อัลตราซาวนด์ช่วยในการสกัดแคปไซซินอยด์จากผลพริก *Capsicum frutescens* : จากห้องปฏิบัติการสู่ขนาดกึ่งอุตสาหกรรม

นายสุเมธ บุญเกิด

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ULTRASOUND-ASSISTED EXTRACTION OF CAPSAICINOIDS FROM *CAPSICUM FRUTESCENS* (FRUIT) : LABORATORY SCALE TO PILOT SCALE

Mr. Sumate Boonkird

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering

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นายสุเมธ บุญเกิด : อัลตราซาวนด์ช่วยในการสกัดแคปไซซินอยด์ของผลพริก *Capsicum frutescens*: จากห้องปฏิบัติการสู่ขนาดกึ่งอุตสาหกรรม. (ULTRASOUND-ASSISTED EXTRACTION OF CAPSAICINOIDS FROM *CAPSICUM FRUTESCENS* (FRUIT) : LABORATORY SCALE TO PILOT SCALE) อ. ที่ปรึกษา : ผศ.ดร. เหมือนเดือน พิศาลพงศ์, อ.ที่ปรึกษาร่วม : ดร. ชฎา พิศาลพงศ์ , 60 หน้า.

ในการศึกษานี้ได้ทำการสกัดแคปไซซินอยด์จากผลพริกแห้ง โดยใช้อัลตราซาวด์ช่วยในการสกัด เปรียบเทียบกับการสกัดแบบเดิมที่ใช้การคนภายใต้อุณหภูมิสูง เปรียบเทียบผลในแง่ของความสะดวกและประสิทธิผล การสกัดแคปไซซินอยด์ทำโดยเครื่องโซนิเคชันแบบผ่านตัวกลาง ในอ่างอัลตราโซนิคที่ความถี่ของคลื่นอัลตราซาวด์คงที่ที่ 35 กิโลเฮิรตซ์ ปัจจัยของการสกัดที่ทำการศึกษาได้แก่ อุณหภูมิ ชนิดของตัวทำละลาย (อะซิโตน และ เอทานอล 50-95 เปอร์เซ็นต์ ปริมาตรโดยปริมาตร) และสัดส่วนของตัวทำละลายต่อผงพริก โดยความเข้มข้นของแคปไซซินอยด์ในสารสกัดวิเคราะห์ด้วยเครื่องโครมาโทกราฟีเหลวสมรรถนะสูง พบว่าสภาวะที่เหมาะสมในการดำเนินการคือ ใช้เอทานอล 95 เปอร์เซ็นต์ (ปริมาตรโดยปริมาตร) เป็นตัวทำละลาย ในอัตราส่วน 1 กรัมผงพริกแห้ง ต่อ 5 มิลลิลิตรตัวทำละลาย ที่อุณหภูมิ 45 องศาเซลเซียส และใช้เวลาสกัด 180 นาที ซึ่งจะได้ค่าการนำกลับ (recovery) ของ แคปไซซินอยด์ 84.8 เปอร์เซ็นต์ เมื่อนำสภาวะดังกล่าวมาใช้ในการสกัดแบบอัลตราโซนิคในระดับกึ่งอุตสาหกรรม พบว่าที่ความถี่ 26 กิโลเฮิรตซ์ และ 70 กิโลเฮิรตซ์ จะได้ค่าการนำกลับ 76.4 และ 69.6 เปอร์เซ็นต์ ตามลำดับ โดยการสกัดแบบอัลตราโซนิคในระดับกึ่งอุตสาหกรรมถึงแม้ว่าจะได้ค่าการนำกลับที่ต่ำกว่า แบบการคนภายใต้อุณหภูมิสูงในระดับอุตสาหกรรม (ได้ค่าการนำกลับ 81. 8 เปอร์เซ็นต์) เล็กน้อย แต่สะดวกกว่าในการดำเนินการ และลดเวลาในการดำเนินการลงอย่างมาก นอกจากนี้ยัง สามารถดำเนินการที่อุณหภูมิที่ต่ำกว่า อีกทั้งผลิตภัณฑ์ที่ได้ยังมีสารปนเปื้อนที่น้อยลง

ภาควิชา.....วิศวกรรมเคมี..... ลายมือชื่อนิสิต.....

สาขาวิชา.....วิศวกรรมเคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา ..... 2549..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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SUMATE BOONKIRD : ULTRASOUND-ASSISTED EXTRACTION OF CAPSAICINOIDS FROM *CAPSICUM FRUTESCENS* (FRUIT) : LABORATORY SCALE TO PILOT SCALE. THESIS ADVISOR : ASST. MUENDUEN PHISALAPHONG,Ph.D THESIS CO-ADVISOR : CHADA PHISALAPHONG, Ph.D., 60 pp.

This study presents the use of ultrasound-assisted extraction (UAE) to extract capsaicinoids from dried ground chili fruits, *capsicum frutescens* and compared the results with a hot maceration method, in the aspect of their conveniences and effectiveness. Capsaicinoids were extracted by the indirect sonication in ultrasonic bath at fixed frequency 35 kHz. The studied parameters were temperature, type of solvents (acetone and 50-95 % v/v ethanol) and ratio of solvent: crude chili pepper. The capsacinoid concentration was analyzed by high performance liquid chromatography. It was found that the optimized procedure for the operation employed 1 gram of ground dried chili fruit per 5 mL of 95 % (v/v) ethanol at 45 °C and 180 minutes of extraction time. The recovery of capsaicinoids was 84.8 %. This operating condition was applied for the ultrasonic extraction in pilot scale. At the fixed frequency of 26 kHz and 70 kHz, the recovery of capsaicinoids was 76.4 and 69.6 %, respectively. Although the recovery of capsaicinoids was slightly lower than that of the industrial hot maceration process (81.8 % recovery), the ultrasonic extraction was more convenient for the operation, gave a signification reduction in extraction time, could be carried out at lower temperature and the obtained product contained less impurities.

Department.....Chemical Engineering ... Student's signature .....

Field of study ....Chemical Engineering . Advisor's signature .....

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CHAPTER I
INTRODUCTION

1.1 Rationale

Chili pepper is a popular plant found in many parts of the world. Because of its sensory and color, this plant has been increasingly used as a feed additive in livestock, food and pharmaceutical industry. Its hot flavor generally caused by capsaicinoids, consisting of three major substances found more than 95% in capsaicinoids (Krajewska, and Powers, 1987). Capsaicinoids normally consists of capsaicin (N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methy-6-nonenamide), dihydrocapsaicin (N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methynonanamide), and nordihydrocapsaicin (N-[(4-hydroxy-3-methoxyphenyl)methyl]-7-methyloctanamide) (Hoffman, Lego and Galetto, 1983) and have been known for pharmacological properties such as chemoprotector against mutagenesis or tumorigenesis (Surh et al., 1995), antimicrobial (Cichewicz, 1996), antioxidant (Handerson and Slickman, 1999), anticancer effect (Surh, 2002), analgesic effects (Kaale et al., 2002), and the effects on the neuronal responsible for pain transmission and neurogenic inflammation (Szolcsanyi, 2004).

The known extraction techniques of capsainoids are maceration (Kirschbaum-Titze, 2002), magnetic stirring (Contreras-Padilla, 1998), soxhlet (Korel et al., 2002), ultrasound-assisted extraction (UAE) (Karnka et al., 2002), and microwave-assisted extraction (Barbero, Palma, and Barroso 2006). Some advantages of UAE over the other techniques are that this technique requires lower amounts of solvents, shorter periods of time, and lower temperatures for extracting the target compounds from herbs. In addition, in many cases the ultrasound-assisted extraction procedure is found to be more convenient to operate than a novel technique like microwave-assisted extraction and can be industrially employed in local company. The usage of ultrasound-assisted extraction can also be applied instead of boiling maceration method due to cavitational phenomenon.

The effects of ultrasound radiation on the extraction of bioactive compound have been recently published in great amount. However, for the extraction of capsaicinoids, only a report using ultrasonic extraction as preparative method for analyzing was performed by Karnka et al. (2002). The optimized procedure employed 0.3 g of capsicum samples: 10 mL of acetonitrile and 60 minutes of extraction time.

The objective of this thesis is to investigate the influence of operating parameters on the ultrasonic extraction of capsaicinoids. Then, the optimized condition is used for the operation in pilot scale ultrasonic extraction at the Government Pharmaceutical Organization (GPO). The results obtained from both procedures are compared with reflux method.

1.2 Objective

The objective of this work is to investigate the optimum condition for laboratory scale ultrasound-assisted extraction and to compare the efficiency of pilot scale ultrasonic assisted extraction with the conventional method.

1.3 Scope of research

1. Investigate the operating parameters for the ultrasonic extraction in the laboratory scale (250 mL); experaimental parameters are solvent types, solvent composition, extraction time, and temperature.

2. Perform ultrasonic extraction in the pilot scale (20 L) using information from the laboratory scale.

3. Perform the conventional extraction and compare the result with the ultrasound-assisted extraction.

CHAPTER II
LITERATURE REVIEW

2.1 The extraction of capsaicinoids

The extraction of capsaicinoids has been studied for a long time because chili is considered a high-valued herb. The pungency, sensory and pigment of chili have fascinated scientists to find an efficient method to extract its components. The old-fashioned, classical, and novel extraction methods have been continuously improved to achieve higher yields, to reduce time and temperatures, to consume less solvent and to be friendly to the environment. The ultrasound-assisted extraction method is promising to be used for chili extraction as it has been proved to require less time, lower temperatures and consumed less extracted solvent. The ultrasonic extraction of capsaicinoids for a sample preparation was previously reporteded (Karnka et al., 2002). The result showed that the optimized procedure to extract capsaicinoids from capsicum samples was obtained by using acetonitrile at the ratio of 0.3 g of capsicum samples : 10 mL of acetonitrile and 60 minutes of extraction time.

2.2 The analyzed condition of capsaicinoid

The analysis of capsaicinoids could be conducted in various methods including spectrophotometer (Romos, 1989), high performance thin-layer chromatography (Suzuki, 1980), gas chromatography (Krajewska and Powders, 1987), mass fragmentography (Lee et al., 1976), low pressure liquid chromatography (Krajweska and Power, 1986), micellar electrokinetic capillary chromatography (Laskaridou-Monnerville, 1999) and high performance liquid chromatography (Saria, Lembeck and Skofitsch, 1981; Hoffman, Lego, and Galetto, 1983; Weaver, Luker, and Neale, 1984; Perucka and Oleszek, 2000). However, the analysis by using HPLC is preferable to another since it provides a reliable, accurate, and efficient procedure.

2.3 The novel technique on capsaicinoid extraction

The new technique, microwave-assisted extraction, has recently been developed by Gerardo, Palma and Barroso (2006) for the extraction of capsaicinoids. In order to investigate the extraction parameters, they performed the extraction with different solvents, temperature, sample amount, solvent volume and extraction time. The results revealed that the optimal parameters were 125^{o}C, 500 W, 25 mL of 100% ethanol as a solvent per 0.5 g of ground fresh peppers, and 5 minutes extraction time.

The advantages of microwave-assisted extraction are that it shortens extraction time, requires less amount of solvents and improves extraction yields but the efficiency of microwaves can be extremely decreased when either the target compounds or the solvent are non-polar. Comparing to ultrasound-assisted extraction, the ultrasound apparatus is cheaper and its operation is easier. Furthermore, using ultrasound can reduce the extraction temperature, rendering this technique very useful for thermolabile compounds extraction.

2.4 The use of capsicum

Badmaev et al. (1997) reviewed the use of capsicum in terms of pharmacological action of capsaicin in the thermoregulatory, gastrointestinal, cardiovascular, respiratory and sensory system. For thermogenic effect, capsicum could increase the metabolic rate and a feeling warmth by activated the sympathetic nervous system to enhance energy metabolism and catecholamine secretion (Watanabe, 1988). For moderate doses of capsicum, capsaicin could stimulate the flow of saliva and gastric juices but the high qualities of capsaicum had induced diarrhea.

Capsicum has also used as a counter-irritant, classified by the FDA, which applied to the skin to relieve irritation and pain. The clinical trials were performed to alleviate cluster headaches (Fusco, 1994), desensitize nasal nerves to offset non-allerginic rhinitis (Riechelmann, 1994), lessen orofacial pain (Adekkmle, 1994), prevent herpes flare-ups (Bernstein, 1981; Watson, 1988), and treat psoriasis (Glinski and Brodecka, 1994).

2.5 The usage of ultrasound-assisted extraction

Ultrasound-assisted extraction is an effective way to extract a large number of bioactive compounds. An overview of ultrasound-assisted extraction of bioactive compounds from herbs was reviewed by Vinatoru (2001). Many published documents have revealed the successful examples of using UAE methods. The related literatures using the application of ultrasound-assisted extraction to extract herbs are shown below.

Comparison of conventional and ultrasonically assisted extractions of pharmaceutically active compounds from *Salvia officinalis.* (Sališvová, Toma and Mason 1997)

Sališvová, Toma and Mason (1997) studied extraction of conventional and ultrasonically assisted extraction of biologically active compounds from *Salvia officinalis* using 65 % ethanol with ultrasonic cleaning bath at 37-42 kHz and with an ultrasonic horn at 20 kHz working frequency. The results indicated that ultrasonically assisted extraction with mechanical stirring at room temperature in a period of 12 hours produced a substantial improvement over conventional methodology. In addition, the extraction using ultrasonic horn was found to be more effective after 2 hours sonication.

Comparison of classical and ultrasound-assisted extraction of polysaccharides from *Salvia officinalis* L. (Hromádková et al., 1999)

Hromádková et al. (1999) compared yields and compositions of polysaccharides extracted from the insoluble residues of *Salvia officinalis*, using conventional and ultrasound-assisted ethanol extraction at 20 kHz. For ultrasound-assisted extraction, the plant residues was first extracted in ethanol and, then with hot water followed by diluted alkali in order to isolate polysaccharide cell wall compounds. The results showed that yields of the hot water extract as well as total extracted polysaccharides were higher in the case of the ultrasound-treated plant in both laboratory and pilot plant experiments.

Feasibility study of repeated harvesting of Menthol from biolobically viable *Mentha x piperata* using ultrasonic extraction. (Shotipruk, Kaufman and Wang 2001)

Shotipruk, Kaufman and Wang (2001) investigated that the conventional destructive extraction process could be replaced by a nonfatal and repeated method. Menthol could be extracted from biologically viable peppermint plants (*Mentha x piperita*) using ultrasound. Their results showed that the amount of released menthol from leaf tissue using ultrasonication for 1 hour at 22 °C in a standard 40 kHz ultrasonic bath was approximately 2 % of total product. The treatment temperature and time had an effected on increasing the amount of menthol released. When the temperature was increased from 22 °C to 39 °C, the total product increased from 2% to 12%. The product released according to the cavitation phenomenon in ultrasound. The treated plants remained viable and were ready for the subsequent ultrasound extraction after approximately 4 days of recovery. However, the amount of product released was reduced in subsequent extractions. While maintaining the plant's viability, a nondestructive extraction method could be applied to continuously release intracellular plant metabolites from the plants by online ultrasonic.

The extraction of rutin from flower buds of *Sophora japonica*. (Paniwnyk, Beaufoy, and Lorimer 2001)

Paniwnyk, Beaufoy, and Lorimer (2001) studied the efficiency of extraction of rutin from *Sophora japonica* by ultrasound (20 kHz ultrasonic probe). Ultrasonic extraction with aqueous solvent was found unsuitable for the compound with antioxidant activity. They found that the application of ultrasound to the methanolic extraction gave a significant reduction in extraction time and an increase in maximum extraction yield.

Ultrasound-assised extraction of ginseng saponins from ginseng roots and cultured ginseng cells. (Wu, Lin, and Chau, 2001)

Wu, Lin, and Chau (2001) evaluated the ultrasound-assisted extraction as a simpler and more effective than conventional extraction methods especially for the isolation of ginsenosides (saponins) from various types of ginseng. The ginseng

samples were extracted with different solvents, under either direct sonication by an ultrasound probe horn (20 kHz) or indirect sonication in an ultrasound cleaning bath (38.5 kHz). The ultrasonic extraction was compared with the conventional method of refluxing boiling solvents in a soxhlet extractor. They found that the sonication-assisted extraction of ginseng saponins was about three times faster than the traditional extraction method. The ultrasonic extraction was not only more efficient but also convenient for the recovery and purification of the active ingredients of plant materials. In addition, the sonication-assisted extraction could be carried out at lower temperatures which were favorable for the thermally unstable compounds.

Optimzation of High-Performance Liquid Chromatographic Parameters for the Determination of Capsaicinoid Compounds Using the Simplex Method. (Karnka et al., 2002)

Karnka et al. (2002) optimized the high-performance liquid chromatographic method for sample preparation of Thai capaicum fruits by using ultrasound-assisted extraction. Higher extraction rate was obtained by using acetonitrile as a solvent instead of acetone or methanol. They found that the separation was achieved in 11 min. using C-8 column of 15-cm length and 4.6 mm diameter. A flow rate was 1.15 mL/min at a column temperature of 43.5 °C using 63.7 % methanol in water.

Comparison of classical and ultrasound-assisted isolation procedures of cellulose from kenaf (*Hibiscus cannabinus L.*) and eucalyptus (*Eucalyptus rodustrus Sm.*). (Pappas, Tarantilis, and Daliani, 2002)

Pappas, Tarantilis, and Daliani (2002) compared classical and ultrasound-assisted extraction for the purification of cellulose from kenaf (*Hibiscus cannabinus L.*) and eucalyptus (*Eucalyptus rodustrus Sm.*) in water bath at the frequency of 35 kHz. The cellulose samples were isolated and studied by diffuse reflectance infrared Fourier transform spectroscopy and ^{13}C nuclear magnetic resonance spectroscopy. The usage of ultrasound decreased the total time of treatment. In addition, the purity of the cellulose obtained from the ultrasound-assisted extraction was greatly high.

Effect of ultrasound on the extractability of corn bran hemicelluloses. (Ebringerová and Hromádková 2002)

Ebringerová and Hromádková (2002) examined the effect of sonication on the extractability and properties of the non-cellulose components of industrial corn bran (CB). The short sonication was used to treat the polysaccharide extraction at the second step. The result showed that potential of ultrasound-assisted extraction was promising for the isolation of industrially polysaccharides from plant materials.

Ultrasonic extraction of plant materials—investigation of hemicellulose release from buckwheat hulls. (Hromádková and Ebringerová, 2003)

Hromádková and Ebringerová (2003) investigated one- and two-step extraction procedures with and without a short application of ultrasound at the beginning of the extraction. The effect of sonication on the extractibility of the hemicellulose components of buckwheat hulls was examined. Ultrasonication was carried out at 20 kHz. Yields and compositions of polysaccharides recovered from the extract composition were determined by chemical methods and spectroscopic techniques. They found that these polysaccharides were comprised of a complex of glucuronoxylan and co-extracted amylose-rich starch in various proportions contaminated with other cell wall components (protein, pectic polysaccharides). The hemicellulose fractions obtained by classical and ultrasound-assisted extraction exhibited significant immunomodulatory activities. The increased yield of ultrasonically extracted hemicelluloses with their preserved structural and molecular properties as well as immunological activity, confirmed the importance and great potential of using ultrasound for polysaccharide in industrial scale.

High intensity ultrasound-assisted extraction of oil from soybeans. (Li, Pordesimo and Weiss, 2004)

Li, Pordesimo and Weiss (2004) studied the application of 20 kHz ultrasound during the extraction of soybeans using hexane, isopropanol and a 3:2 hexane-isopropanol mixture. Using a simplified extraction procedure, ground soybeans

were added to solvent and ultrasonicated between 0-3 hours. The results showed that solvent type influenced the efficiency of the extraction. The highest yield was obtained using ultrasound in combination with mix solvent due to the ultrasonically induced cavitation increasing permeability of plant tissues. The study showed that the high-intensity ultrasound might reduce time required to extract edible oils from sources.

Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for thefood and pharmaceutical industry. (Albu, Joyce and Paniwnk, 2004)

Albu, Joyce and Paniwnk (2004) found that ultrasound could enhance the extraction efficiency of carnosic acid from the herb *Rosmarinus officinalis* using butanone, ethyl acetate and ethanol as solvents in 40 kHz cleaning bath. For dried and fresh leaves of the herb, the yields of carnosic acid extraction were improved for all three solvents (butanone, ethanol, and ethyl acetate) and the extraction times were reduced. Although ethanol is consided to be less efficient compared to the other solvents under conventional conditions, using sonication was also be able to reduce such solvent effects and hence give similar the extraction efficiency. Extraction of dried herb with ethanol was proved to be more efficient than that of fresh material, probably because of the water present in the latter.

Comparison of classical and ultrasound-assisted extractions of steroids and triterpenoids from three *Chresta* spp. (Elisandra et al., 2004)

Elisandra et al. (2004) compared efficiency of classical and ultrasound-assisted extractions of steroids and triterpenoids from stem, leaves and flowers of *Chresta exsucca, C. scapigera* and *C. sphaerocephala*. The extraction was performed with ultrasound cleaning bath 60 Hz. They found that the yields obtained by ultrasound assisted extraction for 30 minutes extraction time were comparable to maceration at 24 h extraction time. Therefore, they have concluded that ultrasound-assisted extraction was more efficient than the classical one in the case of the extractions of steroids and triterpenes.

Kinetics of ultrasonic extraction of extractive substances from garden (Salvia officinalis L.) and glutinous (Salvia glutinosa L.) sage. (Veličković, Milenović and Ristić, 2006)

Veličković, Milenović and Ristić (2006) investigated the kinetics of ultrasonic extraction of extractive substances from dry herbs of garden (*Salvia officinalis L.*) and glutinous (*Salvia glutinosa L.*) sage using petroleum ether, 70% ethanol or water (different polarity) at 40 ^{o}C in cleaning bath operating at 40 kHz frequency. They found that the mechanism of ultrasonic extraction was occurred in two steps: first, dissolution of extractive substances near particle surface (washing) and, second, the diffusion from solid particles to the bulk of the liquid extract (slow extraction). The yields of extractive substances were increased when solvent polarity was raised, and the maximum concentration of extractive substances in liquid extracts was achieved at about 20 minutes. The composition of the extracts depended on both the applied extraction conditions and the plant material.

Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. (Hemwimol, Pavasan and Shotipruk, 2006)

Hemwimol, Pavasan and Shotipruk (2006) investigated the use of ultrasound-assisted extraction (UAE) to improve the extraction efficiency of the classical solvent extraction techniques such as maceration and soxhlet to extract anthraquinones from the root of *Morinda citrifolia*. The effects of extraction conditions were determined, i.e., temperatures (25, 45, 60 ^{o}C), ultrasonic powers, solvent types, and compositions of ethanol in ethanol–water mixtures. An ultrasonic bath was used as an ultrasound source with 38.5 kHz. The results revealed that when the extraction times and temperatures were increased, the yields were greater. Dependening on the solvent types, the percent recovery of anthraquinones were higher in acetone, acetonitrile, methanol, and ethanol respectively. In addition, the use of ethanol–water solution as extraction solvent increased the yield of anthraquinones because of the relative polarity, the swelling effect of plant tissue matrix by water, and increased sound absorption. Ultrasound-assisted extraction required much shorter time when compared with soxhlet extraction and maceration to achieve the same recovery.

Application of ultrasonic technique for extracting chlorogenic acid from *Eucommia ulmodies* Oliv. (*E. ulmodies*). (Li, Chen and Yao, 2005)

Li, Chen and Yao (2005) investigated and optimized ultrasonic method for the extraction of chlorogenic acid from fresh leaves of *Eucommia ulmodies Oliv*. They studied the influence of four extraction variables on extraction efficiency of chlorogenic acid by using cleaning bath at 50 kHz. The optimum extraction conditions were: 70% aqueous methanol; solvent: sample ratio = 20:1 (v/w); extraction time 3 x 30 min. The recovery of chlorogenic acid and the reproducibility of the method were also determined using HPLC. The optimized ultrasonic extraction conditions were applied to extract chlorogenic acid from fresh leaves, fresh bark and dried bark of *E. ulmodies* and four traditional Chinese medicines. The application of sonication method was shown to be highly efficient in the case of extracting of chlorogenic acid from *E. ulmodies* and other Chinese medicines when compared with classical methods.

CHAPTER III
THEORY

3.1 Ultrasonic principle

Sound waves are mechanical vibrations in a solid, liquid and gas. They must travel in a matter differ from electromagnetic waves. Sound waves involve in expansion and compression cycle.

The definition of ultrasound is the sound wave having frequency higher the range of human hearing which is between 16 Hz to 16 kHz. The upper limit of frequency is not sharply defined but it is about 5 MHz for gas and 500 MHz for liquid and solid. The usage of ultrasound can divided into two categories. First, the low range frequency is high power ultrasound with frequency between 20-100 kHz. The low frequency ultrasound is suitable for cleaning, plastic welding, and cell disruption. Second, the high range frequency is low power ultrasound between 2-10 MHz which is used in medical application (Mason and Lormier 1988)

Cavitation is the phenomenon in ultrasound occurred when sufficiently high sound pressures in liquids. Any liquid has a theoretical tensile strength which characterizes the minimum pressure for disruption. Because of the presence of nuclei such as dissolved gases, solid impurities, and rough walls, cavitation occurs at far lower sound pressures than are theoretically necessary. In nearly any liquid, initial nuclei are present which show a distinct size distribution and grow under a certain sound pressure (Mason and Lormier, 1988).

The significance of cavitation is not depended on how they form but depend on what they are after bubbles collapse. Luque de Castro and Priego-Capte (2006) has studied and estimated that the temperature may rise up to 5,000 ^{o}C, the pressure increases more than 2,000 atm, and also the velocity of microjet will be up to 400

kilometer per hour but the bubble size is very small relative to the total liquids volume, so the heat is rapidly dissipated with no substantial change in the environment conditions

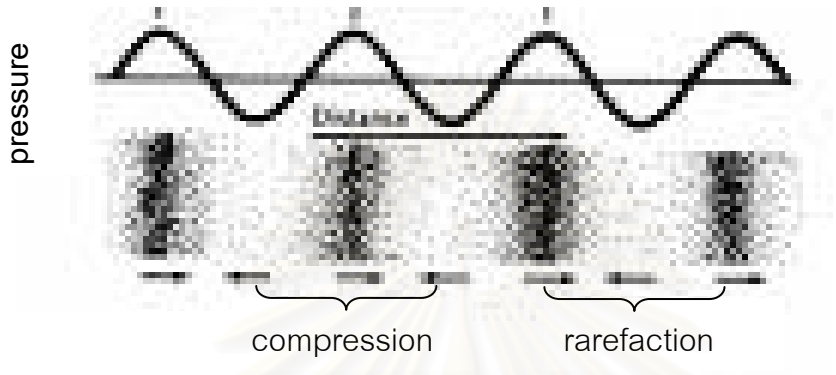


Figure 3.1 Charateristic of sound wave
(Available from : http//www.neurophys.wisc.edu/~ychen/textbase/s1-p2.html)[Apr 27, 2007]

3.2 The Influencing parameters on cavition (Mason and Lorimer 1988, Hoffman 2003).

1. **Dissolved gases.** Dissolved gases are present in most applications of ultrasound. The type and amount of these gases in treated liquid are a very important factor. Highly gas saturated liquids have large numbers of cavitation nuclei and low cavitation thresholds. Degassed liquids need higher sound pressure for cavitation. The bubbles can collect gas from the surrounding liquid, and this diffusion creates large bubbles with high gas contents. On collapsing, these bubbles create moderate temperatures and pressures. Monoatomic gases like argon and xenon exhibit good cavitation intensities, whereas diatomic gases such as nitrogen and oxygen tend to

decrease the observed effects. Gases with high thermal conductivity such as helium decrease the intensity of cavitational collapse to nearly zero.

2. **Vapor pressure.** The high vapor pressure of liquids can reduce the bubble collapse like a high gas content. Vapor in transient bubble can be condensed in the compression cycle and lead to higher cavitation intensities than gas filled bubbles. Experiments with different solvents showed that small vapor pressures are necessary for sufficiently high cavitation intensity. The cavitation of the boiling point of the liquid, higher vapor pressures is nearly zero.

3. **Viscosity.** High viscosity of sonicated liquid increases the cavitation threshold markedly. High viscosity cause the molecular interaction between liquid higher. Viscous liquids generate bubbles only at high sound pressures. Bubble motion is limited by the dissipative effect of the viscosity.

4. **Temperature.** Many physical properties depend on temperature such as viscosity, gas solubility, vapor pressure and surfaced tension. In most case, cavitation is more noticeable at lower temperatures due to the lower vapor pressure of the liquid. This lowers the total gas content of the collapsing bubbles and causes higher cavitation intensities. The mathematic equation described the effect of temperature was developed (Mason and Lorimer, 1988). When the temperature decreases (or the decreases in liquid vapour pressure), either the surface tension or viscosity of liquid increases.

5. **Frequency.** The production and intensity of cavitation in liquid decreases when the ultrasonic frequency is applied increasingly. Power ultrasound, low frequencies in the range from 16 to 100 kHz are principally active in heterogeneous systems with micromixing, cleaning, mechanical action on suspended solids and intense bubble motion. Radicals increase because high frequencies favors high temperatures and pressures in the cavitation bubbles.

6. **Ultrasound intensity.** Higher intensities cause a larger amplitude at the vibrating surface in contact with the liquid. At high intensities, small cavity grow rapidly and collapsed violently during compression cycles. Raising this intensity above the cavitation threshold of the liquid causes oscillating bubbles, and under certain circumstances, the contact between radiating surface and liquid is lost. The motion of the transducer and the liquid are out of phase, and the effect was known as decoupling.

A secondary reason for the nonlinear relationship between intensity is the creation of cavitation zones. Higher intensities create more and larger bubbles, which may merge and lead to less transient events

3.3 Type of ultrasonic equipment

The equipment using electrochemical transducer can be divided 3 groups

1. The cleaning bath. This type is easily found, accessible, and cheapest of ultrasonic equipment. The frequency and power of cleaning bath depends on type and number of transducer used. The disadvantages of cleaning bath are lower power compared with probe system. Moreover, this type cannot change frequency as much as possible.

2. The probe. Sonic horn or velocity transformer are also named. No losses from liquid transfers through vessel walls. It is also difficult to control the temperature. Furthermore, radical species may be formed at the tip.

3. The Cup-horn. This type consists of an ultrasonic bath with the controllable power.

3.4 The effect of Ultrasound on the extraction of herb

Operations such as conventional extraction, percolation, maceration, soxhlet, infusion etc, require to use a long time to obtain the extraction target. Nowadays, the economic aspect is to reduce the time and solvent in the extraction. Therefore, the improved methods supercritical fluid extraction, vortical extraction, extraction by electrical energy, microwave-assisted extraction, and ultrasound-assisted extraction are carried out. In ultrasound-assisted extraction, the mechanism on extraction of dried product may be described in two stages (Veličković, 2006). First, solvent is steeping to vegetal materials to facilitate swelling and hydration process. Ultrasound can facilitate swelling and hydration and cause an enlargement in the pores of the cell wall. Second, the mass of soluble constituents transfers from the material to solvent by diffusion and osmotic processes. The effect of ultrasonic waves on the vegetal material that is ultrasonic waves breaks the cells wall and increase the cell's contents into the extraction medium. Because the ultrasonic wave produces cavitations against the cell wall of material, then the microjet occur during that process which is high energy to penetrate the cell wall.

3.5 The aspect of *Capsicum* spp.

The capsicum refers to the fruits of species pertaining to the plant genus Capsicum in the family of Solanaceae, which includes tomatoes, potatoes, tobacco, and the deadly nightshade (Badmaev et al., 1997).The nomenclature of capsicum like pepper, chili, chilli, paprika, and capsicum are interchangeably. Capsicum consists of only five species currently recognized: *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinensis*, *Capsicum pendulum* and *Capsicum pubescens* (Badmaev et al., 1997). *Capsicum frutescens* is a shrubby perennial with angle stem and branches, broadly ovate-acuminate leaves, axillaries greenish-white or white flowers with peduncles, the calyx cup-shaped, embracing base of fruit multiple pods per nod, the

corolla rotate and often with ocherous making in the throat. The fruit is very pungent and oblong-conical berry, up to 30 mm. in length (Youngken, 1950).

Figure 2 Some kinds of *capsicum frutescens*

(Available from : http//www.halba.de.tabasco2001.jp)[Apr 27,2007]

Capsicum annuum is almost alike *C. frutescens* but different in some aspects. *C. annuum* is generally one year alive plant, single pod node, purple, white or purple white flower. The fruit is not or mild to moderate pungent and up to 150 mm. in length.

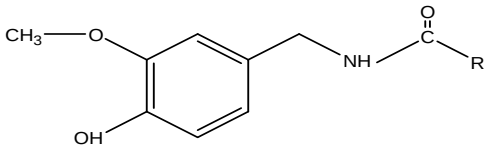
3.6 The chemistry of capsicin

Capsaicin and its analogues, called capsaicinoids, are the pungent compounds of the *Capsicum* fruits. Capsaicin, the major pungent compound of hot pepper fruits, is an amide derivative of vanillylamine and 8-methylnon-trans-6-enoic acid. The hot flavor is caused by nine closely related alkaloids or capsaicinoids, but capsaicin

(8-methyl-N-vanillyl-6-nonenamide), dihydrocapsaicin and nordihydrocapsaicin are responsible for about 95 % of the pungency.

Natural capsaicin [N-[(4-hydroxy-3-methoxy)phenyl-methyl]-8-methyl-(E)-6- nonenamide : $C_{18}H_{27}NO_3$; MW 305.4] is odorless white needle crystal with severe burning pungency, melting point at 64.5 ^{o}C; boiling point at 210-220 ^{o}C at 0.01 mmHg and sublimate at 115 ^{o}C. The ultraviolet maximum absorption was 227, 280 nm. It is easily soluble in ethyl ether, ethyl alcohol, acetone, methyl alcohol and hot alkali, practically insoluble in cold water.

Synthetic capsaicin: [N-[(4-hydroxy-3-methoxy)phenyl–methyl]-nonanamide $C_{17}H_{27}NO_3$; MW 293.4 is sometimes a cheap adulterant in capsaicin and *Capsaicum* oleoresin.



General structure of capsaicinoid

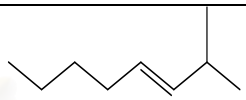
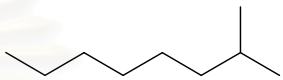
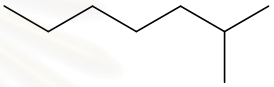
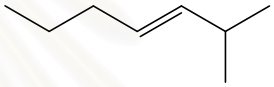
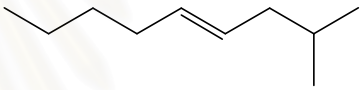
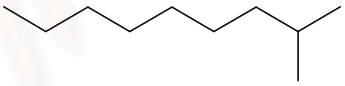
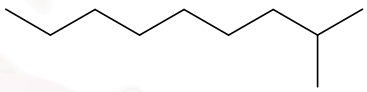
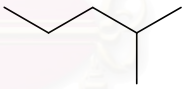
Capsaicinoid and analogs	-R
Capasaicin	
Dihydrocapsaicin	
Nordihydrocapsaicin	
Norcapsaicin	
Homocapasaicin	
Homodihydrocapsaicin	
Nornordihydrocapsaicin	
Nornornordihydrocapsaicin	
Nonivamide	

Figure 3.3 General structure of Capsaicinoids presents in hot pepper (Barbero, Palma, Barroso, 2006)

CHAPTER IV
MATERIALS AND METHODS

4.1 Plant material

Two batches of chili materials were purchased locally. Both were cultivated in Mae Chan district, Chiangrai the northern part of Thailand. The first batch was purchased in the form of dried ground chili and then packed in aluminium foils (purged with nitrogen), and stored in refrigerator at below 10 ^{o}C until used for laboratory scale experiments (figure 4.1).

Figure 4.1 Dried ground chili pepper passing 3 mm opening of Brablender

The second batch of chili was purchased in unground form (figure 4.2). It was used in conduction pilot and industrial scale experiment. Before used, it was ground into coarse powder (figure 4.3) by hammer mill, packed in plastic bag and then stored in cold room until use. The second batch of chili was for pilot scale and industrial scale extraction.

Figure 4.2 Dried chili pepper before grinding

Figure 4.3 Dried ground chili pepper passing 13 mm opening of Hammer mill

4.2 Reagents and Standards

For laboratory:

4.2.1 Acetonitrile (HPLC grade, Mallinckrodt, USA) and ultrapure water used for HPLC mobile phase was passed through 0.45 mm membrane filter before used.

4.2.2 Capsaicin standard (N-Vanillyln-nonanamide) was purchased from Sigma-Aldrich (USA).

4.2.3 Acetic glacial used in mobile phase is AR grade (Labscan, Thailand).

4.2.4. Ethanol for separation and sample preparation is AR grade

4.2.5 Acetone for separation and sample preparation is AR grade.

For pilot scale:

The ethanol for separation and sample preparation is industrial grade.

4.3 Apparatus

4.3.1 Laboratory-scaled ultrasonic bath: Bandelin sonorex super RK 1050, power 600 watt, 35 kHz as working frequency with regtangular container (50 cm x 60 cm x 20 cm) connected to a temperature-controlled bath (polyscience 9610, USA).

4.3.2 Ultrasonic pilot-scaled extractor: 20-L extraction tank consisting of double output of ultrasonic frequencies (26 and 70 kHz ± 3 kHz) and ultrasound electric power 1.08 kW with temperature control (figure 4.4). It can be performed in half and full mode function. The stirrer speed is 16 round/min with an explosion proof motor.

Figure 4.4 The pilot scale 20-L ultrasonic extractor

4.3.3 Industrial scale extractor: The extractor volume was 1500-L(figure 4.5).

Figure 4.5 The industrial scale extractor

4.3.4 In the analysis of AOAC, the sample sizes of chili was reduced by FRITSCH pulverisette 14 (Germany) (Figure 4.6), but in the laboratory scale experiment, the sample size was reduced by brablender(Figure 4.7). For the pilot ultrasound-assisted extraction and industrial scale extraction, the samples were reduced by hammer mill(Figure 4.8).

Figure 4.6 The Fritsch pulverisette 14

Figure 4.7 Blablender

Figure 4.8 Hammer mill

4.3.5 HPLC instrument. Thermo Separation Product (TSP) consisted of the Spectra System P1000 solvent delivery system, and the UV-6000LP diode array UV-visible detector. The data was processed by software PC1000 to evaluate the peak area. Samples were automatically injected by the Spectra System AS3000 autosampler, which was able to inject 120 samples continuously.

4.3.6 HPLC analytical column is (C18) reverse phase column packed with 5 µm. in particle size, 150 x 4.6 mm I.D. (Kanato chemical co., inc)

4.3.7 Vacuum oven. (WTBbinder, VDL 53, Germay)

4.3.8 Soxhlet. Soxhlet apparatus (FDF 3 0250CE, Britain)

4.3.9 Water bath.

4.3.10 Cooling water (LTD 6, England).

4.4 Quantitative analytical method for capsaicinoids

The analysis of capsaicinoids in dried chili powder and in extraction samples followed the method of The Association of Analytical Communities (AOAC) method 995.03 “Capsaicinoids in capsicums and their extractives liquid chromatographic method” (1995).

HPLC conditions for capsaicinoids separation

Column	:	Mightysil C18 reverse phase : 150 x 4.6 mm.I.D., 5 µm particle size.
Mobile phase	:	isocratic 40% acetonitrile in 1% acetic acid (v/v) ultrapure water
runtime	:	30 min
flow rate	:	1.5 mL/min
UV wavelength	:	280 nm.
Injection volume	:	20 µl

Calculation for % capsaicinoids content in dried chili

$$N = \frac{P_n}{p_{std}} \times \frac{C_{std}}{W_{sam}} \times \frac{V}{0.98} \times 100$$

$$C = \frac{P_c}{p_{std}} \times \frac{C_{std}}{W_{sam}} \times \frac{V}{0.89} \times 100$$

$$D = \frac{P_d}{p_{std}} \times \frac{C_{std}}{W_{sam}} \times \frac{V}{0.93} \times 100$$

$$\%Capsaicinoids = N + C + D$$

Calculation for % capsaicinoids content in each conditions, using linear equation in calibration curve, y=ax+b

$$N = \frac{P_n - b}{a} \times \frac{C_{std}}{W_{sam}} \times \frac{V}{0.98} \times 100$$

$$C = \frac{P_c - b}{a} \times \frac{C_{std}}{W_{sam}} \times \frac{V}{0.89} \times 100$$

$$D = \frac{P_d - b}{a} \times \frac{C_{std}}{W_{sam}} \times \frac{V}{0.93} \times 100$$

$$\%Capsaicinoids = N + C + D$$

$$\text{Percent recovery} = \frac{\text{\% capsaicinoids extracted condition X100}}{\text{\% capsaicinoids in dried chili}}$$

whereas

V = Solvent volume (mL)

P_n = Average peak area for nordihydrocapsaicin

P_c = Average peak area for capsaicin

P_d = Average peak area and dihydrocapsaicin

W_{sam}= Weight of sample (mg), in dry basis

P_s = Average peak area of standard solution

C_s = Concentration of standard solution, (mg/mL)

a = constant from linear equation

b = constant from linear equation

N = Content of nordihydrocapsaicin (%w/w)

C = Content of capsaicin (%w/w)

D = Content of dihydrocapsaicin (%w/w)

4.5 Extraction method

4.5.1 Conventional extraction: the conventional extraction method for boiling and refluxing the solvent in a soxhlet extractor was used as a control for initial study and compared with the maceration and ultrasound-assisted extraction methods.

4.5.2 Maceration: the 250 mL Erlenmeyer flask filled with 25 g of chili and 200 mL of 95%v/v ethanol was set in a 45°C water bath placed on a hot plate stirrer (Heidolph, MR 3003 GD Germany) and stirred at 250 round per minutes for 15 hours.

4.5.3 Ultrasonic extraction experiment: an ultrasonic cleaning bath was used as an ultrasound source. The frequency of the cleaning bath was 35 kHz. For each condition, 250 mL Erlenmeyer filled with 25 g of chili and selected solvent was immersed under the controlled water level in controlled-temperature sonicator. The samples were

drawn at 5, 15, 30, 60, 120, and 180 minutes after sonication and were injected to HPLC without evaporation. The extraction profiles of capsaicinoids were established. For extraction with acetone, acetone must be completely removed from the samples and adjusted volume with ethanol prior to being injected to HPLC.

4.5.4 Pilot scale UAE: 3 kg of dried course ground chili pepper was employed with the selected condition from the laboratory experiment. The samples were drawn at 5, 15, 30, 60, 120, and 180 minutes for establishing the extraction profiles of capsaicinoids.

4.5.5 Industrial scale extractor: 90 kg of dry ground chili pepper was added and filled with 500-L 95 %v/v ethanol. It was left overnight at ambient temperature for 19 hours, then opened steam valve until temperature reached 78^{o}C. All procedures followed GPO working record. The samples were collected at 5, 15, 30, 60, 120, and 180 minutes.

4.5.6 Dry weight measurement was performed using a vacuum oven operated at pressure below 100 mbar at 105 ^{o}C. Sample was weighed in the range of 1-2 g and placed in discs. After being dried in the oven for 3 hours, the cooled sample was weighed again. The drying cycle was repeated until the constant weight of the samples achieved.

4.5.7 Soxhlet extraction: the 25 g of sample was placed into the cartridge with 200 mL of 95 %v/v ethanol 250 mL flask. The extraction time was 5 hours.

Table 4.1 Range of experimental variable

Parameters	conditions					
	AOAC method	UAE laboratory scale	maceration	soxhlet	UAE pilot scale	Industrial scale
temperature (°C)	75	30, 45, and 60	45	boiling point	45	78
time (min)	5 hours	3 hours (0, 5, 15, 30, 60, 120, and 180 min)	15 hours (0, 5, 15, 30, 60, 120, and 180 min)	15 hours	3 hours (0, 5, 15, 30, 60, 120, and 180 min)	24 hours
solvent type	ethanol		ethanol	ethanol	ethanol	ethanol
solvent composition (ethanol:water)	95:5	ethanol and acetone 50:50, 75:25, and 95:5	95:5	95:5	95:5	95:5
crude power:solvent ratio	1:8	1:5, 1:6, 1:8	1:8	1:8	1:5	1:5.5
sample size	0.5 mm	sieve: 3 mm aperture			sieve: 13 mm aperture	

CHAPTER V
RESULTS AND DISCUSSIONS

5.1 Experimental study in laboratory scale

5.1.1 The effect of ultrasonication and its extraction time

The effect of ultrasonication and its extraction time at 5, 15, 30, 60, 120 and 180 minutes on the extraction of capsaicinoids was shown in figure 5.1. In the experiment, maceration at 45 ^{o}C was used as a control system. It revealed that when the ultrasound was applied, the recovery of capsaicinoids increased. In ultrasound-assisted extraction, ultrasound could accelerate swelling and hydration and caused an enlargement in the pores of the plant cell wall. Moreover, the cavitational effects provided a better mass transfer of solute constituents from the plant materials to solvent. The disruption of plant cells when impacted by microjet after cavitation bubble collapsed caused the solvent penetrated into plant tissue (Paniwnyk, Beaufoy and Mason, 2001; Toma et al., 2001). In both experiments, the release rate of capsaicinoids was very high during the first 5 minutes because the effect of capsaicinoids concentration gradient between the solvent and plant cells. In addition, the extraction from the outer part of cells might easily occur than the inner one because of cavitational effect as mentioned above. Afterward the release of capsaicinoids was slowly because the remaining capsaicinoids located in the inner part of cells and the concentration gradient was very low.

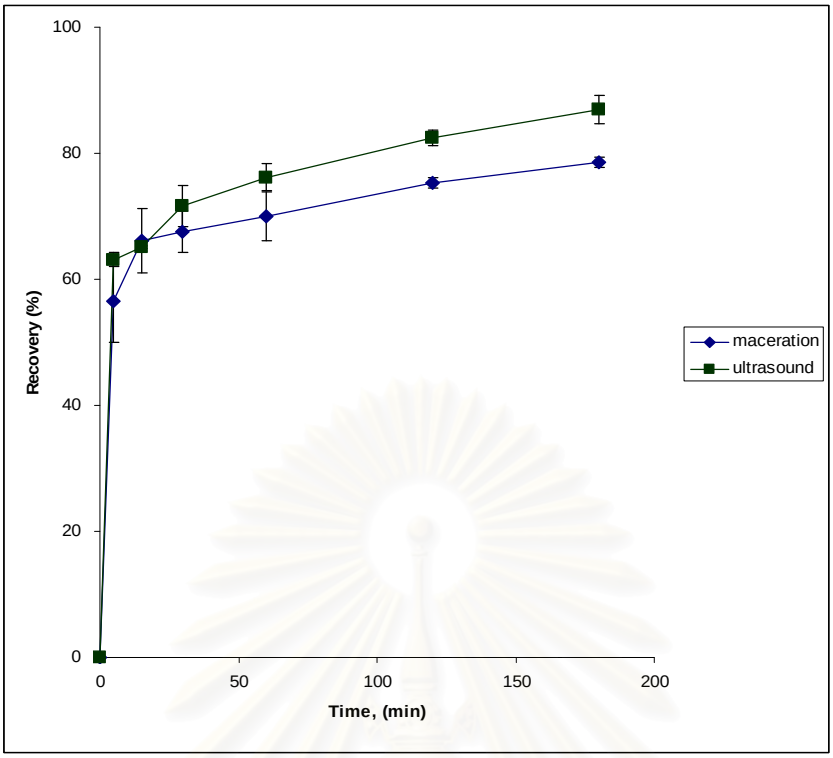


Figure 5.1 The effect of ultrasonication and its extraction time at 45 °C using 95 %v/v ethanol as the solvent on the extraction of capsaicinoids

5.1.2 The effect of extraction temperature

The effect of ultrasonic extraction temperature on the release of capsaicinoids using 95 % ethanol and acetone as a solvent was shown in figure 5.2 and figure 5.3, respectively. It was found that with 95 %v/v ethanol as a solvent, the percent of recovery increased with the increasing temperature from 30 °C to 45 °C owning to the increased solubility of capsaicinoids. Furthermore, at higher temperatures, the solvent viscosity and density decreased resulting in higher mass transfer. However, when the temperature was fixed at 60 °C, the percent recovery of capsaicinoids was slightly lower than that of 45 °C. This demonstrated that to some extent, the increase of temperature did not guarantee the higher product recovery. The numerical values of this experiment were shown in Appendix.

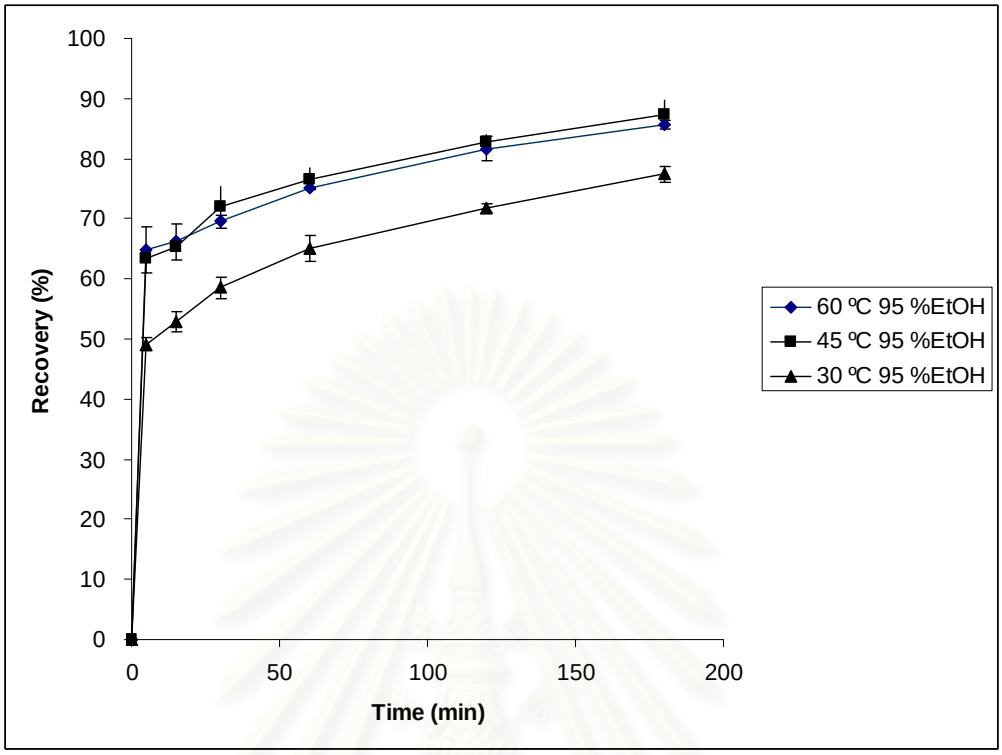


Figure 5.2 Effect of extraction temperature for ultrasonic extraction of capsaicinoids in 95 %v/v ethanol

In figure 5.3, it also revealed that with the ultrasonic extraction using acetone as a solvent, no significant difference was observed when the extraction were carried out at 45 $^{\circ}$C in comparison to at 30 $^{\circ}$C. In this experiment, the temperature at 60 $^{\circ}$C was not conducted because at that point it was above the boiling point of acetone, 58.5 $^{\circ}$C.

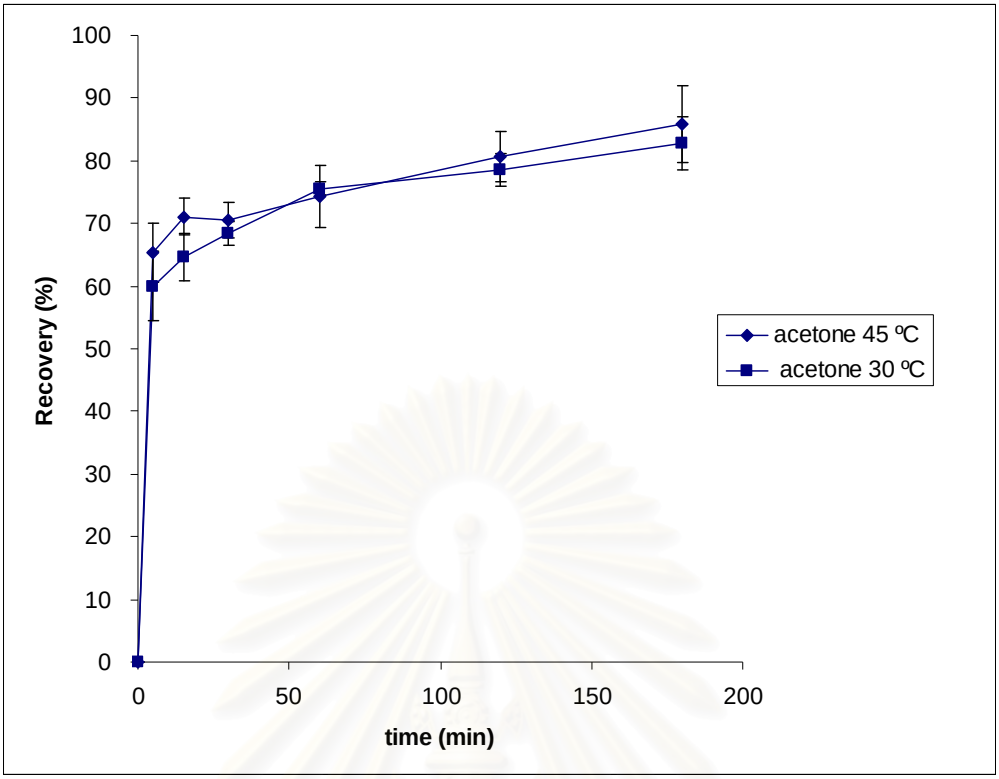


Figure 5.3 Effect of extraction temperature on ultrasonic extraction of capsaicinoids in acetone

5.1.3 The effect of solvent type

The effect of the type of solvents on ultrasonic assisted extraction of capsaicinoids was determined using two solvents, ethanol in water and acetone. Ethanol and acetone are safe and classified in class 3 pharmaceutical level according to the United Stage of Food and Drug Administration (US-FDA) (MeConville, 2002). Although acetonitrile, methanol, and hexane were used as the extracted solvents in some previous studies (Karnka et al., 2002), they were not used in this study because of their high toxicity. The other reasons for using acetone and ethanol were theirs costs, and availability (especially for ethanol, it can be produced in Thailand). Figure 5.4 showed the profile of released capsaicinoids conducting at 45 ^{o}C.

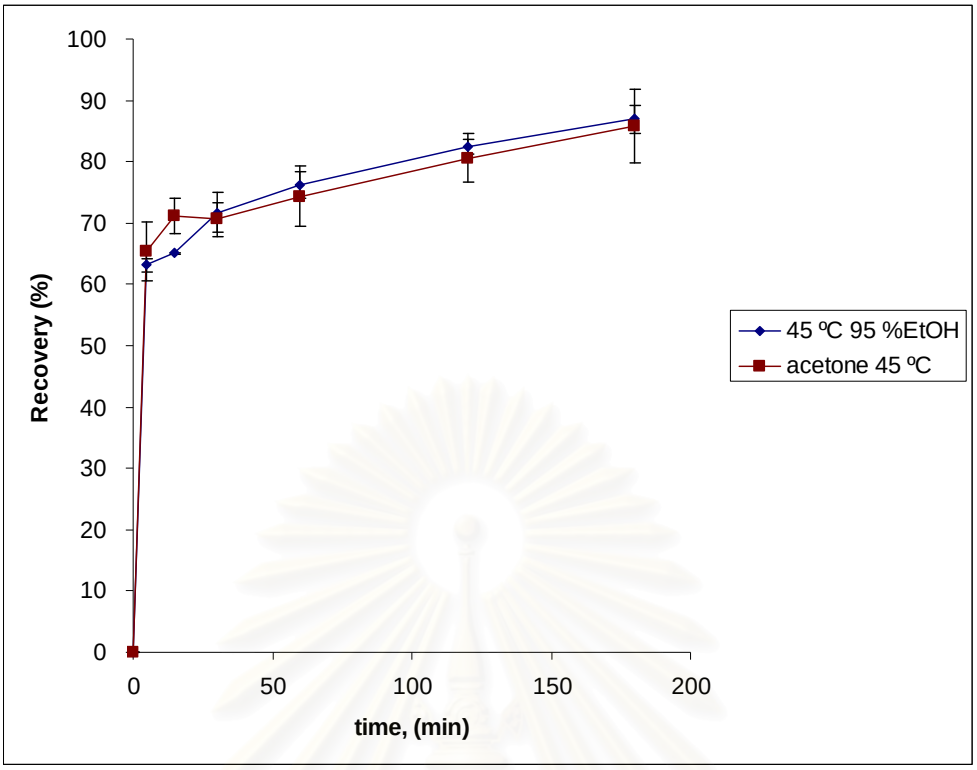


Figure 5.4 Effect of solvents on ultrasound extraction of capsaicionids at 45 °C

As seen in figure 5.4, the recovery of capsaicinoid extracted in 95 %v/v ethanol at 45°C was comparable to that in acetone. In general extraction, compared to 95 %v/v ethanol, the product obtained using acetone as a solvent should be more concentrated because of the lower viscosity and higher diffusivity of acetone (theirs properties were shown in table 5.1). However, with the ultrasound-assisted extraction as demonstrated in this study, the extraction using 75-95 %v/v ethanol was found slightly more effective. One of the influencing parameters on cavitation is vapor pressure. Higher vapor pressure, especially near the boiling point of liquid could reduce the cavitation to nearly zero. From a previous study (Hemvimol et al., 2006), it was explained that at high vapor pressure, more bubble was created but they collapsed with less intensity owing to a smaller internal/external pressure difference. Since vapor pressure of acetone was much higher than ethanol and water, the improved extraction efficiency by the cavitation effect should be less.

Table 5.1 Some physical properties of alcohol, water, and acetone (at 25 °C)

Type of solvents	polarity index	surface tension (mN/cm)	vapor pressure (mmHg)	viscosity (cP)
acetone	5.1	23.7	229.52	0.32
ethanol	5.2	23.7	59.02	1.2
water	9	72	23.8	0.89

5.1.4 The effect of ethanol-water composition

The effect of ethanol-water composition on the extraction of capsaicinoids using ultrasonic assisted extraction was performed at different temperatures (30, 45, and 60°C) as showed in figure 5.5, 5.6 and 5.7. The results revealed that the ratio of water in ethanol had significant effect on the extraction of capsaicinoids. For all applied temperatures, it was found that 50 %v/v ethanol were the least effective solvent for capsaicinoids extraction. This should be due to the differences in the polarity between solvents and the active compounds. Assumed from the structure, capsaicinoids should be less polar than the solvent so that it was not well solubilized in 50 %v/v ethanol. After the extraction time of 5 minutes, the percent recovery of capsaicinoids in 50 %v/v ethanol was almost constant. The influence of solubility, therefore, was suggested as the cause of low extraction efficiency of 50 %v/v ethanol.

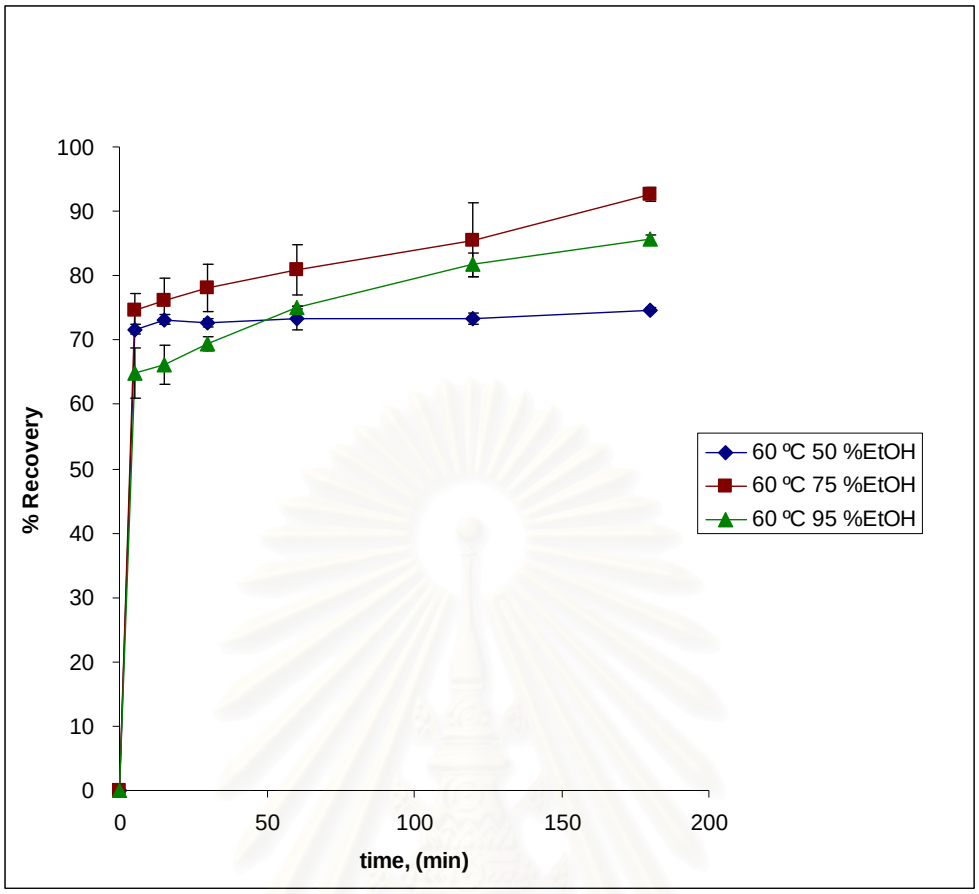


Figure 5.5 Effect of ethanol-water on ultrasound extraction of capsaicinoids at 60 ^{o}C

Figure 5.5 demonstrated that capsaicinoids recovery at 60^{o}C extraction temperature with 75 %v/v ethanol was higher than that of 95 %v/v ethanol. However, figure 5.6 showed that the profile of capsaicinoids recovery at fixed temperature of 45^{O}C in 95 %v/v ethanol was higher than that of 75 %v/v ethanol. Temperature affected many physical properties such as viscosity, diffusivity, solubility, vapor pressure and surface tension. The cavitational effects at the moderated (45 ^{O}C) temperature exhibited a stronger intensity than at 60 ^{O}C owning to higher vapor pressure of ethanol in water at 60 ^{O}C. For solubility, capsaicioids were better solubilized in 95 %v/v ethanol than in 75 %v/v ethanol.

At fixed temperature of 45 ^{O}C, both cavitational effect and solubility effect could enhance percent of capsaicinoid recovery while at 60 ^{O}C, high vapor pressure of

95 %v/v ethanol could decrease capsaicinoids ultrasound-assisted extraction even the solubility in this condition was higher.

At the fixed temperature of 30 ^{O}C (figure 5.7), the percent recovery of capsaicinoids in 75 %v/v ethanol was higher than in 95 %v/v. This was possibly due to the effect of swelling of the plant by water. In addition, ultrasound could facilitate the hydration processes of dried materials (Vinatoru, 2001). This process caused the pores of the cell walls to enlarge so that the diffusion process and mass transfer were improved and also the intensity of ultrasonic cavitation in the solvent mixture in the present of water was increased as a surface tension increased and the viscosity decreased. Hemwimol et al.(2006) studied the effect of water present in solvent. They revealed that to some extent, the extraction efficiency was increased with the amount of water added. However, in this study, adding too much water in solvent (i.e. 50 %v/v ethanol in water) was found decreasing the solubility of capsaicinoids resulting in lower extraction efficiency.

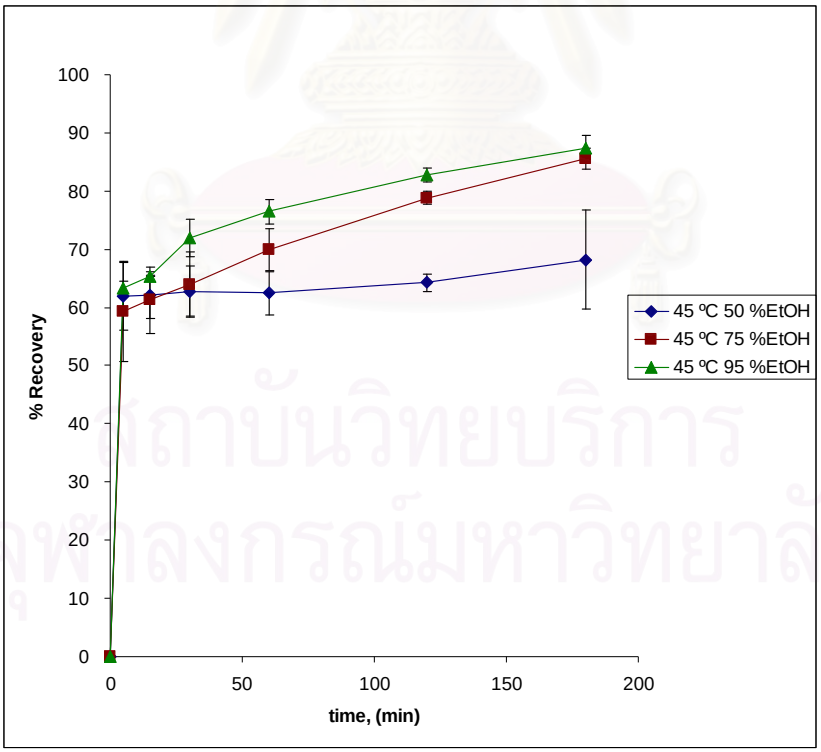


Figure 5.6 Effect of ethanol-water on ultrasound extraction of capsaicinoids at 45 ^{o}C

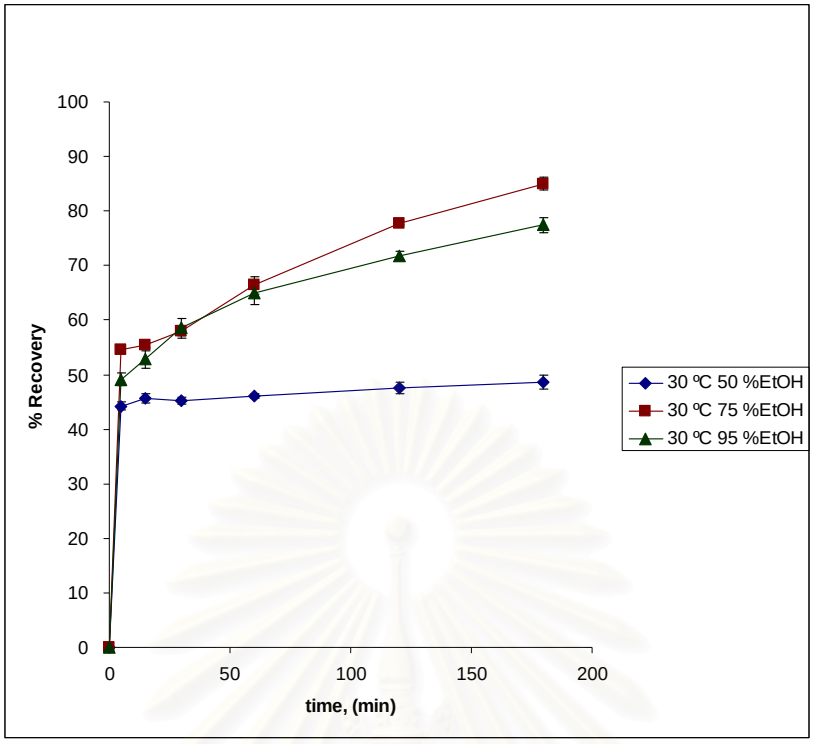


Figure 5.7 Effect of ethanol-water on ultrasound extraction of capsaicinoids at 30 ^{o}C

5.1.5 The Effect of solvent ratio

We also studied extraction efficiency at different ratios of dry weight of chili powder and solvent volume. From figure 5.8, it was found that with the ratios varied from 1:5 -1:8, no significant difference of percent recovery was observed. However, the result differed if it was compared with the extraction efficiency when the large amount of solvent (i.e. with the ratio > 1:30) was employed as in the experiment of Karnka et al.,(2002). Nonetheless, using large amount of solvent was considered not to be economical owing to the high operating cost of solvents and energy consumption for solvent evaporation.

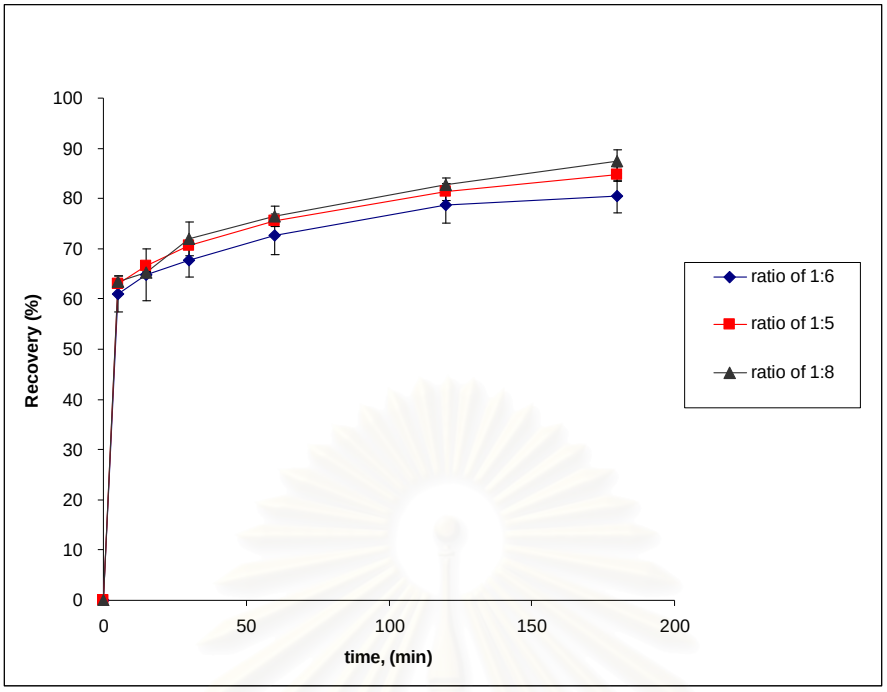


Figure 5.8 Effect of crude and ethanol ratio on ultrasound extraction of capsaicinoids at 45 °C

5.1.5 The comparison of percent recovery for each method

The percent capsaicinoids recovery from the ultrasound-assisted extractions at the fixed temperature of 45°C in this study was compared to those of maceration and soxhlet processes as shown in table 5.2. The recovery from maceration with magnetic stirrer using 95 %v/v ethanol as the solvent was lower than ultrasound-assisted extraction using the same solvent. The soxhlet extraction gave the highest percent recovery because extraction temperature was performed at boiling point temperature and using fresh solvent in each cycle made high concentration gradient. Moreover, the longer time of 5 hours was used for the soxhlet extraction comparing to 3 hours for ultrasound-assisted extraction. The percent recovery of capsaicinoids using 95 %v/v ethanol as solvent by maceration, soxhlet and UAE were 79.36, 92.04 and 87.39, respectively.

Table 5.2 The comparison of percent recovery for each method

Methods	Time (h)	Temperature	Type of solvent	Recovery (%)
Maceration (with magnetic stirrer)	15	45°C	EtOH:water (95:5)	79.36 ± 3.06
Soxhlet	5	Boiling point	EtOH:water (95:5)	92.04 ± 2.32
UAE	3	45	EtOH:water (50:50)	68.24
UAE	3	45	EtOH:water (75:25)	85.58
UAE	3	45	EtOH:water (95:5)	87.39

The optimal condition from laboratory

From the experimental results, all potential conditions were listed in table 5.3. The optimal condition was selected according to the consideration of less consumed energy and high extractability.

It has been known that when higher temperature was employed, the more energy was consumed. In this case, the condition at 45 °C gave percent recovery slightly different from that of 60 °C. However, 15 °C difference in operating temperature should lead on to significantly higher energy cost in industrial scale.

For the ethanol-water composition aspect, it was costly to use 75 %v/v ethanol as a solvent since the removal of solvent at the final process required high energy consumption.

In addition, since the difference in capsaicinoids recovery was not significant with the varied ratio of capsicum dried powder weight and solvent volume in the applied range as demonstrated previously, the lowest ratio was considered more effective. Therefore, the selected condition to apply for the pilot scale ultrasonic

extraction was using 95 %v/v ethanol as a solvent, at extraction temperature of 45 °C with the ratio of 1 g of dried chili powder per 5 mL of solvent.

Table 5.3 The potential conditions obtained from the laboratory scale ultrasonic assisted extraction

condition			Recovery (%)
temperature (°C)	ethanol-water composition	powder: solvent	
45	75:25	1:8	85.58
	95:5	1:8	87.39
	95:5	1:6	80.45
	95:5	1:5	84.84
60	75:25	1:8	92.66
	95:5	1:8	85.77

Table 5.4 The selected condition for the pilot scale extraction

items	condition
temperature	45 °C
solvent	95 % ethanol
crude powder:solvent ratio	1: 5

5.2 Experiment study in pilot and indurtrial (GPO) scale

The second part of this study was conducted in pilot scale and industrial scale at the Government Pharmaceutical Organization (GPO).

5.2.1 The pilot scale ultrasonic assisted capsaicinoids extraction

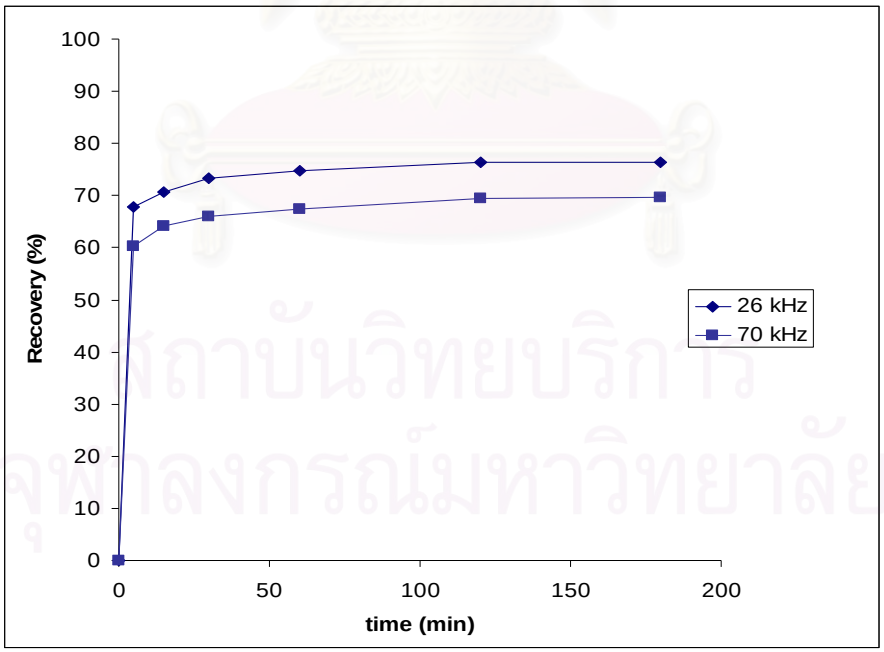


Figure 5.9 the study on pilot ultrasonic assisted extraction of capsaicinoids at 26 and 70 kHz using 95 % ethanol at 45 °C and 1:5 crude powder and solvent ratio

The pilot scale ultrasonic assisted extraction was performed at the selected condition obtained from the laboratory scale experiment and the result was shown in figure 5.9. The extraction was conducted at 26 kHz and 70 kHz. It was found that the percent recovery of capsaicinoids at 26 kHz (76.4%) was higher than that at 70 kHz (69.6%). The reason should be according to the decrease in the amount and intensity of cavitation in liquids at high frequency (Mason, 1988). At higher frequency, the rarefaction (and compression) cycles time for a bubbles to grow to a size sufficient to cause disruption of liquid were shorter.

The main reason of the lower percent recovery from the pilot scale extraction compared with the laboratory scale might be due to the difference in particle size of the chili material. The particle size used in the laboratory scale was 3 mm. but for the pilot scale, the particle of 13 mm. chili was used in order to ease the filtration step.

5.2.2 The Industrial scale capsaicinoids extraction (hot maceration).

The capsaicinoids extraction by hot maceration process in industrial scale was followed the government pharmaceutical organization procedure. The extraction of capsaicinoids was started by overnight soaking of chili powder in 95 %v/v ethanol at 30 ^{o}C , then the hot maceration was proceeded. The solution was heated at boiling point (78^{o}C) for 180 minutes. After that the extract were drawn at the bottom of the extractor.

After that the herbs residues was separated by filtration unit. The obtained solution was well mixed and 15 ml of sample was drawn from the homogenous extract for HPLC analysis. The recovery of capsaicinoids extracted by the hot maceration process was 81.81± 0.42 percent. The yield of capsaicinoids from the industrial scale hot maceration was 7.0% higher than that from the pilot scale UAE.

Although, the yield of capsaicinoids from pilot scale ultrasonic extraction was slightly lower than that from industrial scale hot maceration, the ultrasonic extraction provided many advantages.

The ultrasonic assisted extraction required much shorter extraction time and lower temperature. Moreover, from the chromatogram observation, there were some more peaks in the HPLC chromatogram of the extract obtained from the hot maceration compared with that from the pilot scale ultrasonic extraction as shown in figure 5.10 and figure 5.11 These peaks was considered as impurities which could arise from thermal degradation at high temperature operation.

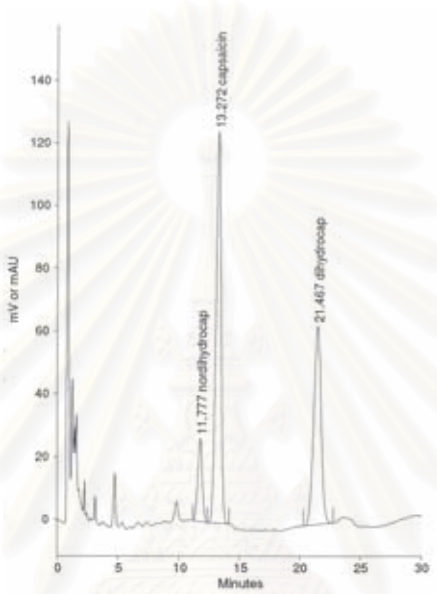


Figure 5.10 The chromatogram of sample conducting using the hot maceration process in industrial scale

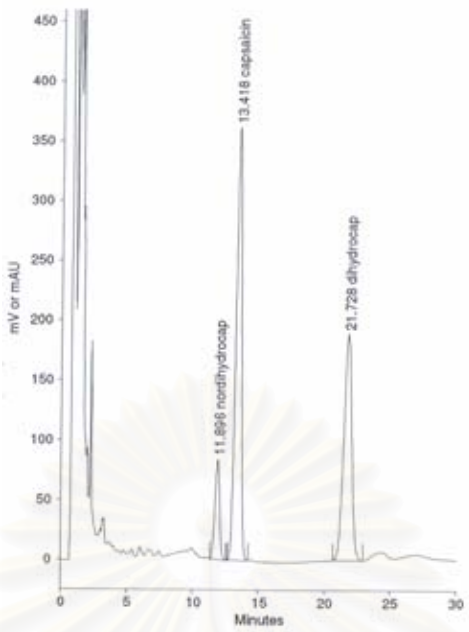


Figure 5.11 The chromatogram of sample conducting using the ultrasonic in pilot scale

CHAPTER VI
CONCLUSIONS

6.1 Conclusions

6.1.1 The rate of capsaicinoids release was very rapidly within the 5 minutes of the extraction and significantly decreased after that.

6.1.2 The rising temperature up to 45°C on ultrasonic extraction enhanced the capsaicinoids release into the extract. However, at 60°C, the amounts of capsaicinoids became slightly lower comparing with those obtained at 45 °C.

5.1.3 Acetone as well as 75 and 95 %v/v ethanol could be used as solvents for ultrasonic assisted extraction of capsaicinnoids without much difference in extractability. The ethanol-water composition at 50 %v/v had the lower capsaicinoids extractability in all cases.

6.1.4 The optimum condition from ultrasonic assisted capsaicinoids extraction in laboratory scale was selected under consideration of extraction efficiency and operating cost. The optimized condition was the ratio of 1 gram dried powder per 5 mL of 95 %v/v ethanol, 45 °C, and 180 minutes of extraction time.

6.1.5 In pilot scale ultrasonic extraction, the operation at 26 kHz gave a higher percent recovery of capsaicinoids than that obtained at 70 kHz. The percent recovery was slightly lower than that obtained in the laboratory scale. This might be due to the difference in particle size of chili.

6.1.6 The percent recovery of capsaicinoids from the hot maceration (GPO method) was slightly higher than that obtained from the pilot scale ultrasonic assisted extraction. However, the advantage of ultrasonic assisted extraction was less time and lower operating temperature. Also the extract from ultrasonic assisted extraction contained lower impurities.

6.1 Suggestion for further study

The further study should be conducted to reduce the energy consumed in ultrasound-assisted extraction. A pulse control should be considered such as 10 minutes for sonication, then, rest it for 5 minutes.

The ultrasound-assisted extraction should be further studied for extracting thermal degraded compounds in other indigenous plants in Thailand, for example; xanthophylls in marigold and lactones in *Andrographais paniculata*. The temperature and the time of extraction can be lower and then the high quality of the extract with lower cost can be achieved.

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Appendix

Standard capsaicinoids using in the experiment

The analytical standard in all experiment was a synthetic substance, N-vanillynonanamide because its properties were similar to natural capsaicinoids and agreed to the analytical method of AOAC. Moreover, the synthetic standard was cheaper and more available than natural capsaicin. The structure and chromatogram of the substances was shown in figure A1 and figure A2.

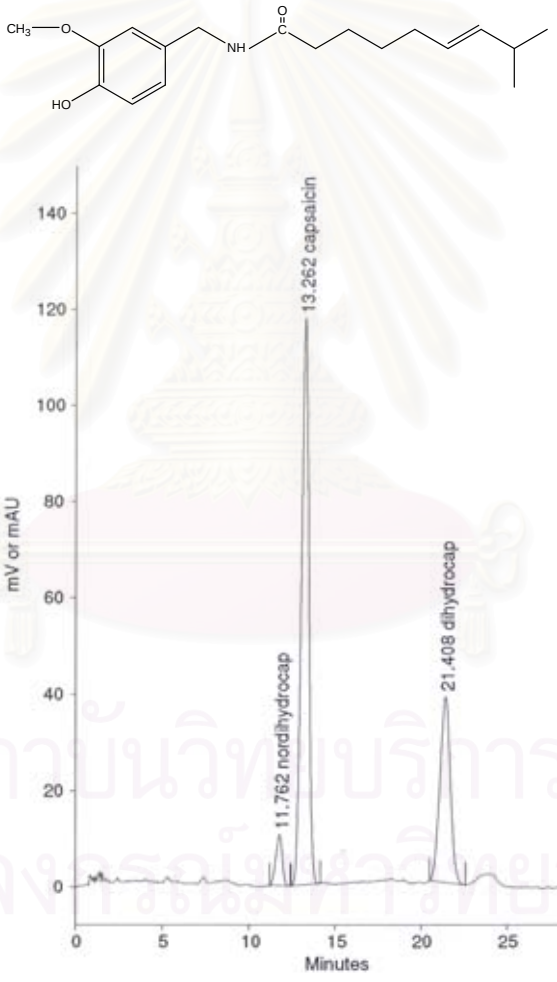
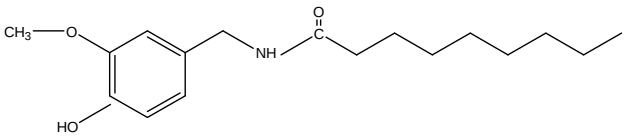


Figure A1 The chromatogram of natural capsaicin standard



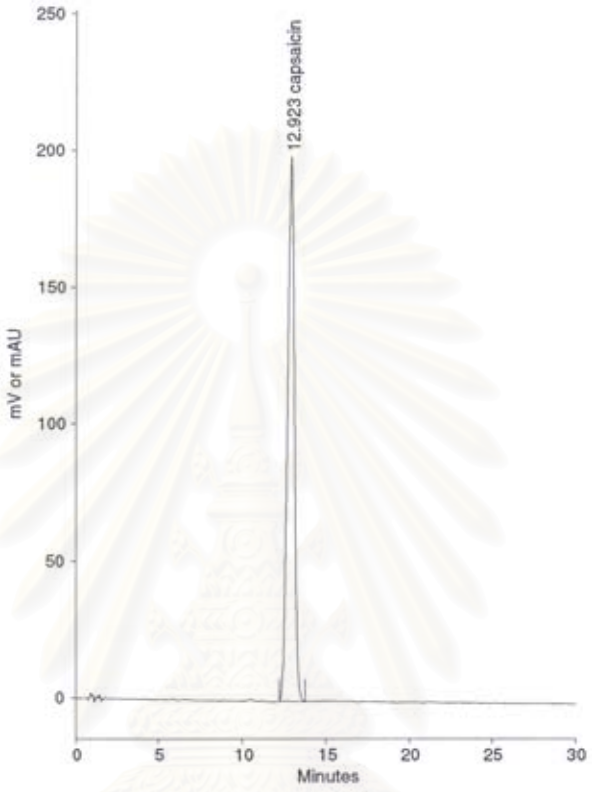


Figure A2 The chromatogram of N-vanillylnonanamide

The relative relation time of N-vanillylnonanamide to natural capsaicin was 0.9744.

Calibration curves of capsaicin

The calibration curve of capsaicin in each experiment performs in 6 concentrations, 0.001, 0.01, 0.015, 0.02, 0.025, 0.03 mg/ml as shown below. The linear regression value (R^2) is 0.99995. (normally, R is not less than 0.9995)

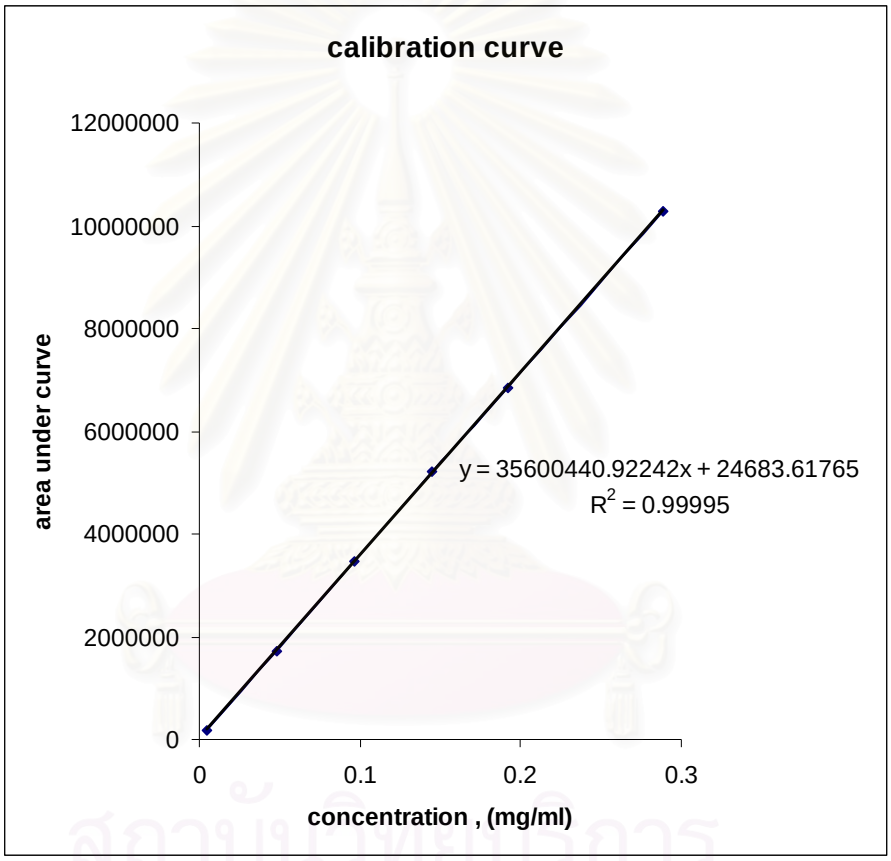


Figure A3 Calibration curve of N-vanillylnonanamide in 95 %v/v ethanol

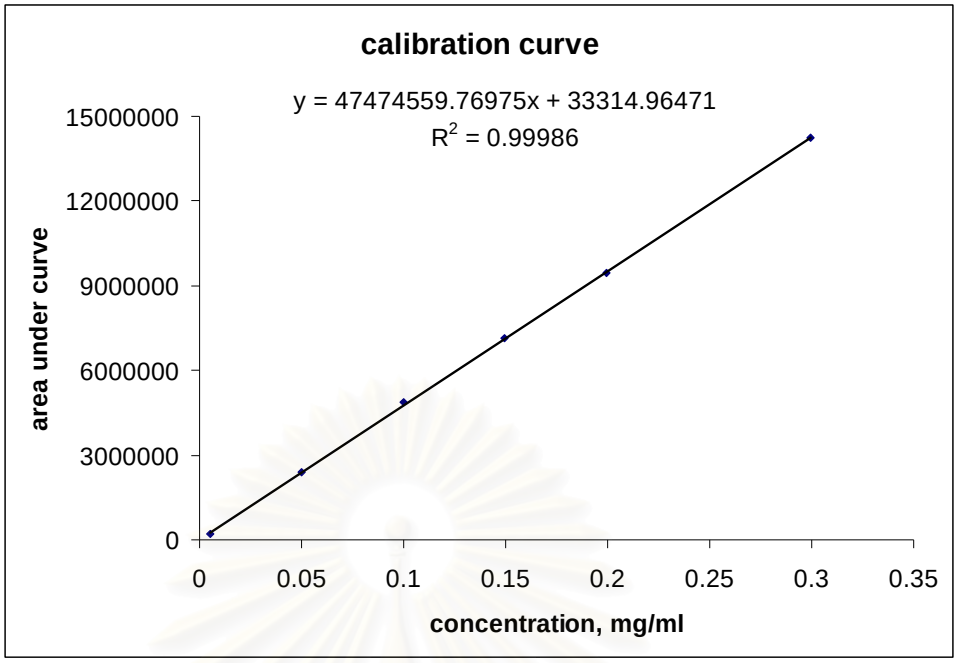


Figure A4 Calibration curve of N-vanillylnonanamide in 75 %v/v ethanol

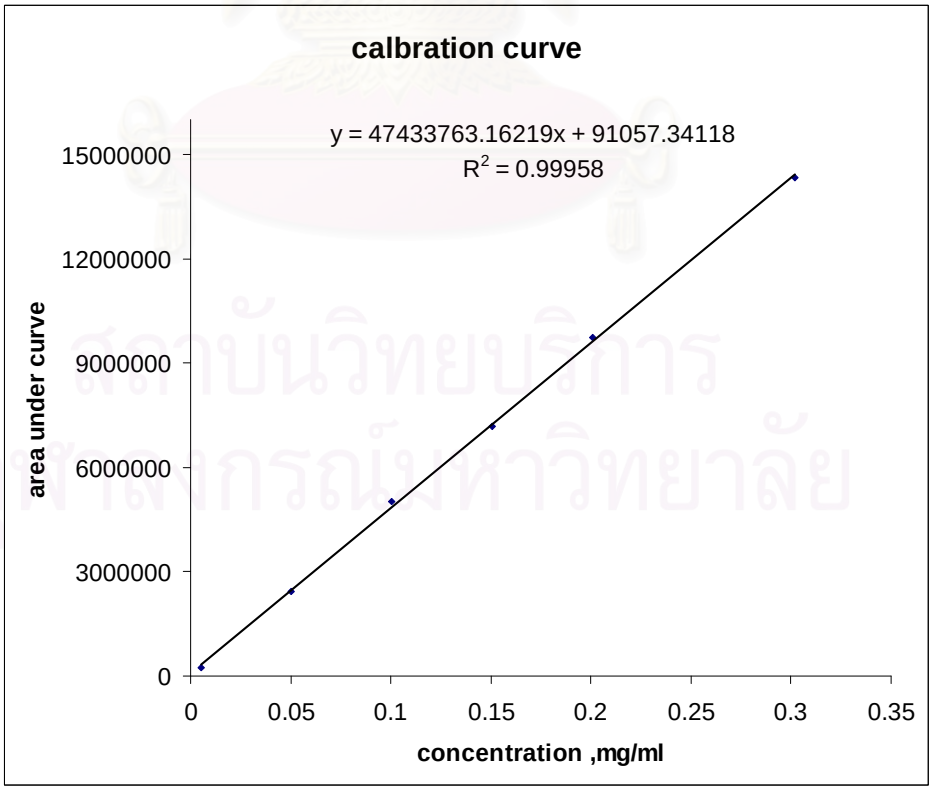


Figure A5 Calibration curve of N-vanillylnonanamide in 50 %v/v ethanol

The content of capsaicinoids in raw material

The raw material, *capsicum frutescens*, was purchased from Mae Chan, Chiang Rai for laboratory and pilot scale. The first part, used in laboratory scale was ground and sieved through 3 mm opening. The latter employed in pilot scale was ground and sieved through 13 mm opening. Both of them were analyzed for the amount of total capsaicinoids.

Table A1 The content of capsaicinoids in raw material

scale	the amount of capsicinoids on dry basis	Drying weight (%)
laboratory scale	0.509	94.31
pilot and industrial scale	0.511	90.03

Table A2 the percent recovery of capsaicinoids by ultrasonic-assisted extraction

time	Recovery of capsaicinoids (%)										
	temperature at 60 ºC			temperature at 45 °C				temperature at 30 ºC			
	50 %EtOH	75 %EtOH	95 %EtOH	50 %EtOH	75 %EtOH	95%EtOH	acetone	50 %EtOH	75 %EtOH	95 %EtOH	acetone
0	0	0	0	0	0	0	0	0	0	0	0
5	71.72	74.63	64.89	61.97	59.30	63.44	65.63	44.29	54.59	49.17	60.26
15	73.24	76.11	66.20	62.20	61.28	65.39	71.38	45.64	55.43	52.84	64.93
30	72.63	78.15	69.55	62.86	63.99	72.02	70.88	45.25	57.93	58.54	68.67
60	73.42	81.02	75.06	62.51	69.97	76.55	74.71	46.09	66.46	65.04	75.83
120	73.36	85.56	81.73	64.35	78.89	82.89	81.01	47.58	77.86	71.88	78.97
180	74.75	92.66	85.77	68.24	85.58	87.39	86.25	48.55	85.07	77.46	83.14

Table A3 The recovery of capsaicinoids by ultrasonic extraction at 26 and 70 kHz, 45 ^{o}C in pilot scale

time (min)	Percent recovery of capsaicinoids	
	Frequency of 26 kHz	Frequency of 70 kHz
0	0.00	0.00
5	67.80	60.36
15	70.69	64.24
30	73.30	65.95
60	74.77	67.44
120	76.37	69.41
180	76.37	69.58

BIOGRAPHY

Mr. Sumate Boonkird was bone on August 25, 1977 in Bangkok, Thailand. He received his Bachelor of Engineering from the School of Engineering, Suranaree University, Nakornrajsima, Thailand in 2000. Currently, he is working as a researcher at Research and Develpoment Institute, The Government Pharmaceutical Organization (GPO).