EFFECT OF FLUORIDE ON CHEMICAL CONSTITUENTS OF TEA LEAVES

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SUMMARY: Seedlings of the tea plant, *Camellia sinensis* (L.), were grown hydroponically for 30 days to study the effect of fluoride (F) on the chemical composition and minerals in the leaves. Polyphenols, total catechins, and protein decreased significantly with increasing exposure to F. Except for epigallocatechin, most of the monomeric catechins also decreased significantly. These changes are not considered beneficial. On the other hand, the content of amino acids and soluble-sugars increased significantly, but the differences in caffeine and water-soluble extracts were not statistically significant. Except for magnesium and manganese, the uptake of most of minerals was inhibited, whereas the content of F increased markedly. These results suggest that the main chemical constituents of tea leaves decreased under F treatment and that individuals who consume these teas may ingest excessive amounts of F. Therefore, the direction of our further study will be to find ways to reduce the levels of F in tea plants.

Keywords: Amino acids in tea; Caffeine in tea; *Camellia sinensis* (L.); Catechins in tea; Fluoride in tea; Fu ding da bai tea; Minerals in tea; Polyphenols in tea.

INTRODUCTION

Tea as a beverage is an infusion of the leaves of the tea plant *Camellia sinensis* (L.). Native to China, today tea is the most widely consumed beverage in the world, aside from water.[1]

The chemistry of tea is complex: polyphenols, alkaloids, amino acids, glucosides, proteins, water-soluble extracts, minerals.[2] Tea infusions not only have a delicate taste and flavour, they also have many physiological and biological effects owing to the fact that they contain such compounds as polyphenols, caffeine, amino acids, etc.[3] Recently, tea has attracted attention for its health benefits, particularly with respect to its potential for preventing and treating cancer, cardiovascular diseases, and low-density lipoprotein oxidation.[4,5] In part because of these facts, the popularity of tea as a beverage has grown all over the world.

As a phytotoxin in air, water, and soil, F is released into the environment from a number of industrial sources,[6,7] application of phosphate fertilizers in agriculture,[8] and weathering of volcanic ashes.[9] For example, in 1999 the total quantity of F released into the atmosphere from industrial production in China was estimated at 2 million metric tons.[10] The F content, 1~20 mg/kg in various plant leaves, is reported to reach 871~1337 mg/kg in mature tea leaves, and even more than 2000 mg/kg in leaves of old tea plants.[11,12] Thus it is well known that tea leaves can

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accumulate massive amounts of F. However, we do not know what changes occur in the chemical composition of tea when F has accumulated in leaves.

In the present study, Fu ding da bai tea, the primary cultivated strain in China, was used as the experimental material. In this study, changes in the chemical composition and mineral content of tea leaves from tea plants grown hydroponically with different F concentrations in the nutrient solution were investigated.

MATERIALS AND METHODS

Materials: The experiment was conducted in a glass greenhouse. One-year-old cutting seedlings of Fu ding da bai were provided by the Fruit and Tea Research Institute of Hubei Agricultural Academy. The standards of (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin gallate (EGCG), (+)-catechin (C), and caffeine were purchased from Fisher Chemical Reagent Co. Ltd., USA. Methanol (chromatographic grade) was purchased from Fisher ChemAlert Guide, New Jersey, USA. Superoxide dismutase reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute, China. All other chemicals used were of analytical grade.

Plant treatments: Camellia sinensis (L.) seedlings were planted in plastic pots containing 1.5 L of one-half strength of Hoagland nutrient solution.[13] The nutrient solution contained the following ingredients (in mmol/L): $Ca(NO_3)_2{\cdot}4H_2O$, 2; KNO_3, 3; $NH_4H_2PO_4$, 0.5; $MgSO_4{\cdot}7H_2O$, 1; and (in µmol/L) H_3BO_3, 50; $MnCl_2{\cdot}4H_2O$, 0.9; $ZnSO_4{\cdot}7H_2O$, 0.76; $CuSO_4{\cdot}5H_2O$, 0.32; $(NH4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.016; EDTAFe, 50. F was supplied as NH_4F with six concentrations (mmol/L): 0, 0.11, 0.21, 0.32, 0.53, and 0.84. For each treatment, 5 pots (with 5 seedlings each) were used, and the pots were arranged in the glasshouse in random design. The seedlings were cultivated in the glass greenhouse under constant conditions (temperature, 25°C during the day and 15°C at night; light:dark, 16:8 h). The liquid solutions (pH 5.8) were ventilated for 1 hr every 3 hr with air pumps. The solutions were replaced completely every five days. The period of growth was 30 days.

Determination chemical constituents: The fresh leaves of tea plants were oven-dried at 80°C for 24 hr, then ground to pass through a 2-mm sieve. For infusion, 1.5-g dry tea samples were extracted by 120 mL of boiling distilled water for 30 min. The infusions were filtered into a standard flask of 250 mL and diluted to the mark with distilled water.

The tea infusions were analyzed for total polyphenols, amino acids, protein, sugar, and water-soluble extracts according to the China National Standard by using Uv-vis spectrophotometry.[14] Catechins and caffeine were determined by high performance liquid chromatography (HPLC) (VAREAN, USA; Column: Agilent TC-C 18, 4.6×150 mm, USA) according to Zhou.[15]

Minerals in the tea leaves were assessed by atomic absorption spectrometry.[16,17] Total F was determined with an ion selective electrode (9609BNWP, Thermo, USA). The ground sample (0.2000g) was extracted with 10 mL of 0.2 M HCl at room temperature for 1 hr. After filtration, 25 mL of TISAB was added. Finally, the volume was fixed to 100 mL with ultra-pure water (GB/T5009.18-2003).

Superoxide dismutase (SOD): For this determination, 0.5000 g of fresh leaves was ground on ice with 0.5 g of quartz sand and 5 mL of 50 mM precooled phosphate buffer (pH 7.8) containing 1 mM ethylenediaminetetracetic acid (EDTA), and 5% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifugated (16,000 g) at 4°C for 15 min. The activity of SOD [EC.1.15.1.1] was determined by the xanthine oxidase method. The measurement was performed following the directions of the reagent kit.

Statistical analysis: All statistical analyses were done using the statistical package of the SAS software computer program. ANOVA followed by LSD tests were conducted to determine statistical significance. Differences at $P<0.05$ were considered significant. Differences at $P<0.01$ were considered highly significant differences. All data were expressed as mean ± standard deviation (SD) of three replicates.

RESULTS AND DISCUSSION

The effect of F treatment on biochemical parameters of tea leaves is shown in Table 1.

Table 1. Biochemical parameters in tea leaves (%). (Values are mean±SD)

F (mM)	Polyphenols	Amino acids	Protein	Soluble-sugar	Water soluble extracts
0	13.63±0.12	2.38±0.08	4.25±0.03	7.64±0.26	32.14±0.60
0.11	13.42±0.29	2.46±0.06	3.96±0.04†	8.18±0.25†	33.41±0.89
0.21	12.61±0.28†	2.62±0.06	3.81±0.05†	8.38±0.26†	32.28±1.18
0.32	12.35±0.25†	2.79±0.05†	3.58±0.05†	8.33±0.31†	33.81±0.70*
0.53	12.12±0.38†	2.81±0.01†	3.28±0.03†	8.47±0.30†	33.41±0.26
0.84	11.78±0.03†	3.02±0.02†	3.18±0.08†	8.51±0.34†	33.40±0.42

Compared with the control group, *$p<0.05$; †$p<0.01$.

Polyphenol compounds are regarded as the key elements that determine both colour and taste qualities of green tea during processing.[18] In addition, tea polyphenols are the most biologically active group of tea components that may affect the pathogenesis of several diseases, especially antioxidative, antimutagenic, and anticarcinogenic.[19-21] The results here showed that the content of polyphenols decreased significantly ($p<0.01$) with increasing exposure to F and had a significant negative correlation with F concentration ($r= –0.9186$). This finding is in agreement with the result of Lv Yi.[22] This decrease in polyphenol content is not considered beneficial.

A high correlation exists between the quality of green tea and the content of amino acids, especially that of theanine. Theanine exists only in the free form and is the most important free amino acid in tea.[23] Besides having a delicate taste, theanine has many important biological effects. It has been reported that theanine is able to decrease norepinephrine and serotonin levels in the brain and to reduce blood pressure.[24] In the present study, there was a significant increase in amino acids in tea leaves ($p<0.01$) with increasing exposure to F during growth, which might be attributed to the degradation of soluble protein. As seen in Table 1, the content of protein decreased significantly ($p<0.01$) from F treatment. The content of soluble sugar increased remarkably ($p<0.01$), while the differences in water-soluble extracts were not statistically significant.

The concentrations of caffeine and catechins are presented in Table 2.

Table 2. Concentrations of caffeine and catechins in tea leave by HPLC-UV (μg/mL). (Values are mean±SD)

F (mM)	EGC	C	CAF	EGCG	EC	ECG	Total catechins
0	182.7±11.9	16.6±0.8	262.2±4.9	296.3±16.6	92.3±2.6	62.1±0.6	661.6±13.6
0.11	180.5±2.3	15.3±0.2*	256.7±19.8	281.3±8.9†	90.8±1.6	44.6±7.2†	612.0±5.5*
0.21	176.1±2.8	9.5±0.2†	245.8±16.3	282.0±0.5†	89.6±1.4	37.0±2.4†	593.9±2.1†
0.32	176.0±10.5	8.6±0.1†	244.5±7.1	281.0±3.9†	88.0±2.1*	33.3±2.1†	587.0±12.3†
0.53	166.3±0.8*	7.5±0.6†	242.2±3.9	274.5±5.9†	85.4±1.5†	30.9±1.6†	564.4±3.7†
0.84	167.3±3.5*	7.3±0.4†	238.4±1.5	275.1±11.6†	76.0±2.0†	16.5±1.9†	550.4±5.7†

Compared with the control group,*$p<0.05$; †$p<0.01$.

Tea polyphenols are the leading functional component and one of the most important parameters of tea quality. They are mainly composed of catechins up to 70-80%. Tea leaves contain five major catechins: C, EC, ECG, EGC, and EGCG. These compounds are mainly responsible for the characteristic astringent and bitter taste of tea brews. Because catechin monomers have different anti-oxidative and health properties, it is important to know the concentration of each of them in the infusions. Our results showed that most of the monomeric catechins (e.g. C, EC, ECG, EGCG) decreased significantly ($p<0.01$) except for EGC with increasing exposure to F. Therefore, the anti-oxidative effect was reduced. In support of this interpretation, as seen in the Figure the activity of SOD decreased significantly ($p<0.01$) with F exposure. EGCG is the catechin present in the largest amount, implicating it as the main active ingredient. It is therefore reasonable to infer that the decrease in superoxide scavenging of reactive oxygen species radicals was due to reduction of EGCG content. Toschi et al. have observed that the antioxidant activity of the green tea is higher in the teas that contain higher levels of EGCG and EGC.[25]

Besides catechins and amino acids, caffeine is the main methylxanthine constituting the tea alkaloids and is another important factor in determining the quality of tea because of its stimulative effect. In contrast to the catechins in green tea leaves, caffeine can noticeably enhance tea flavor.[26] Recent researches also suggest that caffeine in tea leaves may play an important role in free radical scavenging. In our study, the difference in caffeine was not statistically significant with different F exposure.

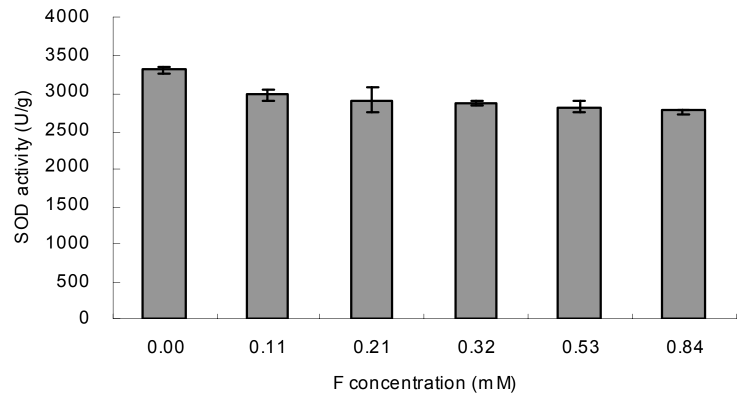

Figure. The effect of F on SOD activity in tea leaves. Values are mean±SD.

Results of the analyses of the chemical elements present in the tea leaves are summarized in Table 3.

Table 3. Content of the elements in tea leaves (as mg/g for Ca, Mg, and K, as mg/kg for Fe, Mn, Na, Cu, Zn, Al, and F). (Values are mean±SD)

F (mM)	Ca	Mg	K	Cu	Mn	Zn	Fe	F
0	9.66±0.10	6.62±0.09	20.09±0.15	13.78±0.30	1.88±0.01	122.87±1.98	470.42±4.23	1283±16
0.11	9.48±0.06	6.41±0.11	19.79±0.87	13.45±0.23	1.89±0.02	107.59±5.00†	538.52±1.57†	1525±6†
0.21	9.39±0.05	6.43±0.05	18.65±0.04†	13.02±0.33	1.98±0.01†	87.77±2.67†	532.46±1.40†	1702±15†
0.32	9.30±0.27	6.38±0.10	18.73±0.52†	12.52±0.49*	2.02±0.01†	80.63±1.89†	480.01±6.57	1761±20†
0.53	9.28±0.03*	6.52±0.15	18.61±0.14†	11.79±0.14†	2.07±0.01†	79.00±1.38†	476.61±5.11	1876±14†
0.84	9.15±0.01†	6.50±0.15	18.46±0.16†	11.40±0.29†	2.11±0.03†	74.69±0.90†	474.50±8.39	2171±31†

Compared with the control group,*p<0.05; †p<0.01.

As can be seen in Table 3, tea can be an important source of some minerals. The content of Mn increased significantly ($p<0.01$) with increasing exposure to F. On the other hand, the content of Cu, Zn, K ($p<0.01$), and Ca ($p<0.05$) decreased significantly, and the content of Fe first increased and then decreased ($p<0.01$), while the content of Mg increased slightly but not significantly.

The tea plant takes up F from the soil and accumulates it in its leaves where it becomes a major source of F. As seen in Table 3, F content in tea leaves showed an increasing tendency as its concentration in the nutrient solution increased with a very high positive correlation ($r=0.9667$, $p<0.01$). A substantial amount of F is released during tea infusion and nearly all (about 94.9%) of the released F is absorbed by consumers.[27]

Since there is increasing evidence that F exposure may actually increase the risk of tooth decay as well as dental fluorosis,[28] it is desirable to seek and develop measures to reduce F levels in tea plants. Also worth noting is the fact that early-stage skeletal as well as dental fluorosis in children has been traced to high-F brick tea,[29] and adult skeletal fluorosis has been reported from excessive consumption of instant tea with 3.3 ppm F.[30]

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