Food Chemistry 114 (2009) 1335–1338

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Caffeine in Chiang Rai tea infusions: Effects of tea variety, type, leaf form, and infusion conditions

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ARTICLE INFO

*Article history:*
Received 6 September 2008
Received in revised form 29 September 2008
Accepted 7 November 2008

*Keywords:*
Caffeine
Tea
Variety
Tea type
Leaf form
Infusion conditions

ABSTRACT

Caffeine in Chiang Rai tea infusions was found to be dependent on infusion conditions (water temperature and infusion time), and leaf form (non-ground or ground) but independent of tea variety and type. For non-ground leaf samples, the higher the water temperature and the longer the infusion time, the higher the caffeine concentrations in tea infusions. After infusing for longer than 15 min, the dissolution rate of caffeine became slower and the concentration was essentially constant. For ground leaves, the caffeine content was not influenced by infusion time. Caffeine concentrations in tea infusions from *Camellia sinensis* var. *sinensis* (26.8 ± 0.81 and 22.3 ± 5.55 mg/100 ml for ground and non-ground samples, respectively) were not significantly different from that of *Camellia sinensis* var. *assamica* (24.4 ± 0.66 and 20.3 ± 5.07 mg/100 ml for ground and non-ground samples, respectively). The difference in caffeine concentration between green tea (28.1 ± 8.19 mg/100 ml) and oolong tea (20.3 ± 1.52 mg/100 ml) was not statistically significant.



## 1. Introduction

Tea is one of the most widely consumed beverages in the world, with the exception of water. Green tea (non-fermented tea) and oolong tea (semi-fermented tea) are produced and consumed in several countries in the Asian regions, such as China, Japan and Thailand, whereas black tea (fermented tea) is mainly consumed in most European countries and North America, and produced in India, Sri Lanka, and Central African countries (Yamauchi et al., 2008). These types of tea are produced from the leaves of the tea plant (*Camellia sinensis* L.), which contain many chemical compounds. The major groups are polyphenols, commonly known as catechins, and methylated xanthine, e.g., caffeine and theobromine. Tea polyphenols have been known for their antioxidant activity and antimutagenic and anticarcinogenic properties (Yang, Lambert, Ju, Lu, & Sang, 2007; Yang, Ye, Xu, & Jiang, 2007). However, drinking large amounts of tea may potentially have adverse affects on health due to the high caffeine intake.

Caffeine is a mild central nervous system stimulant. Although it has been found that caffeine in tea leads to faster digit vigilance reaction time, improved rapid visual information processing accuracy, and attenuated increases in self-reported metal fatigue (Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008), at a sufficiently high dose, it may also cause flushing, chills, agitation, irritability, loss of appetite, weakness, and tremor. Hypertension, hypotension, tachycardia, vomiting, fever, delusions, hallucinations, seizures, arrhythmia, cardiac arrest, coma and death have been reported in cases of overdose (>200 mg/day) (Kerrigan & Lindsey, 2005; Schmidt et al., 2007). Tea normally contains 20–50 mg of caffeine/g dry leaves (Yamauchi et al., 2008) and 24–50 mg/150 ml tea (Dixit, Vaney, & Tandon, 2006). However, it has been shown that the concentration of caffeine in a cup of tea is dependent on brewing conditions, namely, water temperature, brewing duration, and leaf/water ratio (Horie, Nesumi, Ujihara, & Kohata, 2002; Labbé, Tremblay, & Bazinet, 2006; Liang et al., 2007) because brewing conditions influence the dissolution and diffusion rates of caffeine (Spiro & Lam, 1995). Our preliminary work showed that the form of the leaf could affect the infusion rate of caffeine in solution (Butsoongnern, 2006; Butsoongnern, Thanomsilp, & Suteerapataranon, 2005). It was shown that the caffeine concentration in the tea solution prepared from rolled-leaf oolong tea was less than that from loose-leaf green tea, although those tea samples were produced from the same tea variety (*C. sinensis* var. *sinensis*) grown in the same plantation. This clearly indicates the need for further work, given the current interest in the impact of drinking tea on human health.

Some other factors that could affect caffeine concentration in tea infusion have been studied. The tea processing method could

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doi:10.1016/j.foodchem.2008.11.013

have an effect on tea quality (Muthumani & Kumar, 2007a,b). Attempts had been made to study the effect of fermentation on tea quality but work on the effect of processing method on caffeine content is limited. To produce green (non-fermented), oolong (semi-fermented), and black (fermented) teas, dry leaves are steamed, rolled, and withered to different degrees. Caffeine, and also other important components, could be increased or decreased during those processes (Muthumani & Kumar, 2007a,b; Park et al., 2007). The caffeine concentration in fresh tea leaves is another factor affecting caffeine concentrations in tea infusions. Yang, Lambert, et al. (2007), Yang, Ye, et al. (2007) reported the different amounts of caffeine in *C. sinensis* (2.72%w/w) and *C. assamica* var. kucha (0.94%w/w) while Chen, Wang, Xia, Xu, and Pei (2005) found no significant differences among *C. sinensis* var. *sinensis*, *C. talinensis*, *C. sinensis* var. *dehungensis*, *C. crassicolumna* and *C. sinensis* var. *assamica*.

In the present paper, we report on the effects of tea varieties that are normally grown in Thailand (*C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*) and, particularly, type (green and oolong teas), leaf form (rolled/loose and ground/non-ground leaves) and infusion conditions (infusion time and temperature) on the caffeine contents of tea infusions.

## 2. Materials and methods

### 2.1. Materials

Leaf-tea samples, commercially available in Chiang Rai, Thailand, were used for the studies. Green tea samples produced from *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* were employed for the study of the effect of tea variety. To study the effect of tea type, Chiang Rai green and oolong teas produced from *C. sinensis* var. *sinensis* were used. In order to compare the influence of leaf form, ground and non-ground samples of rolled and loose leaf teas were used.

Hydrochloric acid (J.T. Baker, USA), lead(II)acetate (BDH, England) and sulfuric acid (Merck, Germany) were employed to remove tannins which could interfere with the analysis by spectrophotometry (Yao et al., 2006).

### 2.2. Standard solutions

Caffeine (anhydrous) (Sigma, Germany) was dissolved to provide a stock standard solution (1000 mg/l) of caffeine and kept at 4 °C. Working standard solutions (5, 10, 15, 20, and 25 mg/l) were freshly prepared. Absorbance of caffeine was measured at 273 nm with a UV/V is spectrophotometer (Speckol 1200, Analytik Jena AG, Germany). A calibration curve was constructed each day before analysis of the samples. Deionized water was used as the blank.

### 2.3. Preparation of tea solutions

One gramme of leaf-tea sample was infused in 100 ml of hot water (80, 90, and 100 °C) for various periods (from 0.5 up to 60 min). The leaves were separated from the tea infusion by filtering through filter paper (No. 1, Advantec, Japan).

### 2.4. Determination of caffeine

The procedure used for the determination of caffeine in tea infusion was based on the modification of the international standard method by Yao, Chen, Cheng, and Liu (1993), and Yao et al. (2006). To remove tannins, 2.5 ml of 0.01 M HCl were added to the tea infusion, followed by 0.5 ml of 2 M $Pb(CH_3COO)_2$ in a 50 ml volumetric flask. Deionized water was used to adjust the solution to the mark. The solution was then filtered through filter paper. Then 0.3 ml of 3 M $H_2SO_4$ was added to 25 ml of the filtrate in a second 50 ml volumetric flask to eliminate excess lead. After the volume was adjusted to the mark, the solution containing lead precipitate was filtered. Absorbance of this filtrate was measured at 273 nm.

## 3. Results and discussion

### 3.1. Effect of tea variety

Caffeine concentrations in tea infusions prepared from dried, loose leaves of *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* (for ground samples; 26.8 ± 0.81 and 24.4 ± 0.66 mg/100 ml, respectively, and for non-ground samples; 22.3 ± 5.55 and 20.3 ± 5.07 mg/100 ml, respectively) were compared using the *F* test and the Student *t* test. It was found that caffeine concentrations in these solutions were not significantly different (number of samples = 3 with 3 replicates; 95% confidence level) in both ground and non-ground leaves (Fig. 1). This result is in agreement with Chen et al. (2005) who reported that there were no significant differences in the caffeine contents of tea infusions brewed from the two varieties. It should be noted that there are other factors

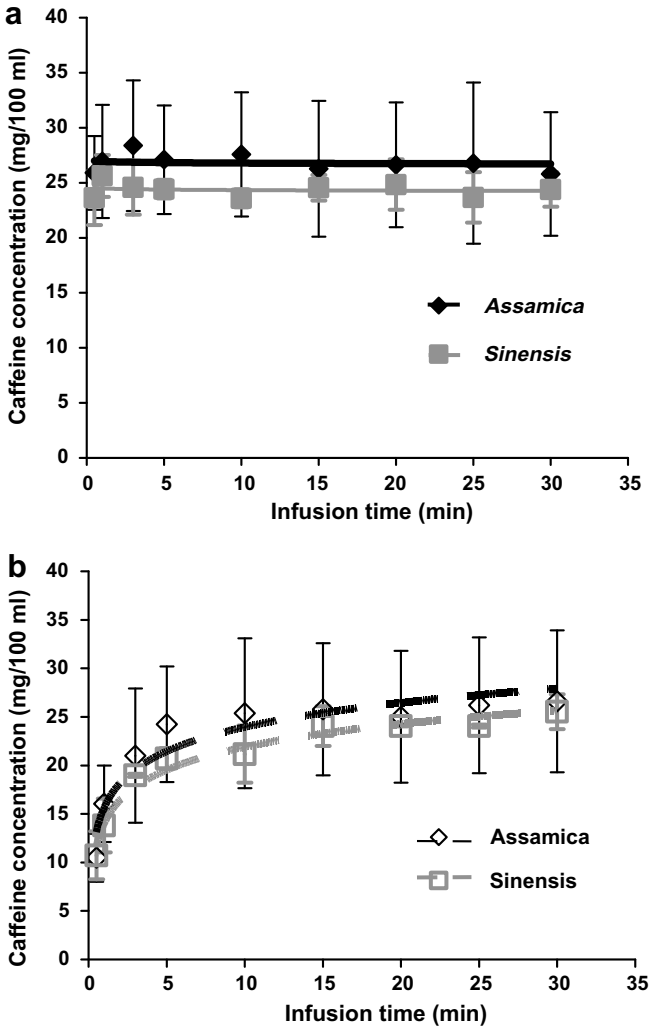


**Fig. 1.** Caffeine concentrations in tea infusions from (a) ground and (b) non-ground leaves of *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis* at various infusion times (error bars are standard deviations ($n = 3$)).

that are related to tea origin (e.g., cultivation method, climate, and soil composition) which could affect the biosynthesis of caffeine in tea (Ashihara & Crozier, 2001). The different-variety tea samples studied in this work were purchased from a manufacturer who cultivated the variety *assamica* in the area near to the variety *sinensis*. Thus the effect of tea origin could usefully be studied in further work in order to clarify the influence of tea varieties on the caffeine content in tea.

### 3.2. Effect of tea type

On average, the green (non-fermented) tea infusions contained a little more caffeine (28.1 ± 8.19 mg caffeine/100 ml) than did the oolong (semi-fermented) tea infusions (20.3 ± 1.52 mg caffeine/100 ml) brewed in water at 90 °C (Fig. 2). However, when the data were treated statistically with the *F* and Student *t* tests ($n = 3$; 95% confidence level), it was found that the concentrations in oolong and green tea infusions were not significantly different. This is in contrast to the findings of Horie, Mukai, and Kohata (1997) who reported that the contents of caffeine were significantly different among various kinds of teas. The tea samples used in the literature were from different sources and were manufactured in different ways but the samples used in this work were manufactured from the same variety (*C. sinensis* var. *sinensis*) by the same factory. Thus, the different tea manufacturing methods used to produce the oolong and green tea samples used here are unlikely to play a major role in influencing the caffeine content in tea products and, therefore, in tea infusion. This result is in agreement with the findings of Kanchi et al. (2007) who showed that black tea and green tea from the same region (Lahijan, Iran) did not contain significantly different amounts of caffeine (19.6 and 17.6 mg/g in black tea and green tea, respectively).

### 3.3. Effect of leaf form

A preliminary study of the effect of leaf form on the caffeine content of tea infusions, obtained using a rolled-leaf and a loose-leaf sample, suggested that the dissolution rate of caffeine from the rolled leaves was slower than that from the loose leaves (Butsoongnern, 2006; Butsoongnern et al., 2005). Fig. 3 shows slight differences in the caffeine concentrations (~3–4 mg/100 ml) in tea infusions brewed from rolled and loose leaves. This agrees with the previous results. It is possible that the rates of dissolution and release of caffeine were affected by the water uptake rate of tea leaves. It might be expected that the rate of water uptake by rolled leaves would be lower. However, when the data were treated with the *F* test and Student *t* tests, it was found that the difference was not statistically significant (95% confidence level). This was due to a high deviation from the mean. More samples should be considered in a further study. However, the ground leaves clearly showed results for the concentration of caffeine in infusions different from those of the non-ground leaves. For the ground leaves, caffeine dissolved very quickly in the bulk solution (i.e., caffeine concentrations were quite stable for the whole range of the infusion times studied; from 0.5 min to 30 min) while, for the non-ground leaves, infusion time showed a significant effect on the dissolution of caffeine (discussed in Section 3.4.).

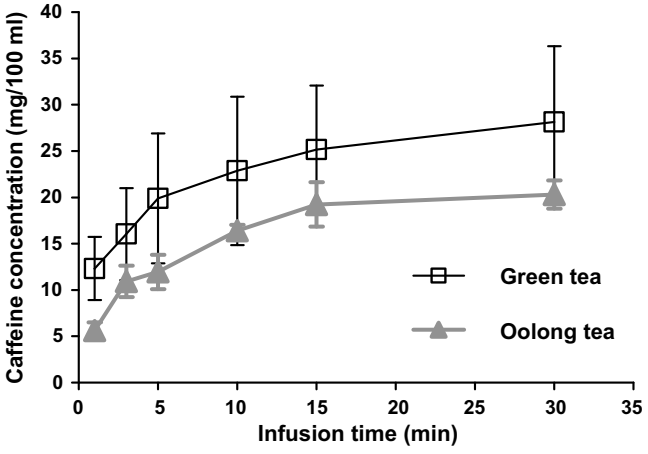


**Fig. 2.** Comparison of caffeine concentrations in green tea and oolong tea infusions at various infusion times (error bars are standard deviations ($n = 3$)).

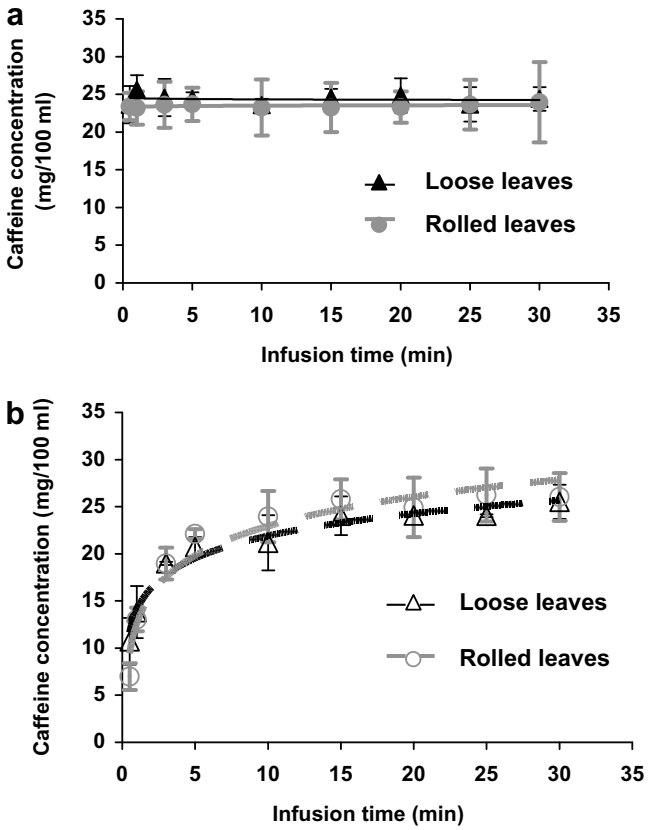


**Fig. 3.** Caffeine concentrations in tea infusions brewed from (a) ground and (b) non-ground leaves of loose- and rolled-leaf samples at various infusion times (error bars are standard deviations ($n = 3$)).

### 3.4. Effect of infusion conditions

As expected, the higher the water temperature, the higher was the caffeine in tea solution (Fig. 4) with the non-ground tea samples. This is due to the effect of temperature on the solubility and diffusion rate of caffeine in the bulk solution. Generally, the solubility and the diffusion coefficient of a substance increase with an increase in the temperature of the solvent (Atkins, 2001). The dissolution and diffusion of caffeine in the aqueous solution, therefore, increased with an increase in water temperature. The infusion time also affected the concentration of caffeine in the infusion. For the period of 0.5–15 min, the concentration of caffeine increased rapidly whereas, for the longer infusion time (15–60 min), the caffeine concentration increased only slightly. It seems likely that the rate of caffeine diffusion depends on the initial concentration at the leaf surface. When hot water is added to tea leaves, the diffusion starts at a fast rate because there is no caffeine in the water. When

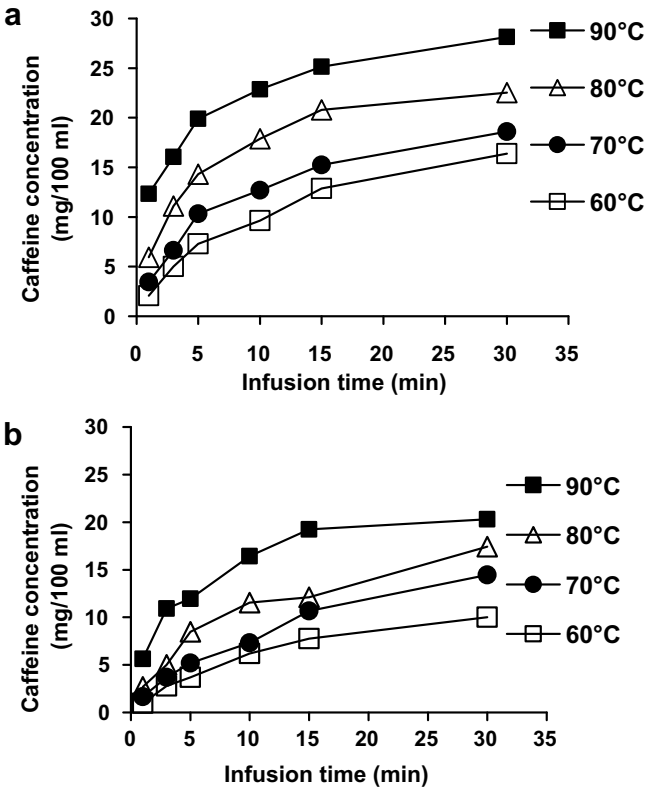


**Fig. 4.** Effect of water temperature and infusion time on caffeine concentrations brewed from (a) green tea and (b) oolong tea samples.

tea leaves are infused for a longer time, the concentration of caffeine in the bulk solution became higher and close to the final concentration and hence caffeine diffuses at slower rate (Spiro & Lam, 1995).

## 4. Conclusions

Caffeine concentrations in tea infusions were highly influenced by infusion conditions, namely, water temperature and brewing duration, and leaf form (ground/non-ground). Because the solubility and the rate of diffusion of caffeine increase with water temperature, the higher the water temperature, the higher is the caffeine concentration in a tea infusion. For non-ground rolled- and loose-leaves and a shorter brewing duration (0.5–10 min), the dissolution of caffeine increased rapidly because there was no caffeine in water while, for a longer brewing time (15–60 min), the dissolution of caffeine became quite stable due to the effect of the high concentration of caffeine in the bulk solution. For ground leaves, caffeine concentration in the tea infusions was independent of infusion time because the higher surface area of ground leaves leads to very quick release of caffeine to the infusion. The dissolution of caffeine from the rolled- and loose-leaves was slightly different but this was not statistically significant.

Tea variety and type did not have much effect on caffeine content in Chiang Rai tea infusions. Caffeine concentrations in different tea-varieties and tea-type infusions were not significantly different. In our study, tea varieties and types do not play a major role. Other factors such as the altitude of the site, climate, soil composition and properties, and cultivation method, which were considered to be similar for the samples studied, might have more influence. Further study of these factors is required. However, drinking more than four cups of Chiang Rai tea per day (1 g tea/100 ml/cup) is likely to lead to a caffeine intake which is too high.

## Acknowledgements

The authors acknowledge the National Research Council of Thailand (NRCT) for full financial support (Project No.: 50201010036). The Tea Institute and the Scientific and Technological Instrument Center (STIC), Mae Fah Luang University are acknowledged for laboratory facilities. Professor Dr. John Keith Syers, Dean, School of Science, Mae Fah Luang University, provided useful discussion.

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