Radio Frequency Assisted Extraction of Phenolic Compounds from Apple Peel

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Abstract

The work presented in this thesis is the step by step approaches taken in the effort of developing radio frequency heating technology for the extraction of apple peel phenolic compounds. The work that has been carried out covers the design of an extraction system, the establishment of the initial extraction conditions, characterization of suitable solvents for the extraction system, process optimization and finally the justification study. Two comprehensive literature reviews encompassing RF heating and apple peel phenolics were also prepared in light of gaining deeper insights in these fields.

A full laboratory scale RF heating system for nutraceutical extraction was conceptualized and constructed. This setup allows for initial evaluation of the potential of RF heating in this area. Special emphasis was given to the design of the applicator as this aspect significantly influenced the efficiency of heat generation and distribution in the system. Vertical positioning of the parallel electrodes with a fixed gap between surfaces was employed in our new applicator since this unique design is suitable for heating liquid samples and also plausibly minimizes the non-uniform heating effect due to the air gap. The function of each component and the importance of this design were also highlighted to provide information to the readers about this new system since related information is lacking in the published literature.

From the preliminary extraction test, the initial conditions for the Radio Frequency Assisted Extraction (RFAE) of apple peel phenolic compounds, involving the moisture content requirement for the sample, the type of acidic solutions and temperature were established. From this study, it was concluded that a high quality apple peel extract can be obtained from RFAE, containing total phenolic contents of 26.11 mg GAE/g dry peel, total flavonoids contents of 14.48 mg CAT/g dry peel, ferric reduction antioxidant property of 2.37 mmol ferrous equivalent/ g dry peel and 76.33 % of DPPH inhibition. This is achieved when the extraction process is performed using a mixture of 17 % moisture content apple peel sample in 1 mM hydrochloric acid solution, at an extraction temperature between 50°C to 60°C. A simple comparative investigation between RFAE and conventional extraction method showed that the apple peel extract obtained via RFAE had 41.84 % higher TPC compound and 6.90 % more capability in scavenging free radicals than the conventional extract. This study provides the basic foundation for RFAE and also highlights the potential and advantage of this new method over the conventional method.

The efficiency of the RF heating is strongly dependable on the compatibility of the material to be heated with the RF energy. Due to this, the dielectric properties and heating rate of hydrochloric acid solution (HCl), sodium hydroxide solution (NaOH), and citric acid solution (CA) for RFAE as influenced by the type of solutions and variations in solution concentration, ethanol content and temperature were investigated and highlighted. The change of these factors directly affected the dielectric properties and heating rate of these solutions. However, there was no definite correlation found between their dielectric constant or dielectric loss with the heating rate. The addition of ethanol was found to improve the heating rate of HCl and NaOH solutions meanwhile it produced a mixed effect on the CA solution. Increasing the concentration of HCl and NaOH yields a negative effect on the heating rates of these solutions, while on the contrary, increasing the concentration of CA has a positive effect on the heating rate of the CA solution. This study has assisted the understanding of the extraction solvents' behavior under RF heating and helps in directing the decision on the best solvent or solvent combination to be used for RF extraction.

An optimized RFAE for recovering phenolic compounds from apple peel was developed and proposed. Through a multi-response optimization approach, the highest quality of apple peel extract was obtained with total phenolic compounds of 90.85 mg GAE/ g DM, ferric reduction antioxidant property of 1100.00 mM ferric (II)/g DW, and 89.25 % DPPH inhibition. This was achieved with considerably efficient energy utilization when the process was performed using a combination mixture of 1 mM hydrochloric acid with 10% ethanol content, 245.60 ml N_2/min of stirring speed, 0.02 g/ml solid to liquid ratio and RF power of 300 W. The success and feasibility usage of a nitrogen bubble stirrer for RFAE for improving yield and heating rate was demonstrated in this study. The positive result from this investigation promotes the potential of RFAE to be developed further as a green extraction method based on RFAE preference towards low solvent utilization.

The feasibility and potential of RFAE as a new extraction method was proven in the comparative study with microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and conventional methods without and with heat (CONOHEAT and CONHEAT). The recovery and the quality of extracts obtained from RFAE (17.48 ± 0.02 mg GAE/ g DM) were higher than for the other selected methods (11.80 ± 0.04 mg GAE/ g DM (CONOHEAT), 15.42 ± 0.03 mg GAE/ g DM (CONHEAT), 16.15 ± 0.02 mg GAE/ g DM (UAE) and 17.18 ± 0.03 mg GAE/ g DM (MAE)). The difference between each extraction method was clearly explained and visualized through high effective diffusivity value, kinetic curves and SEM images. SEM images showed that each extraction method tested imposed different degree of impact on the apple peel samples. It is hypothesized that the excellent performance of RFAE is being driven by the combination of internal superheating from the rapid volumetric heating with the deeper penetration depth of RFAE. The perfect fit between the RFAE experimental kinetic curves with the model suggests that Peleg's equation can be used for modeling RFAE kinetics.

Résumé

Les travaux de recherche présentent une approche progressive visant le développement d'une technologie d'extraction, par fréquence radio (RF), des composés phénoliques des résidus de peau de la pomme. Les travaux effectués ont touché la conceptualisation d'un système d'extraction, l'étude des conditions initiales d'extraction, l'optimisation du procédé et une analyse justificative du projet dans son ensemble. Deux revues de la littérature se consacrant au chauffage RF, et aux composés phénoliques de la pomme, sont également présentées afin de mieux les comprendre.

Un système complet à l'échelle laboratoire adaptant le chauffage RF pour l'extraction de composés nutraceutiques a été conçu et construit. Ce système permet l'évaluation initiale du potentiel du chauffage RF en extraction. L'emphase a été mise sur la conception de l'applicateur lui-même puisqu'il influence significativement l'efficacité du dégagement calorifique et sa distribution. Un positionnement vertical des électrodes parallèles avec un espacement fixe a été utilisé pour ce système unique afin de pouvoir chauffer des échantillons liquides et afin de minimiser l'espace d'air et le chauffage non-uniforme. La fonction de chacune des composantes et l'importance de la conception sont également mises en évidence afin de fournir l'information nécessaire pour la promotion de cette nouvelle technologie.

Les extractions préliminaires effectuées par extraction assistée par fréquence radio (RFAE) des composés phénoliques de la peau de pommes ont établi les paramètres initiaux tels que le taux d'humidité requis, le type de solutions acides et la température. De cette étude il fut conclu qu'un extrait de pelure de pomme de haute qualité peut être obtenu par RFAE, avec un contenu en composés phénoliques de 26.11 mg GAE/g de pelure sèche, en flavonoïdes de 14.48 mg CAT/g de pelure sèche, une propriété anti-oxydante par réduction ferrique de 2.37 mmol équivalent ferreux/g de pelure sèche et 76.33% d'inhibition DPPH. Cet extrait a été obtenu par extraction de pelure de pomme ayant 17% d'humidité, dans une solution de 1 mM d'acide chlorhydrique et une température d'extraction entre 50° et 60°C. Une comparaison entre RFAE et l'extraction traditionnelle a montré que l'extrait obtenu par RFAE avait 41.84% plus de composés phénoliques et 6.90% plus de capacité d'élimination des radicaux libres que l'extrait traditionnel. Ces résultats indiquent les paramètres de base de l'extraction RFAE et soulignent son potentiel par rapport à l'extraction traditionnelle.

L'efficacité du chauffage RF dépend fortement de l'affinité du matériau à être chauffé par l'énergie RF. À cet effet, les propriétés diélectriques et la vitesse de montée en température ont été étudiées pour des solutions d'acide chlorhydrique (HCl), d'hydroxyde de soude (NaOH) et d'acide citrique (CA) selon le type de solutions, les variations de concentrations, la concentration en éthanol et la température. Ces facteurs ont tous eu un effet sur les propriétés diélectriques et la vitesse de montée en température, cependant il n'y a pas eu de corrélation bien définie entre eux. L'addition d'éthanol a amélioré la montée en température des solutions d'HCl et de NaOH, alors que l'effet s'est avéré mixte pour la solution de CA. L'augmentation de la concentration d'HCl et de NaOH entraine un effet négatif sur le taux de chauffage de ces solutions, alors qu'au contraire une augmentation de la concentration de CA a un effet positif sur le taux de chauffage de la solution de CA. Cette étude a permis de mieux comprendre le comportement des solvants d'extraction lorsque sujet au chauffage RF, facilitant de ce fait la sélection de la combinaison adéquate de solvants pour une extraction RF optimale.

Un procédé optimisé RFAE pour la récupération des composés phénoliques des pelures de pommes a été développé et proposé. Grâce à une approche d'optimisation par réponses multiples, un extrait de pelure de pomme de haute qualité a été obtenu avec une concentration en composés phénoliques de 90.85 mg GAE/g matière sèche, une propriété anti-oxydante par réduction ferrique de 1100.00 mmol équivalent ferreux/g matière sèche, et 89.25% d'inhibition DPPH. Cet extrait a été obtenu efficacement en utilisant une combinaison de 1 mM acide chlorhydrique avec 10% d'éthanol, 245.60 ml N_2/min de vitesse d'agitation, 0.02 g/ml ratio solide liquide et une puissance RF de 300 W. Le succès et la valeur pratique de l'agitation en utilisant des bulles d'azote pour améliorer le rendement et la montée en température de l'extraction RFAE ont été démontrés. Les résultats positifs de cette recherche mettent en valeur le potentiel de l'extraction RF et encouragent son développement à titre de méthode d'extraction verte puisqu'elle favorise la réduction de l'utilisation des solvants.

La valeur pratique et le potentiel de la technologie RFAE comme nouvelle méthode d'extraction a été démontrée par une étude comparative avec l'extraction microonde (MAE), l'extraction ultrasonique (UAE) et par méthode traditionnelle sans et avec addition de chaleur (CONOHEAT et CONHEAT). Le rendement et la qualité des extraits RF (17.48 ± 17.48 mg GAE/ g matière sèche) étaient plus élevés que pour les autres méthodes (11.80 ± 0.04 mg GAE/ g matière sèche (CONOHEAT), 15.42 ± 0.03 mg GAE/ g matière sèche (CONHEAT), 16.15 ± 0.02 mg GAE/ g matière sèche (UAE) and 17.18 ± 0.03 mg GAE/ g matière sèche (MAE)). La différence entre chaque méthode d'extraction a été clairement illustrée par une forte diffusivité effective, par les courbes de cinétique et les images par microscopie électronique à balayage (SEM). Les images SEM ont démontré que chacune des méthodes d'extraction a imposé différents degrés d'impact physique sur les échantillons de pelure de pomme. Il est donc supposé que l'excellente performance de l'extraction RF est causée par la combinaison d'une surchauffe interne issue du chauffage volumétrique et de la capacité de pénétration de l'énergie RF. Le meilleur ajustement des modèles des courbes cinétiques de l'extraction RFAE suggère l'équation de Peleg pour la modélisation de l'extraction RF.

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“The only way to do a great work is to love what you do”- Steve Job

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Nomenclature

ABS	energy absorption by sample
ANOVA	analysis of variance
C	concentration at time t
C_0	concentration at time 0
CA	citric acid or chlorogenic acid
CAT	catechin equivalent
CCD	central composite design
C_p	heat capacity
D_{eff}	effective diffusivity
DM	dry mass
d_p	penetration depth
DPPH	2,2-diphenyl-1-picrylhydrazyl
E	electric field
EP	epicatechin
EtOH	ethanol
FEM	finite element modeling
FD	freeze drying
FRAP	ferric reducing antioxidant power
g	gram
GAE	gallic acid equivalent
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HR	heating rate
IL	ionic liquid
K_1	Peleg's rate constant

K_2	Peleg's capacity constant
MAE	microwave assisted extraction
min	minute
MHz	megaHertz
ml	milliliter
mM	milliMolar
MD	microwave drying
NaOH	sodium hydroxide
N_2	nitrogen gas
PEFE	pulsed electric field extraction
PLE	pressurized liquid extraction
RF	radio frequency
RFAE	radio frequency assisted extraction
RP	reversed phase
R^2	coefficient of correlation
SDG	secoisolariciresinol diglucoside
SFE	supercritical fluid extraction
TPC	total phenolic content
TFC	total flavonoids content
UAE	ultrasonic assisted extraction
UV-Vis	ultraviolet-visible
V	voltage
W	watt
ε	permittivity
ε'	dielectric constant
ε''	dielectric loss

σ	electrical conductivity
Ω	ohm
°C	celcius
π	pi

Chapter 1 - Introduction

1.1 Thesis Background

Plant phenolic compounds are gaining popularity in nutraceutical, pharmaceutical, food and beverages industries for their multiple benefits. Studies have proven that plant phenolics are not only essential for plant to maintain good physiology, but also have been demonstrated beneficial for promoting and restoring human's health and preventing degenerative diseases. In plants, phenolic compounds are responsible for plant pigmentation, growth and reproduction, protecting the plants from pathogens, predators, plagues, preharvest seed germination, ultraviolet rays, etc. (Bravo, 1998; Naczk & Shahidi, 2006). These compounds are available abundantly in fruits, vegetables, seeds, cereals and legumes.

One of the fruits that contain a wide variety of beneficial phenolic compounds is the apple. High consumption of apples and apple products with their high health beneficial phenolics profiles has suggested health protective effects in the North American and European populations (Boyer & Liu, 2004; Hyson, 2011). More and more studies have been published recently highlighting the health benefits of apple phenolic which encompass protection against neurodegeneration, cancers, cardiovascular diseases, asthma and pulmonary dysfunction, diabetes, aging, bone weight loss and also weight loss promotion (Heo, Kim, Choi, Shin & Lee, 2004; Hyson, 2011). Based on these encouraging findings, studies on promoting the utilization of these apple compounds, as functional ingredient, have drastically increased. This effort is also in conjunction with the Canada Horticulture Council's strategic planning in promoting local apples production by increasing the utilization of apples as ingredient in a variety of industrial uses such as cosmetics and food additives. Canada is ranked 25th of the world's top apple producing country and produces approximately 340 000 metric tonnes of apples annually. According to the Canadian Apple

Industry's 2010 report produced by Agriculture and Agri-food Canada, the country also imports apples from other countries, an average of 175 466 metric tonnes per year (from year 2006 to 2010). 33.5 % of the available apples in Canada are used for producing juice, sauce, pie filling and canned produced, meanwhile 66.5 % of the apples are consumed fresh.

It is important to know that most of the apple antioxidant compounds are not embedded in the flesh but are within the skin of the apple. This means that the solid waste produced from the apple processing industry still contains high amount of phenolic compounds which is the perfect and inexpensive source for mass recovery of these undervalued compounds. To date, the apple waste is generally used for producing dietary fibre pectin and citric acid, also as animal feed and fertilizer. With modifications in the extraction process, pectin and phenolic compounds from apple can plausibly be extracted simultaneously. Apple has the highest amount of free phenolic compounds that can be easily extracted (Boyer et al., 2004). The full utilization of this waste is projected to reduce waste disposal issues and also provide additional income to apple producers.

Studies showed that these compounds can be incorporated into the sample in the form of ground apple peel powder or extract. In general the usage of an extract is more practical in a variety of applications. The main process required for obtaining the apple peel extract is extraction. There are many extraction methods available which can be classified into two categories namely the conventional solvent methods and novel methods. Conventional extraction methods are normally associated with certain issues such as long extraction duration, low recovery, high extraction solvent and energy utilization and high loss of target compounds due to exposure to heat for a long period. On the other hand, novel extraction methods offer unique extraction mechanisms (e.g. volumetric heating, high pressure impact, cavitational disturbance, etc.) which may offer shorter processing time and produce higher yield, with however some problems which still exist such as degradation of target compounds due to excessive heat or chemical hydrolysis, high solvent utilization, noise contamination, non-uniform heating, requirement of constant contact between sample and the system and expensive operational machinery setup and maintenance costs which are somewhat unavoidable.

Due to these issues, a new extraction process based on radio frequency heating technology is proposed. The commercialization of RF heating can be found in the area of drying and thawing, meanwhile this technology has been explored and investigated for other applications including post-harvest treatment, pasteurization, cooking, medical, etc. In our study, the RF heating is applied for accelerating the extraction rate of phenolic compounds from the apple peel. This new extraction technique, using radio frequency heating, is termed as radio frequency assisted extraction (RFAE). RFAE was first reported in 2009 for recovering a phytoestrogen, podophyllotoxin, by a research group from University of Saskatchewan (Izadifar, 2009).

In general, incorporation of heat in an extraction process significantly improves the rate of extraction or extraction kinetics and that is the reason why most of the extraction methods available these days such as Soxhlet, steam distillation and microwave assisted extraction are heat-driven methods. Nevertheless, the heating concept of RF heating is different from conventional conduction or convection heating because the heat is generated within the sample (volumetric heating) as a result from collisions of molecules or ions in the sample that absorb and dissipate energy from the electric field. Since the heat in RF heating is generated within the material, the time taken to heat the sample completely is relatively shorter than the time taken to heat up the material via conventional heating by conductive or convective heat transfer. The internal superheating produced within the material is also anticipated to assist in breaking the cell walls which allows the movement of more compounds into the extraction solvent, as explained by Wang & Weller (2006) for microwave assisted extraction.

Another reason for proposing RFAE is its heating preference towards dielectric materials with lower moisture content. This is another advantage of RF heating and this special characteristic, highly useful for developing a green extraction system that utilizes low solvent, produces high recovery. RF heating uniformity of a solid-liquid mixture was proven better than for conventional heating which is another plus point for this technology. Besides the volumetric heating, the high penetration depth in RF heating offers better heat uniformity for thick or large material which is a crucial aspect in ensuring consistent product quality or scaling up of the extraction system for practical application, unlike its sister technology microwave assisted extraction which is limited by a low penetration depth.

The RF heating attributes mentioned earlier are expected to offer several benefits to the nutraceutical extraction field such as short process duration, target compounds recovery maximization, minimization of costly extraction solvent usage, optimal heating uniformity and feasibility for scale up. Besides these special characteristics, the encouraging result obtained from our preliminary work on evaluating the RFAE of secoisolariciresinol diglucoside (SDG), a phytoestrogen from flaxseed, contributed to the development of this study. This initial investigation demonstrated that the recovery of SDG via RFAE (18.68 ± 0.48 mg SDG/g DM) was higher than the recovery from the conventional hot plate method (17.51 ± 0.60 mg SDG/g DM) and microwave-assisted extraction (MAE) (17.33 ± 0.030 mg SDG/g DM) (Jusoh & Orsat, 2011). This finding highlighted the potential of RFAE as a new alternative extraction method over the existing extraction methods

The underutilization of apple peel phenolic compounds, with their high antioxidant activity, and the need for an efficient and green extraction process (short time, low solvent, safe solvent, low energy, etc) for recovering these health beneficial compounds has initiated this work.

1.2 Thesis Hypothesis

Radio frequency technology, with its selective and volumetric heating can be developed into an efficient novel extraction method for phenolic compounds. Its longer wavelength, than for microwave extraction, should provide homogeneous volumetric heating.

1.3 Thesis Main Objective and Scopes of Work

The overall aim of this project was to develop an effective and efficient phenolic compound extraction system using radio frequency dielectric heating known as radio frequency assisted extraction (RFAE). In accomplishing this aim, the following detailed scopes of work, relating to the crucial aspects of this method development, were proposed:

i. To present the fundamental and available information on RF heating and apple phenolic compounds and highlight important current advances in these subjects from the published literature. These comprehensive reviews on RF heating and apple phenolic compounds are presented in **Chapter 2** and **Chapter 3,** respectively.

ii. To design and build a radio frequency heating applicator and its overall control system for the study of solid-liquid extraction. This is presented in **Chapter 4.**

iii. To establish the initial extraction conditions for radio frequency assisted extraction of apple peel phenolic compounds by investigating the effects of solvents, sample moisture content and extraction temperature on the quality of the extract. The results of this study are presented in **Chapter 5**.

iv. To investigate the effects of solvent concentration, ethanol content and temperature on the dielectric properties and heating performance of the selected solvents and to determine the most suitable solvent combination for apple peel phenolics extraction by RFAE. The results of this study are presented in **Chapter 6**.

v. To investigate the effects of processing parameters namely ethanol concentration, solid to liquid ratio, stirring speed and RF power on the RFAE performance and to develop an optimized RFAE for apple peel phenolic compounds extraction. The results of this study are presented in **Chapter 7**.

vi. To evaluate the potential of RFAE by comparing its performance with selected existing methods and to study their extraction mechanisms. The results of this study are presented in **Chapter 8**.

The studies that have been carried out chapter by chapter in this thesis were written in the manuscript-based format in preparation for publication.

Connecting Statement to Chapter 2

The background information of this study, encompassing the objectives and scopes of work, was presented in **Chapter 1**. **Chapter 2** highlights the fundamental information relating to RF heating mechanisms, factors associated to the success of this heating method, recent studies and advances together with future work with the intention of building a better understanding on this technology and its capabilities.

Chapter 2 has been written in the manuscript format for submission to the journal of Food and Bioproducts Processing:

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who prepared the experimental design, conducted the laboratory work, analysed the results and prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated ideas, provided the technical advice, suggestions, experimental facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.

Chapter 2 - Radio Frequency Heating Technology: Principles, Applications and Future Studies

2.1 Introduction

Radio frequency (RF) heating is a heating process within a material, dielectric in nature, using energy dissipation which is produced from the interaction between an electric field produced between the electrodes and the polar and ionic charges in the material. In general, RF heating falls under the category of dielectric heating, which also comprises microwave heating. In the electromagnetic spectrum, radio frequency lies between the frequencies of 300 kHz to 300 MHz. Despite its broad frequency range, only three frequencies are legally allowed to be used for various industrial applications i.e. 13 MHz, 27 MHz and 40 MHz to avoid interference with telecommunications.

This technology offers wide advantages over other heating techniques in terms of significant processing time reduction, energy efficiency, no contact heating, more uniform heat distribution, and no surface overheating, thus less damaging towards original material properties. With the benefits offered, this technology seems as an excellent replacement for conventional heating processes.

Due to the high initial investment cost, lack of dielectric properties data and information related to optimized heating parameters and its high sensitivity of performance with heterogeneous products, this technology is not widely commercially developed except for selected applications in the area of drying and thawing. Recently, a growing interest in scaling up the RF heating technology can be seen in various fields (not limited to food processing), indicates that RF heating is closer towards more widespread commercialization. With the assistance of computer modeling and simulation, the feasibility of the system could be assessed quickly and potential problems could be solved prior to the actual development.

This chapter serves as an overview of RF heating and it covers the basic information of this technology including the heating principle, the influencing factors, the applications, the requirements for computer modeling and simulation and last but not least, the prospective areas of investigation for RF heating.

2.2 Radio Frequency Heating Principle

Heating using radio frequency is different from conventional conduction or convection heating. In conduction heating, heat is transferred by diffusion through the solid particles within a material from high temperature to low temperature surfaces. Convection heating is heat transfer through flow of fluid. In radio frequency heating, heat is generated within the sample through frictional energy dissipation caused by the rapid movement of molecules or ions within an alternating electromagnetic energy field. This mechanism is called volumetric heating. In RF heating, the material to be heated is sandwiched between two electrodes. When the RF generator is switched on, a rapid alternating electric field is generated between these electrodes. Due to the rapid change of polarity of the electric field, dipolar polarization and ionic charges in the material react following the changes of the electric field, and as they move and collide with each other, energy dissipation produces heat within the material. In RF heating, heat is dominantly produced through the movement of ionic charges and then followed by dipolar polarization meanwhile in microwave heating, dipolar polarization is more dominant. Undeniably, volumetric heating is faster than conduction and convection heating. Figure 2-1 illustrates the orientation of the molecules and ions due to the changes of polarity of the electric field during RF heating.

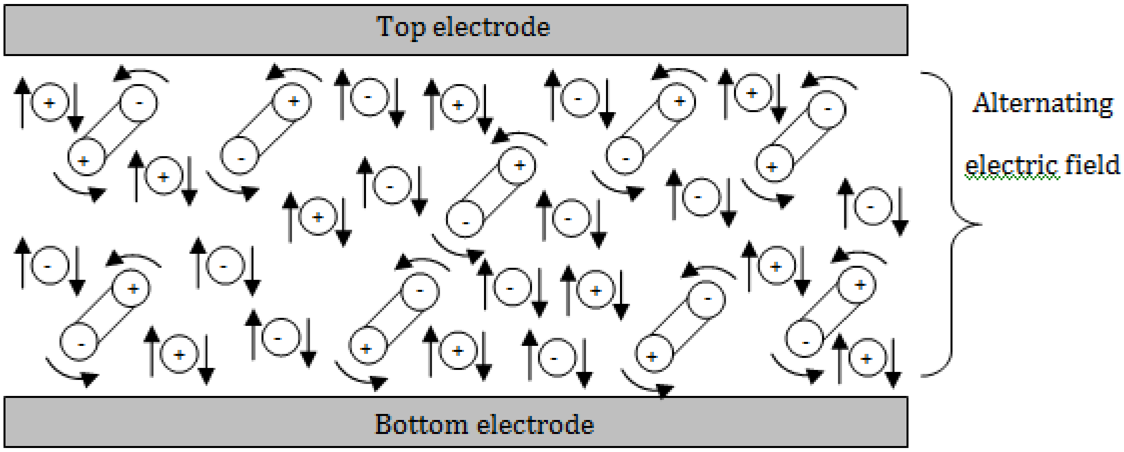
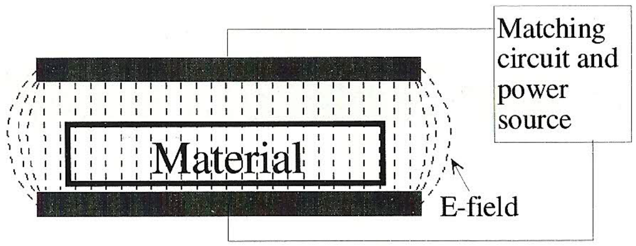
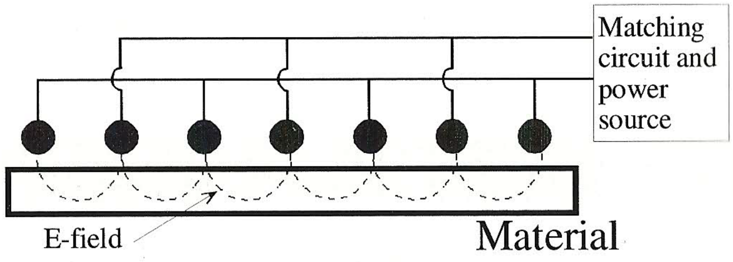


Figure 2-1: Ionic charges and dipolar polarization occurring in the alternating electric field of two RF parallel plates

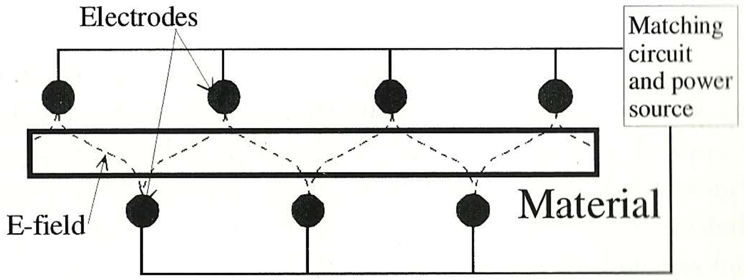
Figure 2-2 illustrates the three basic designs of an RF applicator depending on its use i.e. throughfield, strayfield and stagger-throughfield applicator. The throughfield applicator is commonly utilized in many publications, for examples in Wang et al. (2008), Wang et al. (2006) and Orsat et al. (2001). For liquid products, several alternative designs, as illustrated in Figure 2-3, other than the conventional parallel throughfield applicator, have been proposed by Geveke (2005), Piyasena & Dussault (2003a) and Houben et al. (1991).



a) Throughfield Applicator (parallel plate)



b) Strayfield Applicator



c) Stagger-throughfield Applicator

Figure 2-2 : Basic designs of RF applicators (a) throughfield (b) strayfield (c) stagger-throughfield (Orsat & Raghavan, 2004)

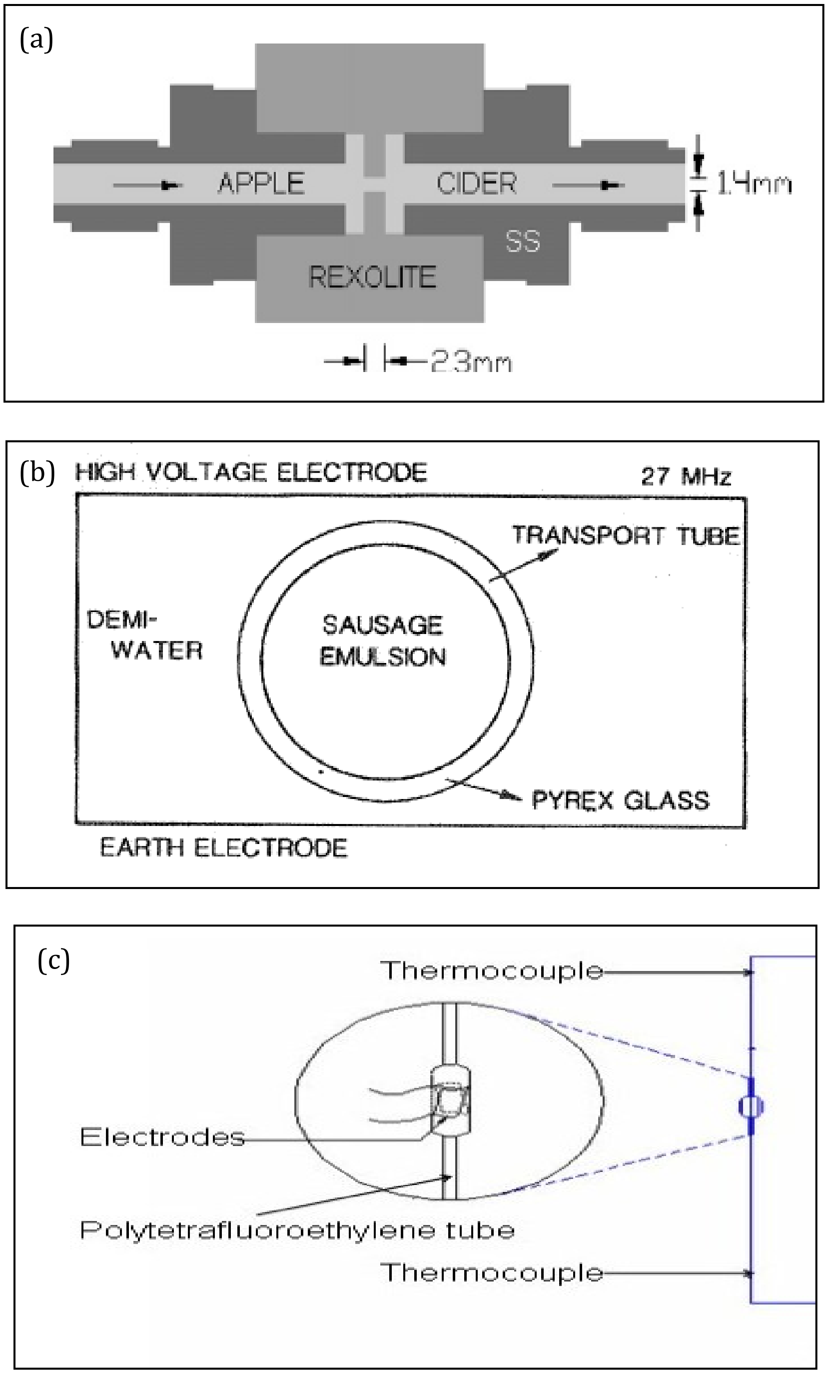


Figure 2-3 : Applicator tubes for RF heating of liquid medium (a) juice and cider (Geveke & Brunkhorst, 2008), (b) sausage emulsion (Houben et al., 1991) and (c) viscous solution (Piyasena et al., 2003a).

2.3 Factors Affecting Radio Frequency Heating

The efficiency of radio frequency heating strongly depends on several factors; namely the electromagnetic properties of the material (dielectric constant, dielectric loss and electrical conductivity) and RF system design (types of materials and shape of electrodes, air gap, gap between electrodes, product size, shape and position between the electrodes). The influences that these factors have on the performance of RF heating will be discussed in detail in this section.

2.3.1 Electromagnetic Properties of the Material

2.3.1.1 Permittivity, Dielectric Constant and Dielectric Loss

The success of RF heating is greatly affected by the dielectric properties of the material to be heated. A material with poor electrical conducting ability is categorized as a dielectric material. Dielectric properties of the materials determine how well energy can be absorbed from the high frequency alternating electric fields by the material and how rapidly the materials will dissipate this energy as heat (Nelson & Trabelsi, 2012). Many experimental studies on the dielectric properties of materials were performed for the purpose of understanding how these properties are affecting the heating process. Besides predicting the heating performance in a dielectric heating apparatus, other beneficial uses of these data are for rapid sensing of moisture content in grains or cereals (Sacilik, Tarimci & Colak, 2006) and for detecting change (Fito, Castro-Giráldez, Fito & Chenoll, 2010; Guo, Zhu, Nelson, Yue, Liu & Liu, 2011b) or adulteration in products (Guo, Liu, Zhu & Wang, 2011a).

The dielectric properties of materials are divided into two parts which are permeability and permittivity, however only permittivity (ε) is considered to be related to the heating process (Marra, Zhang & Lyng, 2009). In general, permittivity describes the ability of a material to absorb, transmit and reflect the electromagnetic energy (Ryynänen, 1995). Permittivity is represented as $\varepsilon = \varepsilon' - j\varepsilon''$, where ε' is the dielectric constant meanwhile ε’’ is the dielectric loss. The dielectric constant (ε’) is associated with the ability of the material to store energy from the electric field in the material meanwhile the dielectric loss (ε’’) is associated with the ability of the material to dissipate that energy as heat (Nelson, 2010).

In general, a material with low ε’’ has higher transparency towards electromagnetic energy thus it will absorb less energy and is expected to heat poorly in the alternating electric field (Marra et al., 2009). On the other hand, too high ε’’ will only heat the surface of the material due to low penetration depth which contradicts the purpose of uniform volumetric heating of RF heating (Orsat & Raghavan, 2005). Metaxas (1988) suggested that only a material within the range of $10^{2} < \varepsilon'' < 2$ is suitable to be processed using this type of heating. Nevertheless, the low-loss host material can be made suitable for dielectric processing by adding selective polar or ionic materials (Orsat & Raghavan, 2005). High heating rate can be achieved when the value of the dielectric constant is close to the dielectric loss and this was experimentally observed by Jiao et al. (2014). One plausible reason for this condition is when the capacity of the material to dissipate energy is similar to its capacity at storing the energy, the material immediately dissipates the energy as heat once it absorbs energy from the electric field. Several work on empirical modeling of dielectric properties as a function of frequency, temperature, moisture content, composition and density for predicting the behavior of these properties have been published in Sacilik & Colak (2010), Sacilik et al. (2006) and Piyasena et al. (2003c). Principles and detailed methods for measuring the dielectric properties of materials can be found in reviews by Nelson (2010) and Venkatesh & Raghavan (2005).

Dielectric properties of materials are greatly influenced by the frequency of the alternating electric field, temperature, moisture content, composition and density of the material. The primary and most common studies published are on the effect of frequency and temperature applied on the change in dielectric properties of materials. For some materials, the dependency of the dielectric properties on frequency and temperature can be described using the Debye relaxation principle. Nelson et al. (2012) and Piyasena et al. (2003a) presented detailed explanations of this phenomena. In many cases, ε’ decreases with an increase in frequency and temperature meanwhile ε” decreases with an increase in frequency but increases with an increase in temperature (Zhu, Guo & Wu, 2012). A comprehensive compilation of dielectric properties data for food products which is useful for food manufacturers can be found in Sosa-Morales et al. (2010).

In general, the higher moisture content present in a material results in higher dielectric constant and loss factor (Sosa-Morales et al., 2010). The manner which water is bound in the material (chemically bound or free) influences the electrical properties of the material (Icier & Baysal, 2004). Free water can orient freely in an applied electric field compared to chemically bound water, which exerts more impact on the dielectric properties of the sample. An increase of the ε’ and ε” with an increase of moisture content in the sample was evidenced in packed bed rhizome particles embedded in extraction solvent (Izadifar & Baik, 2008a) and chick pea flour (Guo et al., 2008). However, it is also important to know that at some frequency ranges, the dielectric loss decreases with an increase in moisture content (Icier & Baysal, 2004).

The chemical composition of a material also exerts strong influence on its dielectric properties. It is however difficult to predict the dielectric properties of a material as a function of its chemical composition as the properties strongly depend on the nature of how these compounds are bound within the material. Most additives which contain free ions can change the dielectric properties of a final product and in turn influence the RF heating process (Marra et al., 2009). Salt is a common ingredient studied for its effect on dielectric properties and RF heating. It was observed that direct or indirect salt addition on a sample could increase its heating rate (Orsat, 1999; Izadifar, Baik & Mittal, 2009). Several studies on the effect of salt addition on the dielectric properties of a material for RF heating have been performed. Piyasena et al. (2003c) demonstrated that ε’ and ε” values of starch solutions increased with addition of salt ranging from 0.2 to 0.5 % (w/w). Similar observation was reported on the effect of salt concentration ranging from 0.20 to 3.30 % on the dielectric properties of sturgeon and salmon caviar (Al-Holy et al., 2005). On contrary, Zhang, Lyng & Brunton (2007) reported that 0.40 to 2.40 % salt addition in meat batters did not significantly affect its ε’, however it significantly increased the ε”. Similar result was observed in Guan et al. (2004). The elevation of ε” was attributed to the addition of conductive charge carriers from the salt addition to the sample (Ryynänen, 1995). It is important to know that with highest conductivity, there is a reduction in the intensity of the voltage and power absorption in the material (Orsat & Raghavan, 2005). Experimental works by Jiao et al. (2014) and Zhang et al. (2007) showed that a high salt content does not always improve the heating rate in RF, as repeatedly mentioned in the literature. Thus it is crucial to have information relating to the amount of table salt or amount and types of ions present prior to their utilization as an additive to improve heating uniformity and heating rate in a RF sample. The effect of fat and starch concentration on the dielectric properties of meat batter and starch solutions are reported in Zhang et al. (2007) and Piyasena et al. (2003c), respectively. Study performed by Zhang et al. (2007) concluded that fat had a significant influence on the dielectric properties of the meat batter nonetheless its impact on the temperature rise of the batter was not significant. While the study by Piyasena et al. (2003c) on starch solutions showed that an increase in starch concentration would cause a significant drop in the relative permittivity of the starch solution while the starch concentration effect on the dielectric properties is dependent on the operating frequency and temperature.

Another important factor that influences the dielectric properties is the density of the material. The physical structure of material affects the dielectric properties of the material (Ryynänen, 1995). The amount of mass per unit volume (density) has certain effect on the interaction of the electromagnetic field and the involved mass (Nelson et al., 2012; Sosa-Morales et al., 2010). In a study on the effect of density on the dielectric properties of flaxseed and corn by Sacilik et al. (2010 & 2006), it was observed that the ε’, ε” and tan δ values increased with increase in density. Samples of an air-particle mixture with high density generally have higher ε’ and ε” because of the lower air incorporation within the samples (Marra et al., 2009).

A compilation of the dielectric properties studies for various products in the RF range is given in Table 2-1.

Table 2-1: Recent published studies on dielectric data for various products at RF range

Product	Authors and year	Factors
Dried fruits	Alfaifi et al. (2013)	Frequency : 27, 40, 915, 1800 MHz
Juice	Zhu et al. (2012)	Frequency : 27, 40, 915, 2450 MHz
Almond shells (ground)	Gao et al. (2012)	Frequency : 27, 40, 915, 1800 MHz
Honey and sucrose syrup	Guo et al. (2011a)	Frequency : 27, 40, 915, 2450 MHz
Seed	Sacilik et al. (2010)	Frequency : 1- 100 MHz
Flour	Guo et al. (2010)	Frequency : 10 – 1800 MHz
Egg	Wang et al. (2009)	Frequency : 27 and 40 MHz
Fruits	Birla et al. (2008)	Frequency : 27, 915, 1800 MHz
Rhizome particles in 70% ethanol and	Izadifar et al. (2008a)	Frequency : 27 and 40 MHz

2.3.2 Loss Tangent

The loss tangent (tan δ) is defined as the power dissipation in the material. It is related to the ability of the material to be penetrated by the electrical field and to dissipate electrical energy as heat (Mudget, 1986). It is the ratio of ε” over ε’ (Equation 1):

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \tag{1}$$

2.3.3 Penetration Depth

Penetration depth (d_p) is defined as the depth of the material where the energy of a plane wave propagating perpendicular to the surface has decreased to 1/e of the surface value (1/e = 1/2.72) (Bengtsson & Risman, 1971). In layman terms, d_p means the practical depth at which an effective electromagnetic heating can occur in the material. If the material is thicker than the allowable d_p, a non-uniform heating is expected in the material which annuls the benefit of dielectric heating. The penetration depth expression is presented below (Piyasena et al., 2003b):

$$d_p = \frac{c}{2\pi f\sqrt{2}\sqrt{\varepsilon'_r}\sqrt{\sqrt{1+(\tan\delta)^2}\ -1}} \tag{2}$$

where c is the speed of propagation of waves in vacuum at 3 x 10^8 m/s; f is the frequency (Hz) and tan δ is the ratio of dielectric loss over dielectric constant. Bengston & Risman (1971) observed that d_p was the greatest when ε’ and ε” were the lowest.

2.3.4 Electrical Conductivity

Electrical conductivity (σ) is an important parameter in RF heating as it is linearly correlated to the dielectric loss as expressed in Equation 3 (Marra et al., 2009):

$$\sigma = 2\pi f\varepsilon'' \tag{3}$$

This parameter indicates the ability of the material to conduct an electric current. From the above equation, one can expect that when a material is having high ionic conductivity, it also has high dielectric loss. In the RF region of the electromagnetic spectrum, it is important to note that ε” is the combination of ionic displacement (ε''_σ) and dipole rotation (ε''_D) of the material (Piyasena et al., 2003b; Mudgett, 1986):

$$\varepsilon'' = \varepsilon''_\sigma + \varepsilon''_D \tag{4}$$

2.3.5 Temperature Rise and Power Absorption

A direct relationship between dielectric properties and the temperature rise in a material heated under electromagnetic heating was described by Orfeuil (1987) and is presented in Equation 5:

$$\Delta T = \frac{2\pi t f\varepsilon_0\varepsilon'_r \tan\delta V^2}{C_p\rho} \tag{5}$$

where ΔT is the temperature increase (°C); t , temperature rise time (s); ε_0, dielectric constant of vacuum (8.85419×10^{-12} F/m); f, frequency (Hz); ε'_r, relative dielectric constant or permittivity of the material to be heated; tan δ, tangent of dielectric loss angle; V, electric field strength (V/cm); C_p, specific heat of the material to be heated (J/kg.C); and ρ, density of the material to be heated (kg/m^3).

From the above equation, the magnitude of the temperature rise can be increased by increasing the ε'', meanwhile a slow heating rate will be expected if the material bears too low of an ε''.

Another parameter which is affected by the dielectric properties directly is the material's power absorption. Nelson (1985) reported the relationship between dielectric loss in the power absorption of material as depicted in Equation 6:

$$P = 55.63 \, f \, E^2 \varepsilon'' \, 10^{-12} \tag{6}$$

where P is the power dissipation per unit volume or power absorbed by the material (W/m^3), f is the frequency applied to the system (Hz), E is the strength of the electric field (V/m) and ε'' is the dielectric loss of the material. This equation clearly shows that a material with higher ε'' will absorb higher power supplied to it, as long as the frequency and E are maintained.

2.4 Design of the RF Heating System

The success of an RF system is greatly dependable on the design of the whole energy transfer system and the impedance matching between the power generator and the applicator (Orsat & Raghavan, 2004). This section briefly presents several recent findings on the design aspects in RF heating. A comprehensive step by step design consideration in developing an RF heating system has been published by Orsat & Raghavan (2004).

2.4.1 Material and Shape for the Electrode Assembly

In RF heating, the heating process takes place within the applicator. As mentioned in Section 2.2, there are several basic types of applicators, and the design is different and tailored according to the requirement and suitability of the materials to be heated. The material selection, design and shape for electrodes and the importance of the air gap between the electrodes and the heated product are some of the key factors in determining the performance of an RF heating system since they are closely related to the evenness of heating within the product.

First of all, care should be given to the choice of material and the length of the electrodes. To produce high electric field, the electrodes must be made from thin sheets of good electrical conducting material such as copper, silver, aluminum and brass (Orsat & Raghavan, 2004). The length of the electrodes, on the other hand, must be smaller than the quarter of the wavelength which is 11 m to prevent standing waves.

In addition, Barber (1983) stated that temperature uniformity in the product could be improved if the electrodes are shaped to fit the surface of the material. This statement is supported by a recent work by Tiwari et al. (2011a) whom performed a simulation study on the effect of electrodes bending position and angle on the uniformity of RF heating of dry samples. Results of their study showed that heating uniformity in the sample was significantly affected and could be maximized with a specific top electrode bending position and angle.

An air gap between the top electrode and the upper surface of the material is introduced in a RF heating applicator set up to avoid the occurrence of a runaway heating effect (Sanders, 1966). However, the distance between the product to be heated and the electrodes must be minimized to avoid energy wastage. When air gap exists between the electrodes and the sample, two electric fields are developed, one in the air gap and the other one in the sample. The electric field distributed in the air gap will always be higher than the one in the sample since the value of the electric field in the air gap is the multiplication between the electric field and the permittivity value of the sample ($E_{airgap} = E_{sample} \times \varepsilon_{sample}$). The value of the electric field of the air gap is directly related to the value of the electric field of the sample. In relation to this, the voltage applied to the system is also divided into two components, one into the air and the other one into the material. That is why the air gap must be minimized to avoid energy wastage through the energy distribution. Slight improvement on heating rate of carrot sticks was observed by increasing the layer of carrot stick from 1-2 to 4-5 cm to reduce the air gap in Orsat et al. (2001). A simulation study by Tiwari et al. (2011a) showed that the uniformity of RF power in the sample was improved by reducing the distance between the sample and the upper electrode.

2.4.2 Product Size, Shape and Position Between Electrodes

Besides the factors mentioned in the previous sections, researchers also have performed a number of studies on the influence of the product shape and position on the heating efficiency and uniformity in RF heating. Romano & Marra (2008) concluded that the shape of the product heated in RF heating has a strong influence on the heating rate and temperature distribution. Through their simulation study using meat batters of three different regular shapes; cube, cylinder and sphere, they concluded that the cubic shape is the most suitable shape for heating in an RF field since rapid and uniform heating with high power absorption was observed within the sample. Sphere was the least favorable shape since low heating rate and power absorption was evident in this shape. For cylindrical product, the position or orientation played a major role in determining the performance. It was evident that only cylindrical product with vertical position demonstrated fast uniform heating with high power absorption comparable to the cubic shape. The same conclusion was also drawn in the work of Izadifar et al. (2009) in developing the shape of an extraction vessel for radio frequency extraction. This is only valid when a cylinder is vertically positioned between two plate electrodes.

Another simulation study, using finite element method, examined the effect of size, shape (cuboid, ellipsoid and cylinder) and relative position of the product between RF electrodes (bottom electrode, middle and top electrode), electrode gap (65 mm and 155 mm) and top electrode configuration on RF power density uniformity index (PUI), was performed by Tiwari et al. (2011a). From the study, it was evident that the RF power density distribution and PUI were different between the three sizes. PUI of the sample depends on amount of electric field entering the sample. The highest PUI was observed when the samples were having lengths and widths of 35-50% of the size of the top electrode and the lowest when the sample had the same size as the top electrode. RF power uniformity in the sample is better when the sample size approaches zero or the maximum size that can be kept completely between RF electrodes. When the sample size is small or approaching zero, the electric field passes directly through the ground electrode without entering sample. As size increases, the electric field starts to enter the sample obliquely. Oblique electric field or edge deflection causes increase of PUI. As the sample size is approaching the size of the top electrode, the electric field starts to enter the sample more uniformly. The highest and lowest power density distributions of a cuboid and cylinder were observed at the edges and in the middle of both shapes, respectively. Ellipsoid, on the contrary, had an opposite trend from the other two shapes.

2.5 Radio Frequency Heating Applications

In recent years, we have seen many reviews on RF heating applications focusing specially on food applications. In this chapter review, for the first time, various additional applications of this novel technology are compiled. Besides food, RF heating application has also been investigated for bioremediation, biomedical, biofuel and nutraceutical extraction purposes which have rarely been highlighted. Recent works on radio frequency heating for various applications are summarized in this section. It is hoped that this summary serves as an encouragement for more potential developments in this field.

2.5.1 Post Harvest Treatment

The earliest work conducted on RF heating was in the area of postharvest treatment. RF heating potential was studied as an alternative to the conventional pest infestation control method i.e. chemical fumigation. The toxicity of the chemicals used for the conventional chemical fumigation has become a serious issue in this area. Dielectric heating was thus studied as an alternative eco-friendly pest control treatment. Even though in general, microwave heating is known to heat faster than RF heating, the latter technology has been demonstrated in various work to be more effective in controlling pest infestation owing to the differential selective nature of RF heating. Nelson & Stetson (1974) revealed the effectiveness of RF heating treatment at 39 MHz in causing complete mortality of adult rice weevils *Sitophilus oryzae* (L.) in red hard winter wheat. In this study, the rice weevils were only exposed to a RF heat treatment at 40°C, however a similar weevil mortality result can only be achieved by microwave heating at 2450 MHz by exposing the wheat to 80°C. As inferred by the authors, the difference in the treatment performance is attributed to some level of selective heating capability in the RF range. At 39 MHz, the dielectric loss factor (ε'') of insect was five times higher than the ε'' of wheat, thus the heating rate is effectively expected to be faster in the weevils. Since there was only a little difference in terms of ε'' between weevil and wheat at 2450 MHz, thus little differential heating was observed when 2450 MHz was applied. Similar findings on high differential heating in RF were also observed in Jiao et al. (2011) and Wang et al. (2003). It was reported that the effective frequency for pest lethality treatment is between 1-100 MHz. Due to the promising results obtained from the laboratory work, several pilot and industrial scale RF heating systems for pest control treatment for various agricultural products have been designed and built by Jiao et al. (2012), Gao et al. (2010) and Wang et al. (2007).

2.5.2 Food and Beverage Processing

Currently, the growing interest and the widest applications of RF heating can be seen in the area of food and beverage processing. Since the year 2000, numerous publications have emerged on the investigation of applications of RF heating for various food processing fields especially for pasteurization, cooking, drying and thawing. The encouraging growth of this technology in food and beverage processing is indebted to its advantages over conventional heating, such as rapid, selective, uniform heating, nondestructive towards quality, and producing higher quality and longer shelf-life products. Nevertheless, in-depth studies are still required. A list of RF heating studies in food and beverage processing since the year 2000 is presented in Table 2-2:

Table 2-2 : RF heating applications in food and beverage industry

Process and Product	Authors and year
Pasteurization	
Juice	(Zhu et al., 2012)
	(Geveke et al., 2008)
	(Geveke et al., 2007)
Vegetable	(Orsat et al., 2001)
Meat, chicken, fish, egg	(Brunton et al., 2005)
	(Dev et al., 2012)
	(Guo et al., 2006)
Pre-packaged food	(Wang et al., 2012)
Cooking	
Meat, Chicken, Fish	(Schlisselberg et al., 2013)
	(Kirmaci & Singh, 2012)
	(Laycock et al., 2003)
	(Al-Holy et al., 2005)
Drying	
Milk	(Chen et al., 2013)
	(Uemura et al., 2010)
	(Awuah et al., 2005)
Bread	(Liu et al., 2011)
Thawing/Defrosting	
Meat and meat products	(Farag et al., 2009)
Freezing	
Meat	(Anese et al., 2012)

2.5.3 Wood Drying and Fungi Treatment

RF heating is not limited to agriculture and food industries in fact its application has extended to other sectors such as the wood industry. The challenge of drying has been an important issue for the wood industry and the potential of using RF drying for wood has been researched and published since the 1980s. Through these studies, it was observed that the proper drying of thick and not-easy-to-dry wood, to the required moisture content standard with exceptional physical qualities can be accomplished using RF vacuum drying (Fang, Ruddick & Avramidis, 2001; Avramidis & Liu, 1994). It is made possible because the dielectric constant of water is 20 times larger than the wood cell which causes the water bound in the wood to heat faster than the wood (Orsat et al., 2005).

Besides the drying issue, RF heating has also been studied as a method for treating wood decay fungi as the fungi is responsible for deterioration and devaluation of wood products. At present, wood decay fungi is controlled using methyl bromide fumigation or conventional heat treatments. Hazardous and inefficient effects of the current available treatments drive the development of RF heating as an alternative wood fungi treatment. The effectiveness of RF heating on treating wood fungi (*Gloeophyyllum trabeum, Ganoderma Lucium, Irpex lacteus, Ceratocystis fimbriata*) on red oak, poplar and southern yellow pine wood was demonstrated by Tubajika et al. (2007). Results showed that 98 to 100 % of RF treated wood samples were completely inhibited from fungus and on top of that, the wood moisture content was reduced. The reliability of RF heating for wood treatment was also evidenced in Lazarescu et al. (2011) where in this study, the pine wood nematodes in pine boards were completely eradicated when the boards were exposed to 30 minutes at 56°C or for 15 minutes at 60°C using RF heating. RF energy was also studied for assisting wood pulp bleaching (Wan et al., 2001) and drying of wood adhesive (Chiozza, Toniolo & Pizzo, 2013).

2.5.4 Soil and Groundwater Remediation

Since 1993, researchers have been working on RF heating for treating hydrocarbon-contaminated soil and groundwater. Some of the early works highlighting the principles can be found in Price et al. (1999) and Edelstein et al. (1994). RF heating was considered for soil remediation because of its rapid volumetric heating and its ability to heat soil up to 400°C which supports variable remediation techniques treatments for short periods. An example of a successful in-situ radio-frequency heating (ISRFH) was reported in Huon et al. (2012) where in this study, RF heating was observed to drastically increase the extraction rate of the contaminants from 2.91 to 31.00 kg d^{-1}. In addition to high recovery of contaminants, it was also reported that with ISRFH, 80% of the conventional cold soil vapour extraction period was reduced. The energy consumption in ISRFH was comparable or lower than the conventional method. The successful finding in Huon et al. (2012) was repeated in another work by Holzer et al. (2013).

2.5.5 Biofuel Production

The hazardous by-products emission from engine combustion (e.g. nitrogen oxide, carbon dioxide and volatile organic compounds) and rapid depletion of the petroleum oil reserves are highly encouraging the usage of renewable energy such as bioethanol over non-renewable fossil fuels. The high production cost of bioethanol made from the fermentation of sugar cane or corn forced a replacement of feedstock to lignocellulosic biomass from wheat, barley, oats and flax among many sources of residue waste. However, this biomass possesses a complex physical structure that is resistant towards biological and chemical degradation which leads to low yield of sugar; the main ingredient required for the fermentation to bioethanol. Alkali or acid pretreatment of this biomass is required to breakdown the structure and the rate of the chemical reaction can be accelerated with heat. Recently, there has been a growing trend in the application of RF heating for this thermal purpose.

Hu, Wang & Wen (2008) applied RF heating in the pretreatment of switchgrass using sodium hydroxide to improve its enzymatic digestibility. From a comparison study they conducted between RF heating and conventional heating, the process heated via RF produced higher amount of the required sugar than the conventional heating. They also observed that uniform heating can be achieved in samples of high solid contents (20-50%) with RF heating, which is difficult to achieve for conventional heating as this sample is highly viscous not allowing proper mixing for proper heat distribution.

In addition to that, RF heating technology was also studied for the production of biodiesel from waste cooking oil and beef tallow (Liu, McDonald & Wang, 2010; Liu et al, 2011). Both studies indicated that radio frequency heating significantly assisted the process that led to high conversion rates of biodiesel from 98.8 % waste cooking oil and 96.3 % in beef tallow. The success of RF heating in these studies strengthens and widens the potential usage of this technology in other fields.

2.5.6 Biomass Gasification

Biomass gasification is a process of producing energy from biomass at very high temperatures, in replacement of fossil fuel. Although biomass gasification produces useful commodities like fuel gases, char and chemicals, it also generates undesirable by-products like fly ash, NOx, SO_2 and tar (Anis & Zainal, 2011). RF heating was studied for biomass gasification to remove tar from the fuel gas or particularly known as the producer gas. High tar content in the producer gas can cause blockage of the downstream pipelines and foul the engines and turbines. According to the study by Jess (1996), high tar content removal in thermocatalytic treatment could be achieved if the process occurs at 1200°C with the residence time below 10 s. The success of RF heating application in removing tar content during thermocatalytic tar treatment process was reported in Anis, Zainal & Bakar (2013). Result from this study demonstrated that the RF heating applied was able to heat the catalytic bed of 120 mm to 1200°C within 20 minutes at a power of 700 W. This strengthens the potential of RF heating as an alternative thermal treatment for tar removal in biomass gasification.

2.5.7 Sand and Shale Oil Extraction

Another interesting application of RF heating is in the field of oil extraction. The feasibility of RF heating for extracting oil from sand was highlighted in Jha (1999) and Kasevich et al. (1994). Raytheon (2006) proposed a fast, cheap, efficient and safe way through a combination of RF heating with supercritical extraction and projected that years taken to actually produce oil using the conventional in-ground heating can be shortened to two months with RFCFE technology, along with higher product yield. In conjunction to the success in site observations of RF heavy oil recovery in Davletbaev et al. (2010), a model to describe the heating in reservoir under the influence of radio frequency electromagnetic radiation was developed by Davletbaev, Kovaleva & Babadagli (2011).

2.5.8 Biomedical

Thermal ablation (TA) is a new technique for destroying small sized tumor cells in cancer patients without undergoing surgery. In TA, the tumor cell is destroyed by heating up the cell using probes which transmit radio frequency or microwave energy. Several studies have shown that RF ablation (RFA) can result in complete tumor eradication in properly selected candidates, and have provided indirect evidence that the treatment improves survival (Lencioni & Crocetti, 2007). A recent successful deployment of RFA was reported by Ding et al. (2013) where a 99% eradication of tumor was observed in 85 liver cancer patients with 98 tumor lesions who received this treatment. At the moment, RFA treatment is limited to lung, kidney, liver, breast and bone cancers, not suitable for deep-seated tumors. For deep-seated tumors, magnetic nanoparticle-mediated hypothermia using nanoparticle combined with RF heating is proposed (Wen, 2013).

2.5.9 Nutraceutical Extraction

Numerous publications on microwave assisted extraction (MAE) for a variety of nutraceutical compounds proved its capacity as an extraction method. As any other methods, MAE is also bound to several limitations such as limited extractor size and degradation of target compounds. Microwave has low penetration depth, thus making it difficult for scaling up of the system for industrial use. As a result of this, an early work on developing radio frequency extraction was initiated by researchers from Saskatchewan University. Their work involved designing and developing a radio frequency extraction apparatus and evaluating the operating parameters (i.e. thermal properties, dielectric properties, sodium chloride concentration) that affect the RF extraction (Izadifar et al., 2009; 2008a; 2008b and 2007).

2.6 The Advantages of Computer Modeling and Simulation in RF Heating System Development

Nowadays, modeling and simulation are essential steps in any industry. Modeling and simulation is a rapid tool that can be used to virtually build and evaluate the feasibility of the new system and through this virtual assembling, operational time and cost will be saved. Recently, for RF heating, modeling and simulation studies have increased, partly owing to the development of sophisticated modeling and simulation software suitable for dielectric heating. The ability to evaluate the feasibility of a new RF heating system to be developed or scaled up, to understand and evaluate the heating distribution and power density performance in the system, and to develop the ability to predict and troubleshoot issues relating to the RF system, are some of the advantages provided via this approach.

Excellent examples of the successful use of modeling were illustrated to effectively assist the evaluation of the feasibility of a new RF heating design as can be found in Dev et al. (2012) and Tiwari et al. (2011b). Dev et al. (2012) applied finite element modeling (FEM) to evaluate the feasibility of an in-shell egg heating process using RF parallel plates and coaxial cavity. Through the simulation results, it was observed that the heating distribution was not uniform in the egg for both RF designs proposed, however the simulated results also demonstrated that the uniformity can be improved by rotating the egg during the RF heating process. The gap between the egg and the electrodes and the orientation of the egg between the electrodes were optimized to achieve high heating uniformity based on the simulation. The simulation results were then validated by experimental results, and the close agreement indicated that FEM is suitable for predicting the heat distribution within RF heated eggs. Information scarcity on the behavior of RF heating of dry food materials drove Tiwari et al. (2011b) to conduct a study. Wheat was selected as the sample to represent dry food. Their simulation study, also using FEM, showed that uneven heating occurred in the sample, where high temperatures concentrated in the middle layer and at the edge of the sample. From their simulation results, it was confirmed that the uneven heating was attributable to the non-uniform power density distribution in the sample.

Besides knowing the feasibility of the technology, it is also important to know and understand how heat, power density and electric field are generated and distributed in an RF heating environment because these properties are directly linked to the efficiency and effectiveness of the system. For example, non-uniform heating, in general, could cause degradation of product quality over time. Romano et al. (2008) developed a 3-dimensional (3D) multi-physics meat batter model using FEM to evaluate the effects of shapes (cube, cylinder and sphere) and sample orientation on the heating rate and temperature distribution in the sample. Their simulation study demonstrated that the sample shape and orientation have significant influence on the mentioned properties. The highest heating rate and uniform heating were observed in the regular cubic shaped products, which inferred that this type of shape is more suitable for RF heating. Meanwhile, a spherical shape is the least favoured geometry for RF heating. The effect of the sample's orientation was greater for a cylindrical sample as vertically oriented sample showed to impose better heating tendency and uniformity than the horizontal placed cylinder. Tiwari et al. (2011a) studied the effect of the sample shape, size, sample position between electrodes, dielectric properties of the sample and the medium, electrode gap and electrode configuration on RF power distribution and power density index using a computational modeling and simulation approach. Among the important findings from this study, the power distribution in all the three shapes was found different, where edge heating was prominent for cubic and cylindrical samples while centre heating was found for the ellipsoid sample. In addition to that, it was also observed that large samples had better heating uniformity when exposed to RF heating. Wang et al. (2012) modeled and simulated the electric field distribution and heating patterns of meat lasagna during RF heating as influenced by the dielectric properties of the components in the lasagna. Their study revealed that the difference in the dielectric properties due to heterogeneity in the meat lasagna components caused large variation in electric field and dissipated energy in the lasagna. However, the actual experiment and simulation results demonstrated that slight uneven heating can still occur even when the components of the meat lasagna in the container were evenly distributed and kept in close contact. Uyar, Erdogdu & Marra (*in press*) developed a 3D multi-physics meat cube model to study the effects of sample size on the heating rate and the power absorption in the sample. From their simulation work, it was evident that when the distance between electrodes was fixed, smaller sample size caused slow heating of the sample, and that simply due to the presence of a large air gap. However, when the distance between the sample and top electrode was limited, faster heating rate was observed with smaller gap between these surfaces.

Nevertheless, for a successful model and simulation approach, accurate data on the properties of the materials is required (for example dielectric properties, heat capacity etc.) and must be available.

2.7 Challenges and Future Studies for RF Heating

RF heating has the potential to be developed for numerous industrial applications however until today the commercialized RF units are really few due to their relative complexity, high initial installation capital investment and insufficient in-depth knowledge about RF heating and its effect on commodities involved. Marra et al. (2009) have comprehensively summarized the prospective work in RF heating which covers the followings areas:

i. evaluation of qualities and shelf-life of various food commodities after RF heating treatment
ii. investigation of the temperature distribution within treated products
iii. design and scale up of RF heating units for industrial level developments to enable related cost estimation and evaluation of system efficiency
iv. production of more dielectric properties data for food commodities and packaging materials
v. wider utilization of mathematical modeling approaches to demonstrate RF heating system's capacity, versatility and efficiency

In addition to the above suggestions, there are additional proposed studies which are necessary in this area based on the recent wider applications developments:

i. To intensify the application of this technology on heating liquid and solid-liquid mixture materials. Most of the published reports available on RF heating focussed on treating dry materials or low moisture content materials. This suggestion is being made since RF heating is shown to be feasible for heating liquid or solid-liquid mixtures. On top of that, this technology is considered as the better alternative over the temperature gradient conventional methods to successfully heat liquid or solid-liquid mixtures without overheating or affecting the original quality of the materials.
ii. To have more studies on the dielectric properties of various potential commodities besides food since this technology has also been investigated intensively for other sectors. In addition to that, it would also be a benefit to search for correlations between dielectric properties and conductivity on heating rate or temperature rise of these materials.
iii. It is also highly imperative to conduct and publish more investigations on the effect of RF heating on the quality of the products. Most of the studies only

highlight on its positive effect on microbial reduction however the information relating to the taste, texture, flavour, etc. after the treatment is still lacking.

iv. To provide more information on the efficiency and economical aspects of RF heating systems that have been applied in various fields which are currently commercially available.

v. To develop the computer aided technology to aid the optimization of system's efficiency and the cost-benefit ratio of the technology through computer simulation.

Connecting Statement to Chapter 3

The mechanism of heating, influential factors, general applications, computational modeling and the importance of simulation aids and future directions of RF heating were discussed in **Chapter 2**. Besides understanding the fundamental of RF heating, it is also crucial to have ample information on the breadth of knowledge available on apple phenolic compounds. In the subsequent chapter, a comprehensive review for apple phenolic compounds which is the key focus of the study, is presented. The review in **Chapter 3** covers the important aspects of apple phenolics namely their health benefits, extractions processes and applications gathered from the available literature. The potential areas of apple phenolic compounds study are also discussed and proposed.

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who prepared the experimental design, conducted the laboratory work, analysed the results and prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated ideas, provided the technical advice, suggestions, experiment facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.

Chapter 3 - Advances in Apple By-products Phenolic Compounds

3.1 Abstract

Scientific evidence is confirming that the phrase “an apple a day keeps the doctor away” is not merely just a saying. As with other fruits, apples contain a lot of healthy nutrients, encompassing the broad range of bioactive compounds namely vitamins, dietary fibers and phenolic compounds. Epidemiological studies are showing the strong relationship between fruits and vegetables consumption and the reduction of health risks and the effects of aging. This relationship encourages incorporation of more fresh fruits and vegetables in the daily diet, together with additional intake of bioactive compounds as supplements whenever possible. The apple processing by-products are rich in bioactive compounds especially phenolic compounds which have high potential use as natural antioxidants, antimicrobials, colorants and therapeutic agents. Recent global research trends from academia and industries are showing increasing interest in exploring and utilizing apple phenolics, therefore the purpose of this review is to bring in most of the available studies related to apple phenolics, explicitly focusing on the types and benefits of phenolic compounds sourced from apples, methods of extraction and quantification of compounds and their prospective applications in food, beverages, pharmaceutical, cosmetics, bio-polymeric films and textile industries. Future studies on apple phenolics are also briefly discussed.

Key words : apple-byproducts , phenolic compounds, natural antioxidants, functional food

3.2 Introduction

Apple is one of the most cultivated tree fruits with oranges, grapes, mango, and bananas. It is reported that more than 7500 cultivars of apples are grown all over the world, and according to the World Market and Trade report from the United States Department of Agriculture, 66.5 million metric tonnes of apples were produced in 2012 and it is estimated to increase up to 67.9 million tonnes in 2013. From the total apple produced in 2012, 11.8 million tonnes were processed meanwhile 54.2 million tonnes were freshly consumed. China is the largest grower of apples with half of the world apple production dominated by this country, followed by the United States of America and European Union countries.

In general, apples contain water, carbohydrates, protein, fat, various vitamins and minerals. Research demonstrated that all parts of an apple are functional for human consumption. Apple flesh is used for making juice, cider, puree, jam or eaten fresh meanwhile its by-product pomace is commonly used for production of pectin, livestock feed, vinegar, citric acid, enzyme, ethanol and fertilizer (Wolfe & Liu, 2003). Apple seed was recently studied as a potential source for natural colorant as an alternative to synthetic petroleum based yellow dye, tartrazine (Guyot & others, 2007; Fromm & others, 2013).

Vegetables and fruits processing industries generate a high volume of wastes which, if not utilized, can cause considerable disposal issue and environmental impact. Wastes from these industries generally contain valuable materials that can be converted into many useful and valuable products such as dietary fiber, gelling agent, natural antioxidant, antimicrobial agent, anti-browning agent, natural flavoring, natural colorant, etc. (Schieber et al., 2001; Balasundram et al., 2006; Ayala-Zavala et al., 2011). The by-products of the apple processing industry contain a high concentration of phenolic compounds since most of these compounds are principally found in the peel (Schieber et al., 2001; Łata, Trampczynska & Paczesna, 2009). Phenolic compounds are considered an important functional food ingredient that could be used in nutraceutical supplements, and functional food and beverage applications. According to a Leatherhead Food Research report published in 2012, the current world market value for phenolic compounds is estimated around $200 million (Wijngaard et al., 2012). Its antioxidant, anti-inflammatory, antimicrobial, anti-cancer, antihypertensive and anti-aging characteristics strengthen the apple by-product potential value in the phenolic compounds market.

3.3 Apple Phenolic Compounds and their Health Benefits

3.3.1 Phenolic Compounds

Phenolic compounds are plant secondary metabolites. Even though traditionally phenolic compounds are known as anti-nutrients and having non-nutritional values, later discovery has showed that these compounds play a significant role in plant development. In plant metabolism, phenolic compounds contribute to the pigmentation, are involved in growth and reproduction and protect plant from ultraviolet radiation, pathogens, predators and other plagues, etc. (Bravo, 1998).

Epidemiological studies have shown the correlation between the lower risks of chronic diseases in human populations with high consumption of fruits and vegetables. Besides vitamins and fiber which are known for their significant human health impact, fruits and vegetables also contain a high amount of phenolic compounds. Apples are rich in antioxidant compounds and most of the antioxidant activity in the apple fruit is contributed by its phenolic compounds (Vieira et al., 2011). Eberhardt et al. (2000) studied the antioxidant activity of fresh apple extract and they concluded that high antioxidant activity of 83.3 ± 8.9 percent in this total oxyradical-scavenging capacity (TOSC) assay was due to the phenolic compounds content and not due to the vitamin C present in the apple extract. Vitamin C in this extract only contributed to 0.32 percent of the total antioxidant activity. The high antioxidant capacity of apples is supported by another study reported by Heo et al. (2004) that 90% of the antioxidant activity of apple extract is contributed by the phenolic compounds present in the apple. Many studies on apple phenolics have been carried out to prove the high antioxidant and high scavenging performance of apple extracts. In comparison, apple is ranked as the fifth highest fruit for its content in total phenolic compounds, after cranberry, blackberry, raisins and blueberry (Balasundram et al., 2006).

Based on the difference in their chemical structures, phenolic compounds are divided into eight groups, namely simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans and lignins (Naczk & Shahidi, 2006). As a general rule, the antioxidant activity of these phenolic compounds depends highly on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings (Balasundram et al., 2006). In apples, the phenolic compounds found are predominantly from the group of phenolic acids (hydroxycinnamic acid derivatives and hydroxybenzoic acids) and flavonoids (flavonols, flavanols flavanones, flavones, dihydrochalcones and anthocyanins) (Reis et al., 2012).

Cultivars, region, environmental exposure, growing period, harvest time and post-harvest activities are the factors that affect the availability and amount of phenolic compounds in apples. Antioxidant activity of the apple is different between different cultivar, and the antioxidant activity is positively correlated to the amount of phenolic compounds present (Boyer & Liu, 2004). Studies by Tsao et al. (2003) and Lata et al. (2009) demonstrated that apple peel contains more types of phenolic compounds and higher amount as compared to flesh. Quercetin glycosides and cyanidin glycosides are example of compounds that are exclusively found in apple peel (Wolfe et al., 2003). Chlorogenic acid, catechin, quercetin and phloridzin, to name a few, are compounds recurrently identified in apples that have been extensively studied and proven to be strong antioxidants which are correlated to numerous health benefits. The common types of phenolic compounds available in apples and apple pomace are listed in Table 3-1.

Table 3-1 : Common phenolic compounds found in apples and apple pomace

Phenolic	Numbers of	Growing Location	Researchers
Chlorogenic acid	8	Canada	Tsao et al. (2003)
Catechin	19	Poland	Łata and et al. (2009)
	Not mentioned	New Zealand	Schieber et al. (2003)
Epicatechin	1	Italy	Mari et al. (2010)
	6	Serbia	Ćetković et al. (2008)
Procyanidin B1	11	Spain	Diñeiro García et al. (2009)
Procyanidin B2	1	Not mentioned	Alonso-Salces et al. (2001)
	Not mentioned	Spain	Alonso-Salces et al. (2005)
Quercetin	6	Spain	Picinelli et al. (1997)
	104	Switzerland	Ceymann et al. (2012)
Quercetin glycosides	Not mentioned	China	Bai et al. (2010)

3.3.2 Health Benefits of Apples

Phenolic compounds exhibit many properties such as antioxidant, anti-inflammatory, anti-carcinogen, antimicrobial, cardio-protective, etc. which are fundamentally important as protective agents against diseases and the effects of aging. Epidemiological studies show the association between the consumption of a diet loaded with phenolic compounds from vegetables, fruits, cereals and tea to lower risks of diseases and a significant slowdown of the aging process. Several evidences related to protective and therapeutic effects of apple phenolics, on three major health issues, namely cognitive decline and neurodegenerative diseases, cancer and cardiovascular diseases will be presented in this paper. A comprehensive review on the published evidence of the effects of apple phenolics against cancer, cardiovascular disease, asthma, diabetes, weight loss and bone health obtained through human or animal trials, *in vivo* and *in vitro* studies can be found in Boyer & Liu (2004) and Hyson (2011).

3.3.2.1 Cognitive Decline and Neurodegenerative Diseases

Growing evidences are showing that neuronal and behavioral decline are unavoidable, owing to the fact that the neurotransmitter receptor sensitivity is altered during the aging process. Eventhough naturally occurring, the aging process could contribute to cognitive decline, however the role of nutrition deficiency in this matter also cannot be ignored (Youdim & Joseph, 2001). Epidemiological studies have showed that a diet rich in fruits and vegetables, containing numerous phytochemicals can assist in preventing diseases, including cognitive decline due to aging and neurodegenerative diseases such as Alzheimer. Heo et al. (2004) reported the anti-neurodegenerative capacity of apple phenolics extracts. Their *in vitro* study elucidated that fresh Red Delicious extract which contained 239.9 mg/100 g vitamin C equivalent antioxidant capacity protected rat pheochromocytoma neuronal cells (PC-12) from H_2O_2 oxidation in a dose dependent manner and increased viability of PC-12 cells. Among all phenolic compounds available in their Red Delicious extract, quercetin was highlighted as the main agent providing protection towards cells oxidation. Quercetin, a compound abundantly found in apple, has been extensively studied for its protective benefits for cognitive decline and neurodegenerative diseases (Dajas, 2012). Other studies by Ortiz & Shea (2004), Rogers et al. (2004) and Tchantchou et al. (2005) showed that apple juice concentrate, high in antioxidants was able to prevent the oxidative stress induced by beta-amyloid in culture test and prevent impaired cognitive performance on aged, genetically and diet deficient mice. Results of these studies support the potential of apple antioxidant compounds as cognitive decline and neurodegenerative protective and therapeutic agents.

3.3.2.2 Cancers

According to the World Health Organization (WHO), in 2008, 13 % or 7.6 million of the world's death in the population were recorded as caused by cancer, making clear this disease as one of the deadliest threat to human beings. Since the medical field has started to unfold the mystery of cancer, extensive work has been laid out in search for cures and protective ways against this deadly disease, including the use of natural remedies such as apple extracts. The ability of apple extracts to inhibit cancer growth has been studied by Eberhardt et al. (2000), Veeriah et al. (2006), McCann et al. (2007) and Sun & Rui (2008). Early work by Eberhardt et al. (2000) demonstrated the positive effect of apple, where they observed that fresh apple extract (from flesh and peel) was shown to inhibit the proliferation of colon and liver cancer cells with no sign of cytotoxicity. They concluded that the strong inhibition of cancer cells proliferation is due to the phenolic compounds (natural antioxidants) in the apple. The anticancer characteristics of apple were also shown in the work of Veeriah et al. (2006) where an apple extract was able to inhibit cell growth of colon cancer. In the *in vitro* study by McCann et al. (2007), the effect of crude apple extract on human colorectal cancer development was examined and it resulted that polyphenols' rich apple extract protected cells against damage of DNA, and caused an improvement on the barrier function and invasion inhibition. In addition to that, an *in vitro* study by Sun et al. (2008) demonstrated the ability of an apple peel extract to inhibit the proliferation of estrogen-dependent and estrogen-independent human breast cancer cells.

3.3.2.3 Cardiovascular Diseases

In 2008, WHO estimated that 17.6 million people died due to cardiovascular diseases and this number is projected to increase to 23.3 million deaths yearly by the year 2030. In relation to this chronic disease, apple extracts have been studied and found to be protective against cardiovascular diseases. The capability of apple extract in curbing cardiovascular diseases has been studied by Balasuriya & Rupasinghe (2012) and Thilakarathna, Rupasinghe & Needs (2013). Flavonoid rich apple peel extract inhibited angiotensin converting enzyme (ACE) from becoming a catalyst in the blood pressure increase mechanism (Balasuriya & Rupasinghe, 2012). ACE is the enzyme responsible for the development of hypertension thus by inhibiting ACE, high blood pressure can be controlled. Another study by Thilakarathna et al. (2013) showed that flavonoid rich apple peel extract could reduce serum total cholesterol and non-high density lipoprotein cholesterol in hamsters. The positive results from these studies open up possibility of utilizing apple extract as part of treatments for cardiovascular diseases.

3.4 Apple Phenolics Extractions

3.4.1 Factors Affecting Extraction

In developing an efficient and safe extraction methodology for bioactive compounds, several factors, for example choice of solvent, extraction mechanism, temperature, time, particle size and energy utilization, must be taken into consideration (Naczk & Shahidi, 2004; Wang & Weller, 2006). The importance of these factors is briefly discussed here.

3.4.1.1 Solvent

Solvent selection is important in the extraction process because it relates to three key aspects; target compound stability, process efficiency and lastly, consumer and environment safety. Increase in awareness and concern about environmental safety drives humans towards green technology, making utilization of non-toxic solvents and low solvent extraction process more attractive and preferable.

Solubility of phenolic compounds in solvent is affected by two factors; firstly the chemical nature or structural diversity of the compound and secondly the polarity of the solvent used. Phenolics structure can vary from simple (eg. phenolic acids) up to highly polymerized (eg. tannins) structure, and also exist as complexes with carbohydrates, proteins and other plant components which increases their insolubility in many solvents (Naczk & Shahidi 2004; 2006). The structure of phenolic compounds plays a major role in affecting the degree of polarity, hydrophilicity, stability and degradation tendency of the compounds. The diverse capacity of a solvent to extract phenolic compounds is strongly contributed by the solvents' unique chemical properties, degree of solvents concentration or degree of combination with other solvents. In general, the usage of a particular solvent depends strongly on the nature of the phenolic compounds to be extracted; polar compounds are to be extracted using polar solvents, and non-polar compounds are to be extracted with non-polar solvents. Solvent not only affect types of phenolic compounds extracted but also affect the bioactive activity and stability of the compounds.

Methanol, acetone, ethanol, water and combinations of these solvents with water, are commonly reported in literature as solvents tested for the extraction of phenolic compounds from apple (Lu & Foo, 1997; Escarpa & González 1998; Alonso-Salces et al., 2001; Pinelo et al, 2008; Wijngaard & Brunton , 2010; Reis et al., 2012). Methanol has been used in numerous extraction studies of phenolic compounds including apple phenolic extraction and has been shown as the most efficient solvent for the extraction of many phenolic compounds. Alonso-Salces et al. (2001) demonstrated that aqueous methanol (50-75% methanol in water) extracted more phenolics from apple, however recent comparative solvent studies are showing that aqueous acetone extraction yielded higher recovery of phenolic compounds than methanol (Michiels et al., 2012). Research observations demonstrated that methanol is more efficient in extracting lower molecular weight phenolic compounds meanwhile aqueous acetone is the better option for extracting higher molecular weight phenolic compounds (Dai & Mumper, 2010). Nevertheless, the works on finding the most efficient solvent still continues and the frequent usage of aqueous ethanol in numerous reports shows this solvent's preference over methanol and acetone due to their toxicity.

Besides methanol, acetone, ethanol and combination of these solvents with water, several works have been performed on studying the feasibility of water only as a solvent for extracting apple phenolic compounds (Çam & Aaby, 2010; Pingret et al., 2012; Reis et al., 2012). It is a reasonable choice since water is safe, inexpensive and easily available, however experimental results have demonstrated that water is not a very effective solvent for extraction. Apples, as in other plants samples, contain a vast range of phenolic compounds with variable degree of polarity and hydrophobicity. Due to water's high polarity, it is probable that only a small amount of highly polar compounds will be extracted from the sample, based on theory of "like dissolves like". Studies by Rupasinghe et al. (2011) and Reis et al. (2012) demonstrated that water was not able to extract flavonols from apple pomace however it extracted other phenolic compounds. In addition to that, water-extraction interaction could trigger the formation of hydroxyl radicals, which promote the degradation of phenolic compounds during extraction (Biesaga, 2011) and lead to low yield of beneficial compounds as observed in the work of Paniwnyk et al. (2001).

Apples contain anthocyanins (cyanidin 3-galactosides), which require an acidified extraction solvent to ensure compound stability and high recovery, therefore an extra criteria of the solvent/solvent mixture must be included which is its acidity level. Varying the pH of the solvent alters the chemical forms of the anthocyanins and in a pH higher than 7, anthocyanins are prone to degradation and are most unstable (Castañeda-Ovando et al., 2009). Tabart et al. (2007) compared the extractability and stability of phenolic compounds from black currant leaves and buds using acidified acetate or glycine buffers from pH 2.2 to 5 and concluded solvent with acidity of pH 3 is the best condition for this extraction. Acidity is also important for hydrolysis (production of a stable form of the compound, aglycones), which will be discussed further in Section 3.4.1.6. Furthermore, the acidic solvent extraction also offers another advantage since the acidic condition can help prevent oxidation of the phenolic compounds during extraction. An example of the successful use of diluted acid on apple pomace for simultaneous extraction of phenolic compounds and pectin was presented in Schieber et al. (2003).

Another type of extraction solvent that is gaining popularity is ionic liquid (IL). These ionic liquids are actually organic salts that exist as liquids below 100°C. Ionic liquids offer several advantages over common volatile solvents due to their negligible vapor release issue, good thermal stability, miscible with water and other organic solvents, tunable viscosity, good dissolving activity and good extract ability, which is mainly contributed by their adjustable chemical structures (Du et al., 2009; Bonny et al., 2011). Ionic liquids have been shown to be more effective than methanol and water in extracting phenolic compounds from brown algae, *Laminara japonica* Aresch (Han et al., 2011).

3.4.1.2 Extraction Method

Another important factor for consideration is the extraction method. Conventional maceration, reflux heating, Soxhlet extraction, hydro-distillation, pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), microwave assisted extraction (MAE), solvent-less microwave assisted extraction, radio frequency assisted extraction (RFAE), pulsed electric field extraction (PEFE), ultrasonic assisted extraction (UAE) and microwave-ultrasonic extraction are extraction systems available nowadays (Wang et al., 2006; Lianfu & Zelong, 2008; Vilkhu et al., 2008; Izadifar et al., 2009; Li et al., 2013; Azmir et al., 2013). Each of these methods operates under different mechanisms and each one may offer different advantages and disadvantages. It is necessary to understand the extraction mechanisms involved as this will assist in determining which method is most suitable to be applied for a given process. The extraction method contributes to the process efficiency since it is directly linked to the quantity and quality of yield obtained, total extraction time requirement, cost of production and maintenance, and last but not least production and operational safety.

Studies have demonstrated that some of the methods mentioned earlier perform exceptionally better and faster than other methods due to their different mechanism of extraction. Table 3-2 below is a short summary of the extraction mechanisms involved. Nevertheless, one must not forget that the nature of the sample to be extracted must also be suitable for the chosen system to ensure high recovery and efficiency.

Table 3-2 : Methods of extraction and mechanisms involved

Extraction Method	Extraction Mechanism	References
Soxhlet extraction	Target compounds diffuse into solvent vapor that passes through bed of sample.	Luque de Castro & Priego-Capote (2012) Zygler et al. (2012)
Hydrodistillation	Using boiling temperature of solvent to break plant matrix and to enhance diffusion of target compounds or materials into the solvent. Target compounds or materials are being separated based on volatility.	Azmir et al. (2013)
Conventional extraction (maceration)	Target compounds diffuse into the solvent due to a concentration gradient, diffusion rate may be increased with temperature increase and mixing.	Azmir et al. (2013)
Pressurized liquid extraction (PLE)	Using high temperature and pressure to modify extraction conditions. High temperature increases extraction rate via increasing solubility of solute in solvent and diffusion rate, disrupts the solid-matrix bond, and decreases solvent viscosity and surface tension. High pressure forces liquid into the solid matrix and helps to maintain high temperature solvent in liquid state.	Kaufmann & Christen (2002) Wijngaard et al. (2012)
Supercritical fluid extraction (SFE)	Using high temperature and pressure to cause supercritical state of selected substance (substance is in a mixed liquid and gas phase). This condition promotes high diffusion due to lower fluid viscosity and surface tension.	Wang & Weller (2006)
Microwave assisted extraction (MAE)	Two mechanisms: Firstly, interaction of water in plant matrix with microwave energy causes internal superheating that	Kaufmann & Christen (2002) Wang & Weller

	disrupts plant matrix, causing liberation of compounds into the solvent. Secondly, there is an increase of solvent penetration into the plant matrix due to migration of ions that leads to releasing of compounds into the solvent.	(2006)
Solvent -free microwave assisted extraction	Utilizing only the available water in plant matrix to interact with microwave energy to cause internal superheating that disrupts plant matrix. Water rich in bioactive compounds is collected in the bottom flask via gravitational force.	Li et al. (2013)
Ultrasonic assisted extraction (UAE)	Expansion and compression produces bubbles with high pressure. These bubbles eruption produce high jet liquids that can cause peeling or erosion of particle surface and particle breakdown.	Wang & Weller (2006) Vilkhu et al. (2008)
Pulse electric field (PEFE)	PEF exposure drastically increases membrane permeability by destroying cell membrane structure. This occurs as the result of charge carrying molecules repulsion that lead to formation of pores at weak area of membrane. Mass transfer increases and helps in speeding up the extraction process.	Azmir et al. (2013)

Comparative study between conventional solid-liquid extraction and PLE on apple pomace extraction performance has been performed by Wijngaard & Brunton (2009) and from their study, PLE application for apple pomace extraction shortened extraction time and reduced the solvent requirement, and on top of that, PLE apple pomace extract had 2.4 times higher antioxidant activity (DPPH assay) than the extract from the other extraction method tested. In a study by Alonso-Salces et al. (2005), PLE was shown to be less effective than UAE in extracting phenolic compounds from apple peel and pulps. Despite this observation, these researchers still suggested that PLE is the better option for industrial application as PLE can be a fully automated system. UAE yielded 30 % more total phenolic compounds than conventional extraction of apple phenolics in the work of Pingret et al. (2012). The advantage of optimized MAE (62.68 ± 0.35 mg GAE/100 g) in producing apple pomace extract which contained higher phenolic compounds value in comparison to UAE (54.76 ± 0.39 mg GAE/100g) and reflux extraction (46.33 ± 0.58 mg GAE/100g) was observed in the study performed by Bai et al. (2010).

Despite their extraction potential, some studies have demonstrated that certain methodologies, e.g. UAE, could cause some degradation of certain phenolic compounds (rutin in this case) during the extraction process when using an aqueous solvent (Paniwnyk et al., 2001). The liberation of reactive hydroxyl radicals that degrade rutin, from the interaction between the aqueous solvent and the sonication energy was identified as the reason to this low yield.

3.4.1.3 Temperature and Time

Increased temperature may increase extraction rate because high temperatures decrease the viscosity of the solvent (Wijngaard et al., 2009) which promotes better penetration of solvent into the matrix and at the same time weakens the solid-matrix interactions (Kaufmann & Christensen, 2002). Although these are valid facts, careful selection of temperature and time is necessary to avoid issues related to degradation and stability of the extracted compound, which later could lead to quality, quantity, toxicity and purification issues for the extract. Hence, it is necessary to perform studies on the effect of extraction temperature and time in relation to yield, bioactivity, stability and degradation in deciding the suitable temperature and time to be applied during extraction.

The positive effect of extraction temperature on yield and mass transfer coefficient was observed in Pinelo et al. (2008). Effect of extraction time on increasing yield of phenolic compounds via MAE was demonstrated by Bai et al. (2010). Generally, an increase in extraction duration will provide more time for the solid-solvent interaction thus allowing more target compound to be liberated into the solvent, however the yield will be constant when the extraction reaches its equilibrium point.

Several examples on the phenolic compounds transformation or degradation as affected by extraction temperature and duration were reported in Liazid et al. (2007), Wijngaard et al. (2009) and Dai et al. (2010). This phenomenon strongly depends on the complex interaction between compound structure with the extraction temperature and time. A study by Liazid et al. (2007) demonstrated that variable degrees of phenolic compounds degradation were experienced during MAE extraction of grapes performed at or beyond 120°C. However, they concluded that no compound degradation would occur if the extraction is performed at 100°C for 20 minutes. In another work, Wijngaard et al. (2009) demonstrated that high extraction temperature plausibly contributed to the formation of unwanted compounds, hydroxymethylfurfuran, in the apple pomace extract recovered using PLE. Effect of long extraction duration on phenolic compound degradation was highlighted in Dai et al. (2010).

3.4.1.4 Particle Size

Small particle size is always favorable in extraction processes as recovery of phenolics could be enhanced as a result of two mechanisms, firstly due to increase of contact area between solid and solvent and secondly due to the shorter diffusion path for the target compound to travel out from the solid matrices into the solvent. Shorter diffusion path means faster rate of extraction as emphasized by Fick's Second Law (Gertenbach, 2002). The effect of smaller particle size in shortening the extraction time which promoted 80% recovery of phenolic compounds from *Echinacea purpurea* roots sample was reported in Gertenbach (2002). A mass transfer kinetics study on the extraction of apple peel by Pinelo et al. (2008) demonstrated that extraction with smaller particle size (1.8 – 2.6$10^{-8}$ m^2/s) led to higher effective diffusivities than larger particle size (0.2 – 1.5 x 10^{-8} m^2/s) which correlates to higher recovery of target compounds. In addition to that, they also highlighted that proper agitation must accompany the extraction process with small particle size to ensure proper contact between solid and solvent, otherwise this could lead to major reduction of mass transfer. Similar results of higher recovery with smaller particle size were also observed in the work done by Herodež et al. (2003), Bucić-

Kojić et al. (2007) and Hojnik et al. (2008). Even though smaller particle size shows positive results on extraction, powder size particle must be avoided to prevent the recovery of unwanted compounds.

3.4.1.5 Liquid to Solid Ratio

The concentration gradient is a driving force for mass transfer. Theoretically, higher liquid to solid ratio may increase yield based on the theory that bulk liquid will wash up all compounds at the surface of the solid, thus creating higher gradient concentration between solid surface and its interior (Gertenbach, 2002). The advantage of using lower sample quantities in relation to solvent volume on increasing extraction yield of bioactive compounds was highlighted in studies by Cacace & Mazza (2003), Pinelo, Sineiro & Nunez (2006) and Bai et al. (2010). On contrary, the yield of bioactive compound was observed to increase with the reduction of liquid to solid ratio in radio frequency assisted extraction (Izadifar & Baik, 2008).

3.4.1.6 Pretreatment (Blanching, Enzymatic or Acid or Base Hydrolysis)

Blanching, enzymatic and acid or base hydrolysis are pretreatment steps prior to the actual extraction process. Incorporation of a pre-treatment step in phenolic compounds extraction process may promote preservation, stabilization and subsequently increase recovery of these compounds.

Blanching or hot water dipping can assist in deactivating oxidative enzymes that can cause phenolic compounds degradation. Wolfe & Liu (2003) studied the effect of blanching on apple peel phenolics and the result showed that blanching apple peel for 10 s was shown to be more effective in preserving phenolic compounds than a citric or ascorbic acid dip. Recent works on blanching of plant materials by Nantitanon et al. (2010) also demonstrated that this process preserves bioactive compounds.

Another pre-treatment step that is gaining popularity is enzymatic treatment. Enzymes assist in breaking cell wall and releasing structurally bound compounds, which enhance the liberation of bioactive compounds into the solvent. Plant phenolic compounds are classified into two categories i.e. cell wall linked phenols and non-cell wall phenolic compounds (Pinelo et al., 2008). The second category mentioned is easier to be extracted into the solvent because it is not bound to any compound. On contrary, the ones bound to cell wall require enzymatic hydrolysis to assist in liberating cell wall linked phenolic compounds into the solvent. Enzymes disintegrate the cell wall matrix to free the cell wall linked phenolic compounds and at the same time may release these compounds in their stable aglycone form. Enzymatic extraction of apple pomace by Young et al. (2005), Zheng et al. (2009) and Ajila et al. (2011) resulted in higher yield and antioxidant activity of the apple pomace extract, inferring that enzymatic hydrolysis facilitated the liberation of carbohydrate bound phenolic compounds. The key challenge in this area is to identify the suitable enzymes that can ensure the higher success of the hydrolysis.

The usage of acid or base hydrolysis as a pre-treatment is also common in extraction. Besides enzymatic hydrolysis, this is an alternative method to remove sugar moiety from the glycosides of flavonoids (Sivam, 2002). Consideration should be given on the concentration of acid or base applied, since a high concentration may cause degradation of naturally occurring glycosides therefore producing more extraction artifacts which later complicates the identification and purification process. The usage of acid hydrolysis in the extraction of apple phenolic compounds can be found in the work of Rupasinghe et al. (2011).

3.4.1.7 Yield and Energy Efficiency

There are many novel extraction methods available now, however most of the studies relating to these new systems emphasize only on the efficiency of recovery. In developing new processes, focus should not only be on efficiency of yield, but good utilization of energy should be considered and reported as well in order to promote commercial use. The information relating to recovery and energy efficiency can help industry to decide whether the process is economically viable. Energy utilization and efficiency of new extraction methods are rarely discussed in literature.

3.5 Published Work on Extraction and Quantification of Apple Phenolic Compounds

Stalikas (2007) listed four general procedures in preparing bioactive compounds encompassing pre-treatment, extraction, isolation and instrumental analysis. Table 3-3 summarizes extraction works on apple phenolic compounds that have been reported in literature. This summary helps in providing a general idea on the extraction methods applied, solvents used, chemical analyses methods explored and finally the types of compounds detected in apples.

Table 3-3 : Extraction methods reported in literature for extracting phenolic compounds from apple pomace/peel/juice

Method	**Extraction Procedure**	**Analysis Method**	**Compounds Detected**	**References**
Liquid-liquid extraction (LLE) (juice)	5 ml of juice was neutralized to pH 7 using sodium hydroxide (NaOH). Then the sample was extracted 3 times using 5 ml of ethyl acetate. The organic solvent fraction was evaporated until dry using rotary evaporator at 35°C and added with 0.5 ml methanol and filtered. Same steps were	**HPLC** C_6 column (250 x 4.6 mm, 5μm) Gradient method for neutral phenolics *Mobile phase A (2mM phosphoric acid).* *Mobile phase B (methanol).* *Flowrate was 0.8 – 1.0 ml/min.* *Detection for neutral phenolics was performed at*	catechin epicatechin procyanidin B1 procyanidin B2 procyanidin B5 chlorogenic acid phloridzin phloretin xyloglucoside hyperin	Picinelli et al. (1997)

	performed for aqueous fraction of the sample.	*280 nm.* Isocratic method for phenolic acids *Mobile phase (6% methanol in 2 mM phosphoric acid).* *Flowrate was 1.2 ml/min.* *Detection for neutral phenolics was performed at 320 nm.*	quercitrin isoquercitrin + rutin avicularin + unknown flavonol	
Solid-phase extraction (SPE) (pomace & cider)	A specific volume of sample was mixed with 10g/ml of ascorbic acid to avoid oxidation. Prior to SPE, this sample was adjusted to pH 7 with sodium hydroxide. This neutral sample was passed through C_{18} neutral fractionating column and washed with 10 ml water. The effluent was adjusted to acidic condition (pH 2) by mixing with 2M hydrochloric acid. Then this acidic effluent was loaded into preconditioned acidic column and washed with 5 ml 0.01 M hydrochloric acid. Compounds retained in columns were eluted using 12 ml 100 % methanol and evaporated till dryness at 35°C. The output from this evaporation was mixed with 0.5 ml methanol and filtered through 0.22μm filter for HPLC analysis.	**HPLC (neutral and acidic compounds from pomace and neutral compounds from cider)** C_{18} column (250 x 4.6 mm, 5μm) Gradient method for phenolics *Mobile phase A (2mM phosphoric acid).* *Mobile phase B (methanol).* *Flowrate was 0.8 – 1.2 ml/min.* **HPLC (acidic compounds in cider)** C_{18} column (250 x 4.6 mm, 3μm) Gradient method for neutral phenolics *Mobile phase A (2% acetic acid).* *Mobile phase B (methanol).* *Flowrate was 0.8 ml/min.*	**Pomace** catechin cinnamic ester procyanidin B1 procyanidin B2 procyanidin B5 epicatechin chlorogenic acid phloridzin phloretin xyloglucoside quercitrin isoquercitrin + rutin avicularin + unknown flavonol ρ-coumaric derivatives hydroxycinnamic acid derivatives **Cider** hydrocoumaric acid	Suárez et al. (1996)

		Detection was performed at 280, 320 and 360 nm.	ρ-coumaric hydroxycinnamic acid	
Solid-phase extraction (pomace)	3g of freeze dried ground pomace was mixed and stirred in 40 ml water for 90 minutes. Then the mixture was filtered. The extract residue was extracted with methanol or acetone. All these extracts were loaded in SPE C18 to remove sugar and isolate the compounds. Sugar was eluted using 300 ml 2% acetic acid and phenolic fraction was eluted using methanol with 0.1% hydrochloric acid. The extract was filtered and kept at -20°C for next analysis.	**Colorimetric/ spectrophotometric determination :** *i) total phenolic compounds* *ii) total flavonoids content* *iii)proanthocyanidin contents* *iv)ferric reducing antioxidant power* *v) antioxidant activity determination using DPPH* *vi) antioxidant activity β-carotene/linoleic acid assay* **LC-ESI/MS** C_{18} column (100 mm x 2.1 mm, 3 μm) Gradient method *Mobile phase A (0.5 % aqueous formic acid)* *Mobile phase B (0.5 % formic acid in 50:50 acetone:methanol (v/v))* Flowrate was 0.2 ml/min. Electrospray mass spectra data was measured for mass range m/z 100 to 1600. Capillary voltage was 3 kV cone voltage was 30 kV. Collision induced dissociation achieved at 10	hydroxycinnamic acid chlorogenic acid feruloylquinic acid quercetin isorhamnetin quercetin 3-O-arabinoside quercetin 3-O-galactoside quercetin 3-O-glucoside quercetin 3-O-rhamnoside quercetin-3-O-rutinoside epicatechin procyanidin A1 procyanidin B1 procyanidin B2 procyanidin C procyanidin D phloretin phloridzin phloretin 2'-O-xylosil-glucoside kaemferol	Reis et al. (2012)

		to 30 eV. Collision gas used was argon.		
Maceration-homogenizer or blender (peel) **Variety** Rome Beauty	25 g dried or fresh sample was extracted in 200g 80% acetone or 80% ethanol for 5 minutes. Extract was rotary evaporated at 45°C until 10 ml left, then mixed with distilled water up to 50 ml. Final extract was kept at -40°C until analysis.	**Colorimetric/ spectrophotometric determination :** *i) total phenolic compounds* *ii) total flavonoids content* *iii) anthocyanin contents* *iv) antiproliferative activity determination using inhibition of human liver cell* **Total antioxidant activity via TOSC assay**	not mentioned	Wolfe et al. (2003)
Maceration-blender (peel and flesh) **Variety** Golden Delicious Red Delicious McIntosh Empire Idared Northern Spy Mutsu Cortland	10 g of sample was mixed with 70 % aqueous methanol and filtered with Whatman filter paper and 0.45 μm syringe filter. Final extract was kept at -20°C until further analysis. For acid hydrolysis and monosaccharide analysis, the HPLC fraction was hydrolyzed using 1 M hydrochloric acid for 1.5 hour at 90°C. Then this sample was diluted with water and filtered using 0.45μm syringe filter.	**HPLC-DAD** C_{18} column Gradient method *Mobile phase A (6% acetic acid in 2mM sodium acetate buffer , pH 2.55)* *Mobile phase B* *(100 % acetonitrile)* Flowrate was 1.0 ml/min and injection volume was 10 μL. Detection was performed at 280, 320, 360 & 520 nm. **LC-MS** *Mobile phase A (6% acetic acid)*	chlorogenic acid *p*-coumaroylquinic acid catechin epicatechin procyanidin B1 procyanidin B2 other procyanidins cyanidin 3-galactoside quercetin 3-O-arabinoside quercetin 3-O-galactoside quercetin 3-O-glucoside quercetin 3-O-rhamnoside quercetin 3-O-rutinoside phloridzin 3-hydroxyphloretin 2’-	Tsao et al. (2003)

		Mobile phase B *(100 % acetonitrile)* **Acid Hydrolysis and Monosaccharide analysis** Dionex ion chromatograph coupled with CarboPac PA1 column (4 x 250 mm) and pulsed amperometric detector (PAD) *Mobile phase A (100 Mm sodium hydroxide)* *Mobile phase B (30 mM sodium hydroxide)* *Mobile phase C (water)* Flowrate was 1.0 ml/min and injection volume was 50 µL.	xylglucoside 3-hydroxyphloretin 2’-glucoside phloretin 2’-xyloglucoside	
Maceration (pomace)	Prior to extraction, 0.25g lyophilized pomace powder was mixed with 15 ml of selected solvent (ethanol/acetone) and homogenized for 30s at 10 000 rpm and 60s at 20 000 rpm. After homogenization, 25 ml of solvent was added and extraction was performed. Optimized extraction conditions proposed was extraction using *i)56% ethanol for 31 minutes at 80 ℃ or* *ii) 65% acetone for 60 minutes at 25 ℃.* Sample was vortexed and centrifuged for 20 and 10 minutes, respectively and kept at -20°C until further	**Spectrophotometric determination** *i) total phenolic compounds via Folin-Ciocalteu Reagent* *ii) antioxidant activity via DPPH assay* **HPLC-DAD** C_{18} column (150 mm x 4.6 mm I.D., 5 µm) Gradient method *Mobile phase A (acetic acid in 2Mm sodium acetate, pH 2.55)* *mobile phase B (100 % acetonitrile)* Flowrate was 1.0 ml/min and injection volume was	chlorogenic acid phloretin glycosides flavonol	Wijngaard et al. (2010)

	analysis.	10 μL. Detection was performed at 280, 320 and 360 nm		
Enzymatic Maceration	1 g of lyophilized apple peel with particle size of 1500 μm was added into liquid enzyme and water (extraction solvent) at solvent to solid ratio 40:1. Then this mixture was allowed to mix at constant stirring rate of 150 rpm and temperature 50°C for specific time interval. Sample was pasteurized at 90°C for 60s to stop the enzymatic process. Then, sample was vacuum filtered for the subsequent analysis step.	**Spectrophotometric method determination :** i) *total phenolic compounds via Folin-Ciocalteu Reagent* **HPLC** C_{18} column (150 mm x 3.9 mm) Detection was performed at 280, 316 and 365 nm.	chlorogenic acid procyanidin B2 epicatechin quercetin 3-O-rutinoside (rutin) phloridzin phloridzin derivatives	Pinelo et al. (2008)
Reflux (pomace)	5 g of dried pomace powder was mixed with 200 ml of 70% ethanol-water mixture, and undergone reflux extraction for 6 hours. Extract was filtered with 0.45 μm membrane and kept at -4°C until further analysis.	**Spectrophotometric method determination :** i) *total phenolic compounds via Folin-Ciocalteu Reagent* **HPLC** C_8 column Gradient method *Mobile phase A (90 % aqueous 0.1% acetic acid)* *Mobile B (10% acetonitrile)* Flowrate was 1.2 ml/min and injection volume was 10 μL. Detection was performed at 280 nm.	chlorogenic acid syrigin procyanidin B2 caffeic acid epicatechin cinnamic acid coumaric acid phloridzin quercetin	Bai et al. (2010)

Ultrasonic bath (peel and flesh) Golden Delicious Red Delicious Granny Smith Green Reineta	5 g fresh peel or 10 g fresh flesh extracted in 5 ml or 10 ml methanol with 1% BHT for 30 -60 minutes, room temperature extraction with absence of light.	**HPLC** C_{18} column (25 cm x 0.46 cm I.D., 5 μm) Gradient method *Mobile phase A (aqueous 0.01 M phosphoric acid), mobile phase B (100 % methanol)* Flowrate was 1.0 ml/min and injection volume was 20 μL. Detection was performed at 280 nm	chlorogenic acid caffeic acid catechin epicatechin procyanidin B1 procyanidin B2 procyanidin B3 phloretin derivative phlodrizin quercetin 3-O-rutinoside (rutin) flavonol glycoside	Escarpa & Gonzalez (1998)
Ultrasonic bath (pomace)	5 g of dried pomace powder was mixed with 200 ml of 70% ethanol-water mixture, and undergone sonication for 1 hour under room temperature. Extract was filtered with 0.45 μm membrane and kept at -4°C until further analysis.	Similar to analysis method in reflux extraction by (Bai et al., 2010) above.	chlorogenic acid syrigin procyanidin B2 caffeic acid epicatechin cinnamic acid coumaric acid phloridzin quercetin	Bai et al. (2010)
Ultrasonic bath (peel) Idared	0.5 g dried peel mixed in 25 ml methanol, acetone, ethyl acetate, chloroform or water. Sample was vortexed and extracted in ultrasonic bath for 60 minutes. Temperature was between	**HPLC** C_{18} column (150mm x 2.1 mm, 5μm) Gradient elution *Mobile phase A (0.1 % formic acid in water)* *Mobile phase B (0.1%*	quercetin quercetion 3-O-galactoside quercetin 3-O-rhamnoside quercetin 3-O-rutinoside	Rupasinghe et al. (2011)

	20 – 24°C. 1 g dried peel mixed in 50 ml methanol – hydrochloric acid mixture at various methanol (60, 70, 80, 90 and 100 %) and acid concentration (0, 0.01, 0.1, 1 and 2%). Sample was vortexed and extracted in ultrasonic bath at various time (15 to 75 minutes). Extract was filtered with 0.45 μm before succeeded to analytical instrument. Ultrasonic bath was at 20 kHz/1000 W.	*formic acid in acetonitrile)* *Flowrate was 0.35 ml/min* **MS/MS** Electrospray ionization in negative mode was used. The settings for negative mode were capillary voltage 3kV, nitrogen gas, flowrate 600 L/h at 375°C.		
Ultrasonic bath (peel and flesh)	Optimized extraction conditions proposed was water as solvent, 40°C extraction temperature, 40 minutes of sonication and 0.764 W/cm^2 ultrasonic intensity.	**Spectrophotometric determination :** *i) total phenolic compounds via Folin-Ciocalteu Reagent* *ii) antioxidant activity via inhibition of linoleic acid peroxidation* **HPLC-DAD** C_{18} column (5 μm) Gradient method *Mobile phase A (aqueous acetic acid 25ml/L)* *Mobile phase B (100%*	catechin epicatechin procyanidins phloretin xyloglucoside phloridzin chlorogenic acid ρ-paracoumaroylquinic acid quercetin 3-O-rutinoside (rutin) quercetin 3-O-galactoside (hyperoside)	Pingret et al. (2012)

		acetonitrile) Detection was performed at 280, 320 and 350 nm.	quercetin 3-O-glucoside (isoquercitrin) quercetin 3-O-xyloside (reynoutrine) quercetin 3-O-arabinopyranoside (guajaverin) quercetin 3-O-arabinoside (avicularin) quercetin 3-O-rhamnoside (quercitrin)	
Pressurized liquid extraction (peel and flesh) Golden Delicious	1 g of dried peel or 3 g of dried flesh was mixed with diatomaceous earth (1:1) ratio and placed in 11 ml or 22 ml stainless steel PLE cell. Extraction conditions proposed was 100% methanol as solvent, extraction temperature at 40°C, static extraction time 5 minutes, pressure 1000 psi and 2 extraction cycles. Extract was filtered with 0.45 μm nylon membrane and stored at -20°C until further analysis.	**HPLC-DAD** C_{18} column (300 x 3.9 mm I.D., 4 μm) Gradient-isocratic method *Mobile phase A (90% acetic acid, 10% water)* *Mobile phase B (100% methanol)* Flowrate was 0.8 ml/min and injection volume was 50 μL Detection was performed at 280, 320 and 370 nm.	catechin epicatechin procyanidin B2 unknown procyanidin phloretin 2'-xylglucoside phloridzin quercetin 3-O-galactoside (hyperoside) quercetin 3-O-glucoside (isoquercitrin) quercetin 3-O-arabinoside (avicularin) quercetin 3-O-rhamnoside (quercitrin) Unknown quercetin glycoside + rutin chlorogenic acid ρ-coumaric acid derivatives	Alonso-Salces et al. (2001)

Pressurized liquid extraction (pomace)	1 g of dried pomace was used for PLE. Extraction conditions proposed was 60 % ethanol-40% water as solvent, extraction temperature at 102°C, static extraction time 5 minutes, pressure 10.3 MPa Extract was centrifuged for 15 minutes, filtered with 0.22 μm syringe filter and stored at -20°C until further analysis.	Spectrophotometric determination : *i) total phenolic compounds* *ii) antioxidant activity determination using DPPH assay* HPLC-DAD C_{18} column (150 x 4.6 mm I.D.,5 μm) Gradient method *Mobile phase A* *(6% acetic acid in 2Mm sodium acetate buffer , pH 2.55)* *Mobile phase B (100% acetonitrile)* Flowrate was 1.0 ml/min and injection volume was 10 μL. Detection was performed at 280, 320 and 360 nm.	chlorogenic acid caffeic acid ρ-coumaric acid quercetin glycosides quercetin 3-O-rutinoside (rutin) phloretin glycoside	Wijngaard et al. (2009)
Microwave assisted extraction (pomace)	1 g of pomace powder was mixed with 22.9 ml of 62.1 % aqueous ethanol, and extracted with MAE for 53.7 seconds, at 70°C with microwave power of 650.4 W.	Similar to analytical method in reflux extraction by (Bai et al., 2010) above.	chlorogenic acid syrigin procyanidin B2 caffeic acid epicatechin cinnamic acid coumaric acid phloridzin quercetin	Bai et al. (2010)

3.6 Phenolic Compounds Applications

Numerous benefits or properties of phenolics e.g. antioxidant, antimicrobial, antiviral, anti-inflammatory, anticancer, antihypertensive and anti-aging effects reported lead to potential multi-purpose use of these phenolics in our daily lives. In this section, we summarize most of the applications for which apple pomace, apple extract, apple juice or specific apple compounds, relating to apple phenolic compounds, have been reported in the literature.

3.6.1 Health Supplements and Ingredients for Healthcare Products and Cosmetics

Gingko biloba (flavonoids and terpenoids), bilberry (anthocyanins), green tea (catechins), green coffee (chlorogenic acid), hawthorne, and tomato extract (lycopene) are several popular plants extracts available as health supplements in the market. Even though apple phenolic compounds are not as popular as the extracts mentioned above, apple extracts are gaining popularity indebted to the many recent findings relating to the health benefits offered by their phenolics. To date, an apple peel powder is available in the market as a health supplement distributed by Apple Poly LLC (Morrill, Nebraska, USA).

Besides consuming this extract as a daily health supplement, apple phenolics are also suitable to be made as ingredients for healthcare products and cosmetics. Yanagida et al. (2000) proposed an apple extract as a tooth paste ingredient after observing its antimicrobial effect against dental microbes. In addition to that, apple phenolics were also observed to be protective against skin pathogens and herpes viruses (Alberto et al., 2006; Suárez et al., 2010). Apple phenolics are also suitable as cosmetic ingredients for skin care because they contain phloridzin, a compound with high antioxidant capacity, antimicrobial and protective properties against ultraviolet radiation. Baldisserotto et al. (2012) have studied the potential of a synthetic phloridzin derivative as a formulation for face cream. A database summarizing apple phenolics applications in commercial cosmetics can be found in Anonymous (2008).

3.6.2 Natural Antioxidants and Colorings

Synthetic antioxidants and colorants have been under scrutiny for a long time due to the hypothesis that these chemicals could pose a potential health hazard. Nevertheless, toxicity studies on the negative effect of synthetic antioxidants and colorants on health are still inconclusive. As a safer option however, antioxidants and colorants from fruits and vegetables are proposed as substitute to these synthetic antioxidants and colorants due to their natural origins.

These natural antioxidants work in two ways, not only to prevent lipid oxidation or to scavenge free radicals, but also to add value to the products and provide a healthy quality. Several investigations have been performed to evaluate apple peel extract or compounds as natural antioxidants for food model systems. Sekhon-Loodu et al. (2013) have evaluated fractionated apple peel phenolics as a stabilizing agent for omega-3 polyunsaturated fatty acid enriched fish oil and the results indicated that the apple phenolics' inhibitory effect on fish oil oxidation was higher than α-tocopherol and synthetic butylated hydroxytoluene (BHT). Their experimental results showed that apple peel fractions containing a mixture of quercetin glycosides, epicatechin, phloridzin and cyanidin-3-0-galactoside have the highest antioxidant activity. Another study was conducted by Sun-Waterhouse et al. (2012) on the stability of encapsulated avocado oil fortified with phloridzin (a phenolic from apple) or BHT. Albeit the synthetic antioxidant BHT showed higher antioxidant activity in preventing avocado oil oxidation, these researchers insisted on the utilization of phloridzin for oil stability because this compound offers another two additional advantages over BHT i.e. it suppresses hydrolytic rancidity and adds nutritional value to the oil. Benzoin, ferulic acid, chlorogenic acid and quercetin have also been tested as lipid oxidation inhibitors in extruded food and results exhibited quercetin as the potent antioxidant for this purpose (Viscidi et al., 2004). Quercetin is abundantly available in apple.

Apples contain varieties of colored phenolic compounds which can also be an excellent source for natural colorants. Natural colorants have vast applications e.g. for food, pharmaceuticals, cosmetics, textile, pH indicator, histological staining, production of UV protective clothing, antimicrobial textile finishing, etc. (Shahid et al, 2013). Work on recovering and characterizing of a variety of colored phenolic compounds from apple has been performed by Guyot et al. (2007) and Fromm et al. (2013). In these studies, enzymatic oxidized apple extracts produced a yellow dye, which can be further explored as an alternative to synthetic yellow dye from petroleum base, tartrazine. However, more work is required to evaluate its stability and toxicology in food matrices. In addition, some plant phenolics have been investigated as dyes and ingredients for development of bio-functional or intelligent textile, a new concept of supplementing human bodies with extra antioxidant by allowing the skin to absorb the antioxidants embedded in the fabric. Flavonoids, tannins and resveratrol have recently been studied for the development of bio-functional textiles (Baliarsingh et al., 2012; Alonso et al., 2013).

3.6.3 Ingredients for Functional Foods or Beverages

The demand for functional food and beverage is increasing, indebted to the increase of awareness on the importance of maintaining good health even with busy lifestyles through healthy diet. Besides being offered in their regular form of health supplements, foods and beverages can be a good medium for delivering the goodness of the fruits and vegetables' phenolic compounds to human. Vegetable and fruit phenolic compounds which contain high antioxidant activity are potential ingredients for functional foods and beverages. Since many diseases development are linked to high amounts of free radicals in the body, this imbalance can possibly be neutralized with sufficient intake of antioxidants.

Recently, a study by Sun-Waterhouse & co-workers (2010) on the utilization of apple phenolic compounds as functional ingredients in a snack bar can be found in literature. In their study, the regular snack bar was transformed into a functional food by fortifying it with apple dietary fiber and phenolic compounds for increasing fiber content and antioxidant value of the product. Sun-Waterhouse et al. (2011) and Sivam et al. (2011) reported their work on the development of bread fortified with fruits phenolic and pectin. From these studies, the addition of these compounds has contributed to the desirable and undesirable effects of the dough and bread physicochemical properties. Yogurt, a world-wide growing popularity food, has also been studied as another quick source of high antioxidant food even though yogurt is already known as an excellent healthy choice as a source for probiotics. Sun-Waterhouse et al. (2012) developed a new yogurt drink incorporated with apple extract and pectin. Their study showed that the addition of the apple extract, which is rich in phenolic compounds, has positive effect on the chemical, physical and microbiological properties of the yogurt. Healthy desserts made from 100 percent apple pomace, rich in phenolic compounds and dietary fiber, have recently been developed by Haghighi & Rezaei (2013).

In addition to food, beverage is another easy medium for antioxidant supplementation. Milk is one example that has been studied as a functional beverage, even though milk itself is naturally nutritious. Wegrzyn et al. (2008) have conducted a study on the fortification of milk with apple phenolic compounds. Their study showed that apple polyphenols fortified milk has good physical stability and at the same time its antioxidant capacity can be retained even after the processing stage. Bioactive drinks, specifically for cardio protection, which comprise a mixture of fruit juices (including apple) and a ginger extract have recently been formulated by Gunathilake et al. (2013).

3.6.4 Ingredients for Development of Active Biopolymer Film (Food and Pharmaceutical)

Increasing evidences are supporting the capability of phenolic compounds as agents for increasing physicochemical and bioactive properties of biopolymer smart films (Wang et al., 2012). Biopolymer films are preferable over the commercial petroleum derived films due to their degradability and other contributions towards minimizing ecological impacts. Biopolymer films have numerous applications in food and pharmaceutical industries as packaging material, material coating, ingredients encapsulation, drug delivery, etc.

In the preparation of foods, the use of edible biopolymer films or coatings is often required to protect the food from pathogenic attack that could lead to food contamination. The usage of apple peel phenolics in the production of edible films, for inhibiting *L. monocytogenes,* was successfully studied by Du et al. (2011). From their observations, the addition of apple peel phenolics, not only works as an antimicrobial, but also increased the elongation capacity and reduced the water vapor permeability of the film, which makes the film more suitable for direct application on food surfaces. In another study, the incorporation of gallic acid and catechin in zein biofilm demonstrated the improvement of the flexibility of the film (Arcan & Yemenicioğlu, 2011).

Another compound from apple, quercetin has also been studied as an active ingredient for wound healing and antibacterial protection in the development of natural-based wound dressings (Dias et al., 2011). Further understanding and the latest information on the utilization and advantages of bioactive plant extract in biopolymer film preparation can be found in Wang et al. (2012).

3.7 Conclusion and Future Studies

Apple phenolics have demonstrated numerous bio-functionalities in *in vitro* studies and displayed great potential in many applications, nevertheless further understanding and knowledge development on apple phenolics are required. Apple is one of the most important fruit crops produced in the world and its processing waste definitely can be explored for production of naturally sourced antioxidants. Even though these compounds are proven useful, more studies must be performed to make these compounds available in the market and most importantly safe for consumers.

The first area that requires further studies is on characterization and determination of compounds, bio-functionalities, stability, toxicity and bioavailability. By knowing this, the compounds can be designed for suitable applications. In order to do this, a common method of extraction must be established. As we already discussed, methods, solvents, temperature and time can interact or alter the compounds, thus making determination of compounds, bio-functionalities, stability and recovery of compounds difficult. Çam et al. (2010) have showed that improper extraction conditions of apple waste may cause the formation of the carcinogenic compound, 5-hydroxymethylfurfural.

Secondly, it is important to know whether the single compound or a mixture of compounds is more effective for the promotional use of only the specific compound or the mixture for target applications. Each compound has different bioactivities and may only be effective for certain applications.

Thirdly, more *in vivo* and human test for apple phenolics extract must be performed to justify its superior performance in *in vitro* studies. Results of *in vitro* studies alone are insufficient to support the application of these extracts as ingredients for food, cosmetics or pharmaceutical uses.

Another area that requires deeper investigation is the study of the effects of processing parameters on the stability and availability of these compounds in food, beverage, cosmetics and pharmaceutical products. The existing reports on the practical use of apple phenolics are not thorough enough to promote the extensive usage of these compounds for industrial applications. Study on encapsulation of these compounds must not be ignored due to the fact that phenolic compounds in general are heat sensitive. Thus to increase their availability in products after processing, encapsulation could be required.

Lastly, it will also be worth to conduct a feasibility study on the economical production of apple phenolic compounds as natural antioxidants. The cost of production is estimated high due to the requirements for several processing steps (e.g. extraction, purification, encapsulation etc.) and this process will only be feasible if the phenolic compounds are proven to have high value in the market. Thus, if feasibility is not the case, another option is to simply convert the dry pomace powder into supplements or increase the phenolic compounds in cider or juice as has been proposed by Gerard & Roberts (2004). These researchers have successfully produced apple juice with higher phenolic compounds by thermally treating the apple mash using microwave heating.

The future focus, as emphasized above, may have been studied to some extent and presented in the literature for various plant samples, however it has not been explored extensively in apples. Therefore the wide commercial utilization of safe apple phenolic compounds is still pending. This brings to conclusion that more work is required to ensure that safe apple compounds are recovered and delivered to consumers.

Connecting Statement to Chapter 4

A comprehensive review on the advances of health benefits, extraction methodologies, applications and prospective studies on apple phenolics was presented in **Chapter 3**, with the purpose to gain further understanding, disseminate information, promote utilization of these compounds and identify research gaps.

In **Chapter 4**, an introduction to the RFAE system setup together with a detailed description of the basic function of each component are presented. The reasons and benefits of the chosen applicator design and the finite element modeling and simulation work to visually predict the distribution of electric field and temperature in the sample are elaborated. The design of the RF applicator was given special emphasis due to its significant contribution towards the effectiveness of an RF heating system dedicated to extraction.

Chapter 4 has been prepared as a book chapter for Universiti Teknologi Malaysia Food and Biomaterial Engineering Book.

Mohd Jusoh, Y.M.[a,b], Orsat, V.[a] and Gariépy, Y.[a] (2014). Introduction to Radio Frequency Assisted Extraction, Its Equipment Setup, Functions and Applicator Design.

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who built the extraction unit and who prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated the ideas, provided the technical advice, suggestions, experiment facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.
- The third author is the engineer in charge of the laboratory who assisted in the machinery setup.

Chapter 4 - Introduction to Radio Frequency Assisted Extraction, Its Equipment Setup, Functions and Applicator Design

4.1 General Introduction

Nowadays, food, beverage and personal care products are supplemented with additional bioactive compounds to meet the demand from the consumers for innovative healthy products. The need for high quality specialized target compounds in large volume for food, beverage and personal care products encourages researchers and manufacturers to search for better extraction processes.

The extraction methods available today are convenient however issues such as lengthy process duration, target compounds unintentional modification or destruction, high usage of extraction solvent, high production and maintenance cost and non-environmentally friendly process are still faced by the manufacturers. Dielectric heating as a means for extraction is in focus these days. The superior performance of dielectric heating extraction particularly using microwave energy (laboratory scale) for various nutraceutical compounds has been frequently reported. Volumetric and differential heating of dielectric heating offers advantages for extraction purpose. Short processing time, high product recovery and low solvent utilization are amongst the benefits of the microwave assisted extraction, however this method also bears several disadvantages, especially suitable only for small size reactor, potential destructive effect towards target compounds, potential overheating and non-uniformity of heat distribution (in relation to large reactor size).

In this chapter, we are interested to highlight a sister technology to microwave heating, which is radio frequency heating as an alternative means for nutraceutical extraction. Radio frequency heating also falls in the category of dielectric heating. The key reasons for selecting this technology for nutraceuticals' extraction is because of its unique heating characteristics which are volumetric, rapid and selective, allowing more uniform heating within a short processing time, which cannot be offered by the existing conventional conduction or convection heating methods. The rapid volumetric heating does not only accelerate the mass transfer, but plausibly causes internal superheating that helps in breaking plant cell wall for liberating more compounds into the extraction solvent. In addition to that, RF has high penetration depth which enables for radio-frequency assisted extraction (RFAE) to be scaled up for industrial commercialization, unlike some of the novel extraction methods that are restricted by their limited reactor size. The RFAE superior performance, in comparison to other methods (microwave assisted extraction and conventional hot plate extraction), was primarily observed in our initial work in 2009 on the recovery of the phytoestrogen, secoisolariciresinol diglucoside (SDG), from flaxseed meal using a low concentration sodium hydroxide (NaOH) solution. The experiment revealed that the highest recovery of SDG was obtained with RF assisted extraction (18.68 ± 0.48 mg SDG/g DM) then followed by the conventional hot plate method (17.51 ± 0.60 mg SDG/g DM) and microwave assisted extraction (17.38 ± 0.03 mg SDG/g DM) (Jusoh and Orsat, 2011). Based on this encouraging finding, a series of comprehensive studies focusing on the development of RF heating for phenolic compounds extraction was performed to evaluate this new method and gain a deeper understanding on the potential of this new method.

The objective of this chapter is to introduce the radio-frequency assisted extraction system that was developed in our laboratory for extraction of phenolic compounds. This introductory RFAE chapter elaborates on the role of each component in this new system, the key reasons or advantages of the applicator design modification and the importance in utilizing a computer modeling and simulation aid in building this RFAE system.

4.2 Radio Frequency Assisted Extraction (RFAE)

Radio frequency heating for nutraceutical extraction or radio frequency assisted extraction (RFAE) was firstly introduced by Izadifar (2009) from University of Saskatchewan in recovering an anti-cancer compound known as Podophyllotoxin. If we take a closer look, the concept of RFAE is relatively similar to the existing RF heating of solid-liquid material for pasteurization or sterilization, the only difference is the objective and the output. For RFAE, the objective and the output lies on the quality and the quantity of the extract, meanwhile in RF pasteurization and sterilization, the focus is on the microbial count reduction and sensory attributes.

The complete radio frequency heating set-up designed for the nutraceutical extraction research in our laboratory is illustrated in Figure 4-1. This radio frequency assisted extraction (RFAE) set-up consists of similar components as the previous set-up developed by Orsat (1999) for thermal heating of agri-food products. The seven fundamental components in our RFAE system are the RF generator, applicator, impedance adapter (matching box), impedance regulator (load and phase), fiber-optic temperature measurement system, voltage regulator and lastly the data acquisition and control system. The basic information on the function of each component is presented in Section 4.3.

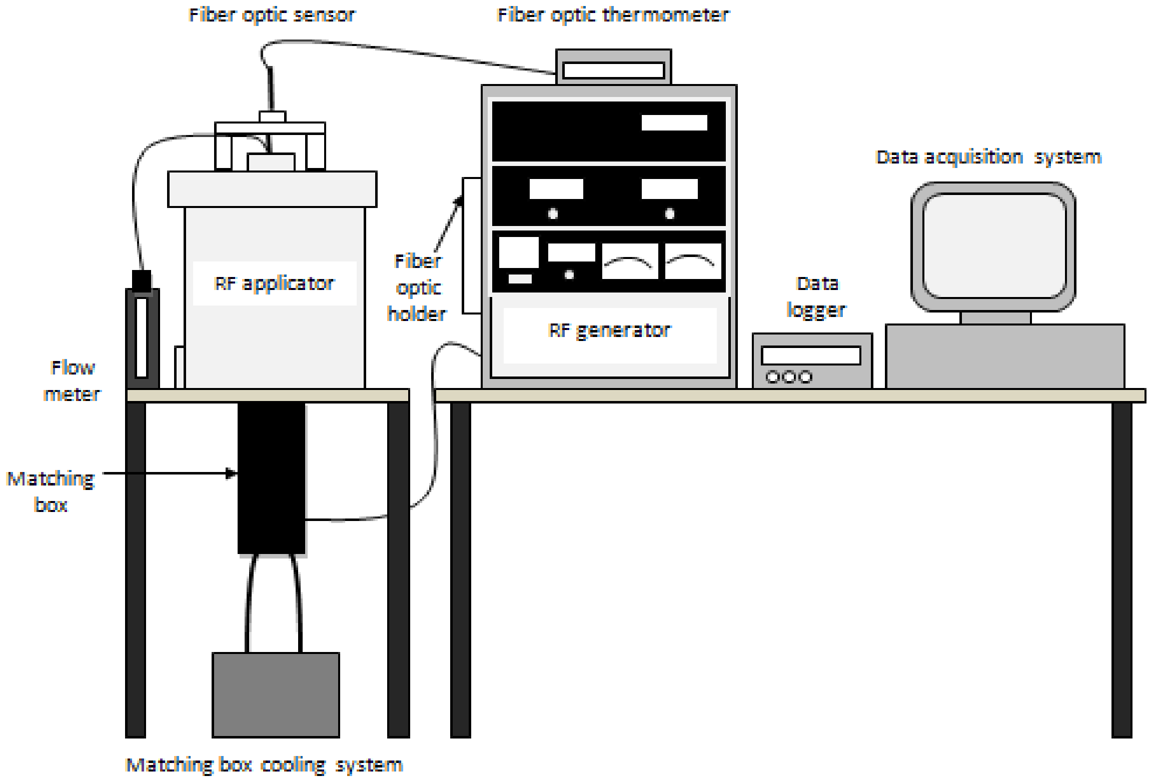


Figure 4-1 : Bioresource Engineering Department (McGill University, Canada) laboratory scale radio frequency assisted extraction (RFAE) system

4.3 The Role of Each Component in RFAE

4.3.1 RF Generator

RF generator is the heart of the RF heating system. The generator that was utilized for RFAE was the same generator that was used previously in Orsat (1999) for treating agri-food products. This RF generator with capability of providing an input power of 600 W was procured from a French microwave company, SAIREM. This Sairem RF generator has the maximum power output of 600 W and it was purchased initially based on the sample treatment size requirement (50 to 300 g) in the previous study by Orsat (1999). It was reused for this RFAE project due to its adequate capacity and capability of heating small volumes of extraction solutions between 50 ml to 200 ml (since this RFAE project is laboratory scale).

This Sairem generator operates on the 50 Ω technology. This 50 Ω generator is expensive however it provides benefit in terms of frequency stability and it is electromagnetic compatibility (EMC) compliant. The 50 Ω generator produces a single frequency that is 27.12 MHz meanwhile the conventional class C oscillators or free-running oscillators commercially available produce high harmonics thus unable to fix the system to a single frequency (Neophytou & Metaxas, 1998, 1999). Nevertheless, the free-running oscillator design is still the most commonly used in the food industry because of the lower cost, simple structure and flexibility (Jiao, Tang, Wang & Koral, 2014). Our generator is equipped with two galvanometers which are located on the front panel; one galvanometer is used to indicate the incident power (power supplied by the generator) meanwhile the other one shows the amount of reflective power (power that is not being absorbed by the load in the applicator and which is returned back to the generator). It is important to note that the reflective power must be kept low to prolong the shelf-life of the generator.

The success of an RF heating set up highly depends on the impedance matching between the generator and the applicator (Orsat & Raghavan, 2004). The output impedance of the generator was set by the manufacturer at 50 Ω. It is very important for the applicator to have a matching impedance of 50 Ω to ensure a maximum transfer of energy from the generator to the applicator (Metaxas & Clee, 1993). In our RFAE system, the impedance matching between the generator and applicator is performed by the impedance adapter or commonly known as the matching box. A brief description on the role of an impedance adapter is provided in Section 4.3.3.

4.3.2 RFAE Applicator

Our RFAE applicator was designed as a metallic box equipped with a metallic cover (Figure 4-2b). Two parallel aluminum electrodes are placed in this applicator and dielectric heating actually takes place between these electrodes. In order to heat a material using this dielectric heating method, the material to be heated needs to be sandwiched between these electrodes. When the RF generator supplies power to the applicator, an alternating electric field is formed between these electrodes. Material sandwiched between the electrodes interacts with the electric field and dissipates energy as heat volumetrically through the dielectric heating mechanism which has been explained in Chapter 2. A comprehensive description on the design of the applicator is elaborated in Section 4.4.

4.3.3 Impedance Adapter (Matching Box)

The matching of impedance between the generator and the applicator is done by the impedance adaptor; which is also known as the matching box. The nominal output impedance of the generator is fixed at 50 Ω. As mentioned previously, it is very crucial for the applicator to carry similar impedance value as the nominal output impedance of the generator in order to ensure a maximum transfer of energy from the supply (generator) to the load (applicator). Improper matching of impedance could lead to energy wastage and shortening of the life of the generator. In reality, the impedance value of the applicator is not constant, it keeps changing due to the changes in material, material composition, moisture content etc. Due to this variability, the impedance adapter is made of two variable capacitors and one fixed resistive coil. With these variable capacitors, different types of material can be heated up in the RF applicator. These variable capacitors are electrically controlled by the impedance regulator for ensuring a maximum transfer of energy and minimization of reflective power.

4.3.4 Impedance Regulator (Load and Phase)

The variation in the capacitors is electrically controlled by the impedance regulator. It is equipped with an automatic feedback control system that automatically corrects any fault detected during module tuning ($\neq 50\ \Omega$) and phase tuning ($\neq 0°$). This auto-correct impedance regulator minimizes the reflected power and maximizes the power transfer.

4.3.5 Fiber-optic Temperature Measurement System

Another important component in the RFAE set up is the fiber-optic thermometer measurement system. With this system, the temperature buildup in the material during heating is monitored and controlled. This temperature sensor is made of a fiber-optic probe which is transparent to electromagnetic energy thus it can be placed directly in the material to be heated.

4.3.6 Voltage Regulator

The role of the voltage regulator is to measure the voltage at the electrodes. It is an advantage to have a voltage regulator in the system because it enables the user to quantify the energy being absorbed by the material by knowing the value of the voltage at the electrodes. Besides that, it can also serve as voltage monitor and control during the heating process.

4.3.7 Data Acquisition and Control System

The last component in the RFAE system is the data acquisition and control system. This system consists of a data logger, a data logging software and a computer. All RFAE components that have been mentioned earlier are electronically connected to this system.

This system has the role to collect, display and store data obtained from the generator, impedance regulator, voltage regulator, fiber-optic thermometer and impedance adapter cooling system. The information displays are the incident and reflected power of the generator (W), voltage of the electrode (V), temperature of the heated product (°C) and temperature in and out of the water cooling system (°C). The computer is used to monitor and control these important parameters during the extraction process.

4.4 The RFAE Applicator Design

The previous and current designs of the applicator are shown in Figure 4-2 and Figure 4-3. Clearly, the dissimilarity between these two set-ups lies in the orientation of the electrodes. Both set-ups employed the most common design which is the through-field applicator design; horizontal parallel electrodes were used in Orsat (1999) meanwhile vertical parallel electrodes are used in the current applicator. Due to the size of our samples, the through-field applicator design was selected basically because of its suitability for block or thick materials (Jun & Irudayaraj, 2009). The full schematic diagram of the RFAE applicator with dimensions is provided in the appendix section (Appendix 1). The design of the RFAE applicator was made according to the guidelines presented in Orsat & Raghavan (2004).

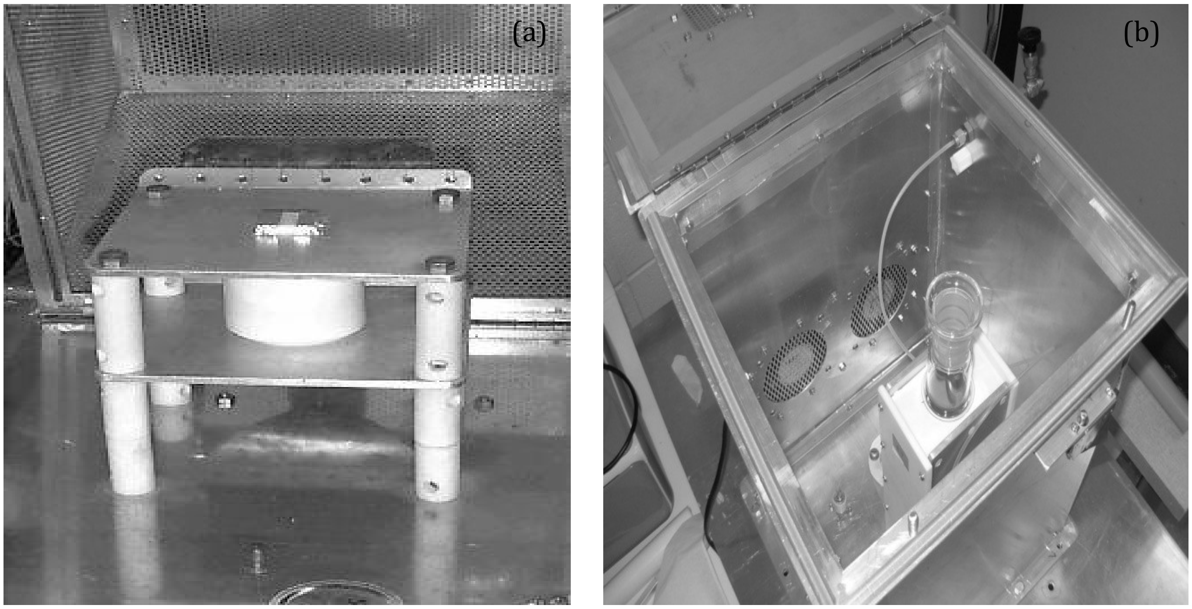
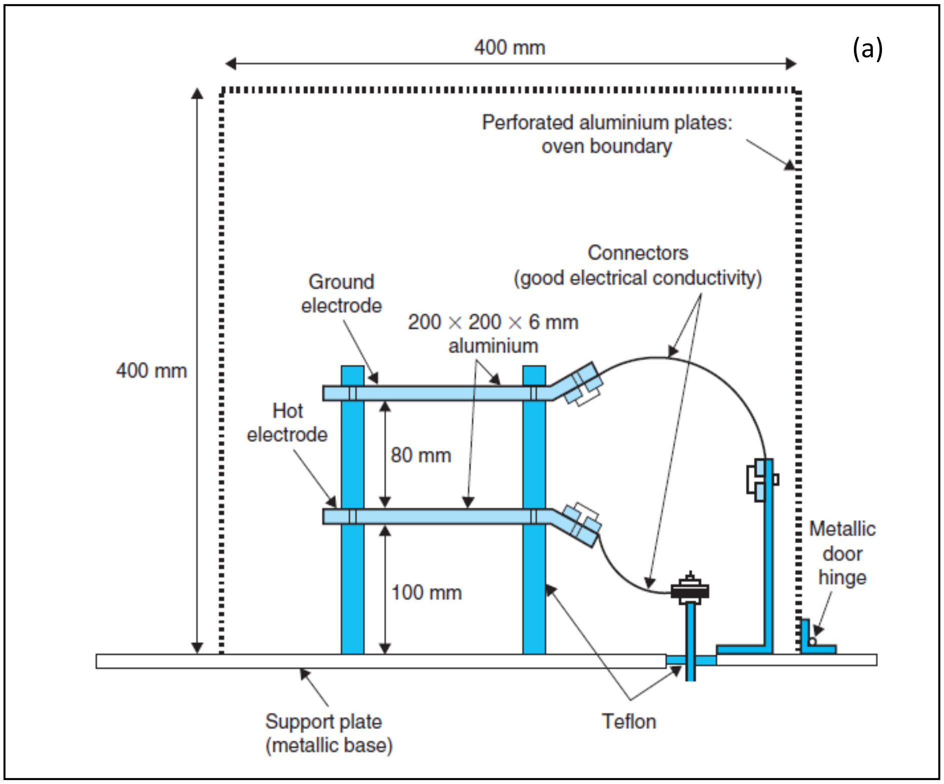


Figure 4-2 : The RF heating horizontal parallel plates setup for agri-food product (a) and the vertical parallel plates setup for nutraceutical extraction (b)



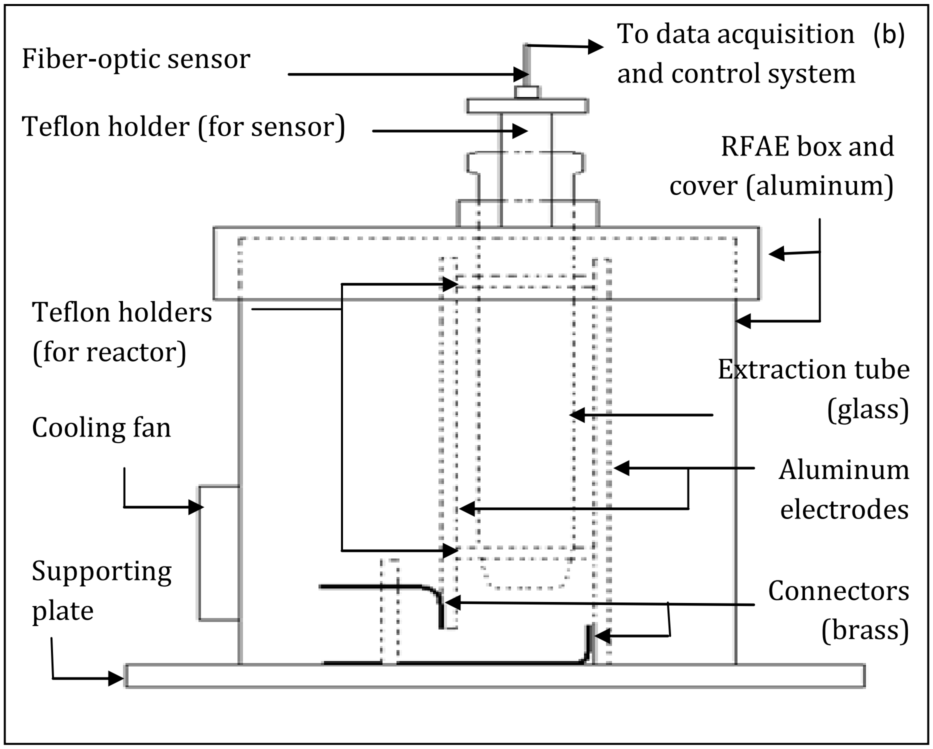


Figure 4-3 : Schematic diagram of RF heating system for agri-food products (Orsat, 1999)(a) and for nutraceutical extraction (b) (side views)

A careful selection of building materials for the electrodes must be made. According to Orsat & Raghavan (2004), the RF heating electrodes must be made of good electrical conducting materials e.g. from copper, silver, aluminum or brass. Our RFAE electrodes are made of aluminum since this material bears three benefits, it is a good electrical conductor, light in weight and flexible. Next, the length of the electrodes must be smaller than a half or a quarter of the wavelength of the applied frequency. This is crucial to avoid the formation of standing waves that could lead to heat non-uniformity in the sample. The frequency that we used is 27 MHz, thus the wavelength is 11 m. Our rectangle electrodes length has dimensions of 0.23 m in length and 0.10 m in width, which are both smaller than the quarter of the wavelength. The thickness of the electrodes was maintained at 6 mm since this thickness is sufficiently thick to prevent electrical sparks in the applicator due to brushing effect.

To ensure safety and prevent leakage or unnecessary irradiation towards the user, the electrodes are enclosed in a metallic box that is incorporated with a metallic cover. The cover needs to be flipped open in order to place the tube (containing sample) or to have access to the inner parts of the applicator. Hinges are used to attach the metallic cover to the metallic box. On the top and middle of the box metallic cover, there is a round opening, 60 mm in diameter, for allowing the insertion of the fiberoptic temperature sensor. The extraction tube, illustrated in Figure 4-4, was custom-made from borosilicate glass (Montreal Glassblower Company). The shape and size of the tube was adapted and modified from the MAE reaction tube of the CEM Star system.

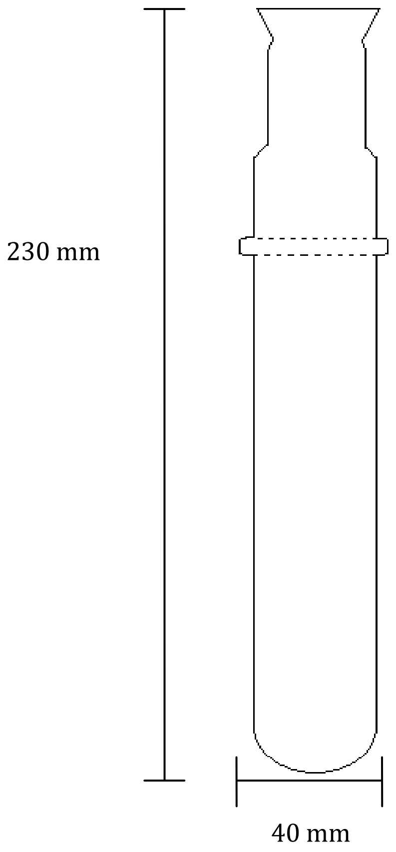


Figure 4-4 : The RFAE borosilicate glass tube

4.5 Objectives and Advantages of Applicator Design Modification

The need for electrodes orientation modification was made based on several important objectives. The rationale behind the modification of the applicator is as the followings:

i. Initial intention was to evaluate the performance, similarities and differences between RFAE and MAE. By maintaining the experimental conditions and solely change the setup of the applicator, valuable information relating to similarity or differences between the RFAE and MAE can potentially be obtained. This type of information is unavailable in the literature. The parallel plates RFAE system was intentionally designed vertically in mimicking the CEM Star system MAE which is available in our laboratory. MAE is one of the most efficient extraction methods available to date.
ii. The second reason for the applicator modification was due to the nature of the sample. In RFAE study, the samples were generally in liquid or slurry form therefore a vertical orientation is highly preferable since this orientation allows the installation of mechanical mixing (which is required), and also favours the dissipation of water vapours.
iii. Next, this orientation is considered practical for industrial application as the space requirement can be minimized. With horizontal electrodes configuration, to increase the volume of the applicator (for bigger sample) means an increase in the horizontal sides (more floor space is required which contributes to cost). Non-uniform heating and process inefficiency may occur if the sample height is increased (vertical expansion) in the horizontal electrode configuration.
iv. The last reason for this modification was to minimize the issue relating to the air gap and sample size. Reports have shown that minimizing the air gap and appropriate sizing of the sample are crucial factors in ensuring an effective uniform heating in RF heating. In this new design, the air gap between the sample (which is designed to be placed in a glass tube) and both electrodes is fixed.

In addition to the above mentioned aspects, this modification also leads to several other benefits for example no direct contact between the samples and the electrodes, more uniform heating and larger sample volume.

4.6 Modeling and Simulation Work Prior to RFAE Applicator Development

The utilization of computer modeling and simulation accelerates the designing process and actual development to lower the cost and can be seen in many publications relating to RF heating development. The results from an accurate modeling and simulation exercise for a RF heating system can assist in making further necessary modifications or in troubleshooting problems in the system.

In this project, finite element modeling using COMSOL Multiphysics was adopted. The objective of this modeling and simulation work was to evaluate virtually the electric field, and the heat and temperature distribution in the new system. This step allows to better understand what happens between the electrodes during a RFAE process. The results of the modeling and simulation work are presented below in Figures 4-5 to 4-9. The first step of this work was to model the RFAE system in COMSOL. This virtual model was built according to the actual dimensions of the sample (mimicking the size and shape of the glass tube) and the electrodes (Figure 4-5a). This model mainly consists of a shielding box and a quartz reactor (diameter = 40 mm by 230 mm long) placed horizontally between an electrode at 6000 V and a ground plate. The frequency is 27 MHz. The dimensions of the electrodes are 230mm long by 100mm wide and the assembly is maintained in place by two teflon sheets (dimension = 60 mm high by 230 mm long). Mesh was added to the model to enable the simulation (Figure 4-5b). The simulation was performed using moist wheat grains since this material is known to heat uniformly under RF heating (good coupling). The dielectric properties of the wheat grains were supplied for the simulation project. The verification and validation of the model and simulation was compared with the actual RF heating system.

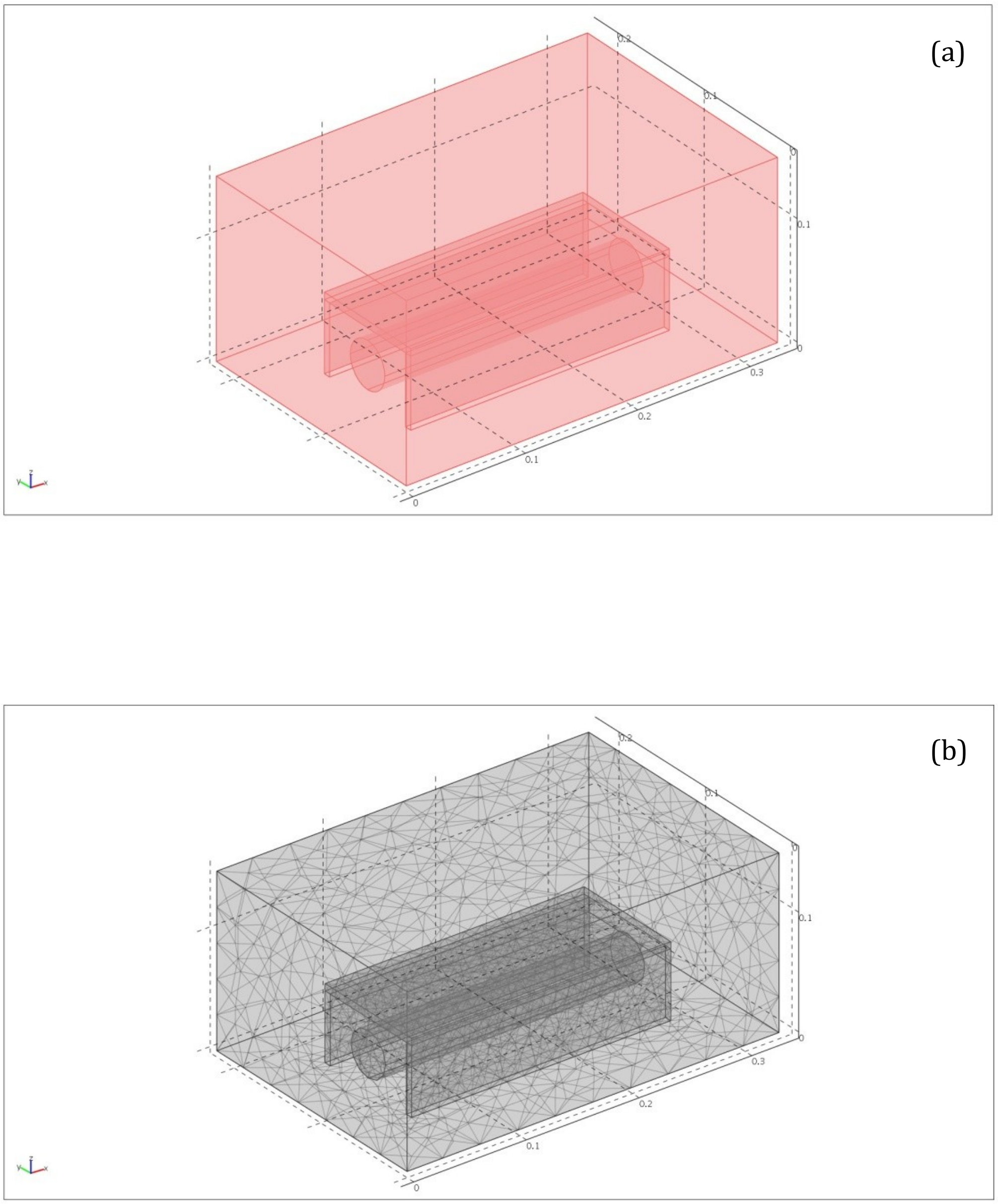


Figure 4-5 : (a) Diagram of the physical model used to simulate the new RF applicator. It mainly consists of a shielding box, a quartz reactor and two electrodes. (b) Similar diagram with meshing effect (via Comsol Multiphysics).

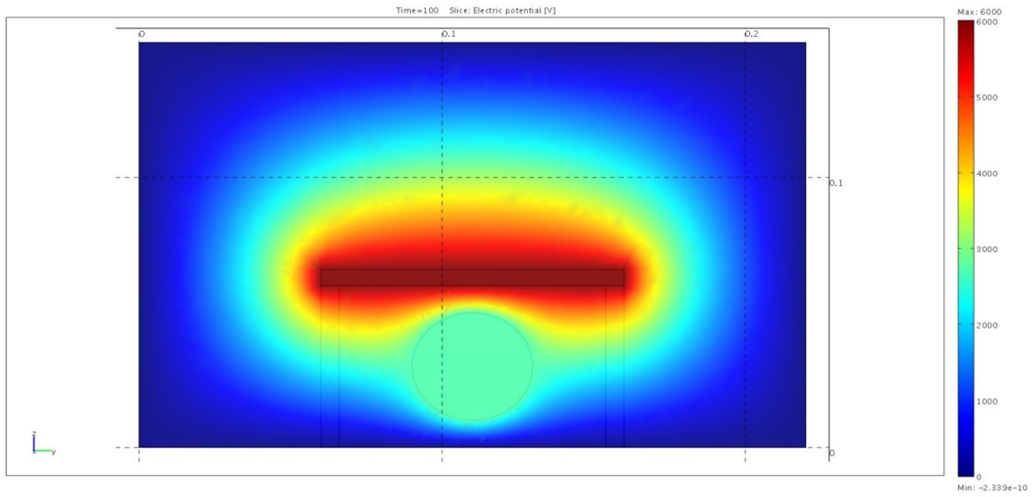


Figure 4-6 : Cross-section of the electric potential (V) distribution in the RF heating system. Voltage ranged between 0 to 6000 V. Coupling was good (3 kV) and evenly distributed in the food material.

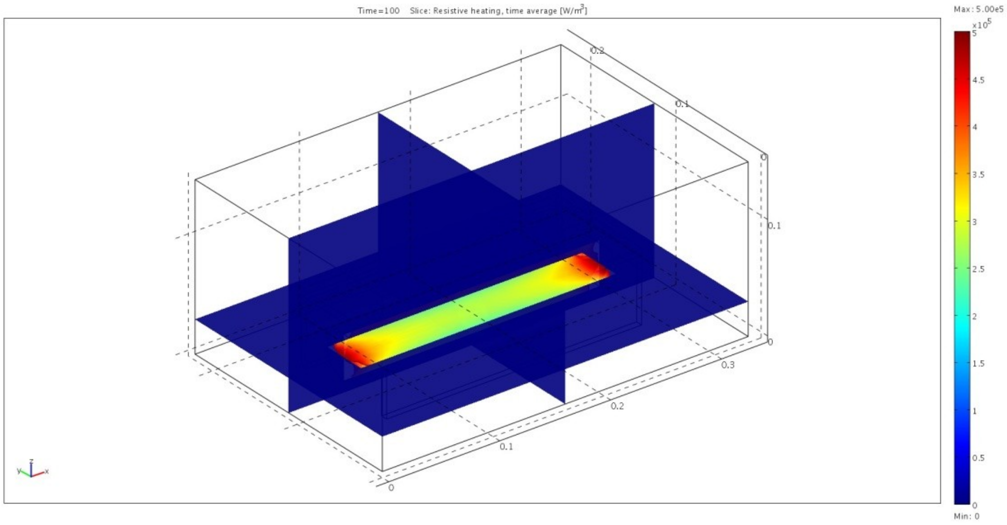


Figure 4-7 : Distribution of resistive heating in the food material placed in RF heating system. Heating was at about 250 W/kg and fairly uniform. However, over-heating occurred at both ends of the sample.

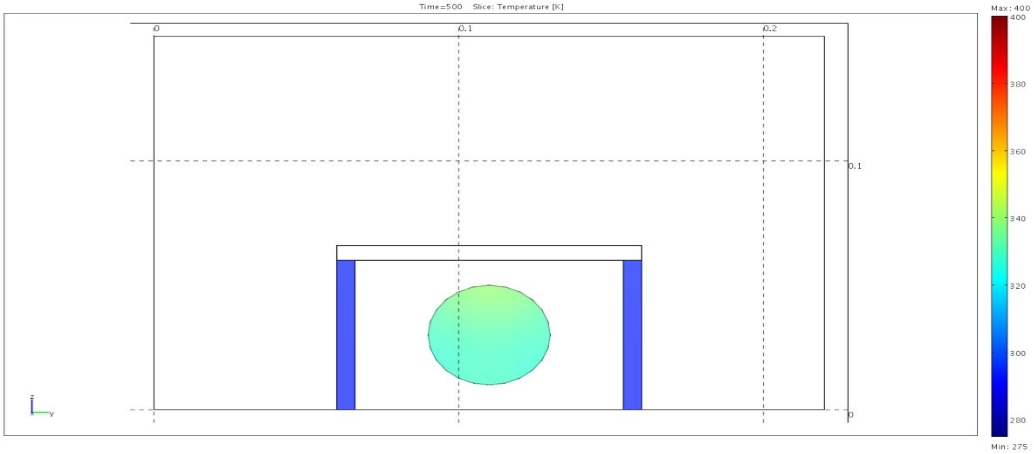


Figure 4-8 : Cross-section of the temperature distribution in the food sample and Teflon edges after 500 s of heating in the RF system. The temperature of the food sample had raised from 293°K to about 340°K and the distribution was uniform. The temperature of the Teflon edges remained unchanged indicating that they can be safely used as electrode holders.

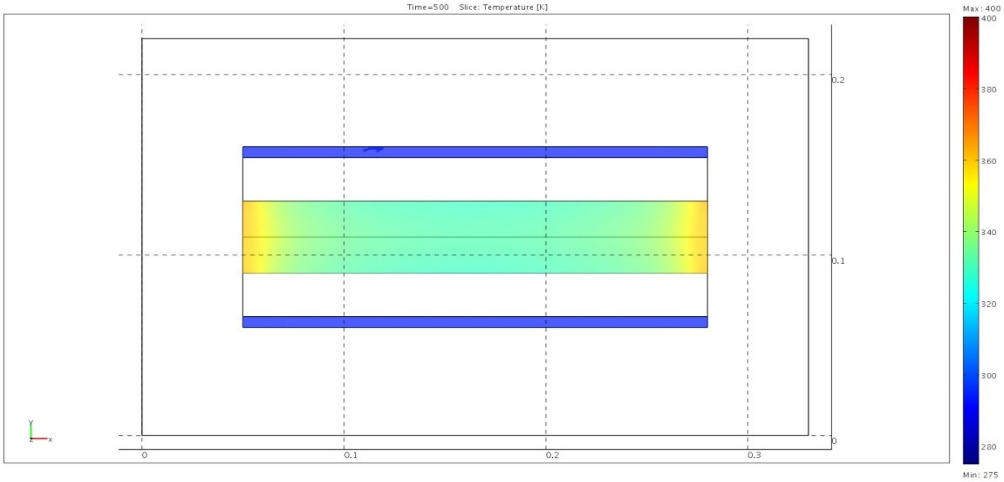


Figure 4-9 : Longitudinal temperature distribution in the food sample and Teflon edges after 500 seconds of heating in the RF system. Over-heating occurred at both ends of the sample.

4.7 Incorporation of Mixing/Agitation in RFAE

Mixing or agitation is a compulsory step in many processes including solid-liquid extraction. The incorporation of mixing or agitation in a process is often required as it potentially promotes homogeneity especially in samples made of multi-components mixture, improves temperature uniformity throughout the sample and induces diffusivity (mixing action provides more surface contact between the sample and the solvent maintaining a concentration gradient).

In RFAE, a nitrogen gas bubble mixer was designed for mixing the sample. This type of mixer is rarely reported in published literature and in addition, this is the first time this type of mixer is applied in RFAE. This mixer setup consists of a nitrogen gas supply, a stainless steel coil (for connecting the nitrogen gas supply outlet to the inlet of the flowmeter), a flowmeter (for controlling the flowrate of the nitrogen gas) and a Teflon tube (for connecting the flowmeter outlet to the extraction tube). The Teflon tube is inserted into the extraction tube by attaching it, using a Teflon ring, to the fiberoptic thermosensor. The complete RFAE system with mixing apparatus is shown in Figure 4-10.

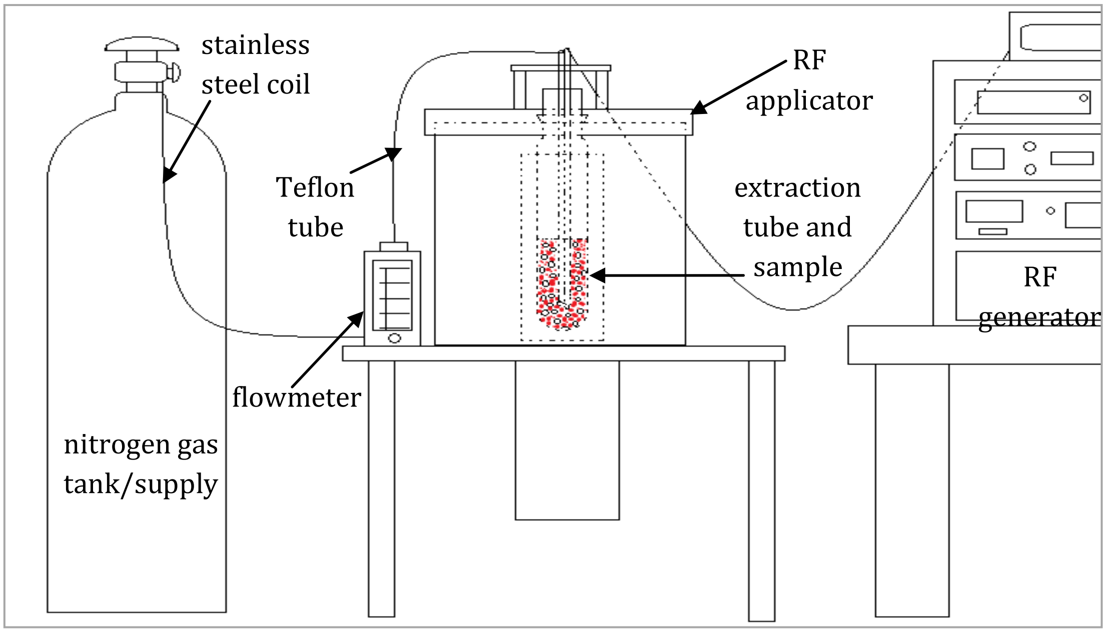


Figure 4-10 : Nitrogen gas mixer/stirrer setup for RFAE system.

4.8 Summary

The focus of this chapter was to introduce a newly developed radio frequency assisted extraction system. This system was modified from an existing horizontal parallel electrodes RF heating system that was used previously for heat-treating agri-food produce. The whole RF heating system which consists of seven components (RF generator, applicator, impedance adapter, impedance regulator, fiber-optic temperature measurement system, voltage regulator and data acquisition and control system) was maintained except for the applicator which was re-designed to meet the new requirements for effective extraction. A bubble mixing system was developed and integrated to the RFAE system to ensure proper agitation to favor extraction. In order to provide deeper understanding on how RFAE system works, the components' functions were highlighted as this information is rarely currently available in the literature. The modeling and simulation results were also presented to emphasize the need for this step prior to the actual physical development of the system since it allows prediction on the performance.

Connecting Statement to Chapter 5

In **Chapter 4**, the full RFAE setup with the function of each of its components were illustrated and described. Special emphasis was given to the applicator with special focus on the layout, purpose and benefits of this new design. This chapter was prepared with the intention of providing fundamental information on this new system to the readers and with hope that the information gathered contributes to new knowledge in this field.

In order to evaluate this new RFAE system, the first attempt for recovering apple peel phenolic compounds using this new method was performed and presented in **Chapter 5**. This chapter has been written in the manuscript style for submission to the Journal of Food Engineering:

Mohd Jusoh, Y.M.[a,b] and Orsat, V.[a], (2014). Effects of Processing Parameters on the Phenolic Compounds obtained by Radio Frequency Assisted Extraction of Apple Peel

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who prepared the experimental design, conducted the laboratory work, analysed the results and prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated ideas, provided the technical advice, suggestions, experiment facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.

Chapter 5 - Effects of Processing Parameters on the Phenolic Compounds obtained by Radio Frequency Assisted Extraction of Apple Peel

5.1 Abstract

The influence of sample drying method (microwave-dryer and freeze dryer), solvent type (0.001 M sulfuric and hydrochloric acid) and extraction temperature (50°C and 70°C) on the quantity and quality of phenolic compounds extracted using a radio frequency (RF) extractor were studied. Total phenolic contents, total flavonoid contents, FRAP and DPPH antioxidant activities were used to measure the quality of the extract. These values were quantified via spectrophotometric method. The total phenolic content, total flavonoid content, FRAP and DPPH antioxidant activities of McIntosh apple peel extract ranged from 20.08 – 26.17 mg GAE/g dry peel, 10.91 – 15.66 mg CAT/g dry peel, 1.95 - 2.51 mmol Ferrous equivalent/ g dry peel and 57 – 77 % DPPH inhibition, respectively. Apple peel extract with high total phenolic contents, total flavonoid contents, FRAP and DPPH antioxidant activities were obtained when extraction was performed with a microwave-dried sample, 0.001 M hydrochloric acid and an extraction temperature of 50°C to 70°C. Increasing temperature had a negative relationship with the DPPH antioxidant activities. A comparison study between RF and conventional extraction showed that RF extraction produced an extract with higher yield and antioxidant capability, revealing its potential as a new extraction method.

Keywords :

Radio frequency extraction, apple peel, total phenolic content, flavonoids

5.2 Introduction

Phenolic compounds are secondary plant metabolites, which exist ubiquitously in every part of plants. Within the plant, phenolics may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants, protective agents against UV light, among others (Naczk & Shahidi, 2004). Research has demonstrated that phenolic compounds not only play an important role in protecting plants from diseases and predators, but also in ensuring plant growth and reproduction, contributing to colours and sensory characteristics in plants (Ignat, Volf & Popa, 2011), while these compounds also offer health protecting benefits to humans. Epidemiological studies have indicated that a diet rich in phenolics may have protective effects against various degenerative diseases (Knekt et al., 2002; Boyer & Liu, 2004; Huntley, 2009).

Phenolic compounds are divided into several groups e.g. simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans and lignins (Naczk & Shahidi, 2004). There is a growing interest in utilizing these compounds in food, cosmetic and pharmaceutical products due to their potent antioxidant, antimicrobial, antiviral, anti-inflammatory, free radical scavenging and metal chelating properties.

Apple is one of the fruits rich in phenolic compounds especially the flavonoid group. Many studies have been done associating flavonoids to a lower risk of degenerative diseases such as cancer, stroke, Alzheimer, dementia, diabetes, etc. Studies by Veeriah et al. (2006) and McCann et al. (2007) demonstrated the ability of a crude apple extract to prevent colon cancer. In addition, apple extract has been shown to reduce the oxidative stress that leads to death of neuron cells (Heo et al., 2004). In North America, apple peel wastes are available abundantly as by-products of the apple sauce and juice industry. Apple peel is an invaluable source of phenolic compounds from apples because the peel has higher concentrations and may have more different types of phenolic compounds than the apple flesh (Escarpa & González, 1998; Tsao et al., 2003; Łata, Trampczynska & Paczesna, 2009). Even though these compounds are embedded within the waste, they can be extracted, purified and used as nutraceutical ingredients.

There are many extraction methods available however, in this study, we are interested in exploring and highlighting the potential of radio frequency (RF) heating as a new extraction method. A rapid extraction process, food grade solvent utilization in small quantity, high recovery and environmental safe technology are some of the advantages that can be offered by this method. However, like any other new method, there are still many aspects of the RF extraction mechanisms (eg. dielectric properties of materials to be heated, equipment design, etc.) that need to be fully understood before this extraction process can be commercialized.

Radio frequency is an electromagnetic wave ranging from 0.003 to 300 MHz. In this technology, volumetric heating or heat is generated within the sample as the result of collisions between ionic and polar molecules under the influence of the alternating electric field. It shares the similar principle as microwave heating, only RF heating functions at a lower frequency. This technology has been successfully applied in several food processing applications such as thawing, tempering, post-baking drying, pasteurization, cooking and roasting of food (Piyasena, et al., 2003).

The ultimate aim of this paper was to establish an initial extraction condition for apple peel phenolic compounds recovery using RFAE method. This main aim was separated into three specific objectives. The first specific objective was to determine the best method of drying apple peel for adequate storage prior to extraction. Ideally, the drying method should be rapid and not be destructive towards the phenolic compounds. The second specific objective was to determine the best extraction solvent to be used in the radio frequency extractor. The interaction between solvent and radio frequency energy should be able to produce an extract with a high yield of phenolic compounds with optimal antioxidant activities within a short extraction period. Aqueous acid as an extraction solvent is not uncommon and it has reportedly been used in the recovery of apple pectin and as a pre-treatment in the extraction of phenolic compounds from apple waste. The third specific objective was to evaluate the effect of the temperature rise on the quantity and quality of phenolic compounds obtained. Total phenolic content (TPC), total flavonoid content (TFC), FRAP and DPPH antioxidant activity were selected as the performance indicators. A short comparative study was performed to highlight the performance of the RF extractor as compared to conventional thermal solvent extraction.

5.3 Materials and Methods

5.3.1 Samples and Chemicals

The McIntosh apples used in this experiment were harvested in Quebec in October 2011. Sulfuric acid (H_2SO_4), hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,4,6-tris-2-pyridyl-1,3,5-triazine (TPTZ), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), ferric chloride hexahydrate ($FeCl_3.6H_20$), methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, ferrous sulfate heptahydrate ($FeSO_4.7H_20$) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

5.3.2 Apple Peel Preparation

Apples were washed and patted dried at room temperature. An apple peeler (StarFrit, Atlantic Promotions Inc., Canada) was used to peel the apple skin meanwhile the apple flesh residue attached to the skin was removed using a spatula. The peel was placed in zip-lock bags for use in subsequent processes.

5.3.3 Drying of Sample

5.3.3.1 Microwave Drying (MD)

The drying system used was a custom made system built at the McGill University Bioresource Engineering Post-Harvest Laboratory. This system consists of two sections, the drying (Panasonic microwave oven equipped with hot air blower) and the control (computer) section (Figure 5-1). Detail information relating to this dryer can be found in Dev et al. (2011) and Changrue et al. (2007). For this drying, the control system was programmed to run at maximum power of 150 watt and sample temperature of 50°C. The control system automatically shuts down the microwave generator when the incident power or the sample temperature exceeds the specified maximum limit. The system turns off when the reflectance power is very high.

In order to dry the sample, 50 g of the moist peel was placed on the integrated microwave dryer weighing scale. Then, the microwave drying system was switched on. The peel was left to dry until the system indicated that the remaining weight of peel was 18.1 g which was equivalent to 17% moisture remaining in the sample. The drying process had to be stopped at this point to avoid overheating of the sample. In this study, overheating is determined by a significant change in the colour of the sample. It took approximately 26 minutes to reach the final mass. The dry sample was removed from the microwave cavity and left to cool down for 3 minutes. Then, the sample was placed in a glass jar and kept in the refrigerator until further use.

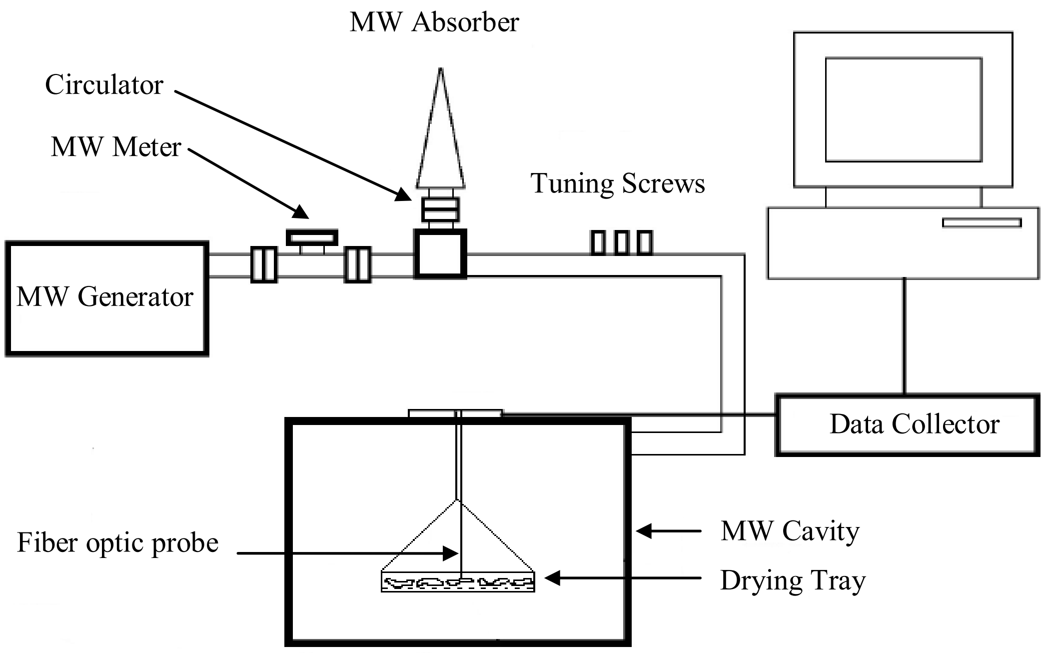

Figure 5-1 : Schematic diagram of microwave hot air dryer for apple peel drying

5.3.3.2 Freeze Drying (FD)

200 g of moist apple peel, placed onto two wax paper plates, were placed onto the two-deck trays of the freeze dryer (LabConco Corporation, Missouri, USA). The purpose of using these plates was to prevent the sample from sticking to the metal trays. The freeze dryer was programmed to dry materials at -40°C and the peel was left to dry for 24 hours.

5.3.4 Radio Frequency Assisted Extraction

The radio frequency assisted extraction was performed by adding 0.5 g of dried sample into 50 ml of solvent in RF extraction glass tube. The mixture was stirred using a stirring rod for 10 seconds to ensure uniformity. Then, the extraction tube was placed between the RF electrodes. The RF power was set at 300 W and each extraction was performed for 10 minutes. After the extraction, the mixture was vacuum-filtered using

Whatman No 1 filter paper and the extract was kept in an aluminum foil wrapped 50 ml centrifuge tube for further chemical analyses. The complete RFAE system and extraction steps using this new method are shown in Figures 5-2 and 5-3.

In establishing an initial condition for extracting phenolic compounds with RFAE, two different extraction solvents, aqueous hydrochloric acid and sulfuric acid, with similar concentration of 0.001 M were selected for investigation as these two acids are commonly used for nutraceutical extraction. In addition, the effect of two more important factors, sample drying methods (freeze drying and microwave drying) and extraction temperatures (50°C and 70°C), were also evaluated. To facilitate temperature effect evaluation under fixed applied power and time as mentioned above, the RF power was continuously supplied to the sample until it reached the targeted temperature. Once the sample reached the target temperature, the RF power was intermittently supplied to the system and this step was manually performed by switching on and off the RF power supply. A temperature difference of ± 2°C due to manual control had to be allowed in order to evaluate the temperature effect under fixed extraction power and time. The information about the experimental design and analysis is explained in Section 5.3.5.6.

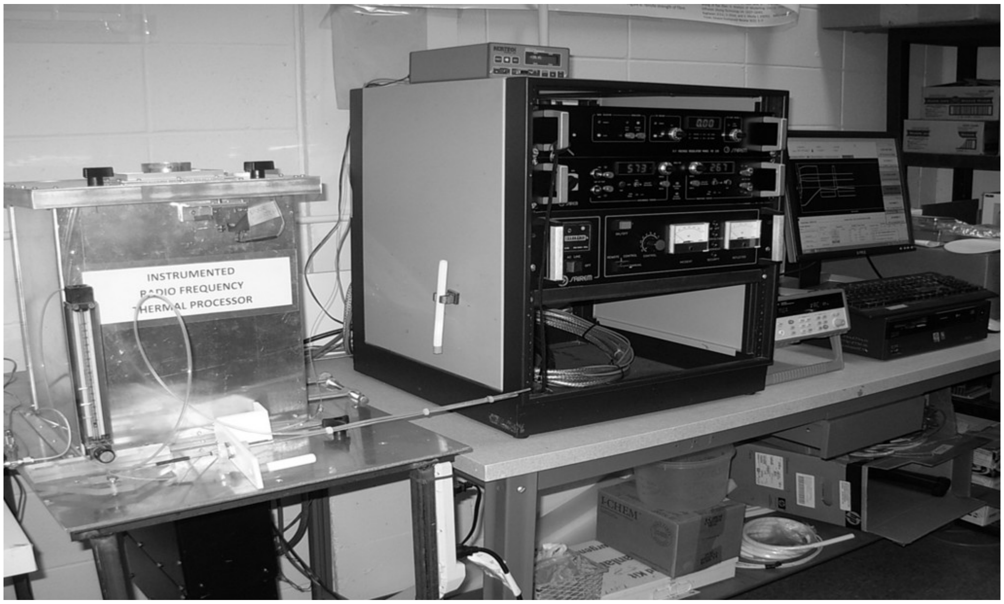

Figure 5-2 : Radio frequency extractor system for RFAE

Figure 5-3 : Extraction of apple peel phenolic compounds using RFAE applicator

5.3.5 Chemical Analysis

5.3.5.1 Total Phenolic Content Determination

The total phenolic contents in the apple peel extract were determined using the method described in Waterhouse (2005) with minor modifications. 0.1 ml of extract was mixed with 7.9 ml of distilled water in a 30 ml centrifuge tube. Then, 0.5 ml Folin-Ciocalteu reagent was added into the mixture. The tube containing the mixture was agitated for 1 minute using a shaker and left aside for 7 minutes. After that, 1.5 ml of $NaCO_3$ was added and mixed again. The mixture was incubated for 2 hours at room temperature. All tubes were properly covered with aluminum foil to protect them from light exposure. Biochrom US 1000 UV/VIS spectrophotometer (Biochrom, Massachusetts) was used to measure the absorbance of the sample with the wavelength set at 765 nm. The quantification of total phenolic compounds in the sample was performed using gallic acid standard. Since this experiment was performed using two types of acids, two gallic acid calibration curves (100, 200, 400, 600 mg/L) were prepared, one in 1 mM sulfuric acid (r^2 = 0.98) and another one in 1 mM hydrochloric acid (r^2 = 0.98). The amount of phenolic content obtained was expressed as milligram gallic acid equivalent (GAE) per gram of dry peel.

5.3.5.2 Total Flavonoid Content Determination

The amount of flavonoids contained in the sample was measured based on the method described in Alothman, Bhat & Karim (2009). 1 ml of extract was mixed with 4 ml of distilled water. At time 0, 0.3 ml of $NaNO_2$ (5% w/v) was added into the mixture. At the 5th minute, 0.3 ml of $AlCl_3$ (10% w/v) was added, followed by 2 ml of 1 M of NaOH at the 6th minute. Then 2.4 ml of distilled water was added into the mixture and the mixture was shaken vigorously to ensure uniformity. The absorbance of the mixture was read at 510 nm using a Biochrom US 1000 UV/VIS spectrophotometer. Two catechin calibration curves of 50, 100, 150 and 200 mg/L in 1 mM sulfuric acid (r^2 = 0.99) and 1 mM hydrochloric acid (r^2 = 0.99) were prepared to quantify the total flavonoid contents in the sample. The amount of flavonoid was expressed as milligram catechin equivalent (CAT) per gram of dry peel.

5.3.5.3 Antioxidant Activity by Ferric Reducing Antioxidant Power (FRAP) Assay

This test was performed according to the method described in Benzie & Strain, (1996) and Alothman, Bhat & Karim (2009) with minor modifications. 0.1 ml extract was mixed with 0.3 ml distilled water and 3.0 ml FRAP reagent. The FRAP reagent was made up of 25 ml 0.3 M sodium acetate buffer of pH 3.6, 2.5 ml of 10 mM TPTZ in HCl and 2.5 ml of 20 mM $FeCl_3.6H_20$. The mixture of distilled water and FRAP reagent was incubated at 37°C for 4 minutes prior to its colour reading. Aforementioned UV/VIS spectrophotometer was used to read the absorbance of the sample and for this test the wavelength was set at 593 nm. The antioxidant activity was determined via the calibration curves of 1, 2 and 3 mM of ferrous sulphate $FeSO_4.7H_20$ in 1 mM sulfuric acid (r^2 = 0.98) and 1 mM hydrochloric acid (r^2 = 0.98). The result was expressed as micromoles of ferrous equivalent Fe (II) per gram of dry peel.

5.3.5.4 Antioxidant Activity by DPPH Assay

Another test to evaluate the antioxidant activity of apple peel extracts was the inhibition of DPPH free radical. Experimental procedures were performed according to Hatano et al. (1988). Buffered methanol DPPH solution as described in Sharma & Bhat (2009) had to be used in this experiment since the original extraction solvent does not contain methanol. 3 ml of sample (40 % extract and 60% methanol) was mixed with 1 ml 100 μM methanolic DPPH solution (40% acid and 60% methanol) in 15 ml tube covered with aluminum foil. This mixture was mixed and left aside for 30 minutes at room temperature. The absorbance of the sample at 517 nm was measured by spectrophotometry. The following equation was used to calculate the percentage inhibition of DPPH by apple peel phenolic compounds:

$$\text{DPPH inhibition } \% = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

Abs control is the absorbance of 100 μM methanolic DPPH solution without extracts.

5.3.5.5 Extraction Comparison

In order to demonstrate the efficiency of the radio frequency extraction process, a single factor comparative study between radio frequency and conventional solvent extraction (using hot plate heating at 50°C) method was conducted. The experiment was performed following the best conditions obtained from the previous experiment i.e. using microwave-dried sample, 0.001 M hydrochloric acid and extraction temperature of 50°C for 10 minutes. In order for the extraction solution to reach 50°C, it took two minutes for RFAE while four minutes were required for conventional hot plate method (with medium heat setting). The extraction temperature was fixed at 50°C since the DPPH inhibition was the highest at that temperature (as observed in results from Section 5.4.2 for RF obtained samples at 50 and 70°C). Total phenolic compounds and percentage of DPPH inhibition were the two responses used to assess the efficiency of the two extraction methods.

5.3.5.6 Experimental Design & Statistical Analysis

Full factorial design of three factors (drying method, solvent type and extraction temperature) with two levels for each factor was applied in this experiment. Each treatment combination was performed in triplicate using biological replicates thus bringing the total of experimental runs to 24. The single factor extraction comparison study was also replicated three times. The significance of the investigated factor is determined by $p<0.05$. The statistical analysis was performed using JMP version 8 (SAS Inc, North Carolina).

5.4 Results and Discussions

5.4.1 Effect of Drying Method, Solvent Type and Extraction Time on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Results showed that TPC of extracts were significantly influenced by the sample drying method ($p<0.05$), extraction temperature ($p<0.05$) and choice of solvent ($p<0.05$) (Figure 5-4). The microwave-dried extract had higher TPC compared to the freeze-dried extract when both were extracted at 50°C and 70°C. The plausible explanation for the difference in the quality of the extracts is due to the interaction between the remaining moisture content in the sample with the electromagnetic field. Microwave-dried sample still contained 17 % moisture content meanwhile the freeze-dried samples were completely dried. Firstly, this interaction may have caused internal cell breakage that released more phenolic compounds into the solvent as described in Wang & Weller (2006). Secondly, the remaining moisture content in the sample would probably have altered the dielectric properties of the mixture, improving the heating rate of RF and indirectly affecting the solubility and diffusivity of the target compounds.

Besides the drying method, increasing the extraction temperature also helps in increasing TPC (Figure 5-4). Heat, as reported in the literature, helps in increasing extraction yield through the enhancement of diffusion rate and solubility of target compounds, modification of solvent surface tension and breakage of plant cell matrix. The effect of heat was more significant on the drier samples prepared by freeze drying since the TPC recovery increased from 12.24 to 12.45 % for FD dried samples with 0 % moisture content sample meanwhile only slight increase from 5.39 to 6.82 % for the MW dried samples with 17% moisture content when the temperature was increased to 70°C. In addition, the extraction using mild hydrochloric acid yielded higher amounts of TPC when compared to the mild sulfuric acid treatment (Figure 5-4).

The influence of sample moisture content, extraction temperature and choice of solvent were also significant ($p<0.05$) on the TFC of the extract. TFC obtained from this experiment ranged from 10.91 to 15.66 mg catechin equivalent/g dry peel which accounted for 45.70% to 47.80% of the total amount of phenolic compounds recovered (Figure 5-5). Radio frequency extraction using higher moisture content sample produced extracts with higher TFC.

From the observation, increasing the extraction temperature, within the range tested, had a positive impact on TFC, in which increasing temperature up to 70°C helped in increasing the amount of flavonoids. In terms of solvent, extraction performed with hydrochloric acid produced extract of higher TFC, which was similar for the TPC. Higher recovery of phenolic compounds or flavonoids can be obtained when the plant sample is extracted with aqueous acid with a pH between 3 to 4 (Tabart et al., 2007; Ruenroengklin et al., 2008). The difference of TFC between solvents was more prominent at the extraction temperature of 70°C.

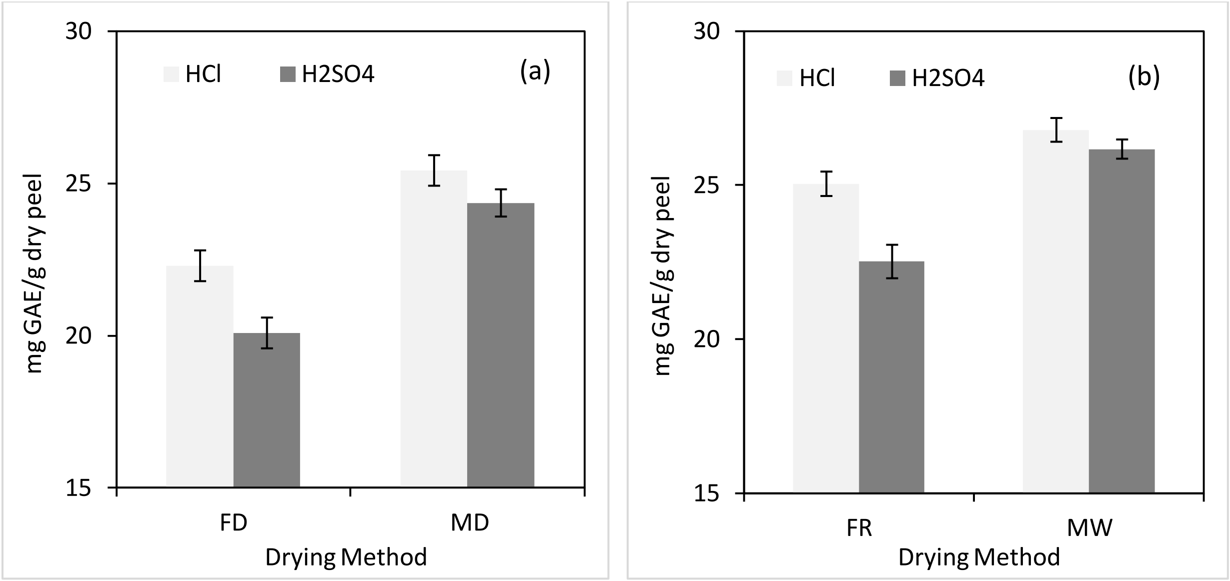

Figure 5-4 : Effect of drying method and solvent type on TPC at (a) 50°C and (b) 70°C

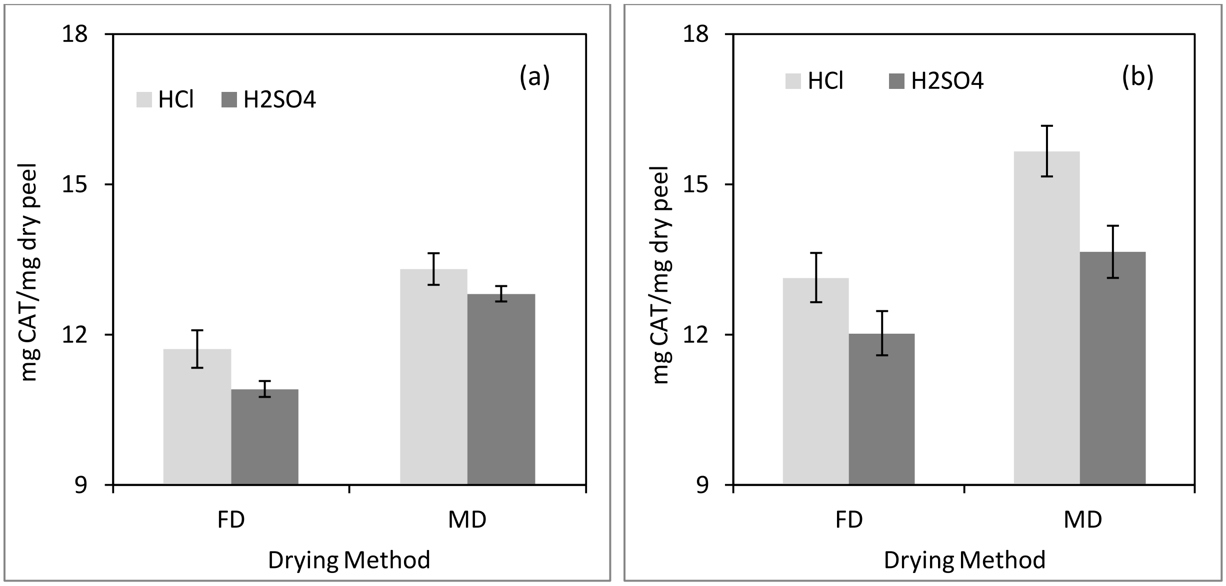

Figure 5-5 : Effect of drying method and solvent type on TFC at (a) 50°C and (b) 70°C

5.4.2 Effect of Drying Method, Solvent Type and Extraction Time on FRAP and DPPH Antioxidant Activities

Besides TPC and TFC, the study of the antioxidant activity of the extract is essential to confirm the strength and quality of the phenolic compounds recovered. The influence of the sample drying method, choice of solvent and extraction time were evaluated with two antioxidant assays i.e. ferric reduction (FRAP) and free radical scavenging (DPPH).

In FRAP assay, extraction temperature and drying method have the most significant effect on the antioxidant activity of FRAP ($p<0.05$), followed by type of solvent ($p<0.05$) (Figure 5-6). Increasing the extraction temperature from 50°C to 70°C caused an increase in FRAP antioxidant activity from 8.65 to 14.50%. In terms of sample drying method, microwave-dried peel extracts showed higher antioxidant activity than the freeze-dried peel extracts. In addition, the higher antioxidant activity of FRAP was observed in apple peel extracted with 0.001 M hydrochloric acid. These observations were expected since the amount of phenolic compounds obtained in microwave-dried peel extract was higher than in freeze-dried extract and in addition, the phenolic compounds were higher when the extraction was performed with mild hydrochloric acid.

The effects of drying method, choice of solvent and temperature on the inhibition of DPPH are showed in Figure 5-7. Solvent type has high significant impact ($p<0.05$) on DPPH inhibition, in which the inhibition of DPPH was significantly lower when H_2SO_4 was used as the extraction solvent. Liberation of different types of phenolic compounds with different antioxidant activities is primarily due to the solvent-target compound affinity and the effect of the solvent on the scavenging activities as highlighted by Sharma and Bhat (2009). Besides that, it was also observed that the drying method of a sample is an important factor in the inhibition of DPPH since the differences between DPPH inhibition of microwave-dried peel extracts and freeze-dried extracts were significant.

In addition to the aforementioned factors, temperature had a significant impact on DPPH inhibition ($p<0.05$). It had a positive relationship with TPC, TFC, FRAP and negative relationship with DPPH inhibition. It was observed that temperature rise reduced DPPH inhibition and similar findings were reported in Larrauri et al. (1998) and Thoo et al. (2010). Our study revealed that DPPH inhibition starts to decrease when extraction temperature goes beyond 50°C. It is possible that at temperatures higher than 50°C, some of the phenolic compounds that scavenge DPPH may have degraded or changed. Another possibility is that temperature may have released specific phenolic compounds that reacted more favorably with the FRAP assay.

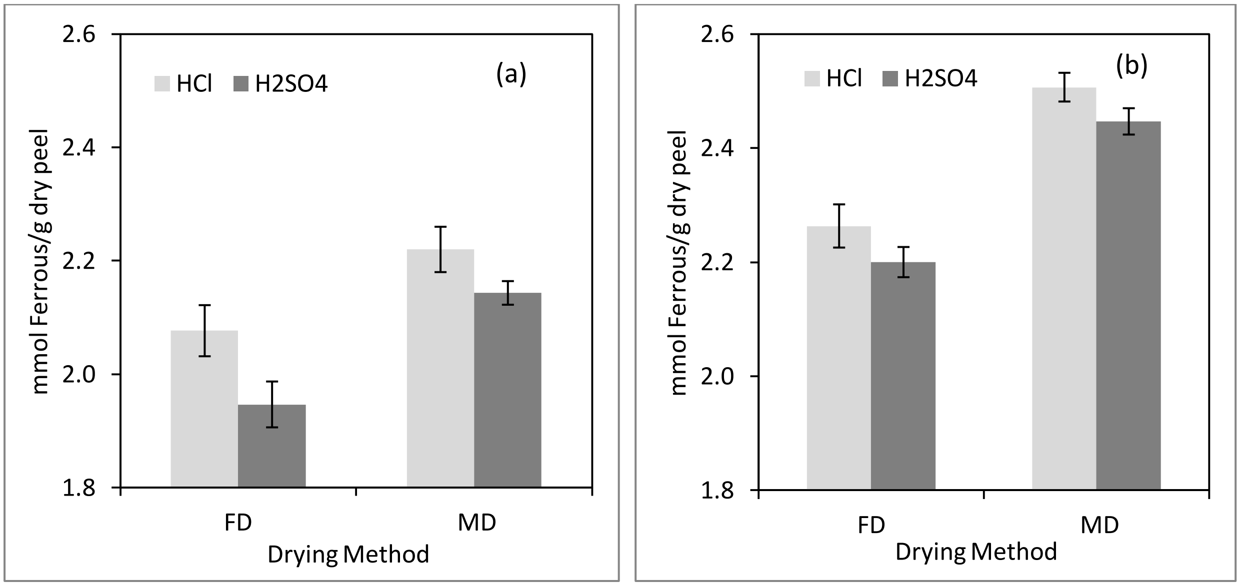

Figure 5-6 : Effect of drying method and solvent type on the antioxidant activity expressed by FRAP assay at (a) 50°C and (b) 70°C

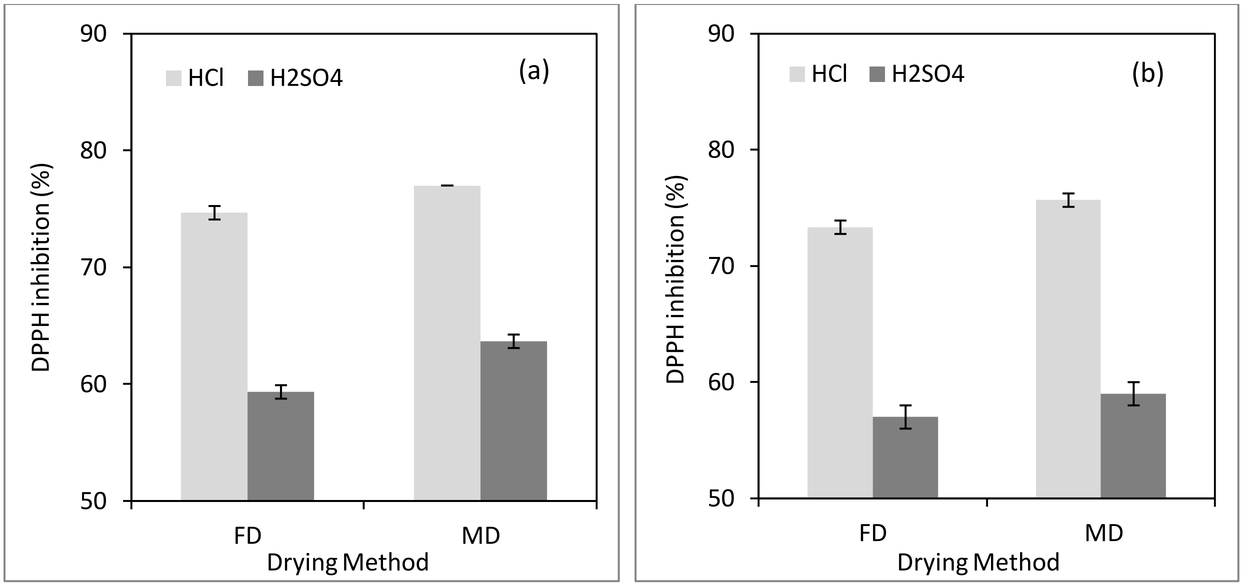

Figure 5-7 : Effect of drying method and solvent type on the antioxidant activity expressed by the DPPH assay at (a) 50°C and (b) 70°C

5.4.3 Selection of the Best Radio Frequency Assisted Extraction Conditions

From this screening study, the microwave-dried samples with 0.001 M hydrochloric acid were the best combination of drying method and solvent for this RF extraction system since their combined effect has produced the highest amount of TPC, TFC, FRAP and DPPH antioxidant activities. Even though the values of TPC, TFC and FRAP would/could be higher at an extraction temperature 70°C, in this case, a compromising point of 50°C to 60°C was selected to avoid the degradation of the many of the compounds that react with DPPH (Figure 5-7).

5.4.4 Comparison Study Between Radio Frequency and Conventional Extraction

A short comparison study to highlight the advantage of the RF heating system as an extraction method in comparison to conventional heating extraction was performed. Both extractions were performed using microwave dried samples and 0.001 M hydrochloric acid for 10 minutes. The study showed that the RF apple peel extract contained 41.84 % higher TPC (25.29 ± 0.40 mg GAE/ g dry peel) compared to the conventional extract (17.83 ± 0.38 mg GAE/g dry peel) (Figure 5-9a). The antioxidant activity of the extract was higher in the RF extract (Figure 5-9b). The study demonstrated the potential of RF heating as a new extraction technique to produce high quality extracts.

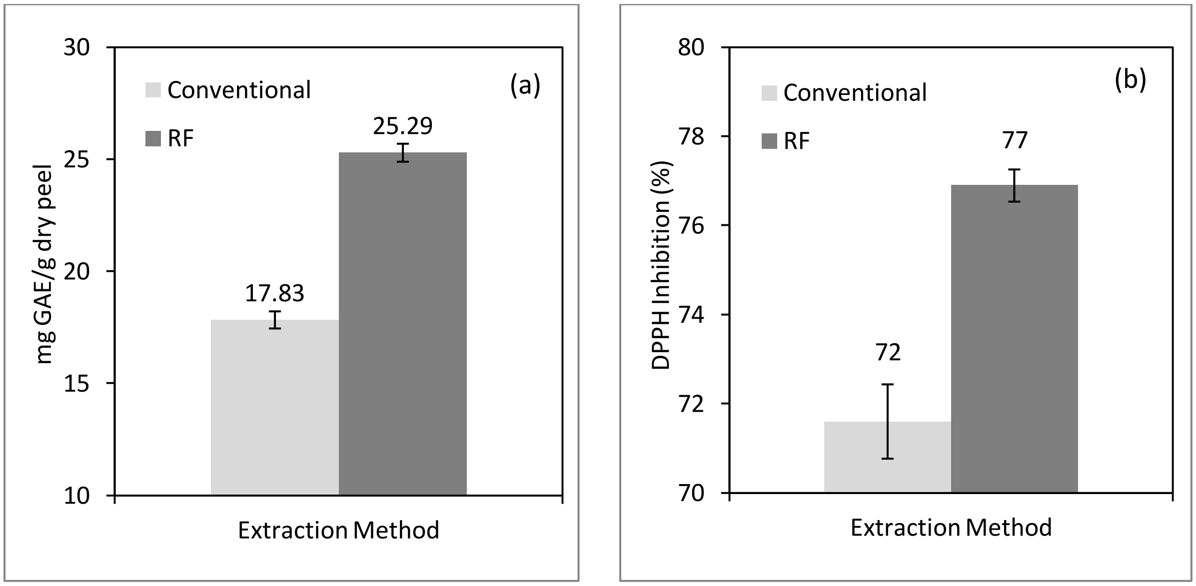

Figure 5-8 : Total phenolic compounds (a) and percentage DPPH inhibition (b) of apple peel extract via two extraction methods, conventional and RF extraction

5.5 Conclusions

The selection of the method of drying for the plant samples, the choice of solvent and the extraction temperature are crucial factors controlling the quantity and quality of apple peel extracts using radio frequency extraction. Extracts with greater concentrations of phenolic compounds and antioxidant activity can be achieved using microwave-dried peel samples and hydrochloric acid at an extraction temperature between 50 to 70°C. Samples with higher moisture content are preferable in RF extraction, and this condition can be achieved with a short duration microwave drying process. Microwave drying is a perfect alternative to freeze drying since the operating cost is cheaper and processing time is much faster. In addition, our comparative study demonstrated that radio frequency extraction obtained greater extract yields than conventional thermal extraction. Information obtained from this study is useful and will be used for further work on apple peel phenolic compounds using radio frequency extraction.

5.6 Acknowledgements

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Connecting Statement to Chapter 6

In **Chapter 5**, a preliminary study on the RFAE of apple peel was conducted to establish an informative foundation regarding solvent, sample condition and extraction temperature requirements for RFAE development. It was clearly demonstrated by the experimental results that the quality of the extract is highly controlled by the selection of solvent, sample condition and temperature.

In conjunction to the previous chapter, the importance of solvent selection was further studied in Chapter 6. The solvent selection is crucial in any extraction as it plays a major role in the extraction recovery owing to 'like attracts like' concept, meanwhile its properties determine the rate of heat generated in a dielectric heating system. It is crucial to understand the dynamic behavior of the solvents under the influence of RF energy. In **Chapter 6**, the changes in dielectric properties and heating rate of the solvents as influenced by solvent concentration, ethanol content, and temperature were investigated and reported as a means to gain better understanding on the behavior of selected solvents and to determine the best solvent for our RFAE of apple peel.

Chapter 6 has been written in the manuscript style for submission to the Journal of Food Engineering:

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who prepared the experimental design, conducted the laboratory work, analysed the results and prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated ideas, provided the technical advice, suggestions, experiment facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.

Chapter 6 - Dielectric Properties and Heating Rate of Solvents for the Development of Novel Radio-Frequency Assisted Extraction of Plants Phenolic Compounds

6.1 Abstract

Understanding the dielectric and heating properties of materials is very important for designing an efficient radio frequency or microwave electro-heating system. In this study, the dielectric properties of solutions of low concentration of hydrochloric acid (HCl), sodium hydroxide (NaOH) and citric acid (CA) at 27.12 MHz were measured using an open-ended coaxial probe and network analyzer. The effects of solvent strength, solvent concentration, ethanol content and temperature on the dielectric constant and dielectric loss of the solvents were determined. Aqueous HCl and NaOH have higher dielectric properties than CA. Solvent concentration and ethanol content play a significant role in increasing and decreasing the dielectric constant and loss. Temperature, on the other hand, has contradicting effect where it reduces dielectric constant while it increases dielectric loss. The heating rates of aqueous HCl and NaOH decrease as their concentrations increase while the heating rate of aqueous CA increases as its concentration increases. From the heating performance study, ethanol addition improves the heating rate of aqueous HCl and NaOH. Empirical models for predicting dielectric properties of the solvents were obtained from this study.

Keywords: *Dielectric properties, radio-frequency assisted extraction, extraction solvents, heating rate, solvent ionic strength*

6.2 Introduction

Many of the fruits and vegetables waste or by-products contain high concentrations of phenolic compounds that have medicinal or nutritional value for humans. For many fruits, these bioactive compounds, which are normally concentrated in the fruit's skin, are the primary protective layer that responds to the climatic changes, ultra violet exposure, predators, pest and diseases. Antiallergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects are several of the beneficial properties of these compounds elucidated from numerous studies (Balasundram, Sundram, & Samman, 2006). Due to these properties and health potentials, the phenolics-rich fruit and vegetable wastes can be further processed into natural antioxidant for daily use in food, functional food, beverage, cosmetics, supplements, packaging materials, etc. Feasibility of some natural antioxidant phenolic compounds in food systems has been published in research articles by numerous groups such as Ajila et al. (2008 & 2010), Martín-Diana et al. (2008 & 2010), Sun-Waterhouse et al. (2004) and Wegrzyn et al. (2008). Utilization of fruits and vegetables waste to obtain these useful compounds would minimize the generated waste from agri-food industries.

Concentrated phenolic compounds from fruits peel can be added into food, beverage, supplements, and cosmetics as ground peel or as a pure extract. On the other hand, pure extracts have broader applications where the users have the choice to select the types of phenolics to use while any unwanted attributes, for example taste, smell, color, anti-nutritive, etc can be minimized via series of purification steps. Although many extraction techniques are available, further research is required to improve or develop better extraction techniques that provide advantages in terms of cost, yield, time, little waste and safety. High volume solvent utilization and long extraction process are two unavoidable issues in conventional extraction methods and these contribute to the increase in operational cost and waste production. Microwave assisted extraction (MAE), an electro-heating technique, is a novel powerful extraction method with promising future for extraction, however its low penetration depth may cause non-uniform heating or low heating performance if the system needs to be scaled up for pilot plant or industrial applications. Non-uniform heating due to the low penetration depth in microwave has been discussed in Al-Harahsheh, Kingman, & Bradshaw (2006).

As an alternative to MAE, a new electro-heating extraction system using radio-frequency (RF) is being developed in our research laboratory based on the longer RF wavelength for its potential and advantages over the existing extraction methods; short extraction time, low solvent volume utilization, ambient pressure environment and possibility of using water as the solvent during extraction. The heating mechanism in radio frequency assisted extraction (RFAE) is similar to MAE, however RF works at a frequency between 1-300 MHz meanwhile microwave operates at 300 MHz to 3 GHz.

The success of an electro-heating heating system depends on several factors especially the permittivity (ε) of the material. Understanding the permittivity of the materials to be heated in electro-heating is important as it represents the material's heating ability and uniformity. In general, permittivity provides information concerning the behavior of a material in an electric field and it is composed of two components, namely known as the dielectric constant (ε') and the dielectric loss (ε''). These two components are also known as the material's dielectric properties. The complex relationship of permittivity with dielectric constant and loss is shown in Equation 1:

$$\varepsilon = \varepsilon' - j\varepsilon'' \quad (1)$$

The ε' indicates the ability of the material to store energy from the electric field in the material meanwhile the ε'' is associated with the energy dissipation in the material or the conversion from electric energy to heat energy (Mudgett, 1986; Icier & Baysal, 2004; Nelson, 2010). Frequency, temperature, density, moisture content, chemical composition and physical structures of materials have been shown to have influence on these properties (Piyasena et al., 2003; Nelson & Trabelsi, 2012; Venkatesh & Raghavan, 2004). Studies of the dielectric properties in RF range encompass food materials such as juice (Zhu, Guo, & Wu, 2012), nuts (Gao et al., 2012), grains (Sacilik & Colak, 2010), oilseeds (Sacilik, Tarimci, & Colak, 2006), meat products (Zhang, Lyng, & Brunton, 2007), caviars (Al-Holy et al., 2005), etc. Although there have been reported studies on dielectric properties in the RF range, reports on the dielectric properties for solvents used for extraction (eg. alcohol, acids, bases) are rare.

The knowledge in dielectric properties of materials has vast applications in the development of the food processing industry. Dielectric properties are used in the development of new and effective electro-heating equipment since these data can be used to model the heating patterns of materials when exposed to an electric field (Birla et al., 2008). Penetration depth, power absorption and heating uniformity of materials are all directly related to the dielectric constant and loss, which contribute to the efficiency of an electro-heating application. Besides that, the sensitivity of a material's dielectric properties, due to changes of chemical compositions, enables this measurement to be extended as a quick method for determining contaminated products or food adulteration as showed in the reported work by Guo et al. (2011). In addition to that, it can also be used as a fruit quality detector for determining food maturity thus allowing assessment of the best storage practice or usage of the fruits as necessary (Fito et al., 2010). Quick moisture content online detector can be designed based on the close relationship between moisture content and the dielectric properties for automated food industry applications (Icier & Baysal, 2004).

The overall aim of this study is to develop a low acidified water extraction system using radio frequency heating. In developing an efficient RF extraction, the choice of solvent is crucial. The solvent must meet the following characteristics; high affinity toward the target compound to be extracted, non-toxicity and most importantly must be able to heat volumetrically when exposed to the RF electric field. Heating within a dielectric material is always associated to its dielectric loss. For the case of RF heating, the dielectric loss of a material is governed by two factors, ionic charge migration and dipole rotation ($\varepsilon'' = \varepsilon''_{ionic} + \varepsilon''_{dipole}$) (Mudgett, 1986). In the case of regular extraction solvents, for example water and ethanol or combination of both, the dielectric loss does not generate sufficient volumetric heating when exposed to an RF electric field simply because they are polar solvents and polarity factor alone is not preferred in RF heating, thus requiring an ionic input. A radio frequency extraction study by Izadifar, Baik, & Mittal (2009) suggested the addition of table salt or sodium chloride in the extraction solvent in order to improve heating rate and uniformity of the extraction system. Salt addition increases the ionic content and subsequently increases the dielectric loss of the solvent in comparison to a water-alcohol system which has lower dielectric loss and low ionic conductivity. Formulating the right solvent mixture that would enable to produce high target yield in a short time of extraction remains a challenge in RF assisted extraction. Based on this requirement, common acid and base in food processing industry was proposed as components of the extraction solvents for the development of RF extraction of phenolic compounds.

The objectives of this study were (1) to establish the dielectric properties data of aqueous hydrochloric acid, sodium hydroxide and citric acid solutions over a specified range of solvent concentrations (0.001 and 0.01 M), ethanol content (0, 30 and 70 %) and temperature (25, 45 and 65°C); (2) to develop empirical models that predict dielectric constant and dielectric loss of solvents as a function of ethanol content and temperature; (3) to develop a correlation between the solvent and heating rate; (4) to find a relationship between dielectric properties and the heating rate in RF; and (5) to determine the best solvent mixture suitable for radio frequency extraction of phenolic compounds.

6.3 Materials and Methods

6.3.1 Chemicals

Hydrochloric acid (HCl), citric acid (CA), sodium hydroxide (NaOH) and ethanol (EtOH) used in this study were purchased from Fisher Scientific, Sigma Aldrich, American Chemicals Limited and Greenfield Ethanol Inc., respectively. Distilled water was used for preparing the solvent solutions according to various concentrations.

6.3.2 Solvents and Sample Preparation

HCl, NaOH and CA solutions of 0.01 M and 0.001 M concentrations were prepared by diluting 1 M stock of each chemical. To prepare 1M HCl, 8.20 ml of HCl was mixed with 91.80 ml water. 1 M CA was prepared by mixing 19.21 g CA in 100 ml water and 1 M NaOH was prepared by mixing 40.00 g of this NaOH pellets into 100.00 ml of water. All mixtures were stirred for 5 minutes to ensure homogeneity. Serial dilutions were applied to obtain samples at the desired concentrations.

6.3.3 Dielectric Properties Measurement

All dielectric properties data were measured using an open-ended coaxial probe connected to an Agilent RF Impedance Network Analyzer (E4991A). The frequency range for this machine is between 1 MHz to 3 GHz and the accuracy of the measurement is ± 0.8 %. For each series of experiments, a two-steps calibration was conducted to ensure the accuracy and consistency of the measurement. The first calibration was for the network analyzer using calibration kits (open, short and load) and the second calibration was for the high temperature probe using 3 calibration conditions (air, short and water at 25°C). The newly calibrated water at 25°C data was compared to published water data. If a big difference was observed between the data, the calibration was repeated. All samples for dielectric properties measurement were prepared in 50 ml tubes. The dielectric properties of the samples were measured at three temperatures 25, 45 and 65°C. A controlled temperature water bath was used to heat up the samples in 50 ml tubes to the specified temperatures. The tube containing the sample was quickly placed in contact with the bottom part of the high temperature probe to measure the properties once the sample reached the desired temperature.

6.3.4 Heating Rate Measurement

Heating rate measurement was performed by heating 50 ml of the solution mixtures to 50°C using the radio frequency extractor (described in details in Chapter 4 and Chapter 5). The solvents were prepared at 3 concentrations (0.001, 0.01 and 0.1M) and ethanol contents (0, 30 and 70 %). Temperature and time taken to heat the solvent was recorded automatically by the RF data acquisition and control system. In order to ensure consistency in the measurement, the temperature reading was made only at one point which was 1 cm from the bottom of the extraction vessel. The heating rate was calculated using the following equation:

$$\text{Heating rate (HR)} = \frac{\text{Final temperature (50°C)} - \text{Initial temperature}}{\text{Stop time} - \text{Start time}} \quad (2)$$

Where initial temperature is the temperature of the solvent at the beginning of the measurement (°C), stop time is the actual time when the experiment was stopped and start time is the actual time recorded at beginning of the experiment and the unit for this time difference is in minute. Heating rate has the unit of °C/min.

6.3.5 Experimental Design and Statistical Analysis

Two separate experiments were conducted in this study. The first experiment was for determining the dielectric properties while the second experiment was for determining the heating rate of the solvent mixtures. A factorial design of four factors was used for the first experiment to investigate the effect of solvent type, solvent concentration, ethanol content and temperature on the dielectric constant and dielectric loss of the solvent mixtures. Alternatively, the heating experiment was conducted based on a factorial design of three factors (solvent type, concentration and ethanol content). All experiments were analyzed using ANOVA from JMP version 8 (SAS Inc, North Carolina) and all the graphs were plotted via Microsoft Office Excel 2007 (Microsoft Corporation, Washington). For both experiments, each experimental treatment combination was biologically replicated for three times. The significance of effect was set at $p < 0.05$.

6.4 Results and Discussions

6.4.1 Effect of Solvent Type

The three solvents investigated in this study have different degree of ionic dissociation in water. HCl and NaOH are respectively a strong acid and base that dissociate completely in water, meanwhile CA is categorized as a weak acid that dissociates only partially in water. These solvents are different in ionic strength, however, at 0.001 M concentration, the dielectric constants for all solvents were not significantly different from each other (Table 6-1). However, at 0.01 M, the dielectric constants for aqueous HCl and NaOH were significantly higher than aqueous CA ($p <0.05$) (Table 6-1).

The dielectric loss was significantly different between these solvent mixtures ($p<0.05$). Aqueous HCl had the highest dielectric loss value, followed by aqueous NaOH and finally aqueous CA (Table 6-2). Strong acid and base like HCl and NaOH, have higher value of dielectric loss values as compared to CA, which possibly contributed to the effect on the dielectric loss by the higher degree of ionic dissociation in water. As mentioned previously, the dielectric loss in the radio frequency range is highly affected by the amounts of ions in the solvent as the dielectric loss is governed by two factors, ionic charge migration and dipole rotation.

6.4.2 Effect of Solvent Concentration

Aqueous HCl, NaOH and CA are ionic solutions. For all types of solutions tested, the dielectric constant increased as the concentration of the solution increased ($p<0.05$)(Table

6-1). The trend we obtained contradicted the usual result trend of salt addition in RF. Nevertheless Izadifar, Baik, & Mittal (2009) obtained a similar trend with their salt addition with an increase of dielectric loss. The strong acid or base (HCl and NaOH) showed a bigger difference on the dielectric constant value between the concentrations (30 to 44 %) as compared to the weak acid CA (10 -17 %).

In addition, the results also showed that the solvent concentration had a significant impact on the dielectric loss ($p < 0.05$). The dielectric loss of the solvent increased as the concentration of solvent increased (Table 6-2). This result was similar to Piyasena et al. (2003) and Zhang, Lyng, & Brunton (2007). The increase in dielectric loss is due to the increase of ions as the concentration of acid or base increases. At low electromagnetic frequency, in the RF range such as 27.12 MHz, the ionic charge migration of dielectric loss is more dominant than the polarity effect on the dissipation of energy as heat, and therefore, increasing the acid or base concentration definitely would contribute to the significant increase of the overall dielectric loss. Nevertheless, since the degree of ionic dissociation differs between strong acid or base to weak acid or base, CA, a weak acid, has the lowest increase in the dielectric loss value as a function of an increase in concentration as compared to the strong acid, HCl.

Another interesting observation was that for solvents at 0.001 M and 25°C, at 30% and 70 % concentration of alcohol, the dielectric constants of all solvents were not significantly different, regardless of the strength of the acid and base (Table 6-1). At 0.01 M, the dielectric constant was significantly affected by the change in solvent and alcohol content. As for the dielectric loss, no similarity was observed between solvents and each solvent with different ethanol concentration behaved uniquely.

6.4.3 Effect of Temperature

The experimental results showed that temperature has a negative effect on the dielectric constant and a positive effect on the dielectric loss. It is observed that as the temperature increased, the dielectric constants of all solvents decreased. This result is similar to work done by Zhu, Guo, & Wu (2012). The temperature dependence of dielectric constant is quite complex, and the dielectric constant may increase or decrease with increasing temperature depending on the material (Venkatesh & Raghavan, 2004;). Nelson & Trabelsi (2012) explained that the dynamic behavior of dielectric properties - temperature dependency is governed by the relaxation process of the materials. In the dispersion (intermediate frequencies) region, an increase in temperature will cause a rise in dielectric constant meanwhile below (low frequencies) and above (high frequencies) the dispersion region, an increase in temperature reduces the dielectric constant, all these owing to the relaxation process (Nelson & Trabelsi, 2012). Gabriel et al. (1998) highlighted the negative relationship between the solvents' temperature and relaxation time which the relaxation times of solvents decreased as the temperature of solvents increased. The relationship between dielectric constant, temperature and relaxation time is perfectly described by Debye's equation where an increase in temperature reduces relaxation time that subsequently leads to higher dielectric constant.

On the other hand, the dielectric losses of all solvents in this study increased with an increase in temperature. The power dissipated per unit volume in a non-magnetic, uniform material exposed to radio-frequency or microwave fields is proportional to the dielectric loss factor of the material (Zhu, Guo, & Wu, 2012). Based on the power absorption equation, a material with high dielectric loss has higher ability to dissipate, as heat, the absorbed power, and as a result higher heating will be produced in the material. However, this high dielectric loss may not always be favorable since it could lead to local overheating also known as runaway heating (Wang et al., 2003).

6.4.4 Effect of Ethanol Concentration

From the obtained results, it is shown that ethanol has a significant effect ($p < 0.05$) on the dielectric constant and loss of the solvent solutions studied (Tables 6-1 and 6-2). The values of the dielectric constant and dielectric loss were observed to reduce significantly by increasing the concentration of ethanol into the solution. This finding is similar to the water-ethanol dielectric properties study performed by Izadifar & Baik, (2008). The dielectric properties of pure ethanol are lower than that of water therefore adding ethanol in the solutions may have contributed to the reduction of dielectric constant and loss of the solutions. Besides that, it was also observed that there was no major difference between the dielectric constants of 0.001M and 0.01M for all solvent solutions at 70 % ethanol concentration, as compared to 30% ethanol. On the contrary, the difference for dielectric loss between samples was governed as the ethanol concentration increased (for all solvent types).

Table 6-1 : Dielectric constant for aqueous HCl, NaOH and CA at 0.001 and 0.01M with 0, 30 and 70% ethanol contents within a temperature range between 25°C to 65°C

Solvent	Ethanol Content (%)	Molarity	Temperature (°C)		
			25	45	65
Hydrochloric acid (HCl)	0	0.001	80.68 ± 2.86	75.82 ± 2.46	71.67 ± 3.20
		0.01	126.59 ± 2.51	121.92 ± 3.21	115.21 ± 2.52
	30	0.001	64.23 ± 1.21	59.61 ± 2.26	54.90 ± 1.87
		0.01	91.28 ± 2.30	86.11 ± 2.55	82.55 ± 2.66
	70	0.001	41.33 ± 2.52	37.46 ± 2.35	35.15 ± 1.23
		0.01	41.14 ± 2.44	38.30 ± 2.83	32.72 ± 2.53
Sodium Hydroxide (NaOH)	0	0.001	77.85 ± 2.55	74.24 ± 1.10	70.17 ± 1.36
		0.01	108.68 ± 1.51	103.07 ± 2.29	96.47 ± 3.01
	30	0.001	63.94 ± 1.29	58.91 ± 1.13	55.65 ± 1.13
		0.01	78.77 ± 2.08	73.23 ± 2.28	70.09 ± 1.72
	70	0.001	40.83 ± 0.09	37.66 ± 0.39	33.69 ± 0.78
		0.01	41.49 ± 3.56	38.32 ± 0.03	35.84 ± 2.32
Citric Acid (CA)	0	0.001	78.83 ± 1.70	73.05 ± 2.27	68.06 ± 1.58
		0.01	84.64 ± 1.62	83.63 ± 0.53	80.46 ± 2.02
	30	0.001	62.82 ± 2.28	57.60 ± 1.10	54.24 ± 1.35
		0.01	66.11 ± 1.55	62.31 ± 2.69	58.16 ± 1.91
	70	0.001	39.87 ± 1.65	37.70 ± 2.19	35.19 ± 1.96
		0.01	39.78 ± 0.18	36.97 ± 0.70	34.91 ± 0.22

± represents the standard deviation of the experiment which was replicated three times

Table 6-2 : Dielectric loss for aqueous HCl, NaOH and CA at 0.001 and 0.01 M with 0, 30 and 70% ethanol within a temperature range between 25°C to 65°C

Solvent	Ethanol Content (%)	Molarity	Temperature (°C)		
			25	45	65
Hydrochloric acid (HCl)	0	0.001	14.16 ± 3.07	23.78 ± 2.01	29.81 ± 2.66
		0.01	247.24 ± 2.68	305.83 ± 2.92	372.83 ± 3.13
	30	0.001	8.45 ± 2.72	14.80 ± 2.20	20.73 ± 1.72
		0.01	156.21 ± 3.97	211.81 ± 3.21	251.86 ± 2.67
	70	0.001	1.26 ± 0.09	4.88 ± 0.64	8.53 ± 1.47
		0.01	41.51 ± 2.83	68.19 ± 3.06	92.18 ± 3.16
Sodium Hydroxide (NaOH)	0	0.001	5.59 ± 0.68	10.77 ± 1.10	15.59 ± 1.36
		0.01	131.75 ± 3.23	193.05 ± 2.53	251.02 ± 3.21
	30	0.001	3.46 ± 1.29	7.04 ± 1.13	10.52 ± 1.13
		0.01	83.89 ± 2.97	126.40 ± 2.51	167.09 ± 3.15
	70	0.001	0.87 ± 0.09	1.89 ± 0.39	4.55 ± 0.78
		0.01	14.33 ± 3.44	33.67 ± 2.44	59.51 ± 2.91
Citric Acid (CA)	0	0.001	3.47 ± 0.66	6.33 ± 0.93	9.56 ± 1.05
		0.01	45.27 ± 2.30	62.42 ± 0.37	71.48 ± 1.19
	30	0.001	1.36 ± 0.04	3.70 ± 0.47	6.10 ± 1.62
		0.01	18.48 ± 0.42	27.83 ± 1.44	36.45 ± 0.89
	70	0.001	0.02 ± 0.03	0.07 ± 0.04	0.41 ± 0.54
		0.01	0.89 ± 0.03	1.25 ± 0.09	2.92 ± 0.07

± represents the standard deviation of the experiment and each experiment was replicated three times

6.4.5 Empirical Models for Predicting Dielectric Properties of Aqueous HCl, NaOH and CA

From the results, empirical models for predicting the dielectric constants and dielectric losses for all three solvent solutions as a function of ethanol concentration and temperature were successfully derived (Table 6-3). The purpose of the models was for estimating the dielectric constants and losses for the solvent solutions without having to perform the actual measurements, in order to save time and cost. Dielectric data is important for calculating other properties in dielectric heating such as penetration depth, power density, temperature difference, etc. The solvent concentration was not considered as a function of the model since the dielectric data range for 0.001 and 0.01 M was significantly different which resulted in a model with high lack of fit.

Table 6-3 : Models to predict dielectric properties of solvents (E for ethanol concentration and T for temperature)

Solvent	Concentration (M)	Empirical Models	R^2
HCl	0.001	$\varepsilon' = 85.19 - 0.54E - 0.20T$	0.99
		$\varepsilon'' = 9.25 - 0.25E + 0.29T - 0.003(E\text{-}33.33)(T\text{-}45)$	0.96
	0.01	$\varepsilon' = 132.45 - 1.20E - 0.24T$	0.99
		$\varepsilon'' = 207.21 - 3.45E + 2.27T - 0.03(E\text{-}33.33)(T\text{-}45)$	0.99
NaOH	0.001	$\varepsilon' = 83.19 - 0.53E - 0.19T$	0.98
		$\varepsilon'' = 2.82 - 0.12E + 0.17T - 0.002(E\text{-}33.33)(T\text{-}45)$	0.97
	0.01	$\varepsilon' = 112.24 - 0.92E - 0.22T + 0.002(E\text{-}33.33)(T\text{-}45)$	0.98
		$\varepsilon'' = 99.19 - 2.23E + 2.07T - 0.03(E\text{-}33.33)(T\text{-}45)$	0.99
CA	0.001	$\varepsilon' = 82.40 - 0.51E - 0.20T + 0.002(E\text{-}33.33)(T\text{-}45)$	0.99
		$\varepsilon'' = 2.24 - 0.09E + 0.09T - 0.002(E\text{-}33.33)(T\text{-}45)$	0.96
	0.01	$\varepsilon' = 88.02 - 0.65E + 0.13T$	0.98
		$\varepsilon'' = 42.57 - 0.82E + 0.35T - 0.008(E\text{-}33.33)(T\text{-}41.67)$	0.96

6.4.6 Heating Rate of Solvents

This experiment was performed to determine the best formulation of a solvent solution for the development of radio frequency extraction of phenolic compounds. In this study, the solvent heating rate was used as an indicator for selecting the best solvent combination. In developing an efficient extraction system driven by heat, the selection of solvent is important because several requirements need to be met; first of all the solvent must be able to produce rapid heating as rapid heating helps to break the plant cell matrix, releases target compound and expedites dissolution of target compound into the extraction solvent. Moreover, heat also modifies solvent surface tension that eases the penetration of target compound into the solvent and the solvent into the plant matrix. Besides being able to generate heat, the solvent must also be compatible with the product to be extracted and remain non-toxic, and preferably food grade.

Results from Tables 6-4 to 6-6 show that, for pure diluted solvents, aqueous HCl has the highest heating rate at 0.001 M meanwhile for aqueous NaOH and CA, the highest heating rate was obtained when the solvent was at 0.01 M. One plausible explanation for this is that NaOH and CA are chemically weaker than HCl, thus these solvents required more ions to dissipate energy as heat in the RF environment. HCl is a strong acid which disassociates completely (acid disassociation constant, $K_a > 1$) in water to release H^+ and Cl^- ions. CA is a weak acid that partially disassociates in water (acid disassociation constant, $K_a < 1$), thus at low CA concentration, not much ions were released that could react with the change of the RF electric field. Thus, it is logical that the heating rate of CA was highest at 0.01M since more CA ions would have been released at this concentration. In addition, the results also demonstrated the significant role of ethanol in improving the heating rate of aqueous HCl and NaOH. However, ethanol addition had mixed impact on aqueous CA, where ethanol addition reduced the heating rate of 0.001 M CA, meanwhile increased the heating rate of 0.1 M CA. The difference in heating rate is mainly attributed to changes of ionic conductivity and polarity of solvents due to the effect of concentration and ethanol content modifications.

Table 6-4 : Heating rate (°C/min) of hydrochloric acid at three concentrations and ethanol contents

Ethanol (%)	Concentration of HCl (M)		
	0.001	0.01	0.1
0	6.78 ± 0.02	4.79 ± 0.02	0.99 ± 0.01
30	7.19 ± 0.02	6.98 ± 0.04	1.28 ± 0.01
70	9.53 ± 0.03	15.27 ± 0.02	2.31 ± 0.03

Table 6-5 : Heating rate (°C/min) of sodium hydroxide at three concentrations and ethanol contents

Ethanol (%)	Concentration of NaOH (M)		
	0.001	0.01	0.1
0	4.21 ± 0.01	6.43 ± 0.03	1.39 ± 0.02
30	4.81 ± 0.01	8.69 ± 0.02	1.71 ± 0.01
70	5.95 ± 0.05	17.03 ± 0.04	4.62 ± 0.03

Table 6-6 : Heating rate (C/min) of citric acid at three concentrations and ethanol contents

Ethanol (%)	Concentration of CA (M)		
	0.001	0.01	0.1
0	4.70 ± 0.02	9.10 ± 0.01	5.05 ± 0.05
30	3.02 ± 0.02	8.20 ± 0.02	9.55 ± 0.05
70	1.99 ± 0.03	5.77 ± 0.05	13.15 ± 0.05

Another interesting observation was found from the plot of the correlation between concentration and heating rate of solvents (Figure 6-1). For HCl and NaOH, the relationship between concentration and heating rate can be best described in the exponential form. The Figure 6-1a and 6-1b demonstrated that the increase in the concentration or molarity of HCl and NaOH (increase of ions in solvents), decreased the heating rates of these solvents in RF heating. The negative effect of salt addition on the temperature rise in the sample during RF heating was also observed in the work done by Piyasena et al. (2003) and Zhang, Lyng, & Brunton (2007). In the study of heating sensitive viscous liquid with 0.27 % guar solution as a replacement of liquid egg by Piyasena et al. (2003b), it was demonstrated that an increase in the salt content, from 0.2 to 0.7 %, caused a reduction in the temperature rise of the guar solution (this was for a fixed liquid flowrate of 12 liter/hour). However, when the flowrate was increased to 48 liter/hour, the relationship between salt content and temperature rise showed a negative quadratic curve. Zhang, Lyng & Brunton (2007) reported that an increase in salt content reduces the temperature rise in meat blends.

On contrary, in the case of CA, positive linear correlations were observed for CA solvents with 30 and 70 % ethanol content meanwhile the exponential relationship, similarly to HCl and NaOH, could only be observed in CA solvent with 0% ethanol content. This observation infers that the ethanol addition and molarity increase in solvent could improve the heating rates of CA in RF heating. The difference observed between CA and HCl and NaOH is probably due to the strength of the acid or base as has been mentioned earlier in Section 6.4.6. The relationships between concentration and heating rate of the investigated solvents and R^2 are listed in Table 6-7.

These correlations can be used to estimate which concentration of aqueous HCl, NaOH and CA would generate the highest heating rate in an RF electric field.

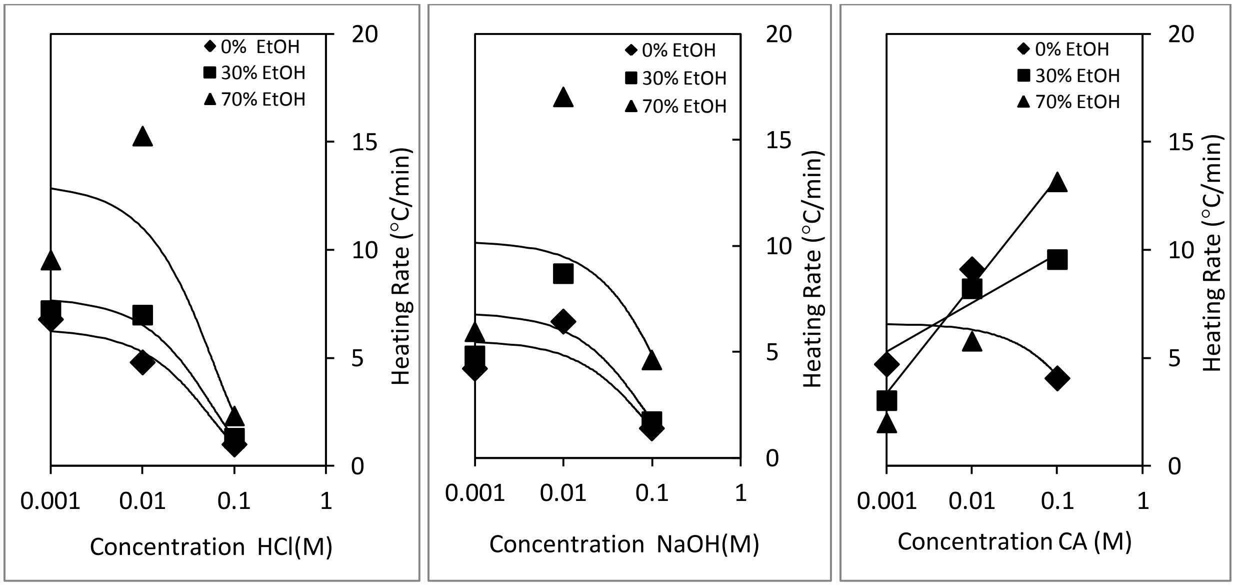

Figure 6-1: Heating rate of HCl (a), NaOH (b) and CA (c) as a function of concentration and ethanol content

Table 6-7 : Relationship between solvent concentration (C) and heating rate of solvents (HR)

Solvent	Ethanol	Equations	R^2
HCl	0	$HR = 6.34e^{-18.66C}$	0.99
	30	$HR = 7.80e^{-18.01C}$	0.99
	70	$HR = 13.05e^{-17.02C}$	0.90
NaOH	0	$HR = 5.53e^{-13.56C}$	0.88
	30	$HR = 6.85e^{-13.53C}$	0.81
	70	$HR = 10.21e^{-7.40C}$	0.34
CA	0	$HR = 6.60e^{-4.54C}$	0.33
	30	$HR = 45.3C + 5.25$	0.52
	70	$HR = 100.27C + 3.26$	0.94

6.4.7 Relationship Between Dielectric Properties and Heating Rate

Attempts to correlate the dielectric properties and heating rates were made however it was not successful at this stage. This strengthens the statement by Marra, Zhang, & Lyng (2009) that the dielectric properties are not the sole factors that influence the magnitude of temperature rise in electro-heating.

6.5 Conclusions

This study of dielectric properties showed that the strength of an acid or base, its concentration, ethanol content and temperature, play a major role in increasing or reducing the dielectric constants and dielectric losses of these solutions at 27.12 MHz. The data obtained is useful for further understanding the reactions of these solvent solutions when exposed to an RF electric field. Dielectric constants and losses of these solvents as a function of ethanol concentration and time can be predicted from the models obtained. Besides that, the heating rate study showed that ionic increase (through increased concentration) has a quadratic effect on the heating rate of the solution mixture. This implies that there is a certain limit of ion addition that can help to improve the heating performance of a given solution in RF heating. Strong acid HCl and basic NaOH heated faster than weak CA at lower concentrations. The heating performance of aqueous HCl and NaOH can be significantly improved by addition of ethanol into the solution; however it has mixed impact on aqueous CA. The heating rate of solvents within the range of the parameters studied can be predicted successfully via the empirical models obtained. There was no definite correlation that could be drawn between the dielectric properties and the heating rate of solvents. Nevertheless, this study assisted in understanding the solvents' behavior in RF and is suggesting choices for best solvent or solvent combination to be used for RF extraction.

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Connecting Statement to Chapter 7

The behavior of acid and base solutions commonly used for phenolic compounds extraction was investigated and reported in **Chapter 6**. The type of solutions, concentration of acid or base, ethanol content and temperature all play a significant role in altering the dielectric properties of the solutions at 27.12 MHz and the heating rate of the solutions under RF heating. In **Chapter 7**, a comprehensive study on the effects of processing parameters on two important aspects in RFAE establishment, i.e. the extract quality and energy utilization was performed. This study was done as a means to gain better understanding on the processing factors and their impact on the system, and subsequently using this information to improve and optimize the process.

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who prepared the experimental design, conducted the laboratory work, analysed the results and prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated ideas, provided the technical advice, suggestions, experiment facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.
- The third author is the engineer in charge of the laboratory who assisted in the machinery setup.

Chapter 7 - Analysis and Optimization of Phenolic Content, Antioxidant Activity, Heating Performance and Energy Efficiency during Radio Frequency Assisted Extraction of Apple Peel

7.1 Abstract

A radio frequency assisted extraction (RFAE) was performed for extracting phenolic compounds from apple peel. Response surface methodology was employed for designing the experiment, analyzing the effects and optimizing the extraction conditions for RFAE of apple peel phenolic compounds. The effects of ethanol concentration (10 – 70%), mixing speed (10 – 320 ml N_2/min), solid to liquid ratio (0.002 - 0.02 g/ml) and RF power (200 – 400 W) on total phenolic compounds, FRAP ferric reducing antioxidant power, DPPH radical scavenging activity, heating performance, sample energy absorption and energy efficiency were investigated in order to understand and improve the RFAE performance. Statistical analysis suggested that highest yield and performance could be achieved by performing the extraction using 10% ethanol in 1mM HCl with stirring speed of 245.60 ml N_2/min, solid to liquid ratio of 0.02 g/ml at 300 W. No significant difference was observed between experimental and predicted values which indicated the adequacy of the optimized models for predicting yield and performance of the system under this experimental range. Solid to liquid ratio and mixing speed could assist in improving RFAE performance. HPLC analysis confirms the availability of chlorogenic acid and epicatechin in the apple peel extract.

7.2 Industrial Relevance

This paper focuses on two interesting subjects; application of radio frequency heating for bioactive compounds extraction and utilization of apple peel (waste material) for isolating phenolic compounds as functional ingredients. Radio frequency heating offers several advantages such as rapid heating, low solvent utilization, no contact with sample, uniform heating, non-destructive towards target compounds and feasible scalability due to its high penetration depth which might be an interest to the industry. Apple peel contains a high amount of strong antioxidants which can be extracted, purified and further used in supplements, food, beverage, cosmetics, textile, biomaterials etc. The design of the experiment, analysis and process optimization was performed using response surface methodology (RSM) for its known accuracy and precision under a smaller number of experimental runs.

7.3 Introduction

Phenolic compounds are becoming important as more and more research findings are showing their association with potential health improvements. Research has shown that these compounds possess several beneficial properties such as antioxidant, antimicrobial, anti-inflammatory, anti-allergenic, anti-atherogenic, anti-thrombotic, cardioprotective and vasolidatory effect (Balasundram, Sundram & Samman, 2006). Phenolic compounds are present in many food sources such as fruits, vegetables, cereals, nuts, honey, maple syrup, etc. Nowadays, much work has been done on recovering phenolic compounds from fruits and vegetables waste during postharvest handling or food processing since these wastes contain large concentrations of phenolic compounds which can be extracted and converted into functional ingredients.

In this paper, the research focuses on recovering numerous phenolic compounds from apple peel waste as apple is one of the largest production of fruits grown in North America which are not solely grown for raw consumption but also for the production of juice, puree, sauce, cider, jam, etc. Tonnes of peels are being discarded each year from the apple processing industry, which, if not processed into useful products, might lead to waste disposal issues. Chlorogenic acid, procyanidins, epicatechin, quercetin, cyanidins, phloretin and phloridzin are among the phenolic compounds detected in apple peel (Tsao et al., 2003). *In vitro*, *in vivo* and human trials have demonstrated the strong antioxidant activity of these apple phenolic compounds and their potential therapeutic effects for neurodegenerative diseases, cancers, cardiovascular diseases, etc. (Boyer & Liu, 2004; Hyson, 2011). To date, apple peel waste is principally used as animal feed. Recently many studies have been published on the utilization and commercialization of apple phenolics as functional ingredients in yogurt, snack bar, beverage, cosmetics, textile, etc.

Apart from highlighting the importance of phenolic compounds from apple peel, the main focus of this paper is to introduce a newly developed radio frequency assisted extraction system (RFAE) as a new method of extraction for bioactive compounds. The key driving force of the developed RFAE method is the rapid heating under the influence of an RF electric field. In order to produce a high quality extract at a reasonable cost, an efficient extraction method must be carefully selected. In any case, short extraction duration with high yield is preferable, however the process must also comply to green technology requirements, be efficient in terms of energy utilization and safe for handling. Radio frequency assisted extraction (RFAE) is being developed because it has several benefits over existing extraction systems. First of all, RFAE can be considered as a green extraction process because it focuses on minimizing the usage of solvent and can restrict the selection of the solvent to food grade solvents only. Rapid heating, low solvent utilization, no product surface burning/overheating, no direct contact between the sample and the heating element, ambient pressure working environment, and better heating uniformity are some of the advantages offered by RFAE. The way RFAE functions is similar to microwave assisted extraction (MAE), however the major difference is that RFAE functions at a lower frequency (27.12 MHz). With lower frequency, RF has higher penetration depth which might be an advantage for a larger scale industrial application as compared to the sample size restriction encountered with MAE and UAE (ultrasound assisted extraction). The principal driving force for extraction in RFAE is heat, which is generated volumetrically within the sample thus accelerating the mass transfer and diffusion coefficients of target compounds into the solvent. This volumetric heat is generated in RF heating due to rapid ions and molecules collision resulting from ionic conduction and polar realignment under the influence of the alternating RF electric field (Orsat & Raghavan, 2005). With RFAE, the extraction time and recovery of target compounds are anticipated to be respectively shortened and maximized.

In developing an efficient extraction system using radio frequency heating, numerous factors were involved and they were shortlisted into a list of four for this work simply based on the results from initial work (Chapter 5, Chapter 6 and Mohd Jusoh & Orsat, 2012). The effect of ethanol concentration, mixing speed, solid to liquid ratio and RF power on the quantity and quality of the extract and RFAE energy consumption were studied. These factors exert a significant effect on the extraction yield and also on the energy efficiency of RFAE.

The usage of an aqueous alcohol mixture as the effective solvent for efficient bioactive compounds extraction has been reported in Zhang et al. (2007) and Prasad et al. (2011). Research findings by Dai & Mumper (2010) and Reis, Rai & Abu-Ghannam (2012) have confirmed that pure alcohol and water are not the best extraction solvents for simultaneous recovery of a broad range of bioactive compounds due to the variable polarity of the compounds of interest. On top of that, in solvent heating experiments under RF and microwave heating presented in Chapter 6 and Routray & Orsat (2013), it was demonstrated that an aqueous alcohol mixture heats faster than either of the pure alcohol or pure water, which would definitely be beneficial for reducing process time.

Another important factor under investigation was the possibility of mixing during extraction. This factor is important in radio frequency heating as non-uniform heating might occur under dielectric heating due to several factors such as non-homogeneity in sample composition, incorrect sample positioning between the electrodes, inappropriate sample size, applicator design, etc. This uneven heating may result in the loss of extraction yield and increased energy consumption in RFAE. Therefore, to overcome this issue, nitrogen bubble mixing has been proposed with the current RFAE system. The addition of a stirring mechanism has proven successful in promoting heat uniformity in RF heating as reported in Wang et al. (2005) and Wang et al. (2007). Turbulence motion, induced by supplied nitrogen bubbles, may improve surface contact between solid particles and the solvent and heat distribution. We would like to highlight that this is the first work reported on the utilization of stirring in RF extraction and the first to use nitrogen bubbles dispersed in the system instead of using a conventional mechanical stirrer.

The effect of solid to liquid ratio is investigated in this study based on its positive result on the heating performance that was observed in the preliminary investigations on RFAE as reported by Mohd Jusoh & Orsat (2012). Increasing the ratio of solid to liquid undeniably changes the dielectric properties of the sample which subsequently affects its ability to generate volumetric heating. Increasing solid to liquid ratio in our extraction is anticipated to improve the heating and energy absorption in RFAE. This hypothesis is based on the quick increase of temperature in RF heating of the sample with low moisture content. However, one has to understand that heating performance and energy efficiency in RF heating is not solely contributed by dielectric properties of the sample, but also strongly relies on other thermal properties of the sample to be heated.

The final factor tested in this study is the RF power supplied as power plays a major role in influencing the heating performance of dielectric heating. By increasing the power, the sample's heating performance is expected to increase as well. However, a suitable power level must be determined as excessive power might cause compound degradation during extraction, and at the same time may incur energy wastage and high production cost in comparison to the output obtained (Song et al., 2011). Thus in order to control energy usage for an optimal process operation, the effect of input power has to be investigated.

It is hoped that the information gathered from this study would be informative for improving RFAE for the purpose of nutraceutical extraction and further the development of radio frequency solid-liquid heating in a variety of other applications.

7.4 Materials and Methods

7.4.1 Apple Peel Sample Preparation

McIntosh apples from Quebec which were harvested in October 2011 were used for this experiment. The apples were washed and surface dried before the skin was removed. The peeling of apple skin was performed using an Apple Pro-Peeler (Starfrit Atlantic Promotions Inc., Canada). The fresh peel was subjected to drying using a microwave-hot air dryer (Post-Harvest Laboratory, McGill University) for 40 minutes at a microwave power of 150 W with maximum temperature of 50°C. The final moisture content of the peel was 17 %. Subsequently, the dried peel was ground using a domestic blender Magic Bullet (Homeland Housewares LLC, USA) and sieved to obtain apple peel powder with particle sizes ranging between 500 - 750 μm. This size was selected based on our previous screening study (Mohd Jusoh & Orsat, 2012).

7.4.2 Chemicals

Hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,4,6-tris-2-pyridyl-1,3,5-triazine (TPTZ), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), ferric chloride hexahydrate ($FeCl_3.6H_20$), methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, ferrous sulfate heptahydrate ($FeSO_4.7H_20$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was obtained from Greenfield Ethanol Inc. (Canada).

7.4.3 Radio Frequency Assisted Extraction

Our RFAE system consists of three major operations that are the RF generator, RF applicator and the data acquisition system. Detailed information relating to this system can be found in Chapter 4. Figure 7-1 schematically presents the RFAE lab-scale set up that was developed and used for the extraction of phenolic compounds from apple peel.

In order to perform the extraction, 50 ml of extraction solvent (a mixture of specific concentration of ethanol with aqueous hydrochloric acid maintained at 1 mM) was added into the RF extraction tube. This borosilicate glass extraction tube is of cylindrical shape with a length of 0.230 m and a diameter of 0.04 m. This tube has an opening at one of its end to allow sample insertion, while the other is rounded. Then specific amounts of ground apple peel samples were added to the solvent and the sample-solvent mixture was stirred for 15 seconds using a stirring rod to enhance mixture uniformity. After that, the extraction tube was placed between the RF electrodes in the applicator (Figures 7-1 and 7-2). To perform the RF heating treatment, the cover of the applicator must be locked properly for safety purpose (to prevent energy leakage). The RF generator was then switched on and tuned accordingly to ensure maximum energy coupling and avoid power reflectance as this significantly impacts the efficiency and lifespan of the generator. The machine has maximum input power of 600 W. The control of RF power was done manually by turning the RF power knob. All extractions were performed for 10 minutes with final temperature of 50°C. It took approximately 2 to 3 minutes for the sample to reach final temperature of 50°C. The samples were continuously stirred during the extraction process using a nitrogen bubble stirrer which was developed solely for this RFAE application (Figure 7-2). Nitrogen gas was used in this extraction process since it is an inert gas that does not participate in causing oxidation of compounds during extraction.

The extraction was performed at three levels of ethanol contents (10, 40 and 70 %), with three different nitrogen bubble stirring speed (10, 165 and 320 ml N_2/min), solid to liquid ratio (0.1, 0.5 and 1.0 g/ml) and RF power (200, 300 and 400 W) to evaluate the effects of these processing factors on the extract quality and also energy consumption and efficiency of the system.

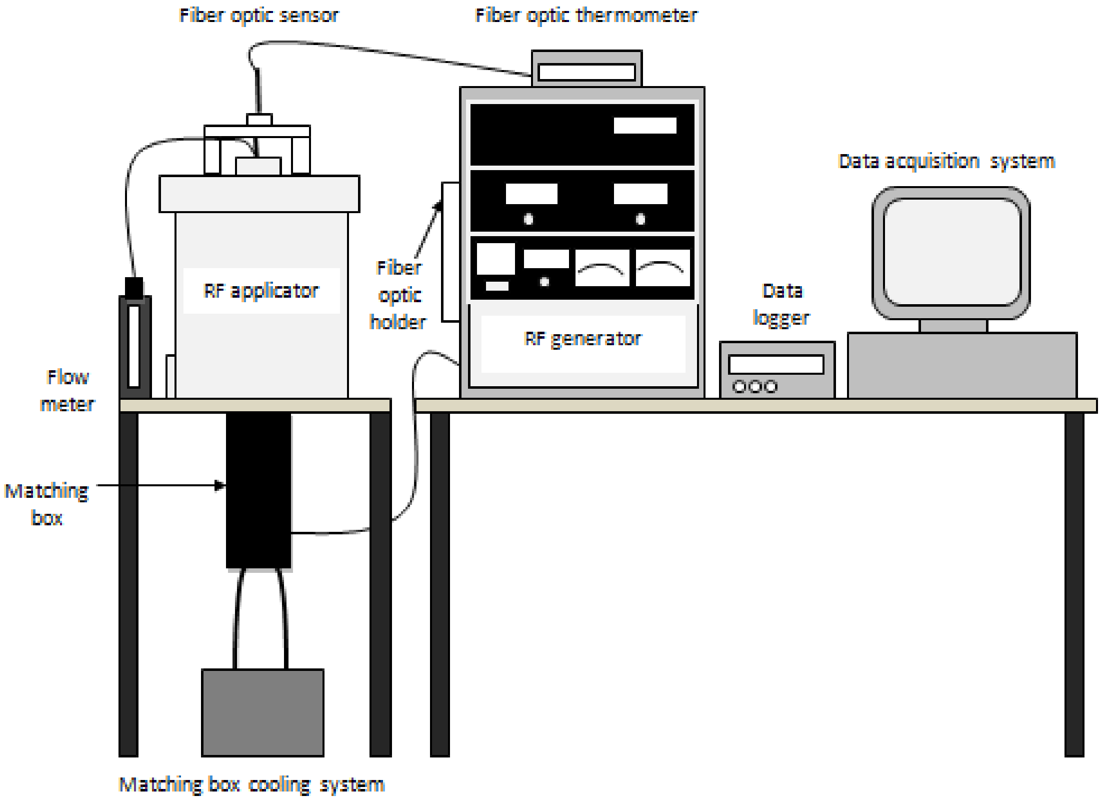

Figure 7-1: Radio frequency assisted extraction (RFAE) lab-scale setup

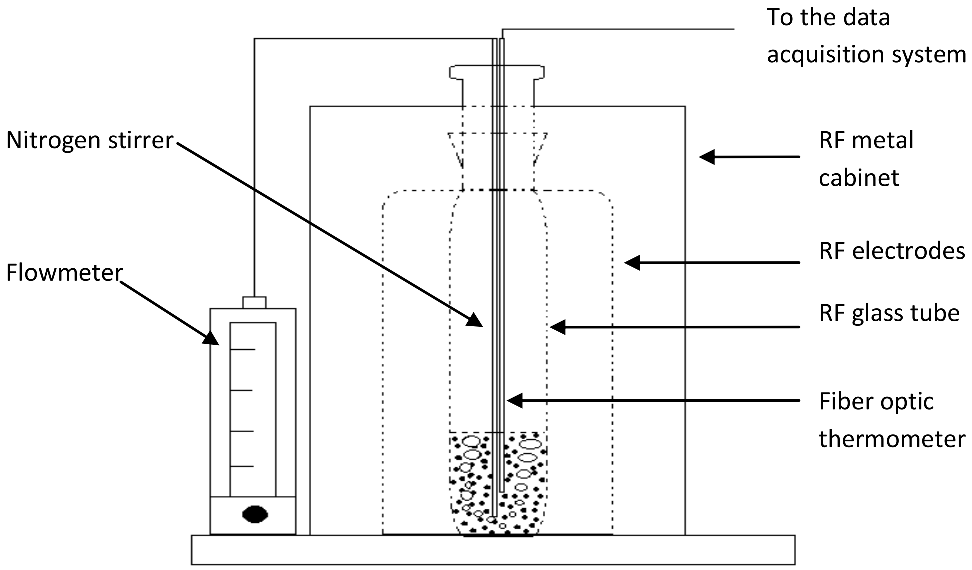

Figure 7-2 : Bubble stirrer for RFAE

7.4.4 Total Phenolic Compounds Determination

The total phenolic contents in the apple peel extract were determined using the method described in Waterhouse (2005) with minor modifications. 0.1 ml of extract was mixed with 7.9 ml of distilled water in a 15 ml centrifuge tube. Then, 0.5 ml Folin-Ciocalteu reagent was added into the mixture. The tube containing the mixture was agitated for 1 minute using a shaker and left aside for 7 minutes. After that, 1.5 ml of $NaCO_3$ was added and mixed again. The mixture was incubated for 2 hours at room temperature. All tubes were properly covered with aluminum foil to prevent them from light exposure. A Biochrom US 1000 UV/VIS spectrophotometer (Biochrom, Massachusetts) was used to measure the absorbance of the sample with the wavelength set at 765 nm. The quantification of total phenolic compounds in the samples was performed using gallic acid standard. Initially, three gallic acid curves in 1 mM hydrochloric acid with different amounts of ethanol content (10, 40 and 70%) were prepared, however all the three curves shared the same equation ($y = 0.0008x$) and the same r^2 value (0.98). The amount of phenolic content obtained was expressed as milligram gallic acid equivalent per gram of dry mass (μg GAE/g DM).

7.4.5 Antioxidant Determination Using Ferric Reducing Antioxidant Power (FRAP) Assay

The first antioxidant activity test performed for evaluating the antioxidant activity of our RFAE apple peel extract was the FRAP assay. This method was carried out according to the method described in Benzie & Strain (1996) and Alothman, Bhat & Karim (2009) with minor modifications. 0.1 ml extract was mixed with 0.3 ml distilled water and 3.0 ml FRAP reagent. This FRAP reagent was made up of 25 ml 0.3 M sodium acetate buffer of pH 3.6, 2.5 ml of 10 mM TPTZ in HCl and 2.5 ml of 20 mM $FeCl_3.6H_20$. The mixture of distilled water and FRAP reagent was incubated at 37°C for 4 minutes prior to its color reading. The same UV/VIS spectrophotometer as mentioned in Section 7.4.4 was used to read the absorbance of the samples at 593 nm. The antioxidant activity of the samples was determined using three sets of calibration curves as the experiment was conducted using 1 mM hydrochloric acid with different ethanol levels (10, 40 and 70%). As with the gallic acid curves, all the three calibration curves for FRAP shared same equation (y = 668.88x) and r^2 value (0.98). Four concentrations of ferrous sulphate $FeSO_4.7H_20$ at 0, 1, 2 and 3 mM were selected for plotting each of the calibration curves. The result was expressed as micromoles of ferrous equivalent Fe (II) per gram of dry mass (μMol Fe (II)/g DM).

7.4.6 Antioxidant Determination Using DPPH Assay

The second method to evaluate the antioxidant activity of our apple peel extract was through DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Experimental procedures were performed by modifying the methods described in Hatano et al. (1988) and Sharma & Bhat (2009). DPPH solution of 100 μM was prepared in extraction solvent of 1 mM HCl with 10, 40 and 70 % ethanol concentrations. 3 ml of extract was mixed with 1 ml DPPH solution in 15 ml tube covered with aluminum foil. This mixture was shaken vigorously and left aside for 30 minutes at room temperature. The absorbance of the sample at 517 nm was measured via spectrophotometry. The following equation (Equation 1) was used to calculate the percentage inhibition of DPPH by the apple peel phenolic compounds:

$$\text{DPPH inhibition } \% = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100 \qquad (1)$$

Abs control is the absorbance of 100 μM DPPH solution without extracts.

7.4.7 Heating Rate (HR) Measurement

The heating rate was calculated by monitoring the temperature rise from the initial state up to 50°C as a function of the time taken for the sample to heat from the initial start temperature up to 50°C under RF heating. Below is the expression (Equation 2) used for calculating the heating rate of the solvent mixture :

$$\text{Heating rate}\left(\frac{{}^\circ\text{C}}{\text{min}}\right)=\frac{\text{End temperature}-\text{Start temperature }(^\circ\text{C})}{\text{Start time}-\text{End time (min)}} \tag{2}$$

Knowing the heating rate of the tested solid-liquid mixture is important as it explains the behavior of the samples under the influence of the electromagnetic field.

7.4.8 Energy Absorption by the Sample (ABS)

Energy absorption by the sample is calculated according to the fundamental engineering expression below (Equation 3):

$$\mathrm{E}=\mathrm{mC_p\Delta T} \tag{3}$$

Where m is the weight of the solid-liquid mixture (g), C_p is the heat capacity of the solid-liquid mixture (J/g.K) which has been measured experimentally and ΔT is the difference in temperature rise of the solid-liquid mixture (°C) during a determined heating duration.

7.4.9 Energy Efficiency (EE)

The purpose of calculating the efficiency of this extraction is to understand better the operating conditions that affect the efficiency of the system and how extraction can be maximized by manipulating the experimental factors. Energy efficiency (EE) of the system is the ratio of energy absorbed by the sample to the energy supplied to the sample as shown in the equation below (Equation 4):

$$\text{Energy efficiency (EE) (\%)}=\frac{\text{Energy absorbed by sample (ABS)}}{\text{Energy supplied to sample}}\text{x }100 \tag{4}$$

Energy supplied to the sample can be calculated from the energy balance from the RFAE system (Equation 5):

$$E_{Generator} = E_{CoolingWater} + E_{Suppliedtosample} \quad (5)$$

where $E_{generator}$ is calculated using $P_{Generator}/\Delta t$, meanwhile $E_{CoolingWater}$ was calculated using $mC_p\Delta T$. $E_{CoolingWater}$ is the amount of energy lost due to continuous change in impedance matching or in other words, the amount of energy which is not supplied to the sample. Its value can be measured by the increase of temperature in the cooling water system that is attached to the matching box (Figure 7-1).

7.4.10 Design of Experiment and Statistical Analysis

A face centered central composite design (CCD) with uniform precision was applied for this experiment. CCD was selected mainly due to the optimization purpose of the study and face centered was chosen due to the limited experimental range. In addition, this design offers a lower number of experimental runs to be performed compared to other designs. Through CCD, the number of experimental runs performed was only 31 including 7 central points. The experimental design and analysis was performed using JMP version 8 (SAS Institute Inc., North Carolina). Significance of each effect was determined by $p < 0.05$. Table 7-1 below summarizes the experimental factors and levels for this experiment.

Table 7-1 : Face Centered CCD experimental design in coded and uncoded form

Factor	Unit	Low level (-1)	Medium level (0)	High level (+1)
Ethanol content (X_1)	%	10	40	70
Stirring speed (X_2)	ml N_2/min	10	165	320
Solid to liquid ratio (X_3)	g/ml	0.002	0.011	0.02
Power (X_4)	W	200	300	400

The experimental responses of TPC, FRAP, DPPH, heating rate, sample energy absorption and energy efficiency are denoted as Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6, respectively.

7.4.11 Verification of the Optimized Model

In order to validate the accuracy of the optimized multi-responses model, another set of experiment was conducted according to the optimized conditions obtained. The experimental data was then compared to the predicted data to evaluate the accuracy of the optimized model in mimicking the real experiment at the optimized extraction condition. This condition is briefly explained in Section 7.5.3.

7.4.12 Phenolic Compounds Determination via High Performance Liquid Chromatography (HPLC)

The compounds available in the RFAE apple peel crude extract were identified using the HPLC method proposed by Tsao et al. (2003). The separation and identification was performed at 25°C by a reversed-phase RP C18 Discovery column (Supelco) (5μm, 25 cm x 4.6 mm) with a Discovery Supelguard cartridge C18 (5μm, 25 cm x 4 mm) using a HPLC Agilent 1100 series equipped with a variable wavelength detector. The injection volume and flowrate were fixed at 10 μL and 1 ml/min, respectively. The HPLC sample was prepared by filtering the RFAE crude apple peel extract through a 0.20 μm nylon syringe filter (Whatman, United Kingdom) into the HPLC vial. The compounds were detected at 280 nm. Catechin, chlorogenic acid, epicatechin and quercetin standards were used for identifying the compounds available in our crude apple peel extract.

7.5 Results and Discussions

The design of the experiment used for this research work together with the actual data obtained from the experiments are presented in Table 7-2.

Table 7-2 : Experimental design with experimental data

Run	Parameters				Responses					
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
1	10	10	0.002	200	72.13	1012	13.00	5.21	5580	14.34
2	10	10	0.002	400	74.38	1018	10.43	10.73	6006	10.75
3	10	10	0.02	200	83.94	1066	81.00	5.56	7151	20.64
4	10	10	0.02	400	85.94	1075	82.88	10.23	7007	18.66
5	10	320	0.002	200	77.50	1011	18.05	6.40	5376	17.19
6	10	320	0.002	400	85.00	1011	14.93	12.98	5797	15.05
7	10	320	0.02	200	83.88	1076	86.28	7.52	7004	23.55
8	10	320	0.02	400	90.19	1074	85.08	15.66	7476	24.31
9	70	10	0.002	200	77.50	1001	6.63	7.71	3072	12.42
10	70	10	0.002	400	80.63	1012	9.21	15.15	3396	10.56
11	70	10	0.02	200	82.38	1051	66.36	6.18	3043	9.47
12	70	10	0.02	400	88.19	1071	74.07	12.69	3447	9.23
13	70	320	0.002	200	90.63	1013	13.15	6.96	2967	9.83
14	70	320	0.002	400	96.88	1019	16.26	15.48	3270	10.09
15	70	320	0.02	200	91.13	1078	80.97	6.76	3127	7.46
16	70	320	0.02	400	95.06	1084	83.75	14.36	2756	9.81
17	10	165	0.011	300	94.53	1064	67.91	8.10	6671	16.14
18	70	165	0.011	300	99.65	1064	61.15	8.95	2908	8.37
19	40	10	0.011	300	111.1	1035	55.00	5.67	4870	10.42
20	40	320	0.011	300	120.2	1041	62.65	6.90	4762	11.18
21	40	165	0.002	300	106.3	1012	19.96	8.54	4330	11.73
22	40	165	0.02	300	110.4	1072	86.91	9.13	5487	15.26
23	40	165	0.011	200	112.3	1055	72.06	4.37	4645	11.68
24	40	165	0.011	400	119.6	1063	73.10	9.87	4876	10.49
25	40	165	0.011	300	116.9	1061	68.19	6.68	4978	11.23
26	40	165	0.011	300	117.6	1048	70.65	7.39	4679	10.56
27	40	165	0.011	300	119.6	1064	75.73	6.56	4880	11.00
28	40	165	0.011	300	116.9	1057	67.19	6.68	4750	10.75
29	40	165	0.011	300	117.6	1058	70.65	7.19	4943	11.21
30	40	165	0.011	300	119.3	1064	75.73	6.56	4698	10.65
31	40	165	0.011	300	118.1	1057	69.86	6.84	4843	10.86

Ethanol content (X_1), Stirring speed (X_2), Solid to liquid ratio (X_3), Power (X_4), TPC (Y_1), FRAP (Y_2), DPPH (Y_3), heating rate (Y_4), sample energy absorption (Y_5) and energy efficiency (Y_6).

7.5.1 Influence of Extraction Parameters on TPC, FRAP and DPPH

Table 7-3 shows the regression coefficient for the predicted TPC, FRAP and DPPH models. Each of the models is uniquely affected by the extraction parameters at the different degrees of significance.

Table 7-3 : Regression coefficients for predicted TPC, FRAP and DPPH models and theory statistical importance

Effect	TPC (Y_1)		FRAP (Y_2)		DPPH (Y_3)	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	117.78	<0.05	1056.77	<0.05	68.91	<0.05
Linear						
X_1	3.03	<0.05	-0.74	0.45	-2.67	<0.05
X_2	4.12	<0.05	3.69	<0.05	3.47	<0.05
X_3	2.79	<0.05	29.82	<0.05	33.65	<0.05
X_4	2.47	<0.05	3.55	<0.05	0.68	0.30
Interaction						
X_1X_2	1.55	<0.05	3.72	<0.05	1.30	0.07
X_1X_3	-1.49	<0.05	-0.04	0.97	-1.18	0.10
X_1X_4	0.07	0.82	1.84	0.09	1.32	0.07
X_2X_3	-1.60	<0.05	2.38	0.03	0.54	0.44
X_2X_4	0.68	0.03	-2.21	0.05	-0.50	0.47
X_3X_4	-0.07	0.82	0.69	0.51	0.70	0.32
Quadratic						
X_1^2	-20.43	<0.05	9.35	<0.05	-3.72	0.04
X_2^2	-1.87	0.02	16.63	<0.05	-9.44	<0.05
X_3^2	-9.20	<0.05	-12.28	<0.05	-14.81	<0.05
X_4^2	1.62	0.04	4.41	0.10	4.33	0.02
Model		<0.05		<0.05		<0.05
Lack of Fit		0.40		0.97		0.89
R^2		0.99		0.98		0.99

Through a multiple regression analysis, a relationship between the extraction parameters to the TPC yield obtained is described in Equation 6. The statistical analysis indicates that this TPC model (Equation 6) is significant ($p<0.05$), and the good fit between the model and observation data was proven through its lack of fit value which was not significant. This also means that the model is reliable for predicting TPC under similar experimental conditions. An R^2 of 0.99 indicates that all variation in the model are well accounted for by the model.

$$Y_1\ (\mathrm{TPC}) = 117.78 + 3.03X_1 + 4.12X_2 + 2.79X_3 + 2.47X_4 + 1.55X_1X_2 - 1.49X_1X_3 - 1.60X_2X_3 + 0.68X_2X_4 - 20.43X_1^2 - 1.87X_2^2 - 9.20X_3^2 - 1.62X_4^2 \tag{6}$$

From Table 7-3 and the response surface plot presented in Figure 7-3, the ethanol concentration and the solid-to-liquid ratio have significant quadratic effects on TPC yield. This significant effect is reflected by the high coefficient value for both factors. Figure 7-3a illustrates the increasing values of these two factors up to a certain point (ethanol 10 – 42 %, solid to liquid ratio 0.002 – 0.0114 g/ml) which yielded the maximum TPC value. If these maximum operational values are increased beyond the stated value, a reduction in TPC yield will be observed. The plausible explanation for the quadratic effects of these two factors is explained by the fact that aqueous ethanol at 42 % may have an intermediate polarity between pure ethanol and water which is more suitable for extracting the phenolic compounds from the apple peel. Work carried out by Bai et al. (2010), Yang & Zhai (2010) and Song et al. (2011) showed that aqueous ethanol is a better solvent rather than pure water or pure ethanol for the extraction of phenolic compounds. Adding some water to ethanol can enhance the rate of extraction by causing the raw material to swell, allowing the solvent to penetrate the solid particles more easily (Gertenbach, 2002). In terms of solid to liquid ratio, theoretically, lowering the solid to liquid ratio increases the concentration gradient between the inside and the surface of the particles which leads to higher extraction rates (Gertenbach, 2002). On the contrary, with RFAE, an increase of TPC up to a maximum point caused by a higher solid to liquid ratio was observed. A logical explanation is that if the appropriate surface contact between the solid and solvent is maintained, an increase in TPC value, caused by a high solid to liquid ratio is possible and expected. As the solvent surface contact subsides due to the high solid to liquid ratio, the TPC recovery reduces. Even though this finding violates the mass transfer principle, nonetheless, an improvement of yield due to the high solid to liquid ratio was also observed and reported by Pinelo et al. (2005) and Gupta, Jaiswal & Abu-Ghannam (2013). On the other hand, increases in the mixing speed and RF power increased the TPC yield as the mixing action increases uniformity and contact between solid and solvent, meanwhile higher power induces faster volumetric heating that plausibly accelerates diffusivity of target compounds into the solvent. Based on the experimental data and mathematical computation, it is suggested that the highest recovery of TPC with a value of 121.87 mg GAE/ g DW can be achieved if the extraction is performed using 42.67% ethanol concentration, mixing speed of 320 ml N$_2$/min, solid to liquid ratio of 0.0114 g/ml and power at 400W.

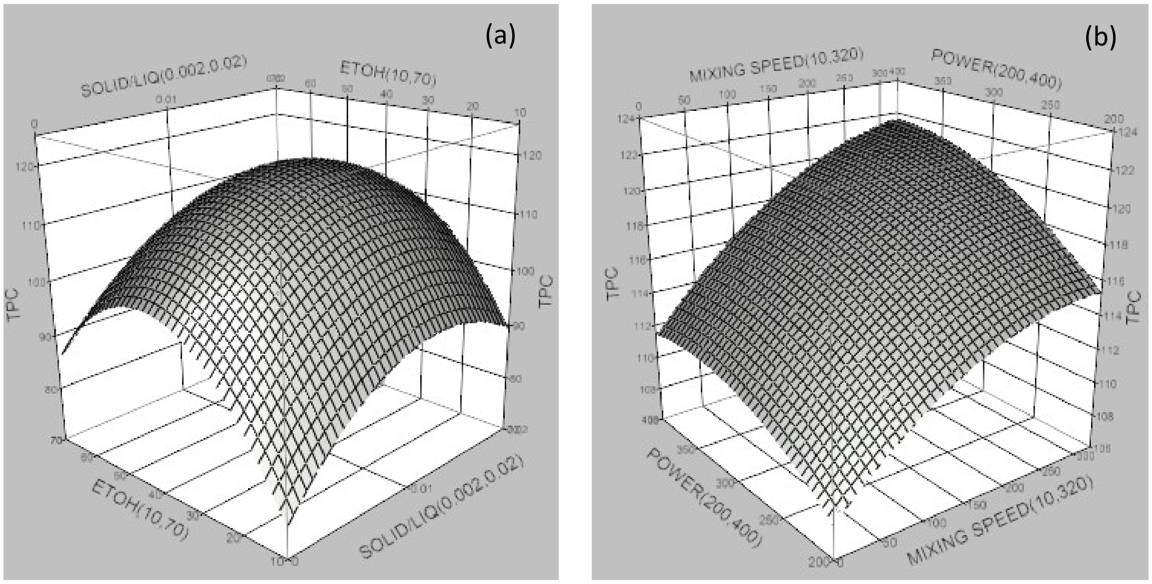

Figure 7-3 : Three dimensional representation of the effects of ethanol concentration and solid to liquid ratio (a) and mixing speed and RF power (b) on TPC yield

As the TPC value alone does not justify the claim that the extract possesses antioxidant properties, therefore two antioxidant activity measurements, using the FRAP and DPPH methods were performed. From the experiment, a model for predicting FRAP was obtained (Equation 7). The value of lack of fit was not significant and R^2 value of the model of 0.98 inferred that the predictive model obtained is well fitted with the data and it can be used for predicting the FRAP value under similar experimental conditions (Table 7-3).

$$\mathrm{Y_2(FRAP)} = 1056.77 + 3.69\mathrm{X_2} + 29.82\mathrm{X_3} + 3.55\mathrm{X_4} + 3.72\mathrm{X_1X_2} + 2.38\mathrm{X_2X_3} - 2.21\mathrm{X_2X_4} + 9.35\mathrm{X_1^2} - 16.63\mathrm{X_2^2} - 12.28\mathrm{X_3^2} \tag{7}$$

The three dimensional plot (Figure 7-4) below is the best representation for explaining the interaction of all parameters with the resulting FRAP values. Ethanol concentration, mixing speed and solid to liquid ratio exert significant quadratic effects on the FRAP value. For ethanol concentration, the highest FRAP values were achieved at both end of the spectrum with ethanol concentrations approaching 10% or 70%. This might be explained by the different types of phenolic compounds, with different antioxidant ability, which are extracted at these two ethanol concentrations having very different polarity which react differently with the FRAP reagent.

For the effect of mixing, increasing the mixing speed up to 199.4 ml N_2/min increases the FRAP value while increasing the speed beyond that causes a negative effect on FRAP which is probably due to an insufficient contact time between the solids and solvent as mentioned in the analysis of TPC. Increasing the solid to liquid ratio increases the FRAP value and this is expected as higher amounts of compounds probably were extracted from a higher amount of sample.

For power, an increase in this factor can assist in increasing the FRAP value as power increments accelerate heating and diffusion of compounds into the solvent. The analysis indicates that the maximum FRAP value (1094.12 Um Ferric (II)/ g DW) can be achieved when the radio frequency assisted extraction is performed using 70.00 % ethanol, with constant stirring of 199.40 ml N_2/min, solid to liquid ratio of 0.02 g/ml at a power of 400 W (the maximum power studied).

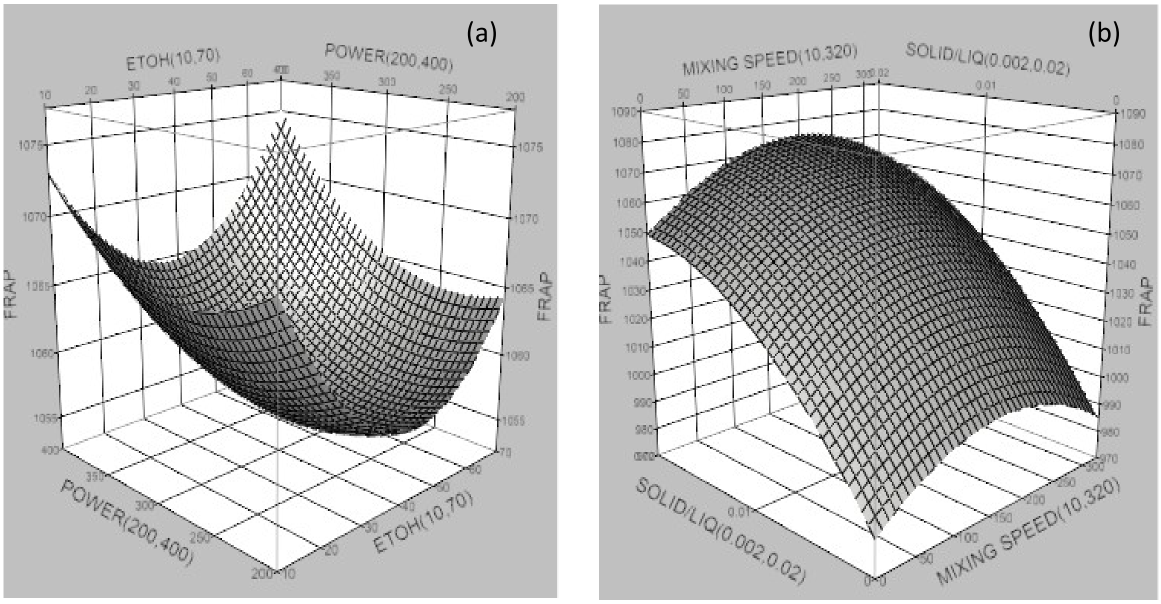

Figure 7-4 : Three dimensional representation on the effect of ethanol concentration and RF power (a) and solid to liquid ratio and mixing speed (b) on FRAP

The relationship between all tested factors and their effect on the DPPH value is formulated in Equation 8. Based on the lack of fit value which was not significant and R^2 value of 0.99 in Table 7-3, it is concluded that the model obtained for DPPH is also well fitted and suitable for predicting the DPPH value.

$$Y_3(\mathrm{DPPH}) = 69.81 - 2.67X_1 + 3.47X_2 + 33.65X_3 - 3.72X_1^2 - 9.42X_2^2 - 14.81X_3^2 + 4.33X_4^2 \tag{8}$$

Through the coefficient analysis, it can be inferred that the most influential factor on DPPH value is the solid to liquid ratio (linear or quadratic terms). Increasing the solid to liquid ratio increases the DPPH value plausibly because more phenolic compounds are liberated in the solvent. Increasing the mixing speed from 10 to 189.1 ml N_2/min causes an increase in the DPPH value however if the speed is increased beyond that, the DPPH value will decrease (Figure 7-5a). This is likely due to the insufficient time of contact between the solid particle and solvent with high intensity agitation as experienced as well in the case of

FRAP. Highest DPPH can be achieved when ethanol concentration is between 10 - 47 %, beyond that value, the DPPH value starts to decrease. One plausible explanation is that by increasing the ethanol content the polarity changes, which thus reduces the tendency of the solvent to aptly extract compounds that can react with DPPH. On the other hand, increasing the power had no significant impact on DPPH. From the optimization analysis for single response, the maximum value of DPPH (94.54%) can be achieved if the extraction is performed using an ethanol concentration, mixing speed, solid to liquid ratio and power of 37.33 %, 165 ml N_2/min, 0.02 g/ml and 400 W, respectively.

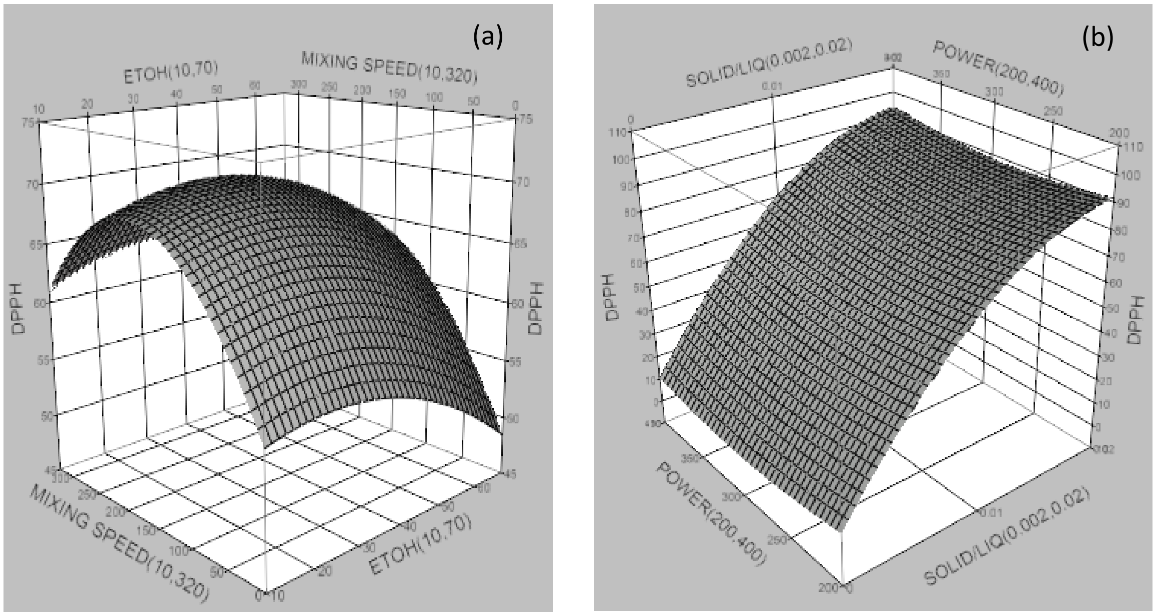

Figure 7-5 : Three dimensional representation on the effect of ethanol concentration and mixing speed (a) and solid to liquid ratio and power (b) on DPPH

7.5.2 Influence of Extraction Parameters on Heating Rate, Sample Energy Absorption and Energy Efficiency

Another focus of this study is aimed to investigate the interaction between the extraction parameters and the heating rate (HR), energy absorption by the sample (ABS) and energy efficiency (EFF) of the process. All these responses (Table 7-4) are indicators for the compatibility between the sample and the RFAE system. This study is vital for understanding and improving the performance of the laboratory scale RFAE and at the same time it provides essential information for the development of efficient RF heating of solid - liquid mixture samples operating under similar conditions for industrial applications. In RF heating, the dielectric properties of the sample to be heated are a critical factor since these properties significantly govern the interactions between the sample and the electromagnetic field, contributing to process efficiency. Ideally, any sample exposed to a RF electromagnetic field should be able to heat up quickly and uniformly within a short time with low energy requirement and power density.

Table 7-4 : Regression coefficients for predicted heating rate (HR), energy absorption by sample (ABS) and energy efficiency (EFF) and their statistical importance.

Effect	HR (Y_4)		ABS(Y_5)		EFF (Y_6)	
	Coefficient	P-value	Coefficient	P-value	Coefficient	P-value
Intercept	6.87	<0.05	4827.34	<0.05	11.02	<0.05
Linear						
X_1	0.66	<0.05	-1671.23	<0.05	-4.08	<0.05
X_2	0.77	<0.05	-57.62	0.26	0.67	<0.05
X_3	-0.06	0.60	372.48	<0.05	1.47	<0.05
X_4	3.36	<0.05	114.79	<0.05	-0.42	<0.05
Interaction						
X_1X_2	-0.56	<0.05	-46.65	0.35	-1.26	<0.05
X_1X_3	-0.56	<0.05	-388.21	<0.05	-2.30	<0.05
X_1X_4	0.3225	<0.05	-32.35	0.51	0.47	<0.05
X_2X_3	0.41	<0.05	22.45	0.65	0.19	0.03
X_2X_4	0.42	<0.05	-11.42	0.82	0.57	<0.01
X_3X_4	-0.07	0.55	-69.73	0.17	0.51	<0.05
Quadratic						
X_1^2	1.61	<0.05	-40.83	0.74	1.09	<0.05
X_2^2	-0.63	<0.05	-14.57	0.91	-0.37	0.07
X_3^2	1.92	<0.05	78.10	0.53	2.33	<0.05
X_4^2	0.20	0.50	-69.97	0.57	-0.08	0.67
Model		<0.05		<0.05		<0.05
Lack of Fit		0.11		0.06		0.29
R^2		0.98		0.98		0.99

Due to the RF selectivity, not all materials interact well with a RF electromagnetic field and dissipate the energy as heat. Heating rate of the material, which is in our case the solid-liquid mixture, is a simple way to determine whether the sample is suitable for RF heating. This method was initiated from the work of Orsat (1999) and Piyasena & Dussault (2003) in measuring the heat rise of their agricultural and food samples under RF heating. In addition, to commercialize a RF heating technology, shorter heating time is preferable as the processing time contributes to the production cost. From this experiment, a significant model to estimate the heating rate of the sample under the experimental range was established (Equation 9).

$$\begin{aligned} \mathrm{Y4\ (HR)} = {} & 6.88 + 0.66\mathrm{X}_1 + 0.77\mathrm{X}_2 + 3.36\mathrm{X}_4 - 0.56\mathrm{X}_1\mathrm{X}_2 - 0.56\mathrm{X}_1\mathrm{X}_3 + 0.32\mathrm{X}_1\mathrm{X}_4 \\ & + 0.41\mathrm{X}_2\mathrm{X}_3 + 1.61\mathrm{X}_1^2 - 0.63\mathrm{X}_2^2 + 1.92\mathrm{X}_3^2 + \end{aligned} \tag{9}$$

The fitness of the model and its reliability is assured by its insignificant lack of fit (p = 0.11) and R^2 value of 0.98 (Table 7-4). Based on the coefficient value and surface plot (Figure 7-6), the linear effect of power and the quadratic effect of mixing speed and solid to liquid ratio have strong impact on the model. The high heating rate of the mixture could be achieved at the highest concentrations of ethanol and solid to liquid ratio (Figure 7-6a). The linear increase of heating rate due to power can be seen in Figure 7-6b. Quadratic effect of mixing speed showed that the maximum heating rate can be achieved when the speed is fixed at 165 ml N_2/ml (Figure 7-6b). From the single response optimization analysis, the maximum heating rate (15.64 °C/min) can be achieved if the experiment is performed using 70% ethanol concentration, mixing speed of 165 ml N_2/min, solid to liquid ratio of 0.002 g/ml and a power of 400W.

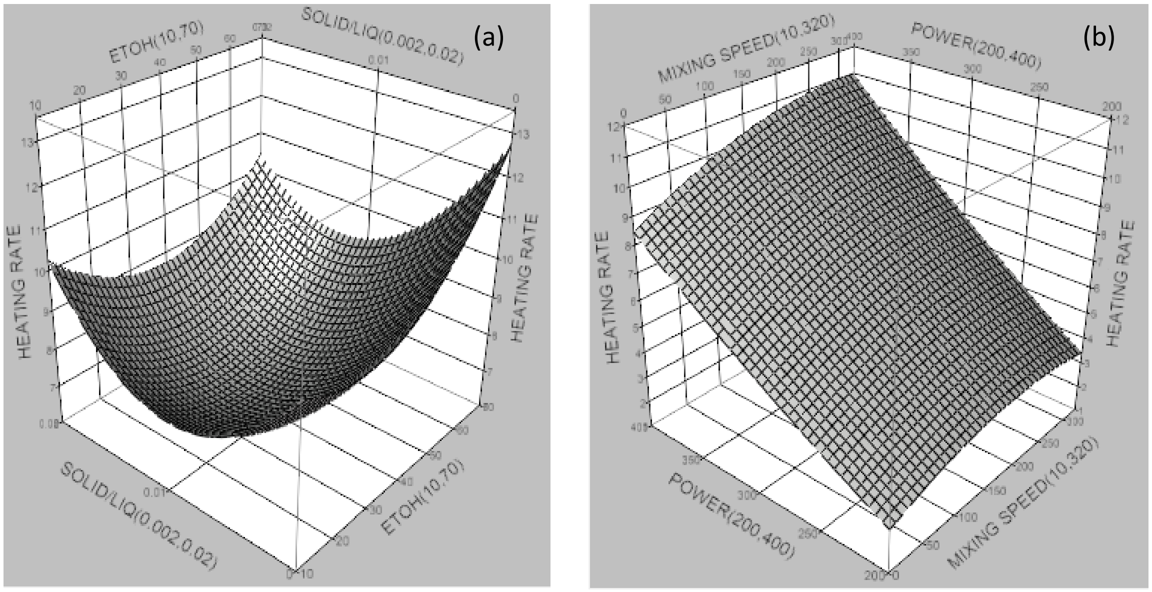

Figure 7-6 : Three dimensional representation on the effect of ethanol concentration and solid to liquid (a) and mixing speed and power (b) on the heating rate

Understanding the ability of the sample to absorb the energy supplied to the sample is another important response in ensuring that the energy supplied by the generator will be properly utilized and not wasted. Heating is generated within the sample if the sample absorbs energy efficiently. Ethanol concentration and solid to liquid ratio definitely affect the dielectric properties of the sample mixture, which in turn significantly affect the ability of the sample to store and dissipate energy as heat. The effect of process parameters on the energy absorption of the sample is given in Equation 10 below:

$$Y_5(\mathrm{ABS}) = 4827.34 - 1671.23X_1 + 372.48X_3 + 114.79X_4 - 388.21X_1X_3 \tag{10}$$

The insignificant lack of fit (p = 0.06) and R^2 value of 0.98 indicate the fitness and reliability of the model to predict energy absorption (Table 7-4). In this study, only linear and interaction terms of ethanol concentration, solid to liquid ratio and power demonstrate a significant effect on the sample energy absorption. Figure 7-7 shows that increasing ethanol content significantly reduced the capability of the sample to absorb energy and on the contrary, increasing the solid to liquid ratio significantly increased the sample's energy absorption. RF heating was initially developed for heating low moisture content sample as dielectric samples with low moisture content react well in a RF electric field. Increasing the dry apple peel content in the solvent increased the solid to liquid ratio. The additional dry apple peel sample may have improved the overall dielectric properties of the solid-liquid mixture and led to an improvement of the sample's energy absorption. Therefore, we can highlight the fact that RFAE is effectively a low solvent extraction method. Mixing speed, on the other hand, had no significant impact on improving the sample's energy absorption. The maximum energy absorption by the sample (7303 Joule) was obtained by performing the extraction with 10% ethanol in water as the solvent, mixing speed of 196 ml N_2/min, solid liquid ratio of 0.02g/ml and RF power of 400 W.

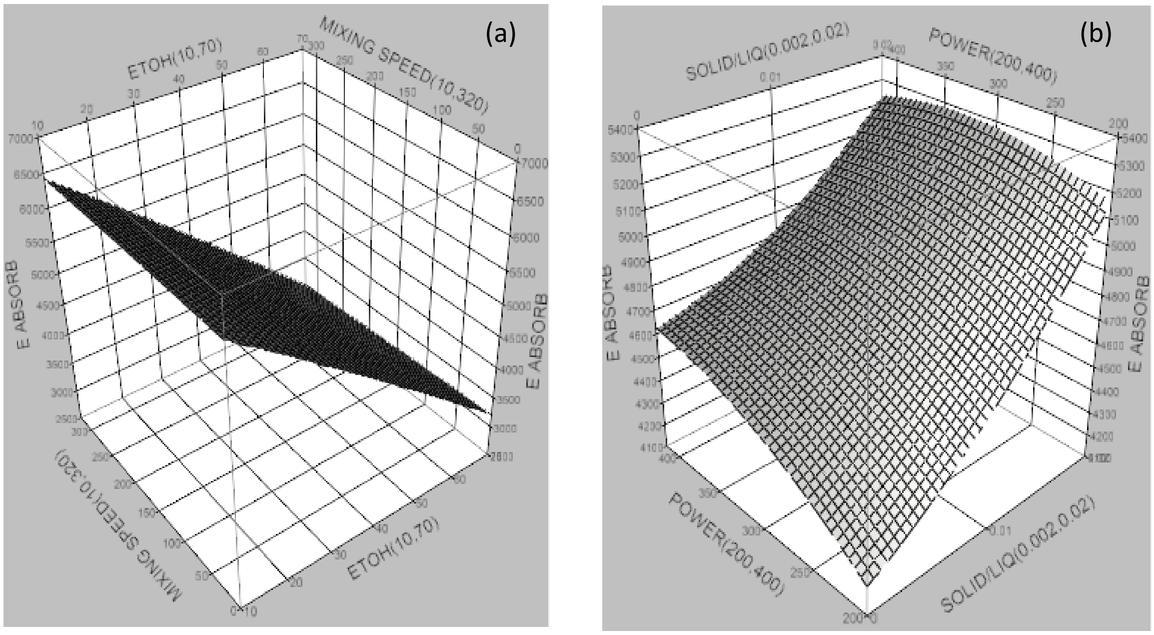

Figure 7-7 : Three dimensional representation of the effect of ethanol concentration and mixing speed (a) and solid to liquid ratio and power (b) on energy absorbed by the sample

The final response investigated was the energy efficiency. Successful conceptualization of a new equipment or process depends on its efficiency. Since sample composition and homogeneity both affect the dielectric properties, this indirectly affects energy efficiency. A model for predicting the energy efficiency of RFAE is shown in Equation 11 below:

$$
\begin{aligned}
Y_6(\mathrm{EFF}) = {} & 11.02 - 4.08X_1 + 0.67X_2 + 1.47X_3 - 0.42X_4 - 1.26X_1X_2 - 2.30X_1X_3 \\
& + 0.47X_1X_4 + 0.19X_2X_3 + 0.57X_2X_4 + 0.51X_3X_4 + 1.09X_1^2 \\
& + 2.33X_3^2
\end{aligned}
\tag{11}
$$

From Figure 7-8a, it is observed that high ethanol content in the extraction solvent does not improve RFAE energy efficiency. On the other hand, increases in the solid to liquid ratio improved the efficiency. Maximum energy efficiency (24.12%) can be obtained by performing the extraction using 10% ethanol concentration in the solvent, a mixing speed of 320 ml N_2/min, solid liquid ratio of 0.02g/ml and 200 W RF power. Results indicate that RFAE energy efficiency can be further improved by increasing the solid to liquid ratio beyond 0.02 g/ml. Increasing solid content may improve the energy absorption and permittivity of the sample therefore improving the overall efficiency. Even though the efficiency currently obtained is low, however it is possible to increase the energy efficiency by increasing the sample size (since increased solid's sample size improves the energy coupling within the applicator) and proper modification on the sample composition (adding more solid content or ionic compounds thus modifying the sample dielectric properties). The large energy losses in our system may have been contributed by small sample size and sample composition of lower permittivity. Higher energy efficiency was observed when low RF power was applied (Figure 7-8b), this condition is parallel with our intention of developing a heating system that functions on low power density to prevent energy wastage and excessive local heating.

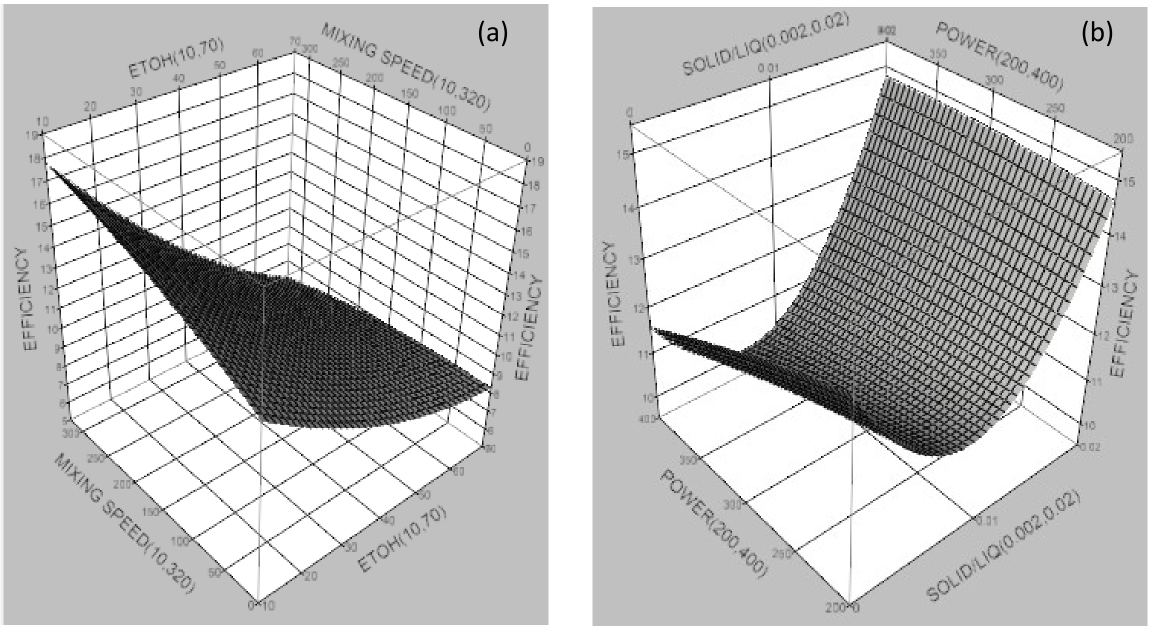

Figure 7-8 : Three dimensional representation of the effect of ethanol concentration and mixing speed (a) and solid to liquid ratio power (b) on energy efficiency

7.5.3 Multi-response Optimization and Desirability

In Section 7.5.1 and Section 7.5.2, individual optimized extraction conditions for each response were suggested based on the obtained results. However, the target of this study is not solely focusing on achieving high recovery of antioxidant but also to ensure that the extraction is performed at high energy efficiency. Thus a compromised optimized point must be selected based on the desirability profiler provided by JMP statistical software (Figure 7-9). Complex interactions between the parameters and multiple responses led to the best optimized point for this experiment with a desirability factor of 73.11 % which corresponds to the extraction conditions using a 10% ethanol concentration solvent, 245.60 ml N_2/min mixing speed, 0.02 g/ml solid to liquid ratio and 300 W RF power. From this optimized condition, TPC, FRAP, DPPH, heating rate, energy absorbed by sample and energy efficiency are predicted to be 89.40 mg GAE/g DM, 1081.64 mM ferric (II)/g DM, 87.64 %, 10.99 °C/min, 7298.56 J, 23.28 %, respectively.

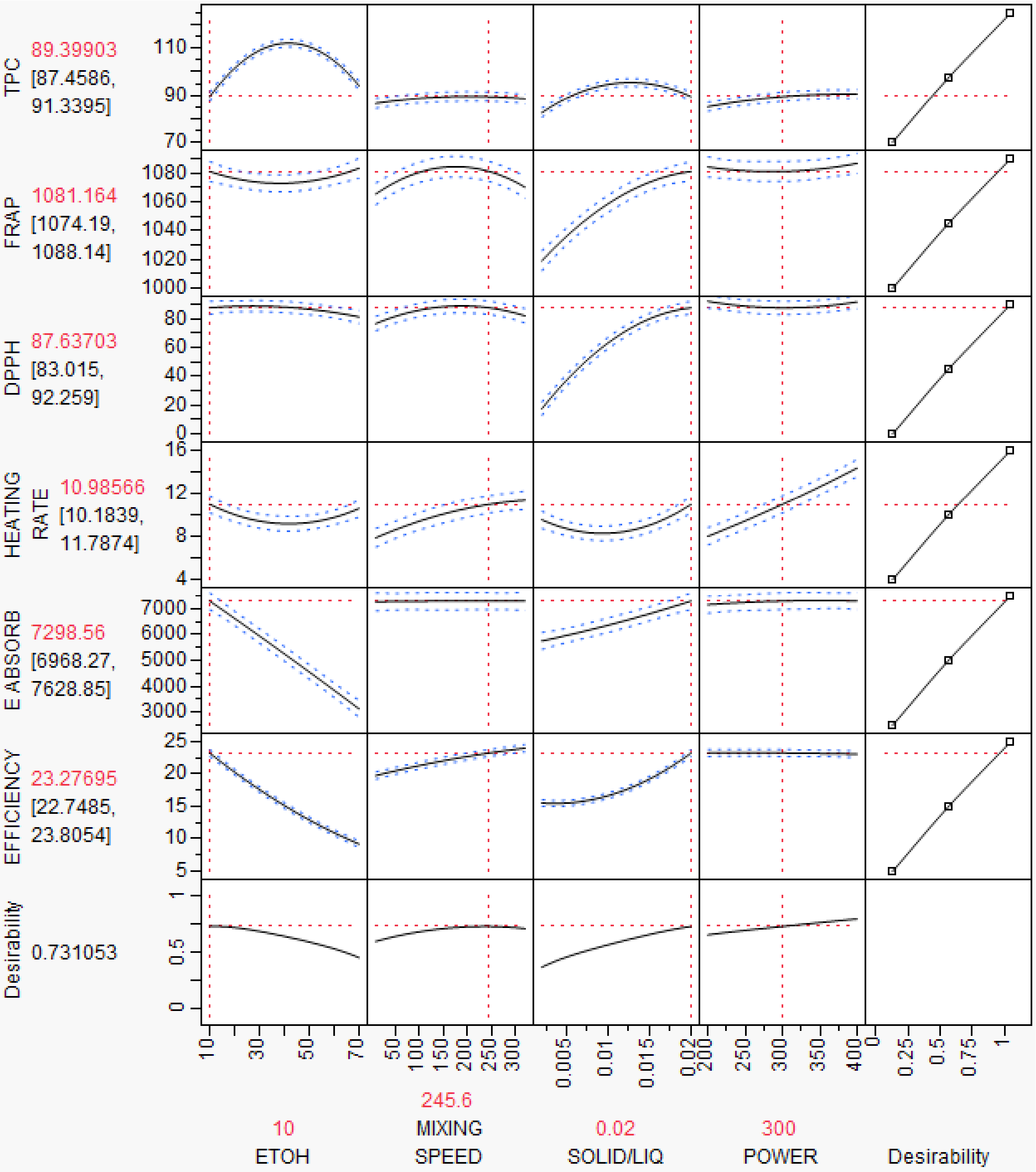

Figure 7-9 : Desirability profiler for multi-response outputs of RFAE apple peel

7.5.4 Verification of the Model

The difference between the predicted and the experimental data of the optimized points was less than 10% and not significant. This concludes that the optimized model is accurate for predicting the responses. The results are presented in Table 7-5 below:

Table 7-5: Experimental and Predicted Values of TPC, FRAP, DPPH, HR, ABS and EE

Extraction Parameters				Responses	Predicted Values	Experimental Values	(%)
X_1 (%)	X_2 (ml N_2/min)	X_3 (g/ml)	X_4 (W)				
10	245.60	0.02	300	TPC	89.40	90.85	1.61
				FRAP	1082	1100	1.73
				DPPH	87.64	89.25	1.77
				HR	10.99	11.15	1.55
				ABS	7299	7401	1.40
				EE	23.28	23.61	1.50

7.5.5 Compounds Determination via HPLC

From the HPLC analysis, two phenolic compounds were identified namely chlorogenic acid and epicatechin. By comparing with the available standards, chlorogenic acid and epicatechin were eluted at 13.52 and 20.92 min, respectively. Figure 7-9 presents the HPLC chromatograph of our crude apple peel extract. By comparing with the reference method (Tsao et al., 2003), the additional peak at 6.63 min could be belonging to procyanidin B1 meanwhile the peaks observed in the range between 39.27 to 53.69 min probably belong to phloretin, phloridzin, quercetin and their derivatives.

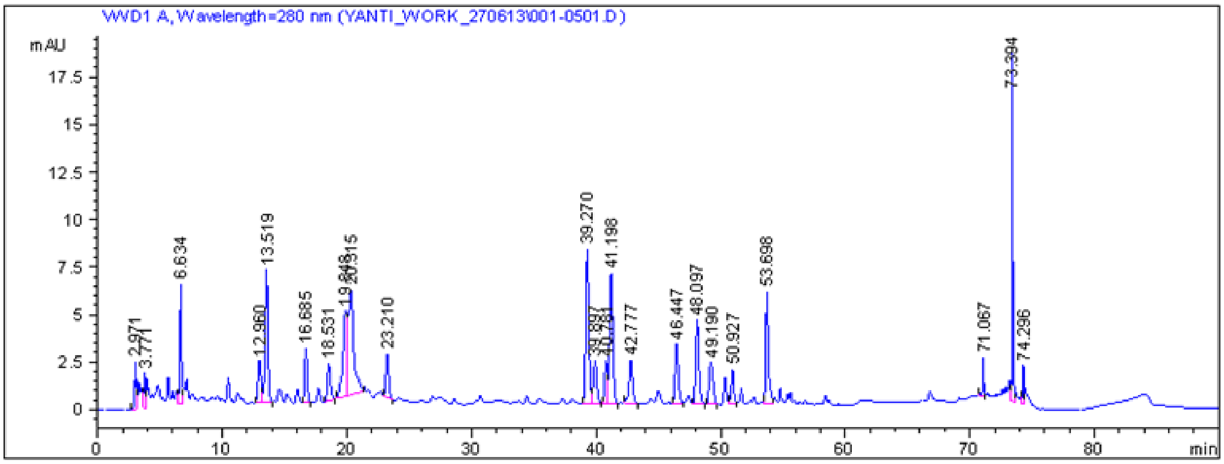

Figure 7-10 : HPLC analysis of crude apple peel extract obtained through RFAE. Chlorogenic acid and -epicatechin were detected at 13.52 and 20.92 min, respectively

7.6 Conclusions

There is a great potential for the development of radio frequency heating applications for the extraction of bioactive compounds from biomass. Ethanol concentration, mixing speed, solid to liquid ratio and power, all exert significant effects on the quantity and quality of the extract obtained and at the same time, these factors also affect the heating performance and efficiency of RFAE. A mixing mode has been introduced with RF heating which can help to improved RFAE performance. Due to combined effects of multiples responses, a realistic optimum point was chosen. The best TPC, FRAP, DPPH, heating rate, sample energy absorption and energy efficiency can be obtained by conducting the RFAE using 10 % ethanol concentration in the solvent, 245.60 ml N_2/min mixing speed, 0.02 g/ml solid to liquid ratio and 300 W RF power. Optimized responses obtained from the experimental and predictive values were in close agreement indicating the accuracy of the model. From this experiment, it is confirmed that RFAE is a low solvent extraction method. Stirring action may improve the heating uniformity in RFAE. In addition, the HPLC analysis confirmed the presence of chlorogenic acid and epicatechin in the crude apple peel extract. Furthermore, RSM was confirmed to be an excellent, precise and reliable tool for designing experiments, analyzing data and to conduct process optimization.

7.7 Acknowledgements

Heartfelt thanks to Professor Vijaya Raghavan for providing the facilities for the experimental work. The authors would also express their gratitude to Universiti Teknologi Malaysia and Ministry of Higher Education Malaysia for funding Mrs. Mohd Jusoh throughout her studies at McGill University. Financial support, for activities in the laboratory, from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Connecting Statement to Chapter 8

In **Chapter 7**, the effects of processing parameters on the RFAE extract quality and energy utilization were investigated. The study shows that it is possible to develop an efficient extraction with RF heating that produces high quality apple peel extract with adequate selection of these significant parameters. The recovery and energy efficiency of RFAE were also demonstrated to be higher with utilization of less solvent. These encouraging results have led to another interesting study in **Chapter 8** with two important objectives; to evaluate the performance of optimized RFAE compared with existing extraction methods and to highlight the extraction mechanisms involved in RFAE.

Chapter 8 has been written in the manuscript style for submission to Journal of Chemical Engineering Research and Design:

Mohd Jusoh, Y.M.[a,b] and Orsat, V.[a] (2014). Evaluating the Performance of Radio Frequency Assisted Extraction By Comparing with Other Extraction Methods Using Extraction Kinetics, Effective Diffusivity, High Performance Liquid Chromatography and Scanning Electron Microscopy

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Contributions made by the different authors are as follows:

- The first author is the Ph.D. student who prepared the experimental design, conducted the laboratory work, analysed the results and prepared the manuscript.
- The second author is the thesis supervisor who initiated and stimulated ideas, provided the technical advice, suggestions, experiment facilities and guided the first author in writing, correcting and preparing the manuscripts throughout the study.

Chapter 8 - Evaluating the Performance of Radio Frequency Assisted Extraction By Comparing with Other Extraction Methods Using Extraction Kinetics, Effective Diffusivity, High Performance Liquid Chromatography and Scanning Electron Microscopy

8.1 Abstract

The objective of this study was to evaluate the performance and investigate the similarities and differences of a novel extraction method, radio frequency assisted extraction, compared to other selected novel and conventional methods namely microwave assisted extraction (MAE), ultrasonic assisted extraction (UAE), conventional extraction with heat (CONHEAT) and conventional extraction without heat (CONOHEAT). Apple peel was chosen as the extraction sample due to the significant amount of its scientifically proven health and the chemical importance of its bioactive compounds. The kinetics of extraction, effective diffusivity, total phenolic compounds, high performance liquid chromatography analysis and SEM images were used to compare the performance of methods tested. RFAE has similar extraction kinetics as other extraction methods tested, however this new method has the highest recovery value of TPC (17.48 ± 0.02 mg GAE/mg DM). The effective diffusivity was also the highest in RFAE (7.32×10^{-10} m^2s^{-1}). The cross-sectional images of dried samples after extraction treatment were successfully obtained via SEM imaging highlighting the different degree of surface cellular destruction caused by each extraction techniques. The images were used to explain the difference in the kinetics, effective diffusivity and chemical analyses between the extraction methods. The experimental kinetic curve of RFAE was perfectly modeled using Peleg's equation. Encouraging findings from this study elevate the potential of RFAE for commercial extraction application.

Keywords : extraction, apple peel, kinetics, effective diffusivity, radio frequency assisted extraction

8.2 Introduction

Extraction is a process to recover important compounds from a material. In the pharmaceutical, nutraceutical and cosmetic industries, extraction is the main unit operation for obtaining the active ingredients from biomass. The increase in awareness on maintaining good health contributes to the growing demand for nutraceutical products, which subsequently leads to a search for better extraction processes for catering to the consumers' needs. High utilization of extraction solvent and energy, low recovery of target compounds and lengthy extraction time are common problems in extraction. In order to overcome these issues, a new extraction method using radio frequency heating known as radio frequency assisted extraction (RFAE) is proposed.

Radio frequency heating is not a new technology, in fact this technology has been previously studied and applied for thermal treatments of various materials for example postharvest treatment, food and beverage processing, wood and textile drying, oil extraction, cancer treatment, etc. (Orsat & Raghavan, 2005; Marra, Zhang & Lyng, 2009). It has similar volumetric heating as its sister technology, microwave heating, however its advantage in terms of higher penetration depth and rapid selective heating entice its application in industry. MAE is an excellent extraction method however its overall efficiency is limited by its uneven heating, low penetration depth and difficulty to control rapid heat generation. On contrary, RFAE is anticipated to offer advantages, for instance rapid volumetric heating (with the selection of suitable material), higher uniformity in heating distribution, low solvent requirement, food grade solvent preference, indirect contact with sample, and low maintenance and service costs. Our preliminary work on extracting secoisolariciresinol diglucoside (SDG) using RFAE in 2009 (Mohd Jusoh & Orsat 2011), elucidated that RFAE recovered higher amount of SDG, than the amount obtained from MAE. Due to this promising finding, we hypothesized that RFAE may perform better than other existing extraction methods, therefore more work in understanding and improving the performance of the radio frequency heating as an extraction technique was performed. The first and only work published on radio frequency extraction was carried out by researchers from Saskatchewan University on the extraction of the anticancer compound podophyllotoxin and can be found in Izadifar & Baik (2007, 2008a & 2008b), Izadifar (2009) and Izadifar, Baik & Mittal (2009).

The rationale for the hypothesis lies on the fact that different extraction methods present different extraction mechanisms, which possibly result in different amounts of compound recovery and types of compounds extracted. Comparing the target compounds recovery is the most common way to evaluate the difference in the involved extraction mechanisms and performance, while the extraction kinetics, effective diffusivity coefficients and image analysis can provide valuable information for deeper understanding. Extraction kinetics and effective diffusivity values are normally used for evaluating the effects of extraction parameters (i.e. temperature, time, solid to liquid ratio, particle size, solvent flowrate, stirring speed) on the mass transfer of compounds and the information obtained is normally utilized for optimizing or scaling up the extraction process. Scanning electron microscope (SEM) is a characterization method gaining popularity as this visual approach is a perfect alternative for studying a mechanism that is complicated to be represented or understood via numerical approach. SEM analysis for understanding extraction mechanisms has been published in works by Xie et al. (2013) and Gujar, Wagh & Gaikar (2010).

In this comparative study, apple peel, the by-product from jam, puree and the ready-to-eat apple industry was selected as the biomass sample. Apples contain a great concentration of phenolic compounds which are mostly located in the peel. The bioactive compounds from apples are in focus nowadays as they possess numerous health beneficial phenolic compounds proven useful for preventing or treating degenerative diseases and gastrointestinal injury, for slowing down aging effects and to promote weight loss (Hyson, 2011). In addition to that, the utilization of apple peel waste for bioactive compounds production is a smart effort towards reducing the environmental impacts of agri-product solid waste disposal. We are interested to recover the natural antioxidants from apple peel, namely the chlorogenic acid and epicatechin. The anticancer, anti-inflammatory, antimicrobial, anti-oxidative effect, neuro-protective effect, ultraviolet protective effect, high free radical scavenging ability of chlorogenic acid and hypertension and diabetic protective effect of epicatechin were reported in Tomaru et al. (2007), Xiang & Ning (2008), Kwon et al. (2010), Gómez-Guzmán et al. (2012) and Luís et al. (2013).

The first objective of this paper was to evaluate the RFAE performance in comparison to other novel extraction methods and analyze the similarities and differences between the tested methods through the extraction kinetics, effective diffusivity, high performance liquid chromatography and scanning electron microscopy results. The second objective was to demonstrate the extraction mechanism of RFAE and finally to model the RFAE extraction kinetics of the apple peel. This comparative study is considered as a feasibility study for promoting RFAE and also the first effort reported for obtaining a deeper understanding of the application potential of radio frequency heating for nutraceutical extraction.

8.3 Materials and Methods

8.3.1 Sample Preparation

Apple samples used for this experiment were purchased from a local grocery store Provigo (Kirkland, Quebec). Three varieties; Cortland, Empire and McIntosh, were selected for this study based on their intense red skin. The apples were washed with 50°C hot water, wiped dry before being peeled using an Apple Pro-Peeler (Starfrit Atlantic Promotions Inc., Canada). The peeler has a fixed distance between the apple and the blade which enables the control of peel thickness at ± 1.00 mm. The fresh peel was quickly stored in airtight container to avoid oxidation. All the peels were properly mixed to prevent non-uniformity during the extraction process. Subsequently, the peels were microwave hot-air dried to 17 % moisture content at 50°C using the drying parameters proposed by Dev et al. (2011) with slight modifications. The dried peels were ground using a Magic Bullet Grinder (Homeland Housewares LLC, USA) and sieved. The powdered peel, with particle size between 500 - 750 μm, was used for the extraction. The suitability of the particle size was determined in our previous work (Mohd Jusoh & Orsat, 2012).

8.3.2 Extraction Procedures and Kinetics

Five different extractions were performed in this experiment and the methods were radio frequency assisted extraction (RFAE), microwave assisted extraction (MAE), ultrasonic assisted extraction (UAE), conventional extraction with heat (CONHEAT) and conventional extraction without heat (CONOHEAT)(control method). The schematic diagrams for all extraction processes are shown in Figures 8-1 to 8-4. All the experiments were performed at the extraction temperature of 50°C ± 2°C using 50 ml solvent mixture of 1 mM hydrochloric acid with 10% ethanol, solid to liquid ratio of 0.02 g/ml and the mixing speed of 245.60 ml N_2/min. These parameters were fixed at the designated optimized value obtained from our previous RFAE optimization work (Chapter 7). The temperature of the mixture during the extraction process was monitored using a digital thermometer (Fisher Scientific, USA) or fiberoptic temperature sensor. For all extractions, the heating started from room temperature. Once the temperature of the solvent mixture reached 50°C, the heating was stitched off to prevent temperature from going above 50°C. The heating was switch on again when temperature went below 49°C. Additional mixing action was not introduced in the UAE since UAE method has its own bubble collapse cavitation impact which also serves as agitation. The recovery of the total phenolic compounds was measured at 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes interval for plotting the extraction kinetics. The separation of the extraction solvent from the residue was performed via vacuum filtration method using Whatman No. 1 filter paper (Whatman, UK).

8.3.2.1 Radio Frequency Assisted Extraction (RFAE)

The RFAE was performed using an RF generator (Sairem, France) with the maximum power capacity and voltage of 600 W and 5000 V, respectively. The system produces single frequency of 27.12 MHz. Initial RF heating setup (with horizontal electrodes) was used in Orsat et al. (2001). A different design of applicator was used in this RFAE study while all other RF heating components, responsible for total operation of the RF heating system such as the RF generator, matching box, impedance regulator, voltage regulator, fiberoptic temperature measurement system and data acquisition and control system were maintained. Details of the new applicator can be found in Chapter 3. The schematic diagram of the RFAE system (displaying only the RFAE applicator) is illustrated in Figure 8-1.

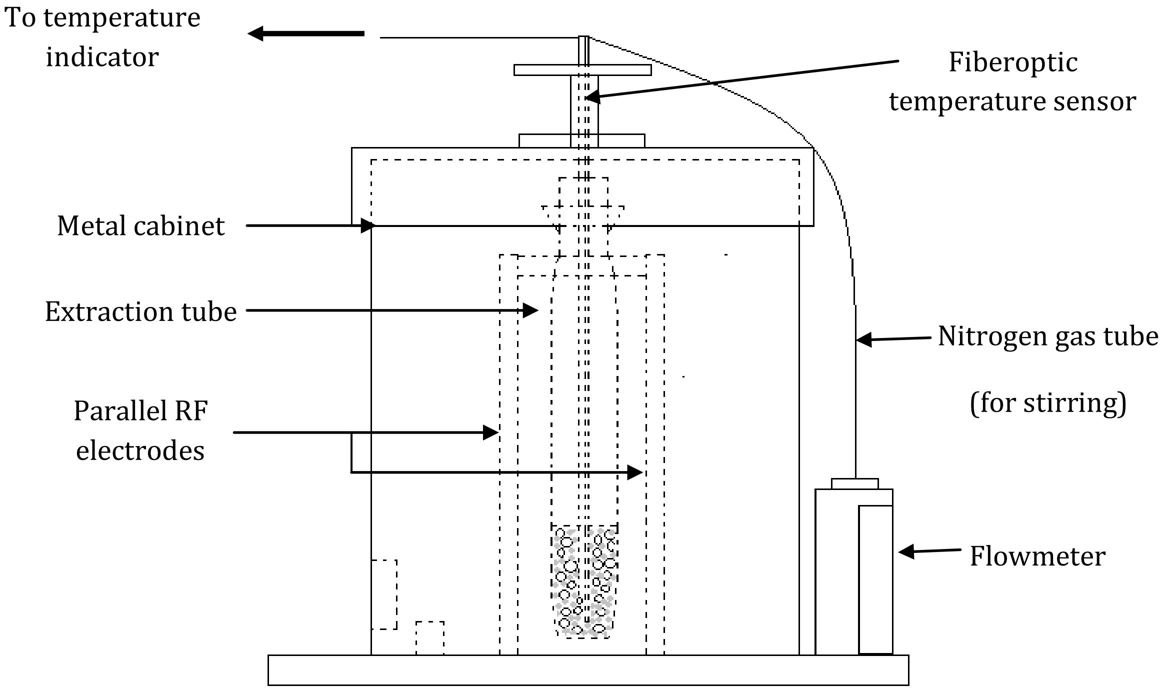


Figure 8-1 : Schematic diagram of the radio frequency assisted extraction (RFAE) setup

8.3.2.2 Microwave Assisted Extraction (MAE)

For MAE, a CEM STAR System 2 microwave digestion system (CEM Corporation, USA) with two heating compartments was used. MAE extractions were consistently performed in compartment 1 to minimize variability during the experiment. The MAE setup used is shown in Figure 8-2. The temperature was maintained at 50°C throughout the process by manual intermittent switching on and off of the microwave power. A low power of 150 W was used to avoid overheating and possibility of target compounds degradation in this system.

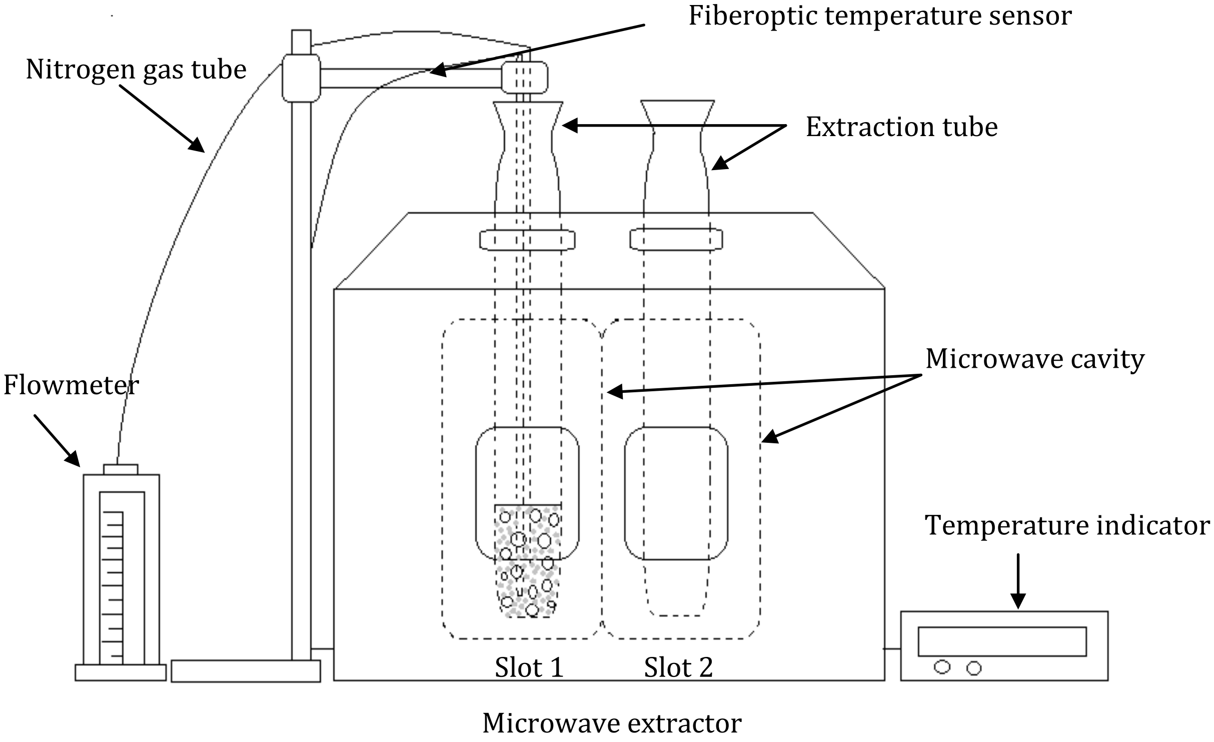


Figure 8-2 : Schematic diagram of the microwave assisted extraction (MAE) setup

8.3.2.3 Ultrasonic Assisted Extraction (UAE)

For UAE, an ultrasonic cleaner (Fisher-Scientific, USA) with a single frequency of 42 Hz was used. Nitrogen gas stirrer was not included in this setup as it was included in RFAE or MAE. The reason for this exclusion was to allow the evaluation of the main effect of ultrasonic treatment which is cavitation agitation on the extraction medium. The ultrasonic tank was continuously supplied with hot water of 50°C manually to maintain the extraction temperature at 50°C throughout the extraction. The temperature monitoring was done using a digital thermometer (Fisher Scientific, USA). The schematic diagram of the UAE system employed is illustrated in Figure 8-3.

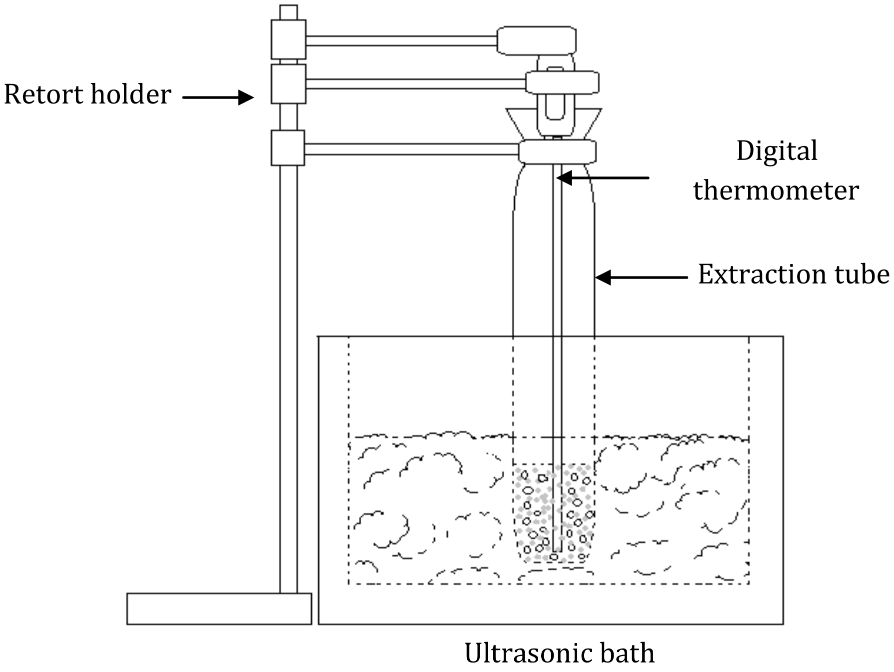


Figure 8-3 : Schematic diagram of the ultrasonic assisted extraction (UAE) setup

8.3.2.4 Conventional Extraction with Heat (CONHEAT) and Conventional Extraction without Heat (CONOHEAT)

The setup for CONHEAT and CONOHEAT is shown in Figure 8-4. A water bath unit from Precision Scientific Incorporated (Model 182, USA) was used for CONHEAT and CONOHEAT. In CONHEAT experiment, the water bath tank was filled with tap water that was heated to 50°C and constantly maintained at that temperature automatically by the unit. The same setup was used for CONOHEAT, with water temperature maintained at an ambient temperature of 25°C. The temperature monitoring was done using a digital thermometer (Fisher Scientific, USA).

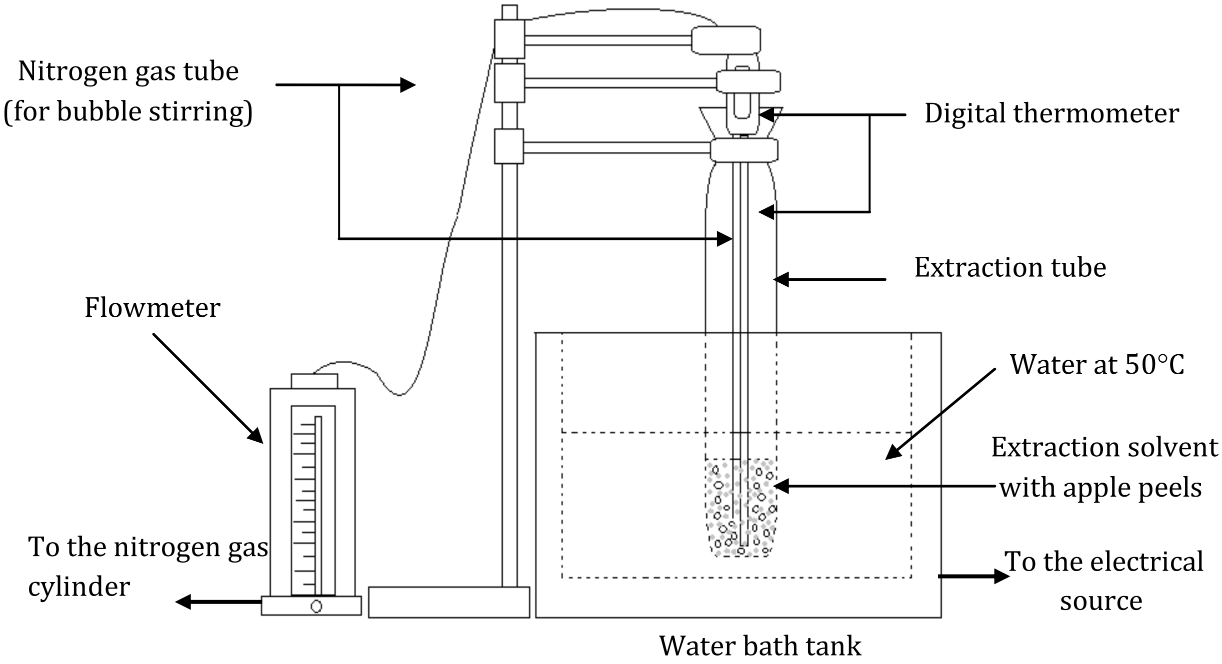


Figure 8-4 : Schematic diagram of the conventional extraction with (CONHEAT) or without heating (CONOHEAT) using a water bath setup

8.3.3 Total Phenolic Compounds Determination

The total phenolic contents in the apple peel extract were determined using the method described in Waterhouse (2005) with minor modifications. 0.1 ml of extract was mixed with 7.9 ml of distilled water in a 15 ml centrifuge tube. Then, 0.5 ml Folin-Ciocalteu reagent was added to the mixture. The tube containing the mixture was agitated for 1 minute using a shaker and left aside for 7 minutes. After that, 1.5 ml of $NaCO_3$ was added and mixed again. The mixture was incubated for 2 hours at room temperature. All tubes were properly covered with aluminum foil to prevent them from light exposure. A

Biochrom US 1000 UV/VIS spectrophotometer (Biochrom, USA) was used to measure the absorbance of the sample with the wavelength set at 765 nm. The quantification of total phenolic compounds in the samples was performed using gallic acid standard. A standard curve of gallic acid diluted in 1 mM hydrochloric acid of 10 % ethanol content was prepared. The amount of phenolic content obtained was expressed as milligram gallic acid equivalent per gram of dry mass (mg GAE/g DM).

8.3.4 Chlorogenic Acid and Epicatechin Determination using High Performance Liquid Chromatography (HPLC)

Compounds of interest, chlorogenic acid and epicatechin, were identified using the HPLC method proposed by Tsao et al. (2003) with minor modifications. The separation and identification was performed at 25°C by a reversed-phase RP C18 Discovery column (Supelco) (5μm, 25 cm x 4.6 mm) with Discovery Supelguard cartridge C18 (5μm, 25 cm x 4 mm) using an HPLC Agilent 1100 series equipped with variable wavelength detector. The injection volume and flowrate were fixed at 10 μL and 1 ml/min, respectively. The HPLC sample was prepared by filtering the RFAE crude apple peel extract with a 0.20 μm nylon syringe filter (Whatman, UK) into the amber HPLC vial. Both compounds were detected at 280 nm. Chlorogenic acid and epicatechin standards were used for identifying and quantifying the compounds available in our crude apple peel extract.

8.3.5 Effective Diffusivity Determination

The result of extraction process depends on how fast the target compound will dissolve and reach equilibrium concentration in the liquid solvent (Cacace & Mazza, 2003). There are four mass transfer steps involved in extraction, however in the case of extracting compounds from the botanical sources, the rate limiting step is the diffusion through the pores within the particles to the surface (Gertenbach, 2005). This rate limiting step can be represented by Fick's Second Law (Equation 1):

(1)

$$\frac{\delta C}{\delta t} = D\frac{\delta^2 C}{\delta r^2}$$

Where C is the concentration of the solute, t is time, D is the diffusivity coefficient or diffusivity and r is the radius of the particles (Cacace & Mazza, 2003; Gertenbach, 2005).

In order to apply this equation, several assumptions must be made such as that the particles must be spherical in shape, there isno variation of diffusion coefficient with solute concentration, while concentration differences only occur in radial direction and no chemical reactions are involved during extraction (Kim & Mazza, 2007; Pinelo et al., 2006).

Rate of extraction can be improved by increasing the diffusivity. In this study, effective diffusivity was calculated using Fick's second law linearization method which was used in Pinelo, Sineiro & Núñez (2006) and Ho, Cacace & Mazza (2008). Through several stages of simplifications and linearization of Equation 1, the final form of equation for calculating effective diffusivity was obtained and applied to the system:

$$\ln Y = \ln\frac{6}{\pi^2} - \frac{\pi^2 D_{eff}}{r^2}t \qquad (2)$$

Y is the ratio of unextracted phenol concentration at time t divided by maximal phenol able to be extracted, (in this case at 120 min); D_{eff} = effective diffusivity, in m^2/s; r = radius of diffusion, in meter (m); t = time, in second (s). The D_{eff} can be obtained from the slope of the graph plotted using Equation 2 (example calculations are presented in Appendix 2).

8.3.6 Scanning Electron Microscope (SEM) Image Analysis

To prepare the samples for image analysis with SEM, the extraction residues (apple peel) were air-dried for 24 hours. Then, a piece of the dried peel was chosen and placed on the SEM specimen holder. The surface of the specimen holder was covered with double sided sticky carbon tape to hold the specimen in place. The sample preparation was simpler since gold coating of the sample was not required for this experiment, using a new tabletop SEM provided by Hitachi-Olympus (Model TM3000, Japan).

8.3.7 Mathematical Modeling of Extraction Kinetics

The RFAE kinetics was modeled using the two parameter, non-exponential Peleg's rehydration model initially used by Bucić-Kojić et al. (2007) for modeling extraction kinetics. Peleg's rehydration or adsorption model was selected as the extraction kinetics have a similar curve as the rehydration curve. The model is given in the equation below:

$$C(t) = C_0 + \frac{t}{K_1 + K_2 t} \tag{3}$$

where C(t) is the concentration of total phenolic compounds at time t (mg GAE/g DM), t is the extraction time (min), C_o is the initial total phenolic compounds at t=0 (mg GAE/g DM), K_1 is Peleg's rate constant (min g DM/mg GAE) and K_2 is Peleg's capacity constant (min g DM/mg GAE). K_1 and K_2 can be determined following the method published by Peleg (1988). C_o is always 0 because at t = 0, there is no compounds extracted yet thus this simplifies Equation 3 to Equation 4 below:

$$C(t) = \frac{t}{K_1 + K_2\, t} \tag{4}$$

8.3.8 Design of Experiment and Statistical Analysis

A single factor experiment was employed for this study and the experiment was designed to have fixed temperature, solid to liquid ratio and mixing intensity for the purpose of highlighting the unique effect of each extraction method tested, with respect to evaluating the performance of our newly developed extraction system using radio frequency heating and explaining the extraction mechanism involved. The experimental design and the t-test were performed using JMP 8 (SAS Institute Inc., USA). Significance value fixed for this study is $p < 0.05$. Excel 2007 (Microsoft Corporation, USA) was used for modeling the experimental kinetics and plotting the experimental results. The error bars in Figures 8-7 and 8-8 represent standard deviation which ranged from 0.1 to 0.2.

8.4 Results and Discussion

8.4.1 Experimental Extraction Kinetics

The extraction kinetics of all methods are shown in Figure 8-5. By comparing the amount of total phenolic compounds (TPC) recovered from CONOHEAT (control method) with the other methods, it can be seen that the incorporation of heat into the extraction process can significantly improve the recovery of total phenolic compounds from 11.80 ± 0.04 mg GAE/ g DM (CONOHEAT) to 15.42 ± 0.03 mg GAE/ g DM (CONHEAT), 16.15 ± 0.02 mg GAE/ g DM (UAE), 17.18 ± 0.03 mg GAE/ g DM (MAE) and 17.48 ± 0.02 mg GAE/ g DM (RFAE). Higher equilibrium value was achieved in extraction processes that involved heat. This is expected as the mass transfer and diffusion of the target compounds into the solvent is influenced by the temperature of the solvent. Elevated temperature in extraction process modifies the surface tension and viscosity of the solvent which subsequently increase the solubility of solute into the solvent which led to an increase in mass transfer rate (Gertenbach, 2002 and Takeuchi et al., 2009).

Another interesting finding to highlight here is the significant difference ($p < 0.05$) in terms of amount of total phenolic compounds recovery as affected by different extraction process. Surprisingly, the highest recovery of TPC was obtained by the new method RFAE, followed by MAE, UAE, CONHEAT and CONOHEAT. The MAE or UAE were expected to produce the highest recovery of TPC since both methods are well known for their superior performance in extraction due to the internal superheating or bubble cavitational impact, however they were both surpassed in this study. There are two possible explanations for this. Firstly, RFAE might have had a better diffusion mechanism than MAE and UAE, which at this point is still not explained. Secondly, it is possible that some of the compounds have thermally degraded or hydrolyzed during MAE or UAE which leads to lower yield in these extractions. Degradation of bioactive compounds due to MAE or UAE was reported in the work of Biesaga (2011) and Paniwnyk et al. (2001). Figure 8-5 also shows, for all methods evaluated, that the TPC reached almost equilibrium state within a short period of 40 minutes, and adding more time to the extraction process did not significantly increase the recovery. The amount of TPC recovered was significantly affected by the method and mechanism of extraction.

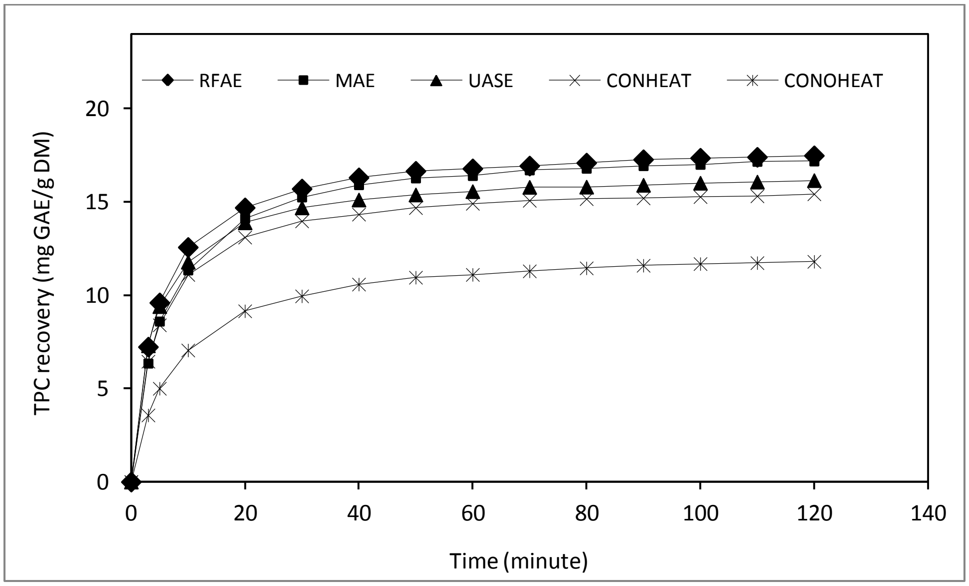


Figure 8-5 : Experimental extraction kinetics of apple peel obtained from RFAE, MAE, UAE, CONHEAT and CONOHEAT

8.4.2 Effective Diffusivity

Besides the experimental kinetics, the effective diffusivity value of each extraction process was calculated to evaluate and differentiate the performance of each extraction methodology. An extraction method with a high diffusivity value means that the mass transfer of compounds occurred at a faster rate during that particular extraction process. Experimental results summarized in Table 8-1 show that RFAE had the highest effective diffusivity value (7.32 x 10^{-10} m^2s^{-1}) compared to the other methods, meanwhile CONOHEAT had the lowest value (9.40 x 10^{-11} m^2s^{-1}). It is interesting to note that RFAE has a higher effective diffusivity value than MAE and UAE, which supports the better extraction diffusion mechanism in RFAE than the other novel methods tested in this study. Incorporation of heat significantly accelerates the mass transfer rate which leads to an increase in the effective diffusivity value via modifying the selectivity of the compounds, modifying the solvent surface tension and providing the energy for compounds to rapidly move into the solvent. The difference in heat deliverance or dissipation in the solvent-sample mix (i.e. conduction, convection or volumetric) could have influenced the effective diffusivity. Internal superheating from MAE or cavitational impact from UAE was predicted to assist in breaking the cell matrix which allows more compounds to solubilize into the solvent. The correlation between SEM image analysis with kinetics and diffusivity presented in Section 8.3.3 will provide somewhat of a better understanding on the degree of apple peel cell destruction due to the effect of the different extraction treatments.

Table 8-1 : Effective diffusivity for different extraction methods

Methods	Effective Diffusivity (m^2s^{-1})
RFAE	7.32×10^{-10}
MAE	4.43×10^{-10}
UAE	2.16×10^{-10}
CONHEAT	1.75×10^{-10}
CONOHEAT	9.40×10^{-11}

8.4.3 Scanning Electron Microscope (SEM) Image Analysis

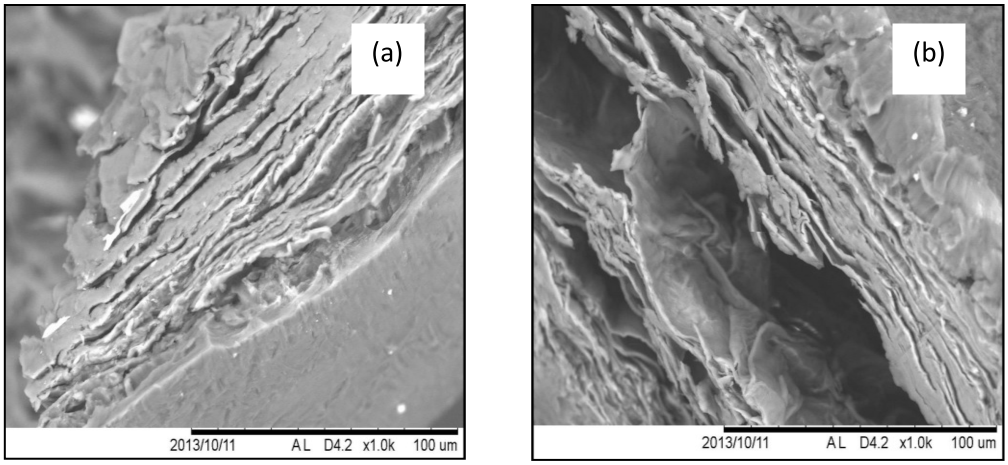
The purpose of using scanning electron microscope (SEM) is to highlight the impact of the different extraction methods tested on apple peel physical structure. These image observations can help to further explain the difference obtained in the amount of target compounds recovered from each of the heating treatments, besides their kinetic and effective diffusivity values. In this study, the cross-sectional view of the apple peel was chosen for this analysis, in contrast with the front view that is normally adopted by other researchers. In this study, the cross-sectional side view provides more sensible information relating to the kinetics and effective diffusivity as it presents more clearly the cellular breakdown. The effect of CONOHEAT, CONHEAT, MAE, RFAE AND UAE on apple peel is presented via SEM images in Figure 8-6a to 8-6e, respectively. Distinctive dissimilarity on the apple peel structures shown by the images led to a conclusion that each of the extraction treatment exerted a different degree of destructive impact on the apple peel cells thus allowing different levels of compound release.

Prior to SEM analysis, all the apple peel samples had undergone extraction and air drying process. Figure 8.6a represents the structure of apple peel extracted using CONOHEAT. The structure of the apple peel was dense. In could be deduced that the conventional solvent extraction, in our study CONOHEAT, only extracted the most accessible and weakly attached phenols from the fruit skins as described in Pinelo, Zornoza & Meyer (2008). This explains the lowest amount of TPC gathered via this method. The weakly bound polysaccharides phenolics compounds can easily be washed away whenever the sample is in contact with the extraction solvent, however the mass transfer proceeded at slower pace since the extraction was not driven by heat. The SEM image for CONOHEAT implies minimal to no destructive impact on the cell structure. With this dense structure, the accessibility of the extraction solvent to penetrate into the sample is also restricted because of limited surface contact.

A slight different observation was visualized from the sample treated with CONHEAT method (Figure 8-6b). In CONHEAT, the structure of the sample was still dense however it can be observed that a small amount of cells from the top most layer were ruptured and opened. This infers that incorporation of conductive and convective heating in CONOHEAT might have triggered minimal destruction on the cells of the apple peel sample which corresponds to a 31 % increase in recovery from 11.80 ± 0.04 mg GAE/ g DM to 15.42 ± 0.03 mg GAE/ g DM. The heat application might have liberated more target compounds which were weakly bound to cells into the solvents and provided energy for the movement of mass hence improved the mass transfer rate.

With the volumetric heating, the structure of apple peel samples extracted via MAE (Figure 8-6c) and RFAE (Figure 8-6d) were less dense and these samples had more ruptured and opened cells. Higher cells destruction infers two things, more compounds were liberated and more sample surface area became in contact with the solvent. On top of that, it is important to note that, visually, RFAE caused more damage towards the cells than MAE. The degree of destruction observed in Figure 8c and 8d confirms the difference in the amount of total phenolic compounds recovered by MAE (17.18 ± 0.03 mg GAE/ g DM) and RFAE (17.48 ± 0.02 mg GAE/ g DM). Because of greater cell rupture, RFAE had higher recovery than MAE as mentioned in the previous sections.

UAE displayed an interesting result (Figure 8-6e). The SEM image actually showed that ultrasonic energy had caused the most damage to the cells with very minimal to no dense structure observed at all. Most of the cells were ruptured and opened. The synergistic effect of ultrasound bubble cavitation and heat might have caused this cell rupture. Although UAE produced the most cell destruction on the apple peel samples, the recovery of target compounds using UAE (16.15 ± 0.02 mg GAE/ g DM) did not surpass MAE or RFAE. This is probably due to the decomposition of the target compounds through hydrolysis as mentioned in Paniwnyk et al. (2001).



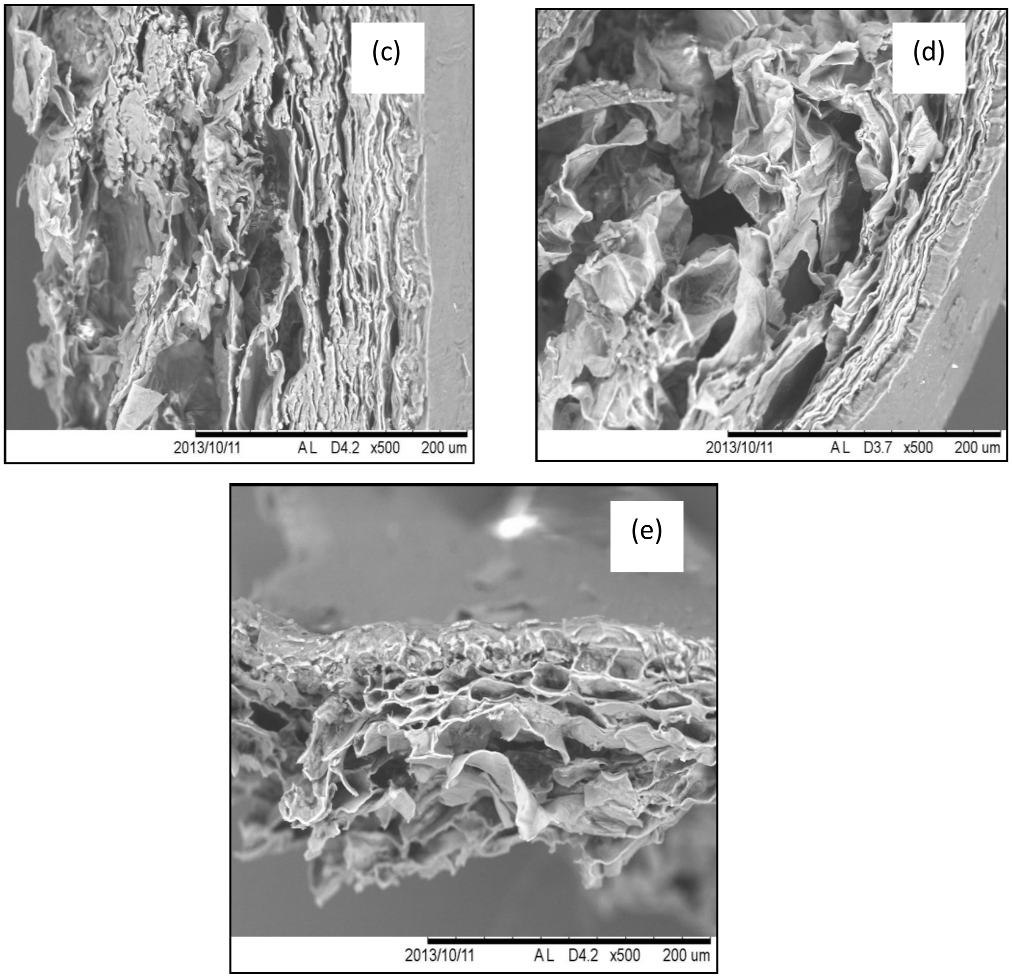


Figure 8-6 : Effect of conventional extraction without heating (CONOHEAT) (a); conventional extraction with heating (CONHEAT)(b); microwave heating (MAE)(c), RF heating (RFAE)(d) and ultrasonic heating (UAE)(e) on the structure of apple peel

8.4.4 High Performance Liquid Chromatography (HPLC)

Besides the analysis of kinetics, effective diffusivity and SEM images, two chemical analyses were also used to describe the performance of the extraction methods. TPC is a common, simple and quick method for roughly estimating the total amount of phenolic compounds available in the extract however its drawback is that it does not provide any detailed information on the types of compounds available in the extract. For identifying and quantifying compounds of interests, such as chlorogenic acid and epicatechin, HPLC analysis was adopted.

The amount of chlorogenic acid (CA) and epicatechin (EP) extracted are presented in Figures 8-7 and 8-8, respectively. Figure 8-7 shows that the highest recovery of chlorogenic acid after 10 and 120 minutes extraction was obtained through RFAE (295 and 315 μg CA/g DM), and followed closely by MAE (292 and 301 μg CA/g DM) then UAE (275 and 260 μg CA/g DM), CONHEAT (267 and 293 μg CA/g DM) and CONOHEAT (246 and 280 μg CA/ g DM). The difference in recovery, due to change of method, was significant. In addition to that, a significant increase was observed by increasing extraction time to 120 minutes in RFAE and both conventional heating methods but not in MAE. On the other hand, a negative impact of increasing the extraction time was observed on UAE where a reduction of 5.50 % from the total amount of chlorogenic acid obtained initially after extending the process to 120 minutes. This reduction could plausibly happen because of compounds hydrolysis during UAE since a high percentage of water was used in the solvent.

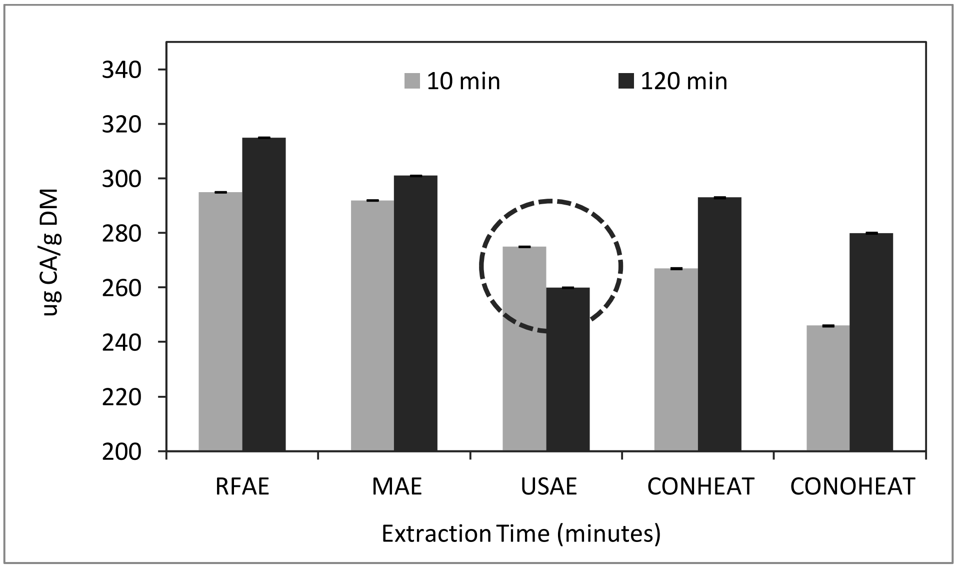


Figure 8-7 : Chlorogenic acid (CA) recovered by all methods after 10 and 120 minutes. The recovery was quantified by HPLC method.

In the case of epicatechin, the recovery of this compound was also the highest by RFAE (Figure 8-8). From the results obtained, the amount of epicatechin extracted via RFAE was significantly higher than other methods tested. This leads to a speculation that plausibly there was some sort of preferential selectivity for EP during the RFAE extraction process. With the advantage of rapid volumetric heating coupled with deeper penetration depth, RFAE had probably caused higher degree of cell permeation through the cell matrix which linked best to epicatechin than any other methods did, and as result it released more of this compound into the solvent. Besides that, a reduction of epicatechin was again associated with the increasing extraction time in UAE, thus strengthening the possibility of compounds destruction during extended extraction time in UAE.

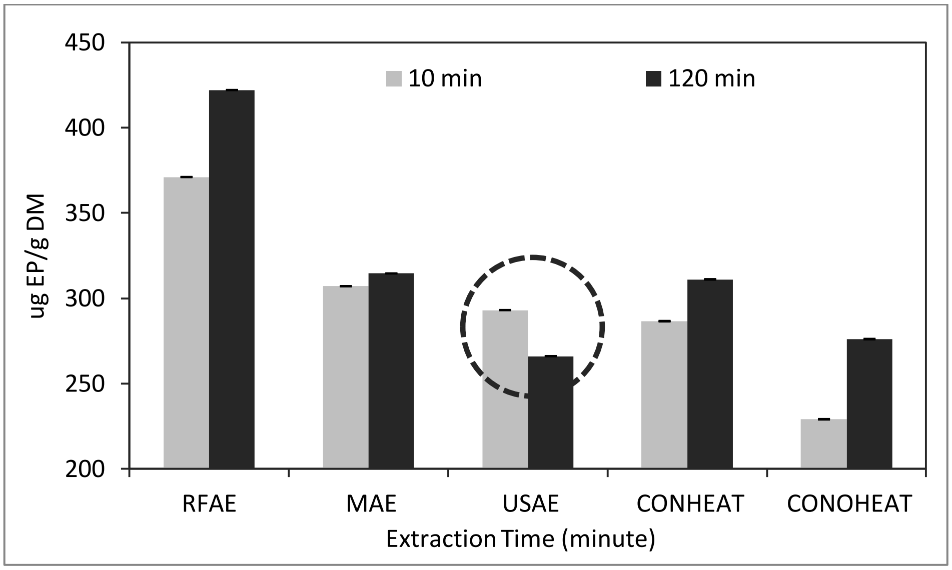


Figure 8-8 : Epicatechin (EP) recovered by all methods after 10 and 120 minutes. The recovery was quantified by HPLC method

Overall, the total recovery of chlorogenic acid and epicatechin contributes to approximately 4.00 to 5.00 % from the total amount of TPC obtained. The rest of the TPC compounds come from a variety of lesser known phenolic compounds present in the apple peel. Traces of procyanidin B1 and quercetin were observed but not reported.

8.4.5 Extraction Kinetics Modeling

The extraction curve of RFAE was modeled and presented here. The RFAE extraction curve was perfectly modeled with the two parameters non-exponential Peleg's equation. This model perfectly fitted the experimental data. From the extraction kinetics curve, it can be deduced that the extraction had occurred in two phases; the first phase is the washing phase (fast rate) and subsequently followed by the molecular diffusion phase (slower rate). The experimental kinetics curve and the kinetics model are plotted together in Figure 8-9. K_1 and K_2 values for this model are 0.2514 min.g DM/mg GAE and 0.0552 min.g DM/mg GAE, respectively.

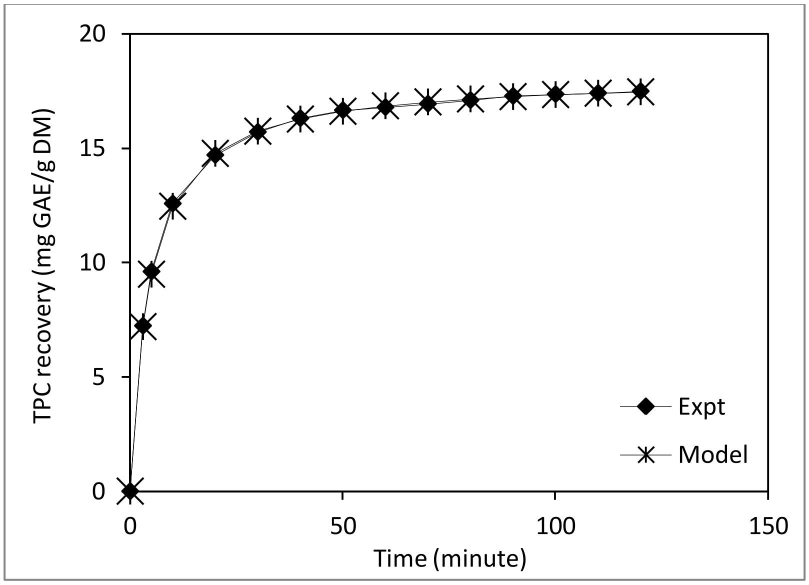


Figure 8-9 : RFAE kinetics experimental curve (Expt) versus model (Model)

The above kinetics model is represented by the modified Peleg's equation below:

$$\mathrm{TPC_{model}} = \frac{t}{0.2514+0.0552t} \tag{4}$$

8.5 Conclusions

In this study, it was shown that novel methods RFAE, MAE and UAE significantly enhanced the recovery of target compounds from apple peel. The importance of heat inclusion in the extraction process for increasing the target compounds recovery and accelerating the process was demonstrated via comparing the TPC recovery of CONHEAT and CONOHEAT (control). The new method RFAE performs better than MAE, UAE, CONHEAT and CONOHEAT, and represents an interesting new application in this field. Epicatechin recovery was significantly higher when extracted in RFAE indicating a probable special interaction of compound with the extraction method due to the nature of the compound and the extraction mechanism. Decomposition of compounds due to the long extraction duration was observed in UAE. Visual study using SEM image analysis was an excellent method for explaining the biomass cell rupturing and the extraction mechanisms in each method and assisted in understanding the similarities and differences between the systems. RFAE kinetic curve can be very well represented by Peleg's model.

8.6 Acknowledgements

Heartfelt thanks to Professor Vijaya Raghavan for providing the facilities and Mr. Yvan Gariépy for the assistance in the machinery set-up. A special thanks to Hitachi-Olympus for providing the SEM TM3000 to Bioresource Engineering Department McGill University for research purposes. Our deepest gratitude to Miss Kiruba Krisnaswamy (McGill University PhD. student) for her assistance in the SEM experiments. Financial support, for activities in the laboratory, from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. Lastly, the authors would also like to express their gratitude to Universiti Teknologi Malaysia and Ministry of Higher Education Malaysia for funding Mrs. Mohd Jusoh throughout her studies at McGill University.

Connecting Statement to Chapter 9

From **Chapter 8**, the higher values of recovery and diffusion rate demonstrated by the optimized RFAE method indicates that this new method has better extraction performance than the selected novel and conventional methods which were tested simultaneously. These encouraging findings highly support further investigations on this new extraction technology. Besides that, the kinetics and diffusion study with visual image evaluation of the destructive impact of each method on the apple peel samples has assisted in understanding the extraction mechanism of RFAE. This was the final chapter involved with experimental work in this thesis.

To recap all the work performed pertaining to the development of RFAE for apple peel phenolics in Chapter 2 to Chapter 8, a summary of the important findings and conclusions drawn from each chapter is presented in **Chapter 9**. The contributions of knowledge gained from this investigation and necessary future work in this area are also included in this closing chapter.

Chapter 9 - Summary of Findings, Conclusions, Contributions to Knowledge and Recommendations for Future Work on RFAE and Apple Peel Phenolic Compounds

9.1 Introduction

The research studies that have been described in this thesis were formulated for assessing the capability of RF technology for its new potential application in the field of nutraceutical extraction and also for gaining a deeper understanding of the RF heating of liquid or solid-liquid mixtures. The summary of findings, general conclusions, contribution to knowledge and recommendations for future work in this area are compiled in this section.

9.2 Summary of Findings and Conclusions

Step-by-step original work on developing a new nutraceutical extraction method using radio frequency heating technology was presented in this thesis. The work done covers several important aspects including: the designing and setting up of the RFAE system; determination of the important factors that influence the extraction process; screening for the best solvents for RFAE of phenolic compounds through a thorough evaluation of their dielectric properties and heating rate; optimizing of the RFAE process for highest recovery and efficiency and finally highlighting of its advantages over the available extraction methods by a comparative study. The important findings and conclusions from each chapter are summarized and presented below.

In **Chapter 4**, the newly developed RFAE system was introduced. This new system was modified from the RF heating system in our laboratory that was used previously for RF heating of agri-food produce. The key focus of this chapter lies on the aspect of RFAE machinery setup and design since not much information can be obtained from the literature. The general information on the application of radio frequency heating for nutraceutical extraction, the information relating to the designing of a complete RFAE setup and the important function of each component in this particular system, the reasons for modification of the parallel electrode RF applicator (from horizontal to vertical electrodes configuration) and the advantages that resulted from this modification were presented to the readers. In addition, the modeling and simulation work, that was carried using computer aided system prior to the actual physical RFAE development, was also presented to show that the computer simulation can aid in providing necessary preliminary information relating to the potential success or failure of a designed system. This chapter was written to provide basic information on RFAE to the readers.

In **Chapter 5**, the effects of the drying methods used for the conditioning of the apple peels, the solvent acid solution selection and extraction temperature, on the quality of the apple peel extract were investigated. The first interesting finding from this study is that the amount of phenolic compounds and antioxidant activity are both significantly affected by the moisture content in the sample. The TPC recovery and antioxidant activity were higher in the RFAE extract from microwave-dried sample (17 % m.c.) than the extract from freeze-dried sample (0 % m.c.). Besides that, it was also observed that the recovery and antioxidant activity are also affected by the choice of acid solution used. The study showed that the highest yield and antioxidant activity (FRAP) of the apple peel phenolic compounds was obtained when the extraction was performed at 50°C to 70°C. However, increasing the temperature has negative effect of DPPH inhibition. From all these important findings, several general conclusions were drawn. The first conclusion is that the RFAE mechanism could be enhanced by utilizing samples with low moisture content samples rather than completely dried samples. The small amount of moisture left in the sample might have absorbed better the energy from the electric field thus generating heat faster as compared to the completely dry sample. The quick heat generation within the solid-solution mixture may have caused higher degree of destruction on the cell matrix and accelerated the mass transfer. Secondly, the selection of acid solution for nutraceutical extraction under RFAE is important as it may affect the target compound recovery, property and availability in the extract. Lastly, the results also implied that the phenolic compounds in the apple peel are heat sensitive thus it is necessary to conduct the RFAE at the designated temperature range to ensure the production of a high quality apple peel extract.

In **Chapter 6**, the effects of solution concentration, ethanol content and temperature on the dielectric properties of the hydrochloric acid (HCl), sodium hydroxide (NaOH) and citric acid (CA) solutions were investigated. All these factors significantly affected the dielectric properties of the studied solutions, which theoretically affect the heating performance under the influence of RF heating. Significant empirical models for predicting the dielectric constant and loss for these solutions were successfully established. The heating rate of the selected solutions during RF heating was investigated in order to choose the best solution for RFAE development. The experimental result showed that the heating rate of the solvents was significantly affected by the concentration and ethanol content. For strong acid (HCl) or base (NaOH), low concentration ranging from 0.001 to 0.01 M of this solution is preferred meanwhile a high concentration of 0.1 M is preferred for weak acid (CA) in RF heating. It was also observed that the ethanol addition improves the heating rate of solutions. There was no correlation found between dielectric properties of the solvents and their heating rate. Another interesting finding is that the heating rates of aqueous HCl and NaOH decrease as their concentration increase but the heating rate of aqueous CA increases as its concentration increases. From this interesting study, several important conclusions for RF liquid heating were drawn. First of all, it is compulsory to distinguish the factors that may have influence on the heating properties of the materials intended for RF heating and it is also imperative to understand the behavior of the materials. This information contributes to the development of an efficient heating system. Secondly, it is possible to predict the dielectric properties of the solutions accurately through the modeled data obtained. The most important conclusion derived from this study is that the dielectric properties alone are insufficient for explaining or predicting the heating behavior of solutions during RF heating. The negative relationship between concentration of HCl and NaOH with heating rate of these solvents infers that excessive presence of ions does not enhance RF heating.

In **Chapter 7**, the effects of ethanol concentration, mixing speed, solid and liquid ratio and RF power on the target compounds recovery, antioxidant activity, heating rate of solid-liquid mixture, energy absorption by sample and energy efficiency were investigated. The experimental results showed that each of the factor exerted unique impact on the responses. The statistical models for predicting target compounds recovery, antioxidant activity, heating rate, energy absorption by sample and energy efficiency were successfully obtained. The complex interaction of the factors and multi-responses led to the best optimized apple peel phenolics via RFAE with desirability factors of 73.11 % when the extraction is performed with a mixture solution of 0.001 M HCl and 10 % ethanol concentration, 245.60 ml N_2/ml stirring speed, 0.02 g/ml solid to liquid ratio and 300 W RF power. Two strong antioxidant compounds namely chlorogenic acid and epicatechin were detected in the RFAE extract. Most importantly, RFAE which can be considered as a low solvent extraction method was successfully developed and demonstrated as a valid extraction alternative. With the right combination of processing parameters, an optimized extraction process can be promoted. The inclusion of stirring in the system is highly recommended as it could help in increasing heat uniformity and extraction recovery. The most important conclusion is that with the appropriate combination of factors, a low solvent extraction via RFAE can be developed which is a strong evidence for further investigation in this area. It is also important to note that in developing an extraction process fit for industrial application, the justification for the new system does not lie solely on the amount and quality of the extract, but also on the energy utilization and efficiency.

In **Chapter 8**, the capability of a newly developed RFAE was assessed by comparing its performance with existing methods namely MAE, UAE and conventional water bath methods. The extraction kinetics, effective diffusivity, SEM images and chemical analysis were the responses used for the comparative study. The first interesting discovery from this study is that the recovery of the target compounds and mass transfer rate of RFAE did not only supersede the conventional methods, but also the novel methods MAE and UAE. The higher value of equilibrium concentration and effective diffusivity for methods incorporated with heat highlight the importance of heat in expediting mass transfer in an extraction process. Each extraction method exerted different degree of destructive impact on the samples and this effect is perfectly illustrated by the SEM images of the different treated biomass. The degree of destruction showed by SEM images followed this sequence

CONOHEAT < CONHEAT < MAE < RFAE < UAE. There exists a correlation between kinetics and effective diffusivity with the degree of destruction on the apple peel cells. The SEM image of UAE showed that this technique had caused the highest destruction on the apple peel cells, however, the yield and the diffusivity constant obtained was lower for UAE than for RFAE and MAE. From these interesting findings, it can be concluded that the different extraction methods help release target compounds at different rate and may produce extract of different quality since it strongly depends on the speed of heat generated and the mechanism of extraction. Peleg's Model can be used for modeling the extraction kinetics of RFAE. This encouraging result also supports the development of RF heating for nutraceutical extraction.

9.3 Contributions to Knowledge

The studies performed for this thesis have generated significant amount of new information in the area of RF heating application for liquid medium and also in the extraction of apple peel phenolic compounds for the production of functional ingredients from biomass. The major contributions to knowledge are:

i. An RF heating set up for batch nutraceutical extraction application was developed and introduced in this study. The new vertical orientation design with fixed gap between the sample surface and both electrodes was highlighted.

ii. New information relating to the sample pre-extraction conditions, suitable potential solvent solutions and extraction temperature for an effective apple peel phenolic compounds extraction using RF heating was established as means for providing information on the conditions of the sample or types of extraction solutions suitable for RFAE of phenolic compounds which is limited in the literature.

iii. Through the investigation of the dielectric properties and heating rate of the solvent solutions, the suitable nutraceutical extraction solutions for RFAE were characterized and determined. The sensitivity of these properties is highly influenced by the change in concentration, ethanol content and temperature. In addition to that, the empirical models for predicting these properties were successfully developed. An attempt to correlate the dielectric properties and heating rate of solutions for generating useful information for RFAE was performed but was not successful.

iv. The effects of ethanol content, solid to liquid ratio, mixing speed and power on the extraction recovery, sample heating rate, sample energy absorption and energy efficiency of the newly proposed RFAE was assessed. This investigation was performed as a means to optimize target compounds, energy usage and efficiency by manipulating the influential factors on the RFAE. Through this experiment, the potential of low solvent RFAE development was highlighted. The application of nitrogen gas bubble stirring is possible in RFAE and the heating rate of the sample can be improved with constant stirring.

v. A comparative study between RFAE and common extraction methods was performed to demonstrate its extractive capability, justify its need for further development and for better understanding of its extraction mechanism. This investigation has demonstrated that RFAE has better extraction capability and thorough extraction mechanisms than the other selected methods.

9.4 Future Recommendations for RFAE and Apple Peel Phenolic Compounds Studies

There is a potential for commercialization of this technology and compounds studied, however for making that target a success, further work for gaining deeper understanding is highly required. In relation to that, several recommendations on the important future studies for knowledge advancement in this field are outlined as the following:

i. Develop pre-treatment method and enzymatic extraction of RFAE apple peel to increase extraction recovery and efficiency.
ii. Develop more suitable solvents for RFAE of nutraceuticals that meet the consumer safety requirement, study the dielectric properties and correlate the loss tangent to heating rate of the solvents for useful engineering application.
iii. Model heat generation and distribution during RFAE using computer-aided software for the purpose of understanding and improving the existing method and system.
iv. Study the potential of RFAE as a low solvent extraction method by manipulating the solid to liquid ratio with appropriate solvent.
v. Study the feasibility of our new applicator for dielectric heating of other solid-liquid mixture food model samples for the development of additional applications using RF heating.
vi. Investigate the availability and effect of apple peel phenolics compounds incorporated in food model systems to evaluate their effective antioxidant activity. The success of this study could lead to investigations on the suitability and availability of the compounds in food model system and promote more efforts on new product development from apple phenolics.
vii. Evaluate the feasibility of RFAE for industrial application through modeling process scale-up and economic analysis.

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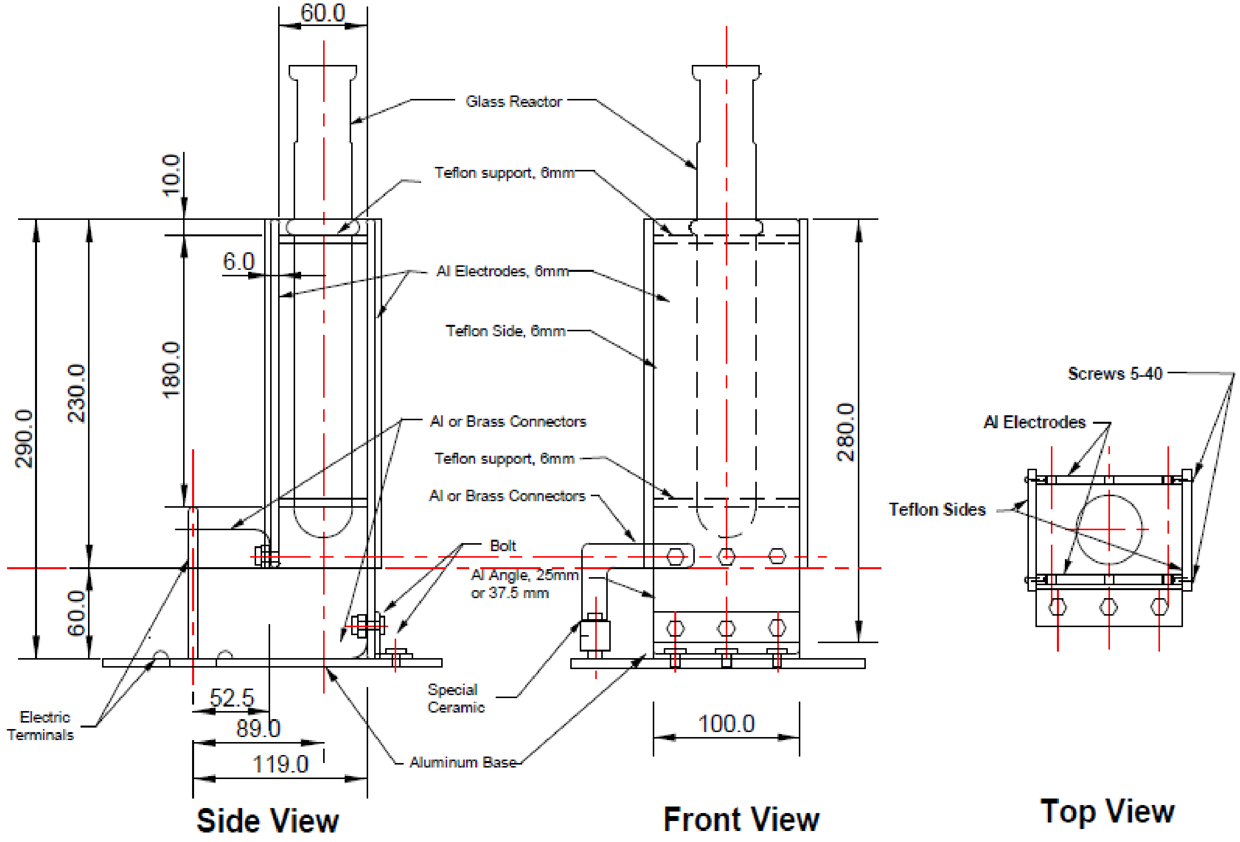
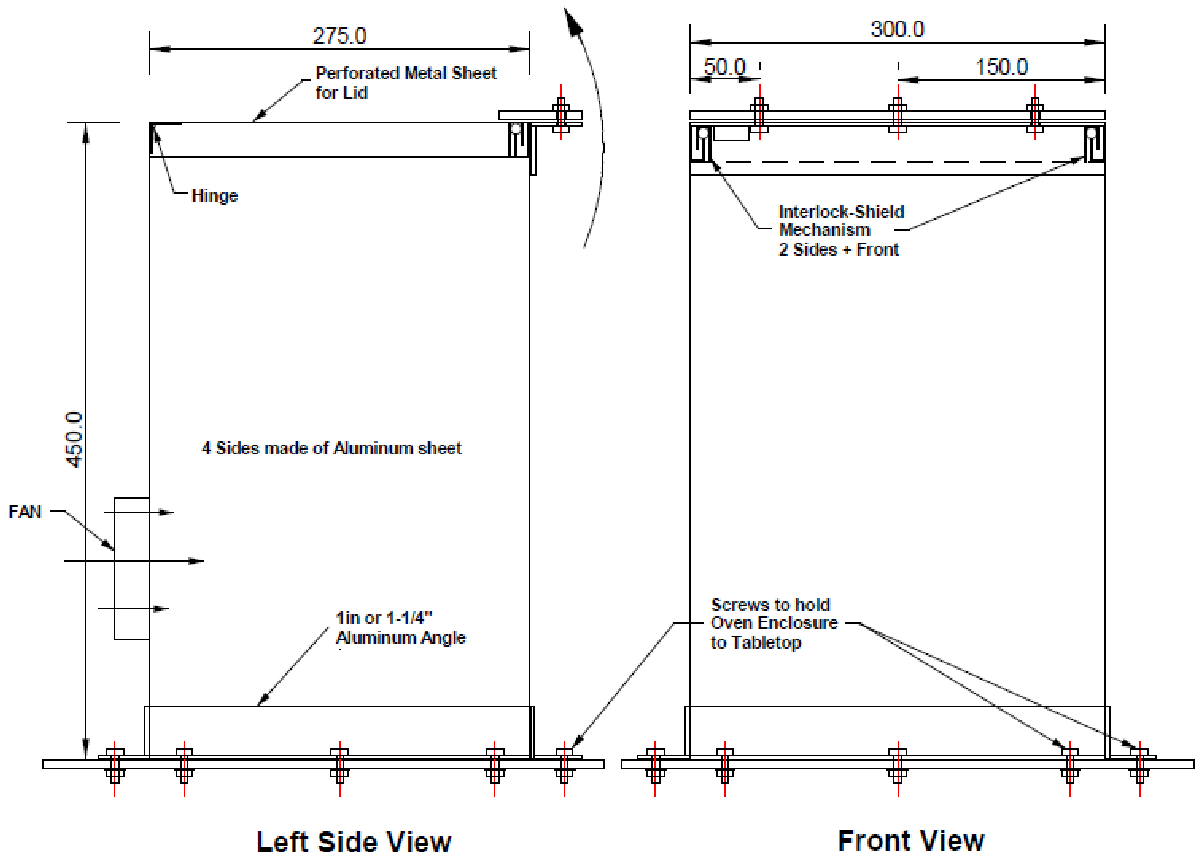
Appendix 1



RF Applicator for SAIREM System
File: RF1.DWG
YG 2009-09-21

Note
No sharpe edges or corners



RF Oven Enclosure
File: RF4.DWG
YG 2009-09-21

Note
No sharpe edges or corners

Appendix 2

(Chapter 8)

Example For Calculating Y and ln Y and Determining Diffusion Coefficient for CONOHEAT CONHEAT, UAE, MAE and RFAE

In Chapter 8, Fick's Second Law Linearization method (equation 1) was used for determining diffusion coefficient (D_{eff}) for all methods evaluated. Data ln Y against t was plotted to obtain the slope of the curve which represents D_{eff}.

$$\ln Y = \ln \frac{6}{\pi^2} - \frac{\pi^2 D_{eff}}{r^2} t \qquad (1)$$

The values of Y were manually calculated by substituting values of C_t and C_∞ into equation 2 obtained from experiment (Table 1):

$$Y = 1 - \frac{C_t}{C_\infty} \qquad (2)$$

Table 1: Kinetics data, values of Y and ln Y for CONOHEAT

Time (t) minute	Concentration (C) mg GAE/g DM	Y	ln Y
0	0	0	0
2	3.57	0.80	- 0.23
5	5.00	0.71	- 0.34
10	7.05	0.60	- 0.52
20	9.15	0.48	- 0.74
30	9.95	0.48	- 0.84
40	10.58	0.39	- 0.93
50	10.95	0.37	- 0.99
60	11.10	0.37	- 1.01
70	11.29	0.35	- 1.04
80	11.45	0.35	- 1.06
90	11.59	0.34	- 1.09
100	11.68	0.33	- 1.10
110	11.73	0.33	- 1.11
120	11.80	0.32	- 1.12
∞	17.48		

Jiao, Y., Tang, J., Wang, S., & Koral, T. (2014). Influence of dielectric properties on the heating rate in free-running oscillator radio frequency systems. *Journal of Food Engineering*, *120*(1), 197-203.

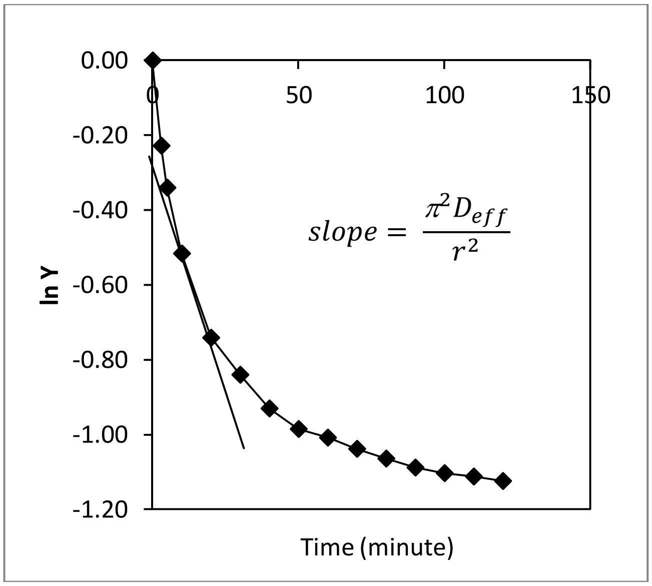


Figure 1: Plot of ln Y against Time for CONOHEAT extraction

From calculation, the value of the slope obtained was 0.0066. By substituting $\pi = 3.1416$ and $r = 0.0004$ m into equation 2, D_{eff} obtained for this method was 9.40×10^{-11} m^2s^{-1}. Example of the calculation is shown below:

Slope = 0.0066, $\pi = 3.1416$, $r = 0.0004$, then

Slope = $\pi^2 D_{eff}/r^2$

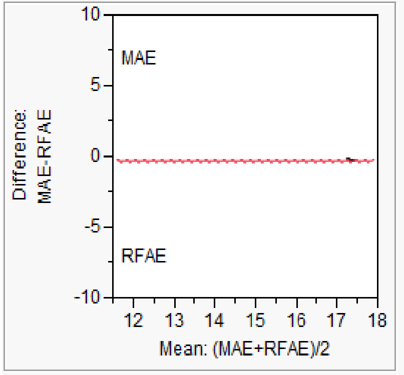
Deff = slope r^2/π^2

= $(0.0066)(0.0004m)^2/(3.1416)^2$

= $\mathbf{9.40 \times 10^{-11}\ m^2/s}$

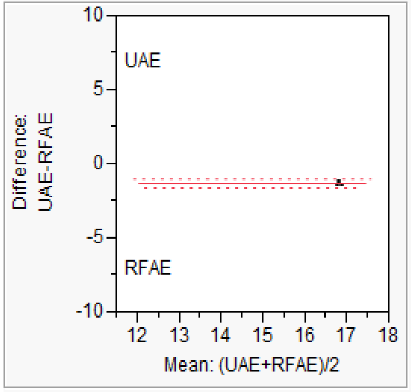
Statistical Analysis (t-test) for Comparing the Extraction Methods

Difference: MAE-RFAE



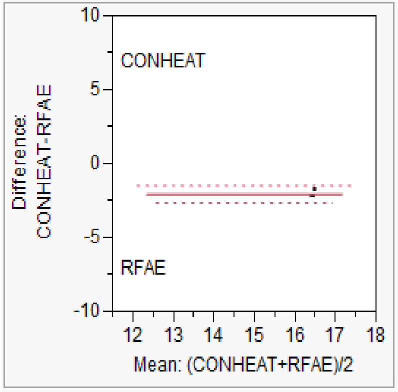
MAE	17.1833	t-Ratio	-14.4115
RFAE	17.4833	DF	2
Mean Difference	-0.3	Prob > |t|	0.0048*
Std Error	0.02082	Prob > t	0.9976
Upper 95%	-0.2104	Prob < t	0.0024*
Lower 95%	-0.3896		
N	3		
Correlation	0.99858		

Difference: UAE-RFAE



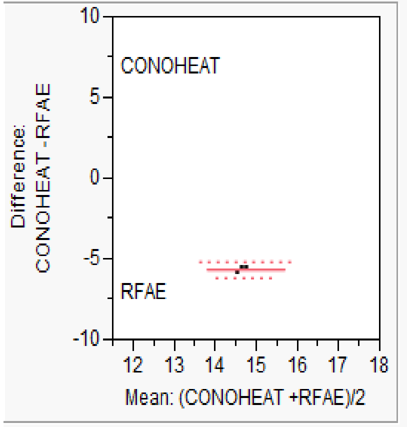
UAE	16.1533	t-Ratio	-17.6163
RFAE	17.4833	DF	2
Mean Difference	-1.33	Prob > |t|	0.0032*
Std Error	0.0755	Prob > t	0.9984
Upper 95%	-1.0052	Prob < t	0.0016*
Lower 95%	-1.6548		
N	3		
Correlation	-0.4558		

Difference: CONHEAT-RFAE



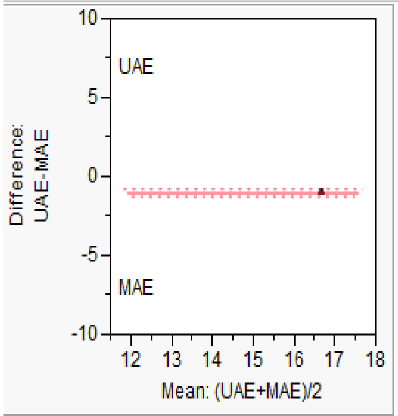
CONHEAT	15.4233	t-Ratio	-15.2558
RFAE	17.4833	DF	2
Mean Difference	-2.06	Prob > |t|	0.0043*
Std Error	0.13503	Prob > t	0.9979
Upper 95%	-1.479	Prob < t	0.0021*
Lower 95%	-2.641		
N	3		
Correlation	-0.8363		

Difference: CONOHEAT -RFAE



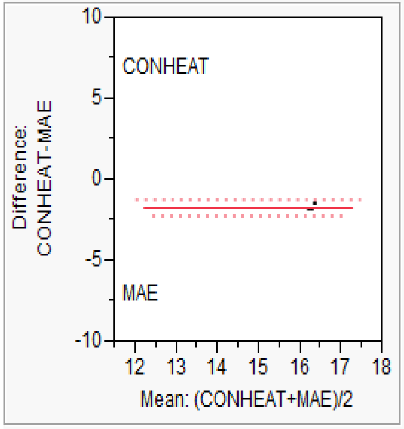
CONOHEAT	11.81	t-Ratio	-49.0508
RFAE	17.4833	DF	2
Mean Difference	-5.6733	Prob > |t|	0.0004*
Std Error	0.11566	Prob > t	0.9998
Upper 95%	-5.1757	Prob < t	0.0002*
Lower 95%	-6.171		
N	3		
Correlation	0.07429		

Difference: UAE-MAE

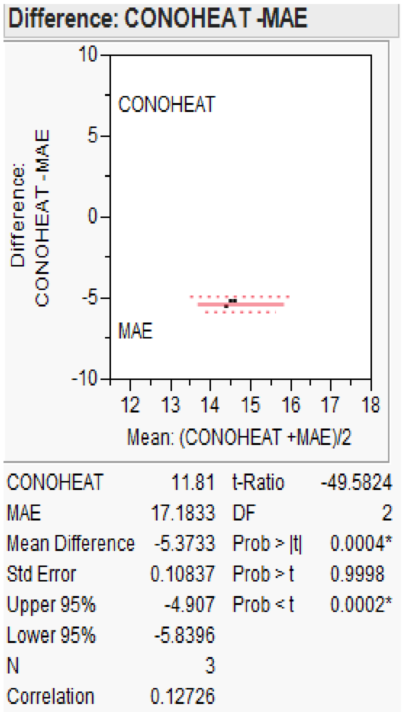


UAE	16.1533	t-Ratio	-18.1139
MAE	17.1833	DF	2
Mean Difference	-1.03	Prob > |t|	0.0030*
Std Error	0.05686	Prob > t	0.9985
Upper 95%	-0.7853	Prob < t	0.0015*
Lower 95%	-1.2747		
N	3		
Correlation	-0.4078		

Difference: CONHEAT-MAE



CONHEAT	15.4233	t-Ratio	-15.2611
MAE	17.1833	DF	2
Mean Difference	-1.76	Prob > |t|	0.0043*
Std Error	0.11533	Prob > t	0.9979
Upper 95%	-1.2638	Prob < t	0.0021*
Lower 95%	-2.2562		
N	3		
Correlation	-0.8059		



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CONOHEAT          11.81    t-Ratio     -49.5824
MAE             17.1833    DF                 2
Mean Difference -5.3733    Prob > |t|   0.0004*
Std Error       0.10837    Prob > t     0.9998
Upper 95%        -4.907    Prob < t     0.0002*
Lower 95%       -5.8396
N                     3
Correlation     0.12726
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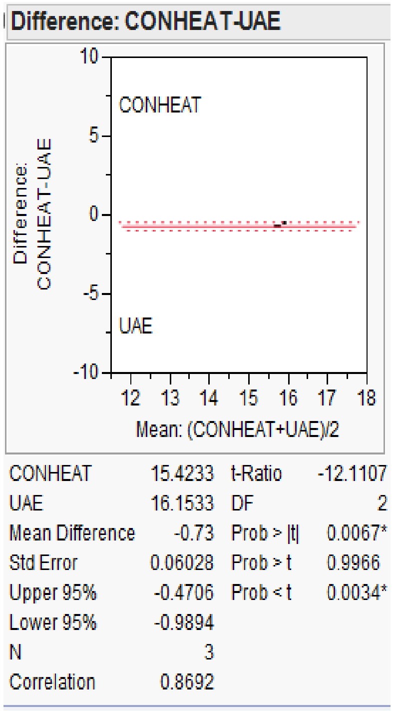

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CONHEAT         15.4233    t-Ratio     -12.1107
UAE             16.1533    DF                 2
Mean Difference   -0.73    Prob > |t|   0.0067*
Std Error       0.06028    Prob > t     0.9966
Upper 95%       -0.4706    Prob < t     0.0034*
Lower 95%       -0.9894
N                     3
Correlation      0.8692
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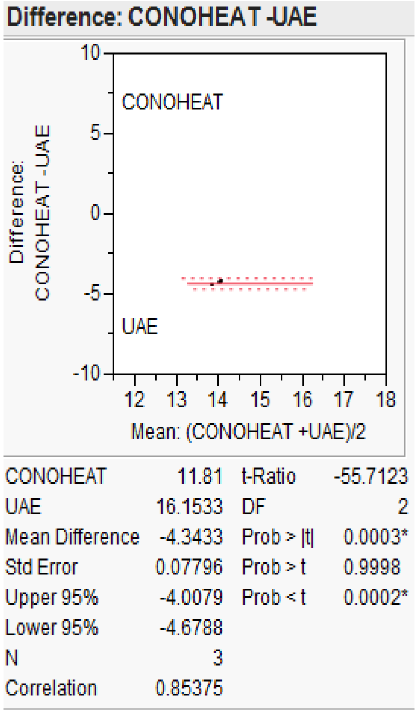

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CONOHEAT          11.81    t-Ratio     -55.7123
UAE             16.1533    DF                 2
Mean Difference -4.3433    Prob > |t|   0.0003*
Std Error       0.07796    Prob > t     0.9998
Upper 95%       -4.0079    Prob < t     0.0002*
Lower 95%       -4.6788
N                     3
Correlation     0.85375
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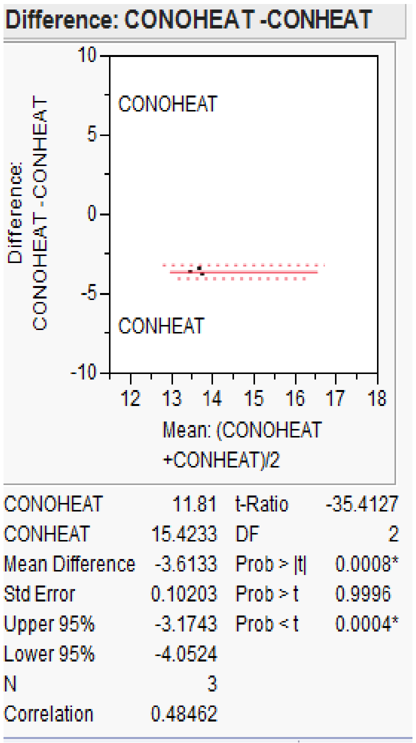

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CONOHEAT          11.81    t-Ratio     -35.4127
CONHEAT         15.4233    DF                 2
Mean Difference -3.6133    Prob > |t|   0.0008*
Std Error       0.10203    Prob > t     0.9996
Upper 95%       -3.1743    Prob < t     0.0004*
Lower 95%       -4.0524
N                     3
Correlation     0.48462
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