

DETERMINATION OF OXALATE CONTENT IN TEA SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: Oxalate is one of the major risk factors for kidney stones which become more common in industrialized countries including Thailand. Tea is a significant source of oxalate. There have been a few published reports about oxalate content in tea samples in Thailand. Therefore, the purpose of the present study was to determine oxalate content in tea samples and study the effect of steeping duration on the oxalate content. One-hundred and twenty samples of tea (white, green, oolong and black tea) were purchased from markets in Thailand from February to June 2011. The oxalate content was measured by the high performance liquid chromatography (HPLC) method with an anion exchange column using 0.85 mM sodium bicarbonate and 0.9 mM sodium carbonate as a mobile phase. The highest of oxalate content was found in black tea (1.36-4.42 mg/g tea), followed by oolong tea (0.74-3.94 mg/g tea), green tea (0.44-2.18 mg/g tea) and the lowest was found in white tea (0.40-1.79 mg/g tea). The forms of tea did not have significant influence on the amount of oxalate content. Steeping the tea for a longer duration provided more oxalate content. People in a high risk group should avoid drinking high amounts of black tea.

Keywords: oxalate, hyperoxaluria, tea

INTRODUCTION

Oxalate, a salt form of oxalic acid, is widely distributed in a number of plant tissues [1]. Oxalate is not significantly metabolized in the human body; therefore, it is the end product of metabolism excreted in urine. It is suggested that about 10-20% of urinary oxalate is derived from food [2] and the recent study showed that dietary oxalate contributed up to 53% of urinary oxalate [3].

Consumption of foods with high amounts of oxalate may cause adverse health effects. Oxalate combines with several essential minerals including calcium, iron, and magnesium to form insoluble compounds in the gastrointestinal tract; therefore, it may decrease the bioavailability of these minerals [4, 5]. A high dietary oxalate intake also increases the risk of kidney stone formation [6] which is becoming more common in industrialized countries including Thailand. About 80% of stones are composed of calcium oxalate [7]. The major risk factor of its formation is hyperoxaluria (urinary oxalate excretion above 0.5 mmol/24 h) [8].

Tea is a beverage consumed throughout the world [9]. It appears that tea is a significant source of oxalate intake in English diets [10]. White, green, oolong, and black teas are all made by brewing leaves of the tropical evergreen *Camellia sinensis* (L.) O. Kuntze in hot water [11, 12]. White tea is obtained from steaming and drying buds and young tea leaves with the minimum amount of processing.

Green tea is prepared from unfermented leaves, whereas oolong and black tea are partially and fully fermented respectively [12]. Due to the difference of its fermentation process, tea differs in its appearance, organoleptic taste, chemical content, and flavor [13].

Oxalate can be determined by various methods. The AOAC method analyzes oxalate based on calcium oxalate precipitation [14]. Oxalate is extracted with acid, precipitated as the calcium salt, and then analyzed with colorimetric, gas chromatography or atomic absorption.

There are some limitations in terms of sensitivity, precision and time consumption. The other methods include ion electrophoresis, capillary electrophoresis, enzymatic assays using oxalate oxidase and high performance liquid chromatography (HPLC) [15-17]. The HPLC has been shown to be accurate and reliable for determination of oxalate in plant materials [18, 19].

Although several studies have been published for the amount of oxalate in tea, the data on oxalate content of commercial tea available in Thailand is limited, especially in white tea. Therefore, the aims of the present study were to determine the oxalate content in tea samples including white, green, oolong, and black tea available in Thailand and to study the effect of steeping duration on the oxalate content of these four types of tea.

MATERIALS AND METHODS

Tea samples One-hundred and twenty samples of tea-white, green, oolong and black teas from 40 brands-were used for analysis of the oxalate

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content. Tea samples were purchased from markets in Thailand from February to June 2011.

Reagents Oxalate standard solution was IC grade and purchased from Alltech (USA). Sodium carbonate and sodium bicarbonate were obtained from BDH & Prolabo (UK). Ultra pure water, ω-18 (Maxima Ultra Pure Water, Elga, UK) was used for standard and mobile phase preparation. Filtered water, Coolly fresh® (Thailand) was used for sample preparation.

Instruments HPLC system was composed of a model 626 pump, model 630 autosampler, a model 650 conductivity detector with a model 640 suppressor, all from Alltech, USA. Chromatographic separation was carried out using anion exchange column (Allsep™, 7 μm, 100 x 4.6 mm, USA). PeakSimple chromatography data system SRI model 302 was used for data integration.

Preparation of tea samples

Tea sample from tea leaves Tea leaves (3.50 g) were accurately weighed into a 250-mL beaker. The sample was cleaned by rinsing in hot water. After cleaning, 200 mL of hot filtered water (80±2°C) was added into the beaker and left it for 3 minutes. Then the solution was filtered through a tea strainer to remove tea leaves. Each tea sample was cooled to room temperature. For analysis, 20 mL of tea infusion was pipetted into a 100- mL volumetric flask and made up to volume with filtered water. After that, the sample was filtered through a 0.45 μm cellulose acetate membrane syringe filter and then analyzed by HPLC.

Tea sample from tea bags Each tea bag was placed into the 250-mL beaker. 200 mL of hot filtered water (80±2°C) was added for infusion. After 3 minutes of infusion, the tea bag was taken out. When the tea solution reached room temperature, the 20 mL of tea solution was pipetted in a volumetric flask to make up 100-mL volume. After that, each tea sample was filtered through a cellulose acetate membrane syringe filter with a pore size of 0.45 μm and then analyzed by HPLC. To study the effect of steeping duration on the oxalate contents, tea samples were steeped in hot water in different steeping times (0.5, 1, 3, 5 and 7 minutes).

Preparation of standard solutions A 100 μg/mL of oxalate standard stock solution was prepared in ultrapure water and stored at 4°C. For analysis, 1, 5, 10, 20 and 30 μg/mL of standard solutions were prepared from stock oxalate standard solution by appropriate dilution with ultrapure water. All standard solution should be filtered through a 0.45 μm cellulose acetate membrane syringe filter and protected from light.

Validation of the method The precision was investigated by five injections of each oxalate standard solution (1, 5, 10, 20 and 30 μg/mL) in the

same day (Intraday-precision) and the analysis was continued for 3 consecutive days (Interday-precision). The accuracy was determined by standard addition technique. Known oxalate standard solutions containing 1, 5, 10, 20 and 30 μg/mL were added to know aliquot of sample solution. Oxalate in the added sample was analyzed in triplicate by HPLC. The accuracy was expressed as % recovery.

Oxalate determination The experiment was performed with HPLC. The chromatographic conditions were set up according to the manufacturer's recommendations (Application note #A0016). The isocratic mobile phase consisting of 0.85 mM sodium bicarbonate and 0.9 mM sodium carbonate was used. The HPLC column was run at a flow rate of 1 mL/min. The volume for each injection was 20 μL.

Data analysis The oxalate standard calibration curve was obtained by plotting the peak area against the concentration of oxalate standard. The linear regression equation and the correlation coefficient (r^2) were calculated from calibration curve. The data were analyzed using SPSS (version 17.0, SPSS Inc. Chicago, IL, USA). The effect of tea type was calculated by Kruskal-Wallis H test and the effect of tea form was calculated by Mann-Whitney U test.

RESULTS

The standard calibration curve had a correlation coefficient of 0.9999. The relative standard deviations (RSD) were 0.59% and 1.37% for Intraday-precision and Interday-precision respectively. Percentage of the recovery was 94.41. HPLC chromatogram of oxalate standard solution and tea sample are shown in Figure 1.

Oxalate content in tea samples

The amount of oxalate in tea samples was presented as range and mean ± SD (Table 1). The oxalate contents of black tea both in leaf and bag forms were significantly different from those of white tea and green tea. Only bag form, oxalate contents in black tea differed from those in oolong tea significantly. The oxalate contents in leaf form and

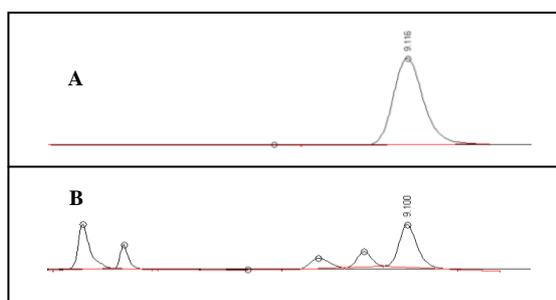


Figure 1 HPLC chromatogram of oxalate standard solution (A) and tea sample (B)

Table 1 Oxalate content in tea samples

Tea samples	Oxalate content (mg/g tea)		Oxalate content (mg/cup tea)*	
	Range	Mean \pm SD	Range	Mean \pm SD
White tea				
Leaf form (n=15)	0.85-1.69	1.29 \pm 0.33 ^a	2.98-5.92	4.51 \pm 1.14
Bag form (n=15)	0.40-1.79	1.25 \pm 0.45 ^a	0.80-3.23	2.19 \pm 0.76
Green tea				
Leaf form (n=15)	0.44-2.18	1.41 \pm 0.56 ^a	1.54-7.63	4.95 \pm 1.97
Bag form (n=15)	0.55-1.99	1.24 \pm 0.48 ^a	1.11-3.98	2.48 \pm 0.95
Oolong tea				
Leaf form (n=15)	0.74-2.82	1.62 \pm 0.79 ^{a, b}	2.59-9.87	5.69 \pm 2.77
Bag form (n=15)	1.00-3.94	1.88 \pm 1.04 ^a	2.26-7.89	3.69 \pm 2.05
Black tea				
Leaf form (n=15)	1.36-4.42	2.32 \pm 1.10 ^b	4.76-15.47	8.10 \pm 3.83
Bag form (n=15)	1.45-3.82	2.37 \pm 0.80 ^b	3.45-6.69	4.65 \pm 1.13

* 1 cup = 200 mL

^{a, b} = Items with different letter in the same column are significantly different ($p < 0.05$).**Table 2** Oxalate content in tea samples when steeping in various duration

Steeping duration (minute)	Oxalate content (mg/g tea)			
	White tea (n=12)	Green tea (n=12)	Oolong tea (n=12)	Black tea (n=12)
0.5	0.49-0.86	0.24-1.65	0.41-1.42	0.84-2.36
1	0.65-1.35	0.77-2.12	0.79-1.98	1.60-3.75
3	1.43-1.79	1.56-2.16	1.85-3.94	2.35-4.42
5	1.63-2.31	2.26-4.69	2.42-4.62	2.90-5.00
7	1.97-2.59	2.35-5.17	2.51-4.86	2.92-5.47

bag form were not significantly different for every type of tea. In leaf form, the highest oxalate content was found in black tea (1.36 to 4.42 mg/g tea), then in oolong tea (0.74 to 2.82 mg/g tea), green tea (0.44 to 2.18 mg/g tea) and lowest in white tea (0.85 to 1.69 mg/g tea). In bag form, the oxalate content in black tea (1.45 to 3.82 mg/g tea) was higher than those of oolong tea (1.00 to 3.94 mg/g tea), followed by green tea (0.55 to 1.99 mg/g tea) and white tea (0.40 to 1.79 mg/g tea), respectively.

Effect of steeping duration on oxalate content in tea samples

As the tea samples were steeped in hot water for 0.5, 1, 3, 5 and 7 minutes, the level of oxalate content was found to increase with longer steeping time (Table 2). Black tea contained the highest amount of oxalate in all steeping durations.

DISCUSSION

Consumption of tea, which is made from the leaves of *Camellia sinensis* plants, has shown many health benefits because of its potent ingredients, polyphenols [20]. Polyphenols, particularly catechins act as antioxidants scavenging free radicals both *in vitro* and *in vivo*, reduce the risk of cardiovascular diseases, fight against various types of cancer and prevent diabetes [12]. However, oxalate in tea is thought to be a major risk factor for kidney stones. Kidney stones are correlated with increased urinary oxalate. High urinary oxalate results from increased dietary oxalate intake. Gasinska and Gajewska [21] found that the main sources of food oxalate in patients with kidney oxalate stones were tea and

coffee (80-85%). One of them drank 10 cups of black tea per day. Moderate consumption of tea is considered as a tea drinker who consumes six cups of tea per day [11]. In the result of present study, if people consume in moderate, they will consume oxalate 20.70-92.82 mg/day for black tea while consumption of oolong tea, green tea and white tea will result in an intake of oxalate 13.56-59.22, 6.66-45.78 and 4.80-35.52 mg/day respectively. The American Dietetic Association (ADA) [22] recommended that patients who had kidney stones should consume dietary oxalate not more than 40-50 mg/day. If the tea is consumed six cups per day, this may cause recurrent stone formation, especially with black tea.

The present results indicated that the amount of oxalate found in black tea was significantly higher than that in the other types of tea. These differences may result from the degree or period of fermentation [23]. Black tea is made from tea leaves that are allowed to ferment for several hours to undergo a full oxidation. So, it is confirmed that oxalate occurs as metabolic end products of the oxaloacetate, glycolate and glyoxylate pathways in many plants [24]. As the oxalate contents in leaf form and bag form were not significantly different, it indicated that the form of tea did not influence on the amount of oxalate.

In addition, steeping duration had an influence on the amount of oxalate in tea samples, especially for first 3 minutes and slightly increased after 5 minutes. The longer the tea infusion time, the more oxalate extracted. These results were similar to the

study of McKay et al. [25]. It was found that 5-minutes steeping black tea had more oxalate content than 1-minute steeping black tea.

This study consisted of simple methods for preparing tea samples. The process of stirring tea leaves or squeezing tea bags were not included. As the previous study, stirring the tea leaves leads to significant increase in the oxalate extraction [25]. Thus, the result of this study showed the lowest amount of oxalate. Moreover, based on a real situation, filtered water was used for sample preparation. So tea drinkers could adjust suitable tea preparation for themselves.

CONCLUSION

Different types of tea and steeping durations had an influence on the amount of oxalate in tea. Black tea contained the highest amount of oxalate while white tea contained the lowest. People who have risk of kidney stones should avoid drinking high amounts of black tea and consume white tea as an alternative. Steeping tea for a short duration is also recommended.

ACKNOWLEDGEMENT

The authors would like to thank Scientific and Technological Research Equipment Centre, Chulalongkorn University for laboratory analysis. This study was supported by a grant from Graduate School of Chulalongkorn University, Graduate thesis grant in academia year 2011.

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