Z Lebensm Unters Forsch (1992) 195:108–111

Zeitschrift für
Lebensmittel-Untersuchung und -Forschung


*Original paper*

# Influence of catechins and theaflavins on the astringent taste of black tea brews

**Zhang Ding** *, **Susanne Kuhr, and Ulrich H. Engelhardt**

Institut für Lebensmittelchemie der Technischen Universität,
Schleinitzstrasse 20, W-3300 Braunschweig, Federal Republic of Germany

Received March 31, 1992

**Einfluß der Catechin- und Theaflavingehalte auf den adstringierenden Geschmack von Teeaufgüssen**

**Zusammenfassung.** Die Korrelation der Gehalte an den fünf mengenmäßig bedeutendsten Catechinen und den vier Haupttheaflavinen zum sensorisch ermittelten adstringierenden Geschmack wurde untersucht. Catechine und Theaflavingehalte wurden mittels HPLC bestimmt. Es ergaben sich signifikante Korrelationen zum adstringierenden Geschmack für die Summe der Catechine und alle Einzelkomponenten außer Catechin. Zwischen Theaflavingehalten und adstringierendem Geschmack ergab sich keine Korrelation.

**Summary.** The concentrations of the five main catechins (flavanols), epigallocatechin gallate, epicatechin gallate, epigallocatechin, epicatechin, catechin and four main theaflavins, theaflavin, theaflavin-3′-gallate, theaflavin-3-gallate, theaflavin-3,3′-digallate in black tea brew and their effects on astringency were investigated. The catechins and theaflavins were assayed by HPLC. Astringency, which was scaled from 1 to 9, was estimated by a trained panel. The correlations between astringency and content of total catechins and individual catechins, except catechin itself, were significant, whereas no correlation between theaflavins and astringency was observed.

## Introduction

The quality assessment and the price evaluation of tea is determined by professional tea tasters. For a long time attempts have been made to find a correlation between tea tasters ' results and the constituents of tea as determined by chemical analysis. In particular the content of theaflavins (TFs) and thearubigins (TRs) have been claimed to correlate with tea tasters' results (e.g. [1, 2]).

* *Present address:* Horticultural Section, Agriculture and Forestry Department of Jiangsu Province, 34 North Zhongshan Road, Nanjing, People's Republic of China
*Correspondence to:* U.H. Engelhardt

One of the criteria tea tasters use in determining quality is the astringent taste. It is established that polyphenols account for the astringency. The objective of this study was to find out whether a correlation of astringency (determined by sensory analysis) and the content of the main catechins (Cs) and/or TFs (both determined by HPLC methods) exists or not.

## Material and methods

### *Samples*

Fifteen commercial black teas from different producing area were obtained from local retail shops and the China National Native Produce and Animal By-products Import and Export Shanghai Tea Branch: 1, Feinster Tippy-Yünnan; 2, Darjeeling first flush; 3, Darjeeling second flush; 4, Darjeeling autumnal; 5, Assam Broken; 6, Assam Leaf; 7, Ceylon Lowgrown; 8, Ceylon Uva Highland; 9, Kenya; 10, China Black Tea 1; 11, China Black Tea 2; 12, China Black Tea 3; 13, China Black Tea 4; 14, China Black Tea 5; 15, China Black Tea 6.

### *Chemical analysis*

*Catechins.* Tea brew preparation and clean-up by solid-phase extraction were as described previously [3]. The HPLC equipment was a solvent delivery module Beckman 110 B (San Ramon, USA), with an Altex 210 injection valve (San Ramon, USA) with a 20 µl loop, variable wavelength Knauer monitor (Berlin, FRG) (wavelength range from 190 to 600 nm) set at 278 nm, LDC/Milton Roy CI-10 integrator (Shannon, Ireland); stainless steel column (250 × 4.6 mm i.d.) with Nucleosil C-18, 5 µm (Melz VDS, FRG). The eluents were (1) acetonitrile : 2% acetic acid aq. = 13 : 87 (v : v) for 6 min; (2) acetonitrile: 2% acetic acid aq. = 25 : 75 (v : v) for 16 min both at a flow rate of 1.0 ml/min.

*Theaflavins.* Tea was brewed by infusing 1.5 g untreated tea with 90 ml boiling water on a hot plate for 5 min. The brew was filtered, transferred to a 100-ml flask and made up to volume with water while still hot.

For liquid extraction, 50 ml hot brew (see above) was mixed with 30 ml isobutyl methyl ketone (IBMK) in a separatory funnel and shaken for 2 min. The water layer was extracted once more in the same way. The second water layer was discarded. Two extracts were pooled and centrifuged at 3000 rpm for 3 min. The supernatant was evaporated to dryness to remove IBMK completely. The residue was dissolved in 25% acetonitrile (aq.), transferred to a 10-ml flask and made up to volume with 25% acetonitrile (aq.). This solution was used for HPLC determination. The HPLC equipment was as described above using as eluent acetonitrile : 2% acetic acid aq. = 26 : 74 (v : v). To check peak identity an HPLC device with a photo diode array detector was used at the beginning of the study. Calibration and calculation was carried out using theaflavin isolated and purified by HPLC on a semipreparative scale, cf. [4].

### *Sensory analysis*

*Panel.* Eight tasters were selected from the institute staff by sensory tests. The tasters who gave sensitive and reproducible results were selected and trained to distinguish the astringency from other factors of tea taste.

*Conditions.* The sensory analysis was undertaken in a special sensory room that was kept dark and only a yellow light was given to each taster. Using brown cups the effect of colour of the tea brew was excluded.

*Tea brews.* Tea (2 g) was infused by 150 ml boiling water for 5 min [5]. Infused leaves were discarded. The infusions were cooled down to 40–45° C and used for tasting.

*Astringency scale.* A 1–9 scale was used to score the astringency as follows: 1 = absent; 3 = slightly astringent; 5 = moderately astringent; 7 = very astringent; 9 = extremely astringent. All samples were analysed in duplicate.

## Results and discussion

The total amount of catechins in the black teas analysed varied from 2.02% to 12.48% (based on dry weight). Figure 1 shows an HPLC trace of a black tea. The results of HPLC analysis (including the amounts of theogallin, gallic acid and caffeine, which are not discussed here) and the corresponding sensory scores can be found in Table 1. The difference in the amounts of Cs partly depends on the origin of samples and partly depends on the processing methods and season.

As precursors of TFs and TRs, Cs have often been analysed in black tea (e.g. [6–9]). Their role in green tea taste has also been researched in detail [10]. In the research here, we find that Cs also play an important part in black tea taste. The linear correlations between astringency and the amount of total Cs and individual Cs except C are significant (Table 2). The contents of total Cs and epicatechin gallate (ECG) have highly significant effects on the astringency of black tea. The correlations between these two parameters and astringency are conform more closely to a logarithmic function. The former appears in: $y = 0.21296 + 2.7742 \ln X$ ($r = 0.91520$) (see Fig. 2),

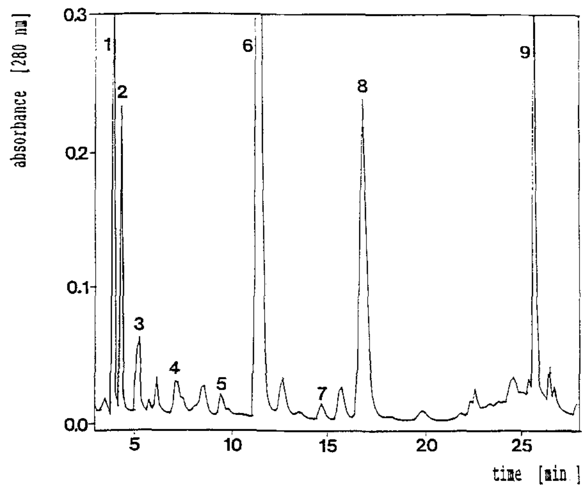


**Fig. 1.** HPLC trace of a black tea sample (Darjeeling). For clean-up and HPLC parameters, see text: *1* theogallin; *2* gallic acid; *3* theobromine; *4* EGC; *5* C; *6* caffeine; *7* EC; *8* EGCG; *9* ECG

**Table 2.** Correlation coefficient between catechins and astringency

| Catechin | Correlation coefficient ($r$) | Significant level |
|---|---|---|
| C | 0.5050 | $r_{0.05} = 0.5139$ |
| EC | 0.6903[b] | $r_{0.01} = 0.6411$ |
| EGC | 0.6378[a] | $r_{0.001} = 0.7603$ |
| ECG | 0.7615[c] | |
| EGCG | 0.6723[b] | |
| Σ Catechins | 0.8672[c] | |

[a] Weakly significant
[b] Significant
[c] Highly significant

**Table 1.** Catechins in black tea (g/100 g dry matter) and astringency (scale 1–9, see text) Sen, sensory score; TG, theogallin; GA, gallic acid; Caf, caffeine; EGC, epigallocatechin; C, catechin; EC, epicatechin; EGCG, epigallocatechin gallate; EGG, epicatechin gallate; Σ Cat, total catechins

| Sample No. | TG | GA | Caf | EGC | C | EC | EGCG | ECG | Σ Cat | Sen |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.93 | 0.42 | 2.94 | 2.15 | 0.46 | 0.51 | 0.14 | 1.98 | 5.24 | 4.6 |
| 2 | 1.09 | 0.18 | 2.55 | 2.13 | 0.13 | 0.39 | 4.32 | 1.19 | 8.16 | 5.6 |
| 3 | 0.81 | 0.25 | 2.39 | 1.50 | 0.10 | 0.26 | 2.79 | 1.23 | 5.88 | 4.7 |
| 4 | 1.22 | 0.27 | 2.37 | 1.51 | 0.11 | 0.21 | 2.70 | 0.99 | 5.52 | 4.9 |
| 5 | 1.33 | 0.45 | 3.52 | 2.72 | 0.21 | 0.14 | 1.21 | 1.03 | 5.31 | 5.3 |
| 6 | 1.40 | 0.37 | 3.05 | 2.13 | 0.15 | 0.13 | 1.02 | 0.79 | 4.22 | 3.8 |
| 7 | 0.79 | 0.36 | 2.33 | 1.05 | 0.07 | 0.06 | 0.40 | 0.46 | 2.04 | 2.2 |
| 8 | 1.18 | 0.23 | 2.32 | 3.87 | 0.28 | 1.10 | 5.09 | 2.19 | 12.48 | 7.3 |
| 9 | 1.52 | 0.40 | 2.23 | 2.21 | 0.22 | 0.19 | 0.84 | 0.74 | 4.20 | 4.0 |
| 10 | 1.40 | 0.29 | 2.64 | 1.07 | 0.30 | 0.58 | 0.42 | 2.38 | 4.75 | 6.0 |
| 11 | 1.06 | 0.35 | 2.45 | 1.08 | 0.20 | 0.35 | 1.12 | 1.61 | 4.36 | 5.1 |
| 12 | 0.76 | 0.26 | 2.29 | 1.14 | 0.13 | 0.32 | 0.56 | 1.11 | 3.26 | 2.6 |
| 13 | 0.48 | 0.32 | 2.59 | 1.23 | 0.07 | 0.08 | 0.36 | 0.28 | 2.02 | 2.3 |
| 14 | 0.67 | 0.27 | 1.99 | 1.26 | 0.17 | 0.29 | 0.78 | 0.70 | 3.20 | 2.7 |
| 15 | 0.37 | 0.27 | 2.09 | 1.23 | 0.13 | 0.17 | 0.37 | 0.46 | 2.36 | 3.0 |

where y = sensory score and X = Σ catechins (g/100 g dry matter). The latter is given by: y = 4.3382 + 2.0091 ln X ($r$ = 0.81529), where y = sensory score and x = ECG (g/100 g dry matter). From the viewpoint of the threshold values, the concentrations of individual Cs are low (Table 3), especially that of C, which is much lower than its threshold value. So it is reasonable for C not to have any effect on the astringency of black tea. It is notable that the concentration of epicatechin (EC), which has a significant correlation with astringency is also very low. It should be stressed that these threshold values were determined in pure water. In a tea brew system the situation might be different. There exists a certain amount of interaction among all the compounds in the system. In the case here EC may enhance the effects of other compounds with astringency although itself not contributing to the astringent taste [11]. The concentrations of epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) are still low, but for each compound there are several samples with a concentration higher than its threshold value and more than half the samples have a concentration higher than its half-threshold value. Especially for ECG, twelve out of fifteen samples had concentrations higher than its half threshold value. With respect to possible interactions, they all had significant correlations with astringency.

The levels of TFs in black tea were very low (Table 4), especially in Darjeeling tea samples in which the concentrations of total TFs were from 9.5 to 14.7 mg $L^{-1}$. The patterns of the four main TFs in teas from Kenya, Sri Lanka lowlands and China tended to be similar. Those in teas from Darjeeling, Sri Lanka highlands and Assam were distinct from the other. The former two (samples 2, 3, 4, 8) had a high amount of TF and lower amounts of theaflavin-3,3′-digallate (TF-3,3′-dig). In contrast, Assam teas (samples 5, 6) had lower amounts of TF and higher amounts of TF-3,3′-dig. For all the samples, the amounts of theaflavin-3′-gallate (TF-3′-g) and theaflavin-3-gallate (TF-3-g) were less different. Further work is necessary to clarify this aspect.

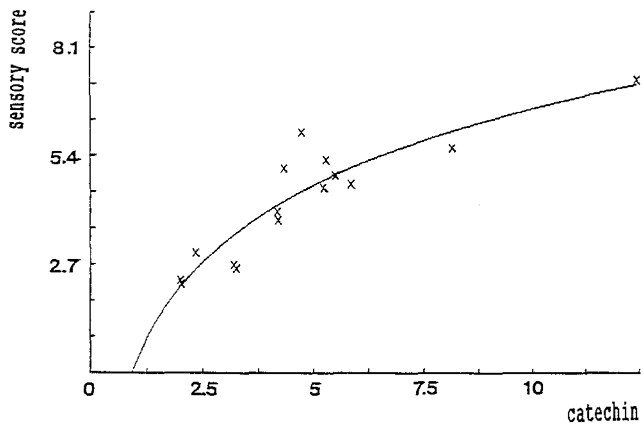


**Fig. 2.** Graphic plot of the correlation between catechins (sum, g/100 g dry matter) and sensory score (astringency), see text

**Table 3.** Concentrations of catechins in black tea brew (mg $L^{-1}$). For sensory scores see Table 1

| Sample | C | EC | EGC | ECG | EGCG | Σ Catechins |
|---|---|---|---|---|---|---|
| 1 | 61 | 68 | 287 | 264 | 19 | 699 |
| 2 | 17 | 52 | 284 | 159 | 576 | 1088 |
| 3 | 13 | 35 | 200 | 164 | 372 | 784 |
| 4 | 15 | 28 | 201 | 132 | 360 | 736 |
| 5 | 28 | 19 | 363 | 137 | 161 | 708 |
| 6 | 20 | 17 | 284 | 105 | 136 | 362 |
| 7 | 9 | 8 | 140 | 61 | 53 | 271 |
| 8 | 37 | 146 | 509 | 292 | 679 | 1663 |
| 9 | 29 | 25 | 295 | 99 | 112 | 560 |
| 10 | 40 | 77 | 143 | 317 | 56 | 633 |
| 11 | 27 | 47 | 144 | 215 | 149 | 582 |
| 12 | 17 | 43 | 152 | 148 | 75 | 435 |
| 13 | 9 | 11 | 164 | 37 | 48 | 269 |
| 14 | 23 | 39 | 168 | 93 | 104 | 427 |
| 15 | 17 | 23 | 164 | 61 | 49 | 314 |
| Threshold value[a] | 510 | 460 | 350 | 180 | 200 | |

[a] Adopted from M. Nakagawa [11]

**Table 4.** Concentrations (Conc) of theaflavins (TF) in black tea brew (mg $L^{-1}$) and relative amounts of TF (percentage of total TF determined); Sensory scores cf. Table 1: TF-3′-g, Theaflavin-3′-gallate; TF-3-g, theaflavin-3-gallate; TF-3,3′-dig, theaflavin-3,3′-digallate

| Sample | TF | | TF-3′-g | | TF-3-g | | TF-3,3′-dig | | Σ TF Conc |
|---|---|---|---|---|---|---|---|---|---|
| | Conc | % | Conc | % | Conc | % | Conc | % | |
| 1. | 12 | 25.5 | 19 | 40.4 | 5 | 10.7 | 11 | 23.4 | 47 |
| 2. | 6 | 60.0 | 2 | 20.0 | 1 | 10.0 | 1 | 10.0 | 10 |
| 3. | 6 | 40.0 | 5 | 33.4 | 2 | 13.3 | 2 | 13.3 | 15 |
| 4. | 6 | 46.2 | 4 | 30.8 | 1 | 7.6 | 2 | 15.4 | 13 |
| 5. | 21 | 19.1 | 32 | 29.1 | 16 | 14.5 | 41 | 37.3 | 110 |
| 6. | 16 | 17.8 | 28 | 31.1 | 14 | 15.6 | 32 | 35.5 | 90 |
| 7. | 8 | 22.2 | 14 | 38.9 | 4 | 11.1 | 10 | 27.8 | 36 |
| 8. | 23 | 59.0 | 9 | 23.1 | 5 | 12.8 | 2 | 5.1 | 39 |
| 9. | 15 | 23.8 | 24 | 38.1 | 8 | 12.7 | 16 | 25.4 | 63 |
| 10. | 19 | 26.0 | 29 | 39.7 | 8 | 11.0 | 17 | 23.3 | 73 |
| 11. | 21 | 25.9 | 29 | 35.8 | 10 | 12.3 | 21 | 25.9 | 81 |
| 12. | 20 | 29.4 | 25 | 36.8 | 9 | 13.2 | 14 | 20.6 | 68 |
| 13. | 9 | 23.0 | 15 | 38.5 | 4 | 10.3 | 11 | 28.2 | 39 |
| 14. | 12 | 27.9 | 15 | 34.9 | 6 | 14.0 | 10 | 23.2 | 43 |
| 15. | 18 | 31.6 | 20 | 35.1 | 7 | 12.3 | 12 | 21.0 | 57 |
| Threshold value[a] | 800 | | | 360 | | | 125 | | 600 |

[a] Adopted from Sanderson [12]

Table 5. Correlation coefficient between TFs and astringency

| Theaflavin | Correlation coefficient ($r$) | Significance level |
|---|---|---|
| TF | 0.3001 | $r_{0.05}$ = 0.5139 |
| TF-3′-g | −0.1084 | $r_{0.01}$ = 0.6411 |
| TF-3-g | 0.1160 | $r_{0.001}$ = 0.7603 |
| TF-3,3′-dig | −0.0741 | |
| Σ TF | −0.0067 | |

The relationship between the content of TFs and quality or price of black tea has often been investigated. In some papers a significant correlation between price and TF content has been reported [13–15]. Other authors found no statistically significant correlation [1]. This was probably due to different sample origin, different index of quality and to the fluctuation of market price. In our research here, no correlation with tea price or tea tasters' assessment should be checked. The objective of this study was not an attempt to set up another correlation between tea tasters' assessment and the content of a group of tea constituents. Among all the sensory parameters, only one factor, the astringent taste, was considered. The sensory method used here excluded the effects of other sensory factors such as brightness, bitterness, colour and appearance. The results indicate that neither concentration of total TFs nor those of the four main TFs (TF, TF-3′-g, TF-3-g, TF-3,3′-dig) has any correlation with astringency (Table 5). Comparing their concentrations with the threshold values, it is obvious that they are too low to have a strong influence on astringent taste.

In black tea brews, the concentrations of Cs are much higher than that of TFs, although there are large differences among samples. On the other hand, the astringent threshold values of Cs are lower than those of TFs. Therefore Cs are more important when black tea taste is researched. The concentrations of Cs should be one of the major chemical parameters of black tea taste.

## Conclusions

Catechins remain in black tea in a considerable amount, although they decrease during the processing procedure due to oxidation and polymerisation. Additionally they are highly soluble in water, so their concentration in black tea brews is still high.

Theaflavins are one of the principle products of polyphenol oxidation during black tea manufacture. It is undoubted that TFs are characteristic substances that might affect tea tasters' results due to their contribution to colour, brightness and formation of "cream" in black tea brews, but the concentration in black tea brews is very low. The total amount of Cs has a signifcant effect on astringency, while TFs have no statistical correlation with astringency.

When the effect of certain compounds on tea taste is estimated, the threshold values of these compounds may not be ignored. It is not difficult to interpret the abovementioned phenomena. The concentrations of TF are low compared with their threshold values for astringent taste. Therefore they are much less important in the astringency of black tea.

The concentrations of Cs are much higher than those of TFs in black tea brews; moreover, their astringent threshold values are lower than those of TFs. Catechins are more important when tea taste is researched and strongly influence black tea astringency.

*Acknowledgements.* The project was granted by the Bundeswirtschaftsministerium via the Arbeitskreis industrieller Forschungsvereinigungen (AIF-FV 8160). The authors gratefully acknowledge the support of the German Tea Association. We also thank Mr. A. Vollers for advice. Ms. Zhang Ding was financed by the State Educational Commission of People's Republic of China.

## References

1. Owuor PO, Reeves SG, Wanyoko K (1986) J Sci Food Agric 37:507–513
2. Roberts EAH, Smith RF (1961) Analyst 86:94–98
3. Kuhr S, Engelhardt UH (1991) Z Lebensm Unters Forsch 192:526–529
4. Steinhaus B, Engelhardt UH (1989) Z Lebensm Unters Forsch 188:509–511
5. Bundesgesundheitsamt (1988) Amtliche Methodensammlung nach § 35 LMBG, L 47.00.07
6. Cartwright RA, Roberts EAH (1954) Chem Ind 1389–1391
7. Roberts EAH, Myers M (1959) J Sci Food Agric 10:172–176
8. Collier PD, Bryce T, Mallows R, Thomas PE, Frost DJ, Korver O, Wilkins CK (1973) Tetrahedron 29:125–142
9. Robertson A, Bendall DS (1983) Phytochemistry 22:883–887
10. Zhaopeng S, Zhonghua L (1987) Proceedings of International Tea Quality-Human Health Symposium 108–116
11. Nakagawa M (1970) Bull Tea Res Stat 6:65–166
12. Sanderson GW, Ranadive AS, Eisenberg LS, Farell FJ, Simons R, Manley CH, Coggon P (1976) In: Charalambous G, Katz I (eds) ACS Symposium Series 26:14–46
13. Wood DJ, Roberts EAH (1984) J Sci Food Agric 15:19–25
14. Millin DJ et al. (1969) J Agric Food Chem 17:717–722
15. Hilton PJ, Palmer-Jones RW (1975) J Sci Food Agric 26:1681–1687