

# Comparative studies on the qualities of green teas in Karst and non-Karst areas of Yichang, Hubei Province, PR China

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## Abstract

Green teas (*Camellia sinensis*) from the non-Karst and the Karst areas of Yichang, Hubei Province, PR China, have been compared as regards L-proline content of the green tea and the quality, as well as the soil properties. The results showed that some qualities of green tea from the Karst region, such as a higher content of L-proline and a lower contents of aluminum and fluoride were beneficial. Other qualities of green tea from the Karst region, for example, the lower content of tea polyphenols, were not beneficial. The differences in amino acids and caffeine contents were not statistically significant.

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**Keywords:** Green tea; Karst; Proline; Tea polyphenols; Caffeine; Aluminum; Fluoride

## 1. Introduction

Tea (*Camellia sinensis*) is a popular beverage in the world. Tea is an evergreen shrub. The favoured conditions for tea cultivation include a suitable temperature (15–25 °C), high relative humidity (80–90%) and high annual rainfall (around 1500–2000 mm). Most of the tea plantations in China are found on mountains with mist, and the high humidity ensures that leaves grow more slowly and remain tender (Shu, Zhang, Lan, & Wong, 2003; Dong, Xie, & Du, 2001; Cao, Zhao, & Liu, 1998). The soil in Karst areas is unsuitable for plant by tea unless a special improving method is employed (Wang & Chen, 1994). Evidently there are no statistically significant differences between the qualities of green teas from the Karst and the non-Karst areas (Wu, Pan, Yin, & Zhou, 2003).

It has been claimed that Alzheimer's disease (AD) is associated with the Al content in the human brain (McLachlan, 1995), so the high Al content of tea is a concern.

L-Proline accumulation in plant cells exposed to salt or water stress is a widespread phenomenon (Chandler & Thorpe, 1986; Buchanan, Gruijsem, & Jones, 2000). L-Proline is believed to protect plant tissues against stress by acting as N-storage compound, osmotic-solute and hydrophobic protectant for enzymes and cellular structures (Tripathi & Gaur, 2004). L-Proline accumulation appears to be an additional defence against metal-induced oxidative stress (Lin & Kao, 1996). L-Proline residues are responsible for affinity toward proanthocyanidins and take part in the production of aromatic compounds (Outtrup, 1989). The L-proline content differs significantly between beers, depending on the raw materials and enzymic reactions used in beer technology, but the reasons are not detailed (Gorinstein et al., 1999).

Yichang, Hubei Province, China, is one of the popular regions for tea production. The China Three Gorges Project is built in Yichang, Hubei Province, China. The construction of the three Gorges reservoir may bring, not only new opportunities for regional development, but also new problems in relation to the ecological environment (Wu, Huang, Han, Xie, & Gao, 2003). The tea plants in the three Gorges area mainly grow on the non-Karst area,

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but some grow on the Karst area. We do not know what changes occurred in quality of green tea when the China Three Gorges Project was built.

In this paper, we compare the soil properties, the L-proline content of the green tea and the other qualities of green tea between the Karst and the non-Karst areas in Yichang, Hubei Province, China.

## 2. Materials and methods

Qian-zhang-yan tea plantations (shortened form: Q) and Deng-cun tea plantations (shortened form: D) were located in the main tea production regions in Yichang, Hubei Province, in southwestern China. There is abundant plant biodiversity in the healthy vegetation next to these two tea plantations. Q is to the south of the Chang-Jiang river, whereas D is to the north. The distance between the two tea plantations is about 100 km. The study sites have a subtropical climate. Their elevations are both 800 m above sea level, where the frost-free period is 280 days in a year. The annual average temperature there is 13 °C and the annual rainfall is 1400–1500 mm. The difference in temperature is great between day and night. It is far away from the industrial area. At Q, the soil is a little acidic and yellow, part of the Karst region, where the bedrock is dolomite. At D, the soil is an acidic sandy loam, part of the non-Karst region, where the bedrock is granite (Yichang Institute of Geology & Mineral Resources, Chinese Academy of Geological Sciences, 1987). Organic fertilizer, urea and special fertilizer, which included some N, P, K and trace elements, were used in these two tea plantations. The fertilizer was applied at the same rate in all treatments.

The experiment, laid out in a randomized block design, had five replications. The soil samples were taken from the tea plantations according to the literature (Shu et al., 2003), at a depth of 10–20 cm below the surface. The tea flush (bud with two leaves) was gathered at the study sites, at distances not more than 200 cm from the location of the soil sample. The green tea products were produced from the tea flush by the same procedure (Chen, 1992; The first research institute of China Standards Publisher, 2003). The green tea and soil were taken on April 4, 2005 (i.e., spring green tea).

Soil samples were air-dried and passed through a 2-mm sieve to determine the following (Shu et al., 2003; Li, Yu, Li, & Li, 2005): pH (pH meter, soil:distilled water 1:1); total F (extracted with 0.2 M HCl for 4 h × 5 times, and tested with an ion-selective electrode). Dried soil samples (0.1000 g), 7 ml concentrated HNO<sub>3</sub> and 3 ml concentrated HF were placed in PTFE vessels of the microwave digestion system. After digestion, the contents of Al, Ca, Mg, Zn, Cu, Fe, Mn, K, Na (ICP-AES for Al, FAAS for Ca, Mg, Zn, Cu, Fe, Mn, K, Na) were determined by the standard addition method (Li et al., 2005; Li, Yu, Li, & Li, 2006). The organic C content was corrected for carbonate, which was determined by subjecting the samples to 10% hydrochloric acid (HCl) and measuring the CO<sub>2</sub> evolution. The organic

C and nitrogen contents of all samples were measured with a VarioEL III analyser (Rosenberg et al., 2003).

The samples of tea products were oven-dried at 60 °C for 24 h, then ground to pass through a 2 mm sieve. Total Al, Ca, Mg, Zn, Cu, K (ICP-AES for Al, AAS for Ca, Mg, Zn, Cu, K after digestion with 16 M HNO<sub>3</sub> + 12 M HClO<sub>4</sub>) and total F (extracted with 0.2 M HCl for 4 h × 5 times, and tested with an ion-selective electrode) were determined (Shu et al., 2003; Li et al., 2005; Li et al., 2006).

A portion of the collected green tea products was subjected to laboratory analysis to assess total polyphenols, caffeine and amino acids according to the China National Standard by using UV-vis spectrometry and HPLC (The first research institute of China Standards Publisher, 2003). L-Proline was extracted and its concentration was determined by the method of (Bates, Waldren, & Teare, 1973). Leave were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged. The supernatant was treated with acetic acid and acid ninhydrin and the whole boiled for 1 h. The absorbance at 520 nm was determined. Levels of L-proline were expressed as µg/g dry weight.

Five replicate measurements were made and the mean values and the standard deviations calculated. All samples were used for experiments within one week.

## 3. Results and discussion

Some chemical properties of the surface soils at the sample sites are presented in Table 1. The soil at Q was a little acidic and yellow, part of the Karst region, where the bedrock is dolomite. The soil moisture content was only 11–16%. The soil at D was an acidic sandy loam, part of the non-Karst region, where the bedrock is granite. The soil moisture content was 17–24%. The *T*-test was used in this study, in which a *P*-value smaller than 0.05 is obtained at the 95% confidence level (Dean, 1995) and showed the soil moisture contents, the contents of organic C and the pH in these two tea plantations to be statistically significant different. The contents of total N showed no statistically significant differences.

The mineral elements of the surface soils at the collection sites are presented in Table 2. The contents of Ca, Mn, Na, Cu and Zn in soil from Q are significantly higher than those from D. The contents of Mg, Fe, Al and F in soil from Q are significantly lower than those from D. The K content showed no statistically significant difference.

The mineral elements of green tea from the two tea plantations are presented in Table 3. The contents of Ca, Mn and Cu in green tea from Q are significantly higher than those from D. The contents of Mg, K, Fe, Na, Al and F in green tea from Q are significantly lower than those from D.

The content of Zn showed no statistically significant difference, although, the content of Zn in soil from Q was significantly higher than that from D. The content of K in green tea from Q was significantly lower than that from D, although, the content of K in these two soils was not statistically significantly different.

Table 1  
Some chemical properties of surface soils at Qian-zhang-yan and Deng-cun tea plantations in Yichang, Hubei Province, PR China

Site	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	C/N ratio	pH	Soil moisture content (%)
Q	8.74 ± 0.30	2.45 ± 0.25	3.57 ± 0.54	6.46	11–16
D	9.36 ± 0.41	2.41 ± 0.22	3.88 ± 0.58	5.87	17–24

Q is Qian-zhang-yan tea plantation.

D is Deng-cun tea plantation.

The results are mean values,  $n = 5$ ,  $p < 0.05$ .

Table 2  
The contents of some elements (as mg/g for Ca, Mg, K, Fe, Mn and Al, as mg/kg for Na, Cu, Zn and F) in surface soils from Qian-zhang-yan and Deng-cun tea plantations in Yichang, Hubei Province, PR China

No.	Q	D
Ca	4.16 ± 0.22	3.01 ± 0.19
Mg	2.61 ± 0.20	4.30 ± 0.25
K	4.70 ± 0.31	4.78 ± 0.31
Fe	32.9 ± 1.8	40.0 ± 2.1
Mn	1.92 ± 0.09	0.273 ± 0.022
Al	47.4 ± 1.1	61.0 ± 1.3
Na	66.8 ± 5.0	43.6 ± 4.5
Cu	57.4 ± 3.1	19.8 ± 1.0
Zn	139 ± 9	72.2 ± 5.3
F	277 ± 5	289 ± 5

Q is Qian-zhang-yan tea plantation.

D is Deng-cun tea plantation.

The results are mean values,  $n = 5$ ,  $p < 0.05$ .

Table 3  
The contents of some elements (as mg/g for Ca, Mg and K, as mg/kg for Fe, Mn, Na, Cu, Zn, Al and F) in green tea from Qian-zhang-yan and Deng-cun tea plantations in Yichang, Hubei Province, PR China

No	Q	D
Ca	3.15 ± 0.18	2.85 ± 0.18
Mg	1.26 ± 0.09	1.33 ± 0.11
K	19.5 ± 0.22	24.1 ± 0.27
Fe	147 ± 6	197 ± 9
Mn	517 ± 19	389 ± 19
Na	373 ± 11	676 ± 19
Cu	26.1 ± 0.9	19.9 ± 0.9
Zn	63.1 ± 3.0	62.6 ± 3.1
Al	263 ± 4	277 ± 5
F	28.6 ± 1.1	33.2 ± 1.4

The results are mean values,  $n = 5$ ,  $p < 0.05$ .

According to the concept of bioavailability, the soil texture, pH, organic matter, the metal concentration, cation exchange capacity (CEC), the organism living in contact with the soil and even the presence of competing ions, e.g. Na and Ca, are the major factors in assessing the impact of plant acquisition and transport of mineral elements on terrestrial ecosystems. Recently, emphasis has also been on those essential mineral elements for which researchers are beginning to understand the molecular mechanisms of plant acquisition and transport. Experimental and technological advances have enabled researchers to clone the gene encoding mineral ion transporters (Buchanan et al., 2000).

Table 4  
Biochemical parameters in green tea from Qian-zhang-yan and Deng-cun tea plantations in Yichang, Hubei Province, PR China

Site	Polyphenols (%)	Caffeine (%)	Amino acids (%)	Proline (μg/g)
Q	31.04 ± 0.50	4.08 ± 0.22	4.27 ± 0.20	234 ± 12.3
D	34.06 ± 0.50	4.06 ± 0.20	4.21 ± 0.19	180 ± 10.2

The results are mean values,  $n = 5$ ,  $p < 0.05$ .

Upon increasing Ca concentration (especially in true limestone, calcium carbonate, CaCO<sub>3</sub>), soil pH is increased, resulting in lower concentrations of Al and F in tea plant (Woog, Fung, & Carr, 2003). At Q, the soil has little acidity and belongs to the Karst region, the bed-rock being dolomite, the main component of which is CaMg(CO<sub>3</sub>)<sub>2</sub> (Yichang Institute of Geology & Mineral Resources, Chinese Academy of Geological Sciences, 1987). The soil content of Ca from Q was higher than that from D and the soil pH from Q was also higher than that from D. The contents of Al and F in soil from Q were lower than those from D. The content of Al and F of green tea from Q in the Karst region was lower because the pH of the soil in the Karst region is higher and the contents of Al and F were lower.

The biochemical constituents, such as total tea polyphenols, caffeine, amino acids and proline, are presented in Table 4.

The tea polyphenol content of green tea from D was higher than that from Q, but the contents of amino acids and caffeine exhibit no significant differences.

L-Proline accumulation in plant cells exposed to salt or water stress is a widespread phenomenon (Buchanan et al., 2000). The L-proline content of green tea from Q was clearly higher than that from D. The soil moisture content at Q was only 11–16%, whereas that at D is 17–24%. The L-proline accumulation appears to be an additional defence against water stress in *C. sinensis* at Q. There are other reasons for L-proline accumulation, which may be related to the accumulation of mineral elements.

#### 4. Conclusion

Some qualities of green tea from the Karst region are beneficial, e.g., the higher content of L-proline and the lower contents of Al and F. Some other qualities of green tea from the Karst region are not beneficial, e.g., the lower content of tea polyphenols. Green tea from the

Karst region might be useful because of its special qualities.

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### References

- Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, *39*, 205–207.
- Buchanan, B. B., Gruissem, W., & Jones, R. L. (2000). *Biochemistry and molecular biology of plant*. USA: American Society of Plant Physiologists, pp. 407–409, 1204–1247.
- Cao, J., Zhao, Y., & Liu, J. W. (1998). Safety evaluation and fluorine concentration of Pu'er brick tea and Bianxiao brick tea. *Food and Chemical Toxicology*, *36*, 1061–1063.
- Chandler, S. F., & Thorpe, T. A. (1986). Variation from plant tissue cultures: biotechnological application to improving salinity tolerance. *Biotechnology Advances*, *4*, 117–135.
- Chen, Z. (1992). *China tea classics*. PR China: Shanghai Culture Press, pp. 386–400, 467–477.
- Dean, J. A. (1995). *Analytical chemistry handbook*. McGraw-Hill Book Company, pp. 14.1, 20.1.
- Dong, D., Xie, Z., & Du, Y. (2001). The bioavailability of Al in soils to tea plants. *Applied Geochemistry*, *16*, 1413–1418.
- Gorinstein, S., Zemser, M., Vargas-Albores, F., Ochoa, J-L., Paredes-Lopez, O., Scheler, C., et al. (1999). Proteins and amino acids in beers, their contents and relationships with other analytical data. *Food Chemistry*, *67*, 71–78.
- Li, T., Yu, L., Li, M., & Li, W. (2006). A new approach to the standard addition method for the analysis of F, Al and K content in green tea. *Microchimica Acta*, *153*, 109–114.
- Li, T., Yu, L., Li, W., & Li, M. (2005). Analysis of zinc in *Vitex Negundo* foliage by AAS and application of a new kind of standard addition method. *Microchimica Acta*, *150*, 153–157.
- Lin, C. C., & Kao, C. H. (1996). Proline accumulation is associated with inhibition of rice seedling root growth caused by NaCl. *Plant Science*, *114*, 121–128.
- McLachlan, D. R. C. (1995). Aluminium and the risk for Alzheimer's disease. *Environmentrics*, *6*, 233–275.
- Outtrup, H. (1989). Haze active peptides in beer. *EBC Congress*, 609–616.
- Rosenberg, W., Nierop, K. G. J., Knicker, H., de Jager, P. A., Kreutzer, K., & Weiß, T. (2003). Liming effects on the chemical composition of the organic surface layer of a mature Norway spruce stand (*Picea abies* [L.] Karst). *Soil Biology and Biochemistry*, *35*, 155–165.
- Shu, W. S., Zhang, Z. Q., Lan, C. Y., & Wong, M. H. (2003). Fluoride and aluminium concentrations of tea plants and tea products from Sichuan Province, PR China. *Chemosphere*, *52*, 1475–1482.
- The first research institute of China Standards Publisher. (2003). *Standards Collection for Tea* (pp. 1–238). PR China, Beijing: China Standards Publisher.
- Tripathi, B. N., & Gaur, J. P. (2004). Relationship between copper and zinc-induced oxidative stress and proline accumulation in *Scenedesmus* sp. *Planta*, *219*, 397–404.
- Wang, X., & Chen, H. (1994). Relationship between soil chemical characteristics and tea quality in tea gardens in the three Gorges area of the Yangtze river. *Acta Phytocologica Sinica*, *18*(3), 253–260.
- Woog, M. H., Fung, K. F., & Carr, H. P. (2003). Aluminium and fluoride contents of tea, with emphasis on brick tea and their health implications. *Toxicology Letters*, *137*, 111–120.
- Wu, J., Huang, J., Han, X., Xie, Z., & Gao, X. (2003). Three-Gorges Dam—Experiment in habitat fragmentation? *Science*, *300*, 1239–1240.
- Wu, X., Pan, G., Yin, L., & Zhou, Y. (2003). Soil taxonomy of tea garden soil in limestone region. *Soil (PR China)*, *33*, 58–61.
- Yichang Institute of Geology and Mineral Resources, Chinese Academy of Geological Sciences. (1987) (pp. 1–79). *Stratigraphic Excursion Guidebook in the Yangtze Gorges Area*, (PR China), Geological Publishing House.