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Article

A temperature-based comparison of compounds found in Bao Chong tea, green tea, and black tea.

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SUMMARY

Tea is a widely-consumed beverage, and different types of tea can be produced depending on their oxidation levels. However, how different types of tea and brewing water temperature can affect health has not been considered. This investigation studied how compounds in tea such as caffeine, catechins, and L-theanine, were different in green tea, Bao Chong tea, and black tea, as well as in Bao Chong tea brewed with different water temperatures (25ºC, 45ºC, 75ºC, 90ºC) using NMR analysis. The results showed that Bao Chong Tea had the highest caffeine, catechins, and L-theanine content, followed by green tea and then black tea. The best temperature for brewing tea to produce caffeine, catechins and L-theanine was between 45ºC and 75ºC, and all chemical contents were reduced greatly at 90ºC. A better understanding of the relationship between tea and its chemical contents will allow people to have better knowledge of their food intake, and possibly allow people to choose accordingly.

INTRODUCTION

The ability of a small green leaf to weave itself into cultures and societies throughout history is remarkable. From Imperial China to Victorian Britain, from Emperors to builders, tea is a rich, complex beverage that has affected numerous decisions and people. Since tea is a healthy and popular drink, this research aims to investigate tea. As people care more about their health, the benefits of tea have been more widely explored. People no longer only drink hot tea; cold tea has become a trending beverage as well [1]. However, the impacts of drinking various teas brewed in different temperatures remain uncertain.

Tea, the common name for *Camellia sinensis*, contains thousands of chemical compounds. Different compounds are released depending on how the tea is processed and brewed [2]. To make different types of tea that people like to drink, tea leaves are subjected to distinct processes [3]. The most known types of tea are: green tea, Bao Chong tea, and black tea. All tea starts off as *Camellia sinensis* leaves but are then distinguished further based on different levels of oxidation and processing [3]. Tea leaves are first harvested and withered, reducing the water content and allowing the antioxidants to develop and amino acids to degrade [4]. Maceration is then performed to allow oxidation by the physical release of oxidative enzymes [3,4]. Oxidation is the natural chemical reaction by which new compounds are unlocked. The tea leaves begin to turn brown and new flavors are generated through biochemical modifications within the leaves [3]. When the desired oxidation process is over, fixation and drying are performed to reduce the activity of oxidative enzymes. Green tea is un-oxidized, black tea is fully-oxidized, and Bao Chong tea is semi-oxidized [2].

Out of the thousands of compounds found in tea, caffeine, catechins, and L-theanine are the most well-known ones [2]. Caffeine is popularly known for its ability to increase alertness and to keep people awake (**Figure 1A**). Catechins are famous for their antiviral properties, which can, for example, interfere with the replication cycle of DNA viruses and keep people healthier (**Figure 1C**) [5]. L-theanine is recognized for its anxiety-relieving effects as it increases dopamine levels and increases the production of alpha waves, which makes people more relaxed (**Figure 1B**) [6,7].

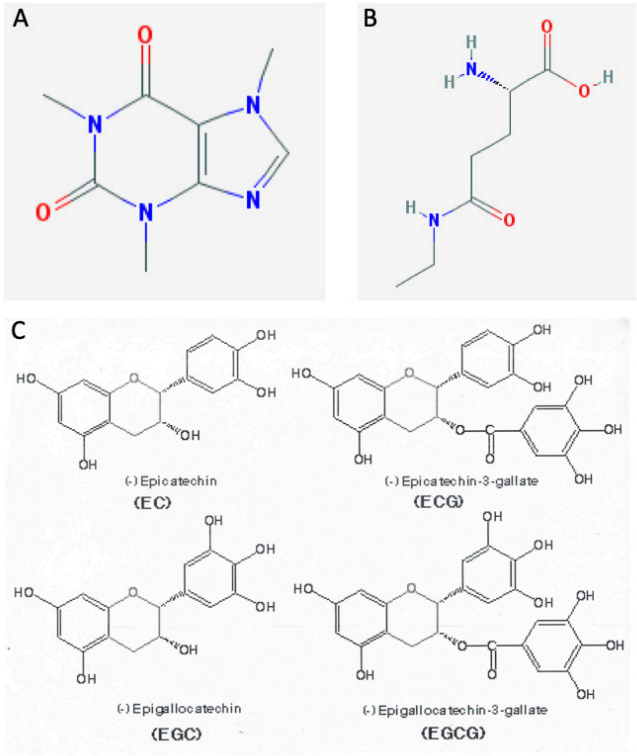

Figure 1. Chemical structures compunds found in tea.
A. Chemical structure of caffeine (8). B. Chemical structure of L-theanine (10). C. Chemical structure of catechins (9).

It is important to study the relationship between tea contents and the types of tea because tea is a widely consumed beverage, and a better understanding of its composition will allow people to choose their preferences accordingly. Also, a better understanding of chemical contents in tea could lead to more developed applications in health. For example, green tea is suggested to be beneficial in reducing the risk for cardiovascular diseases [11]. Therefore, this research aims to gain a better understanding of how green tea, Bao Chong tea, and black tea can affect health, and how brewing temperature can affect the release of caffeine, catechins, and L-theanine. This research investigated how caffeine, catechins, and L-theanine are different in green tea, Bao Chong tea, and black tea and how water temperature affects the levels of caffeine, catechins, and L-theanine in Bao Chong tea. It was hypothesized that the levels of caffeine, catechins, and L-theanine in Bao Chong tea would be in between the levels found in non-oxidized green tea and fully-oxidized black tea because it is semi-oxidized. It was also hypothesized that higher water temperatures used to brew tea would result in higher concentrations of caffeine, catechins, and L-theanine because the high water temperature causes the breakdown of cell walls and the release of compounds.

RESULTS

Comparing Caffeine, Catechins and L-theanine in Green tea, Bao Chong tea, and Black tea

Three types of tea samples, green tea, Bao Chong tea and black tea, were brewed with 75ºC water for three minutes and analyzed using NMR to compare the levels of caffeine, catechins, and L-theanine in tea. This experiment was conducted to test the hypothesis that semi-oxidized tea produces levels of tea compounds between non-oxidized and fully-oxidized tea.

The peaks are the highest in Bao Chong tea than in green tea and black tea. Bao Chong tea contained the most caffeine, catechin, and L-theanine content, followed by green tea and then black tea (**Figure 2**).

Comparing Bao Chong tea under 45º, 75º, and 90ºC Brewing Water

Bao Chong tea samples were brewed in 45ºC, 75ºC, and 90ºC water for three minutes. The supernatant liquid was extracted and analyzed using NMR to answer the research question of this investigation "How does water temperature affect the levels of caffeine, catechins, and L-theanine in green tea, Bao Chong tea, and black tea?"

The experiment showed that lower water temperatures resulted in higher chemical content (**Figure 3**). Both catechins and L-theanine content decreased as the brewing water temperature increased. The caffeine content remained about the same for 45ºC and 75ºC, then decreased for 90ºC. Both caffeine and catechins peaks shifted upfield for 45ºC and 75ºC. There was also an unordered shifting pattern of caffeine peak in 7.60 ppm.

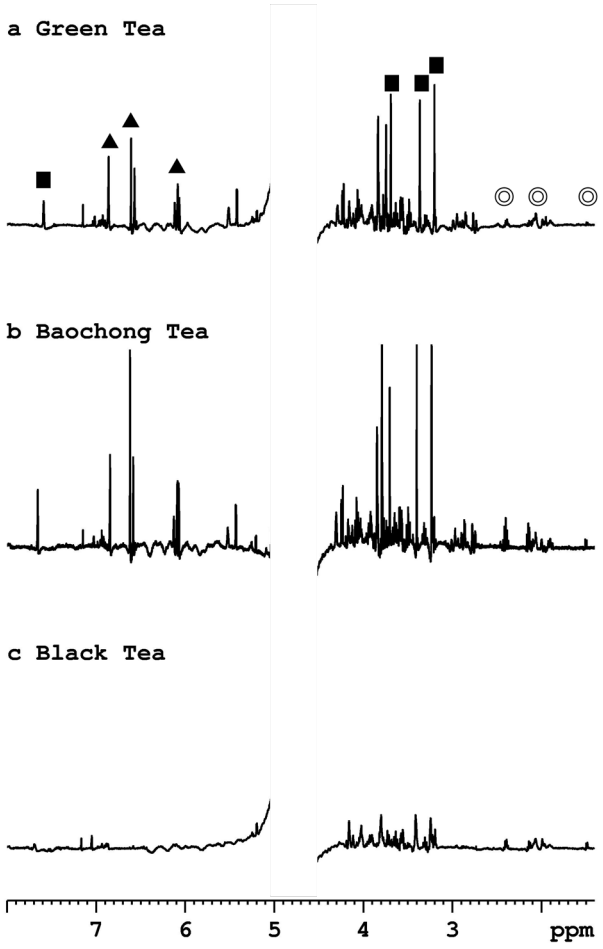

Figure 2. NMR analysis of the three tea samples. The spectra have been modified for easier reading. Corresponding peaks are denoted by ■ [caffeine]; ▲ [catechins]; circle [L-theanine]. Caffeine, catechins, and L-theanine are labeled according to the literature [12].

Through this experiment, it was found that levels of all three compounds tested: caffeine, catechins and L-theanine, remained the same for 45ºC and 75ºC, then decreased for 90ºC (**Table 1**).

Comparing Bao Chong tea under 25º and 45ºC Brewing Water

Bao Chong tea samples that were brewed under 25ºC and 45ºC were also analyzed using NMR. The 25ºC trial used tea leaves and was immersed for 12 hours, while the 45ºC trial used tea leaf powder and was brewed for only 3 minutes.

	25ºC	45ºC	75ºC	90ºC
Brewing Time	12 hrs	3 mins	3 mins	3 mins
Caffeine	4:4:4:1	5:5:4:1	5:5:4:1	4:4:4:1
Catechin	1:4:1	1:3:1	1:3:1	1:2:1
L-theanine	4:1:1	4:1:1	4:1:1	2:1:1

Table 1. Ratio of Caffeine, Catechins, and L-theanine content integrated peaks. Each number represents a different peak.

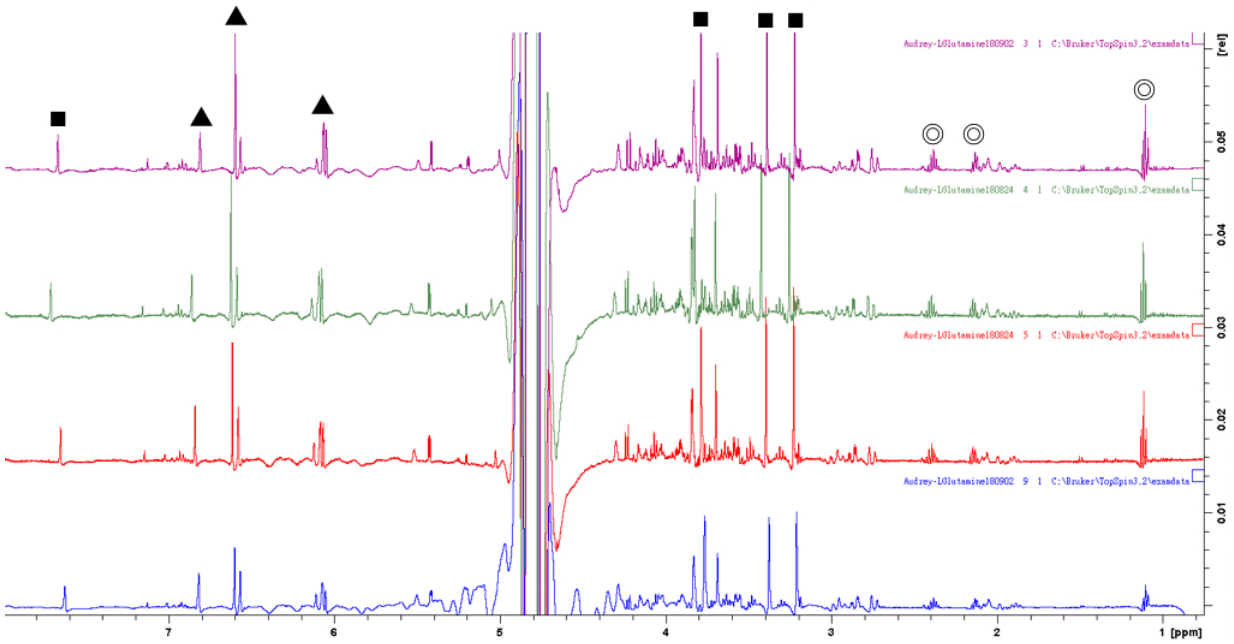

Figure 3. NMR Spectra of Bao Chong tea brewed in different temperatures of water. Tea was brewed at 25ºC for 12hrs (purple), 45ºC for 3 mins (green), 75ºC for 3 mins (red), and 90ºC for 3 mins (blue).. Corresponding peaks are denoted by ■ [caffeine], ▲ [catechins], and circle [L-theanine].

The different conditions were designed to match how people typically make cold tea, so the sample will be in a condition similar to when it is normally consumed [13]. This expands the brewing water temperature range and compares the two samples in a more applicable condition.

In this experiment, it was found that the amount of caffeine increased, catechins decreased, and L-theanine remained the same from 25ºC to 45ºC (**Figure 4-6**). However, there is a suspected structural change to either caffeine or a type of catechin (**Figure 5,6**). It was also found that the Caffeine and catechins peaks shifted upfield for about 0.01 ppm, and the peaks remain about the same in L-theanine (**Figure 5,6**). Besides the shifts, there was also a peak structural change at 3.8 ppm (**Figure 5**). The implications of the results are further clarified in the discussion section.

DISCUSSION

Comparing Caffeine, Catechins and L-theanine in Green tea, Bao Chong tea, and Black tea

A better understanding of the amounts of caffeine, catechins and L-theanine in different types of tea will allow people to choose their preferred type of beverage. For example, elders might want to consume less caffeine to prevent sleep disturbance [14]. The signals of the studied compounds are clearly detected: L-theanine within 1 to 2 ppm, caffeine within 3 to 4 and 7 to 8 ppm, and catechins within 6 to 7 ppm (parts per million) (**Figure 2**). Bao Chong tea had the highest caffeine, catechin, and L-theanine content, followed by green tea and then by black tea (**Figure 2**).

However, the findings are not supported by existing literature. From the literature review, many studies suggested that green tea, which is un-oxidized tea, has the highest content of catechins and L-theanine, while black tea, which is the fully-oxidized tea, has the highest caffeine content [1,15,16]. The literature also suggests that half-oxidized tea has the highest caffeine content [17]. The outcome of this study, however, suggests that Bao Chong tea is the richest in all three compounds. A possible explanation of the differences could be the different production process or tea leaves used. Therefore, various tea brands or origins could be compared in future studies.

For all three tea samples, no phase shifts were observed, indicating there were no environmental changes to hydrogen and therefore no structural changes to the chemical contents in tea [18,19]. This is important as any slight changes to the chemical structure might not produce the same health effects [18]. As tea contains more than thirty thousand compounds, it is crucial to understand that tea processing does not change chemical structures and is safe for human consumption [1].

Comparing Bao Chong tea under 45º, 75º, and 90ºC Brewing Water

Tea is valued not only for its taste, but also for its antioxidant content, which can bring health benefits. Besides the production of tea, temperature of the brewing water could also cause great differences in the taste of tea or even the released compounds. Based on the results of this study, the best temperature for brewing tea to produce caffeine, catechins and L-theanine was between 45ºC and 75ºC. Caffeine, catechins and L-theanine levels remained the same for 45ºC to 75ºC but reduced greatly when the temperature increased to 90ºC (**Figure 3, Table 1**). The varying amounts

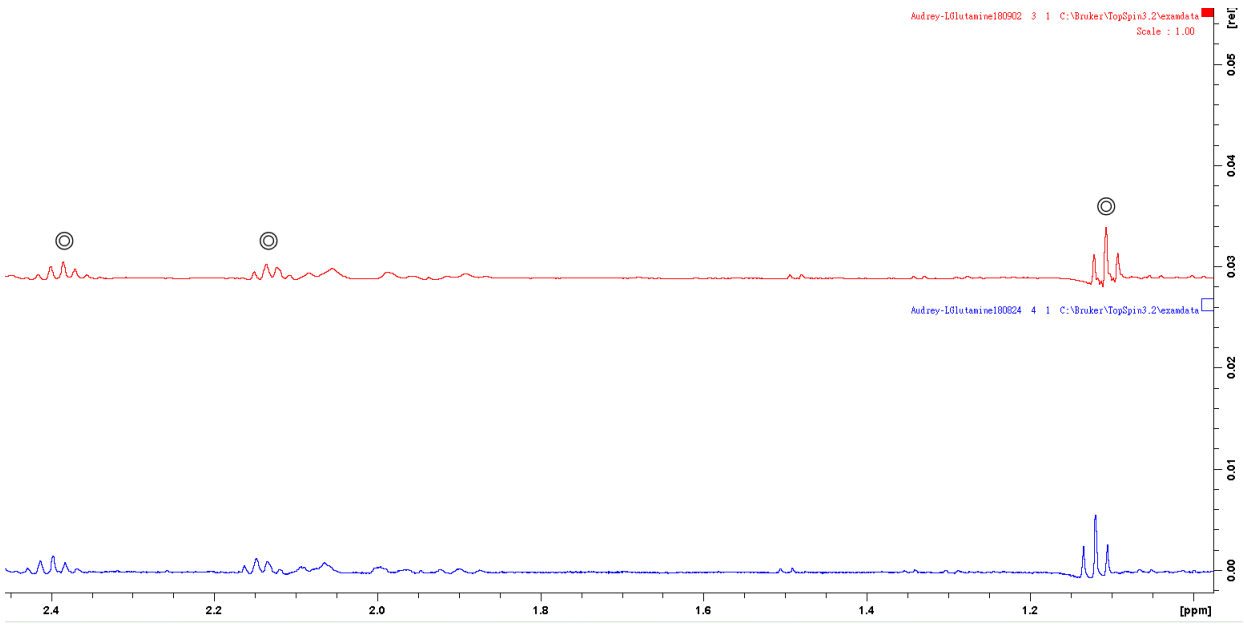

Figure 4. 0 to 2.5 ppm NMR spectra of 25ºC and 45ºC Bao Chong Tea. Spectra represent brewing temperatures 25ºC [red] and 45ºC [blue].Corresponding peaks are denoted by ■ [caffeine], ▲ [catechins], and circle [L-theanine].

of compounds in the tea when brewed with different water temperatures might be due to the fact that there are certain ranges of temperatures that are best for the compounds to be released [20-22].

However, the literature suggests that the optimum conditions to extract L-theanine is 80ºC, 85ºC for catechins, and 90ºC or 100ºC for caffeine [20-22]. This difference might be due to different measuring methods. For further research, various types of tea could be brewed at different temperatures, followed by analysis of the levels of different compounds.

Another finding in this study was that the caffeine and catechins peaks shifted upfield to different amounts for 45ºC and 75ºC compared to 90ºC (**Figure 3**). Also, the shifting pattern of the caffeine peak to 7.60 ppm is unordered (**Figure 3**). The shifting peaks might indicate slight changes in the NMR magnetic field or changes in concentration or additives [19]. It could be concluded that there were hydrogen related environmental changes, but the compound itself did not change [19]. Since the shifting was not appreciable, it can be assumed that the changes were minimal [19]. Further investigation is required to identify the nature of the changes that occurred.

All the peaks that do not indicate caffeine, catechins and L-theanine represent different compounds found in tea. There were not many significant changes observed for those other peaks besides the area between 5.20 ppm to 5.40 ppm, which is suspected to be sugar, indicating that most compounds were not sensitive to brewing water temperature (**Figure 3**) [12]. An explanation to this could be that sugar becomes very sensitive to molecular changes when it reaches around 90ºC and will be released greatly at 90ºC. However, an in-depth literature search found no supporting evidence to prove or disprove the explanation, so this could be a direction for future research.

Comparing Bao Chong tea under 25º and 45ºC Brewing Water

Cold-brewed tea is also a common drink and can be made easily by immersing tea leaves in bottled water for 12 hours [13]. Therefore, this experiment aimed to compare 25ºC and 45ºC brewing water. However, the experiment between 25ºC and 45ºC Bao Chong tea is distinct from the previous two because the two samples were prepared differently. The 25ºC trial used tea leaves that were immersed for 12 hours, while the 45ºC trial used tea leaf powder that was immersed for 3 minutes. Thus, the outcome cannot be attributed to the temperature change.

Although the 25ºC and 45ºC trials differed by their preparation, the changes were noteworthy. The amount of caffeine increased, catechins decreased, and L-theanine remained the same from 25ºC to 45ºC (**Figure 2**). The general pattern remained as well, indicating that the length of time which tea leaves are immersed in water will not affect the overall contents in the tea. However, there are two independent variables in this experiment, which makes the cause of the changes uncertain. Therefore, future research could compare 25ºC and 45ºC brewing temperature for the same amount of time, or brewing tea at the same temperature for various amount of times. This would clarify the changes within this experiment.

Additionally, shifted peaks were found for caffeine, catechins, and L-theanine (**Figures 4-6**). The shifting peaks indicate changes in NMR magnetic field, concentration, or additives [19]. This could be due to different temperatures or brewing time. However, a more in-depth investigation is

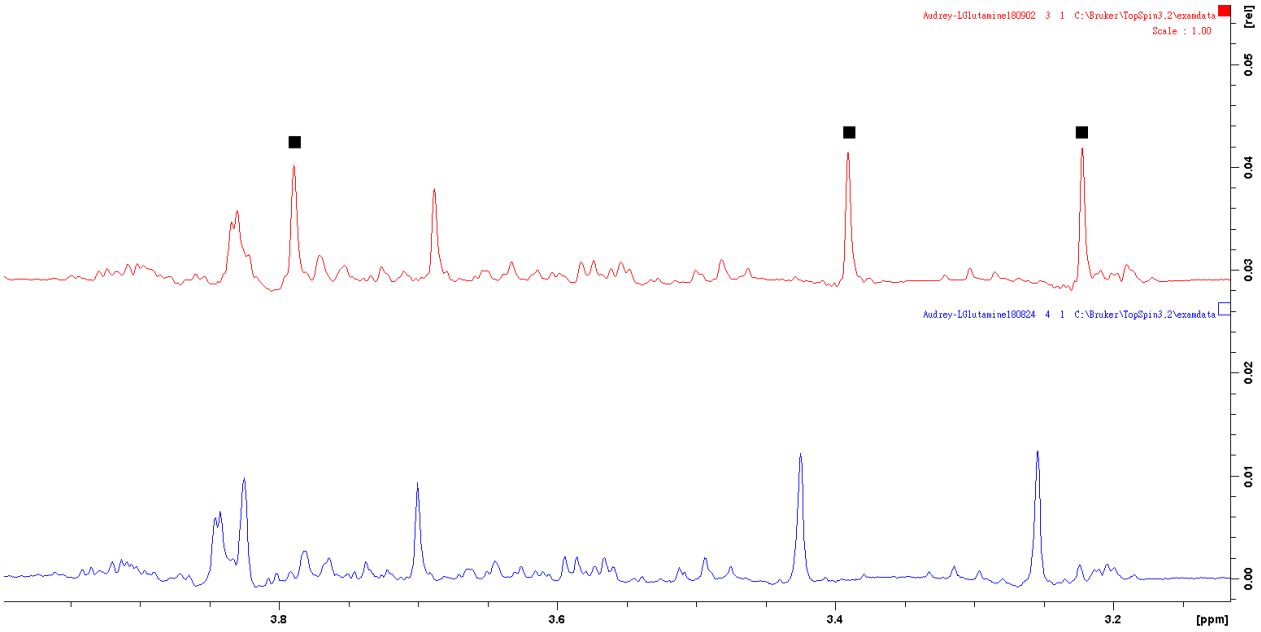

Figure 5. 3.1 to 4.0 ppm NMR of 25ºC and 45ºC Bao Chong Tea. Spectra represent brewing temperatures of 25ºC [red] and 45ºC [blue]. Corresponding peaks are denoted by ■ [caffeine], ▲ [catechins], and circle [L-theanine].

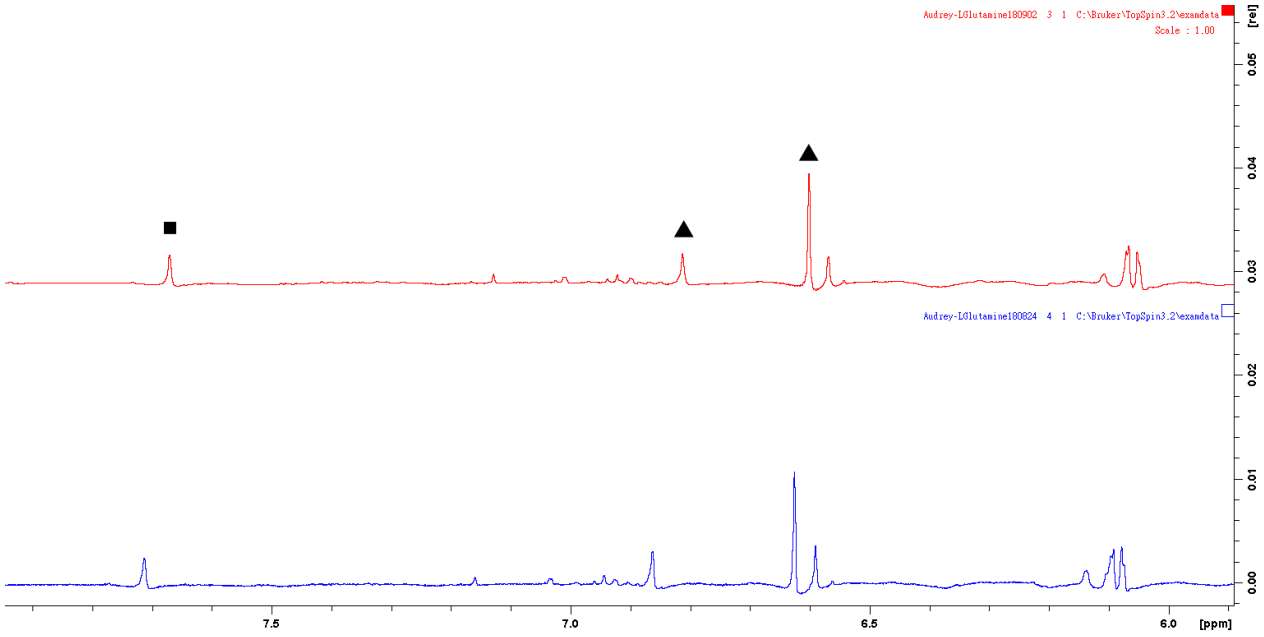

Figure 6. 6.0 to 8.0 ppm NMR of 25ºC and 45ºC Bao Chong Tea. Spectra represent brewing temperatures of 25ºC [red] and 45ºC [blue]. Corresponding peaks are denoted by ■ [caffeine], ▲ [catechins], and circle [L-theanine].

required to better understand the reason behind the changes.

A structural difference at 3.8 ppm was also found (**Figure 5**). The compound was suspected to be caffeine or a type of catechin [12]. It is a conformational change, which means the changes in the environment have caused changes in shape of the macromolecule [23]. Although most of the changes seen in this experiment may be caused by external variations such as hydrogen related environmental changes, the compound at 3.8 ppm had structural changes because of its sensitivity towards temperature. This sensitivity might be due to the compound structure where the hydrogens in caffeine compounds are more widely spread compared to the unchanged L-theanine and are more easily affected by water temperature.

Besides the limitations mentioned in previous sections, there are also possible errors within this research. Only one trial was conducted for each water temperature, and the lack of replication might lead to unidentified errors. The measurements made during the experiment might contain random errors because of the variable measurements, including the milligram balance, pipet, and the NMR machine. The temperature control during the experiment is unstable because it is

controlled by a hot plate without a precise temperature and was monitored by a thermometer, causing the temperature of the water to be variable. Therefore, a few improvements for future research include conducting more trials of the same temperature to ensure accuracy and reduce errors, using a hot plate with a temperature control along with a covered beaker, and other additions when brewing water to prevent heat loss. Another aspect to improve upon in future research is the NMR set up. NMR spectra can be highly accurate when all the sources of errors are properly addressed [24]. The getprosol would need to be set correctly to get the probe head and solvent to the corresponding acquisition parameter [25]. Moreover, the number of scans would need to be set appropriately to ensure the level of accuracy, and the relaxation times would need to be long enough for the spectrometer to adjust measurements in order to produce accurate results [24]. To ensure that the collected data was accurate, various setting conditions should be conducted and the results should be compared to ensure the outcomes are the same [24].

Specific future investigations could be performed on the basis of this research. Various brands of tea could be tested to investigate if the result is suitable regardless of the brand. Samples can be analyzed using other NMR techniques such as ^{13}C-NMR or ^{15}N-NMR or the two-dimensional nuclear magnetic resonance spectroscopy (2D NMR) to offer a more precise understanding of the structural change of caffeine, catachins and L-theanine. A more comprehensive investigation can be conducted by extracting the compounds in the tea for more accurate comparison. A brewing water temperature comparison could be conducted on different types of tea, for example, green tea or black tea. Also, the relationship between brewing time and the number of times tea leaves are brewed and the levels of chemicals that make up tea content can be investigated. Additionally, how the environmental conditions of tea farms affect chemical contents of tea could also be studied. Various types of compounds and their correlation with brewing water temperature, types of tea, brewing time could also be investigated. For example, specific types of catechins such as EGCG and ECG, various kinds of sugar, or flavanols such as kaempferol and quercetin glycoside [21], could all be future research topics.

In conclusion, drinking Bao Chong tea was suggested to be more beneficial than green tea or black tea as it has the highest content of the investigated compounds, where black tea has the lowest for all. Therefore, Bao Chong tea could be selected to drink if the consumer is looking to drink tea for its health benefits. Also, brewing water between 45ºC and 75ºC was suggested as the best for releasing caffeine, catechins, and L-theanine. Therefore, in the future, people could measure their tea brewing water temperature and make tea according to their desired beverages. Lastly, although a relationship between caffeine, catechins, and L-theanine and 25ºC and 45º C brewing water cannot be drawn directly, there are significant changes in the NMR results which require future investigation.

METHODS

^{1}H NMR is a technology used to detect the different hydrogen environments in the compound [25]. By using NMR, the changes in compounds caused by the different brewing temperatures can be detected.

Preparation for Tea Samples

Green tea, Bao Chong tea, and black tea leaves, depending on the experiment, were first ground into powder and 100mg was measured and transferred into an Eppendorf tube. Distilled water was added into a second Eppendorf tube and placed into a beaker with water and “cooked” on a hot plate for ten minutes after reaching the desired temperature (75ºC for tea type comparison and 45ºC, 75ºC, and 90ºC for temperature comparaison). 1.2 mL of cooked distilled water was taken from the second Eppendorf tube and added into the Eppendorf tube with tea powder as mentioned above, and the Eppendorf tube with tea powder was then put into the same beaker and “cooked” on the hot plate for three minutes. After that, the Eppendorf tube was removed from the beaker and put into a centrifuge for ten minutes. After centrifugation, 0.4 mL of the supernatant liquid was extracted and mixed with 0.1 mL of D2O in an NMR tube. NMR samples were vortexed for thirty seconds before being placed into a 500Hz NMR to be analyzed.

Preparation for Bao Chong tea under 25º C Brewing Water

An extra trial of 25ºC was prepared the day before the experiment. 1g of tea leaves was added to 600 mL of water for 12 hours [13].

The Use of NMR

To compare the different chemical contents of tea under various conditions, proton nuclear magnetic resonance spectroscopy (^{1}H NMR) was utilized. Access to this technology was provided by the Department of Chemistry at Academia Sinica. The set-up of NMR was as listed: the solvent selected as D2O, the acquisition time set as 0.5 per second, the number of scans set as 128, time domain data size set as 5000, and spectra width in hertz set as 5000.

NMR Peak Integration

After combining the NMR spectra, the magnifications were increased on a computer for measurements. A ruler was used to measure the length of the desired peaks. After that, peaks of the same temperature were divided by the smallest measure and written as a ratio.

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