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Tea preparation and its influence on methylxanthine concentration

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The amount of tea or coffee estimated from the number of cups consumed is frequently used as an indication of caffeine consumption in epidemiologic studies. However, this alone may be an inadequate indication of intake since drinking practices of tea varies. In this study, 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. On a dry leaf weight basis, total caffeine after three brews was highest in black (32.8 mg/g) and green (36.6 mg/g) tea leaves and lowest in Formosa oolong tea 2 (23.8 mg/g). Total theobromine was highest in black teas (1.64 and 1.69 mg/g) and least in oolong teas (0.65 and 0.71 mg/g). Caffeine and theobromine were not detected in either herbal tea samples, and theophylline was not detected in any tea tested. The overall average caffeine released in the first through third brews were 69%, 23%, and 8%, respectively. Three cups of tea brewed using three tea bags (Western culture) have approximately twice the amount of methylxanthines as the same volume prepared by three successive brews of loose tea leaves (Asian culture). These differences should be accounted for by the epidemiologic studies evaluating the effect of methylxanthines on health. Copyright © 1996 Canadian Institute of Food Science and Technology. Published by Elsevier Science Ltd

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INTRODUCTION

Tea is the most popular beverage in the world and its popularity is increasing, especially in the United States. Due to the popularity of tea, much concern exists regarding health implications associated with its consumption. Tea leaves contain many compounds, two groups of which are polyphenols and methylxanthines (caffeine, theobromine, and theophylline). The antioxidant properties of polyphenols in tea appear to help reduce cancer risks (Wang *et al.*, 1994; Chen, 1992; Yu *et al.*, 1994). Thus, Fujiki *et al.* (1992) suggested drinking green tea in large amounts, such as ten cups per day, as a form of possible cancer prevention for the general population. However, consuming high amounts of methylxanthines have been shown to have negative effects upon premenstrual syndrome (PMS) (Rossignol *et al.*, 1989; Rossignol & Bonnlander, 1990), fertility and pregnancy (Wilcox *et al.*, 1988; Caan & Golhaber, 1989; Fenster *et al.*, 1991; Mills *et al.*, 1993), and even cancer (Welsch *et al.*, 1988*a*,*b*; Phelps & Phelps, 1988).

Many epidemiological studies measuring caffeine consumption use the approximate average methylxanthine content per cup of coffee or tea multiplied by the number of cups consumed per day rather than the actual amount of caffeine in beverages. Fenster *et al.* (1991) calculated caffeine consumption by taking the average caffeine content of beverages and multiplying this by the number of cups consumed while Rossignol (1985) did not include specific caffeine concentrations, only the number of cups consumed. Results of such studies may be misleading because methylxanthine concentration in tea can be altered depending on tea preparation methods. For example, Stavric *et al.* (1988) studied the methylxanthine content of brewed teas prepared as bags versus loose leaves and brewing for

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2 versus 5 min. Not only did the teas brewed for different amounts of time have significantly different methylxanthine concentrations, but the extraction of caffeine and theobromine was higher from the tea bags than the loose leaves. Previous epidemiological studies did not take into account the variability of methylxanthine concentrations associated with different brewing methods and drinking practices.

While black tea bags are most commonly used in Western countries, green tea and oolong tea leaves are much more popular in Asia. In Asian countries, it is common practice to brew the same leaves three or more times whereas in Western countries tea bags are used once and discarded. There is no data available that shows methylxanthine concentration in second and third brews of tea leaves, which is the preferred brewing method in Asia. It is crucial to accurately determine methylxanthine concentrations in brewed teas before making generalizations about the amount of tea consumed and its effect on health.

The objectives of this study were to quantify the methylxanthine content in three brews of four types of commonly used teas (black, green, oolong, and herbal) in both tea bags and loose tea leaves and to determine the approximate total concentration of each methylxanthine present in tea according to different brewing methods. The results are important for determining the effects of tea brewing techniques on the total methylxanthine concentration in brewed tea as well as for providing more accurate methylxanthine concentration data for use in epidemiological studies.

MATERIALS AND METHODS

Reagents

HPLC grade acetonitrile was purchased from Fisher Scientific Company (Norcross, GA). Caffeine, theobromine, and theophylline were obtained from Sigma Chemical Company (St. Louis, MO). Purified water from the Milli-Q reagent water system (Millipore, Bedford, MA) was used to prepare standards and samples in this study.

Samples and sample preparation

Eight teas were analyzed for caffeine, theobromine, and theophylline. These included two black teas (store-bought Lipton bag tea and Asian black tea leaves), two oolong teas (Formosa oolong tea leaves #1 and #2, both from Taiwan), two green teas (store-bought Lipton bag tea and imported Korean green tea leaves), and two naturally caffeine free herbal teas (Celestial Seasoning Lemon Mist and International Bazaar Cinnamon Apple, both purchased at the supermarket).

One tea bag was placed in a beaker, and 177 ml (6 fl. oz.) of boiling water was poured over the bag; the tea was allowed to brew for 5 min. The tea bag was removed and allowed to drain into the beverage for 30 s. The brewed tea was cooled to room temperature. A portion of the tea was filtered through a 0.45 μm syringe filter (Fisher Scientific) and diluted to an appropriate concentration prior to analysis of the methylxanthines.

For the loose black tea leaves and green tea leaves, approximately the same amount of leaves were measured out as was contained in the tea bags (i.e. 2.3 to 2.5 g). The standard amount of oolong tea for brewing, according to label directions, was 3 g of leaves in 150 ml of boiling water, which calculated to be 3.5458 g/177 ml. The leaves were drained for 30 s using Fisher filter paper (qualitative P8-Coarse:Fast); the sample was prepared for analysis as described previously.

For each tea, the same bags or leaves were brewed three times to produce a first, second, and third brew, using the previously described brewing method for each brew. Samples were analyzed in duplicate for caffeine, theobromine, and theophylline. The entire experiment was repeated in triplicate.

High-performance liquid chromatographic analysis

Quantitative high-performance liquid chromatography (HPLC) analysis was accomplished using a 250 × 4.6 mm C-18 reverse phase column (Phenomenex, Torrance, CA) and a mobile phase consisting of 10% acetonitrile/90% water (v/v). The flow rate was 1.0 ml/min, the injected volume was 20 μl, and the detector was set at 254 nm. Retention times were approximately 5.5 min for theobromine, 8 min for theophylline, and 15 min for caffeine.

Statistical analysis

All data were analyzed by the General Linear Models Procedure of SAS (SAS, 1985). Analysis of variance (ANOVA) was employed to evaluate treatment effects. Where differences were significant, Tukey's Standardized Range test was used to compare means at the $\alpha = 0.01$ level.

RESULTS AND DISCUSSION

Methylxanthine concentrations were examined on a volume (177 ml per 6 oz. cup) basis to determine the actual amount of methylxanthines present in tea according to various brewing and drinking practices. Table 1 lists caffeine and theobromine concentrations for each brewed tea. Each type of tea was prepared according to label instructions rather than an equal weight basis. Caffeine concentration from the first brew was significantly higher in Formosa oolong tea 1 than in the other teas. Formosa oolong tea 1 had 67.7 ±

Table 1. Mean caffeine and theobromine contents (n = 6) of selected brewed teas on a volume* and a dry weight basis

Tea	Dry weight of leaves (g)	Caffeine					Theobromine				
		Brew 1	Brew 2	Brew 3	Total		Brew 1	Brew 2	Brew 3	Total	
		(mg/cup)	(mg/cup)	(mg/cup)	(mg/3 cups)	(mg/g)	(mg/cup)	(mg/cup)	(mg/cup)	(mg/3 cups)	(mg/g)
Lipton black bag	2.36 ± 0.05**	58.8 ± 0.41^{b}	13.9 ± 2.31^{d}	4.54 ± 0.37^{d}	77.2 ± 2.74^{d}	32.8 ± 0.60^{b}	3.17 ± 0.05^{a}	0.63 ± 0.12^{b}	0.17 ± 0.02^{a}	3.97 ± 0.14^{a}	1.69 ± 0.03^{a}
Black leaves (loose)	2.47 ± 0.02	54.8 ± 6.44^{b}	24.5 ± 2.74^{ab}	10.9 ± 1.30^{a}	90.3 ± 2.72^{b}	36.6 ± 1.07^{a}	2.50 ± 0.45^{b}	1.11 ± 0.15^{a}	0.43 ± 0.05^{a}	4.04 ± 0.32^{a}	1.64 ± 0.12^{a}
Formosa oolong 1 (loose)	3.54 ± 0.00	67.7 ± 5.25^{a}	26.1 ± 1.66^{a}	8.26 ± 0.40^{b}	102 ± 7.06^{a}	28.8 ± 1.97^{c}	1.38 ± 0.37^{d}	0.66 ± 0.11^{b}	0.48 ± 0.14^{a}	2.52 ± 0.42^{b}	0.71 ± 0.12^{c}
Formosa oolong 2 (loose)	3.54 ± 0.00	60.6 ± 2.49^{b}	18.9 ± 0.32^{c}	4.97 ± 0.16^{d}	84.5 ± 2.62^{c}	23.8 ± 0.74^{d}	1.42 ± 0.37^{cd}	0.62 ± 0.20^{b}	0.31 ± 0.35^{a}	2.30 ± 0.57^{b}	0.65 ± 0.16^{c}
Lipton green bag	2.29 ± 0.07	44.5 ± 1.99^{c}	13.5 ± 1.15^{d}	4.85 ± 0.26^{d}	62.9 ± 1.19^{e}	27.5 ± 0.23^{c}	1.78 ± 0.18^{cd}	0.47 ± 0.11^{b}	0.17 ± 0.06^{a}	2.42 ± 0.29^{b}	1.06 ± 0.14^{b}
Green leaves (loose)	2.32 ± 0.02	56.1 ± 2.61^{b}	21.7 ± 1.18^{cb}	6.90 ± 0.21^{c}	86.7 ± 2.76^{c}	36.6 ± 0.96^{a}	1.95 ± 0.17^{cb}	0.72 ± 0.05^{b}	0.21 ± 0.05^{a}	2.87 ± 0.18^{b}	1.24 ± 0.07^{b}

*Cup = 177 ml (6 fl. oz.).
**Mean (n = 3) ± SD.
^{a,b,c,d,e}Means in columns with a different letter are significantly different ($P < 0.01$).

5.25 mg/177 ml in the first brew and 26.1 ± 1.66 mg/177 ml in the second brew. The higher caffeine content in oolong tea is due to the fact that more tea leaves were brewed according to the standard brewing procedures. Lipton green bag tea contained significantly less caffeine in the first brew with only 44.5 ± 1.99 mg/177 ml. In the second brew, Lipton green bag tea as well as Lipton black bag tea contained the least amounts of caffeine with 13.5 ± 1.15 and 13.9 ± 2.31 mg/177 ml, respectively. In the third brew, black leaves contained significantly more caffeine (10.9 ± 1.30 mg/177 ml) than the other teas. Lipton black bag, Formosa oolong tea 2, and Lipton green bag teas contained the least amounts of caffeine, with all concentrations ranging between 4.5 and 5.0 mg/177 ml.

Lipton black bag tea contained significantly more theobromine (3.17 ± 0.05 mg/177 ml) in the first brew than the other teas (Table 1). Formosa oolong tea 1, Formosa oolong tea 2, and Lipton green bag tea contained significantly less theobromine in the first brew than the other teas. In the second brew, black tea leaves contained significantly more theobromine (1.11 ± 0.15 mg/177 ml) than the other teas. In the third brew, there were no significant differences between the theobromine concentrations of the teas.

There have been no previous studies quantifying methylxanthines in second and third brews; the only available data is for first brews. Stavric *et al.* (1988) compared methylxanthines content of tea prepared from a 5 min brew of bags versus loose tea leaves in 175 ml of water. Caffeine concentrations varied between 0.40 and 0.41 mg/ml for bagged teas and between 0.20 and 0.34 mg/ml for loose leaf tea. Bunker & McWilliams (1979) found that caffeine concentrations in 5 min brewed bagged teas (black, green, and oolong) were 0.19 to 0.36 mg/ml while in loose leaf tea (black, green, and oolong) were 0.14 to 0.29 mg caffeine/ml. Their study used 1 teaspoon of dry leaves (the weight of which is unknown) per 140 ml of water. The present data was converted to units consistent with the above studies and found 0.25 to 0.34 mg/ml caffeine in bagged teas and 0.31 to 0.39 mg/ml in loose leaf teas. Bagged teas in the present study contained approximately the same concentrations of caffeine as determined by Bunker & McWilliams (1979), but the concentrations were less compared to results found by Stavric *et al.* (1988). Brewed loose tea leaves in the present study contained more caffeine than the other studies. This is surprising because Stavric *et al.* (1988) used 3.98 g leave per 175 ml of water while the present study used a smaller ratio of leaves to water, with tea leaf weights ranging between 2.32 to 3.54 g/177 ml.

Blauch & Tarka (1983) measured the amount of caffeine in black teas (American and Imported) when brewed for 5 min; the caffeine contents of their teas ranged between 55.0 and 67.4 mg/177 ml which are also similar to those from the present study (54.8 to 58.8 mg/177 ml). They also measured the amount of theobromine in black teas. The theobromine concentrations ranged between 2.7 and 4.4 mg/177 ml which is comparable with the findings of the present study (2.50 to 3.17 mg/177 ml). The differences in methylxanthine concentrations between teas of same kind may be affected by factors such as growing conditions, which include climate and origin of the teas (Roberts & Barone, 1983) and age of the tea leaves (Ashihara & Kubota, 1986). There is not much information available about oolong teas and their methylxanthine concentrations.

Total extracted methylxanthine concentrations (i.e. sum of the three brews) for each type of tea (Table 1) showed that the caffeine content was significantly higher in Formosa oolong tea 1 (102 ± 7.06 mg) while Lipton green bag tea contained significant lower amounts (62.9 ± 1.19 mg). Formosa oolong teas contain more caffeine because about 50% more tea leaves were brewed, thus giving a stronger tea, as compared to the other teas. Oolong tea is usually prepared strong, served in smaller cups (2 to 4 oz.), and sipped in small amounts. Both black teas contained significantly more total theobromine than the other teas (bag, 3.97 ± 0.14 mg; leaves, 4.04 ± 0.32 mg). Theobromine is found in younger leaves, which are the choice leaves used in the production of black tea because of their high polyphenol concentrations (Ashihara & Kubota, 1986; Graham, 1992). Therefore, the black teas should contain more theobromine than other teas. In the present study, the black teas did contain significantly higher theobromine concentrations than the other teas. In addition, more total caffeine was extracted from both types of leaves than their bagged counterparts. No significant differences in the total theobromine content were observed between loose tea leaves and tea bags for a given type of tea.

Total mean caffeine and theobromine contents per gram of tea leaves are also shown in Table 1. On a dry weight basis, Formosa oolong tea 2 had significantly less caffeine (23.8 ± 0.74 mg/g) and theobromine (0.65 ± 0.16 mg/g) than the other teas. Formosa oolong 1 also had low caffeine (28.8 ± 1.97 mg/g) and theobromine (0.71 ± 0.12 mg/g) levels. Therefore, Formosa oolong tea may have a less stimulatory effect as compared to black and green teas when prepared with the same quantity of leaves. Black and green tea leaves contained significantly more caffeine on a dry weight basis than both Formosa oolong teas as well as both bagged teas. There were no significant differences between the theobromine content in leaves and bags. Both black teas contained the most theobromine on a dry weight basis (bag, 1.69 ± 0.03 mg/g; leaves, 1.64 ± 0.12 mg/g) followed by both green teas, and both oolong teas, which contained the least.

The percentage of caffeine released in each brew was calculated based on a total of three brews and is shown in Table 2. In brew 1, Lipton black bag tea had the

Table 2. Percent caffeine and theobromine (n = 6) extracted from brewed teas

Tea	Caffeine			Theobromine		
	% Brew 1	% Brew 2	% Brew 3	% Brew 1	% Brew 2	% Brew 3
Lipton black bag	76.3 ± 2.52^{a}	17.9 ± 2.32^{c}	5.9 ± 0.03^{c}	80.0 ± 2.87^{a}	15.8 ± 2.56^{d}	4.19 ± 0.35^{d}
Black leaves (loose)	60.6 ± 5.25^{d}	27.3 ± 3.80^{a}	12.2 ± 1.77^{a}	61.6 ± 6.27^{cd}	27.8 ± 5.13^{a}	10.6 ± 1.77^{b}
Formosa oolong 1 (loose)	66.3 ± 0.91^{c}	25.6 ± 0.87^{ab}	8.1 ± 0.12^{b}	54.1 ± 6.32^{a}	26.5 ± 4.60^{a}	19.4 ± 5.85^{a}
Formosa oolong 2 (loose)	71.7 ± 0.77^{b}	22.4 ± 0.44^{b}	5.9 ± 0.35^{c}	62.9 ± 13.6^{cd}	27.0 ± 6.44^{a}	10.0 ± 13.6^{bc}
Lipton green bag	70.8 ± 2.10^{b}	21.5 ± 2.02^{b}	7.7 ± 0.52^{ab}	73.6 ± 4.18^{b}	19.3 ± 2.84^{c}	7.13 ± 2.12^{c}
Green leaves (loose)	66.2 ± 1.29^{c}	25.7 ± 1.34^{ab}	8.2 ± 0.46^{b}	67.8 ± 3.28^{c}	24.9 ± 1.97^{ab}	7.24 ± 1.79^{c}
Average	69	23	8	67	23	10

^{a,b,c,d}Means in columns with different letters are significantly different ($P < 0.01$).

highest percentage of extraction at 76.3 ± 2.52% and loose black tea leaves had the least with 60.6 ± 5.25%. The opposite was the case with the second brew because loose black tea leaves had the highest percentage of extraction (27.3 ± 3.80%) and Lipton black bag tea had the least (17.9 ± 2.32%). In the third brew, loose black tea leaves had the highest percentage of extraction (12.2 ± 1.77%) and Lipton black bag tea had the least (5.87 ± 0.03%). The overall average caffeine extraction from all types of tea in brews 1 through 3 were 69%, 23%, and 8%, respectively. Caffeine extraction greatly decreased with each respective brew.

The percentage of theobromine extracted in each brew was also determined (Table 2). Lipton black bag tea had the highest percentage of extraction in brew 1 and the lowest percentage in brews 2 and 3. Formosa oolong tea 1 had the lowest percentage in brew 1 (54.1 ± 6.32%) and the highest percentage in brew 3 (19.4 ± 5.85%). Black loose tea leaves had the least percentage extracted in brew 2 (27.8 ± 5.13%). The overall average percent theobromine released in the first through third brews were 67%, 23%, and 10%, respectively. As with caffeine, the percentage of theobromine extraction greatly decreased between each brew.

Caffeine, theobromine, and theophylline were not detected in either herbal tea. Theophylline was not detected in any teas by the method used, indicating its concentration is less than 0.1 mg/177 ml of beverage.

It was observed that the flavor remains in the second and third brews of tea brewed from loose whole tea leaves as opposed to those brewed from tea bags. As was shown in Table 1, the second and third brews contained significantly more caffeine when prepared using loose tea leaves as compared to bags. Caffeine contributes to tea flavor as do numerous other compounds which may also extract from the tea leaves in a similar manner to that of caffeine. In addition, the total caffeine extracted from the three brews of loose tea leaves was significantly higher than tea bags for both black and green teas. Thus, it would be expected that tea prepared from loose leaves would maintain its flavor during the various brews.

The results of this study indicate the differences in methylxanthine concentrations in tea that may be consumed depending on the preferred brewing method. Fujiki *et al.* (1992) suggest drinking 10 cups of green tea daily as a form of cancer prevention. They did not specify how many milliliters were considered a cup. If one were to consume 10 cups of tea per day by brewing green or black tea the Western or bagged tea method, one would approximately consume 445 or 588 mg caffeine and 17.8 or 31.7 mg theobromine respectively. Someone brewing green tea or black tea by the loose leaf method would approximately consume 289 or 301 mg caffeine and 9.6 or 13.5 mg theobromine per day respectively, which is about a third to a half less methylxanthines as compared to the Western brewing method. Therefore, the epidemiological studies that base methylxanthine consumption on the average methylxanthine content in a cup of tea can be very misleading because of different brewing methods. Methylxanthine consumption causes decreased drowsiness and lessened fatigue at doses between 50 and 200 mg/day and can cause such health problems as headache, tremors, nervousness, and irritability at doses between 200 and 500 mg/day (Stephenson, 1977). Caan & Golhaber (1989) found that the consumption of > 300 mg/day was harmful to the fetus by increasing the risk of low birthweight. Methylxanthine consumption also causes increased PMS symptoms (Rossignol *et al.*, 1989; Rossignol & Bonnlander, 1990), and doses of 250 mg/day causes cardiovascular stimulation (Robertson *et al.*, 1978). Therefore, the consumption of the higher levels of methylxanthines, such as 10 cups of bag tea could cause health problems as mentioned above, while drinking 10 cups of repeatedly brewed loose tea leaves may have no noticeable health effects from the methylxanthine consumption. Further studies are needed to determine the extraction of tea polyphenols as compared to the extraction of methylxanthines. This would help determine whether it is more beneficial to consume three or more brews from the same tea leaves as compared to consuming three or more brews from different tea bags.

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