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# Molecular and Sensory Studies on the Umami Taste of Japanese Green Tea

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Aimed at defining the key drivers for the quality-determining umami taste of a high-grade powdered green tea, called mat-cha, a bioactivity-guided fractionation using solvent extraction, solvent precipitation, preparative chromatographic separations, and human psychophysical experiments was applied on freshly prepared mat-cha. Liquid chromatography−tandem mass spectrometry and one-/two-dimensional nuclear magnetic resonance studies on isolated fractions led to the identification of L-theanine, succinic acid, 3,4,5-trihydroxybenzoic acid (gallic acid), and (1*R*,2*R*,3*R*,5*S*)-5-carboxy-2,3,5-trihydroxycyclohexyl-3,4,5-trihydroxybenzoate (theogallin) as umami-enhancing compounds in the green tea beverage, and it can be shown by sensory studies that these compounds are able to raise the umami intensity of sodium L-glutamate proportionally.

**KEYWORDS: Taste; taste enhancer; umami; green tea; mat-cha; theogallin; L-theanine**

## INTRODUCTION

The freshly prepared infusion of the dried leaves and buds of the plant *Camellia sinensis* has been consumed by humans for thousands of years as a desirable beverage. Whereas fermented black tea, accounting for about 75% of the world tea production, is consumed mainly in the Western countries, nonfermented green tea is traditionally preferred as one of the most popular beverages in Japan. Historically consumed during Japanese tea ceremonies, so-called mat-cha, literally powdered green tea, is a precious, jewel-green powder made from hand-picked, high-grade Japanese green tea grown under shade conditions in the absence of direct sunlight. Mat-cha is whisked with water at a relatively low temperature of 70−80 °C in a special bowl to make a creamy, frothy, healthful beverage exhibiting a unique taste profile centering around a rich and complex umami-like taste sensation (*1*) in addition to fresh vegetable-like, green, and roast notes.

Because the taste quality is one of the key criteria used by the tea tasters to describe the quality of tea beverages, multiple attempts have been made to correlate the sensory results of the tea tasters and the molecules exhibiting the typical taste of tea infusions, but the data reported so far on the molecules imparting the umami taste of mat-cha are very contradictory. Removal of the amino acids from infusions of green tea by means of preparative ion exchange chromatography, followed by sensory analysis of the remaining tea extract depleted in amino acids, suggested that approximately 70% of the umami taste intensity of green tea is due to amino acids (*2*). Besides L-glutamate, the amino acid 5-*N*-ethylglutamine (theanine), accounting for about two-thirds of the total amino acid content in green tea leaves, is reported as a key contributor to the umami taste of green tea (*3*). In addition, some nucleotides such as adenosine 5′-monophosphate (AMP) were identified as umami-tasting compounds (*4*) and have been suggested to synergistically enhance the taste of monosodium L-glutamate (*5*). In contradiction, the taste contribution of nucleic acids to green tea taste was reported to be neglectable due to their low concentrations in the tea infusion (*6*).

Aimed at discovering the compounds contributing to the umami taste of mat-cha, the objectives of the present investigation were, therefore, to screen a freshly prepared mat-cha beverage for its umami contributors by combining instrumental analysis and human psychobiological tools, to isolate and identify the key compounds, and to evaluate their taste impact on the basis of taste reconstruction and omission experiments.

## MATERIALS AND METHODS

**Chemicals.** The following compounds were obtained commercially: L-glutamic acid, L-aspartic acid, potassium hydroxide, sodium hydroxide, gallic acid monohydrate (Wako Pure Chemical Industries, Japan), gallustannic acid (0.05%) and quercetin-3-*O*-*β*-D-glucopyranoside (Roth, Karlsruhe, Germany), L-theanine (Tokyo Kasei Kogyo Co., Japan), succinic acid (Nakarai Chemicals, Japan), 5′-adenosine monophosphate, 5′-guanosine monophosphate disodium salt (Kohjin Co., Japan), magnesium hydroxide, and hydrochloric acid (Kanto Chemical Co., Japan). Solvents were of high-performance liquid chromatography (HPLC) grade (Merck, Germany). Deuterated solvents were obtained from Euriso-Top (Gif-Sur-Yvette, France). Mat-cha (Aiya Co., Aichi, Japan, high-grade, Omatcha Gokujohin) was obtained from Aiya Co.

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Ltd. Gallustannic acid and quercetin-3-*O*-*β*-D-glucopyranoside were obtained from Roth and purified by means of RP18 chromatography to a final purity of more than 99.9%.

**Sensory Analyses.** *Training of the Sensory Panel.* Twelve subjects with no history of known taste disorders were trained to evaluate the taste of aqueous solutions (2 mL each) of the following standard taste compounds in bottled water (pH 6.0) by using a triangle test as described in the literature (*7*): sucrose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (20 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, monosodium L-glutamate (8 mmol/L; pH 5.7) for umami taste, and gallustannic acid (0.05%) and quercetin-3-*O*-*β*-D-glucopyranoside (0.01 mmol/L) for the puckering astringency and the velvety astringent, mouth-drying oral sensation. The assessors had participated earlier at regular intervals for at least 2 years in sensory experiments and were, therefore, familiar with the techniques applied. Sensory analyses were performed in a sensory panel room at 19−22 °C in three different sessions.

*Taste Profile Analysis*. A freshly prepared mat-cha beverage was presented to the sensory panel, who was asked to score the taste qualities astringent, bitter, sour, sweet, salty, and umami on a scale from 0 (not detectable) to 5 (strong detectable). To achieve this, the samples (2 mL) were applied with a plastic pipet, swirled around in the mouth briefly, and expectorated.

*Evaluation of Taste Intensities.* Taste intensities of test solutions in bottled water adjusted to pH 6.0 by adding trace amounts of aqueous formic acid or potassium hydroxide solution (0.1 mM) were rated by trained sensory panels using a five-point scoring method. To evaluate the potential of individual tea fractions or purified compounds, respectively, in enhancing the umami taste of monosodium glutamate (MSG), the umami taste intensity of a binary mixture of monosodium l-glutamate (3 mmol/L) and the fraction or compound under investigation was compared to that of an aqueous solution of monosodium L-glutamate (3 mmol/L) as the control.

*Determination of Recognition Threshold Concentrations.* Taste thresholds were determined by means of a triangle test with two samples of bottled water (pH 6.0) as the control. To prevent excessive fatigue, tasting began at a concentration level two steps below the threshold concentration that had been determined in a preliminary taste experiment. Whenever the panelist selected incorrectly, the next trial took place at the next higher concentration step. When the panelist selected correctly, the same concentration was presented again besides one blank as a proof for the correctness of the data. The geometric mean of the last and the second last concentration was calculated and taken as the individual recognition threshold. The values between individuals, and between five separate sessions, differed by not more than two dilution steps.

*Biomimetic Taste Reconstruction and Omission Experiments.* To reconstruct the umami taste of mat-cha, the compounds summarized in **Table 2** were dissolved in natural concentrations in distilled water, which was adjusted to pH 6.0. The umami taste intensity of an aliquot (2 mL) of that cocktail was evaluated by each panellist by means of a five-point scoring method and was compared to the umami taste of the authentic mat-cha beverage. To confirm the taste contribution of individual compounds, omission experiments were done by comparing the umami intensity of the total recombinate with the intensity of partial recombinates lacking in single compounds.

*Determination of Isointensity of Umami Solutions.* The umami taste intensity of aqueous solutions (pH 6.0) containing constant amounts of monosodium L-glutamate (3.0 mmol/L) but increasing concentrations of (1*R*,2*R*,3*R*,5*S*)-5-carboxy-2,3,5-trihydroxycyclohexyl-3,4,5-trihydroxybenzoate (theogallin), l-theanine, or succinic acid, respectively, was compared to the taste intensity of a dilution series of monosodium L-glutamate by means of a comparative duo test. The concentration at which no difference between both solutions could be detected was selected as the iso-intense solution.

**Preparation of a Mat-Cha Infusion.** Powdered mat-cha leaves (100 g) were infused with distilled water (1 L) at 80 °C for 4 min while stirring with a magnetic stirrer. The green homogeneous suspension was then rapidly cooled to room temperature in an ice bath, the nonsoluble materials were removed by the taste-active solubles by centrifugation (3000 rpm) for 10 min at room temperature, and the soluble part was filtered and freeze-dried to give the lyophylized mat-cha solubles in a yield of 17%. Aliquots (600 mg) of the lyophylisate obtained were taken up in bottled water (100 mL) and used for sensory experiments, chemical analysis, and fractionation experiments.

**Liquid−Liquid Extraction of the Mat-Cha Solubles.** The lyophylized mat-cha solubles (15 g) were dissolved in distilled water (500 mL) and then sequentially extracted with dichloromethane (2 × 500 mL) and ethyl acetate (2 × 500 mL). The individual fraction was used for a sensory experiment. After the solvents were removed in high vacuum (<5 mPa) and freeze-dried twice, the dichloromethane solubles (fraction I; yield, 0.6 g), the ethyl acetate extractables (fraction II; yield, 1.0 g), and the remaining aqueous layer (fraction III; yield, 12.8 g) were stored in a desiccator at −18 °C prior to use and then used for sensory analyses as well as additional fractionation experiments.

**Solvent Precipitation of the Mat-Cha Fraction III.** The umami-tasting aqueous fraction III isolated from the mat-cha infusion was suspended in a methanol/water mixture (50/50, v/v) and maintained for 10 min at room temperature. The white precipitate formed (fraction IIIa) was removed by filtration, and the filtrate (fraction IIIb) was freed from solvents by high vacuum distillation (<5 mPa) and lyophylization.

**Preparative Separation of Fraction IIIb on RP18 Material.** Aliquots (9.2 g) of the mat-cha fraction IIIb were taken up in water (50 mL) acidified with formic acid to pH 4.0 and then further separated by column chromatography (100 mm × 40 mm) on RP18 material ODS RP18 25−40 *μ*m (Phenomenex, Aschaffenburg, Germany) conditioned with water (pH 4.0). After application of the crude material, chromatography (flow rate, 1.1 mL/min) was performed with water (10 × 50 mL, fraction IIIb-A), followed by methanol (10 × 50 mL, fraction IIIb-B). Both fractions were collected, freed from solvent in high vacuum, freeze-dried, and finally used for sensory analysis as well as HPLC analysis.

**HPLC Tasting of Mat-Cha Fraction IIIb-A Separation.** An aliquot (117 mg) of fraction IIIb-A was taken up in aqueous formic acid (0.1% in water, 0.8 mL), membrane filtered, and then analyzed by RP18-HPLC on a ODS-Hypersil 100-5C18 column, 250 mm × 10 mm i.d. (ThermoHypersil, Kleinostheim, Germany). Monitoring the effluent at 220 nm, chromatography was performed isocratically with aqueous formic acid (0.1% in water) or 60 min at a flow rate of 3.0 mL/min, and then, the methanol content was increased to 100% within 10 min. In total, 22 peaks (fractions IIIb-A/1−IIIb-A/22) were collected individually in several runs, the eluates of the corresponding fractions were combined and freeze-dried, and the residues obtained were then taken up in water (18 mL) in order to evaluate the taste quality as well as the taste intensity of these fractions on a five-point scale or in an aqueous solution of monosodium L-glutamate (3.0 mmol/L; 18 mL) in order to determine the umami taste-enhancing activity of these fractions.

**Isolation and Identification of Umami-Enhancing Compounds in Fraction IIIb-A.** Fractions IIIb-A/8, IIIb-A/14, and IIIb-A/21, for which the umami taste-enhancing activity has been detected (**Table 1**), were purified by means of reversed phase (RP)-HPLC following the method described above. liquid chromatography-tandem mass spectrometry (LC-MS/MS), one-dimensional (1D)/1D NMR spectroscopy, and sensory studies led to the unequivocal identification of the umami enhancer in fraction IIIb-A/21 as theogallin. In addition, HPLC-diode array detection (DAD), HPLC-MS/MS, and comparison of chromatographic, spectroscopic, and sensory data with those obtained for the corresponding reference compounds led to the unequivocal identification of gallic acid and L-theanine as the taste modifiers in fraction IIIb-A/14 and fraction IIIb-A/8, respectively.

**Theogallin.** UV/vis (water): 280 nm. LC-MS/MS ($ESI^+$): *m*/*z* 345 (72), 327 (40), 153 (100). $^1$H NMR (400 MHz, DMSO-$d_6$; COSY): $\delta$ 1.80 [dd, 1H, $^2J$ = 13.0 Hz, $^3J$ = 7.8 Hz, $H_{ax}$-C(2)], 1.95 [dd, 1H, $^2J$ = 13.3 Hz, $^3J$ = 3.7 Hz, $H_{ax}$-C(6)], 2.06 [m, 2H, $H_{eq}$-C(2), $H_{eq}$-C(6)], 3.59 [m, 1H, H-C(4)], 3.96 [m, 1H, H-C(5)], 4.77 [d, 1H, $^3J$ = 6.0 Hz, HO-C(5)], 4.93 [d, 1H, $^3J$ = 5.2 Hz, HO-C(4)], 5.10 [ddd, 1H, $^3J$ = 6.9 Hz, $^3J$ = 4.1 Hz, H-C(3)], 5.55 [s, 1H, HO-C(1)], 6.91 [s, 2H, H-C(2′), H-C(6′)], 8.93 [s, 1, HO-C(4′)], 9.19 [s, 2H, HO-C(3′), HO-C(5′)]. $^{13}$C{$^1$H} NMR (100 MHz, DMSO-$d_6$; HMQC, HMBC): $\delta$ 36.1 [C(2)], 37.0 [C(6)], 68.1 [C(5)], 70.5 [C(4)], 71.1 ([C(3)]), 73.4 [C(1)], 108.7 [C(2′), C(6′)], 119.7 [C(1′)], 138.3 [C(4′)], 145.3 [C(3′), C(5′)], 165.1 [C(7′)], 174.7 [C(7)].

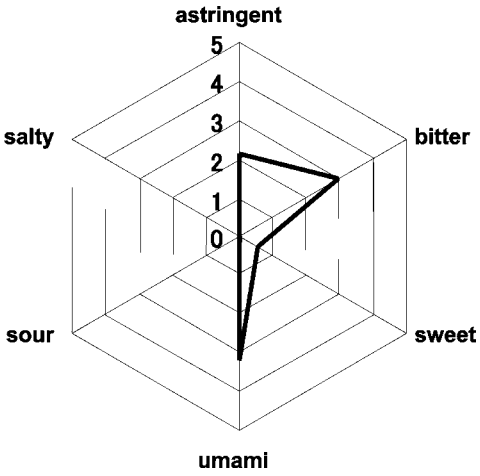


**Figure 1.** Taste profile of a freshly prepared high-grade mat-cha beverage.

**3,4,5-Trihydroxybenzoic Acid (Gallic Acid).** UV/vis, LC-MS, and $^1$H NMR data of the compound isolated from the mat-cha infusion were identical to those found for the reference compound.

***N*-Ethylglutamine (Theanine).** LC-MS and $^1$H NMR data of the compound isolated from the mat-cha infusion were identical to those found for the reference compound.

**Quantitative Analyses.** Amino acids including theanine were quantified by means of an amino acids analyzer L-8500A (Hitachi, Tokyo, Japan) with ninhydrin detection (*8*). Organic acids were quantified by means of an CAPCELL Pak $NH_2$ column, 4.6 mm × 250 mm i.d. (Shiseido, Tokyo, Japan) as reported earlier (*9*). Nucleotides were quantified by HPLC on a Develosil C30-UG column, 4.6 mm × 250 mm i.d. (Nomura Chemical Co., Aichi, Japan) (*7*). Sodium, potassium, and magnesium were quantitatively determined by means of atomic absorption spectroscopy, and chloride was determined by means of potentiometry (*10*). After their identities were confirmed by means of LC-MS, quantification of theogallin and gallic acid was performed by analytical RP-HPLC on a ODS-Hypersil 100-5C18 column, 250 mm × 4.6 mm i.d. (ThermoHypersil) by comparing the peak areas obtained at 280 nm with those of defined standard solutions of each reference compound in methanol.

**HPLC.** The HPLC apparatus (BIO-TEK Kontron Instruments, Eching, Germany) consisted of two pumps (type 522), a Rheodyne injector (100 $\mu$L loop), and a DAD (type 540) monitoring the effluent in a wavelength of 220 nm.

**LC/MS.** Electrospray ionization (ESI) spectra were acquired on an API 4000 Q-Trap (Applied Biosystems, Darmstadt, Germany) with direct flow infusion. The spray voltage was set at 5500 V, and the declustering potential was set at 30 V in the $ESI^+$ mode using nitrogen, which served as the curtain gas (20 psi). For HPLC-MS/MS, an Agilent 1100 series HPLC equipped with an analytical Synergi Fusion-RP column, 2 mm × 150 mm i.d., 4 $\mu$m (Phenomenex), was coupled to the mass spectrometer operating in the ESI mode. After injection of the sample (10 $\mu$L), analysis was performed using a mixture (98/2, v/v) of aqueous formic acid (0.1%) and methanol as the solvent.

**NMR Spectroscopy.** The $^1$H, $^{13}$C, COSY, HMQC, and HMBC spectroscopic experiments were performed on a Bruker DMX-400 spectrometer (Bruker, Rheinstetten, Germany). DMSO-$d_6$ was used as the solvent, and chemical shifts were measured using tetramethylsilane as the internal standard.

## RESULTS AND DISCUSSION

The freshly prepared aqueous suspension of the mat-cha tea imparted the expected complex taste and was therefore used for taste profile analysis. To achieve this, the trained sensory panel was asked to rate the intensity of the taste qualities given in **Figure 1** on a scale from 0 (not detectable) to 5 (very strong sensation). By far, the highest scores were found for the intensity of the umami-like taste (3.1) as well as the bitter taste (3.0), followed by an astringent oral sensation judged with a somewhat lower intensity of 2.2. In comparison, sweetness was just evaluated with a rather low intensity score of 0.6 and the taste qualities sourness and saltiness were not detectable at all.

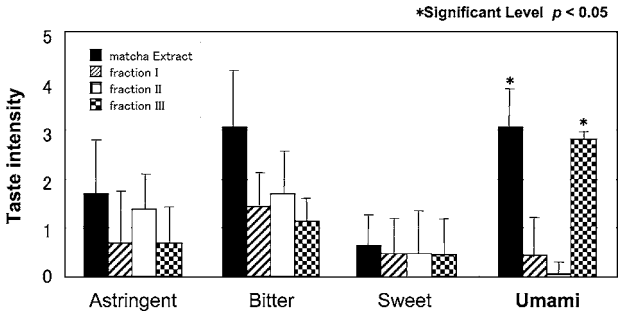


**Figure 2.** Intensity of taste qualities of mat-cha extract and fractions I–III obtained by solvent fractionation.

**Screening for Umami Taste Contributors in Mat-Cha.** To get first insights into the hydrophilicity of the key drivers of the typical umami sensation imparted by the mat-cha, the freshly prepared beverage was sequentially extracted with dichloromethane and ethyl acetate. After the solvent was removed by high vacuum distillation and freeze-drying, aliquots of the dichloromethane solubles (fraction I), the ethyl acetate extractables (fraction II), and the water soluble material (fraction III) were dissolved in water in their "natural" concentrations, and the taste of these aqueous solutions was sensorially compared to the taste profile of the authentic mat-cha infusion using a five-point intensity scale (**Figure 2**). The aqueous solution of fractions I and II, respectively, just showed rather high intensities in bitter taste and astringency, whereas umami taste and sweetness were not perceivable. In contrast, the hydrophilic fraction III imparted an umami taste (2.8) closely matching the umami taste intensity of the mat-cha infusion (3.1) and, in addition, exhibited a minor impact in sweetness (0.5), bitterness (1.1), and astringency (0.8). These data clearly indicate that the umami compounds in mat-cha seem to be hydrophilic, whereas astringent and bitter taste drivers belong to the group of hydrophobic tea components (**Figure 2**).

To enrich the taste compounds responsible for the umami-like taste of mat-cha, polysaccharides were removed from the highly viscous aqueous solution of the umami-like tasting fraction III by precipitation with 50% aqueous methanol. After the solvent was removed in high vacuum, followed by freeze-drying, sensory analysis on aliquots of the precipitate (fraction IIIa) as well as the soluble fraction IIIb revealed that the umami taste is exclusively present in fraction IIIb, whereas the precipitate was tasteless (data not shown). To achieve a further concentration of the umami taste contributors, the taste-active fraction IIIb was separated by preparative column chromatography on RP18 material. After application of the crude fraction, subfractions IIIb-A and IIIb-B were collected after flushing the column with water and methanol, respectively. The solvents were then removed in high vacuum, followed by freeze-drying, and aliquots of these fractions dissolved in water in their "natural" concentrations were evaluated sensorially. As given in **Figure 3**, fraction IIIb-A exhibited an umami-like and a sweet taste only, the intensity of which matched that of the mat-cha fraction III. In contrast, fraction IIIb-B just contained the compounds contributing to the bitter and astringent taste of fraction III.

To identify the key drivers of the umami-like taste of mat-cha, the umami-like tasting fraction IIIb-A was separated into 22 subfractions by means of semipreparative RP-HPLC monitoring the effluent at 220 nm (**Figure 4**). After freeze-drying,

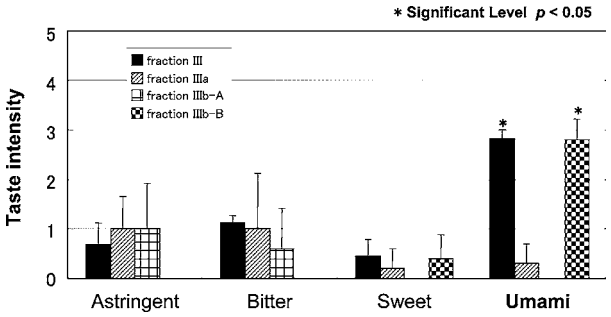


**Figure 3.** Intensity of taste qualities of subfractions obtained by solvent precipitation and RP18 chromatography of solvent fraction III.

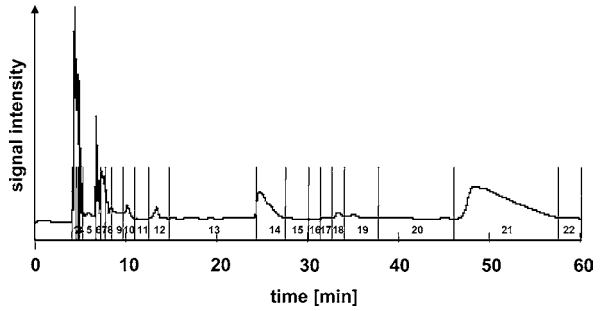


**Figure 4.** RP18-HPLC chromatogram of fraction IIIb-A isolated from mat-cha.

**Table 1.** Taste Quality, Taste Intensity, and Umami Enhancement Activity of HPLC Fractions Isolated from Mat-Cha Fraction IIIb-A

| fraction no. | taste quality | taste intensity | umami intensity in the presence of MSG[a] |
|---|---|---|---|
| 1 | astringent | 1 | 1.0 |
| 2 | astringent sweet | 2 | 1.0 |
| 3 | astringent | 3 | 2.0 |
| | umami | 1 | |
| 4 | umami | 1 | 2.0 |
| 5 | umami | 1 | 1.0 |
| | astringent | 1 | 1.0 |
| 6 | tasteless | <1 | 1.0 |
| 7 | astringent | 1 | 1.3 |
| 8 | tasteless | <1 | 3.0 |
| 9 | tasteless | <1 | 1.0 |
| 10 | tasteless | <1 | 1.0 |
| 11 | tasteless | <1 | 1.0 |
| 12 | astringent | 1 | 1.3 |
| 13 | tasteless | <1 | 3.0 |
| 14 | astringent | 3 | 2.0 |
| 15 | tasteless | <1 | 1.0 |
| 16 | tasteless | <1 | 1.0 |
| 17 | tasteless | <1 | 1.0 |
| 18 | tasteless | <1 | 1.4 |
| 19 | tasteless | <1 | 1.0 |
| 20 | bitter | 2 | 1.0 |
| 21 | astringent | 2 | 2.5 |
| | tasteless | <1 | 1.0 |

[a] The fractions were added to a solution of monosodium L-glutamate (MSG, 3 mmol/L; umami intensity, 1.0), and the umami intensity of these binary solutions was evaluated on a scale from 0 (not detectable) to 5 (strong sensation).

an aliquot of each individual subfraction was dissolved in water, the taste quality was evaluated by the trained sensory panel, and the intensity of the taste perceived was judged on a five-point scale as described above. As given in **Table 1**, fractions IIIb-A/3 to IIIb-A/5 exhibited the umami taste with an intensity of 1 or 2. In addition, fraction IIIb-A/20 was evaluated as bitter and eight fractions exhibited the astringent taste with fractions IIIb-A/3 and IIIb-A/14 judged with a rather high-intensity score of 3 (**Table 1**).

Because amino acid analysis revealed L-glutamate to be responsible for the umami taste of fractions IIIb-A/3 to IIIb-A/5, we believed that additional compounds in fraction IIIb-A might contribute to the umami-like taste of mat-cha by intensifying the umami stimulus induced by L-glutamate. By using the recently developed half-mouth test (*7*), aliquots of the 22 HPLC fractions were therefore dissolved in an aqueous solution of 3 mmol/L MSG and the umami intensity of these binary mixtures was compared to that of a 3 mmol/L MSG solution, which was evaluated with an umami intensity score of 1.0. As given in **Table 1**, the presence of the fractions IIIb-A/8, IIIb-A/13, IIIb-A/14, or IIIb-A/21, which did not exhibit any umami taste on their own, resulted in a significant increase of the umami taste intensity of the MSG solution. On the basis of these findings, it was concluded that there are polar compounds in these fractions, which are able to intensify the umami taste of MSG and contribute to the umami-like taste sensation of mat-cha.

**Identification of Umami Modifiers.** After isolation and HPLC purification and $^1$H NMR and LC/MS measurements, comparison of the spectroscopic data with those obtained for the commercially available reference compounds, followed by cochromatography, led to the identification of theanine and gallic acid as the umami modifiers in fractions IIIb-A/8 and IIIb-A/14, respectively. Whereas L-theanine has already been reported as an umami taste compound in tea (*2*,*11*), the influence of gallic acid on the umami taste of green tea has as yet not been reported.

LC-MS and MS/MS analysis of the compound in fraction IIIb-A/21 showed a pseudomolecular ion $[M + H]^+$ with *m*/*z* 345 as well as the ion *m*/*z* 153 as the base peak, thus indicating the presence of a galloyl group in the molecule. This was further confirmed by means of $^1$H NMR spectroscopy showing a resonance signal at 6.91 ppm integrating for the two aromatic protons H-C2′ and H-C6′ as part of the galloyl moiety (**Figure 5**). In addition, two resonance signals were detectable at a low magnetic field at 9.19 and 8.93 ppm, respectively, which disappeared upon addition of minor amounts of deuterium oxide and were therefore assigned as the phenolic hydroxyl protons HO-C(3′), HO-C(4′), and HO-C(5′) in the molecule, the structure of which is outlined in **Figure 5**. Additional hydroxyl protons HO-C(1), HO-C(4), and HO-C(5), being part of a quinic acid moiety, resonated at 5.50, 4.93, and 4.77 ppm, respectively. The chemical shifts and the assignment of the protons H-C(2), H-C(4), H-C(5), and H-C(6) were achieved by means of homonuclear (COSY) and heteronuclear (HMQC, HMBC) correlation experiments. The resonance signal at 5.10 ppm could be assigned as the proton linked to carbon atom C(3) bearing the galloyl moiety. HMBC experiments allowed the detection of a connectivity between this proton and the corresponding carbonyl atom C(7′) of the galloylester moiety (**Figure 6**), thus confirming the position of the galloyl substitution of the quinic acid and enabling the unequivocal identification of theogallin as the umami modifier in fraction IIIb-A/21. Although this compound has been reported earlier as a tea constituent (*12*), to the very best of our knowledge, its intensifying effect on the umami taste of L-glutamate has as yet not been reported.

**Quantification of Taste Compounds and Calculation of Dose over Threshold (DOT) Factors.** To evaluate the umami taste contribution of theanine, theogallin, and gallic acid besides all the compounds that have been previously reported to contribute to umami taste (*4*, *5*, *13*−*17*), namely, L-glutamic acid, L-theanine, L-aspartic acid, succinic acid, AMP, guanosine 5′-monophosphate (GMP), sodium, potassium, magnesium, and chloride ions, the concentrations of these compounds were

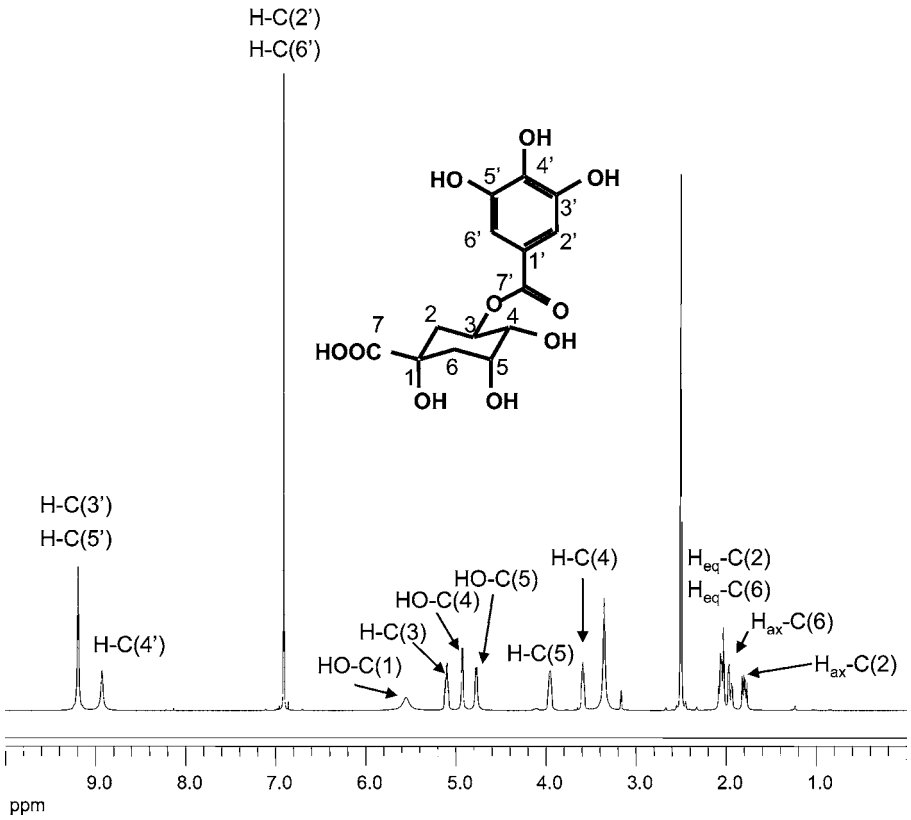


**Figure 5.** $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) and chemical structure of theogallin.

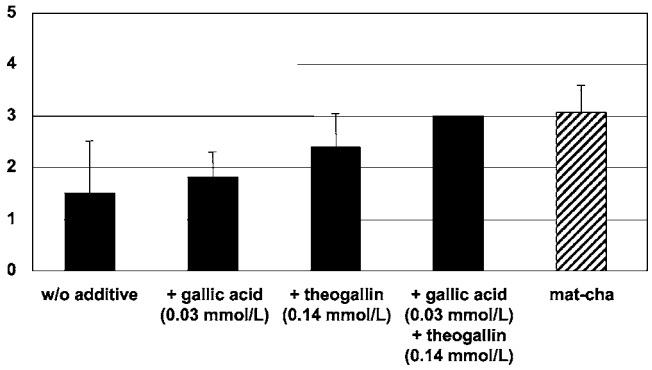


**Figure 6.** Influence of gallic acid and theogallin, respectively, on the umami taste intensity of an aqueous solution (pH 6.0) containing L-glutamic acid, L-theanine, L-aspartic acid, succinic acid, AMP, GMP, sodium, potassium, magnesium, and chloride (concentrations are given in **Table 2**).

quantitatively measured in mat-cha and their taste recognition threshold concentrations were determined by means of triangle tests. To rate these compounds in their taste impact, DOT factors were calculated as the ratio of the concentration of an individual compound in mat-cha beverage and its corresponding taste recognition threshold. As given in **Table 2**, L-glutamic acid was the only umami-like tasting compound evaluated with a DOT factor above one and exceeding its threshold concentration in mat-cha by a factor of 7. As the concentration of all of the other umami-tasting compounds were significantly below their corresponding umami detection thresholds, it was assumed that some of these compounds might increase the taste intensity of L-glutamic acid already in subthreshold concentrations. Aimed at investigating the umami-enhancing activity of each individual compound, taste reconstruction as well as taste omission experiments have been performed in the following.

**Reconstruction of Mat-Cha Umami Taste and Omission Experiments.** To confirm the sensory contribution of theogallin and gallic acid to the umami taste of mat-cha, a biomimetic taste recombinate was prepared containing natural amounts of all of the compounds that have been previously reported to contribute to umami taste of tea, namely, L-glutamic acid, L-theanine, L-aspartic acid, succinic acid, AMP, GMP, sodium, potassium, magnesium, and chloride ions (**Table 2**), and the umami taste intensity of this solution was evaluated in the absence or the presence of theogallin and/or gallic acid. As given in **Figure 7**, the umami taste intensity of the basic taste recombinate evaluated in the absence of any further additive was judged with an intensity of 1.5 on the five-point score applied. After the addition of the natural amount of gallic acid (0.03 mmol/L) or theogallin (0.14 mmol/L), respectively, the umami taste was intensified to reach scores of 1.8 and 2.4. Moreover, in the presence of gallic acid and theogallin, the umami taste of the tastant cocktail was evaluated with an intensity of 3.0 closely matching the umami taste intensity of the authentic mat-cha. These data clearly demonstrated both trihydroxybenzene compounds, theogallin and gallic acid, as umami taste modifiers and key contributors to the typical taste of mat-cha.

To further investigate the contribution of the individual tea compounds to the umami taste of mat-cha, a total taste recombinate was prepared by dissolving the 12 compounds in their natural concentrations as listed in **Table 2** and comparing the umami taste intensity of this solution with partial recombinates lacking individual umami compounds. As given in **Figure 8**, the umami intensity of the total reconstitute dropped from 3.0 to 0.7 when L-glutamic acid was omitted, thus demonstrating the key importance of that amino acid for the umami taste of mat-cha. Furthermore, the lack of minerals, theogallin, succinic acid, and L-theanine, respectively, led to a strong decrease of the umami taste intensity from 3.0 to $<2.0$, thus identifying these compounds as important umami taste modifiers in mat-cha. In contrast, the omission of aspartic acid,

**Table 2.** Concentrations, Taste Thresholds, and DOT Factors of Selected Components in Mat-Cha

| compounds | taste quality | threshold concn (mmol/L) | concn in mat-cha leaves (mmol/kg) | concn in matcha infusion (mmol/L) | DOT factor[a] |
|---|---|---|---|---|---|
| L-glutamic acid | umami | 0.20 | 41 | 1.4 | 7.0 |
| L-theanine | astringent | 6.0 | 103 | 3.4 | 0.6 |
| | umami, sweet | 24.0 | | | 0.1 |
| L-aspartic acid | umami | 7.5 | 35 | 1.2 | 0.2 |
| succinic acid | umami | 0.9 | 19 | 0.10 | 0.1 |
| AMP | umami | 2.0 | 0.0089 | 0.00030 | <0.1 |
| GMP | umami | 0.3 | 0.015 | 0.00056 | <0.1 |
| sodium | salty | 7.5[b] | 0.33 | 0.011 | <0.1 |
| potassium | salty | 15.0[b] | 538 | 18.0 | 1.2 |
| magnesium | salty | 4.0[b] | 17 | 0.57 | 0.1 |
| chloride | | 7.5[c] | 0.70 | 0.023 | <0.1 |
| theogallin | astringent | 0.09 | 3.9 | 0.14 | 1.5 |
| gallic acid | astringent | 0.20 | 0.82 | 0.03 | 0.2 |

[a] The DOT factor was calculated from the ratio of the concentration and the taste threshold concentration of the compound. [b] The taste threshold was determined for the corresponding chloride. [c] The taste threshold was determined for the corresponding sodium salt.

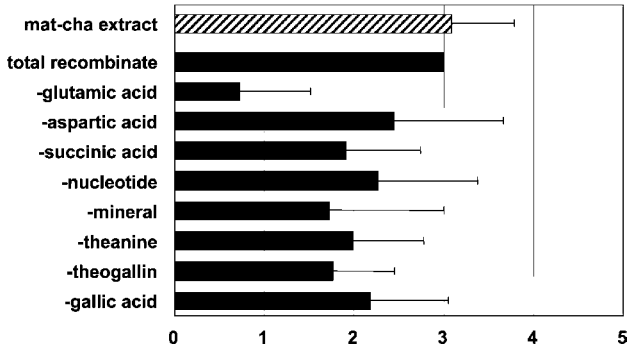


**Figure 7.** Contribution of individual tea components to the umami taste intensity of mat-cha.

nucleotides, and gallic acid from the total taste recombinate induced just a minor reduction in umami taste intensity (**Figure 8**).

**Influence of Selected Compounds on the Umami Taste of L-Glutamate.** To further investigate the umami taste-modifying effect of gallic acid, theogallin, succinic acid, and L-theanine, increasing amounts of the individual compounds were added to an aqueous solution of MSG (1.4 mmol/L), and the umami taste intensity of these binary mixtures was compared to that of a dilution series of MSG by means of a duo test until the iso-intensity in the umami sensation was reached. Although gallic acid showed an astringent taste sensation above the threshold concentration of 0.2 mmol/L (**Table 2**), the umami taste intensity of the 1.4 mmol/L MSG solution containing gallic acid just in a concentration of 0.06 mmol/L was iso-intense to a 1.8 mmol/L MSG solution (**Figure 8A**). Also, theogallin (**Figure 8B**), L-theanine (**Figure 8C**), and succinic acid (**Figure 8D**) induced an increase in umami taste intensity of the MSG solution already at levels below their threshold concentrations (**Table 2**), but the taste intensity of MSG rose with increasing concentration of the modifiers (**Figure 8B**).

In summary, application of a sensory-guided fractionation of mat-cha using solvent extraction, solvent precipitation, followed by preparative chromatographic separations, revealed that the typical and quality-determining umami-like taste of high-grade mat-cha is due to an interplay between the umami-tasting L-glutamate and several umami modifiers. Among these, although being present in subthreshold concentrations, theogallin, L-theanine, and succinic acid have been identified for the first time as umami taste enhancers in high-grade mat-cha.

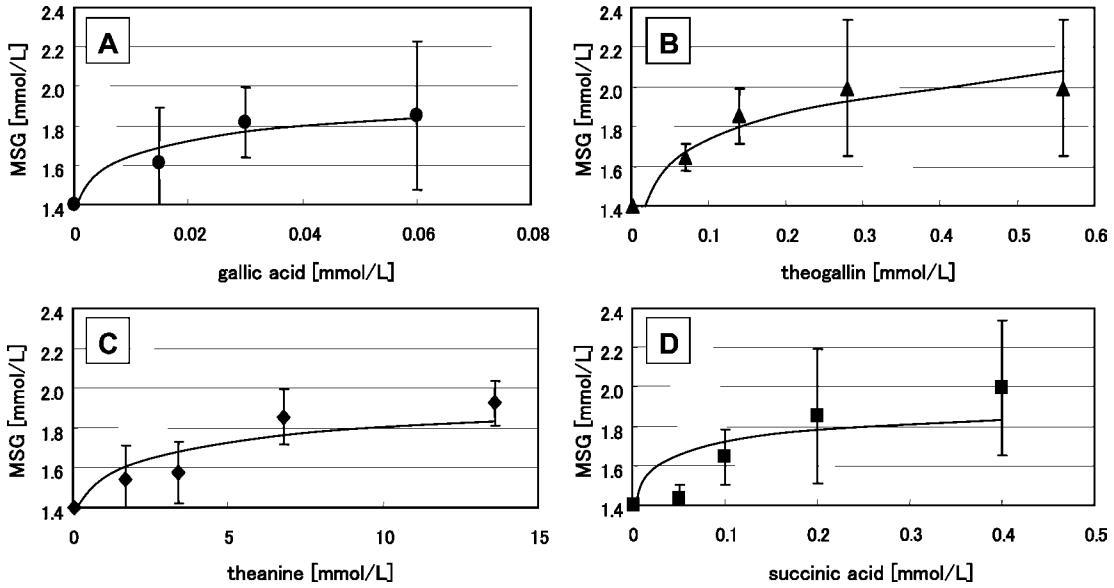


**Figure 8.** Influence of gallic acid (**A**), theogallin (**B**), L-theanine (**C**), and succinic acid (**D**), respectively, on the umami intensity of an aqueous solution (pH 6.0) of MSG (1.4 mmol/L).

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