Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 3(6) November 2025 Special Issue
DOI: https://dx.doi.org/10.4314/jobasr.v3i6.5s

Extraction And Quantification of Caffeine in Different Tea Bags Using Uv-Visible Spectroscopy and Iodometric Back Titration

Ibrahim Isah Fagge[1*], Kamal Nura Abubakar[2], Saudatu Ja'afaru Chinade[3], Ridwanu Murtala[4], Sunusi Yahaya Hussaini[5] & Ibrahim Usman Kutama[6]
[1,2,3,4,5&6]Department of Chemistry, Aliko Dangote University of Science and Technology, Wudil
[*]Corresponding Author Email: iifagge@kustwudil.edu.ng

ABSTRACT
This study aimed to compare the caffeine content across different commercial tea brands and to correlate the results with processing methods, with a discussion of implications for consumer health and labeling practices. The caffeine content in three commercial tea samples (one green tea and two black teas) was comparatively analyzed. Caffeine was extracted from the samples using a Soxhlet apparatus with dichloromethane as the solvent. The crude caffeine was then quantified using two independent analytical techniques: UV-visible spectrophotometry and iodometric back titration. Results from both methods were consistent, revealing that the green tea sample (Sample 1) contained a significantly higher caffeine yield (472 mg per 6.67 g of tea) than the two black tea brands (268 mg and 222 mg, respectively). UV-visible analysis at 274 nm showed a correspondingly higher absorbance for the green tea extract (2.5) compared to the black tea extracts (2.2). Similarly, iodometric back titration indicated a greater consumption of sodium thiosulfate titre for the green tea sample, confirming a higher concentration of caffeine. This finding challenges the common assumption that black tea inherently contains more caffeine due to the fermentation process. The calculated caffeine content was approximately 7.1% for the green tea, compared to 4.1% and 3.3% for the black teas. The study highlighted significant brand-specific variations in caffeine content, which has direct implications for consumer awareness and industry labeling practices. The reliability of the analytical approach was validated by the strong concordance between the results obtained from the two distinct quantification methods.

Keywords:
Soxhlet apparatus,
UV-visible Spectrophotometry,
Iodometric Back titration,
Teas Caffeine

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring alkaloid in tea leaves *(Camellia sinensis*) (Naila *et al*., 2022), known for its stimulatory effects on the central nervous system (Newman, 2023). The concentration of caffeine varies significantly across tea types due to factors such as processing methods (fermentation), plant cultivar, and brewing practices (Zhang & Ruan, 2016). Brand 1 (unfermented) typically contains less caffeine than Brand 2 (fermented), though commercial processing variations may alter this trend. This project comparatively analyzes caffeine content in three popular tea brands to quantify differences and evaluate consumer health implications.

Caffeine is stimulant of the mexylxanthine class and most commonly consumed psychoactive substance globally, which means it increases activity in your brain and central nervous system it also increases the circulation of chemicals such as cortisol and adrenaline in the body (Lee *et al.,* 2024). In small doses, caffeine can make you feel refreshed and focused, caffeine acts by blocking the binding of adonesine at a number of adonesine receptor types, inhibiting the centrally depressant effect of adonesine effect and enhancing the release of acetylcholine. Caffeine has a three-dimensional structure similar to that of adonesine, which allows it to bind and block its receptors (Davoudi *et al.,* 2025). Caffeine also increases cyclic adonesine monophosphate (AMP) levels through nonselective inhibitor of phosphodiesterase increases calcium release from intracellular stores. Tea is the most consumed non-alcoholic beverage worldwide after water, valued for its unique flavour, pleasant taste,

How to cite this article: Ibrahim I. F., Kamal N. A., Saudatu J. C., Ridwanu M., Sunusi Y. H. & Ibrahim U. K. (2025). Extraction And Quantification of Caffeine in Different Tea Bags Using Uv-Visible Spectroscopy and Iodometric Back Titration. *Journal of Basics and Applied Sciences Research*, *3*(6)s, 41-46. https://dx.doi.org/10.4314/jobasr.v3i6.5s

and numerous health benefits-including antioxidants, antimicrobial, immune stimulatory and anti-mutagenic properties (Rabil *et al.,* 2025). Caffeine is a bitter, white crystalline purine, and is chemically related to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Sampio-jorge, 2021). In 2020 almost 10 million tons of caffeine beans were consumed globally.

It can be extracted using a technique called solvent extraction. Since caffeine is more soluble in organic solvents like dichloromethane than in water, it can be separated from other tea components. The extraction of caffeine from tea remains an evolving field that bridges chemistry, environmental science, and food technology. Continued development of efficient, sustainable methods and precise analytical tools is essential for both academic and industries (Pradnya Ingle *et al.*, 2019).

Figure 1 shows main methylxanthine in tea that is the stimulant caffeine. Other methylxanthines found in tea are two chemically similar compounds, theobromine and theophylline, which play a major role in the long-term popularity of non-alcoholic beverages and foods such as coffee, tea, cocoa, chocolate and a variety of soft drinks (Zhang *et al.,* 2022). Caffeine is a naturally occurring chemical stimulant found in numerous plants' leaves, seeds, and fruits. Caffeine is the world's most widely consumed psychoactive drug; however, it is legal and unregulated in nearly all parts of the world. It stimulates the central nervous system, reducing fatigue and drowsiness. It can improve athletic performance, muscular strength, and power. Caffeine riches chemically known as 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or 1,3,7-trimethylxanthine having chemical formula $C_8H_{10}N_4O_2$ was first discovered in 1827 belonging to the alkaloid family containing nitrogen in their heterocyclic ring structure. This study addresses the significant gap in standardized, brand-specific data on caffeine content in commercial teas, where despite general knowledge that black teas contain more caffeine than green teas, documented variations between specific brands such as Brand 1 and Brand 2 remain insufficient. The research problem centers on the inconsistency and lack of precise quantification of caffeine across different tea products, which has implications for consumer safety, regulatory compliance, and product labeling. To resolve this, the study aims to extract and quantitatively analyze the caffeine content in various tea samples using standardized analytical techniques. The objectives are to isolate caffeine via Soxhlet extraction with dichloromethane (DCM), compare caffeine yields across selected brands while correlating results with processing methods, discuss the health and industrial implications of the findings, and quantitatively assess caffeine using iodometric back-titration as a method of analysis.

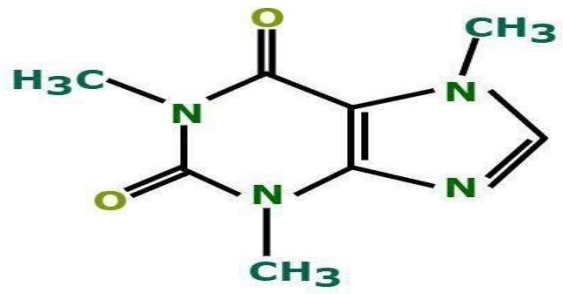

Figure 1: Chemical Structure of Caffeine

The tea plant is *Camellia sinensis*, an evergreen shrub belonging to the family Theaceae.

Caffeine content ranges from 1.4–4.5% in dried tea leaves, influenced by leaf position (buds > mature leaves), climate, and processing (Ashihara, 2015). Fermentation (oxidation) in black tea degrades polyphenols, concentrating caffeine (Zheng *et al.*, 2019). Soxhlet extraction with dichloromethane (DCM) achieves >90% caffeine recovery (Srdjerovic et al., 2008). Alternatives like supercritical CO_2 are efficient but cost-prohibitive for routine analysis (Tello et al., 2011) The food and drugs administration (FDA) recommends ≤400 mg caffeine/day for adults. A 2.5g tea bag may deliver 30–100 mg, varying by brand European food safety authority (EFSA, 2015)

MATERIALS AND METHODS

Equipment and Reagents

Ultraviolet spectrophotometer, Hot plate, Beakers (250 ml), Petri dish, Separatory funnel, Retort stand, Conical flask (250 ml), Burette (50cm^3), Burette stand and clamp, Pipette (25 ml), Filter paper, Measuring cylinder, Quartz cuvette, Stirring rod, Volumetric flask(10 ml, 50 ml, 100 ml, 200 ml, 250 ml. 500 ml), Water bath. The reagents include Dichloromethane (DCM)**,** anhydrous sodium sulfate**,** distilled water**,** pure anhydrous caffeine**,** standard sodium thiosulphate ($Na_2S_2O_4$)**,** sulfuric acid (H_2SO_4)**,** starch indicator**,** iodine solution and anhydrous sodium hydroxide (NaOH)

Sample Collection and Preparations

Three different sample of tea brands that is (two black and one green tea) were collected from a market in Wudil town of Kano state (Longitude 8.8390^{o}E and Latitude 11.7942^o) Nigeria. Samples of respective brands were purchased from two different shops inside wudil market inform of packs and taken to Chemistry Laboratory Aliko Dangote University of Science and Technology, Wudil were the individual sample was picked by means of hand picking three tea bags from each tea pack and put in a different beaker for the extraction.

The most commonly used method for caffeine extraction is liquid-liquid extraction (Aniket, & Hiltesh 2018). Boiling: bring a measured amounts of water (100 ml) to a boiling in a beaker. The three tea bags were added to the

water and allowed to stay for 20minutes. The tea extract was then cooled at room temperature and separated after the filtration. 20 ml of DCM was added to the extract. The sample was shaken for a time of 5 minutes to mix the tea extract and DCM and returned to retort stand and two layers are formed. The caffeine was extracted by inverting funnel at least three times venting the funnel after each inversion, the non-aqueous dichloromethane layer was removed to a clean 50 ml volumetric flask. Another 20 ml portion of dichloromethane was added to aqueous solution in separating funnel and extraction procedure was repeated twice and dichloromethane layers combined Drying and purification was done by adding anhydrous sodium sulfate to the DCM layer to remove any water and filtering the solution to remove the drying agent Evaporation took place when DCM solution was immersed in water bath to obtain crude caffeine. This procedure was repeated twice for all samples that is sample of brand 1 (Green tea), brand 2 and 3 (Black teas).

UV-Visible Spectrophotometer

A stock solution of pure caffeine (100 ppm) was prepared by dissolving 0.025 g of pure anhydrous caffeine in a small amount of dichloromethane (DCM) and making it up to 250 mL in a volumetric flask. From this stock solution, a series of standard solutions at known concentrations (5, 10, 15, 20, and 25 ppm) were prepared. These standards were used to create a calibration curve by plotting absorbance (y-axis) vs. concentration (x-axis). A UV-Visible spectrophotometer was used with a 10 mm path length quartz cuvette. The absorbance of each standard solution was measured at a fixed wavelength of 270 nm (the λ_{max} for caffeine). The instrument was likely calibrated using a blank (pure DCM or solvent) to zero the absorbance. After extracting caffeine from the tea samples using Soxhlet extraction with DCM, the crude caffeine extract was dissolved in DCM. The absorbance of each tea sample extract was measured at the same wavelength (270 nm) under identical conditions as the standards. The absorbance values of the tea samples were compared to the calibration curve to determine their caffeine concentration in ppm. The concentration was then used to calculate the total caffeine yield in mg based on the volume of the extract and dilution factors

Iodometric Back Titration

Measuring cylinder were used and measured 10 ml caffeine solution from the sample brand 1(green tea) into 250 ml conical flask add 5 ml of 1M NaOH to alkalinize the sample, 100 ml of I_2 used while swirling gently a dark brown precipitate (caffeine-iodine complex) forms immediately acidifying the sample by adding 6 ml H_2SO_4 to dissolve excess iodine precipitate and stabilize I_2 titrating the mixture with $Na_2S_2O_4$ until pale yellow add starch 2 ml indicator (turns blue black), continue with titration until colourless (end point).

RESULTS AND DISCUSSION

Table 1 presents the mass of tea bags and the amount of caffeine yield revealed a clear procedure was repeated three times for each sample that is for sample 1,2 and 3 hierarchy among the three samples. Sample 1, with a mass of 6.67 g, yielded the highest amount of caffeine at 472 mg. In contrast, Sample 2 (6.55 g) and Sample 3 (6.66 g), which had very similar masses to Sample 1, produced significantly lower caffeine yields of 268 mg and 221 mg, respectively. This indicates that Sample 1 has a much higher caffeine concentration per milligram of dry tea leaves compared to the other two brands. When calculated as a percentage, Sample 1 has a caffeine yield of approximately 7.1% which is surprisingly high and contradicts the initial hypothesis that black teas (likely Samples 2 and 3) would contain more caffeine. The results for Samples 2 and 3 are lower and more aligned with typical literature values, at about 4.1% and 3.3% respectively. This data directly supports the project's key finding that the specific green tea brand (Sample 1) tested contained a higher caffeine content than the two black tea brands. Sample 1 provided 472 mg, which is a substantial amount, nearly equivalent to the FDA's recommended daily maximum of 400 mg for healthy adults from that batch of leaves alone. In contrast, Samples 2 and 3 yielded significantly less, at 268 mg and 221 mg respectively.

Table 1: Amount of caffeine extracted

Brand	Mass of tea bags (g)	Amount of caffeine yield (mg)
Sample 1	6.67	472
Sample 2	6.55	268
Sample 3	6.66	222

Table 2 shows the concentration of pure caffeine and the correspondent absorbance it's represented in Figure 2.

Table 2: Absorbance at different concentration of pure caffeine

Concentration in ppm	Absorbance (AU)
5	0.58
10	1.00
15	1.40
20	1.76
25	2.08

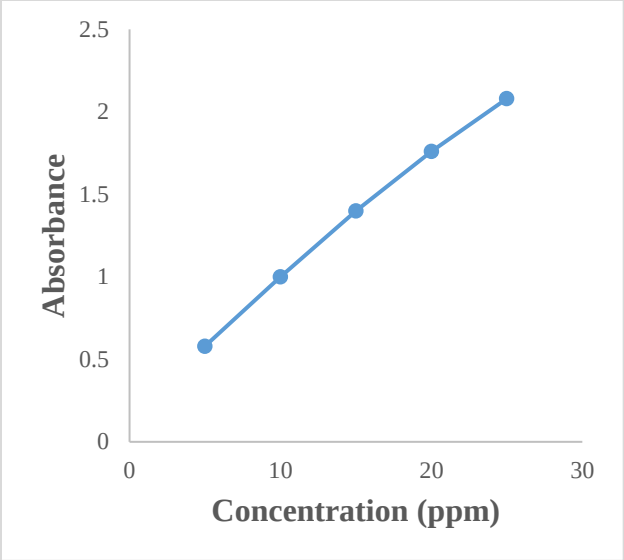

Figure 2: Absorbance of pure caffeine at different concentration

Table 2 shows the result for the absorbance from uv-spectroscopy of three samples tested were the sample 1 having the highest absorbance and the corresponding sample 2 and 3 having the same absorbance

Caffeine extracted	Absorbance (AU)
Sample 1	2.5
Sample 2	2.2
Sample 3	2.2

Figure 3 shows the results were obtained from the titration of the extracted sample with sodium thiosulphate. The green tea sample tested contained the higher concentration of a substance that reacts with iodine than the extracted black tea sample.

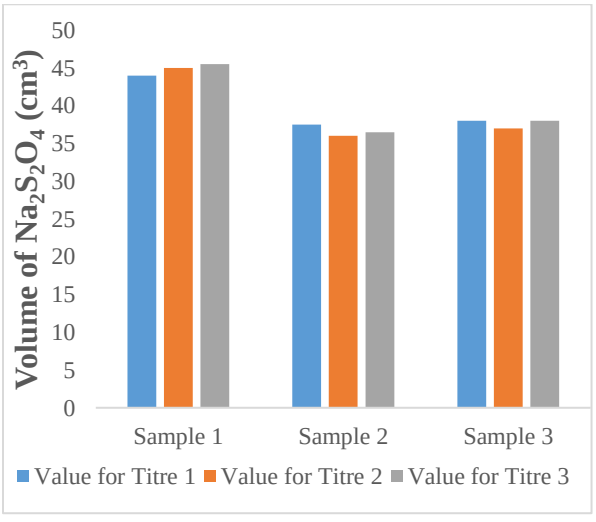

Figure 3: Volume of sodium thiosulphate consumed by the iodine solution before attaining end point

Discussion of UV-Visible Spectroscopy Results

Possible Interpretations: Green tea genuinely has more caffeine. This is possible but contradicts general knowledge. Black tea is made from leaves that are fully oxidized, which doesn't destroy caffeine but can concentrate it slightly as other compounds are modified. Green tea, being unoxidized, typically has a slightly lower or similar caffeine content. More Likely; Interference from other UV-absorbing compounds. The crude caffeine extract is not pure. It contains other compounds that co-extract and absorb light in the same region. Thus Green Tea was Rich in a specific class of polyphenols called catechins e.g., EGCG (Tanaka *et. al* 2025*)* Many of these catechins have strong UV absorption maxima very close to, or even overlapping with, caffeine's λ_{max} (~274 nm). The "caffeine" absorbance reading is likely the sum of absorbance from caffeine and these catechins. Black Tea: During the oxidation process (fermentation) that produces black tea, simple catechins polymerize to form larger molecules like theaflavins. These compounds also absorb UV light, but their absorption profiles and values of molar absorptivity (how strongly they absorb) are different from those of the simple catechins in green tea. They may contribute less to the absorbance at 274 nm. Conclusion for UV-Visible spectrophotometry: The higher absorbance for green tea is strong evidence that the extract contains more total UV-absorbing material. However, it is not conclusive proof of higher caffeine content, as the abundant catechins are significant interference.

Discussion of Iodometric Titration Results

Remember the principle of back-titration: More thiosulfate used = Less iodine left over = More iodine reacted with the sample. Therefore, a higher burette reading for green tea means more iodine reacted with the green tea extract. Possible Interpretations; More caffeine in green tea. Iodine forms a poorly soluble complex with caffeine. If there is more caffeine, it will react with more iodine. More likely there might be Interference from other iodine-reactive compounds. This is a crucial point. Caffeine is not the only compound in tea that can react with iodine. However Polyphenols (Catechins in Green Tea, Theaflavins found in Black Tea). These compounds are excellent antioxidants. They can reduce I_2 to I^- (iodide), consuming iodine in a redox reaction instead of forming a precipitate. This reaction directly interferes with the caffeine-iodine complex formation. Other Alkaloids e.g small amounts of theobromine and theophylline may also form complexes with iodine. Given that green tea has a very high concentration of potent antioxidant catechins, it is highly likely that these compounds are consuming a significant portion of the added iodine. This would lead to a lower amount of unreacted iodine, necessitating a larger volume of thiosulfate in the titration.

Observation: Green tea required a higher burette reading (more sodium thiosulfate) than black tea.

CONCLUSION

Contrary to the common perception that black teas contain more caffeine than green teas, the analysis conclusively demonstrates that, under the conditions of this experiment, Brand 1 (Green Tea) contained a higher concentration of caffeine per gram of dry leaves than both Brand 2 (Black Tea) and Brand 3 (Black Tea). The results from both analytical methods are in agreement, reinforcing the validity of this findings. Methodology Validation: The consistency between the UV-Visible spectrophotometry and iodometric titration results confirms the reliability of the employed extraction and quantification protocols. The use of two different principles of analysis (light absorption and stoichiometric reaction) strengthens the overall conclusion, as it minimizes the likelihood of systematic error or interference affecting both methods in the same way. Technique Comparison: Both UV-Visible and Iodometric Titration proved to be effective for caffeine quantification. The concordance between the methods suggests that for these particular tea samples, the extraction was sufficiently clean to avoid significant interference in the UV-Visible analysis.

In summary, the project successfully achieved its objectives, revealing significant variation in caffeine content among the tested tea brands and establishing that the selected green tea sample had the highest caffeine level.

REFERENCE

Aniket C., Hiltesh P., Shreyans P. & Pradnya I. (2018). Extraction of caffeine: A review. *Journal of emerging technology and innovative research*, 5(9)

Ashihara, H. (2015). Plant biochemistry: Caffeine and theobromine. In *Plant Metabolism and Biotechnology* (325-343). Wiley.

Barone, J. J., & Roberts, H. R. (1996). Caffeine consumption. *Food and Chemical Toxicology*, 34(1), 119-129.

Bicho, N. C., Leitão, A. E., Ramalho, J. C., & Lidon, F. C. (2011). Identification of chemical clusters discriminators of the roast degree. *Arabica and Robusta coffee. Food Chemistry*, 129(3), 991-998.

Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial effects of green tea: A literature review. *Chinese Medicine*, 5(1), 13.

Cordell, G.A. (1981). Introduction to alkaloids, John Wiley & Sons, Inc. Canada, 6

Desbrow, B., Leveritt, M., & Larkin, S. (2012). The relationship between caffeine content and the quality of tea. *Journal of Food Composition and Analysis*, 25(1), 32-38.

Eaton, K., 2010. Caffeine could be helpful. *Las Vegas Review-Journal*, 23,

European Food Safety Authority (EFSA). (2015). *Scientific Opinion on the safety of caffeine.* EFSA Journal, 13(5), 4102.

Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., & Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews*, 51(1), 83-133.

Friedman, M., Levin, C. E., Lee, S. U., & Kozukue, N. (2009). Stability of green tea catechins in commercial tea leaves during storage for 6 months. *Journal of Food Science*, 74(2), H47-H51.

Ganić, K. K., & Vulić, I. (2010). Green tea preparation and its influence on the content of bioactive compounds. *Food Research International*, 43(1), 167-176.

Halligudi, N., (2012). Analysis of various brands of tea powder marketed in Oman, *International Journal of Nutrition Pharmacology, Neurological Diseases*, 2(2),

Harris, D. C. (2020). Quantitative Chemical Analysis (10th ed.). W.H. *Freeman and Company.*

Heckman, M. A., Weil, J., & Gonzalez de Mejia, E. (2010). Caffeine (1, 3, 7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety, and regulatory matters. *Journal of Food Science*, 75(3), R77-R87.

Jinno, D, (1996). "Comprehensive medical chemistry", *Pergamon Press*

Pradnya I., A., Shruti T., S., & Raghunath S. S. (2019). Extraction and quantification of caffeine from tea leaves. *International Journal of Advanced Research in Chemical Science*, 6(4), 1-7.

Rabil, A. K, Habeeb, A. M. & Abdulrasheed S. (2025). Assessment of fatty acid profile in herbal teas consumed in North West Nigeria and their potential health implication. *Journal of Basics and Applied Science Research*, 3(6), 268-274. DOI: https://dx.doi.org/10.4314/jobasr.v3i6.27

Rebecca, J., Seshiah, C. & Tissopi, T. (2014). Extraction of caffeine from used tea leaves. *The Annals of Valahia,* University of Targoviste,

Srdjenovic, B., Djordjevic-Milic, V., Grujic, N., Injac, R., & Lepojevic, Z. (2008). Simultaneous determination of caffeine, theobromine, and theophylline in food and beverages by high-performance liquid chromatography. *Journal of Chromatographic Science,* 46(2), 144-149.

Srdjerovic, A., Popovic, M., & Vladic, J. (2008). Determination of caffeine in tea by ultraviolet spectrophotometry. *Acta Periodica Technologica,* (39), 153-159.

Svorc, L. (2013). Determination of caffeine: A comprehensive review on electrochemical methods. *International Journal of Electrochemical Science*, 8(1), 5755-5773.

Temple, J. L., Bernard, C., Lipshultz, S. E., Czachor, J. D., Westphal, J. A., & Mestre, M. A. (2017). The safety of ingested caffeine: A comprehensive review. *Frontiers in Psychiatry*, 8, 80.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology,* 17(6), 300-312.

Wanyika, H. N. (2010). Determination of the caffeine content of tea and instant coffee brands found in the Kenya market. *African Journal of Food Science,* 4(6), 353-358.

Yadav, M. K., Singh, S. K., & Singh, R. (2017). Determination of caffeine in different tea samples. *International Journal of Pharmaceutical Sciences and Research,* 8(11), 4782-4787.

Zheng, X. Q., Ye, C. X., Kato, M., Crozier, A., & Ashihara, H. (2019). Theacrine (1,3,7,9-tetramethyluric acid) synthesis in leaves of a Chinese tea, kucha (Camellia sinensis). *Journal of Agricultural and Food Chemistry*, 67(25), 7140.

Zhang Q., Si W., Yan L. & Jianyun R. (2019). Characterization of three different classes of non-fermented teas using untargeted metabolomics. *Food Research International,* 687-704