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ORIGINAL PAPER

Oxalate content of green tea of different origin, quality, preparation and time of harvest

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Abstract The purpose of the study was to analyse the oxalate content of green tea (*Camellia sinensis*) depending on origin, quality, time of harvest and preparation. Fifty-two green tea samples were received from different regions of China. The oxalate content of each tea infusion was measured using a validated HPLC-enzyme-reactor method. The soluble oxalate content of green tea ranged from 8.3 to 139.8 mg/l. In samples from known provenances, the highest oxalate concentration was found in green tea from Zhe Jiang. Low grade tea showed a tendency to lower oxalate concentration. Leaves reaped in the autumn when grown to full size yielded more oxalate than small and young leaves reaped in the spring. Modifications in steeping duration of tea leaves had no significant influence on the oxalate content of the beverage. Patients at risk for recurrent stone formation should take into account the oxalate content of green tea.

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Introduction

Green tea is a popular beverage worldwide with most frequent consumption in Asian countries, especially China and Japan. In European countries the popularity of green tea has increased within the last years.

Green tea is derived from the leaves of *Camellia sinensis*. The tea contains large amounts of polyphenols, monomeric flavonoids of the catechin family. The polyphenols polymerize after harvest due to an enzymatic oxidation. Whereas black tea is allowed to completely oxidize, oolong tea undergoes partial enzymatic oxidation. In contrast, for green tea, oxidation is stopped directly after harvest before drying. In China, the polyphenol oxidase enzyme is inactivated by dry heating the fresh leaves in hot pans. In Japan, the deactivation is carried out by applying hot water steam. Jasmine tea is green tea with jasmine flowers as a flavouring agent.

Green tea has recently gained scientific interest for its potential health benefits. The main interest focuses on the effects of green tea on cancer prevention due to its high content of polyphenols, such as epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG). Most of the effects of green tea are associated with their strong antioxidant potential [1, 2]. At present, epidemiological evidence of the effect of tea consumption against the development of human cancers is inconclusive, which may be attributed to variables related to individual differences in tea preparation and consumption patterns, as well as seasonal and geographic differences in tea production [3]. In addition, recent studies found that antioxidative effects of green tea might have an inhibitory effect on calcium oxalate stone formation [4, 5].

Calcium oxalate is the major constituent of about 75% of all urinary stones [6]. Hyperoxaluria is a primary risk factor for calcium oxalate stone formation. Urinary oxalate is derived from endogenous production and exogenous sources. Dietary oxalate absorbed from the gastrointestinal tract may contribute up to 50% of urinary oxalate excretion already in normal subjects [7]. Moreover, intestinal hyperabsorption of oxalate, defined as an absorption exceeding 10%, can make a considerable contribution to urinary oxalate, even in the absence of gastrointestinal disorders. A study using [$^{13}C_2$]oxalate reported an increased oxalate absorption in 46% of patients with calcium oxalate stone disease [8].

It is suggested that a significant source of soluble oxalate such as tea could increase urinary oxalate excretion, since soluble oxalate appears to have a higher bioavailability than insoluble oxalate. In contrast to black tea, oolong and green tea have been reported to be lower in oxalate [9, 10]. However, only a few samples of green tea have been analysed so far. Therefore, the aim of the present study was to determine the oxalate content of green tea depending on origin, quality, time of harvest and preparation.

Table 1 Oxalate content of Chinese green tea infusions (3.5 g/200 ml; 90°C; 10 min)

Tea	Oxalate (μM/l)	Oxalate (mg/l)
Cuan In 1	277	24.9
Cuan In 2	199	17.9
Da Zo	224	20.2
Fan San	533	48.0
Fu An	151	13.6
Fu Din	208	18.7
Gau Ga Nau	363	32.7
Jan Si Lu San In Wa	636	57.2
Jin Can Green	220	19.8
Lin De	281	25.3
Mao San Green	574	51.7
Sa Cha Longjing	133	12.0
Su Chu Bi Lu Zan 1	208	18.7
Su Chu Bi Lu Zan 2	200	18.0
Way Shvei Shan	299	26.9
Wu Lu	92	8.3
Zan Ho	160	14.4
Zan Zan	965	86.9
Green tea	697	62.7
Jasmine tea	532	47.9

Materials and methods

Samples

Fifty-two samples of Chinese green tea were received from Zhenjiang Medical College of Shanghai (Tables 1, 2, 3, 4). Thirty-two samples from known provenances were collected in four regions of China. The territories are (from north-east to south-west): Jiang Su, Zhe Jiang, Yun Nan and Guang Xi. Samples varied in their quality from extra to low grade (Table 3). For the investigation of the time of harvest, samples plucked in the spring and autumn, respectively, were analysed (Table 4, samples I–IV). Because only soluble oxalate is extracted during tea preparation, the soluble oxalate content of green tea samples was determined.

Brewing process and sample preparation

The two different methods for preparation of samples I–IV (Table 4) are shown in Table 5. Therefore, the samples were prepared by steeping 3.5 g of leaves in 200 ml 90°C water for 5 (A) or 10 min (B), respectively. Tea samples of Tables 1, 2 and 3 were prepared according to preparation method (B).

Each tea infusion was cooled to room temperature. For analysis samples were diluted 1:20 with distilled water (J.T. Baker Water; HPLC-reagent, Deventer, The Netherlands) and acidified with 50 μl 0.25 N hydrochloric acid (p.a.; Merck, Darmstadt, Germany) to stabilize potentially contained ascorbic acid. For determination 10 μl of the filtrated solution were injected twice.

Determination

For the determination of the oxalate content a selective and sensitive HPLC-enzyme-reactor method has been developed [11]. This method combines enzymatic conversion of oxalate to hydrogen peroxide and its amperometric detection with the selectivity of a chromatographic separation. HPLC-system (Gynkotek Modell 300, Gina 50, Germering, Germany) consisted of an anion exchange column (AS4A-DIONEX 4 × 50 mm, Sunnyvale, CA), a mobile phase of an aqueous EDTA solution (2.0 g/l, adjusted to pH 5.0 with NaOH; flow rate: 0.6 ml/min) (p.a.; Merck, Darmstadt, Germany), an enzyme reactor containing 5 units of immobilized oxalate oxidase (oxalate oxidase: E.C. 1.2.3.4.; Sigma Diagnostics, St. Louis, USA; carrier: VA Epoxy Biosynth, Riedel-de-Häen, Seelze, Germany) and an amperometric platinum detector (potential: +0.5 V; silver–silver chloride electrode; Gynkotek PED 300, Germering, Germany).

Table 2 Oxalate content of Chinese green tea infusions (3.5 g/200 ml; 90°C; 10 min) of different regions

Tea	Region	Oxalate (µM/l)	Oxalate (mg/l)
Xue Ja Cha	Guang Xi	1,300	117.0
Yin Sha	Guang Xi	231	20.8
Shi Shang Mao Feng	Guang Xi	847	76.2
Jin Mei Cha	Guang Xi	844	76.0
Da Ye Lu	Yun Nan	133	12.0
Yin Zhu	Yun Nan	223	20.1
Tup Cha	Yun Nan	244	22.0
Dain Jiang	Yun Nan	221	19.9
Hao Cha	Wu Xi/Jiang Su	225	20.3
Bi Luo Chun	Su-Zhou/Jiang Su	291	26.2
Mao Shan Qing Feng	Mao-Shan/Jiang Su	105	9.5
Yi Xing Ming Cha	Yi-Xing/Jiang Su	202	18.2
Nan Jing	Jiang Su	345	31.1
Nie Chin	Jiang Su	281	25.3
Wu Si	Jiang Su	1,024	92.2
Yang Zhou	Jiang Su	863	77.7

Table 3 Oxalate content of Chinese green tea infusions (3.5 g/200 ml; 90°C; 10 min) of different quality

Tea	Region	Quality	Oxalate (µM/l)	Oxalate (mg/l)
Tie Guan Yin	Zhe Jiang	Extra	322	29.0
Tie Guan Yin	Zhe Jiang	First class quality	397	35.7
Mei Cha	Zhe Jiang	High grade	603	54.3
Mei Cha	Zhe Jiang	Medium grade	633	57.0
Mei Cha	Zhe Jiang	Low grade	689	62.0
Se Zhong	Zhe Jiang	Extra	359	32.3
Se Zhong	Zhe Jiang	First class quality	341	30.7
Jian Cha	Zhe Jiang	High grade	931	83.8
Jian Cha	Zhe Jiang	Medium grade	805	72.5
Jian Cha	Zhe Jiang	Low grade	701	63.1
Dian Jiang	Zhe Jiang	High grade	1,500	135.0
Dian Jiang	Zhe Jiang	Medium grade	1,553	139.8
Dian Jiang	Zhe Jiang	Low grade	1,319	118.7
Qi Jiang	Zhe Jiang	High grade	1,405	126.5
Qi Jiang	Zhe Jiang	Medium grade	1,107	99.6
Qi Jiang	Zhe Jiang	Low grade	1,163	104.7

Results

The soluble oxalate content of Chinese green tea varied widely from 8.3 to 139.8 mg/l (Tables 1, 2, 3, 5). In samples from known provenances (Tables 2, 3), the highest oxalate concentration was found in green tea from Zhe Jiang (Table 3). Oxalate levels were lowest in tea samples from Yun Nan compared to samples from Zhe Jiang, Guang Xi and Jiang Su.

Specified quality had only a slight influence on the oxalate concentration of green tea (Table 3). The low grade quality showed a tendency to lower oxalate concentration in three of the four types of tea for which a high grade was compared to a low grade quality.

The amount of soluble oxalate in green tea samples varied with time of harvest (Tables 4, 5). Leaves reaped in the autumn seemed to contain more oxalate than leaves reaped in the spring.

Modifications in steeping duration of tea leaves between 5 and 10 min had no significant influence on the oxalate content of the beverage (Table 5). Unexpectedly, a longer extraction time yielded only a slight increase in oxalate concentration.

Table 4 Green tea from Jiang Su

	Leaf size	Time of harvest	Tea
I	Small	Spring	Nan Jing
II	Small	Spring	Nie Chin
III	Large	Autumn	Wu Si
IV	Large	Autumn	Yang Zhou

Table 5 Oxalate content of Chinese green tea infusions by time of harvest and preparation (3.5 g/200 ml; 90°C)

Extraction time	I		II		III		IV	
	μM/l	mg/l	μM/l	mg/l	μM/l	mg/l	μM/l	mg/l
A (5 min)	318.1	28.6	269.1	24.2	990.1	89.1	733.8	66.0
B (10 min)	345.2	31.1	281.0	25.3	1,024.4	92.2	862.9	77.7

Discussion

Hyperoxaluria, defined as urinary oxalate excretion above 0.500 mmol/24 h, is a major risk factor for calcium oxalate stone formation. Increased urinary oxalate may be caused by high dietary oxalate as well as by intestinal hyperabsorption of oxalate from normal dietary intake [8, 12]. Even small increases in urinary oxalate concentration have pronounced effects on crystallization and stone formation in the urine [13]. Patients at risk for calcium oxalate stone formation are therefore advised to avoid foods and beverages rich in oxalate. A high dietary oxalate intake does not only increase the risk for calcium oxalate stone formation, but may also decrease the bioavailability of minerals and trace elements essential for human health due to their ability to form complexes with divalent cations in the gastrointestinal tract [14–16].

The present results show, that the consumption of 1 l of Chinese green tea would result in an intake of between 8.3 and 139.8 mg soluble oxalate per day. Therefore, most of the levels of oxalate found in the green teas consumed in China are significantly higher than the values reported for four green teas available in New Zealand (1.3–9.1 mg/l) [10] and partly exceed the amounts of oxalate found in black tea [9, 10, 17]. Studies of oxalate bioavailability from brewed black tea without milk in normal healthy subjects have yielded absorption values from 0.08% over an 8-h postload period [18] to values of 1.9 and 4.7% calculated over a 6-h collection period for two tea brands [19], using the increment in urinary oxalate above basal levels as a measure of oxalate bioavailability. A recent study reported a mean absorption rate of 2.4% during a 6-h urine collection period after administration of 25 mg of $[^{13}C_2]$oxalic acid with four cups of black tea, containing 10.0 mg oxalate/150 ml cup [20]. However, it should be emphasized that oxalate bioavailability in these studies is an underestimate of true absorption rate because additional oxalate would have been recovered if the urine collection period would have been extended to 24 h. Accordingly, a previous study revealed an oxalate bioavailability from instant tea of 6.2% within a 24-h period [21]. A further limitation of the studies that assessed oxalate bioavailability from black tea is that they were carried out in healthy subjects. A study using $[^{13}C_2]$oxalate revealed intestinal hyperabsorption of oxalate, defined as an absorption exceeding 10%, in 46% of patients with calcium oxalate stone formation [8].

Assuming therefore that approximately 10% of the oxalate from green tea is absorbed by the gastrointestinal tract in calcium oxalate stone patients, 24-h urinary oxalate excretion may increase by up to 155 μmol in persons who drink four cups (1 l) of Chinese green tea per day. Compared with green tea, consumption of a similar volume of any herbal tea e.g. camomille (3 mg/l), fennel (13 mg/l), peppermint (6 mg/l) or fruit tea (6 mg/l), would contribute substantially less to the daily oxalate intake [17].

In samples from known provenances, the highest oxalate concentration was found in green tea from Zhe Jiang. These findings indicate that the oxalate content of Chinese green tea does not derive from the area of cultivation like south or north. The low grade tea showed a tendency to lower oxalate concentration. Green tea from lower quality is usually made from a higher part of stems, whereas tea from higher grade is made only from leaves. This could explain the slightly higher oxalate content of the higher grades. Moreover, the amount of oxalate in green tea may vary with time of harvest. Leaves reaped in the autumn when grown to full size yielded more oxalate than small and young leaves reaped in the spring. However, data on seasonal effects are limited because of the low number of samples. Finally, infusion of tea for a shorter duration has been found to extract less oxalate [9, 22]. Our results showed, however, only a weak effect of steeping duration on the oxalate content of the final extraction. The weak effect of steeping duration on oxalate content may have been at least partially due to comparing only 5 and 10 min duration. If a shorter steeping period had been compared a greater effect may have been observed. In contrast, stirring the tea has been shown to have a significant effect on oxalate extraction [9]. In conclusion, the results showed that the soluble oxalate content of Chinese green tea may vary strongly depending on the origin, quality, time of harvest and brewing process of the tea.

Two possible positive aspects of green tea consumption regarding calcium oxalate stone formation are worthy of note: the antioxidant potential and the contribution of tea intake to the increase in urinary volume. First, polyphenols, important constituents of green tea have been reported to have a potent antioxidative effect and might affect urinary stone formation. Itoh et al. [4] and Jeong et al. [5] found an inhibitory effect of green tea on crystallization and urinary stone formation. Their studies in rats, which involved administration of either sodium oxalate or ethylene glycol to induce calcium oxalate deposition, showed that urinary oxalate excretion and the number of crystals within kidneys decreased significantly in rats fed green tea instead of water. However, the application of these results to calcium oxalate stone formers is questionable because patients do not ingest such high doses of oxalate or ethylene glycol, respectively. Second, due to the wide range of oxalate in the brewed green tea, an increase in the daily intake of green tea is not generally advisable for calcium oxalate stone patients.

According to the findings, patients at risk for recurrent stone formation should take into account the oxalate content of green tea. Especially the declared grade of quality, origin and time of harvest of green tea should be considered. Further investigations should clarify whether oxalate concentration and catechin content of green tea leaves are associated.

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