EXTRACTION OF CAFFEINE FROM PBC123

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SUPERVISOR’S DECLARATION

“I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.”

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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 $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$. 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 plantations 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 1 hour. Then, the dried seeds will blend and sieved into 5 different particles size which is 2000, 1000, 800, 630 and 315 $\mu$m. 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 315$\mu$m (0.27% w/w caffeine or 2.739 mg/g cocoa), solvent/feed ratio of 1:1 (0.26% w/w caffeine or 2.637 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 315$\mu$m 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 $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$. 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 alkaloïd(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. Objetif 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 refluxus haba selama 15, 30, 45, 60, 90 minit. Bagi pengekstrakan cecair-cecair, pengekstrakan pelarut dilakukan dengan menggunakan etil asetat pada nisbah pelarut/sampel 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

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPERVISOR’S DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>STUDENT’S DECLARATION</td>
<td>iii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vii</td>
</tr>
<tr>
<td>TABLE OF CONTENT</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF ABREVIATIONS</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xiii</td>
</tr>
</tbody>
</table>

## CHAPTER 1    INTRODUCTION

1.1 Background 1  
1.2 Problem of statement 4  
1.3 Research objectives 4  
1.4 Research scope 4

## CHAPTER 2    LITERATURE REVIEW

2.1 Cocoa 5  
2.1.1 History of cocoa 7  
2.1.2 Characteristics of cocoa tree 7  
2.1.3 Types of cocoa 7  
2.2 Caffeine 8  
2.2.1 Properties 8  
2.2.2 Applications 9  
2.2.3 Advantages 10  
2.2.4 Disadvantages  
2.3 Extraction of Caffeine 11  
2.3.1 Types of solvent 12
CHAPTER 3 METHODOLOGY

3.1 Materials 15
3.2 Flow chart 15
3.3 Methods 17
   3.3.1 Preparation of sample 17
   3.3.2 Preparation of solution 17
   3.3.3 Solid liquid extraction of caffeine 18
   3.3.4 Liquid liquid extraction of caffeine 18
   3.3.5 Separation of caffeine 18
   3.3.6 Analysis of caffeine 19

CHAPTER 4 RESULT AND DISCUSSION

4.1 Standard curve of caffeine 20
4.2 The effect of PBC123 particles size on the caffeine yield 20
4.3 The effect of PBC123 solvent/feed ratio on the caffeine yield 22
4.4 The effect of PBC123 extraction time on the caffeine yield 24
4.5 Kinetic constant relation to extraction time of caffeine 26

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion 28
5.2 Recommendation 29

REFERENCES 29

APPENDICES
A MSDS of ethyl acetate 33
B Result data 38
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Chemical composition of cocoa beans</td>
<td>2</td>
</tr>
<tr>
<td>B-1</td>
<td>Absorbance of standard curve concentration of caffeine</td>
<td>39</td>
</tr>
<tr>
<td>B-2</td>
<td>Percentage of caffeine yield for different PBC123 particle size</td>
<td>39</td>
</tr>
<tr>
<td>B-3</td>
<td>Percentage of caffeine yield for different PBC123 solvent/feed ratio</td>
<td>40</td>
</tr>
<tr>
<td>B-4</td>
<td>Percentage of caffeine yield for different PBC123 extraction time</td>
<td>40</td>
</tr>
<tr>
<td>B-5</td>
<td>Kinetic relation to extraction time</td>
<td>40</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The structure of caffeine</td>
<td>2</td>
</tr>
<tr>
<td>1.2</td>
<td>Heat reflux extractor</td>
<td>3</td>
</tr>
<tr>
<td>4.1</td>
<td>Standard curve of caffeine</td>
<td>20</td>
</tr>
<tr>
<td>4.2</td>
<td>Caffeine yield in percentage for different particle size</td>
<td>21</td>
</tr>
<tr>
<td>4.3</td>
<td>Caffeine yield in percentage for different solvent/feed ratio</td>
<td>23</td>
</tr>
<tr>
<td>4.4</td>
<td>Caffeine yield in percentage for different extraction time</td>
<td>25</td>
</tr>
<tr>
<td>4.5</td>
<td>The relation of rate constant with extraction time</td>
<td>27</td>
</tr>
</tbody>
</table>
**LIST OF ABREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>PBC123</td>
<td>Prang Besar Cocoa 123</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>Ultraviolet/Visible</td>
</tr>
<tr>
<td>Appendix</td>
<td>Title</td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>A</td>
<td>MSDS of ethyl acetate</td>
</tr>
<tr>
<td>B</td>
<td>Result data</td>
</tr>
</tbody>
</table>
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-1H-purine-2,6(3H,7H)-dione, with chemical formula C_{8}H_{10}N_{4}O_{2} (Arnaud, 1987). The structure of caffeine is shown in Figure 1, below (Mumin et al., 2006).
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

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

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. Nowadays, 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°C, point at which caffeine sublimes is 178°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°C, the solubility in water is 2.17g per 100ml water at 25°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 (Kramer, 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°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°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 KH₂PO₄ (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.
http://www.unctad.org/infocomm/anglais/cocoa/characteristics.htm


http://www.livestrong.com/article/518058-the-disadvantages-of-caffeine/#ixzz1jQw9QAEO


