Determination of Caffeine Content in Tea and Maté Tea by using Different Methods

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Abstract: Caffeine-containing products have been consumed for hundreds of years for their pleasant flavor and stimulating effects. In recent years, caffeine received increasing attention in food and pharmaceutical industries, due to its pharmacological properties which comprise stimulation of the central nervous system, peripheral vasoconstriction, relaxation of the smooth muscle and myocardial stimulation. The aim of this study was to determine the content of caffeine in five types of tea (white, yellow, green, oolong, black) and two types of maté tea (green maté and roasted maté tea). The content of caffeine was determined by using four different methods: extraction with chloroform, micromethod, method with lead-acetate and high performance liquid chromatography method (HPLC-PDA). The antioxidant capacity of teas as well as of the extracted ("raw") caffeine was determined by using two methods: reactions with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical (ABTS assay) and Ferric reducing antioxidant power (FRAP assay). The content of caffeine has been associated with plant origin and growth conditions, as well as processing conditions. By applying all four methods, the highest content of caffeine was determined in white tea, whereas maté and roasted maté tea were characterised with the lowest content of caffeine. Spectrophotometric micromethod has proven to be the best alternative to the HPLC method. The highest antioxidant capacity was determined in yellow tea, while the lowest was determined in roasted maté tea. In comparison to the antioxidant capacity of teas, the antioxidant capacity of extracted ("raw") caffeine is almost negligible, and does not contribute to the overall antioxidant properties of tea.

Keywords: antioxidant capacity; caffeine; HPLC; maté tea; tea

INTRODUCTION

Caffeine (1,3,5-trimethylxanthine) and two of its minor isomeric dimethylxanthines, theobromine and theophyline, belong to a group of methylxantines. The widespread occurrence of caffeine in a variety of plants played a major role in the long-standing popularity of caffeine-containing products. The most important sources of caffeine are coffee (*Coffea* spp.), tea (*Camellia sinensis*), guarana (*Paullinia cupana*), maté (*Ilex paraguariensis*), cola nuts (*Cola vera*), and cocoa (*Theobroma cacao*). The amount of caffeine found in these products varies – the highest amounts are found in guarana (4–7%), followed by tea leaves (3.5%), maté tea leaves (0.89–1.73%), coffee beans (1.1–2.2%), cola nuts (1.5%), and cocoa beans (0.03%) (Clifford *et al*. 1990).

Caffeine has pharmacological effects on central nervous system, heart, peripheral and central vasculature, renal, gastrointestinal and respiratory system. Due to the widespread consumption of methylxanthines, it is important to collect precise information on their content in foods. Most research activities have been focused on chromatographic methods, however, spectrophotometric determination is preferred because of its rapidity, high accuracy and reproducibility. Therefore, it is important to develop more reliable, simpler and faster methods for the determination of caffeine from different sources in order to find a more precise relationship between the amounts of consumed

caffeine and its physiological effects (Georga *et al*. 2001; De Aragão *et al*. 2005).

The aim of this study was to compare four different methods for determination of caffeine and two methods for antioxidant capacity estimation in tea (*Camellia sinensis*) and maté (*Ilex paraguariensis*) products. Results of this study could be used to facilitate selection of the appropriate method in order to obtain satisfactory data on caffeine content in plant materials.

MATERIALS AND METHODS

Sample preparation. Five types of tea in loose leaf form: Pai Mu Tan-superior (white tea), Sencha (green tea), Formosa Fine Oolong (oolong tea), Lingia (black tea), Yin Zhen (yellow tea) and two types of loose leaf maté tea (maté tea and roasted maté tea) were purchased on a local market. In order to simulate household brewing conditions, teas were prepared using an aqueous extraction. Tea samples (2.5 g) were poured with 200 ml of boiling water and stirred for 10 minutes. Extracts were filtered through a cotton wool, cooled at a room temperature, diluted to 250 ml with distilled water, and used for spectrophotometric analyses.

Caffeine isolation with chloroform. The caffeine isolation procedure was performed according to a modified method described by Rapić (1994). Briefly, 20 g of tea and 90 ml of distilled water was refluxed for 30 min, and filtered under vacuum. The residue was again refluxed and filtered. Obtained filtrates were combined, 12.5 ml of $Pb(CH_3COO)_2$ solution was added, boiled (5 min), and filtered through a Büchner funnel with silica gel layer. The filtrate was extracted four times with chloroform (40 ml). Combined chloroform phases were washed with KOH solution and then with distilled water. Chloroform was removed from extracts by rotary evaporator. After evaporation, extracted caffeine was weighed and expressed in mg/l.

Caffeine determination using the lead acetate solution. This procedure is based on international standards with some modifications (Yao *et al*. 1992, 1993). Tea extract was treated with HCl solution (5 ml), $Pb(CH_3COO)_2$ and H_2SO_4 solution. Absorbance of obtained extracts was measured at 274 nm. The content of caffeine (mg/l) was calculated using a standard curve derived from caffeine (0–250 mg/l). All measurements were performed in triplicate.

The micromethod for the determination of caffeine. The teas were also analysed for their caffeine content according to the method reported by Groisser (1978). Briefly, tea extracts (pH = 8–9) were extracted with benzene and H_2SO_4. Absorbance of extracts was read at 273 nm against a blank (H_2SO_4). Results, obtained from triplicate analyses, were calculated using a standard curve and expressed as mg/l.

HPLC analysis of caffeine. Filtered tea extracts and caffeine solutions were injected for HPLC analysis according to the method reported in our previous study (Horžić *et al*. 2009). Equipment used consisted of a Varian Pro Star Solvent Delivery System 230 and a Photodiode Array detector Varian Pro Star 330 (Varian, Walnut Creek, USA) with a reversed-phase column Pinnacle II C-18 (Restek, USA) (250 × 4.6 mm, 5 µm i.d.). Caffeine was identified by comparing the retention times and spectral data with those of authentic standards. All analyses were repeated three times.

Antioxidant capacity. Antioxidant capacity of tea extracts and caffeine solutions (100 mg/l) was determined using the ABTS radical scavenging assay according to the method reported by Re *et al*. (1999), and ferric reducing/antioxidant power (FRAP) assay, carried out according to the original method by Benzie and Strain (1996).

RESULTS AND DISCUSSION

Caffeine content of five teas and two maté teas determined by the extraction procedure with chloroform ranged from 0.69% (black tea) to 1.33% (white tea) (Figure 1). Ashihara and Kubota (1986) stated that caffeine biosynthesis is the most active in young tea leaves and buds, which implies that white tea, produced from the youngest tea buds should contain the highest caffeine content. Our results confirm this thesis, since white tea contains the highest caffeine content, and black tea, the most processed tea type, contains the lowest caffeine content.

In comparison to all methods used in this study for the determination of caffeine content, the micromethod with benzene exhibited the highest results. White tea contained the highest caffeine content (4.55%) and maté tea the lowest (1.05%). The content of caffeine in green, black and oolong tea ranged from 2.04% (green tea) to 3.86% (black tea), which is in agreement with the

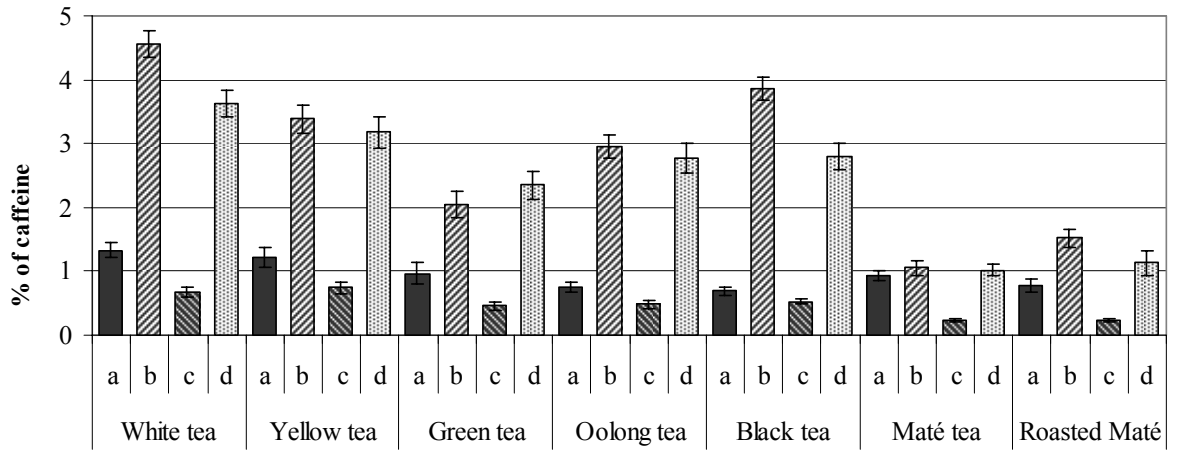

Figure 1. Caffeine contents of teas and maté teas determined by four different methods (a – chloroformic isolation; b – micromethod; c – method with lead acetate; d-HPLC)

results obtained by Groisser (1978), who found 2.47% caffeine in black tea, 3.45% in green tea and 1.79% in oolong tea. Although there were no significant differences between the order of caffeine content among the tested teas, the method with lead acetate exihibited significantly lower results with regard to other methods applied for the determination of caffeine. Reversed-phase and normal phase HPLC methods with UV detection have been successfully applied for the separation and determination of methylxanthines in a wide range of samples. Some of the reported methods require tedious pretreatment or do not allow separation and quantitation of different derivatives in the same sample (Georga *et al*. 2001). The gradient method developed in this study has shown to be appropriate for an efficient simultaneus analysis of both methylxanthines and polyphenols in tea (Horžić *et al*. 2009). The caffeine content of tested teas determined by the HPLC analysis was decreasing in the following order: white tea (3.62%) > yellow tea (3.18%) > black tea (2.79%) > oolong tea (2.77%) > green tea (2.35%) > roasted maté tea (1.13%) > maté tea (1.02%). According to these results the caffeine content obtained with HPLC analysis is the most comparable to the ones obtained by the micromethod with benzene.

Earlier studies revealed that caffeine content is associated to origin, genetic and environmental variability, harvest time and processing manner of plant material (Athayde *et al*. 2000), and can range from 24% to 40%. The displayed results confirmed that caffeine content depends on the age of tea leaves and processes involved in the production of tea. Since the caffeine content was the lowest in both types of analysed maté tea, it is also obvious that caffeine content depends on plant origin, indicating that the use of maté tea as a beverage provides a milder effect in comparison to other tea types.

Table 1. The antioxidant capacity of tea extracts and caffeine evaluated by two antioxidant assays

	ABTS (mM Trolox)		FRAP (mM Fe(II))	
	tea extract	extracted caffeine	tea extract	extracted caffeine
White tea	6.66 ± 0.12	0.05 ± 0.01	7.77 ± 0.02	0.03 ± 0.00
Yellow tea	11.18 ± 0.12	0.07 ± 0.01	14.98 ± 0.10	0.05 ± 0.00
Green tea	8.85 ± 0.11	0.06 ± 0.01	10.20 ± 0.15	0.02 ± 0.00
Oolong tea	8.51 ± 0.12	0.07 ± 0.01	4.16 ± 0.06	0.02 ± 0.00
Black tea	4.46 ± 0.14	0.07 ± 0.01	9.02 ± 0.04	0.05 ± 0.01
Maté tea	3.23 ± 0.07	0.08 ± 0.01	5.69 ± 0.04	0.04 ± 0.00
Roasted maté tea	2.43 ± 0.10	0.07 ± 0.01	3.25 ± 0.06	0.05 ± 0.00

The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging assay and Ferric reducing antioxidant power (FRAP) assay were applied for the evaluation of antioxidant capacity of tea extracts and the extracted caffeine (determined by the isolation method with chloroform). As could be seen in Table 1, yellow tea exhibited the highest antioxidant capacities, in both assays. The antioxidant capacities of extracted caffeine was almost negligible in comparison to the antioxidant capacities of tea, indicating that caffeine itself does not contribute to the overall antioxidant properties of tea.

CONCLUSION

The content of caffeine varies depending on tea type, which is directly attributed to their processing and leaf maturity. White tea, made from the youngest tea leaves contained the highest caffeine content, and maté and roasted maté teas the lowest. The spectrophotometric micromethod proved to be the best alternative for the determination of caffeine content, exhibiting the most similar results to the HPLC analysis. According to the obtained results, all studied teas exhibited high antioxidant capacity, as opposed to caffeine, indicating that the contibution of caffeine to the antioxidant properties of these beverages is irrelevant.

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