EFFECT OF FLUORIDE ON THE AMINO ACID COMPOSITION OF TEA LEAVES

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ABSTRACT: Hydroponic solution culture experiments were carried out on seedlings of the Fu ding da bai variety of tea (*Camellia sinensis*, L.) for 30 days in order to investigate the influence on the leaf amino acid composition of the fluoride ion (F) in concentrations of 0–16 mg F/L. The results indicated that, as the F ion concentration increased, the total amino acid content and the contents of the individual amino acids, apart from Tyr and Cys, generally increased initially and then decreased. In contrast, as the F concentration increased, the Tyr content decreased significantly while the Cys content fluctuated with an initial increase followed by a decline and then a further increase. With increasing F concentrations, the bitter amino acid content generally decreased, while the sweet, delicious, and flowery amino acid contents initially increased, reaching a maximum at 4 mg F/L, and then declined. In conclusion, low concentrations of F, up to 4 mg F/L, can promote the synthesis of the amino acids with sweet, delicious, and flowery tastes, without increasing the bitter amino acid content, resulting in an improved tea quality, while higher F concentrations, 6–16 mg F/L, tend to reduce amino acid synthesis and lower the tea quality.

Keywords: *Camellia sinensis* (L.); Fluoride effect on tea leaves; Fu ding da bai tea; Hydroponic tea growth; Tea amino acid composition.

INTRODUCTION

In previous studies, we investigated the effect of the fluoride ion (F) on the composition of the leaves of hydroponically grown seedlings of the Fu ding da bai tea plant (*Camellia sinensis*, L.), including polyphenols, amino acids, caffeine, catechins, minerals, and aroma.[1,2] The objective of the present study was to further investigate the effect of fluoride on the leaf amino acid composition of hydroponically grown Fu ding da bai tea plants (*Camellia sinensis*, L.) in order to provide additional information on the effects of F on the quality of tea that, together with the earlier work, might provide a basis for further studies.

MATERIALS AND METHODS

Plant treatments: The method of processing the seedlings of the Fu ding da bai variety of tea (*Camellia sinensis*, L.) was the same as previously reported with some modifications.[2] F was supplied as NH_4F with six concentrations of F: 0, 2, 4, 6, 10, and 16 mg F/L. The pH adjusted to 5.5±0.1 and the N content was leveled by adding urea solution (Table 1). For each 30-day treatment, 15 pots with 8 seedlings each were used.

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Amino acid analysis: The amino acid components were detected with a HITACHI L-800 high speed amino acid analyzer and measured with a GB/T5009.124-2003. The analysis was performed with a separation column (ion exchange column 2622 sc.ph, 4.6×60 mm) and an amino removal column (ion exchange column 2622 sc.ph, 4.6×60 mm). The temperatures of the separation and reaction columns were 57ºC and 135ºC, respectively, and the column pressures were 10.0 Mpa in pump one and 1.07 MPa in pump two. The flow rate was 0.40 mL/min in pump one and 0.35 mL/min in pump two.

Table1. Levelling of the N content of the NH_4F treatments by the addition of urea $CO(NH_2)_2$

Parameters	NH_4F treatments					
	1	2	3	4	5	6
F concentration of each NH_4F treatment (mg/L)	0	2	4	6	10	16
F concentration of each NH_4F treatment (mMol/L)	0	0.105	0.211	0.316	0.526	0.842
N concentration of each NH_4F treatment (mg/L)	0	1.47	2.95	4.42	7.37	11.79
Total volume of each NH_4F treatment (L)	22.5	22.5	22.5	22.5	22.5	22.5
N content of each NH_4F treatment of 22.5 L (mg)	0	33.1	66.4	99.5	165.4	265.3
Volume of urea solution $CO(NH_2)_2$ with 1 g N/L added (mL)	265	232	199	166	99.5	0
N content in the added urea solution $CO(NH_2)_2$ (mg)	265	232	199	166	99.5	0
Total N content of each NH_4F treatment after levelling (mg)	265	265	265	265	265	265

Theanine analysis: For the theanine analysis, a HPLC (Varean, high performance liquid chromatograph, USA) and an Agilent TC-C18 column (particle size 5 µm, 150 length × 4.6 mm i.d.) were used.[3] The mobile phase was composed of acetonitrile-0.1% phosphoric acid solution (containing 10 mL sodium dodecyl sulfate [SDS]/L), the proportion was 64:36, the flow rate was 1 mL/min, the temperature was 32°C, the detective wavelength was 200 nm, and the injection volume was 20 µL.

Statistical analysis: All statistical analyses were done using the statistical package of the SAS software computer program. ANOVA followed by the LSD test were carried out to test the significance.

RESULTS AND DISCUSSION

Free amino acids in tea leaves are the important biochemical components that determine the aroma and taste of tea. At present, 26 kinds of amino acid have been separated and identified in tea and usually account for 1.5–4% of the dry weight.[4] In this study, 18 kinds of amino acid were tested (Table 2).

Table 2. Effect of fluoride on the amino acid composition of tea leaves (mg/g)

Amino acids	Fluoride concentrations (mg/L)					
	0	2	4	6	10	16
Asp	11.74^{ab} ±0.09	11.82^{ab} ±0.20	11.97^{a} ±0.17	11.69^{ab} ±0.14	11.66^{ab} ±0.11	11.54^{b} ±0.17
Thr	5.07^{c} ±0.01	5.22^{b} ±0.07	5.44^{a} ±0.07	5.23^{b} ±0.06	5.06^{c} ±0.01	5.06^{c} ±0.04
Ser	7.81^{b} ±0.15	7.82^{b} ±0.04	8.12^{a} ±0.03	8.05^{a} ±0.08	7.69^{bc} ±0.01	7.61^{c} ±0.07
Glu	19.90^{b} ±0.23	19.99^{ab} ±0.29	20.42^{a} ±0.37	20.12^{ab} ±0.13	19.66^{b} ±0.06	18.93^{c} ±0.15
Pro	5.9^{b} 8±0.12	6.22^{ab} ±0.26	6.37^{a} ±0.22	6.30^{ab} ±0.14	6.06^{ab} ±0.17	6.28^{ab} ±0.07
Gly	7.24^{bc} ±0.08	7.32^{abc} ±0.11	7.41^{a} ±0.02	7.35^{ab} ±0.05	7.19^{cd} ±0.02	7.09^{d} ±0.02
Ala	7.17^{d} ±0.20	7.46^{bc} ±0.08	7.70^{a} ±0.01	7.60^{ab} ±0.01	7.40^{bc} ±0.04	7.31^{cd} ±0.11
Cys	0.46^{c} ±0.03	0.99^{a} ±0.06	0.40^{c} ±0.03	0.36^{c} ±0.04	0.75^{b} ±0.10	0.68^{b} ±0.06
Val	7.23^{abc} ±0.04	7.41^{a} ±0.14	7.28^{ab} ±0.03	7.20^{bc} ±0.03	7.07^{cd} ±0.11	6.99^{d} ±0.07
Met	1.39^{abc} ±0.02	1.30^{bcd} ±0.03	1.49^{a} ±0.08	1.46^{ab} ±0.10	1.22^{d} ±0.01	1.23^{cd} ±0.12
Ile	5.56^{bcd} ±0.19	5.65^{bc} ±0.11	5.94^{a} ±0.06	5.72^{b} ±0.04	5.51^{cd} ±0.03	5.38^{d} ±0.01
Leu	11.39^{bc} ±0.24	11.47^{ab} ±0.17	11.70^{a} ±0.01	11.61^{ab} ±0.06	11.15^{cd} ±0.13	10.95^{d} ±0.03
Tyr	5.18^{a} ±0.23	4.27^{b} ±0.21	4.09^{b} ±0.02	3.55^{c} ±0.10	3.62^{c} ±0.25	3.31^{c} ±0.09
Phe	7.08^{c} ±0.03	7.16^{bc} ±0.23	7.43^{a} ±0.06	7.36^{ab} ±0.15	7.12^{bc} ±0.01	7.02^{c} ±0.06
Lys	9.74^{ab} ±0.10	9.86^{a} ±0.28	9.81^{ab} ±0.07	9.81^{ab} ±0.06	9.53^{bc} ±0.07	9.36^{c} ±0.01
His	3.01^{ab} ±0.04	3.04^{ab} ±0.02	3.07^{a} ±0.01	2.97^{bc} ±0.05	2.99^{b} ±0.02	2.91^{c} ±0.04
Arg	16.64^{b} ±0.25	16.71^{ab} ±0.31	17.10^{a} ±0.02	16.87^{ab} ±0.10	16.71^{ab} ±0.26	15.24^{c} ±0.13
Thea	5.21^{d} ±0.27	5.59^{c} ±0.18	6.65^{b} ±0.22	6.99^{a} ±0.09	6.89^{a} ±0.13	6.54^{b} ±0.06
Total amino acids	137.81^{b} ±1.68	139.30^{ab} ±2.53	142.3^{a} ±0.88	140.25^{ab} ±0.85	137.27^{b} ±1.32	133.43^{c} ±0.75

Note: Values are mean±SD of three measurements (an initial analysis and two replicate analyses). Different lower case letters in each row represent significant differences at $p<0.05$, based on LSD's multiple range test.

As the F ion concentration increased, the total amino acid content and the contents of the individual amino acids, apart from Tyr and Cys, generally increased initially and then decreased (Table 2). In contrast, as the F concentration increased, the Tyr content decreased significantly while the Cys content fluctuated with an initial increase followed by a decline and then a further increase. The maximum concentrations for Val and Lys were reached at a F concentration of 2 mg F/L, for Thea at 6 mg F/L, and for the other amino acids at 4 mg F/L. The total amino acid content also showed an initial increase with a maximum at 4 mg F/L followed by a decrease. Previous studies showed that 6 mg F/L is the critical value for the effect of F on the physiological index of tea and the cell ultra-structure.[5] In the present study, the critical value for the effect of F on the amino acid components was found to be 4 mg F/L. This suggests that the effect of F on the amino acid content may result from the effect of F on the physiological metabolism. In a pot experiment by Tang Qian et al., with F concentrations of 20, 50, 100, 150, and 200 mg F/kg, as the F concentration increased the component amino acids and the total amino acid content of Fu ding da bai tea and Ming shan bai hao tea both decreased.[6] The Qian et al. study differed from ours by having far higher F concentrations but the decrease in the component and total amino acid content was consistent with our results for F concentrations exceeding 4 mg F/L.

Free amino acids usually produce different taste sensations, such as delicious, sweet, bitter, etc. The differences in the amino acid contents and their ratios cause the different flavor characteristics of tea infusions. Amino acids also contribute to the tea aroma and the interaction of free amino acids and carbohydrate could enhance the fragrance and taste of tea in the processes of drying and extracting perfume.[7] Bitterness was produced by five amino acids: Ile, Leu, Phe, Tyr, and Val; sweetness by five amino acids: Ser, Ala, Gly, Pro, and Thr; and a flower fragrance by three amino acids: Glu, Ala, and Phe.[7] Methyl pyruvate sulfide, hydrolyzed by methionine, is an important component of the aroma of new tea, so Met was classified as a floral amino acid.[7] In the present study, the bitter amino acid content tended to decrease with increasing F concentrations, contributing to improved flavor quality, while the contents of the sweet, delicious, and flowery amino acids showed an initial increase, with a maximum 4 mg F/L, followed by a decrease (Figure).

CONCLUSION

In conclusion, low concentrations of F, up to 4 mg F/L, can promote the synthesis of the amino acids with sweet, delicious, and flowery tastes, without increasing the bitter amino acid content, resulting in an improved tea quality, while higher F concentrations, 6–16 mg F/L, tend to reduce amino acid synthesis and lower the tea quality.

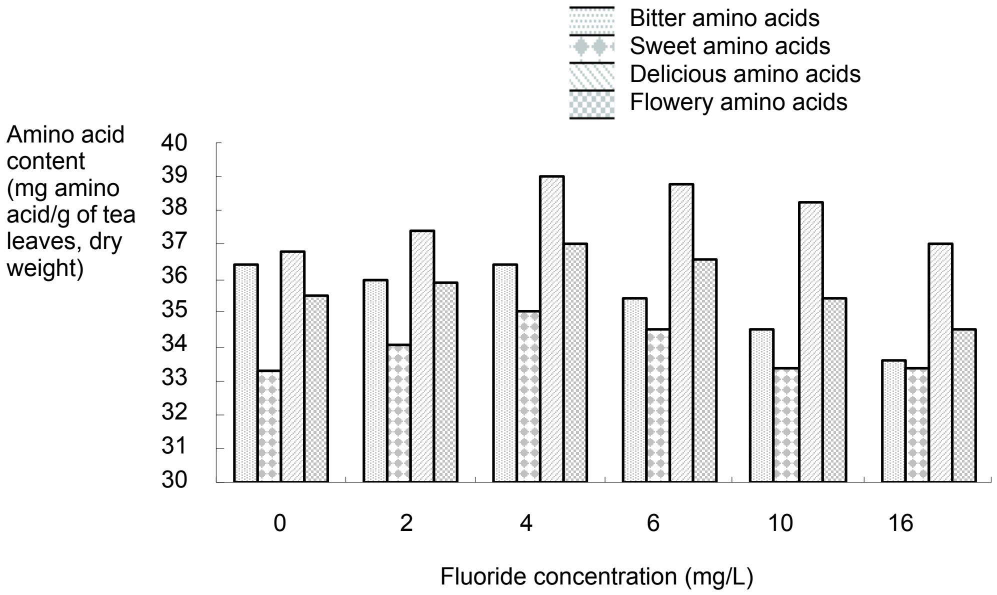

Figure. Effect of fluoride on the tea leaf content of amino acids with bitter, sweet, delicious, and flowery tastes. For each group of four columns, for each fluoride concentration, the column order, from left to right, is: bitter amino acids, sweet amino acids, delicious amino acids, and flowery amino acids.

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