EXTRACTION OF CAFFEINE FROM PBC123

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EXTRACTION OF CAFFEINE FROM PBC123

NUR FARAHIN BT MOHD RAZLAN

A report submitted in partial fulfillment of the

requirements for the award of the degree of

Bachelor of Chemical Engineering

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Special dedication to my beloved family

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ABSTRACT

Caffeine is one of the few plant products with which the general public is readily familiar because of its occurrence in beverages such as coffee and tea, as well as various soft drinks. Its chemical formula is $C_8H_{10}N_4O_2$. Moreover, caffeine can naturally be found in cocoa seeds such as *Prang Besar Cocoa (PBC) 123*. PBC 123 one of cocoa clone that can be found in Malaysian Cocoa Board plantaion at Jengka, Pahang. Commonly, the alkaloid contents (caffeine, theobromine, and theophylline) in cocoa are extracted before the cocoa is processed and the caffeine are discarded without use whereas the caffeine has its own benefits. For example, it is used for pharmaceutical purposes and the caffeine has increasing demand in the world. Moreover, an effective and low cost solvent is also needed for better extraction of caffeine. This will increase the profit from the sales of caffeine. Thus, this research will find the best solvent for the high extraction yield and also will optimize the parameters that affect the extraction yield of caffeine. The purpose of this research is to extract caffeine from *Prang Besar Cocoa (PBC) 123*. Besides that, this research is also to investigate the effect of sample particle size, extraction time and solvent/feed ratio on the yield of caffeine. Firstly, the seeds dried at 60˚C for 1hour. Then, the dried seeds will blend and sieved into 5 different particles size which is 2000, 1000, 800, 630 and 315 µm. 5g of powdered seeds will boiled with 250mL distilled water in batch heat reflux extractor for 15,30,45,60 and 90 minutes. For liquid-liquid extraction, solvent extraction will done using ethyl acetate at solvent or feed ratio of 1:5, 1:4, 1:3, 1:2 and 1:1. The lower layer that contains caffeine will collect. Rotary evaporator will used to evaporate ethyl acetate at 78˚C. Finally, the yields of caffeine in the solution will analyze by using UV/Vis Spectrophotometric method. The caffeine yield was highest at sample particle size of 315µm (0.27% w/w caffeine or 2.739 mg/g cocoa), solvent/feed ratio of 1:1 (0.26% w/w caffeine or 2.614 mg/g cocoa), and extraction time of 90 minutes (0.26% w/w caffeine or 2.637 mg/g cocoa). The best conditions for the highest yield of caffeine from *PBC123* were 315µm of sample particle size, 1:1 of solvent/feed ratio and 90 minutes of extraction time.

ABSTRAK

Kafein adalah salah satu daripada beberapa produk tumbuhan yang kebanyakan orang awam biasa kerana terdapat dalam minuman seperti kopi dan teh serta pelbagai minuman ringan. Formula kimia kafein adalah $C_8H_{10}N_4O_2$. Selain itu, kafein adalah sebuah zat yang dijumpai secara semulajadi dalam biji koko seperti *Prang Besar Cocoa (PBC) 123*. PBC 123 adalah salah satu dari klon koko yang boleh didapati dari Lembaga Koko Malaysia di Jengka, Pahang. Biasanya, kandungan alkaloid(kafein, teobromina dan teofilin) dalam koko diekstrak sebelum koko diproses dan kafein yang dibuang tanpa digunakan sedangkan kafein mempunyai manfaat yang tersendiri. Sebagai contoh, ia digunakan untuk tujuan farmaseutikal dan kafein mempunyai permintaan di dunia. Selain itu, kos pelarut yang efektif dan rendah juga diperlukan untuk pengekstrakan kafein yang lebih baik. Ini akan meningkatkan keuntungan daripada jualan kafein. Oleh itu, kajian ini akan mencari pelarut yang terbaik untuk hasil pengekstrakan kafein yang tinggi dan juga akan mengoptimumkan parameter yang member kesan kepada hasil pengekstrakan kafein. Objektif kajian ini adalah untuk mengekstrak kafein dari *Prang Besar Cocoa (PBC) 123*. Disamping itu, kajian ini juga adalah untuk mengkaji pengaruh saiz zarah koko, nisbah pelarut/sampel dan masa ekstraksi terhadap hasil kafein. Pertamanya, biji benih dikeringkan pada 60°C selama 1 jam. Kemudian, biji benih yang dikeringkan diadun dan disaring ke 5 zarah yang berlainan saiz iaitu 2000,1000, 800, 630 dan 315 µm. 5g serbuk koko akan direbus dengan 250mL air suling di dalam refluks haba selama 15, 30, 45, 60, 90 minit. Bagi pengekstrakan cecair-cecair, pengekstrakan pelarut dilakukan dengan menggunakan etil asetat pada nisbah pelarut atau suapan 1:5, 1:4, 1:3, 1:2 and 1:1. Lapisan yang lebih rendah yang mengandungi kafein akan dikumpulkan. "Rotary evaporator" akan digunakan untuk menyejat etil asetat pada 78°C. Hasil kafein yang tertinggi diperolehi pada saiz zarah sampel 315µm (0.27% w/w kafein atau 2.739 mg/g koko), nisbah pelarut/sampel 1:1 (0.26% w/w kafein atau 2.614 mg/g koko), dan masa ekstraksi 90 minit (0.26% w/w kafein atau 2.637 mg/g koko). Keadaan terbaik untuk mendapatkan hasil tertinggi kafein dari *PBC123* adalah pada saiz zarah sampel 315µm, nisbah pelarut/sampel 1:1 dan masa ekstraksi 90 minit.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVATIONS

HPLC	High Performance Liquid Chromatography
PBC123	Prang Besar Cocoa 123
UV	Ultraviolet
UV/Vis	Ultraviolet/Visible

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Caffeine (1,3,7-trimethylxanthine) is one of the few plant products with which the general public is readily familiar, because of its occurrence in beverages such as coffee and tea as well as various soft drinks. A growing belief that the ingestion of caffeine can have adverse effect on health has resulted in an increased demand for decaffeinated beverages. Unpleasant short-term side effects from caffeine include palpitations, gastrointestinal disturbances, anxiety, tremor, increased blood pressure and insomnia. In spite of numerous publications on the long-term consequences of caffeine consumption on human health, no clear picture has emerged, with reports of both protective and deleterious effects. (Ashihara and Crozier.2001)

Caffeine is also an alkaloid of the methylxanthine family. Caffeine is an intensely bitter white powder in its pure state. Its IUPAC name is 1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione, with chemical formula $C_8H_{10}N_4O_2$ (Arnaud, 1987). The structure of caffeine is shown in Figure 1, below (Mumin *et al*., 2006).

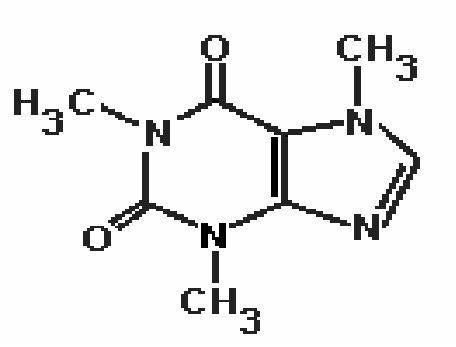

Figure 1.1: The structure of caffeine

One analysis of the chemical composition of cocoa beans after fermentation and drying is shown in Table 1.1, below:

Table 1.1: Chemical compositions of cocoa beans

Contents	Nib % Maximum	Shell % Maximum
Water	3.2	6.6
Fat (cocoa butter, shell fat)	57	5.9
Ash	4.2	20.7
Total Nitrogen	2.5	3.2
Theobromine	1.3	0.9
Caffeine	0.7	0.3
Starch	9	5.2
Crude Fibre	3.2	19.2

Source: Minifie, 1989

This table shows that caffeine is one of the compositions of cocoa. The indication of chemical composition of cocoa are depends on the type of beans, quality of fermentation and drying and also subsequent processing of beans.

There have several methods that can be used to extract caffeine from cocoa such as supercritical carbon dioxide extraction, water extraction and organic solvent extraction. Solvents such as chloroform, methyl chloride, ethanol, and ethyl acetate are commonly used for the solvent extraction of caffeine (Anonym., 2010). Besides that, Soxhlet extraction, Ultrasonic extraction and Heat Reflux extraction are example of several methods that can be used for this extraction purpose. The Heat Reflux extraction is one of the common methods used to extract caffeine from cocoa seed on a laboratory scale.

Figure 1.2: Heat reflux extractor

Several chromatographic methods have been proposed for the determination of these methylxanthines or caffeine in a variety of matrices such as High Performance Liquid Chromatography and UV/Vis Spectrophotometer. However, only a few of them permit the complete separation of the three compounds, require sample pretreatment before the determination step or do not show very low detection limits. UV/Vis Spectrophotometric methods is suitable be using in laboratory scale.

PBC123 is a new type of cocoa clone that have been produced by Malaysian Cocoa Board at Jengka, Pahang. This type of clone has probability to produce not less than two to four tones per hectares in Malaysia. Since this is a new brand of cocoa

clone, we not analyzed yet the composition of caffeine in this cocoa seeds. Therefore, it gives some benefit to Malaysian Cocoa Board to analyze the new breed of cocoa clone.

1.2 PROBLEM STATEMENT

Malaysia is one of the country that generally tropical and subtropical countries that produced cocoa other than Brazil, Africa and Hawaii. Malaysia also was recognised as the one of largest cocoa producer and Malaysia is ranked 11th in the list of cocoa cultivating countries, worldwide. Commonly, the alkaloid contents (caffeine, theobromine, and theophylline) in cocoa are extracted before the cocoa is processed and the caffeine are discarded without use whereas the caffeine has its own benefits. For example it is used for pharmaceutical purposes and the caffeine has increasing demand in the world. Moreover, an effective and low cost solvent is also needed for better extraction of caffeine. This will increase the profit from the sales of caffeine. Thus, this research will find the best solvent for the high extraction yield and also will optimize the parameters that affect the extraction yield of caffeine.

1.3 RESEARCH OBJECTIVE

To extract the caffeine from PBC123 cocoa seeds using heat reflux extraction method by the variation of particle size, solvent/feed ratio and extraction time.

1.4 SCOPE OF RESEARCH

The scope of this research is:

1) Extraction of caffeine from PBC123 cocoa seeds.
2) Evaluating the selected parameter for particle size, solvent/feed ratio and extraction time.
3) Analyzing the caffeine yield by using UV/Vis Spectrophotometer.
4) Identifying the affect of particle size, solvent/feed ratio and extraction time on caffeine yield.
5) Kinetic model approval of caffeine yield.

CHAPTER 2

LITERATURE REVIEW

2.1 COCOA

2.1.1 History of Cocoa

Cocoa or the cocoa tree's botanical name, 'Theobroma cacao' has translated from the Greek means "food of the gods" has a history rooted in the mists of time as far back as 1662. In the early days, the native belief that cocoa tree was of divine origin and resulted in a holy ritual being performed whenever cocoa trees were planted.

Cocoa has successfully conquered all countries and continents of the world in just over 500 years since its first discovery in the ancient civilization of the Mayas and Aztecs in South America. In South America, the Aztecs considered the beverage a royal drink served in ceremonial golden goblets. The Mayas of the Yucatán and the Aztecs of Mexico cultivated cocoa and the Aztec emperor Montezuma is said to have regularly consumed a preparation called chocolatl, a mix of roasted cocoa nibs, maize, water and spice. (Anonym., 2010)

The cocoa tree soon began to appear in Spanish colonies some 20 years after it had been brought back by the early explorers. However, the processing of cocoa beans began in earnest but under a veil of secrecy in monasteries. Chocolate was restricted to nobility and the recipes were kept secret for nearly 100 years. Hernando Cortez brought back the first cocoa and chocolate drink recipe to the Court of King of Spain in 1528. Gradually a transformation began. Cane sugar was added. Newly discovered spices such as vanilla and cinnamon were also used as flavourings.

The Spanish court soon fell under the spell of this exotic elixir and adapted it to their taste, adding cane sugar, vanilla, cinnamon and pepper. Initially Spain reserved cocoa for its exclusive use, carefully guarding its existence from the rest of the world. They were so successful keeping cocoa secret that when a group of English pirates captured a Spanish galleon, not recognizing the value of the weighty cargo of beans, they burned them!.

In 1585, the first cargo of cocoa beans arrived on the Iberian Peninsula from New Spain, launching the trade in cocoa, and resulting in the establishment of the first chocolate shops, thus, ushering in a new era of rapidly growing demand for this mysterious nectar from the new world.

Then, on 17th century, cocoa began arriving in other ports throughout Europe, effortlessly conquering every region's palate. Chocolate beverages were first embraced by the French court following the royal marriage of King Louis XIII to the Spanish Princess Anne of Austria in 1615.

In 1650 chocolate beverages first appeared in England coinciding with the arrival of tea from China and coffee from the Middle East. For many years it remained a treat reserved for the upper classes. In 1659 the first chocolate-confection maker opened in Paris. In 1720, Italian chocolate-makers received prizes in recognition of the quality of their products. Finally, in 1765, North America discovered the virtues of cocoa. Then, it was believed that the cocoa tree was later brought to Indonesia and Sabah in the early 18th century.

In Malaysia, the first cocoa planted area was found in Malacca in 1778. Subsequently, the cocoa planting was started in a plotted area at Serdang Agriculture Station and Silam Agriculture Research Center, Sabah. The earliest cocoa commercialization started between 1853 to 1959 where cocoa types Amelonado was first planted at Jerangau, Terengganu. The planted area was 403 hectarages. Cocoa trial was further undertaken at Serdang, Cheras, Kuala Lipis and Temerloh between 1936 to 1940. However, cocoa was only actively planted after World War II. Cocoa officially came to Quoin Hill, Tawau and Sabah in 1960. (Varma, 2010)

2.1.2 Characteristics of Cocoa Tree

According to UNCTAD information, the cocoa tree is usually small tree with 4 to 8 metres tall. However, it may reach up to 10 meters in height. The stem is straight, the wood is light and white while the bark is thin, smooth and brownish. The fruit can reach up to 15 until 25 cm in length. Each pod contains about 30 to 40 seeds which after drying and fermentation. The seeds are reddish brown externally and covered by white and sweet pulp. For ideal production, cocoa trees need rainfall between 1150 and 2500 mm per year and temperature between 21°C and 32°C. Besides that, each cocoa tree will yield 20 to 30 pods per year and the peak times for harvesting are around the months April and September in Malaysia (Varma and Nurdin, 2010).

2.1.3 Types of Cocoa

There are three varieties of cocoa trees. Firstly is Criollo which means 'Creole' in Spanish. This type of cocoa was recorded in 17th century as earliest plantations. It also produces "fine and flavour" beans. Criollo originally grown in Venezuela, Central America and Mexico. Nowdays, it also can be found in Ecuador, Nicaragua, Guatemala and Sri Lanka. Besides that, Criollo has a reputation for fitness and an intense aroma. This type of cocoa also represents 5% of global production in part due to its vulnerability to insects and disease. Secondly is Forastero which means foreigner in Spanish. This type is very diverse and more resistant to disease and pests. Because of that, it more productive compare to Criollo type. It originally grown in the high Amazon region and now predominant variety cultivated in Africa. It also account for 90% of the cocoa beans produced in the world. Forastero also considered being of ordinary quality which is very slight aroma, strong, short and bitter taste. Lastly is Trinitario which from word "Trinidad". This type of cocoa tree is natural biological hybrid between the Criollo and Forestario. The Spanish colonists had established Trinitario. Besides that, it quality is between average and superior with strong cocoa butter content. It also represents 15% of world production.(Anonym., 2010)

2.2 CAFFEINE

2.2.1 Properties

Pure caffeine act as odourless, white, fleecy masses, glistening needles of powder in physically. Its molecular weight is 194.19g/gmol, melting point at 236^{o}C, point at which caffeine sublimes is 178^{o}C, at atmospheric pressure, pH is 6.9 (1% solution), the specific gravity is 1.2, volatility is 0.5%, for vapour pressure is 760 mm Hg at 178^{o}C, the solubility in water is 2.17g per 100ml water at 25^{o}C, and vapour density is 6.7 (Clementz and Dailey, 1988).

The nitrogen atoms in the structure of caffeine are all planar which is in sp^2 orbital hybridization and resulting in the aromatic characteristics of caffeine. Caffeine is a readily available by-product of decaffeination and it is not usually synthesized (Anonym., 2001). But if desired, caffeine also can be synthesized from dimethylurea and malonic acid (Wilson and Norman, 2004).

2.2.2 Applications

Caffeine is most commonly used to improve mental alertness but it has many other uses. Caffeine can use by directly to mouth or rectally in combination with painkillers such as aspirin, acetaminophen and ergotamine, a chemical for treating migraine headaches. It is also used with painkillers for simple headaches and preventing and treating headaches after epidural anesthesia.

According to other WebMD article, some people use caffeine for asthma, gallbladder disease, attention deficit-hyperactivity disorder (ADHD), shortness of breath in newborns and low blood pressure. Caffeine is also used for weight loss and type of diabetes. Besides that, it is often used in combination with ephedrine as an alternative to illegal stimulants.

Caffeine is also one of the most commonly used as stimulants among athletes. National Collegiate Athletic Association (NCAA) is allowed taking caffeine within limits. Urine concentrations over 15mcg/mL are prohibited. It takes most people about eight cups of coffee to providing 100mg/cup to reach this urine concentration.

Caffeine has been found in creams that are applied to the skin to reduce redness and itching in dermatitis. Healthcare providers sometimes give caffeine intravenously for headache after epidural anesthesia, breathing problems in newborns and to increase urine flow. (Anonym., 2011)

In food industry, caffeine is used as an ingredient in soft drinks, energy drinks and beverages. People with voice disorders, singers and other voice professionals are often advised against using caffeine. However, until recently, this recommendation was based only on hearsay. Now developing research seems to indicate that caffeine may actually harm voice quality. But further study is necessary to confirm these early findings.

Caffeine is used as a drug on the basis of its effect on the respiratory, cardiovascular and the central nervous system. Caffeine is included with aspirin in some preparations for treatment of headaches as it decreases cerebral eye blood flow. Caffeine is also included with ergotamine in some anti migraine preparations, in order to produce a mildly agreeable sense of alertness (Lawrence, 1986).

2.2.3 Advantages

Using caffeine is more of an art than a science. Caffeine safely offers a wider range of benefits than any other drug in the pharmaceutical. The very scope of these benefits requires us to learn something about the scientific studies that describe these effects if we are to use it strategically. Because of that, the range of individual responses to caffeine is so great, we also need experience, self-testing, and reasoned judgment to enable us to enjoy all the benefits caffeine offers. Caffeine's benefits are very real, and yet they are complex and variable. Because caffeine is a generic drug, the profit on the sale of each caffeine pill or capsule is low. Caffeine has been consistently included by

the Food and Drug Administration (FDA) on the list of substances Generally Recognized As Safe (GRAS) for over twenty-five years. That is the reason it can be legally added to foods and drinks. But the FDA, while acknowledging that caffeine is safe, allows caffeine to be promoted only as an "alertness aid." People who sell products that include caffeine as an ingredient aren't legally permitted to make any other claims for it. (Anonym, 2002)

2.2.4 Disadvantages

Consumption of caffeine in large amounts, and especially over extended periods of time, can lead to a condition known as caffeinism (Mackay and Rollins, 1989). Caffeinism usually combines caffeine dependency with a wide range of unpleasant physical and mental conditions including nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations (Leson et al., 1988).

Although caffeine has their own advantages, but there are also have some disadvantages. Especially when the caffeine is taking too much in our life. For example, the disadvantages of caffeine is can cause hypersensitivity. Body should be able to tolerate about 200 to 300 mg of caffeine in a day where equivalent to about 2 to 4 cups of brewed coffee. Unless you are sensitive to caffeine. You might be more sensitive to caffeine if you don't usually consume much, are young, have a small frame, are a man, take certain medications or you have a health condition such as an anxiety disorder. If you are extra-sensitive to caffeine, a single cup of coffee could prompt undesirable effects, such as restlessness. (Anonym, 2011)

Besides that, caffeine can give a pregnancy risks. Pregnant woman who are have a lot of caffeine are increasing their risk of fertility problems. This is because caffeine can reduces muscle activity in Fallopian tubes, which are responsible for delivering eggs from ovaries to womb. Caffeine also can increase pregnancy complications because it crosses the placenta and reaches the fetus. Because the fetus has an immature metabolism, caffeine may linger in its system and build up to toxic levels. You may be

at a higher risk of miscarriage and delivery of a low birth weight infant if your caffeine intake exceeds 200 to 300 mg per day. (Anonym, 2011)

When start out the habit of drinking coffee every day, it will notice that in the long run, body will be craving for it every day too. This is because caffeine is addictive. When skip it, will become irritable, tired, depressed or even have headaches.

2.3 EXTRACTION OF CAFFEINE

Extraction of caffeine or Decaffeination is a popular term to analyze the caffeine contents in various sources. There have many types of solvents which are available to be use to extract that caffeine. For example are chloroform, methyl chloride, ethyl acetate and super critical carbon dioxide.

The extraction of caffeine is also done with extraction with water. In a water process, a batch of fresh green beans is heated within a previously decaffeinated concentrate or extract of oils and water. The caffeine in each bean is drawn out and becomes part of the water. The solution and the beans are then separated. The beans are moved for rinsing and drying. The solution is run through charcoal filtration to remove the caffeine and is used for another batch of beans. This process is done without chemicals and removes 96 percent of the caffeine in beans.

For ethyl acetate solvent extraction, ethyl acetate is streamed into beds of moistened beans to specifically extract the caffeine, which is salvaged when the solvent is evaporated. Ethyl acetate solvates caffeine more effectively than water and extracts the caffeine. The remaining ethyl acetate is removed from the cocoa solution by steaming. The cocoa solution is then combined with the beans which reabsorb the cocoa oils as they are dried. 2-Propanol is also used as extraction solvent rather than ethyl acetate as it is less hazardous to human health (Hampp, 1996).

Supercritical carbon dioxide also have been using recently. When a sealed vial containing both gaseous and liquid carbon dioxide under high pressure is heated, the liquid density drops while the gas density rises. If the pressure is above 72.8 atm, and

the temperature rises above 304.2 K, the density of the liquid and the density of the gas become identical. The meniscus between the liquid and gas phases vanishes. The carbon dioxide becomes a supercritical fluid which has both gas and liquid properties. The fluid fills the container like a gas but can dissolve substances like a liquid. Supercritical fluid carbon dioxide is an excellent non polar solvent for many organic compounds, including caffeine. The extraction process is simple. Supercritical carbon dioxide is forced through green cocoa beans. Gas behavior allows it to penetrate deep into the beans, and it dissolves 97-99% of the caffeine present. (Anonym, 1999)

2.3.1 Types of solvent

The common solvents used in this extraction method are chloroform, methyl chloride, ethyl acetate, super critical carbon dioxide and many more. Methylene chloride is also used to extract caffeine from cocoa and it is highly effective. However, methylene chloride is potentially dangerous under certain circumstances. This is because it can cause faintness, dizziness, and headache if inhaled at high concentrations (Kirmer, 1988). In the other hand, ethyl acetate is another compound used to extract caffeine from cocoa. It removes caffeine from cocoa effectively too and it extracts other chemical components from the cocoa as well. Ethyl acetate is much less hazardous to health and environment compared to chlorinated solvents (Johnson *et al.*, 1988).

2.3.2 Methods of Extraction of Caffeine

In a research done by Hu *et al*. (1997), caffeine was extracted from tea using ethanol solvent by heat reflux extraction. A 50% ethanol in water was refluxed at 85^{o}C for 45 minutes. Then it was filtered through a filter paper and the filtered solution was centrifuged for 10 minutes, at a speed of 4000rpm. The supernatant was then analyzed to determine the caffeine composition.

Hu *et al*. (1997) has also done a research of extracting caffeine from tea using ultrasonic extraction method. 50% ethanol in water was used as solvent to extract the caffeine from tea and the solution was sonicated for 90 minutes in an ultrasonic bath (frequency 50Hz, power 250W) at 20-40^{o}C. Then the extract was filtered and the

filtered solution was centrifuged for 10 minutes at a speed of 4000rpm. Finally, the supernatant collected was analyzed to know the caffeine composition.

Ramli *et al*. (2000) also has analyzed the total polyphenols, epicatechin, catechin, theobromine and caffeine contents in Commercial cocoa and chocolate products such as cocoa powder, cocoa beans, cocoa liquor and chocolate using High Performance Liquid Chromatography (HPLC). The methylxanthines were identified and quantified using Bondapak column and mobile phase of methanol:water:acetic acid at ratio 20:79:1. 32 samples of chocolate products were analyzed and the levels of caffeine and theobromine were 0.62-1.14 mg/g and 0.026-0.153 mg/g, respectively. The chocolate coating made from fat substitute had theobromine and caffeine levels which is the ranged between 0.36-0.70 mg/g and 0.027-0.061 mg/g respectively. The mean theobromine and caffeine levels in local chocolates respectively were 0.72 mg/g and 0.04mg/g in milk chocolate and 0.85 mg/g and 0.06 mg/g in dark chocolate. In imported chocolates, the mean theobromine and caffeine levels respectively were 1.05 mg/g and 0.12 mg/g in dark chocolate, 0.76 mg/g and 0.04 mg/g in milk chocolate and 0.74 mg/g and 0.03 mg/g in white chocolate. From that we can know that the imported chocolates have higher level of theobromine and caffeine compared with the local chocolates.

Mumin *et al*. (2006) has done a research on determination and characterization of caffeine in tea, coffee, and soft drinks by Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC). Caffeine which is a mild addicting drug was isolated, purified and characterized from tea (black and green) and coffee. The isolation of caffeine was done by liquid-liquid extraction using chloroform as the extracting solvent. Four steps of extraction were carried out such as leaching, dye removal, liquid extraction and recrystallization. Toluene and petroleum ether were the solvent used for recrystallization. The crude caffeine was purified by SPE method. For the characterization of pure caffeine by HPLC, 50mM KH_2PO_4 (pH=2), acetonitrile, and methanol at ratio 40:8:2 was used as solvent as well as mobile phase at ratio. The amount of caffeine in various soft drinks (Cola) that commercially available in Bangladesh were also determined by HPLC method.

Li S *et al.* (1989) have developed a method for the determination of theobromine and caffeine in cocoa beans using UV spectrophotometer. They have presented a rapid, simple and accurate method for individually determining theobromine and caffeine in cocoa beans. Caffeine alone was completely extracted into chloroform from an aqueous solution at a pH between 12.5 and 12.7, and analyzed by UV spectrophotometer at 275.9nm. For the remaining theobromine in the aqueous solution, a wavelength of 272.7nm was used. A result with relative standard deviation of about 0.65% was obtained.

Varma and Nurdin (2010) also had done the research for extraction of caffeine. They are presented extraction of caffeine method by using heat reflux extractor and analyzed with UV/Vis Spectrophotometer. The MCBC2 cocoa seeds were tested in laboratory scale. The cocoa that been used in this research was extracted with ethyl acetate solvent. There are also consists two types of extraction that done by these researcher which are solid-liquid extraction and liquid-liquid extraction. Solid-liquid extraction is done by extract the sample with distilled water. While, liquid-liquid extraction was done by using ethyl acetate. Caffeine was analyzed with UV/Vis Spectrophotometer at wavelength 275.9nm.

CHAPTER 3

METHODOLOGY

3.1 MATERIALS

The *PBC123* cocoa seeds were bought from Malaysian Cocoa Board research station in Jengka, Pahang. Three parameters were set up to investigate its effect on the caffeine yield. The equipments and apparatus used in this research were beaker, heater, vacuum filter, separatory funnel, pH meter, rotary evaporator, electronic balance, oven, batch heat reflux extractor, Buchner funnel, and sieve shaker. The chemicals and reagents used in this research were distilled water, 10% lead ethanoate solution, ethyl acetate, anhydrous sodium hydrogen carbonate, 1M sodium hydroxide solution, and anhydrous sodium sulphate.

3.2 FLOWCHART

PBC 123 cocoa tree.

PBC 123 cocoa seeds.

The seeds dried at 60°C for 2 hours.

The seeds were blended to get powder sample.

The powdered seeds were sieved into 2000, 1000, 800, 613 and 315µm size.

5g of powdered seeds were boiled with 250mL distilled water by using heat reflux extractor for 15, 30, 45, 60 and 90 minutes.

Solvent extraction was done by using ethyl acetate at solvent/feed ratio 1:1, 1:2, 1:3, 1:4 and 1:5. The lower layer was collected.

Ethyl acetate was evaporated by using rotary evaporator at 78°C.

The caffeine yield was analyzed using UV-Vis Spectrophotometer.

3.3 METHODS

3.3.1 Preparation of Sample

50 grams of the *PBC123* cocoa seeds were weighed and dried in incubator at 60°C for 2 hours. This is to remove the moisture in the seeds. Then, the seeds were blended to get the powdered sample. Next, the powdered sample was sieved into 5 different particle sizes which are 2000, 1000, 800, 630 and 315μm.

3.3.2 Preparation of Solutions

10% (w/v) lead acetate solution was prepared by adding 10 grams of anhydrous lead acetate into 100mL of distilled water. While, 1M sodium hydroxide solution was prepared by adding 4 grams of anhydrous sodium hydroxide into 100mL of distilled water.

3.3.3 Solid-Liquid Extraction of Caffeine

5 grams of the prepared powdered sample of *PBC123* cocoa seeds were put in a 500mL beaker and subsequently 250mL of distilled water was added into the beaker. The mixture was boiled in a batch heat reflux extractor for 5 different extraction times which are 15, 30, 45, 60, and 90 minutes. The mixture was filtered using Buchner funnel and the filtrate was collected. Then, 25mL of 10% (w/v) lead acetate solution was added to the filtrate.

The purpose of adding 10% (w/v) lead acetate solution is to convert tannins and other acids into anions (base) that will not be soluble in water and ethyl acetate. This also helps to avoid an emulsion. Next, the solution was boiled for 10 minutes. The lead acetate formed a precipitate and this precipitate was removed by filtering it in vacuum filter. 1 gram of anhydrous sodium hydrogen carbonate was added to the filtrate. The solution will become clear by adding the anhydrous sodium hydrogen carbonate because of removing the Pb^{2+} ions in the solution, in a form of white precipitate of

$PbCO_3$. The solution was filtered repeatedly using vacuum filter until a clear solution is obtained.

3.3.4 Liquid-Liquid Extraction of Caffeine

The clear solution obtained was transferred into a 500mL separatory funnel. The pH of the solution was measured using pH meter. 5.5mL of 1M sodium hydroxide solution was added if the pH of the solution is not between 12.5 and 12.7 until it regulate in that range. The addition of sodium hydroxide is because to maintain the basicity of the solution, so that tannins and other acids do not soluble in water and ethyl acetate. Basic condition also increases the water polarity and the caffeine in least polar form will be more readily solvated in ethyl acetate than in water. Then, the caffeine in the solution was extracted with 5 different solvent/feed ratios which are 1:5, 1:4, 1:3, 1:2, and 1:1. The mixture was shaken uniformly while the stopcock is opened to expel vapours. The layers were allowed to separate and the lower layer was collected into a 100ml beaker.

Ethyl acetate is a highly flammable liquid that is moderately hazardous. Therefore, the Material Safety Data Sheet of ethyl acetate is referred when dealing with this chemical during the research. The Material Safety Data Sheet of ethyl acetate is shown in Appendix A.

3.3.5 Separation of Caffeine

Anhydrous sodium sulphate was then added into the collected solution that containing caffeine. The purpose of adding anhydrous sodium sulphate is to remove any water and water-soluble salts that were retained in the ethyl acetate or accidentally transferred during decantation of solution. The ethyl acetate appeared a bit cloudy because the anhydrous sodium sulphate clumped when water present. The mixture was shaken gently until no more clumping is observed. Next, the ethyl acetate solvent in the caffeine containing solution was evaporated using rotary evaporator and the temperature of the water bath was controlled low enough between 76°C and 78°C because to avoid

caffeine decomposition. After 1 hour, a solution that saturated with caffeine was obtained.

3.3.6 Analysis of Caffeine

The caffeine containing solution was analyzed using UV/Visible Spectrophotometric method. A standard curve of absorbance versus concentration was prepared at wavelength of 275.9 nm. The absorbance was measured for caffeine concentrations of 0, 5, 10, 15, 20, 25, and 30 mg/L for the standard curve preparation. Then, the absorbance of all the caffeine containing solution samples was measured in UV/Visible Spectrophotometer at 275.9 nm. The concentrations of caffeine in the solution were read from the standard curve using the absorbance value.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 STANDARD CURVE OF CAFFEINE

The value of absorbance for each concentration of caffeine had shown in the Appendix B. The graph of standard curve of caffeine is plotted as below.

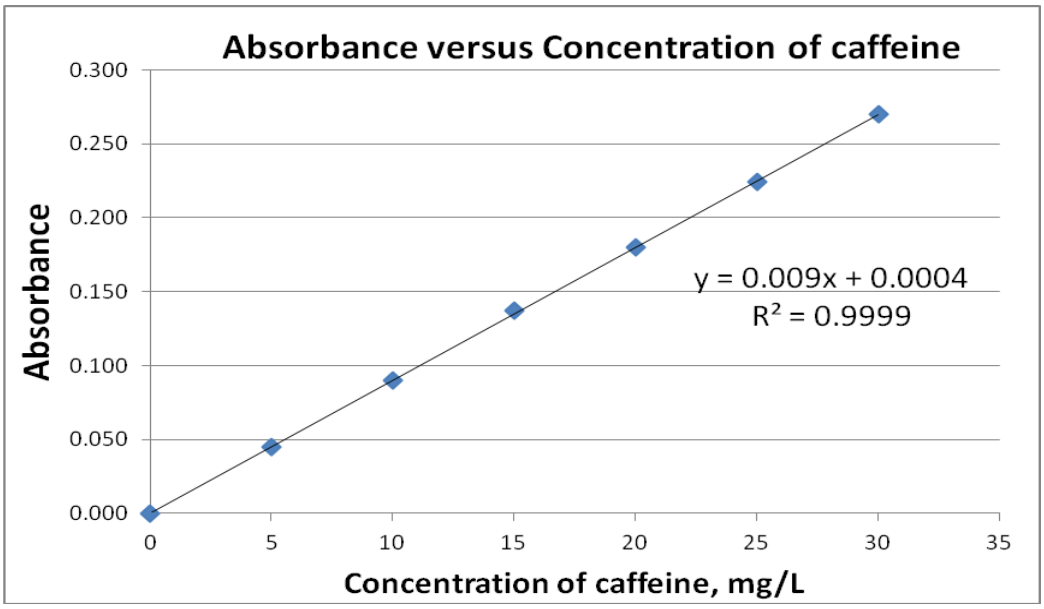

Figure 4.1: Standard curve of caffeine

4.2 THE EFFECT OF PBC 123 PARTICLE SIZE ON THE CAFFEINE YIELD

The effect of PBC123 particle size on the percentage of caffeine yield was analyzed and the data obtained had shown in Appendix B (Table B.2).

The absorbance value was read at 275.9nm for every caffeine solution that obtained 5 different particle sizes of *PBC 123*. The concentration of caffeine was read from the standard curve of caffeine by using the value of absorbance. From that, the amount of caffeine in 1 gram of *PBC123* sample was calculated. Firstly, determining the concentration of *PBC123* sample solution was done. Since 5 grams of *PBC123* is in 100mL of water, thus the concentration of *PBC123* solution is 50 g/L. Then, the amount of caffeine is calculated by dividing concentration of caffeine (mg/L) by *PBC123* concentration (g/L). The percentage of caffeine yield is calculated by changing the amount of caffeine to mg per mg sample unit and then multiplies it with 100%.

By using the data from Table B.2, the graph of caffeine yield versus PBC 123 particle size was plotted as below.

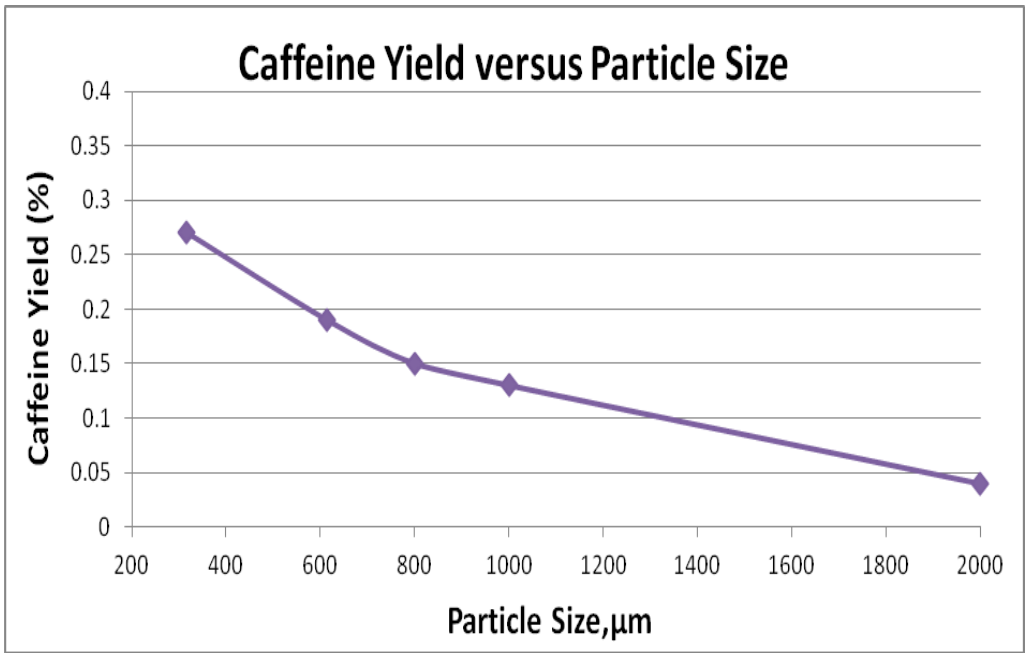

Figure 4.2: Caffeine yield in percentage for different particles size

From the graph, it shows that the percentage of caffeine yield is higher at small particle size. When the PBC 123 particles size is increase, the caffeine yield also increase. This is because, when the particle size in smaller, the surface area of particles

become larger. So that, more caffeine can diffuse out from cocoa and extracted. In addition, the length of diffusion path for caffeine becomes shorter. Because of that, the caffeine can easily diffuse out from inside of cocoa to the surface and extracted by solvent. Therefore, the highest percentage of caffeine yield is at particle size of 315µm which is 0.27% of caffeine or 2.739 mg/g cocoa.

According to research done by Li *et al.* (1990), an average caffeine yield of 2.316 mg/g sample was obtained for the cocoa beans tested. The research resulted that at smaller particle size of cocoa, the caffeine found was higher.

Varma and Nurdin (2010) were done the research and had found that the percentage of caffeine yield of *MCBC2* particle size is 0.35% and it is feasible. This also shows that at smaller particle size, the caffeine yield is also high. So that the results obtained in this research using *PBC123* are acceptable.

4.3 THE EFFECT OF PBC 123 SOLVENT/FEED RATIO ON THE CAFFEINE YIELD

The effect of solvent/feed ratio on the percentage of caffeine yield was analyzed and the data obtained had shown in Appendix B (Table B.3).

The absorbance value was analyzed for every caffeine solution that obtained for 5 different solvent/feed ratios which at 275.9 nm. The caffeine concentration (mg/L), amount of caffeine (mg per g sample) and the percentage of caffeine yield was calculated likely as the calculation of effect of particle size.

Solvent/feed ratio of 1:5 shows that the feed is 5 times of the solvent, whereby for 200mL of feed, 40mL of solvent is used. Similarly for solvent/feed ratio of 1:3 and 1:2, the feed is 3 times of the solvent, whereby for 200mL of feed, 66.67mL of solvent is used and the feed is 2 times of the solvent, whereby for 200mL of feed, 100mL of solvent is used, respectively. For solvent/feed ratio of 1:1, the amounts of solvent and feed are equal. The solvent used here is ethyl acetate.

By using the data shown in Table B.3, a graph of percentage of caffeine yield against solvent/feed ratio was plotted as shown in the following Figure 4.3 below.

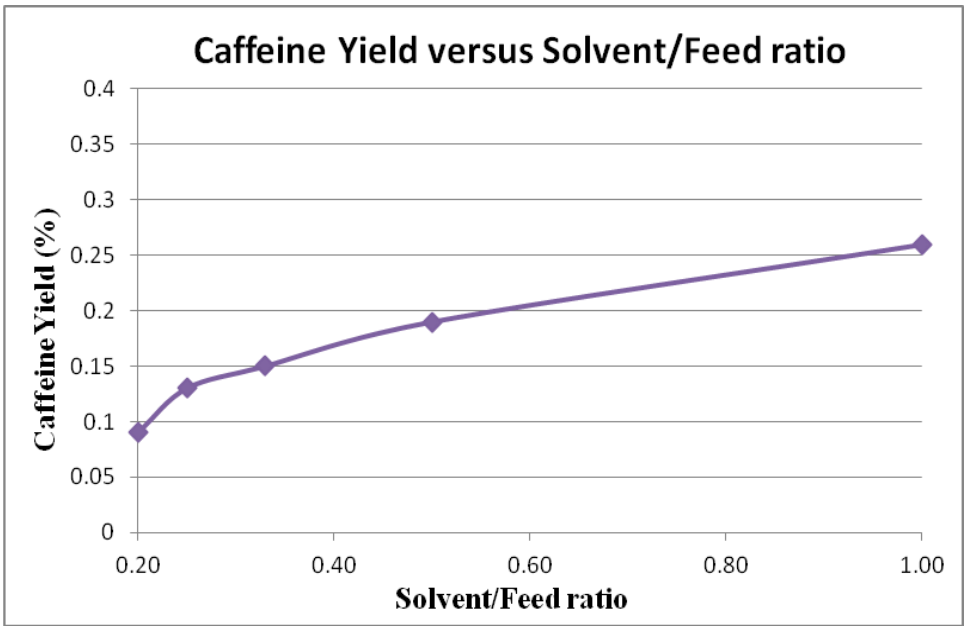

Figure 4.3: Caffeine yield in percentage for different solvent/feed ratio

The graph in Figure 4.3 shows that the percentage of caffeine yield is higher at higher solvent/feed ratio. When the solvent/feed ratio increases, the percentage of caffeine yield is also increases. This is because, at high solvent/feed ratio, the volume of solvent becomes higher per volume of feed. As the solvent (ethyl acetate) volume becomes higher in the solution, the contact between solvent and solute which is caffeine becomes more frequent. So that, more caffeine is extracted from the solution. Thus, in the tested range between 1:5 and 1:1 of solvent/feed ratio, the highest percentage of caffeine yield is at 1:1 solvent/feed ratio, which is 0.26% of caffeine or 2.614 mg/g cocoa.

Theoretically, high amount of caffeine is extracted with high amount of solvent, but economically, it is not relevant. This is because high amount of solvent requires

high cost. Therefore, an optimum amount of solvent/feed ratio that is between 1:5 and 1:1 should be used.

Hameed *et al.* (2003) was states that percent of extraction increases as the solvent/feed ratio increases. In this research, using of *PBC123* also shows the similar result, whereby percent of caffeine yield increases as the solvent/feed ratio increases.

Besides that, according to Varma and Nurdin (2010) research, the percentage of caffeine yield was obtained at higher solvent/feed ratio which is 0.35%. Because of that, the result shows in this research is acceptable.

4.4 THE EFFECT OF PBC123 EXTRACTION TIME ON THE CAFFEINE YIELD

The effect of extraction time on the percentage of caffeine yield was analyzed and the data obtained is shown in Appendix B (Table B.3).

The absorbance value was analyzed for every caffeine solution that obtained for 5 different extraction time which at 275.9 nm. The caffeine concentration (mg/L), amount of caffeine (mg per g sample) and the percentage of caffeine yield was calculated similarly as the calculation of effect of particle size.

The extraction time is the time where the solid-liquid extraction (leaching) of caffeine from *PBC123* by using distilled water as solvent. The solid-liquid extraction was done for 5 different extraction time which is 15, 30, 45, 60 and 90 minutes.

By using the data shown in Table B.3, a graph of percentage of caffeine yield against extraction time was plotted as shown in the following Figure 4.4 below.

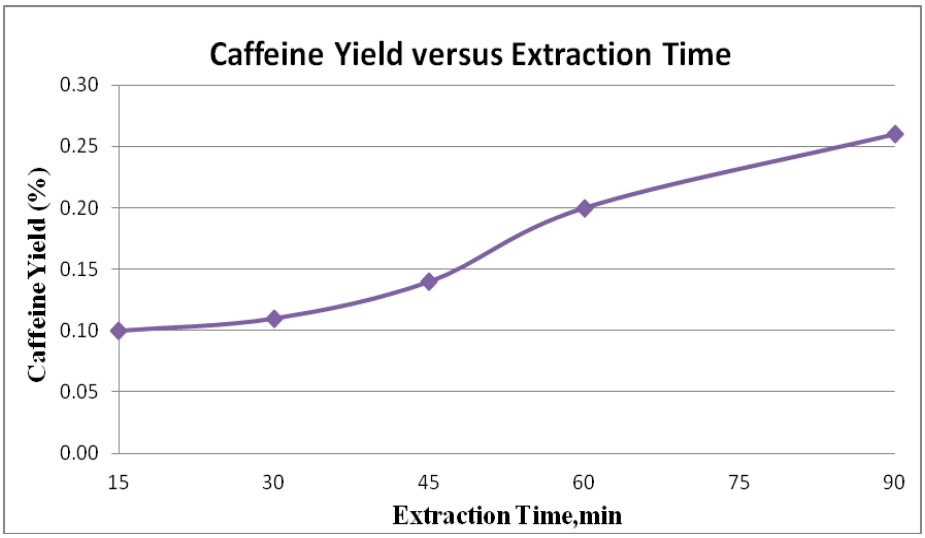

Figure 4.4: Caffeine yield in percentage for different extraction time

The above graph, Figure 4.4 shows that the percentage of caffeine yield is higher at longer extraction time. When the extraction time increases, the percentage of caffeine yield is also increases. This is because the longer the extraction time, the longer the *PBC123* particles spend time in the solvent, water. The diffusion of caffeine from inside of cocoa to the surface of cocoa, and then to the solvent, will takes place for longer time. So that, the caffeine yield increases by time. In the tested range between 15 and 90 minutes of extraction time, the highest percentage of caffeine yield is obtained at 90 minutes which is 0.26% of caffeine or 2.637 mg/g cocoa.

In a research done by Dinesh and Nurdin (2010) stated that percent of extraction is increases as the extraction time is increases. The *PBC123* in this research also shows similar result, whereby percent of caffeine yield increases as the extraction time increases.

Besides that, according to Dinesh and Nurdin (2010), the increasing in caffeine yield becomes less after the 60^{th} minute. This is because, the solvent, water that saturated with caffeine, further diffusion of caffeine is decreasing into the solvent. In

industrial productions, time is an important factor. The more the time saved, the more the production. This applies also in extraction, where longer time will yield more caffeine. However, an optimum time should be used. So that long time is not consumed and at the same time high caffeine yield is obtained.

In a research done by Ramli *et al.* (2001), the amount of caffeine in cocoa bean sample tested was 4.12 mg/g sample. The amount of caffeine in *PBC123* was approximately 2.637 mg/g sample. This shows the results obtained in this research are acceptable.

4.5 KINETIC CONSTANT RELATION TO EXTRACTION TIME OF CAFFEINE

(Jaganyi and Ndlovn, 2001) state that infusion of caffeine from tea, through the tea bag membrane, can be quantified using Eq. (4.5). It was firstly derived by Spiro and Jago (1982) for loose tea. The variation of concentration, c, with time, t, was again found to fit the first-order kinetic equation with small intercepts, a:

$$\ln\left(\frac{C\infty}{C\infty - C)}\right) = k_{obs}t + a \qquad \text{Eq. (4.5)}$$

It also has been shown by Jaganyi and Mdletshe (2000), where c is the equilibrium concentration and k_{obs} is the observed rate constant. The concentration values used in Eq. (4.5) were all corrected for volume lost due to evaporation and sampling (Spiro & Jago, 1982).

Since $C\infty$ this is the same type of extraction with this research, this equation is available to be used. The value of is used at 90 minutes because at 90 minutes, the reaction is starting equilibrium. The data have been shown in Appendix B (Table B.5).

The linear equation of Eq. (4.5) can be plotted for $\ln\left(\frac{C\infty}{C\infty - C}\right)$ versus k_{obs}, as below:

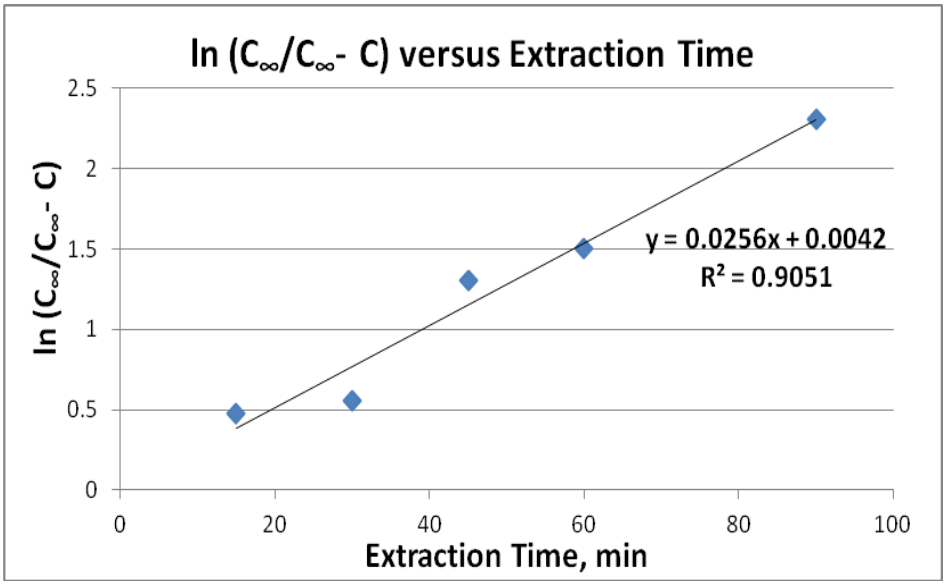

Figure 4.5: The relation of rate constant with extraction time

From the equation in the graph, which is y = 0.0256x + 0.0042, the gradient of the graph is 0.0256. So, the value of rate constant, k, is 0.0256 min^{-1}.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The caffeine has been resulted from *PBC123* using batch solvent extraction method, effectively. The affectivity of caffeine yield is influence by the particle size, solvent/feed ratio and extraction time. The highest caffeine yield was obtained at PBC123 particle size of 315μm which is 0.27% of w/w caffeine or 2.739 mg/g of cocoa, solvent/feed ratio of 1:1 which is 0.26% of w/w caffeine or 2.614mg/g of cocoa and extraction time of 90 minutes which is 0.26% of w/w caffeine or 2.637mg/g of cocoa.

This research is an important practice for the potential of extracting caffeine from *PBC123* and at the same time to get decaffeinated cocoa at low cost and high efficiency.

5.2 RECOMMENDATIONS

For recommendation, it is recommended to repeat this research by using different solvents for liquid-liquid extraction such as supercritical carbon dioxide, hexane, *etc*. Different solid-liquid extraction method should be tried, such as microwave extraction. The analysis of caffeine yield should be tried using high performance liquid chromatography (HPLC). Besides that, during the filtration process, it more suitable to use micro fibre and nylon membrane filter paper rather than common filter paper. This is because, the smallest pore of filter paper can get more clear solution.

REFERENCES

Anonymous(2000). “Caffeine Overview Information”.*http://www.webmd.com/vitamins-supplements/ingredientmono979CAFFEINE.aspx?activeIngredientId=979&activeIngredientName=CAFFEINE*

Anonymous (2011). “Cocoa Facts”. *http : // www. Hawaiian chocolate. com /growing_ chocolate_ cocoafacts.html*

Anonymous (2005).“Countries by Commodity, Cocoa Beans”.*http://www.fao. org/es/ess/top/commodity.html?lang=en&item=661&year=2005*

Anonymous (2010)."Gastroesophageal Reflux Disease (GERD)". Cedars-Sinai.*http:// www.csmc.edu/pf_5543.html*

Anonymous(2001).“Hictory of Cocoa”.*http://www. koko.gov.my/lkm/loader. cfm?page =Industry/History.cfm*

Anonymous (2010).“History of Cocoa”.*http://www.worldagroforestry.org/treesand markets/inaforesta/history.htm*

Anonymous (2010). “How is Caffeine Removed From Coffee”.http://www.livestrong. com/article/241229-how-is-caffeine-removed-from-coffee/#ixzz1jRDauHuI

Anonymous (2005). “MSDS of Ethyl Acetate”*http://www.sciencelab.com/msds.php? msdsId=9927165*

Anonymous (2010). “Reflux”.*http://www.chem-ilp.net/images/photos/reflux.jpg*

Anonymous (1997). "The Characteristics of Cocoa".*http://www.unctad.org/infocomm/anglais/cocoa/characteristics.htm*

Anonymous (2000). "The Cocoa Tree".*http://www.zchocolat.com/chocolate/chocolate/cocoa-tree.asp*

Anonymous (2011). "The Disadvantages of Caffeine".*http://www.livestrong.com/article/518058-the-disadvantages-of-caffeine/#ixzz1jQw9QAEQ*

Anonymous (2009). "The Latest Trend: The use of Caffeine in Aesthetics". *http://www.iapam.com/the-latest-trend-the-use-of-caffeine-in-aesthetics.html*

Anonymous (2010) "*Theobroma Cacao*".*http://en.wikipedia.org/wiki/Theobroma_cacao.*

Abourashed, E. A., Mossa, J. S. (2004). "HPTLC Determination of Caffeine in Stimulant Herbal Products and Power Drinks". *Journal of Pharmaceutical and Biomedical Analysis* 36, 617–620.

Arnaud, M. J. (1987). "The Pharmacology of Caffeine". *Prog Drug*, 31, 273.

Clementz, G. L., Dailey, J. W. (1988). *"Psychotropic Effects of Caffeine"*, Amer. Fan Physician. 37, 167.

Hampp, A. (1996). "The Extraction of Caffeine from Tea: A Modification of Procedure Murray and Hansen". *J. Chem. Educ.*, (73) 1172

Hiroshi Ashihara and Alan Crozier (2001). *"Caffeine : A Well Known but Little Mentioned Compound in Plant Science".* Trends in Plant Science, **Vol. 6**, No. 9.

Hu, Q. H., Jiang, M., Zhu, J. C. (1997*). "Research on Technology of Extraction of Tea Caffeine and Polyphenols".* Natural Product Research and Development of China 9, 63–66.

Islam, M. S., Rahman, M. M., Abedin, M. Z. (2002). "Isolation of Caffeine from Commercially Available Tea and Tea Waste". *Jahangirnagar Uni. J. Sci.*, 25, 9.

Jaganyi, D., Ndlovu, T. (2001). "Kinetics of Tea Infusion. Part 3: The Effect of Tea Bag size and Shape on the Rate of Caffeine Extraction from Ceylon Orange Pekoe Tea". *Food Chemistry*, 75 (2001) 63-66.

Jefferson, J. W. (1998). "Lithium Tremor and Caffeine Intake: Two Cases of Drinking Less and Shaking More". *J. Clin Psychiatry.*, 49, 72.

Johnson, G. D., Fatis, M., Sonnek, D. (1988) "A Survey of Caffeine Use and Associated Side Effects in a College Population". *J Drug Educ.*, 18(3), 211.

Kirmer, D. A. (1988). "Caffeine Use and Abuse in Psychiatric Clients". *J. Psychosoc Nurs Ment Health Serv.*, 26, 20.

Leson, C. L., McGuigan, M. A., Bryson, S. M. (1988). "Caffeine Overdose in an Adolescent Male". *J. Toxicol. Clin. Toxicol.* 26 (5-6): 407–15.

Li, S., Berger, J., Hartland, S. (1990). "UV Spectrophotometric Determination of Theobromine and Caffeine in Cocoa Beans". *Analytrca Chrmrca Acta*, 232, 409-412.

Mackay, D. C., Rollins, J. W. (1989). "Caffeine and Caffeinism". *Journal of the Royal Naval Medical Service* 75 (2): 65–7.

Minifie, B. W. (1989). "Chocolate, Cocoa, and Confectionery Science and Technology". *Third edition. Van Nostrand Reinhold.*

Mumin, M. A., Akhter, K. F., Abedin, M. Z., Hossain, M. Z. (2006). "Determination and Characterization of Caffeine in Tea, Coffee and Soft Drinks by Solid Phase Extraction and High Performance Liquid Chromatography (SPE – HPLC)". *Malaysian Journal of Chemistry.* Vol. 8, No. 1, 045 – 051.

Murray, S.D., Hansen, P.J. (1995). "The Extraction of Caffeine from Tea". *J. Chem. Educ.* 72, 851–852

Ramli, N., Yatim, A. M., Said, M., Hok, H. C. (2001). "HPLC Determination of Methylxanthines and Polyphenols Levels In Cocoa and Chocolate Products". *Malaysian Journal of Analytical Sciences,* Vol. 7, No. 2, 377-386.

Varma, D., Nurdin, S. (2010). *"Batch Solvent Extraction of Caffeine from MCBC2".* Degree Thesis. University Malaysia Pahang, Malaysia.

Wilson T., Norman J. T. (2004). *"Beverages in Nutrition and Health"*. Humana Press. p. 172.

APPENDICES

Appendix A: Material Safety Data Sheet of Ethyl Acetate

Chemical Product and Company Identification

Product Name: Ethyl acetate
CAS#: 141-78-6
Synonym: Acetic Acid, Ethyl Ester Acetic Ether
Chemical Name: Ethyl Acetate
Chemical Formula: C4-H8-O2

Hazards Identification

Potential Acute Health Effects:
Hazardous in case of ingestion, of inhalation. Slightly hazardous in case of skin contact (irritant, permeator), of eye contact (irritant).

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH. MUTAGENIC EFFECTS: Not available.
TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance is toxic to mucous membranes, upper respiratory tract. The substance may be toxic to blood, kidneys, liver, central nervous system (CNS).
Repeated or prolonged exposure to the substance can produce target organs damage.

First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.

Skin Contact:
Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

Serious Inhalation:

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek medical attention.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

Fire and Explosion Data

Flammability of the Product: Flammable.
Auto-Ignition Temperature: 426.67°C (800°F)
Flash Points: CLOSED CUP: -4.4°C (24.1°F). (TAG) OPEN CUP: 7.2°C (45°F) (Cleveland).
Flammable Limits: LOWER: 2.2% UPPER: 9%
Products of Combustion: These products are carbon oxides (CO, CO2).

Fire Hazards in Presence of Various Substances:
Highly flammable in presence of open flames and sparks, of heat. Slightly flammable to flammable in presence of oxidizing materials, of acids, of alkalis. Non-flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of static discharge: Not available. Slightly explosive in presence of heat. Nonexplosive in presence of shocks.

Fire Fighting Media and Instructions:
Flammable liquid, soluble or dispersed in water. SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use alcohol foam, water spray or fog.

Special Remarks on Fire Hazards:
Vapor may travel considerable distance to source of ignition and flash back. When heated to decomposition it emits acrid smoke and irritating fumes.

Special Remarks on Explosion Hazards:
The liquid produces a vapor that forms explosive mixtures with air at normal temperatures. Explosive reaction with lithium tetrahydroaluminate.

Accidental Release Measures

Small Spill:
Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

Large Spill:

Flammable liquid. Keep away from heat. Keep away from sources of ignition. Stop leak if without risk. Absorb with DRY earth,sand or other non-combustible material. Do not touch spilled material. Prevent entry into sewers, basements or confined areas; dike if needed. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Handling and Storage

Precautions:
Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as oxidizing agents, acids, alkalis.

Storage:
Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Moisture sensitive.

Exposure Controls/Personal Protection

Engineering Controls:
Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Personal Protection:
Safety glasses. Lab coat. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
TWA: 400 (ppm) from OSHA (PEL) [United States] TWA: 400 from ACGIH (TLV) [United States] TWA: 1400 (mg/m3) from NIOSH [United States] TWA: 400 (ppm) from NIOSH [United States] TWA: 400 (ppm) [Canada] TWA: 1440 (mg/m3) [Canada] TWA: 1400 (mg/m3) from OSHA (PEL) [United States]3 Consult local authorities for acceptable exposure limits.

Physical and Chemical Properties

Physical state and appearance: Liquid.
Odor: Ethereal. Fruity. (Slight.)
Taste: Bittersweet, wine-like burning taste

Molecular Weight: 88.11 g/mole
Color: Colorless.
Boiling Point: 77°C (170.6°F)
Melting Point: -83°C (-117.4°F)
Critical Temperature: 250°C (482°F)
Specific Gravity: 0.902 (Water = 1)
Vapor Pressure: 12.4 kPa (@ 20°C)
Vapor Density: 3.04 (Air = 1)
Odor Threshold: 3.9 ppm
Water/Oil Dist. Coeff.: The product is more soluble in oil; log(oil/water) = 0.7
Dispersion Properties: See solubility in water, diethyl ether, acetone.
Solubility: Soluble in cold water, hot water, diethyl ether, acetone, alcohol, benzene.

Stability and Reactivity Data

Stability: The product is stable.
Conditions of Instability: Heat, ignition sources (flames, sparks, static), incompatible materials
Incompatibility with various substances: Reactive with oxidizing agents, acids, alkalis.
Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity:
Also incompatible with nitrates, chlorosulfonic acid, oleum, potassium-tert-butoxide, and lithium tetrahydroaluminate. Moisture sensitive. On storage, it is slowly decomposed by water.
Polymerization: Will not occur.

Toxicological Information

Routes of Entry: Absorbed through skin. Eye contact. Inhalation. Ingestion.
Toxicity to Animals:
WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE. Acute oral toxicity (LD50): 4100 mg/kg [Mouse]. Acute toxicity of the vapor (LC50): 45000 mg/m3 3 hours [Mouse].
Chronic Effects on Humans:
CARCINOGENIC EFFECTS: A4 (Not classifiable for human or animal.) by ACGIH. Causes damage to the following organs: mucous membranes, upper respiratory tract. May cause damage to the following organs: blood, kidneys, liver, central nervous system (CNS).

Other Toxic Effects on Humans:
Hazardous in case of ingestion, of inhalation. Slightly hazardous in case of skin contact (irritant, permeator).

Special Remarks on Toxicity to Animals: LD50 [Rabbit] - Route: skin; Dose >20,000 ml/kg

Special Remarks on Chronic Effects on Humans:

May affect genetic material (mutagenic). May cause adverse reproductive effects. based on animal test data. No human data found at this time.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects: Skin: May cause skin irritation. Eyes: Causes eye irritation. May cause irritation of the conjunctivia. Inhalation: May cause respiratory tract and mucous membrane irritation. May affect respiration and may cause acute pulmonary edema. May affect gastrointestinal tract (nausea, vomiting). May affect behavior/central nervous system (mild central nervous system depression - exhilaration, talkativeness, boastfulness, belligerancy, vertigo, diplopia, drowsiness,
slurred speech, slowed reaction time, dizziness, lightheadedness, somnolence, ataxia, unconciousness, irritability, fatigue,sleep disturbances, reduced memory and concentration, stupor, coma), cardiovascular system (peripheral vascular collapse (shock) - rapid pulse, hypotension, cold pale skin, hypothermia). Other symptoms may include: flushing of face and sweating.
Ingestion: May cause gastrointestinal tract irritation with nausea and vomiting. May affect blood, behavior/central nervous system (CNS depression - effects may be similar to that of inhalation). Chronic Potential Health Effects: Skin: Repeated or prolonged skin contact may cause drying and cracking of the skin. IngestIon: Prolonged or repeated ingestion may affect the liver. Inhalation: Prolonged inhalation may affect behavior/central nervous system (symptoms similar to those of acute inhalation), and cause liver, kidney, lung, and heart damage. It may also affect metabolism, and blood (anemia, leukocytosis).

Ecological Information

Ecotoxicity:
Ecotoxicity in water (LC50): 220 mg/l 96 hours [Fish (Fathead minnow)]. 212.5 ppm 96 hours [Fish (Indian catfish)].
BOD5 and COD: Not available.
Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.
Toxicity of the Products of Biodegradation: The product itself and its products of degradation are not toxic.
Special Remarks on the Products of Biodegradation: Not available.

Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Appendix B: Result data

Table B.1: Absorbance of standard concentration of caffeine

Caffeine Concentration (mg/L)	Absorbance
0	0.000
5	0.045
10	0.090
15	0.137
20	0.180
25	0.224
30	0.270

Table B.2: Percentage of caffeine yield for different PBC123 particles size

Particle size (μm)	Absorbance	Concentration of Caffeine (mg/L)	Amount of Caffeine (mg/g sample)	Caffeine Yield (%)
2000	0.176	19.511	0.390	0.04
1000	0.604	67.067	1.341	0.13
800	0.679	75.41	1.508	0.15
613	0.846	93.966	1.879	0.19
315	1.233	136.956	2.739	0.27

Table B.3: Percentage of caffeine yield for different *PBC123* solvent/feed ratio

Solvent/feed ratio	Absorbance	Concentration of Caffeine (mg/L)	Amount of Caffeine (mg/g sample)	Caffeine Yield (%)
0.20	0.389	43.178	0.864	0.09
0.25	0.573	63.650	1.273	0.13
0.33	0.651	72.289	1.446	0.15
0.50	0.895	99.4	1.988	0.19
1.00	1.1765	130.678	2.614	0.26

Table B.4: Percentage of caffeine yield for different *PBC123* extraction time

Extraction time (min)	Absorbance	Concentration of Caffeine (mg/L)	Amount of Caffeine (mg/g sample)	Caffeine Yield (%)
15	0.451	50.067	1.001	0.10
30	0.509	56.511	1.130	0.11
45	0.632	70.178	1.404	0.14
60	0.924	102.622	2.052	0.20
90	1.187	131.850	2.637	0.26

Table B.5: Kinetics relation to extraction time

Extraction Time, t (min)	ln (C∞/C∞ - C)
15	0.4776
30	0.5597
45	1.3073
60	1.5065
90	2.3082