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Characterization of the Key Aroma Compounds in the Beverage Prepared from Darjeeling Black Tea: Quantitative Differences between Tea Leaves and Infusion

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By application of the aroma extract dilution analysis on the volatile fraction isolated from a black tea infusion (Darjeeling Gold Selection), vanillin (vanilla-like), 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (caramel), 2-phenylethanol (flowery), and (*E*,*E*,*Z*)-2,4,6-nonatrienal (oat-flake-like) were identified with the highest flavor dilution (FD) factors among the 24 odor-active compounds detected in the FD factor range of 4−128. Quantitative measurements performed by means of stable isotope dilution assays and a calculation of odor activity values (OAVs; ratio of concentration to odor threshold in water) revealed, in particular, the previously unknown tea constituent (*E*,*E*,*Z*)-2,4,6-nonatrienal as a key odorant in the infusion and confirmed the important role of linalool and geraniol for the tea aroma. An aroma recombinate performed by the 18 odorants for which OAVs > 1 were determined in their "natural" concentrations matched the overall aroma of the tea beverage. In the black tea leaves, a total of 42 odorants were identified, most of which were identical with those in the beverage prepared thereof. However, quantitative measurements indicated that, in particular, geraniol, but also eight further odorants were significantly increased in the infusion as compared to their concentration in the leaves.

KEYWORDS: Black tea; (*E*,*E*,*Z*)-2,4,6-nonatrienal; aroma extract dilution analysis; aroma reconstitution; Darjeeling

INTRODUCTION

The tea plant is of great economic importance and, after water, the tea infusion is the most consumed beverage worldwide. Today about 3.1 million metric tons of different types of tea are produced, with India closely followed by China being the main producers of tea.

The common process of black tea production consists of four stages, namely, withering, rolling, fermentation, and firing. Besides being differentiated by their origin, three main types of tea are generally produced: unfermented (green), semifermented (oolong), and fermented (black) tea. Because in black tea processing enzymatic processes are active prior to the drying process, many biochemical reactions are known to occur.

Besides the typical astringent, bitter taste, in particular, the characteristic aroma of the tea beverage is an important criterion in the evaluation of the tea quality.

Investigations on the volatile compounds of tea have been performed since the 1930s, and this area of research is still very active. The first studies were performed already more than 60 ago, leading to the identification of linalool, geraniol, and (*Z*)-3-hexenol in the volatile fraction of tea leaves or the tea beverage, respectively (cf. review in ref *1*). Studies performed later by Bandarovich et al. (*2*), Renold et al. (*3*), Vitzthum et al. (*4*), Mick et al. (*5*), and Mick and Schreier (*6*) have tremendously increased the number of volatiles identified in tea, and to date about 600 constituents have been characterized in tea leaves or in the beverage prepared thereof, respectively. The current knowledge in tea flavor research has been comprehensively reviewed by Robinson and Owour (*7*), Constantinides et al. (*8*), and Yamanishi and Kobayashi (*1*).

In particular for foods, the aromas of which are generated from odorless precursors during a sophisticated manufacturing process, it is important to know those volatiles, which really evoke the characteristic aroma by inducing a bioresponse occurring at the human odor receptors. Only the quantitative changes of such compounds and, in addition, extended knowledge on the pathways of their formation during processing would enable tea manufacturers to either improve the aroma of tea or avoid the formation of undesired odors.

Dilution to odor threshold techniques, such as the aroma extract dilution analysis (AEDA) (*9*), are useful tools to separate the odor-active volatiles from the bulk of odorless food volatiles. However, surprisingly, only two applications of such methods on black tea or a black tea beverage are available in the current literature. Guth and Grosch (*10*) were the first to study the odor-active volatiles in a Chinese black tea powder by application of the AEDA. They identified 3-hydroxy-4,5-dimethyl-2(*5H*)-

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furanone, (*E*)-β-damascenone, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, and linalool with the highest flavor dilution (FD) factors in an aroma distillate prepared by extraction of the tea powder followed by high-vacuum sublimation. By applying the same technique on retorted, black tea drinks manufactured from Darjeeling tea, Masuda and Kumazawa (*11*) confirmed linalool and β-damascenone to be the most odor-active constituents in the tea beverage. In addition, the high FD factors determined for geraniol, dimethyl trisulfide, 2-methoxy-4-vinylphenol, methyl salicylate, phenylacetaldehyde, (*E*,*Z*)-2,6-nonadienal, methional, and 3-methylbutanal suggested these aroma compounds as further key contributors to the aroma of the tea drink.

Although dilution to odor threshold techniques are useful screening methods to select odorants potentially contributing to a food aroma, such data are not directly related to the food aroma itself, because during gas chromatography−olfactometry (GC-O), the entire amount of each odorant is completely volatilized, but its volatility from a matrix such as water is not taken into account (*9*). Therefore, quantitative measurements and a correlation with odor thresholds are necessary further experiments to link analytical data with the overall tea aroma perception. Finally, aroma reconstitution experiments based on the natural concentrations of the key aroma compounds selected by this approach, which can be assigned as "molecular sensory evaluation", are needed to confirm the aroma relevance of the single compounds quantified (*12*).

Because no such data are available in the literature, the purpose of this investigation was, first, to compare the key aroma compounds in a hot water infusion prepared from Darjeeling black tea and in the black tea leaves used for beverage preparation by means of GC-O and AEDA. Second, the concentrations of the odorants showing high FD factors in the leaves and an infusion should be compared to indicate, for example, the extraction efficacy. Third, the aroma of the infusion should be mimicked on the basis of the quantitative data and the use of reference compounds.

MATERIALS AND METHODS

Tea Sample. Darjeeling tea from India (Darjeeling Gold Selection) was purchased from the tea trade (Tee Handelskontor, Bremen, Germany). The black tea had the grade TGFOP. The packaging size was 250 g, and the tea bags were stored under vacuum at −70 °C immediately after purchase. For comparison of the overall aromas of the tea infusions another 10 black tea types were purchased in a local shop.

Chemicals. The following reference aroma compounds were purchased from the companies given in parentheses: 2,3-butanedione, (*E*,*E*)-2,4-decadienal, (*S*)-ethyl 2-methylbutanoate, 3-ethylphenol, (*E*,*E*)-2,4-heptadienal, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, (*R*)-linalool, methional, 2-methylbutanal, 3-methylbutanal, 3-methylbutanoic acid, methyl octanoate, 2-methylpropanal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,6-nonadienal, (*E*,*Z*)-2,6-nonadienol, γ-nonalactone, (*E*)-2-nonenal, phenylacetaldehyde, phenylacetic acid, and vanillin (Aldrich, Steinheim, Germany); butanoic acid, hexanoic acid, (*R*)/(*S*)-linalool, pentanoic acid, and 2-phenylethanol (Fluka, Neu-Ulm, Germany); bis-(2-methyl-3-furyl) disulfide (Acros Organics, Geel, Belgium); geraniol and β-ionone (Roth, Karlsruhe, Germany); 1-octen-3-one (Lancaster, Mühlheim, Germany); 2-methoxyphenol (Merck, Darmstadt, Germany). (*E*)-β-Damascenone and (*Z*)-4-heptenal were gifts from Symrise (Holzminden, Germany).

Diethyl ether, methylene chloride, pentane, silica gel 60, sodium sulfate, and sulfuric acid were obtained from Merck, lithium aluminum hydride was obtained from Aldrich, and Dess−Martin periodinane was obtained from Lancaster. Diethyl ether, methylene chloride, and pentane were distilled prior to use.

Syntheses. The following chemicals were synthesized according to the literature cited: *trans*-4,5-epoxy-(*E*)-2-decenal (*13*); (*Z*)-3-hexenal (*14*), 3-methylnonane-2,4-dione (*15*); (*E*,*E*,*E*)-2,4,6-nonatrienal, (*E*,*E*,*Z*)-2,4,6-nonatrienal, and (*E*,*Z*,*E*)-2,4,6-nonatrienal (*16*); and (*Z*)-1,5-octadien-3-one (*17*).

[$^{13}C_2$]-2-Phenylethanol. [$^{13}C_2$]Phenylacetic acid (500 mg, 3.7 mmol) was dissolved in anhydrous diethyl ether (20 mL), and lithium aluminum hydride (10-fold molar access) was added with stirring. After 1 h at room temperature, water was added cautiously until the hydrogen generation stopped. The precipitate formed was dissolved by adding aqueous sulfuric acid (4 mol/L), and the organic phase was separated. The remaining water phase was extracted three times with diethyl ether (total volume = 80 mL), and the combined organic phases were dried over anhydrous sodium sulfate. The analyte was characterized by the following mass spectral data: MS-EI, *m*/*z* (%) 92 (100), 93 (62), 144 (33), 65 (15), 39 (7), 51 (6), 77 (5); MS-CI, *m*/*z* (%) 107 (100).

[$^{13}C_2$]-2-Phenylacetaldehyde. Dess−Martin periodinane (5.5 mmol, 2500 mg) was added dropwise to a solution of [$^{13}C_2$]-2-phenylethanol (3.7 mmol, 460 mg) in methylene chloride (20 mL) and stirred at room temperature. After 12 h, the solvent was removed, and the residue was suspended in pentane and filtered. The [$^{13}C_2$]phenylacetaldehyde formed was separated from the unreacted [$^{13}C_2$]phenylethanol by means of column chromatography (water-cooled column, i.d. = 1.5 cm) using modified silica gel 60 (*32*). After the column had been flushed with *n*-pentane (50 mL), the aldehyde was eluted with pentane/diethyl ether (100 mL; 95+5). The target compound was analyzed by mass spectrometry, yielding the following data: MS-EI, *m*/*z* (%) 92 (100), 93 (27), 122 (19), 66 (14), 65 (9), 64 (6); MC-CI, *m*/*z* (%) 123 (100).

Isotopically Labeled Internal Standards. The following isotopically labeled internal standards were synthesized according to the literature cited: [$^2H_6$]-(*E*)-β-damascenone (*18*), [$^2H_4$]-(*E*,*E*)-2,4-decadienal (*19*), [$^2H_3$]ethyl 2-methylbutanoate (*20*), [$^2H_2$]-(*Z*)-4-heptenal (*21*), [$^2H_4$]-hexanal (*20*), [$^2H_2$]hexanoic acid (*22*), [$^2H_2$]-(*E*)-2-hexenal (*20*), [$^2H_2$]-(*Z*)-3-hexenal (*19*), [$^2H_2$]-(*Z*)-3-hexenol (*19*), [$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(*5H*)-furanone (*23*), [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (*24*), [$^2H_2$]linalool (*25*), [$^2H_2$]-3-methylbutanal (*26*), [$^2H_3$]-3-methylnonane-2,4-dione (*19*), [$^2H_7$]-2-methylpropanal (*27*), [$^2H_2$]-(*E*,*E*)-2,4-nonadienal (*28*), [$^2H_2$]-(*E*,*Z*)-2,6-nonadienal (*19*), [$^{13}C_2$]-(*E*,*E*,*E*)-2,4,6-nonatrienal (*16*), [$^2H_2$]-(*E*)-2-nonenal (*19*), [$^2H_2$]-1-octen-3-one (*19*), and [$^2H_3$]vanillin (*29*). [$^{13}C_2$]Phenylacetic acid was purchased from Aldrich.

Determination of the Concentrations of the Synthesized Labeled Compounds. Because the syntheses were performed on a microscale basis, it was not possible to purify the compounds, for example, by distillation, and, thus, it was not possible to determine their yields, for example, by weight. Therefore, the concentrations of the labeled compounds were determined using the following procedure: First, an FID response factor was determined in solutions containing defined amounts of the respective unlabeled compound and methyl octanoate. In a second run, to a defined volume taken from a stock solution of the labeled compound, a defined amount of methyl octanoate was added. From the GC peak areas and using the FID response factor determined for the unlabeled compound, the concentration of the labeled compound was finally calculated.

The concentrations of the following labeled compounds were determined using the compounds in enclosures as reference and assuming the FID response factor to be 1.0: [$^2H_3$]-3-methylnonane-2,4-dione (methyl octanoate), [$^2H_2$]-(*Z*)-3-hexenal [(*E*)-2-hexenal], and [$^{13}C_2$]-(*E*,*E*,*E*)-2,4,6-nonatrienal [(*E*,*Z*)-2,4-nonadienal].

Isolation and Identification of Tea Volatiles. *Tea Leaves.* The material (50 g) was frozen in liquid nitrogen, then powdered in a Waring blender and extracted three times using the following procedure: The first extraction step was performed with methylene chloride (200 mL) for 3 h, followed by another extraction period of 16 h (200 mL of methylene chloride). In the last extraction step, the tea powder was extracted with diethyl ether (200 mL) for a period of 3 h. After filtration, the organic layers were combined, dried over anhydrous sodium sulfate, and finally concentrated to 50 mL at 42 °C by means of a Vigreux column. From the resulting solution (150 mL) the compounds fraction was isolated using the solvent-assisted flavor evaporation (SAFE) method (*30*).

Tea Infusion. Tea leaves (20 g) were suspended in hot water (95 °C; 1.7 L). After 150 s, the leaves were separated by filtration, and the infusion was cooled to room temperature in an ice bath. The beverage was then extracted repeatedly with methylene chloride (3 × 100 mL) followed by diethyl ether (2 × 100 mL). The organic phases were combined, dried over anhydrous sodium sulfate, and concentrated to 100 mL by distilling off the solvent at 42 °C using a Vigreux column (50 × 1 cm). To remove the nonvolatiles, the extract was distilled at 40 °C using the SAFE technique (*30*). Finally, the distilled extract was concentrated to 0.5 mL by distilling off the solvent at 42 °C using a Vigreux column (50 × 1 cm) followed by microdistillation.

High-Resolution Gas Chromatography (HRGC) and HRGC−Olfactometry (GC-O). HRGC was performed by means of a gas chromatograph type 8160 (Fisons Instruments, Mainz, Germany) using the following fused silica capillary columns: DB-5, 30 m × 0.32 mm, film thickness = 0.25 μm (J&W Scientific, Folsom, CA); DB-FFAP, 30 m × 0.32 mm, film thickness = 0.25 μm (Phenomenex, Torrance, CA); and DB-1701, 30 m × 0.32 mm, film thickness = 0.25 μm (J&W Scientific). The samples were injected at 40 °C using the cold on-column technique. The oven was held at this temperature for 2 min and then raised at 6 °C/min to 150 °C and finally at 20 °C/min to 230 °C. For GC-O, the effluent was split evenly at the end of the column between a flame ionization detector (FID) and a sniffing port. Retention indices (RI) were calculated from the retention times of *n*-alkanes by linear interpolation (*31*).

Static Headspace Olfactometry (SHO). An aliquot of the freshly prepared tea infusion (50 mL) was equilibrated in a 300 mL septum-sealed flask at 50 °C for 10 min. Twenty milliliters of the headspace was then withdrawn by means of a gastight syringe and injected into the precooled (−100 °C) injection port of a Chrompack. After injection, the inlet was rapidly heated to 200 °C, and the volatiles were flushed onto a DB-5 column (50 m × 0.53 mm, film thickness = 1.5 μm). The oven was held at 0 °C for 2 min and then raised at 6 °C/min to 200 °C. The volatiles were detected by FID and GC-O as described above.

AEDA. AEDA was performed using the FFAP capillary. Extracts were diluted stepwise with methylene chloride (1+1 by volume) and analyzed by HRGC-O (injection volume = 0.5 μL).

HRGC−Mass Spectrometry (HRGC-MS). For compound identification, mass spectra were generated using the capillaries and oven programs mentioned above by means of a mass spectrometer MAT 95 S (Finnigan, Bremen, Germany) at 70 eV in the electronic impact mode (MS-EI) and at 115 eV in the chemical ionization mode (MS-CI; reagent gas, isobutane).

Quantitation of Odorants by Stable Isotope Dilution Assays (SIDA). *Tea Infusion.* The tea powder was brewed for 150 s using 83.3 mL of water (95 °C) per 1 g of tea powder (= 12 g/L). Different amounts of the infusion (from 2 mL to 2 L) were used depending on the concentrations of the aroma compounds present in the tea infusion, which were determined in preliminary runs. The idea was to end up with an absolute amount of 2−5 μg of each aroma compound to be quantified. The tea leaves were removed by filtration, and the infusion was cooled to room temperature in an ice bath. Finally, the tea infusion was spiked with defined amounts of the labeled internal standards listed in **Table 1**. The solvent extraction and the SAFE distillation were performed as described above.

The extraction of 2- and 3-methylbutanal and 2-methylpropanal was performed using diethyl ether instead of methylene chloride.

Tea Leaves. Different amounts of the tea leaves (1, 2, 10, 20, or 50 g) were used depending on the concentrations of the aroma compounds present, and the powder was spiked with the labeled internal standards prior to extraction. The isolation technique used was the same as for the isolation of volatiles for AEDA. For 1 and 2 g of tea powder 50 mL of solvent for each extraction step was used; for 10 g, 100 mL, and for 20 and 50 g, 200 mL were used. Extraction of 2- and 3-methylbutanal and 2-methylpropanal was performed using diethyl ether instead of methylene chloride.

Mass Spectrometry−SIDA. Quantitation was performed by means of two different HRGC-MS systems using the capillaries mentioned above. For compounds occurring in concentrations >10 μg/kg, quantitation was performed by means of a Varian gas chromatograph 3800 (Varian, Darmstadt, Germany) coupled to a Saturn 2000 mass spectrometer (Varian). The majority of the measurements was, however, performed by means of a two-dimensional gas chromatography system. For this purpose, a CP 3800 gas chromatograph (Varian) was coupled on-line to a Trace 2000 gas chromatograph (Thermoquest, Egelsbach, Germany), and MS analyses were performed using a Saturn 2000 mass spectrometer (Varian).

The spectra were generated in the MS-CI mode using methanol as the reactant gas. The cut time intervals were determined by injection of the respective reference compound. For all analyses, the oven temperature program mentioned above was used, except for 2-methylpropanal and 2- and 3-methylbutanal, which were quantified isothermally at 35 °C.

Table 1. Isotopically Labeled Standards, Selected Mass Fragments, and Response Factors Used for Quantitation by Means of Stable Isotope Dilution Assays

internal standard used	ion (*m*/*z*)[a]	ion (*m*/*z*)[b]	CF[c]
[$^2H_{3-7}$]-(*E*)-β-damascenone	191	194–198	1.02
[$^2H_{3-5}$]-(*E*,*E*)-2,4-decadienal	153	156–158	1.03
[$^2H_3$]ethyl 2-methylbutanoate	131	134	0.99
[$^2H_2$]-(*Z*)-4-heptenal	95	97	0.67
[$^2H_4$]hexanal	101	105	0.82
[$^2H_2$]hexanoic acid	131	133	0.80
[$^2H_2$]-(*E*)-2-hexenal	99	101	0.95
[$^2H_2$]-(*Z*)-3-hexenal	99	101	0.92
[$^2H_2$]-(*Z*)-3-hexen-1-ol	85	87	0.98
[$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(*5H*)-furanone	129	131	0.99
[$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	129	131	1.00
[$^2H_2$]linalool	137	139	0.96
[$^2H_2$]-2- and 3-methylbutanal	69	71	0.91
[$^2H_3$]-3-methylnonane-2,4-dione	171	174	0.86
[$^2H_7$]-2-methylpropanal	73	80	0.86
[$^2H_2$]-(*E*,*E*)-2,4-nonadienal	139	141	0.81
[$^2H_2$]-(*E*,*Z*)-2,6-nonadienal	139	141	0.61
[$^{13}C_2$]-2,4,6-nonatrienal	137	139	1.01
[$^2H_3$]-(*E*)-2-nonenal	141	143	0.75
[$^2H_2$]-(*Z*)-1,5-octadien-3-one	125	127	0.95
[$^2H_2$]-1-octen-3-one	127	129	0.97
[$^2H_5$]phenol	95	100	0.98
[$^{13}C_2$]phenylacetaldehyde	121	123	1.00
[$^{13}C_2$]-2-phenylethanol	105	107	1.00
[$^2H_3$]vanillin	153	156	0.99

[a] Ion used to monitor the intensity of the analyte. [b] Ion used to monitor the intensity of the internal standard. [c] MS calibration factor.

Determination of MS Calibration Factors. An MS calibration factor for each labeled compound was determined by analyzing mixtures of known amounts of the labeled and unlabeled compounds in three different mass ratios (2+1, 1+1, and 1+2) by GC-MS. The factors, which were calculated as described previously (*18*), are summarized in **Table 1**.

Chiral Analysis. The enantiomeric ratio of linalool was determined by two-dimensional gas chromatography−mass spectrometry (TD-GC-MS) as described recently (*25*).

Aroma Profile Analysis. Assessors for sensory evaluations were recruited from the German Research Centre for Food Chemistry. All panelists were trained weekly in orthonasal recognition based on a selection of aroma compounds taken from a stock of more than 100 odor-active compounds. Aroma descriptors for each aroma compound were defined, and according to these descriptors aroma compounds representing the following aroma attributes were chosen (in enclosures): rose-like/honey-like (geraniol, phenylacetaldehyde); flowery, cooked apple [(*R*)-linalool, (*E*)-β-damascenone]; green (hexanal); malty (3-methylbutanal); hay-like (3-methylnonane-2,4-dione); fatty [(*E*,*E*)-2,4-decadienal]; fishy [(*Z*)-4-heptenal]; caramel [4-hydroxy-2,5-dimethyl-3(*2H*)-furanone]; oat-flake-like/sweet [(*E*,*E*,*Z*)-2,4,6-nonatrienal]. In all sensory evaluations a minimum of 10 assessors participated. Sensory evaluations were performed in a sensory panel room at 21 ± 1 °C.

Reconstitution Experiment. At tea infusion was freshly prepared according to the procedure described above. In parallel, a tea flavor model was set up by dissolving the following 19 compounds in water on the basis of the same concentrations as determined in the tea infusion: (*E*)-β-damascenone, (*E*,*E*)-2,4-decadienal, geraniol, (*Z*)-4-heptenal, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, β-ionone, (*R*)/(*S*)-linalool, 2-methylbutanal, 3-methylbutanal, 3-methylnonane-2,4-dione, 2-methylpropanal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*,*Z*)-2,4,6-nonatrienal, (*E*)-2-nonenal, and phenylacetaldehyde.

For (*E*,*E*,*Z*)-2,4,6-nonatrienal a mixture consisting of the (*E*,*E*,*E*) and (*E*,*E*,*Z*) isomers in a ratio of 2:1 was used. Due to the much higher odor threshold of the (*E*,*E*,*E*) isomer (*16*), a contribution of this isomer to the entire aroma could be excluded.

The fresh tea and the flavor model were kept in a water bath at 50 °C for 5 min and were then evaluated by the panelists. The assessors were asked to rate the intensities of the given odor qualities on a linear, seven-point scale from 0 (weak) to 3.0 (strong). The descriptors had been selected previously as the predominant, characteristic aroma attributes of black tea.

Omission Experiments. An aroma recombinate omitting (*E*,*E*,*Z*)-2,4,6-nonatrienal from the model was prepared and presented to the panelists in comparison to the complete model in a triangle test (*32*). The panelists were asked to identify the sample that differed from the other two samples. The significance α of the difference was calculated as described in ref *33*.

RESULTS AND DISCUSSION

Odor-Active Compounds in the Tea Infusion. In a preliminary study, tea infusions were prepared from 10 different black tea types, purchased as intact leaves, and a hedonic evaluation was done by a group of 25 trained panelists. As a result, the Darjeeling Gold Selection was given the highest aroma preference and, thus, was selected for this investigation. To be independent of seasonal variations several packages of the same batch of tea leaves were mixed and kept at −70 °C.

First, a distillate containing the whole set of volatiles was prepared from an infusion (12 g of tea leaves in 1 L of hot water) by solvent extraction and SAFE. A drop of the distillate in diethyl ether gave a very rich, tea-like aroma when evaluated on a strip of filter paper.

Afterward, GC-O was applied to a distillate obtained from 1.7 L of tea (= 20 g of tea leaves). Twenty-four odor-active areas were detected during GC-O by three panelists, among which a variety of different odor qualities, such as oat-flake-like, honey-like, caramel-like, or vanilla-like could be located with high intensity. By sniffing serial dilutions of the extract by means of the AEDA approach, four compounds appeared with the highest FD factor of 128 among the 24 odor-active areas detected in the range of 4−128 (**Table 2**).

To identify the compounds responsible for these odors, first, the odor quality perceived and, also, the retention index were used for comparison with an in-house database built on more than 500 reference aroma compounds previously identified by us in different foods or food model systems. In addition, to obtain enough material for mass spectral measurements, 5 L of tea (= 60 g of tea leaves) was extracted, and the extract was separated into fractions according to polarity using silica gel and a pentane/diethyl ether gradient for elution (*13*).

The odor-active constituents could, thus, be enriched and then located in the respective fractions by GC-O and their retention indices and, finally, mass spectra (MS-EI, MS-CI) were recorded. On the basis of the structural suggestions obtained by comparison of the odor quality, the retention index, and the mass spectra with those of an in-house database, the respective reference compounds were selected for final confirmation of the structure. This was done by also comparing retention indices on at least one additional GC stationary phase and, also, the odor activity (odor threshold) of the analyte and the respective reference compound.

On the basis of this procedure, 2-phenylethanol, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, and 2-methoxy-4-hydroxybenzaldehyde (vanillin) were identified with the highest FD factors of 128 (**Table 2**). The mass spectrum obtained for a compound eliciting a very intense oat-flake-like aroma in combination with a high FD factor is shown in **Figure 1**. The mass spectrum, the retention indices, and the odor properties were in agreement with data for (*E*,*E*,*Z*)-2,4,6-nonatrienal recently identified by us as the character impact odorant in oat flakes (*16*). The nonatrienal was previously unknown as a constituent of black tea and exhibits the extremely low odor threshold of 0.026 μg/L in water.

Table 2. Important Aroma Compounds (FD ≥ 4) Identified in the Extract of an Infusion Prepared from Darjeeling Black Tea

aroma compound[a]	aroma quality[b]	RI on[c] FFAP	OV-1701	SE-54	FD[d]
(*Z*)-4-heptenal	fishy	1240	988	900	8
(*Z*)-1,5-0ctadien-3-one	geranium-like	1373	1086	981	4
(*E*,*E*)-2,4-heptadienal	fatty	1489	1135	1012	16
(*E*)-2-nonenal	fatty, green	1527	1275	1153	8
(*R*)/(*S*)-linalool	citrus	1541	1198	1098	64
(*E*,*Z*)-2,6-nonadienal	cucumber-like	1583	1275	1130	8
phenylacetaldehyde	honey-like	1642	1178	1038	64
3-methylbutanoic acid	sweaty	1663	1046	870	4
(*E*,*E*)-2,4-nonadienal	fatty, green	1695	1348	1213	16
3-methylnonane-2,4-dione	hay-like	1719	1398	1242	32
(*E*,*E*)-2,4-decadienal	fatty, fried	1804	1453	1318	16
(*E*)-β-damascenone	fruity	1815	1500	1387	32
hexanoic acid	sweaty	1840	1192	1020	4
geraniol	rose-like	1850	1371	1256	32
2-methoxyphenol	smoky	1859	1231	1089	16
(*E*,*E*,*Z*)-2,4,6-nonatrienal	oat-flake-like	1877	1426	1269	128
2-phenylethanol	honey-like	1910	1270	1116	128
β-ionone	violet-like	1948	1617	1490	64
trans-4,5-epoxy-(*E*)-2-decenal[e]	metallic	2006	1563	1379	64
4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	caramel	2025	1248	1071	128
3-ethylphenol	phenolic	2205	1395	1169	4
3-hydroxy-4,5-dimethyl-2(*5H*)-furanone	seasoning-like	2220	1356	1112	64
phenyl acetic acid	honey-like	2582	1531	1262	16
vanillin	vanilla-like, sweet	2605	1661	1400	128

[a] Identification was performed on the basis of a comparison with reference compounds using the following criteria: retention index on three different columns, odor quality and odor threshold at the sniffing port, and mass spectra obtained by MS-EI and MS-CI. [b] Odor quality perceived at the sniffing port. [c] Linear retention index. [d] Flavor dilution factor. [e] No unequivocal mass spectrum was obtained. Identification is based on the remaining criterias given in footnote *a*.

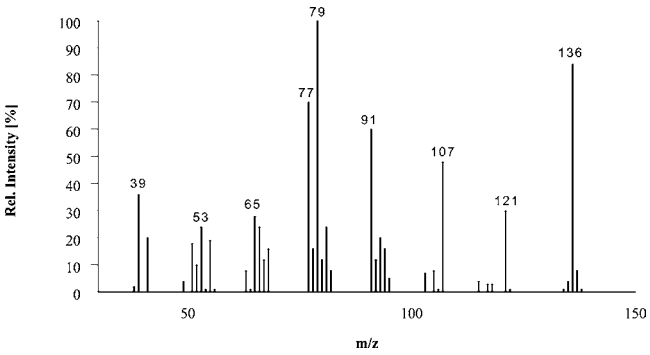

Figure 1. Mass spectrum (MS-EI) of a constituent of the tea infusion eliciting an intense oat-flake-like aroma.

Besides these four aroma compounds showing the highest FD factors, linalool, phenylacetaldehyde, β-ionone, *trans*-4,5-epoxy-(*E*)-2-decenal, and 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone, showing somewhat lower FD factors, were identified as further odor-active constituents in the extract from the black tea infusion (**Table 2**). Linalool was determined to occur as a racemate of (*R*)- and (*S*)-linalool in a ratio of 54:46.

The aroma contribution of linalool, geraniol, phenylacetaldehyde, and (*E*)-β-damascenone was in agreement with recent data of Masuda and Kumazawa (*11*) on the odorants of a black tea drink, but these authors did not report either (*E,E,Z*)-2,4,6-nonatrienal or 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone or 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone as aroma contributors. This difference is probably caused by the lower temperature of the water used for the preparation of the infusion (70 °C) and, also, the additional retorting process (121 °C for 10 min) used by these authors.

During AEDA, losses in highly volatile compounds might occur. Therefore, static headspace olfactometry was additionally applied to the tea infusion. By means of this method, 2-methylpropanal, 2- and 3-methylbutanal, hexanal, ethyl 2-methylbutanoate, and 1-octen-3-one were characterized as additional odor-active compounds of the tea infusion (data not shown).

As indicated in the Introduction, the application of dilution to odor threshold techniques, such as AEDA, does not immediately answer the question of the aroma contribution of single odor-active constituents to the food itself, because the matrix significantly influences the volatility of an odorant and, thus, its concentration in the headspace above the food. To address this issue, 24 aroma compounds were quantified in the tea beverage by means of SIDAs using synthesized, isotopically labeled internal standards, and their odor activity values (OAVs: ratio of concentration to odor threshold) were calculated.

The highest concentrations were determined for hexanoic acid followed by linalool, geraniol, 2-phenylethanol, and (*Z*)-3-hexenol, which all were present in amounts >100 μg/L of the infusion (**Table 3**). On the other hand, very low concentrations of <1 μg/L were determined for eight odorants, for example, ethyl 2-methylbutanoate, 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone (sotolon), (*E*)-2-nonenal, or (*E*)-β-damascenone. Analysis of two to eight different infusions, prepared in separate experiments from the same batch of tea, revealed only minor differences in the concentrations amounting to not more than 20%, for example, for 2-methylbutanal (**Table 3**).

In further experiments, the odor thresholds of the tea aroma compounds under consideration were determined in water using the respective reference compounds and a trained panel of 10 expert assessors. The odor thresholds determined for (*E,E,Z*)-2,4,6-nonatrienal, (*E,Z*)-2,6-nonadienal, and 3-methylnonane-2,4-dione were among the lowest odor thresholds and lay in the area of 10−20 ng/L water (**Table 4**).

Finally, the OAVs of the 24 aroma compounds were calculated. From the data given in **Tables 3** and **4**, the highest OAV was calculated for linalool, which was present in the infusion at ∼140 times above its odor threshold in water (**Table 4**). Next in rank was geraniol with an OAV of 45. Surprisingly, the OAV of the newly identified (*E,E,Z*)-2,4,6-nonatrienal was also very high and was close to those of geraniol and (*E*)-β-damascenone. In addition, the three malty-smelling aldehydes methylpropanal and 2-methyl- and 3-methylbutanal, as well as the hay-like-smelling 3-methylnonane-2,4-dione followed with OAVs of 37. The latter compound has previously been identified as an important odorant also in green tea leaves (*10*).

Table 3. Concentrations of Important Aroma Compounds in the Black Tea Infusion

aroma compound	concn (μg/L)	NR[a]	range (μg/L)
hexanoic acid	344	2	331–356
(R)/(*S*)-linalool	142	4	136–149
geraniol	142	4	134–149
2-phenylethanol	131	2	122–139
(*Z*)-3-hexen-1-ol	95	3	92–97
2-methylbutanal	82	3	74–92
(*E*)-2-hexenal	77	3	74–79
2-methylpropanal	69	3	65–76
phenylacetaldehyde	57	4	56–64
hexanal	55	3	50–56
3-methylbutanal	42	3	41–43
4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	26	2	26–27
vanillin	22	2	22–23
(*E,E*)-2,4-decadienal	2.9	2	2.9
β-ionone	1.5	2	1.44–1.56
(*E,E,Z*)-2,4,6-nonatrienal	1.1	8	0.49–1.56
(*Z*)-4-heptenal	0.66	2	0.65–0.68
(*E,Z*)-2,6-nonadienal	0.56	4	0.52–0.58
3-methylnonane-2,4-dione	0.48	2	0.47–0.49
(*E,E*)-2,4-nonadienal	0.45	2	0.44–0.46
(*E*)-2-nonenal	0.39	2	0.37–0.42
(*E*)-β-damascenone	0.15	2	0.14–0.17
3-hydroxy-4,5-dimethyl-2(*5H*)-furanone	0.12	2	0.12
ethyl 2-methylbutanoate	0.02	2	0.02

[a] Number of replicates analyzed.

Table 4. Orthonasal Odor Thresholds (OT) and Odor Activity Values (OAV) of Important Aroma Compounds in the Black Tea Infusion

aroma compound	OT[a] (μg/L in water)	OAV
(R)-linalool	0.6	140
geraniol	3.2	45
(*E,E,Z*)-2,4,6-nonatrienal	0.026	41
(*E*)-β-damascenone	0.004	38
2-methylbutanal	4.4[b]	37
2-methylpropanal	1.9[b]	37
3-methylbutanal	1.2[b]	37
3-methylnonane-2,4-dione	0.01	37
(*E,Z*)-2,6-nonadienal	0.03	22
(*E,E*)-2,4-decadienal	0.16	18
(*Z*)-4-heptenal	0.06	11
phenylacetaldehyde	6.3	9
(*Z*)-3-hexen-1-ol	13[b]	7
β-ionone	0.20	7
hexanal	10[b]	5
(*E,E*)-2,4-nonadienal	0.16	3
2-phenylethanol	1000	<1
hexanoic acid	890	<1
(*E*)-2-hexenal	190[b]	<1
4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	30	<1
vanillin	25	<1
3-hydroxy-4,5-dimethyl-2(*5H*)-furanone	20	<1
(*E*)-2-nonenal	0.4	<1
ethyl 2-methylbutanoate	0.06[b]	<1

[a] Odor thresholds were determined by the triangle test as described previously (*20*) using tap water. Odor activity values were calculated by dividing the concentration by the odor threshold. [b] Threshold data were taken from ref *36*.

By contrast, the OAVs of eight aroma compounds assigned as "odor-active" on the basis of AEDA results, namely, ethyl 2-methylbutanoate, (*E*)-2-hexenal, (*E*)-2-nonenal, hexanoic acid, 2-phenylethanol, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, and 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone, showed OAVs below 1. Thus, by definition, these odorants should not contribute significantly to the aroma of the tea infusion. The reason for this is undoubtedly the fact that during GC-O the entire amount

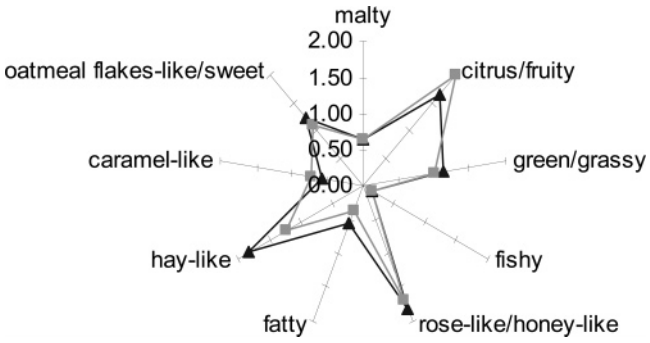

Figure 2. Aroma profile of the tea infusion (▲) and the aroma recombinate (■) consisting of 19 odorants.

of the respective odorant present in the extract used is volatilized, whereas the calculation of OAVs is based on odor threshold in a matrix and, thus, only considers the respective amount of an odorant present in the headspace above the matrix.

It is well accepted that the overall aroma of a food consists of different aroma compounds, none of which might bear the typical aroma of the food itself. Because, up to now, the aroma of a complex mixture of volatiles cannot be predicted from the set of odor-active compound present, aroma reconstitution experiments are a useful tool to confirm the analytical data obtained by the OAV concept. For this purpose, the odor-active compounds quantified are mixed together using pure reference compounds in the same concentrations as they occur in the food under investigation (*9*). Comparison of the original food aroma with the odor of the mixture thus allows differences or similarities to be determined.

The results of the application of this approach on the aroma of the tea infusion are summarized in **Figure 2**. The aroma profiles of the infusion and the recombinate were nearly identical but with some differences in the hay-like odor note, which was less intense in the recombinate compared to the infusion, whereas the citrus-like aroma quality was higher in the model. These data confirmed that the key aroma compounds of the tea infusion have been successfully identified.

Because (*E*,*E*,*Z*)-2,4,6-nonatrienal was identified with a quite high OAV in the tea infusion (**Table 4**), in particular its importance was checked by an omission experiment. For this purpose, the aroma of the entire mixture containing all 19 aroma compounds was compared to the aroma of a mixture from which only the nonatrienal was omitted. Because 9 of the 12 sensory panel assessors were able to detect a clear difference between the samples in a triangle test, this compound can be regarded as one of the most important odorants of the black tea beverages.

In a recent investigation, the nonatrienal was also characterized as a key odorant in oat flakes, and it was shown that it is formed during an autoxidation of linolenic acid (*16*). It might, therefore, be assumed that this odorant is formed by either an enzymatic or a chemical lipid oxidation during fermentation of the tea leaves. The total content of lipids in black tea varies between 4 and 9%, with glycolipids containing high levels of linolenic acid predominating (*8*).

Odorants in Black Tea Leaves. It is known that certain tea odorants such as linalool, (*Z*)-3-hexenol, and geraniol are chemically released during processing of green tea leaves by an enzymatic hydrolysis of the respective glycosides (cf. review in ref *1*). Because the final firing procedure completely inactivates the enzymes, the hot water extraction of the black tea leaves should influence only the extraction yields, without forming additional amounts of aroma compounds from precursors in the leaves.

Table 5. Important Aroma Compounds (FD ≥ 4) in the Extract Prepared from the Black Tea Leaves

		retention index on[c]			
aroma compound[a]	aroma quality[b]	FFAP	OV-1701	SE-54	FD[d]
3-methylbutanal	malty	950	735	652	4
2,3-butanedione	buttery	967	690	587	4
ethyl 2-methylbutanoate	fruity	1049	906	848	4
hexanal	grassy, green	1079	879	802	4
(*Z*)-3-hexenal	green	1148	884	800	8
(*Z*)-4-heptenal	fishy	1240	988	900	128
octanal	citrus	1283	1087	1003	4
1-octen-3-one	muhroom-like	1298	1071	979	8
(*Z*)-1,5-octadien-3-one	geranium-like	1373	1086	981	16
(*Z*)-3-hexen-1-ol	green	1381	971	858	4
methional	cooked potato-like	1439	1040	905	16
(*E*,*E*)-2,4-heptadienal	fatty	1489	1135	1012	4
unknown	green	1512	nd[e]	nd	4
(*E*)-2-nonenal	fatty, green	1527	1275	1153	16
R/*S*-linalool	citrus	1541	1198	1098	256
unknown	foral	1564	nd	nd	4
(*E*,*Z*)-2,6-nonadienal	cucumber-like	1583	1275	1130	32
(*E*,*E*)-2,4-octadienal	fatty	1596	1247	1115	16
butanoic acid	sweaty	1625	984	821	4
phenylacetaldehyde	honey-like	1642	1178	1038	4
3-methylbutanoic acid	sweaty	1663	1046	870	16
(*E*,*E*)-2,4-nonadienal	fatty, green	1695	1348	1213	64
3-methylnonane-2,4-dione	hay-like	1719	1398	1242	128
pentanoic acid	sweaty	1730	1086	911	8
(*E*,*Z*)-2,6-nonadienol	cucumber-like	1759	1270	1173	16
(*E*,*E*)-2,4-decadienal	fatty, fried	1804	1453	1318	32
(*E*)-β-damascenone	fruity	1815	1500	1387	64
hexanoic acid	sweaty	1840	1192	1020	16
geraniol	rose-like	1850	1371	1256	64
2-methoxyphenol	smoky	1859	1231	1089	8
(*E*,*E*,*Z*)-2,4,6-nonatrienal	oat-flake-like	1877	1426	1269	256
2-phenylethanol	honey-like	1910	1270	1116	64
β-ionone	violet-like	1948	1617	1490	256
4,5-epoxy-(*E*)-2-decenal	metallic	1986	1563	1374	32
trans-4,5-epoxy-(*E*)-2-decenal	metallic	2006	1563	1379	128
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	2025	1248	1071	256
γ-nonalactone	coconut-like	2053	1563	1363	8
bis(2-methyl-3-furyl) disulfide[g]	meaty	2153	1639	1534	16
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	seasoning	2220	1356	1112	256
phenylacetic acid	honey-like	2582	1531	1262	256
vanillin	vanilla-like, sweet	2605	1661	1400	256

[a] Identification is based on comparison with reference compounds using the following criteria: retention index on the different columns, odor quality and odor threshold at the sniffing port, mass spectra obtained in the MS-CI and MS-EI. [b] Odor quality perceived at the sniffing port. [c] Linear retention index. [d] Flavor dilution factor. [e] Not determined. [f] No mass spectrum could be obtained. Identification is based on the remaining criteria given in footnote *b*.

To investigate the influence of the hot water treatment on changes in odorant concentrations, the odor-active compounds present in the same black tea leaves used for the preparation of the infusion were identified, and then the odorants showing the highest FD factors were quantified.

By application of the AEDA, a total of 41 odor-active areas were detected in the extract prepared from the black tea leaves displaying FD factors between 4 and 256. The results of the identification experiments in combination with the FD factors resulted in the following seven aroma compounds with the highest FD factors (**Table 5**): linalool, (*E*,*E*,*Z*)-2,4,6-nonatrienal, β-ionone, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone, 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone, 2-phenylacetic acid, and hydroxy-2-methoxybenzaldehyde. Further compounds showing high FD factors were (*Z*)-4-heptenal, 3-methylnonane-2,4-dione, and *trans*-4,5-epoxy-(*E*)-2-decenal. The enantiomeric ratio of linalool was identical with that found in the beverage and was in agreement with data of Wang et al. (*34*), who also found ratios of 60:40 or 53:47, respectively, in black teas from Ceylon.

Table 6. Concentrations of Important Aroma Compounds in Black Tea Leaves

aroma compound	concn (μg/kg)	NR[a]	range (μg/kg)
hexanoic acid	12000	6	9900–13400
(*R*)/(*S*)-linalool	6600	6	6000–7100
2-phenylethanol	2200	4	2100–2400
hexanal	1600	2	1400–1800
(*Z*)-3-hexen-1-ol	1600	2	1500–1600
phenylacetaldehyde	650	2	650–660
vanillin	570	8	500–600
2-methylbutanal	540	2	520–560
geraniol	370	4	360–380
3-methylbutanal	320	2	300–340
(*E*)-2-hexenal	270	2	260–280
2-methylpropanal	250	2	230–270
β-ionone	170	2	160–180
(*E*,*E*,*Z*)-2,4,6-nonatrienal	160	4	160–170
4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	100	3	89–110
(*E*,*E*)-2,4-nonadienal	87	4	63–110
(*E*,*E*)-2,4-decadienal	73	6	62–98
3-methylnonan-2,4-dione	62	8	58–69
(*Z*)-4-heptenal	51	6	39–61
(*E*,*Z*)-2,6-nonadienal	38	2	37–39
(*E*)-2-nonenal	32	4	27–36
(*E*)-β-damascenone	9.8	2	9.6–10.1

[a] Number of replicates analyzed.

A comparison of the aroma compounds with those identified in the tea beverage (cf. **Tables 2** and **5**) revealed that most of the compounds in the leaves and the infusions produced thereof were identical from a qualitative point of view, but differences in the FD factors, that is, the concentrations, occurred. Furthermore, several odorants were detected in only the extract prepared from the tea leaves, but were not present in the infusion, such as γ-nonalactone, 2,3-butanedione, methional, butanoic acid, and bis(2-methyl-3-furyl) disulfide. However, the latter compounds showed low FD factors.

Guth and Grosch (*10*) had reported linalool and (*E*)-β-damascenone as well as 4-hydroxy-2,5-dimethyl-3(2*H*)- and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone also as key odorants in Chinese black tea leaves and, thus, our data corroborate the importance of these compounds for the aroma of black tea.

Because the AEDA of the powder was performed on an extract prepared from 50 g of tea leaves and the AEDA of the infusion was performed on an extract from 1.7 L of tea prepared from 20 g of powder, a comparison of the FD factors between both samples is not appropriate to determine exact differences in concentrations caused by the hot water extraction of the leaves. Therefore, 22 aroma compounds, which had also been quantified in the infusion, were quantified in the black tea leaves by means of SIDAs.

The results revealed the highest concentrations for hexanoic acid, linalool, 2-phenylethanol, hexanal, and (*Z*)-3-hexenol, which were all present in the milligrams per kilogram range (**Table 6**). (*E*)-β-Damascenone occurred with the lowest concentration (9.8 μg/kg) in the black tea leaves.

To statistically confirm the results, two to eight replicates of the quantitations were performed, always starting from a new sample of tea leaves. The concentration ranges determined (**Table 6**) indicated that the deviation from the mean value calculated from the replicates amounted to not more than 25% [for (*E*,*E*)-2,4-nonadienal].

A direct comparison of the quantitative data between the infusion (**Table 3**) and the powder (**Table 6**) does, however, not reflect the differences caused by the hot water treatment of tea leaves, because the data are given as concentration per liter of water or per kilogram of leaves, respectively. Thus, to indicate the influence of the water extraction on the concentrations of the tea aroma compounds, the amount of each aroma compound present in 12 g of black tea leaves was calculated from the data given in **Table 6** and was then contrasted to the amounts present in 1 L of the infusion prepared from 12 g of the same black tea leaves. By dividing both amounts, a clear indication is given of whether a compound was not sufficiently extracted (values below 1) or was released/newly formed during the hot water treatment.

Table 7. Changes in the Concentrations of Important Aroma Compounds of the Black Tea Leaves Induced by the Preparation of the Infusion

aroma compound	concn[a] (μg/12 g of tea leaves)	concn[b] (μg/L of infusion)	ratio I/L[c]
geraniol	4.4	142	32
(*E*)-2-hexenal	3.2	77	24
2-methylpropanal	3.0	69	23
4-hydroxy-2,5-dimethyl-3(*2H*)-furanone	1.2	26	22
2-methylbutanal	6.5	82	13
3-methylbutanal	3.8	42	11
phenylacetaldehyde	7.8	57	7.3
(*Z*)-3-hexenol	19	95	5.1
2-phenylethanol	26	131	5.0
(*E*,*E*)-2,4-decadienal	0.88	2.9	3.3
vanillin	6.8	22	3.1
hexanal	19	55	2.9
hexanoic acid	144	344	2.3
(*R*)/(*S*)-linalool	79	142	1.8
(*E*)-β-damascenone	0.12	0.15	1.3
(*E*,*Z*)-2,6-nonadienal	0.46	0.56	1.2
(*Z*)-4-heptenal	0.61	0.66	1.1
(*E*)-2-nonenal	0.38	0.39	1.0
β-ionone	2.0	1.5	0.7
(*E*,*E*,*Z*)-2,4,6-nonatrienal	1.9	1.1	0.6
3-methylnonane-2,4-dione	0.74	0.48	0.6
(*E*,*E*)-2,4-nonadienal	1.0	0.45	0.4

[a] Calculated from the amounts determined in 12 g of the leaves (**Table 3**). [b] One liter of infusion was prepared using 12 g of tea leaves. [c] Ratio of the concentration in the tea infusion (I) and the leaves (L).

Table 8. Concentrations of Selected Aroma Compounds in an Aqueous Extract of Black Tea Leaves Depending on the Temperature of the Water and the Extraction Time[a]

extraction time (s)	temp (°C)	concn (μg/L)		
		linalool	geraniol	4-HDF[b]
150	95	142	142	26
150	20	5	5	1.7
900	20	35	9	2.3

[a] Powdered black tea (4 g) was extracted with water (333 mL) and, after the addition of the labeled internal standards, with diethyl ether. [b] 4-Hydroxy-2,5-dimethyl-3(*2H*)-furanone.

As expected, some of the nonpolar compounds, such as (*E*,*E*)-2,4-nonadienal, 3-methylnonane-2,4-dione, or (*E*,*E*,*Z*)-2,4,6-nonatrienal, were obviously not fully extracted during the hot water treatment, because their amounts in the beverage amounted to only 40−60% as compared to the amounts present in the leaves (**Table 7**). Contrarily, nine compound were clearly increased in the infusion as compared to the amounts in the black tea leaves. The most significant increase was found for geraniol, the concentration of which was by a factor of 32 higher as compared to the amounts determined in the tea leaves. Other alcohols, such as (*Z*)-3-hexenol, 2-phenylethanol, or 4-hydroxy-

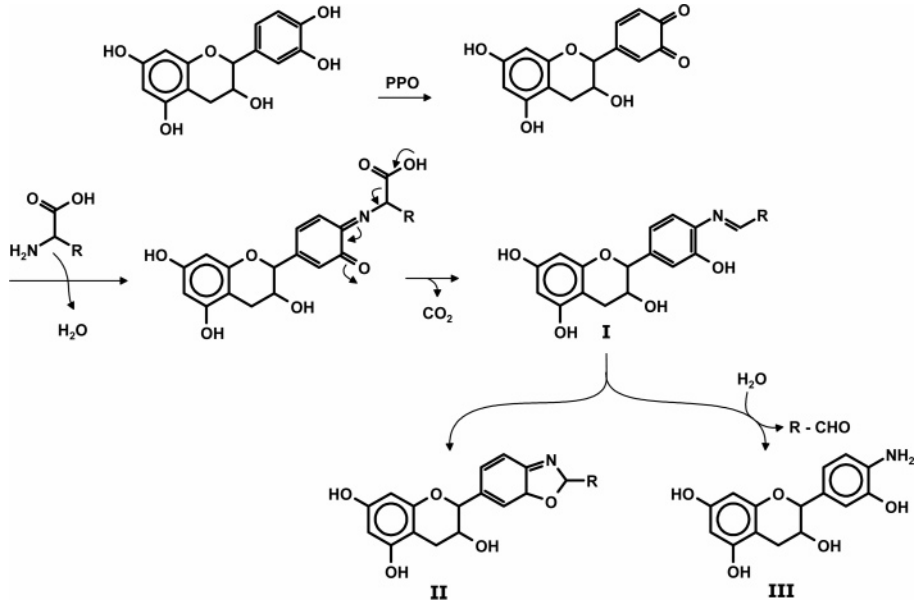

Figure 3. Hypothetical formation pathway leading to Strecker aldehyde formation during fermentation/preparation of the black tea infusion.

2,5-dimethyl-3(*2H*)-furanone, were significantly increased in comparison to the amounts measured in the leaves.

Because, in particular, geraniol and (*Z*)-3-hexenol are known to occur as glycosides in green tea leaves (*1*), it might be assumed that the increase in the concentrations of the aglycons might be attributed to a further hydrolysis of the glycosidic precursors during the hot water treatment. However, because the process of firing should have inactivated all enzymes, it might be probable that other, yet unknown, precursors of the odorants are present in the black tea leaves, which might be released by hydrolytic processes.

A simpler explanation for the increase in their concentrations might be an incomplete extraction of the aroma compounds under consideration from the powder as compared to the infusion. However, although this is not very probable, because the extracted tea leaves were checked to be odorless by sniffing, Guth and Grosch (*10*) had observed that a mixture of water and solvent showed higher extraction yields of certain tea volatiles. To shed more light on this point, the amounts of linalool, geraniol, and 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone were determined in extracts obtained by cold and hot water extraction (**Table 8**). The data clearly indicated that the hot water treatment liberated the highest amounts of the three alcohols, for example, even after 15 min only <10% of geraniol determined in the hot water extract after 90 s (the infusion) was measured in an extract prepared with water of 20 °C. These data suggested that yet unknown precursors are present in black tea.

It is interesting to note that besides the alcohols, also aldehydes known as Strecker degradation products such as 3-methylbutanal were increased. Because a Strecker degradation involving carbohydrates is very unlikely to occur within the 150 s of the hot water treatment, it can be assumed that yet unknown precursors are involved in the formation of the Strecker aldehydes during preparation of the infusion.

A possible route leading to such precursors in proposed in **Figure 3**. Green tea leaves contain several flavonoids, such as catechin, in quite high amounts. A distinct amount of these polyphenols is easily oxidized during black tea fermentation into *o*-chinone structures by phenoloxidases (PO). These *o*-chinones might be able to initiate a Strecker-type degradation of amino acids as shown in **Figure 3**. However, because during drying and firing of the tea leaves, water is no longer available for the hydrolysis of the decarboxylated imine **I**, thereby forming the Strecker aldehyde, the formation of a 3-oxazoline may be possible by a nucleophilic attack of the phenolic hydroxyl group at the imine bond. Intermediates such as **II** (**Figure 3**) might be stable in the dry tea leaves, but might be hydrolyzed during the hot water treatment and, thus, might liberate the Strecker aldehyde. Although to our knowledge, compounds such as **III** have never been identified in tea or other foods, 3-oxazolines have previously been identified by Rizzi (*35*), when α-dicarbonyls, such as 2,3-butanedione, were reacted with α-amino acids under water-free conditions in a model system.

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