EXTRACTION OF CAFFEINE FROM USED TEA LEAVES

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1. INTRODUCTION

Caffeine and other purine alkaloids, including theobromine and theophylline, have played a major role in the long-standing popularity of non-alcoholic beverages and foods such as coffee, tea, cocoa, mate, chocolate and a wide range of soft drinks (Asahihara, 2008). Caffeine is a naturally occurring chemical stimulant found in the leaves, seeds and fruits of a numerous plant species of a group of compounds called trimethylxanthine. Its chemical formula is C_{x}H_{y}N_{z}O_{w}. In its pure form, caffeine is a white crystalline powder that tastes very bitter. It is medically useful to stimulate the heart and also serves as increasing the rate of urine excretion. It is one of the most studied ingredients in the food supply. The most commonly known sources of caffeine are coffee and cocoa beans, guarana, and tea leaves. The amount of caffeine in food and beverage products varies depending on the serving size, the type of product and preparation method. Tea which we generally drink is made from the leaves of an Asian evergreen known as Camellia sinensis. White tea, green tea, red tea, and black tea all come from this plant, and all contain caffeine. The presence of caffeine in plants helps to prevent them from insects and other herbivores with the compound’s bitter taste and stimulating qualities. The growing buds and young leaves of tea plants manufacture the highest amounts of caffeine. Caffeine can be isolated from teas with liquid-liquid extraction and quantitated by gas chromatography with nitrogen-phosphorus detection. The decaffeinated teas contained less than 12 mg of caffeine per serving, and caffeine was not detected in the herbal tea varieties. The steep time affect the caffeine concentration of the tea. Most brewed teas contain less caffeine per serving than brewed coffee. (Jenna et al., 2008). A special ultrasound method with high-frequency, well penetrating power and the sound wave can be used to extract the caffeine. The quantity of caffeine is increased with temperature raised. (Shane-Rong Sheu et al., 2009).

The caffeine content of tea leaves depends on the variety and where they were grown; most tea has 3-5% by weight. The optical transition properties of caffeine were measured in different solvents (dichloromethane, water, chloroform and ethyl acetate). Caffeine has highest optical transitions in dichloromethane than the other solvents. Caffeine can be extracted more at the boiling temperature than at 30°C (Atomiss, Gholap, 2011). Besides, at 280 nm, the limits of detections of catechins and caffeine are 10–6 mol/L, which is suitable for the real sample determination. Using this analytical method, the extraction of these compounds from the tea leaves with hot water is compared under different temperatures. The effects of temperature on the amount of catechins and caffeine extracted are evident, showing that (−)-epigallocatechin gallate is the most easiest to be extracted at 100°C (Guanqun et al., 2003). Spectrophotometric micromethod has proven to be the best alternative to the HPLC method. The highest antioxidant capacity was determined in yellow tea, while the lowest was determined in roasted mate tea (Groiesser, 1978; Komes et al., 2009).

Extraction yield increased with increasing of extraction time and also with the size of the cocoa bean (Nawrot et al., 2003). Methylxanthine (caffeine, theobromine, and theophylline) contents in three brews of four types of tea (black, oolong, green, and herbal) in both bags and loose leaf forms were investigated to determine the actual amount of methylxanthines present in tea as a function of different brewing methods (Brunetto et al., 2005). Moreover, in some tea infusions like Chiang Rai tea infusions caffeine was found to be dependent on infusion conditions (Siripat Suteerapataranon et al., 2008).

Solvent extraction method is found to be dependent on parameters like type of solvent, temperature, pH of the solution, solid-liquid ratio, particle size etc. The number of extraction stages depends on the efficiency of the equipment used (Bharadwaz and Bhattacharjee, 2012). The caffeine from the Camellia sinensis (green tea) leaves was isolated which is obtained from the Sikkim
Caffeine had been widely used in the food and pharma industry. The cost of extraction of caffeine from natural source is more. Research has been taken to extract it from natural source more economically. Alteration of the fermentation process by use of microorganisms is one such attempt (Wang et al., 2005).

The present study was carried out to study the extract and estimate the amount of caffeine from used tea leaves of black, white, green and red tea using dichloromethane as solvent.

2. MATERIALS AND METHOD

Tea samples namely, black tea, red tea, white tea and green tea were obtained from a supermarket. Tea was prepared using the standard procedure namely; addition of tea leaves after the water comes to boiling and leaving it to stand for 5 minutes before filtering.

**Extraction of caffeine:**

About 10g of the tea leaves after first, second and third usage was placed inside a beaker and 4.8g of calcium carbonate was added to it along with 100 ml of distilled water. The mixture was allowed to boil on a hot plate for about 15 minutes. The mixture was then filtered using a funnel and filter paper. The filtrate was cooled to about 15-20°C and transferred to a separating funnel. About 15ml of dichloromethane was added to the filtrate and the funnel was stoppered. The contents were mixed vigorously and allowed to stand for 15 minutes. The mixture separated into two layers. The lower layer containing dichloromethane was drained and collected in a conical flask. Another 15ml of dichloromethane was added to the funnel and the process was repeated. Anhydrous magnesium sulphate was added to the conical flask containing dichloromethane and was allowed to stand for 10 minutes. This mixture was then filtered using a funnel and filter paper.

The weight of the filtrate was measured as the fresh weight. The dichloromethane was evaporated by placing the beaker on a water bath. After evaporation, light green coloured caffeine powder was visible. The weight of caffeine powder was considered as the final weight. On subtracting the initial weight from the final weight, the amount of caffeine extracted was found.

3. RESULT AND DISCUSSION

The procedure for this experiment proved to be quite interesting although a few changes were required in certain aspects. The extraction procedure using dichloromethane proved to be highly efficient (Figure 1).

The total amount of caffeine present initially in the four samples is listed in Table 1. The caffeine content is maximum in green tea and minimum in red tea. It has been suggested that the change of caffeine content in tea leaves during the pile-fermentation depended not only on the growth and reproduction of microorganisms, but also on the tea composition (Wang et al., 2005, Wang et.al., 2008). White tea is the least fermented and is followed by green tea, red tea and black tea. As the fermentation progresses there is an increase in caffeine content and decrease in antioxidant content. The amount of caffeine also varies depending on the variety of tea, brand of tea and is also directly attributed to the processing and leaf maturity (Komes et al., 2009). Green tea contained less caffeine content when compared to black tea and hence was is good for health (Kranthi Kumar and Kiran Kumar, 2014).
However, in the present study the content of caffeine was more in green tea and was followed by white tea, black tea and red tea. It has been stated that generalizing the caffeine content in different varieties of teas is quite misleading as there is a general belief that caffeine content is more in black tea and less in green tea and that white tea has the least caffeine content (Friedman et al., 2005).

The amount of caffeine in the used tea leaves namely after first, second and third usage was studied. It was found that the retention percentage of white tea was more when compared to the other teas (Table 1). It was followed by green, black and red tea. Similarly, after the second usage green tea showed the maximum percentage retention than the other teas (Table 2).

The percentage retention of green tea continued to be at 51% even after the third time of usage (Table 3). Thus it had been concluded that the retention percentage of green tea continued to be at the higher side when compared to the other teas. Red tea showed zero retention percentage after the third usage. More work has to be carried out to substantiate these results.

### Table 1: Amount of caffeine after first use

<table>
<thead>
<tr>
<th>Type of tea</th>
<th>Initial caffeine content (mg/100g of tea)</th>
<th>Amount of caffeine (mg/100g of tea)</th>
<th>Percentage retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>green tea</td>
<td>75</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>white tea</td>
<td>55</td>
<td>48</td>
<td>88</td>
</tr>
<tr>
<td>black tea</td>
<td>20</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>red tea</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
</tbody>
</table>

### Table 2: Amount of caffeine after second use

<table>
<thead>
<tr>
<th>Type of tea</th>
<th>Initial caffeine content (mg/100g of tea)</th>
<th>Amount of caffeine (mg/100g of tea)</th>
<th>Percentage retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>green tea</td>
<td>75</td>
<td>55</td>
<td>73</td>
</tr>
<tr>
<td>white tea</td>
<td>55</td>
<td>34</td>
<td>62</td>
</tr>
<tr>
<td>black tea</td>
<td>20</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>red tea</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
</tbody>
</table>

### Table 3: Amount of caffeine after third use

<table>
<thead>
<tr>
<th>Type of tea</th>
<th>Initial caffeine content (mg/100g of tea)</th>
<th>Amount of caffeine (mg/100g of tea)</th>
<th>Percentage retention</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>white tea</td>
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<td>36</td>
</tr>
<tr>
<td>black tea</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>red tea</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

Tea is very rich in antioxidants. It is the most widely used beverage all over the world. It also has medicinal properties. In this study teas were decaffeinated using dichloromethane as a solvent. This study was carried out to check the amount of caffeine in used tea leaves. It was noticed that the amount of caffeine decreased with every use. Green tea is estimated to have more amount of caffeine among the other three tea leaves followed by white tea, black tea and red tea.

5. REFERENCES


