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Key Odor-Active Compounds in Raw Green and Red *Toona sinensis* (A. Juss.) Roem. and Their Changes during Blanching

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ABSTRACT: Application of aroma extract dilution analysis and headspace aroma dilution analysis revealed 52 odorants in raw green *Toona sinensis* and 54 odorants in raw red *T. sinensis* in the flavor dilution factor range of 8-4096. (*E*,*E*)-2,4-Decadienal, nonanal, 2,3,5-trimethylpyrazine, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl trisulfide, 2-methoxyphenol, and 4-ethylphenol were firstly identified as key odorants of *T. sinensis*. Clear differences between green and red *T. sinensis* in aroma profiles, flavor dilution factors, quantitative data, and odor activity values verified that (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and dimethyl sulfide caused the distinct sulfury odor note of each variety. Further, hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal led to the green odor note in green *T. sinensis*, while 2-methoxyphenol and 4-ethylphenol contributed to the intense phenolic aroma note in red *T. sinensis*. Quantitation experiments and triangle tests in blanched *T. sinensis* verified that the quick loss of the abovementioned sulfur-containing compounds, aldehydes, the alcohol (*E*)-2-hexen-1-ol, and phenols were responsible for the changes in the overall aroma profile during blanching.

KEYWORDS: *T. sinensis*, sensomics concept, aroma extract dilution analysis, stable isotope dilution analysis, odor activity values, aroma recombination, heat-processing, sulfides, phenols.

INTRODUCTION

Toona sinensis (A. Juss.) Roem. is a popular cultivated tree due to its usage in pharmacology based on anti-cancer and antioxidative properties as well as in traditional Chinese diet because of beneficial nutrient contents.[1,2] Nowadays, the planting area of *T. sinensis* is > one billion square meters, which is used for the cultivation of > 800 billion kilograms of fresh *T. sinensis* buds every year.[3] Studies on the volatiles of *T. sinensis* started about two decades ago, and until now, > 100 volatiles have been identified. Among them, Liu et al. reported on *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (both with a *T. sinensis*-like smell) as major contributors to the characteristic aroma of fresh *T. sinensis* (Shanxi, China) via gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O).[6] Yang et al. verified (*E*,*E*)-di-1-propenyl disulfide, (*E*,*Z*)-di-1-propenyl disulfide, hydrogen sulfide, methyl thiirane, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol responsible for the unique and pleasant flavor of fresh *T. sinensis* (Beijing, China) based on the application of headspace solid phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS), GC-MS, and GC-O.[7] Very recently, Zhai and Granvogl characterized dimethyl sulfide, eugenol, hexanal, *β*-ionone, 2-isopropyl-3-methoxypyrazine, 2-methylbutanal, 3-methylbutanal, and 3-methylnonane-2,4-dione as key aroma-active compounds for the first time in two commercially dried *T. sinensis* products (Hubei and Anhui, China) by the application of the molecular sensory science concept.[8]

According to the color of the young buds, *T. sinensis* is classified into red *T. sinensis* and green *T. sinensis*. Red *T. sinensis*, which is matured earlier, has more fuchsia leaves, less fiber, and more grease. Young red leaves are considered to have a better flavor compared to young green leaves, indicating clear differences in key

odorants between red and green *T. sinensis*. However, to the best of our knowledge, molecular differences of the overall aromas and key odorants between red and green *T. sinensis* have not yet been clarified using the comprehensive approach of the molecular sensory science concept.

Although young *T. sinensis* buds, picked in early spring (April and May) in China, are very delicious vegetables, raw buds are rarely used directly as food ingredients. Thus, blanching of raw *T. sinensis* sprouts in boiling water is an essential process prior to further cooking steps, due to the fact that raw sprouts contain some toxic ingredients (e.g., nitrites). After blanching, no matter if starting with red or green *T. sinensis*, the buds are green colored because of the loss of anthocyanines at the applied temperature (~100 °C), and the aroma is less intense. To date, only one report identified the composition of odorants in blanched *T. sinensis* buds using static headspace aroma dilution analysis (SH-ADA) and aroma extract dilution analysis (AEDA) based on GC-O and GC-MS.[7] Thereby, (*E*,*E*)-di-1-propenyl disulfide and (*E*,*Z*)-di-1-propenyl disulfide were found to be potent key odorants. However, no systematic sensory analysis has been applied to elucidate the changes in the overall aroma of blanched *T. sinensis* at a molecular level.

Thus, the aim of the present study was first to elucidate the differences in key aroma-active compounds between green and red *T. sinensis* buds and secondly to reveal the molecular background of aroma changes occurring during blanching by means of the molecular sensory science concept. Thereby, the key odorants were i) identified by comparative AEDA (cAEDA) based on GC-O in combination with GC-MS and ii) quantitated by stable isotope dilution assays (SIDAs) as well as semiquantitated by internal standard method. Next, iii) odor thresholds were determined to calculate odor activity values (OAVs; ratio of concentration to respective odor threshold) and iv) the overall aromas of the respective samples were

simulated by recombination experiments. Finally, triangle tests were applied to confirm the analyzed data and get deeper insights into the influence of the changes induced by blanching of the raw samples on the overall aroma.

MATERIALS AND METHODS

***T. sinensis* Samples.** Raw green and red *T. sinensis* buds were purchased in a local vegetable market (Anhui, China) in April 2018. Fresh *T. sinensis* buds were frozen by liquid nitrogen, crashed into small pieces, and then powdered by SPEX SamplePrep 6870 Freezer/Mill (Metuchen, NJ). Finally, the powder was filled into brown glass bottles and stored at -24 °C prior to analysis. To obtain the respective blanched samples, *T. sinensis* buds were heat-treated in boiling water (100 °C) for 1 min, cooled to room temperature, and then prepared as described for the raw sample.

Reference Odorants. The following reference odorants were commercially available: acetic acid, 2-acetylpyrrole, benzyl alcohol, caryophyllene oxide, decanoic acid, (*E*,*E*)-2,4-decadienal, dimethyl sulfide, dipropyl disulfide, dipropyl trisulfide, 2-ethyl-3,5-dimethylpyrazine, 4-ethylphenol, eugenol, hexanal, hexanoic acid, (*E*)-2-hexenal, (*E*)-3-hexenoic acid, (*E*)-2-hexen-1-ol, *α*-humulene, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, *β*-ionone, isocaryophyllene, 2-isopropyl-3-methoxypyrazine, limonene, linalool, menthol, 2-methoxyphenol, 2-methylbutanoic acid, 3-methylbutanoic acid, methyl 2-methylbutanoate, methylpyrazine, *γ*-nonalactone, 1-octen-3-ol, phenol, phenylacetic acid, 2-phenylethanol, *α*-pinene, propanoic acid, and 2,3,5-trimethylpyrazine (Sigma-Aldrich Chemie, Taufkirchen, Germany); cyclopentadecanone, 2-methylbutanal, 3-methylbutanal, 5-methyl-2-methoxyphenol, and 1-octen-3-one (Alfa Aesar, Karlsruhe, Germany); aromadendrene, butyrolactone, *β*-caryophyllene, (*E*)-2-decenal, heptanoic acid, methyl hexanoate, nonanoic acid, and valencene (Fluka, Neu-Ulm, Germany); 2-ethyl-3-methylpyrazine and nonanal (Acros Organics;

Thermo Fisher Scientific, Schwerte, Germany); isoeugenol and (*E*,*Z*)-2,6-nonadienal (Lancaster, Mühlheim/Main, Germany); 2-ethyl-6-methylpyrazine (Pyrazine Specialties, Ellenwood, GA); vanillin (Merck, Darmstadt, Germany); 3-methylnonane-2,4-dione (Chemos, Regenstauf, Germany); and 2-pyrrolidone (TCI, Eschborn, Germany). 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene was a gift from Firmenich (Geneva, Switzerland).

Chemicals. Methyl octanoate, potassium hydroxide, [$^2H_3$]-propyl bromide, propyl iodide, sulfur, and tetrahydrofuran were obtained from Sigma-Aldrich Chemie. Ethanol, hydrochloric acid, sodium carbonate, sodium chloride, and anhydrous sodium sulfate were from Merck. Deionized water used for HPLC was prepared using a MiliQ Advantage A10 Water Purification System (Milipore S.A.S., Molsheim, France). Acetonitrile used for HPLC analysis was of HPLC grade (Merck). Dichloromethane, diethyl ether, and *n*-pentane (Merck) were freshly distilled prior to use. All chemicals were at least of analytical grade.

Stable Isotopically Labeled Internal Standards. The following stable isotopically labeled internal standards were commercially obtained: [$^{13}C_2$]-acetic acid, [$^2H_5$]-benzyl alcohol, [$^2H_6$]-dimethyl sulfide, [$^2H_3$]-hexanoic acid, [$^2H_{3\text{-}5}$]-1-octen-3-one, [$^{13}C_2$]-phenylacetic acid, [$^{13}C_2$]-2-phenylethanol, and [$^2H_2$]-propanoic acid (Sigma-Aldrich Chemie); [$^2H_{2\text{-}3}$]-decanoic acid, [$^2H_2$]-nonanoic acid, and [$^2H_6$]-2-pyrrolidone (C/D/N Isotopes, Quebec, Canada); [$^2H_4$]-*cis*-isoeugenol (aromaLAB, Planegg, Germany); and [$^2H_9$]-2-methylbutanoic acid (EQ Laboratories, Augsburg, Germany).

The following standards were prepared as previously described: [$^2H_{3\text{-}5}$]-(*E*,*E*)-2,4-decadienal,[9] [$^2H_5$]-2-ethyl-3,5-dimethylpyrazine,[10] [$^2H_{3\text{-}4}$]-4-ethylphenol,[11] [$^2H_{4\text{-}6}$]-hexanal,[12] [$^2H_2$]-(*E*)-2-hexenal,[13] [$^2H_2$]-(*E*)-2-hexen-1-ol,[9] [$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one,[14]

143 [$^2H_3$]-*β*-ionone,[15] [$^2H_3$]-2-isopropyl-3-methoxypyrazine analogue to
144 [$^2H_3$]-2-isobutyl-3-methoxypyrazine,[16] [$^2H_{2-3}$]-linalool,[17] [$^2H_2$]-2-methylbutanal,[18]
145 [$^2H_{6-8}$]-5-methyl-2-methoxyphenol analogue to 5-ethyl-2-methoxyphenol,[19]
146 [$^2H_3$]-methyl 2-methylbutanoate,[12] [$^2H_3$]-3-methylnonane-2,4-dione,[13]
147 [$^2H_{2-3}$]-methylpyrazine,[20] [$^2H_2$]-(*E*,*Z*)-2,6-nonadienal,[13] [$^2H_{2-3}$]-*γ*-nonalactone,[21]
148 [$^2H_4$]-nonanal,[22] [$^2H_{2-3}$]-*γ*-octalactone,[23] [$^2H_{3-6}$]-1-octen-3-ol,[24]
149 [$^2H_{3-4}$]-2,3,5-trimethylpyrazine,[17] and [$^2H_3$]-vanillin.[19]

150 The concentrations of the stable isotopically labeled compounds were determined
151 as recently described.[25]

152 **Syntheses.** *Dipropyl Disulfide and Dipropyl Trisulfide*.[26] Finely powdered
153 potassium hydroxide (1 g) was added to tetrahydrofuran (THF; 14 mL, containing
154 0.2% of water), and a white suspension was obtained. After adding powdered sulfur
155 (0.256 g, 1 mmol) to this vigorously stirred suspension, the reaction mixture was
156 stirred for another 5 min. A brown coloration was observed, which disappeared upon
157 addition of a solution of propyl iodide (1.36 g, 8 mmol) in THF (4 mL, containing 0.2%
158 of water). The mixture was stirred at room temperature for another 2 h, and then
159 filtered. After evaporation of the solvent via a rotary evaporator (50 °C, 380 mbar), the
160 residue was dissolved in *n*-pentane (~2 mL), and purification was performed via
161 column chromatography using purified silica gel 60 (20 g, 0.040-0.063 mm; Merck) in
162 a water-cooled glass column (12 °C; 25 cm × 1 cm id) using *n*-pentane (200 mL). The
163 reaction yield was 50%, with a dipropyl disulfide/dipropyl trisulfide ratio of 29/71,
164 based on area counts obtained via gas chromatography-flame ionization detection
165 (GC-FID). The purity of the mixture was 97%, and the odorants were finally
166 characterized via GC-MS.

167 Dipropyl disulfide: MS (EI): *m*/*z* (%): 150 (100), 43 (96), 108 (50), 73 (30), 41 (24),
168 66 (20), 39 (19), 40 (18), 45 (18), 74 (10), 48 (8), 79 (8), 151 (8), 152 (8), 110 (5).

MS (CI): *m*/*z* (%): 151 (M + 1, 100).

Dipropyl trisulfide: MS (EI): *m*/*z* (%): 182 (100), 75 (98), 41 (40), 43 (40), 73 (30), 45 (13), 47 (13), 184 (12), 44 (11), 117 (11), 39 (10), 98 (10), 140 (10), 183 (10), 64 (8), 105 (8).

MS (CI): *m*/*z* (%): 183 (M + 1, 100).

[$^2H_6$]-Dipropyl Disulfide and [$^2H_6$]-Dipropyl Trisulfide.[27] The same procedure as for the synthesis of dipropyl disulfide and dipropyl trisulfide was applied, except that [$^2H_3$]-propyl bromide (1.01 g, 8 mmol) in THF (4 mL, containing 0.2% of water) was used as alkylating reagent. The products obtained were characterized by GC-MS.

[$^2H_6$]-Dipropyl disulfide: MS (EI): *m*/*z* (%): 156 (100), 46 (78), 40 (45), 44 (43), 43 (40), 111 (28), 112 (25), 45 (20), 73 (15), 47 (13), 75 (13), 76 (13), 41 (12), 67 (9), 66 (8), 158 (8), 42 (7), 157 (7).

MS (CI): *m*/*z* (%): 157 (M + 1, 100).

[$^2H_6$]-Dipropyl trisulfide: MS (EI): *m*/*z* (%): 188 (100), 78 (78), 46 (75), 79 (52), 40 (45), 44 (40), 43 (30), 73 (25), 45 (22), 190 (16), 189 (13), 64 (10), 41 (8), 48 (8), 123 (8), 75 (6), 108 (6), 99 (5).

MS (CI): *m*/*z* (%): 189 (M + 1, 100).

The concentrations of the isotopically labeled standards were determined by a Trace 2000 gas chromatograph (Thermo, Egelsbach, Germany) equipped with an FID using methyl octanoate as the internal standard. First, the FID response factor was determined for each unlabeled reference compound (dipropyl disulfide and dipropyl trisulfide were commercially bought) and methyl octanoate. Then, the concentration of the labeled standard was calculated via the peak areas of the labeled compound and methyl octanoate using the FID response factor determined for the unlabeled compound.[25]

Isolation of the Volatiles. *T. sinensis* powder (20 g) was extracted with

dichloromethane (3 × 200 mL) by vigorously stirring (3 × 0.5 h) at room temperature. The organic extracts were combined, filtered, and dried over anhydrous sodium sulfate (10 g). The volatiles were separated from the nonvolatile fraction by high vacuum distillation using the solvent assisted flavor evaporation (SAFE) technique.[28]

Fractionation of the Volatiles. The SAFE distillate obtained was separated into the acidic fraction (AF) and the neutral/basic fraction (NBF) by liquid-liquid extraction with an aqueous Na_2CO_3 solution (0.5 mol/L; 3 × 50 mL). The organic phase containing the neutral/basic fraction (NBF) was washed with a saturated sodium chloride solution (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated at 42 °C to a volume of ~3 mL using a Vigreux column (50 cm × 1 cm id) and finally to ~200 µL by microdistillation.[29] The aqueous phase containing the acidic fraction (AF) was adjusted to a pH value of 2−3 using hydrochloric acid. Afterward, the odorants were extracted with diethyl ether (1+1 by vol., 3 × 50 mL). The combined organic phases were washed again with a saturated sodium chloride solution and dried over anhydrous Na_2SO_4. After filtration, the fraction was concentrated as described above for NBF.

For an unequivocal compound identification, NBF was further fractionated by column chromatography using purified silica gel 60 (20 g, 0.040-0.063 mm; Merck) in a water-cooled glass column (12 °C; 30 cm × 1 cm id) with the following *n*-pentane/diethyl ether mixtures: 100:0, 80:20, 50:50, and 0:100 (v:v; 50 mL each). Each portion obtained (50 mL) was dried over anhydrous Na_2SO_4, and then concentrated to a final volume of ~200 µL (silica gel fractions, SGF 1-4) as described above.[30]

Isolation of Di-1-Propenyl Disulfides and Di-1-Propenyl Trisulfides. The isolation of di-1-propenyl disulfides and di-1-propenyl trisulfides from SGF 1 obtained from 4 kg of raw green *T. sinensis* was performed via high performance liquid

chromatography (HPLC) using a PU-2089 Plus Quaternary pump and an UV-2075 Plus HPLC UV-VIS detector; both Jasco, Pfungstadt, Germany). Aliquots (100 µL) of SGF 1 (dissolved in acetonitrile (ACN)) were injected onto a Nucleosil 100-5 C18 AB column (250 mm × 4.6 mm; Macherey-Nagel, Düren, Germany) and eluted with the following gradient: 50/50 ACN/H_2O for 20 min, then 100/0 ACN/H_2O for further 20 min. Two sulfury smelling fractions obtained after 21 and 29 min were collected, dried with anhydrous sodium sulfate, and the purity of these two fractions were checked by GC-O/FID and GC-MS (95% and 92%, respectively). Subsequently, these two fractions were characterized as di-1-propenyl disulfide (3 isomers) and di-1-propenyl trisulfide (3 isomers) by GC-MS.

(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-Di-1-propenyl disulfide: MS (EI): *m*/*z* (%): 146 (100), 45 (60), 113 (38), 73 (35), 74 (33), 82 (31), 67 (21), 39 (20), 41 (20), 59 (19), 71 (19), 72 (19), 69 (18), 47 (11), 101 (8), 147 (8), 148 (8), 85 (7).

MS (CI): *m*/*z* (%): 147 (M + 1, 100).

(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-Di-1-propenyl trisulfide: MS (EI): *m*/*z* (%): 114 (100), 178 (62), 45 (60), 105 (45), 73 (33), 41 (31), 61 (31), 39 (24), 71 (24), 99 (23), 106 (20), 112 (20), 118 (18), 47 (12), 180 (12), 64 (10), 116 (9).

MS (CI): *m*/*z* (%): 179 (M + 1, 100).

High-Resolution Gas Chromatography-Olfactometry/Flame Ionization Detection (HRGC-O/FID). HRGC-O/FID was performed using a TRACE GC 2000 (ThermoQuest) equipped with either a DB-FFAP capillary column (30 m × 0.25 mm id, 0.25 µm film thickness) or a DB-5 capillary column (30 m × 0.32 mm id, 0.25 µm film thickness) (both J&W Scientific; Agilent Technologies, Waldbronn, Germany). Helium was used as the carrier gas (flow rate = 1.9 mL/min). Two minutes after manual cold on-column injection of an aliquot of the sample (2 µL) at 40 °C, the oven temperature was raised with 6 °C/min to 240 °C and then held for 10 min. A Y-type quick-seal

glass splitter (Chrompack, Frankfurt, Germany) was used at the end of the column to separate the effluent into two equal parts to a sniffing port held at 230 °C and an FID held at 250 °C, which enabled a simultaneous detection of the respective odor qualities and the FID chromatogram. Linear retention indices for each compound were calculated using the retention times of a series of *n*-alkanes (C6-C26 (DB-FFAP) and C6-C18 (DB-5), respectively).

Comprehensive High-Resolution Gas Chromatography-Time-of-Flight Mass Spectrometry (HRGC×HRGC-TOF-MS) for Identification. The instrument consisted of a gas chromatograph 6890N (Agilent, Böblingen, Germany) equipped with a DB-FFAP capillary column (30 m × 0.25 mm id, 0.25 µm film thickness) in the first dimension and a DB-5 capillary column (2 m × 0.15 mm id, 0.30 µm film thickness) in the second dimension (both J&W Scientific). The front part of the DB-FFAP capillary column passed a liquid nitrogen-cooled dual stage quad-jet thermal modulator (Leco, St. Joseph, MI), and the end part was connected via a heated (250 °C) transfer line to the inlet of a Pegasus III TOF-MS (Leco). Sample injections were performed by a GC-PAL autosampler (CTC Analytics, Zwingen, Switzerland). Helium was applied as the carrier gas (flow rate = 2 mL/min). The following temperature program was used: for the first oven, 40 °C held for 2 min, then raised with 6 °C/min to 230 °C, held for 5 min. The modulation time was set to 4 s. For the second oven, the temperature started at 70 °C (2 min), raised with 6 °C/min to 250 °C, held for 10 min. Mass spectra were recorded in electron ionization (EI) mode at 70 eV at a rate of 100 spectra/s. The scan range was set at *m*/*z* 35-350. Data was analyzed by means of GC Image (Lincoln, NE).

Comparative Aroma Extract Dilution Analysis (cAEDA). The flavor dilution (FD) factors of the odorants were determined via cAEDA as previously reported.[8]

Comparative Static Headspace Aroma Dilution Analysis (cSH-ADA) Based

on Static Headspace High-Resolution Gas Chromatography−Olfactometry/Mass Spectrometry (SH-HRGC-O/MS). To detect very volatile components and the compounds which were co-eluting with the solvent during AEDA, SH-HRGC-O/MS was performed as previously reported.[8] FD factors of the odorants were determined via cSH-ADA as previously reported.[8]

Stable Isotope Dilution Assays (SIDAs). The stable isotopically labeled internal standards (0.1-400 µg, dissolved in dichloromethane; amount depending on the concentration of the respective analyte determined in preliminary experiments) and dichloromethane (30-300 mL) were added to the powdered materials (1-10 g). After equilibration, the samples were worked-up as described for isolation of the volatiles. The SAFE distillate obtained was concentrated to ~ 200 µL as described above and was used for high-resolution gas chromatography-mass spectrometry (HRGC-MS) or two-dimensional heart-cut high-resolution gas chromatography-mass spectrometry (HRGC/HRGC-MS) (Table 1).

High-Resolution Gas Chromatography-Mass Spectrometry (HRGC-MS). HRGC-MS was performed by a gas chromatograph 431 (Varian, Darmstadt) equipped with a DB-FFAP capillary column (30 m × 0.25 mm id, 0.25 µm film thickness; J&W Scientific). The initial oven temperature (40 °C) was held for 2 min, then raised with 6 °C/min to 230 °C, and held for 10 min. The respective response factors (R_f) were determined via analyzing known mixtures of the respective unlabeled analyte and the corresponding stable isotopically labeled internal standard in five different mass ratios (5:1, 3:1, 1:1, 1:3, 1:5) (Table 1).

Two-Dimensional Heart-Cut High-Resolution Gas Chromatography-Mass Spectrometry (HRGC/HRGC-MS). The instrument consisted of a TRACE GC 2000 (ThermoQuest) equipped with a cold on-column injector and a DB-FFAP capillary column (30 m × 0.32 mm id, 0.25 µm film thickness; J&W Scientific) in the first

dimension, and a gas chromatograph CP-3800 (Varian) equipped with a DB-1701 capillary column (30 m × 0.25 mm id, 0.25 μm film thickness; J&W Scientific) in the second dimension. Sample injections were performed by a CombiPal autosampler (CTC Analytics). The target compound was transferred via a moving column stream switching (MCSS) system (ThermoQuest) onto the second column via a cold trap (-100 °C; cooled by liquid nitrogen), and was finally analyzed by a Saturn 2000 ion trap mass spectrometer (Varian). The individual temperature programs for each analyte in both dimensions were optimized according to its heart-cut time in the first dimension and the retention time in the second dimension. Mass spectra were recorded in CI mode at 105 eV with methanol as the reactant gas.

Headspace Solid Phase Microextraction Combined with High-Resolution Gas Chromatography-Mass Spectrometry (HS-SPME-HRGC-MS). Due to their low boiling points, dimethyl sulfide, 2-methylbutanal, and 3-methylbutanal were quantitated by SIDAs using HS-SPME-HRGC-MS as previously reported.[8]

High-Resolution Gas Chromatography-Flame Ionization Detection (HRGC-FID) for Semiquantitation. A Trace GC Ultra (ThermoQuest) equipped with a DB-FFAP (30 m × 0.32 mm id, 0.25 μm film thickness; J&W Scientific) was used for semiquantitation of aromadendrene, *β*-caryophyllene, caryophyllene oxide, *α*-humulene, isocaryophyllene, and valencene via internal standard method as previously reported (internal standard: cyclopentadecanone).[8]

Determination of Orthonasal Odor Thresholds (OTs) in Water. Orthonasal OTs in water of di-1-propenyl disulfide (3 isomers), di-1-propenyl trisulfide (3 isomers), and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (2 isomers) were newly determined by means of triangle tests according to a previously published protocol.[31]

Determination of OTs in Air. OTs in air of (*E*,*E*)-di-1-propenyl disulfide, (*E*,*Z*)-di-1-propenyl disulfide, (*Z*,*Z*)-di-1-propenyl disulfide, (*E*,*E*)-di-1-propenyl

trisulfide, (*E*,*Z*)-di-1-propenyl trisulfide, (*Z*,*Z*)-di-1-propenyl trisulfide, *cis*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene were determined by the following procedure:[32] isomers of di-1-propenyl disulfide, di-1-propenyl trisulfide, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene were dissolved in diethyl ether. After adding (*E*)-2-decenal as reference compound, stepwise with diethyl ether diluted solutions (1+1, v+v) were analyzed by HRGC-O (DB-FFAP capillary column) until no odorant was detectable at the sniffing port. The odor thresholds in air were then calculated with the previously published odor threshold of (*E*)-2-decenal (2.7 ng/L).[33]

Aroma Profile Analysis (APA). Aqueous solutions of each of the nine reference compounds in concentrations 50-fold above their respective odor thresholds were prepared to define the odor descriptors: acetic acid (vinegar-like), eugenol (clove-like), hexanal (green), *β*-ionone (flowery), 2-isopropyl-3-methoxypyrazine (earthy), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (cooked onion-like, TS-like), 2-methoxyphenol (phenolic), 2-methylbutanal (malty), and 3-methylnonane-2,4-dione (hay-like, aniseed-like, fishy). Aliquots of the samples (1 g) were presented in covered odorless Teflon® vessels (id = 40 mm). Sensory analysis was carried out in a sensory room at 21 ± 1 °C equipped with individual booths. The sensory panel, consisting of 15-20 weekly trained members able to describe and recognize odor qualities, and thus, to perform a comparative APA, evaluated the odor intensities of the aroma attributes of each *T. sinensis* sample from 0 (not perceivable) to 3 (strongly perceivable) on a seven-point linear scale by steps of 0.5.

Aroma Recombination. Raw green and red *T. sinensis* (10 g each) were extracted with dichloromethane (100 mL each) several times until the residues were odorless. These odorless residues were used as matrices for the recombinates of raw green and red *T. sinensis* as well as the respective blanched samples. Aqueous

solutions of all odorants with an OAV ≥ 1 were prepared from ethanolic stock solutions and were added to the respective matrices in their original concentrations determined in the samples. After vigorous shaking for 30 min, the recombinates were evaluated in the same way as described above for APA.

Triangle Tests. To confirm the data obtained before and after blanching, and to get deeper insights into the blanching-induced changes of the key odorants, and thus, of the overall aroma, triangle tests were designed and interpreted according to the method ISO 4120:2004. Therefore, spiking experiments to green and red blanched *T. sinensis* samples were performed by adding reference aroma compounds to obtain their initial concentrations in the respective raw samples. Then, these spiked samples were compared to the original raw *T. sinensis* samples. First, aqueous solutions of all analyzed aroma compounds with OAVs ≥ 1 were added to blanched *T. sinensis* samples in the way that their naturally occurring concentrations determined in raw *T. sinensis* were obtained. Subsequently, different compounds eliciting a certain odor quality were added to blanched *T. sinensis* sample, again in the amounts to obtain their naturally occurring concentrations in raw *T. sinensis*. For “cooked onion-like/TS-like”, the following odorants were chosen: dimethyl sulfide, (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide, and *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene. The “green” group included hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal, the “phenolic” group 4-ethylphenol and 2-methoxyphenol, the “earthy” group the three pyrazines 2-ethyl-3,5-dimethylpyrazine, 2-isopropyl-3-methoxypyrazine, and 2,3,5-trimethylpyrazine, the “malty” group 2-methylbutanal and 3-methylbutanal, and “vinegar-like” was represented by acetic acid. Finally, aqueous solutions of the remaining analyzed aroma compounds with OAVs ≥ 1 were added to blanched *T. sinensis* in the same way.

RESULTS AND DISCUSSION

Aroma Profiles of Raw Green and Red *T. sinensis*. First of all, aroma profile analysis was performed to evaluate the overall aroma of green and red *T. sinensis* using nine odor descriptors. Thereby, the cooked onion-like/TS-like (2.3) odor impression was the most intense attribute in green *T. sinensis*, followed by green (1.7), hay-like/aniseed-like/fishy (1.7), earthy (1.6), malty (1.3), vinegar-like (1.0), phenolic (0.7), clove-like (0.6), and flowery (0.5). The aroma profile of red *T. sinensis* revealed clearly lower intense cooked onion-like/TS-like (1.8) and green (1.1) odor notes, whereas the phenolic attribute was ranked with a higher intensity (1.3) (Figure 1).

Odorant Screening in Raw Green and Red *T. sinensis*. Application of cAEDA to the SAFE distillate obtained from buds of green and red *T. sinensis* revealed 57 aroma-active areas present in the FD factor range between 8 and 4096 in at least one of the two *T. sinensis* aroma extracts (Table 2, Figure 2). For structure identification, the retention indices on two columns of different polarities (DB-FFAP and DB-5) as well as the odor qualities and intensities detected at the sniffing port during AEDA were compared to data available in an in-house database containing > 1000 odorants. Subsequently, authentic reference compounds were analyzed by GC-O with two different columns to match the retention indices and odor descriptors. The final step of identification was based on mass spectrometry in EI and CI mode in comparison to data obtained from the respective reference compounds. For an unequivocal identification (avoiding a possible overlap of minor odorants by (aroma-active) compounds present at higher concentrations), the SAFE distillates were fractionated by liquid-liquid extraction into the acidic fraction (AF) and the neutral/basic fraction (NBF). The NBF was further fractionated into four subfractions by silica gel chromatography (SGF 1-4). All the fractions were analyzed by GC-O and GC-MS to identify the odorants.

Following the abovementioned procedure, the highest FD factors were obtained for (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide (**21-23**, FD factors of 4096 (green) and 2048 (red), roasted onion-like), *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (**30** and **31**, 2048 and 1024, cooked onion-like/TS-like), and eugenol (**57**, 1024 and 2048, clove-like) in both raw green and red *T. sinensis* samples (Table 2).

Differences in FD factors between green and red *T. sinensis* were e.g. found for dimethyl sulfide (**1**, FD factors of 32 (green) and 16 (red), cabbage-like), hexanal (**6**, 128 and 32, green/grassy), (*E*)-2-hexenal (**9**, 128 and 32, green apple-like), (*E*)-2-hexen-1-ol (**15**, 64 and 16, green/fruity), acetic acid (**19**, 16 and 64, vinegar-like), linalool (**25**, 16 and 64, citrus-like/flowery), (*E*,*Z*)-2,6-nonadienal (**27**, 128 and 32, green/cucumber-like), 2-methylbutanoic acid (**35**, 8 and 32, fruity/sweaty), 3-methylbutanoic acid (**36**, 8 and 32, sweaty), (*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl trisulfide (**39**-**41**, 512 and 128, cooked onion-like), 2-methoxyphenol (**44**, 8 and 256, smoky/phenolic), 2-phenylethanol (**46**, 4 and 32, flowery/honey-like), and 4-ethylphenol (**56**, < 4 and 64, fecal-like/phenolic) (Table 2).

The presence of the very volatile dimethyl sulfide (**1**), 3-methylbutanal (**2**, malty), and 2-methylbutanal (**3**, malty) in both raw samples was confirmed via cSH-ADA.

Based on FD factors, nine sulfur-containing compounds including dimethyl sulfide (**1**), (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide (**21**-**23**), *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (**30** and **31**), and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide (**39**-**41**) should contribute to the intense cooked onion-like/TS-like aroma note of green *T. sinensis*. Their FD factors in green *T. sinensis* were all higher than the respective FD factors in red *T. sinensis*. In addition, the green smelling aldehydes hexanal (**6**), (*E*)-2-hexenal (**9**), and (*E*,*Z*)-2,6-nonadienal (**27**) as well as the green smelling (*E*)-2-hexen-1-ol (**15**) showed

higher FD factors in green *T. sinensis* compared to red *T. sinensis*, probably responsible for the intense green aroma note of the green variety. 2-Methoxyphenol (**44**) and 4-ethylphenol (**56**), both present at higher FD factors in red TS (256 and 64) compared to green *T. sinensis* (8 and < 4), should lead to the specific phenolic aroma note of red *T. sinensis* (Table 2).

Differences in Odorant Concentrations in Raw Green and Red *T. sinensis*. To get knowledge about the role of the individual *T. sinensis* odorants discussed above and to clarify the aroma differences between the two *T. sinensis* varieties, quantitative analysis of aroma compounds revealing FD factors ≥ 32 in at least one sample as well as of compounds characterized as key odorants in dried *T. sinensis* in our previous study[8] was performed by SIDAs (Table 1). Aromadendrene, *β*-caryophyllene, caryophyllene oxide, *α*-humulene, isocaryophyllene, and valencene were semiquantitated by GC-FID via internal standard method using cyclopentadecanone as standard. Concentrations of the nine sulfur-containing compounds dimethyl sulfide (14000 µg/kg (green) vs 11000 µg/kg (red)), sum of di-1-propenyl disulfide isomers (1360 µg/kg vs 595 µg/kg), sum of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene isomers (230 µg/kg vs 51.1 µg/kg), and sum of di-1-propenyl trisulfide isomers (1300 µg/kg vs 230 µg/kg)) in green *T. sinensis* were much higher than those in red *T. sinensis* (Table 3). For hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal, quantitative results revealed higher concentrations in green *T. sinensis* compared to those in red *T. sinensis*. Quantitation experiments also showed much higher concentrations of 2-methoxyphenol and 4-ethylphenol in red *T. sinensis*, both present at amounts ≥ factor of 60 compared to raw green *T. sinensis* (Table 3), confirming the data obtained during cAEDA.

Also differences in other aroma-active components of green and red *T. sinensis*

samples were found. For example, green *T. sinensis* revealed clearly higher concentrations for 2-methylbutanal (2470 µg/kg vs 1770 µg/kg), nonanoic acid (1760 µg/kg vs 1140 µg/kg), decanoic acid (1570 µg/kg vs 742 µg/kg), 1-octen-3-ol (14.5 µg/kg vs 7.78 µg/kg), and 1-octen-3-one (3.47 µg/kg vs 1.16 µg/kg). All other compounds, such as acetic acid (772 mg/kg vs 2750 mg/kg), caryophyllene (4.51 mg/kg vs 15.4 mg/kg), propanoic acid (2.26 mg/kg vs 16.7 mg/kg), 2-methylbutanoic acid (1200 µg/kg vs 7860 µg/kg), 3-methylbutanoic acid (1120 µg/kg vs 10600 µg/kg), aromadendrene (178 µg/kg vs 8230 µg/kg), phenylacetic acid (90.8 µg/kg vs 1140 µg/kg), linalool (55.4 µg/kg vs 301 µg/kg), 2-phenylethanol (50.6 µg/kg vs 2150 µg/kg), and 2-isopropyl-3-methoxypyrazine (29.1 µg/kg vs 136 µg/kg) were present at clearly higher concentrations in red *T. sinensis* (Table 3).

Differences of Odor Activity Values (OAVs) in Raw Green and Red *T. sinensis*. To get information about the contribution of a respective odorant to the overall aroma of the two *T. sinensis* samples, OAVs were calculated for each odorant. Due to the lack of pure isomers of di-1-propenyl disulfide, di-1-propenyl trisulfide, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, their OAVs were calculated with their respective OTs in water based on their isomer mixtures. In green *T. sinensis*, 36 odorants were present in concentrations higher than the respective odor thresholds. Thereby, the highest OAV was calculated for the three isomers of di-1-propenyl disulfide (400000), followed by dimethyl sulfide (47000), 2-isopropyl-3-methoxypyrazine (7500), *β*-ionone (6500), (*E*,*Z*)-2,6-nonadienal (6300), the two isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (5900), the three isomers of di-1-propenyl trisulfide (5000), eugenol (2800), 2-methylbutanal (1600), 3-methylbutanal (1400), and hexanal (780) (Table 4).

For red *T. sinensis*, 41 odorants showed OAVs ≥ 1. The highest OAV was obtained, again, for the three isomers of di-1-propenyl disulfide (170000), dimethyl

sulfide (37000), 2-isopropyl-3-methoxypyrazine (35000), and *β*-ionone (7000), followed by eugenol (3800), 3-methylbutanal (1400), the two isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (1300), 2-methylbutanal (1200), the three isomers of di-1-propenyl disulfide (890), and (*E*,*Z*)-2,6-nonadienal (770) (Table 4).

A comparison between both raw *T. sinensis* revealed the sulfur-containing compounds di-1-propenyl disulfide, dimethyl sulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and di-1-propenyl trisulfide with clearly lower OAVs in red *T. sinensis*. Moreover, (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, and (*E*)-2-hexen-1-ol showed clearly lower OAVs in red *T. sinensis*. In contrast, 2-methoxyphenol and 4-ethylphenol resulted in clearly higher OAVs in red *T. sinensis* (440 and 32) compared to those in green *T. sinensis* (7 and <1) (Table 4). Obvious differences in other compounds between green and red *T. sinensis* were also found for 2-isopropyl-3-methoxypyrazine (7500 (green) and 35000 (red)), 1-octen-3-one (220 and 73), acetic acid (140 vs 490), linalool (96 and 520), valencene (8 and 27), caryophyllene (4 and 13), 3-methylbutanoic acid (2 and 22), aromadendrene (1 and 24), phenylacetic acid (1 and 17), and 2-phenylethanol (<1 and 15) (Table 4).

OTs in Air of Sulfur-Containing Isomers. Due to the good separation of each isomer of di-1-propenyl disulfide, di-1-propenyl trisulfide, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene during GC-O using a DB-FFAP capillary column, the odor qualities and odor thresholds in air of the respective isomers were determined. Thereby, (*E*,*E*)-di-1-propenyl disulfide (roasted onion-like, 0.015 ng/L), *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (cooked onion-like/TS-like, 0.089 ng/L), (*E*,*Z*)-di-1-propenyl disulfide (roasted onion-like, 0.092 ng/L), and (*Z*,*Z*)-di-1-propenyl disulfide (roasted onion-like, 0.26 ng/L) showed

extremely low odor thresholds in air. In contrast, the odor thresholds in air of *cis*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (cooked onion-like/TS-like), (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide (all cooked onion-like) were ≥ 1.7 ng/L (Table 5). Interestingly, the odor qualities did not differ between the isomers.

Aroma Recombination Experiments of Raw Green and Red *T. sinensis*. To validate the data obtained after identification and quantitation, aroma recombinates of both samples were prepared in respective odorless residues obtained after solvent extraction of the initial sample material. To those matrices, all odorants showing OAVs ≥ 1 (Table 4) were added in their natural occurring concentrations. APA showed very good similarities between both raw *T. sinensis* samples and the respective recombinate, proving the successful characterization of the key aroma compounds for raw green and red *T. sinensis* (Figure 3).

Influence of the Blanching Process on Key Odorants in Blanched Green and Red *T. sinensis*. To characterize the aroma-active compounds in blanched green and red *T. sinensis*, cAEDA and cSH-ADA were applied to *T. sinensis* blanched at 100 °C for 1 min. The odorant screening demonstrated that all the odorants identified in raw green *T. sinensis* in the first part of the actual study could be identified again. Lowered FD factors (30 compounds within the FD factor range of 8-1024) determined in green *T. sinensis*, for example, for most of the sulfury and green smelling odorants, gave first hints, that the loss of these odorants might be responsible for the aroma profile change after blanching (Table 2 and Figure 4A). Lower FD factors were also found for, e.g., 3-methylbutanal (32 vs 4), 2-methylbutanal (32 vs 4), 2-isopropyl-3-methoxypyrazine (512 vs 128), eugenol (1024 vs 256), and vanillin (32 vs 4) (Table 2).

A comparison of FD factors in raw and blanched red *T. sinensis* showed the same decreasing trends as for green *T. sinensis*. Additionally, the FD factors of

2-methoxyphenol (256 vs 64) and 4-ethylphenol (64 vs < 4) were lower, which might also indicate the lower phenolic odor note after blanching (Table 2 and Figure 4B).

Quantitation of Key Odorants in Blanched Green and Red *T. sinensis*. To get a deeper insight into the changes of the overall aroma in blanched *T. sinensis*, concentrations of selected odorants with high FD factors or compounds showing obvious differences in their FD factors after blanching were determined via SIDAs or internal standard method and their OAVs were calculated. Compared to raw green *T. sinensis*, the concentrations of dimethyl sulfide (14000 µg/kg (raw) vs 1710 µg/kg (blanched)), (*E*)-2-hexenal (3290 µg/kg vs 433 µg/kg), 2-methylbutanal (2470 µg/kg vs 213 µg/kg), hexanal (1880 µg/kg vs 329 µg/kg), 3-methylbutanal (723 µg/kg vs 18.3 µg/kg), (*E*)-2-hexen-1-ol (340 µg/kg vs 31.9 µg/kg), and 4-ethylphenol (3.92 µg/kg vs 0.53 µg/kg) decreased by 83 to 97% (Table 3) in blanched green *T. sinensis*. The concentrations of *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene and (*E*,*Z*)-2,6-nonadienal decreased between 37 and 50%, of the three isomers of di-1-propenyl disulfide between 4 and 28%, and of di-1-propenyl trisulfide between 5 and 70%. In general, nearly all odorants were present at decreased concentrations after blanching, e.g., acetic acid, eugenol, decanoic acid, aromadendrene, *β*-ionone, 2-phenylethanol, and methyl 2-methylbutanoate. In contrast, only a few compounds increased: nonanal (+16%), 3-methyl-2,4-nonadione (+18%), (*E*,*E*)-2,4-decadienal (+112%), and 1-octen-3-one (+118%) (Table 3).

Calculation of OAVs in blanched green *T. sinensis* showed the three isomers of di-1-propenyl disulfide with the highest OAV of 320000, followed by 2-isopropyl-3-methoxypyrazine (6400), dimethyl sulfide and *β*-ionone (both 5700), the three isomers of di-1-propenyl trisulfide (4300), (*E*,*Z*)-2,6-nonadienal (3600), the two isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (3100), and eugenol (1600) (Table 4). A comparison of the OAVs in raw and blanched green *T. sinensis* confirmed

559 that the processing step resulted in clearly lower OAVs for di-1-propenyl disulfide (3
560 isomers), dimethyl sulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (2 isomers),
561 di-1-propenyl trisulfide (3 isomers), (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, and
562 (*E*)-2-hexen-1-ol (Table 4).

563 To indicate whether similar aroma losses also occur in blanched red *T. sinensis*,
564 quantitation of the same aroma compounds as analyzed in raw red *T. sinensis* was
565 performed (Table 3). Thereby, the same trends as already seen for green *T. sinensis*
566 were found for the abovementioned odorants. Monitoring the changes in the
567 concentrations of 2-methoxyphenol (-60%) and 4-ethylphenol (-70%) indicated that
568 their clear decreases caused the loss in intensity of the phenolic odor attribute after
569 blanching. Further, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, hexanal,
570 *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and dimethyl sulfide showed
571 high losses of 99, 83, 59, 63, and 46%, respectively. Also the amounts of
572 di-1-propenyl disulfide (3 isomers), di-1-propenyl trisulfide (3 isomers), and
573 (*E*,*Z*)-2,6-nonadienal decreased. Additionally, most other analyzed compounds, such
574 as 2-methylbutanoic acid, 3-methylbutanoic acid, isocaryophyllene, 3-methylbutanal,
575 butyrolactone, benzyl alcohol, aromadendrene, 2-phenylethanol,
576 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, methylpyrazine, and
577 methyl 2-methylbutanoate decreased by > 65%. On the other hand, identical to the
578 green variety, nonanal (+14%), 3-methylnonane-2,4-dione (+4%),
579 (*E*,*E*)-2,4-decadienal (+62%), and 1-octen-3-one (+141%) were again the only four
580 compounds present at higher concentrations after blanching.

581 **Aroma Profiles and Recombinates of Blanched Green and Red *T. sinensis*.**
582 To clarify the changes in the quantitative pattern of the key odorants and in the overall
583 aroma occurring during the blanching process of *T. sinensis*, APA of blanched green
584 and red *T. sinensis* was performed (Figure 4). For green *T. sinensis*, the sensory

panel analyzed clearly lower scores of the cooked onion-like/TS-like (1.9) and green (1.3) aroma attributes compared to those of raw *T. sinensis* (Figure 4A). For red *T. sinensis*, obviously lower cooked onion-like/TS-like (1.4), green (1.0), and especially phenolic (0.7) odor notes were perceived after blanching (Figure 4B).

Aroma recombination experiments revealed very good similarities with the original blanched *T. sinensis* samples, both for blanched green and red *T. sinensis* (Figures 5A and 5B). Thus, all key odorants in both blanched samples were successfully characterized. In addition, the change to less intensive cooked onion-like/TS-like (for both *T. sinensis*), green (especially for green *T. sinensis*), and phenolic (for red *T. sinensis*) aroma notes after blanching was successfully confirmed on a molecular level for the first time in this study.

Sensory Experiments to Elucidate the Contribution of Single Odorants to the Overall Aroma. During the blanching process of both green and red *T. sinensis*, clear reductions of nearly all aroma-active compounds were found with an exemption of only four compounds. Therefore, spiking blanched *T. sinensis* with the amounts of the odorants lost during blanching should enable a “reconstitution” of the aroma profile of raw *T. sinensis*. To verify this assumption, the following triangle experiments were performed (Table 6). First of all, the respective amounts (equivalent to the losses during blanching) of odorants with OAVs ≥ 1 were added to the blanched *T. sinensis* samples (Table 6, tests S1 and S8) to obtain their concentrations determined in the original raw *T. sinensis* samples. While the blanched *T. sinensis* and the raw *T. sinensis* samples were clearly distinguishable by their overall aroma profiles (Figure 4), the spiked blanched *T. sinensis* could not significantly be distinguished anymore from the raw *T. sinensis* (p = 0.5; Table 6), proving the abovementioned assumption.

In a second part of experiments, aroma compounds were grouped according to six aroma notes, namely “cooked onion-like/TS-like” (S2 and S9), “green” (S3 and

S11), “phenolic” (S10), “earthy” (S4 and S13), “malty” (S5 and S12), and “vinegar-like” (S6 and S14), and added to the blanched *T. sinensis* samples. In a last set of experiments, the odorants with OAVs ≥ 1, but not included in the abovementioned groups, were added to the blanched *T. sinensis* samples (remaining compounds; S7 and S15).

The blanched *T. sinensis* samples spiked with “earthy”, “malty”, “vinegar-like”, and remaining compounds could significantly be differentiated from the raw *T. sinensis* samples (all $p < 0.001$; only for test S12, $p < 0.008$; Table 6), indicating that changes in different odorants are (additionally) responsible for the overall aroma shift. Interestingly, already after administering only the “cooked onion-like/TS-like” group, the panelists could not discriminate the spiked blanched samples from the raw samples ($p = 0.3$; significantly different not before $p < 0.05$; Table 6), proving that the losses of these compounds during blanching is the crucial factor for the change of the overall aroma of both blanched *T. sinensis* varieties and that the groups “malty”, “earthy”, “vinegar-like”, and “remaining compounds” did not have very important effects. In addition, also spiking of hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal (“green” group) to the blanched samples ended up with no discrimination ($p = 0.3$ and 0.2; Table 6). Due to the low OAVs of 2-methoxyphenol and 4-ethylphenol (7 and <1) in raw green *T. sinensis* and high OAVs in raw red *T. sinensis* (440 and 32), the “phenolic” group was only spiked to blanched red *T. sinensis* and this partial recombinate could also not be differentiated from raw red *T. sinensis* ($p = 0.3$; Table 6).

These results clearly corroborated the importance of the sulfur-containing compounds di-1-propenyl disulfide, dimethyl sulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and di-1-propenyl trisulfide as well as the aldehydes (*E*,*Z*)-2,6-nonadienal, hexanal, and (*E*)-2-hexenal and the alcohol

637 (*E*)-2-hexen-1-ol for the overall aroma of both *T. sinensis*. In addition, the two phenolic
638 compounds 2-methoxyphenol and 4-ethylphenol were proven to be key odorants of
639 red *T. sinensis*.

640 **Possible Sources of Key Odorants in *T. sinensis*.** *Sulfur-Containing*
641 *Compounds.* (*E*,*Z*)- and (*Z*,*Z*)-Di-1-propenyl trisulfide were identified for the first time
642 as aroma-active compounds in *T. sinensis*. In addition, eight sulfur-containing
643 compounds including (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, *cis*- and
644 *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiopnene, and (*E*,*E*)-, (*E*,*Z*)-, and
645 (*Z*,*Z*)-di-1-propenyl trisulfide were quantitated for the first time in *T. sinensis* via SIDA
646 and showed extremely high OAVs in both *T. sinensis*, causing the cooked
647 onion-like/TS-like odor note. Hydrogen sulfide and methyl thiirane, recently reported
648 in *T. sinensis* by Yang et al.,[7] were not found as key odorants in this study, neither in
649 the raw nor in the blanched samples, which was additionally confirmed by the very
650 good similarities of both recombinates to the respective original samples.
651 Di-1-propenyl disulfides and di-1-propenyl trisulfides, also contributing to the
652 characteristic aroma of onion, garlic, and other *Alliaceae*, are usually formed during
653 crushing by a very fast enzymatic degradation of *S*-(1-propenyl)-L-cysteine sulfoxide
654 (precursor) cleaved by allinase, followed by a cascade of chemical reactions.[34,35]
655 However, Li et al. reported that nonvolatile precursors of di-1-propenyl disulfides and
656 di-1-propenyl trisulfides in *T. sinensis* are different from those in *Alliaceae*.[36] Based on
657 their findings, *T. sinensis* contains metabolites with *S*-alkylnorcysteine moieties such
658 as (*S*,*S*)-γ-glutamyl-(*cis*-*S*-1-propenyl)thioglycine and
659 (*S*,*S*)-γ-glutamyl-(*trans*-*S*-1-propenyl)thioglycine, which may release thiols via
660 cleavage of the amide bond by proteases of *T. sinensis*, followed by a spontaneous
661 decomposition of the resulting unstable alk(en)yl norcysteine moiety. Moreover,
662 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene is a thermal degradation product of

di-1-propenyl disulfide.[7] Dimethyl sulfide can be formed from the amino acid *S*-methylmethionine, which was recently proven as a possible precursor.[37] However, disruption of the plant cells occurs unavoidably during blanching at 100 °C, resulting in premature release and subsequent loss of these sulfur-containing compounds[35] which are essential for the overall aroma of both raw (more pronounced) and blanched (less pronounced) *T. sinensis*.

Green Smelling Aldehydes and (E)-2-Hexen-1-ol. Many lipid-derived odorants with green odor notes were identified and quantitated via SIDAs in all four *T. sinensis* samples. While these compounds have already been identified[7,8] and partially quantitated[8] either in raw, cooked, and/or dried *T. sinensis*, (*E*,*Z*)-2,6-nonadienal was quantitated for the first time in *T. sinensis* via SIDA in the present study. Hexanal is a common secondary product arising from peroxidation of linoleic acid by a hydroperoxide cleavage enzyme system.[38] (*E*)-2-Hexenal is produced by isomerization of (*Z*)-3-hexenal that can enzymatically be formed from linolenic acid in the presence of oxygen.[39] Subsequently, (*E*)-2-hexenal can be converted into (*E*)-2-hexen-1-ol via alcohol dehydrogenase in plant leaves.[39] In addition, (*E*,*Z*)-2,6-nonadienal was characterized as key odorant in *T. sinensis* and was present at OAVs of 6300 in raw green *T. sinensis* and 770 in raw red *T. sinensis*. It can be enzymatically formed from linolenic acid by a sequence of enzyme reactions containing lipoxygenase, hydroperoxide lyase, and isomerase.[40]

Phenols. The amounts of 2-methoxyphenol and 4-ethylphenol have obvious impact on the different overall aroma of green and red *T. sinensis*. The smoky and phenolic smelling 2-methoxyphenol was found in raw red *T. sinensis* with an OAV of 440, 63 times higher compared to raw green *T. sinensis* (OAV only 7). Rahouti et al. showed a metabolism starting from ferulic acid via 4-vinylguiacol to vanillin by the low phenol oxidase producer *Paecilomyces variotii*. Subsequent oxidation leads to vanillic

acid and, again, in a final decarboxylation step to 2-methoxyphenol.[41] 4-Ethylphenol with a fecal-like and phenolic note was characterized not to be a key odorant in green *T. sinensis* due to its OAV < 1, whereas an OAV of 32 was obtained for raw red *T. sinensis*. The origin of 4-ethylphenol might be related to a sequential activity of two enzymes which can reduce and decarboxylate hydroxycinnamic acids (e.g., ferulic acid, *p*-coumaric acid, and caffeic acid) into hydroxystyrenes (e.g., vinylphenols), followed by a reduction to the corresponding ethyl derivatives.[42]

In conclusion, this study characterized 36 key odorants in raw green *T. sinensis* and 41 key odorants in raw red *T. sinensis*, among which (*E*,*E*)-2,4-decadienal, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl trisulfide, 4-ethylphenol, 2-methoxyphenol, nonanal, and 2,3,5-trimethylpyrazine were identified for the first time. Furthermore, the intense cooked onion-like/TS-like odorants characterized the green *T. sinensis* aroma, namely (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, dimethyl disulfide, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide. The obvious green note in green *T. sinensis* was caused by (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, and (*E*)-2-hexen-1-ol. 2-Methoxyphenol and 4-ethylphenol evoked the specific phenolic odor note in red *T. sinensis*. Recombination experiments of the overall aroma of four samples including raw green and red *T. sinensis* as well as the respective blanched buds proved the successful identification and quantitation of their respective key odorants. As confirmed by sensory experiments, the clear losses of abovementioned sulfur-containing compounds, aldehydes, (*E*)-2-hexen-1-ol, and phenols are responsible for the overall aroma changes during blanching. Thus, these new findings provide knowledge of molecular differences not only between green and red *T. sinensis*, but also between raw and blanched *T. sinensis* (for both varieties), which can be used in the future to improve the final overall aroma of *T. sinensis* after the

715 blanching process.

716

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720 isomers and di-1-propenyl trisulfide isomers from *T. sinensis* via preparative HPLC.

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853 # FIGURE CAPTIONS

854 **Figure 1.** Aroma profiles of raw green *T. sinensis* (solid line) and raw red *T. sinensis*
855 (broken line).

856

857 **Figure 2.** Flavor dilution chromatograms on a polar DB-FFAP capillary column
858 obtained by cAEDA from raw green (**A**) and red (**B**) *T. sinensis*. Odorants with an FD
859 factor ≥ 32 are displayed. Numbering is identical to that in Table 2.

860

861 **Figure 3.** Aroma profiles of raw green *T. sinensis* (solid line) and the respective
862 recombinate (broken line) (**A**), and aroma profiles of raw red *T. sinensis* (solid line)
863 and the respective recombinate (broken line) (**B**).

864

865 **Figure 4.** Aroma profiles of raw green *T. sinensis* (solid line) and blanched green *T.*
866 *sinensis* (broken line) (**A**), and aroma profiles of raw red *T. sinensis* (solid line) and
867 blanched red *T. sinensis* (broken line) (**B**).

868

869 **Figure 5.** Aroma profiles of blanched green *T. sinensis* (solid line) and the respective
870 recombinate (broken line) (**A**), and aroma profiles of blanched red *T. sinensis* (solid
871 line) and the respective recombinate (broken line) (**B**).

TABLES

Table 1. Selected Ions (*m/z*) of Analytes and Stable Isotopically Labeled Standards, Response Factors (R_f), and Systems Used in Stable Isotope Dilution Assays.

odorant	isotope label	ion (*m/z*)[a]		R_f[b]	system[c]
		analyte	standard		
acetic acid	[$^{13}C_2$]-acetic acid	61	63	1.00	I
benzyl alcohol	[$^{2}H_5$]-benzyl alcohol	91	96	0.87	I
butyrolactone[e]	[$^{2}H_{2-3}$]-*γ*-octalactone[e]	87	145+146[d,e]	1.12	II
(*E*,*E*)-2,4-decadienal	[$^{2}H_{3-5}$]-(*E*,*E*)-2,4-decadienal	153	156-158[d]	0.97	II
decanoic acid	[$^{2}H_{2-3}$]-decanoic acid	187	189+190[d]	0.93	II
dimethyl sulfide	[$^{2}H_6$]-dimethyl sulfide	63	69	0.96	III
(*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl disulfide[f]	[$^{2}H_6$]-dipropyl disulfide[f]	147	157[f]	1.00	II
(*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl trisulfide[g]	[$^{2}H_6$]-dipropyl trisulfide[g]	179	189[g]	0.72	II
2-ethyl-3,5-dimethylpyrazine	[$^{2}H_5$]-2-ethyl-3,5-dimethylpyrazine	137	142	1.00	II
4-ethylphenol	[$^{2}H_{3-4}$]-4-ethylphenol	123	126+127[d]	1.00	II
eugenol[h]	[$^{2}H_4$]-*cis*-isoeugenol[h]	165	169[h]	0.67	I

odorant	isotope label	ion (*m/z*)[a]		R_f[b]	system[c]
		analyte	standard		
hexanal	[$^2H_{4-6}$]-hexanal	101	105-107[d]	0.92	II
hexanoic acid	[$^2H_3$]-hexanoic acid	99	102	0.94	I
(*E*)-2-hexenal	[$^2H_2$]-(*E*)-2-hexenal	99	101	0.58	III
(*E*)-2-hexen-1-ol	[$^2H_2$]-(*E*)-2-hexen-1-ol	83	85	1.00	II
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	[$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	129	131	0.95	II
β-ionone	[$^2H_3$]-*β*-ionone	193	196	0.94	I
2-isopropyl-3-methoxypyrazine	[$^2H_3$]-2-isopropyl-3-methoxypyrazine	153	156	0.88	II
linalool	[$^2H_{2-3}$]-linalool	137	139+140[d]	0.90	II
cis- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene[i]	[$^2H_6$]-dipropyl disulfide[i]	147	157[i]	1.00	II
2-methoxyphenol[j]	[$^2H_{6-8}$]-5-methyl-2-methoxyphenol[j]	125	145-147[d,j]	0.59	II
2-methylbutanal	[$^2H_2$]-2-methylbutanal	87	89	1.00	III
3-methylbutanal[k]	[$^2H_2$]-2-methylbutanal[k]	87	89[k]	1.00	III

odorant	isotope label	ion (*m/z*)[a]		R_f[b]	system[c]
		analyte	standard		
methyl 2-methylbutanoate	[$^2H_3$]-methyl 2-methylbutanoate	117	120	0.98	II
2-methylbutanoic acid	[$^2H_9$]-2-methylbutanoic acid	103	112	0.96	II
3-methylbutanoic acid[l]	[$^2H_9$]-2-methylbutanoic acid[l]	103	112[l]	0.96	II
3-methylnonane-2,4-dione	[$^2H_3$]-3-methylnonane-2,4-dione	171	174	0.95	II
methylpyrazine	[$^2H_{2-3}$]-methylpyrazine	95	97+98[d]	0.75	II
(*E*,*Z*)-2,6-nonadienal	[$^2H_2$]-(*E*,*Z*)-2,6-nonadienal	139	141	0.82	II
γ-nonalactone	[$^2H_{2-3}$]-*γ*-nonalactone	157	159+160[d]	1.00	II
nonanal	[$^2H_4$]-nonanal	143	147	0.82	II
nonanoic acid	[$^2H_2$]-nonanoic acid	173	175	0.98	II
1-octen-3-ol	[$^2H_{3-6}$]-1-octen-3-ol	111	114-117[d]	0.75	II
1-octen-3-one	[$^2H_{3-5}$]-1-octen-3-one	127	130-132[d]	0.75	II
phenylacetic acid	[$^{13}C_2$]-phenylacetic acid	137	139	0.92	II
2-phenylethanol	[$^{13}C_2$]-2-phenylethanol	105	107	0.91	I
propanoic acid	[$^2H_2$]-propanoic acid	75	77	1.00	II

odorant	isotope label	ion (*m/z*)[a]		R_f[b]	system[c]
		analyte	standard		
2-pyrrolidone	[$^2H_6$]-2-pyrrolidone	86	92	0.82	I
2,3,5-trimethylpyrazine	[$^2H_{3-4}$]-2,3,5-trimethylpyrazine	123	126+127[d]	0.93	II
vanillin	[$^2H_3$]-vanillin	153	156	0.93	II

[a]Ions used for quantitation in chemical ionization (CI) mode. [b]Response factor (R_f) was determined by analyzing mixtures of known amounts of unlabeled analyte and corresponding labeled internal standard. [c]I: HRGC-MS(CI); II: HRGC/HRGC-MS(CI); III: HS-SPME-HRGC-MS(CI). [d]Internal standard was used as a mixture of isotopologues. [e]Butyrolactone was quantitated using [$^2H_{2-3}$]-γ-octalactone as the internal standard. [f](*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl disulfide were quantitated using [$^2H_6$]-dipropyl disulfide as the internal standard. [g](*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl trisulfide were quantitated using [$^2H_6$]-dipropyl trisulfide as the internal standard. [h]Eugenol was quantitated using [$^2H_4$]-*cis*-isoeugenol as the internal standard. [i]*cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene[i] were quantitated using [$^2H_6$]-dipropyl disulfide as the internal standard. [j]2-Methoxyphenol was quantitated using [$^2H_{6-8}$]-5-methyl-2-methoxyphenol as the internal standard. [k]3-Methylbutanal was quantitated using [$^2H_2$]-2-methylbutanal as the internal standard. [l]3-Methylbutanoic acid was quantitated using [$^2H_9$]-2-methylbutanoic acid as the internal standard.

Table 2. Aroma-Active Compounds in Raw and Blanched Green and Red *T. sinensis*, Their Odor Qualities, Retention Indices, Flavor Dilution (FD) Factors, and Separated Fractions.

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]				fraction[f]
					green TS		red TS		
			DB-FFAP	DB-5	raw	blanched	raw	blanched	
1	dimethyl sulfide[g]	cabbage-like	nd[h]	511	32	16	8	8	HSF
2	3-methylbutanal[g]	malty	933	652	32	4	16	16	HSF
3	2-methylbutanal[g]	malty	934	657	32	4	16	16	HSF
4	methyl 2-methylbutanoate	fruity	1006	775	16	4	16	4	SGF 2
5	α-pinene	resin, fir needle-like	1010	939	8	<4	8	<4	SGF 1
6	hexanal	green, grassy	1090	769	128	32	32	16	SGF 2
7	methyl hexanoate	fruity, musty	1199	922	8	4	4	4	SGF 2
8	limonene	citrus-like, carrot-like	1206	1030	<4	8	<4	<4	SGF 1
9	(*E*)-2-hexenal	green apple-like	1216	851	128	16	32	8	SGF 2

Table 2. Continued

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]				fraction[f]
					green TS		red TS		
			DB-FFAP	DB-5	Raw	blanched	raw	blanched	
10	methylpyrazine	green, roasty	1273	822	<4	<4	<4	<4	SGF 4
11	1-octen-3-one	mushroom-like	1293	979	16	32	8	8	SGF 2
12	nonanal	citrus-like, soapy	1381	1103	32	32	32	32	SGF 3
13	2-ethyl-6-methylpyrazine	roasty	1382	1001	<4	<4	8	<4	SGF 4
14	2-ethyl-3-methylpyrazine	roasty	1382	1010	<4	<4	8	<4	SGF 4
15	(*E*)-2-hexen-1-ol	green, fruity	1403	860	64	4	16	8	SGF 3
16	2,3,5-trimethylpyrazine	earthy	1410	1003	32	32	32	32	SGF 4
17	2-isopropyl-3-methoxypyrazine	earthy, pea-like	1421	1094	512	128	1024	256	SGF 4
18	2-ethyl-3,5-dimethylpyrazine	earthy	1430	1079	64	32	64	32	SGF 4
19	acetic acid	vinegar-like	1441	612	16	8	64	32	AF
20	1-octen-3-ol	mushroom-like	1442	975	4	<4	<4	<4	SGF 3
21	(*E*,*E*)-di-1-propenyl disulfide	roasted onion-like	1448	1121	4096	1024	2048	1024	SGF 1
22	(*E*,*Z*)-di-1-propenyl disulfide	roasted onion-like	1467	1130	4096	1024	2048	1024	SGF 1

Table 2. Continued

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]				fraction[f]
					green TS		red TS		
			DB-FFAP	DB-5	raw	blanched	raw	blanched	
23	(*Z*,*Z*)-di-1-propenyl disulfide	roasted onion-like	1490	1142	4096	1024	2048	1024	SGF 1
24	propanoic acid	sour, sweaty	1535	706	16	8	8	4	AF
25	linalool	citrus-like, flowery	1539	1100	16	8	64	16	SGF 2
26	isocaryophyllene	citrus-like	1556	1407	16	4	32	4	SGF 1
27	(*E*,*Z*)-2,6-nonadienal	green, cucumber-like	1571	1153	128	32	32	4	SGF 2
28	*β*-caryophyllene	moldy	1577	nd[h]	8	<4	8	<4	SGF 1
29	aromadendrene	eucalyptus-like	1591	nd[h]	16	4	32	16	SGF 1
30	*cis*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like/TS-like	1618	1119	2048	1024	1024	256	SGF 1
31	*trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like/TS-like	1633	1127	2048	1024	1024	256	SGF 1
32	butyrolactone	sweet, aromatic	1638	900	128	32	32	4	SGF 4
33	menthol	mint-like	1641	nd[h]	4	4	4	4	AF

Table 2. Continued

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]				fraction[f]
					green TS		red TS		
			DB-FFAP	DB-5	raw	blanched	raw	blanched	
34	*α*-humulene	balmy	1654	1455	8	<4	8	<4	SGF 1
35	2-methylbutanoic acid	fruity, sweaty	1660	870	8	<4	32	<4	AF
36	3-methylbutanoic acid	sweaty	1662	870	8	<4	32	<4	AF
37	valencene	fruity, flowery	1702	1497	4	<4	8	<4	SGF 1
38	3-methylnonane-2,4-dione	hay-like, aniseed-like, fishy	1716	1251	128	128	128	128	SGF 4
39	(*E*,*E*)-di-1-propenyl trisulfide	cooked onion-like	1741	1341	512	512	128	64	SGF 1
40	(*E*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	1759	1355	512	512	128	64	SGF 1
41	(*Z*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	1788	1378	512	512	128	64	SGF 1
42	(*E*,*E*)-2,4-decadienal	fatty, deep-fried	1801	1371	16	32	16	32	SGF 2
43	hexanoic acid	sweaty	1839	1010	8	8	8	8	AF
44	2-methoxyphenol	smoky, phenolic	1848	1090	8	<4	256	64	SGF 2
45	benzyl alcohol	bitter almond-like, fruity	1873	1036	8	<4	32	nd[h]	SGF 3
46	2-phenylethanol	flowery, honey-like	1900	1117	4	<4	32	16	SGF 3

Table 2. Continued

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]				fraction[f]
					green TS		red TS		
			DB-FFAP	DB-5	raw	blanched	raw	Blanched	
47	*β*-ionone	flowery, violet-like	1923	1488	256	128	512	128	SGF 2
48	heptanoic acid	rancid, sweaty	1942	1074	8	<4	<4	<4	AF
49	(*E*)-3-hexenoic acid	cheese-like	1947	986	8	<4	<4	<4	AF
50	caryophyllene oxide	citrus-like, soapy	1969	1578	16	4	16	4	SGF 2
51	2-acetylpyrrole	musty	1989	1066	8	<4	8	<4	SGF 2
52	phenol	ink-like, phenolic	2016	981	8	<4	8	<4	SGF 2
53	*γ*-nonalactone	coconut-like	2029	1360	32	8	32	8	SGF 3
54	2-pyrrolidone	fruity	2054	nd[h]	8	<4	8	<4	SGF 4
55	nonanoic acid	moldy, pungent	2150	nd[h]	64	16	32	16	AF
56	4-ethylphenol	fecal-like, phenolic	2163	1077	<4	nd	64	<4	SGF 3
57	eugenol	clove-like	2167	1359	1024	256	2048	512	SGF 2
58	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like, spicy	2200	1108	8	<4	8	<4	SGF 4

Table 2. Continued

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]				fraction[f]
					green TS		red TS		
			DB-FFAP	DB-5	raw	blanched	raw	blanched	
59	decanoic acid	soapy, musty	2250	1369	64	32	32	16	AF
60	phenylacetic acid	beeswax-like, honey-like	2552	1261	<4	<4	8	<4	AF
61	vanillin	vanilla-like, sweet	2571	1403	32	4	32	4	SGF 4

[a]Retention indices, calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [b]Flavor dilution factor: highest dilution of the concentrated SAFE distillate in which the odorant was detected during GC-O for the last time; average of three trained panelists (two females, one male). [c]Odorants were consecutively numbered according to their retention indices on a DB-FFAP capillary column. [d]Odorants were identified by comparing their odor qualities and intensities, retention indices on capillary columns DB-FFAP and DB-5, and mass spectra (EI and CI mode) to data of reference compounds. [e]Odor quality perceived at the sniffing port during HRGC-O. [f]Fraction, in which the odorant was detected by HRGC-O or SH-HRGC-O after fractionation of the initial extract: AF = fraction of acidic volatiles, HSF = fraction of headspace volatiles, SGF 1-4 = silica gel subfractions 1-4 of fraction of neutral/basic volatiles (NBF). [g]FD factors of these odorants were determined via cSH-ADA. [h]Not determined.

Table 3. Concentrations of Important Aroma-Active Compounds in Raw and Blanched Green and Red *T. sinensis* and Their Losses During Blanching.

odorant	green TS			red TS		
	conc. (μg/kg)[a]		loss (%)	conc. (μg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
acetic acid	772000	322000	58	2750000	1340000	51
dimethyl sulfide	14000	1710	88	11000	5900	46
eugenol	5120	2800	45	6830	3410	50
caryophyllene	4510	3310	27	15400	10500	32
(*E*)-2-hexenal	3290	433	87	1480	256	83
2-methylbutanal	2470	213	91	1770	1030	42
propanoic acid	2260	1090	52	16700	8470	49
hexanal	1880	329	83	579	237	59
nonanoic acid	1760	884	50	1140	753	34
decanoic acid	1570	470	70	742	162	78

Table 3. Continued

odorant	green TS			red TS		
	conc. (μg/kg)[a]		loss (%)	conc. (μg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
2-methylbutanoic acid	1200	432	64	7860	1230	84
3-methylbutanoic acid	1120	516	54	10600	1480	86
isocaryophyllene	1080	1030	5	2190	565	74
caryophyllene oxide	1010	879	13	1620	680	58
hexanoic acid	914	439	52	1290	531	59
3-methylbutanal	723	18.3	97	690	165	76
(*Z*,*Z*)-di-1-propenyl trisulfide	656	588	10	87.3	72.6	17
(*E*,*Z*)-di-1-propenyl disulfide	643	460	28	366	355	3
butyrolactone	574	209	64	456	145	68
valencene	546	431	21	1770	1420	20
(*E*,*E*)-di-1-propenyl trisulfide	534	506	5	81.5	67.4	17
(*E*,*E*)-di-1-propenyl disulfide	461	393	15	199	178	11
α-humulene	438	366	16	1230	947	23

Table 3. Continued

odorant	green TS			red TS		
	conc. (μg/kg)[a]		loss (%)	conc. (μg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
2-pyrrolidone	423	190	55	535	248	54
benzyl alcohol	377	159	58	1290	331	74
(*E*)-2-hexen-1-ol	340	31.9	91	134	1.99	99
(*Z*,*Z*)-di-1-propenyl disulfide	259	249	4	29.8	25.2	15
aromadendrene	178	60.7	66	8230	430	95
vanillin	176	77.4	56	159	88.7	44
trans-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	167	83.6	50	41.0	15.3	63
β-ionone	137	119	13	146	92.0	37
(*E*,*Z*)-di-1-propenyl trisulfide	112	33.1	70	61.4	27.1	56
phenylacetic acid	90.8	56.6	38	1140	986	14
cis-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	62.6	39.2	37	10.1	7.81	23
linalool	55.4	30.2	45	301	107	64
2-phenylethanol	50.6	17.3	66	2150	692	68

Table 3. Continued

odorant	green TS			red TS		
	conc. (µg/kg)[a]		loss (%)	conc. (µg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
2,3,5-trimethylpyrazine	41.2	16.0	61	46.3	19.5	58
nonanal	37.6	43.7	+16[b]	41.7	47.7	+14[b]
2-isopropyl-3-methoxypyrazine	29.1	24.8	15	136	43.7	68
(*E*,*Z*)-2,6-nonadienal	28.3	16.0	43	3.47	2.34	33
γ-nonalactone	24.4	14.7	40	52.2	29.0	44
2-ethyl-3,5-dimethylpyrazine	21.0	7.79	63	23.6	4.90	79
1-octen-3-ol	14.5	6.88	53	7.78	4.00	49
3-methylnonane-2,4-dione	13.7	16.2	+18[b]	17.0	17.6	+4[b]
methylpyrazine	10.9	nd[c]	nc[d]	29.8	9.69	67
2-methoxyphenol	5.67	1.87	67	365	146	60
(*E*,*E*)-2,4-decadienal	4.62	9.81	+112[b]	3.51	5.68	+62[b]
4-ethylphenol	3.92	0.53	86	418	124	70
1-octen-3-one	3.47	7.57	+118[b]	1.16	2.79	+141[b]

Table 3. Continued

odorant	green TS			red TS		
	conc. (µg/kg)[a]		loss (%)	conc. (µg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
methyl 2-methylbutanoate	2.72	0.68	75	4.27	0.76	82
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	1.10	0.90	18	1.16	1.01	13

873 [a]Mean values of triplicates, differing not more than ±15%. [b]Concentration of odorant was higher in blanched *T. sinensis* compared to

874 raw *T. sinensis*. [c]Not determined. [d]Not calculable.

Table 4. Orthonasal Odor Thresholds (OTs) and Odor Activity Values (OAVs) of Important Aroma-Active Compounds of Raw and Blanched Green and Red *T. sinensis*.

odorant	OT (µg/kg)	OAV[a]			
		green TS		red TS	
		raw	blanched	raw	blanched
di-1-propenyl disulfide	0.0034[b]	400000	320000	170000	160000
dimethyl sulfide	0.3[c]	47000	5700	37000	20000
2-isopropyl-3-methoxypyrazine	0.0039[d]	7500	6400	35000	11000
β-ionone	0.021[c]	6500	5700	7000	4400
(*E*,*Z*)-2,6-nonadienal	0.0045[c]	6300	3600	770	520
2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	0.039[b]	5900	3100	1300	590
di-1-propenyl trisulfide	0.26[b]	5000	4300	890	640
eugenol	1.8[c]	2800	1600	3800	1900
2-methylbutanal	1.5[d]	1600	140	1200	690
3-methylbutanal	0.5[d]	1400	37	1400	330
hexanal	2.4[d]	780	140	240	99

Table 4. Continued

odorant	OT (µg/kg)	OAV[a]			
		green TS		red TS	
		raw	blanched	raw	blanched
decanoic acid	3.5[c]	450	130	210	46
3-methylnonane-2,4-dione	0.046[c]	300	350	370	380
1-octen-3-one	0.016[c]	220	470	73	170
(*E*)-2-hexenal	17[c]	190	25	87	15
(*E*,*E*)-2,4-decadienal	0.027[c]	170	360	130	210
acetic acid	5600[c]	140	58	490	240
linalool	0.58[c]	96	52	520	180
(*E*)-2-hexen-1-ol	3.9[c]	87	8	34	<1
2-ethyl-3,5-dimethylpyrazine	0.28[c]	75	28	84	18
nonanoic acid	26[c]	68	34	44	29
isocaryophyllene	20[d]	54	52	110	28
caryophyllene oxide	22[d]	46	40	74	31
nonanal	2.8[c]	13	16	15	17

Table 4. Continued

odorant	OT (μg/kg)	OAV[a]			
		green TS		red TS	
		raw	blanched	raw	blanched
butyrolactone	50[c]	11	4	9	3
valencene	66[d]	8	7	27	22
2-methoxyphenol	0.84[c]	7	2	440	170
caryophyllene	1190[d]	4	3	13	9
2,3,5-trimethylpyrazine	11[c]	4	1	4	2
α-humulene	130[d]	3	3	9	7
γ-nonalactone	9.7[c]	3	2	5	3
vanillin	53[d]	3	1	3	2
3-methylbutanoic acid	490[d]	2	1	22	3
aromadendrene	337[d]	1	<1	24	1
phenylacetic acid	68[c]	1	<1	17	15
methyl 2-methylbutanoate	2.5[c]	1	<1	2	<1
4-ethylphenol	13[c]	<1	<1	32	10

Table 4. Continued

odorant	OT (µg/kg)	OAV[a]			
		green TS		red TS	
		raw	blanched	raw	blanched
2-phenylethanol	140[c]	<1	<1	15	5
2-methylbutanoic acid	3100[c]	<1	<1	3	<1
benzyl alcohol	620[c]	<1	<1	2	<1
propanoic acid	16000[c]	<1	<1	1	<1
1-octen-3-ol	45[c]	<1	<1	<1	<1
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	1.7[c]	<1	<1	<1	<1
methylpyrazine	110[c]	<1	<1	<1	<1
hexanoic acid	4800[c]	<1	<1	<1	<1
2-pyrrolidone	2100[d]	<1	<1	<1	<1

875 [a]Odor activity value were calculated as ratio of the determined concentrations to the respective odor thresholds in water. [b]Orthonasal
876 odor threshold in water of isomer mixture was newly determined in this study according to literature.[31] [c]Threshold from in-house
877 database. [d]Orthonasal odor threshold in water as reported previously.[31]

Table 5. Odor Qualities and Orthonasal Odor Thresholds in Air of Sulfur-Containing Isomers.

odorant	odor quality	odor threshold (ng/L)
(*E*,*E*)-di-1-propenyl disulfide	roasted onion-like	0.015
(*E*,*Z*)-di-1-propenyl disulfide	roasted onion-like	0.092
(*Z*,*Z*)-di-1-propenyl disulfide	roasted onion-like	0.26
cis-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like/TS-like	3.6
trans-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like/TS-like	0.089
(*E*,*E*)-di-1-propenyl trisulfide	cooked onion-like	1.7
(*E*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	7.7
(*Z*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	8.7

Table 6. Triangle Tests for Spiking Experiments of Raw Red and Green *T. sinensis* Compared to Blanched Red and Green *T. sinensis* to which Reference Aroma Compounds were Added in Concentrations Compensating the Losses during Blanching.

test	reference sample	spiked sample	correct answers/ panelists[a]	statistical significance
S1		blanched green TS + all odorants with OAVs ≥ 1	7/19	$p = 0.5$
S2		blanched green TS + “cooked onion-like/TS-like”	8/19	$p = 0.3$
S3	raw	blanched green TS + “green”	7/16	$p = 0.3$
S4	green TS	blanched green TS + “earthy”	15/16	$p < 0.001$
S5		blanched green TS + “malty”	14/16	$p < 0.001$
S6		blanched green TS + “vinegar-like”	13/19	$p < 0.001$
S7		blanched green TS + remaining compounds	14/16	$p < 0.001$
S8		blanched red TS + all odorants with OAVs ≥ 1	6/16	$p = 0.5$
S9		blanched red TS + “cooked onion-like/TS-like”	6/15	$p = 0.3$
S10		blanched red TS + “phenolic”	7/16	$p = 0.3$
S11	raw	blanched red TS + “green”	8/16	$p = 0.2$
S12	red TS	blanched red TS + “malty”	5/15	$p = 0.008$
S13		blanched red TS + “earthy”	15/15	$p < 0.001$
S14		blanched red TS + “vinegar-like”	15/15	$p < 0.001$
S15		blanched red TS + remaining compounds	15/15	$p < 0.001$

[a]Number of correct answers resulting from the triangle tests and total number of panelists participating.

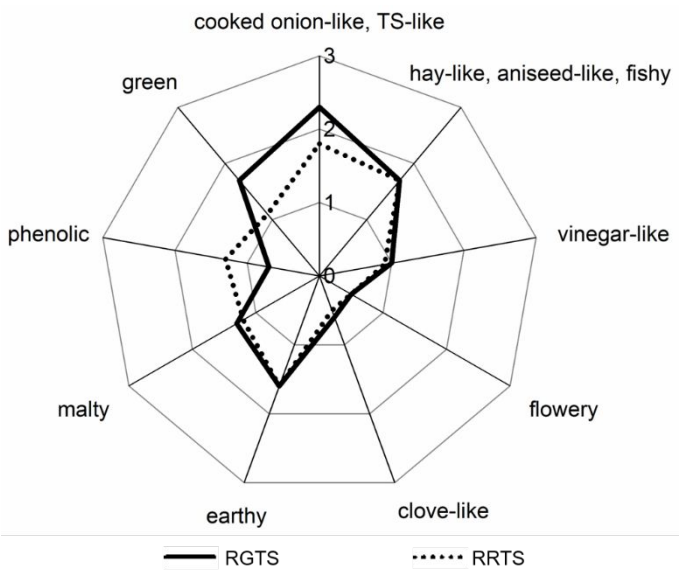
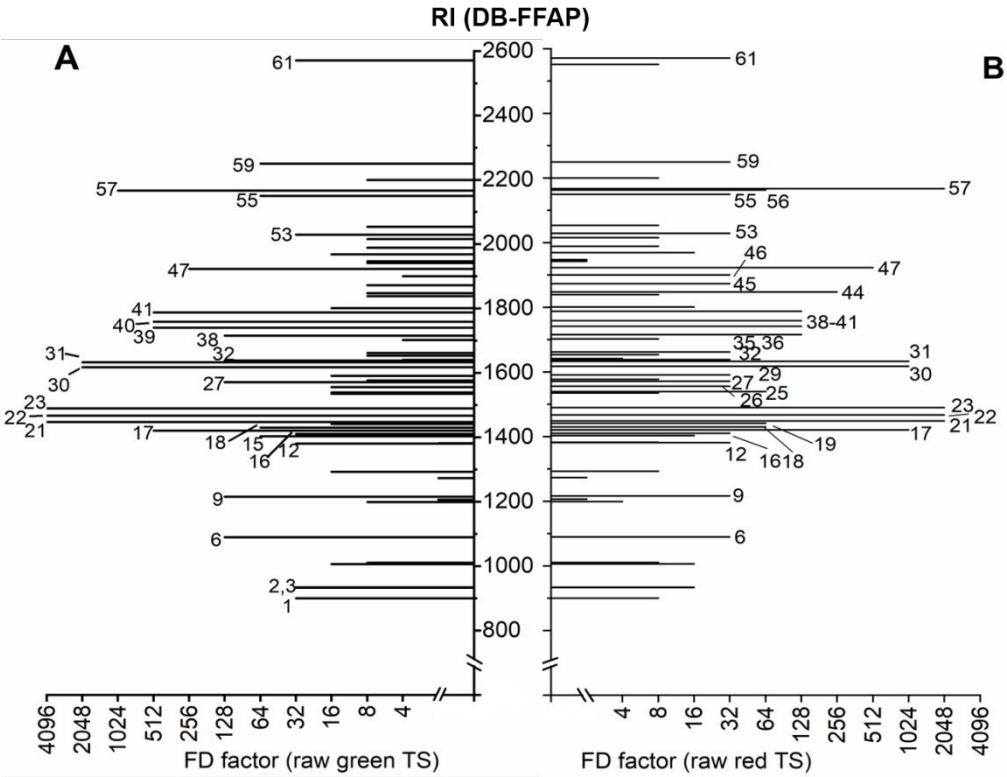

cooked onion-like, TS-like
hay-like, aniseed-like, fishy
vinegar-like
flowery
clove-like
earthy
malty
phenolic
green
3
2
1
0
RGTS
RRTS

Figure 1.


RI (DB-FFAP)
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B
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2400
2200
2000
1800
1600
1400
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1000
800
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256
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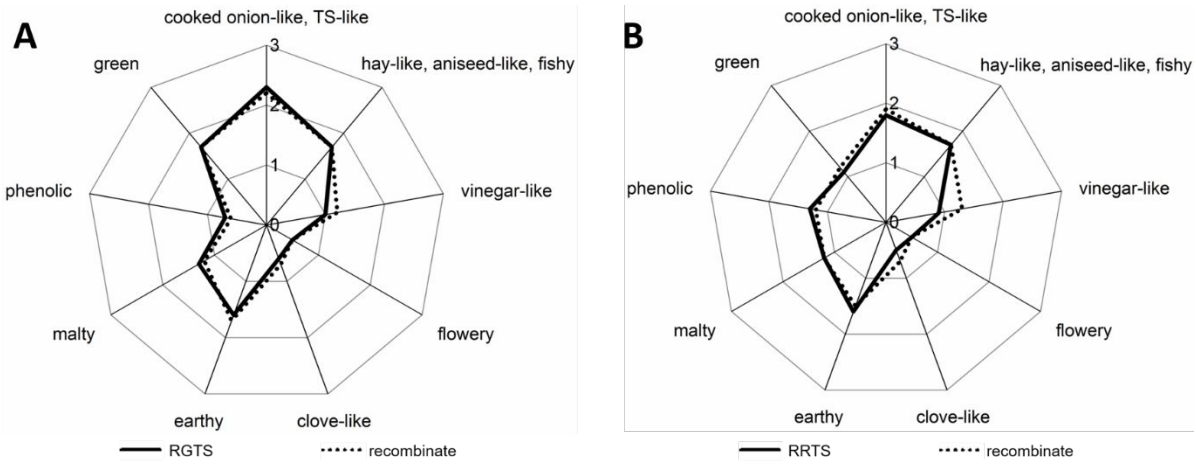
32
16
8
4
FD factor (raw green TS)
FD factor (raw red TS)

Figure 2.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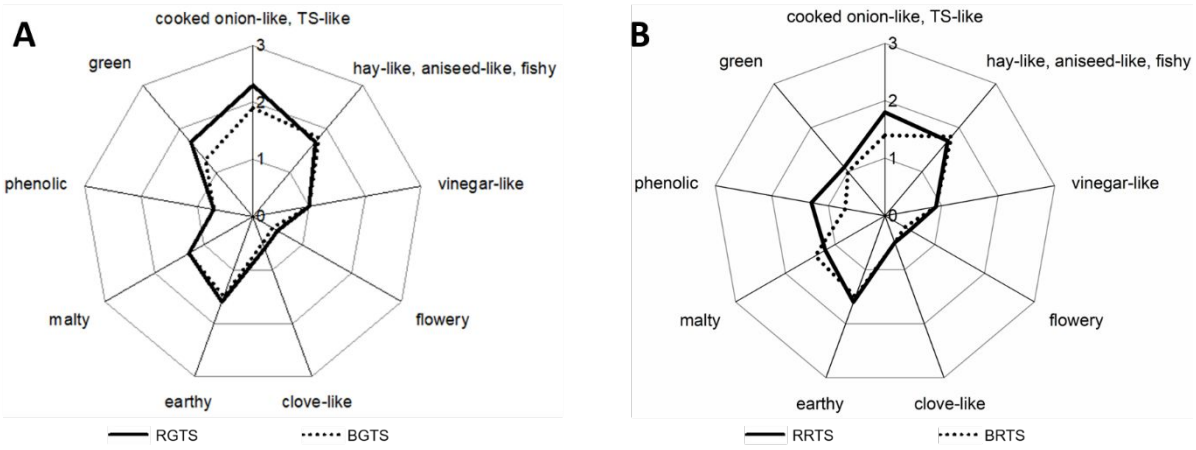
green
hay-like, aniseed-like, fishy
phenolic
vinegar-like
malty
flowery
earthy
clove-like
3
2
1
0
RGTS
recombinate
B
cooked onion-like, TS-like
green
hay-like, aniseed-like, fishy
phenolic
vinegar-like
malty
flowery
earthy
clove-like
3
2
1
0
RRTS
recombinate

Figure 3.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hay-like, aniseed-like, fishy
phenolic
vinegar-like
malty
flowery
earthy
clove-like
0
1
2
3
RGTS
BGTS
B
cooked onion-like, TS-like
green
hay-like, aniseed-like, fishy
phenolic
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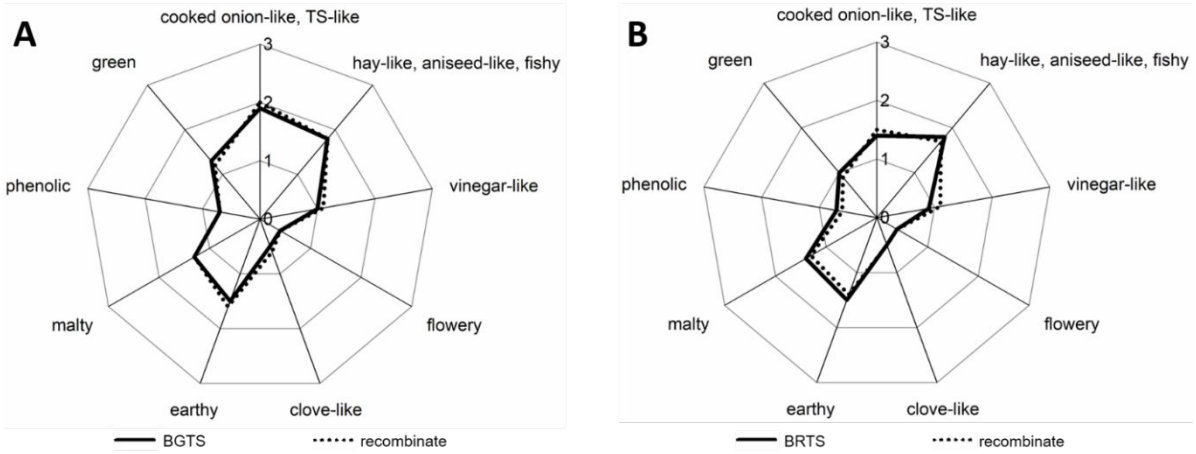
malty
flowery
earthy
clove-like
0
1
2
3
RRTS
BRTS

Figure 4.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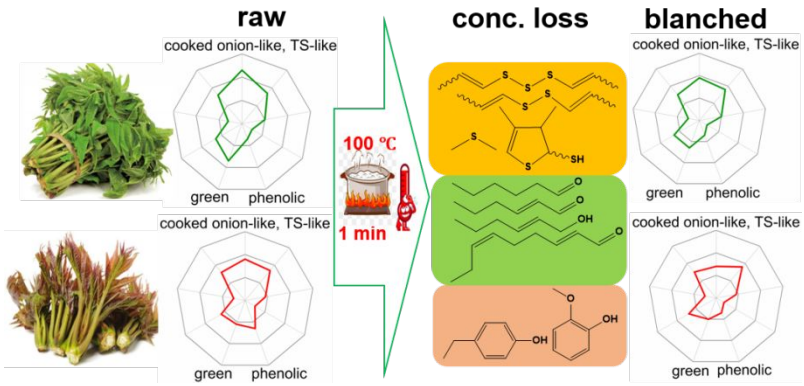
hay-like, aniseed-like, fishy
vinegar-like
flowery
clove-like
earthy
malty
phenolic
green
3
2
1
0
BGTS
recombinate
B
cooked onion-like, TS-like
hay-like, aniseed-like, fishy
vinegar-like
flowery
clove-like
earthy
malty
phenolic
green
3
2
1
0
BRTS
recombinate

Figure 5.

TOC Graphic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conc. loss
blanched
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phenolic
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1 min
SH
OH