EXTRACTION OF CURCUMIN FROM TURMERIC VIA AQUEOUS TWO-PHASES SYSTEM

By

MISS Pinutta KASEMWATTANAROT

A Thesis Submitted in Partial Fulfillment of the Requirements

for Master of Engineering (CHEMICAL ENGINEERING)

Department of CHEMICAL ENGINEERING

Graduate School, Silpakorn University

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Field of Study	(CHEMICAL ENGINEERING)
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MISS PINUTTA KASEMWATTANAROT : EXTRACTION OF CURCUMIN FROM TURMERIC VIA AQUEOUS TWO-PHASES SYSTEM THESIS ADVISOR : ASSOCIATE PROFESSOR PRAKORN RAMAKUL, D.Eng.

Turmeric is widely distributed in East and South East Asia. Turmeric is currently consumed heavily in Asian countries in the of 200-1000 mg/day due to potential therapeutic applications resulting in the extraction of important bioactive compounds such as curcumin has attracted much attention. Curcumin is a natural polyphenol compound found in turmeric rhizomes, which is frequently used in the food and pharmaceutical industries. However, curcumin's limitations greatly affect extraction efficiency: not stable to visible light, degrades at high temperatures, and poor water solubility, and decomposes very quickly in aqueous solutions at alkaline pH, resulting in the choice of extraction method important to maximum extraction yield. Curcumin was separated successfully from turmeric rhizomes using a new and easy-to-industrialized extraction method called "Aqueous two-phase system (ATPS)". In this work, the effect of ethanol concentration, ammonium sulfate concentration, solvent to solid ratio, and extraction temperature were stuied via ATPS consisting of ammonium sulfate/ethanol/water. The extracted curcumin samples was confirmed of curcumin was carried out by UV-vis spectroscopy and High-performance liquid chromatography (HPLC). An experimental design and response surface method (RSM) were utilized to determine the optimal values and attain maximum extraction yield.

The optimum extraction conditions were 15% ethanol concentration, 30.697% $(NH_4)_2SO_4$ concentration, a solid to solvent ratio of 5:100, extraction temperature of 55 °C, and extraction time of 140 min which extraction yield of curcumin was accumulated in the upper phase of 93.96% or 98.370 mg/g of turmeric. In addition, equilibrium and kinetic extraction characteristics were studied and found that the pseudo-second order kinetic model correlates well with the experimental data.

มหาวิทยาลัยศิลปากร

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CHAPTER I

INTRODUCTION

1.1 Background of the study

Turmeric is a biennial plant in the ginger family. There is an underground rhizome; the flesh of the rhizome is yellow with a unique aroma. Turmeric is extensively disseminated across East and South-East Asia. Turmeric is currently consumed in Asian countries in the of 200-1000 mg/day or 160-440 g/person/year [1]. Moreover, as of 2018, the dietary supplements containing turmeric supplements attracted some of the highest sales in the United States [2]. The most important bioactive ingredients in turmeric are curcuminoids, which containing curcumin (diferuloylmethane, $C_{21}H_{20}O_6$) 77%, demethoxycurcumin 17%, and bisdemethoxycurcumin 3% [3, 4]. In addition, sugar and essential oils were found, accounting for 0.1 to 2 % of the total dry weight [5]. Turmeric should not be harvested when the turmeric begins to sprout, because low among curcumin. The age of harvested turmeric is 9-12 months and must not be stored too long, important not to be exposed to sunlight. Due to its unique properties, turmeric has been used extensively from ancient times to the present, often used fresh or extracted for use functional food, preservative, dietary supplement ingredient, and cosmetic ingredient such as moisturizers, soaps, etc. In addition, the consumption of turmeric containing curcumin is claimed to alleviate numerous health problems [6] and it is known that curcumin is safe for humans to take at doses as high as 8 g/day, and in the case of animal research 12 g/day, which refers to very low toxicity [4].

Curcumin can be exploited as an antioxidant, antiviral, anti-HIV agent, antimicrobial or antibacterial agent, anti-aging and wrinkles, healthy skin, eliminate toxins from the body by reducing cholesterol, reducing hyperlipidemia and insulin

resistance, treatment of Alzheimer's disease (AD) and Parkinson's disease (PD) in the aged population, anti-inflammatory, inhibits the release of inflammatory cytokines that cause pneumonia, inhibits the pro-inflammatory mediators in osteoarthritis (OA) and rheumatoid arthritis (RA), treatment of psoriasis, dermatitis, nourishes the lungs, treatment of liver diseases by decreasing liver damage and fibrosis. Thus, curcumin is used to treat gastric ulcers, relieve pain and reduce joint inflammation, instead of non-steroidal anti-inflammatory drugs (NSAIDs). Curcumin can protect against complications in patients with chronic diseases such as colon cancer, breast cancer, diabetes, obesity, cardiovascular, pulmonary, neurological, autoimmune diseases, and other chronic disorders [5, 7-13]. Turmeric's properties are enormous, but its characteristic smell is strong both during and after consumption Including being absorbed into the body of curcuminoids is low in quantity. Since curcuminoids are well fat-soluble, it has to be consumed in large quantities to have real therapeutic benefits. Therefore, many methods have been invented to make it easier to consume and to improve the efficiency of absorption into the body.

Curcumin was first extracted in 1815 [14]. After that, the development of curcuminoid extraction methods is many occurrences, such as microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), supercritical fluid (SCF), supercritical carbon dioxide extraction, subcritical water extraction (SWE), aqueous two-phase system (ATPS), Soxhlet extraction and high-efficient column chromatographic extraction. However, each type of extraction has different advantages and disadvantages. The limitations in the curcumin extraction process are not stable to visible light [15, 16], degrades at high temperatures [5], and poor water solubility and decomposes relatively quickly in aqueous solutions at alkaline pH [17]. Therefore, the least impacted technique that affects to curcumin structure should be chosen and green sustainable technologies were emphasized.

An interesting process for extracting curcumin from turmeric is an aqueous two-phase system (ATPS). ATPS are biphasic systems that can be generated by mixing components in water until when the concentration of two hydrophilic polymers up to a certain value, the insoluble phenomenon will be observed. In 1956, Albertsson discovered ATPS for the first time, experimenting with chlorophyll extraction [18]. Due to the unique advantages of ATPS, research about ATPS sprang up very much later. In recent research, numerous investigations regarding ATPS containing short-chain alcohol and salt, because it has substantial advantages: lower cost, benign extraction conditions, quicker recovery of alcohol, and simple scale-up.

The response surface method (RSM) is a method of optimizing the response (output variable) by helps to find the optimum conditions of the process with decreased experimental trials, highlight the interactions between independent variables (input variables), reduce the experiment duration, and reduce the cost of analysis methods. The main experimental designs utilized in response surface methodology are the Box-Behnken design (BBD) and the central composite design (CCD). The experiments were designed using MINITAB software version 19 to create the CCD concept matrix, to achieve a better evaluation of the independent parameters, analysis of variance (ANOVA), and process optimization.

In this work, the important point is a selection of aqueous two-phase system (ATPS) techniques for extraction and the use of the MINITAB19 program to the central composite design (CCD) for experimentation to acquire the best extraction.

1.2 Objective of the study

The objectives of this study were to evaluate the parameters that affect the solvent extraction of curcuminoid from turmeric utilizing the important point is a selection of aqueous two-phase system (ATPS) techniques for extraction and the use of the MINITAB19 program to design an experiment to obtain the optimum extraction.

1.3 Scope of the study

The scopes of this study include the following topic:

1. Extraction of curcumin from turmeric.

2. Optimization employing Central composite design in response surface methodology to evaluate the influence of the extraction such as ethanol concentration, $(NH_4)_2SO_4$ concentration, solvent to solid ratio, and extraction temperature at various values as shown in **Table 1**.

3. Study the kinetic model for extraction of curcumin from turmeric utilizing non-toxic solvent extraction.

4. Analysis of curcumin extracted utilizing ultraviolet-visible spectrophotometry (UV-Vis) and high-performance liquid chromatography (HPLC).

Table 1 *Scope of the research.*

Factors	Range
Ethanol concentration (%)	15 - 23
$(NH_4)_2SO_4$ concentration (%)	25 – 37
Solid to solvent ratio (g/ml)	3:100 – 5:100
Extraction temperature (°C)	35 - 55

5. Determination of antioxidant activity utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

CHAPTER II

THEORY

2.1 Properties of curcumin

Curcumin is a natural polyphenolic compound, which has a crystal structure has a surface tension of roughly 54 mN/m and a molecular weight of 368.37 Da. Curcumin is present in the yellow spice turmeric (Curcuma longa L) [4]. Curcumin properties under environmental conditions; density of 1.3 g/cm^3, a refractive index of 1.643, the water solubility of curcumin at acidic and neutral conditions is 11 ng/ml, a melting point of 183 ºC, and boiling point of 521 ºC. Usually, in food manufacturing, high temperature is not used. Curcumin dissolves in oil or water phase below 100 ºC [19]. The most important bioactive ingredients in turmeric are curcuminoids, which curcumin [diferuloylmethane; 1,7-bis(4-hydroxy-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione], demethoxycurcumin, and bisdemethoxycurcumin. Although there is research to suggest that curcumin was the most effective in the group, bisdemethoxycurcumin might be equally effective; more importantly, it is the combination of the three analogs that actually exerts the best action [20]. The chemical structure of curcuminoids is shown in **Figure 1** [21] and the composition of turmeric and three main curcuminoids is shown in **Table 2** [1].

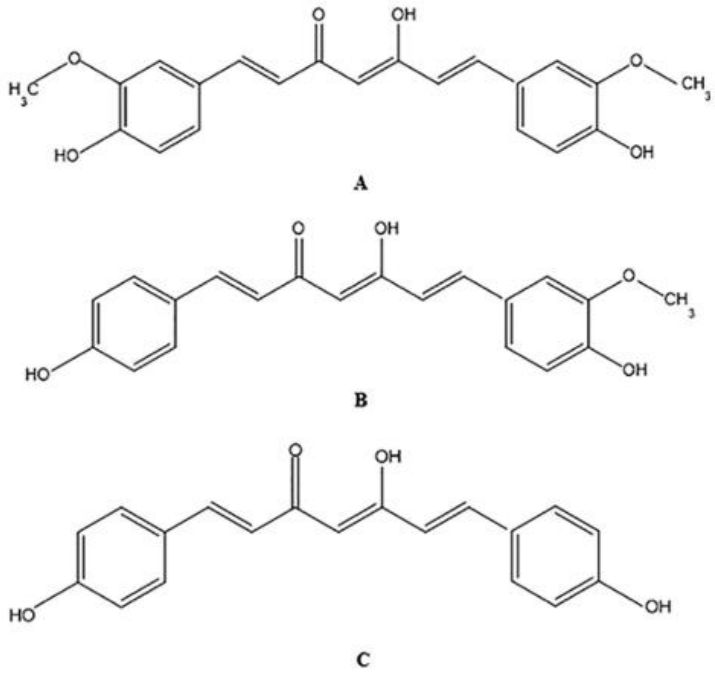

Figure 1 *The chemical structure of (A) curcumin, (B) demethoxycurcumin, and (C) bisdemethoxycurcumin.*

From the possibility of tautomerism, there are two potential structures of curcumin in an aqueous solution; the β-diketone and keto-enol tautomers as shown in **Figure 2** [4]. The diketone tautomer dominates in the acidic aqueous environment, and the enolic form dominates in an alkaline aqueous environment (pH > 8). However, curcumin is chemically stable in acidic circumstances than neutral or alkaline environments [17]. Contrary facts, at alkaline pH (pH> 8) the phenolate ions formed by deprotonation of the phenol group of curcumin (the acidic phenol group in curcumin gives its hydrogen) resulting in curcumin being readily soluble in water, but curcumin is not stable at neutral and alkaline pH, where it rapidly degrades to the molecules such as dicyclopentadiene, vanillin, and ferulic acid, yet the bioactivity of these chemicals are less than curcumin [4]. Overall, curcumin crystallizes at acidic pH values and may chemically be destroyed in neutral and alkaline pH values [17]. Other investigations demonstrate that the chemical stability of curcumin is temperature-dependent; curcumin is heat-stable up to 70 ºC for 10

minutes. Curcumin begins degrading when the temperature is over 70 ºC, and 20 min of boiling caused 32% partial loss of curcumin [22].

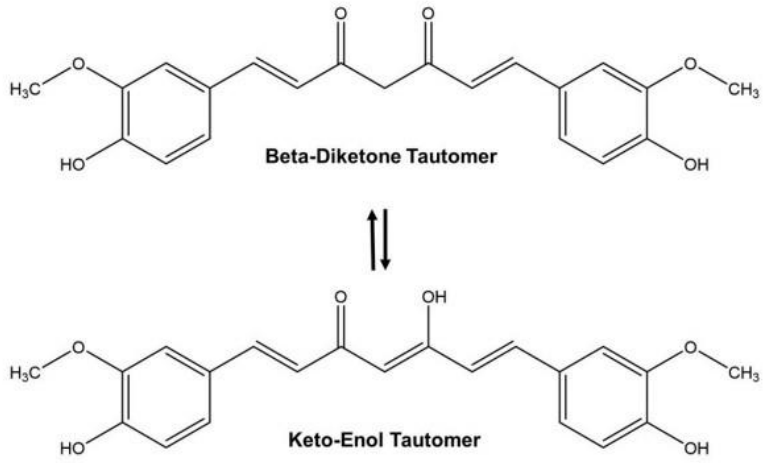

Figure 2 *Potential structures of curcumin in an aqueous solution.*

Table 2 *Composition of turmeric and three major curcuminoids.*

Turmeric constituents %(w/w)		Curcuminoids (%)	
Curcuminoids	1 - 6	Curcumin	60 – 70
Essential oils (TEO)	3 – 7	Demethoxycurcumin	20 – 27
Fiber	2 – 7	Bismethoxycurcumin	10 – 15
Minerals	3 – 7		
Fat	5 – 10	Turmerosaccharide	10
Protein	6 – 8		
Carbohydrate	60 – 70		
Moisture	6 - 13		

TEO, turmeric essential oil.

Curcumin was shown to be the most active component extracted from turmeric; however, curcumin is unstable in the presence of oxygen, highly hydrophobic, very poor bioavailability, not stable to visible light, degrades at high temperatures, and poor water solubility, and decomposes very quickly in aqueous solutions at alkaline pH.

2.2 Benefits of curcumin

2.2.1 Role of curcumin in the human body

Overall bioavailability: Studies have indicated that when large amounts of curcumin (8 g) were provided to human volunteers, the concentration of free curcumin in the plasma was very low, reaching only 41 ng/mL after six hours [19]. It was shown that the bioavailability of curcumin affects the efficacy that occurs in the body. According to The Nutraceutical Bioavailability Classification Scheme (NuBACS), factors affecting the overall bioavailability (BA) of bioactive compounds (e.g., curcumin) can be divided into absorption (A*); biological accessibility (B*); transformation (T*); distribution (D*); and excretion (E*). Therefore, the overall bioavailability (BA) of curcumin may be determined by the following formula [23]:

$$BA = B^* \times A^* \times T^* \times D^* \times E^* \quad (1)$$

Where BA: The fractions of bioactive compounds in active forms that are ingested reach the site of action within the human body (e.g., heart, brain, and lung).

B*: The fractions of the amount of bioactive compound initially ingested, which is released and solubilized within the gastrointestinal fluid.

A*: The fraction of bioactive compounds that absorbed by enterocyte cells.

T*: The fractions of the active substance in a biologically active form at the site of action.

D*: The fractions of the bioactive substances that distributed amongst the various tissues, after that absorbed to the site-of-action (e.g., brain or heart).

E*: Excretion processes (breath, sweat, urine, and feces)

According to NuBACS, the symbol '(+)' for each bioactive in each step cannot reduce the bioavailability by more than 25%; whereas, the symbol '(–)' signifies that this step can significantly reduce the bioavailability of bioactive compounds (i.e., more than 25%).

Each category is classified into sub-categories such as sub-categories for bio-accessibility: inadequate liberation from the original food matrix (L), poor solubilization in the gastrointestinal fluids (S), and interactions that promote bioactive insolubility (I). Transformation-driven limited bioavailability can be separated into the two sub-categories of chemical (C) degradation limited and metabolic (M) degradation limited processes. According to the Biopharmaceutical Classification Scheme (BSC), curcumin has low solubility in gastrointestinal fluids but it has significant permeability across epithelium cells, resulting in the NuBACS characteristics of curcumin present within only powdered turmeric would be B*(–) L, S A*(+) T*(–) C, M. Therefore, to build a delivery system with characteristics B * (+) A * (+) T * (+) is to increase curcumin's bioavailability.

Diffusion Coefficient: Diffusion of curcumin inside the gastrointestinal tract is related to its release properties, stability, and absorption. The diffusion coefficient of the molecule can be determined using the following expression:

$$D = kT/(6\pi\eta r) \quad (2)$$

Where k: Boltzmann's constant

T: The absolute temperature (°K)

η: The shear viscosity of the solvent (mPa.s)

r: The hydrodynamic radius of the molecule, for curcumin is 4.3×10^{-10} m.

It is determined that the diffusion coefficient of curcumin depends on the medium (oil or water). From the chemical stability; that curcumin is dissolved in oil but decomposes swiftly when dissolved in water. As a result, over time part of the curcumin may diffuse out of the oil droplets and into the surrounding aqueous phase as a result of the concentration gradient, which encourages additional deterioration.

2.2.2 Safety and toxicity evaluation of turmeric/curcumin

Currently, curcumin is increasingly being used as a treatment. Although curcumin is generally known to be a safe substance, its use must also be studied for some other toxic effects. An examination of the safety and toxicity of turmeric/curcumin in human double-blind, randomized, placebo-controlled clinical trials are shown in **Table 3** [24]. According to the table data, it was revealed that turmeric and curcumin in the form of standardized powder and extract are harmless in humans, and furthermore, bioavailable formulations such as nano-formulations where safe however studies on these formulations are few and additional studies are needed.

Table 3 *Safety and toxicity of turmeric/curcumin in human double-blind, randomized, placebo controlled clinical trials.*

Patient/healthy	*Duration and route of administration, curcumin/turmeric dose*	*Toxicity*	*Reference*
Patients with ulcerative colitis	*6 months orally 2 g/day curcumin*	No major toxicity but *flatulence was observed*	*Hanai et al., 2006*
Patients with Alzheimer's disease	*6 months orally 1 or 4 g/day curcumin*	*No toxicity*	*Baum et al., 2008*
Patients with Alzheimer's disease	*24 weeks orally 0.2 or 4 g/day curcumin*	*No toxicity*	*Ringman et al., 2012*
Patients after laparascopic cholecystectomy surgery	*3 weeks orally 2 g/day curcumin*	*No toxicity*	*Agarwal et al., 2011*
Patients with osteoarthritis	*3 weeks orally 1 g/day curcumin*	*No toxicity*	Pinsornsak & Niempoog, *2012*

Table 3 (cont.) *Safety and toxicity of turmeric/curcumin in human double-blind, randomized, placebo controlled clinical trials.*

Patient/healthy	*Duration and route of administration, curcumin/turmeric dose*	*Toxicity*	*Reference*
Patients with anxiety and fatigue	*30 days orally 1,000 mg/day curcumin (bioavailable formulation)*	*No toxicity*	Pandaran Sudheeran *et al., 2016*
Healthy women	3 cycles of premenstrual syndrome (each cycle lasted 10 days) orally *200 mg/day curcumin*	*No toxicity*	*Fanaei et al., 2016*
Patients with leukoplakia	*6 months orally 3.6 g/day curcumin*	*No toxicity*	*Kuriakose et al., 2016*
Patients with leukoplakia	*6 months orally 3.6 g/day curcumin*	*No toxicity*	*Kuriakose et al., 2016*
Patients with osteoarthritis	*8 weeks orally 180 mg/day curcumin*	No major toxicity was reported but *hypertension, tachycardia, and redness of tongue were reported*	*Nakagawa et al., 2014*
Patients with osteoarthritis	*6 weeks orally 1,500 mg/day curcumin*	*No toxicity*	*Rahimnia et al., 2015; Panahi, Rahimnia, et al., 2014*

Table 3 (cont.) *Safety and toxicity of turmeric/curcumin in human double-blind, randomized, placebo controlled clinical trials.*

Patient/healthy	*Duration and route of administration, curcumin/turmeric dose*	*Toxicity*	*Reference*
Prediabetic patients	*6 months orally 1.5 g/day curcumin*	*Safe but itching in one patient and constipation in two patients were reported*	*Chuengsamarn et al., 2012*
Patients with Type 2 *diabetes mellitus*	*6 months orally 1,500 mg/day curcumin*	*No major toxicity but nausea and constipation were observed*	*Chuengsamarn et al., 2014*
Patients with breast cancer	*4–7 weeks orally 6 g/day curcumin*	*No toxicity*	*Ryan et al., 2013*
Healthy postmenopausal and *not active women*	*8 weeks orally 150 mg/day curcumin*	*No toxicity*	*Sugawara et al., 2012*
Diabetic and obese patients	*3 months orally 300 mg/day curcumin*	*No major toxicity but changes in liver enzymes and biochemical parameters of blood were observed*	*Na et al., 2013*
Patients with mild to moderate increased alanine transaminase (ALT)	*12 weeks orally 4 g/day of fermented turmeric*	*No toxicity*	*Kim et al., 2013*

Table 3 (cont.) *Safety and toxicity of turmeric/curcumin in human double-blind, randomized, placebo controlled clinical trials.*

Patient/healthy	*Duration and route of administration, curcumin/turmeric dose*	*Toxicity*	*Reference*
Healthy young people	*8 weeks orally 200 mg/day curcumin*	*No toxicity*	*Oliver et al., 2016*
Healthy individuals	Intravenous single dose of 120 mg/m^2 *liposomal curcumin*	*Safe but morphology of red blood cells was changed at higher doses*	*Storka et al., 2015*
Patients with metabolic *syndrome*	12 weeks orally 1,890 mg *curcumin extract*	No major toxicity but nausea *and diarrhea were reported*	*Yang et al., 2014*
Patients with coronary *artery disease*	*8 weeks orally 2 g/day curcumin*	*No major toxicity but diarrhea was observed*	*Mirzabeigi et al., 2015*
Healthy mature individuals	*4 weeks orally 400 mg/day curcumin*	*No toxicity*	*Cox et al., 2015*
Patients with prostate cancer	*9 weeks orally 3 g curcumin*	*No toxicity*	*Hejazi et al., 2013*
Patients with solid tumors	*8 weeks orally 900 mg/day curcumin*	*No toxicity*	*Panahi, Saadat, Beiraghdar, Nouzari, et al., 2014; Panahi, Saadat, Beiraghdar, & Sahebkar, 2014*

Table 3 (cont.) *Safety and toxicity of turmeric/curcumin in human double-blind, randomized, placebo controlled clinical trials.*

Patient/healthy	*Duration and route of administration, curcumin/turmeric dose*	*Toxicity*	*Reference*
Patients with pulmonary problems induced by sulphur mustard	*4 weeks orally 1.5 g/day curcumin*	*No toxicity*	*Panahi et al., 2015*
Patients with depression	*5 weeks orally 500 mg/day curcumin*	*No toxicity*	*Bergman et al., 2013*
Patient with major depressive disorder (MDD)	*8 weeks orally 1,000 mg/day curcumin*	*No major toxicity but stomachache and flatulence were observed*	*Lopresti et al., 2014*
Patients with pruritus caused by sulphur-mustard	*4 weeks orally 1 g/day curcumin*	*No major toxicity but gastrointestinal side effects were reported*	Panahi, Sahebkar, Amiri, *et al., 2012*

2.2.3 Potential concerns of curcumin with the changes to food composition

Research studies of the last few years, three key issues of employing excipient meals to boost the bioavailability of nutraceuticals. First, the use of permeability enhancers and efflux inhibitors boosts the absorption of bioactive compounds. It can also boost the absorption of hazardous or even toxic chemicals in the food matrix. Secondly, increased plasma concentrations leading to a toxic level might be because the boosted bioavailability of bioactive compounds. Third, modifying the gastrointestinal fate of the bioactive components might to cause the meal structure and the concentration that enters the large intestine are altered, which in turn may alter function of the colon microbiome resulting in health

problems. Therefore, before using permeability enhancers and efflux inhibitors, the problems and limits should be properly evaluated [4].

2.3 Extraction methods

2.3.1 Solvent extraction

Solvent extraction also called liquid-liquid extraction (LLE): The extraction process is a method of quantitative separation of compounds in which a component transfers from one solvent to another based on the difference in solubility or the distribution coefficient between these two immiscible (or barely soluble) solvents. Water and organic solvents are often liquids that cannot get mixed up together. Therefore, the solvent separates into layers when stirring is stopped. In general, organic compounds are soluble in organic solvents and these commonly used solvents are immiscible with water, such as benzene, chloroform, and ether. Finally, this non-aqueous layer is removed and further distilled to yield the pure chemical.

The properties of the solvent used for the solvent extraction Including the solvent should be miscible with the liquid to be extracted, the solvent should not react with the solute, and the boiling point of the solvent should be lower than the melting point of the solute so that it can be easily removed later. Studies have shown that LLE methods can separate compound and selectivity better than ion-exchange methods. The LLE method has many advantages including low energy usage, huge production capacity, and easy continuous operation.

The aqueous two-phase system (ATPS) is proposed as an alternative to the liquid-liquid extraction (LLE) process. ATPS are biphasic systems that can be generated by combining components in water until when the concentration of two hydrophilic polymers up to a certain value, the insoluble phenomenon will be observed. The type of aqueous two-phase system (ATPS) includes two polymers

(polymer/polymer: polyethylene glycol (PEG) and dextran), polymer with salt (polymer/salt: phosphate, sulfate, or citrate), two different salts (salt/salt), and other types of ATPS (ionic liquids and short-chain alcohols). Many of these combinations can create a biphasic regime within a particular concentration range, wherein the phase separation is regulated by the water solubility of the phase-forming agent.

Polymers/salts ATPS are created by the solubility of water-soluble polymer and inorganic (or organic) salt above the critical concentration, resulting in a salt-rich, polymer-poor bottom phase and a polymer-rich, salt-poor top phase. Formation of Polymers/salts ATPS can mix various polymers and salts. In general, PEG and PPG polymers are reported to be the most regularly employed polymer phase-forming agents, and inorganic phosphate, sulfate-based salts are the most commonly ionic components.

The effect on phase separation in ATPS is caused by many factors such as polymer concentration and molecular weight, salt concentration and composition, etc. The force involved acts on the drop during phase separation and determines its movement are related to 3 forces: gravitational, flotation, and frictional, where gravity force depends on the density of drops while flotation and frictional forces depend on the flow characteristics of phases. The determinant of the partitioning between two phases depends on the surface qualities of the materials and components of the ATPS.

The key elements driving partitioning behavior in ATPS are:

(1) Molecular weight (MW) and concentration of polymer: In a polymer–salt system, partition towards polymer-rich phase decreases upon increasing the concentration of polymer because the increase in the MW of the polymer increases hydrophobicity by reducing the hydrophilic groups/hydrophobic area.

(2) Hydrophobicity: A crucial role in the partitioning of protein. In polymer-salt systems, hydrophobicity may be regulated by altering the TLL, MW of the polymer,

and by the addition of salt. These salts are composed of ions with different hydrophobicity and the hydrophobic ions force the partitioning of the counter to a higher hydrophobic phase. The salting-out effect transports the biomolecule from the salt-rich phase to the polymer-rich phase. On the other hand, the salting effect migrates biomolecules from the salt-rich phase to the polymer-rich phase.

(3) pH: The pH may modify the charge and surface properties of the solute, with the net charge of the protein becoming negative in case of greater pH than the isoelectric point (pI) and positive if lesser than pI. Moreover, the negatively charged biomolecules in the higher pH environment enhance the partition coefficient, and the target biomolecule favours the top phase.

(4) Temperature: The temperature affects the change of Physico-chemical properties (i.e., density, viscosity, and interfacial tension), resulting in temperature considerably affects partition behavior of biomolecule and phase separation rate.

In this research, interest in the extraction of curcumin from turmeric using ATPS of short-chain alcohol and salt. This method provides advantages over conventional extraction procedures such as environment-friendly, low cost, easy ingredient recovery, decreased settling times, and many proteins are not compatible with the alcohol-rich phase.

2.3.2 Soxhlet extraction

The Soxhlet extractor is used for liquid-solid extractions when the desired component has limited solubility in the solvent and the impurity is insoluble in that solvent. The working principle of the Soxhlet extractor is that after heating the round bottom flask, the solvent is vaporized through the side glass tube up to the condenser. The vapor is then continually condensed into a liquid flood into the chamber housing the thimble, which contains a solid sample to be extracted (Observing from the liquid level in the chamber will increase steadily) by the thimble guarantees that the rapid motion of the solvent does not convey any the solid

material to the still pot. Therefore, the solid matter is immersed and extracted by a pure solvent every time for a while, until when the liquid level exceeds the highest point of the siphon, siphoning occurs and the solution is refluxed into the distillation flask so that a portion of the substance dissolved in the solvent can be extracted and the extraction efficiency is high.

2.3.3 Supercritical fluid extraction (SFE)

The technique of separating one component (the extractant) from another (solid or liquid matrix) utilizing supercritical fluids as the extracting solvent, the supercritical state of a fluid is that its density is similar to that of a liquid, while its viscosity and diffusion are similar to that of a gas. SFE can be strip undesired material from a product or gather a desired product, it is commonly used in the pharmaceutical, food, chemical, and cosmetic materials business also in the areas of toxicology, chemistry, environment, textile, petrochemical, polymers, among others.

SFE is a diffusion-based extraction technique, in which the solvent is required to diffuse into the matrix and the extracted material to diffuse out of the matrix into the solvent, where the diffusivities in the supercritical fluids are substantially faster than in the liquid. Therefore, supercritical fluid extraction can occur faster than organic liquid extraction and less use of organic solvents. Moreover, this extraction method is acknowledged as an environmentally safe technology.

Carbon dioxide (CO_2) is the most used supercritical fluid due to its safety, low cost, non-toxic, non-flammable, non-corrosive, and operation at low pressures and near room temperature, sometimes modified by co-solvents such as ethanol or methanol to improve the polarity of supercritical SC-CO_2. The solubility of the solute in SC-CO2 relies on temperature and pressure, since increasing the pressure leads to a liquid-like density of SC-CO2, thus increasing the probability of interaction between the solute and solvent resulting in rising in solubility.

On the other hand, increasing the temperature causes the SC-CO_2 density to drop [25]. In the extraction process, the system must contain a valve to control the flow of the crucial fluid into a heated extraction cell, an exit valve leading to a flow restrictor that depressurizes the fluid and transfers it into a collection device, and raw material in an extractor vessel, which incorporates temperature and pressure controllers to maintain the desired conditions. The basket is then placed into the extraction vessel and the jar is shut appropriately, the system is filled with CO_2 at tank pressure while the depressurization valve is closed, verifying that there are no leaks in the system, when the extractor temperature and pressure have stabilized, the depressurization valve can be opened to start the flow of SC-CO_2 through the cell. Once the fluid and the dissolved chemicals are carried to separators at lower pressure, and the recovered material settles out. The CO_2 can then be cooled, re-compressed, and recycled throughout an experiment, or vented to the atmosphere.

2.3.4 Enzyme-assisted extraction (EAE)

Enzyme-assisted extraction is the extraction of bio-actives by employing enzymes that can damage the structural integrity of the plant cell wall. The most commonly used enzymes are cellulases, pectinases, and hemi-cellulase, resulting in the bio-actives from plants needed to come out of the cell more easily, thereby higher extraction yields of bio-actives, which method are often used together with chemical extraction. Most researches are used enzymes to extract carotenoids, vanillin, flavonoids, lycopene, and polysaccharide, polyphenols, etc. Typically, the source of the enzyme can be obtained from bacteria, fungi, animal organs, and vegetable/fruit extracts. Factors affecting the efficiency of the enzyme include the part of the plant to be extracted due to differences of plant affects the choice of the appropriate enzyme type, the type of enzyme used in the extraction, and optimum conditions for extraction and enzyme activity (Enzyme concentration, pH value, the time required for enzymes fermented with plants, and temperature) [26].

The advantages of employing enzymes for extraction include reduction in extraction time, minimizing utilization of solvents in the extraction, increasing yield and quality of output, and more environmentally friendly. However, there may be some limitations, such as enzymes being relatively expensive, the enzymes used may cannot completely hydrolyze plant cell walls, and the stability of the enzymes in the extraction solvents, especially in organic solvents that result in reduced enzyme efficiency. Another important problem is that once the extraction is complete, the enzyme cannot be reused, thus immobilization technology on the supporter has been developed to immobilize the enzyme within the insoluble supporter by the enzyme retains its catalytic ability. In addition, enzyme immobilization increases the stability of the enzyme in the used condition and allows the enzyme to be reused, thereby reducing the cost of using enzymes in the extraction. The enzyme-assisted extraction of bioactive compounds from plant sources is shown in **Figure 3**.

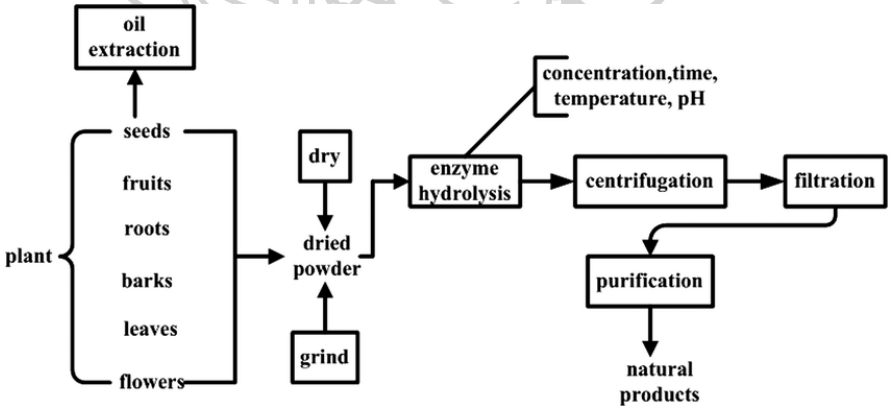

Figure 3 *The enzyme-assisted extraction of bioactive compounds from plant sources [27].*

2.3.5 Microwave-assisted extraction (MAE)

Currently, extraction using MAE is an increasingly popular method. The principle of microwave extraction can be described as the use of microwaves, which are electromagnetic forces in the frequency range from 300 MHz to 300 GHz and wavelengths in the range of 0.01 to 1 m with organic solvents for extraction of bioactive compounds. The microwave extraction method converts to heat following ionic conduction and dipole rotation methods.

During the ionic conduction mechanism, heat is formed because the solution offers resistance to this migration of ions, friction is generated and the solution is heated, which impacts plant cells and causes the extraction of bioactive substances.

The extraction mechanism of microwave-assisted extraction consists of three steps: first, the separation of solutes from the active sites of the sample matrix under increased temperature and pressure; second, the diffusion of the solvent through the sample matrix, thirdly, the release of solute from the sample matrix to the solvent [27]. Factors affecting the efficiency of extraction include solvent is very important, solvent nature and solvent feed ratio, microwave power (maximum power consumed ranges between 600W and 1000W for closed systems and roughly 250Wfor open systems), temperature (sufficient for compound solubility and good solvent penetration into the plant matrix.), plant matrix characteristic, stirring, and extraction time and cycle.

The advantages of MAE include a simple approach, minimizes the usage of organic solvent, rapid extraction of bioactive compounds, and better recovery than standard extraction procedures. Moreover, MAE is acknowledged as a green technology. The MAE technique can be used to extract natural substances in different groups including glycosides, alkaloids, carotenoids, terpenoids, polyphenols, and essential oils.

2.3.6 Ultrasound-assisted extraction (UAE)

Ultrasound is a sound wave with a frequency range of 20 kHz to 100 MHz, it can flow through a medium by causing compression and expansion, a phenomenon known as "cavitation". This process causes the creation and collapse of bubbles, which in turn produces very high shear energy waves and turbulence as a result of variations in temperature and pressure. The change in these factors is used to expedite the mass transfer in the extraction process.

The mechanism of extraction by ultrasonic operations involves two basic types of physical phenomena: the diffusion across the cell wall and washing of cell contents after shattering the walls [27].

Ultrasonic-assisted extraction is a method that uses ultrasonic or ultrasonic waves with organic solvents or water to extract antioxidants from raw materials. The instrument is a cylindrical rod of varying length and frequency, the instrument emits high-frequency sound waves in a carrier (water or organic solvents), when the gas bubbles expand, the antioxidants inside the material will be dissolved in the solvent. When the bubbles implode, in turn, results in the tissue tearing of the material, and the higher the temperature, the better the antioxidants required to be extracted dissolved in the solvent. Factors affecting extraction efficiency include the frequency of sound waves used, raw materials (particle size and moisture content), extraction time, solvent properties, vapor pressure, extraction temperature, and sound wave intensity. The UAE method is generally used to extract antioxidant, phenolic compounds such as naringin, naringenin, quercetin, ellagic acid, kaempferol, etc.

The advantages of the UAE include saving energy and extraction time, reduced solvent consumption, lower extraction temperatures, faster energy transfer, and showing the potential for the utilization of ultrasonic extraction on an industrial scale.

2.3.7 Surfactant-free microemulsions (SFME)

Surfactant-free microemulsion (SFMEs), in other words, traditional microemulsions in the absence of surfactants. SFME can be defined as a thermodynamic stable and optically isotropic transparent dispersion consisting of at least two immiscible fluids (aqueous-oil phase) and co-solvents or hydrotropes (solvents that are completely or at least partially miscible with both the aqueous and oil phases), resulting in a macroscopically homogenous liquid.

According to research studies, SFME comprised of water, triacetin (TriA) as the oil phase, and ethanol (EtOH) as hydrotrope can be used to extract curcumin from Curcuma Longa [5]. The results showed that the extraction power of SFMEs was superior compared to a commonly used aqueous solvent mixture due to the enhanced solubility of curcumin in SFME mixes. Furthermore, the components of the SFME are entirely food agreed, sustainable, and green, giving the SFMEs a superb alternative that is particularly ideal for the food industry. However, SFME must be aware of complex separation and purification procedures, and high material costs.

2.4 Optimization using response surface methodology

The response surface methodology (RSM) is a method used to determine the optimal conditions of a system or production process by employing a mix of mathematical and statistical approaches for empirical model building and evaluating a process in which the response of interest is affected by various variables from careful design of experiments. The purpose of this strategy is to optimize a response (output variable) by helps to reach the optimum circumstances of the process with reduced experimental trials, highlight the interactions between independent variables (input variables), minimize the experiment time, and reduce the cost of analysis methods [28].

An experiment is a sequence of tests known as run, which adjusts the input variable to determine the causes for the change in the output response. An example of Optimization of Response, Let Y is the response (dependent variable), X_1 and X_2 are independent variables and is the experimental error, then it may be represented in equation form as follows:

$$Y = f(x_1,x_2) + \boldsymbol{\varepsilon} \quad (3)$$

The response can be represented graphically, either in the three-dimensional space or as contour plots that assist visualize the shape of the response surface, hence the major goals of an RSM study are to understand the topography of the response surface and determine the location where the best appropriate response occurs.

The problem with response surfaces is that the pattern of the relationship between the response and the factors is often unknown. Therefore, the first step is to estimate the relationship between the response and the components. In general, the first-order model is used to evaluate the correlation, but in the case of surface curvature is estimated using second-order polynomial, then it can be written in equation form as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (4)$$

where Y is the experimental response (dependent variable), β_0 is the intercept term, β_i is linear coefficient, β_{ii} is squared coefficient, β_{ij} is interaction regression coefficient, and x_i and x_j are the independent variables ($i \neq j$).

RSM is an experimental design (DoE), with the objective of the selection of the most suitable areas where the response should be evaluated. The mathematical model of the process is related to the experimental design. Therefore, the choice of experimental design has a significant effect on the precision of response surface construction. In a DoE, screening experiments are performed in the early phases of the process, if there are several variables that have little or more effect on the response, the variables which have considerable effects on response are identified for further investigation. The main experimental designs utilized in response surface technique are the Box-Behnken design (BBD) and the central composite design (CCD). Experimental data are examined to fit statistical models using analysis of variance (ANOVA) in the mean test for statistical significance. The model was adequacies when its P-value < 0.05 (Probability), R^2 > 0.9 (The coefficient of determination), and Adeq Precision > 4.

In this study, the Central composite design method was used to increase the extraction efficiency, which Central composite design is the most common design. Central composite designs (CCD) are an experimental model suitable for studying or modeling the Second-order or Quadratic Model and most effective when compared to other trial plans. The CCD is a first-order (2N) design, supplemented with additional center and axial points to allow efficient estimate of parameters of the second-order model. The CCDs are ideal for sequential testing and require sufficient data to identify a lack of fit, while an extraordinarily large number of design points are not involved. The experiments were designed using MINITAB software version 19 to create the CCD concept matrix, to acquire a better evaluation of the independent factors, data analysis, and process optimization. Moreover, replications were performed to compute the lack of fitness, sum of square error, and generally, evaluate the validation of the prior results.

For example, the Central Composite Design for a 3-variable study found that the CCD had a design including 2N factorial points, 2N axial points, and 1 center point, as shown in **Figure 4**, which can be seen that it consists of 3 components:

(1) Factorial Points, which take 2-Level Full Factorial as part of the experiment.

(2) Axial Points is an adjust one variable while keeping the other variable at the mean or zero value.

(3) Center Points is an adjust of all variables at the mean or zero value.

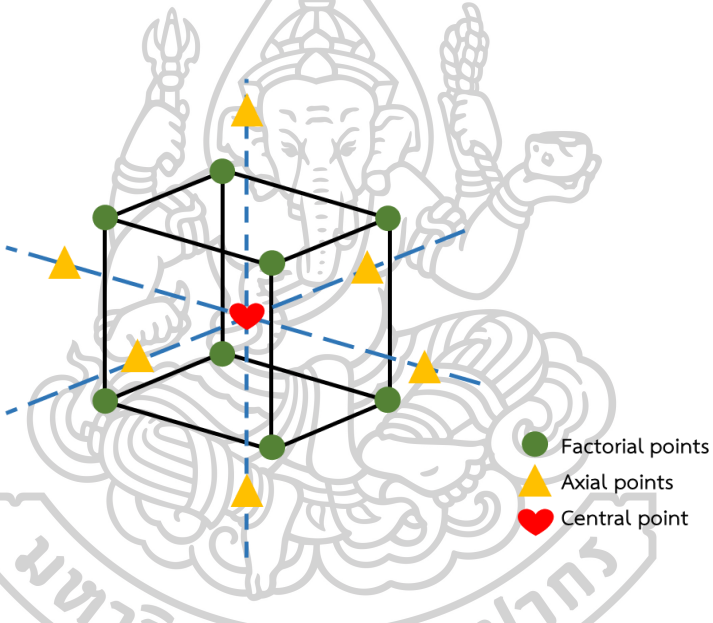

Figure 4 *Central composite design for a 3-variable study.*

The CCD offers an alternative to 3^N design in the creation of second-order models since the number of trials is reduced compared to the complete factorial design, with the designed experimental and the associated findings presented in **Table 4**.

Table 4 *The experimental design of the central composite design in the case of 3-variables.*

Point types	Order in the experiment	Significant experimental factors		
		X_1	X_2	X_3
Factorial Point (F)	1	-1	-1	-1
	2	-1	-1	+1
	3	-1	+1	-1
	4	-1	+1	+1
	5	+1	-1	-1
	6	+1	-1	+1
	7	+1	+1	-1
	8	+1	+1	+1
Center Points (C)	9	0	0	0
	10	0	0	0
	11	0	0	0
	12	0	0	0
	13	0	0	0
	14	0	0	0
Axial Points (A) Let α = 1.682	15	$-\alpha$	0	0
	16	$+\alpha$	0	0
	17	0	$-\alpha$	0
	18	0	$+\alpha$	0
	19	0	0	$-\alpha$
	20	0	0	$+\alpha$

The processes for using the Response Surface Method in the extraction investigation is shown in **Figure 5**.

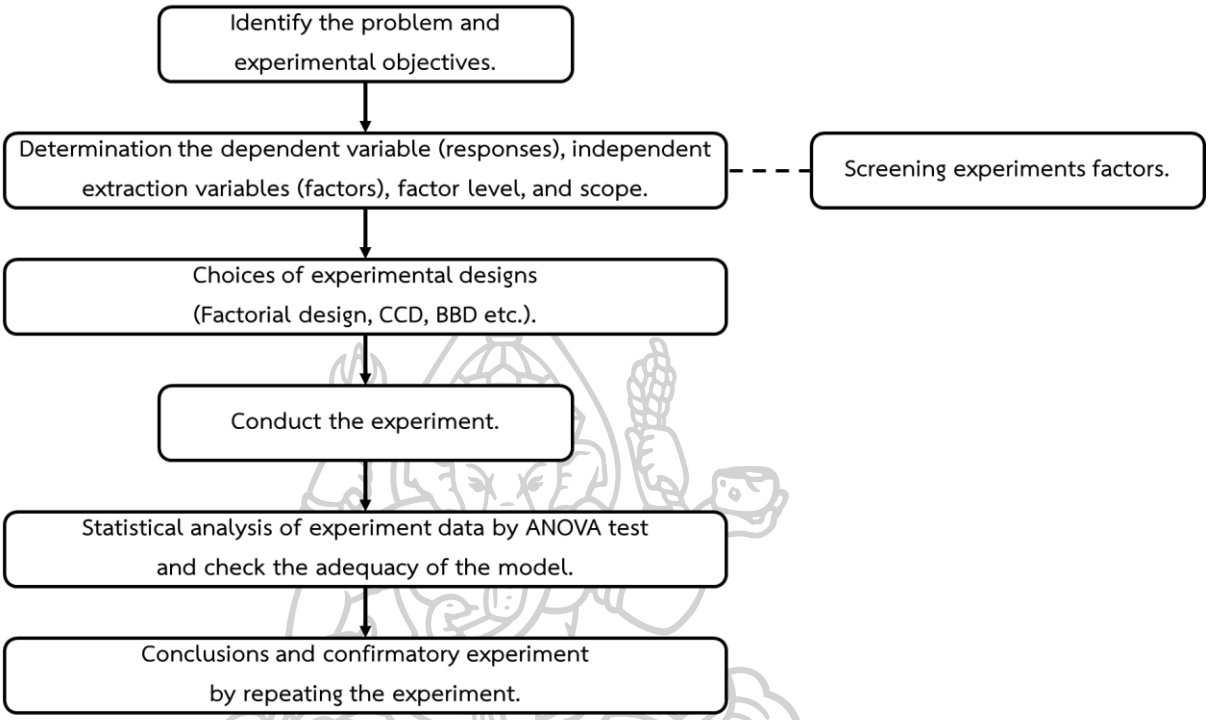

Figure 5 The steps for using the Response Surface Method.

CHAPTER III

LITERATURE REVIEWS

Ramya Jagannathan et al. [29], studied the temperature and dielectric environment of curcumin molecules on the solubility affecting deep spectroscopy. It was found that solvent and temperature play a vital impact in the curcumin absorption spectrum. In this experiment, when comparing different percentages of solvent mixture: (1) 50% methanol, (2) 20% methanol, and (3) 10% methanol in water, wherein each case, the amount of curcumin is the same. It was found that the absorption spectra of these three samples showed different positions, and the temperature resulted in different absorption peak for each condition as shown in **Figure 6**, where the change in position is completely attributable to the change in the dielectric environment, while the structure of the curcumin molecule remains unchanged.

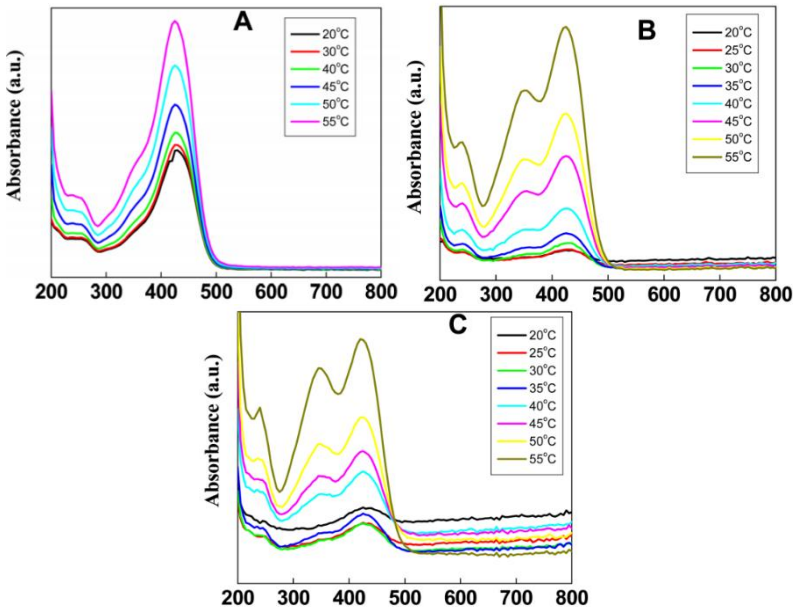

Figure 6 UV-visible spectroscopy of curcumin in water and methanol at different vol% (A, 50% methanol; B, 20% methanol; C, 10% methanol in water).

In addition, the HPLC analysis revealed that the solution contains not only curcumin but there is also bisdemethoxycurcumin. **Figure 7** shows that before heating and after heating, curcumin did not undergo any chemical change, only the intensity of the peak of bisdemethoxycurcumin A slight increase, which can be explained by the absence of a non-polar methoxy group in the molecule. As the absence of a non-polar methoxy group in this molecule increases its availability in polar water compared to curcumin with up to two methoxy groups.

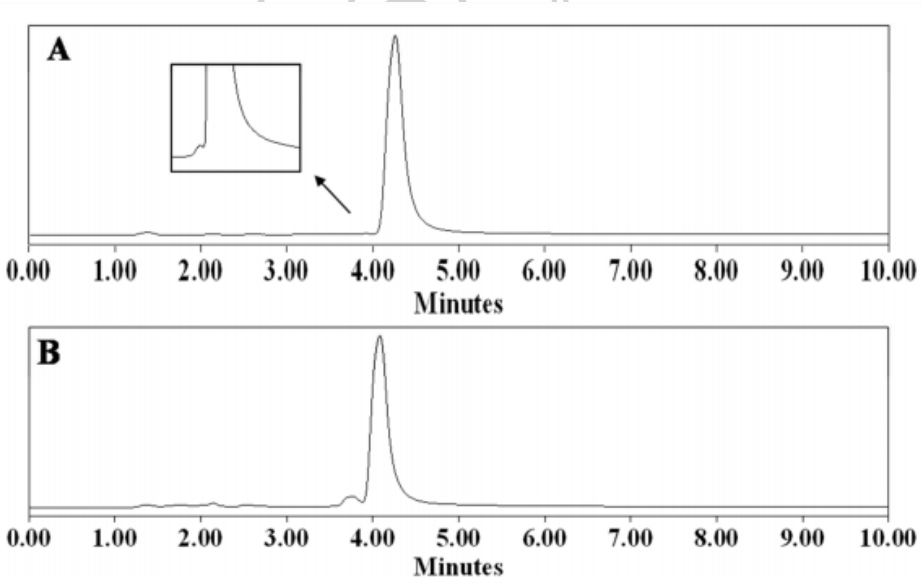

Figure 7 *HPLC chromatograms of curcumin (A) before heating and (B) after heating in water, drying, and redissolving in methanol.*

Mahesh Kharat et al. [17], studied the effects of pH affecting the physical and chemical stability of curcumin in aqueous solutions. The experiment was divided into 2 cases; (1) In the absence of continuous stirring (unmixed) (2) in case of stirring (mixed). In the absence of continuous agitation, the absorption of the curcumin buffer solution decreases over time as shown in **Figure 8(A)**, which can be observed that under alkaline conditions, absorption decreased rapidly over the first 7 minutes

with predicted degradation rates of 92, 135 and 125 cm^{-1}/min at pH 7.0, 7.4 and 8.0, respectively. These results suggest showed curcumin is more chemically stable under acidic environments. Interestingly, samples containing curcuminoids appeared to have greater stability at pH 7 than pure curcumin samples, according to previous studies that the composition of curcuminoids has an effect on the stability of different pH. In a comparison of 2 cases, the continuous stirring resulted in a decrease in absorbance for the acidic curcumin solutions than in the absence of stirring, indicates that stirring influences the change in the physical or chemical stability of the curcumin as shown in **Figure 8(B)**.

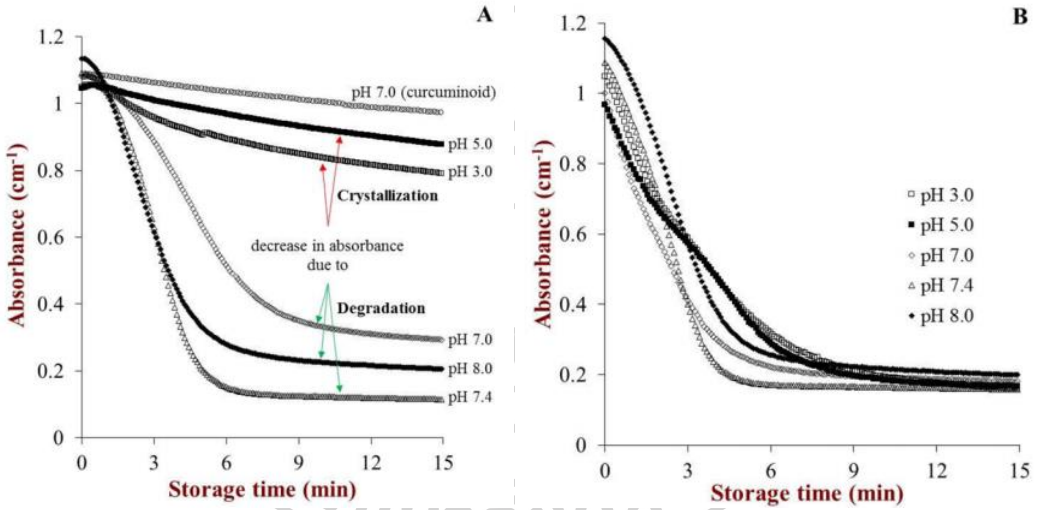

Figure 8 Stability of curcumin in phosphate buffer solutions of various pH values when the system was unmixed (A) and mixed (B).

Pierre Degot et al. [30], found that the most popular alternative in the industry is the use of ethanol and aqueous ethanol solution, the frequently used reference mixture is ethanol/water 80/20 (in weight). Although these mixes are not hazardous to humans. But to extract refined curcuminoids powder from C. longa, the essential oils included in turmeric must be eliminated, which involves the use of

hazardous and volatile solvents again. Therefore, a surfactant-free microemulsion (SFME) was selected as the extraction medium, containing NaSal as the hydrotrope, water, and ethyl acetate (EtOAc) as the oil phase to stabilize them against oxidation. It was found that the highest effective powder to the solvent ratio for curcuminoid extraction was 1:15, with yields a result ~ 6% higher than curcuminoid content compared to Soxhlet extraction. Due to the amount of bisdemethoxycurcumin in the SFME extraction is higher than that of the Soxhlet extraction, arising from the fast breakdown of the molecule at higher temperatures. But the ratio applied to 1:10 is because it meets Chemat's six principles of green extraction, and the lower extraction efficiency of ~ 18% is an acceptable value because the industry can save up to ~ 35% solvent as well. In addition, the stability of curcumin in the solution was studied over time, and NaSal's antioxidant properties were found to slow down curcumin degradation and may act as a UV filter to protect curcumin because NaSal can absorb light from the ultraviolet spectrum, this can be explained by the intermolecular interaction between the two molecules that the weak interaction of the salycilate acid group and curcumin in a keto-enol form can produce a hetero synthon resulting in a eutectic system and maintain the molecules closely together in solution so that NaSal can effectively protect curcumin. An experiment was then to compare the effects of NaSal with NaSal inactivity, indicating that a small concentration of NaSal was adequate to protect curcumin from degradation, as shown in **Figure 9**.

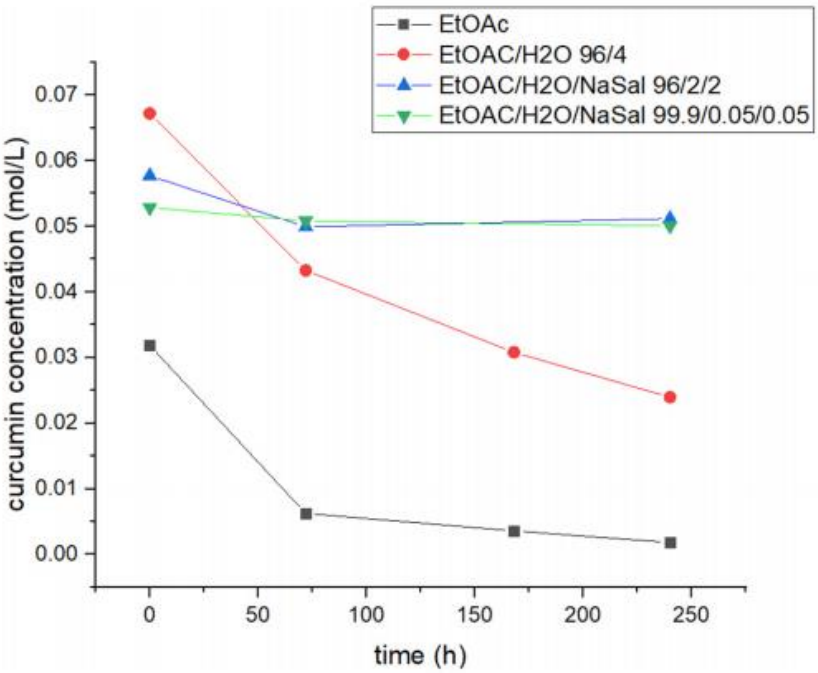

Figure 9 Investigation of the stability of curcumin in a mixture of various solvents.

Pierre Degot et al. [31], studied the effect of solvent and extraction process of curcuminoid extraction by using a green solvent. The comparison between ethanol/Triacetin (TriA) and Diacetin (DiA)/Triacetin (TriA) was studied. The solubility of curcumin in a binary mixture of ethanol in high, due to the viscosity of ethanol is lower than DiA resulted in the higher extraction yield of curcuminoid with ethanol/TriA binary extraction mixture with the ratio of 1:4. The addition of water to a binary mixture of ethanol/TriA resulted in the increase of curcuminoid extraction yield, especially of bisdemethoxycurcumin that good in polar solvent. The extraction of curcumin by using trinary mixture that uses ethanol as an extraction media showed superior extraction yield compare to the mixture of DiA or common extraction medium ethanol/water binary mixture.

In case of the extraction process, the Soxhlet method and surfactant-free microemulsion (SFME) were studied. Soxhlet extraction method plays an important

role in curcumin extraction yield but it cannot confirm the content of curcuminoids because the rising of temperature is the cause of degradation of curcuminoids. SFME is the best choice of curcuminoids extraction. This process was produced at room temperature with a short extracting time and no need to remove the solvent. Moreover, the solvent in this process, ethanol, and TriA is completely food grade and edible. So, it can be concluded that the SFME with ethanol showed the best extraction yield of curcuminoids.

Khadija Doldolova et al. [32], studied optimization and modeling of microwave-assisted extraction of curcumin by using natural deep eutectic solvents (NADES) because eutectic solvents are green chemistry. Also, NADES has low toxicity, biocompatibility, low cost, and simple preparation. The NADES constituents used in this study were choline chloride (non-toxic quaternary ammonium salt), lactic acid, and several simple sugars (sucrose and fructose) as shown in **Table 5**.

Table 5 *NADESs constituents for the studied.*

Solvent Code	Constituents	Molar Ratio
NADES-1[1]	Fructose: choline chloride: water	2:5:5
NADES-2[2]	Sucrose: choline chloride: water	1:4:4
NADES-3	Fructose: lactic acid: water	1:5:5
NADES-4	Sucrose: lactic acid: water	1:5:7
NADES-5	Lactic acid: choline chloride: water	1:1:2

[1] Diluted with water at a ratio of 9:1 (v:v) before extraction.

[2] Diluted with water at a ratio of 8:2 (v:v) before extraction.

In addition, the variables used for microwave-assisted extraction (MAE) were simulated and optimized using the response surface methodology (RSM). According to research, it was found that the viscosity of eutectic solvent had an effect on mass transfer. The higher the viscosity, the lower the efficiency of mass transfer. Therefore,

the viscosity is decreased by dilution with water in the eutectic solvent preparation, but be careful, excessive water may decrease the extraction yield. Therefore, a suitable value must be selected. As a result of the extraction, it was found that higher temperatures cause the TAC and CC of the extract to increase, cautioning that very high temperatures induce antioxidant compounds degradation. In studies the optimum extraction temperatures were 64.7–71.8 °C. Extraction time conditions, it was found that with long time extraction it may cause some bioactive components to degrade due to long exposure to heat, in studies, the optimum extraction time was 15.4–21.6 min. In extraction solvent-to-solid ratio conditions, it was found that the TAC and CC values of the extract increased with an increase in the ratio. As the increased ratios resulted in higher concentrations and mass transfer, in studies the optimum solvent to solid ratio was 14.5–16.5 mL/0.2 g-DS. **Figure 10** shows the conclusion to the results of the experiment, NADES-4 was able to extract the highest amount of curcuminoids and NADES-3 extracted the highest antioxidant, depending on the structure of the NADES determine its physicochemical properties. However, all NADESs except NADES-1 provide an extraction yield greater than 80% methanol: water mixture. Therefore, it is better to choose a natural deep eutectic solvent because it is green chemistry.

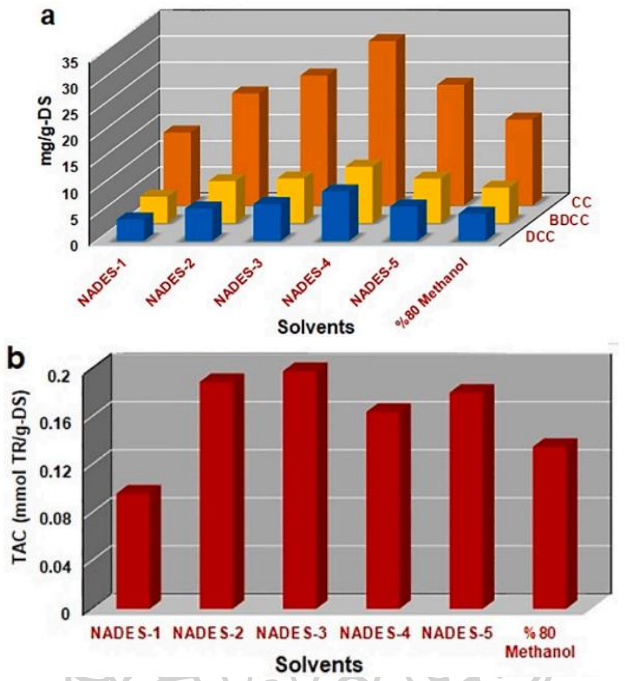

Figure 10 *Effect of type of solvent on the MAE of (a) curcuminoids (CC: curcumin content; BDCC: bisdemethoxycurcumin content; DCC: demethoxycurcumin content) and (b) TAC.*

Ming Li et al. [33], studied the optimization and kinetic of the curcuminoids extraction by pulsed ultrasonic and microwave-assisted using the response surface methodology. This approach was chosen because it has excellent extraction efficiency, low energy, and solvent consumption. The 1:200 mass to solvent ratio was employed in both procedures before the experiment. It was found that the response surface plot of the curcuminoid yield of PUAE showed that the most influential variables were ethanol concentration, AMP, and pulsed duration time, respectively as shown in **Figure 11.** The results of the pulsed ultrasonic-assisted extraction (PUAE) showed that optimum conditions were 83.40% ethanol concentration, 60% AMP, 3/1 (s/s) pulsed duration/interval time, and 10 min irradiation time, where the predicted yield was 0.95 g/100 g. In addition, experiments were performed to show that interval duration had a great influence on extraction, where reducing the interval

time to high extraction yields. As a result, pulsed ultrasonic extraction is more effective than CUAE.

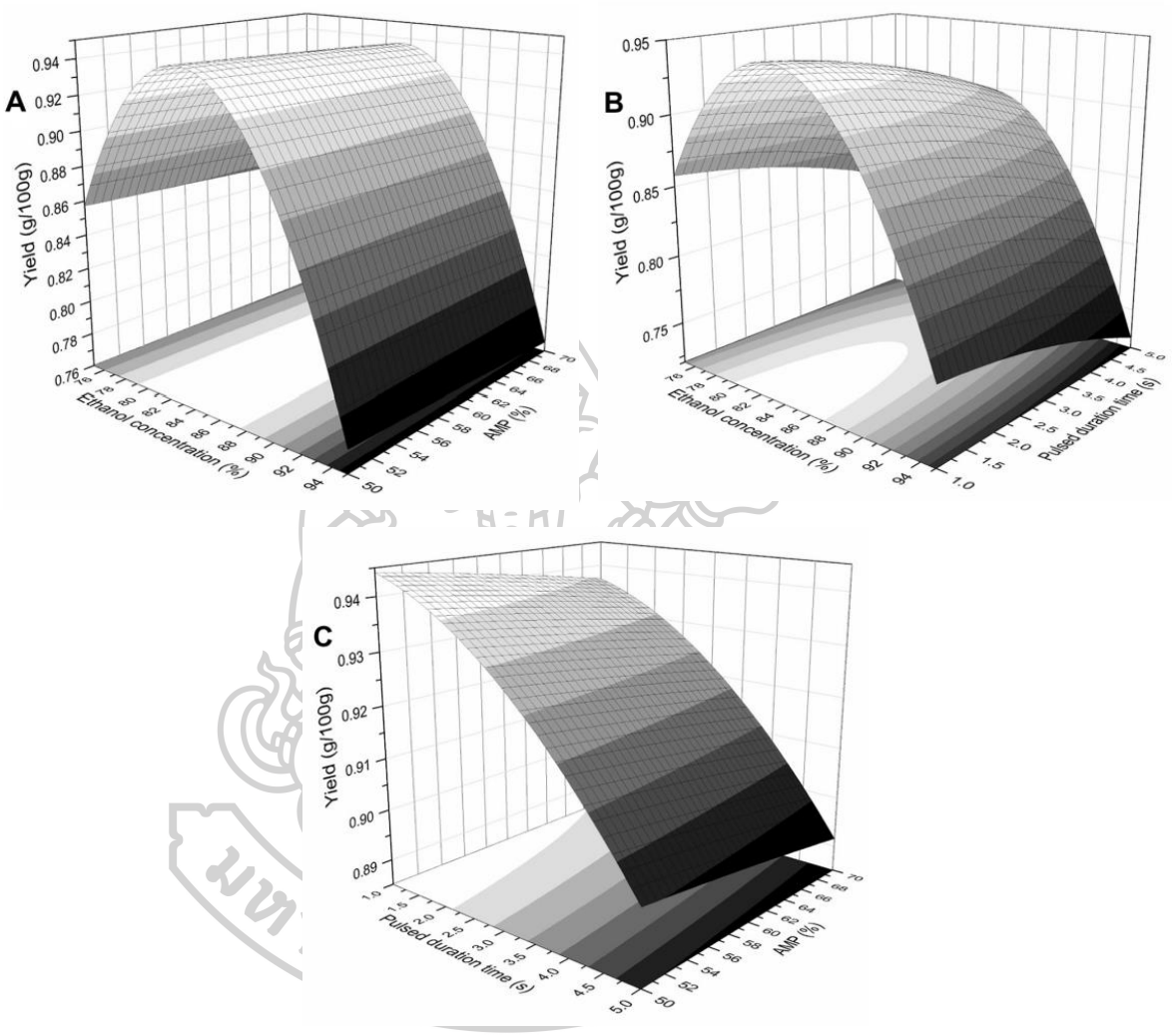

Figure 11 *Response surface plot of the curcuminoid yield of PUAE for the effect of (A) with 3 s pulsed duration time, (B) with 60% AMP and (C) with 85% ethanol concentration (v/v).*

It was found that the response surface plot of the curcuminoid yield of MAE showed that the most influential variables were extraction time, ethanol concentration, and power level, respectively as shown in **Figure 12**. The results of

the microwave-assisted extraction (MAE) showed that optimum conditions were 72.06% ethanol concentration, 10.45% power level, and 7 min extraction time, where the anticipated yield was 0.94 g/100 g.

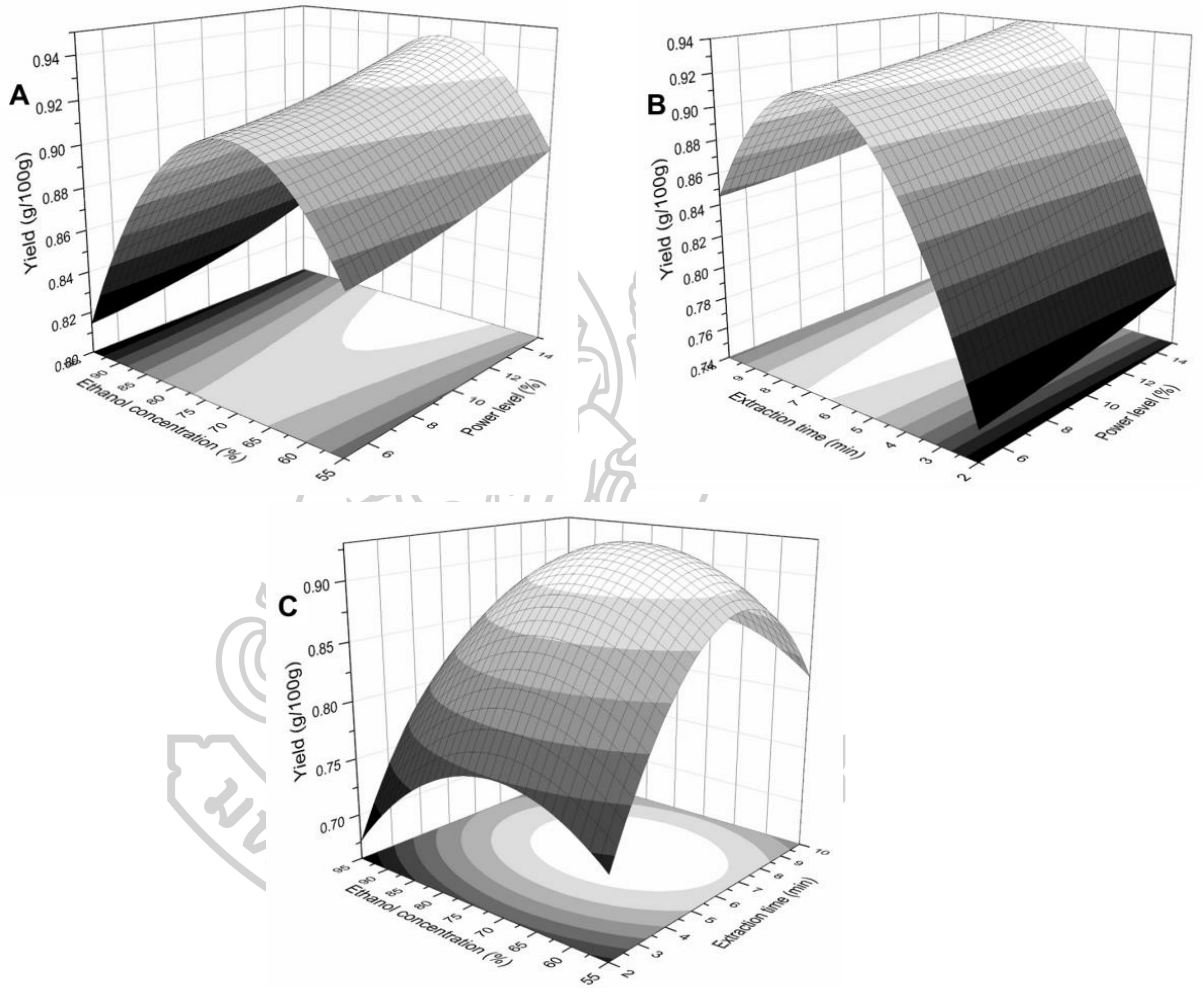

Figure 12 *Response surface plot of the curcuminoid yield of MAE for the effect of (A) with 6 min of extraction time, (B) with 75% ethanol concentration (v/v), and (C) with 10% power level.*

In addition, the kinetic study was performed according to the optimal conditions obtained in the response surface experiments. The rate is constant for

PUAE at 60% AMP higher than 20% AMP indicating that at 60% AMP is more efficient in extracting curcuminoids according to the preliminary single factor experiment as shown in **Figure 13(A)**. Meanwhile, the rate constant for MAE at 10% AMP higher than 5% AMP indicating that at 10% AMP is more efficient in extracting curcuminoids as shown in **Figure 13(B)**. The kinetic results can be concluded that MAE had the same efficiency as PUAE and was superior than CUAE in the extraction of curcuminoids.

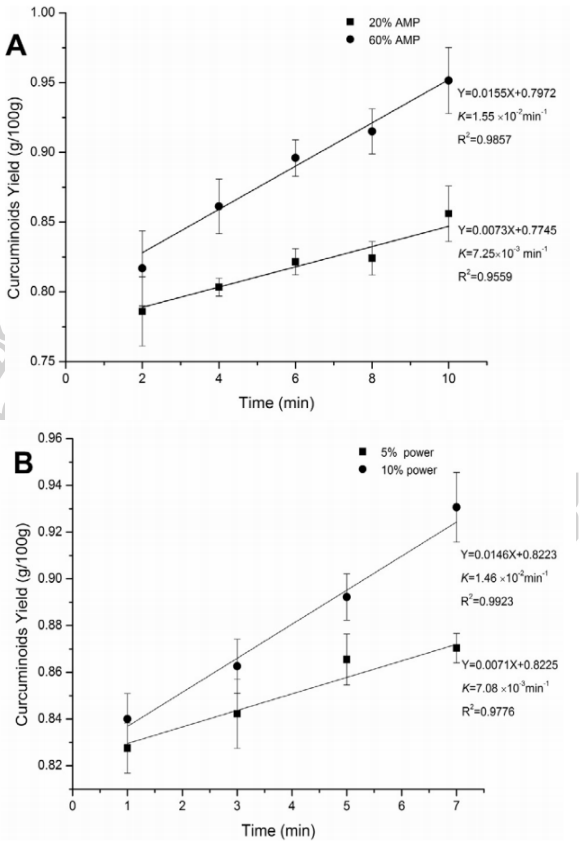

Figure 13 *The kinetic study of PUAE (A) and MAE (B) under their optimal conditions.*

Mustafa Bener et al. [34], studied the optimization of the Curcuma longa L. extraction by microwave-assisted. The variables used to investigate were solvent (methanol in water and ethanol in water), temperature, and extraction time. The

effect of both solutions at different concentrations showed that MeOH extracted curcumin higher than EtOH and extracted well at high concentrations, but the TAC value found that optimum extraction conditions at 80% (v/v) MeOH as shown in **Figure 14.** Therefore, 80% (v/v) MeOH concentration in water was the best choice in the following experiments.

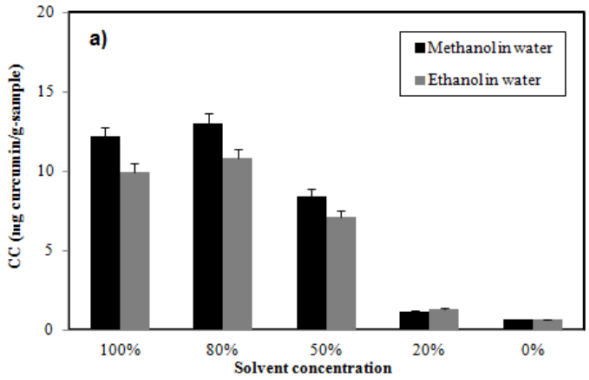

Figure 14 *The effect of solvents used on the extraction of (a) curcumin and (b) antioxidant compounds.*

The effect of the temperature showed that the optimum extraction temperature of curcumin and TAC was 80 ºC. When the temperature is above 80 ºC, less curcumin is extracted because curcumin is degraded while other phenolic antioxidants were formed to stabilize the TAC. Similarly, curcumin and TAC extraction increased with increasing time and became stable at 5 min. Therefore, the optimum

conditions for extraction by microwave-assisted were 80% (v/v) MeOH concentration in water, 80 ºC temperature, and 5 min extraction time.

Sujata S. Patil et al. [35], A comparison was performed between batch extraction and novel three-phase partitioning (TPP) in the extraction of curcuminoids from Curcuma longa. In the TPP system, the effects of solvent, time, ammonium sulfate loading, slurry to solvent ratio, solute to solvent ratio, and temperature were studied. It was found that tert-butanol is the best solvent because it demonstrated kosmotropic and crowding effect at above temperature than other co-solvents. The tert-butanol solvent was further used to determine the extraction time, the yield of curcuminoids extraction was gradually increased and reached equilibrium at 150 minutes. Subsequently, studied the effect of the ammonium sulfate concentration, it was found that at 30% (w/v) ammonium sulfate solution resulted in the highest curcuminoid yield. Subsequently, studied the effect of slurry to tert-butanol ratio, it was found that at 1:1 (v/v) slurry to tert-butanol ratio resulted in the highest curcuminoid yield because the lower ratios may not be able to properly synergize with ammonium sulfate solutions, and larger ratios may be the transfer of tert-butanol into the aqueous phase which led in a dispersion of curcuminoids into both the phases. Subsequently, studied the effect of amount of turmeric powder on water, it was found that at 1:40 (w/v) ratio resulted in the optimum curcuminoid yield. The temperature was the final effect studied for TPP; it was found that at 40 °C resulted in the maximum yield of 58.38 mg/g due to the higher temperature which permits the more solvent to permeate into the cellular matrix resulting in an enhanced rate of extraction. Then studied the variable effects of batch extraction as well as TPP, it was found that the optimum extraction conditions were ethanol solvent, 180 min extraction time, 40 °C temperature, 1:40 (w/v) amount of turmeric powder to ethanol ratio, and speed of agitation 400 rpm, which batch extraction yield curcuminoids of 52.77 mg/g. According to the study, although Soxhlet

extraction is higher than TPP and batch extraction, Soxhlet and batch have many disadvantages compared to TPP. The TPP method for the extraction of curcuminoids is also available on a small scale, so further studies are being conducted for further industrial use.

Foozie Sahne et al. [36], compared different methods for extracting turmeric, to study the advantages and disadvantages of each method that affect yield. The extractable curcumin content (% w / w) from turmeric was then calculated to compare the extraction efficiency. The results of the experiment showed the following. The Soxhlet extraction at 60 °C extraction temperature and 8 h extraction time obtain the total yield of curcumin and oleoresin extracted equal to 6.9 and 8.29%, respectively. The microwave-assisted extraction at the power of 300 W and irradiation time of 2 min obtain the total yield of curcumin and oleoresin extracted equal to 5.19 and 3.72%, respectively. The ultrasound-assisted extraction at 30 min sonication time and 35 °C sonication temperature obtains the total yield of curcumin and oleoresin extracted equal to 5.72 and 3.92%, respectively. The enzyme-assisted extraction (by conditions for enzyme pretreatment: enzyme loading 3% and incubation time of 6 h) at 4 h extraction time obtain the total yield of curcumin and oleoresin extracted equal to 6.27 and 4.1%, respectively. In conclusion, Soxhlet extraction is the most efficient method, followed by enzyme-assisted extraction, microwave-assisted extraction, and ultrasound-assisted extraction exhibited roughly the same performance. Although the Soxhlet extraction method is highly efficient, a long extraction time, high extraction temperature and uses a lot of solvents. Therefore, choosing another extraction method appears to be a more promising trend in future studies.

Pierre Degot et al. [5], have improved the concentration, purity, and stability in food-approved by surfactant-free microemulsions (SFME) consisting of water, ethanol, and triacetin. It was found that the powder to solvent ratio of 1:24 (the

binary EtOH/TriA (40/60)) resulted in the highest curcuminoid extraction. The results show that a modest amount of water (5 wt%) has a large influence on the extraction power, as water has the potential to expand the cells to allow higher solvent permeability. Also, increasing to 30 wt% resulted in better extraction power, but after 30 wt% showed very similar results thus suggesting water could no longer improve extraction. After four cycles of extraction, the saturation is reached, due to the limited solubility of curcuminoids, wherein a mass of roughly 150 mg of curcuminoids in the extraction solvent could be attained, shown in **Figure 15**.

The extract's purity enhancement was then examined by comparing three methods: lyophilization, steam distillation, and vacuum distillation. It was found that steam distillation was the fastest method of purification and provided a purity of ~ 82%, but operating at high temperatures resulted in curcuminoid degradation. The vacuum distillation provides purity results similar to that of three steam distillation cycles. Meanwhile, Lyophilization could be attained ~ 94% purity, despite losing less than 20% of curcuminoids, making it the preferred rhizome pre-treatment. To test the stability of aqueous solutions in general, storage in darkness is important to preserve the original color intensity of the extracts and aqueous solution of curcumin. It found that the solution exhibits a strong yellow coloration, transparent and stable for at least 14 days without precipitation of curcuminoid or only slight precipitation.

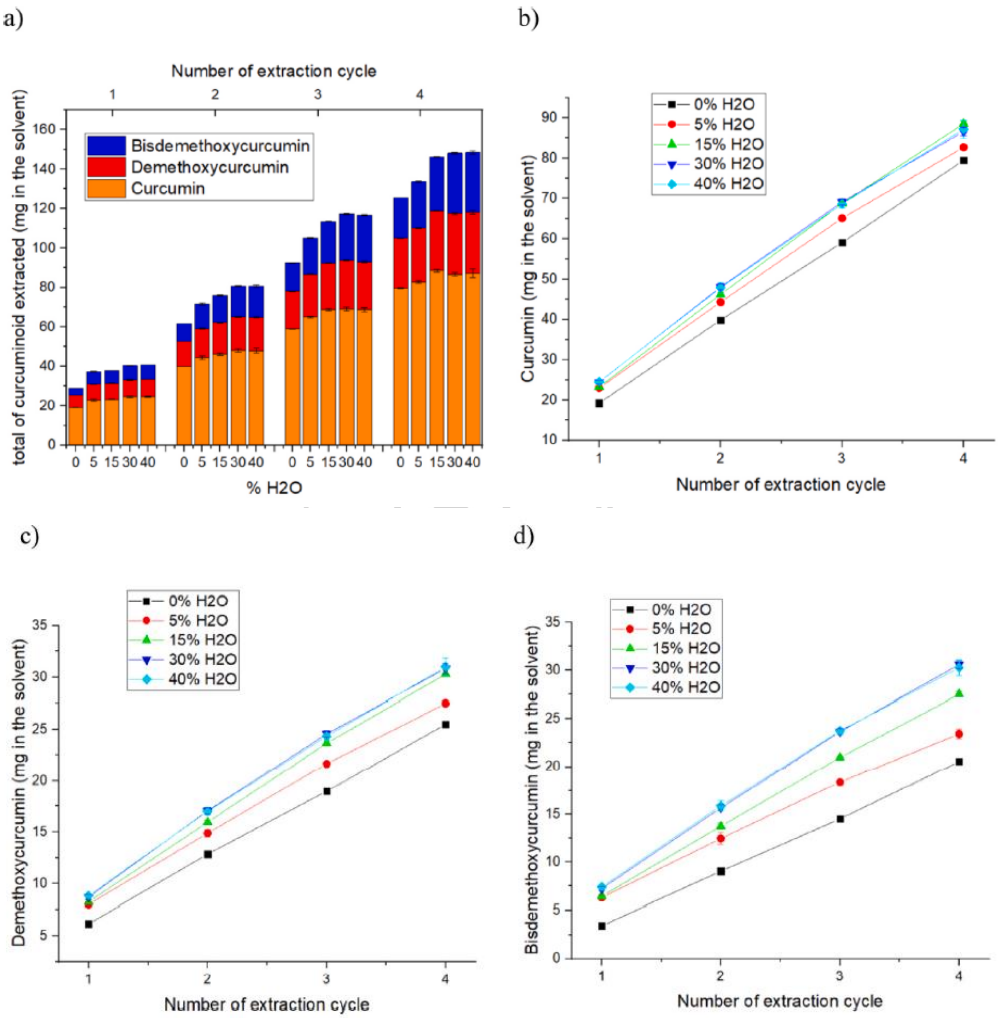

Figure 15 *a) Overview over the extraction cycles for extraction mixtures with a varying water content b) curcumin, c) demethoxycurcumin and d) bisdemethoxycurcumin for each extraction mixture and cycle of extraction for the ratio 1:24.*

S.R. Shirsath et al. [37], examined the variables affecting the extraction of curcumin using an ultrasound-assisted method. It found that ultrasound can be used to improve the extraction rate by increasing the mass transfer rate and resulting in the rupture of the cell wall. In addition, the transmission of sound waves through solvents and the implosion of cavitation bubbles reduces particle size due to micron cavity formation resulting in higher yields by the reduced processing time and

solvent use. The study variables were: extraction time, type of solvent, solid to solvent ratio, particle size, extraction temperature, ultrasonic power, and ultrasonic frequency. In this research, Peleg's model was used to describe the extraction kinetics can be written as follows:

$$C_t = t/K_1+K_2t \quad (5)$$

where C_t is the concentration of curcumin at time t (mg curcumin/g C. amada powder), K_1 is Peleg's rate constant (min. g/mg) and K_2 is Peleg's capacity constant (g/mg).

The results of the extraction time and the type of solvent showed that the curcumin extraction was very fast for the first 15 minutes. After that, the production of curcumin grew gradually over time until 1 hour will become stable, coupled with ethanol as a solvent, the maximum curcumin extraction is 72% (9.18 mg/g) as shown in **Figure 16(A)**. Due to its stronger polarity, lower viscosity, and surface tension, ethanol is a good solvent. In addition, ethanol is generally recognized as a safe (GRAS) solvent as well. The effect of extraction temperature showed that increasing temperature resulted in higher extraction yield as the solute diffusivity as well as solubility rises. A temperature of 55 °C resulted in yielded the highest curcumin extraction, but the degradation of the active ingredient could occur. Therefore, the optimum temperature for the experiment was 35 ° C, resulting in a curcumin extraction of 9.18 mg/g as shown in **Figure 16(B)**. The effect of the solid to the solvent ratio in extraction showed that when the ratio increased from 1:15 to 1:25, the extraction increased and then decreased when the ratio was higher than 1:25, which possibly due to the limitation of mass transfer. Therefore, the optimum solid to solvent ratio was 1:25 resulted in yielded the highest curcumin extraction of 9.18 mg/g (72%) as shown in **Figure 16(C)**. The effect of particle size on extraction was

found that as the particle size increased, the extraction yield decreased as the available surface area decreased resulting in less contact with the solvent. Therefore, the particle size suitable for the experiment was 0.09 mm, resulting in the highest extraction yield as shown in **Figure 16(D)**. The effect of ultrasonic power on extraction was found that as the ultrasonic power increased, the yield of curcumin in extraction increased. Due to the increased ultrasonic power, the cell wall is cracked and the diffusion of the solvent is increased. Although high ultrasonic power resulting in the highest extraction yield if the ultrasonic power is too high, the system temperature cannot be controlled. Therefore, in the present research, the optimal ultrasonic power for the experiment is 250 W as shown in **Figure 16(E)**. All experiments were performed at a frequency of 22 kHz and Peleg's model can be used to accurately and satisfactorily describe the extraction kinetics of curcumin. This research compares the extraction methods. Soxhlet can extract curcumin up to 12.75 mg / g (considered to be 100%), while ultrasound-assisted extraction, curcumin extracted 9.18 mg / g (72%), but Soxhlet extraction is performed at much higher temperatures and for a very long time. Therefore, using ultrasound to assist extraction is more advantageous in terms of extraction time and energy.

Hui Wang [38] et al. [38], were interested in the extraction of bioactive compounds (piceid, resveratrol, and emodin) in Polygonum cuspidatum by microwave-assisted aqueous two-phase extraction (MAATPE). Considering the partition behavior of piceid, resveratrol, and emodin in ethanol/$(NH_4)_2SO_4$ aqueous two-phase system, it was found that the partition coefficient and recovery of piceid, resveratrol, and emodin were significantly increased with the increasing concentration of $(NH_4)_2SO_4$ and ethanol, indicating that almost all of the three compounds are ethanol-soluble chemicals that tend to be concentrated in the top phase. Therefore, the ethanol/$(NH_4)_2SO_4$ system is suitable for all three components. Therefore, the

variables used to investigate were phase composition (concentration of $(NH_4)_2SO_4$ and ethanol) and extraction time.

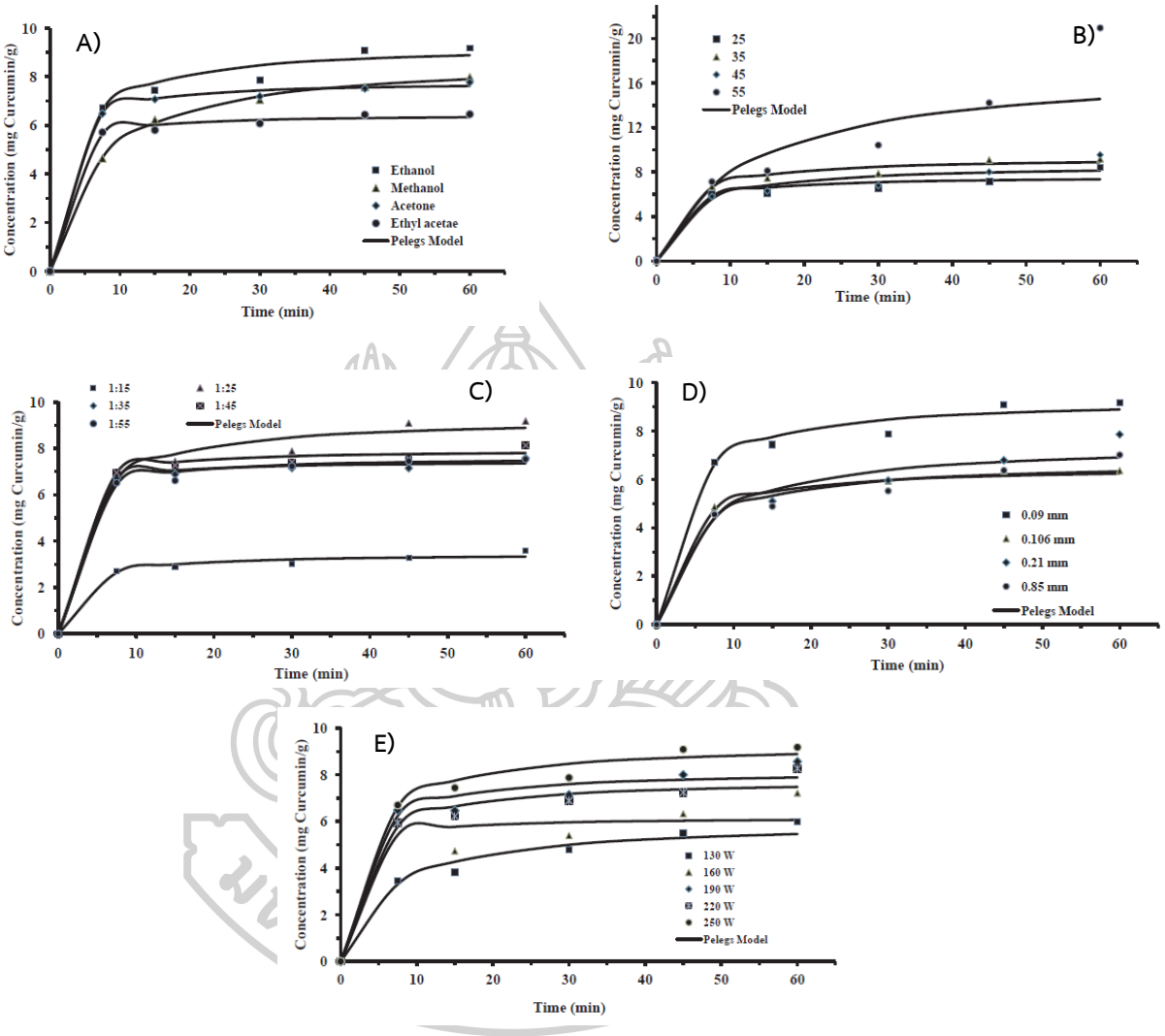

Figure 16 *Effect of different variables on curcumin extraction A) solvents and time B) temperature C) solid to solvent ratio D) particle size and E) ultrasonic power.*

The effect of phase composition showed that the system composed of 25% (w/w) ethanol and 21% (w/w) $(NH_4)_2SO_4$, the highest yield of piceid and resveratrol to 86% and emodin 55%. The yield of emodin is lowest because emodin is more hydrophobic than piceid and resveratrol, and its solubility is substantially lower in ethanol. The influence of extraction time showed that the yield of piceid and

resveratrol slightly increased with increasing extraction time, while that of emodin significantly increased until 60 s. Therefore, 60 s extraction time is optimal for the highest yield. In addition, MAATPE, MAE, and heat-reflux extraction were compared. By comparison, the piceid yields of all three methods were equal, but the yields of resveratrol and emodin were highest in the MAATPE method as shown in **Figure 17**.

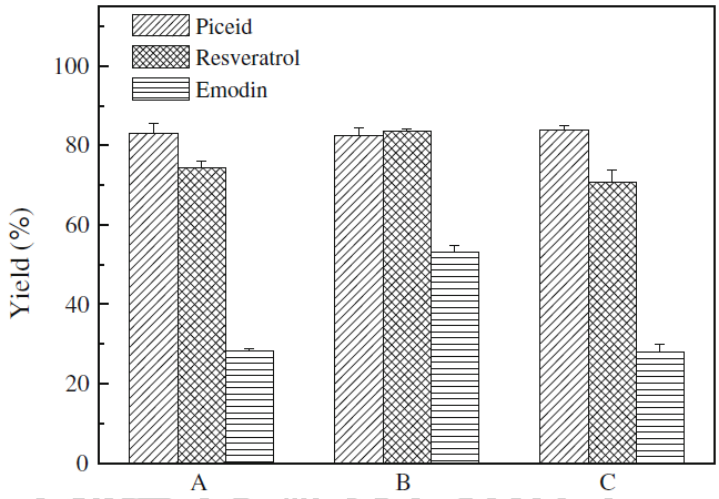

Figure 17 *Comparison of extraction methods A) MAE, B) MAATPE and C) Heat reflux extraction.*

However, the use of MAATPE has more advantages compared to heat-reflux extraction and MAE such as quick time, utilizing little solvent, low cost, high efficiency, and easy alcohol recovery by evaporation. Therefore, MAATPE is a prospective method of extraction and subsequent purification of the target product suitable for future development.

Tao Guo et al. [39], interested in the extraction of lignans (fargesin, sesamin, and L-asarinin) from Zanthoxylum armatum by the ultrasound-assisted aqueous two-phase system. Optimal conditions for this extraction were identified via the response

surface method (RSM) for process optimization and an artificial neural network (ANN) for process modeling.

The ATPS system was selected based on the phase composition (concentration of $(NH_4)_2SO_4$ and n-propanol). The effect of the $(NH_4)_2SO_4$ concentration showed that the recovery of lignans increased with increased concentration of $(NH_4)_2SO_4$ and highest at 24% $(NH_4)_2SO_4$ and decreased, maybe that the water entered the lower phase, while the concentration of n-propanol became too high in the upper phase with the increasing salt in the ATPS. The effect of the n-propanol concentration showed that the ATPS with 24% $(NH_4)_2SO_4$ and 20% n-propanol gained the highest recovery of lignans, maybe that the ATPS reached a sufficient upper phase/lower phase ratio leading the lignans to partition into the upper phase containing n-propanol readily. Therefore, ATPS with 24% $(NH_4)_2SO_4$ with 20% n-propanol was selected for further investigation.

Once the required ATPS has been obtained, studied the variables for the ultrasonic to enhance the extraction efficiency were ultrasonic time, ultrasonic temperature, and solvent to solid ratio using the response surface method and an artificial neural network, the results of which are shown in **Table 6.** From the results of the experiment, it was concluded indicated the solvent to solid ratio was the most critical factor controlling the yield of the three lignans.

In addition, a comparison between UAE-ATPS and heat reflux extraction. Extraction conditions of ultrasound-assisted aqueous two-phase system: 24% $(NH_4)_2SO_4$/20% n-propanol, solvent to solid ratio 15:1 (w/w), 55 min, 40 °C and 250 W, while extraction conditions of heat reflux extraction were extracted twice: 95% ethanol with a solvent to solids ratio of 12:1 (v/w) for 1 hr. The results showed that the use of ATPS resulted in a higher lignan yield than the use of reflux extraction as shown in **Table 7**, thus concluding that ATPS was a good alternative for the efficient extraction and enhancement of Z. armatum lignans.

Table 6 *Experimental design of the BBD of the yield of lignans and the ANN predicted values.*

Test Set	Extraction Conditions			Actual Yield (mg/g Sample)			ANN Predicted Values		
	X_1	X_2	X_3	Far	Ses	Asa	Far	Ses	Asa
1	1	0	-1	1.541	0.751	0.953	1.541	0.751	0.952
2	0	0	0	1.358	0.686	0.922	1.398	0.703	0.949
3	0	0	0	1.386	0.719	0.961	1.398	0.703	0.949
4	0	1	1	1.194	0.649	0.83	1.193	0.649	0.831
5	0	0	0	1.423	0.692	0.961	1.398	0.703	0.949
6	1	1	0	1.358	0.727	1.062	1.355	0.751	0.124
7	0	-1	1	1.190	0.641	0.897	1.189	0.641	0.896
8	0	1	-1	1.259	0.697	0.868	1.259	0.697	0.869
9	0	-1	-1	1.243	0.638	0.845	1.301	0.715	0.738
10	1	0	0	1.305	0.727	1.124	1.264	0.727	1.124
11	1	-1	0	1.349	0.751	1.093	1.305	0.741	1.100
12	-1	1	0	1.100	0.588	0.729	1.126	0.583	0.704
13	0	0	0	1.423	0.706	0.977	1.398	0.703	0.949
14	-1	0	-1	1.258	0.625	0.701	1.161	0.582	0.702
15	0	0	0	1.391	0.712	0.915	1.398	0.703	0.948
16	-1	-1	0	1.309	0.582	0.711	1.314	0.582	0.711
17	-1	0	1	1.190	0.597	0.716	1.122	0.583	0.704

X_1: Solvent to Solid, X_2: Ultrasound Temperature, and X_3: Ultrasound Time.

Table 7 *Comparison of ATPS and heat reflux extraction.*

Method	Yield of Extract (mg/g)	Fargesin		Sesamin		Asarinin	
		Yield (mg/g)	Purity (%)	Yield (mg/g)	Purity (%)	Yield (mg/g)	Purity (%)
UAE-ATPS	71.57 ± 1.82	1.591 ± 0.16	2.222	0.763 ± 0.07	1.066	1.133 ± 0.10	1.583
Heat reflux	89.80 ± 1.33	1.382 ± 0.12	1.539	0.771 ± 0.05	0.858	1.125 ± 0.09	1.253

CHAPTER IV

RESEARCH METHODOLOGY

4.1 Materials and apparatus

Turmeric was purchased from a vegetable garden, Nakhon Pathom, Thailand. Curcumin standard (99.5%) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were bought from Sigma-Aldrich. Ethanol Absolute (99.93%) and Ammonium sulfate (≥ 99.5%) were bought from CT Chemical Co., Ltd. The deionized water from the Department of Chemical Engineering, Faculty of Engineering and Industrial Technology, Silpakorn University.

The hotplate & magnetic stirrer was purchased from Misung scientific Co., Ltd., Korea. The Centrifuge was purchased from China. The detecting instrument was HPLC (YL9150 HPLC) was purchased from YL Instruments Co., Ltd., Korea, and UV-VIS spectrophotometer (T92+ UV spectrometer) was purchased from PG Instruments Ltd., UK.

4.2 Extraction of curcumin

4.2.1 The effect of parameter

The turmeric was dried in the oven at 60 °C for 48 hr. The dried turmeric was blend thoroughly with blender and sorted size with a 60-mesh sieve (less than 250 microns). First step, extract curcumin by using ethanol, ammonium sulphate, and DI water in the ratio of 20:32:48 %w/w to find the equilibrium time for using in another extraction batches. Second step, the ratio of turmeric powder, ethanol, ammonium sulphate, DI water, and temperature was depended on research scope and the experimental design of Central Composite Design. Ammonium sulphate was dissolved with DI water with a stirrer until the mixture was homogeneous. Poured

ethanol into the mixture and heated up the mixture up to the design temperature then put the turmeric powder, stirred according to the time obtained from the experimental design. After the extraction, the mixture was held split into two phases fully. The top phase was an ethanol-rich phase, and the bottom phase was a salt-rich phase. The top phase should be centrifuged for 15 min to separate the turmeric powder. The top phase was again separated with a 0.45 μm nylon membrane filter before analysis using UV-Vis and HPLC.

The experimental design of Central Composite Design determines factors with variables: ethanol concentration represented by A, $(NH_4)_2SO_4$ concentration represented by B, solvent to solid ratio represented by C, and extraction temperature represented by D. The determination of the values of the experimental variables and the experimental design of Central Composite Design as shown in **Table 8** and **Table 9** respectively.

Table 8 *The determination of the values of the experimental variables.*

Factors	Variable	Level				
		-2	-1	0	1	2
Ethanol concentration (%)	A	15	17	19	21	23
$(NH_4)_2SO_4$ concentration (%)	B	25	28	31	34	37
Solid to solvent ratio (g/ml)	C	3:100	3.5:100	4:100	4.5:100	5:100
Extraction temperature (°C)	D	35	40	45	50	55

Table 9 *The experimental design of Central Composite Design.*

	Ethanol concentration (A)	$(NH_4)_2SO_4$ concentration (B)	Solvent to solid ratio (C)	Extraction temperature (D)
1	-1	-1	-1	-1
2	1	-1	-1	-1
3	-1	1	-1	-1
4	1	1	-1	-1
5	-1	-1	1	-1
6	1	-1	1	-1
7	-1	1	1	-1
8	1	1	1	-1
9	-1	-1	-1	1
10	1	-1	-1	1
11	-1	1	-1	1
12	1	1	-1	1
13	-1	-1	1	1
14	1	-1	1	1
15	-1	1	1	1
16	1	1	1	1
17	-2	0	0	0
18	2	0	0	0
19	0	-2	0	0
20	0	2	0	0
21	0	0	-2	0
22	0	0	2	0
23	0	0	0	-2
24	0	0	0	2
25	0	0	0	0

Table 9 (cont.) *The experimental design of Central Composite Design.*

	Ethanol concentration (A)	$(NH_4)_2SO_4$ concentration (B)	Solvent to solid ratio (C)	Extraction temperature (D)
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0

****Repeat the experiment 3 times for accurate results.*

4.2.2 The kinetic studied

The experiment was performed using the best conditions from the previous experiment by divided the experiment into 6 batches, extracted the curcumin for 30 min to 180 min., held the mixture, centrifuged, and filtered, respectively. The top phase was analysis using UV-Vis.

4.3 Determination of total curcumin content and percentage yield (% Yield)

To determine the total curcumin content in turmeric powder, 1 g of turmeric powder was extracted several times repeatedly with 20 ml of ethanol absolute until absorbance of the extract at 426 nm was lower than the instrumental noise which suggests that the curcumin in the solvent was very close to zero. Finally, total curcumin content was measured by adding curcumin content in the solvent from every repeated sample. The percentage yield (% Yield) was derived using the following equation:

$$\%\text{Yield} = \frac{\text{Concentration (mg/mL) of curcumin of the upper phase x Volume (mL) of the upper phase}}{\text{The mass of curcumin in turmeric powder (mg)}} \times 100$$

4.4 Analysis of extracted curcumin

The extracted curcumin sample was analyzed using ultraviolet-visible spectrophotometry (UV-Vis) and high-performance liquid chromatography (HPLC). To avoid deterioration and isomerization, curcumin standard stock solution was stored in amber bottles, wrapped in aluminum foil, and stored in the freezer at 5 °C. All analysis was done in duplicate and the absence of direct sunlight.

4.4.1 Ultraviolet-Visible spectroscopy (UV-VIS) analysis

The curcumin content in the extracts was measured using UV-VIS spectrophotometer with ethanol 50 %v/v as the blank at 427 nm to minimize the interference from other carotenoids. For calibration, a stock solution of commercially standard curcumin was prepared by dissolving 0.7 mg of standard curcumin in 7 mL ethanol 50 %v/v (100 mg/L of stock standard solution). The stock solution was diluted with ethanol 50 %v/v to obtain a concentration between 2.5 – 15 mg/L. The calibration curve was plotted between absorbance and concentration with a near linearity equal to 1.

4.4.2 High-performance liquid chromatography (HPLC) analysis

The purity of curcumin in the extracts was evaluated using HPLC equipment equipped with a C18 column (250 mm x 4.6 mm x 5 µm, Phenomenex, USA) including C18 guard column (10 mm x 4.6 mm x 5 µm). The mobile phase was comprised of acetonitrile and acetic acid (45:55, v/v). Before injecting the samples into HPLC, the mobile phase was filtered through a 0.45 µm nylon membrane filter. The mobile phase flow rate was 1.0 ml/min, the injection volume was 20 µL. All measurements were performed at room temperature and a detection wavelength at 425 nm, the column temperature was 35 °C.

4.5 Determination of antioxidant activity

The antioxidant activity of the extracts obtained under optimized conditions of the extraction process was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, for which the readings of the absorbance of the reaction mixtures at 517 nm. The reference compound used was gallic acid. The percentage of free radical scavenging was determined for different concentrations of the extract was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where $A_{control}$ = Absorbance of the control solution as a control; A_{sample} = Absorbance of sample solution.

CHAPTER V

RESULTS AND DISCUSSION

This research aimed to optimize the conditions for the extraction of curcumin, an important biological activity of turmeric using ATPS. The results were analyzed using Minitab19 program to analyze the factors affecting the extraction compared to the data obtained from the regression equation, showing the optimum conditions of extraction and the effect of the main factor on the extraction of curcumin from turmeric. Also, the antioxidant and kinetic on extraction were evaluated.

5.1 Effect of extraction variables on curcumin yield.

After extraction, the curcumin in the upper phase was examined by the HPLC. The experimental results obtained by central composite design are shown in **Table 10.**

Table 10 Extraction yields of the curcuminoids at different conditions according to the central composite design. All extractions were analyzed via HPLC.

Run	Curcumin (mg/g)	Demethoxycurcumin (mg/g)	Bisdemethoxycurcumin (mg/g)
1	21.006	13.470	13.017
2	21.165	10.963	8.972
3	24.526	13.767	12.578
4	23.234	10.934	11.088
5	26.253	13.540	14.834
6	27.997	12.987	13.227
7	27.594	12.865	13.245
8	23.690	10.526	10.734
9	28.559	10.642	10.575
10	27.865	12.777	12.932

Table 10 (cont.) Extraction yields of the curcuminoids at different conditions according to the central composite design. All extractions were analyzed via HPLC.

Run	Curcumin (mg/g)	Demethoxycurcumin (mg/g)	Bisdemethoxycurcumin (mg/g)
11	29.871	13.839	14.109
12	26.851	12.015	12.592
13	29.066	19.342	21.844
14	29.229	13.192	13.307
15	34.270	14.878	16.101
16	35.306	15.839	16.480
17	32.859	19.528	21.583
18	28.340	12.987	13.313
19	24.721	12.759	13.791
20	20.580	9.324	9.578
21	27.704	12.605	12.950
22	30.692	13.753	14.525
23	20.464	11.590	12.166
24	41.846	21.041	17.298
25	29.676	13.757	14.097
26	27.468	12.765	13.047
27	27.267	12.605	12.848
28	26.251	12.169	12.385
29	31.690	14.607	15.009
30	29.169	13.444	13.715
31	29.200	13.474	13.655

The results obtained for statistical analysis (ANOVA) with Minitab19 program will show the results of the main effects affecting the extraction of curcumin from turmeric using ATPS as shown in **Figure 18.**

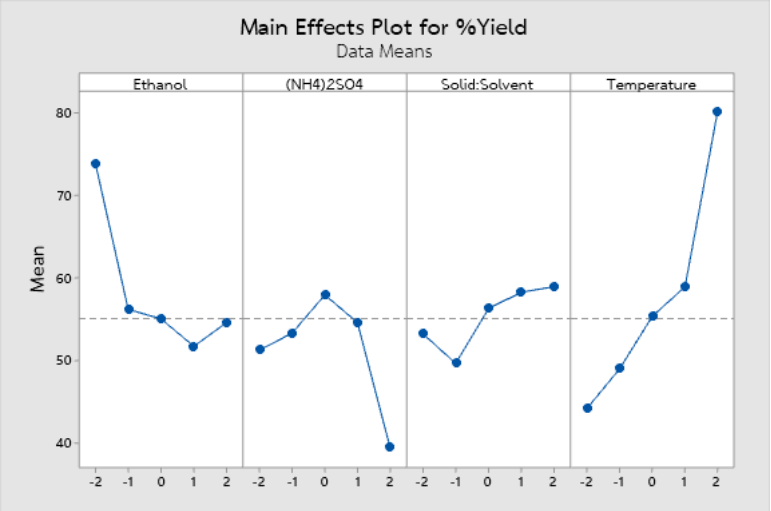

Figure 18 *The main effects of curcumin extraction from turmeric.*

The main effect on the extraction of curcumin from turmeric using ATPS consisted of 4 factors: ethanol concentration, ammonium sulfate concentration, solid to solvent ratio, and extraction temperature.

The effect of ethanol concentration was studied at different concentrations such as 15, 17, 19, 21, 23% (w/w) with experimental conditions according to the experimental design of CCD. In this study, ethanol was used as a suitable solvent for the extraction of a desired natural constituent [31]. Ethanol was selected on solubility, polarity, overall cost, safety, and ethanol can break down the cell walls as well [37]. The results showed that lower ethanol concentrations resulted in higher extraction yield, which could mean higher water content. That means water is a suitable curcumin extractant. Therefore, the higher water content has a beneficial

effect on the extraction capacity of curcumin, but the extracted curcumin is not soluble in the aqueous phase and result in the curcumin substantially transported to the ethanol-rich phase.

At the same time, the effect of ammonium sulfate concentration was examined at different concentrations such as 25, 2 8, 3 1, 34, 37% (w/w) with experimental conditions according to the experimental design of CCD. The increased ammonium sulfate concentration results in high extraction yield. As the increased ammonium sulfate concentration resulted in a greater polarization difference between the upper phase and lower phase, curcumin was better transferred to the ethanol-rich phase. The increased concentration of ammonium sulfate also helps to exclude or crowd proteins and carbohydrates from the products resulting in higher purity [40].

The extraction yield was improved when the ammonium sulfate concentration was greater. From **Figure 18**, the extraction yield was highest when the ammonium sulfate concentration was 3 0 . 7 0 % and the extraction yield was decreased when the ammonium sulfate concentration was more than 3 0 . 7 0 % because ammonium sulfate is saturated until it cannot be completely dissolved in the salt-rich phase.

The effect of solid (amount of turmeric powder) to the solvent ratio in ATPS extraction by varying this ratio as 3:100, 3.5:100, 4:100, 4.5:100, and 5:100 (w/v) with experimental conditions according to the experimental design of CCD. From the experimental results shown in **Figure 18**, curcumin extraction tended to increase when the solid (amount of turmeric powder) to the solvent ratio increased because increasing the amount of turmeric powder increases the amount of curcumin that can be extracted as well. In other words, if the solid (amount of turmeric powder) to the solvent ratio was increased above 5:100 at optimal extraction conditions using

ATPS, extraction of curcumin was increased [37]. Thus, 5 : 1 0 0 (w/v) ratio of solids (turmeric powder content) to solvent was the optimum value for this experiment.

To study the effect of extraction temperature using ATPS, experiments were carried out over the range of 35 - 55 °C with experimental conditions according to the experimental design of CCD. From the experimental results shown in **Figure 18**, An increase in temperature has a beneficial effect on the extraction capacity because the increase in temperature results in a decrease in the density and viscosity of the solvent, and may result in the opening of cell matrix helps the solvent more easily into the turmeric and better mass transfer resulting in higher extraction rates [37, 41-43]. Hence, 55 °C was the optimum value for this experiment.

From the main effects study, the solid (amount of turmeric powder) to the solvent ratio significantly affected the extraction yield, but not significantly compared to the main effects of ethanol concentration, ammonium sulfate concentration, and extraction temperature. Thus, it was concluded that the determination of the four factors; ethanol concentration, ammonium sulfate concentration, solid (amount of turmeric powder) to the solvent ratio, and extraction temperature affect the extraction yield. Therefore, further consideration in the Interaction Plot is that if the interaction between the factors is significant, it can influence the effect analysis of the main effects as shown in **Figure 19**.

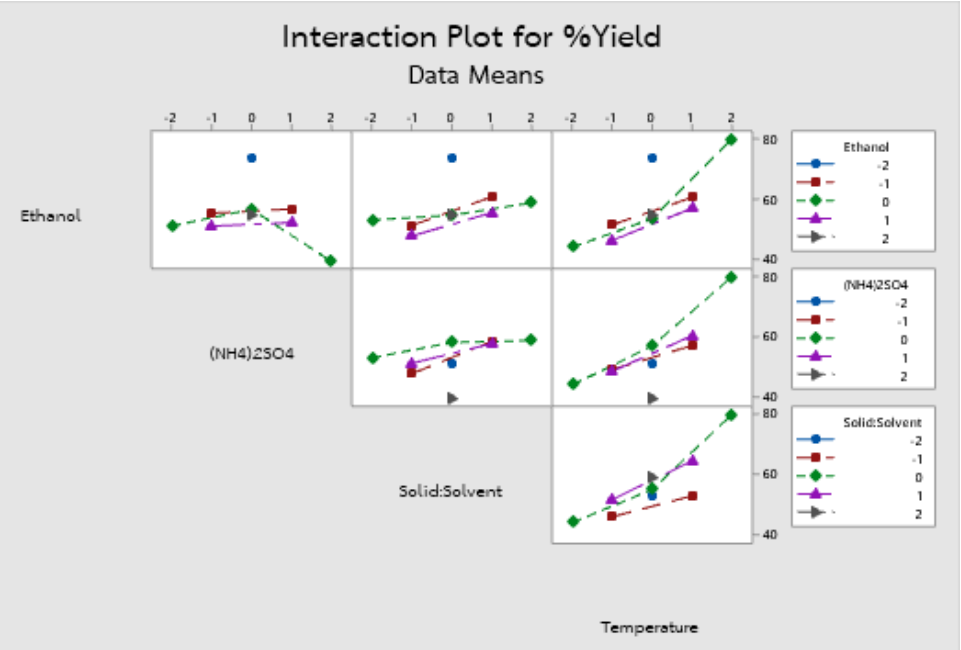

Figure 19 *Interaction Plot of curcumin extraction from turmeric.*

The interaction between ethanol concentration and ammonium sulfate concentration, ethanol concentrations of 17% and 21% with increasing ammonium sulfate concentration resulted in a slight increase in extraction yield. As for the ethanol concentration of 19%, the extraction capacity was higher when the ammonium sulfate concentration was increased up to 31% and the extraction capacity was worse when the ammonium sulfate concentration was above 31%. Whereas the ethanol concentration of 15% and 23% when increasing the ammonium sulfate concentration did not change the extraction yield.

The interaction between ethanol concentration and the solid (amount of turmeric powder) to the solvent ratio, ethanol concentrations of 17%, 19%, and 21% with increasing solid to solvent ratio resulted in a slight increase in extraction yield. Whereas the ethanol concentration of 15% and 23% when increasing solid to solvent ratio did not change the extraction yield.

The interaction between ethanol concentration and extraction temperature, ethanol concentrations of 17% and 21% with increasing extraction temperature resulted in a slight increase in extraction yield. For an ethanol concentration of 19%, extraction capacity was slightly increased at an extraction temperature range of 35°C - 45°C and extraction capacity was very high at an extraction temperature range of 45°C - 55°C. Whereas the ethanol concentration of 15% and 23% when increasing extraction temperature did not change the extraction yield.

The interaction between ammonium sulfate concentration and solid (amount of turmeric powder) to solvent ratio, ammonium sulfate concentration of 28%, 31%, and 34% with increasing solid to solvent ratio resulted in a slight increase in extraction yield. Whereas the ammonium sulfate concentration of 25% and 37% when increasing solid to solvent ratio did not change the extraction yield.

The interaction between ammonium sulfate concentration and extraction temperature, ammonium sulfate concentration of 28%, and 34% with increasing extraction temperature resulted in a slight increase in extraction yield. As for an ammonium sulfate concentration of 31%, extraction capacity was slightly increased at an extraction temperature range of 35°C - 45°C and extraction capacity was very high at an extraction temperature range of 45°C - 55°C. Whereas the ammonium sulfate concentration of 25% and 37% when increasing solid to solvent ratio did not change the extraction yield.

The interaction between solid (amount of turmeric powder) to solvent ratio and extraction temperature, solid to solvent ratio of 3.5:100, and 4.5:100 with increasing extraction temperature resulted in a slight increase in extraction yield. As for the solid to solvent ratio of 4:100, extraction capacity was slightly increased at an extraction temperature range of 35°C - 45°C and extraction capacity was very high at an extraction temperature range of 45°C - 55°C. Whereas the solid to solvent ratios of

3:100, and 5:100 when increasing solid to solvent ratio did not change the extraction yield.

In the analysis of the main effect and the interaction of factors affecting the extraction of curcumin from turmeric using ATPS, the regression equation was created using the calculation from the Minitab19 program to obtain the coefficient of each factor. Therefore, yield prediction equations for curcumin extraction using ATPS are obtained as shown in **Eq (8)**.

$$\%Yield = 55.47 - 3.11A - 0.56B + 3.35C + 6.30D + 1.71A^2 - 3.03B^2 - 0.34C^2 + 1.18D^2 - 0.05A*B - 0.42A*C + 0.40A*D - 1.05B*C + 0.97B*D + 1.47C*D \quad (8)$$

5.2 Optimization of extraction of curcumin from turmeric using ATPS

The previously obtained regression equations can be eliminated for statistically insignificant variables by determining variables with statistically significant values less than 0.05 (P-value < 0.05), which can be seen in the analysis of variance (ANOVA) table as shown in **Table 11**. Therefore, when statistically insignificant variables were eliminated, yield prediction equations for curcumin extraction using ATPS were obtained as follows:

$$\%Yield = 55.47 - 3.11A + 3.35C + 6.30D - 3.03B^2 \quad (9)$$

where A, B, C, and D are ethanol concentration, $(NH_4)_2SO_4$ concentration, solvent to solid ratio, and extraction temperature.

Table 11 Analysis of variance (ANOVA) for a second order model.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	1967.32	140.523	4.46	0.003
Linear	4	1461.04	365.261	11.59	0.000
A	1	231.56	231.560	7.35	**0.015**
B	1	7.49	7.492	0.24	0.632
C	1	269.47	269.473	8.55	**0.010**
D	1	952.52	952.518	30.23	**0.000**
Square	4	433.88	108.471	3.44	0.033
A*A	1	83.15	83.146	2.64	0.124
B*B	1	261.97	261.973	8.31	**0.011**
C*C	1	3.35	3.351	0.11	0.749
D*D	1	39.80	39.798	1.26	0.278
2-Way	6	72.39	12.065	0.38	0.879
Interaction	1	0.04	0.042	0.00	0.971
A*B	1	2.85	2.846	0.09	0.768
A*C	1	2.62	2.615	0.08	0.777
A*D	1	17.55	17.548	0.56	0.466
B*C	1	14.96	14.956	0.47	0.501
B*D	1	34.38	34.384	1.09	0.312
C*D					
Error	16	504.17	31.511		
Lack-of-Fit	10	430.28	43.028	3.49	0.070
Pure Error	6	73.90	12.316		
Total	30	2471.49			

When the statistically insignificant variables of the equations were eliminated and the accuracy of the predictive equations was checked, the equations were accurate to 92.45% as shown in **Table 12**.

Table 12 *The accuracy of the prediction equations compared to the experimental results.*

Run	A	B	C	D	%Yield (mg/g turmeric)		Accuracy (%)
					Exp.	Predicted	
1	-1	-1	-1	-1	*47.493*	*45.900*	*96.53*
2	1	-1	-1	-1	*41.100*	*39.680*	*96.42*
3	-1	1	-1	-1	*50.871*	*45.900*	*89.17*
4	1	1	-1	-1	*45.257*	*39.680*	*85.95*
5	-1	-1	1	-1	*54.628*	*52.600*	*96.15*
6	1	-1	1	-1	*54.211*	*46.380*	*83.12*
7	-1	1	1	-1	*53.703*	*52.600*	*97.90*
8	1	1	1	-1	*44.950*	*46.380*	*96.92*
9	-1	-1	-1	1	*49.776*	*58.500*	*85.09*
10	1	-1	-1	1	*53.574*	*52.280*	*97.53*
11	-1	1	-1	1	*57.819*	*58.500*	*98.84*
12	1	1	-1	1	*51.458*	*52.280*	*98.43*
13	-1	-1	1	1	*70.252*	*65.200*	*92.25*
14	1	-1	1	1	*55.729*	*58.980*	*94.49*
15	-1	1	1	1	*65.248*	*65.200*	*99.93*
16	1	1	1	1	*67.624*	*58.980*	*85.34*
17	-2	0	0	0	*73.970*	*61.690*	*80.09*
18	2	0	0	0	*54.639*	*49.250*	*89.06*
19	0	-2	0	0	*51.271*	*43.350*	*81.73*
20	0	2	0	0	*39.482*	*43.350*	*91.08*
21	0	0	-2	0	*53.259*	*48.770*	*90.80*
22	0	0	2	0	*58.970*	*62.170*	*94.85*
23	0	0	0	-2	*44.220*	*42.870*	*96.85*

Table 12 (cont.) *The accuracy of the prediction equations compared to the experimental results.*

Run	A	B	C	D	%Yield (mg/g turmeric)		Accuracy (%)
					Exp.	Predicted	
24	*0*	*0*	*0*	*2*	*80.185*	*68.070*	*82.20*
25	0	0	0	0	*57.529*	*55.470*	*96.29*
26	0	0	0	0	*53.281*	*55.470*	*96.05*
27	0	0	0	0	*52.721*	*55.470*	*95.04*
28	0	0	0	0	*50.805*	*55.470*	*91.59*
29	0	0	0	0	*61.307*	*55.470*	*89.48*
30	0	0	0	0	*56.327*	*55.470*	*98.45*
31	0	0	0	0	*56.328*	*55.470*	*98.45*

5.2.1 Response surface analysis

The contour plots based on response surface analysis in **Figure. 20** revealed the effect of variable pairs indicating more than 80% extraction yield was the effect of extraction temperature and ethanol concentration, apparently at ethanol concentrations decreased and increased extraction temperatures resulting in curcumin extraction yields greatly increased. Therefore, concluded that the effect of extraction temperature and ethanol concentration had the greatest effect on extraction yield.

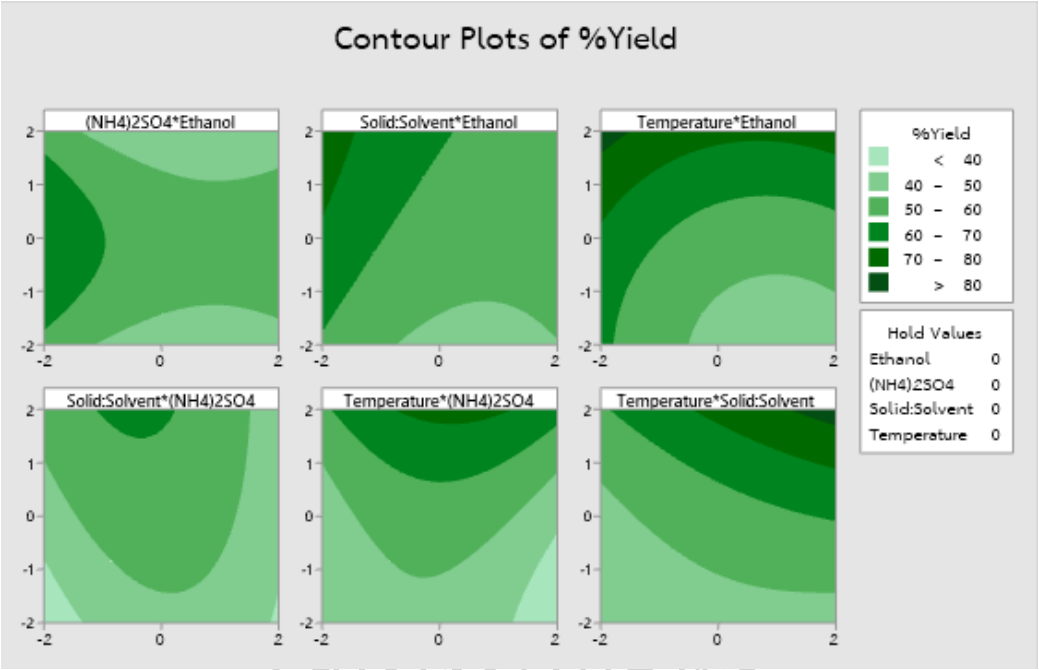

Figure 20 Contour plot of curcumin extraction from turmeric.

5.2.2 Optimization of extraction conditions

The optimal extraction conditions for curcumin extraction can be obtained from the response optimization plot for the extraction parameters using the Response Optimizer function of Minitab19, shown in **Figure 21.**

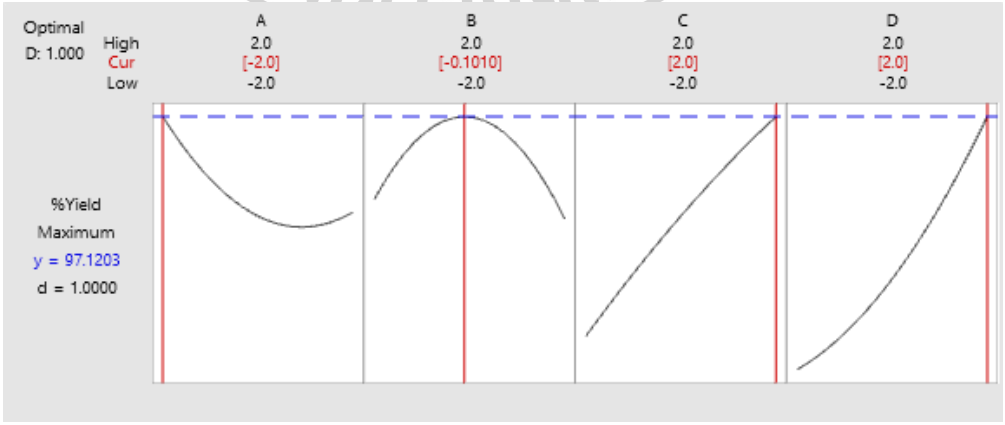

Figure 21 Response optimization plot of curcumin extraction from turmeric.

The values of the independent variables at the optimum extraction conditions were A = -2, B = -0.1010, C = 2, and D = 2. This means that the optimum extraction conditions were 15% ethanol concentration, 30.697% $(NH_4)_2SO_4$ concentration, a solid to solvent ratio of 5:100, and extraction temperature of 55 °C predicting an extraction yield of curcumin was accumulated in the upper phase of 97.12% or 101.673 mg/g of turmeric.

The experiment was carried out under the predicted optimal extraction condition, the extraction of curcumin accumulated in the upper phase was actually 93.96% or 98.370 mg/g of turmeric. Thus, the optimum extraction conditions anticipated by the model was in good agreement with the actual experimental results to be the best extraction conditions in the research.

5.3 Antioxidant activity of curcumin from turmeric using ATPS

In this work, the antioxidant activity was considered in terms of %inhibition. Examine the %inhibition of 3 samples with the highest curcumin extraction yield: Run17, Run24, and RunMaximum, as shown in **Table 13**. When comparing the inhibition percentages between Run17 and Run24, the percentage inhibition of Run24 was lower than that of Run17, while curcumin concentrations from the extraction were close together. This was due to Run24 using a higher extraction temperature, as a result, the temperature-sensitive curcumin was degraded in large quantities. However, the RunMaximum experiment with extraction temperatures higher than Run24 showed the highest inhibition percentages since RunMaximum was able to extract the highest amount of curcumin that enough to be able to remain in large quantities even after being destroyed by temperature with the percentage inhibition was as high as 86.47%.

Therefore, it can be stated that the extraction conditions of RunMaximum was able to provide the highest curcumin extraction yield and the highest antioxidant activity.

Table 13 %inhibition of curcumin extraction from turmeric.

Run	% Inhibition					
	1	2	3	Mean	SD	%RSD
17	81.12	80.95	80.46	80.84	0.34	0.43
24	78.55	79.11	78.90	78.85	0.28	0.35
Maximum	86.41	86.44	86.55	**86.47**	0.07	0.08

5.4 Kinetic study on extraction of curcumin from turmeric using ATPS

The kinetic description of solid-liquid extraction assisted in the design, optimization, and simulate the extraction process. The kinetics were studied where the variable studied was temperature and determined another variable is constant. temperatures studied were 35, 45, and 55 °C, respectively. The experimental results are shown in **Figures 22 - 23**.

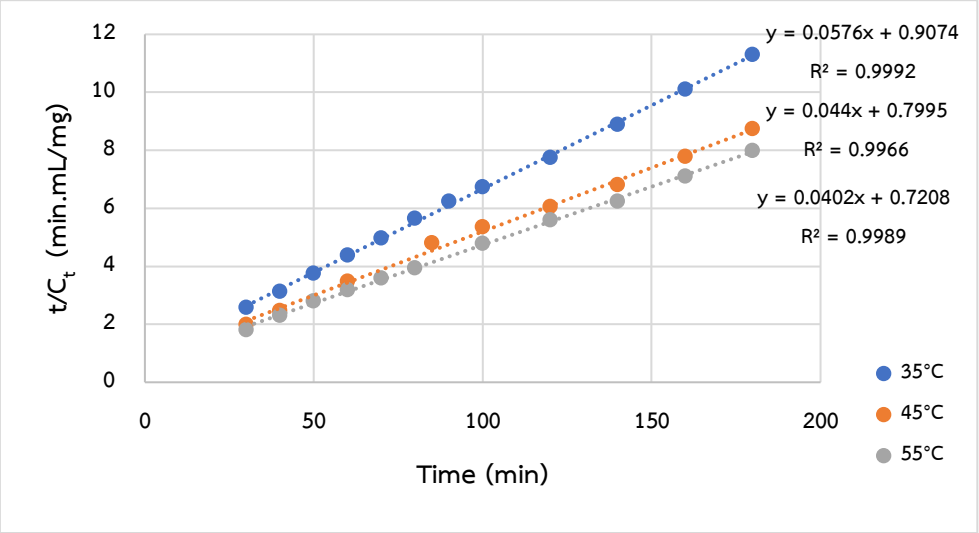

***Figure 22** Linear equations of kinetics at different temperatures of curcumin extraction from turmeric.*

The study of extraction kinetics according to various researches revealed that the solid-liquid extraction process can be described from the pseudo-second-order model. The general form can be written as follows;

$$\frac{dC_t}{dt} = k(C_e - C_t)^2 \quad (10)$$

where k is the extraction constant (g/mg.min)

C_e is the amount of curcumin at the equilibrium point (mg/1 g turmeric)

C_t is the amount of curcumin at any time (mg/1 g turmeric).

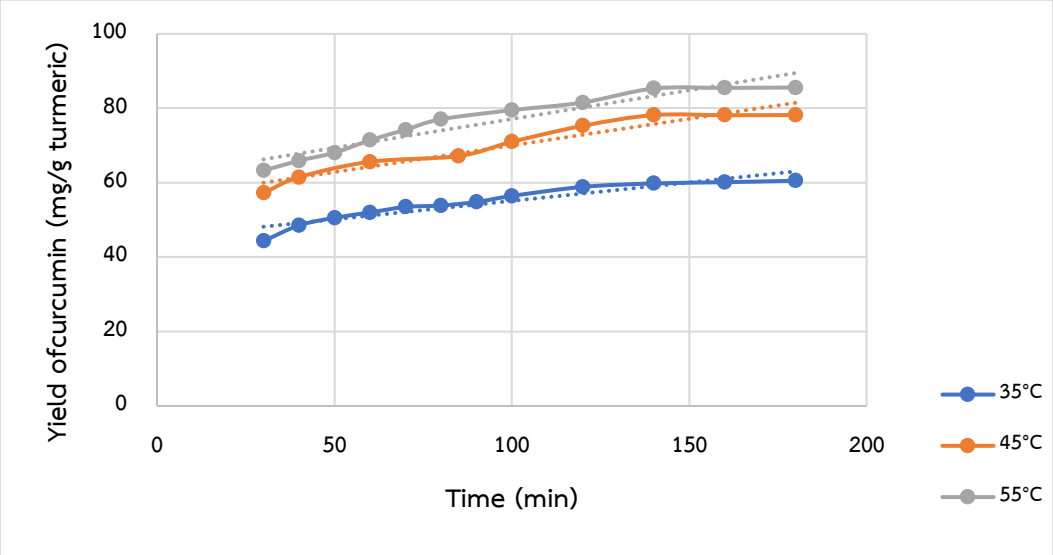

Figure 23 *Effect of temperature on yield of curcumin extraction from turmeric.*

When integrating the equation or writing it as a linear equation as follows:

$$\frac{t}{C_t} = \frac{1}{k \cdot C_e^2} + \frac{t}{C_e} \quad (11)$$

When linear equations were obtained at different extraction temperatures, the extraction constants were obtained as shown in **Table 14**.

Table 14 *The extraction kinetic parameters by the model and results from real experiments of curcumin from turmeric.*

Temperature (°C)	k_2 (mg/ml-min)	C_{eq} (mg/ml)[a]	C_{eq} (mg/ml)[b]	R^2	% Error
35	0.00435	17.361	16.944	0.9992	2.405
45	0.00295	22.727	21.592	0.9966	4.995
55	0.00273	24.876	23.538	0.9989	5.379

[a]Second-order model.

[b]Real experiments.

From the above information it can be concluded that the equation obtained from the experiment is the second order equation. This second-order equation can be used to calculate the equilibrium concentration. From **Table 14**, it is found that the equilibrium concentration calculated from the experiment is accurate and close to the value obtained from the equation with percentage error was less than 6% in all experiments. It may be concluded that the second-order equation is suitable for predicting the extraction capacity of curcumin from turmeric via ATPs process.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Study on the extraction of curcumin from turmeric using ATPS, experiments were performed using a central composite design, and results were analyzed using the Minitab 19 statistical program, including the analysis of factors affecting the extraction compared to the data obtained from the regression equations to help in explaining the effect of the main effect and interaction of variables from curcumin extraction from turmeric The conclusion from the experimental results can be written as following.

1. Experimental results from curcumin extraction from turmeric, the effect of ethanol concentration and extraction temperature had a significant effect on the extraction yield but the effect of ammonium sulfate concentration and the solid (amount of turmeric powder) to solvent ratio affects the extraction yield less. Analyzing the main effects and interaction of variables, yield prediction equations for curcumin extraction using ATPS were obtained as follows:

$$\%Yield = 55.47 - 3.11A + 3.35C + 6.30D - 3.03B^2$$

2. The optimal extraction conditions for curcumin extraction can be obtained using the Response Optimizer function of Minitab19, the optimum extraction conditions were 15% ethanol concentration, 30.697% (NH4)2SO4 concentration, a solid to solvent ratio of 5:100, extraction temperature of 55 °C, and extraction time 140 min which extraction yield of curcumin was accumulated in the upper phase of 93.96% or 98.370 mg/g of turmeric.

3. The results from the kinetic study showed that the extraction of curcumin from turmeric could be described by a pseudo-second order kinetic model and the slope of the linear kinetic equation curve can be calculated for the k-value for each temperature.

6.2 Recommendations

1 . The solvent used in the experiment for natural extraction should be a food-grade solvent. Therefore, solvents other than ethanol may be a good choice for future experiments, as the change of solvent results in a change in extraction yields and optimum extraction conditions.

2. Study the scope of experimental design to be more comprehensive, which may result in extraction yields higher than 93.96%.

3 . The extracted curcumin is in the form of a solution. In the future, the transformation should be studied for easier and more practical use.

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APPENDIX A

Calibration curve

Calibration curve for standard curcumin using UV-VIS spectrometer

The calibration curve for standard curcumin using UV-VIS spectrometer was measured at 426 nm. The stock standard solution was prepared by dissolving standard curcumin in 50% (v/v) ethanol. Preparing the stock solution for the calibration curve.

1. Preparation of 200 mg/l stock solution, prepared using 0.0012 g curcumin and 6 ml of solvent.

$$C_1 = \frac{1.2 \text{ mg}}{0.006 \text{ l}} = 200 \text{ mg/l}$$

2. The dilution of the stock solution to a concentration between 2.5 - 15 mg/l using the equation:

$$C_1V_1 = C_2V_2$$

Example: The dilution of stock solution from 200 mg/L to 15 mg/L.

C_1 = 200 mg/l C_2 = 15 mg/l

V_1 = volume of C_1 to be used V_2 = volume of C_2 to be used

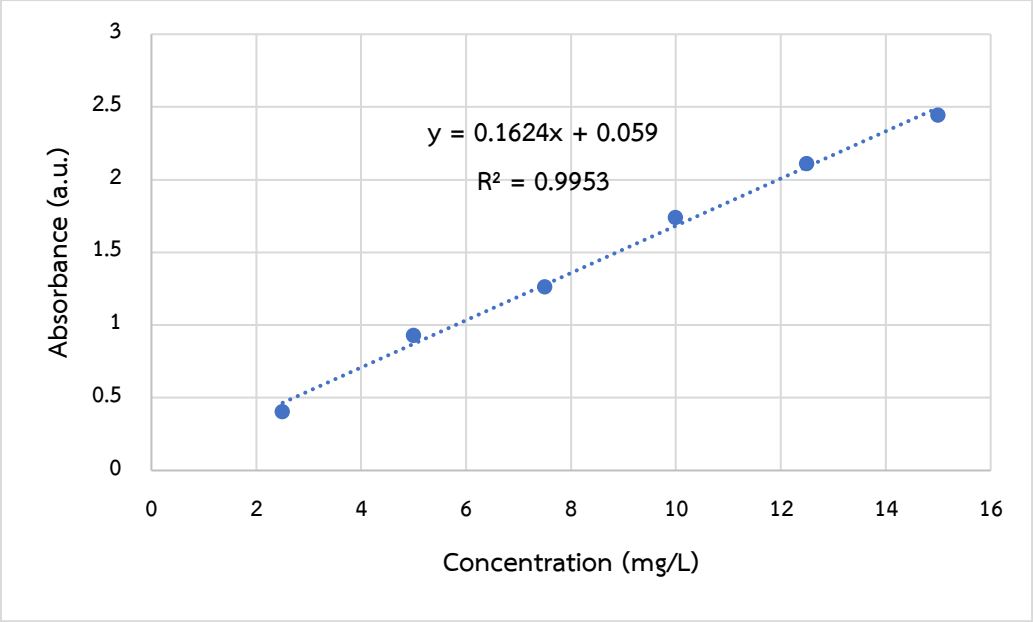
$$(200 \text{ mg/l})(75 \text{ ml}) = (15 \text{ mg/l})(V_2)$$

$$V_2 = 1000 \text{ ml}$$

Therefore, 75 ml of C_1 is mixed with 925 ml of 50% (v/v) ethanol to obtain C_2.

3. The stock solution was diluted at various concentrations and absorbance was determined using a UV-vis spectrophotometer.

The absorbance values at different concentrations were then plotted between the absorbance and concentration values.



APPENDIX B

Experimental data

B.1 An experiment to determine the optimal extraction time.

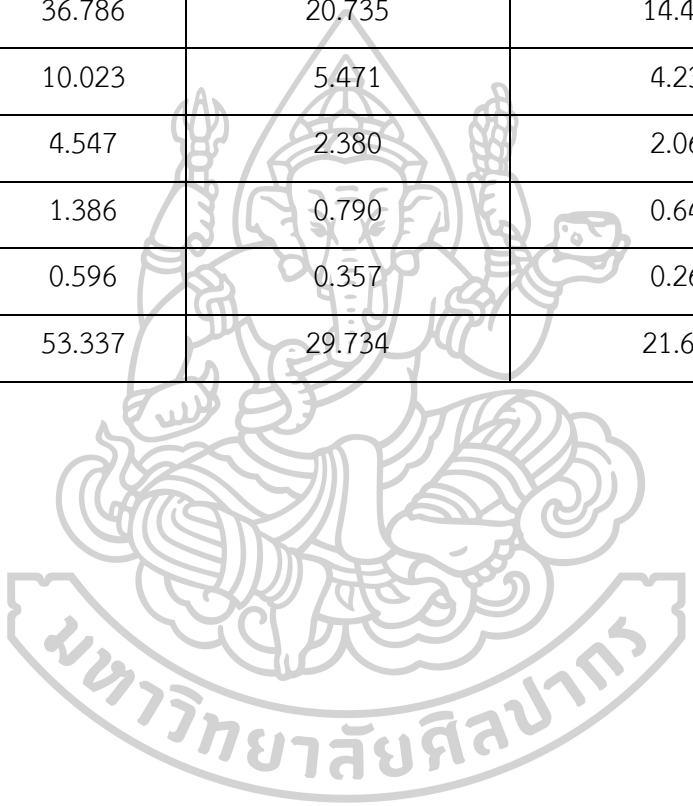
The experiments were performed to determine the optimum extraction time at 35, 45, and 55 ℃. The experimental results are shown in the table.

Time (min)	Curcumin (mg/g Turmeric)		
	35 °C	45 °C	55 °C
30	44.305	57.224	63.245
40	48.532	61.485	65.905
50	50.559	63.439	68.070
60	52.006	65.626	71.425
70	53.476	66.013	74.227
80	53.869	67.170	77.053
90	54.826	68.592	78.459
100	56.438	70.986	79.502
120	58.882	75.298	81.553
140	59.804	78.169	85.312
160	60.135	78.141	85.483
180	60.545	78.197	85.585

B.2 An experiment to determine the amount of curcuminoids in 1 g of turmeric.

The experiments were performed to determine the amount of curcuminoids in 1 g for use in the calculation of extraction yield, the results are shown in the following table.

Run	Amount of curcuminoids/ 1 g of turmeric (mg/g)			
	Curcumin	Demethoxycurcumin	Bisdemethoxycurcumin	
1	36.786	20.735	14.419	
2	10.023	5.471	4.230	
3	4.547	2.380	2.060	
4	1.386	0.790	0.640	
5	0.596	0.357	0.269	
Total	53.337	29.734	21.618	**104.688 mg/g**



APPENDIX C

The calculation for yield of curcumin extract

The calculation for yield of curcumin extracted from turmeric using ATPS.

In the curcumin extraction solution from turmeric, curcumin content was determined using UV-VIS spectrometer.

Example: Experimental of 17% ethanol, 28% ammonium sulfate, 3.5:100 for solid to solvent ratio, and extraction temperature 40 °C, the concentration of curcumin in the upper phase was 7724.593 mg/l.

$$\%\text{Yield} = \frac{\text{Concentration (mg/mL) of curcumin of the upper phase x Volume (mL) of the upper phase}}{\text{The mass of curcumin in turmeric powder (mg)}} \times 100$$

Concentration of curcumin = 7724.593 mg/l

Volume of the upper phase (V_{EtOH}) = 0.0215 l

The mass of curcumin in turmeric powder = 104.688 mg

$$\%\text{Yield} = \frac{7724.593 \text{ mg/l} \times 0.0215 \text{ l}}{(104.688 \text{ mg}) \times 3.5} \times 100 = \mathbf{45.37\%}$$

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