Caffeine Content and Sleep Implications of CHAGEE Milk Tea: A Back Iodometric Titration Study

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Abstract. Caffeine is a potent psychoactive compound that occurs naturally in some drinks, such as tea, coffee, and milk tea, that works to either improve alertness or encourage sleep. This research aims to present the caffeine levels in CHAGEE milk tea and indicate potential outcomes on sleep. The study used the much-esteemed back iodometric titration as the approach of analysis due to its validity and reliability in science. The measured levels of caffeine in CHAGEE were higher than predicted, and caffeine levels might collectively be equivalent to or higher than a full cup of traditional tea. The consumption of CHAGEE has the potential for too much caffeine consumption from the beverage, thus negatively affecting the quality of sleep. This research contains useful information for consumers about making healthy choices in beverages that they may consume, and indicates features for future research to expand food safety and nutrition aspects. Additionally, this research could assist product developers in improving product formulas and labeling in a way that supports public health awareness from consumption. Future studies may also explore the caffeine interaction with other substances in milk tea and their combined biological effects.

Keywords: Caffeine; Milk Tea; Back Iodometric Titration; Sleep Quality; Quantitative Analysis.

1. Introduction

The rapid development of society has increased the pressure on young people today, while promoting consumption. Milk tea has become an essential drink in the daily life of contemporary young people. For the fast-paced life nowadays, milk tea can not only bring people instant happiness, but also slow down their pressure [1]. But at the same time, it seems that people are not fully aware of the bad effects of drinking milk tea. It is not difficult to find that milk tea is addictive, and people may feel lost without it for a long time. What's more, milk tea can also cause insomnia, which can lead to more serious emotional and health problems [2]. In fact, all of this is closely related to the caffeine in milk tea. Caffeine is a widely consumed psychoactive substance and is extremely abundant in beverages [3, 4], which is popular among adolescents, raising sociological concerns regarding the abuse of caffeine through such beverages [2]. Given that the overdose of caffeine may lead to poor sleep quality [3], anxiety, flushing, palpitations, headache, and tremors [4], it is important to qualify the caffeine levels in milk tea. This article aims to examine the caffeine content in milk tea CHAGEE by using iodometry [5-7]. We compare our titration result to the label on the type of tea we bought, which presents the caffeine content, to determine whether CHAGEE has false reporting or under-reporting the caffeine content.

2. Literature Review

2.1 Pharmacology of Caffeine

According to Figure 2, Caffeine acts at A1 and A2A receptors as a non-selective competitive antagonist [8,9]. It ensures wakefulness by seizing adenosine receptors, mainly blocking A2A in the ventrolateral preoptic area. When adenosine binds to the A1 receptor, it inhibits the enzyme adenyl cyclase via a Gi protein, leading to a decrease in intracellular cAMP levels. In contrast, binding to the A2A receptor activates adenyl cyclase through a Gs protein, resulting in increased cAMP production. This rise in cAMP can trigger various metabolic, cardiovascular, and neural responses [8]. PDE inhibition occurred when caffeine was consumed in high doses (6.1mg/kg), causing excitatory signaling. Caffeine inhibits PDE enzymes, resulting in higher intracellular cAMP/cGMP levels, leading to enhancement in excitatory signaling, which may consequently cause anxiety, palpitation, and tremor [7,10].

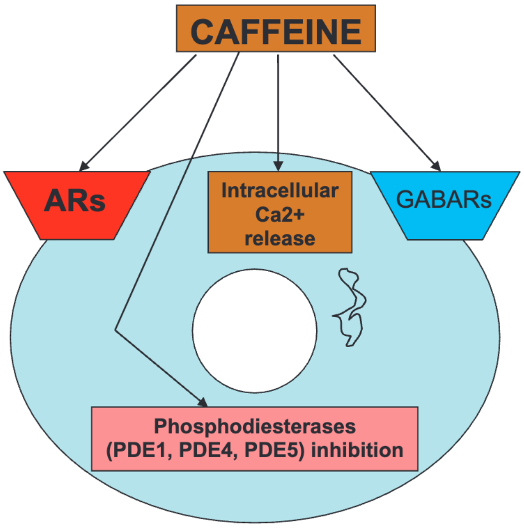

Figure 1. Sites/mechanisms of action of caffeine [11].

Ryanodine receptors (RYR) can be activated by caffeine, promoting the release of sarcoplasmic and endoplasmic reticulum Ca^{2+}. Increasing concentration of Ca^{2+} can promote muscle and neuronal excitability [12], which shows enhancement in the performance of alertness and motility. Caffeine can increase alertness and decrease fatigue by blocking adenosine receptors through CNS stimulation. This causes the observation of mild increases in heart rate and blood pressure via A1 receptor antagonism. The A1 receptor blockade attributed to caffeine in the kidneys leads to increased urine output. Ryanodine receptor activation causes an increase in muscle and neuron excitability and A1 blockade in the heart and kidney, causing a mild increase in BP/HR, contributing to tolerance [8]. Tolerance can develop after chronic caffeine use through the modulation of adenosine receptors and the release of adenosine in the neurosystem [12]. Caffeine may also alter the dopamine regulation system by chronic A2A receptor antagonism, which contributes to dependence. Withdrawal symptoms, including headache, fatigue, and decreased alertness, can be observed at 12-24 h, peak at 20-48 h, and occur in ~50% of daily coffee consumers who intake more than 100 mg caffeine per day on average [13, 14]. The figure below shows the overall Pharmacokinetics of caffeine.

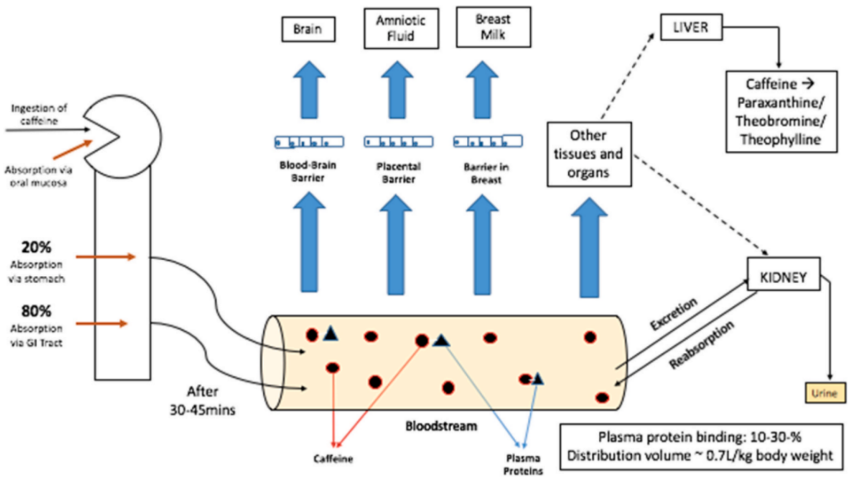

Figure 2. The overall Pharmacokinetics of caffeine [8].

Due to the high water-solubility of caffeine, it is rapidly and almost completely absorbed from the gastrointestinal tract [15]. Approximately 99% of caffeine consumed is absorbed within 45 minutes, with peak plasma concentrations occurring between 15–120 minutes [16], depending on gastric emptying and the presence of food. Bioavailability is nearly 100% after oral administration, presenting no significant first-pass metabolism [17]. Caffeine distributes widely throughout the body, with a volume of distribution of 0.5–0.75 L/kg [8], indicating its water and lipid solubility. It crosses the blood-brain barrier and placenta easily because of its structure. Plasma protein binding is around 10–30% [18], primarily to albumin. As a result, massive tissue penetration is detected.

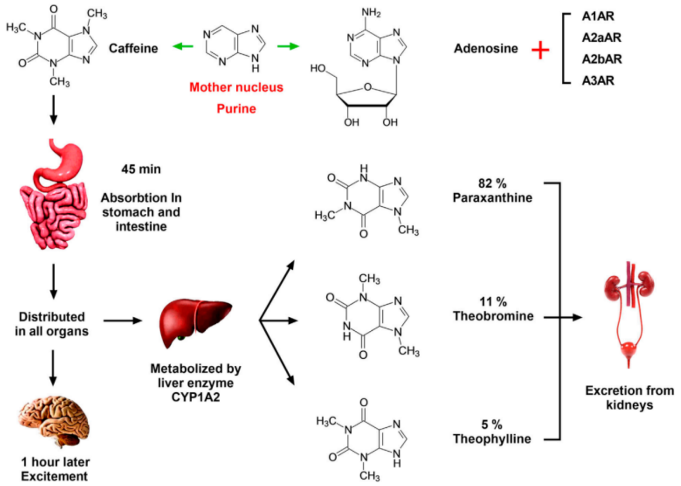

Figure 3. Metabolism of Caffeine and Its Major Metabolites in Humans [19].

According to Figure 3, Caffeine is metabolized in the liver primarily by cytochrome CYP1A2, which accounts for ~90% of its metabolic clearance [20]. Through metabolism, caffeine undergoes oxidative N-demethylation [21] to produce the main mono-demethylated metabolites like theophylline, theobromine, and paraxanthine, which are distributed as follows in Table 1:

Table 1. Caffeine Metabolites and Their Physiological Effects [19].

Metabolite	Proportion of Caffeine Metabolism	Primary Effect (s)
Paraxanthine	80–84%	Increases lipolysis (fat breakdown)
Theobromine	11–12%	Vasodilator, diuretic
Theophylline	4–7%	Bronchodilator

Caffeine and its metabolites are primarily excreted through the kidneys. Only about 3-5% of caffeine is excreted unchanged in the urine [22, 23]. The rest is excreted in urine as paraxanthine (80-84%), theobromine (11-12%), and theophylline (4-7%) [19].

2.2 Method to Analyse Caffeine

There are common methods for analysing the content in milk tea. Potassium iodate precipitation gravimetric method by Wallrabe [24], titration in a chloroform-benzene mixed solvent, using 1% α-naphthylbenzylaminein acetic acid solution as the indicator by Fritz [25], HPLC [26], UV-Vis [27], and ICP-MS [28]. Due to the limitations of lab conditions, we chose to determine the concentration by using back iodometric titration.

3. Method

3.1 Materials and Reagents

Reagents

- Tea sample: 100 ml
- Ethyl acetate: 450 ml
- 0.9825 M iodine solution with 0.1 M potassium iodide solution: 10 ml

- 0.05 M sodium thiosulfate
- Extracted crude caffeine sample: 10 ml
- 0.1 M sulfuric acid: 10 ml
- Distilled water
- Starch indicator (0.5%)

Apparatus

- Cylinders and beakers
- Test tubes
- Droppers
- Burette
- Pipette (10 ml)
- Conical flask
- Volumetric flask (250 ml)
- Ceramic tile
- Separatory funnel
- Rotary evaporator
- A glass rod
- Water bath kettle
- Funnel

3.2 Experimental Procedure

1. Transfer the protein-free supernatant (about 100 ml) into a separatory funnel. Add 150 ml of ethyl acetate (equal volume to the milk tea sample) to ensure sufficient solvent for caffeine extraction.
2. Shake the separatory funnel gently to mix the phases, allowing the two layers to come into contact. After shaking, allow the layers to separate by standing.
3. Repeat the extraction process three times, each time adding 150 ml of ethyl acetate and collecting the organic phase (ethyl acetate layer).
4. Carefully separate the organic (ethyl acetate) phase and transfer it to a new container. Discard the aqueous phase.
5. Transfer the collected ethyl acetate solution to a rotary evaporator. Set the rotary evaporator to low temperature (40–50°C) and reduced pressure to evaporate ethyl acetate. Evaporate the solvent completely, leaving behind the caffeine precipitate.
6. Dissolve the caffeine precipitate in 50 mL of distilled water. Gently stir with a glass rod and use a water bath at 60°C to ensure complete dissolution.
7. Transfer 10 ml of the crude caffeine solution into a conical flask. Acidify the solution using 10 ml of dilute sulfuric acid.
8. Add 10 ml of iodine solution along with potassium iodide to the caffeine solution. Allow the mixture to react completely; a pale brown color indicates the presence of excess iodine.
9. Titrate the excess iodine using a standardized sodium thiosulfate solution until the solution turns pale brown. Add starch solution as an indicator; the solution will turn dark blue. Continue titrating until the solution becomes colorless — this marks the endpoint.
10. Repeat the titration three times and calculate the average caffeine content.

3.3 Calculation

Calculate the amount of unreacted iodine from the thiosulfate used. Subtract this from the total iodine added to determine the iodine that reacted with caffeine. Use the stoichiometry (1 mol caffeine: 2 mol iodine) to determine the caffeine content.

4. Results

To obtain the caffeine content in the sample, we first need to understand the two redox reactions involved in the titration process. Caffeine reacts with iodine in an acidic solution:

$$C_8H_{10}N_4O_2 + 2I_2 + KI + H_2SO_4 \rightarrow C_8H_{10}N_4O_2 \cdot HI \cdot I_4 + KHSO_4$$

This means one mole of caffeine will consume two moles of iodine.

The excess iodine that does not react with caffeine is titrated using sodium thiosulfate:

$$I_2 + 2NA_2S_2O_3 \rightarrow 2NaI + NA_2S_4O_6$$

We first measured how much iodine was added in total, and then how much was left after reacting with caffeine. The difference corresponds to the amount of iodine that reacted with caffeine. In our titration, we used the volume of sodium thiosulfate (in milliliters) and its concentration to calculate how many moles of iodine were left. Subtracting this from the total amount added, we get the amount of iodine that reacted with caffeine. Because one molecule of caffeine reacts with two molecules of iodine, we divide the reacted iodine amount by 2 to get the amount of caffeine. Then, we multiplied the amount of caffeine in moles by its molecular weight (194.19 g/mol) to get the mass of caffeine in the extracted solution. Finally, we used the dilution factor (due to the small sample volume used in titration and the total beverage volume) to calculate how much caffeine was present in the entire drink.

$$\text{Content} = [39.3 - (37.10 + 37.10 + 37.05) + 3] \times 10^3 \times 0.05 \div 2 \div 2 \times 194.19 \times 5 \times 4.7 \times 10^3$$
$$\text{mg} = 126.46\text{mg}$$

Through research [29], it is considered that the efficiency of caffeine extraction utilizing ethyl acetate is approximately 79%, the final content in the sample of the beverage is 138.11mg, which fits the label on the beverage (141.2mg), with a percentage difference of 2.1876%.

Table 2. Titration readings for iodine solution.

Trial	Initial Reading (cm)	Final Reading (cm)	Volume (cm³)	Comments
With iodine only	0.00	39.30	39.30	✓
Accurate 1	0.00	37.40	37.40	✓
Accurate 2	0.10	37.45	37.35	✓
Accurate 3	0.25	37.60	37.35	✓
Accurate 4	0.70	38.15	37.45	✓

5. Discussion

During the course of our experiments, there are a number of factors that can influence our final results, the most obvious of which is the time of the reaction and the extraction efficiency of the dispensing funnel. The timing of the reaction can affect the size of the final titre data. If we start dropping sodium thiosulfate immediately after mixing the solution to be titrated, the overall titre data may be large. This is because there is still some iodine left over from the first reaction, so it will be counted as part of the iodine left over from the first reaction. At the same time, the extraction efficiency of the dispensing funnel can also affect the results of our experiments. Even if we repeated the extraction many times, some caffeine would still be left in the funnel, so we could not guarantee that all the caffeine was extracted. This results in a smaller overall caffeine content, and in our actual results, the caffeine content is indeed less than the caffeine content labelled by CHAGEE. Through the previous research on caffeine, the actual caffeine content in CHAGEE merchandise is known. We've come up with some recommendations on caffeine intake and alternative ways to keep people’s spirits up. For most healthy adults, no more than 400 mg of caffeine per day is usually safe. Based on the caffeine content labeled by CHAGEE and the actual measurements, it can be concluded that people can drink up to 1,400ml of milk tea per day to remain healthy and safe. That is to say, it is safe to drink two cups of milk tea a day and below. However, this does not mean that it is safe for people to drink two cups of milk tea every day. People should still try to drink less milk tea and consume less caffeine. The main purpose of people's caffeine intake is to stay awake so that they can enhance their efficiency at work and school. However, excessive intake of caffeine can bring about a host of problems such as insomnia, anxiety, and even death. Therefore, it is vital to find some alternatives that can equally keep people awake. One of the very common ways is to do more outdoor exercise to promote blood circulation and ensure that the body has enough oxygen, thus reducing people's sleepiness. In addition to this, people can also tackle the root cause of the problem. Adopting a good routine and getting enough sleep can keep energy levels high in daily life without the need for caffeine.

Throughout the experiment, we had many deficiencies and areas for improvement. In general, we can analyse our shortcomings in three aspects: measurement, experimental operation and current conditions. In terms of measurement, any measurement of liquids we are not able to fully ensure that our line of sight is completely parallel to the liquid surface, so the actual data we measured and the planned data will have a certain degree of error, which leads to our final results not being completely accurate. Then come to the experimental operations. To begin with, in the step of dissolving caffeine, we could not be sure whether the caffeine we extracted was completely and uniformly dissolved in the distilled water, which might have some influence on our subsequent titration. In addition, when we were titrating, we were unable to capture the exact moment when the color of the reagent changed to colorless due to human reaction time, so our measurements may have been large overall. Finally, the conditions available in the laboratory also limit the accuracy of our data. Specifically, the most common method of measuring caffeine in beverages today is HPLC (High Performance Liquid Chromatography). However, this instrumentation is not available in our lab, so we designed an experiment using redox titration to detect caffeine. As mentioned before, we need to configure a lot of reagents, so there will be unavoidable errors between the resultant data and the planned data.

6. Conclusion

In conclusion, caffeine is an antagonist of the adenosine receptor, which blocks the transmission of messages so that people do not feel sleepy and thus become refreshed and motivated. At the same time, however, it brings with it a host of problems, the most common of which is insomnia, and a host of emotional problems secondary to insomnia. We chose one of the most popular milk tea brands, CHAGEE, as our research subject to test whether the caffeine content of its product matches its labeled content. We describe the structure, absorption, and pharmacological effects of caffeine to give a comprehensive introduction to the substance and how it keeps people awake. Then, due to the limitations of the laboratory equipment, we chose to test the caffeine content in our milk tea samples by redox titration. Although the first experiment was unsatisfactory, we got satisfactory results in the second experiment. Our experiment proved that CHAGEE is a safe and trustworthy brand, as the data labeled on its products are not falsified. People can measure how much milk tea they are allowed to drink in a day according to the ingredient content of the products provided by CHAGEE. Through the previous research, we have learnt that caffeine can make people energetic, but it is also highly addictive and dependent. People who become dependent on caffeine are very likely to develop symptoms such as anxiety and depression once their caffeine intake is restricted. Therefore, in the field of psychology, when faced with a patient with depression or anxiety, doctors can consider whether the patient has developed a strong dependence on caffeine-containing products. This can provide a good idea for symptom relief as well as precise drug administration.

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