STUDENT STUDY PROJECT

2021-2022

Title of the project: **EXTRACTION OF CAFFEINE FROM USED TEA LEAVES**

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EXTRACTION OF CAFFEINE FROM USED TEA LEAVES

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 C8 H10 N4 O2. 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

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 (Atomssa, 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 1000C (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 (Groisser, 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

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.

Materials and Methods

Different varieties of Indian tea samples were purchased from the market. Tea waste was collected from tea stall. The physical properties of tea powder and tea waste were analyzed.

Tea acidity and taste

Twenty-five grams of each sample was taken in a beaker and 100 ml of water was poured to it. The solution was heated till it boiled, after which the beaker was kept covered and allowed to stand for 5 minutes. A drop was taken from each beaker to spot on a pH meter. After recording the pH of the different tea samples we also tasted a sample of the tea from each beaker8.

Analysis of color

The 20grams of the sample was taken in a beaker added 200 ml of distilled water to it. The colour intensity was measured using a spectrophotometer at 450 nm.

Analysis of bulk density:

A weighed quantity of tea powder was introduced into a graduated measuring cylinder. The measuring cylinder was tapped manually till a constant volume was obtained. This volume is known as the bulk volume of the tea powder. The same procedure was followed for each brand of tea powder. Bulk density of powder is defined as the ratio of the mass of the powder to its bulk volume of distilled water.

Bulk density = mass of the powder / bulk volume) 10

Extraction of caffeine:

Tea bags are used as the source of caffeine for this experiment. Different varieties of 5 tea bags (20 g of each) were taken and boiled with 200 ml of distilled water for 10 minutes. This was allowed to cool for 5 minutes and then decant the mixture into another beaker. Then the tea bags were squeezed to liberate the rest of the water. The collected aqueous solution was cooled and mixed with 30ml of dichloromethane followed by 1%sodium sulphide. This mixture was mixed and poured into separation funnel. Two layers were separated and extracted filtrate was further heated to get the white crystals of caffeine. The extracted caffeine was measured spectrophotometerically at 645nm. The physical properties of caffeine were analyzed11.

Antioxidant assay:

Antioxidant capacity of tea extracts and caffeine solutions (100 mg/l) were determined using the ABTS radical scavenging assay according to the method reported by Re etal. (1999)11, and ferric reducing/antioxidant power (FRAP) assay, carried out according to the original method by Benzie and Strain (1996)12 **.**

Results and Discussion:

The different varieties of tea samples and tea waste were subjected in to analysis of pH and bulk density. Most Tea has the slightly acidic pH and they possess a mildly bitter taste (Table1). Red Label Tea had the pH of 5.5, with a strong bitter taste13. Tetley tea was found to be low pH and mild bitter in taste. The colour of the different tea samples were observed in range of light brown to dark brown. The bulk density of Red label and Lipton tea were fall in the range of 0.42g to 0.43g. Green tea of Tetley had the lowest absorbance of 0.29 but Red label showed highest absorbance of 0.53. There is a linear relationship between color and bitter taste.

Table 1: Physical characteristics of tea powders and tea waste.

Sample	pH	Bulk density	Taste	Colour
3ROSES	**4.7**	0.33g±0.01	Bitter	Brown
TETLEY	**4.0**	0.23g ±0.001	Mild bitter	Light brown
LIPTON	**4.8**	0.42g±0.02	Bitter	Brown
TEA WASTE	**4.3**	0.32g±0.003	Mild Bitter	Brown
RED LABEL	**5.5**	0.43g±0.014	Strong bitter	Dark Brown

The caffeine content was extracted from tea samples and its concentration was observed by using spectrophotometer. Ashihara and Kubota (1986) reported that caffeine production is most active in young tea leaves and buds, which should contain the highest caffeine content1. The maximum concentration of caffeine was found in Tetley and less in 3Roses. The tea waste also contained less amount of caffeine. Earlier studies reported that caffeine content is associated to origin, genetic and environmental variability, harvest time and processing manner of plant material3. According to the obtained results, all studied teas exhibited high antioxidant activity ,reflecting that the contribution of caffeine to the antioxidant roperties.The maximum amount of caffeine crystals was extracted from 3roses tea powder to be 2.20gms.Lipton and tetly tea powders had 1.25and 1.5 gm of caffeine per 250kgof sample.

Conclusion

The caffeine was extracted from tea powder and tea waste samples. Caffine is a good neuro stimulant and also inhibit the growth of cancer cells. Thus caffeine plays an important role in cancer research for my future research.