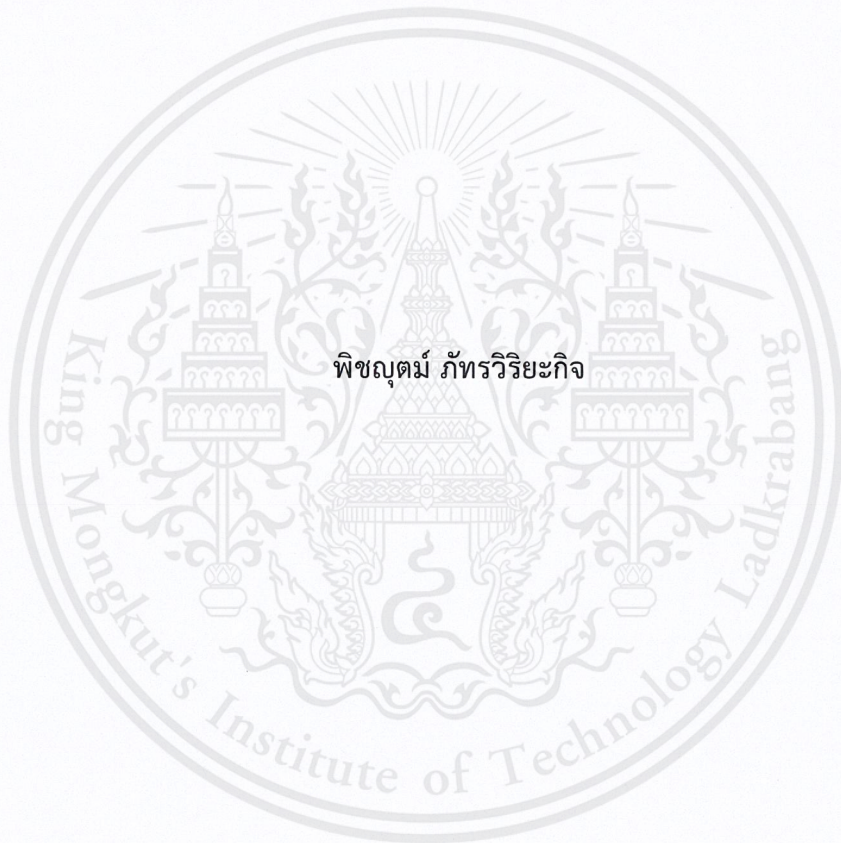


**Kinetics Model for Antioxidant Extraction  
from Coffee Silver Skin using Ultrasonic Assisted Extraction**



**A Report Submitted in Partial Fulfillment of the Requirements  
for the Degree of Bachelor of Engineering (Petrochemical Engineering)  
Department of Chemical Engineering, Faculty of Engineering,  
King Mongkut's Institute of Technology Ladkrabang  
Academic Year 2018**

สมการคณิตศาสตร์การสกัดของสารต้านอนุมูลอิสระจากเห็ดห่มเมลิ็ดกาแพ  
โดยวิธีการสกัดแบบอัลตราโซนิก



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### **Abstract**

Coffee silver skin (CS) is the main residue from the roasting step of the coffee process. It consists of antioxidant components, which can be utilized as valuable commodity in food, cosmetics, and pharmaceutical industries. The aims of this work were to study the important parameters; frequency of extraction, sample to solvent ratio, and solvent type to find the suitable condition of total phenolic compounds (TPC) extraction using ultrasonic-assisted extraction (UAE). The samples were analyzed by Diphenyl-picrylhydrazyl (DPPH) radical scavenging assay and total phenolic compound (TPC) method. Moreover, the three mathematical kinetics models (Peleg's law, second-order rate law, and two-site kinetic model) were explored to properly predict the effect of each parameter. This investigation provides useful information for scale up. The study of various variables in extraction showed that using the frequency of 28 kHz, 1/30 solid to solvent ratio and 50% ethanol : 50% water by weight is the most suitable condition used to extract the TPC from coffee silver skin. The Peleg's law is the best model for phenolic extraction from CS.

**Keywords:** Coffee Silver Skin (CS), Total Phenolic Compounds (TPC), Ultrasonic-Assisted Extraction (UAE).

ปริญญาานิพนธ์เรื่อง	สมการคณิตศาสตร์การสกัดของสารต้านอนุมูลอิสระจากเยื่อหุ้มเมล็ดกาแฟโดย วิธีการสกัดแบบอัลตราโซนิก
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### บทคัดย่อ

เปลือกหุ้มเมล็ดกาแฟ (Coffee silver skin: CS) เป็นของเสียหลักที่ได้จากขั้นตอนการคั่วของกระบวนการผลิตกาแฟ มันมีส่วนประกอบของสารต้านอนุมูลอิสระซึ่งสามารถนำมาทำเป็นผลิตภัณฑ์ที่มีมูลค่าทั้งทางด้านอุตสาหกรรมอาหาร เครื่องสำอาง และยาได้ วัตถุประสงค์ของงานวิจัยครั้งนี้คือการศึกษาตัวแปรที่มีความสำคัญ เช่น ความถี่ที่ใช้ในการสกัด สัดส่วนของเปลือกหุ้มเมล็ดกาแฟต่อตัวทำละลาย และชนิดตัวทำละลาย เพื่อหาสภาวะที่เหมาะสมต่อการสกัดสารประกอบฟีนอลทั้งหมดจากเปลือกหุ้มเมล็ดกาแฟโดยใช้การสกัดแบบอัลตราโซนิก (Ultrasonic-assisted extraction: UAE) สารตัวอย่างจะถูกวิเคราะห์โดยวิธีการทดสอบการกำจัดอนุมูลอิสระ Diphenyl-picrylhydrazyl (DPPH assay) และการทดสอบหาปริมาณสารประกอบฟีนอลทั้งหมด (TPC method) นอกจากนี้แบบจำลองจลนศาสตร์ทางคณิตศาสตร์ทั้ง 3 แบบ (Peleg's law, second-order rate law, and two-site kinetic model) จะถูกตรวจสอบอย่างเหมาะสมเพื่อทำนายผลของแต่ละตัวแปร การตรวจสอบนี้จะให้ข้อมูลที่เป็นประโยชน์ต่อการขยายขนาดทางอุตสาหกรรมได้ การศึกษาตัวแปรต่างๆในการสกัดแสดงให้เห็นว่า การใช้ความถี่ 28 kHz, สัดส่วนของเปลือกหุ้มเมล็ดกาแฟ 1 กรัมต่อตัวทำละลาย 30 ml และชนิดตัวทำละลายเอทานอล 50% ต่อน้ำ 50% คือสภาวะที่สามารถสกัดสารประกอบฟีนอลได้เหมาะสมที่สุด และแบบจำลองจลนศาสตร์ Peleg's law เป็นแบบจำลองที่ดีที่สุดต่อการสกัดสารประกอบฟีนอลในเปลือกหุ้มเมล็ดกาแฟ

**คำสำคัญ:** เยื่อหุ้มเมล็ดกาแฟ, สารประกอบฟีนอล, การสกัดแบบอัลตราโซนิก

## Acknowledgements

The success and final outcome of this project required guidance and assistance from many people and I am extremely thankful to my project adviser, Asst. Prof. Dr. Tanawan Pinnarat, for providing a good support and some suggestion along the completion of my project.

I would like to thank Assoc. Prof. Dr. Chamroon Laosinwattana for allowing the use of his laboratory at the Faculty of Agriculture and I heartily thank graduate students, Ms. Pattharin Wichittrakarn and Mr. Nguyen Huy Thinh for antioxidant analysis training.

I respect and thank Mr. Pisan Polpoh, Mrs. Pimjai Puchanakij, and Ms. Sukanpirom Sriwong, the staff of Department of Chemical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang, who helped with documentation for equipment request in lab equipment utilization.

I would also like to thank Khao Shong Industry 1979 Company Limited for the coffee silver skin samples.

The most indispensable thank you to my parents and families for their support and encouragement throughout the course of my educational journey until graduation.

Any benefit derived from this work devoted to my parents, teachers and all benefactors, who have provided the knowledge and training to make this work successfully accomplish.

Pitchayut Pataraviriyakit

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## CHAPTER I

### INTRODUCTION

#### 1.1 Background

Coffee is one of the most widely consumed beverage all over the world and be produced in at least 70 countries. Globally, the total coffee consumption in the year 2018, according to an International Coffee Organization (ICO) report, was estimated that 161.93 million of 60 kg bags with an increase of 1.8% compared to the year 2017[2]. In accordance with coffee bean processing, a large number of residues (e.g. coffee silver skin and spent coffee grounds) is generated. These residues have not completely utilized and being usually used for composting and soil fertilization or burn as fuel and hence release pollution. In the last 5 years, many researchers interested in turning these residues into valuable commodities [3].

Coffee silver skin (CS) is the main residue from roasting step of coffee processing. It is an interesting residue because it contains high content of phenolic compounds, the antioxidant substances [4]. Recently, the total phenolic compounds (TPC), which have strong antioxidant and metal-chelating properties, have received considerable attention because of numerous evidence and research suggesting that their consumption promote abundant benefits for human health such as agent free radical and reduce the risk of diseases such as atherosclerosis, cancer, diabetes, and neurodegenerative diseases etc.[5] Therefore, this natural antioxidant substances can be applied in food, pharmaceutical, cosmetics and nutraceutical industries [6].

The first step in the isolation and utilization of these phenolic compound is their extraction. Chemically active extraction of CS is the liquid/solid extraction in which the liquid phase TPC is extracted out from solid material by mass transfer of the solvent (hydro alcoholic mixture). In the past, the conventional technique is widely used for extraction of the chemical substance from herbs and fruits due to easy operation; nevertheless, it is not only consume high solvent but also require long time and causing high energy consumption. In fact, there are others extraction techniques other than conventional method such as supercritical fluid extraction, microwave-assist extraction, and ultrasonic-assisted extraction etc. Currently, the newer technique like ultrasonic-assisted extraction (UAE) received many attention because it is relatively cheap when compared to other technique and require less instrument.[7]

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The efficiency of the extraction of all techniques depends on the process operating parameters, for instance, solvent type, extraction time, temperature, and solid/liquid ratio. In order to select the appropriate condition, several empirical kinetics models have been utilized for explaining the effect of each parameter. The mathematical kinetics models, which use to explain the extraction system are Peleg's law [8], second-order rate law [9], and two-site kinetic model [10] in which provide the useful information for designing, optimizing and controlling the instruments in industrial scale production.

There are many researchers who studied the optimization of antioxidant from extracted CS and only a few studies have dealt with the kinetic model of phenolic compounds from CS using UAE. Therefore, this present study will investigate the important parameters: frequency of UAE, sample to solvent ratio, solvent type, temperature and time of extraction to find the optimal condition to extract phenolic compound using UAE then determining and analyzing the kinetics through mathematical model; Peleg's law, second-order rate law, two-site kinetic model were selected to describe the extraction kinetics.

## 1.2 Objectives

1) To investigate the important parameters (sample to solvent ratio, solvent type, frequency of UAE, temperature and time of extraction) affect on total phenolic compound of coffee silver skin using the ultrasonic-assisted extraction.

2) To determine the kinetics model for the extraction of phenolic compounds from coffee silver skin.

## 1.3 Scope of work

1) Study important parameters affect on total phenolic compound (TPC) of coffee silver skin (CS) using the ultrasonic-assisted extraction.

1.1) Frequency of ultrasonic (kHz): 28, 40

1.2) Solid to solvent ratio (g CS/v solvent): 1/5, 1/30, 1/50, 1/70

1.3) Solvent type: ethanol/water (%v/v): 100/0, 75/25, 50/50, 25/75, 0/100

1.4) Extraction temperature (°C): 40, 60, 80

1.5) Time of extraction (min): 5, 10, 15, 30, 60, 90

2) Study three kinetics models (Peleg's law, second-order rate law, and two-site kinetic model) for the extraction of TPC from CS.

## CHAPTER II

### THEORY AND LITERATURE REVIEW

#### 2.1 Coffee silver skin

Coffee is native to tropical Africa and South Asia. Arabica and Robusta are only two main economically important coffee species: 70 and 30% of the coffee traded in the world are Arabica and Robusta, respectively. Coffee fruit is a drupe containing the pericarp and seed. In mature fruit, the thick pericarp consist of outer skin, pulp and pectin layer as shown in figure 2.1. The latter is coffee seed, which is elliptical or egg shape. It is covered by the hard pale brown external layer as known as “parchment”. Under this parchment, there is a thin seed coat (around 70  $\mu\text{m}$  thick), called “silver skin” [11].

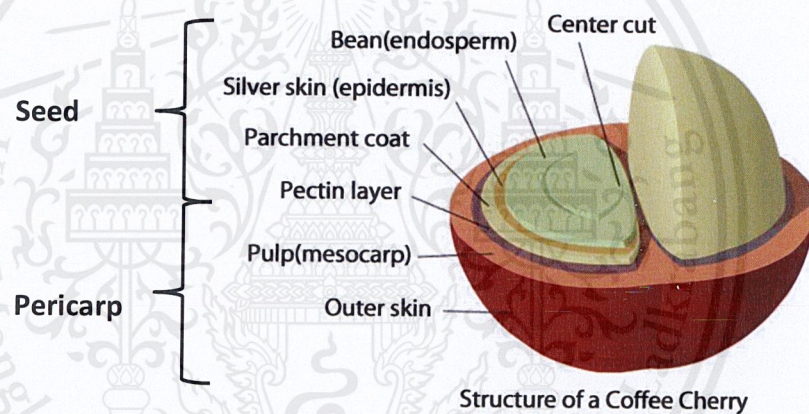


Figure 2.1. Anatomy of the coffee fruit and bean [12]

In coffee production, the silver skin is waste in the roasting step as the result of swelling and expansion due to loss of moisture content at high temperature, and also make the CS detach from the seed as chaff showed as in figure 2.2. In addition, considering that more than 50% of the fruit is discarded, coffee production leads to an elevated amount of this waste, which has very nutritious and beneficial to human health. therefore, many researchers are interested in the extraction of bioactive compound from silver skin [13].

According to the results of Castillo et al.,2016 [1], the extract silver skin is a good source of caffeine (3%), melanoidins (17% to 23%) and polyphenols, particularly chlorogenic acid (1% to 6%); including total dietary fiber (28% to 36%).

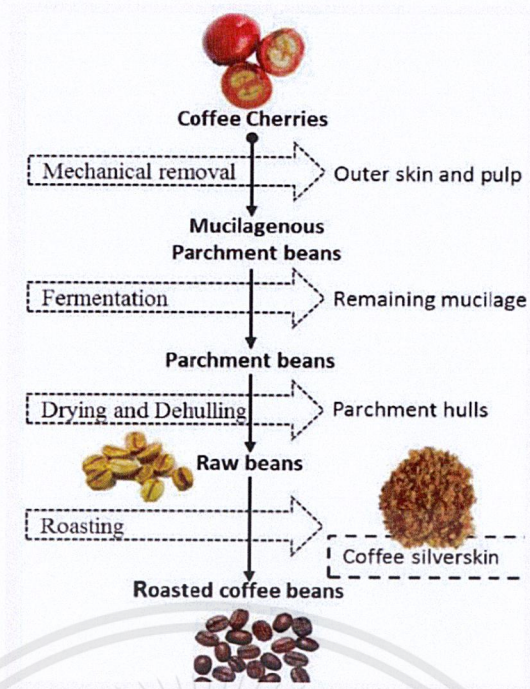


Figure 2.2. Diagram of coffee silver skin production from wet processing [13]

Martinez-saez et al.,2014 [14] reported that the new antioxidant beverage from roasted CS extract with right nutritional composition and sensory quality has the potential for health promoting application in term of fat reduction or the prevention of excess weight and obesity, especially type 2 diabetes.

In use of cosmetic, the result of Rodrigues et al.,2015 [15] explained that the total phenolic compounds (TPC) and the total flavonoids compounds (TFC ) of extracts were 18.33-35.25 mg gallic acid equivalent per g dry basis (mg GAE/gdb) and 1.08-2.47 catechin equivalents per g dry basis (gCE/g db), respectively. The antioxidant activity was high, with values ranging between 95.95 and 216.40 mmol  $\text{Fe}^{2+}/\text{g}$  for aqueous and alcoholic samples, respectively. Preliminary assays for antimicrobial potential showed that extracts display antibacterial activity. Consequently, CS is a safe source of natural antioxidants with antifungal and antibacterial activity and no toxicity, with potential usefulness for cosmetic applications.

## 2.2 Antioxidant activity

Free radicals are unpaired electrons in atoms or molecules that found everywhere in the environment, in living organisms, and in cells, especially in the production of energy within the cell or metabolism. The electrons move from the oxygen molecules, causing the unbalance of electrons in the oxygen molecules, which

become active free radicals; furthermore, it can draw electrons from other molecules to replace missing electrons to make it balanced and stable. This reaction is a continuous as chain reaction and appear in the cell all the time.

The most important free radicals in cells compose of oxygen radical, Derivative of oxygen radical (e.g. superoxide radical ( $O_2^{\cdot-}$ ) and hydroxyl radical ( $OH^{\cdot}$ )), hydrogen peroxide, transition metals (transition metals), carbonate radical ( $CO_3^{\cdot-}$ ), peroxy radical ( $ROO^{\cdot}$ ), reactive oxygen species (ROS) etc. In addition, free radicals can destroy all types of biological molecule in both of cell and organelle of organism such as lipid, protein, carbohydrate, collagen and DNA etc. However, whether it is a plant or animal, there is a system called “antioxidant defense system” to maintain balance of free radicals in the body. The antioxidant defense system is caused by the work of various substances called “antioxidant”, for example, enzyme (catalase, glutathione peroxidase and superoxide dismutase) and/or derivative protein (glutathione, urate, and albumin). On the other hand, if there are excess free radicals to inhibit with the protection system, oxidative stress condition will occur. In this condition, the remaining free radicals may impact to organ and tissue of human body and then cause cancer, aging, coronary heart disease, Alzheimer’s disease, and arthritis etc. [16] Thus, the synthesis antioxidant is one solution to control and prevent the effect of free radicle. Even though it is high efficiency, it has limitations and issues of safety in consumption; as a result, the natural antioxidant is interesting.

The antioxidant is divided into two categories as follows:

2.2.1 The synthesis antioxidant; there are 5 types of phenolic compounds such as propyl gallate, 2-butylated hydroxyanisole, 3-butylate hydroxyanisole, BHT (butylated hydroxytoluene) and tertiary butylhydroquinone. All of this antioxidant are usually used in food industrial for blocking the oxidation of lipid, which cause the smell, change of color, and food taste changing.

2.2.2 The natural antioxidant; this group has caught attention and has been developed intensively in the present owing to return to nature issue. It is found in the microorganisms, animal and plants, which are vitamins, such as, vitamin C, vitamin E and Beta carotene. Not only the vitamins are found but also the amount of phenolic compounds, especially flavonoids, are found. The phenolic compounds consist of aromatic hydroxyl group that plays an important role in trapping free radicals in order not to stimulate or cause oxidative reactions [17].

## 2.3 Extraction techniques

There are many extraction techniques to extract phenolic compounds from natural sources. In this section, several methods are reviewed.

### 2.3.1 Soxhlet extraction and solid-liquid extraction (SLE)

Soxhlet extraction and solid-liquid extraction (SLE) are the oldest simple techniques commonly used for the isolation of nonpolar and semi-polar organic substances (phenolic compounds in natural sources) from solid matrix. SLE is a technique that extract the substance out from solid material by using liquid solvent. Soxhlet extractor is continuous SLE, which usually use in the laboratory. Easily use and good yield results are the strength of this technique. The main drawback of this technique are high solvent consumption, which also mean that the solvent may be evaporated, and high energy consumption because of the long extraction time.

Lina F. Ballesteros et al.,2014 [18] obtained the total phenolic compounds of 13 mg GAE/g CS when using 60% ethanol in a 35 ml/ g CS, at 60-65°C in 30 minutes. The antioxidant activity were 18.24  $\mu\text{mol}$  Trolox equivalents/g CS (DPPH assay) and 0.08 mmol Fe (II)/g CS (FRAP assay). This result gave the high value of antioxidant from CS, thus, SLE is still a good choice of extraction.

### 2.3.2 Supercritical fluid extraction (SFE)

Supercritical fluid extraction is the extraction with the use of substances at critical temperature and pressure conditions. In this state, the solvent has a diffusion coefficient as gas and viscosity lower than that of liquid, thus dissolving like liquid. Carbon dioxide is the most commonly used as supercritical fluid because of its many advantages such as non-toxic, environmentally friendly, high purity, low cost, and low critical temperature (31.1 °C), suitable for thermo-sensitive substances.

The advantages of SFE are no residue of organic solvents after extraction. The limitation of this technique is the high price of equipment and operating cost.

There are many researchers including Andrade et al.,2012 [19] use the SFE technique to extract the phenolic compounds and reported that the total phenolic compounds (TPC) of extract from spent coffee ground (SCG) was the highest, 56.7 mg Chlorogenic acid equivalent per g extract (mg CAE/g extract), at pressure of 100

bar, temperature of 50 °C in 2.5 hr by using CO<sub>2</sub> as supercritical fluid. The antioxidant activity was 18% of 2,2'-azino-bis, 3-ethylbenzothiazoline-6 sulfonic acid (ABTS) inhibition at the same condition.

### 2.3.3 Microwave-assisted extraction (MAE)

The technology of MAE is widely applied in the extraction of biological compounds from natural resources. This process is effective in extracting, reducing the preparation time and reducing the volume of solvent used when compared to conventional extraction techniques but it consumes high energy. In the conventional heat extraction process, it is necessary to pass heat through the container before it enters the solution (heat transfer), which takes a longer time for any of the molecules of solvent and sample to heat up to the required condition.

The heating of the MAE is based on the direct extraction of microwave per molecules. Microwave energy infiltrates the container, causing the molecules to move, resulting in a volumetric heat source. The ionization of the ions and the rotation of the polarized pairs results in the electric field of the molecule in both the solvent and in the sample. As a result, the temperature of the solvent is higher than its boiling point.

The MAE extraction system is divided into two systems: Closed and open system. The closed system is mainly used in semi-volatile extraction due to the high solvent temperature, which increases the mass transfer efficiency of substances from the sample to the solvent used. The open system is used to extract low volatiles such as insecticides and provide a reflux process to aid in extraction[20].

### 2.3.4 Ultrasonic-assisted extraction (UAE)

The ultrasonically assisted extraction is an attractive option in extraction process by the reason of higher yield, faster kinetics, less expensive and simple in comparison with conventional methods. This technique uses the elastic mechanical vibration wave or ultrasound energy produce strong cavitation effect, the disturbance effect, breaking and mixing effect and molecular motion frequency. After that the expansion and compression are occur then cause a large number of micro bubble, which lead to particle dispersion and increase surface area between solid and liquid phase for extraction.

Al-Dhabi et al.,2017 [21] used UAE method to extract the waste spent coffee ground and found the optimum condition (244 w ultrasonic power, 40°C temperature, 40 min and 1:17 g solid /ml liquid), which resulted in  $33.84 \pm 0.59$  mg GAE/g SCG (in term of TPC),  $5.04 \pm 0.07$  mg quercetin equivalent (QE)/g SCG (in term TFC). From this results, it can conclude that UAE is one of the useful technique.

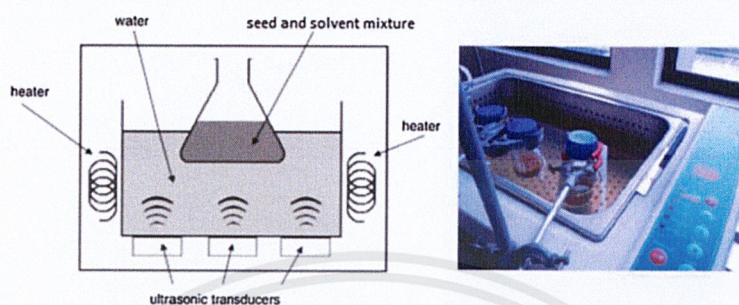


Figure 2.3. Ultrasonic bath extractor [22]

The comparison of the advantage and disadvantage of each extraction method is given in table 2.1.

Table 2.1 Comparison of extraction techniques.

Method	Condition mechanism	Advantage	Disadvantage	Reference
SLE	- Used solvent pass through the solid materials and extract the substances.	- Easy to use - good yield results	- high solvent consumption - high energy consumption - long extraction time	[18]
SFE	- Used the supercritical fluid, which have the properties like liquid and gas to extract.	- no residue of organic solvents after extraction	- high investment for equipment - high operating cost	[19]
MAE	- Used the microwave energy to heat the solid particles.	- high yield - short extraction time - low solvent consumption	- high instrument cost - filtration requirement after extraction	[20]

Table 2.1 Comparison of extraction techniques (continues).

Method	Condition mechanism	Advantage	Disadvantage	Reference
UAE	- Used the ultrasound energy in the form of wave to produce strong cavitation effect and thermal effect.	- high yield - fast kinetics - inexpensive - simple		[21]

## 2.4 The extraction parameters

The efficiency of extraction depends on many parameters as follows:

### 2.4.1 Coffee species

The most commercially coffee species are Arabica and Robusta. The characteristic properties of two coffee species (e.g. aroma and flavor) are different including amount of the antioxidant substance. According to Tan et al.2016 [23], they presented the information that Robusta extract has higher value of TPC, TFC and antioxidant activity than Arabica extract at 40 °C for 60 min using 50%v ethanol /50%v water. The antioxidant activity of Robusta extract were 816.75 mg GAE/L sample (in term of TPC) and 54.8% inhibition of DPPH, while for Arabica extract were 473.51 mg GAE/L sample (in term of TPC) and 26.3% inhibition of DPPH.

### 2.4.2 Particle size

The small particle size of solid particle can be more effective in extraction than the larger size owing to the small particle size have more surface area than the larger size. Pinelo et al.2005 [24] investigated the effect of grape peel size on phenolic compounds content in the extraction. Researcher reported that the size of grape peel affects the duration of extraction. The smaller grape peel sample sizes (diameter of 0.5 mm) have more surface area than the larger size (diameter of 5 mm), which results in shorter extraction times.

### 2.4.3 Solvent type

The choice of solvent is the main parameter influencing to the extraction efficiency. The polarity of the targeted compound, boiling point, environmentally safety and human toxicity must also be considered in solvent selection. The solvent like ethanol, pure distilled water, methanol, or hydro-alcoholic mixtures in different ratios and an isopropanol /water mixture [25] were usually used to extract the

antioxidant substance because these polar solvents easily solubilize the phenolic compounds.

Cvitanovic et al. 2017 [26] reported that the equimolar (50 v/50 v) mixture of ethanol and water is the best solvent for phenolic compound extraction. But when they use pure ethanol, it provided lowest phenolic content and lower than using pure methanol or 100% water. Furthermore, the results of Costa et al., 2014 [27] showed that 50% ethanol is the most appropriate for phenolic extraction from coffee silver skin gave the phenolic concentration and the antioxidant activity of  $302.5 \pm 7.1$  mg GAE/L sample and  $326.0 \pm 5.7$  mg Trolox equivalent (TE)/L, respectively. According to Bravo et al., 2013 [28], methanol is the least effective solvent comparing water, ethanol and their mixture due to the small amount of TPC ( $7.37 \pm 0.66$  mg GAE/g CS) was founded. Moreover, methanol is toxic to human health and require additional cost in methanol separation for food grade production.

#### 2.4.4 Temperature and time

The temperature and time are the importance parameters. Many research groups found the optimal conditions that use temperature of  $40^\circ$  and  $60^\circ\text{C}$  for extraction of phenolic compounds from plant [26]. When the temperature increases, the solubility and mass transfer rate increases because the viscosity and surface tension decreases, which helps the solvents easily reach the sample matrices and improve the extraction rate. Panusa et al., 2013 [29] reported that the temperature  $60^\circ\text{C}$  give high value of TPC (11-28 mg GAE/g spent coffee ground).

In term of extraction time, Costa et al. 2014 [27] suggested that  $40^\circ\text{C}$  for 60 min using 50%v ethanol /50%v water is the optimal condition for extraction (optimal time), while Cvitanovic et al. 2017 [26] use the time of range 30 - 90 min and reported that no differences in the phenolic yield.

#### 2.4.5 solid/liquid ratio

Pinelo et al. 2005 [24] found that when the amount of solvent was increased, more phenolic compounds are extracted. However, the phenolic compounds extracted will start to stabilize when adding solid / liquid ratio to the saturated value. The optimal condition of this research was 1 g/ 10 ml solvent comparing 1 g/ 5 ml solvent.

## 2.5 Test assay

### 2.5.1 DPPH radical scavenging assay

Diphenyl-picrylhydrazyl (DPPH) radical scavenging assay is a chemically-based test with substances that are free radicals. DPPH radical is a violet synthetic compound that has the highest absorption at the wavelength of 517 nm by using spectrophotometer. When DPPH is reacted with ethanol-soluble antioxidants, the purple color fades to yellow because of oxidative reaction. Therefore, the antioxidant capacity of the sample can be determined by calculating the color of DPPH radical inhibition. The standard antioxidant used is trolox, 6-hydroxyl 2,5,7,8-tetramethylchlorman-2-carboxylic acid, expressed in units of mmol/mg or  $\mu\text{mol/mg}$ .

The advantages of this method are simple, easy and fast. The disadvantage is that it is relatively stable, not sensitive to the reaction like free radicals that occur in the body, thus the reaction is slow[30].

### 2.5.2 Determination of total phenolic compound

Folin-Ciocalteu reagent method is used to determine the total phenolic contents by the means of redox reaction of molybdate tungstate ion reagent containing sodium tungstate, sodium molybdate, Phosphoric acid, and Sodium carbonate. When yellow Mo (VI) ions receive electrons from the antioxidants, they turn into blue Mo (V). The absorbance at a wavelength of 765 nm was measured and reported as the total phenolic contents in the form of gallic acid equivalent (mg GAE / g solid) [31].

## 2.6 Kinetic models

There are different mathematical models for extraction. In this study, three models will be considered as follows:

### 2.6.1 Peleg's law model

Many researchers including Bucić-Kojić et al. 2007 [32] selected this model to describe the extraction of bioactive compounds because it is an empirical form and successfully predicted. The model is shown as follows:

$$C_t = \frac{t}{k_1 + k_2 t} \quad (2.1)$$

where  $k_1$  is the Peleg's rate constant at the beginning (min g dry basis/mgGAE).

$k_2$  is the Peleg's capacity constant (g dry basis/mgGAE).

As  $t$  approaches 0,  $k_1$  is related to the extraction rate  $B_0$  (mgGAE/gdb min):  $B_0 = 1/k_1$ . And when  $t$  approaches  $\infty$ ,  $k_2$  is related to the equilibrium concentration  $C_e$  (mgGAE/gdb) of the phenolics:  $C_e = 1/k_2$ .

### 2.6.2 Second-order rate law model

Numerous researches have been studied the suitable kinetics of the extraction process and second-order rate law model is one of the most used models. It was found that the second-order kinetic model is appropriate for bioactive compound extraction, especially polyphenol [8,32,33].

The general second-order rate law model for the TPC can be written as in equation (2.1)

$$\frac{dC_t}{dx} = k(C_s - C_t)^2 \quad (2.2)$$

where  $k$  is the second-order extraction rate constant (L/g min).

$C_s$  is the equilibrium/saturated concentration of total phenolic compounds in the liquid extract (mg/L).

$C_t$  is the total phenolic concentration in the liquid extract at a given extraction time  $t$  (mg/L).

From the initial and boundary conditions,  $t = 0$  to  $t$  and  $C_t = 0$  to  $C_t$ , the integrated rate law for a second-order extraction and a linear form was obtained as in equation (2.2) and (2.3), respectively.

$$C_t = \frac{C_s^2 kt}{1 + C_s kt} \quad (2.3) \quad C_t = \frac{t}{(1/kC_s^2) + (t/C_s)} \quad (2.4)$$

The initial extraction rate,  $h$  (g/L min), when  $t$  approaches 0, can be defined as:  $h = kC_s^2$ . And then rearrangement as in equation (2.4)

$$C_t = \frac{t}{(1/h) + (t/C_s)} \quad (2.5) \quad \frac{t}{C_t} = \frac{1}{h} + \frac{t}{C_s} \quad (2.6)$$

The constants:  $h$ ,  $C_s$  and  $k$  were determined experimentally from the slope and intercept by plotting  $t/C_t$  versus  $t$ .

### 2.6.3 Two - site kinetic model

This model assume that there are 2 step of extraction, first is the lower rate step desorption and the latter is the higher rate step desorption. The two-site kinetic model proposed by Yang Tao et al.2014 [10] is given as follows:

$$\frac{M_t}{M_\infty} = 1 - Fe^{-k_1t} - (1 - F)e^{-k_2t} \quad (2.7)$$

Where  $M_t$  is the concentration of TPC extracted (mg/g) from the raw material at time t.

$M_\infty$  is the concentration of TPC extracted (mg/g) from the raw material at infinite time.

F is the portion of solute released rapidly.

(1 - F) is the portion of solute released slowly.

$k_1, k_2$  are the first-order rate constants for representing both the stages during the extraction process( $\text{min}^{-1}$ ).

In the present study, the important parameters (sample to solvent ratio, solvent type, frequency of UAE, temperature and extraction time) will be investigated to find the optimal condition to extract phenolic compound using UAE. The DPPH radical scavenging assay and Folin-Ciocalteu reagent method (total phenolic compound) are used to find antioxidant activity. The extraction of phenolic compounds will be described using mathematical model like Peleg's law, second-order rate law and two-site kinetic model.

### 2.6.4 The Arrhenius equation

This equation was implemented which is expressed in equation (2.8)

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \quad (2.8)$$

where  $k_0$  is the temperature-independent factor ( $\text{min}^{-1}$ )

R is the gas constant (J/mol K),

$E_a$  is the activation energy (kJ/mol)

T is the extraction temperature (K).

To determine the values of  $E_a$  and  $k_0$ ,  $\ln(k)$  was plotted against  $1000/T$ . The linearized form of the equation is given in equation (2.8)

$$\ln k = \ln k_0 - \frac{E_a}{RT} \quad (2.9)$$

## CHAPTER III

### RESEARCH METHODOLOGY

#### 3.1 Preparation of Coffee silver skin

##### Equipment

- 1) Blender
- 2) Test sieve size 250 to 450  $\mu\text{m}$

##### Material

Coffee silver skin from Khao Shong Industry 1979 Co., Ltd

##### Procedure

- 1) Reduce the size of the coffee silver using a blender.
- 2) Sizing by test sieve to diameter of 250 to 450  $\mu\text{m}$ .
- 3) Store the coffee silver skin in a dry place at room temperature.

#### 3.2 Extraction of antioxidants using the ultrasonic probe system extraction

##### Equipment

- 1) Ultrasonic probe system
- 2) Erlenmeyer flask with lid 250 ml
- 3) Filter paper grade F1001 (10-13  $\mu\text{m}$ )
- 4) Dark color sample bottle 50 ml

##### Chemicals

- 1) Coffee silver skin (diameter of 250-425  $\mu\text{m}$ ).
- 2) Solvent (99.9% pure ethanol, distilled water)

##### Procedure

- 1) Weight the coffee silver skin and mixed with the desired solid/solvent ratio as the studied parameters shown in table 3.1 into 250 ml Erlenmeyer flask.
- 2) Immerse the solvent to the flask from 1) to ultrasonic probe system machine and set the frequency and extraction time as shown in table 3.1
- 3) Phenolic compound extract is obtained in the liquid part by filtering
- 4) Take the sample in the dark color sample bottle and store in refrigerator at temperature of 20  $^{\circ}\text{C}$ .

Table 3.1 Studied parameters of phenolic extraction from coffee silver skin.

Parameter	Condition
Solid/liquid ratio (g CS/v solvent)	1/5, 1/30, 1/50, 1/70
Solvent type (%v ethanol/v water)	100/0, 75/25, 50/50, 25/75, 0/100
Frequency of ultrasonic (kHz)	28, 40
Extraction temperature (°C)	40, 50, 60
Time of extraction (min)	5, 10, 15, 30, 60, 90

### 3.3 Determination of Total Phenolic Compounds and Antioxidants

#### 3.3.1 Determination of Total Phenolic Compounds

##### Equipment

- 1) Test tube 15 ml
- 2) Micro pipes size 100 - 1000  $\mu$ l and size 1-5 ml.
- 3) Vortex Mixer
- 4) Centrifuge
- 5) UV-VIS Spectrophotometer

##### Chemicals

- 1) Extract samples from CS
- 2) Gallic acid
- 3) 2N Folin-Ciocalteu reagent
- 4) Sodium carbonate solution (7.5% by w/v)
- 5) Solvent (99.9% pure ethanol or distilled water)

##### Procedure

- 1) Pipette a sample of 0.5 ml into 0.5 ml of solvent (same solvent used in extraction) in a test tube.
- 2) Pipette 4.5 ml distilled water, 0.5 ml 2N Folin-Ciocalteu reagent and sodium carbonate solution (7.5% by weight / volume) and mixed.
- 3) Shake each test tube in a voltex mixer for 30 second.
- 4) Leave the test tube in the dark at room temperature for 1 hr.
- 5) Put the test tube in the centrifuge for 5 minutes.
- 6) Measure the absorbance of the sample using UV-VIS Spectrophotometer at 765 nm.
- 7) Compare the absorbance values of sample with the standard gallic acid calibration curve.

### 3.3.2 Determination of antioxidant content (DPPH assay)

#### Equipment

- 1) Test tube 15 ml
- 2) Micro Pipette size 1-5 ml.
- 3) Vortex Mixer
- 4) UV-VIS Spectrophotometer

#### Chemicals

- 1) Sample from 3.2: extract from CS
- 2) Trolox standard
- 3) DPPH (2,2-diphenyl-1-picrylhydrazyl) 4.82  $\mu\text{M}$
- 4) Solvent (99.9% pure ethanol, distilled water)

#### Procedure

- 1) Dilute the sample to 0.1% 0.08% 0.06% 0.02% using solvent.
- 2) Pipettes 2 ml of diluent and DPPH 2 ml.
- 3) Shake each test in a voltex mixer for 30 second.
- 4) Leave the test tube in the dark at room temperature for 30 minutes.
- 5) Measure the absorbance of the sample using UV-VIS Spectrophotometer at 517 nm.
- 6) Calculate the percent of radical scavenging activity relative to the standard log which express in equation (3.1)

$$\text{Antioxidant Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (3.1)$$

Where  $A_{\text{control}}$  is the absorbance of DPPH radical + methanol.

$A_{\text{sample}}$  is the absorbance of DPPH radical + sample extract.

- 7) Calculate the concentration of the extract that decreases the amount of DPPH by 50% (IC<sub>50</sub>) from the graph of the percent antioxidants and the concentration of the extract.

## CHAPTER IV

### RESULTS AND DISCUSSION

The influence of important parameters; frequency of extraction, sample to solvent ratio, and solvent type were studied to find the proper condition of total phenolic compounds using ultrasonic-assisted extraction (UAE). The DPPH assay and TPC method were used to analyze.

#### 4.1 The effect of frequency

Ultrasound-assisted extraction technique use ultrasound wave (frequency) to generate the micro-bubbles as a result of compression resulting in cavitation. In this study, the frequency of 28 kHz and 40 kHz were compared for suitable condition selection and other conditions were fixed (temperature of 60 °C, time of 60 min, solid/solvent ratio of 1g/50 ml, and 75% v ethanol / 25% v water).

From figure 3.1, It was shown that the antioxidant activity of the sample in 28 kHz and 40 kHz in the term of IC<sub>50</sub> are 0.0389 and 0.0443, respectively. The total phenolic content of 28 kHz and 40 kHz are 7.8854 mgGAE/gCS and 7.6236 mgGAE/gCS, respectively. According to this results, it was found that the extraction of TPC using 28 kHz gave slightly higher amount of TPC compared to 40 kHz. In accordance with the results of IC<sub>50</sub>, the antioxidant activity of the sample in 28 kHz result in low value of IC<sub>50</sub>, it means that the concentration of antioxidant, which reduce the free radical to 50% is 0.0389 (use only 0.0389 to reduce free radical). Therefore, the condition 28 kHz produce slightly higher antioxidants than 40 kHz and was selected for further experiment.

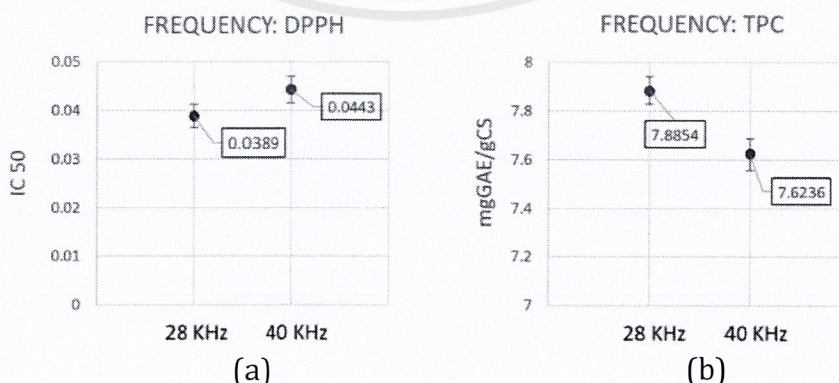


Figure 4.1. The effect of frequency on antioxidant extraction from CS at T = 60 °C, time = 60 min, solid/solvent ratio = 1g/50 ml, 75% v ethanol / 25% v water a) IC<sub>50</sub>, b) Total phenolic compounds

#### 4.2 The effect of sample to solvent ratio

Sample to solvent ratios of 1/5, 1/30, 1/50, 1/70 (in unit g/ml) were investigated in this study at the fix condition of 60°C, 60 min, 75/25 % v ethanol/v water, and 28 kHz

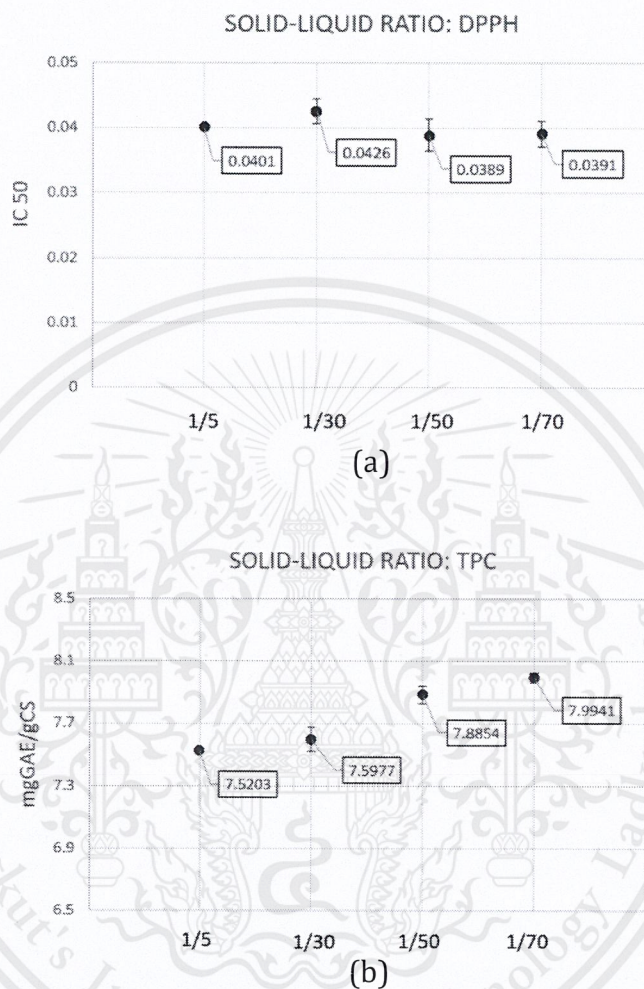


Figure 4.2. The effect of sample to solvent ratio on antioxidant extraction from CS at T = 60°C, time = 60 min, 75% v ethanol / 25% v water, 28 kHz

a) IC<sub>50</sub>, b) Total phenolic compounds

From the results in figure 4.2 showed that the value of IC<sub>50</sub> of 1/5, 1/30, 1/50, 1/70 were 0.0401, 0.0426, 0.0389 and 0.0391, respectively. All results gave the similar value and close to 0.04. The value of total phenolic content was slightly increased when adding more solvent and 1/70 g/ml gave the highest value of 7.9941 mgGAE/gCS, which similar to Pinelo's results.

Pinelo et al.,2005 [24] reported that when the amount of solvent was increased, more phenolic compounds are extracted and rise to stabilize value when adding solid / liquid ratio to the saturated point. They studied in the range of 1/5 to 1/50

The study of sample to solvent ratio in this work found that increasing the amount of solvent results in more phenolic compounds being extracted. The presence of more solvent gave higher interaction surface which help in mass transfer. However, the 1/30 g/ml was selected because the value of IC50 and TPC do not have a significant increase when using more solvent. Therefore, we chose the lowest amount to save cost and in consider the separation at the end of process.

#### 4.3 The effect of solvent type

This study, the mixture of ethanol and water was used (100/0, 75/25, 50/50, 25/75, 0/100 %v/v ethanol/water). The other condition like temperature, time, frequency and solid/liquid ratio were fixed at 60°C, 60 min, 28 kHz, and 1 g CS /30 ml solvent.

From figure 4.3, it was shown that the value of IC50 of 100/0, 75/25, 50/50, 25/75, 0/100 %v/v ethanol/water were 0.0967, 0.0426, 0.1166, 0.1428, and 0.1820, respectively. The value of TPC of 100/0, 75/25, 50/50, 25/75, 0/100 %v/v ethanol/water were 2.7520, 7.5977, 9.1531, 7.4473, and 5.5322 mgGAE/gCS, respectively. According to this results, the solvent type giving highest antioxidant is 75/25 %v/v ethanol/water that against with Cvitanovic's [26] and Costa's [27] report. All of them report in same way that the equimolar (50 v/50 v) mixture of ethanol and water is the most appropriate solvent for phenolic compound extraction at the condition of 60°C, 60 min, which relate to the results in the term of TPC (50/50 %v/v ethanol /water is the best solvent type and give 9.1531 mgGAE/gCS).

The inconsistency of results may be caused by different coffee species, coffee species Costa's report is 40%Robusta and 60% Arabica but this study use pure Robusta. In addition, the dielectric constant for water is 78.5, while the constant for ethanol is 24.3, thus water is more polarity than ethanol.

However, the 50/50 %v/v ethanol /water was selected because the value of TPC will be used in kinetics part for further experiment.

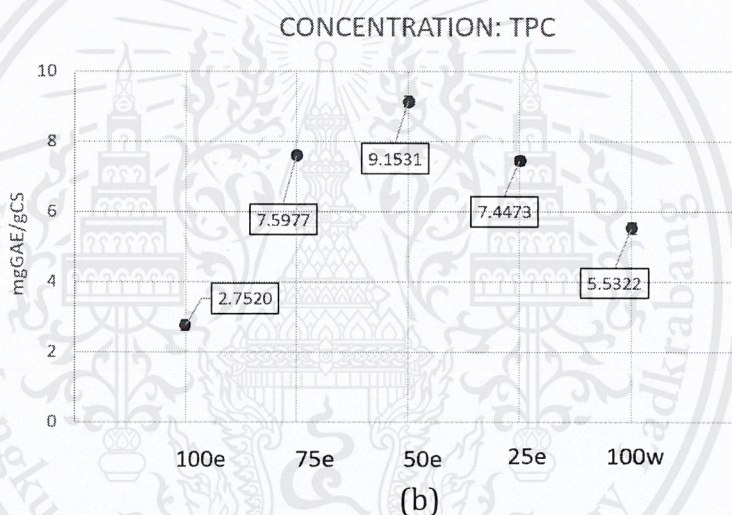
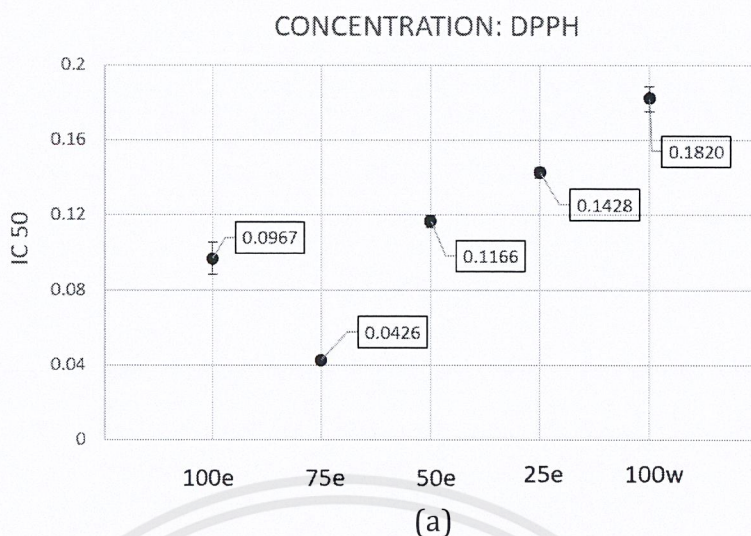


Figure 4.3. The effect of solvent type on antioxidant extraction from CS at  $T = 60\text{ }^{\circ}\text{C}$ , time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml  
a) IC<sub>50</sub>, b) Total phenolic compounds

For kinetics study, the peleg's law, second-order rate law, and two-site kinetic model were used to describe the kinetics for extraction of phenolic antioxidants from coffee silver skin. In the part of important parameters study, it was conclude that the suitable condition for phenolic extraction is 28 kHz, solid/solvent ratio of 1g/30 ml and 50/50 %v/v ethanol/water, which gave the TPC of 9.1531 mgGAE/gCS. The value of TPC in kinetics study part should be like that value but it was decrease because of the degradation of CS which stored in the long time.

#### 4.4 Peleg's model

The Peleg's model is empirical form which used to describe the extraction of bioactive compound in many research. The study also use this model to predict the phenolic extraction and vary three different temperatures (40°C, 60°C and 80°C).

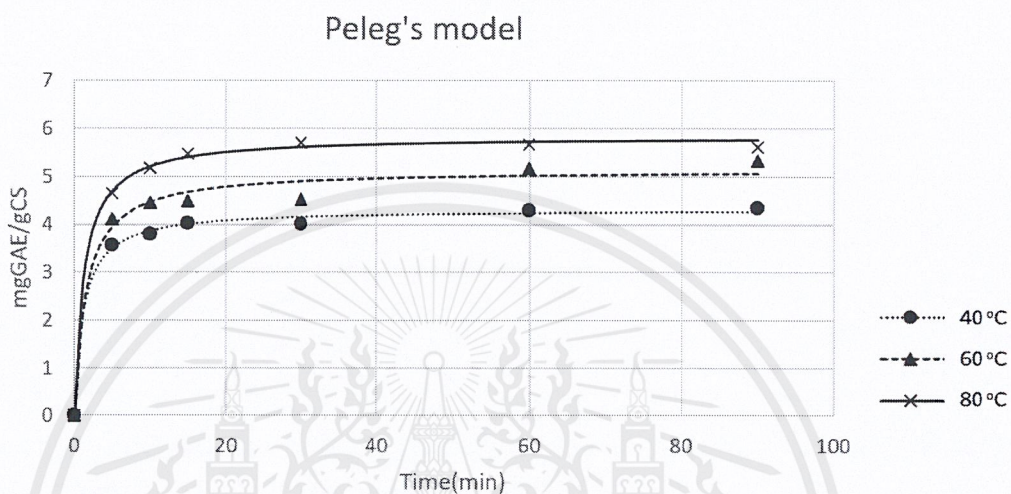


Figure 4.4. Experimental and predicted values of total phenolic content at different temperature using Peleg's model.

From the results in figure 4.4, it was shown that the temperature of 80°C gave highest total phenolic compound (5.621 mgGAE/gCS), followed by the temperature of 60°C and 40 °C at any same time. The increasing temperature can extract more phenolic compound due to it affect to the diffusion and solubility of solvent. The values from experiment is quite close to the Peleg's model values.

Table 4.1 Peleg's model for the ultrasound-assisted extraction of phenolic compounds from coffee silver skin for different extraction temperature.

Temperature (°C)	Peleg's rate constant, $k_1$ (min gCS/mgGAE)	Peleg's rate capacity, $k_2$ (gCS/mgGAE)	$B_0$ (mgGAE/gdb min)	$R^2$	RMSE
40	0.2647	0.2318	3.7779	0.9967	0.0885
60	0.2877	0.1947	3.4762	0.9849	0.2238
80	0.2058	0.1720	4.8595	0.9985	0.0809

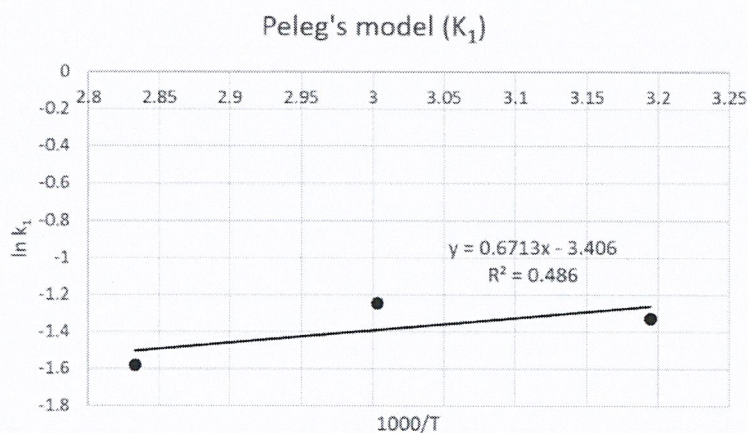


Figure 4.5. The relationship between  $\ln k_1$  and  $1000/T$  from the Arrhenius equation of Peleg's model.

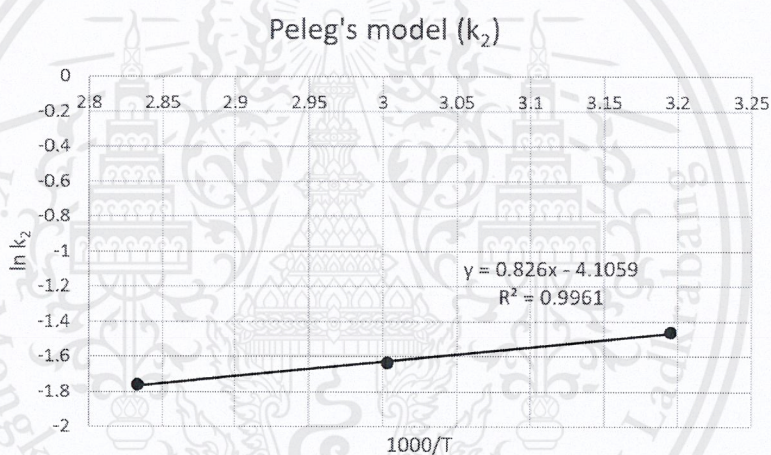


Figure 4.6. The relationship between  $\ln k_2$  and  $1000/T$  from the Arrhenius equation of Peleg's model.

From table 4.1, it was shown that the Peleg's rate constant,  $k_1$ , increased at the temperature of 40 °C to 60 °C, then, decreased to 0.2058 min gCS/mgGAE at 80 °C. In accordance with this results, it means that the extraction rate ( $B_0$ ) slightly increased with high temperature because  $B_0 = 1/k_1$ . The Peleg's rate capacity,  $k_2$ , decreased with increasing temperature, it means that high temperature produced high saturated concentration of phenolic compounds due to  $C_e = 1/k_2$ , which related to experimental result as shown in figure 4.4. Moreover, the value of  $R^2$  is in the range of 0.9849 – 0.9985. The root mean square error is in the range of 0.0809 – 0.2238.

#### 4.5 The second-order rate law

The second-order rate law is ideal for quantification of the extraction of TPC. The temperatures of 40°C, 60°C and 80°C were used to study. From the results in figure 4.7, it was shown that the experimental values is quite close to predicted values.

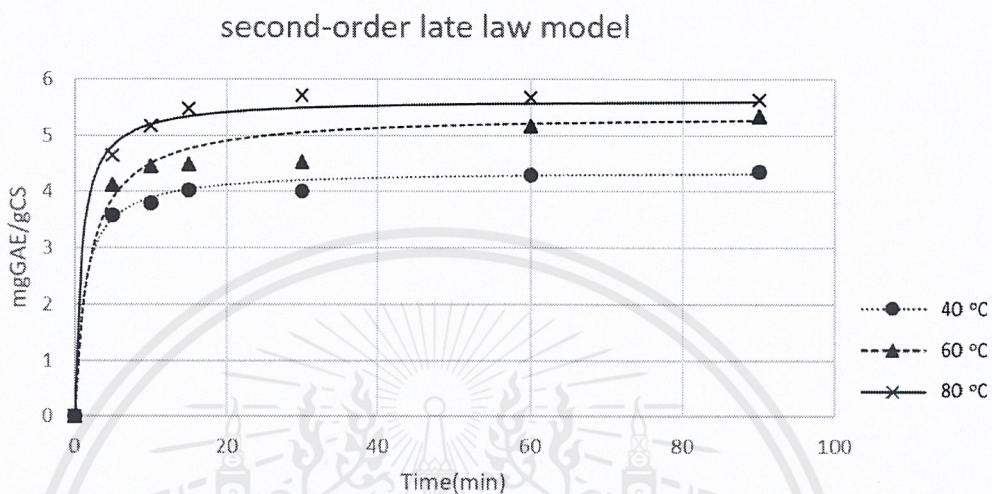


Figure 4.7. Experimental and predicted values of total phenolic content at different temperature using second-order rate law model.

Table 4.2 Second-order rate law for the ultrasound-assisted extraction of phenolic compounds from coffee silver skin for different extraction temperature.

Temperature (°C)	initial extraction rate, $h$ (g/L min)	Extraction rate constant, $k$ (L/g min)	Equilibrium concentration of phenolics, $C_e$ (g/L)	$R^2$	RMSE
40	3.5685	0.1869	4.3511	0.9965	0.0912
60	2.7642	0.09698	5.3388	0.9808	0.2524
80	6.4828	0.2052	5.6210	0.9950	0.1457

At the temperature 40 °C, the extraction rate constant ( $k$ ) was 0.1869 L/g min and then decreased to 0.09698 L/g min at the temperature of 60 °C, however, it increase to 0.2052 L/g min after temperature reach to 80 °C as shown in table 4.2. Moreover, the value of  $R^2$  is in the range of 0.9808 – 0.9965. The root mean square error is in the range of 0.0912 – 0.2524.

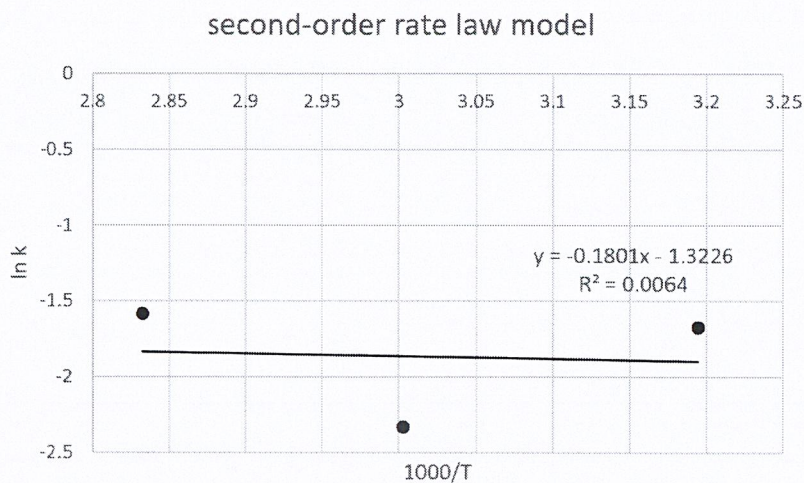


Figure 4.8. The relationship between  $\ln k$  and  $1000/T$  from the Arrhenius equation of second-order rate law

#### 4.6 Two-site kinetic model

This model assume that there are 2 step of extraction, first is the lower rate step desorption and the latter is the higher rate step desorption. This study used this model for phenolic extraction prediction and the temperatures (40°C, 60°C and 80°C).were varied. From the results in figure 4.9, it was shown that the experimental values is quite close to predicted values.

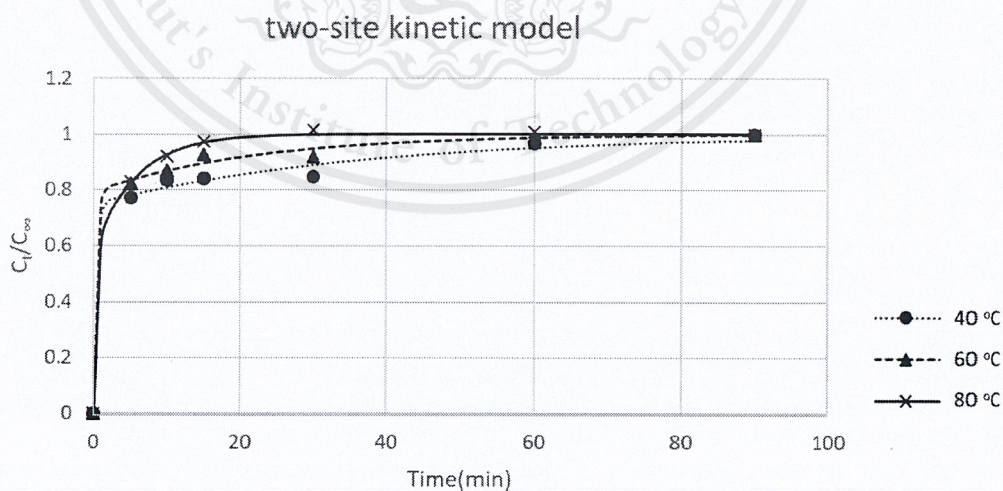


Figure 4.9 Experimental and predicted values of total phenolic content at different temperature using two-site kinetic model.

Table 4.3 Two-site kinetic model for the ultrasound-assisted extraction of phenolic compounds from coffee silver skin for different extraction temperature.

Temperature (°C)	F	1-F	Equilibrium concentration of phenolics, $M_{\infty}$ (mg/g)	First-order rate constant, $k_1$ ( $\text{min}^{-1}$ )	First-order rate constant, $k_2$ ( $\text{min}^{-1}$ )	$R^2$	RMSE
40	0.2081	0.7919	4.3511	0.0458	2.9763	0.9974	0.0181
60	0.2504	0.7496	5.3388	0.0274	2.7996	0.9955	0.0232
80	0.4166	0.5834	5.621	0.1742	2.9125	0.9996	0.0090

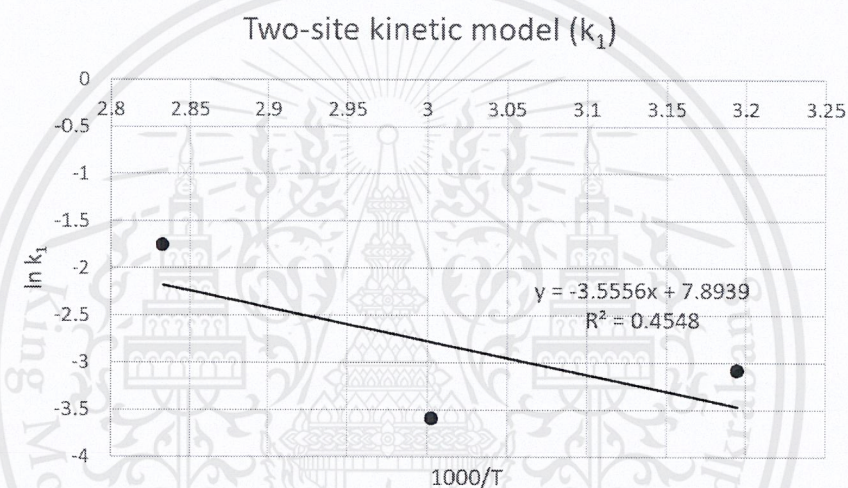


Figure 4.10. The relationship between  $\ln k_1$  and  $1000/T$  from the Arrhenius equation of two-site kinetic model.

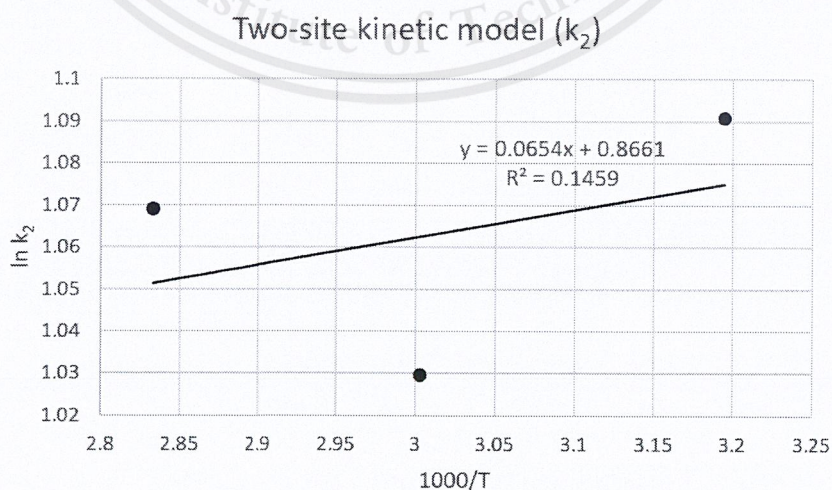


Figure 4.11. The relationship between  $\ln k_2$  and  $1000/T$  from the Arrhenius equation of two-site kinetic model.

From table 4.3,  $F$  representing the solute released rapidly (fast washing stage) is low for all temperatures. It mean that a more effective recovery of phenolics took place during the slower dissolution stage of extraction. Moreover, the value of  $R^2$  is in the range of 0.9955 – 0.9996. The root mean square error is in the range of 0.0090 – 0.0181.

Table 4.4 Kinetic variables from the Arrhenius equation for all models.

Model	Temperature-independent factor, $k_o$ ( $\text{min}^{-1}$ )	Activation energy, $E_a$ (J/mol)	$R^2$	RMSE
Peleg's law	0.000393	-5.5812	0.4860	0.1021
	7.84E-05	-6.8674	0.9961	0.0077
second-order rate law	78324927	29.5613	0.0064	0.3324
Two site kinetic law	7.34686	-0.5437	0.4548	0.5757
	0.047577	1.4974	0.1459	0.0234

The Arrhenius law was utilized to determine the two coefficients,  $E_a$  (activation energy) and  $k_o$  (temperature-independent factor). Table 4.4 showed the kinetics parameters obtained from Arrhenius equation for all three models. It showed that the Arrhenius relationship can be used only for Peleg's law model. It is not fit to the second-order rate law model and two-site kinetic model.

From the all results in kinetics study, it was concluded that the Peleg's law model is the best model for phenolic extraction due to  $R^2$  is in the range of 0.9849 – 0.9985 and RSME is in the range of 0.0809 – 0.2238.

## CHAPTER V

### CONCLUSION

#### 5.1 Summary of the results

This senior project studied the independent parameters like solvent type, sample to solvent ratio, and frequency of extraction to find the proper condition for phenolic extraction using ultrasound wave. The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was used for testing of antioxidant activity. The Folin-Ciocalteu reagent was used to find the phenolic content. In addition, the three kinetic models (Peleg's law, second-order rate law, and two-site kinetic model) have been utilized to find the information which use in the industry at a later time.

From the experimental results, it was concluded that the most suitable condition for phenolic extraction from coffee silver skin is at the frequency of 28 kHz, 1/30 solid to solvent ratio and 50% ethanol : 50% water by weight, which gave  $IC_{50} = 0.1166$ ,  $TPC = 9.1531$  mgGAE/gCS. The best kinetic model is Peleg's law model.

#### 5.2 Suggestions

5.2.1 The analysis of coffee silver skin extract to determine the specific phenolic compounds should be performed to gain more insight of the useful component

5.2.2 The coffee silver skin should keep in the dry space to avoid antioxidant degradation.

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## APPENDIX A

### SAMPLE OF CALCULATION

#### A.1 Calculation of IC50

Firstly, the samples were dilute to 5 concentration (0.1%, 0.08%, 0.06%, 0.04% and 0.02%). After that this dilute samples reacted with DPPH. If there are a plenty of antioxidant activity, the color of DPPH will chance to yellow shade. This samples were then measure the absorbance with the ultraviolet wavelength spectrophotometer at a wavelength of 517 nm and apply the measured absorbance value to calculate the antioxidant activity percentage according to the equation (A.1)

$$\text{Antioxidant activity} = \frac{(A - B) - (C - D)}{(A - B)} \times 100 \quad (\text{A.1})$$

Where (A-B) is absorbance control (DPPH radical + solvent)

(C-D) is absorbance sample (DPPH radical + sample)

**Example:** To calculate the percent of antioxidant activity of 0.1% concentration, the sample data was shown in table A.1

$$\begin{aligned} \text{Antioxidant activity} &= \frac{(0.450 - 0.039) - (0.107 - 0.057)}{(0.450 - 0.039)} \times 100 \\ &= \frac{0.411 - 0.050}{0.411} \times 100 \\ &= 87.91565 \% \end{aligned}$$

Table A.1 the absorbance value (517 nm) at condition of 28 kHz, 50% ethanol, 1/50 mg/L, 60°C and 60 min for DPPH testing.

Item	REP I	REP II	REP III	A - B
Tube A :	0.450			0.411
Tube B :	0.039			
				Avg
Tube C : 0.1%	0.106	0.107	0.107	0.107
Tube C : 0.08%	0.131	0.126	0.126	0.128
Tube C : 0.06%	0.169	0.179	0.174	0.174
Tube C : 0.04%	0.236	0.244	0.243	0.241
Tube C : 0.02%	0.344	0.332	0.339	0.338
			C-D	
Tube D : 0.1%	0.057		0.050	
Tube D : 0.08%	0.052		0.076	
Tube D : 0.06%	0.050		0.124	
Tube D : 0.04%	0.045		0.196	
Tube D : 0.02%	0.042		0.296	

After calculate the percent of antioxidant activity of five concentration as shown in table A.2, the graph between %antioxidant and log (concentration) was plotted.

Table A.2 Show %antioxidant and log (concentration)

%antioxidant	%concentration	Log (concentration)
87.91565	0.1	-1
81.58962	0.08	-1.09691
69.82968	0.06	-1.22185
52.31144	0.04	-1.39794
27.89943	0.02	-1.69897

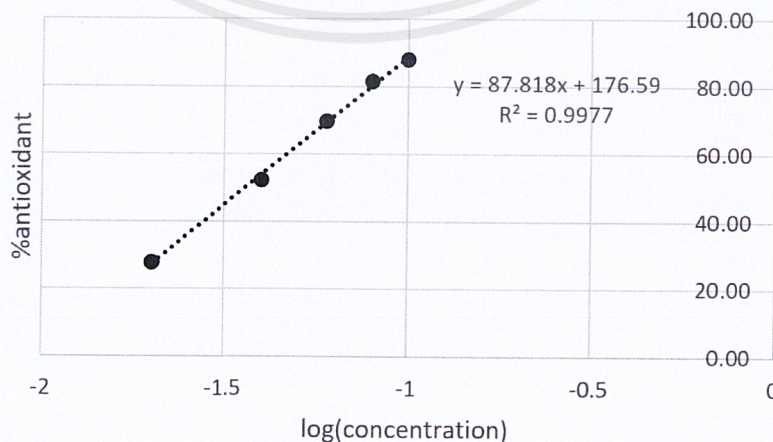


Figure A.1. the relationship between %antioxidant and log of concentration

After that, the equation from graph in figure A.1 were substituted by  $y= 50$  for finding the concentration which reduce free radical to 50%

$$y = 87.818x + 176.59$$

$$50 = 87.818x + 176.59$$

$$x = -1.4415$$

The value of  $x$  is log of concentration, so the concentration which reduce free radical to 50% is  $\log x = -1.4415 \Rightarrow x = 0.0362$

## A.2 Calculation of Total phenolic content (TPC)

After extraction part, the samples were add with Folin-Ciocalteu reagent, sodium carbonate and water. When yellow Mo (VI) ions receive electrons from the antioxidants, they turn into blue Mo (V). This samples were then measure the absorbance with the ultraviolet wavelength spectrophotometer at a wavelength of 765 nm and apply the measured absorbance value to calculate the TPC by gallic standard curve.

Example: To calculate the total phenolic compound, the sample data was shown in table A.3. Substituting the absorbance value to equation from gallic standard curve ( $y = \text{absorbance}$ ,  $x = \text{mg Gallic acid}$ ) as shown in figure A.2

$$y = 11.463x + 0.1262$$

$$1.0237 = 11.463x + 0.1262$$

$$x = 0.0783$$

the amount of gallic acid in sample, which have 0.01% concentration, is 0.0783

so this sample have TPC =  $0.0783 \text{ mgGAE}/0.01 \text{ gCS} = 7.83 \text{ mgGAE/gCS}$

Table A.3 the absorbance value (765 nm) at condition of 28 kHz, 50% ethanol, 1/50 mg/L, 60°C and 60 min for TPC testing.

% concentration	REP I	REP II	REP III	AVG
0.01	1.018	1.023	1.03	1.0237

Table A.4 show value of absorbance and amount of gallic acid

Stock (mg/ml)	Amount of gallic acid (mg) in reaction (us 1 ml)	60 min (OD 765)			Mean
		Rep I	Rep II	Rep III	
0.0125	0.0125	0.241	0.239	0.276	0.252
0.025	0.025	0.407	0.412	0.43	0.416
0.05	0.05	0.72	0.75	0.706	0.725
0.1	0.1	1.273	1.25	1.26	1.261

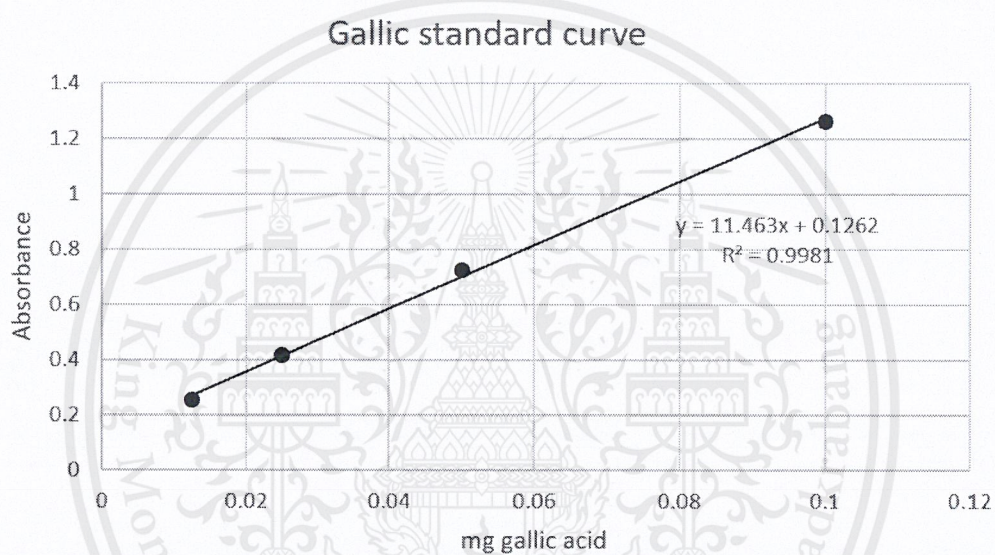


Figure A.2 gallic standard curve

**APPENDIX B**  
**EXPERIMENTAL DATA**

**B.1 the frequency**

The results from the antioxidant analysis (DPPH assay) and Total phenolic compounds method (TPC method) were shown in table B.1-B.4

Table B.1 the IC50 results at condition of at T = 60 °C, time = 60 min, solid/solvent ratio = 1g/50 ml, 75% v ethanol / 25% v water and 25 kHz.

Frequency	Rep	Concentration (%)	Tube	Mean absorbance	IC50	Mean IC50	
28 kHz	1		solvent	0.450	0.0362	0.0389	
		0.1	1	0.106			
		0.08	2	0.127			
		0.06	3	0.174			
		0.04	4	0.241			
		0.02	5	0.338			
	2			solvent	0.491		0.0396
		0.1	1	0.119			
		0.08	2	0.146			
		0.06	3	0.193			
		0.04	4	0.278			
		0.02	5	0.387			
	3			solvent	0.506		0.0409
		0.1	1	0.141			
		0.08	2	0.162			
		0.06	3	0.224			
		0.04	4	0.281			
		0.02	5	0.401			

Table B.2 the IC50 results at condition of at T = 60 °C, time = 60 min, solid/solvent ratio = 1g/50 ml, 75% v ethanol / 25% v water and 40 kHz.

Frequency	Rep	Concentration (%)	Tube	Mean absorbance	IC50	Mean IC50	
40 kHz	1		solvent	0.566	0.0435	0.0443	
		0.1	1	0.160			
		0.08	2	0.191			
		0.06	3	0.261			
		0.04	4	0.340			
		0.02	5	0.447			
	2			solvent	0.493		0.0421
		0.1	1	0.130			
		0.08	2	0.154			
		0.06	3	0.235			
		0.04	4	0.285			
		0.02	5	0.375			
	3			solvent	0.602		0.0473
		0.1	1	0.180			
		0.08	2	0.229			
		0.06	3	0.284			
		0.04	4	0.375			
		0.02	5	0.487			

Table B.3 the TPC results at condition of at T = 60 °C, time = 60 min, solid/solvent ratio = 1g/50 ml, 75% v ethanol / 25% v water and 28 kHz.

frequency	Rep	%concentration	mean absorbance	TPC	Mean TPC
28 kHz	1	0.02	0.1477	7.3835	7.8854
		0.01	0.0783	7.8282	
	2	0.02	0.1535	7.6773	
		0.01	0.0794	7.9416	
	3	0.02	0.1509	7.5449	
		0.01	0.0789	7.8863	

Table B.4 the TPC results at condition of at T = 60 °C, time = 60 min, solid/solvent ratio = 1g/50 ml, 75% v ethanol / 25% v water and 40 kHz.

frequency	Rep	%concentration	mean absorbance	TPC	Mean TPC
40 kHz	1	0.02	0.1433	7.1667	7.6236
		0.01	0.0756	7.5606	
	2	0.02	0.1409	7.0476	
		0.01	0.0769	7.6915	
	3	0.02	0.1441	7.2061	
		0.01	0.0762	7.6188	

## B.2 the solid to solvent ratio

The results from the antioxidant analysis (DPPH assay) and Total phenolic compounds method (TPC method) were shown in table B.5-B.8

Table B.5 the IC<sub>50</sub> results at condition of at T = 60 °C, time = 60 min, 75% v ethanol / 25% v water, 28 kHz and solid/solvent ratio = 1g/30 ml.

Solid to solvent ratio	Rep	Concentration (%)	Tube	Mean absorbance	IC <sub>50</sub>	Mean IC <sub>50</sub>	
1/30	1		solvent	0.566	0.0429	0.0426	
		0.1	1	0.140			
		0.08	2	0.189			
		0.06	3	0.253			
		0.04	4	0.338			
		0.02	5	0.447			
	2			solvent	0.499		0.0443
		0.1	1	0.148			
		0.08	2	0.183			
		0.06	3	0.217			
		0.04	4	0.298			
		0.02	5	0.404			
	3			solvent	0.507		0.0405
		0.1	1	0.139			
		0.08	2	0.174			
		0.06	3	0.227			
		0.04	4	0.289			
		0.02	5	0.387			

Table B.6 the IC50 results at condition of at T = 60 °C, time = 60 min, 75% v ethanol / 25% v water, 28 kHz and solid/solvent ratio = 1g/70 ml.

Solid to solvent ratio	Rep	Concentration (%)	Tube	Mean absorbance	IC50	Mean IC50	
1/70	1		solvent	0.545	0.0413	0.0391	
		0.1	1	0.141			
		0.08	2	0.183			
		0.06	3	0.238			
		0.04	4	0.323			
		0.02	5	0.421			
	2			solvent	0.537		0.0382
		0.1	1	0.129			
		0.08	2	0.163			
		0.06	3	0.217			
		0.04	4	0.298			
		0.02	5	0.403			
	3			solvent	0.539		0.0377
		0.1	1	0.130			
		0.08	2	0.158			
		0.06	3	0.217			
		0.04	4	0.295			
		0.02	5	0.406			

Table B.7 the TPC results at condition of at T = 60 °C, time = 60 min, 75% v ethanol / 25% v water, 28 kHz and solid/solvent ratio = 1g/30 ml.

Solid to solvent ratio	Rep	%concentration	mean absorbance	TPC	Mean TPC
1/30	1	0.0167	0.1204	7.2235	7.5977
		0.0083	0.0626	7.5128	
	2	0.0167	0.1195	7.1084	
		0.0083	0.0639	7.6629	
	3	0.0167	0.1199	7.1991	
		0.0083	0.0635	7.6175	

Table B.8 the TPC results at condition of at T = 60 °C, time = 60 min, 75% v ethanol / 25% v water, 28 kHz and solid/solvent ratio = 1g/70 ml.

Solid to solvent ratio	Rep	%concentration	mean absorbance	TPC	Mean TPC
1/70	1	0.0143	0.1084	7.5908	7.9941
		0.0071	0.0569	7.9751	
	2	0.0143	0.1115	7.8065	
		0.0071	0.0569	7.9751	
	3	0.0143	0.1096	7.6721	
		0.0071	0.0574	8.0321	

### B.3 the solvent type

The results from the antioxidant analysis (DPPH assay) and Total phenolic compounds method (TPC method) were shown in table B.9-B.16

Table B.9 the IC<sub>50</sub> results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 100% ethanol.

Solvent type	Rep	Concentration (%)	Tube	Mean absorbance	IC <sub>50</sub>	Mean IC <sub>50</sub>	
100% ethanol.	1		solvent	0.641	0.1067	0.0967	
		0.25	1	0.175			
		0.20	2	0.215			
		0.15	3	0.293			
		0.10	4	0.375			
		0.05	5	0.484			
	2			solvent	0.565		0.0909
		0.25	1	0.124			
		0.20	2	0.154			
		0.15	3	0.203			
		0.10	4	0.297			
		0.05	5	0.420			
	3			solvent	0.601		0.0926
		0.25	1	0.165			
		0.20	2	0.201			
		0.15	3	0.268			
		0.10	4	0.345			
		0.05	5	0.447			

Table B.10 the IC50 results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 50% v ethanol/50% v water.

Solvent type	Rep	Concentration (%)	Tube	Mean absorbance	IC50	Mean IC50	
50/50 %v/v ethanol/water	1		solvent	0.552	0.1145	0.1166	
		0.25	1	0.164			
		0.20	2	0.230			
		0.15	3	0.282			
		0.10	4	0.339			
		0.05	5	0.432			
	2			solvent	0.565		0.1153
		0.25	1	0.172			
		0.20	2	0.245			
		0.15	3	0.274			
		0.10	4	0.352			
		0.05	5	0.467			
	3			solvent	0.557		0.1200
		0.25	1	0.168			
		0.20	2	0.241			
		0.15	3	0.292			
		0.10	4	0.345			
		0.05	5	0.441			

Table B.11 the IC50 results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 25%v ethanol/ 75%v water.

Solvent type	Rep	Concentration (%)	Tube	Mean absorbance	IC50	Mean IC50	
25/75 %v/v ethanol/water	1		solvent	0.549	0.1427	0.1428	
		0.50	1	0.119			
		0.40	2	0.128			
		0.30	3	0.172			
		0.20	4	0.253			
		0.10	5	0.369			
	2			solvent	0.540		0.1456
		0.50	1	0.109			
		0.40	2	0.119			
		0.30	3	0.165			
		0.20	4	0.247			
		0.10	5	0.356			
	3			solvent	0.565		0.1402
		0.50	1	0.127			
		0.40	2	0.147			
		0.30	3	0.202			
		0.20	4	0.275			
		0.10	5	0.402			

Table B.12 the IC50 results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 100% water.

Solvent type	Rep	Concentration (%)	Tube	Mean absorbance	IC50	Mean IC50	
100% water	1		solvent	0.561	0.1814	0.1820	
		0.50	1	0.125			
		0.40	2	0.161			
		0.30	3	0.234			
		0.20	4	0.304			
		0.10	5	0.416			
	2			solvent	0.554		0.1890
		0.50	1	0.117			
		0.40	2	0.152			
		0.30	3	0.223			
		0.20	4	0.289			
		0.10	5	0.396			
	3			solvent	0.559		0.1820
		0.50	1	0.120			
		0.40	2	0.157			
		0.30	3	0.228			
		0.20	4	0.295			
		0.10	5	0.402			

Table B.13 the TPC results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 100% ethanol.

Solvent type	Rep	%concentration	mean absorbance	TPC	Mean TPC
100% ethanol	1	0.0167	0.6567	2.7758	2.7519
		0.0083	0.3590	2.4352	
	2	0.0167	0.6211	2.5897	
		0.0083	0.0342	2.2475	
	3	0.0167	0.6786	2.8905	
		0.0083	0.0358	2.4862	

Table B.14 the TPC results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 50%v ethanol/ 50%v water.

Solvent type	Rep	%concentration	mean absorbance	TPC	Mean TPC
50/50 %v/v ethanol/water	1	0.0167	1.7743	8.6264	9.1631
		0.0083	0.9997	9.1426	
	2	0.0167	1.7526	8.5281	
		0.0083	0.9856	9.0023	
	3	0.0167	1.7974	8.8956	
		0.0083	1.145	9.3142	

Table B.15 the TPC results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 25%v ethanol/ 75%v water.

Solvent type	Rep	%concentration	mean absorbance	TPC	Mean TPC
25/75 %v/v ethanol/water	1	0.0167	1.4873	7.1241	7.5977
		0.0083	0.8387	7.4569	
	2	0.0167	1.4843	7.1083	
		0.0083	0.8583	7.6628	
	3	0.0167	0.1502	7.1990	
		0.0083	0.8540	7.6175	

Table B.16 the TPC results at condition of at T = 60 °C, time = 60 min, 28 kHz, solid/solvent ratio = 1g/30 ml and 100% water.

Solvent type	Rep	%concentration	mean absorbance	TPC	Mean TPC
25/75 %v/v ethanol/water	1	0.0167	1.1830	5.5309	5.5322
		0.0083	0.5420	5.2060	
	2	0.0167	1.0291	5.3672	
		0.0083	0.5023	5.1482	
	3	0.0167	1.2875	5.6984	
		0.0083	0.5869	5.4791	

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