.92	3.45	49.19	3.45	47.79	3.27	49.72	3.44	49.48	3.44	47.82	3.3	50.71	3.67	50.41	3.67
140	50.24	1.31	49.83	0.14	50.36	1.31	51.7	3.22	53.17	3.47	52.02	3.22	50.49	3.13	52.13	3.21	52.4	3.21	51.01	3.22	53.26	3.54	53.02	3.54	50.96	3.14	54.52	3.81	54.22	3.81
160	50.09	0.15	51.01	1.18	50.21	0.15	54.97	3.27	56.58	3.41	55.29	3.27	53.6	3.11	55.61	3.48	55.88	3.48	54.22	3.21	56.65	3.39	56.41	3.39	54.33	3.37	58.15	3.63	57.85	3.63
180	50.09	0	50.93	0.08	50.21	0	58.19	3.22	60.01	3.43	58.51	3.22	56.59	2.99	58.97	3.36	59.24	3.36	57.38	3.16	60.12	3.47	59.88	3.47	57.4	3.07	61.77	3.62	61.47	3.62
200	50.12	0.03	50.86	0.07	50.24	0.03	61.36	3.17	59.63	0.38	61.68	3.17	59.62	3.03	62.39	3.42	62.66	3.42	60.48	3.1	63.43	3.31	63.19	3.31	60.39	2.99	65.31	3.54	65.01	3.54
220	50.1	0.02	50.85	0.01	50.22	0.02	60.9	0.46	60.19	0.56	61.22	0.46	62.58	2.96	65.47	3.08	65.74	3.08	63.51	3.03	66.69	3.26	66.45	3.26	63.47	3.08	68.7	3.39	68.4	3.39
240	50.08	0.02	50.74	0.11	50.2	0.02	60.65	0.25	60.01	0.18	60.97	0.25	65.41	2.83	68.71	3.24	68.98	3.24	66.45	2.94	69.86	3.17	69.62	3.17	66.52	3.05	71.96	3.26	71.66	3.26
260	50.01	0.07	50.63	0.11	50.13	0.07	60.47	0.18	59.88	0.13	60.79	0.18	68.3	2.89	69.41	0.7	69.68	0.7	69.33	2.88	72.98	3.12	72.74	3.12	69.17	2.65	75.18	3.22	74.88	3.22
280	50	0.01	50.55	0.08	50.12	0.01	60.29	0.18	60.61	0.73	60.61	0.18	71.49	3.19	69.48	0.07	69.75	0.07	72.12	2.79	75.97	2.99	75.73	2.99	72.04	2.87	78.19	3.01	77.89	3.01
300							60.11	0.18	60.44	0.17	60.43	0.18	70.96	-0.5	69.51	0.03	69.78	0.03	74.81	2.69	78.88	2.91	78.64	2.91	74.75	2.71	81.11	2.92	80.81	2.92
320							59.9	0.21	60.24	-0.2	60.22	0.21	70.47	-0.5	70.21	0.7	70.48	0.7	77.46	2.65	80.57	1.69	80.33	1.69	77.49	2.74	83.69	2.58	83.39	2.58
340							60.79	0.89	60.05	0.19	61.11	0.89	70.11	-0.4	69.83	0.38	70.1	0.38	80	2.54	80.34	0.23	80.1	0.23	80.04	2.55	86.59	2.9	86.29	2.9
360													69.77	-0.3	70.33	0.5	70.6	0.5	78.29	1.71	79.64	-0.7	79.4	-0.7	82.6	2.56	89.09	2.5	88.79	2.5
380													70.75	0.98	69.96	0.37	70.23	0.37	81.5	3.21	80.76	1.12	80.52	1.12	83.79	1.19	89.49	0.4	89.19	0.4
400													70.25	-0.5	70.21	0.25	70.48	0.25	80.73	0.77	80.13	0.63	79.89	0.63	86.26	2.47	89.82	0.33	89.52	0.33
420													70.58	0.33	70.11	-0.1	70.38	-0.1	80.16	0.57	79.59	0.54	79.35	0.54	88.59	2.33	90.23	0.41	89.93	0.41
440																			78.67	1.49	79.92	0.33	79.68	0.33	90.06	1.47	90.31	0.08	90.01	0.08
460																			81.31	2.64	80.62	0.7	80.38	0.7	90.24	0.18	90.03	0.28	89.73	0.28
480																			80.6	-0.1	79.94	0.68	79.7	0.68	89.24	-1	90.03	0	89.73	0
500																									90.59	1.35	90.71	0.68	90.41	0.68
520																									88.96	1.63	90.63	0.08	90.33	0.08
540																									91.9	2.94	90.52	0.11	90.22	0.11
560																									90.75	1.15	90.37	0.15	90.07	0.15
580																									89.96	0.79	90.11	0.26	89.81	0.26

	MICROWAVE POWER																													
	140 Watt						280 Watt						420 Watt						560 Watt						700 Watt					
	1	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**
0	28.71		28.79		28.41		28.23		27.26		28.04		28.83		30.70		30.55		28.96		28.96		28.80		28.97		28.99		28.90	
20	29.52	0.81	30.07	1.28	29.22	0.81	30.47	2.24	29.26	2.00	30.28	2.24	32.21	3.38	33.62	2.92	33.47	2.92	35.03	6.07	35.40	6.44	34.87	6.07	36.52	7.55	37.64	8.65	36.45	7.55
40	30.37	0.85	31.00	0.93	30.07	0.85	32.57	2.10	31.58	2.32	32.38	2.10	35.14	2.93	36.80	3.18	36.65	3.18	39.31	4.28	40.25	4.85	39.15	4.28	42.60	6.08	44.48	6.84	42.53	6.08
60	31.24	0.87	32.00	1.00	30.94	0.87	34.75	2.18	33.80	2.22	34.56	2.18	39.42	4.28	39.36	2.56	39.21	2.56	43.94	4.63	44.69	4.44	43.78	4.63	48.80	6.20	50.80	6.32	48.73	6.20
80	32.10	0.86	33.01	1.01	31.80	0.86	36.94	2.19	36.04	2.24	36.75	2.19	41.71	2.29	42.95	3.59	42.80	3.59	48.50	4.56	49.32	4.63	48.34	4.56	54.76	5.96	57.00	6.20	54.69	5.96
100	33.02	0.92	33.97	0.96	32.72	0.92	39.16	2.22	38.35	2.31	38.97	2.22	44.75	3.04	46.20	3.25	46.05	3.25	53.02	4.52	53.99	4.67	52.86	4.52	60.67	5.91	63.09	6.09	60.60	5.91
120	33.86	0.84	34.91	0.94	33.56	0.84	41.37	2.21	40.73	2.38	41.18	2.21	47.99	3.24	49.43	3.23	49.28	3.23	57.58	4.56	58.48	4.49	57.42	4.56	65.81	5.14	69.15	6.06	65.74	5.14
140	34.76	0.90	35.87	0.96	34.46	0.90	43.66	2.29	42.97	2.24	43.47	2.29	50.89	2.90	52.84	3.41	52.69	3.41	61.98	4.40	62.93	4.45	61.82	4.40	70.56	4.75	70.99	1.84	70.49	4.75
160	35.59	0.83	36.80	0.93	35.29	0.83	45.87	2.21	45.27	2.30	45.68	2.21	54.95	4.06	55.64	2.80	55.49	2.80	66.39	4.41	67.01	4.08	66.23	4.41	70.79	0.23	70.61	0.38	70.72	0.23
180	36.48	0.89	37.70	0.90	36.18	0.89	48.10	2.23	47.53	2.26	47.91	2.23	57.17	2.22	59.25	3.61	59.10	3.61	70.82	4.43	70.04	3.03	70.66	4.43	70.44	0.35	70.20	0.41	70.37	0.35
200	37.32	0.84	38.69	0.99	37.02	0.84	50.30	2.20	49.79	2.26	50.11	2.20	60.28	3.11	62.28	3.03	62.13	3.03	70.36	0.46	70.98	0.94	70.20	0.46	70.25	0.19	69.88	0.32	70.18	0.19
220	38.20	0.88	39.61	0.92	37.90	0.88	52.46	2.16	51.99	2.20	52.27	2.16	63.00	2.72	65.32	3.04	65.17	3.04	70.02	0.34	70.34	0.64	69.86	0.34	70.07	0.18	69.59	0.29	70.00	0.18
240	39.10	0.90	40.50	0.89	38.80	0.90	54.60	2.14	54.19	2.20	54.41	2.14	66.01	3.01	68.00	2.68	67.85	2.68	70.97	0.95	70.02	0.32	70.81	0.95	70.98	0.91	70.43	0.84	70.91	0.91
260	39.87	0.77	41.50	1.00	39.57	0.77	56.78	2.18	56.36	2.17	56.59	2.18	68.81	2.80	70.46	2.46	70.31	2.46	70.63	0.34	70.22	0.20	70.47	0.34	70.58	0.40	69.97	0.46	70.51	0.40
280	40.66	0.79	42.35	0.85	40.36	0.79	58.85	2.07	58.50	2.14	58.66	2.07	71.71	2.90	69.36	1.10	69.21	1.10	70.38	0.25	70.17	0.05	70.22	0.25	70.26	0.32	70.10	0.13	70.19	0.32
300	41.49	0.83	43.15	0.80	41.19	0.83	60.92	2.07	60.57	2.07	60.73	2.07	70.92	0.79	72.57	3.21	72.42	3.21	70.11	0.27	70.07	0.10	69.95	0.27						
320	42.30	0.81	44.08	0.93	42.00	0.81	62.91	1.99	62.61	2.04	62.72	1.99	70.43	0.49	74.57	2.00	74.42	2.00	71.22	1.11	69.93	0.14	71.06	1.11						
340	43.11	0.81	45.00	0.92	42.81	0.81	64.85	1.94	64.60	1.99	64.66	1.94	70.09	-0.4	73.96	0.61	73.81	0.61												
360	43.85	0.74	45.82	0.82	43.55	0.74	66.76	1.91	66.55	1.95	66.57	1.91	69.75	0.34	73.64	0.32	73.49	0.32												
380	44.70	0.85	46.64	0.82	44.40	0.85	68.62	1.86	68.32	1.77	68.43	1.86	70.72	0.97	73.43	0.21	73.28	0.21												
400	45.46	0.76	47.52	0.88	45.16	0.76	70.39	1.77	69.34	1.02	70.20	1.77	70.22	0.50	72.94	0.49	72.79	0.49												
420	46.24	0.78	48.31	0.79	45.94	0.78	69.54	0.85	69.43	0.09	69.35	0.85	70.90	0.68																
440	47.00	0.76	49.11	0.80	46.70	0.76	71.33	1.79	69.06	0.37	71.14	1.79																		
460	47.76	0.76	49.86	0.75	47.46	0.76	70.62	0.71	69.93	0.87	70.43	0.71																		
480	48.49	0.73	50.67	0.81	48.19	0.73	70.24	0.38	71.54	1.61	70.05	0.38																		

500	49.26	0.77	51.47	0.80	48.96	0.77	69.93	0.31	70.58	0.96	69.74	0.31
520	49.97	0.71	52.35	0.88	49.67	0.71	71.21	1.28	70.15	0.43	71.02	1.28
540	50.72	0.75	53.04	0.69	50.42	0.75	70.56	0.65	70.08	0.07	70.37	0.65
560	51.43	0.71	53.66	0.62	51.13	0.71	70.21	0.35			70.02	0.35
580	52.16	0.73	54.39	0.73	51.86	0.73						
600	52.81	0.65	55.09	0.70	52.51	0.65						
620	53.53	0.72	55.72	0.63	53.23	0.72						
640	54.17	0.64	56.56	0.84	53.87	0.64						
660	54.85	0.68	57.13	0.57	54.55	0.68						
680	55.49	0.64	57.80	0.67	55.19	0.64						
700	56.17	0.68	58.41	0.61	55.87	0.68						
720	56.77	0.60	59.02	0.61	56.47	0.60						
740	57.45	0.68	59.54	0.52	57.15	0.68						
760	58.04	0.59	60.11	0.57	57.74	0.59						
780	58.66	0.62	60.62	0.51	58.36	0.62						
800	59.25	0.59	61.18	0.56	58.95	0.59						
820	59.86	0.61	61.81	0.63	59.56	0.61						
840	60.41	0.55	62.42	0.61	60.11	0.55						
860	61.00	0.59	63.01	0.59	60.70	0.59						
880	61.54	0.54	63.65	0.64	61.24	0.54						
900	62.09	0.55	64.06	0.41	61.79	0.55						
920	62.63	0.54	64.71	0.65	62.33	0.54						
940	63.18	0.55	65.22	0.51	62.88	0.55						
960	63.66	0.48	65.82	0.60	63.36	0.48						
980	64.19	0.53	66.36	0.54	63.89	0.53						
1000	64.66	0.47	67.00	0.64	64.36	0.47						
1020	65.17	0.51	67.38	0.38	64.87	0.51						
1040	65.63	0.46	67.85	0.47	65.33	0.46						
1060	66.14	0.51	68.28	0.43	65.84	0.51						
1080	66.58	0.44	68.84	0.56	66.28	0.44						
1100	67.08	0.50	69.20	0.36	66.78	0.50						
1120	67.51	0.43	69.73	0.53	67.21	0.43						
1140	67.98	0.47	69.69	0.04	67.68	0.47						
1160	68.42	0.44	70.03	0.34	68.12	0.44						
1180	68.88	0.46	69.76	0.27	68.58	0.46						
1200	69.27	0.39	69.63	0.13	68.97	0.39						
1220	69.53	0.26	70.09	0.46	69.23	0.26						
1240	70.11	0.58	69.60	0.49	69.81	0.58						
1260	70.54	0.43	69.83	0.23	70.24	0.43						
1280	70.60	0.06	70.37	0.54	70.30	0.06						
1300	70.66	0.06	70.11	0.26	70.36	0.06						
1320	70.73	0.07			70.43	0.07						
1340	70.70	0.03			70.40	0.03						
1360	70.69	0.01			70.39	0.01						
2070	70.66	0.03			70.36	0.03						
2100					70.37	0.01						

	EXTRACTION TIME																													
	0 minutes						**2 minutes**						**4 minutes**						**6 minutes**						**8 minutes**					
	1	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**	**1**	**Δ T**	**2**	**Δ T**	**3**	**Δ T**
0	28.07		28.32		28.21		28.03		28.15		28.27		28.17		28.35		28.40		28.09		28.18		28.01		28.10		28.52		28.39	
20	31.57	3.50	32.66	4.34	31.71	3.50	31.57	3.54	32.00	3.85	31.81	3.54	31.45	3.28	32.50	4.15	31.68	3.28	31.48	3.39	32.04	3.86	31.69	3.68	31.57	3.47	32.56	4.04	31.86	3.47
40	34.91	3.34	36.33	3.67	35.05	3.34	34.89	3.32	35.69	3.69	35.13	3.32	34.70	3.25	35.86	3.36	34.93	3.25	34.49	3.01	35.57	3.53	34.70	3.01	34.91	3.34	36.32	3.76	35.20	3.34
60	38.17	3.26	39.77	3.44	38.31	3.26	38.25	3.36	39.15	3.46	38.49	3.36	37.85	3.15	39.21	3.35	38.08	3.15	37.61	3.12	39.06	3.49	37.82	3.12	38.23	3.32	39.94	3.62	38.52	3.32
80	41.50	3.33	43.37	3.60	41.64	3.33	41.56	3.31	42.70	3.55	41.80	3.31	41.00	3.15	42.52	3.31	41.23	3.15	40.63	3.02	42.47	3.41	40.84	3.02	41.54	3.31	43.59	3.65	41.83	3.31
100	44.87	3.37	46.77	3.40	45.01	3.37	44.95	3.39	46.18	3.48	45.19	3.39	44.17	3.17	45.70	3.18	44.40	3.17	43.85	3.22	46.02	3.55	44.06	3.22	44.87	3.33	47.19	3.60	45.16	3.33
120	48.21	3.34	50.27	3.50	48.35	3.34	48.30	3.35	49.72	3.54	48.54	3.35	47.36	3.19	49.36	3.66	47.59	3.19	47.18	3.33	49.46	3.44	47.39	3.33	48.26	3.39	50.73	3.54	48.55	3.39
140	51.47	3.26	53.78	3.51	51.61	3.26	51.68	3.38	53.10	3.38	51.92	3.38	50.49	3.13	52.49	3.13	50.72	3.13	50.52	3.34	52.87	3.41	50.73	3.34	51.48	3.22	54.23	3.50	51.77	3.22
160	54.84	3.37	57.28	3.50	54.98	3.37	54.97	3.29	56.53	3.43	55.21	3.29	53.60	3.11	56.05	3.56	53.83	3.11	53.71	3.19	56.27	3.40	53.92	3.19	54.72	3.24	57.65	3.42	55.01	3.24
180	58.11	3.27	60.75	3.47	58.25	3.27	58.23	3.26	59.91	3.38	58.47	3.26	56.59	2.99	59.28	3.23	56.82	2.99	56.87	3.16	59.64	3.37	57.08	3.16	58.09	3.37	61.17	3.52	58.38	3.37
200	61.31	3.20	63.99	3.24	61.45	3.20	61.32	3.09	63.21	3.30	61.56	3.09	59.62	3.03	62.75	3.47	59.85	3.03	59.96	3.09	62.86	3.22	60.17	3.09	61.29	3.20	64.48	3.31	61.58	3.20
220	64.52	3.21	67.26	3.27	64.66	3.21	64.44	3.12	66.46	3.25	64.68	3.12	62.58	2.96	65.81	3.06	62.81	2.96	63.12	3.16	66.09	3.23	63.33	3.16	64.36	3.07	67.80	3.32	64.65	3.07
240	67.55	3.03	68.65	1.39	67.69	3.03	67.48	3.04	69.60	3.14	67.72	3.04	65.41	2.83	69.05	3.24	65.64	2.83	65.93	2.81	69.15	3.06	66.14	2.81	67.34	2.98	69.80	2.00	67.63	2.98
260	68.37	0.82	70.21	1.56	68.51	0.82	69.46	1.98	71.93	2.33	69.70	1.98	68.30	2.89	69.73	0.68	68.53	2.89	69.03	3.10	69.84	0.69	69.24	3.10	70.35	3.01	70.03	0.23	70.64	3.01
280	70.42	2.05	70.97	0.76	70.56	2.05	70.17	0.71	71.31	0.62	70.41	0.71	71.49	3.19	69.46	0.27	71.72	3.19	69.98	0.95	70.06	0.22	70.19	0.95	70.09	0.26	70.68	0.65	70.38	0.26
300							70.63	0.46	70.88	0.43	70.87	0.46	70.96	0.53	69.21	0.25	71.19	0.53	70.78	0.80	70.31	0.25	70.99	0.80	70.76	0.67	70.25	-0.3	71.05	0.67
320							70.25	0.38	70.51	0.37	70.49	0.38	70.47	0.49	70.20	0.99	70.70	0.49	70.44	0.34	69.98	0.33	70.65	0.34	70.32	0.44	70.40	0.15	70.61	0.44
340													70.11	0.36	69.82	0.38	70.34	0.36	70.23	0.21	70.16	0.18	70.44	0.21	69.98	0.34	70.01	0.39	70.27	0.34
360													69.77	0.34	70.31	0.49	70.00	0.34	70.02	0.21	70.46	0.30	70.23	0.21	70.86	0.88	70.44	0.43	71.15	0.88
380													70.75	0.98	70.03	0.28	70.98	0.98	70.75	0.73	70.15	0.31	70.96	0.73	70.37	0.49	70.00	0.44	70.66	0.49
400													70.25	0.50	69.84	0.19	70.48	0.50	70.36	0.39	69.83	0.32	70.57	0.39	69.98	0.39	70.59	0.59	70.27	0.39
420													70.58	0.33	70.61	0.77	70.81	0.33	70.06	0.30	69.82	0.01	70.27	0.30	70.77	0.79	70.30	0.29	70.98	0.71
440																			70.31	0.25	69.81	0.01	70.52	0.25	70.74	0.03	70.56	0.26	70.95	0.03
460																			70.41	0.10	69.80	0.01	70.62	0.10	70.72	0.02	70.77	0.21	70.93	0.02
480																			70.03	0.38	69.89	0.09	70.24	0.38	70.69	0.03	70.82	0.05	70.90	0.03
500																			70.79	0.76	70.12	0.23	71.00	0.76	70.68	0.01	70.82	0.00	70.89	0.01
520																									70.67	0.01	70.81	0.01	70.88	0.01
540																									70.65	0.02	70.80	0.01	70.86	0.02
560																									70.63	0.02	70.78	0.02	70.84	0.02

APPENDIX V

Table V: Volume Rate of Heat Generation of Various Extraction Parameter

No	**(%)**	**(%)**	**(^{0}C.s^{-1})**					**(*Watts.mL^{-1}*)**				
			60	**120**	**180**	**240**	**300**	**60**	**120**	**180**	**240**	**300**
1	**Extraction time (minutes)**	**0**	0.147	0.159	0.154	0.148	0.120	0.617	0.668	0.646	0.622	0.503
2		**2**	0.304	0.277	0.213	0.157	0.123	0.525	0.478	0.367	0.272	0.213
3		**4**	0.305	0.324	0.226	0.160	0.124	0.538	0.572	0.398	0.283	0.218
4		**6**	0.243	0.284	0.221	0.161	0.125	0.471	0.551	0.428	0.312	0.243
5		**8**	0.214	0.215	0.180	0.132	0.105	0.645	0.648	0.542	0.399	0.316
6	**Microwave Power (Watts)**	**140**	0.042	0.043	0.043	0.043	0.042	0.176	0.179	0.181	0.181	0.177
7		**280**	0.109	0.110	0.110	0.110	0.109	0.454	0.458	0.461	0.459	0.455
8		**420**	0.176	0.172	0.157	0.155	0.140	0.733	0.717	0.658	0.647	0.586
9		**560**	0.250	0.239	0.233	0.175	0.137	1.043	0.997	0.972	0.731	0.573
10		**700**	0.331	0.307	0.270	0.195	0.158	1.381	1.283	1.130	0.815	0.659
11	**Extraction Temperature (°C)**	**50C**	0.157	0.174	0.174	0.144	0.112	0.521	0.577	0.577	0.478	0.372
12		**60C**	0.308	0.310	0.210	0.153	0.121	0.636	0.639	0.433	0.316	0.250
13		**70C**	0.223	0.251	0.261	0.194	0.150	0.460	0.518	0.538	0.400	0.310
14		**80C**	0.242	0.261	0.214	0.156	0.122	0.524	0.565	0.464	0.338	0.264
15		**90C**	0.204	0.218	0.212	0.158	0.125	0.501	0.534	0.520	0.388	0.307

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