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Characterization of the Key Aroma Compounds in Raw *Toona sinensis* (A. Juss.) Roem. Buds and Their Products by Application of the Molecular Sensory Science Concept

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Contents

1 Summary

Toona sinensis (TS), a popular seasonal vegetable with a strong and typical odor, is originally from China and widely distributed in China. According to the color of the young buds, TS is classified into the two varieties red TS and green TS. Red TS buds are considered to have a more intense aroma compared to green TS, indicating obvious differences in key odorants between red and green TS.

To clarify the molecular background of the different aromas, a comparative aroma extract dilution analysis (cAEDA) was carried out on the entire set of the volatiles isolated from TS bud powder by solvent extraction and high vacuum distillation using the solvent assisted flavor evaporation (SAFE) technique. Comparative static headspace aroma dilution analysis (cSH-ADA) was applied to the original TS bud powder to analyze highly volatile odorants either coeluting with the solvent during cAEDA or getting lost during sample preparation for cAEDA. Results manifested (*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, *β*-ionone, eugenol, and 2-isopropyl-3-methoxypyrazine as the most potent aroma-active compounds in both samples. Among them, the sulfur-containing compounds showed clearly higher flavor dilution (FD) factors in green TS. Further, the green smelling compounds (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, and (*E*)-2-hexen-1-ol were perceived with higher FD factors in green TS, while 2-methoxyphenol and 4-ethylphenol with a phenolic odor note showed obviously higher FD factors in red TS. Further, it is noteworthy that 1-octen-3-one, nonanal, 2,3,5-trimethylpyrazine, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-decadienal, 2-methoxyphenol, and 4-ethylphenol were firstly identified as aroma-active compounds in TS. Quantitation experiments via stable isotope dilution assays (SIDAs) and internal standard method (ISM) and subsequent calculation of odor activity values (OAVs; ratio of the concentration of an odorant to its respective odor threshold) confirmed the results obtained during cAEDA and cSH-ADA. In green TS, (*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 were present at clearly higher OAVs compared to red TS, causing the intense, typical onion-like/TS-like odor note. Further, high OAVs of green smelling hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal were found in green TS, leading to the intense green odor note, while 2-methoxyphenol and 4-ethylphenol were proven to contribute to the intense phenolic aroma note in red TS. Aroma profiles of both original TS powders matched those of the respective recombinates very well, corroborating the successful identification and quantitation of all key odorants in raw green and red TS.

To elucidate aroma changes occurring during blanching of TS, odorant screening, quantitation, and aroma recombination experiments on the basis of the sensomics concept were performed. Results revealed clear reductions of the concentrations of nearly all odorants after blanching, with an exemption of only four compounds. High OAVs were found for di-1-propenyl disulfide, dimethyl sulfide, 2-isopropyl-3-methoxypyrazine, *β*-ionone, (*E*,*Z*)-2,6-nonadienal, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, di-1-propenyl trisulfide, eugenol, and 2-methylbutanal in both blanched green and red TS buds. Spiking experiments to blanched TS were performed and

verified that the loss of the abovementioned sulfur-containing compounds, hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*,*Z*)-2,6-nonadienal, 2-methoxyphenol, and 4-ethylphenol contributed to the changes in the overall aroma profile during blanching.

To get an insight into the influences of the drying methods on the overall aromas of green and red TS, freeze-dried TS (FDTS), solar-dried TS (SDTS), and oven-dried TS (ODTS) (all lab-dried) were subjected to a screening for aroma-active compounds using cAEDA and cSH-ADA. Results showed eugenol and *β*-ionone with the highest FD factors for all six dried samples. In addition, high FD factors in green FDTS were found for (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide and *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene. In SDTS, also (*E*,*Z*)-2,6-nonadienal and vanillin were found with high FD factors. In general, FDTS revealed the highest FD factors for nearly all odorants, while ODTS showed the lowest FD factors. Data of dried red TS revealed the same pattern. To verify the screening results, again, quantitation experiments via SIDAs and ISM were carried out for odorants with high FD factors as well as for compounds previously characterized as key odorants in raw TS. In combination with the calculation of OAVs, the results confirmed the odorant screening data. Thereby, di-1-propenyl disulfide, dimethyl sulfide, *β*-ionone, eugenol, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, di-1-propenyl trisulfide, 2- and 3-methhylbutanal, (*E*,*Z*)-2,6-nonadienal, 2-isopropyl-3-methoxypyrazine, and 3-methyl-2,4-nonandione showed the highest OAVs in FDTS. In contrast, abovementioned sulfides showed much lower OAVs in SDTS, while 3-methyl-2,4-nonanedione, phenylacetic acid, and 1-octen-3-ol showed clearly higher OAVs. In ODTS, *β*-ionone revealed the highest OAV, followed by dimethyl sulfide, eugenol, 2-isopropyl-3-methoxypyrazine, di-1-propenyl disulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and 3-methylbutanal. In addition, concentrations of the key odorants differed in dried green and red TS buds. In conclusion, FDTS revealed the highest OAVs for most of the quantitated odorants and ODTS showed the lowest ones, proving the advantage of gentle freeze-drying at cryogenic temperature to obtain a product of high sensorial quality. Interestingly, the overall aroma of solar-dried TS did not differ too much from freeze-dried TS, and thus, solar-drying - as much easier and cheaper technique - might be the most convenient choice for drying.

To verify the overall aroma of commercially dried TS products, the characterization of key odorants and sensory tests were applied to two dried products obtained from the online market: vacuum-dried TS (CVDTS) and solar-dried TS (CSDTS). The odorant screening, quantitative data, and aroma recombination experiments indicated, e.g., 2-isopropyl-3-methoxypyrazine, eugenol, *β*-ionone, dimethyl sulfide, 2- and 3-methylbutanal, linalool, 3-methylnonane-2,4-dione, and hexanal as key aroma-active compounds. Interestingly, most of the abovementioned important sulfur-containing compounds including di-1-propenyl disulfide, di-1-propenyl trisulfide, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene were not found in these two commercial products. Subsequently, four further commercially dried products from different regions and markets were analyzed to focus on these missing aroma-potent sulfur-containing compounds. Thereby, di-1-propenyl disulfide and di-1-propenyl trisulfide were only found in two samples at clearly different concentrations, while 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene was only found in one sample at a very low concentration. The application of the different drying parameters on the one

side and the fact that all commercial products were blanched before drying on the other side, might have resulted in high losses of these sulfur-containing compounds.

2 Zusammenfassung

Toona sinensis (TS), ein beliebtes saisonales Gemüse mit einem intensiven und typischen Geruch, stammt ursprünglich aus China und ist dort weit verbreitet. Je nach Farbe der jungen Knospen unterscheidet main zwei Varietäten, rotes TS und grünes TS. Rote TS-Knospen haben im Vergleich zu grünen TS-Knospen einen intensiveren Geruch, was auf offensichtliche Unterschiede bei den Schlüsselaromastoffen zwischen rotem und grünem TS hinweist.

Um den molekularen Hintergrund der verschiedenen Aromen aufzuklären, wurde eine vergleichende Aromaextraktverdünnungsanalyse (vAEVA) mit den aus TS-Knospenpulver durch Lösungsmittelextraktion und anschließender Hochvakuumdestillation mittels der „solvent assisted flavor evaporation"-Technik (SAFE) isolierten flüchtigen Stoffen durchgeführt. Zusätzlich wurde eine vergleichende statische Headspace-Aromaverdünnungsanalyse (vSH-AVA) mit dem TS-Knospenpulver durchgeführt, um hochflüchtige Stoffe wahrzunehmen, die während der vAEVA mit dem Lösungsmittel coeluieren oder während der Aufarbeitung hohe Verluste erfahren können. Hierbei zeigten sich (*E*,*E*)-, (*E*,*Z*)- und (*Z*,*Z*)-Di-1-propenyldisulfid, (*E*,*E*)-, (*E*,*Z*)- und (*Z*,*Z*)-Di-1-propenyltrisulfid, *cis*- und *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen, *β*-Ionon, Eugenol und 2-Isopropyl-3-methoxypyrazin als potenteste Aromastoffe in beiden Proben, wobei die schwefelhaltigen Verbindungen deutlich höhere FD-Faktoren in grünem TS zeigten. Weiterhin wurden für die grün riechenden Verbindungen (*E*,*Z*)-2,6-Nonadienal, Hexanal, (*E*)-2-Hexenal und (*E*)-2-Hexen-1-ol höhere FD-Faktoren bei grünem TS festgestellt, während 2-Methoxyphenol und 4-Ethylphenol, beide mit einer phenolischen Note, deutlich höhere FD-Faktoren in rotem TS zeigten. Dabei wurden 1-Octen-3-on, Nonanal, 2,3,5-Trimethylpyrazin, (*E*,*Z*)-2,6-Nonadienal, (*E*,*E*)-2,4-Decadienal, 2-Methoxyphenol und 4-Ethylphenol das erste Mal als Aromastoffe in TS identifiziert. Quantifizierungsexperimente mittels Stabilisotopenverdünnungsassays (SIVAs) und interner Standard-Methode (ISM) sowie anschließende Berechnung von Aromawerten (AWs; Quotient aus der Konzentration eines Geruchsstoffs und seiner jeweiligen Geruchsschwelle) bestätigten die Ergebnisse der cAEVA und cSH-AVA. Die schwefelhaltigen Verbindungen (*E*,*E*)-, (*E*,*Z*)- und (*Z*,*Z*)-Di-1-propenyldisulfid, (*E*,*E*)-, (*E*,*Z*)- und (*Z*,*Z*)-Di-1-propenyltrisulfid, *cis*- und *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen und Dimethylsulfid waren in deutlich höheren Konzentrationen in grünem TS im Vergleich zu rotem TS vorhanden und verursachten die intensive gekochte zwiebelartige/TS-ähnliche Geruchsnote. Weiterhin wurden hohe Konzentrationen von den grün riechenden Verbindungen Hexanal, (*E*)-2-Hexenal, (*E*)-2-Hexen-1-ol und (*E*,*Z*)-2,6-Nonadienal in grünem TS gefunden, die zu der intensiven grünen Geruchsnote führten, während 2-Methoxyphenol und 4-Ethylphenol zur intensiven phenolischen Geruchsnote in rotem TS beitrugen. Die Aromaprofile der beiden ursprünglichen TS-Pulver stimmten mit denen des jeweiligen Rekombinats sehr gut überein und bestätigten somit die erfolgreiche Identifizierung und Quantifizierung aller Schlüsselaromastoffe in rohem grünen und roten TS.

Um die während des Blanchierens von TS auftretenden Aromaveränderungen aufzuklären, wurden ein Geruchsstoffscreening, Quantifizierungen sowie Aromarekombinationsexperimente basierend auf dem Sensomics Concept durchgeführt. Die Ergebnisse zeigten, dass die Konzentrationen von nahezu allen Geruchsstoffen, mit Ausnahme von nur vier Verbindungen,

nach dem Blanchieren deutlich reduziert waren. Hohe AWs sowohl in blanchierten grünen als auch roten TS-Knospen wurden für Di-1-propenyldisulfid, Dimethylsulfid, 2-Isopropyl-3-methoxypyrazin, *β*-Ionon, (*E*,*Z*)-2,6-Nonadienal, 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen, Di-1-propenyltrisulfid, Eugenol und 2-Methylbutanal gefunden. Dotierungsexperimente zu blanchiertem TS wurden durchgeführt und bestätigten, dass der Verlust der oben genannten schwefelhaltigen Verbindungen, Hexanal, (*E*)-2-Hexenal, (*E*)-2-Hexen-1-ol, (*E*,*Z*)-2,6-Nonadienal, 2-Methoxyphenol und 4-Ethylphenol zu den Veränderungen des gesamten Aromaprofils während des Blanchierens beitrug.

Um einen Einblick in die Einflüsse der Trocknungsmethoden auf das Gesamtaroma von TS zu erhalten, wurden gefriergetrocknetes TS (FDTS), sonnengetrocknetes TS (SDTS) und ofengetrocknetes TS (ODTS) (alle laborgetrocknet) einem Screening auf aromaaktive Verbindungen unter Verwendung von cAEVA und cSH-AVA unterzogen. Die Ergebnisse zeigten Eugenol und β-Ionon mit den höchsten FD-Faktoren in allen sechs getrockneten Proben. Darüber hinaus wurden hohe FD-Faktoren in grünem FDTS für (*E*,*E*)-, (*E*,*Z*)- und (*Z*,*Z*)-Di-1-propenyldisulfid und *cis*- und *trans*-2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen gefunden. In SDTS wurden (*E*,*Z*)-2,6-Nonadienal und Vanillin mit hohen FD-Faktoren analysiert. Generell wurden die höchsten FD-Faktoren für fast alle Geruchsstoffe in FDTS und die niedrigsten FD-Faktoren in ODTS gefunden. Screening-Daten von getrockneten roten TS-Knospen ergaben dasselbe Muster. Um die Screening-Ergebnisse zu bestätigen, wurden Quantifizierungsexperimente über SIVAs und ISM für Geruchsstoffe mit hohen FD-Faktoren sowie für Verbindungen, die zuvor bereits als Schlüsselaromastoffe in unverarbeiteten TS-Knospen charakterisiert wurden, durchgeführt. Die Quantifizierungexperimente, in Kombination mit der Berechnung der AWs, bestätigten die Geruchsstoff-Screeningdaten, mit Di-1-propenyldisulfid, Dimethylsulfid, *β*-Ionon, Eugenol, 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen, Di-1-propenyltrisulfid, 2- und 3-Methylbutanal, (*E*,*Z*)-2,6-Nonadienal, 2-Isopropyl-3-methoxypyrazin und 3-Methyl-2,4-nonandion als Verbindungen mit den höchsten AWs in FDTS. Im Gegensatz dazu zeigten die oben genannten Sulfide in SDTS deutlich niedrigere AWs im Vergleich zu FDTS, während 3-Methyl-2,4-nonandion, Phenylessigsäure und 1-Octen-3-ol deutlich höhere AWs aufwiesen. In ODTS zeigte *β*-Ionon den höchsten AW, gefolgt von Dimethylsulfid, Eugenol, 2-Isopropyl-3-methoxypyrazin, Di-1-propenyldisulfid, 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen und 3-Methylbutanal. Zudem waren die Konzentrationen der jeweiligen Schlüsselaromastoffe in getrockneten grünen und roten TS-Knospen unterschiedlich. Zusammenfassend zeigte FDTS die höchsten AWs für die meisten quantifizierten Geruchsstoffe und ODTS die niedrigsten AWs, was den Vorteil der schonenden Gefriertrocknung bei tiefen Temperaturen zum Erhalt eines Produkts mit hoher sensorischer Qualität zeigte. Interessanterweise unterschied sich das Gesamtaroma von SDTS jedoch nicht sehr stark von FDTS, und daher könnte die Sonnentrockung als die viel einfachere und billigere Technik die beste Wahl bzgl. einer Trocknung sein.

Um das Gesamtaroma kommerziell getrockneter TS-Produkte zu verifizieren, wurden die Schlüsselaromastoffe in zwei getrockneten Produkten aus dem Online-Markt charakterisiert: vakuumgetrocknetes TS (CVDTS) und sonnengetrocknetes TS (CSDTS). Das Aromastoff-Screening, die quantitativen Daten und die Aromarekombinationsexperimente zeigten 2-

Isopropyl-3-methoxypyrazin, Eugenol, *β*-Ionon, Dimethylsulfid, 2- und 3-Methylbutanal, Linalool, 3-Methylnonan-2,4-dion und Hexanal als aromaaktive Schlüsselverbindungen. Interessanterweise wurden einige der oben genannten wichtigen schwefelhaltigen Verbindungen, z.B. Di-1-propenyldisulfid, Di-1-propenyltrisulfid und 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen, in diesen beiden kommerziellen Produkten nicht gefunden. Deshalb wurden weitere vier kommerziell erhältliche getrocknete Proben aus verschiedenen Regionen und Märkten auf das Fehlen dieser aromapotenten schwefelhaltigen Verbindungen analysiert. Die Ergebnisse zeigten, dass Di-1-propenyldisulfid und Di-1-propenyltrisulfid nur in zwei Proben gefunden wurden und dabei deutlich unterschiedliche Konzentrationen aufwiesen, während 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophen nur in einer Probe in sehr geringen Mengen vorhanden war. Eine Erklärung dafür könnten einerseits unterschiedliche Trocknungsparameter sein oder andererseits die Tatsache, dass die im Handel erhältlichen Produkte vor dem Trocknen blanchiert wurden, wodurch hohe Verluste an diesen schwefelhaltigen Verbindungen entstanden sein könnten.

3 Abbreviations and common names

Abbreviations:

AEDA	aroma extract dilution analysis
cAEDA	comparative aroma extract dilution analysis
AF	acidic fraction
3-AFC	3-alternative forced choice
APA	aroma profile analysis
CI	chemical ionization
CSDTS	commercially solar-dried *Toona sinensis*
CVDTS	commercially vacuum-dried *Toona sinensis*
EI	electron ionization
FD	flavor dilution
FDGTS	freeze-dried green *Toona sinensis*
FDRTS	freeze-dried red *Toona sinensis*
FDTS	freeze-dried *Toona sinensis*
FFAP	free fatty acid phase
FID	flame ionization detector
G_{olf}	olfactory G-protein
GDP	guanosine bisphosphate
GPCR	G-protein coupled receptor
GTP	guanosine triphosphate
HRGC	high-resolution gas chromatography
HRGC-FID	high-resolution gas chromatography–flame ionization detector
HRGC/HRGC-MS	two-dimensional heart-cut high-resolution gas chromatography-mass spectrometry
HRGC-MS	high-resolution gas chromatography–mass spectrometry
HRGC-O	high-resolution gas chromatography–olfactometry
HRGC×HRGC-TOF-MS	comprehensive two-dimensional high-resolution gas chromatography-time-of-flight-mass spectrometry
HS-SPME	headspace solid phase microextraction
ISM	internal standard method
MCSS	moving column stream switching
NBF	neutral-basic fraction
NIST	National Institute of Standards and Technology
OAV	odor activity value
ODGTS	oven-dried green *Toona sinensis*
ODRTS	oven-dried red *Toona sinensis*
ODTS	oven-dried *Toona sinensis*
OT	odor threshold
R_f	response factor

Abbreviations:

RGTS	raw green TS
RI	retention index
RRTS	raw red TS
SAFE	solvent assisted flavor evaporation
SDGTS	solar-dried green *Toona sinensis*
SDRTS	solar-dried red *Toona sinensis*
SDTS	solar-dried *Toona sinensis*
SGF	silica gel fractions of neutral and basic volatiles
cSH-ADA	comparative static headspace aroma dilution analysis
SH-HRGC-O/MS	static headspace high-resolution gas chromatography-olfactometry/mass spectrometry
SIDA	stable isotope dilution assay
SPME	solid phase microextraction
TS	*Toona sinensis* (A. Juss.) Roem

Common names:

aromadendrene	1,1,7-trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene
β-caryophyllene	(1*R*,4*E*,9*S*)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene
caryophyllene oxide	(1*R*,4*R*,6*R*,10*S*)-4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.$0^{4,6}$]dodecane
1,8-cineole	1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane
eugenol	2-methoxy-4-(prop-2-en-1-yl)phenol
farnesene	3,7,11-trimethyl-1,3,6,10-dodecatetraene
α-humulene	2,6,6,9-tetramethyl-1,4-8-cycloundecatriene
β-ionone	4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one
isocaryophyllene	(1*R*,4*Z*,9*S*)-4,11,11-trimethyl-8-methylenbicyclo[7.2.0]undec-4-en
limonene	1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene
linalool	3,7-dimethylocta-1,6-dien-3-ol
menthol	(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexanol
α-pinene	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
valencene	(3*R*,4a*S*,5*R*)-4a,5-dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene
vanillin	4-hydroxy-3-methoxybenzaldehyde

4 Introduction

4.1 Molecular sensory science concept

4.1.1 Odor-active compounds and aroma perception

Aroma is an important quality criterion of a foodstuff. The perception of aroma is the result of a multitude of interactions between a wide variety of odor-active compounds and sensory receptors of the human olfactory system. Based on the fact that each food product elicits its characteristic aroma, the aroma has an impact not only on food selection, identification of food, differentiation from other food products, but also on detection of food spoilage. Thus, the odor-active compounds contribute to food quality and are very important for food products.

Odor-active compounds are volatiles which are capable of interacting with odor receptors on the surface of the olfactory epithelium, and thus, induce an aroma impression (Figure 1).[1,2] However, not every volatile substance can evoke a typical odor note. The prerequisites for interacting with an odor receptor are a certain water solubility, a relatively low polarity to be soluble in fat, as well as sufficient vapor pressure, and a corresponding surface activity.[3] A few odorants, on the one hand, can be recognized by more than one type of distinct olfactory receptor and cause different activation levels. On the other hand, one receptor can specifically be activated by multiple odorants.[4-6]

The human olfactory system consists of olfactory epithelium and olfactory bulb. They are spatially separated by the ethmoid plate (bone). The olfactory epithelium is constituted by a large number of olfactory sensory cells and the cilia (sensory hairs) with specific odorant receptors located at the ends of the bipolar olfactory sensory cells. The odorants reach the cilia of olfactory epithelium either through the nasal cavity during inhalation (orthonasally) or through the mouth during the chewing and swallowing process (retronasally). The conformation of the odor receptor changes due to the binding of an odorant, which causes an intracellular reaction cascade, resulting in the depolarization of the cell membrane. The depolarization activates olfactory receptor cells and sends electric signals as a neural impuls which is transfers to the axon of the olfactory neuron. Odorant receptors are known as seven-transmembrane G protein-coupled receptors. However, only one type of receptor is expressed per cell. Thus, axons of receptor cells with the same receptor type, bundled as fila olfactoria, pass through the ethmoid bone, and finally proceed to a glomerulus in the olfactory bulb. A set of axons activates the glomerulus, resulting in specific signals which are transmitted via activated mitral cells to higher sensory regions of the brain, where a characteristic aroma can be assigned to the activation pattern of the receptors (Figure 1).[5-10]

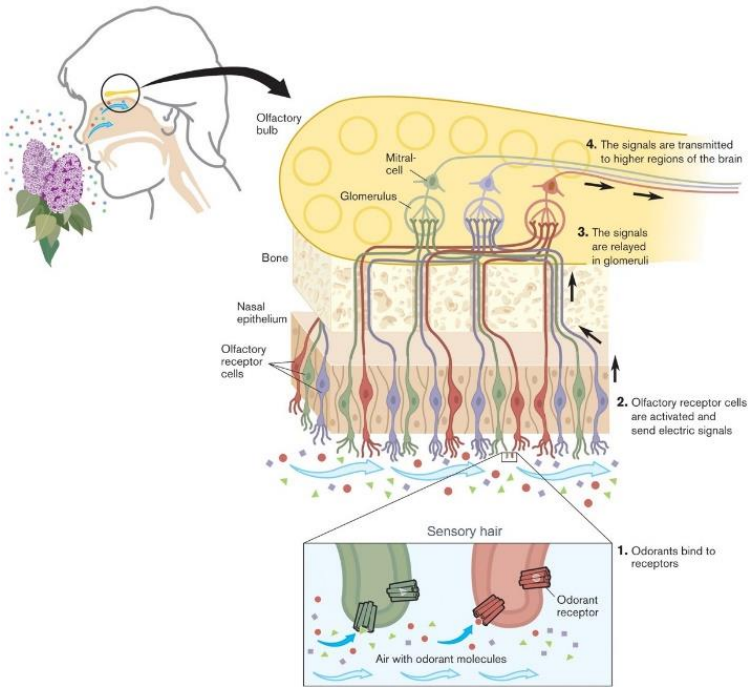

Figure 1: Odorant receptors and the organization of the human olfactory system.[2]

Olfactory receptors, located in the end of the cilia, are the so-called G-protein coupled receptors (GPCRs) which belong to the superfamily of seven transmembrane domain proteins. The N-terminal of the receptor is located extracellularly, and its C-terminal is inside of the cell. A characteristic spatial interior is formed due to the transmembrane domains, leading to the binding of the odorants (Figure 2).[10]

Depending on the structure and functional groups of the respective receptors as well as the affinity of the aroma compound, the odorant binds and interacts with one or more receptors, which results in a change in conformation of the receptor molecule and triggers a G-protein-mediated cascade. The olfactory G-protein (G_{olf}) has three subunits *α*, *β*, and *γ*. In inactive form, GDP (guanosine bisphosphate) is bound to the *α*-subunit and coupled to the *β*- and *γ*-subunits. The binding of an odorant leads to the activation of the G protein, which in turn results in the binding of the *α*-subunit bounds to GTP (guanosine triphosphate) and the detachment of *β*- and *γ*-subunits. The release of the GTP-coupled *α*-subunit stimulates adenylyl cyclase (phospholipase) to produce elevated levels of cyclic adenosine monophosphate (cAMP) via catalyzing the conversion of adenosine triphosphate (ATP). cAMP opens cyclic nucleotide-gated cation channels in the plasma membrane, causing an influx of cations including sodium and calcium ions. The inflowing cations open chloride channels, leading to the outflow of chloride ions, and consequently, create action potential on the axon hill of the olfactory receptor cell (Figure 2).[3,10,11]

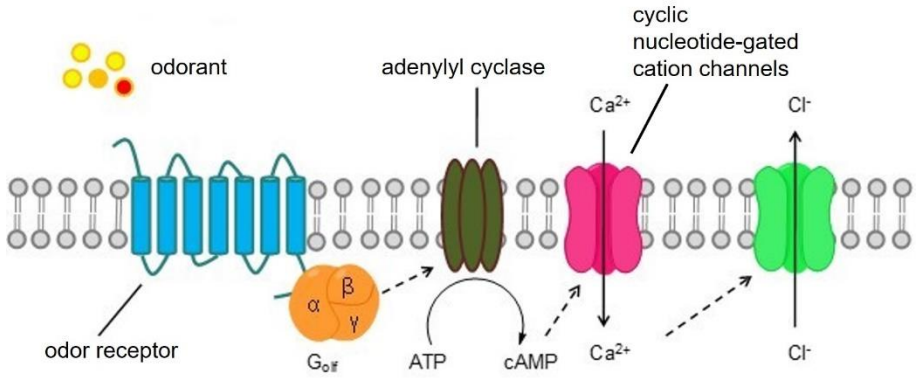

Figure 2: The pathway of signal transduction in G-protein coupled receptors.[12]

Food-specific chemosensory perceptions are critically influenced by food aroma-characteristic combinations and concentration ratios of odor-active compounds. The specific release characteristics of a compound depends on the chemical structure and properties as well as on the food matrix. In addition, the odor threshold can be determined to describe the sensory perception of a compound in a certain matrix. It is well-known that the odor thresholds of the odorant present in different food matrices are obviously different. For example, green and grassy smelling hexanal has an odor threshold of 2.4 µg/kg in water[13], while its odor threshold is 276 µg/kg in oil[14]. In addition, the odor thresholds of different odorants in the same matrix are also extremely different, e.g., the odor threshold of 2-isopropyl-3-methoxypyrazine with an earthy and pea-like odor note is 0.0039 µg/kg in water[15], whereas the threshold of ethanol is 990000 µg/kg[16]. Combining the concentration and the odor threshold in food, only < 3% of about 10000 volatiles appear in concentrations above their odor thresholds and can, thus, be considered as key food odorants.[17,18] Thus, the aim of aroma research is to characterize the limited numbers of key odorants out of thousands of volatiles in foods.[19]

4.1.2 Molecular sensory science concept / sensomics concept

The molecular sensory science concept is well accepted in aroma research as the state-of-the-art methodology to systematically characterize the key odorants out of the bulk of odorless volatiles.[17,20] It involves the following steps (Figure 3).

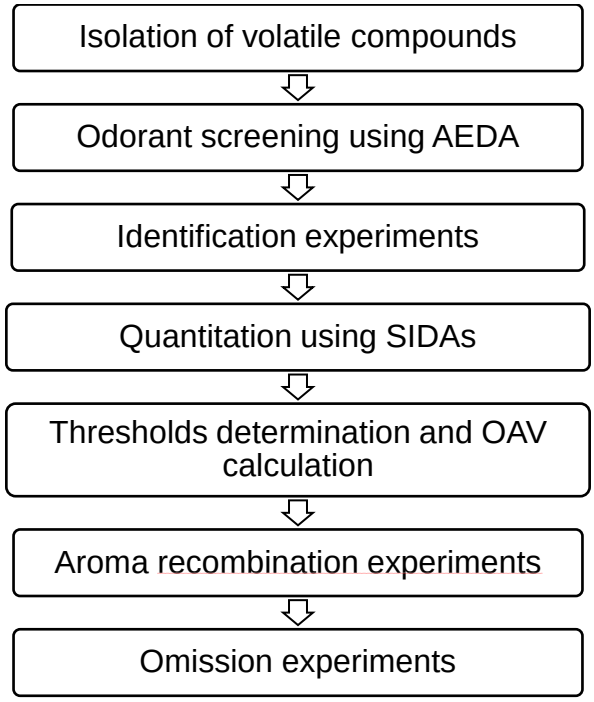

Figure 3: The molecular sensory science concept according to Schieberle, 1995[20] and Grosch, 2001[17] (modified).

4.1.2.1 Isolation of volatile compounds

The isolation of volatile compounds via cold solvent extraction with subsequent high vacuum distillation is the first step of the sensomics concept. After extracting the food sample with a low-boiling organic solvent, such as diethyl ether or dichloromethane, the volatiles are separated from nonvolatile components by means of the so-called solvent assisted flavor evaporation (SAFE) technique (Figure 4).[21] Compared to other isolation technologies, e.g., simultaneous steam distillation and extraction (SDE),[22] SAFE-distillation has significant advantages. During SAFE distillation, a low distillation temperature (40 °C) is applied to minimize any compound degradation of compounds as well as any artifact formation. High vacuum (10^{-4} Pa) allows the isolation of the volatiles from various and complex samples, extracts, aqueous foods, food suspensions, as well as oil samples.

To avoid a possible overlook of compounds during high-resolution gas chromatography-olfactometry (HRGC-O) analysis, the SAFE distillate obtained should be separated into the acidic fraction (AF) and the neutral-basic fraction (NBF) by liquid-liquid extraction. The NBF should further be fractionated into several silica gel fractions (SGF) by column chromatography based on the polarity of the compounds.[23]

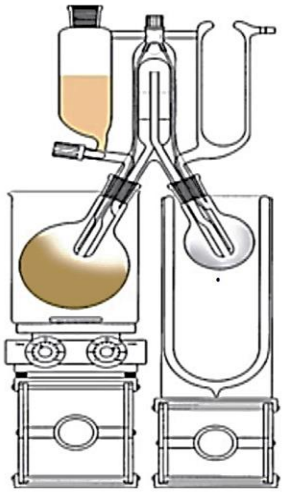

Figure 4: The SAFE apparatus according to Engel et al., 1999[21]

The SAFE-distillate, AF, NBF as well as SGF are concentrated on a Vigreux column at approx. 40 °C. The concentrates were applied to HRGC-O to distinguish aroma-active components from a large number of odorless volatiles (Figure 5). After the gas chromatographic separation on the capillary column, the carrier gas stream is split into two equal parts via a Y-splitter. One part is directed to a flame ionization detector (FID), the second part is transferred to a heated sniffing port where the odor quality and intensity can be simultaneously perceived and marked on the chromatogram (Figure 5).[24]

Figure 5: HRGC-O, basic principle (left) and application (right) (illustration: Martin Steinhaus)

4.1.2.2 Aroma extraction dilution analysis (AEDA) and identification experiments

An aroma extraction dilution analysis (AEDA) is carried out as a screening method to get a first idea about the importance of the aroma of each odorant for the overall food aroma.[25] Therefore,

the concentrated initial extract was diluted stepwise with solvent (1 + 1, v + v). Consequently, the initial extract and the dilutions are subjected to HRGC-O until no aroma-active compound was perceivable at the sniffing port anymore. Each odor quality is assigned an FD factor, representing the highest dilution in which the odorant was detected for the last time at the sniffing port. The undiluted extract is defined as FD 1 (Figure 6). To determine the FD factors of highly volatile compounds and compounds coeluting with the solvent during AEDA, static headspace high-resolution gas chromatography-olfactometry (SH-HRGC-O) was applied to the original sample of different headspace volumes.

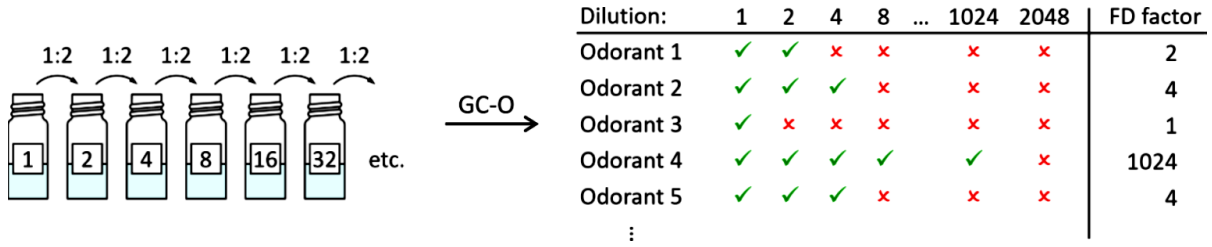

Figure 6: Aroma extract dilution analysis (illustration: Martin Steinhaus)

The identification of the aroma-active compounds is accomplished by comparison of their odor qualities and intensities perceived at the sniffing-port, retention indices determined on two capillary columns of different polarities (DB-FFAP and DB-5), and mass spectra obtained in chemical ionization (CI) mode as well as in electron ionization (EI) mode with data of the respective reference compounds analyzed under the same conditions.

4.1.2.3 Quantitation using stable isotope dilution analysis (SIDA)

AEDA is a preliminary screening method to get a first information which aroma-active compounds within a large number of odorless volatiles should be important for the overall aroma. However, the odor intensity perceived at the sniffing-port depends on the amount of sample extracted, the chemical properties of the odorant such as solubility, extractability, and stability, as well as different interactions of the odorant with the specific food matrix. Thus, differences in aroma release are not considered. Therefore, in a next step, the concentrations of the odor-active compounds are exactly quantitated via stable isotope dilution analysis (SIDA).[26,27] For this purpose, [^{2}H]- or [^{13}C]-stable isotopically labeled internal standards of the target analytes were added to the food sample and homogenized with the food material prior to the work-up procedure (Figure 7).[28] Due to the nearly same physical and chemical properties of the isotopically labeled standard and its corresponding analyte, the final ratio of the concentrations of the two compounds maintain the same as the initial ratio was even if during work-up losses might occur, proving SIDA as the most accurate quantitative methodology. The prepared sample is subjected to high-resolution gas chromatography-mass spectrometry (HRGC-MS) analysis to determine the peak area ratio of the analyte and the isotopically labeled standard. Furthermore, mixtures of the labeled standards and the respective unlabeled analytes in different mass ratios were analyzed

for the calculation of response curves. Finally, the concentration of the analyte in food is calculated from the peak area ratio of the labeled standard and unlabeled analyte, the amount of the labeled standard added, the obtained response curve, and the amount of the sample extracted.

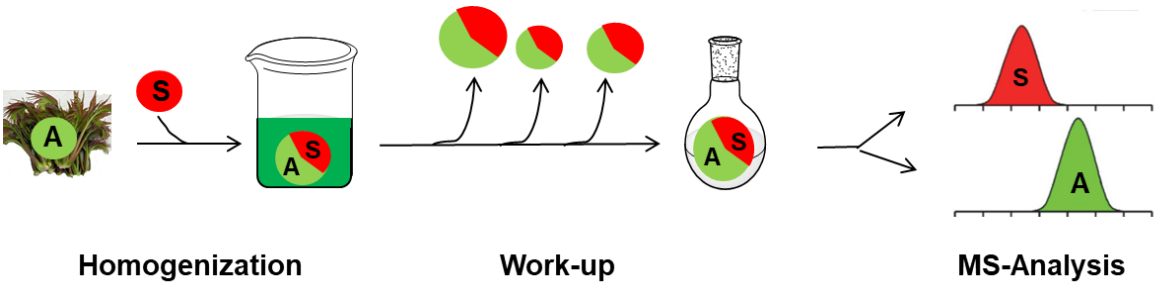

Figure 7: Principle of stable isotope dilution analysis according to Rychlik and Asam, 2008[26]

4.1.2.4 Odor activity value (OAV) calculation

To evaluate the contribution of each compound to the overall aroma of the food, the odor activity value (OAV: ratio of the concentration of an odorant in the food divided by the respective odor threshold) is calculated.[29,30] The odor threshold is determined in a matrix, which should be as much similar as possible to the matrix of the analyzed food material.[31] According to the definition, aroma substances with an OAV < 1 do not contribute to the overall aroma, whereas only odor-active compounds exhibiting an OAV ≥ 1 can be considered as key food odorants in a defined food, contributing to the overall aroma.[30]

4.1.2.5 Aroma recombination and omission experiments

To validate the qualitative and quantitative data obtained, aroma recombination experiments are performed by adding all aroma-active compounds showing OAVs ≥ 1 in their naturally occurring concentrations to an odorless matrix mimicking the situation in the original food.[20] The aroma of the recombinate is then compared to that of the original food in an aroma profile according to the intensities of single odor attributes. A good similarity in the profiles of the recombinate and food indicates the successful characterization of the key aroma-active compounds in the defined food.[32]

To elucidate the contribution of a single aroma-active compound or a group of odorants to the overall aroma, omission tests are carried out as the final step of the molecular sensory science concept. Thus, the single compound or the group of compounds is omitted from the recombinate. The overall aroma of the “incomplete” model is then compared to that of the “complete” recombinate model in a 3-alternative forced choice (3-AFC) test group of compounds. A significant difference of the 3-AFC test result indicates the relevance of the tested compound for the overall aroma of the food as vice versa.[33]

4.2 *Toona sinensis* (A. Juss.) Roem. (TS)

4.2.1 The TS plant

Toona sinensis (A. Juss.) Roem. (TS), also commonly called *Cedrela sinensis* A Juss., Chinese mahogany, Chinese toon, and xiāngchūn (in Chinese), is a perennial, deciduous tropical and subtropical tree native to eastern and southeastern Asia. Due to its frost tolerance, it is widely cultivated from northern Korea, China, Nepal to northeastern India, Myanmar, Thailand, Malaysia, and western Indonesia. This plant belongs to Meliaceae family and is the only *Toona* species in which the margins of the leaflet can be serrate to serrulate. It can grow up to 25 m with a trunk up to 70 cm diameter. All the floral parts are completely glabrous with the sepal margins, the male stamens alternate with sterile staminodes, and the seeds have only one wing at the end (Figure 8).[34-37] TS has a cultivation and consumption history > 2000 years. Nowadays, > 1 billion square meters of planting acreage results in > 800 billion kg of fresh TS buds per year in China.[38]

Figure 8: The whole tree of TS (A)[36], the leave (B)[36], the truck (C)[37], the root (D)[38], and the seed of TS (E)[39]

4.2.2 TS economic values

TS provides various economic values, e.g., very high-quality timber for furniture, boat, and bridge construction in India, based on the fact that the wood is very durable and easy to work. In China, it is commonly called Chinese mahogany for making furniture, window frames, door joists, and grade carpentry. The TS tree has also been used as an avenue tree in botanic gardens and parks in many cities.[40] In addition, the traditional medicinal use of TS was firstly recorded in Tang dynasty in China and has been widely used as a traditional Chinese medicine for thousands of years.[41] Various parts of tissues of this woody plant have been used for many diseases. For example, the stems and leaves of TS are traditionally used for the treatment of dysentery, enteritis, and skin itchiness, etc. The powdered root is a kind of corrective, the bark is used as astringent and depurative, and the fruits are used to treat eye infections.[42,43] The modern researches reported the extract of TS leaves possesses various pharmacological effects including anti-tumor, hypoglycemic, antioxidant, anti-inflammatory, hepatoprotective, antibacterial, antiviral,

anticoagulation, and anti-gout effects, as well as male reproductive system protection and ischemia-reperfusion injury protection.[38,44-63]

Besides its traditional medical usages, the leaves of TS are also used as animal fodder in India. The young buds of TS harvested in early spring enjoy a great popularity as a “tree vegetable” in Chinese and Malaysian diet due to their abundant nutrients (carotene, vitamins B and C, etc.), crispy and juicy taste, as well as an intense and unique flavor. For example, the young buds are popularly used as salad, stir-fried with other foodstuffs, and boiled with noodle soup. While the old leaves are not edible because of the fibrous texture and toxicologically relevant ingredients such as nitrites.[42,64]

4.2.3 TS varieties and the harvest of TS buds

According to the color of the young buds, TS is divided into a green and red variety, each possessing different characteristics. Generally, red TS has a wide canopy, gray-brown bark, and purple-brown spores, while green TS has an upright tree crown and green or dark green bark. Compared to green young buds, the red variety, which is matured earlier, has more fuchsia leaves, less fiber, and more grease. Red young leaves are considered to have a better flavor compared to young green leaves. Thus, more red TS buds and respective products are consumed. [65,66]

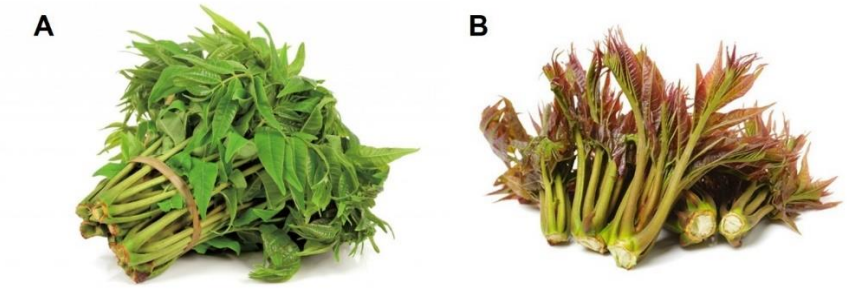

Figure 9: The young buds of green (A) and red (B) TS varieties

In China, the whole harvest period of TS buds is from end of March until middle of May. Every spring, the first buds of TS trees are generated from the branched stem tissue and then grow up to 10-15 cm in length prior to harvest. The growing speed of the buds depends on the conditions of soil, climatic, and light / sun exposure. The buds are removed from the bottom of the buds in the morning, leaving the bases of the buds in the tree for the new coming buds. About 20 days after harvest, the new shoots emerged from the bases of the first buds are about 10 cm in length and ready to harvest for the second time. Two or three leaves of one bud on each branch should be kept for supplying nutrients to the tree. The time frame for harvesting TS buds of an individual variety is between 10 and 14 days. After picking 2-4 times, the whole harvest of this branch is completed. To optimize the harvest time of the buds, nitrite contents, fiber content, length, color, taste, and aroma of TS buds are evaluated (Figure 10). In general, the first buds offer the most

crispy and juicy taste, and best aroma as well as lowest contents of nitrites which are harmful for human health.[67-71]

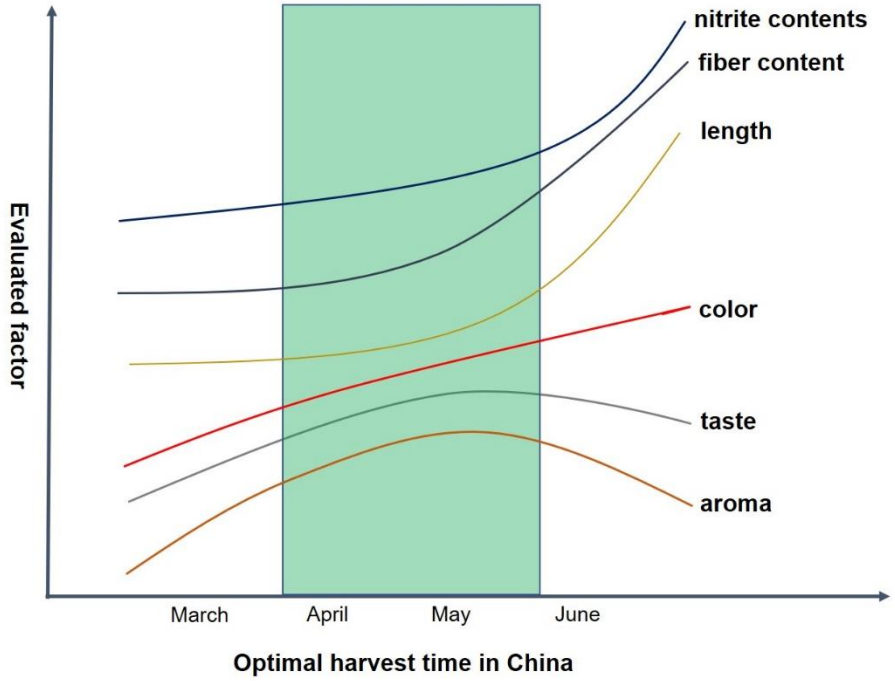

Figure 10: The schematic diagram of the optimal harvest period of TS buds according to Wang 2004[67]

4.2.4 Composition of TS buds

Fresh TS buds contain approximately 85% of water. The total content of cellulose, sugar, protein, lipids, vitamins, and minerals is approximately 13%, proving the high nutrition content of TS buds. From the early 1970s on, scientists have paid attention on the phytochemical constituents of TS buds. Up to now, > hundred compounds have been isolated and identified from this plant, including 3% of flavonoids, 2% of polyphenols, 1% of terpenoids, and 1% of essential oil (Table 1).[36, 72-82]

Table 1. Composition of Fresh TS Buds[70]

ingredient	%
water	80
cellulose, sugar, protein, amino acids, lipids, vitamins, minerals	13
terpenoids	2
flavonoids	3
polyphenols	1
essential oil	1

It is reported that TS buds contain various important amino acids, such as glutamic acid and aspartic acid, abundant minerals, including potassium, iron, zinc, calcium, magnesium, and phosphorus, as well as vitamins B1, B2, and C. Among them, the contents of protein and phosphorus are at the top of common vegetables.[72-76]

The buds of this plant contain abundant terpenoids including triterpenoids, diterpenes, and sesquiterpenes, with triterpenoids as the main components among them. The predominant flavonoids in TS buds are identified as procyanidin B3, procyanidin B4, quercetin, rutin, quercitrin, kaempferol, astragalin, isoquercitrin, and quercetin-3-*O*-(2”-*O*-galloyl)-*β*-D-glucopyranoside. In addition, gallic acid and protocatechuic acid have been found as main polyphenols in the leaves of TS, related to many pharmacological activities.[73,76-82]

Essential oil contains many compounds, which are for the characteristic aroma of TS buds. Until now, > 200 volatile components were identified for the tender buds of TS. The ingredients of the volatile oil depend on the TS variety, the cultivating area and condition, as well as the harvest time.[36]

4.2.5 TS buds processing

4.2.5.1 Blanching

Fresh TS buds are the main product in vegetable markets due to their high water content, and thus, the intolerance to long storage. However, raw buds are rarely used directly because of the high content of toxicologically relevant ingredients such as nitrites. Thus, blanching of raw buds in boiling water for about 1 min is an essential step before further processing. After blanching, the buds of either green or red TS are green colored because of the loss of anthocyanins at the elevated temperature, and the aroma is not that intense anymore.[83-85]

4.2.5.2 Salting

Salting is a traditional food processing technology in China to make it a product available all year. Fresh TS buds are a kind of seasonal vegetable since its extremely short harvest period. Thus, tender buds of TS are often salted. After blanching, the buds are cooled to room temperature, and further salted in a sealed container with some water for about two weeks. With the extension of salting time, the color of buds turns to dark green. [69,75]

4.2.5.3 Drying

Except salting, drying is another popular processing technology of TS buds to decrease the water content of food material for transportation and long-term storage. During this process, the original aroma and nutritional components should be maintained. There are various drying methods for the buds of this plant including convection oven drying, natural solar drying, vacuum drying, spray

drying, and microwave drying. The most popular and traditional drying process for tender TS buds in China is oven drying and natural solar drying. Besides, freeze-drying, a separation process based on the sublimation phenomenon at low temperature, is gaining more and more attention by producers. Previous studies compared the effects of abovementioned drying methods on the quality and volatiles of TS buds and demonstrated that the contents of vitamin C and chlorophyll of the buds maintained very well during oven drying treatment, while microwave-oven drying offered the highest rehydration ratio in boiling water.[86] Solar drying retained more volatiles but with lower relative contents, whereas vacuum drying ended up with higher contents of the original volatiles.[87] A comparison of the total quality of spray-dried TS sprout powder, freeze drying offered an overall high-quality TS powder. The contents of protein, saponins, and alkaloids in the powder prepared by freeze drying were significantly higher than those obtained by spray drying; however, the contents of vitamin C, flavonoids, and polysaccharides had no significant difference in both freeze-dried and spray-dried TS buds.[86-89] In the market, a popular processing for dried TS buds is a combination of salting with drying for a special product quality and flavor.[69,75]

4.2.5.4 Other processing

In some regions of China, TS buds are also processed into powder and sealed stored with oil for two months to obtain a TS sauce. For example, pieces of TS buds (45 g) are mixed with garlic (1 kg), ginger (25 g), as well as salt (3 g). The prepared mixture can be stored for long time as a delicious sauce for e.g., salads.[69,75]

4.2.6 Volatiles of TS buds

Due to its characteristic and intense aroma, a large number of studies have been carried out on the composition of TS buds essential oil since 1990s. So far, > two hundred volatile compounds in TS buds and tender leaves have been identified by the application of GC-MS. Among them, hydrocarbons, sulfur compounds, and alcohols are the main groups (Table 2).[90-111]

Table 2. Summary of Classes of Volatile Compounds Reported in TS Buds[90-111]

substance class	compounds	substance class	compounds
hydrocarbons	98	acids	7
sulfur-containing compounds	57	phenols	5
alcohols	40	further nitrogen compounds	2
aldehydes	17	halogenated compounds	2
ketones	15	thiazoles	2
esters	13	furans	1
epoxides, pyrans, coumarins	11	pyrazines	1

Previous studies presented that the essential oil of fresh TS buds contained approximately 60% hydrocarbons which were allocated to 40% triterpenoids, 7% aromatic hydrocarbons, 5%

sesquiterpenes, 5% diterpenes, 2% monoterpenes, and 1% aliphatic hydrocarbons. About 29% of the volatile compounds belong to the substance class of oxygenated derivates, such as aldehydes, esters, and alcohols, while the remaining 10% are sulfur-containing compounds (Table 3).[81]

Table 3. Main Volatile Classes and Subclasses Identified as well as Respective Relative Contents in TS Buds[81]

substance class	subclass	relative content (%)
hydrocarbons	triterpenoids	40
	aromatic hydrocarbons	7
	sesquiterpenes	5
	diterpenes	5
	monoterpenes	2
	aliphatic hydrocarbons	1
oxygenated derivates	aldehydes	20
	esters	5
	alcohols	4
	acids	< 1
	ketones	< 1
	terpene oxides	< 1
sulfur-containing compounds	sulfides	5
	thiols	5
	further sulfur-containing compounds	< 1

Region-specific differences of fresh TS buds were investigated by several researchers, proving that terpenes, aldehydes, and sulfur-containing compounds were present at significant different amounts in different regions.[73,81,98]

Yang et al. focused on the influence of the blanching process on the volatile compounds in fresh TS buds and demonstrated that relative contents of sulfur-containing compounds were extremely reduced while the relative amounts of alkene components clearly increased.[83]

Wang et al. identified 49 volatile compounds in vacuum-dried TS buds powder and 41 compounds in spray-dried powder. For most of the investigated volatiles, such as (*E*)-2-hexenal, nonanal, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, the relative contents in vacuum-dried TS were much higher than those in spray-dried powder.[88] Chen et al. compared the volatile constituents of TS buds treated with different drying methods using SPME-GC-MS. As major volatile components, caryophyllene, aromadendrene, nonadecane, and phytol were found. Compared with convection oven drying and natural solar drying, vacuum drying and microwave drying obtained higher relative contents of the investigated volatiles.[88]

4.2.7 Aroma-active compounds in TS buds

Although numerous studies focused on the volatiles of TS sprout oil, the identification of the key odorants out of the bulk of odorless volatiles have rarely attracted the attention of scientists. Up to now, only three researches reported on potent aroma-active compounds in TS buds extract via HS-SPME, GC-O, and GC-MS.[109-111]

The first investigation on the identification of five aroma-active compounds in TS buds cultivated in Beijing, China, was accomplished by Li et al. in 2011. By application of GC-O and GC-O-MS, analysis of condensed volatiles of TS buds resulted in the identification of propylene sulfide (garlic, pungent odor note), hexanal (pungent, rubber), (*E*)-2-hexenal (milk), 1,5,5-trimethyl-1,3,6-heptatriene (floral), and *β*-selinene (hay-like). However, some of the odor notes are questionable.[109]

Two years later, Liu et al. identified 26 aroma-active components in TS buds, cultivated in Shanxi, China, using HS-SPME followed by GC-O and GC-MS. These odorants were classified into four groups with specific odor note including 14 sulfur smelling compounds, 5 flowery smelling compounds, 4 green smelling compounds, 2 fruity smelling compounds, and 1 mint-like smelling compound. Most of these odorants were firstly identified in TS buds, for example, (*Z*)- and (*E*)-1-(methylsulfanyl)-1-propene, (*E*,*E*)- and (*E*,*Z*)-di-1-propenyl sulfide, as well as *cis*- and trans-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene. Further, the relative contents were determined via GC-MS and the odor intensity of each odorant was evaluated by the panelist. Among them, cooked onion-like/TS-like smelling compounds *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene showed the highest odor intensities and were seen as two major contributors to the characteristic aroma of TS.[110]

Very recently, using HS-SPME and SAFE distillation combined with GC-O and GC-MS, Yang et al. characterized potent odorants in TS buds, harvested in Beijing, China, leading to the identification of 83 odorants in either raw or cooked TS buds. Application of static headspace dilution analysis and AEDA to the volatiles obtained from raw TS buds revealed high FD factors for (*E,E*)-di-1-propenyl disulfide, (*Z*)-isoeugenol, phenylacetic acid, and vanillin. Further aroma-active compounds including hydrogen sulfide, (*E*,*Z*)-di-1-propenyl disulfide, *β*-caryophyllene, 4,5-trimethylthiazole, 2,4-dimethylthiophene, as well as borneol were also present at high FD factors in cooked TS buds. In agreement with results of aroma profile analysis, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol were suggested as important contributors to the green, grassy aroma of TS, while hydrogen sulfide, methyl thiirane, (*E,E*)- and (*E*,*Z*)-di-1-propenyl disulfide contributed to the sulfurous note in TS buds. However, the work was only based on identification experiments and no quantitative data was available.[111]

Although abovementioned studies attempted to characterize the aroma-active compounds in TS buds, all approaches did not completely fulfill the state-of-the-art methodology according to the molecular sensory science approach described in **4.1.2**.[17,20] First, except (*E,E*)-, (*E*,*Z*)-, and (*Z,Z*)-di-1-propenyl disulfide, most of the investigated odorants were only tentatively identified by comparison of their mass spectra to those published in a mass spectral library database instead

of comparing their RIs, odor qualities and intensities as well as mass spectra to data obtained from respective reference substances. Secondly, the prerequisite of a key odorant in a given food is its amount, which must be above its respective odor threshold, meaning precise quantitative analysis and OAV calculation are necessary for the characterization of key aroma-active compounds. Third, aroma recombination experiments must be performed to verify the obtained data and to confirm that all key odorants were successfully identified and quantitated.

To the best of our knowledge, until now, no accurate data on the key odorants of TS buds and no systematic molecular sensory science analysis of the odorants predominately contributing to the overall aroma of TS buds are available. Thus, the role of potent odorants for the overall aroma of TS buds is still unclear.

5 Objectives

TS is a popular vegetable in China, because its sensory properties combine a pleasant crispy consistency, a pronounced umami taste, and a strong, typical odor, not comparable to those of any other kind of vegetable. In northern and southwestern China, TS is deeply appreciated and often cooked with various foodstuffs, whereas some people in southeastern China regard the TS odor as offensive and nauseous. Although the overall aroma of raw TS is very intense, TS has to be blanched in boiling water to remove toxicological relevant nitrites before cooking. Since fresh TS buds are only available in early spring, TS producers often dry them to enable longer storage time and an easier transportation. However, both blanched TS and dried TS products elicit an aroma, which is different to that of fresh TS. In addition, blanching and further salting are two common and essential steps prior to drying of fresh TS buds as commercial products, leading to a clear change of the key odorants and overall aroma. Food chemists have already paid attention to the unique odor properties of TS for decades. However, most studies focused on the volatiles of TS and it is still unclear which odorants predominately are responsible for its characteristic aroma as well as for the changes of the overall aroma during blanching and drying processes.

Thus, the first aim of the current research was to characterize the key aroma-active compounds in two TS varieties, green TS and red TS, and to elucidate the molecular background of aroma changes occurring during blanching. Sensory tests were performed to elucidate the quantitative data. Further research was focused on the elucidation of the impact of different drying processes on the odorants in TS buds and the characterization of the key odorants in commercially dried TS products. The influences of different raw TS materials and drying processes were assessed based on the quantitative results and sensory experiments.

6 Results and discussion

This thesis is a publication-based dissertation. Results were summarized in three articles published in the well-known international scientific peer-reviewed Journal of Agricultural and Food Chemistry. For each publication, a copy of the original, a summary including the individual contributions of the authors, and the reprint permission of the publisher were attached in the appendix.

6.1 Characterization of odor-active compounds in raw and blanched TS

6.1.1 Screening and identification experiments

Odorant screening via cAEDA and cSH-ADA were applied to samples of raw green TS (RGTS), raw red TS (RRTS), as well as respective blanched TS (BGTS and BRTS). Fresh buds, harvested in Anhui, China, in April 2018, were frozen by liquid nitrogen, and then ground into powder. To obtain the respective blanched samples, fresh buds were heat-treated in boiling water for 1 min, cooled to room temperature, finally frozen, and also ground. The powdered samples were filled into brown glass bottles and stored at -24 °C prior to analysis. The organic extracts obtained from TS samples were distilled via high vacuum distillation using the solvent assisted flavor evaporation (SAFE)[21] technique to isolate the volatile compounds from the nonvolatiles. SAFE distillates were used for cAEDA. In addition, the raw samples were filled into sealed headspace vials and different headspace volumes were subjected to cSH-ADA. For an unequivocal identification, the SAFE distillate obtained was first separated into acidic fraction (AF) and the neutral/basic fraction (NBF) by liquid-liquid extraction, and the NBF was further fractionated into four subfractions by silica gel chromatography (SGF1-4). Finally, SAFE distillate, AF, as well as SGF1-4 were concentrated and used for HRGC-O, HRGC-MS as well as comprehensive high-resolution gas chromatography-time-of-flight mass spectrometry (HRGC×HRGC-TOF-MS) for structure identification. Odor qualities and intensities perceived at the sniffing port during HRGC-O, the retention indices on two columns of different polarities, as well as mass spectra obtained in both EI and CI mode were compared to data obtained from the respective reference compounds analyzed under the same conditions.

In total, 61 odorants were unequivocally identified in all four samples. Among them, 1-octen-3-one, nonanal, 2,3,5-trimethylpyrazine, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-decadienal, 2-methoxyphenol, and 4-ethylphenol were firstly identified as odorants in TS. The cAEDA and cSH-ADA revealed 58 odorants present in the FD factor range between 8 and 4096 in at least one of the four aroma extracts (Table 4). The highest FD factors in all four samples were determined for (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide (**21-23**; roasted onion-like; 1024-4096), *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (**30** and **31**; cooked onion-like/TS-like; 256-2048), eugenol (**57**; clove-like; 256-2048), and 2-isopropyl-3-methoxypyrazine (**17**; earthy/pea-like; 128-1024). Further odor-active compounds showing high FD factors (≥ 64) in at least one of the four samples were the green smelling hexanal (**6**), (*E*)-2-hexenal (**9**), (*E*)-2-hexen-1-ol (**15**),

and (*E*,*Z*)-2,6-nonadienal (**27**), as well as 2-ethyl-3,5-dimethylpyrazine (**18**; earthy), acetic acid (**19**; vinegar-like), linalool (**25**; citrus-like/flowery), butyrolactone (**32**; sweet/aromatic), 3-methylnonane-2,4-dione (**38**; hay-like/aniseed-like/fishy), (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide (**39-41**; cooked onion-like), 2-methoxyphenol (**44**; smoky/phenolic), *β*-ionone (**47**; flowery/violet-like), nonanoic acid (**55**; moldy/pungent), 4-ethylphenol (**56**; fecal-like/phenolic), and decanoic acid (**59**; soapy/musty).

Differences in FD factors between RGTS and RRTS were found for hexanal (128 and 32), (*E*)-2-hexenal (128 and 32), (*E*)-2-hexen-1-ol (64 and 16), acetic acid (16 and 64), linalool (16 and 64), (*E*,*Z*)-2,6-nonadienal (128 and 32), 2- and 3-methylbutanoic acid (8 and 32), (*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-di-1-propenyl trisulfide (512 and 128), dimethyl sulfide (32 and 8), 2-methoxyphenol (8 and 256), 2-phenylethanol (4 and 32), and 4-ethylphenol (<4 and 64). This data confirmed the intense green and cooked onion-like/TS-like odor notes in the overall aroma of RGTS as well as the intense phenolic odor note in the overall aroma of RRTS. A comparison of FD factors in raw and respective blanched TS showed decreasing FD factors for the sulfur-containing compounds dimethyl sulfide, the three isomers of di-1-propenyl disulfide, and the two isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, as well as the green smelling hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal. In addition, the FD factors of 2-methoxyphenol and 4-ethylphenol were also lower in blanched red TS. After blanching, lower FD factors were also found for butyrolactone, eugenol, 2-isopropyl-3-methoxypyrazine, 2- and 3-methylbutanal, *γ*-nonalactone, and vanillin. The odorant screening data gave the first hints that the loss of these odorants may be responsible for the aroma profile change after blanching.

Table 4. Major Aroma-Active Compounds in Raw and Blanched Green and Red TS, Their Odor Qualities, Retention Indices, Flavor Dilution (FD) Factors, and Separated Fractions.[112]

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	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	SGF2
5	α-pinene	resin, fir needle-like	1010	939	8	<4	8	<4	SGF1
6	hexanal	green, grassy	1090	769	128	32	32	16	SGF2
7	methyl hexanoate	fruity, musty	1199	922	8	4	4	4	SGF2
8	limonene	citrus-like, carrot-like	1206	1030	<4	8	<4	<4	SGF1
9	(*E*)-2-hexenal	green apple-like	1216	851	128	16	32	8	SGF2
10	methylpyrazine	green, roasty	1273	822	<4	<4	<4	<4	SGF4
11	1-octen-3-one	mushroom-like	1293	979	16	32	8	8	SGF2
12	nonanal	citrus-like, soapy	1381	1103	32	32	32	32	SGF3
13	2-ethyl-6-methylpyrazine	roasty	1382	1001	<4	<4	8	<4	SGF4
14	2-ethyl-3-methylpyrazine	roasty	1382	1010	<4	<4	8	<4	SGF4
15	(*E*)-2-hexen-1-ol	green, fruity	1403	860	64	4	16	8	SGF3
16	2,3,5-trimethylpyrazine	earthy	1410	1003	32	32	32	32	SGF4
17	2-isopropyl-3-methoxypyrazine	earthy, pea-like	1421	1094	512	128	1024	256	SGF4
18	2-ethyl-3,5-dimethylpyrazine	earthy	1430	1079	64	32	64	32	SGF4
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	SGF3
21	(*E*,*E*)-di-1-propenyl disulfide	roasted onion-like	1448	1121	4096	1024	2048	1024	SGF1
22	(*E*,*Z*)-di-1-propenyl disulfide	roasted onion-like	1467	1130	4096	1024	2048	1024	SGF1
23	(*Z*,*Z*)-di-1-propenyl disulfide	roasted onion-like	1490	1142	4096	1024	2048	1024	SGF1
24	propanoic acid	sour, sweaty	1535	706	16	8	8	4	AF

Table 4. Major Aroma-Active Compounds in Raw and Blanched Green and Red TS, Their Odor Qualities, Retention Indices, Flavor Dilution (FD) Factors, and Separated Fractions.[112]

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	
25	linalool	citrus-like, flowery	1539	1100	16	8	64	16	SGF2
26	isocaryophyllene	citrus-like	1556	1407	16	4	32	4	SGF1
27	(*E*,*Z*)-2,6-nonadienal	green, cucumber-like	1571	1153	128	32	32	4	SGF2
28	*β*-caryophyllene	moldy	1577	nd	8	<4	8	<4	SGF1
29	aromadendrene	eucalyptus-like	1591	nd	16	4	32	16	SGF1
30	*cis*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like, TS-like	1618	1119	2048	1024	1024	256	SGF1
31	*trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like, TS-like	1633	1127	2048	1024	1024	256	SGF1
32	butyrolactone	sweet, aromatic	1638	900	128	32	32	4	SGF4
33	menthol	mint-like	1641	nd	4	4	4	4	AF
34	*α*-humulene	balmy	1654	1455	8	<4	8	<4	SGF1
35	3-methylbutanoic acid	sweaty	1662	870	8	<4	32	<4	AF
36	2-methylbutanoic acid	fruity, sweaty	1662	870	8	<4	32	<4	AF
37	valencene	fruity, flowery	1702	1497	4	<4	8	<4	SGF1
38	3-methylnonane-2,4-dione	hay-like, aniseed-like, fishy	1716	1251	128	128	128	128	SGF4
39	(*E*,*E*)-di-1-propenyl trisulfide	cooked onion-like	1741	1341	512	512	128	64	SGF1
40	(*E*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	1759	1355	512	512	128	64	SGF1
41	(*Z*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	1788	1378	512	512	128	64	SGF1
42	(*E*,*E*)-2,4-decadienal	fatty, deep-fried	1801	1371	16	32	16	32	SGF2
43	hexanoic acid	sweaty	1839	1010	8	8	8	8	AF
44	2-methoxyphenol	smoky, phenolic	1848	1090	8	<4	256	64	SGF2
45	benzyl alcohol	bitter almond-like, fruity	1873	1036	8	<4	32	nd	SGF3

Table 4. Major Aroma-Active Compounds in Raw and Blanched Green and Red TS, Their Odor Qualities, Retention Indices, Flavor Dilution (FD) Factors, and Separated Fractions.[112]

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	
46	2-phenylethanol	flowery, honey-like	1900	1117	4	<4	32	16	SGF3
47	*β*-ionone	flowery, violet-like	1923	1488	256	128	512	128	SGF2
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	SGF2
51	2-acetylpyrrole	musty	1989	1066	8	<4	8	<4	SGF2
52	phenol	ink-like, phenolic	2016	981	8	<4	8	<4	SGF2
53	*γ*-nonalactone	coconut-like	2029	1360	32	8	32	8	SGF3
54	2-pyrrolidone	fruity	2054	nd	8	<4	8	<4	SGF4
55	nonanoic acid	moldy, pungent	2150	nd	64	16	32	16	AF
56	4-ethylphenol	fecal-like, phenolic	2163	1077	<4	nd	64	<4	SGF3
57	eugenol	clove-like	2167	1359	1024	256	2048	512	SGF2
58	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like	2200	1108	8	<4	8	<4	SGF4
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	SGF4

[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 HRGC-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 neutral and basic volatiles (NBF). [g]FD factor was determined via SH-ADA. nd: Not determined.

6.1.2 Quantitation experiments of fresh TS

To verify the odorant screening results and to get knowledge about the role of the individual odorants discussed above, the concentrations of aroma compounds revealing FD factors ≥ 32 in at least one sample were determined mainly via stable isotope dilution assays (SIDAs). Aromadendrene, *β*-caryophyllene, caryophyllene oxide, *α*-humulene, isocaryophyllene, and valencene were semiquantitated by high-resolution gas chromatography-flame ionization detection (HRGC-FID) by internal standard method using cyclopentadecanone as standard. Defined amounts of stable isotopically labeled internal standards and cyclopentadecanone were added to the powdered sample and worked-up as described in section **6.1.1** for isolation of the volatiles. Concentrated SAFE distillates were subjected to HRGC-FID, HRGC-MS (CI), or two-dimensional heart-cut high-resolution gas chromatography-mass spectrometry (HRGC/HRGC-MS) (CI). Dimethyl sulfide and 2- and 3-methylbutanal were quantitated via SIDAs by headspace solid phase microextraction combined with high-resolution gas chromatography-mass spectrometry (HS-SPME-HRGC-MS (CI)).

The quantitative data revealed concentrations between 1.10 µg/kg and 2.75 g/kg, and acetic acid was present at the highest concentration in both two samples. The results verified the odorant screening data. Concentrations of the nine sulfur-containing compounds including dimethyl sulfide, di-1-propenyl disulfide isomers, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene isomers, and di-1-propenyl trisulfide isomers in RGTS were much higher than those in RRTS. The same trends were found for hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal. In contrast, concentrations of 2-methoxyphenol and 4-ethylphenol were determined much higher in RRTS. Further concentration differences between RGTS and RRTS were obtained for higher concentrations of 2-methylbutanal and 3-methylbutanal, butyrolactone, nonanoic acid, decanoic acid, and vanillin in RGTS. All other compounds showed much higher concentrations in RRTS (Table 5).

Table 5. Concentrations of Important Aroma-Active Compounds in Raw and Blanched Green and Red *T. sinensis* and Their Losses During Blanching.[112]

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
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
2-pyrrolidone	423	190	55	535	248	54

Table 5. Concentrations of Important Aroma-Active Compounds in Raw and Blanched Green and Red *T. sinensis* and Their Losses During Blanching.[112]

odorant	green TS			red TS		
	conc. (µg/kg)[a]		loss (%)	conc. (µg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
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
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	nc	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

Table 5. Concentrations of Important Aroma-Active Compounds in Raw and Blanched Green and Red *T. sinensis* and Their Losses During Blanching.[112]

odorant	green TS			red TS		
	conc. (μg/kg)[a]		loss (%)	conc. (μg/kg)[a]		loss (%)
	raw	blanched		raw	blanched	
1-octen-3-one	3.47	7.57	+118[b]	1.16	2.79	+141[b]
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

[a]Mean values of triplicates, differing not more than ±15%. [b]Concentration of odorant in blanched TS was higher than that in raw *T. sinensis*. nd: Not determined. nc: Not calculated.

To get deeper insight into the contribution of a respective odorant to the overall aroma of the two raw samples, OAVs (ratio of concentration to respective odor threshold) were calculated for each odorant. In RGTS, 36 odorants showed OAVs ≥ 1. The highest OAV was calculated for the three isomers of di-1-propenyl disulfide, followed by dimethyl sulfide, 2-isopropyl-3-methoxypyrazine, *β*-ionone, (*E*,*Z*)-2,6-nonadienal, the two isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, the three isomers of di-1-propenyl trisulfide, eugenol, 2-methylbutanal, 3-methylbutanal, and hexanal (OAV ≥ 500). In RRTS, 41 odorants were present in concentrations above the respective odor thresholds in FRTS. The highest OAVs were calculated again for the three isomers of di-1-propenyl disulfide, dimethyl sulfide, 2-isopropyl-3-methoxypyrazine, *β*-ionone, followed by eugenol, 3-methylbutanal, the two isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, 2-methylbutanal, the three isomers of di-1-propenyl disulfide, and (*E*,*Z*)-2,6-nonadienal (OAV ≥ 500). A comparison of OAVs between RGTS and RRTS revealed clearly higher OAVs 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 green smelling (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, and (*E*)-2-hexen-1-ol, resulting in intense cooked onion-like/TS-like and green odor notes in the overall aroma of RGTS. However, clearly higher OAVs of 2-methoxyphenol and 4-ethylphenol in RRTS proved the intense phenolic odor note in RRTS (Table 6).

Table 6. Orthonasal Odor Thresholds (OTs) and Odor Activity Values (OAVs) of Important Odorants of Raw and Blanched Green and Red *T. sinensis*.[112]

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
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

Table 6. Orthonasal Odor Thresholds (OTs) and Odor Activity Values (OAVs) of Important Odorants of Raw and Blanched Green and Red *T. sinensis*.[112]

odorant	OT (µg/kg)	OAV[a]			
		green TS		red TS	
		raw	blanched	raw	blanched
(*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
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
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

[a]Odor activity values were calculated as ratio of the concentration to the respective odor threshold. [b]Orthonasal odor threshold in water of the isomer mixture was newly determined in this study according to literature.[15] [c]Orthonasal odor threshold in water from in-house database. [d]Orthonasal odor threshold in water as reported previously.[15]

6.1.3 Concentration losses of key odor-active compounds in blanched TS

To get a deeper insight into the changes of the overall aroma in blanched TS, quantitation of odorants with high FD factors or components showing obvious differences in their FD factors after blanching (Table 4) were determined via SIDAs and internal standard method (Table 5) and their

OAVs were calculated in the respective blanched TS (Table 6). The results showed that nearly all odorants, both in green and red TS, were present in decreased concentrations after blanching. Only a few compounds increased, especially nonanal, (*E*,*E*)-2,4-decadienal, 3-methylnonane-2,4-dione, and 1-octen-3-one (Table 5). A comparison of the concentrations and OAVs in raw and blanched TS confirmed that the blanching processing step resulted in clear losses of di-1-propenyl disulfide (3 isomers), dimethyl sulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (2 isomers), di-1-propenyl trisulfide (3 isomers), hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*,*Z*)-2,6-nonadienal, 4-ethylphenol, and 2-methoxyphenol (Table 6).

6.1.4 Sensorial experiments

At first, aroma profile analysis (APA) was carried out to evaluate the overall aroma of RGTS, RRTS, BGTS, and BRTS using 9 main odor attributes evaluated by the sensory panel in preliminary sessions. The odor intensities were then illustrated in a spider diagram. The results revealed the cooked onion-like/TS-like and green odor impressions as the two most intense attributes in RGTS and were present at an obviously higher intense compared to those in RRTS, whereas the aroma profile of RRTS elicited a clearly higher phenolic odor note. After blanching, the intensities of the selected odor attributes decreased by varying degrees for both green and red TS. In particularly, the cooked onion-like/TS-like, green, and phenolic attributes were clearly lower pronounced. To validate the analytical data, aroma recombinates of all samples were prepared in respective odorless residues obtained after solvent extraction of the raw sample material. To those matrices, all odorants with an OAV ≥ 1 were added in the concentrations determined in the respective samples. APA showed very good overall similarities between all the samples and the respective recombinates, proving the successful characterization of the key aroma-active compounds for both raw and blanched TS. In addition, the change to less intensive cooked onion-like/TS-like, green, and phenolic aroma notes in TS after blanching could be confirmed on a molecular level for the first time in this study (Figure 11).

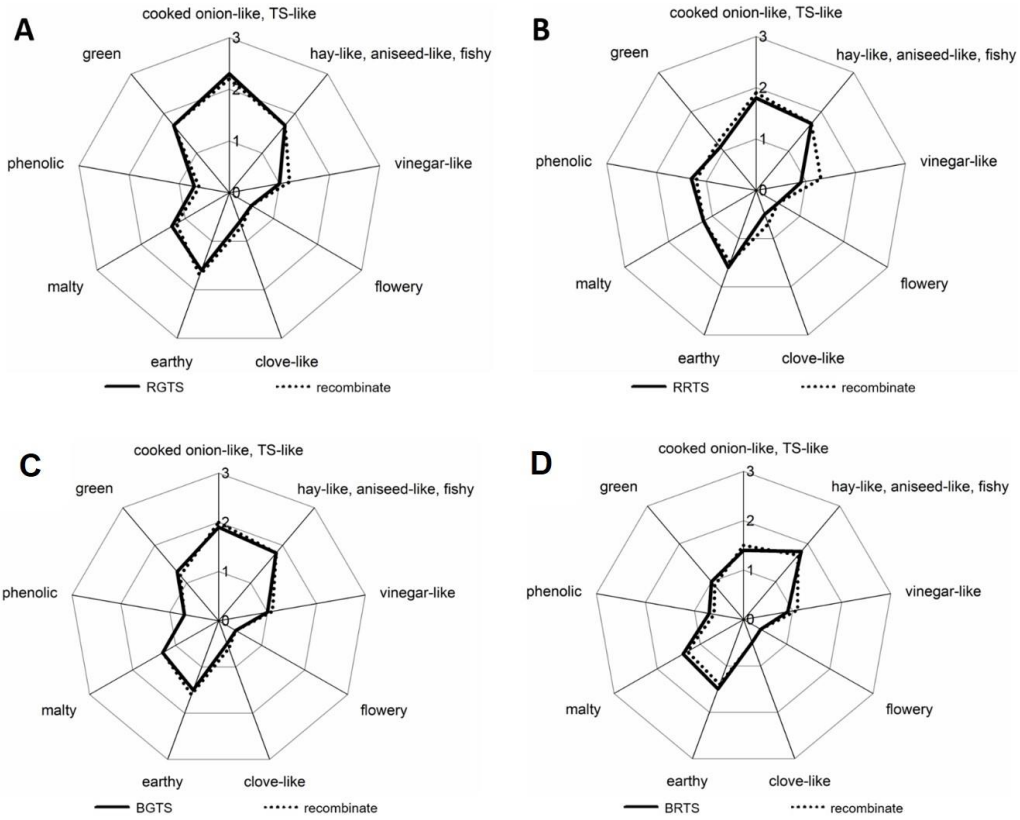

Figure 11. Aroma profiles of samples (solid line) and respective recombinates (broken line): raw green *T. sinensis* (A), raw red *T. sinensis* (B), blanched green *T. sinensis* (C), and blanched red *T. sinensis* (D).[112]

During the blanching process of both green and red TS, clear reductions of nearly all aroma-active compounds were found with an exemption for only 4 compounds. Therefore, spiking blanched TS with the amounts of the odorants lost during blanching should enable a “reconstitution” of the aroma profile of raw TS. To verify this assumption, triangle experiments for eight odor attribute groups including “all compounds”, “cooked onion-like/TS-like”, “green”, “phenolic”, “earthy”, “malty”, “vinegar-like”, and “remaining compounds” were performed. The results revealed that the spiked TS with “all compounds”, “cooked onion-like/TS-like”, “green”, as well as “phenolic” could not significantly be distinguished from the raw TS by the sensory panel, proving the abovementioned assumption. These results corroborated the importance of the sulfur-containing compounds (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, dimethyl sulfide, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide, the aldehydes hexanal, (*E*)-2-hexenal, and (*E*,*Z*)-2,6-nonadienal, as well as (*E*)-2-hexen-1-ol for the overall aroma of both green and red TS. In addition, 2-methoxyphenol and 4-ethylphenol were also vital phenols for the aroma of red TS (Table 7).

Table 7. Triangle Tests for Spiking Experiments of Raw Green and Red *T. sinensis* Compared to Blanched Green and Red *T. sinensis* to Which Reference Aroma Compounds were Added in Concentrations Compensating the Losses during Blanching.[112]

test	reference sample	spiked sample	correct answers/panelists[a]	statistical significance
S1	raw green TS	blanched green TS + all compounds with OAV ≥1	7/19	$p = 0.5$
S2		blanched green TS + cooked onion-like/TS-like	8/19	$p = 0.3$
S3		blanched green TS + green	7/16	$p = 0.3$
S4		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	raw red TS	blanched red TS + all compounds with OAV ≥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		blanched red TS + green	8/16	$p = 0.2$
S12		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.

6.2 Elucidation of the impact of different drying processes on the odorants in TS and commercially dried TS products

6.2.1 Screening and identification experiments of differently dried TS

Due to the popularity of dried TS sprout products in China, the sensomics approach was applied to both green and red TS varieties treated with three different drying methods (freeze drying, natural solar drying, and oven drying) to characterize the key aroma-active compounds in dried TS buds and further to elucidate the influences of different drying processes on the odorants. Thus, freeze-dried TS (FDTS) were prepared by freeze drying of raw green and red TS buds (**6.1.1**). Solar-dried TS (SDTS) was prepared after drying fresh buds in sunlight for 3 days. Oven-dried TS (ODTS) was obtained by drying fresh buds in an oven (40 °C) for two days. All the six groups of dried TS buds were frozen with liquid nitrogen, crashed into small pieces, powdered, then filled into brown glass bottles, and stored at -24 °C prior to analysis as described in section **6.1.1**.

Firstly, a total of 61 aroma-active compounds were successfully identified in at least one of the six dried samples. Among them, 33 odorants in FDGTS, 28 in SDGTS, and only 18 in ODGTS were present in the FD factor range between 16 and 4096, determined by application of cAEDA and cSH-ADA. In both FDGTS and SDGTS, eugenol showed the highest FD factor. High FD factors were further found for (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, *cis*- and *trans*-2-

mercapto-3,4-dimethyl-2,3-dihydrothiophene, *β*-ionone, (*E*,*Z*)-2,6-nonadienal, and vanillin (all ≥ 256), while only eugenol (1024) and *β*-ionone (128) were perceived with high FD factors in ODGTS. Although eugenol showed the highest FD factor in each sample, the respective FD factors were clearly different. Further, compared to ODGTS, di-1-propenyl disulfide isomers, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene isomers, di-1-propenyl trisulfide isomers, (*E*)-2-hexenal, 1-octen-3-one, 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, and (*E*,*E*)-2,4-decadienal were present at obviously higher FD factors in FDGTS and SDGTS. In addition, compounds with obviously higher FD factors only in SDGTS were (*E*,*Z*)-2,6-nonadienal, 2- and 3-methylbutanoic acid, phenylacetic acid, and vanillin.

In the red TS variety, the odorant screening revealed again eugenol and *β*-ionone with the highest FD factors in all three samples. Further compounds with high FD factors in FDRTS and SDRTS were (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, vanillin, (*E*,*Z*)-2,6-nonadienal, 2-phenylethanol, 2-isopropyl-3-methoxypyrazine, and *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene. However, most of the odorants were perceived with clearly lower FD factors in ODRTS, higher FD factors, beside eugenol and *β*-ionone, were only obtained for di-1-propenyl disulfide isomers, 2-phenylethanol, vanillin, and nonanoic acid. Odorant screening suggested clear FD factor differences between the three dried samples. For example, (*E*)-2-hexen-1-ol, linalool, and 2- and 3-methylbutanoic acid with much higher FD factors in FDRTS might contribute to its intense overall aroma. Compounds such as 2-isopropyl-3-methoxyprazine, di-1-propenyl disulfide (3 isomers), (*E*,*Z*)-2,6-nonadienal, and 2-methoxyphenol might have an effect on the changes of the overall aroma of ODRTS due to their respective lower FD factors.

6.2.2 Quantitation experiments of differently dried TS

To verify the odorant screening data of all dried samples in both green and red TS varieties, the quantitation of 51 odorants showing high or clearly different FD factors in the dried samples was accomplished by means of SIDAs in combination with GC-MS or internal standard method in combination with GC-FID analysis. Further, the OAV of each quantitated odorant was calculated to evaluate the contribution of the respective odorant to the overall aroma.

For green TS, FDTS showed the highest concentrations for most of the aroma-active compounds and ODTS showed the lowest amounts. Specifically, the highest concentration (0.82-1.69 g/kg) was found for acetic acid in all three samples. Further compounds including dimethyl sulfide (up to 44.7 mg/kg in FDTS), eugenol (up to 32.4 mg/kg in SDTS), and 2-methylbutanal (up to 10.4 mg/kg in FDTS) were found at high amounts in all samples. High concentrations were also determined for the sesquiterpenes caryophyllene, aromadendrene, isocaryophyllene, *α*-humulene, valencene, and caryophyllene oxide (all > 1.09 mg/kg), as well as for the monocarboxylic acids propanoic acid, 2- and 3-methylbutanoic acid, and decanoic acid (all > 1.82 mg/kg). Various important sulfur-containing compounds revealed specific differences in their concentrations depending on the respective drying method, which confirmed the results of cAEDA. For example, (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide in FDTS were present in amounts 7-49 times higher compared to those in SDTS and 180-920 times higher compared to those in

ODTS. Concentrations of (*E*,*E*)- and (*Z*,*Z*)-di-1-propenyl trisulfide in FDTS were 7-31 times higher compared to those in SDTS and even several hundred times higher compared to those in ODTS. Compared with SDTS, the same pattern was also found for *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene. Clear differences were also analyzed for phenylacetic acid, 3-methyl-2,4-nonandione, and 1-octen-3-ol showing the highest concentrations in SDTS.

Calculation of OAVs revealed 40 odorants above their respective odor thresholds in FDGTS, 43 in SDGTS, and only 37 in ODGTS. In FDGTS, di-1-propenyl disulfide isomers showed the highest OAV (970000), followed by dimethyl sulfide (150000), *β*-ionone (100000), (*E*,*Z*)-2,6-nonadienal (22000), eugenol (12000), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers, 12000), di-1-propenyl trisulfide (three isomers, 7500), 2-methylbutanal (6900), 2-isopropyl-3-methoxypyrazine (3700), 3-methylbutanal (2700), and 3-methyl-2,4-nonandione (1200). In SDGTS, dimethyl sulfide was obtained with the highest OAV of 95000, followed by di-1-propenyl disulfide (three isomers, 53000), *β*-ionone (38000), eugenol (18000), (*E*,*Z*)-2,6-nonadienal (13000), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers, 9400), 3-methyl-2,4-nonandione (6300), 2-isopropyl-3-methoxypyrazine (4900), 3-methylbutanal (3800), 2-methylbutanal (2100), and di-1-propenyl trisulfide (three isomers, 1600). In ODGTS, the highest OAV was obtained for *β*-ionone (150000), followed by OAVs > 1000 for dimethyl sulfide, eugenol, 2-isopropyl-3-methoxypyrazine, di-1-propenyl disulfide (three isomers), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers), as well as 3-methylbutanal. In conclusion, FDGTS revealed the highest OAVs for most odorants and ODGTS showed the lowest OAVs, verifying the results of odorant screening. In conclusion, a comparison between FDGTS and SDGTS illustrated that almost all odorants showed obviously lower OAVs in SDGTS, except for eugenol, 2-isopropyl-3-methoxypyrazine, 3-methylbutanal, 3-methyl-2,4-nonandione, isocaryophyllene, caryophyllene oxide, caryophyllene, phenylacetic acid, and 1-octen-3-ol, while clearly lower OAVs were found for di-1-propenyl disulfide (three isomers), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers), di-1-propenyl trisulfide (three isomers), 2-methylbutanal, (*E*,*Z*)-2,6-nonadienal, 3-methyl-2,4-nonandione, 1-octen-3-one, (*E*)-2-hexenal, valencene, and caryophyllene in ODGTS.

In red TS, the quantitative results followed the trend of the respective dried green TS samples. Acetic acid was revealed as the compound with the highest amount in all samples (>2.58 g/kg). Additionally, compounds with high concentrations in the three samples were caryophyllene, aromadendrene, isocaryophyllene, valencene, *α*-humulene, and caryophyllene oxide (all ≥ 30.9 mg/kg). In accordance with green TS, the monocarboxylic acids showed also high amounts in red TS, such as propanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, hexanoic acid, nonanoic acid, phenylacetic acid, and decanoic acid (all ≥ 2.36 mg/kg). Further, the amounts of the following compounds including eugenol (all > 16.8 mg/kg), butyrolactone (all > 1.3 mg/kg), 3-methylbutanal (all > 1.15 mg/kg), hexanal (all > 1.13 mg/kg), *β*-ionone (all > 0.68 mg/kg), and nonanal (all > 0.11 mg/kg) were determined in high concentrations in the three samples. However, the typical sulfur-containing compounds revealed obvious differences in these dried TS samples. For example, dimethyl disulfide was present at concentrations of 83.9 mg/kg in FDRTS, 26.0 mg/kg in SDRTS, and 23.2 mg/kg in ODRTS. (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-Di-1-propenyl disulfide in FDGTS were present in amounts 2-64 times higher compared to those in SDGTS and 65-1860 times higher compared to those in ODGTS. (*E*,*E*)-, (*E*,*Z*)- and (*Z*,*Z*)-Di-1-propenyl trisulfide in

FDGTS showed 2-10 times higher concentrations compared to SDGTS, while they were present at concentrations > 21 times higher compared to those in ODGTS. The concentrations of *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene were highest in SDRTS and lowest in ODRTS. Further compounds showing clearly higher amounts in FDRTS compared to the other two samples were 2-methylbutanal, 2-phenylethanol, benzyl alcohol, 4-ethylphenol, 2-methoxyphenol, (*E*)-2-hexen-1-ol, linalool, and 2-ethyl-3,5-dimethylpyrazine. In contrast, 3-methyl-2,4-nonandione, vanillin, 1-octen-3-ol, (*E*,*E*)-2,4-decadienal, and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one were present at highest concentrations in SDRTS.

Forty-two odorants were determined in amounts above their respective odor thresholds in FDRTS, 43 in SDRTS, and 39 in ODRTS. Odorants with highest OAVs in FDRTS were di-1-propenyl disulfide (three isomers, 400000), dimethyl sulfide (280000), 2-isopropyl-3-methoxypyrazine (54000), *β*-ionone (39000), eugenol (21000), 2-methylbutanal (15000), 3-methylbutanal (7200), (*E*,*Z*)-2,6-nonadienal (6600), isocaryophyllene (3500), di-1-propenyl trisulfide (three isomers, 2800), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers, 2600), 3-methyl-2,4-nonandione (1500), linalool (1100), 2-methoxyphenol (1100), and acetic acid (1000). In SDRTS, dimethyl sulfide revealed the highest OAV of 87000. Further compounds with high OAVs (> 1000) were 2-isopropyl-3-methoxypyrazine (32000), *β*-ionone (32000), di-1-propenyl disulfide (three isomers, 29000), eugenol (14000), 3-methyl-2,4-nonandione (4700), 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers, 4600), 3-methylbutanal (4200), (*E*,*Z*)-2,6-nonadienal (3800), 2-methylbutanal (2300), caryophyllene oxide (1400), as well as di-1-propenyl trisulfide (three isomers, 1300). In ODRTS, dimethyl sulfide revealed again the highest OAV (77000), followed by *β*-ionone (41000), eugenol (9300), 2-isopropyl-3-methoxypyrazine (8900), 3-methylbutanal (2300), 2-methylbutanal (1400), and di-1-propenyl disulfide (three isomers, 1100). In general, a comparison of the three samples revealed highest OAVs for most compounds in FDRTS and lowest OAVs in ODRTS. In contrast, clearly higher OAVs in SDRTS compared to the other two samples were calculated for 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers), (*E*,*E*)-2,4-decadienal, caryophyllene oxide, caryophyllene, phenylacetic acid, and 1-octen-3-ol. Finally, only three compounds including *β*-ionone, decanoic acid, and valencene were found with the highest OAVs in ODRTS.

6.2.3 Sensorial experiments of differently dried TS

As the last step of the sensomics concept, the contribution of the quantitated aroma compounds to the overall aroma is evaluated on the basis of an aroma recombination using the aroma profile analysis as described in section **6.1.4**. First, six of nine odor notes including vinegar-like, phenolic, flowery, clove-like, malty, and fishy/hay-like attributes were evaluated at similar intensities in all green TS samples, while the cooked onion-like/TS-like odor impression showed an intensity of 2.2 in FDGTS, 1.7 in SDGTS, and 1.3 in ODGTS. Further, both green and earthy attributes were assessed as two characteristic odors for FDGTS, whereas the intensities were much lower in SDGTS and ODGTS. Second, in the three red TS samples, the cooked onion-like/TS-like and hay-like/aniseed-like/fishy impressions were the two major odor attributes. Although the intensity of the cooked onion-like/TS-like odor note was highest in each sample, it was evaluated with the

highest intensity in FDRTS, lower in SDRTS, and lowest in ODRTS. Additionally, earthy and green odor notes showed similar intensities in FDRTS and SDRTS, but a lower intensity in ODRTS. Finally, all the aroma profiles of the recombinates of FDTS, SDTS, and ODTS (both green and red varieties) prepared in a respective odorless matrix matched the aroma profiles of the respective samples very well, proving the successful characterization of all key odorants in FDTS, SDTS, and ODTS.

The characterization of key aroma-active compounds in both green and red TS varieties processed with different lab-drying methodologies verified the influences of conventional natural solar drying and oven drying, as well as novel freeze drying on these odorants, which finally resulted in the change of the overall aroma of differently dried TS. Specifically, freeze drying of pre-concentrated TS maintained the highest amounts of most odorants, confirming the advantage of the application of low temperature and low pressure to obtain a product of high sensorial quality. Natural solar drying process led to losses of key odorants at different extents. However, considering the high costs and the much more complex handling of freeze drying, natural solar drying might be a more suitable drying process of TS buds according to the similar overall aroma compared to that of FDTS despite some odorant losses. In addition, due to extremely losses of most key aroma-active compounds, especially the typical sulfur-containing substances, oven-drying definitely resulted in obvious changes of the overall aroma, and thus, leading to the lowest product quality of dried TS.

6.2.4 Characterization of the key odorants in commercially dried TS products

Due to the additional blanching and salting steps before the drying process, the key odorants and overall aromas of commercially TS products might differ in comparison to the directly dried TS buds. Thus, to get a deeper insight into the aroma quality of commercially dried TS products, the characterization of key odor-active compounds of two dried TS samples, either commercially solar-dried TS (CSDTS) or commercially vacuum-dried TS (CVDTS), harvested in April 2016 from two Chinese provinces, were carried out by means of the molecular sensory science concept. After blanching in boiling water for 1 min, CSDTS were cooled to room temperature, salted, and finally dried in sunlight for 3 days, while CVDTS was cooled to room temperature after blanching, salted in a closed container with water for 2 weeks, and then vacuum freeze-dried. The products obtained were ground into powder and stored at -24 °C as described in section **6.1.1**.

A total of 64 aroma-active compounds were identified via HRGC-O and HRGC-MS in both CSDTS and CVDTS products. The odorant screening experiments by application of AEDA and SH-ADA revealed 39 odorants in CSDTS and 32 odorants in CVDTS with FD factors from 8 to 4096, with the highest for vanillin and eugenol in both samples. High FD factors were also found for 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, acetic acid, linalool, isocaryophyllene, *β*-caryophyllene, butyrolactone, *α*-humulene, valencene, 3-methylnonane-2,4-dione, *β*-ionone, and decanoic acid in at least one product. Compared to the identification data of lab-dried TS, many key odorants found in lab-dried TS including dimethyl sulfide, 2- and 3-methylbutanal, 2-isopropyl-3-methoxypyrazine, 3-methylnonane-2,4-dione, *β*-ionone, eugenol, and vanillin were

again found in both products. Interestingly, some compounds, e.g., di-1-propenyl disulfides, di-1-propenyl trisulfides, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophenes, nonanal, 1-octen-3-one, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-decadienal, 2,3,5-trimethylpyrazine, 4-ethylphenol, and 2-methoxyphenol were not found in these two commercial products.

Due to the fact that AEDA and SH-ADA were only preliminary screening methods, precise quantitation via SIDAs and semiquantitation via internal standard method of 42 compounds with high FD factors were performed. Thereby, acetic acid showed the highest concentration in CSDTS (3.75 g/kg), whereas 1,8-cineole was present the lowest concentration (3.5 µg/kg). High concentrations of >5 mg/kg in CSDTS were found for eugenol, *β*-caryophyllene, 2-pyrrolidone, propanoic acid, 2- and 3-methylbutanoic acid, phenylacetic acid, *α*-humulene, valencene, and butyrolactone. In CVDTS, acetic acid was also present in the highest concentration, followed by eugenol, *β*-caryophyllene (all >33 mg/kg), propanoic acid, butyrolactone, decanoic acid, and nonanoic acid (all >2 mg/kg), followed by caryophyllene oxide, 2-methylbutanal, 2-pyrrolidone, valencene, *α*-humulene, phenylacetic acid, and hexanoic acid (all >1 mg/kg), whereas 2-ethyl-3,5-dimethylpyrazine and 1,8-cineole were present in lowest concentrations (<10 *µ*g/kg). To identify the most potent odorants, OAVs were calculated for all 42 quantitated compounds. The results indicated 35 compounds in CSDTS with an OAV ≥ 1. The highest OAVs were found for 2-isopropyl-3-methoxypyrazine, eugenol, and *β*-ionone, followed by 3-methylnonane-2,4-dione, 3-methylbutanal, 2-methylbutanal, dimethyl sulfide, linalool, hexanal, acetic acid, 2-ethyl-3,5-dimethylpyrazine, and decanoic acid. In CVDTS, 29 compounds resulted in OAVs ≥ 1: eugenol, 2-isopropyl-3-methoxypyrazine, and *β*-ionone revealed the highest OAVs, followed by dimethyl sulfide, 3-methylbutanal, 2-methylbutanal, linalool, and decanoic acid.

To verify the qualitative and quantitative data of both commercially dried products, two aroma recombinates were prepared as described in **6.1.4**. Comparative APA of the recombinates and the respective original dried products revealed very good similarities, proving that all key aroma-active compounds were successfully characterized. However, compared to the lab-scaled dried TS samples (FDTS, SDTS, and ODTS; see section **6.2.1**), some important sulfur-containing compounds including di-1-propenyl disulfide, di-1-propenyl trisulfide, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene as well as (*E*,*Z*)-2,6-nonadienal were not found in these two commercially dried products. The following three reasons were hypothesized regarding this result. First, fresh TS buds were blanched with boiling water (100 °C) prior to processing in commercial products whereupon premature release and subsequent losses of these compounds during blanching at elevated temperature have been proven in section **6.1.3**.[112] Secondly, section **6.2.2** illustrated again that drying processes at elevated temperatures could lead to losses of these compounds in both green and red TS.[113] In addition, we assumed that different varieties and cultivating regions might also lead to the differences of some odorants, e.g., 4-ethylphenol and 2-methoxyphenol, in green and red TS. Thus, to validate the abovementioned assumption, further four commercially dried products from different regions and markets were analyzed, especially to focus on the missing potent sulfur-containing compounds. The results showed di-1-propenyl disulfides and di-1-propenyl trisulfides only in two of these four samples, and at clearly different concentrations, while 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene was only present in one

sample with a very low concentration. Thus, the additional quantitative data confirmed our assumption.

7 References

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8 Appendix

8.1 Publication 1: Key Odor-Active Compounds in Raw Green and Red *Toona sinensis* (A. Juss.) Roem. and Their Changes during Blanching

8.1.1 Bibliographic data

Tiltle:	Key odor-active compounds in raw green and red *Toona sinensis* (A. Juss.) Roem. and their changes during blanching
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8.1.2 Publication reprint

A reprint of publication 1, key odor-active compounds in raw green and red *Toona sinensis* (A. Juss.) Roem. and their changes during blanching, follows starting with the next page.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

Xiaoting Zhai and Michael Granvogl*

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ABSTRACT: Application of aroma extract dilution analysis and headspace aroma dilution analysis revealed 51 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 first 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 was 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 its anticancer and antioxidative properties as well as in traditional Chinese diet because of its beneficial nutrient contents.[1,2] Currently, the planting area of *T. sinensis* is >1 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 2 decades ago, and to date, >200 volatiles have been identified.[4−7] 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 to be 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 matures 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 in 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 has identified the composition of odorants in blanched *T. sinensis* buds using static headspace aroma dilution analysis (SH-ADA) and aroma

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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 second 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 an internal standard method. Next, (iii) odor thresholds (OTs) were determined to calculate odor activity values (OAVs; ratio of concentration to the respective odor threshold) and (iv) the overall aromas of the respective samples were simulated by recombination experiments. Finally, triangle tests were performed to confirm the analyzed data and obtain 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, crushed into small pieces, and then powdered by the 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 high performance liquid chromatography (HPLC) was prepared using a Mili-Q Advantage A10 water purification system (Millipore 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, [$^2H_{3-5}$]-1-octen-3-one, [$^{13}C_2$]-phenylacetic acid, [$^{13}C_2$]-2-phenylethanol, and [$^2H_2$]-propanoic acid (Sigma-Aldrich Chemie); [$^2H_{2-3}$]-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: [$^2H_{3-5}$]-(*E*,*E*)-2,4-decadienal,[9] [$^2H_5$]-2-ethyl-3,5-dimethylpyrazine,[10] [$^2H_{3-4}$]-4-ethylphenol,[11] [$^2H_{4-6}$]-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] [$^2H_3$]-β-ionone,[15] [$^2H_3$]-2-isopropyl-3-methoxypyrazine analogue to [$^2H_3$]-2-isobutyl-3-methoxypyrazine,[16] [$^2H_{2-3}$]-linalool,[17] [$^2H_2$]-2-methylbutanal,[18] [$^2H_{6-8}$]-5-methyl-2-methoxyphenol analogue to 5-ethyl-2-methoxyphenol,[19] [$^2H_3$]-methyl 2-methylbutanoate,[12] [$^2H_3$]-3-methylnonane-2,4-dione,[13] [$^2H_{2-3}$]-methylpyrazine,[20] [$^2H_2$]-(*E*,*Z*)-2,6-nonadienal,[13] [$^2H_{2-3}$]-γ-nonalactone,[21] [$^2H_4$]-nonanal,[22] [$^2H_{2-3}$]-γ-octalactone,[23] [$^2H_{3-6}$]-1-octen-3-ol,[24] [$^2H_{3-4}$]-2,3,5-trimethylpyrazine,[17] and [$^2H_3$]-vanillin.[19]

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

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

Dipropyl disulfide: MS (EI): *m*/*z* (%): 150 (100), 43 (96), 108 (50), 73 (30), 41 (24), 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 the 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

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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] analyte	ion (m/z)[a] standard	R_f[b]	system[c]
acetic acid	[$^{13}C_2$]-acetic acid	61	63	1.00	I
benzyl alcohol	[$^2H_5$]-benzyl alcohol	91	96	0.87	I
butyrolactone[d]	[$^2H_{2\text{-}3}$]-γ-octalactone[d]	87	145 + 146[d,e]	1.12	II
(*E*,*E*)-2,4-decadienal	[$^2H_{3\text{-}5}$]-(*E*,*E*)-2,4-decadienal	153	156−158[e]	0.97	II
decanoic acid	[$^2H_{2\text{-}3}$]-decanoic acid	187	189 + 190[e]	0.93	II
dimethyl sulfide	[$^2H_6$]-dimethyl sulfide	63	69	0.96	III
(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide[f]	[$^2H_6$]-dipropyl disulfide[f]	147	157[f]	1.00	II
(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide[g]	[$^2H_6$]-dipropyl trisulfide[g]	179	189[g]	0.72	II
2-ethyl-3,5-dimethylpyrazine	[$^2H_5$]-2-ethyl-3,5-dimethylpyrazine	137	142	1.00	II
4-ethylphenol	[$^2H_{3\text{-}4}$]-4-ethylphenol	123	126 + 127[e]	1.00	II
eugenol[h]	[$^2H_4$]-*cis*-isoeugenol[h]	165	169[h]	0.67	I
hexanal	[$^2H_{4\text{-}6}$]-hexanal	101	105−107[e]	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	II
(*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	[$^2H_{2\text{-}3}$]-linalool	137	139 + 140[e]	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]	[$^2H_{6\text{-}8}$]-5-methyl-2-methoxyphenol[j]	125	145−147[e,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
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
methyl 2-methylbutanoate	[$^2H_3$]-methyl 2-methylbutanoate	117	120	0.98	II
3-methylnonane-2,4-dione	[$^2H_3$]-3-methylnonane-2,4-dione	171	174	0.95	II
methylpyrazine	[$^2H_{2\text{-}3}$]-methylpyrazine	95	97 + 98[e]	0.75	II
(*E*,*Z*)-2,6-nonadienal	[$^2H_2$]-(*E*,*Z*)-2,6-nonadienal	139	141	0.82	II
γ-nonalactone	[$^2H_{2\text{-}3}$]-γ-nonalactone	157	159 + 160[e]	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	[$^2H_{3\text{-}6}$]-1-octen-3-ol	111	114−117[e]	0.75	II
1-octen-3-one	[$^2H_{3\text{-}5}$]-1-octen-3-one	127	130−132[e]	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
2-pyrrolidone	[$^2H_6$]-2-pyrrolidone	86	92	0.82	I
2,3,5-trimethylpyrazine	[$^2H_{3\text{-}4}$]-2,3,5-trimethylpyrazine	123	126 + 127[e]	0.93	II
vanillin	[$^2H_3$]-vanillin	153	156	0.93	II

[a]Ions used for quantitation in the chemical ionization (CI) mode. [b]Response factor (R_f) was determined by analyzing mixtures of known amounts of the unlabeled analyte and the corresponding labeled internal standard. [c]I, HRGC-MS(CI); II, HRGC/HRGC-MS(CI); III, HS-SPME-HRGC-MS(CI). [d]Butyrolactone was quantitated using [$^2H_{2\text{-}3}$]-γ-octalactone as the internal standard. [e]Internal standard was used as a mixture of isotopologues. [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 were quantitated using [$^2H_6$]-dipropyl disulfide as the internal standard. [j]2-Methoxyphenol was quantitated using [$^2H_{6\text{-}8}$]-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.

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 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

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containing 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 HPLC using a PU-2089 Plus Quaternary pump and a UV-2075 Plus HPLC UV−VIS detector (both Jasco, Pfungstadt, Germany). Aliquots of SGF 1 (100 μL; 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 a further 20 min. Two sulfury smelling fractions obtained after 21 and 29 min were collected and dried with anhydrous sodium sulfate, and the purities 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 (three isomers) and di-1-propenyl trisulfide (three 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, Egelsbach, Germany) 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 at 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 rear 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 used 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 at 6 °C/min to 230 °C, and 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 at 6 °C/min to 250 °C, and held for 10 min. Mass spectra were recorded in the electron ionization (EI) mode at 70 eV at the 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 that were coeluting 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, raised at 6 °C/min to 230 °C, and then 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 the CI mode at 105 eV with methanol as the reagent 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 capillary column (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 the internal standard method as previously reported (internal standard: cyclopentadecanone).[8]

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Determination of Orthonasal Odor Thresholds (OTs) in Water. Orthonasal OTs in water of di-1-propenyl disulfide (three isomers), di-1-propenyl trisulfide (three isomers), and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two 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 the 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). 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 obtain 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* samples, 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 included 4-ethylphenol and 2-methoxyphenol, the "earthy" group included the three pyrazines 2-ethyl-3,5-dimethylpyrazine, 2-isopropyl-3-methoxypyrazine, and 2,3,5-trimethylpyrazine, the "malty" group included 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, 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).

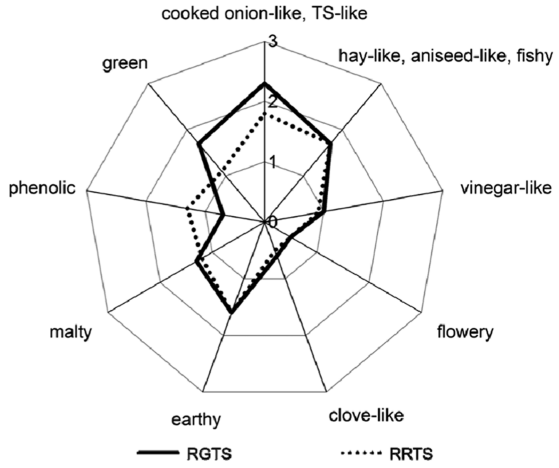

Figure 1. Aroma profiles of raw green *T. sinensis* (solid line) and raw red *T. sinensis* (broken line).

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 and 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 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-

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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

			RI[b]		FD factor[a]				
					green TS		red TS		
no.[c]	odorant[d]	odor quality[e]	DB-FFAP	DB-5	raw	blanched	raw	blanched	fraction[f]
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
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
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
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
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[h]	64	<4	SGF 3

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Table 2. continued

			RI[b]		FD factor[a]				
					green TS		red TS		
no.[c]	odorant[d]	odor quality[e]	DB-FFAP	DB-5	raw	blanched	raw	blanched	fraction[f]
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
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]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). [b]Retention indices, calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [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 the fraction of neutral/basic volatiles (NBF). [g]FD factor was determined via cSH-ADA. [h]Not determined.

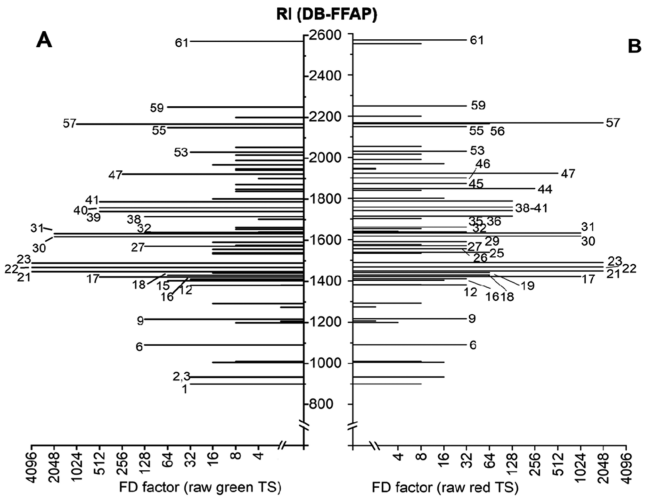

Figure 2. Flavor dilution chromatograms on a polar DB-FFAP capillary column obtained by cAEDA from raw green (A) and red (B) *T. sinensis*. Numbering is identical to that in Table 2.

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 and Figure 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 8 (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 for the overall aroma 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 the internal standard method using cyclopentadecanone as the standard. Concentrations of the nine sulfur-containing compounds dimethyl sulfide (14 000 μg/kg (green) vs 11 000 μg/kg (red)), sum of di-1-propenyl disulfide isomers (1360 vs 595 μg/kg), sum of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene isomers (230 vs 51.1 μg/kg), and sum of di-1-propenyl trisulfide isomers (1300 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.

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

	green TS			red TS		
	conc. (μg/kg)[a]			conc. (μg/kg)[a]		
odorant	raw	blanched	loss (%)	raw	blanched	loss (%)
acetic acid	772 000	322 000	58	2 750 000	1 340 000	51
dimethyl sulfide	14 000	1710	88	11 000	5900	46
eugenol	5120	2800	45	6830	3410	50
caryophyllene	4510	3310	27	15 400	10 500	32
(*E*)-2-hexenal	3290	433	87	1480	256	83
2-methylbutanal	2470	213	91	1770	1030	42
propanoic acid	2260	1090	52	16 700	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
2-methylbutanoic acid	1200	432	64	7860	1230	84
3-methylbutanoic acid	1120	516	54	10 600	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
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
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]
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

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

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 vs 1770 μg/kg), nonanoic acid (1760 vs 1140 μg/kg), decanoic acid (1570 vs 742 μg/kg), 1-octen-3-ol (14.5 vs 7.78 μg/kg), and 1-octen-3-one (3.47 vs 1.16 μg/kg). All

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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*

		OAV[a]			
		green TS		red TS	
odorant	OT (μg/kg)	raw	blanched	raw	blanched
di-1-propenyl disulfide	0.0034[b]	400 000	320 000	170 000	160 000
dimethyl sulfide	0.3[c]	47000	5700	37 000	20 000
2-isopropyl-3-methoxypyrazine	0.0039[d]	7500	6400	35 000	11 000
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
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
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
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	16 000[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

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

other compounds, such as acetic acid (772 vs 2750 mg/kg), caryophyllene (4.51 vs 15.4 mg/kg), propanoic acid (2.26 vs 16.7 mg/kg), 2-methylbutanoic acid (1200 vs 7860 μg/kg), 3-methylbutanoic acid (1120 vs 10 600 μg/kg), aromadendrene (178 vs 8230 μg/kg), phenylacetic acid (90.8 vs 1140 μg/kg), linalool (55.4 vs 301 μg/kg), 2-phenylethanol (50.6 vs 2150 μg/kg), and 2-isopropyl-3-methoxypyrazine (29.1 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

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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 (400 000), followed by dimethyl sulfide (47 000), 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 (170 000), dimethyl sulfide (37 000), 2-isopropyl-3-methoxypyrazine (35 000), 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) and (*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).

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

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 odorants, except methylpyrazine, identified in raw green *T. sinensis* in the first part of the actual study could be identified again in blanched green *T. sinensis*. 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* and Calculation of OAVs. To obtain 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 the internal standard method and their OAVs were calculated. Compared to raw green *T. sinensis*, the concentrations of dimethyl sulfide (14 000 μg/kg (raw) vs 1710 μg/kg (blanched)), (*E*)-2-hexenal (3290 vs 433 μg/kg), 2-methylbutanal (2470 vs 213 μg/kg), hexanal (1880 vs 329 μg/kg), 3-methylbutanal (723 vs 18.3 μg/kg), (*E*)-2-hexen-1-ol (340 vs 31.9 μg/kg), and 4-ethylphenol (3.92 vs 0.53 μg/kg) decreased by 83−97% (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 the three isomers 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-methylbuta-

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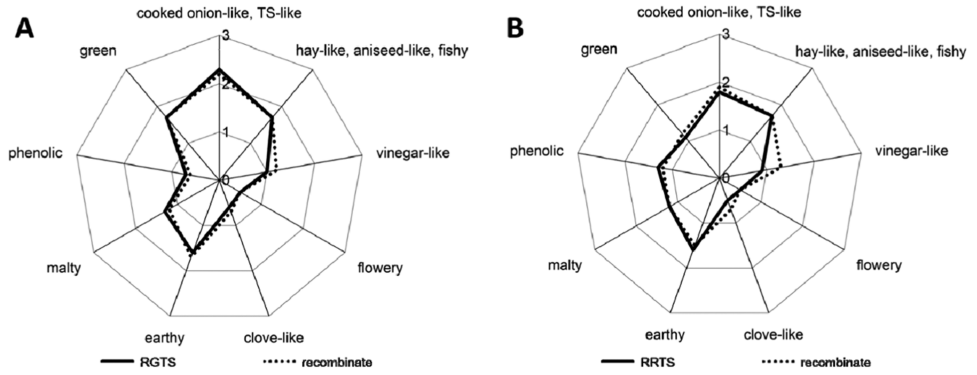

Figure 3. Aroma profiles of raw green *T. sinensis* (solid line) and the respective recombinate (broken line) (A), and aroma profiles of raw red *T. sinensis* (solid line) and the respective recombinate (broken line) (B).

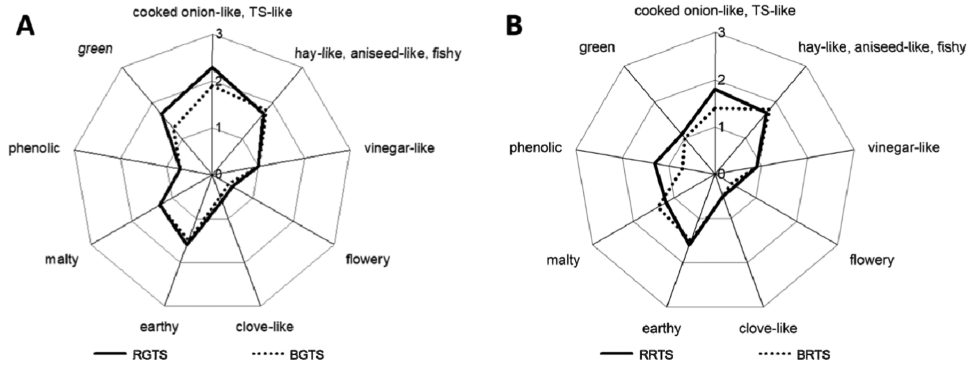

Figure 4. Aroma profiles of raw green *T. sinensis* (solid line) and blanched green *T. sinensis* (broken line) (A), and aroma profiles of raw red *T. sinensis* (solid line) and blanched red *T. sinensis* (broken line) (B).

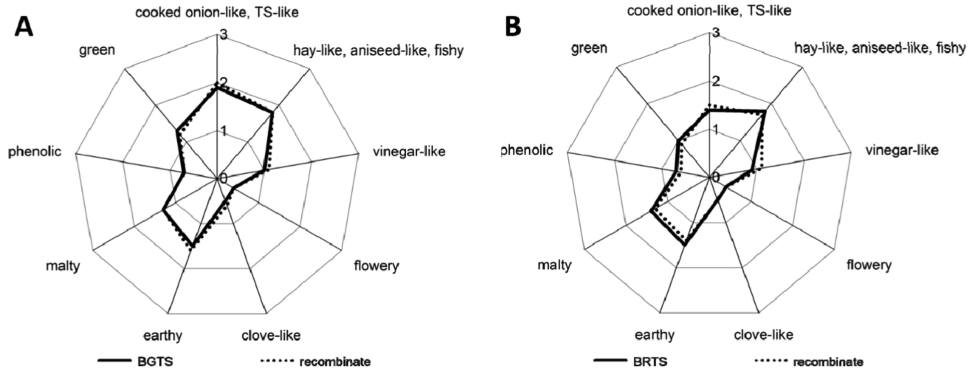

Figure 5. Aroma profiles of blanched green *T. sinensis* (solid line) and the respective recombinate (broken line) (A), and aroma profiles of blanched red *T. sinensis* (solid line) and the respective recombinate (broken line) (B).

noate. 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 320 000, 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 that the processing step resulted in clearly lower OAVs for di-1-propenyl disulfide (three isomers), dimethyl sulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (two isomers), di-1-propenyl trisulfide (three isomers), (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, and (*E*)-2-hexen-1-ol (Table 4).

To indicate whether similar aroma losses also occur in blanched red *T. sinensis*, quantitation of the same aroma compounds as analyzed in raw red *T. sinensis* was performed (Table 3). Thereby, the same trends as already seen for green *T. sinensis* were found for the abovementioned odorants. Monitoring the changes in the concentrations of 2-methoxyphenol (−60%) and 4-ethylphenol (−70%) indicated that their clear decreases caused the loss in intensity of the phenolic odor attribute after blanching. Further, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, hexanal, *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and dimethyl sulfide showed high losses of 99, 83, 59, 63, and 46%, respectively. Also, the amounts of di-1-propenyl disulfide (three isomers), di-1-propenyl trisulfide

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Table 6. Triangle Tests for Spiking Experiments of Raw Green and Red *T. sinensis* Compared to Blanched Green and Red *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	raw green TS	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		blanched green TS + green	7/16	$p = 0.3$
S4		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	raw red TS	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		blanched red TS + green	8/16	$p = 0.2$
S12		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.

(three isomers), and (*E*,*Z*)-2,6-nonadienal decreased. Additionally, most other analyzed compounds, such as 2-methylbutanoic acid, 3-methylbutanoic acid, isocaryophyllene, 3-methylbutanal, butyrolactone, benzyl alcohol, aromadendrene, 2-phenylethanol, 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, methylpyrazine, and methyl 2-methylbutanoate, decreased by >65%. On the other hand, identical to the green variety, nonanal (+14%), 3-methylnonane-2,4-dione (+4%), (*E*,*E*)-2,4-decadienal (+62%), and 1-octen-3-one (+141%) were again the only four compounds present at higher concentrations after blanching.

Aroma Profiles and Recombinates of Blanched Green and Red *T. sinensis*. To clarify the changes in the quantitative pattern of the key odorants and in the overall aroma occurring during the blanching process of *T. sinensis*, APA of blanched green and red *T. sinensis* was performed. 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* (Figure 5A,B). 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, 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 differentiate 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 are the crucial factor for the change in 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-

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1-propenyl trisulfide as well as the aldehydes (*E*,*Z*)-2,6-nonadienal, hexanal, and (*E*)-2-hexenal, and the alcohol (*E*)-2-hexen-1-ol for the overall aroma of both *T. sinensis*. In addition, the two phenolic compounds 2-methoxyphenol and 4-ethylphenol were proven to be key odorants of red *T. sinensis*.

Possible Sources of Key Odorants in *T. sinensis*. *Sulfur-Containing Compounds.* (*E*,*Z*)- and (*Z*,*Z*)-Di-1-propenyl trisulfide were identified for the first time as aroma-active compounds in *T. sinensis*. In addition, eight sulfur-containing compounds including (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiopnene, and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide were quantitated for the first time in *T. sinensis* via SIDA and showed extremely high OAVs in both *T. sinensis*, causing the cooked onion-like/TS-like odor note. Hydrogen sulfide and methyl thiirane, recently reported in *T. sinensis* by Yang et al.,[7] were not found as key odorants in this study, neither in the raw nor in the blanched samples, which was additionally confirmed by the very good similarities of all recombinates to the respective original samples.

Di-1-propenyl disulfides and di-1-propenyl trisulfides, also contributing to the characteristic aroma of onion, garlic, and other Alliaceae, are usually formed during crushing by a very fast enzymatic degradation of *S*-(1-propenyl)-L-cysteine sulfoxide (precursor) cleaved by allinase, followed by a cascade of chemical reactions.[34,35] However, Li et al. reported that nonvolatile precursors of di-1-propenyl disulfides and di-1-propenyl trisulfides in *T. sinensis* are different from those in Alliaceae.[36] Based on their findings, *T. sinensis* contains metabolites with *S*-alk(en)ylnorcysteine moieties such as (*S*,*S*)-γ-glutamyl-(*cis*-*S*-1-propenyl)thioglycine and (*S*,*S*)-γ-glutamyl-(*trans*-*S*-1-propenyl)thioglycine, which may release thiols via cleavage of the amide bond by proteases of *T. sinensis*, followed by a spontaneous decomposition of the resulting unstable alk(en)yl norcysteine moiety. Moreover, 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] 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 a 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 of 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* and an OAV of 10 for blanched 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 blanching process.

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Notes

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8.1.3 Summary and individual contributions

Due to various nutrient contents and unique pleasant aroma, *Toona sinensis* (TS), which has a red and green variety, is a popular seasoning vegetable in China. The aim of the study was first to elucidate the differences in key aroma-active compounds between green and red TS buds, and secondly, to reveal the molecular background of aroma changes occurring during blanching.

Application of cAEDA and cSH-ADA revealed 52 odorants in raw green TS and 54 odorants in raw red TS in the flavor dilution (FD) factor range between 8 and 4096. High FD factors in both varieties were found for (*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, *β*-ionone, eugenol, and 2-isopropyl-3-methoxypyrazine. Among them, the sulfur-containing compounds revealed clear differences in FD factors in both samples. 1-Octen-3-one, nonanal, 2,3,5-trimethylpyrazine, (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-decadienal, 2-methoxyphenol, and 4-ethylphenol were identified as aroma-active compounds in TS for the first time. In addition, obvious differences of FD factors were also found for acetic acid, (*E*,*Z*)-2,6-nonadienal, hexanal, (*E*)-2-hexenal, 2-methoxyphenol, and 4-ethylphenol. In both blanched samples, the same compounds were identified; however, most of them revealed lower FD factors compared to those in the respective raw samples.

To verify the results of cAEDA, 51 selected odorants were quantitated/semiquantitated in all four samples by stable isotope dilution analysis (SIDA) / internal standard method. Quantitation data confirmed the results of the odorant screening and revealed concentrations between 0.53 µg/kg and 2.75 g/kg. During the blanching process, clear reductions of nearly all aroma-active compounds were found for both green and red TS, which was also consistent with the screening results. Clear differences between green and red TS in aroma profiles, flavor dilution factors, quantitative data, and odor activity values proved that on the one hand, (*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 typical cooked onion-like/TS-like odor note. On the other hand, hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal led to the green odor note in green TS, while 2-methoxyphenol and 4-ethylphenol contributed to the intense phenolic aroma note in red TS.

To get a deeper insight into the molecular background of aroma changes occurring during blanching, spiking experiments to blanched TS were performed and confirmed that the loss of the abovementioned sulfur-containing compounds, aldehydes, (*E*)-2-hexen-1-ol, and phenols were responsible for the changes in the overall aroma profile during blanching.

Miss Xiaoting Zhai designed and performed all experiments including isolations of di-1-propenyl disulfide and di-propenyl trisulfide via preparative HPLC, volatiles isolation, HRGC-O screenings, identification, quantitation, and sensory experiments. Miss Zhai evaluated all results and prepared the manuscript. Prof. Dr. Michael Granvogl guided Miss Zhai's work, conceived and directed the study, and revised the manuscript. In addition, Prof. Granvogl participated in the sensory tests.

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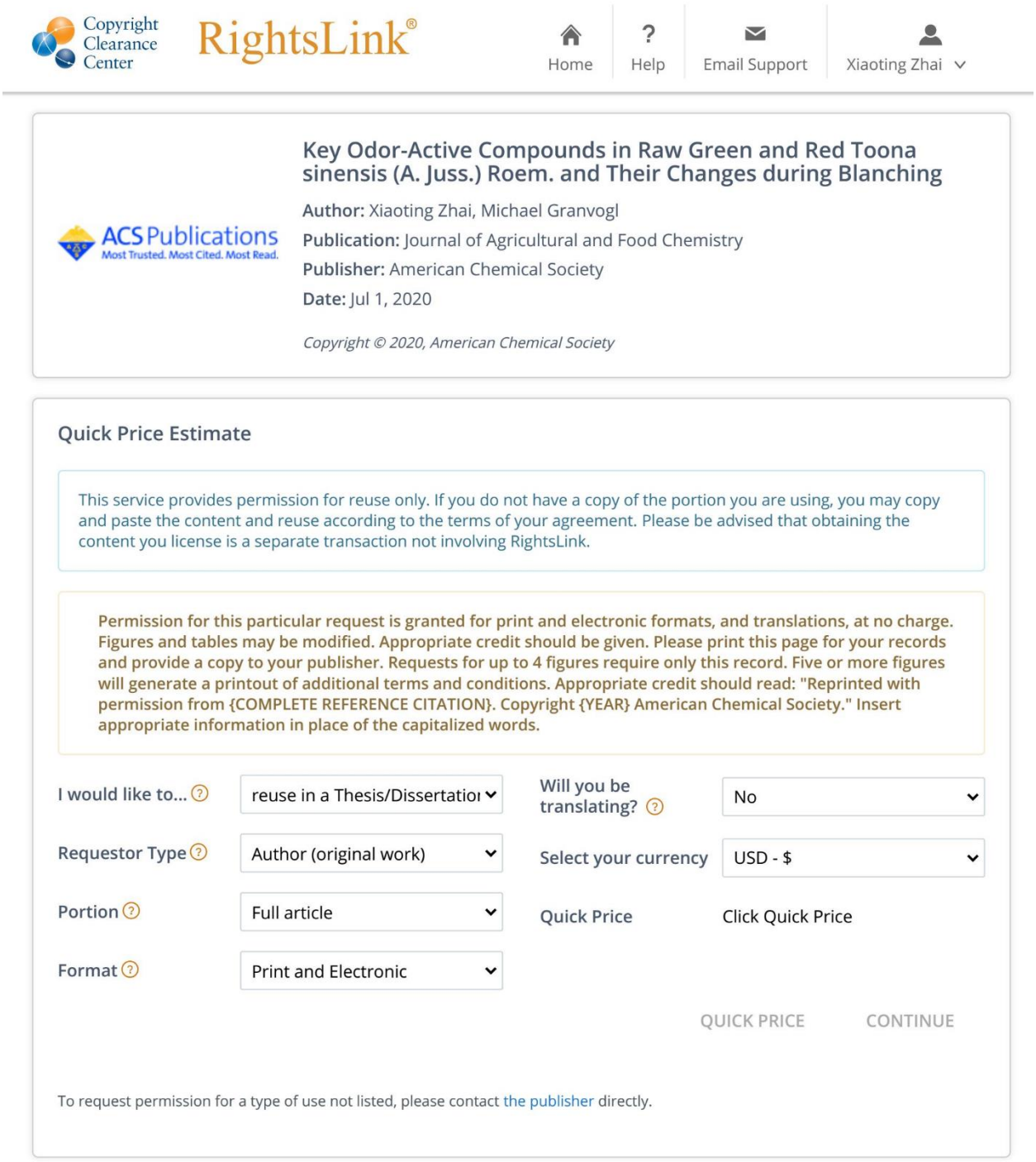

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Key Odor-Active Compounds in Raw Green and Red Toona sinensis (A. Juss.) Roem. and Their Changes during Blanching

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8.2 Publication 2: Elucidation of the Impact of Different Drying Methods on the Key Odorants in *Toona sinensis* (A. Juss.) Roem. Using the Sensomics Approach

8.2.1 Bibliographic data

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8.2.2 Publication reprint

A reprint of publication 2, elucidation of the impact of different drying methods on the key odorants in *Toona sinensis* (A. Juss.) Roem. using sensomics approach, follows starting with the next page.

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Elucidation of the Impact of Different Drying Methods on the Key Odorants of *Toona sinensis* (A. Juss.) Roem. Using the Sensomics Approach

Xiaoting Zhai and Michael Granvogl*

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ABSTRACT: The sensomics approach was applied to both green and red *Toona sinensis* (TS) varieties dried with different methods (freeze drying, solar drying, and oven drying) to elucidate their influences on the key odorants in TS. Odorant screening via comparative aroma dilution analysis revealed eugenol with the highest flavor dilution factor in all six samples. Quantitation of 44 odorants via stable isotope dilution assays and semiquantitation of six compounds via an internal standard method showed (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, dimethyl sulfide, *β*-ionone, eugenol, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, 2- and 3-methylbutanal, and 2-isopropyl-3-methoxypyrazine with high odor activity values (OAVs) in all samples. Differences were found for (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide, 3-methylnonane-2,4-dione, and (*E*)-2-hexenal with clearly higher OAVs in freeze-dried (FDTS) and solar-dried TS (SDTS) compared to those in oven-dried TS (ODTS). Linalool, 2-methoxyphenol, and 4-ethylphenol (the latter two only for red TS) were obtained with high OAVs only in FDTS. In general, ODTS showed the lowest OAVs, whereas FDTS as the gentlest drying process revealed the highest OAVs for most odorants and, consequently, the highest aroma quality. However, the overall aroma of SDTS did not differ too much from that of FDTS, and thus, solar drying as a much easier and cheaper technique might be the best choice.

KEYWORDS: *Toona sinensis, sensomics concept, aroma extract dilution analysis, stable isotope dilution analysis, odor activity values, aroma recombination, drying process*

INTRODUCTION

Fresh young sprouts, picked in early spring from *Toona sinensis* (A. Juss.) Roem. tree, are a popular seasonal vegetable in China owing to its beneficial nutrient contents (e.g., vitamins and trace elements) and unique pleasant aroma.[1,2] Up to now, >800 billion kg of only fresh *T. sinensis* and, in addition, various *T. sinensis* products were consumed in China every year.[3] Generally, the first sprouts possess the best aroma and the lowest contents of nitrites. Two weeks after emerging, the tender leaves are not edible anymore since they become fibrous and enriched in nitrites. To remove these toxicologically relevant nitrites from the sprouts, blanching in boiled water is an essential step before cooking. Nowadays, *T. sinensis* buds are mainly used in salads, stir-fried with other food ingredients, or boiled within soups. Usually, fresh *T. sinensis* sprouts are mainly offered in vegetable markets because of their high water content and, thus, the intolerance to long storage. Consequently, due to popularity and extremely short harvest period, drying processes of tender *T. sinensis* leaves are gaining more and more attention by producers to make them a product available all year.

However, during the drying process, e.g., traditional natural solar drying and oven drying, the original aroma and further bioactive compounds might be degraded. Such drying methods at elevated temperatures may not only reduce the final food quality but may also result in products not easy to rehydrate.[4] In contrast, freeze-drying—a very gentle drying method based on the sublimation phenomenon at low temperature—is a novel process for *T. sinensis* producers. Previous studies on other foods, e.g., apples, strawberries, or mushrooms,[5−7] showed that freeze drying could lead to a final food product of excellent quality.

During the last two decades, >200 volatiles have been identified in *T. sinensis* via headspace solid phase microextraction−gas chromatography−mass spectrometry (HS-SPME−GC−MS), gas chromatography−mass spectrometry (GC−MS), and gas chromatography−olfactometry (GC−O).[8−12] Among them, sulfides including di-1-propenyl disulfides, di-1-propenyl trisulfides, dimethyl sulfide, and methyl thiirane, as well as cooked onion-like/*T. sinensis*-like (TS-like) smelling *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, were reported as potent major contributors to the characteristic aroma of raw *T. sinensis* (Shanxi, Beijing, and Anhui; China).[10−12] Besides, Yang et al.[11] reported that hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol were responsible for the unique and pleasant flavor of fresh *T.*

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sinensis (Beijing, China). Very recently, the molecular sensory science concept was applied to both raw green and red *T. sinensis* (Anhui, China) for the first time. Zhai and Granvogl[12] showed 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 evoked the typical sulfury odor note. Further, hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*E*,*Z*)-2,6-nonadienal were responsible for the green odor note in green *T. sinensis*, while 2-methoxyphenol and 4-ethylphenol led to the intense phenolic aroma note in red *T. sinensis*.

To the best of our knowledge, until now, only a few studies evaluated the influence of different drying methods on the volatiles of *T. sinensis*.[13−15] Chen et al.[13] determined 14 volatiles of *T. sinensis* (Tianjin, China) including α-pinene, aromadendrene, and caryophyllene via HS-SPME−GC−MS. In their study, natural solar drying led to more volatiles but in lower amounts, whereas vacuum drying ended up with higher contents of the original volatiles. Wang et al.[14] verified 49 volatiles in vacuum-dried leaves and 41 volatiles in spray-dried leaves (Henan, China). Further, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene was identified in vacuum-dried leaves via HS-SPME−GC−MS. Very recently, Zhai and Granvogl[15] unraveled the overall aroma of both vacuum-dried and solar-dried commercial *T. sinensis* products, cultivated in Hubei and Anhui Province, China. Applying the molecular sensory science concept, 2-isopropyl-3-methoxypyrazine, eugenol, β-ionone, 3-methylnonane-2,4-dione, 3-methylbutanal, 2-methylbutanal, dimethyl sulfide, linalool, and hexanal were proven to be key odorants.[15] However, in comparison with raw samples, some typical sulfur-containing compounds, aldehydes, and phenols with low odor thresholds were not identified in the two commercially blanched and dried samples.[12,15]

Until now, no comprehensive approach including systematic sensory experiments has been used to clarify the effects of different raw materials and drying methods on the aroma components predominantly contributing to the overall aroma of dried *T. sinensis*.

Thus, the aim of the present study was to characterize the key aroma-active compounds in two varieties of *T. sinensis* buds (green and red *T. sinensis*) treated with different drying methods (freeze drying, solar drying, and oven drying) to elucidate the influence of the drying process on the final aroma of dried *T. sinensis* leaves by means of the molecular sensory science concept. Thereby, the key odorants were (i) identified by comparative aroma extraction dilution analysis (cAEDA) based on GC−O combined with GC−MS and (ii) quantitated by stable isotope dilution assays (SIDAs) as well as semiquantitated by an internal standard method. Next, (iii) odor activity values (OAVs) were calculated, and finally, (iv) the overall aroma profiles of the respective samples were simulated by recombination experiments.

MATERIALS AND METHODS

Materials. Fresh green and red *T. sinensis* buds were purchased from a local vegetable market (Anhui, China) in April 2018. Freeze-dried *T. sinensis* (FDTS) was obtained after freeze drying (Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) the fresh *T. sinensis* buds. Solar-dried *T. sinensis* (SDTS) was prepared by drying the fresh buds in sunlight for 3 days. Oven-dried *T. sinensis* (ODTS) was obtained by drying the fresh buds in a drying oven (40 °C) for two days. All six dried *T. sinensis* samples were frozen with liquid nitrogen, crashed into small pieces, and then powdered by a SPEX SamplePrep 6870 Freezer/Mill (Metuchen, NJ). Finally, the powder was filled into brown glass bottles and stored at −24 °C prior to analysis.

Reference Odorants. The following reference odorants were commercially available: acetic acid, 2-acetylpyrrole, benzyl alcohol, caryophyllene oxide, (*E*,*E*)-2,4-decadienal, decanoic acid, 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, trimethylamine, 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, 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); 3-methylnonane-2,4-dione (Chemos, Regenstauf, Germany), 2-pyrrolidone (TCI, Eschborn, Germany); and vanillin (Merck, Darmstadt, Germany). 2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene was a gift from Firmenich (Geneva, Switzerland).

(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-Di-1-propenyl disulfide and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide were isolated from raw green *T. sinensis* buds as previously described.[12]

Chemicals. Ethanol, hydrochloric acid, sodium carbonate, sodium chloride, and sodium sulfate were from Merck. *n*-Alkanes (C_6−C_{26}) and methyl octanoate were obtained from Sigma-Aldrich. Dichloromethane, diethyl ether, and 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 synthesized as previously described: [$^2H_{3\text{-}5}$]-(*E*,*E*)-2,4-decadienal,[16] [$^2H_6$]-dipropyl disulfide,[12,17] [$^2H_6$]-dipropyl trisulfide,[12,17] [$^2H_5$]-2-ethyl-3,5-dimethylpyrazine,[18] [$^2H_{3\text{-}4}$]-4-ethylphenol,[19] [$^2H_{4\text{-}6}$]-hexanal,[20] [$^2H_2$]-(*E*)-2-hexenal,[21] [$^2H_2$]-(*E*)-2-hexen-1-ol,[16] [$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one,[22] [$^2H_3$]-β-ionone,[23] [$^2H_3$]-2-isopropyl-3-methoxypyrazine analogue to [$^2H_3$]-2-isobutyl-3-methoxypyrazine,[24] [$^2H_{2\text{-}3}$]-linalool,[25] [$^2H_2$]-2-methylbutanal,[26] [$^2H_{6\text{-}8}$]-5-methyl-2-methoxyphenol analogue to [$^2H_{2\text{-}4}$]-4-propyl-2-methoxyphenol,[27] [$^2H_3$]-methyl 2-methylbutanoate,[20] [$^2H_3$]-3-methylnonane-2,4-dione,[21] [$^2H_2$]-(*E*,*Z*)-2,6-nonadienal,[21] [$^2H_{2\text{-}3}$]-γ-nonalactone,[28] [$^2H_4$]-nonanal,[29] [$^2H_{2\text{-}3}$]-γ-octalactone,[30] [$^2H_{3\text{-}6}$]-1-octen-3-ol,[31] [$^2H_{3\text{-}4}$]-2,3,5-trimethylpyrazine,[25] and [$^2H_3$]-vanillin.[27]

Isolation of the Volatiles. *T. sinensis* powder obtained (5 g) was extracted with dichloromethane (3 x 50 mL) by vigorously stirring (3 × 0.5 h) at room temperature. The combined organic extracts were subjected to high vacuum distillation by means of the solvent assisted flavor evaporation (SAFE) technique[32] to separate the volatiles from the nonvolatile fraction. The SAFE distillate obtained was dried with anhydrous sodium sulfate and further concentrated to ∼1 mL using a Vigreux column (50 cm × 1 cm id) and a microdistillation device. Afterward, the distillate was used for cAEDA as recently described.[15]

High-Resolution Gas Chromatography−Olfactometry/Flame Ionization Detection (HRGC−O/FID). HRGC−O/FID was performed as recently described for the detection of the respective odor qualities and odor intensities in parallel to the FID chromatogram.[12] A series of *n*-alkanes (C_6−C_{26} (DB-FFAP) and C_6−

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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] analyte	ion (m/z)[a] standard	R_f[b]	system[c]
acetic acid	[$^{13}C_2$]-acetic acid	61	63	1.00	I
benzyl alcohol	[$^2H_5$]-benzyl alcohol	91	96	0.87	I
butyrolactone[d]	[$^2H_{2-3}$]-γ-octalactone[d]	87	145 + 146[d,e]	1.12	II
(*E*,*E*)-2,4-decadienal	[$^2H_{3-5}$]-(*E*,*E*)-2,4-decadienal	153	156−158[e]	0.97	II
decanoic acid	[$^2H_{2-3}$]-decanoic acid	187	189 + 190[e]	0.93	II
dimethyl sulfide	[$^2H_6$]-dimethyl sulfide	63	69	0.96	III
(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide[f]	[$^2H_6$]-dipropyl disulfide[f]	147	157[f]	1.00	II
(*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl trisulfide[g]	[$^2H_6$]-dipropyl trisulfide[g]	179	189[g]	0.72	II
2-ethyl-3,5-dimethylpyrazine	[$^2H_5$]-2-ethyl-3,5-dimethylpyrazine	137	142	1.00	II
4-ethylphenol	[$^2H_{3-4}$]-4-ethylphenol	123	126 + 127[e]	1.00	II
eugenol[h]	[$^2H_4$]-*cis*-isoeugenol[h]	165	169[h]	0.67	I
hexanal	[$^2H_{4-6}$]-hexanal	101	105−107[e]	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	II
(*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[e]	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[e,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
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
methyl 2-methylbutanoate	[$^2H_3$]-methyl 2-methylbutanoate	117	120	0.98	II
3-methylnonane-2,4-dione	[$^2H_3$]-3-methylnonane-2,4-dione	171	174	0.95	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[e]	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[e]	0.75	II
1-octen-3-one	[$^2H_{3-5}$]-1-octen-3-one	127	130−132[e]	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
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[e]	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 the mixtures of known amounts of the unlabeled analyte and the corresponding labeled internal standard. [c]I, HRGC−MS(CI); II, HRGC/HRGC−MS(CI); III, HS-SPME−HRGC−MS(CI). [d]Butyrolactone was quantitated using [$^2H_{2-3}$]-γ-octalactone as the internal standard. [e]Internal standard was used as a mixture of isotopologues. [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 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.

C_{18} (DB-5), respectively) was used to determine linear retention indices (RIs) for each compound on the abovementioned two capillary columns of different polarities.[33]

Comparative Aroma Extract Dilution Analysis (cAEDA). The concentrated SAFE distillates were analyzed by three experienced panelists via HRGC−O to avoid a potential overlooking of odor-active compounds. The distillate was stepwise diluted with dichloromethane (1 + 1, v + v), and the original distillate as well as all the dilutions were analyzed until no odorant was perceivable at the sniffing port to determine the flavor dilution (FD) factor of each analyte.

Comprehensive High-Resolution Gas Chromatography−Time-of-Flight Mass Spectrometry (HRGC×HRGC−TOF-MS) and High-Resolution Gas Chromatography−Mass Spectrometry (HRGC−MS) for Identification. In addition to the RIs determined for each odorant on DB-FFAP and DB-5 capillary columns and their odor qualities and intensities perceived at the sniffing port, mass spectra, obtained both in electron ionization (EI) mode at 70 eV at a rate of 100 spectra/s by HRGC×HRGC−TOF-MS and in chemical ionization (CI) mode at 105 eV using methanol as the reagent gas by HRGC−MS, were used to compare the data

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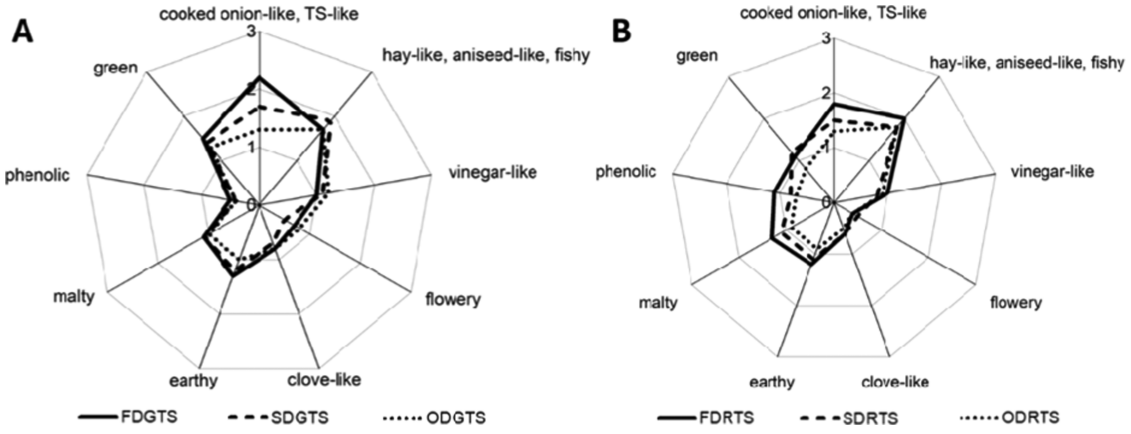

Figure 1. Aroma profile analyses of FDGTS (solid line), SDGTS (broken line), and ODGTS (dotted line) (A) and aroma profile analyses of FDRTS (solid line), SDRTS (broken line), and ODRTS (dotted line) (B).

obtained from the samples to the data available in an in-house database containing >1000 odorants.[12]

Comparative Static Headspace Aroma Dilution Analysis (cSH-ADA) via Static Headspace High-Resolution Gas Chromatography−Olfactometry (SH-HRGC−O) and Identification Experiments via Static Headspace High-Resolution Gas Chromatography−Mass Spectrometry (SH-HRGC−MS). For an unequivocal identification of highly volatile aroma-active compounds, cSH-ADA via SH-HRGC−O and SH-HRGC−MS was used as previously reported.[15]

Quantitation of Odorants by Stable Isotope Dilution Assays (SIDAs). Defined amounts of stable isotopically labeled standards (amounts depending on concentrations of the respective analyte determined in preliminary experiments) and dichloromethane (30−200 mL) were added to *T. sinensis* powder (1−15 g). After equilibration, the sample was worked-up as described for isolation of the volatiles. The concentrated SAFE distillate was used for HRGC−MS or two-dimensional heart-cut high-resolution gas chromatography−mass spectrometry (HRGC/HRGC−MS). Mass spectra were recorded in CI mode at 105 eV using methanol as the reagent gas.

The concentrations of acetic acid, benzyl alcohol, eugenol, hexanoic acid, β-ionone, 2-phenylethanol, propanoic acid, and 2-pyrrolidone were determined by HRGC−MS.[12] A Varian gas chromatograph 431 (Darmstadt, Germany) was equipped with a DB-FFAP capillary column (30 m × 0.25 mm id, 0.25 μm film thickness; J&W Scientific; Agilent Technologies, Waldbronn, Germany). Aliquots of the samples (2 μL) were injected by a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland). The initial temperature was held at 40 °C for 2 min, raised with 6 °C/min to finally 230 °C, and then held for 10 min. Mass spectra were recorded by an ion trap mass spectrometer 220-MS (Varian) running in CI mode at 70 eV with methanol as the reagent gas.

The remaining compounds were quantitated by means of HRGC/HRGC−MS. The instrument consisted of a TRACE GC 2000 (ThermoQuest, Egelsbach, Germany) coupled to a gas chromatograph CP-3800 (Varian) and a Saturn 2000 ion trap mass spectrometer (Varian) as recently described.[12] The first GC was equipped with a DB-FFAP capillary column (30 m × 0.32 mm id, 0.25 μm film thickness; J&W Scientific) and the second GC with a DB-1701 capillary column (30 m × 0.25 mm id, 0.25 μm film thickness; J&W Scientific).

Quantitation of dimethyl sulfide, 2-methylbutanal, and 3-methylbutanal was performed by static headspace solid phase microextraction combined with high-resolution gas chromatography−mass spectrometry (HS-SPME−HRGC−MS).[12] The sample (0.1−0.3 g) was weighed into a 20 mL headspace vial, defined amounts of [$^2H_6$]-dimethyl sulfide (~1 μg) and [$^2H_2$]-2-methylbutanal (~1.2 μg; both dissolved in ethanol; amounts depending on concentrations of the respective analytes determined in preliminary experiments) were added, and the vial was immediately sealed with a gastight septum. After equilibration by continuous stirring for 30 min at 35 °C, the samples were analyzed via a gas chromatograph 7890B (Agilent Technologies) coupled to an ion trap mass spectrometer Saturn 2200 (Varian). Mass spectra were recorded in CI mode at 105 eV using methanol as the reagent gas.

Response factors (R_f) of all compounds were accordingly determined by either HRGC−MS, HRGC/HRGC−MS, or HS-SPME−HRGC−MS 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).

High-Resolution Gas Chromatography−Flame Ionization Detection (HRGC−FID) for Semiquantitation. For the semiquantitation of aromadendrene, β-caryophyllene, caryophyllene oxide, α-humulene, isocaryophyllene, and valencene, HRGC−FID was performed on a Trace GC Ultra (ThermoQuest) using the internal standard method as recently described (internal standard: cyclopentadecanone).[15]

Aroma Profile Analysis (APA). For a comparative APA, dried *T. sinensis* samples were presented in covered odorless Teflon vessels (id = 40 mm) and orthonasally evaluated by 15−20 weekly trained sensory panelists able to describe and recognize odor qualities and, thus, to perform a comparative APA, in a climate-controlled (21 ± 1 °C) sensory room equipped with individual booths. The odor descriptors used in sensory experiments were defined on the basis of the odor of aqueous solutions of each reference compound at concentrations 50-fold above the respective odor threshold: 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). The sensory panelists evaluated the odor intensities of each aroma attribute for all *T. sinensis* samples from 0 (not perceivable) to 3 (strongly perceivable) on a seven-point linear scale by steps of 0.5.

Aroma Recombination Experiments. The respective dried *T. sinensis* (10 g each) was extracted with dichloromethane several times until the residue was odorless. Then, the aroma model was prepared in this odorless residue homogenized with odorless refined sunflower oil (10 g/kg)[34] containing all odorants with OAVs ≥ 1 in the concentrations determined in each sample. The dried samples (1 g each) and the respective recombinates were placed into covered odorless Teflon vessels, and the aroma profiles were evaluated according to the same procedure as mentioned above for APA.

RESULTS AND DISCUSSION

Aroma Profiles of Differently Dried *T. sinensis*. To get a first impression of the differences in the overall aroma

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Table 2. Aroma-Active Compounds in Differently Dried Green and Red *T. sinensis*, Their Odor Qualities, Retention Indices (RIs), and Flavor Dilution (FD) Factors

			RIs[a]		FD factors[b]					
no.[c]	odorant[d]	odor quality[e]	DB-FFAP	DB-5	FDGTS	SDGTS	ODGTS	FDRTS	SDRTS	ODRTS
1	dimethyl sulfide[f]	cabbage-like	nd[g]	511	32	16	8	32	16	16
2	3-methylbutanal[f]	malty	933	652	16	8	4	32	16	8
3	2-methylbutanal[f]	malty	934	657	16	8	4	32	16	8
4	methyl 2-methylbutanoate	fruity	1006	775	8	4	2	16	8	4
5	α-pinene	resin-like, fir needle-like	1010	939	8	16	1	8	8	4
6	hexanal	green, grassy	1090	769	64	32	32	64	64	32
7	methyl hexanoate	fruity, musty	1199	922	2	4	2	8	2	4
8	limonene	citrus-like, carrot-like	1206	1030	4	2	2	2	4	2
9	(*E*)-2-hexenal	green apple-like	1216	851	32	32	4	16	16	4
10	methylpyrazine	roasty	1273	822	2	nd[g]	nd[g]	2	2	1
11	1-octen-3-one	mushroom-like	1293	979	32	32	8	16	8	8
12	nonanal	citrus-like, soapy	1381	1103	16	4	2	4	2	4
13	2-ethyl-6-methylpyrazine	roasty	1382	1001	2	2	nd[g]	2	4	2
14	2-ethyl-3-methylpyrazine	roasty	1382	1010	2	2	nd[g]	2	4	2
15	(*E*)-2-hexen-1-ol	green, fruity	1403	860	8	4	2	64	16	16
16	2,3,5-trimethylpyrazine	earthy	1410	1003	32	16	4	16	8	8
17	2-isopropyl-3-methoxypyrazine	earthy, pea-like	1421	1094	64	128	16	128	128	16
18	2-ethyl-3,5-dimethylpyrazine	earthy	1430	1079	32	32	8	128	64	8
19	acetic acid	vinegar-like	1441	612	64	32	8	64	32	32
20	1-octen-3-ol	mushroom-like	1442	975	2	8	2	4	16	4
21	(*E*,*E*)-di-1-propenyl disulfide	roasted onion-like	1448	1121	2048	1024	32	2048	1024	128
22	(*E*,*Z*)-di-1-propenyl disulfide	roasted onion-like	1467	1130	2048	1024	32	2048	1024	128
23	(*Z*,*Z*)-di-1-propenyl disulfide	roasted onion-like	1490	1142	2048	1024	32	2048	1024	128
24	propanoic acid	sour, sweaty	1535	706	4	2	2	2	4	4
25	linalool	citrus-like, flowery	1539	1100	32	4	4	128	16	4
26	isocaryophyllene	citrus-like	1556	1407	16	4	2	64	8	2
27	(*E*,*Z*)-2,6-nonadienal	green, cucumber-like	1571	1153	64	256	16	256	256	8
28	β-caryophyellene	moldy	1577	nd[g]	8	2	2	2	8	8
29	aromadendrene	eucalyptus-like	1591	nd[g]	16	8	4	8	32	16
30	*cis*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like/TS-like	1618	1119	512	128	16	64	128	32
31	*trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	cooked onion-like/TS-like	1633	1127	512	128	16	64	128	32
			1641	nd[g]	2	4	2	4	nd[g]	2
32	butyrolactone	sweet, aromatic	1638	900	4	8	8	8	8	4
33	menthol	mint-like	1641	nd[g]	2	4	2	4	nd[g]	2
34	α-humulene	balmy	1654	1455	4	8	4	8	4	4
35	2-methylbutanoic acid	sweaty	1660	870	32	128	4	256	32	32
36	3-methylbutanoic acid	fruity, sweaty	1662	870	32	128	4	256	32	32
37	valencene	fruity, flowery	1702	1497	8	4	4	8	16	16
38	3-methylnonane-2,4-dione	hay-like, aniseed-like, fishy	1716	1251	32	64	32	128	256	64
39	(*E*,*E*)-di-1-propenyl trisulfide	cooked onion-like	1741	1341	128	64	16	64	64	16
40	(*E*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	1759	1355	128	64	16	64	64	16
41	(*Z*,*Z*)-di-1-propenyl trisulfide	cooked onion-like	1788	1378	128	64	16	64	64	16
42	(*E*,*E*)-2,4-decadienal	fatty, deep-fried	1801	1371	32	32	8	64	128	64
43	hexanoic acid	sweaty	1839	1010	2	2	1	4	1	2
44	2-methoxyphenol	smoky, phenolic	1848	1090	8	4	4	1024	32	32
45	benzyl alcohol	bitter almond-like, fruity	1873	1036	4	4	4	32	32	8
46	2-phenylethanol	flowery, honey-like	1900	1117	8	8	32	256	128	128
47	β-ionone	flowery, violet-like	1923	1488	256	512	128	1024	256	512
48	heptanoic acid	rancid, sweaty	1942	1074	2	1	1	2	nd[g]	nd[g]
49	(*E*)-3-hexenoic acid	cheese-like	1947	986	2	2	1	4	1	2
50	caryophyllene oxide	citrus-like, soapy	1969	1578	8	2	2	8	16	4
51	2-acetylpyrrole	musty	1989	1066	nd[g]	2	nd[g]	2	2	2
52	phenol	ink-like, phenolic	2016	981	2	1	nd[g]	nd[g]	2	nd[g]
53	γ-nonalactone	coconut-like	2029	1360	16	32	16	16	16	8
54	2-pyrrolidone	fruity	2054	nd[g]	2	nd[g]	nd[g]	2	4	4

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Table 2. continued

			RIs[a]		FD factors[b]					
no.[c]	odorant[d]	odor quality[e]	DB-FFAP	DB-5	FDGTS	SDGTS	ODGTS	FDRTS	SDRTS	ODRTS
55	nonanoic acid	moldy, pungent	2150	nd[g]	16	2	4	64	32	64
56	4-ethylphenol	fecal-like, phenolic	2163	1077	2	nd[g]	nd[g]	256	32	32
57	eugenol	clove-like	2167	1359	4096	2048	1024	4096	2048	1024
58	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like, spicy	2200	1108	4	2	1	4	8	4
59	decanoic acid	soapy, musty	2250	1369	4	8	8	32	32	32
60	phenylacetic acid	beeswax-like, honey-like	2552	1261	8	64	4	128	32	16
61	vanillin	vanilla-like, sweet	2571	1403	64	256	32	512	512	128

[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 (DB-FFAP capillary column) 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 GC−O. [f]FD factor was determined via SH-ADA. [g]Not detected.

between freeze-dried green *T. sinensis* (FDGTS), solar-dried green *T. sinensis* (SDGTS), and oven-dried green *T. sinensis* (ODGTS), aroma profile analyses were performed. The cooked onion-like/TS-like odor impression was present most intensely in FDGTS, while it was clearly lower in SDGTS and ODGTS. All other odor notes were very similar in their respective intensities within the three *T. sinensis* samples (Figure 1A).

To get knowledge about the role of these odor attributes in different varieties of dried *T. sinensis*, APA was also performed for the three dried red *T. sinensis*. Cooked onion-like/TS-like and hay-like/aniseed-like/fishy were the two major odor attributes described in all dried red *T. sinensis* samples and nearly all odor notes were evaluated highest in freeze-dried red *T. sinensis* (FDRTS). Interestingly, similar intensities were found for the attributes earthy and green in FDRTS and SDRTS, while they were clearly lower in ODRTS. The remaining odor notes showed the same trends as in dried green *T. sinensis* (Figure 1B).

Odorant Screening in Differently Dried Green and Red *T. sinensis*. A total of 61 aroma-active compounds detected by HRGC−O and SH-HRGC−O were successfully identified in at least one of the six dried samples by comparison of their retention indices determined on two capillary columns of different polarities, their odor qualities and intensities perceived at the sniffing port, and their mass spectra obtained in EI and CI mode to data of the respective reference compounds. Using cAEDA and cSH-ADA, 32 odorants in FDGTS, 27 in SDGTS, and only 17 in ODGTS were present in the FD factor range between 16 and 4096 (Table 2).

In FDGTS, eugenol (**57**) with a clove-like odor showed the highest FD factor of 4096, followed by (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide (**21**−**23**, all roasted onion-like, all FD factors of 2048), *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (**30** and **31**, both cooked onion-like/TS-like, both FD factors of 512), and *β*-ionone (**47**, flowery/violet-like, 256).

In SDGTS, eugenol was also present at the highest FD factor of 2048, followed by the three isomers of di-1-propenyl disulfide (all 1024), *β*-ionone (512), (*E*,*Z*)-2,6-nonadienal (**27**, green/cucumber-like, 256), and vanillin (**61**, vanilla-like, 256).

In ODGTS, compounds with the highest FD factors were eugenol (1024) and *β*-ionone (128). Although eugenol showed the highest FD factor in each sample, compounds like *α*-pinene (**5**, resin-like/fir needle-like), (*E*)-2-hexenal (**9**, green apple-like), 1-octen-3-one (**11**, mushroom-like), 2,3,5-trimethylpyrazine (**16**, earthy), 2-isopropyl-3-methoxypyrazine (**17**, earthy/pea-like), 2-ethyl-3,5-dimethylpyrazine (**18**, earthy), acetic acid (**19**, vinegar-like), the three isomers of di-1-propenyl disulfide, (*E*,*Z*)-2,6-nonadienal, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, 3-methylbutanoic acid (**35**, sweaty), 2-methylbutanoic acid (**36**, fruity/sweaty), the three isomers of di-1-propenyl trisulfide (**39**−**41**, all cooked onion-like), and (*E*,*E*)-2,4-decadienal (**42**, fatty/deep-fried) were found with clearly higher FD factors in FDGTS and SDGTS compared to those in ODGTS (Table 2).

Application of cAEDA and cSH-ADA to the red *T. sinensis* variety showed in all three samples high FD factors for eugenol (**57**, 1024−4096), (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide (**21**−**23**, 128−2048), and *β*-ionone (**47**, 256−1024) (Table 2). Further compounds with high FD factors in FDRTS and SDRTS were 2-isopropyl-3-methoxypyrazine (**17**), 2-ethyl-3,5-dimethylpyrazine (**18**), (*E*,*Z*)-2,6-nonadienal (**27**), the isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (**30** and **31**), 3-methylnonane-2,4-dione (**38**, hay-like/aniseed-like/fishy), 2-phenylethanol (**46**, flowery/honey-like), and vanillin (**61**). In addition, (*E*)-2-hexen-1-ol (**15**, green/fruity), linalool (**25**, citrus-like/flowery), isocaryophyllene (**26**, citrus-like), 3-methylbutanoic acid (**35**), 2-methylbutanoic acid (**36**), 2-methoxyphenol (**44**, smoky/phenolic), *β*-ionone, and phenylacetic acid (**60**, beeswax-like/honey-like) were found with higher FD factors in FDRTS compared to those in SDRTS (Table 2).

Besides eugenol and *β*-ionone, only a few odorants, including the three isomers of di-1-propenyl disulfide, 3-methylnonane-2,4-dione, 2-phenylethanol, nonanoic acid (**55**, moldy/pungent), and vanillin were present at higher FD factors (from 64 to 128) in ODRTS. Clearly lower FD factors for several odorants, especially in comparison to those in FDRTS, were already a hint for the lowered intensities in the single aroma notes and the overall aroma of ODRTS (Table 2 and Figure 1B).

A comparison of green and red *T. sinensis* treated with the same drying method confirmed that the two phenolic smelling compounds 2-methoxyphenol (**44**) and 4-ethylphenol (**56**) showed clearly higher FD factors in red dried *T. sinensis*, which was consistent with the data of raw green and red *T. sinensis* obtained in a very recent study.[12] For example, 2-methoxyphenol and 4-ethylphenol were detected with FD

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Table 3. Concentrations of Important Aroma-Active Compounds in Differently Dried Green and Red *T. sinensis*

odorant	concentrations (μg/kg)[a]					
	FDGTS	SDGTS	ODGTS	FDRTS	SDRTS	ODRTS
acetic acid	1 690 000	690 000	817 000	5 760 000	2 580 000	4 730 000
dimethyl sulfide	44 700	28 500	7710	83 900	26 000	23 200
caryophyllene	22 300	115 000	386 000	64 800	610 000	261 000
eugenol	21 000	32 400	23 000	37 800	25 200	16 800
2-methylbutanal	10 400	3120	748	21 900	3400	2070
aromadendrene	9870	4920	13 900	183 000	32 900	49 100
propanoic acid	6300	17 400	1820	60 200	7730	8210
2-methylbutanoic acid	5390	4050	4670	13 700	1820	6210
3-methylbutanoic acid	4050	4460	4630	14 000	1790	6110
decanoic acid	3410	2010	2190	1730	1480	2360
isocaryophyllene	2530	3080	1500	69 700	5370	937
α-humulene	2510	886	1220	3370	1120	5090
β-ionone	2140	802	3090	817	680	863
valencene	1940	1090	39 900	4690	10 100	56 200
hexanal	1890	1200	347	1900	1130	1160
hexanoic acid	1860	3720	354	4430	4100	502
(*E*,*Z*)-di-1-propenyl disulfide	1790	36.5	1.95	1060	16.5	0.57
caryophyllene oxide	1720	3230	2530	2860	30 900	1120
(*E*)-2-hexenal	1460	1150	18.8	759	788	37.2
nonanoic acid	1390	946	939	2400	648	1230
3-methylbutanal	1350	1900	553	3600	2100	1150
butyrolactone	1120	1380	1400	1300	1420	1900
(*Z*,*Z*)-di-1-propenyl trisulfide	1120	148	3.40	172	81.5	6.29
2-pyrrolidone	1080	4880	654	1830	4470	1780
benzyl alcohol	1070	1240	316	4470	1220	292
(*E*)-2-hexen-1-ol	828	66.7	4.39	78.3	68.7	53.0
(*Z*,*Z*)-di-1-propenyl disulfide	824	44.0	1.70	66.6	23.8	1.03
(*E*,*E*)-di-1-propenyl disulfide	673	100	3.75	233	57.0	2.12
(*E*,*E*)-di-1-propenyl trisulfide	665	21.4	1.32	199	20.8	1.40
vanillin	463	662	75.6	495	976	122
trans-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	314	279	33.8	87.3	146	15.1
2,3,5-trimethylpyrazine	253	286	11.4	265	146	23.4
nonanal	242	145	60.8	191	134	107
(*E*,*Z*)-di-1-propenyl trisulfide	165	234	12.6	368	225	16.9
cis-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	160	89.2	19.3	12.2	31.5	3.80
2-phenylethanol	155	221	1290	10 700	834	662
phenylacetic acid	125	11 900	168	1780	6510	3050
linalool	116	16.8	8.36	657	9.55	12.0
(*E*,*Z*)-2,6-nonadienal	98.9	59.5	0.13	29.8	17.3	0.25
3-methylnonane-2,4-dione	57.1	291	13.5	70.9	217	27.8
γ-nonalactone	47.2	39.7	23.2	58.1	49.4	73.9
2-ethyl-3,5-dimethylpyrazine	35.1	52.5	41.5	243	24.4	9.36
1-octen-3-ol	27.8	431	4.50	42.9	266	2.57
2-methoxyphenol	17.5	4.62	13.7	896	82.3	26.7
(*E*,*E*)-2,4-decadienal	15.3	14.8	5.98	7.20	22.9	6.43
1-octen-3-one	14.5	5.06	0.88	3.74	5.25	0.79
2-isopropyl-3-methoxypyrazine	14.4	19.1	16.1	211	123	34.7
methyl 2-methylbutanoate	4.91	2.90	0.85	10.3	2.67	1.59
4-ethylphenol	4.02	2.14	10.6	1740	76.5	91.0
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	1.76	7.16	0.19	3.99	7.40	0.15

[a]Mean values of triplicates, differing not more than ±15%.

factors of 8 and 2 in FDGTS, while the respective FD factors were 1024 and 256 in FDRTS (Table 2).

Quantitation and Semiquantitation of Key Odorants and Calculation of Odor Activity Values (OAVs) in Differently Dried *T. sinensis*. cAEDA and cSH-ADA are appropriate screening methods to select the odorants most likely contributing to the overall aroma of foods but do not consider the influence of the food matrix on the odor release. Thus, accurate quantitative measurements were used to finally elucidate the respective key odorants. Consequently, a total of 50 aroma-active compounds, either showing high or clearly different FD factors in the dried samples or being characterized as key odorants of commercially dried *T. sinensis* products in a previous study,[15] were selected and quantitated or semi-

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

odorant	OT (μg/kg)	OAVs[a]					
		FDGTS	SDGTS	ODGTS	FDRTS	SDRTS	ODRTS
di-1-propenyl disulfide	0.0034[b]	970 000	53 000	2200	400 000	29 000	1100
dimethyl sulfide	0.3[c]	150 000	95 000	26 000	280 000	87 000	77 000
β-ionone	0.021[c]	100 000	38 000	150 000	39 000	32 000	41 000
(*E*,*Z*)-2,6-nonadienal	0.0045[c]	22 000	13 000	29	6600	3800	56
eugenol	1.8[c]	12 000	18 000	13 000	21 000	14 000	9300
2-mercapto-3,4-dimethyl-2,3-dihydrothiophene	0.039[b]	12 000	9400	1400	2600	4600	480
di-1-propenyl trisulfide	0.26[b]	7500	1600	66	2800	1300	94
2-methylbutanal	1.5[c]	6900	2100	500	15 000	2300	1400
2-isopropyl-3-methoxypyrazine	0.0039[c]	3700	4900	4100	54 000	32 000	8900
3-methylbutanal	0.5[c]	2700	3800	1100	7200	4200	2300
3-methylnonane-2,4-dione	0.046[c]	1200	6300	290	1500	4700	600
decanoic acid	3.5[c]	970	570	630	490	420	670
1-octen-3-one	0.016[c]	910	320	55	230	330	49
hexanal	2.4[c]	790	500	150	930	470	480
(*E*,*E*)-2,4-decadienal	0.027[c]	570	550	220	270	850	240
acetic acid	5600[c]	300	120	150	1000	460	850
(*E*)-2-hexen-1-ol	3.9[c]	210	17	1	20	18	14
linalool	0.58[c]	200	29	14	1100	16	21
isocaryophyllene	20[d]	130	150	75	3500	270	47
2-ethyl-3,5-dimethylpyrazine	0.28[c]	130	190	150	870	87	33
nonanal	2.8[c]	86	52	22	68	48	38
(*E*)-2-hexenal	17[c]	86	68	1	45	46	2
caryophyllene oxide	22[d]	78	150	120	130	1400	51
nonanoic acid	26[c]	53	36	36	92	25	47
valencene	66[d]	29	17	610	71	150	850
aromadendrene	337[d]	29	15	41	540	98	150
2,3,5-trimethylpyrazine	11[c]	23	26	1	24	13	2
butyrolactone	50[c]	22	28	28	26	28	38
2-methoxyphenol	0.84[c]	21	6	16	1100	98	32
α-humulene	130[d]	19	7	9	26	9	39
caryophyllene	1190[d]	19	97	320	54	510	220
vanillin	53[c]	9	12	1	9	18	2
3-methylbutanoic acid	490[e]	8	9	9	29	4	12
γ-nonalactone	9.7[c]	5	4	2	6	5	8
phenylacetic acid	68[c]	2	180	2	26	96	45
benzyl alcohol	620[c]	2	2	<1	7	2	<1
methyl 2-methylbutanoate	2.5[c]	2	1	<1	4	1	1
2-methylbutanoic acid	3100[c]	2	1	2	4	1	2
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	1.7[c]	1	4	<1	2	4	<1
2-phenylethanol	140[c]	1	2	9	76	6	5
1-octen-3-ol	45[c]	<1	10	<1	<1	6	<1
2-pyrrolidone	2100[d]	<1	2	<1	<1	2	<1
propanoic acid	16 000[c]	<1	1	<1	4	<1	<1
hexanoic acid	4800[c]	<1	<1	<1	<1	<1	<1
4-ethylphenol	13[c]	<1	<1	<1	130	6	7

[a]Odor activity values were calculated as the ratio of the determined concentrations to the respective odor thresholds in water. [b]Orthonasal odor threshold in water as reported previously.[12] [c]Orthonasal odor threshold in water from in-house database. [d]Orthonasal odor threshold in water as reported previously.[15] [e]Orthonasal odor threshold in water as reported previously.[42]

quantitated in all dried green and red *T. sinensis* via SIDA using different gas chromatography−mass spectrometry systems (Table 1) or via an internal standard method using GC−FID.

Odorant Concentrations. For green *T. sinensis*, the highest concentrations (690−1690 mg/kg) were found for acetic acid in all three samples. Dimethyl sulfide (up to 44.7 mg/kg in FDGTS), eugenol (up to 32.4 mg/kg in SDGTS), propanoic acid (up to 17.4 mg/kg in SDGTS), and 2-methylbutanal (up to 10.4 mg/kg in FDGTS) were also present at high amounts in the samples. High concentrations were further determined for the sesquiterpenes caryophyllene (22.3−386 mg/kg), aromadendrene (4.92−13.9 mg/kg), isocaryophyllene (1.50−3.08 mg/kg), α-humulene (0.89−2.51 mg/kg), valencene (1.09−39.9 mg/kg), and caryophyllene oxide (1.72−3.23 mg/kg) as well as for three further monocarboxylic acids, namely, 2- and 3-methylbutanoic acid, and decanoic acid (all ≥1.82 mg/kg) (Table 3).

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Various sulfur-containing compounds revealed specific differences in their concentrations depending on the dried samples. For example, the amounts of (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide in FDGTS (673, 1790, and 824 μg/kg) were 7−49 times higher compared to those in SDGTS (100, 36.5, and 44.0 μg/kg) and 180−920 times higher compared to those in ODGTS (3.75, 1.95, and 1.70 μg/kg). Further, the concentrations of (*E*,*E*)- and (*Z*,*Z*)-di-1-propenyl trisulfide in FDGTS (665 and 1120 μg/kg) were 7 and 31 times higher compared to those in SDGTS (21.4 and 148 μg/kg) and even several hundred times higher compared to those in ODGTS (1.32 and 3.40 μg/kg). Differences in the concentrations of *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene were not so much pronounced but followed the same pattern (Table 3).

In contrast, concentrations of phenylacetic acid, 1-octen-3-ol, and 3-methylnonane-2,4-dione were clearly higher in SDGTS compared to those in the other two samples. Overall, FDGTS revealed the highest concentrations for most of the quantitated aroma-active compounds and ODGTS showed the lowest amounts (Table 3).

In red *T. sinensis*, acetic acid revealed the highest amounts in all samples (≥2580 mg/kg). Further compounds present at high concentrations were the sesquiterpenes caryophyllene (up to 610 mg/kg in SDRTS), aromadendrene (up to 183 mg/kg in FDRTS), isocaryophyllene (up to 69.7 mg/kg in FDRTS), valencene (up to 56.2 mg/kg in ODRTS), α-humulene (up to 5.09 mg/kg in ODRTS), and caryophyllene oxide (up to 30.9 mg/kg in SDRTS). Also, the monocarboxylic acids including propanoic acid (up to 60.2 mg/kg in FDRTS), 3-methylbutanoic acid (up to 14.0 mg/kg in FDRTS), 2-methylbutanoic acid (up to 13.7 mg/kg in FDRTS), hexanoic acid (up to 4.43 mg/kg in FDRTS), nonanoic acid (up to 2.40 mg/kg in FDRTS), phenylacetic acid (up to 6.51 mg/kg in SDRTS), and decanoic acid (up to 2.36 mg/kg in ODRTS) were determined with high amounts in red *T. sinensis* samples (Table 3).

Quantitated sulfur-containing compounds mostly revealed clearly higher amounts in FDRTS, lower amounts in SDRTS, and lowest amounts in ODRTS. For example, dimethyl sulfide was present at 83.9 mg/kg in FDRTS, at 26.0 mg/kg in SDRTS, and at 23.2 mg/kg in ODRTS. (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-Di-1-propenyl disulfide (233, 1060, and 66.6 μg/kg) in FDRTS were found in amounts 3−64 times higher compared to those in SDRTS (57.0, 16.5, and 23.8 μg/kg) and 65−1860 times higher compared to those in ODRTS (2.12, 0.57, and 1.03 μg/kg). (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-Di-1-propenyl trisulfide showed 1.6−10 times higher concentrations in FDRTS (199, 368, and 172 μg/kg) compared to those in SDRTS (20.8, 225, and 81.5 μg/kg) and 21−142 times higher concentrations compared to those in ODRTS (1.40, 16.9, and 6.29 μg/kg). Interestingly, the concentrations of *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene were the highest in SDRTS (31.5 and 146 μg/kg), about 2 times higher compared to those in FDRTS (12.2 and 87.3 μg/kg) and about 10 times higher compared to those in ODRTS (3.80 and 15.1 μg/kg) (Table 3).

Further compounds showing clearly higher amounts in FDRTS compared to those in the other two samples were 2-methylbutanal, benzyl alcohol, 2-phenylethanol, linalool, 2-ethyl-3,5-dimethylpyrazine, 2-methoxyphenol, and 4-ethylphenol. In contrast, caryophyllene, caryophyllene oxide, 2-pyrrolidone, vanillin, phenylacetic acid, 3-methylnonane-2,4-dione, 1-octen-3-ol, (*E*,*E*)-2,4-decadienal, 1-octen-3-one, and 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one were present at the highest concentrations in SDRTS (Table 3).

The quantitative data of the differently dried red *T. sinensis* showed a similar pattern compared to the data of green *T. sinensis*, proving the repeatability of the drying methods on the one side and a similar influence of each method on the aroma changes on the other side.

Odor Activity Values (OAVs) of Key Aroma Compounds. To estimate the contribution of each odorant to the overall aroma, OAVs (ratio of concentration to the respective odor threshold) were calculated for all quantitated compounds in all six samples.

In FDGTS, 40 out of 50 quantitated odorants revealed OAVs ≥ 1 and consequently contributed to its overall aroma. In contrast, 43 odorants in SDGTS and only 37 in ODGTS ended up with OAVs ≥ 1. Thereby, FDGTS revealed the highest OAVs of most selected compounds, confirming the highest odor intensities already described during APA (Table 4 and Figure 1A). Specifically, in FDGTS, the isomers of di-1-propenyl disulfide showed the highest OAV (970 000), which was consistent with data obtained for raw green *T. sinensis* in a very recent study,[12] followed by dimethyl sulfide (150 000), β-ionone (100 000), (*E*,*Z*)-2,6-nonadienal (22 000), eugenol (12 000), the isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (12 000), the isomers of di-1-propenyl trisulfide (7500), 2-methylbutanal (6900), 2-isopropyl-3-methoxypyrazine (3700), 3-methylbutanal (2700), and 3-methylnonane-2,4-dione (1200) (Table 4).

In SDGTS, the highest OAV was calculated for dimethyl sulfide (95 000), followed by the isomers of di-1-propenyl disulfide (53 000), β-ionone (38 000), eugenol (18 000), (*E*,*Z*)-2,6-nonadienal (13 000), the isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (9400), 3-methylnonane-2,4-dione (6300), 2-isopropyl-3-methoxypyrazine (4900), 3-methylbutanal (3800), 2-methylbutanal (2100), and the isomers of di-1-propenyl trisulfide (1600) (Table 4). Compared to those in FDGTS, almost all quantitated odorants showed obviously lower OAVs in SDGTS, except for eugenol, 2-isopropyl-3-methoxypyrazine, 3-methylbutanal, 3-methylnonane-2,4-dione, isocaryophyllene, 2-ethyl-3,5-dimethylpyrazine, caryophyllene oxide, 2,3,5-trimethylpyrazine, butyrolactone, caryophyllene, vanillin, phenylacetic acid, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 2-phenylethanol, 1-octen-3-ol, 2-pyrrolidone, and propanoic acid (Table 4).

In ODGTS, the highest OAV was calculated for β-ionone (150 000), followed by dimethyl sulfide (26 000), eugenol (13 000), 2-isopropyl-3-methoxypyrazine (4100), the isomers of di-1-propenyl disulfide (2200), the isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (1400), and 3-methylbutanal (1100) (Table 4). Compared to those in the other two samples, clearly lower OAVs were found in ODGTS for di-1-propenyl disulfide isomers, (*E*,*Z*)-2,6-nonadienal, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene isomers, di-1-propenyl trisulfide isomers, 2-methylbutanal, 3-methylnonane-2,4-dione, 1-octen-3-one, (*E*)-2-hexenal, 2,3,5-trimethylpyrazine, (*E*)-2-hexen-1-ol, and vanillin (Table 4).

In summary, FDGTS revealed the highest OAVs for most of the quantitated odorants and ODGTS showed the lowest, which was in agreement with APA and the odorant screening via cAEDA. Only five compounds (β-ionone, valencene, aromadendrene, caryophyllene, and 2-phenylethanol) were present at the highest OAVs in ODRTS (Table 4).

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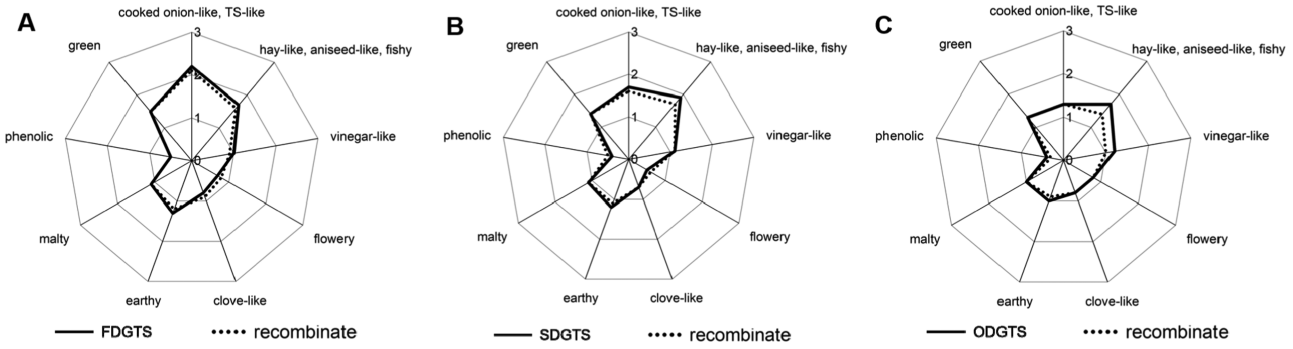

Figure 2. Aroma profiles of differently dried green *T. sinensis* (solid line) and the respective recombinate (dotted line): FDGTS (A), SDGTS (B), and ODGTS (C).

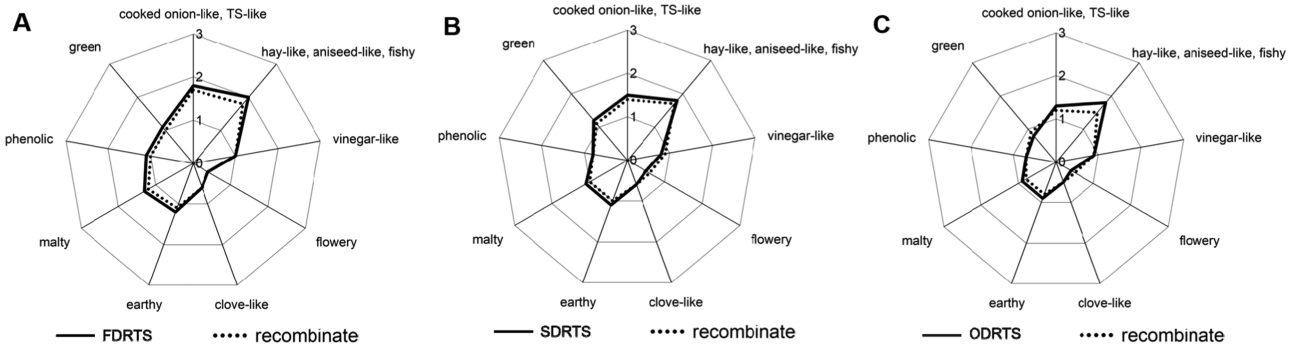

Figure 3. Aroma profiles of differently dried red *T. sinensis* (solid line) and the respective recombinate (dotted line): FDRTS (A), SDRTS (B), and ODRTS (C).

In red *T. sinensis*, OAVs widely showed the same trends as for green *T. sinensis*. Forty-two of the quantitated odorants in FDRTS, 43 in SDRTS, and 39 in ODRTS were present in amounts above their respective odor thresholds. Odorants with the highest OAVs (>1000) in FDRTS were the isomers of di-1-propenyl disulfide (400 000), dimethyl sulfide (280 000), 2-isopropyl-3-methoxypyrazine (54 000), β-ionone (39 000), eugenol (21 000), 2-methylbutanal (15 000), 3-methylbutanal (7200), (*E*,*Z*)-2,6-nonadienal (6600), isocaryophyllene (3500), the isomers of di-1-propenyl trisulfide (2800), the isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (2600), 3-methylnonane-2,4-dione (1500), linalool (1100), 2-methoxyphenol (1100), and acetic acid (1000) (Table 4).

In SDRTS, dimethyl sulfide revealed the highest OAV of 87 000, followed by β-ionone (32 000), 2-isopropyl-3-methoxypyrazine (32 000), the isomers of di-1-propenyl disulfide (29 000), eugenol (14 000), 3-methylnonane-2,4-dione (4700), the isomers of 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene (4600), 3-methylbutanal (4200), (*E*,*Z*)-2,6-nonadienal (3800), 2-methylbutanal (2300), caryophyllene oxide (1400), and the isomers of di-1-propenyl trisulfide (1300) (Table 4).

In ODRTS, dimethyl sulfide also showed the highest OAV (77 000). Further compounds with high OAVs were β-ionone (41 000), eugenol (9300), 2-isopropyl-3-methoxypyrazine (8900), 3-methylbutanal (2300), 2-methylbutanal (1400), and the isomers of di-1-propenyl disulfide (1100) (Table 4).

A comparison of the three samples illustrated the highest OAVs of most odorants in FDRTS and the lowest OAVs in ODRTS, especially for the compounds di-1-propenyl disulfide, (*E*,*Z*)-2,6-nonadienal, eugenol, di-1-propenyl trisulfide, linalool, isocaryophyllene, 2-ethyl-3,5-dimethylpyrazine, (*E*)-2-hexenal, and 2-methoxyphenol. In contrast, clearly higher OAVs were found for 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, 3-methylnonane-2,4-dione, (*E*,*E*)-2,4-decadienal, caryophyllene oxide, caryophyllene, phenylacetic acid, and 1-octen-3-ol in SDRTS compared to those in FDRTS and ODRTS. Interestingly, six compounds (β-ionone, decanoic acid, valencene, butyrolactone, α-humulene, and γ-nonalactone) showed the highest OAVs in ODRTS (Table 4).

In summary, the sensorial results obtained during APA were widely proven by the analytical data obtained after quantitation. For example, the concentrations of 2-methoxyphenol and 4-ethylphenol in the corresponding green and red dried *T. sinensis* confirmed the more intense phenolic odor note in red *T. sinensis* samples recognized during APA (Figure 1). Especially for sulfur-containing compounds and the green smelling aldehydes and (*E*)-2-hexen-1-ol, proven as important odorants for a well pronounced aroma of (green) *T. sinensis*,[12] higher losses were found in *T. sinensis* samples prepared at elevated temperatures. In general, FDTS (produced via the gentlest drying method) contained the highest amounts of the abovementioned compounds, while SDTS (prepared at a higher temperature) revealed lower amounts and ODTS (highest temperature impact) showed the lowest amounts.

Aroma Recombination Experiments of Differently Dried *T. sinensis*. As a final step of the sensomics concept, the contribution of the quantitated aroma compounds with OAVs ≥ 1 to the overall aroma is evaluated on the basis of an aroma recombination experiment. Aroma recombinates of all six samples were prepared containing the reference compounds of all odorants with OAVs ≥ 1 in the prepared odorless matrices in the concentrations measured in the respective original dried samples. All aroma profiles of the samples were nearly identical

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to the aroma profiles of the respective recombinates of FDTS, SDTS, and ODTS (both green and red varieties), proving the successful identification and quantitation of all key odorants in FDTS, SDTS, and ODTS (Figures 2 and 3).

Key Aroma-Active Compounds in Differently Dried *T. sinensis*. *Sulfur-Containing Compounds.* In a previous study, nine sulfur-containing compounds including 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 were characterized as key odorants in both raw and blanched *T. sinensis*.[12] The present study reports again on extremely high OAVs of these sulfur-containing compounds and higher amounts in green *T. sinensis* compared to those in red *T. sinensis* (except for dimethyl sulfide), explaining the more intense cooked onion-like/TS-like odor note in green *T. sinensis*. The highest OAVs of these sulfur-containing compounds were obtained in FDTS, while the lowest OAVs were found in ODTS. In addition, different final overall aromas were obtained for the dried green and red *T. sinensis* samples. Thus, the formation of key aroma-active compounds in dried *T. sinensis* was influenced by the processing conditions on the one side and also by the raw material on the other side. This data might also explain why some sulfur-containing compounds were not found in previously studied commercially dried *T. sinensis* products.[15]

Green Smelling Aldehydes and (E)-2-Hexen-1-ol. All green smelling compounds in raw green and red *T. sinensis*,[12] including hexanal, (*E*)-2-hexenal, (*E*,*Z*)-2,6-nonadienal, and (*E*)-2-hexen-1-ol, were again characterized as key odorants in all dried green and red *T. sinensis* samples in the present study. Clearly decreased OAVs in SDGTS and ODGTS indicated their lower contribution to the overall aroma compared to those in FDGTS and the instability of these odorants at elevated temperatures. The lowered concentrations of (*E*,*Z*)-2,6-nonadienal in the dried red samples might explain their lowered green odor note.

Earthy Smelling Pyrazines. The three pyrazines 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine, eliciting an earthy odor note, were identified as important aroma compounds in all samples. Consistent with commercially dried *T. sinensis*,[15] 2-isopropyl-3-methoxypyrazine revealed high OAVs in all dried samples. 2-Isopropyl-3-methoxypyrazine and 2-ethyl-3,5-dimethylpyrazine were present in similar amounts in all dried green *T. sinensis*. While their amounts were the highest in SDGTS, followed by ODGTS and FDGTS, the amount of 2,3,5-trimethylpyrazine in ODGTS was much lower than those in the other two samples. In red *T. sinensis*, the amounts of all three pyrazines followed the same pattern as mentioned above for the sulfur-containing compounds and green smelling compounds, present at the highest amounts in FDTS and at the lowest amounts in ODTS. Interestingly, the OAVs of 2-ethyl-3,5-dimethylpyrazine and 2,3,5-trimethylpyrazine were more or less comparable for the green and red varieties in the respective dried samples, whereas 2-isopropyl-3-methoxypyrazine was present at much higher amounts in the dried samples of the red variety, confirming recently data of raw and blanched *T. sinensis*.[12]

Phenols. 2-Methoxyphenol and 4-ethylphenol, proven as two key odorants in raw and blanched red *T. sinensis* with a phenolic odor note,[12] were characterized as key aroma-active phenols in all dried red *T. sinensis* samples, verifying again their important impact on the overall aroma of red *T. sinensis*. In addition, the comparison of OAVs of 2-methoxyphenol (1100 for FDRTS vs 98 for SDRTS and 32 for ODRTS) clearly proved the losses during the latter two drying methods (Table 4). 4-Ethylphenol revealed an OAV of 130 in FDRTS, at least 18 times higher compared to those in SDRTS (OAV of 6) and ODRTS (OAV of 7) (Table 4). Their quick losses at elevated temperatures in dried samples were consistent with previously reported data obtained for commercially dried *T. sinensis* products[15] and blanched *T. sinensis*.[12] In the dried green *T. sinensis* samples, only 2-methoxyphenol was present in amounts above its odor threshold (OAV of 6-21), while 4-ethylphenol showed OAVs < 1 for all three samples (Table 4), corroborating the much lower intensity of the phenolic odor note during APA (Figure 1) and the lower FD factors during cAEDA (Table 2) for the green *T. sinensis* samples compared to the red counterparts.

Fishy Smelling Compounds. 3-Methylnonane-2,4-dione with a hay-like/aniseed-like/fishy odor note was found in all six samples as a key odorant and showed the highest OAV in SDTS, followed by FDTS and ODTS. During APA, all recombinates showed very good similarities to the respective samples. However, a slightly lowered odor impression for the hay-like/aniseed-like/fishy aroma note was detected in some recombinates, especially for green and red ODTS (Figures 2 and 3). Thus, there might be another fishy smelling compound or a combination of odorants present in *T. sinensis* samples, evoking this odor note that is missing in the recombinates. As previously reported, a fishy smell in some foodstuffs was evoked by a combination of certain odorants such as (i) (*Z*)-1,5-octadien-3-one and 3-(methylthio)propanal;[35] (ii) (*E*,*E*)-2,4-heptadienal, (*Z*)-4-heptenal, (*E*,*Z*)-2,6-nonadienal, and 1-penten-3-one;[36,37] or (iii) 1-penten-3-one, 1-octen-3-one, (*Z*)-4-heptenal, (*E*,*Z*)-2,6-nonadienal, (*E*,*Z*)-2,4-heptadienal, and (*E*,*Z*,*Z*)-2,4,7-decatrienal.[38] However, some odorants in the abovementioned three combinations were not detected during cAEDA. Thus, they could be excluded as explanation for the fishy odor in *T. sinensis* samples. Based on earlier studies, trimethylamine (TMA) is also well-known as an odorant evoking a fishy odor in foodstuffs.[39,40] A very recent study of Matheis and Granvogl[41] reported on TMA responsible for the fishy off-flavor in steam-treated rapeseed oil using ion exchange chromatography and SPME-HRGC−MS techniques. Nevertheless, TMA was not detected in *T. sinensis* samples via SPME in the present study. In addition, during APA, the panelists did not determine a similarity of the original fishy smell in *T. sinensis* samples and the fishy smell elicited from recombinates spiked with different amounts of TMA, indicating that the fishy smell in *T. sinensis* does obviously not come from TMA. Thus, studies will be continued on *T. sinensis* to elucidate the possible fishy smelling odorant.

In conclusion, the key odorants of both green and red *T. sinensis* treated with different drying methodologies/processes (freeze drying, solar drying, and oven drying) were successfully identified and quantitated. Data obtained were validated by sensory recombination experiments, which showed high similarities to the respective aroma profiles of the original dried *T. sinensis* samples. Clear differences in the key odorants and in the overall aroma between green and red *T. sinensis* samples treated with the same drying method were obtained. The typical sulfur-containing odorants and green smelling compounds were mostly present at higher amounts in green *T. sinensis*, while two phenols and 2-isopropyl-3-methoxypyrazine were determined at clearly higher concentrations in red *T. sinensis*. Further, the amounts of key odorants in the same *T.*

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sinensis variety but processed with different drying methodologies clearly changed, leading to a varying overall aroma of differently dried *T. sinensis*. Specifically, freeze-dried *T. sinensis* showed the highest OAVs for most of the quantitated key aroma-active compounds, confirming the advantage of the application of low temperature and low pressure to obtain a product with high sensorial quality. Although the natural solar drying process caused losses of odorants at different extents, according to APA, the overall aroma of SDTS did not differ very much compared to FDTS. Thus, considering the high costs and much more complex handling of freeze drying, natural solar drying might be the best suitable drying process of *T. sinensis* sprouts in practice. Finally, due to an extremely high loss of most odorants, especially the typical sulfur-containing compounds, conventional oven drying resulted in the weakest overall aroma and, consequently, in the lowest product quality of dried *T. sinensis*.

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Notes

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8.2.3 Summary and individual contributions

Drying of tender TS leaves is important to make it a product available all year due to its extremely short harvest period and relatively high water content of about 80%. The aim of the present study was the characterization of odorants in both green and red TS buds treated with different lab-drying methods (freeze drying, solar drying, and oven drying) to compare the influences of the drying methods on the final aromas.

First, odorant screening via cAEDA and cSH-ADA were applied to three dried green TS and showed a total of 33 odorants with FD factors between 16 and 4096. High FD factors in FDGTS were found for eugenol, (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-di-1-propenyl disulfide, *cis*- and *trans*-2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and *β*-ionone. In SDGTS, beside abovementioned compounds, also (*E*,*Z*)-2,6-nonadienal and vanillin were present at high FD factors. However, compared to FDGTS and SDGTS, odorants in ODGTS were generally perceived at clearly lower FD factors, only eugenol and *β*-ionone showed high FD factors. To get knowledge about the role of these odorants in the different varieties of TS, odorant screening was also performed in three dried red TS samples and high FD factors were obtained for eugenol and *β*-ionone in all three samples. High FD factors in FDRTS and SDRTS were also found for di-1-propenyl disulfide, vanillin, (*E*,*Z*)-2,6-nonadienal, 2-phenylethanol, 2-isopropyl-3-methoxypyrazine, and 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene. Only a few odorants in ODRTS, including di-1-propenyl disulfide, 2-phenylethanol, vanillin, and nonanoic acid, were perceived with high FD factors.

To elucidate the screening data, quantitation experiments via SIDA and semiquantitation via internal standard method were performed, and then OAVs were calculated to characterize the key odorants. In FDGTS, di-1-propenyl disulfide, dimethyl sulfide, *β*-ionone, eugenol, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, di-1-propenyl trisulfide, 2-methylbutanal, (*E*,*Z*)-2,6-nonadienal, 2-isopropyl-3-methoxypyrazine, 3-methylbutanal, and 3-methyl-2,4-nonandione were present at highest OAVs (> 1000). In comparison to FDGTS, sulfides including dimethyl sulfide, di-1-propenyl disulfide, and di-1-propenyl trisulfide showed much lower OAVs, in SDGTS while 3-methyl-2,4-nonandione, phenylacetic acid, and 1-octen-3-ol showed clearly higher OAVs. In ODGTS, *β*-ionone revealed the highest OAV, followed by dimethyl sulfide, eugenol, 2-isopropyl-3-methoxypyrazine, di-1-propenyl disulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and 3-methylbutanal (OAVs > 1000). In general, FDGTS revealed the highest OAVs for most of the quantitated odorants and ODGTS showed the lowest OAVs, demonstrating the advantage of the gentle freeze drying methodology at a low temperature. OAVs in differently dried red TS samples followed the same trend as seen for green TS. However, OAVs of the odorants were different in dried green and red TS. For example, 2-isopropyl-3-methoxypyrazine and β-ionone showed ten times higher OAVs in red TS compared to green TS.

Miss Xiaoting Zhai designed and performed all experiments including volatiles isolation, HRGC-O screenings, identification, quantitation, and sensory experiments. Miss Zhai evaluated all results and prepared the manuscript. Prof. Dr. Michael Granvogl guided Miss Zhai's work, conceived and directed the study, and revised the manuscript. In addition, Prof. Granvogl participated in the sensory tests.

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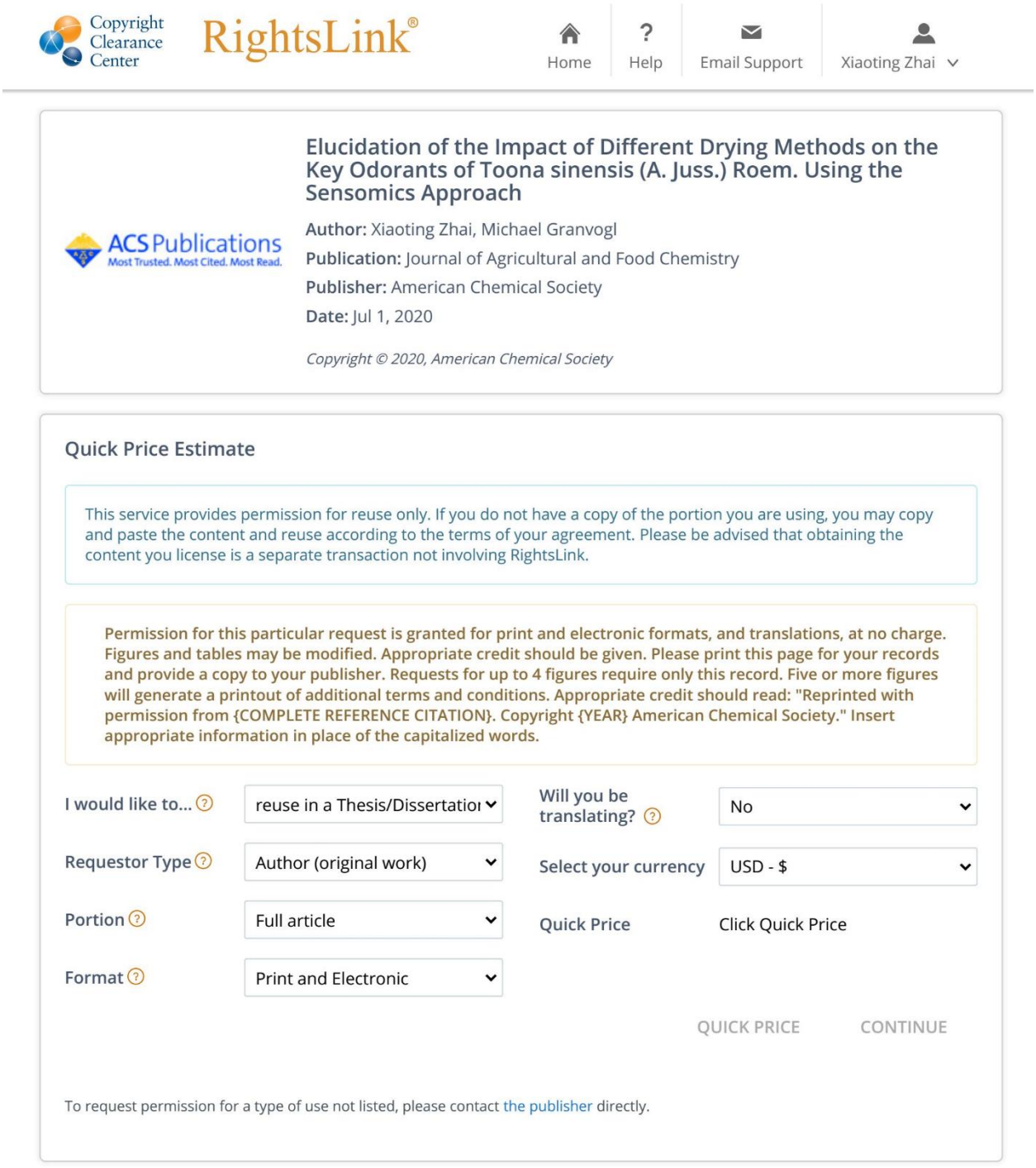

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8.3 Publication 3: Characterization of the Key Aroma Compounds in Two Differently Dried *Toona sinensis* (A. Juss.) Roem. by Means of the Molecular Sensory Science Concept

8.3.1 Bibliographic data

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8.3.2 Publication reprint

A reprint of publication 3, characterization of key aroma-active compounds in two differently dried *Toona sinensis* (A. Juss.) Roem. by means of the molecular sensory science concept, follows starting with the next page.

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Characterization of the Key Aroma Compounds in Two Differently Dried *Toona sinensis* (A. Juss.) Roem. by Means of the Molecular Sensory Science Concept

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ABSTRACT: A systematic approach for the characterization of the key aroma-active compounds in sun-dried *Toona sinensis* (SDTS) and vacuum-dried *T. sinensis* (VDTS) was performed by means of the molecular sensory science concept. A total of 64 aroma-active compounds were identified via gas chromatography−olfactometry (GC-O) and gas chromatography−mass spectrometry (GC-MS). Aroma extract dilution analysis (AEDA) and static headspace dilution analysis revealed 39 odorants in SDTS and 32 odorants in VDTS with flavor dilution (FD) factors from 8 to 4096, with the highest for vanillin and eugenol in both samples. Stable isotope dilution analysis (SIDA) and an internal standard method were applied to quantitate 42 odorants, revealing 35 compounds in concentrations above their respective odor thresholds in SDTS and 29 compounds in VDTS, respectively. Calculation of odor activity values (OAVs) indicated 2-isopropyl-3-methoxypyrazine, eugenol, and β-ionone with the highest OAVs in both samples. Recombination experiments of the overall aromas of SDTS and VDTS by mixing the odorants with OAVs $\geq$1 in their naturally occurring concentrations proved the successful identification and quantitation of the respective key odorants.

KEYWORDS: *Toona sinensis, molecular sensory science concept, aroma extract dilution analysis, stable isotope dilution analysis, odor activity value, aroma recombination*

INTRODUCTION

Toona sinensis, also commonly called Chinese mahogany or Xiang Chun, is a perennial, deciduous tree belonging to the family of Meliaceae, and it is widely distributed in China.[1] The tender leaves of *T. sinensis* have a long history of usage as a traditional Chinese medicine because of its various biological and pharmacological functions, including anticancer, antidiabetic, antiviral, and antioxidant properties.[2−6] In addition, the buds of *T. sinensis* enjoy great popularity as a vegetable and flavoring of food products in the Chinese diet because of their strong, unique aroma and abundant nutrients.[5,6] *T. sinensis* sprouts are mainly used in salads, stir-fried with other foodstuffs, and boiled with noodle soup. Raw *T. sinensis* sprouts need to be blanched in boiled water to remove toxicologically relevant compounds (nitrites) before cooking. The economic importance of *T. sinensis* can exemplarily be seen in the fact that >100 000 people, including farmers and producers, are actually working on *T. sinensis* products in China. Its planting area is more than 1 billion square meters, with more than 800 billion kilograms of fresh *T. sinensis* buds cultivated per year.[7]

Up to now, more than 70 volatile compounds, most of them sulfides, terpenes, hydrocarbons, acids, alcohols, esters, and phenols, are known in fresh *T. sinensis* on the basis of experiments performed by gas chromatography−olfactometry (GC-O), solid phase microextraction (SPME), and gas chromatography−mass spectrometry (GC-MS).[8−10] For example, Liu et al.[9] reported 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, with an odor similar to that of cooked *T. sinensis*, and di-1-propenyl sulfide, with a garlic-like and onion-like odor, as important compounds to the overall aroma profile of fresh *T. sinensis* (Shanxi, China).

The new growth and tender sprouts of *T. sinensis* trees are only available in early spring (April and May). About 2 weeks after emerging, the tender leaves become fibrous, not suitable for consumption any more. Thus, fresh *T. sinensis* leaves can be regarded as a seasonal vegetable. To make it a product available all year, leaves are often salted and dried. Generally, the drying process is performed to decrease the moisture contents of the shoots and leaves for transportation and long-term storage. During this process, the original aroma and nutritional components should be maintained. The most popular and traditional drying process for tender *T. sinensis* leaves in China is salting and natural sun-drying. However, vacuum freeze-drying is gaining more and more attention by producers.

Up to now, most studies paid attention to the composition of odorants in fresh *T. sinensis* buds. However, no accurate data on the aroma components of dried *T. sinensis* and no systematic molecular sensory analysis of the odorants predominately contributing to the overall aroma of dried *T. sinensis* are

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available. To the best of our knowledge, only Chen et al.[11] focused on the evaluation of different drying methods, including oven-drying, microwave-drying, vacuum-drying, natural sun-drying, and spray-drying, in regard to volatiles in *T. sinensis* leaves (Tianjin, China). They determined that sun-drying retained more volatiles but with lower relative contents, whereas vacuum-drying ended up with higher contents of the original volatiles.

Thus, the aim of the present study was to characterize the key aroma compounds of dried *T. sinensis* buds obtained from China using the molecular sensory science concept. The key odorants were (i) identified by aroma extract dilution analysis (AEDA) based on GC-O in combination with GC-MS and (ii) quantitated by stable isotope dilution assays (SIDAs) and semiquantitated by an internal standard method. Afterward, (iii) the odor thresholds were determined to calculate the odor activity values (OAVs), and finally, (iv) the overall aroma was simulated by recombination experiments.

MATERIALS AND METHODS

***Toona sinensis* Samples.** On the basis of the above-mentioned drying methods, sun-dried *T. sinensis* (SDTS) and vacuum-dried *T. sinensis* (VDTS) buds were bought from two internet suppliers in China (Zhaowantechan superstore and Lvyuan food superstore, respectively). SDTS was cultivated in Hubei, China, and harvested in April 2016. After being blanched in boiling water for 2 min, SDTS was cooled to room temperature, salted, and finally dried in sunlight for 3 days. VDTS belonging to the Taihe cultivar (Anhui, China) was harvested in April 2016. After being blanched in boiling water for 2 min, VDTS was cooled to room temperature, salted in a closed container with some water for 2 weeks, and then vacuum freeze-dried.

Chemicals. The following reference odorants were commercially available: acetic acid, 2-acetylpyrrole, benzyl alcohol, 2,3-butanediol, caryophyllene oxide, 1,8-cineole, decanoic acid, 2,3-dimethylpyrazine, dimethyl sulfide, 2,5-dimethylthiophene, 2-ethyl-3,5(6)-dimethylpyrazine, ethylpyrazine, eugenol, farnesene, hexanal, hexanoic acid, (*E*)-2-hexenal, (*E*)-3-hexenoic acid, (*E*)-2-hexenol, α-humulene, 4-hydroxy-2-butanone, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 4-hydroxy-4-methyl-2-pentanone, 3-hydroxy-2-methyl-4*H*-pyran-4-one, β-ionone, 2-isopropyl-3-methoxypyrazine, limonene, linalool, menthol, 3-methylbutanenitrile, 2-methylbutanoic acid, 3-methylbutanoic acid, 6-methyl-5-hepten-2-one, methyl 2-methylbutanoate, 4-methylpentanoic acid, methyl propanoate, methylpropanoic acid, methylpyrazine, 1-octen-3-ol, pentanoic acid, phenol, phenylacetic acid, 2-phenylethanol, α-pinene, propanoic acid, and 2,3,5-trimethylpyrazine (Sigma-Aldrich Chemie, Taufkirchen, Germany); cyclopentadecanone, 2-ethylfuran, 2-methylbutanal, and 3-methylbutanal (Alfa Aesar, Karlsruhe, Germany); aromadendrene, butanal, butyrolactone, β-caryophyllene, heptanoic acid, 1-hydroxy-2-propanone, methyl hexanoate, nonanoic acid, and valencene (Fluka, Neu-Ulm, Germany); butanoic acid and vanillin (Merck, Darmstadt, Germany); 2-ethyl-3-methylpyrazine (Acros Organics; Thermo Fisher Scientific, Nidderau, Germany); 2-ethyl-6-methylpyrazine (Pyrazine Specialties, Ellenwood, GA); isocaryophyllene (Aldrich; Sigma-Aldrich Chemie); isoeugenol (Lancaster, Mühlheim/Main, Germany); 3-methylnonane-2,4-dione (Chemos, Regenstauf, Germany); and 2-pyrrolidone (TCI, Eschborn, Germany).

Liquid nitrogen was from Linde (Munich, Germany). Dichloromethane and pentane (Merck) were freshly distilled prior to use. Hydrochloric acid, sodium carbonate, sodium chloride, and sodium sulfate were from Merck. 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, [$^{2}H_5$]-benzyl alcohol, [$^{2}H_6$]-dimethyl sulfide, [$^{2}H_3$]-hexanoic acid, [$^{13}C_6$]-phenol, [$^{13}C_2$]-phenylacetic acid, [$^{13}C_2$]-2-phenylethanol, and [$^{2}H_2$]-propanoic acid (Sigma-Aldrich Chemie); [$^{2}H_4$]-*cis*-isoeugenol (AromaLab, Planegg, Germany); [$^{2}H_9$]-2-methylbutanoic acid (EQ Laboratories, Augsburg, Germany); and [$^{2}H_{2-3}$]-decanoic acid, [$^{2}H_7$]-methylpropanoic acid, [$^{2}H_2$]-nonanoic acid, and [$^{2}H_6$]-2-pyrrolidone (C/D/N Isotopes, Quebec, Canada).

The following standards were prepared as previously described: [$^{2}H_2$]-butanoic acid,[12] [$^{2}H_2$]-1,8-cineole,[13] [$^{2}H_5$]-2-ethyl-3,5-dimethylpyrazine,[14] [$^{2}H_{4-6}$]-hexanal,[15] [$^{2}H_2$]-(*E*)-2-hexenal,[16] [$^{2}H_2$]-(*E*)-2-hexenol,[17] [$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one,[18] [$^{2}H_3$]-β-ionone,[19] [$^{2}H_3$]-2-isopropyl-3-methoxypyrazine analogue to [$^{2}H_3$]-2-isobutyl-3-methoxypyrazine,[20] [$^{2}H_{2-3}$]-linalool,[21] [$^{2}H_2$]-2-methylbutanal,[17] [$^{2}H_3$]-methyl 2-methylbutanoate,[16] [$^{2}H_3$]-3-methylnonane-2,4-dione,[17] [$^{2}H_{2-3}$]-methylpyrazine,[22] [$^{2}H_{2-3}$]-γ-octalactone,[23] [$^{2}H_{3-5}$]-1-octen-3-ol,[24] [$^{2}H_3$]-pentanoic acid,[25] [$^{2}H_{3-4}$]-trimethylpyrazine,[20] and [$^{2}H_3$]-vanillin.[26]

The concentrations of the stable isotopically labeled compounds were determined as recently described.[27]

Isolation of the Volatiles. The TS samples were frozen with liquid nitrogen, crushed into small pieces, powdered with a SPEX SamplePrep 6870 Freezer/Mill (Metuchen, NJ), and stored in a freezer at −24 °C prior to the next steps. Then, an aliquot of the sample (20 g) was extracted with dichloromethane (3 × 200 mL, 1 h each) at room temperature using a magnetic stirrer. The organic extracts were combined, filtered, and concentrated to ∼100 mL using a Vigreux column (50 × 1 cm). After drying over anhydrous sodium sulfate, the extract was subjected to high vacuum distillation using the solvent-assisted-flavor-evaporation (SAFE) technique[28] to separate the volatiles from the nonvolatiles. To avoid a possible overlap of compounds during GC-O, the distillate obtained was separated into the acidic fraction (AF) and the neutral−basic fraction (NBF), as described earlier.[29] The NBF was further fractionated by column chromatography, on the basis of the polarity of the compounds. The different eluates were collected in 50 mL portions, and each portion was concentrated to a final volume of ∼200 μL by a microdistillation apparatus (silica-gel fractions, SGF 1−6).

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). HRGC-O was performed using a type 8000 gas chromatograph (Fisons Instruments, Mainz, Germany) and two fused silica capillary columns with different polarities: DB-FFAP (30 m × 0.25 mm i.d., 0.25 μm film thickness) or DB-5 (30 m × 0.32 mm i.d., 0.25 μm film thickness; both J&W Scientific; Agilent, Waldbronn, Germany). Aliquots of the samples (2 μL) were manually injected by the cold-on-column technique at 40 °C, and the following temperature program was used: 40 °C, held for 2 min, raised at 6 °C/min to 240 °C, and held for 5 min. A Y-type quick-seal glass splitter (Chrompack, Frankfurt, Germany) was applied to separate the effluent equally to a flame ionization detector (FID) held at 250 °C and a sniffing port held at 230 °C, enabling similar detection of the FID chromatogram and the odor qualities. Retention indices (RIs) were calculated using the retention times of *n*-alkanes as references (C6−C26 for DB-FFAP and C6−C18 for DB-5).

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS) for Identification. Identification experiments based on HRGC-MS were performed as recently described.[30]

Two-Dimensional Heart-Cut High-Resolution Gas Chromatography−Olfactometry−Mass Spectrometry (HRGC/HRGC-O/MS) for Identification. To obtain unequivocal mass spectra of the odor-active trace components and to avoid overlap of compounds, HRGC/HRGC-O/MS was performed with a gas chromatograph Mega 2 (Fisons Instruments) coupled via a moving column stream switching (MCSS) system (Fisons Instruments) to a gas chromatograph CP 3800 (Varian, Darmstadt). In the first dimension, the samples were manually injected onto a DB-FFAP capillary column (30 m × 0.32 mm, 0.25 μm film thickness; J&W Scientific), and the compounds could be detected via an FID and a sniffing port by means of a Y-type splitter at the end of the column. Heart-cuts were then transferred onto a DB-5 column (30 m × 0.25 mm, 0.25 μm film thickness; J&W Scientific) in the second gas chromatograph. The target compounds were simultaneously monitored by a second sniffing-port and a Saturn 2000 mass spectrometer (Varian) via a Y-type splitter. Mass spectra in electron ionization (EI) mode were generated at 70 eV and in chemical ionization (CI) mode at 105 eV using methanol as the reactant gas.

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

odorant	isotope label	ion (m/z)[a] analyte	ion (m/z)[a] internal standard	system[b]	R_f[c]
acetic acid	$^{13}C_2$	61	63	I	1
benzyl alcohol	$^{2}H_5$	91	96	I	0.87
butanoic acid	$^{2}H_2$	89	91	I	1
butyrolactone	—[d]	87	145 + 146[d,e]	II	1.12
1,8-cineole	$^{2}H_2$	155	157	II	0.99
decanoic acid	$^{2}H_{2-3}$[e]	187	189 + 190[e]	II	0.94
dimethyl sulfide	$^{2}H_6$	63	69	II	0.96
2-ethyl-3,5-dimethylpyrazine	$^{2}H_5$	137	142	II	1
2-ethyl-3-methylpyrazine	—[f]	123	126 + 127[e,f]	II	0.51
2-ethyl-6-methylpyrazine	—[f]	123	126 + 127[e,f]	II	0.52
eugenol	—[g]	165	169[g]	I	0.67
hexanal	$^{2}H_{4-6}$[e]	101	105−107[e]	II	0.92
hexanoic acid	$^{2}H_3$	99	102	I	1
(*E*)-2-hexenal	$^{2}H_2$	99	101	II	0.58
(*E*)-2-hexenol	$^{2}H_2$	83	85	II	1
β-ionone	$^{2}H_3$	193	196	I	0.94
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	$^{13}C_2$	129	131	II	0.95
2-isopropyl-3-methoxypyrazine	$^{2}H_3$	153	156	II	0.88
linalool	$^{2}H_{2-3}$[e]	137	139 + 140[e]	II	0.9
2-methylbutanal	$^{2}H_2$	87	89	III	1
3-methylbutanal	—[h]	87	89[h]	III	1
2-methylbutanoic acid	$^{2}H_9$	103	112	II	0.96
3-methylbutanoic acid	—[i]	103	112[i]	II	0.96
methyl 2-methylbutanoate	$^{2}H_3$	117	120	II	0.98
3-methylnonane-2,4-dione	$^{2}H_3$	171	174	II	0.95
methylpropanoic acid	$^{2}H_7$	89	96	I	1
methylpyrazine	$^{2}H_{2-3}$[e]	95	97 + 98[e]	II	0.75
nonanoic acid	$^{2}H_2$	173	175	II	0.98
1-octen-3-ol	$^{2}H_{3-5}$[e]	111	114−116[e]	II	0.96
pentanoic acid	$^{2}H_3$	103	106	I	0.93
phenol	$^{13}C_6$	95	101	I	1
phenylacetic acid	$^{13}C_2$	137	139	II	0.92
2-phenylethanol	$^{13}C_2$	105	107	I	0.91
propanoic acid	$^{2}H_2$	75	77	I	1
2-pyrrolidone	$^{2}H_6$	86	92	I	0.82
vanillin	$^{2}H_3$	153	156	II	0.93

[a]Ions used for quantitation in chemical ionization (CI) mode. [b]System I, GC-MS(CI); system II, GC/GC-MS(CI); system III, HS-SPME-GC-MS(CI). [c]Response factor (R_f), determined by analyzing mixtures of known amounts of analyte and internal standard. [d]The quantitation of butyrolactone was performed using [$^{2}H_{2-3}$]-γ-octalactone as the internal standard. [e]The internal standard was used as a mixture of isotopologues. [f]The quantitation of 2-ethyl-3-methypyrazine and 2-ethyl-6-methylpyrazine was performed using [$^{2}H_{3-4}$]-2,3,5-trimethylpyrazine as the internal standard. [g]The quantitation of eugenol was performed using [$^{2}H_4$]-*cis*-isoeugenol as the internal standard. [h]The quantitation of 3-methylbutanal was performed using [$^{2}H_2$]-2-methylbutanal as the internal standard. [i]The quantitation of 3-methylbutanoic acid was performed using [$^{2}H_9$]-2-methylbutanoic acid as the internal standard.

Aroma Extract Dilution Analysis (AEDA). AEDA was applied to determine the flavor dilution (FD) factors of the odor-active compounds. Aliquots of the distillate (1 mL each) obtained from 10 g of each *T. sinensis* sample were diluted stepwise with dichloromethane (1 + 1, by volume). Next, aliquots (2 μL) of the concentrated distillates as well as of the dilutions were applied to HRGC-O until no odor impression was perceived at the sniffing-port. The FD factors, representing the highest dilution in which the odorant was detected for the last time, were analyzed three times and differed by not more than two dilution steps.

Static Headspace Aroma Dilution Analysis (SH-ADA) Based on Static Headspace High-Resolution Gas Chromatography−Olfactometry/Mass Spectrometry (SH-HRGC-O/MS). To detect very volatile components and compounds coeluting with the solvent during AEDA, SH-HRGC-O/MS was performed using a Trace Ultra gas chromatograph (Thermo Scientific, Dreieich, Germany) equipped with a DB-5 thick-film capillary column (30 m × 0.25 mm i.d., 1.00 μm film thickness; J&W Scientific) coupled to an ion trap mass spectrometer Saturn 2100 T (Varian). Injection was performed with a gastight syringe using a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) and a cryo-trap (−150 °C) cooled with liquid nitrogen. Subsequently, the odorants were transferred onto a DB-5 capillary column by heating the trap to 250 °C. Again, odor impressions and mass spectra were simultaneously recorded. To determine the FD factors, different headspace volumes (5, 2.5, 1.25, 0.62, 0.31, and 0.15 mL) were injected.

Quantitation of Key Odorants by Stable Isotope Dilution Assays (SIDAs). The stable isotopically labeled internal standards

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Table 2. Aroma-Active Compounds in the SAFE Distillate Obtained from SDTS and VDTS

no.[c]	odorant[d]	odor quality[e]	RI[a]		FD factor[b]		fraction[f]	refs[g]
			DB-FFAP	DB-5	SDTS	VDTS		
1	α-pinene	resin-like, fir needle-like	1056	939	4	16	SGF1	8, 32
2	hexanal	green, grassy	1110	796	8	4	SGF2	8, 9, 32
3	3-methylbutanenitrile	smoky, turpentine-like	1144	nd[h]	2	1	AF	8
4	2,5-dimethylthiophene	fried onion-like, rubber-like	1173	nd	2	1	SGF3	8, 9, 32
5	1,8-cineole	terpene-like	1196	1035	4	4	SGF5	
6	methyl hexanoate	fruity, musty	1199	922	8	8	SGF2	8
7	limonene	citrus-like, carrot-like	1206	1030	4	2	SGF1	8, 9, 32
8	(*E*)-2-hexenal	green apple-like, bitter almond-like	1224	851	1	4	SGF3	8, 33
9	methylpyrazine	green, roasty	1273	822	2	16	SGF6	
10	1-hydroxy-2-propanone	green, malty	1307	nd	2	8	SGF5	
11	6-methyl-5-hepten-2-one	mushroom-like, pepper-like	1331	986	4	1	SGF3	
12	ethyl pyrazine	roasty	1337	914	4	1	SGF6	
13	2,3-dimethylpyrazine	nut-like, leather-like	1350	900	2	2	SGF6	
14	4-hydroxy-4-methyl-2-pentanone	earthy	1360	842	1	4	SGF3	
15	2-ethyl-6-methylpyrazine	roasty	1382	1001	16	2	SGF5	
16	2-ethyl-3-methylpyrazine	roasty	1382	1001	16	2	SGF5	
17	(*E*)-2-hexenol	green, fruity	1398	860	2	1	SGF4	32
18	2-isopropyl-3-methoxypyrazine	earthy, pea-like	1423	1094	1024	512	SGF4	
19	2-ethyl-3,5-dimethylpyrazine	earthy	1432	1079	256	16	SGF5	
20	acetic acid	vinegar-like	1441	612	512	256	AF	
21	1-octen-3-ol	mushroom-like	1442	975	8	8	SGF4	
22	propanoic acid	sour-like, sweaty	1535	706	2	8	AF	
23	linalool	citrus-like, flowery	1539	1100	256	64	SGF3	32
24	isocaryophyllene	citrus-like	1556	1407	1024	128	SGF1	9, 32
25	methylpropanoic acid	sweaty, cheesy	1560	824	64	16	AF	
26	2,3-butanediol	butter-like, sweet	1573	793	16	4	SGF4	34
27	β-caryophyllene	rubber-like, moldy	1582	nd	256	256	SGF4	9, 33, 34
28	aromadendrene	eucalyptus-like	1591	nd	32	32	SGF3	
29	butanoic acid	sweaty	1621	809	4	2	AF	
30	butyrolactone	sweet, flowery	1631	900	512	64	SGF6	
31	menthol	mint-like	1631	nd	4	4	AF	
32	α-humulene	balmy	1654	1455	2048	64	SGF1	8, 15
33	3-methylbutanoic acid	sweaty	1663	870	64	16	AF	
34	2-methylbutanoic acid	fruity, sweaty	1663	870	64	16	AF	
35	valencene	fruity, flowery	1709	1497	128	32	SGF1	
36	3-methylnonane-2,4-dione	hay-like, aniseed-like, fishy	1716	1251	512	64	SGF6	
37	pentanoic acid	sweaty, fruity	1723	914	32	16	AF	
38	farnesene	sweet, tea-like	1738	1506	4	2	SGF1	33
39	4-methylpentanoic acid	sweaty	1788	939	2	2	AF	
40	hexanoic acid	sweaty	1839	1010	16	4	AF	
41	benzyl alcohol	bitter almond-like, fruity	1878	1036	128	16	SGF5	
42	2-phenylethanol	flowery, honey-like	1911	1117	4	4	SGF5	
43	β-ionone	flowery, violet-like	1931	1488	512	512	SGF4	
44	heptanoic acid	rancid, sweaty	1942	1074	4	2	AF	
45	(*E*)-3-hexenoic acid	flowery	1947	986	8	2	AF	
46	3-hydroxy-2-methyl-4*H*-pyran-4-one	caramel-like	1953	1108	2	4	AF	
47	caryophyllene oxide	citrus-like, soapy	1975	1578	8	8	SGF3	8, 9
48	2-acetylpyrrole	musty, walnut-like	1989	1066	16	4	SGF3	
49	phenol	ink-like, phenolic	2016	981	16	2	SGF3	
50	2-pyrrolidone	fruity	2054	nd	32	4	SGF5	34
51	nonanoic acid	moldy, pungent	2163	nd	128	64	AF	
52	eugenol	clove-like	2172	1359	2048	4096	SGF3	
53	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like, spicy	2200	1108	32	8	SGF5	
54	decanoic acid	soapy, musty	2267	1369	1024	1024	AF	
55	phenylacetic acid	beeswax-like, honey-like	2559	1261	2	2	AF	
56	vanillin	vanilla-like, sweet	2591	1403	4096	2048	AF	

[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; dilution factor of the 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

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Table 2. continued

indices on a DB-FFAP capillary column. [d]Odorants were identified by comparing their odor qualities and intensities, retention indices on the 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 GC-O. [f]Fraction in which the odorant was detected by GC-O after fractionation of the initial extract. AF, fraction of acidic volatiles; SGF 1−6, silica gel fractions 1−6 of neutral and basic volatiles (NBF). [g]References previously reporting the compound in *T. sinensis*. [h]Not determined.

(0.1−400 μg, dissolved in dichloromethane; amounts depending on concentrations of the respective analytes determined in preliminary experiments) were added to the powdered material of SDTS and VDTS (1−20 g each). After the addition of dichloromethane (50 mL), the sample was stirred for 15 min at room temperature for equilibration, and further workup was done as described above for the isolation of the volatiles. The final distillate was concentrated to ∼200 μL using a Vigreux column (50 cm × 1 cm), followed by microdistillation. Aliquots of the aroma isolates (2 μL) were used for high-resolution gas chromatography−mass spectrometry (HRGC-MS) or two-dimensional high-resolution gas chromatography−mass spectrometry (HRGC/HRGC-MS).

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS) and Two-Dimensional High-Resolution Gas Chromatography−Mass Spectrometry (HRGC/HRGC-MS) for Quantitation. Quantitation experiments via HRGC-MS and HRGC/HRGC-MS were performed as recently described,[30] including the analysis of mixtures of the respective unlabeled analyte and the labeled standard in five different mass ratios (5:1, 3:1, 1:1, 1:3, and 1:5) to calculate the respective response factors (R_f) (Table 1).

Quantitation of Dimethyl Sulfide, 2-Methylbutanal, and 3-Methylbutanal by SIDAs via Headspace Solid Phase Microextraction High-Resolution Gas Chromatography−Mass Spectrometry (HS-SPME-HRGC-MS). Quantitation of dimethyl sulfide, 2-methylbutanal, and 3-methylbutanal was performed by SIDA based on headspace solid phase microextraction (HS-SPME) using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco, Bellefonte, PA). Aliquots of SDTS and VDTS (0.5 g each) were weighed into a headspace vial (20 mL), spiked with [$^2H_6$]-dimethyl sulfide (∼2 μg, dissolved in ethanol; amounts depending on concentrations of the respective analytes determined in preliminary experiments) and [$^2H_2$]-2-methylbutanal (∼0.15 μg), and equilibrated by stirring for 30 min at 35 °C. After extraction for 8 min at 35 °C, thermo-desorption was performed for 30 s at 250 °C. Separation was done with a Trace GC Ultra (ThermoQuest, Egelsbach, Germany) equipped with a VF-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific) coupled to an ion trap mass spectrometer Saturn 2100 T (Varian). Mass spectra were generated in CI mode at 105 eV using methanol as reactant gas. Response factors of these compounds were accordingly determined via HS-SPME.

Semiquantitation of Aromadendrene, β-Caryophyllene, Caryophyllene Oxide, α-Humulene, Isocaryophyllene, and Valencene. Semiquantitation of aromadendrene, β-caryophyllene, caryophyllene oxide, α-humulene, isocaryophyllene, and valencene was performed by the internal-standard method using cyclopentadecanone as standard. Aliquots of the SAFE distillates (2 μL) were analyzed by a trace GC Ultra (Thermo Scientific) equipped with a cold-on-column injector, a DB-FFAP capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific), and an FID to detect the respective peak areas. To calculate the response factors (R_f) of each component, the respective analyte and cyclopentadecanone were mixed in five different mass ratios (5:1, 3:1, 1:1, 1:3, and 1:5) and analyzed in the same way, ending with response factors of 0.83 (aromadendrene), 1.18 (β-caryophyllene), 0.91 (caryophyllene oxide), 0.79 (α-humulene), 0.43 (isocaryophyllene), and 0.77 (valencene).

Determination of Odor Thresholds (OTs) in Water. The OTs in water of aromadendrene, β-caryophyllene, caryophyllene oxide, α-humulene, isocaryophyllene, 2-pyrrolidone, and valencene were newly determined as previously described.[31] All other thresholds used were from an in-house database or from the literature.[31]

Aroma-Profile Analysis (APA). For APA, the respective aqueous solutions of the following reference compounds diluted from ethanolic stock solutions were prepared to define the odor descriptors: 2-isopropyl-3-methoxypyrazine (earthy, pea-like), 2-methylbutanal (malty), acetic acid (vinegar-like), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (seasoning-like, spicy), β-ionone (flowery, violet-like), hexanal (green, grassy), 3-methylnonane-2,4-dione (hay-like, aniseed-like, fishy), and eugenol (clove-like). The concentration of each aroma compound was 50-fold above its respective odor threshold. In addition, the final concentration of ethanol was <500 μg/kg, ensuring that the ethanol could not be smelled (on the basis of its odor threshold of 990 000 μg/kg of water). Sensory analysis was performed with 20 panelists who had been trained weekly in a climate-controlled (21 ± 1 °C) sensory room. The panel was asked to rank the odor intensity of each aroma attribute from 0 to 3 (0, not perceivable; 1, weak; 2, significant; and 3, strong) on a seven point linear scale in steps of 0.5.

Aroma Recombination. For aroma recombination, both SDTS and VDTS (1 g each) were extracted with dichloromethane several times until the residues were odorless. These odorless residues were used as matrices for the SDTS and VDTS recombinates. Therefore, aqueous solutions of all analyzed aroma compounds with an OAV ≥ 1 were added to the respective matrix in their naturally occurring concentrations determined in the samples. Then, the recombinates were evaluated in the same way as described above for APA.

RESULTS AND DISCUSSION

Identification of Key Odorants in SDTS. Concentrated distillates obtained by extraction, SAFE distillation, and fractionation (AF, SGF 1−6) were subjected to GC-O. Separation into the acidic fraction (AF) and the neutral−basic fraction (NBF) was performed to avoid a possible overlap of compounds during GC-O. Because of the high number of compounds in the NBF, further fractionation based on the polarity of the odorants was performed using silica gel column chromatography with different ratios of pentane/dichloromethane as eluents (Table 2).

A total of 34 odor-active areas were detected in the flavor dilution (FD) factor range of 8−4096 in SDTS. Among them, the highest FD factor of 4096 was found for **56** (vanilla-like, sweet), followed by **32** (balmy) and **52** (clove-like) with an FD factor of 2048, and **18** (earthy, pea-like), **24** (citrus-like), and **54** (soapy, musty) with an FD factor of 1024. In addition, intense smells (FD factor of 512) were evoked by vinegar-like (**20**), sweet and aromatic (**30**), hay-like, aniseed-like, and fishy (**36**), and flowery and violet-like (**43**) compounds (Table 2).

For identification of these odor-active areas perceived at the sniffing-port, the sensory and analytical data, including odor quality, odor intensity, and retention indices on two capillary columns of different polarities, were compared to an in-house database consisting of >1000 odor-active reference standards. Then, the mass spectra in both EI and CI mode were recorded to confirm the possible chemical structures of these odor-active areas. Compound **56** with the highest FD factor of 4096 was identified as vanillin, followed by α-humulene (**32**), eugenol (**52**), 2-isopropyl-3-methoxypyrazine (**18**), isocaryophyllene (**24**), decanoic acid (**54**), acetic acid (**20**), butyrolactone (**30**), 3-methylnonane-2,4-dione (**36**), and β-ionone (**43**) (Table 2).

To determine the highly volatile compounds, headspace GC-O/MS was performed and led to the identification of eight additional odorants in SDTS: dimethyl sulfide (**HS1**; asparagus-

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Table 3. Highly Volatile Odorants Detected during Static Headspace Dilution Analysis of SDTS and VDTS

			RI[a]	FD factor[b]		
no.[c]	odorant[d]	odor quality[e]	DB-5	SDTS	VDTS	refs[f]
HS1	dimethyl sulfide	asparagus-like, putrid	511	16	8	
HS2	4-hydroxy-2-butanone	butter-like, carrot-like	588	2	4	
HS3	butanal	malty, sweaty	596	4	2	
HS4	methyl propanoate	fruity	603	4	4	
HS5	3-methylbutanal	malty	640	>32	>32	
HS6	2-methylbutanal	malty	649	8	8	34
HS7	2-ethylfuran	butter-like, caramel-like	705	4	2	
HS8	methyl 2-methylbutanoate	fruity	775	8	2	
HS9	unknown	fruity	842	8	4	

[a]Retention index, calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [b]Flavor dilution factor; the headspace dilution factor of the lowest headspace volume 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-5 capillary column. [d]Odorants were identified by comparing their odor qualities and intensities, retention indices on a DB-5 capillary column, and mass spectra (EI mode) to data of reference compounds. [e]Odor quality perceived at the sniffing-port during SH-GC-O. [f]References previously reporting the compound in *T. sinensis*.

like, putrid), 4-hydroxy-2-butanone (**HS2**; garlic-like, onion-like), butanal (**HS3**; malty, sweaty), methyl propanoate (**HS4**; fruity), 3-methylbutanal (**HS5**; malty), 2-methylbutanal (**HS6**; malty), 2-ethylfuran (**HS7**; butter-like, caramel-like), and methyl 2-methylbutanoate (**HS8**; fruity). The compounds with the highest FD factors were **HS5** (>32), **HS1** (16), **HS6** (8), and **HS8** (8) (Table 3).

Identification of Key Odorants in VDTS. After AEDA and GC-MS experiments were applied to VDTS, 29 odorants in the FD factor range between 8 and 4096 were identified. No additional odorants were found compared with those found in SDTS, and most aroma compounds had lower FD factors. Eugenol (**52**; clove-like) was identified as the compound with the highest FD factor of 4096, followed by vanillin (**56**; FD factor of 2048; vanilla-like, sweet), decanoic acid (**54**; 1024; soapy, musty), 2-isopropyl-3-methoxypyrazine (**18**; 512; earthy, pea-like), β-ionone (**43**; 512; flowery, violet-like), acetic acid (**20**; 256, vinegar-like), and β-caryophyllene (**27**; 256; rubber-like, moldy) (Table 2).

HS-ADA revealed 3-methylbutanal (**HS5**; malty) with the highest FD factor of >32, followed by dimethyl sulfide (**HS1**; asparagus-like, putrid) and 2-methylbutanal (**HS6**; malty), both with an FD factor of 8 (Table 3).

Comparison of the aroma-active compounds identified in this study with those in previous literature revealed that 47 of the 64 odorants were identified in dried TS for the first time, and all the identified compounds were present in both TS samples. Interestingly, most studies on fresh TS leaves reported that the most important odorants were sulfur-containing compounds. However, only some of them were found in this study: 2,5-dimethylthiophene (**4**; fried onion-like, rubber-like) with FD factors of only 2 and 1 and dimethyl sulfide (**HS1**; asparagus-like, putrid) with FD factors of 16 and 8. This is in accordance with literature, which reports big differences in the aroma-active compounds between dried and fresh TS buds.[11] An explanation therefore might be the degradation of the potent sulfur compounds during the drying process.

Quantitation and Semiquantitation of the Key Odorants and Calculation of Odor Activity Values (OAVs). AEDA was used to screen for the odorants that should contribute to the aroma of TS. However, the disadvantage of AEDA is the fact that interactions of the odorants with the matrix, and thus, differences in aroma release are not considered. Therefore, quantitation of 36 compounds with high FD factors was performed by SIDA. Six further compounds, namely, aromadendrene, β-caryophyllene, caryophyllene oxide, α-humulene, isocaryophyllene, and valencene, were semiquantitated by the internal standard method via GC-FID using cyclopentadecanone as the internal standard.

Concentrations of Key Odorants. Acetic acid showed the highest concentration in SDTS (3 750 000 μg/kg), followed by eugenol, β-caryophyllene, 2-pyrrolidone, and propanoic acid (all >10 000 μg/kg). Lower concentrations (>5000 μg/kg) were found for 3-methylbutanoic acid, phenylacetic acid, α-humulene, valencene, 2-methylbutanoic acid, and butyrolactone, whereas 1,8-cineole showed the lowest concentration (3.5 μg/kg) (Table 4).

In VDTS, the compounds present at the highest concentrations were acetic acid, eugenol, β-caryophyllene (all >33000 μg/kg), propanoic acid, butyrolactone, decanoic acid, and nonanoic acid (all >2000 μg/kg), followed by caryophyllene oxide, 2-methylbutanal, 2-pyrrolidone, valencene, α-humulene, phenylacetic acid, and hexanoic acid (all >1000 μg/kg), whereas 2-ethyl-3,5-dimethylpyrazine and 1,8-cineole were only present in concentrations <10 μg/kg (Table 5).

OAVs of Key Odorants. Concentrations do not entirely determine if a certain odorant contributes to the overall aroma. Thus, quantitative data have to be combined with the respective odor thresholds, leading to so-called odor activity values (ratios of the concentrations divided by the odor thresholds).

In SDTS, calculation of OAVs indicated 35 of the 42 compounds had OAVs ≥1. The highest OAVs were found for 2-isopropyl-3-methoxypyrazine (200 000), eugenol (71 000), and β-ionone (20 000); they were followed by those of 3-methylnonane-2,4-dione (2600), 3-methylbutanal (1700), 2-methylbutanal (1200), dimethyl sulfide (1100), linalool (1000), hexanal (980), acetic acid (670), 2-ethyl-3,5-dimethylpyrazine (620), and decanoic acid (510) (Table 4).

In VDTS, a lot of compounds also revealed OAVs ≥1: eugenol (37 000), 2-isopropyl-3-methoxypyrazine (26 000), and β-ionone (11 000) resulted in the highest OAVs, followed by dimethyl sulfide (2100), 3-methylbutanal (1700), 2-methylbutanal (1200), linalool (660), and decanoic acid (630) (Table 5).

Aroma Simulation. First, aroma profile analysis of the two TS samples was carried out to evaluate the impression of the

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Table 4. Concentrations, Relative Standard Deviations (RSDs), Orthonasal Odor Thresholds (OTs), and Odor Activity Values (OAVs) of Important Aroma-Active Compounds in SDTS

odorant	concn (μg/kg)	RSD (%)	OT (μg/kg)	OAV
2-isopropyl-3-methoxypyrazine	773	10	0.0039[a]	200 000
eugenol	127 000	9	1.8[b]	71 000
β-ionone	410	10	0.021[b]	20 000
3-methylnonane-2,4-dione	120	4	0.046[b]	2600
3-methylbutanal	832	8	0.5[a]	1700
2-methylbutanal	1770	7	1.5[a]	1200
dimethyl sulfide	339	8	0.3[b]	1100
linalool	581	2	0.58[b]	1000
hexanal	2340	8	2.4[a]	980
acetic acid	4×10^6	7	5600[b]	670
2-ethyl-3,5-dimethylpyrazine	174	7	0.28[b]	620
decanoic acid	1780	12	3.5[b]	510
nonanoic acid	4350	14	26[b]	170
caryophyllene oxide	3570	11	22[c]	160
isocaryophyllene	2280	10	20[c]	110
phenylacetic acid	6950	12	68[b]	100
butyrolactone	5090	4	50[b]	100
methyl 2-methylbutanoate	251	14	2.5[b]	100
(*E*)-2-hexenol	378	8	3.9[b]	97
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	163	10	1.7[b]	96
valencene	5900	18	66[c]	89
α-humulene	6800	4	130[c]	52
β-caryophyllene	49 600	4	1200[c]	41
3-methylbutanoic acid	7760	1	490[a]	16
2-phenylethanol	1260	1	140[a]	9
1-octen-3-ol	325	17	45[b]	7
benzyl alcohol	4470	1	620[b]	7
2-ethyl-6-methylpyrazine	287	11	40[b]	7
2-pyrrolidone	13 200	13	2100[b]	6
methylpyrazine	639	4	110[b]	6
(*E*)-2-hexenal	90	5	17[b]	5
2-ethyl-3-methylpyrazine	2430	5	500[b]	5
vanillin	228	3	53[a]	4
aromadendrene	1010	6	340[c]	3
2-methylbutanoic acid	5100	1	3100[b]	2
1,8-cineole	3.53	11	4.6[a]	<1
propanoic acid	12 600	4	16 000[b]	<1
hexanoic acid	3090	4	4800[b]	<1
butanoic acid	905	9	2400[a]	<1
methylpropanoic acid	1850	4	16 000[b]	<1
pentanoic acid	1160	4	11 000[a]	<1
phenol	255	12	3400[b]	<1

[a]Orthonasal odor threshold in water as reported previously.[31] [b]Orthonasal odor threshold from an in-house database. [c]Orthonasal odor threshold was newly determined in this study, according to the literature.[31]

Table 5. Concentrations, Relative Standard Deviations (RSDs), Orthonasal Odor Thresholds (OTs), and Odor Activity Values (OAVs) of Important Aroma-Active Compounds in VDTS

odorant	concn (μg/kg)	RSD (%)	OT (μg/kg)	OAV
eugenol	66 700	6	1.8[a]	37 000
2-isopropyl-3-methoxypyrazine	100	8	0.0039[b]	26 000
β-ionone	230	2	0.021[a]	11 000
dimethyl sulfide	639	5	0.3[a]	2100
3-methylbutanal	830	7	0.5[b]	1700
2-methylbutanal	1760	10	1.5[b]	1200
linalool	385	5	0.58[a]	660
decanoic acid	2190	12	3.5[a]	630
3-methylnonane-2,4-dione	18.1	9	0.046[a]	390
acetic acid	2×10^6	12	5600[a]	270
hexanal	340	9	2.4[b]	140
caryophyllene oxide	1870	7	22[c]	85
nonanoic acid	2110	10	26[a]	81
butyrolactone	2610	8	50[a]	52
isocaryophyllene	639	2	20[c]	32
2-ethyl-3,5-dimethylpyrazine	8.72	11	0.28[a]	31
β-caryophyllene	33 200	6	1200[c]	28
valencene	1490	8	66[c]	23
phenylacetic acid	1120	2	68[a]	16
methyl 2-methylbutanoate	33	12	2.5[a]	13
(*E*)-2-hexenol	44.4	6	3.9[a]	11
vanillin	516	7	53[b]	10
α-humulene	1210	16	130[c]	9
1-octen-3-ol	392	10	45[a]	9
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	12.8	10	1.7[a]	8
2-phenylethanol	404	5	140[b]	3
(*E*)-2-hexenal	41.3	11	17[a]	2
aromadendrene	673	13	340[c]	2
3-methylbutanoic acid	639	5	490[b]	1
1,8-cineole	3.9	3	4.6[b]	<1
2-pyrrolidone	1530	10	2100[a]	<1
benzyl alcohol	366	9	620[a]	<1
2-ethyl-6-methylpyrazine	16.3	8	40[a]	<1
2-methylbutanoic acid	898	6	3100[a]	<1
propanoic acid	3920	11	16 000[a]	<1
hexanoic acid	1040	6	4800[a]	<1
methylpyrazine	15.4	6	110[a]	<1
butanoic acid	227	5	2400[b]	<1
methylpropanoic acid	817	4	16 000[a]	<1
2-ethyl-3-methylpyrazine	16.3	10	500[a]	<1
pentanoic acid	300	12	11 000[b]	<1
phenol	44.7	11	3400[a]	<1

[a]Orthonasal odor threshold from an in-house database. [b]Orthonasal odor threshold in water as reported previously.[31] [c]Orthonasal odor threshold was newly determined in this study, according to the literature.[31]

overall aroma. SDTS showed intense seasoning-like/spicy, earthy, malty, vinegar-like, and hay-like/grassy odor attributes (Figure 1A), whereas VDTS was evaluated as being higher in the hay-like/grassy and flowery/violet-like aroma notes (Figure 1B).

To validate the qualitative and quantitative data of both kinds of dried TS, two aroma recombinates were prepared. Therefore, all odorants showing OAVs ≥1 were mixed in their naturally

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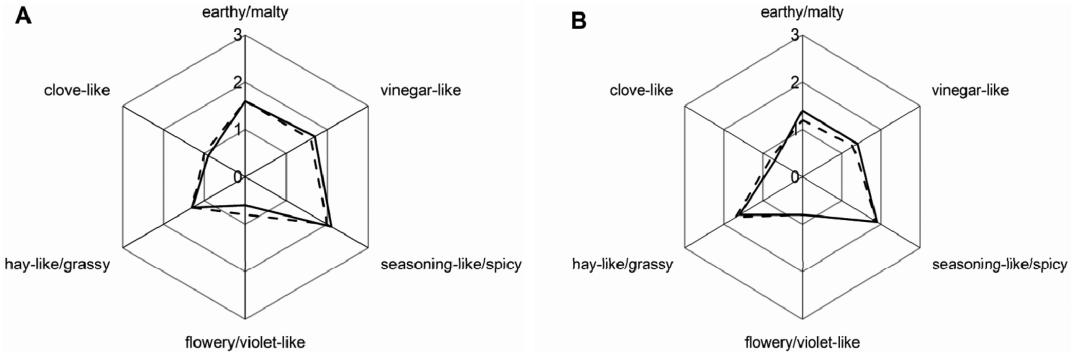

Figure 1. (A) Aroma profiles of SDTS (solid line) and the respective recombinate (broken line) and (B) aroma profiles of VDTS (solid line) and the respective recombinate (broken line).

occurring concentrations in an aqueous solution, which was then added to a deodorized TS matrix obtained after several extraction steps from the respective dried TS. Both recombinates revealed typical intense aromas very similar to those of the original dried TS samples, proving that all key aroma-active compounds were successfully characterized (Figures 1A and B).

Sources of Key Odorants in SDTS and VDTS. 2-Isopropyl-3-methoxypyrazine is known to be widespread in vegetables, plants, and seeds.[35−37] However, its formation pathway has not been clearly elucidated so far. In this study, 2-isopropyl-3-methoxypyrazine resulted in nearly the highest OAVs in both kinds of TS and was found as an important aroma-active compound in TS buds for the first time. Murray et al. proposed a biosynthetic origin in plant tissue.[37] According to this hypothesis, the odorant derives from α-amino acids and α-dicarbonyl compounds. In contrast, Nursten and Sheen insisted that 2-isopropyl-3-methoxypyrazine could not be biosynthesized without glyoxal or α-amino acid amides.[38] However, there has been no evidence that glyoxal really exists in plant tissues and that α-amino acid amides can be formed from α-amino acids so far. They suggested glyoxylic acid plays a key role during the enzymatic or *O*-methylated reaction for ring formation. The second ring-nitrogen may come from Murray's assumption, from a mono-nitrogen derivative of the dicarbonyl, or from a direct involvement of ammonia.

As a protective substance against predators on the one hand and a floral attractant of pollinators on the other, eugenol has a very intense clove-like odor and possesses an extremely low odor threshold in water, leading to an important aroma-active compound in many plants. With its additional antimicrobial properties, eugenol-rich clove has been extensively applied as a spice to flavor food or as a food-preserving substance. In this study, eugenol also showed high OAVs in both kinds of TS. Despite a well-recognized biosynthesis theory, the formation pathway of eugenol in plants has not yet been completely proven. In 1975, Klischies et al. proposed that phenylpropene is synthesized from substituted phenylpropenol, although they could not explain the unusual nature of the reaction mechanism involved in this transformation, mainly because enzymatic procedures were not taken into consideration.[39] About 30 years later, on the basis of a biochemical experiment, Koeduka et al. pointed out that glandular trichomes of sweet basil (*Ocimum basilicum*), which synthesize and accumulate phenylpropenes, possess an enzyme that can use coniferyl acetate and NADPH to form eugenol.[40]

The flowery and violet-like smelling β-ionone is a well-known aroma compound in fruits, vegetables, and ornamentals.[41,42] Because of its low odor threshold in water (0.021 μg/kg), β-ionone was also a key odorant in both kinds of TS, showing the third highest OAVs. Owing to its importance, several formation pathways have already been described. Isoe et al. assumed that β-carotene, the most widespread tetraterpene in plants, is the direct precursor of β-ionone.[41] It could be formed by photooxygenation of β-carotene solution in benzene and methanol without sensitizer. After oxygenation and irradiation, β-ionone was isolated as the main product from the distilled residue. However, Simkin et al. suggested two carotenoid cleavage dioxygenase genes, *LeCCD1A* and *LeCCD1B*, playing an important role in the formation of β-ionone.[42] Although a lot of biochemical evidence was presented in their study, details on the mechanisms of gene expression of *LeCCD1* were not clearly explained.

The malty smelling 2-methylbutanal is well-known to be formed by Strecker degradation of L-isoleucine.[43] Moreover, 2-methylbutanal can also be formed by transamination of L-isoleucine, followed by decarboxylation of the intermediate 2-oxo-3-methylpentanoic acid via the so-called Ehrlich pathway.[44] 2-Methylbutanoic acid can also be formed from L-isoleucine via the Ehrlich pathway, either by oxidative decarboxylation of 2-oxo-3-methylpentanoic acid or by oxidation of the formed 2-methylbutanal. Esterification of 2-methylbutanoic acid with methanol in microbial fermentation can finally lead to the fruity smelling methyl 2-methylbutanoate. The formation of 3-methylbutanal and 3-methylbutanoic acid is based on the similar reactions starting from L-leucine.

The hay-like, aniseed-like, and fishy smelling 3-methylnonane-2,4-dione can be formed by an oxidative-degradation pathway of furan fatty acids.[45]

Dimethyl sulfide, eliciting a putrid and asparagus-like odor at a very low odor threshold of 0.3 μg/kg in water, was previously reported as an odorant in numerous cooked vegetables.[46] During thermal processing and storage of foods, dimethyl sulfide can be formed from the amino acid *S*-methylmethionine, which was recently proven to be a precursor using a stable isotope dilution assay.[47] Thus, dimethyl sulfide in TS might also be formed from *S*-methylmethionine.

The precursor of β-caryophyllene is farnesyl diphosphate, which is endogenously produced by many biological systems. Reinsvolda et al. inserted the β-caryophyllene synthase gene from *Artemisia annua* into the genome of the cyanobacterium *Synechocystis* via double homologous recombination and synthesized β-caryophyllene successfully.[48] Due to the fact that the structures of aromadendrene, caryophyllene oxide, α-humulene, isocaryophyllene, and valencene are similar to that of

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β-caryophyllene, it might be possible that these compounds can also be produced by the above-mentioned biological pathway. Furthermore, Lavy et al. found a linalool synthase gene in transgenic carnation flowers that could produce linalool, another citrus-like and flowery smelling aroma-active compound in TS.[49]

In conclusion, a total of 47 aroma-active compounds were characterized in dried *T. sinensis* for the first time, with 2-isopropyl-3-methoxypyrazine, eugenol, and β-ionone as important key odorants for the overall aroma of both kinds of dried *T. sinensis* buds. Furthermore, clear differences in the amounts of the aroma-active components were found for SDTS and VDTS. SDTS showed a more intense aroma, with more compounds present at higher OAVs compared with VDTS. Thus, the formation of key aroma-active compounds in both kinds of dried TS buds was influenced by the raw material and by the processing conditions, which resulted in different final aromas. Studies will be continued on fresh leaves and leaves dried with different drying methods to elucidate the influences of the raw materials and processing parameters on the final aroma of dried *T. sinensis* leaves in more detail.

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Notes

The authors declare no competing financial interest.

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8.3.3 Summary and individual contributions

Our recent investigations on raw[112] (section **8.3.1**) and differently dried TS buds[113] (section **8.3.2**) revealed high OAVs for di-1-propenyl disulfide, dimethyl sulfide, *β*-ionone, eugenol, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, di-1-propenyl trisulfide, 2- and 3-methylbutanal, (*E*,*Z*)-2,6-nonadienal, 2-isopropyl-3-methoxypyrazine, 3-methyl-2,4-nonandione, and hexanal and indicated these compounds to be key aroma-active components in raw green and red TS varieties as well as in dried TS. However, blanching and further salting are two common and essential steps prior to drying TS buds as commercial products. Thus, the key odorants and overall aromas might change a lot. To get a deeper insight into the aroma qualities of commercially dried TS products on the market, the sensomics approach for the characterization of the key odorants in commercially solar-dried TS (CSDTS) and vacuum-dried TS (CVDTS) was applied.

A total of 64 aroma-active compounds were identified. Thirty-nine odorants in CSDTS and 32 odorants in CVDTS revealed FD factors from 8 to 4096 via AEDA and SH-ADA. The highest FD factors in both samples were found for vanillin, eugenol, 2-isopropyl-3-methoxypyrazine, acetic acid, isocaryophyllene, 3-methylnonane-2,4-dione, *β*-ionone, and decanoic acid. To elucidate the screening data, stable isotope dilution analysis (SIDA) / internal standard method were applied to quantitate / semiquantitate 42 odorants, revealing 35 compounds in CSDTS and 29 compounds in CVDTS with OAVs ≥ 1. Among them, 2-isopropyl-3-methoxypyrazine, eugenol, and *β*-ionone were present at the highest OAVs in both samples. In addition, other compounds with high OAVs in CSDTS were 3-methylnonane-2,4-dione, 3-methylbutanal, 2-methylbutanal, dimethyl sulfide, linalool, hexanal, acetic acid, 2-ethyl-3,5-dimethylpyrazine, decanoic acid, nonanoic acid, caryophyllene oxide, isocaryophyllene, phenylacetic acid, butyrolactone, and methyl 2 methylbutanoate (OAVs ≥ 100). Compounds with high OAVs in CVDTS were dimethyl sulfide, 3-methylbutanal, 2-methylbutanal, linalool, decanoic acid, 3-methylnonane-2,4-dione, acetic acid, and hexanal (OAVs ≥ 100).

However, some sulfur-containing compounds, typical for a well pronounced cooked onion-like/TS-like aroma, including di-1-propenyl sulfide, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, and di-1-propenyl trisulfide were not identified in these two commercial products. Recombination experiments of the overall aromas of CSDTS and CVDTS by mixing the odorants with OAVs ≥ 1 in their naturally occurring concentrations were performed to verify the identification and quantitation data. Thereby, aroma profiles of both recombinates showed very good similarities with the respective sample, proving the successful identification and quantitation of all key odorants. The results illustrated both the raw material and the processing conditions influenced the formation of key odorants in both products, which resulted in different final aromas.

Miss Xiaoting Zhai designed and performed all experiments including volatiles isolation, HRGC-O screenings, identification, quantitation, and sensory experiments. Miss Zhai evaluated all results and prepared the manuscript. Prof. Dr. Michael Granvogl guided Miss Zhai's work, conceived and directed the study, and revised the manuscript. In addition, Prof. Granvogl participated in the sensory tests.

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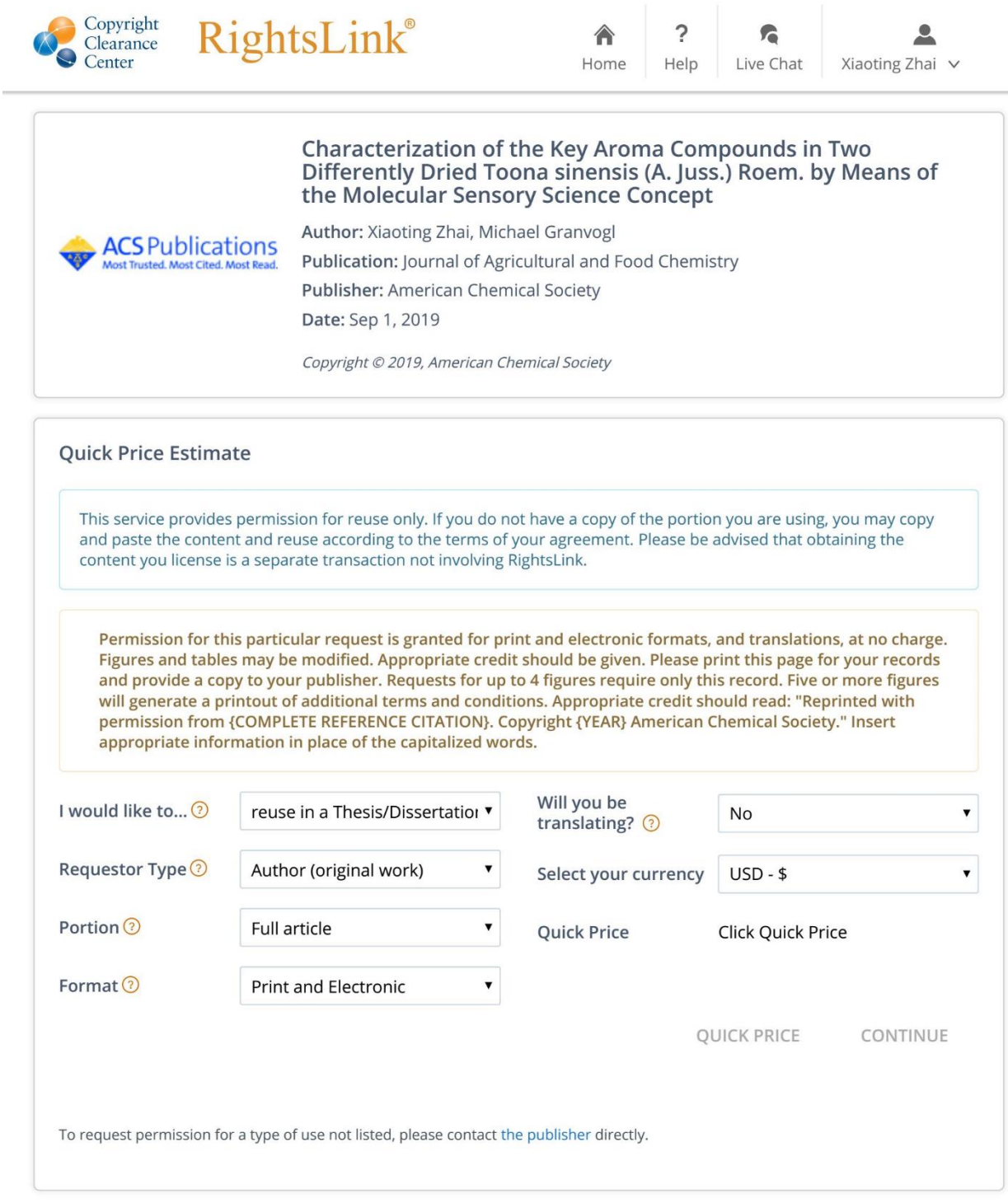

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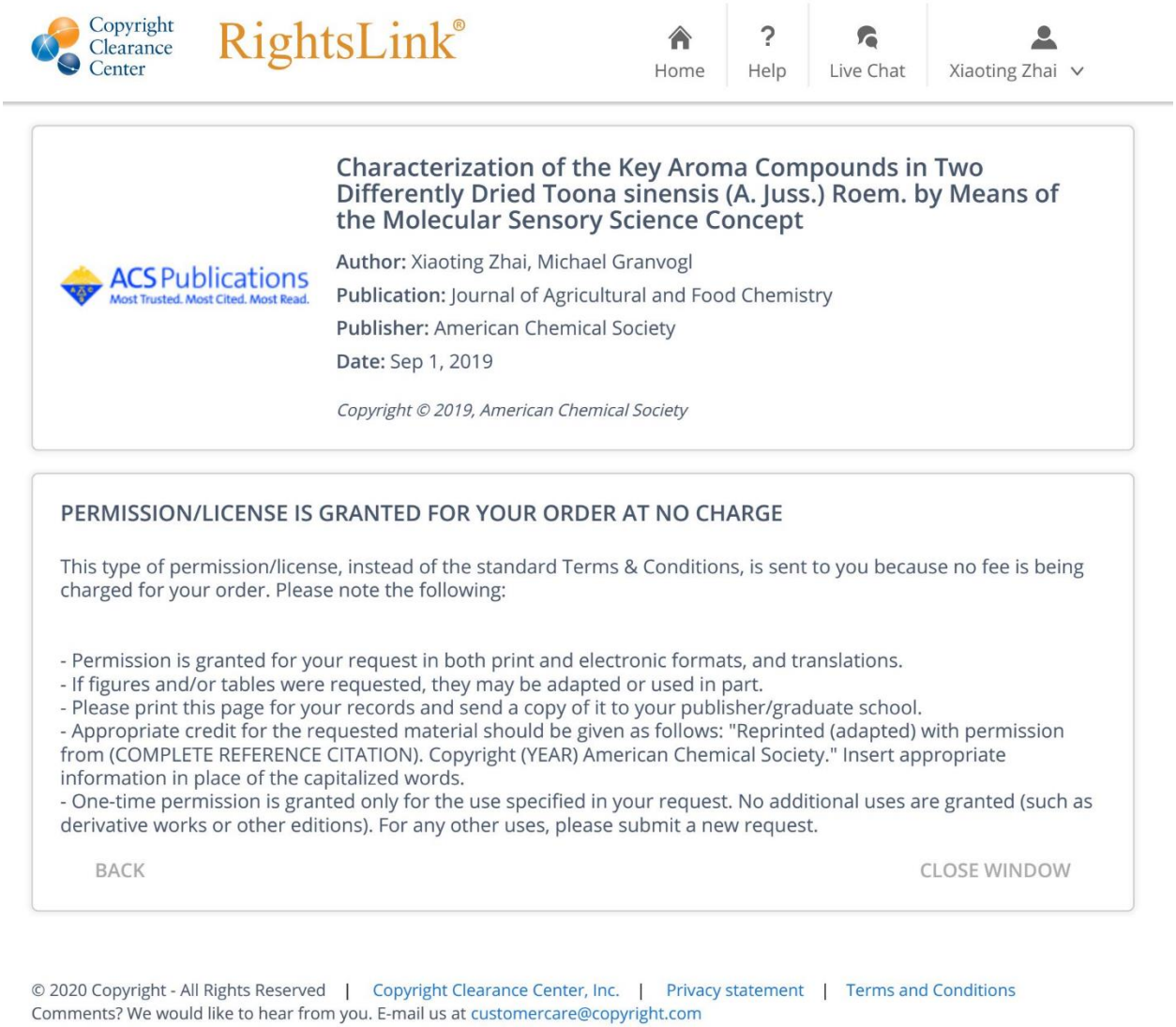

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8.4 List of publications and poster presentations

Publications

Zhai, X.; Granvogl, M. Characterization of the key aroma compounds in two differently dried *Toona sinensis* (A. Juss.) Roem. by means of the molecular sensory science soncept. *J. Agric. Food Chem*. **2019**, *67*, 9885-9894. DOI: 10.1021/acs.jafc.8b06656

Zhai, X.; Granvogl, M. Key odor-active compounds in raw green and red *Toona sinensis* (A. Juss.) Roem. and their changes during blanching. *J. Agric. Food Chem*. **2020**, *68*, 7169-7183. DOI: 10.1021/acs.jafc.0c02012

Zhai, X.; Granvogl, M. Elucidation of the impact of different drying methods on the key odorants in *Toona sinensis* (A. Juss.) Roem. using the sensomics approach. *J. Agric. Food Chem*. **2020**, *68*, 7697-7709. DOI: 10.1021/acs.jafc.0c02144

Poster presentations

Characterization of the key aroma compounds in dried *Toona Sinensis* (A. Juss.) Roem. using the molecular sensory science concept. The 2nd International Flavor and Fragrance Conference. Wuxi, China, May 28-31, 2018.