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Molecular Definition of Black Tea Taste by Means of Quantitative Studies, Taste Reconstitution, and Omission Experiments

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Recently, bioresponse-guided fractionation of black tea infusions indicated that neither the high molecular weight thearubigens nor the theaflavins, but a series of 14 flavon-3-ol glycopyranosides besides some catechins, might be important contributors to black tea taste. To further bridge the gap between pure structural chemistry and human taste perception, in the present investigation 51 putative taste compounds have been quantified in a black tea infusion, and their dose-over-threshold (Dot) factors have been calculated on the basis of a dose/threshold relationship. To confirm these quantitative results, an aqueous taste model was prepared by blending aqueous solutions of 15 amino acids, 14 flavonol-glycosides, 8 flavan-3-ols, 5 theaflavins, 5 organic acids, 3 sugars, and caffeine in their "natural" concentrations. Sensory analyses revealed that the taste profile of this artificial cocktail did not differ significantly from the taste profile of the authentic tea infusion. To further narrow the number of key taste compounds, finally, taste omission experiments have been performed, on the basis of which a reduced recombinate was prepared containing the bitter-tasting caffeine, nine velvety astringent flavonol-3-glycosides, and the puckering astringent catechin as well as the astringent and bitter epigallocatechin-3-gallate. The taste profile of this reduced recombinate differed not significantly from that of the complete taste recombinate, thus confirming these 12 compounds as the key taste compounds of the tea infusion. Additional sensory studies demonstrated for the first time that the flavanol-3-glycosides not only impart a velvety astringent taste sensation to the oral cavity but also contribute to the bitter taste of tea infusions by amplifying the bitterness of caffeine.

KEYWORDS: Tea taste; astringency; bitterness; dose-over-threshold (Dot) factors; taste enhancement; taste recombination; theanine

INTRODUCTION

For centuries, the aqueous infusion of the dried leaves and buds of the plant *Camellia sinensis* has been consumed by humans as a highly desirable beverage. Besides green tea and oolong tea, black tea is one of the major tea products, accounting for >75% of the world tea production. Taste quality is one of the key criteria used by the tea tasters to describe the quality of tea liquors, and descriptors such as "strong", "hard", and "harsh" are used by professional tea tasters to describe the intensity and quality of the taste sensation perceived.

Although multiple attempts have been made to correlate the sensory results of the tea tasters and the molecules exhibiting the typical taste of tea infusions, the data reported so far on the key tastants are very contradictory. For example, the orange low molecular weight theaflavins as well as the red-brown polymeric thearubigins, both generated during tea fermentation upon polyphenol oxidase catalyzed oxidation of flavan-3-ols (*1*−*4*), are believed to be responsible for the briskness and astringency of black tea infusions and have been recommended as a measure of tea quality (*5*, *6*). In contradiction, other researchers could not find any statistical correlation between the overall astringent taste of tea infusions and the theaflavin concentration, but did indicate a relationship between oral astringency and some flavan-3-ols such as epigallocatechin-3-gallate (*7*, *8*). Besides these phenolic components, the amino acid 5-*N*-ethyl-L-glutamine, named theanine, is reported to exhibit sweet (*9*), sweetish-brothy, and umami-like taste qualities (*10*), respectively, and is believed to contribute to the sweet and broth-like taste modality of tea infusions.

To answer the puzzling question as to which nonvolatile, key taste compounds are responsible for the typical astringent and bitter taste of tea infusions, we have recently analyzed a black tea infusion by means of the so-called taste dilution analysis

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(*11*). Application of this bioresponse-guided screening procedure on freshly prepared tea infusions revealed neither the high molecular weight thearubigen-like polyphenols nor the flavan-3-ols and theaflavins, but a series of 14 flavon-3-ol glycopyranosides with high taste dilution factors, thus indicating these glycosides as important contributors to black tea taste.

To bridge the gap between pure structural chemistry and human taste perception, the objectives of the present investigation were therefore to quantify putative taste compounds in a freshly prepared black tea infusion, to rate them on the basis of a dose/activity relationship, and, finally, to confirm the taste contribution of the key compounds by means of taste reconstitution and omission experiments.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: caffeine (Fluka, Neu-Ulm, Germany); catechin, catechin-3-gallate, epicatechin, epicatechin-3-gallate, epigallocatechin-3-gallate, gallocatechin, gallocatechin-3-gallate (Sigma, Steinheim, Germany); kaempferol-3-*O*-*β*-D-glucopyranoside, kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside], quercetin-3-*O*-*β*-D-galactopyrano-side, quercetin-3-*O*-*β*-D-glucopyranoside, and quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside] (Roth, Karlsruhe, Germany). Solvents were HPLC grade (Merck, Darmstadt, Germany) theanine (Roth, Karlsruhe, Germany) and *γ*-aminobutyric acid (Sigma, Steinheim, Germany).

Theaflavin, theaflavic acid, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate were synthesized closely following the procedures reported recently (*12*). Kaempferol-3-*O*-*β*-D-galactopyranoside, 3-*O*-*β*-D-glucopyranoside, 3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside], 3-*O*-[*β*-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside], 3-*O*-[*β*-D-gluco-pyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-galactopyranoside], myricetin-3-*O*-*β*-D-galactopyranoside, 3-*O*-*β*-D-glucopyranoside, 3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside], quercetin-3-*O*-*β*-D-galactopyrano-side, 3-*O*-*β*-D-glucoside, 3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-galactopyranoside], and 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside] were isolated following the procedures reported recently (*11*).

Preparation of the Black Tea Infusion. The black tea drug Darjeeling Gold-Auslese, TGFOP, Summer (Tee-Handelskontor, Bremen, Germany), was infused with boiling tap water (1 g/100 mL) and maintained for 4 min prior to filtration using a cellulose filter. For sensory experiments the tea infusion was used directly. For quantitative analysis of taste compounds, the tea infusion was stabilized by the addition of ascorbic acid (50 mg/100 mL), membrane filtered, and analyzed by means of HPLC.

Quantitative Analyses. *Flavon-3-ol Glycosides.* The stabilized tea infusion was analyzed by a semipreparative 250 × 10.0 mm i.d. HPLC column (ODS-Hypersil 100-5C18, ThermoHypersil, Kleinostheim Germany). The chromatography was performed with aqueous formic acid (0.1% in water; pH 3.5) and acetonitrile as the eluent. For glycoside analysis, the gradient started with an acetonitrile content of 13%, which was increased to 17% within 35 min, held for 15 min, and, finally, increased to 100% within 5 min. The flavon-3-ol-glycosides were identified by comparison of the retention times (HPLC) and LC-MS spectra as well as cochromatography with the synthetic reference compounds. Quantification was performed by analytical RP-HPLC by comparing the peak areas obtained at 339 nm with those of defined standard solutions of each reference compound in methanol.

Catechins. The stabilized tea infusion was applied on an analytical RP-8 HPLC column (250 × 4.6 mm i.d., Grom Sil 120 octyl-5-CP, Grom, Rottenburg-Hailfingen, Germany). Starting with a mixture (10:90, v/v) of methanol and aqueous formic acid (0.1% in water, pH 3.5), the methanol content was increased to 20% within 8.5 min, then to 60% within 31 min, and, finally, to 100% within 5 min. The catechins were identified by comparison of the retention times (HPLC) and LC-MS spectra as well as cochromatography with the synthetic reference compounds. Quantification was performed by analytical RP-HPLC by comparing the peak areas obtained at 280 nm with those of defined standard solutions of each reference compound in methanol.

Theaflavins. The concentration of theaflavin derivatives was determined on an analytical RP-8 HPLC column (250 × 4.6 mm i.d., Grom Sil 120 octyl-5-CP, Grom) as reported recently (*12*). Starting with a mixture (20:80, v/v) of acetonitrile and aqueous formic acid (0.1% in water, pH 3.5), the acetonitrile content was increased to 60% within 60 min and then to 100% within 5 min. Theaflavin, theaflavic acid, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate were identified by comparison of the retention times (HPLC) and LC-MS spectra as well as cochromatography with the synthetic reference compounds. Quantification was performed by analytical RP-HPLC by comparing the peak areas obtained at 370 and 460 nm with those of defined standard solutions of each reference compound in methanol.

Caffeine. For the quantification of caffeine, magnesium oxide (5 g) was added to the tea infusion (250 mL), the mixture was maintained at 90 °C for 20 min, and, after filtration, the filtrate was analyzed by means of analytical RP-8 HPLC using a 250 × 4.6 mm i.d., Grom Sil 120 octyl-5-CP column (Grom). Starting with a mixture (10:90, v/v) of methanol and aqueous formic acid (0.1% in water, pH 3.5), the methanol content was increased to 20% within 8.5 min, then further increased to 40% within 31 min, and, finally, raised to 100% within 5 min. Quantification of caffeine was performed by comparing the peak areas obtained at 254 nm with those of defined standard solutions of the reference compound in methanol.

Soluble Carbohydrates and Organic Acids. Sugars and organic acids were quantitatively determined in the freshly prepared tea infusion using commercially available enzymatic test kits closely following the protocols of the manufacturer (R-Biopharm GmbH, Darmstadt Germany). Oxalic acid was quantitatively determined by means of an enzymatic test purchased from Sigma-Aldrich (Deisenhofen, Germany).

Amino Acids. For amino acid analysis, the membrane-filtered tea infusion was analyzed either directly or after 1+1 dilution with an aqueous buffer solution (0.1 mol/L) containing sodium acetate (8.2 g/L), methanol (7.5%), formic acid (0.3%), acetic acid (1.5%), and octanoic acid (0.001%) by means of an LC 3000 amino acid analyzer (Biotronic, Maintal Germany) equipped with a BTC F guard column (75 × 6.0 mm i.d., Eppendorf-Netheler-Hinz, Maintal Germany) and a BTC 2410 main column (145 × 3.2 mm i.d., Eppendorf-Netheler-Hinz). For amino acid analysis, the column temperature was maintained at 47 °C for 26.5 min and then adjusted to 49 °C from 26.5 to 41.8 min, to 50 °C from 41.8 to 55.8 min, to 52 °C from 55.8 to 70.8 min, to 56 °C from 70.8 to 80.8 min, and, finally, to 60 °C from 80.8 to 98.8 min. After postcolumn derivatization with ninhydrin, the amino acids were quantified by monitoring the effluent at 440 and 570 nm.

High-Vacuum Distillation. The volatile fraction of the freshly prepared tea infusion was carefully and directly isolated at 25 °C using the so-called SAFE (solvent-assisted flavor evaporation) apparatus closely following the procedure reported in the literature (*13*).

Sensory Analyses. *Panel Training.* Using triangle tests, 15 assessors were trained to recognize and quantify the taste of aqueous solutions (5 mL each) of the following standard compounds dissolved in bottled water (Vitel, low mineralization, 405 mg/L) adjusted to pH 6.0 with aqueous hydrochloric acid (0.1 mol/L): saccharose (50 mmol/L) for sweet taste; lactic acid (20 mmol/L) for sour taste; NaCl (12 mmol/L) for salty taste; caffeine (1 mmol/L) for bitter taste; and sodium glutamate (8 mmol/L, pH 5.7) for umami taste. For puckering astringency and velvety-like astringency, the panel was trained by using gallustannic acid (0.001%) and quercetin-3-*O*-*β*-D-galactopyranoside (0.01 mmol/L), respectively, using the half-tongue test. Sensory analyses were performed in a sensory panel room at 19−22 °C in three different sessions.

Recognition Thresholds Concentrations. Threshold concentrations for sour-, sweet-, bitter-, salty-, and umami-tasting compounds were determined by means of triangle tests as reported earlier (*14*). To overcome memory effects of astringent compounds, threshold concentrations of astringent compounds were determined by means of the recently developed half-tongue test (*11*, *12*).

Table 1. Taste Qualities, Taste Thresholds, Concentrations, and Dose-over-Threshold (Dot) Factors of Selected Tea Tastants

tastant	threshold[a] (μmol/L)	concn (μmol/L)	Dot factor[b]
Group I: Compounds Imparting Puckering Astringency and Rough Oral Sensation			
epigallocatechin-3-gallate[c]	190.0	328.0	1.7
theaflavin	16.0	11.0	0.7
catechin	410.0	221.0	0.5
theaflavin-3,3′-digallate	13.0	6.7	0.5
epicatechin-3-gallate	260.0	113.0	0.4
theaflavin-3-gallate	15.0	6.4	0.4
theaflavin-3′-gallate	15.0	4.3	0.3
epigallocatechin	520.0	131.0	0.3
gallocatechin	540.0	131.0	0.3
epicatechin[c]	930.0	84.0	0.1
catechin-3-gallate	250.0	11.0	<0.1
gallocatechin-3-gallate[c]	390.0	11.0	<0.1
theaflavic acid	24.0	0.009	<0.1
Group II: Compounds Imparting Mouth-Drying and Velvety-like Astringency			
quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside]	0.00115	11.1	9652.0
kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside]	0.25	6.5	26.0
quercetin-3-*O*-β-D-galactopyranoside	0.43	5.4	12.6
quercetin-3-*O*-β-D-glucopyranoside	0.65	6.0	9.2
kaempferol-3-*O*-β-D-glucopyranoside	0.67	4.9	7.3
myricetin-3-*O*-β-D-glucopyranoside	2.10	9.3	4.4
quercetin-3-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-galactopyranoside]	1.36	3.3	2.4
myricetin-3-*O*-β-D-galactopyranoside	2.70	6.5	2.4
kaempferol-3-*O*-β-D-galactopyranoside	6.70	3.0	0.4
kaempferol-3-*O*-[β-D-glucopranosyl- (1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside]	19.80	8.6	0.4
quercetin-3-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-glucopyranoside]	18.40	7.1	0.4
apigenin-8-*C*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside]	2.80	0.9	0.3
myricetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside]	10.50	2.2	0.2
kaempferol-3-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-galactopyranoside]	5.80	0.8	0.2
5*N*-ethyl-L-glutamine (theanine)	6000.00	281.0	< 0.1
γ-aminobutyric acid	20.00[e]	0.4	<0.1
Group III: Bitter-Tasting Compounds			
caffeine	500.0	990.0	2.0
L-isoleucine	11000.0	0.2	<0.1
L-leucine	12000.0	0.4	<0.1
L-phenylalanine	58000.0	0.6	<0.1
L-tyrosine	5000.0	0.4	<0.1
L-valine	21000.0	0.8	<0.1
Group IV: Sour Compounds			
succinic acid[d]	900.0	160.0	0.2
oxalic acid	5600.0	860.0	0.2
malic acid	3700.0	160.0	<0.1
citric acid	2600.0	110.0	<0.1
ascorbic acid	700.0	150.0	<0.1
Group V: Sweet-Tasting Compounds			
glucose	90000.0	340.0	<0.1
saccharose	24000.0	100.0	<0.1
fructose	52000.0	110.0	<0.1
L-serine	30000.0	0.8	<0.1
L-alanine	8000.0	1.1	<0.1
glycine	30000.0	0.1	<0.1
L-ornithine	3500.0	0.3	<0.1
L-proline	26000.0	0.1	<0.1
L-threonine	40000.0	0.3	<0.1
Group VI: Umami-like Taste Compounds			
aspartic acid	4000.0	0.7	<0.1
glutamic acid	3000.0	1.0	<0.1

[a] Taste threshold concentrations were determined by means of the comparative duo test in bottled water as reported recently (*11*). [b] The Dot factor is calculated as the ratio of concentration and taste threshold. [c] Epigallocatechin-3-gallate exhibits bitter taste at the threshold concentration of 380 μmol/L, epicatechin and gallocatechin-3-gallate exhibit bitter taste at their threshold level of astringency. [d] This compound exhibits umami-like taste at the threshold level of 700 μmol/L, corresponding to a taste activity value of 0.23 for umami-like taste quality. [e] Taste threshold concentration was taken from the literature (*16*).

Taste Reconstitution. To prepare an artificial taste imitate of the tea infusion, the "natural" amounts of the 51 taste compounds, summarized in **Table 1**, were dissolved in tap water, and the pH value of the solution was then adjusted to 7.0 by the addition of hydrochloric acid (0.1 mmol/L). After an equilibration time of 10 min, the overall taste quality was evaluated by means of the taste profile analysis. To avoid degradation of unstable polyphenols, exclusively fresh preparations were tested.

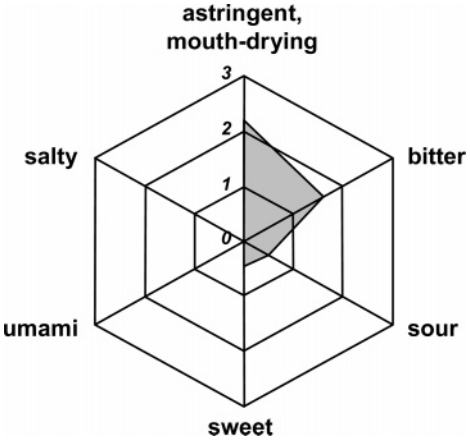

Figure 1. Taste profile of a freshly prepared Darjeeling black tea infusion.

Taste Profile Analysis. Freshly prepared tea infusions, complete taste recombinates, and partial taste recombinates were presented to members of the sensory panel, who were asked to score the taste qualities astringent, bitter, sour, sweet, salty, and umami on a scale from 0 (not detectable) to 3 (strongly detectable). To achieve this, the samples were swirled around in the mouth briefly and expectorated.

Omission Experiments. To investigate the taste contribution of the individual taste compounds, 14 partial taste recombinates were one by one prepared by omitting either individual tastant groups or single tastants from the complete taste reconstitute. Each of the 14 partial recombinates was presented to the panelists in comparison with the complete taste reconstitute by using a triangle test. Panelists were asked to evaluate whether the solutions were identical in the overall taste or not. Those panelists who detected the taste difference correctly were asked to rate the intensity of the given taste descriptors on a scale from 0 to 3.

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (BIO-TEK Kontron Instruments, Eching, Germany) consisted of two pumps (type 522), a Rheodyne injector (100 μL loop), and a diode array detector (DAD type 540), monitoring the effluent in a wavelength range between 220 and 500 nm.

Liquid Chromatography−Mass Spectrometry (LC-MS). The sample (2−20 μL) was injected onto an analytical HPLC column (Grom Sil 120 octyl-5-CP, Grom) coupled to an LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionization.

RESULTS AND DISCUSSION

To evaluate the taste profile of the Darjeeling tea infusion, the trained sensory panel was asked to rate the intensity of the taste qualities astringency, bitterness, sweetness, sourness, and umami taste on a scale from 0 (not detectable) to 3 (strongly detectable). By far the highest scores of 2.2 and 1.6 were observed for the intensity of the astringent, mouth-drying, taste quality and the bitterness, respectively (**Figure 1**). In comparison, the intensities of sourness (0.5) and sweetness (0.4) were significantly lower, and salty as well as umami taste qualities could not be detected at all in the tea infusion.

As reported recently (*11*), application of the taste dilution analysis on Darjeeling tea infusions led to the identification of a group of flavon-3-ol glycopyranosides with the highest taste dilution factors, followed by catechins, theaflavins, and caffeine, which were evaluated with significantly lower taste impacts.

To confirm the importance of flavon-3-ol glycopyranosides, catechins, theaflavins, and caffeine as key tastants of the black tea infusion and to demonstrate a correlation between single-taste compounds and individual taste qualities, we aimed at preparing an taste imitate containing these taste compounds in their "natural" concentrations and to compare the taste profile of this biomimetic taste model to that of the authentic Darjeeling tea infusion. To achieve this, first, all of the individual taste compounds needed to be quantified in the freshly prepared tea infusion and the taste recognition thresholds needed to be determined.

Quantification and Calculation of Dose-over-Threshold (Dot) Factors. With the aim of evaluating the taste contribution of the individual taste compounds, 13 flavonol-3-glycosides, 1 apigenin glycoside, 8 catechins, 5 theaflavins, caffeine, and, in addition, 13 amino acids, 5 organic acids, and 3 soluble carbohydrates have been quantitatively determined in the Darjeeling tea infusion, and the taste quality as well as the taste recognition threshold of each substance was evaluated by the trained sensory panel. As we aimed to elucidate the key contributors for each individual taste quality, the single-taste compounds were grouped into six classes differing in their taste qualities (**Table 1**).

Tastant group I, classifying compounds imparting a puckering astringency and roughness to the oral cavity, contained eight catechins and five theaflavins (**Table 1**). By means of the so-called half-tongue test, the human sensory recognition thresholds of these compounds were determined (*11*). Fitting well with our previous data, the oral sensation imparted by the catechins was described as astringent with relatively high threshold concentrations spanning from 190 to 930 μmol/L. In comparison, the theaflavins induced a mouth-drying, rough-astringent, and long-lasting oral sensation at significantly lower threshold concentrations between 13 and 24 μmol/L; for example, the threshold concentration of 16 μmol/L determined for theaflavin was found to be 58 times lower than the threshold of epicatechin (**Table 1**). Among this group of taste compounds, the catechins were found in highest concentrations; for example, 328 and 221 μmol/L of epigallocatechin-3-gallate and catechin, respectively, were present in the fresh tea infusion. In comparison, the black tea specific theaflavins were determined in low amounts with a maximum of 11 μmol/L for the theaflavin (**Table 1**). To gain first insights into the taste contribution of these compounds, these were rated in their sensory impact on the basis of the ratio of the concentration and the taste recognition threshold of a compound (*15*). Calculation of these Dot factors revealed that exclusively the concentration of the epigallocatechin-3-gallate in the tea infusion exceeded its astringency threshold concentrations by a factor of 1.7 (**Table 1**). In contrast, the concentrations of all of the other catechins and theaflavins in tastant group I were found to be below their taste threshold concentrations. Among these, the concentrations of the flavan-3-ols catechin, epicatechin-3-gallate, epigallocatechin, and gallocatechin as well as the benzotropolons theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate were <10-fold below their taste threshold, whereas for epicatechin, catechin-3-gallate, gallocatechin-3-gallate, and theaflavic acid Dot factors of <0.1 were calculated (**Table 1**).

Group II, summarizing the compounds imparting a mouth-drying and velvety astringent sensation to the oral cavity, contained six flavonol-3-monoglycosides, three flavonol-3-diglycosides, four flavonol-3-triglycosides, and apigenin-8-*C*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside], as well as the amino acids theanine and γ-aminobutyric acid (**Table 1**). Compared to the catechins and theaflavins in group I, the flavon-3-ol-glycosides were found to induce a mouth-drying and mouth-coating sensation at very low threshold concentrations spanning from 0.001 to 19.8 μmol/L (**Table 1**). In particular, the oral threshold of 0.001 μmol/L determined for the quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] is extraordinarily low. Sensory analysis of the amino

acids revealed that purified theanine does not exhibit any sweet or umami-like taste as reported in the literature (*9*, *10*), but imparted a velvety astringent sensation to the oral space at the rather high threshold concentration of 6000 μmol/L (water). In comparison, the recognition taste threshold of the γ-aminobutyric acid was significantly lower; for example, a threshold concentration of 20 μmol/L (water) has been reported recently (*16*). Quantitative analysis of these taste compounds revealed by far the highest concentrations for theanine; for example, 281 μmol/L of that amino acid has been detected in the Darjeeling tea infusion. In comparison, all of the other taste compounds summarized in group II were present in rather low concentrations ranging between 11.1 μmol/L found for quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] and 0.4 μmol/L found for γ-aminobutyric acid (**Table 1**).

Calculation of the Dot factors of these compounds revealed rather high values for some of the flavonol-3-glycosides. Among these, quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] was evaluated with by far the highest taste activity value of 9652. With somewhat lower Dot factors, also kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside], quercetin-3-*O*-β-D-galactopyranoside, quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-[β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-β-D-galactopyranoside], myricetin-3-*O*-β-D-glucopyranoside, and myricetin-3-*O*-β-D-galactopyranoside were evaluated with high taste impacts for the velvety astringent taste sensation. The concentrations of all of the other taste compounds in group II were below their taste threshold concentration. Although theanine was present in huge concentrations, this amino acid was >20-fold below its taste threshold concentration and, therefore, cannot not be considered as an important contributor to black tea taste (**Table 1**).

The alkaloid caffeine as well as the amino acids L-valine, L-leucine, L-isoleucine, L-phenylalanine, and L-tyrosine were classified into group III, representing compounds imparting a clean bitter taste (**Table 1**). Quantitative analysis revealed caffeine as the quantitatively predominating bitter tastant; for example, nearly 1.0 mmol/L of this alkaloid has been found in the freshly prepared tea infusion. In contrast, the concentrations of the bitter-tasting amino acids were rather low; for example, 0.2 and 0.8 μmol/L L-isoleucine and L-valine have been determined. Relating these concentrations to the taste recognition thresholds of these bitter compounds revealed that exclusively the caffeine showed a taste activity value above 1.0, thus demonstrating caffeine as one of the key bitter compounds in black tea. In contrast, the concentration of none of the amino acids exceeded the corresponding bitter threshold concentrations.

Tastant group IV, representing sour-tasting compounds, contained succinic acid, oxalic acid, malic acid, ascorbic acid, and citric acid (**Table 1**). By far the highest concentration of 860 μmol/L was found for oxalic acid, whereas succinic acid, malic acid, ascorbic acid, and citric acid were present in somewhat lower amounts of 160 or 110 μmol/L. On the basis of quantitative data and taste threshold concentrations, each of these compounds was evaluated with a Dot of <1, thus implying that these organic acids might not contribute significantly to tea taste.

Finally, soluble carbohydrates and sweet-tasting amino acids were grouped into the sweet-tasting group V, and aspartic acid and glutamic acid were grouped into the umami-like-tasting group VI (**Table 1**). Quantitative analysis and sensory studies revealed that the "natural" concentration of each of these compounds in the tea infusion was <100-fold below the corresponding recognition threshold concentration. Taking these data into consideration, the sweet- as well as the umami-like-tasting compounds might not be of major importance for the typical taste of a black tea infusion.

Table 2. Influence of the Volatile Tea Fraction on the Taste Profile of the Reconstituted Tea

	intensities of individual taste qualities of the taste recombinate[a]		
taste quality	without additive	+ tea volatiles[b] (with nose clamp)	+ tea volatiles[b] (without nose clamp)
astringent, mouth-drying	2.2	2.2	2.3
bitter	1.5	1.5	1.7
sour	0.5	0.5	0.5
sweet	0.4	0.4	0.6
salty	0	0	0
umami	0	0	0

[a] Intensities were judged on a scale from 0 (not detectable) to 3 (strongly detectable). [b] Volatile fraction was carefully isolated from a freshly prepared Darjeeling tea infusion by means of high-vacuum distillation using the recently developed SAFE device (*13*).

Biomimetic Reconstitution of Tea Taste. To confirm the results of the quantitative analysis and to check whether the compounds already identified can create the typical taste of the Darjeeling tea, we prepared an aqueous taste reconstitute containing the "natural" concentrations of the 51 compounds as given in **Table 1** and compared the taste profile of that complete taste recombinate with that of the authentic, freshly prepared Darjeeling tea infusion. To achieve this, all of the compounds summarized in taste groups I−VI were dissolved in bottled water in their natural concentrations, and the pH value and color tone were adjusted to those of the authentic tea infusion by adding some trace amounts of NaOH as well as sugar couleur. The trained sensory panel was then asked to evaluate the taste profile of these samples by scoring the taste descriptors, given in **Table 2**, on a scale from 0 (not detectable) to 3 (strongly detectable). Sensory evaluation of this complete taste recombinate as well as the authentic tea infusion revealed the highest intensity for astringency (2.2), closely followed by the bitter taste quality, which was judged with scores of 1.6 and 1.5 for the authentic tea infusion and the artificial taste imitate, respectively (**Table 2**). Also, sourness (0.5) and sweetness (0.4) were evaluated with the same intensities for both solutions. The trained panelists concluded that the typical taste of the freshly prepared tea infusion could be completely reconstituted by the blend of the 51 compounds summarized in **Table 1**.

Influence of Volatiles on the Sensory Perception of the Taste Recombinate. To answer the question as to how the odor-active volatile fraction of the tea infusion modulates the taste perception of tea nonvolatiles, the volatile fraction was carefully isolated from a freshly prepared Darjeeling tea infusion by means of high-vacuum distillation using the recently developed SAFE device (*13*). The volatile distillate was mixed with the 51 taste compounds in natural concentrations (cf. **Table 1**), and, after the pH value and color tone had been adjusted as reported above, the sensory profile of the taste recombinate spiked with natural tea volatiles was compared to the taste profile of the recombinate lacking the volatiles. To differentiate between taste and smell phenomena, two procedures were used for the sensory evaluation. To prevent taste perception from being influenced by olfactory responses, the sensory panelists used nose clamps in the first set of experiments. In the second set of experiments, the sensory panel was asked to rate the same solutions without

using nose clamps, thus opening the possibility of detecting olfactory responses. For all of these sensory experiments, the intensities of the taste descriptors, given in **Table 2**, were rated by the trained panel on a scale from 0 (not detectable) to 3 (strongly detectable). When the taste profile of the taste recombinate was compared to the taste recombinate containing the tea volatiles while wearing a nose clamp, the sensory panelists were not able to detect any differences in the intensities of the individual taste qualities. However, when the experiment was repeated in the absence of nose clamps, the presence of the tea volatiles induced a slight increase in the perception of astringency (+0.1), bitterness (+0.2), and sweetness (+0.2) when compared to the nonvolatile recombinate (**Table 2**). To investigate whether this effect of the volatile tea fraction is due to the taste activity of some volatiles or due to an interplay of olfactory and gustatory perception, the taste profile of the high-vacuum tea distillate was evaluated by sensory analysis with and without the use of nose clamps, respectively. While wearing nose clips, the sensory panel evaluated the tea volatiles as being tasteless (data not shown). In contrast, the same solution was rated as being slightly bitter with an intensity of (+0.2) when the nose clamps were omitted (data not shown). These findings clearly demonstrate that olfactory responses to tea volatiles are modifying the perception of taste-active tea nonvolatiles.

Evaluation of Taste Contribution by Omission Experiments. After the taste activity of 51 nonvolatiles in the Darjeeling infusion had been evaluated on the basis of Dot factors as well as taste reconstitution experiments, the following experiments aimed to confirm the taste contribution of the six tastant groups and individual compounds, respectively, by means of so-called omission experiments. To achieve this, individual taste recombinates either lacking one or more taste compounds or lacking a tastant group were evaluated by means of triangle tests using two samples of the complete taste recombinate as the control. Those panelists who detected the taste difference correctly were asked to rate the intensity of the taste descriptors astringent, bitter, sour, sweet, salty, and umami on a scale from 0 to 3.

In a first set of experiments, the complete tastant group I, containing all of the puckering astringent catechins as well as theaflavins, was omitted from the taste recombinate. As given in **Table 3**, seven of eight panelists were able to detect this sample in a traingle test with two samples of the complete recombinate as the reference. These panelists rated the partial recombinate as being less astringent (−0.5) and less bitter (−0.9) than the total recombinate. To investigate the taste contribution of the individual taste classes in tastant group I, additional partial recombinates were prepared lacking either catechins or the theaflavin-type compounds. Six of eight panelists successfully detected the omission of the catechins from the complete recombinate and described this partial recombinate as being less astringent (−0.5) and less bitter (−0.9) than the total recombinate (**Table 3**). This is well in line with the astringent oral sensation imparted by the catechins as well as the additional bitter taste of epigallocatechin-3-gallate, epicatechin, and gallocatechin-3-gallate (cf. **Table 1**). In contrast, the lack of theaflavins could not be significantly detected. Only two panelists observed a taste difference between the recombinate lacking the theaflavins and the total taste recombinate, but they were not able to describe the taste difference in the taste profile analysis. Consequently, the catechins have been demonstrated to contribute to the astringent and bitter tastes of black tea infusions as suggested earlier in the literature (*7*, *8*). In contrast, the calculated Dot factors as well as the results of the sensory studies did not show any taste impact of the benzotropolone-type theaflavins. In contradiction to the literature (*1*−*4*), these theaflavins can be excluded as key taste compounds of the tea infusion.

Table 3. Influence of Tastant Groups or Individual Taste Compounds on the Taste Profile of the Taste Recombinate

tastant omitted	no. of panelists[a] detecting a difference	description of taste difference[b] (change in intensity)
total group I	7	less astringent (2.2→1.7) less bitter (1.5→0.6)
catechins	6	less astringent (2.2→1.6) less bitter (1.5→0.6)
theaflavins	2	no describable difference
total group II	8	less astringent (2.2→1.5) less bitter (1.5→0.5)
flavanol-glycosides	8	less astringent (2.2→1.5) less bitter (1.5→0.5)
theanine	0	no describable difference
total groups I + II	8	less astringent (2.2→0.5) less bitter (1.5→0.1)
flavonol-glycosides + catechins	8	less astringent (2.2→0.7) less bitter (1.5→0.1)
total group III	6	less astringent (2.2→1.7) less bitter (1.5→0.6)
caffeine	5	less astringent (2.2→1.7) less bitter (1.5→0.6)
amino acids	0	no describable difference
total group IV	0	no describable difference
total group V	0	no describable difference
total group VI	0	no describable difference

[a] Eight panelists were asked to detect the recombinate lacking certain tastants by means of a triangle test. [b] If the sample was detected correctly (cf. *a*), the changes in taste intensities were evaluated on a scale from 0 (not detectable) to 3 (strongly detectable).

In a second experiment, the velvety astringent and mouth-drying tastant group II, containing the flavonol-3-glycosides as well as the amino acids theanine and γ-aminobutyric acid, was omitted from the complete taste recombinate. As shown in **Table 3**, all eight panelists were able to detect this sample by means of a triangle test using two samples of the complete recombinate as the control. The panelist rated this partial recombinate as being strongly less astringent and mouth-drying (−0.7) than the total recombinate, thus fitting well with the high Dot factors calculated for most of the flavonol-3-glycosides and, in particular, the quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-glucopyranoside]. It is interesting to note that the omission of tastant group II resulted also in a significant reduction of bitterness (−1.0), although none of the compounds present in tastant group II exhibits bitter taste on its own. To further clarify the role of the individual tastant classes in group II, partial recombinates lacking either the flavonol-3-glycosides, the theanine, or the γ-aminobutyric acid were compared to the complete recombinate by means of triangle tests. Omission of the flavonol-3-glycosides led to a significant reduction of the intensity of astringency and bitter taste evaluated with the same ratings as observed for the recombinate lacking the total taste group II (**Table 3**). In an additional experiment, we prepared a partial recombinate lacking just the theanine. None of the panelists was able to sensorially detect the omission of that amino acid from the taste recombinate. On the basis of these findings, it could be confirmed that theanine and γ-aminobutyric acid do not contribute to tea taste at all and that the flavonol-3-glycosides are key contributors to the astringency and bitter taste of tea infusions.

To finally confirm the flavanol-3-glycosides and catechins as the key players in the astringent taste sensation of the tea infusion, taste recombinates lacking either a mixture of tastant groups I and II or the mixture of flavonolglycosides and catechins were prepared and sensorially compared to the complete taste recombinate consisting of 51 compounds. As given in **Table 3**, all panelists significantly detected the change in the overall taste of these partial recombinates. The panelists evaluated both partial recombinates with just very low intensity scores of 0.5 and 0.1 for the astringent/mouth-drying sensation and the bitter taste, respectively, thus demonstrating the flavonol-3-glycosides besides the catechins as major contributors to the taste of the black tea infusion.

To investigate the importance of purely bitter-tasting compounds for tea taste, either the bitter tasting group III, the alkaloid caffeine, or the bitter amino acids were omitted from the complete taste recombinate (**Table 3**). Six or five of eight panelists were able to detect a taste difference when the total group III or caffeine alone was omitted from the complete taste recombinate. The panelists described both partial recombinates as being less bitter (−0.7) and less astringent (−0.5) compared to the total taste recombinate, thus indicating that caffeine is influencing not only the bitterness but also the astringency of tea infusions. In contrast, omission of all bitter-tasting amino acids from the taste recombinate was not detectable by any of the sensory panelists, thus excluding these amino acids as contributors to tea taste.

Finally, partial recombinates have been prepared lacking either the sour-tasting group IV, the sweet-tasting group V, or the umami-like-tasting group VI. As given in **Table 3**, none of the eight panelists was able to detect any taste difference between these partial recombinates and the complete taste recombinate. Consequently, the sour-tasting organic acids, the sweet-tasting amino acids and soluble carbohydrates as well as the umami-like-tasting amino acids do not contribute to the taste of the tea infusion.

When all of these findings are taken into account, it can be concluded that flavonol-3-glycosides, catechins, and caffeine are the key tastants of the tea infusion, whereas theaflavins, soluble carbohydrates, organic acids, and amino acids do not have any significant taste impact. To further narrow the number of key taste compounds, finally, a reduced recombinate was prepared containing the bitter-tasting caffeine and those flavonol-3-glycosides and catechins that have been evaluated with Dot factors of >0.5 (cf. **Table 1**), namely, the velvety astringent quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside], kaempferol-3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside], quercetin-3-*O*-*β*-D-galactopyranoside, quercetin-3-*O*-*β*-D-glucopyranoside, kaempferol-3-*O*-*β*-D-glucopyranoside, myricetin-3-*O*-*β*-D-glucopyranoside, quercetin-3-*O*-[*β*-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→6)-*O*-*β*-D-galactopyranoside], myricetin-3-*O*-*β*-D-galactopyranoside, and kaempferol-3-*O*-*β*-D-galactopyranoside and the puckering astringent catechin as well as the astringent and bitter-tasting epigallocatechin-3-gallate. The taste profile of the partial recombinate containing these 12 taste compounds was then evaluated by the sensory panel. As given in **Table 4**, the taste profile of this partial recombinate differed not significantly from that of the complete taste recombinate containing all 51 taste compounds; only the astringent taste modality was rated with a slightly lower score (−0.2). In conclusion, the nine astringent/mouth-drying flavonol-3-glycosides, the puckering astringent catechin, the astringent and bitter-tasting epigallocatechin-3-gallate, and the bitter alkaloid caffeine have been successfully identified as the key taste compounds of the Darjeeling tea infusion.

Table 4. Taste Profile Analysis of the Authentic Tea Infusion, the Complete Taste Recombinate Containing 51 Compounds, and a Reduced Taste Recombinate Containing 12 Compounds

	intensities of individual taste qualitites in[a]		
taste quality	authentic tea infusion	complete taste recombinate	reduced taste recombinate
astringent, mouth-rying	2.2	2.2	2.1
bitter	1.6	1.5	1.5
sour	0.5	0.5	0.5
sweet	0.4	0.4	0.4
salty	0	0	0
umami	0	0	0

[a] Intensities were judged on a scale from 0 (not detectable) to 3 (strongly detectable).

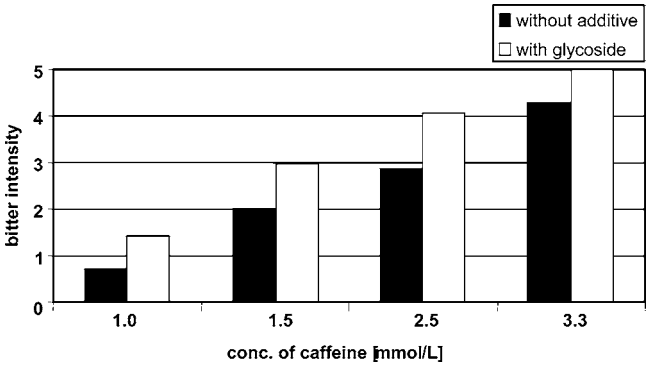

Figure 2. Influence of the astringent quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside] (0.011 mmol/L) on the bitter taste of aqueous caffeine solutions.

Influence of Flavonol-3-glycosides on the Taste Perception of Caffeine. Although the flavonol-3 glycosides do not exhibit any bitter taste on their own, the omission of these compounds from the complete taste recombinate led to a reduction of the bitter taste intensity by ∼50% (cf. **Table 3**). This prompted us to study whether flavanol-3-glycosides are able to modulate the taste activity of the key bitter compound caffeine. Aimed at investigating the influence of flavonol-3-glycosides on the perception of the caffeine bitterness, the bitter intensity of an aqueous solution of 1.0 mmol/L caffeine matching the caffeine concentration in the Darjeeling black tea was rated in the absence or presence of 0.011 mmol/L of quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside], representing its concentration in black tea, on a scale from 0 (not detectable) to 5 (strongly detection). As given in **Figure 2**, the bitterness of the caffeine solution was significantly modulated in the presence of the flavonol-3-glycoside; for example, the bitter intensity increased from 0.8 to 1.4 upon addition of the glycoside. In an additional experiment, aqueous solutions of 1.5, 2.5, and 3.3 mmol/L caffeine and 0.011 mmol/L of the glycoside were evaluated sensorially and compared to the corresponding caffeine solutions lacking the glycoside. As given in **Figure 2**, the presence of the flavonol-3-glycoside significantly enhanced the bitterness of the alkaloid also at higher caffeine concentrations, however, to a somewhat lower extent.

In conclusion, nine astringent/mouth-drying flavonol-3-glycosides, the puckering astringent catechin, the astringent and bitter-tasting epigallocatechin-3-gallate, and the bitter-tasting alkaloid caffeine have been successfully identified as the key taste compounds of the Darjeeling tea infusion by means of

quantitative studies and taste reconstitution experiments as well as taste omission studies. In addition, it could be shown for the first time that the flavanol-3-glycosides not only impart a velvety astringent taste sensation to the oral cavity upon tea consumption but also contribute to the bitter taste of tea infusions upon amplifying the bitterness of caffeine.

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