THE EXTRACTION OF FISH OIL FROM EEL BY USING MICROWAVE EXTRACTOR

MEGAT AHMAD FAIZ BIN ABDUL AZIZ

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

APRIL 2010

ABSTRACT

The eel is rich in calcium and iron as well as vitamin B and D, also believed by many people to be cure for kidney disease, asthma, heart palpitations and impotency as well as hastening healing of surgical wounds. In this research, fish oil was extracted from eel (*Monopterus albus*) by using microwave extractor. Effects of different solvent volume, different temperature and different extraction time on extraction yields were investigated. Then the extracted fish oil was analyzed by using 785 DMP Titrino and Gas Chromatography Mass Spectrometer (GCMS). The solvent volumes used were 25, 50, 75 and 100ml. The microwave temperatures used are 110, 130, 150 and 170°C while the extraction times that were set are 8, 12, 16 and 20 minutes. From the results obtained, the best operating condition to extract the fish oil from eel is at the solvent volume of 75ml for 10g of eel, microwave temperature of 110°C and 20 minutes of extraction time. The highest extraction yield obtained from the quantitative analysis was 11.25%. The quality of the extracted fish oil was highest at 75ml of solvent volume, microwave temperature of 110°C with the extraction time in the range of 16 to 20 minutes. At this operating condition, the lowest value obtained for FFA is 0.36% with acid value of 0.20mg KOH/1g. From the qualitative analysis by using GCMS, results show that the FAME's that were mostly detected in the fish oil are palmitic acid, linoleic acid, oleic acid and also stearic acid. As a conclusion, the objective of this study which is to extract the fish oil from eel by using microwave extraction method is achieved. Besides that, the effects of different solvent volumes, microwave temperatures and extraction times on extraction yields were also determined.

ABSTRAK

Belut kaya dengan kalsium dan zat besi beserta juga vitamin B dan D, ia juga dipercayai ramai dapat memulihkan penyakit buah pinggang, lelah, serangan jantung dan berkemampuan dalam menyingkatkan proses pemulihan luka selepas pembedahan. Di dalam kajian ini, minyak ikan telah diekstrak daripada belut (*Monopterus albus*) dengan menggunakan pengekstrak gelombang mikro. Kesan daripada perubahan isipadu pelarut, suhu gelombang mikro dan masa ekstrak ke atas hasil ekstrak telah disiasat. Kemudian, minyak ikan yang telah diekstrak dianalisis dengan menggunakan 785 DMP Titrino dan Gas Kromatografi Berjisim Spektrometer. Isipadu pelarut yang digunakan adalah 25, 50, 75 dan 100ml. Suhu gelombang mikro yang digunakan adalah 110, 130, 150 dan 170°C manakala masa ekstrak yang ditetapkan adalah 8, 12, 16 and 20 minit. Berdasarkan keputusan yang diperolehi, keadaan terbaik untuk mengekstrak minyak ikan daripada belut adalah pada 75ml isipadu pelarut bagi 10g belut, suhu gelombang mikro 110°C dengan jangka masa ekstrak 20 minit. Hasil ekstrak yang paling tinggi yang dicapai dalam analisis kuantitatif adalah 11.25%. Kualiti minyak ikan yang diekstrak adalah paling tinggi pada isipadu pelarut 75ml bagi 10g belut, suhu gelombang mikro 110°C dengan jangka masa ekstrak antara 16 ke 20 minit. Pada keadaan ini, nilai terendah bagi kandungan asid lemak bebas adalah 0.36% dengan nilai asid 0.20mg KOH/1g. Daripada analisis qualitatif menggunakan Gas Kromatografi Berjisim Spektrometer (GCMS), kandungan asid lemak metil ester (FAME) yang kerap dikesan di dalam minyak ikan adalah asid palmitik, asid linoleik, asid oleik dan asid stearik. Sebagai kesimpulannya, objektif di dalam pembelajaran ini untuk mengekstrak minyak ikan daripada belut dengan menggunakan kaedah ekstrak gelombang mikro tercapai. Selain itu, kesan isipadu pelarut, suhu gelombang mikro dan masa ekstrak ke atas hasil ekstrak juga dikenal pasti.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

AA	-	Arachidonic Acid
ALA	-	Alpha-linolenic Acid
cm	-	centimeter
DHA	-	Docosahexaenoic Acid
DPA	-	Docosapentaenoic Acid
EPA	-	Eicosapentaenoic Acid
FAME	-	Fatty Acid Methyl Ester
FFA	-	Free Fatty Acid
g	-	gram
GCMS	-	Gas Chromatography Mass Spectrometer
h	-	Hour
Hz/GHz	-	Hertz/Gigahertz
KOH	-	Kalium Hydroxide
LA	-	Linoleic Acid
MAE	-	Microwave Assisted Extractor
mg	-	milligram
min	-	minute
ml	-	milliliter
PLE	-	Pressurized Liquid Extraction
PUFA	-	Polyunsaturated Fatty Acids
SFE	-	Supercritical Fluid Extraction
SOX	-	Soxhlet Extraction
USE	-	Ultrasonic Extraction
W	-	Watt
°C	-	Degree Celsius
%	-	Percentage
µL	-	microlitre

CHAPTER 1

INTRODUCTION

1.1 Research Background

During the last two decades polyunsaturated fatty acids (PUFA) have attracted great interest among scientists for their medicinal and nutritional properties. Among the common sources of these PUFAs are fish oils. The PUFA composition in fish oils are affected by several factors, such as geographical location, temperature and water salinity. The oils extracted from the northern hemisphere coldwater fishes are rich in n-3 PUFA, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). An increase in environmental temperature and decrease in water salinity will bring about a higher content of arachidonic acid, which is an n-6 PUFA, in replacement of EPA. Thus, tropical freshwater fishes are expected to contain high levels of arachidonic acid (AA) and DHA. These fatty acids have been approved as essential fatty acids (Razak *et al*., 2000).

At present, arachidonic acid is produced from certain marine fish, mammals and microorganisms while DHA is obtained from certain coldwater fish. Little has been done to study the PUFA content of Malaysian freshwater fish. Endinkeau and Tan Kim Kiew studied the lipid and fatty acid content of several Malaysian freshwater fishes. They discovered that these fishes contain low level of EPA and DHA. However, the eel, Monopterus albus contain a high level of DHA. The study was based on the oil extracted from the fillet of the fishes (Razak *et al*., 2000).

Even though many people are quite reluctant to eat the eel, it cannot be denied that this freshwater fish has many nutritional benefits. Its nutritional values are said to be on par with that of the 'tenggiri' (spanish mackerel) and 'selar' (crevalle) which have 18.6 per cent protein and 15 per cent fat. The eel is also rich in calcium and iron as well as vitamins B and D. Hence, it is no surprise that the eel's flesh is believed by many people to be the cure for kidney disease, asthma, heart palpitations and impotency as well as hastening healing of surgical wounds. According to traditional medicine practitioners, regular consuming of eels helps to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power (Nurul, BERNAMA).

For the extraction method, Microwave-assisted Extractor (MAE) seems to be a viable alternative to conventional extraction techniques for a variety of solid matrices, either spiked or containing native compounds. Moreover, MAE offers great reductions in time and solvent consumption, and increased sample throughput. Optimization of MAE conditions is rather easy, because there are few parameters (matrix moisture, nature of solvent, time, power and temperatures in closed vessels), and it is cheaper than other modern techniques, such as Supercritical Fluid Extraction (SEF) and Pressurized Liquid Extraction (PLE). On the other hand, selectivity may be less, so a clean-up procedure may be required before analysis. Although most existing applications of MAE deal with solid samples, the results from liquid matrices are promising, suggesting that this field of application will expand in the near future. (Ahmed, 2003).

In this study, fish oil was extracted separately from the body of Monopterus albus and the fatty acid composition was determined by gas chromatography mass spectrometer. Subsequently, the oil was saponified to isolate the free fatty acids (FFA) and the fatty acid composition of the FFA was determined (Razak *et al*., 2000).

1.2 Identification of Problem

Eventhough the fish nutrients can be obtained directly by eating cold water fish such as salmon, cod or even eel, there is always the danger of mercury contamination as well as other toxins. This is the main reason why pregnancy women and young children are advised against eating fish. The other issue is that the contents of EPA and/or DHA in the fish or eel are uncertain. Therefore, consumers might not obtain the maximum fish oil benefits. It is always possible that the concentration of these important nutrients is reduced during the manufacturing or handling process. It is reported that taking concentrated fish oil supplement is essential to reap the health benefits from the fish, one that is molecularly distilled, devoid of contaminants and contains high concentrations of DHA and EPA (http://www.herbal-supplements-guide.com/benefits-of-fish-oil.html).

1.3 Statement of Objectives

The main objective of this study is to extract the fish oil from eel by using microwave extraction method. Besides that, the effects of different solvent volumes, temperatures and extraction times on extraction yields were also determined.

1.4 Research Scope

The research scopes for this study are:

i. To study the effects of different solvent volumes at 25, 50, 75 and 100ml with constant microwave power of 1000W, constant extraction time of 20 minutes and constant temperature of 150°C on extraction yields.

ii. To examine the effects of different temperatures at 110, 130, 150 and 170°C with the best solvent volume obtained from 1.4 (i) at constant microwave power of 1000W and constant extraction time of 20 minutes on extraction yields.

iii. To study the effects of different extraction time at 8, 12, 16 and 20 minutes with the best solvent volume obtained from 1.4 (i) and the best temperature obtained from 1.4 (ii) at constant microwave power of 1000W.

iv. To analyze the fish oil by using 785 DMP Titrino and Gas Chromatography Mass Spectrometer (GCMS).

1.5 Rationale and Significance of Study

Experts have found out that eel contains vitamins A, B1, B2, D and E, which are effective agent for the body's wellbeing. Eels are also abundant with unsaturated fatty acids like Palmitoleic acid, Docosahexaenoic acid (DHA) and Eiconsapentaenoic acid (EPA). According to medical research, eel has been shown in certain studies to significantly reduce the chances of the development of type 2 diabetes among certain groups. Because of eel's high omega 3 content, a high intake of eel may delay the development of diabetes in glucose intolerant individuals. It is also safe for individuals with type 2 diabetes and can help ease cardiovascular disease risk factors such as high triglyceride levels. Today it has been made as all-natural food supplement in powdered form and whose health benefits are known and accepted in Japan and some parts of Europe. As food supplement, it has been specifically developed for elderly people who wanted to live with renewed strength, vitality and energy. The powdered form of eel, is made up of the skin, flesh and bones capsulated to make it more concentrated, thus ensuring that every bit of vitamins and minerals is optimized (http://affleap.com/blog/the-benefits-of-eating-eel/).

CHAPTER 2

LITERATURE REVIEW

2.1 Eel

The eel, scientifically known as *Monopterus Albus*, is included in the fish genus. It has a scaleless cylindrical snake-like body with tapered tail and small eyes. It grows to meter or less, usually 25 to 40 cm as an adult. Its body color is brown above and white or light-brown below. In adults, paired fins are lacking, and the dorsal, caudal and anal fins are reduced. The gill openings are merged into a single slit underneath the head while the mouth is large and protractile and both upper and lower jaws have tiny teeth for eating fishes, worms, crustaceans, and other small aquatic animals at night. Asian swamp eels are considered voracious, generalized predators. They lay their eggs into a bubble nest in shallow water. Adults breathe air through the mucosa lining of the gill arch. They move over dry land or create mud burrows in response to lack of water. While it can survive temperatures below freezing, months without water, or saline waters; it is mostly found in warm fresh-waters locations such as muddy ponds, swamps, canals, and rice fields (http://en.wikipedia.org/wiki/Monopterus_albus).

2.1.1 The Benefits of Eel

Even though many people are quite reluctant to eat the eel, it cannot be denied that this freshwater fish has many nutritional benefits. Its nutritional values are said to be on par with that of the 'tenggiri' (spanish mackerel) and 'selar' (crevalle) which have 18.6 per cent protein and 15 per cent fat. The eel is also rich in calcium and iron as well as vitamins B and D. Hence, it is no surprise that the eel's flesh is believed by many people to be the cure for kidney disease, asthma, heart palpitations and impotency as well as hastening healing of surgical wounds. According to traditional medicine practitioners, regular consuming of eels helps to boost the body's immune system, stabilizes the blood pressure, smoothens the skin texture, prevents hepatitis and enhances the memory power (Nurul, BERNAMA).

Experts have found out that eel contains vitamins A, B1, B2, D and E, which are effective agent for the body's wellbeing. Eels are also abundant with unsaturated fatty acids like Palmitoleic acid, Docosahexaenoic acid (DHA) and Eiconsapentaenoic acid (EPA). According to medical research, eel has been shown in certain studies to significantly reduce the chances of the development of type 2 diabetes among certain groups. Because of eel's high omega 3 content, a high intake of eel may delay the development of diabetes in glucose intolerant individuals. It is also safe for individuals with type 2 diabetes and can help ease cardiovascular disease risk factors such as high triglyceride levels. Today it has been made as all-natural food supplement in powdered form and whose health benefits are known and accepted in Japan and some parts of Europe. As food supplement, it has been specifically developed for elderly people who wanted to live with renewed strength, vitality and energy. The powdered form of eel, is made up of the skin, flesh and bones capsulated to make it more concentrated, thus ensuring that every bit of vitamins and minerals is optimized (http://affleap.com/blog/the-benefits-of-eating-eel/).

2.2 Fatty Acids

2.2.1 Essential Fatty Acid

The human body can produce all but two of the fatty acids it needs. These two, linoleic acid (LA) and alpha-linolenic acid (ALA), are widely distributed in plant oils. In addition, fish oils contain the longer-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

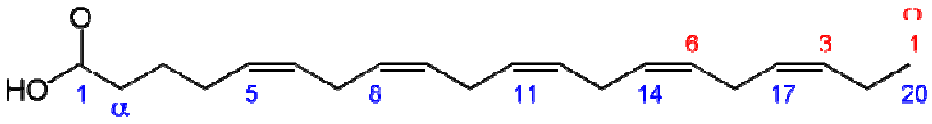

Figure 2.1: Chemical structure of eicosapentaenoic acid (EPA)

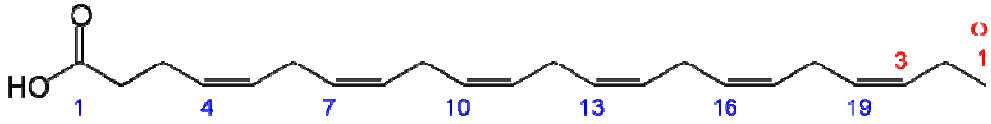

Figure 2.2: Chemical structure of docosahexaenoic acid (DHA)

(http://en.wikipedia.org/wiki/Omega-3_fatty_acid)

Other marine oils, such as from seal, also contain significant amounts of docosapentaenoic acid (DPA), which is also an omega-3 fatty acid. Although the body to some extent can convert ALA into these longer-chain omega-3 fatty acids, the omega-3 fatty acids found in marine oils help fulfill the requirement of essential fatty acids (and have been shown to have wholesome properties of their own). Since they cannot be made in the body from other substrates and must be supplied in food, they are called essential fatty acids. Mammals lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10. Hence linoleic acid and alpha-linolenic acid are essential fatty acids for humans.

In the body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection. Essential fatty acids are polyunsaturated fatty acids and are the parent compounds of the omega-6 and omega-3 fatty acid series, respectively. They are essential in the human diet because there is no synthetic mechanism for them. Humans can easily make saturated fatty acids or monounsaturated fatty acids with a double bond at the omega-9 position, but do not have the enzymes necessary to introduce a double bond at the omega-3 position or omega-6 position.

The essential fatty acids are important in several human body systems, including the immune system and in blood pressure regulation, since they are used to make compounds such as prostaglandins. The brain has increased amounts of linoleic and alpha-linolenic acid derivatives. Changes in the levels and balance of these fatty acids due to a typical Western diet rich in omega-6 and poor in omega-3 fatty acids is alleged to be associated with depression and behavioral change, including violence. The actual connection, if any, is still under investigation. Further, changing to a diet richer in omega-3 fatty acids, or consumption of supplements to compensate for a dietary imbalance, has been associated with reduced violent behavior and increased attention span, but the mechanisms for the effect are still unclear. So far, at least three human studies have shown results that support this: two school studies as well as a double blind study in a prison. Fatty acids play an important role in the life and death of cardiac cells because they are essential fuels for mechanical and electrical activities of the heart (http://en.wikipedia.org/wiki/Fatty_acid).

2.2.2 Free Fatty Acid

Fatty acids can be bound or attached to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. The uncombined fatty acids or free fatty acids may come from the breakdown of a triglyceride into its components (fatty acids and glycerol). However as fats are insoluble in water they must be bound to appropriate regions in the plasma protein albumin for transport around the body. The levels of "free fatty acid" in the blood are limited by the number of albumin binding sites available. Free fatty acids are an important source of fuel for many tissues since they can yield relatively large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids. The brain cannot use fatty acids as a source of fuel; it relies on glucose, or on ketone bodies. Ketone bodies are produced in the liver by fatty acid metabolism during starvation, or during periods of low carbohydrate intake.

Fatty acids react just like any other carboxylic acid, which means they can undergo esterification and acid-base reactions. Reduction of fatty acids yields fatty alcohols. Unsaturated fatty acids can also undergo addition reactions, most commonly hydrogenation, which is used to convert vegetable oils into margarine. With partial hydrogenation, unsaturated fatty acids can be isomerized from cis to trans configuration. In the Varrentrapp reaction certain unsaturated fatty acids are cleaved in molten alkali, a reaction at one time of relevance to structure elucidation (http://en.wikipedia.org/wiki/Fatty_acid).

The following table gives the fatty acid, vitamin E and cholesterol composition of some common dietary fats.

Table 2.1: The composition of fatty acid, vitamin E and cholesterol in some common dietary fats

	Saturated	**Monounsaturated**	**Polyunsaturated**	**Cholesterol**	**Vitamin E**
	g/100g	g/100g	g/100g	mg/100g	mg/100g
Animal fats					
Lard	40.8	43.8	9.6	93	0.00
Butter	54.0	19.8	2.6	230	2.00
Vegetable fats					
Coconut oil	85.2	6.6	1.7	0	0.66
Palm oil	45.3	41.6	8.3	0	33.12
Cottonseed oil	25.5	21.3	48.1	0	42.77
Wheat germ oil	18.8	15.9	60.7	0	136.65
Soya oil	14.5	23.2	56.5	0	16.29
Olive oil	14.0	69.7	11.2	0	5.10
Corn oil	12.7	24.7	57.8	0	17.24
Sunflower oil	11.9	20.2	63.0	0	49.0
Safflower oil	10.2	12.6	72.1	0	40.68
Hemp oil	10	15	75	0	
Rapseed / Canola oil	5.3	64.3	24.8	0	22.21

2.2.3 The Benefits of Omega-3 Fatty Acids

For over 20 years, clinical evidence have shown that Omega-3 fatty acids, specifically DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) are an essential part of our nutrition. The primary source of DHA and EPA is fish oil, which is why fish oil benefits have received so much attention in recent years.

There are so much benefits of fish oil that have been proven nowadays. Some of them are;

1) Higher Intelligence and Brain Function – Omega 3 fish oil has been found to improve memory, recall, reasoning and focus. It even protects against Alzheimer's and senility. And mothers can have a great impact on the intelligence of their babies by supplementing with fish oil while pregnant and nursing.
2) Less Pain from Inflammation – Omega 3 fatty acids regulate your body's inflammatory response and help prevent and relieve arthritis, prostatitis, cystitis, or any other condition ending in "itis." Plus, it reduces pain caused by injuries.
3) Feel Better with Less Depression – Making you smarter isn't all fish oil health benefits do for your brain. Numerous studies show omega 3 fatty acids alleviate mental anguish. This includes bipolar, depression and even schizophrenia.
4) Lower Blood Pressure – Omega 3 oils have been shown to work wonders for your cardiovascular system. This includes improvements in your heart, arteries and veins, plus it lowers blood pressure.
5) Reduced Cholesterol and Triglycerides – Even your cholesterol, LDLs and triglycerides will go down. And, at the same time, good HDLs will go up. This can add years to your life.
6) Protection Against Heart Attack and Stroke – When plaque breaks loose from arterial walls, it creates a thrombosis – a fancy way of saying "clot." If a clot gets stuck in the brain, it causes a stroke and when it plugs arteries, it causes a heart attack. Fish oil for heart health

research shows omega 3 fatty acids break up blood clots before they can cause any damage.

7) Protection Against Cancer – Omega 3 has been shown to help prevent breast, colon, lung and prostate cancer. Science tells us it accomplish this in three ways – by stopping the alteration from a normal healthy cell to a cancerous mass, by inhibiting unwanted cellular growth and by killing off cancer cells.

8) Lower Incidence of Childhood Disorders. Just to show how fish oil fatty acids leave nobody out, studies show that children (and adults) with ADD and ADHD experience a greatly improved quality of life. And those with dyslexia, dyspraxia and compulsive disorders have gotten a new lease on life thanks to omega 3 oils (Michael Byrd, EzineArticles.com).

2.3 Extraction Methods

Based on the researched done on extraction of fish oil from fish waste from Surimi Processing Plant, there are three different extraction methods were used to extract fish oil from fish waste and leaching fish waste. These were Bligh and Dryer method, acetone extraction method and wet reduction method. In Bligh and Dryer method, water, chloroform and methanol mixture was used as the solvent in the ratio of 1:4:4. In the acetone extraction, only acetone is used as the solvent in order to extract the fish oils. Meanwhile, for the wet reduction method, four combinations of heating temperatures (85 and 100°C) and times (30 and 50min) were used in order to obtain higher yields of fish oils. From the results obtained, there is a significant difference in the fish oil obtained from the respective extraction methods used. It can be concluded that the Bligh & Dryer method was the most effective extraction method with respect to the amount of oil obtained followed by the wet reduction method. Whilst the acetone extraction method was the least effective the lowest yield among the other methods (Nuraini *et al*., 2008).

Soxhlet Extraction (SOX) is one of the most frequently used liquid-solid extraction method developed in the late nineteenth century, is still routinely used for extraction of PCBs from several food matrices. However, among its disadvantages are their requirements for large volumes of solvent (10-200 ml for 1-100 g of tissue). Besides that, it's use of highly-purified polar and non-polar organic solvents (dichloromethane, hexaneacetone, hexane-dichloromethane) and the long extraction time (~18 h) as a result of slow analyte diffusion. Other disadvantages are desorption from the sample matrix to the extraction fluid, dilution of the extracts obtained, generation of dirty extracts that require extensive clean-up and also it cannot be automated. Animal and fish tissue are first macerated then ground with sodium sulfate and silica to reduce the water content and rupture cell walls; this method results in higher concentrations of analyte than freeze drying. Extraction with non-polar solvents, such as n-alkanes, takes a considerable time (> 6 h) and was not as effective as polar solvents, such as dichloromethane. Although higher recoveries were obtained by initial saponification of the tissue with 40% potassium hydroxide in ethanol (1:1) at 90^{o}C for 4h prior to extraction, this treatment often leads to loss of some CBs, and dechlorination and hydrolysis of highly chlorinated CBs (Ahmed, 2003).

Ultrasonic Extraction (USE) is a simple extraction technique, in which the sample is immersed in an appropriate organic solvent in a vessel and placed in an ultrasonic bath. The efficiency of extraction depends on the polarity of the solvent, the homogeneity of the matrix and the ultrasonication time. The mixture of sample and organic solvent is separated by filtration and washing with the solvent. USE is optimized, takes > 60 min to perform, is easy to use and does not require expensive instruments. However, it requires a large volume of solvent (50-200 ml), it may require successive extractions, extracts are usually diluted and therefore require filtration, and the method is not automated (Ahmed, 2003).

2.3.1 Microwave Extraction Method

In recent years, Microwave Extraction Method has attracted researcher's interest, as it allows rapid extraction of solutes from solid matrices by employing microwave energy as a source of heat, with extraction efficiency comparable to that of the classical techniques. The partitioning of the analytes from the sample matrix to the extractant depends upon the temperature and the nature of the extractant. Unlike classical heating, microwaves heat the entire sample simultaneously without heating the vessel; thus, the solution reaches its boiling point very rapidly, leading to a very short extraction time. Microwave energy causes molecular motion by migration of ions and rotation of dipoles. Dipole rotation refers to the alignment of molecules in the solvent and samples that have dipole moments. As the field decreases, thermal disorder is restored resulting in the release of thermal energy. At 2.45 GHz (the only frequency used in commercial systems), the alignment of the molecules followed by their return to disorder occurs $4.9X10^9$ times per second, which results in rapid heating (Ahmed, 2003).

During the extraction of oils from plant materials, MAE allows the migration of the compounds out of the matrix by selective interaction between microwaves and free water molecules present in the vascular systems, leading to localized heating and boiling of water. As the system expands, the cell walls rupture, allowing essential oils to flow towards the organic solvent. This process is different from classical solvent extraction, where the solvent diffuses into the matrix and extracts the components by solubilization. Moreover, in MAE, a wider range of solvents could be used, as the technique is less dependent on a high solvent affinity (Ahmed, 2003).

The application of microwave energy to the sample may be performed using two technologies which are closed vessels under controlled pressure and temperature, a process referred to as pressurized MAE (PMAE) or open vessel under atmospheric pressure, referred to as focused MAE (FMAE). The most commonly used closed system, the CEM MES 1000, allows extraction of 12 samples simultaneously under controlled temperatures. The main drawbacks of such a system are: loss of more volatile solutes if the temperature inside the vessel rises rapidly; and, the vessels need to be cooled to room temperature after extraction and before they can be

opened, thus increasing the overall extraction time. In open systems, as extractions proceed under atmospheric pressure, the maximum possible temperature is determined by the boiling point of the solvent. Sample heating is carried out homogeneously and efficiently. The most commonly used system is the Prolabo Soxwave 100. Losses of vapor are prevented by the presence of a reflux system on top of the extraction vessel. The system offers increased safety of sample handling compared to extraction in a pressurized, closed vessel, and a larger sample may be extracted in such a system than in a closed vessel system (Ahmed, 2003).

MAE seems to be a viable alternative to conventional extraction techniques for a variety of solid matrices, either spiked or containing native compounds. Moreover, MAE offers great reductions in time and solvent consumption, and increased sample throughput. Optimization of MAE conditions is rather easy, because there are few parameters (matrix moisture, nature of solvent, time, power and temperatures in closed vessels), and it is cheaper than other modern techniques, such as SEF and PLE. On the other hand, selectivity may be less, so a clean-up procedure may be required before analysis. For some applications, a filtration step only is needed, whereas, for others, SPE, GPC or additional LLE steps may have to be carried out. Although most existing applications of MAE deal with solid samples, the results from liquid matrices are promising, suggesting that this field of application will expand in the near future (Ahmed, 2003).

2.3.2 Microwave Extraction Principle

Even though dried plant material is used for extraction in most cases, but still plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect, evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell. The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptures cells to the surrounding solvent thus improving the yield of phytoconstituents. This phenomenon can even be more intensified if the plant matrix

is impregnated with solvents with higher heating efficiency under microwave (higher tan δ value). Higher temperature attained by microwave radiation can hydrolyze ether linkages of cellulose, which is the main constituent of plant cell wall, and can convert into soluble fractions within 1 to 2 min. The higher temperature attained by the cell wall, during MAE, enhances the dehydration of cellulose and reduces its mechanical strength and this in turn helps solvent to access easily to compounds inside the cell (Vivekananda Mandal *et al*., 2007).

In order to study cell damage during the MAE experiments, tobacco leaf samples were examined by scanning electron microscopy. Scanning electron micrographs of the untreated sample, heat-reflux extraction sample and MAE sample revealed that there were no structural difference between heat-reflux extraction and those of untreated samples, except few slight ruptures on the surface of the sample. However, the surface of the sample was found greatly destroyed after MAE. This observation suggests that microwave treatment affects the structure of the cell due to the sudden temperature rise and internal pressure increase. During the rupture process, a rapid exudation of the chemical substance within the cell into the surrounding solvents takes place. This mechanism of MAE based on exposing the analytes to the solvent through cell rupture is different from that of heat-reflux extraction that depends on a series of permeation and solubilization processes to bring the analytes out of the matrix. Destructive changes in the plant tissue of fresh orange peel due to microwave treatment was also observed using scanning electron micrographs. These changes in the plant tissue due to microwave heating gave a considerable increase in the yield of extractable pectin. Furthermore, the migration of dissolved ions increases solvent penetration into the matrix and thus facilitates the release of chemicals. Evidence has also been presented that during the extraction of essential oils from plant materials, MAE allows the desorption of compounds of interest out of the plant matrix. This occurs due to the targeted heating of the free water molecules present in the gland and vascular systems; this leads to localized heating causing dramatic expansion, with subsequent rupture of their walls, allowing essential oil to flow towards the organic solvent (Vivekananda Mandal *et al*., 2007).