

Temporal Changes in Aroma Release of Longjing Tea Infusion: Interaction of Volatile and Nonvolatile Tea Components and Formation of 2-Butyl-2-octenal upon Aging

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The temporal change in the headspace composition of an aroma model mimicking Longjing green tea aroma was studied in the presence of nonvolatile Longjing green tea constituents. Upon storage at 50 °C, the formation of 2-butyl-2-octenal was found, which increased with time. This enal was generated by crotonization of hexanal as demonstrated in model experiments. The formation of 2-butyl-2-octenal was also detected in Longjing tea infusions and Longjing tea leaves upon storage at 50 °C. The presence of nonvolatiles induced a strong decrease in aroma release. These effects were mainly due to catechins, major constituents of green tea infusion. Free amino acids, that is, glycine, contributed only to significantly decrease α,β -unsaturated carbonyl aroma compounds, that is, 1-octen-3-one and geranial. Model reaction containing a mixture of 1-octen-3-one and glycine indicated on the basis of NMR and MS data the formation of the tentatively identified *N*-1-(3-oxooctyl)glycine resulting from a 1,4-addition. The perceived aroma of green tea infusion is very likely to be affected by the formation of new aroma compounds and the changes in aroma release affected by interactions with tea nonvolatile components. This deserves further investigations on the sensory level.

KEYWORDS: Volatile aroma component; nonvolatile component; green tea; interaction; temporal change; 2-butyl-2-octenal; Longjing tea

INTRODUCTION

Tea is the most consumed beverage in the world after water. Annual production of dried tea leaves is estimated at 1.8 million tons, providing 40 L of tea beverage per capita worldwide. Compared to Oolong and black tea, the consumption of green tea has been increasing during recent years, which is likely due to the more valuable health-related properties of green tea.

In China, green tea is frequently prepared in the morning and consumed throughout the day. Such a way of consumption could influence the aroma profile of the beverage, which has not yet been investigated, although tea aroma is in general well documented in the literature (1–4) including the aroma of Longjing green tea (5–8), one of the most famous Chinese traditional tea blends (9, 10). Therefore, it appeared interesting to study the temporal change of the aromatic profile of a freshly prepared green tea infusion, in particular of Longjing green tea. Also interesting is to understand the aroma change at the

molecular level induced by green tea nonvolatile components, which was scarcely investigated in the past.

Accordingly, the purpose of this work was to study the temporal change of the headspace release of aroma compounds in a model mimicking Longjing green tea aroma in the presence of nonvolatile constituents of Longjing green tea. Therefore, it was necessary to first characterize the aroma composition of the Longjing green tea infusion. The effects of nonvolatiles on aroma release were investigated at the molecular level. Focus was given to selected aroma compounds, which were strongly affected, as well as to a newly identified volatile compound in Longjing tea and its formation upon storage of a Longjing tea infusion model.

EXPERIMENTAL PROCEDURES

Materials. The chemicals, that is, 3-methylbutanal, hexanal, 2,3-butanedione, ethyl 3-methylbutanoate, 2,5-dimethyl-3-ethylpyrazine, 1-octen-3-ol, linalool, benzaldehyde, geraniol, and geranial were from Aldrich (Buchs, Switzerland). 1-Octen-3-one was from Lancaster (Cardiff, U.K.), and (*E*)- β -damascenone was from Firmenich (Geneva, Switzerland). Green tea polyphenol extracts, that is, Sunphenon epigallocatechin gallate (94% EGCG) and Sunphenon LB90 (85% total catechins, 49% of which being EGCG), were provided by Taiyo Green Powder (Wuxi, China), a company of Taiyo Kagaku Co., Ltd. (Gunma,

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Table 1. Composition and Concentration of the Aroma Model

no.	compound	concn ($\mu\text{L/L}$)
1	3-methylbutanal	3
2	2,3-butanedione	32
3	ethyl 3-methylbutanoate	0.75
4	hexanal	4
5	1-octen-3-one	2.6
6	2,5-dimethyl-3-ethylpyrazine	120
7	1-octen-3-ol	7.5
8	benzaldehyde	7.5
9	linalool	7
10	geranial	37.5
11	(<i>E</i>)- β -damascenone	15
12	geraniol	120

Japan). High grade Longjing green tea (*Camellia sinensis* var. *sinensis*) leaves were kindly provided by Dr. Tang Xinjun (Daming Food Co., Zhangzhou, China). Dichloromethane and ethanol from Merck (Darmstadt, Germany) were distilled before use. Aqueous sodium hydroxide was from Merck. Water was treated with a Milli-Q (Millipore, Bedford, MA) filtration system prior to use. Deuterium oxide (99.9 atom % D, containing 0.05 wt % TSP) was from Sigma-Aldrich (Steinheim, Germany). All standards for aroma identification mentioned in **Table 1** were from Sigma-Aldrich or Merck.

Isolation of Nonvolatile Components from Longjing Green Tea Infusion. Longjing green tea leaves were frozen in liquid nitrogen and crushed to powder. Hot deionized Millipore water (80 °C, 800 mL) was added to 20 g of tea powder. After 5 min of gentle stirring at room temperature, the solid was removed by filtration. The filtrate was filtered again and then concentrated using Rotavapor (Buchi, Flawil, Switzerland) under reduced pressure (40 mbar) at 40 °C. The procedure was applied twice. The residue was freeze-dried, yielding 3 g of a green-yellowish powder with a weak tea smell. The powder was stored at –30 °C until use.

Isolation of Volatile Components from Longjing Green Tea Infusion. Forty grams of Longjing green tea leaves was infused with 600 mL of hot water (ca. 80 °C). The infusion was allowed to cool to room temperature. After 5 min at 20 °C, the leaves were removed by filtration. The filtrate was distilled under vacuum ($\sim 10^{-4}$ mbar) at 38 °C using the SAFE technique (11). The distillate was extracted with diethyl ether (3 \times 400 mL). The organic fractions were combined, dried over anhydrous sodium sulfate, and reduced to 20 mL by evaporation at 38 °C using a Vigreux column (50 \times 1 cm) and finally to 400 μL by microdistillation. The concentrate was kept at –30 °C until GC-MS and GC-O analysis.

Preparation of the Longjing Tea Infusion. Twenty grams of Longjing green tea leaves was infused with 250 mL of hot water (ca. 80 °C). The infusion was allowed to cool to room temperature. After 5 min at 20 °C, the leaves were removed by filtration. Eight milliliters of the filtrate was transferred into 20 mL clear glass vials (Sigma), capped with PTFE-faced silicone septum (Sigma). After heating at 50 °C at various time intervals, the samples were cooled to 25 °C and analyzed by SPME/GC-MS.

Aging of Longjing Tea Leaves. Half a gram of Longjing tea leaves was weighed into a 20 mL clear glass vial (Sigma), capped with PTFE-faced silicone septum (Sigma), and stored in a Binder thermostat incubator (Tuttlingen, Germany) at 50 °C for 24 h. The vials were cooled to room temperature using tap water, and 8 mL of hot water (80 °C) or ambient water (20 °C) was then added. After 5 min, the aqueous mixtures were centrifuged and cooled again to 20 °C using tap water. Two milliliters of the clear supernatant was transferred into a 20 mL clear glass vial (Sigma), capped as above, and analyzed by SPME/GC-MS (as described below except that the equilibration temperature was 30 °C instead of 50 °C). Similarly, the headspace of the aged tea leaves was also analyzed prior to water treatment.

Preparation of the Aroma Model. Aliquots of stock solutions containing selected aroma compounds in ethanol were transferred into 200 mL volumetric flasks and made up with water. The final solution of aroma model was composed of 12 aroma compounds, and their

concentrations are summarized in **Table 1**. Aliquots of aroma solution were sampled. Optionally, nonvolatile green tea constituents were directly weighed into the flask containing an aliquot of the aqueous solution of the aroma model followed by 1 h of stirring. All samples were adjusted to pH 6 with aqueous sodium hydroxide (0.01 M). Eight milliliters of the resulting solution was then transferred into a 20 mL clear glass vial (Sigma), capped with PTFE-faced silicone septum (Sigma), and analyzed by headspace GC-MS. The linearity of the peak area detected by GC-MS for each odorant in the concentration range of interest was checked by an external calibration curve using successive dilutions (1–10-fold).

Gas Chromatography–Mass Spectrometry (GC-MS). GC-MS analyses were performed using an HP 5890 series 2 gas chromatograph (Hewlett-Packard, Wilmington, DE) coupled to an HP 5972 series mass spectrometer (Hewlett-Packard) and equipped with a Gerstel MPS2 autosampler (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). Fused silica capillary columns (DB-1 and DB-Wax) were used, both of 30 m \times 0.32 mm i.d. and film thickness 0.25 μm (J&W Scientific, Folsom, CA). The oven temperature program for the DB-1 column was as follows: 35 °C (1 min), raised at 70 °C/min to 60 °C (1 min), and raised at 5 °C/min to 280 °C (15 min). That for the DB-Wax column was 20 °C (1 min), raised at 60 °C/min to 70 °C, and raised at 6 °C/min to 240 °C (20 min). The injected volume was 1 μL using the splitless mode. The injector port temperature was 250 °C. The flow rates of the helium carrier gas were 1 and 1.2 mL/min for the DB-Wax and DB-1 columns, respectively. The mass spectrometer was operated in electron ionization mode at 70 eV. The interface temperature was set at 230 °C and the source temperature at 150 °C.

Headspace SPME GC-MS. This was performed using similar equipment as mentioned above for GC-MS. The sample was equilibrated at 50 °C under stirring (300 rpm) for 30 min. A SPME fiber coated with polydimethylsiloxane/divinylbenzene (PDMS/DVB) of 65 μm thickness (Supelco) was then inserted into the headspace and allowed to equilibrate for 30 min at 50 °C. Aroma compounds were desorbed into the injector port of the GC for 5 min at 250 °C (3 min in splitless mode before opening the split). A DB-Wax fused silica capillary column (J&W Scientific, 30 m, 0.25 mm i.d., 0.25 μm film thickness) was used. The oven temperature program was 20 °C (3 min) raised at 6 °C/min to 240 °C (20 min). The flow rate of the helium carrier gas was 1.2 mL/min.

Static Headspace GC-MS. This was performed using similar equipment as mentioned above for GC-MS. The sample was equilibrated at 50 °C under stirring (300 rpm) for 1 h. One milliliter of headspace was injected into an injector set at 250 °C with a split ratio of 4:1. A similar DB-Wax fused silica capillary column as for headspace SPME GC-MS was used. The oven temperature program was 20 °C (3 min), raised at 6 °C/min to 100 °C, and raised at 10 °C/min to 240 °C (15 min). The flow rate of the helium carrier gas was 1 mL/min.

Gas Chromatography–Olfactometry (GC-O). GC-O was performed by five assessors. It was carried out on a Hewlett-Packard 5890 and a Fison 8000 series gas chromatograph, both equipped with a flame ionization detector (FID) and a sniffing port. DB-Wax and DB-1 fused silica capillary columns and oven temperature programs similar to those used for GC-MS analysis were used. The column effluent was split 1:1 (by volume) into the FID and a heated sniffing port, with moist air pumped into the sniffing port at 100 mL/min.

Aroma Extract Dilution Analysis (AEDA). The original concentrated extract was stepwise diluted with diethyl ether to 1:10, 1:20, 1:100, and 1:1000. The odor-active compounds were detected by GC-O and the flavor dilution (FD) factors of the odorants determined accordingly. The FD factor values were selected according to the maximum values obtained by the best sniffer for each component (12).

Identification of Volatile Components. Identification of the components was achieved by comparison of the Kovats linear retention indices, EI mass spectra, and odor quality to those previously reported in the literature using mass spectra databases (Wiley7N, NIST05, Pal600K, Oceane), the Flavornet database (13), and an in-house flavor database. For the identification of some compounds, authentic reference compounds were used whenever possible.

Table 2. Odor-Active Compounds of Longjing Green Tea (FD Factors ≥ 20)

compound			RI measured		RI reported (ref)		
no.	name	odor quality (GC/O)	FD factor	DB-Wax	DB-1	DB-Wax	DB-1
1	ethyl 3-methylbutanoate ^c	fruity	20	1054	843	1050 ^e	835 (13)
2	heptanal ^b	grass, mushroom	20	1189	879	1177 (14)	885 (13)
3	1-hepten-3-one ^c	grass, metallic	20	1200		1214 ^e	
4	unknown	cooked fruit-like	20	1235			
5	(Z)-4-heptenal ^d	fatty, fish	20	1247	871	1265 (13)	
6	2-methyl-3-furanthiol ^c	meaty, roasty	100	1308		1313 ^e	
7	1-octen-3-one ^a	mushroom-like	100	1309	955	1301 (7)	955 ^e
8	2-acetyl-1-pyrroline ^a	roasty	20	1338	892	1345 ^e	898 (13)
9	(Z)-1,5-octadien-3-one ^c	metallic	1000	1378	961	1375 (7)	
10	(Z)-3-hexen-1-ol ^b	woody, mushroom	20	1389	839	1390 (6)	844 (13)
11	4-mercapto-4-methyl-2-pentanone ^a	sulfury, roasty	20	1389	913	1377 ^e	
12	nonanal ^b	floral, green	20	1389	1083	1385 (14)	1079 (13)
13	2,5-dimethyl-3-ethylpyrazine ^b	sweaty, roasty	20	1433	1072	1439 (14)	1062 (13)
14	acetic acid ^a	acidic	100	1454		1445 ^e	
15	15 Methional ^a	cooked potato-like	1000	1461	862	1458 (13)	856 ^e
16	2-ethyl-3,5-dimethylpyrazine ^a	roasty	100	1473	1072	1470 (7)	1061 ^e
17	(E,Z)-3,5-octadien-2-one ^b	green	100	1514	1046	1493 (14)	
18	2-isobutyl-3-methoxypyrazine ^a	bell pepper, green	100	1532	1174	1519 ^e	1163 ^e
19	(E)-2-nonenal ^a	leather-like, green	100	1533		1533 ^e	1136 ^e
20	linalool ^a	floral	100	1551	1106	1545 (14)	1085 ^e
21	(E,Z)-2,6-nonadienal ^a	fatty, cucumber-like	20	1595	1122	1578 ^e	1137 (13)
22	γ -butyrolactone ^a	caramel	20	1622	858	1647 (13)	852 ^e
23	2-acetylpyrazine ^a	roasty, popcorn-like	20	1633	997	1621 ^e	1003 (13)
24	phenylacetaldehyde ^a	honey-like	100	1650	1015	1651 ^e	1007 ^e
25	2-methylbutanoic acid ^a	cheesy	100	1677	852	1674 (6)	856 (13)
26	3-methylbutanoic acid ^a	cheesy	20	1687	838		838 ^e
27	methyl geranate ^a	woody, leaf, floral	100	1690	1296		1301 ^e
28	(E,E)-2,4-nonadienal ^a	fatty, green	100	1709	1214	1693 ^e	1200 (13)
29	geranial ^a	floral, leaves	1000	1726	1252	1725 ^e	1258 (13)
30	2-acetyl-2-thiazoline ^c	peanut, roasty, rice	100	1765	1067	1764 (7)	1088 (13)
31	(E,E)-2,4-decadienal ^b	green, fatty	100	1818		1815 (7)	
32	(E)- β -damascenone ^a	floral, honey-like	100	1820		1811 ^e	1361 ^e
33	2-phenylethyl acetate ^a	floral, honey-like	100	1831		1821 (6)	1228 ^e
34	geranylacetone ^b	tea leaves, floral	100	1845	1424	1859 (7)	1431 (13)
35	geraniol ^a	floral, woody	1000	1853	1234	1851 ^e	1258 (13)
36	α -ionone ^b	woody, floral	1000	1853	1400	1866 (7)	1408 (13)
37	guaiacol ^a	spicy, smoky	20	1866	1066	1872 (7)	1058 ^e
38	2-phenylethanol ^b	floral, honey, sweet	20	1920	1081	1925 (13)	1080 (13)
39	β -ionone ^a	tea leaves, woody	100	1941	1360	1934 ^e	1363 ^e
40	(Z)-jasmone ^a	woody, floral	100	1946	1460	1953 (7)	1460 ^e
41	3-hexenoic acid ^b	sour, cheesy	100	1947		1954 (14)	
42	benzothiazole ^a	burnt, sulfury, meaty	20	1950	1191	1973 (14)	1184 ^e
43	β -ionone-5,6-epoxide (cis type) ^b	tea leaves, woody	100	1980	1460	2007 (14)	
44	4-hydroxy-2,5-dimethyl-3(2H)-furanone ^b	caramel-like	1000	2049	1030	2041 (7)	1023 (13)

^a Identification based on RI, MS, odor quality (GC/O) and comparison with the authentic reference compound. ^b Tentatively identified based on RI, MS, odor quality (GC/O). ^c Tentatively identified as the MS data were too weak, but the remaining data were consistent with the authentic reference material. ^d Tentatively identified based on RI and odor quality (GC/O) and MS data were too weak for unequivocal identification. ^e RI values obtained with the authentic reference material.

Model Reaction of 1-Octen-3-one with Glycine. A mixture of 1-octen-3-one (18.2 mg, 0.144 mmol), glycine (10.9 g, 145 mmol), and 1-octanol (18.9 mg, 0.145 mmol, used as internal standard) dissolved in water (150 mL) was heated at 50 °C for 1 h. After cooling to 25 °C, the reaction mixture was extracted with dichloromethane (3 \times 50 mL). The organic extracts were combined, dried over Na₂SO₄, and concentrated to 10 mL. The concentrate was analyzed using GC-MS as described above. The ratio of 1-octen-3-one/1-octanol was determined on the basis of their peak area, as well as the loss of 1-octene-3-one, and compared to those similarly obtained from the reaction of 1-octen-3-one (18.0 mg, 0.143 mmol) and 1-octanol (18.9 mg, 0.145 mmol) in the absence of glycine. The aqueous layer was lyophilized, yielding a white powder (10.74 g), which was redissolved in methanol (100 μ g/mL) for LC-MS analysis.

The LC conditions were as follows: solvent A, H₂O acidified with 0.1% formic acid; solvent B, methanol; column, Macherey-Nagel Nucleosil C18 ec 3 μ m, 250 \times 4 mm, 120 Å. The gradient given in **Table 3** was applied.

The MS conditions were as follows: A Micromass Quattro and Q-TOF II hybrid tandem mass spectrometer were used, both in electrospray positive mode. Parameters on the Quattro instrument were

Table 3. LC Gradient

time (min)	solvent A (%)	solvent B (%)	flow rate (μ L/min)	curvevalue
0	99.5	0.5	400	1
3	99.5	0.5	400	6
4	35	65	400	6
10	5	95	400	6
10.1	0	100	400	6
13	0	100	400	6
13.1	99.5	0.5	400	6
20	99.5	0.5	400	6

as follows: capillary voltage, 2.75 kV; cone voltage, 40 V; extractor, 4 V; hexapole, 0.2 V; source block temperature, 100 °C; desolvation temperature, 300 °C. Collisional energy was varied to obtain a representative fragmentation pattern. In the MRM mode, the following transitions were selected: 202.2/42.4, 202.2/60.4, and 202.2/88.2. Parameters on the Q-TOF instrument were as follows: capillary voltage, 2.75 kV; cone voltage, 20 V; extractor, 4 V; hexapole, 0.5 V; source block temperature, 150 °C; probe temperature, 300 °C; LM and HM resolution, 4 units; aperture, 5 V; flight time, 88 μ s; mass 1, 2 amu;

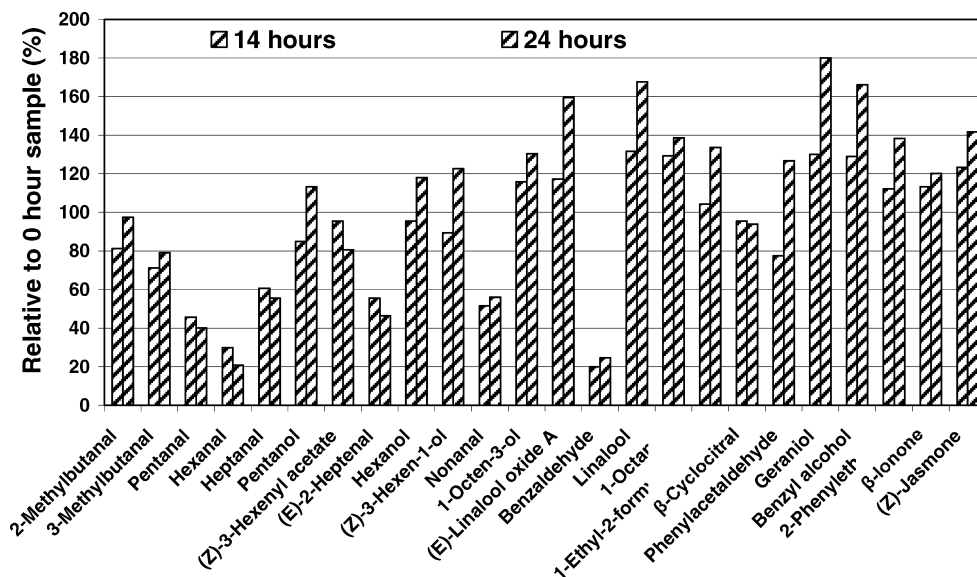


Figure 1. Aging effect on aroma compounds of Longjing tea infusion. Data were obtained by SPME/GC-MS analysis (at least duplicates; CV < 15%).

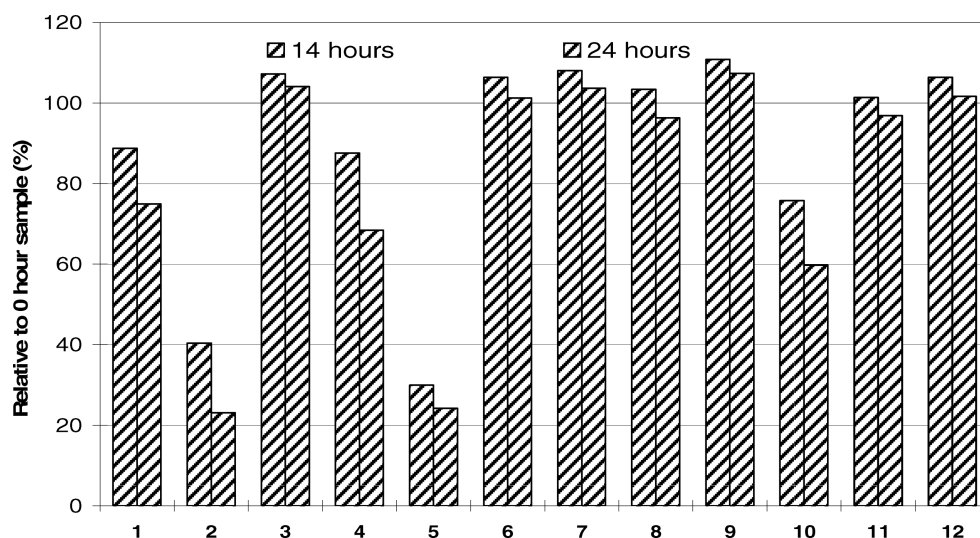


Figure 2. Aging at 50 °C of an aroma model with 1% nonvolatiles isolated from Longjing tea infusion (at least duplicates; CV < 10%). Numbering refers to that in Table 1.

time 1, 10%; ramp 1, 5%; mass 2, 170 amu; time 2, 85%; ramp 2, 0%; mass 3, 170 amu; mass range, 100–1000 Th; scan duration, 1 s; interscan delay, 0.1 s. The Q-TOF was calibrated with phosphoric acid (0.1% H_3PO_4 in H_2O /acetonitrile 1:1).

MS/MS fragmentation: 202 ($\text{M} + \text{H}^+$); 88 ($\text{CH}_3\text{NHCH}_2\text{CH}_2\text{CHO} + \text{H}^+$) and/or ($\text{CH}_2=\text{N}-\text{CH}_2-\text{COOH} + \text{H}^+$); 60 ($\text{CH}_3\text{NHCH}_2\text{CH}_3 + \text{H}^+$); 42 ($\text{CH}_2=\text{N}=\text{CH}_2^+$).

NMR Spectroscopy. A mixture of 1-octen-3-one (0.001 M) and glycine (0.1, 0.01, and 0.001 M, respectively) dissolved in D_2O containing 0.05% of 3-trimethylsilyl-[2,2,3,3- $^2\text{H}_4$]propionic acid sodium salt (TSP) as internal standard was recorded at 25 °C with a Bruker Avance DPX 360 spectrometer (Bruker GmbH, Ettlingen, Germany) operating at the frequency of 360.13 MHz using TopSpin (version 1.3, Bruker GmbH). Chemical shifts (δ) are given in parts per million (ppm).

RESULTS AND DISCUSSION

Aroma Composition. The aroma composition of an infusion of Longjing green tea was characterized first in order to identify the odor-active compounds that may contribute to the overall aroma of the Longjing green tea used in this study. As shown in Table 2, 44 odorants were found by GC-O having FD factors of at least 20. Six odorants could be detected at the dilution of

1:1000: (Z)-1,5-octadien-3-one (metallic, **9**), methional (cooked potato-like, **15**), geraniol (floral, **29**), geraniol (floral, **35**), α -ionone (floral, **36**), and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like, **44**). However, most of the odorants in Table 2 have already been reported in Longjing tea (5–8) or in other tea varieties (4, 12). Differences in composition and FD factors from previous work may be due to the raw material used and/or analytical procedure applied. However, the purpose of this work was to define those odorants that occur in the material used in this study and might be affected by aroma release phenomena, rather than to perform a complete characterization of the aroma composition.

To study the aroma changes at the molecular level, an aroma model mimicking Longjing green tea was defined. It was composed of 12 aroma compounds (Table 1), which were selected among odor-active compounds detected in the SAFE volatile concentrate isolated from Longjing green tea infusion (Table 2), and other volatile constituents that can easily be detected by GC-MS in the SAFE extract and by SPME/GC-MS in Longjing tea infusion. The selection criteria for the compounds to be included in the model were functional group,

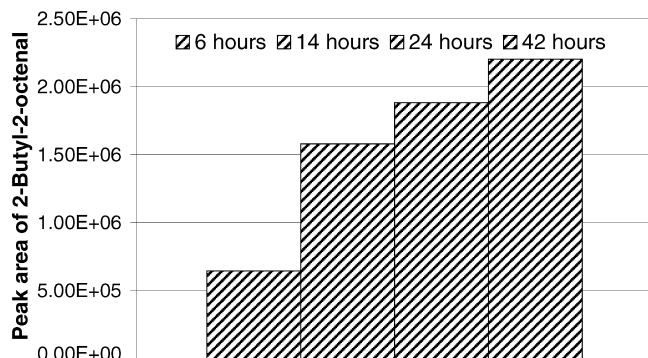


Figure 3. Evolution of 2-butyl-2-octenal formation in an aroma model with 1% nonvolatiles isolated from Longjing tea infusion (at least duplicates; CV < 10%).

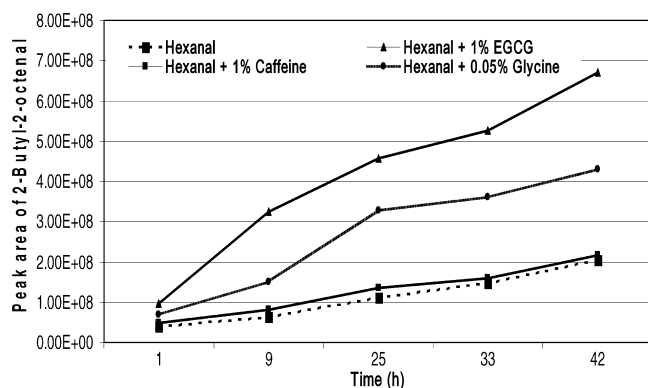


Figure 4. Effect of nonvolatile green tea constituents on 2-butyl-2-octenal formation in an aroma model during storage at 50 °C (duplicate analysis; CV < 15%).

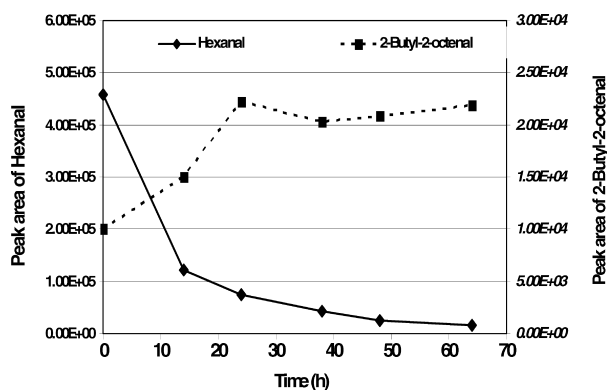


Figure 5. Dynamic change of hexanal and 2-butyl-2-octenal in Longjing tea infusion during storage at 50 °C (at least duplicates; CV < 15%).

FD factor, odor threshold in air, air/water partition, and peak area obtained by headspace GC-MS as well as RI avoiding GC signal overlapping between the selected aroma compounds. Considering the data previously reported on green tea infusion (14, 15), the concentrations of the aroma compounds in the model were 100–10000 times higher than in the Longjing tea infusion to facilitate their monitoring by headspace GC-MS.

Temporal Changes upon Aroma Release. Temporal changes of 24 volatile compounds were monitored in the headspace of the Longjing tea infusion during an aging period of up to 24 h. As shown in **Figure 1**, most of the aldehyde compounds, except cyclic aldehydes, that is, β -cyclocitral, β -ionone, and (Z)-jasnone, decreased in concentration, whereas alcohol derivatives increased. The release of alcohols may be due to hydrolysis of the corresponding glycosides known to occur in tea (16–22). Some glucosides have previously been reported to occur in

tea (23–25), which can release the corresponding alcohols by chemical and/or enzymatic hydrolysis thanks to the occurrence of the required enzymes in green tea leaves (26–28). To better mimic the Longjing tea infusion, the nonvolatile Longjing green tea components isolated from Longjing green tea infusion were added to the aroma model for studying the temporal change during aging. Similarly to the Longjing green tea infusion, the aroma model showed a decrease in not only aldehydes but also ketone compounds upon aging in the presence of Longjing green tea nonvolatiles (**Figure 2**). The exception was with benzaldehyde and β -damascenone, which remained almost unchanged. The aroma model without added nonvolatile tea components but adjusted to the native pH of Longjing tea infusion, that is, pH 6.0, also showed a decrease in similar carbonyl compounds as above, however, to a much lesser extent in comparison to the presence of nonvolatile tea components (data not shown). This difference may be accounted for by the occurrence of the interaction and/or the chemical transformation of the volatile aroma compounds with the nonvolatile tea components. Contrarily to Longjing green tea infusion, alcohol compounds in the model system did not show any change, due likely to the lack of the corresponding glycosides.

Formation of 2-Butyl-2-octenal in Model Systems. Upon storage at 50 °C of the aroma model in the presence of nonvolatiles from Longjing tea infusion, a new component, namely, 2-butyl-2-octenal, was found, which increased with time as shown in **Figure 3**. Its identification was confirmed by the authentic compound obtained by synthesis as previously reported (29).

2-Butyl-2-octenal was suspected to originate from crotonization of hexanal present in the aroma model at pH 6.0. Indeed, a solution of hexanal in water adjusted to pH 6.0 confirmed the formation of 2-butyl-2-octenal, which increased upon time. Similar observations were found with the complete aroma model. The formation of 2-butyl-2-octenal in the aroma model system was also investigated in the presence of major nonvolatile tea components, such as epigallocatechin gallate (EGCG), glycine (a free amino acid), and caffeine, which were reported to occur in green tea (30–32). EGCG and glycine, but not caffeine, accelerated the formation of 2-butyl-2-octenal (**Figure 4**). These effects might hypothetically be explained (i) by the catalytic effect of the nucleophilic character of the amino group (i.e., a free amino acid) in combination with the acidic character of the carboxylic group of glycine and (ii) by the acidic character of the hydroxyl groups of EGCG.

Formation of 2-Butyl-2-octenal in Longjing Green Tea.

2-Butyl-2-octenal was also found in the headspace of the fresh Longjing tea infusion and increased with time upon tea infusion aging. Besides 2-butyl-2-octenal, other enals could also be detected by SPME/GC-MS in green tea infusion upon aging, that is, 2-isopropyl-2-octenal, 2-isopropyl-5-methyl-2-hexenal, and 2-butyl-5-methyl-2-hexenal, however, in much lower amounts.

The increasing formation of 2-butyl-2-octenal correlated with the gradual decrease of hexanal upon aging of Longjing tea infusion (**Figure 5**). By comparing, respectively, the peak area, the loss of hexanal was 10 times larger than the formation of 2-butyl-2-octenal. This finding would indicate that the decrease of hexanal in Longjing tea infusion was linked not only to the formation of 2-butyl-2-octenal but also to its gradual formation by lipid oxidation and its degradation to hexanol, hexanoic acid, 2-butyl-2-octenal, and other related enals.

To get an insight into the formation of 2-butyl-2-octenal in green tea, aging of the Longjing tea leaves was carried out at 50 °C for 24 h. 2-Butyl-2-octenal was detected by SPME/GC-

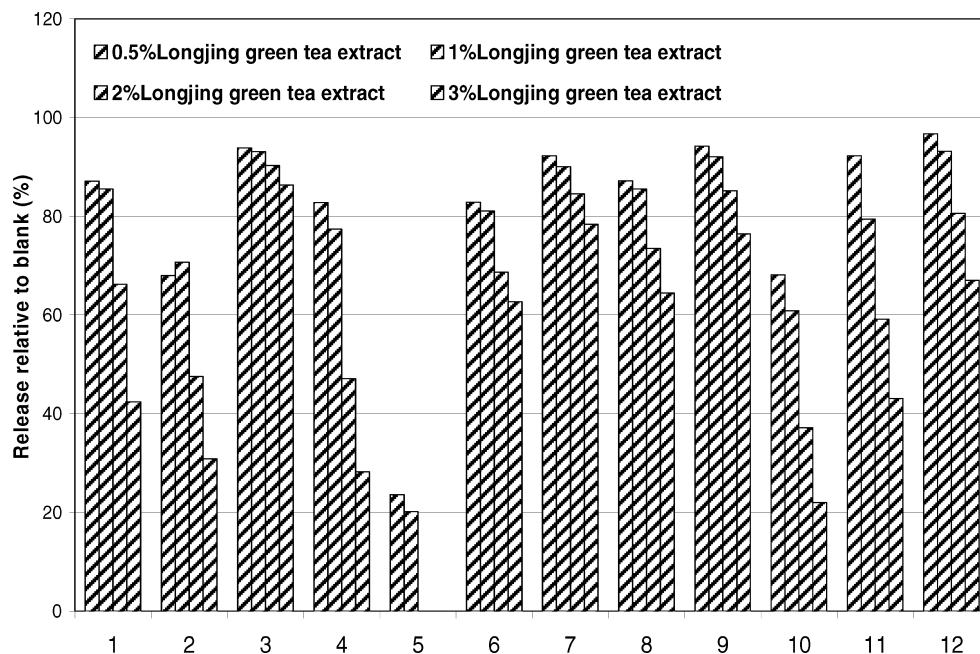


Figure 6. Effect of the added level of nonvolatiles isolated from Longjing tea infusion on the release of aroma compounds in the model (at least duplicates; CV < 15%). Numbering refers to that in Table 1.

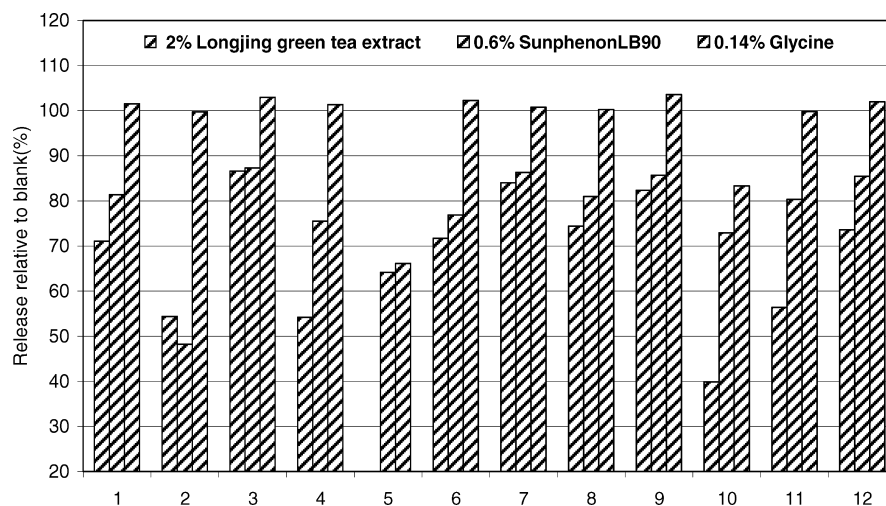


Figure 7. Effect on release of the aroma model in the presence of tea catechins, glycine, and nonvolatile components in Longjing tea infusion (at least duplicates; CV < 15%). Numbering refers to that in Table 1.

MS in the headspace of the tea leaves before and after aging. Larger amounts were detected in the headspace of the infusion prepared from both aged and nonaged tea leaves with boiled water and also with water at 20 °C (data not shown). However, the difference was smaller with ambient water than with boiled water. These results indicate that 2-butyl-2-octenal was already formed in the processed green tea leaves, likely during pan-firing, and it increased further upon accelerated aging. It was bound to the leave matrices, and water is needed to break such an interaction for liberating the native 2-butyl-2-octenal. The release difference of 2-butyl-2-octenal found with boiled and ambient water could be accounted for by the temperature effect on the interaction with the green tea matrix rather than the air/water partition because the infusion samples were cooled to room temperature and the headspace was equilibrated for 30 min at 30 °C prior to SPME sampling.

Influence of Green Tea Nonvolatile Components on Tea Aroma Release in Model Systems. To study the influence of green tea nonvolatiles on the release of aroma compounds in the headspace of a Longjing tea infusion, 1% of nonvolatiles

isolated from Longjing tea infusion was added to the aroma model. It is worth noting that this 1% level of nonvolatiles corresponds to the order of magnitude occurring in a true Longjing tea infusion (between 0.2 and 5.4% depending on the infusion conditions). However, the ratio of nonvolatiles over volatiles in the model is much lower than in a true Longjing tea infusion as more volatiles were added to facilitate their detection.

As shown in Figure 6, the release of all aroma volatiles decreased with the increasing level of Longjing tea nonvolatiles, that is, 0.5, 1, 2, and 3%. The release is strongly dependent on the chemical nature of the aroma compounds. Carbonyl compounds and, in particular, 1-octen-3-one were very much affected with the exception of benzaldehyde. The effects on pyrazines were smaller and even much smaller for esters and alcohols.

To better understand the contribution of green tea nonvolatiles to the interaction with green tea aroma compounds at the molecular level, the release of the model aroma was investigated in the presence of tea catechins and free amino acids (e.g.,

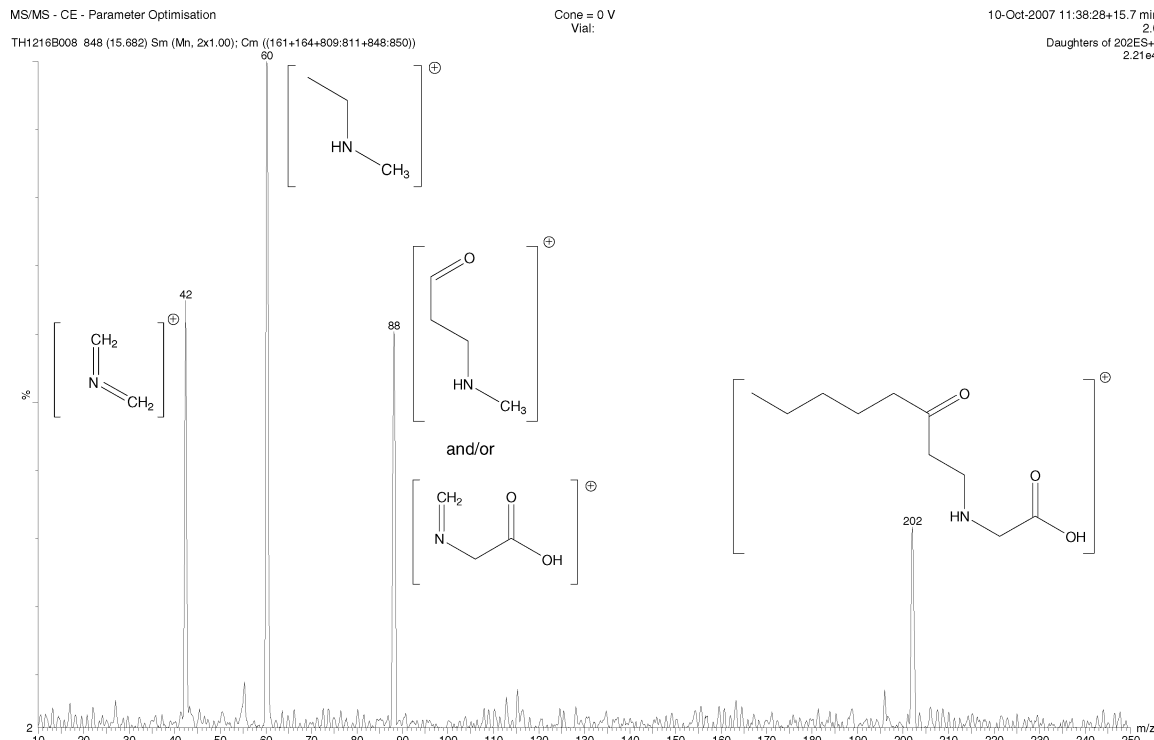


Figure 8. Fragmentation pattern (MS/MS spectrum) of the newly formed product and structure proposal.

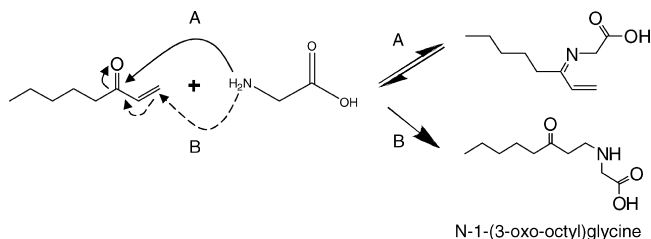


Figure 9. Proposed mechanism of the formation of *N*-1-(3-oxo-octyl)glycine from glycine and 1-octen-3-one.

glycine), which are the major nonvolatile components in green tea. Accordingly, Sunphenon LB90 (i.e., a green tea extract enriched in almost 100% polyphenols, of which 85% is catechins) and glycine (representative of free amino acid in green tea) were added to the aroma model at levels of 0.6 and 0.14%, respectively, which corresponds approximately to the level of catechins and total free amino acids in 2% of nonvolatiles in green tea infusion as calculated on the basis of the previously reported chemical composition of green tea leaves (30–32). As shown in **Figure 7**, the catechin-enriched fraction affected the aroma release to a similar order of magnitude as the Longjing tea nonvolatile fraction except for 1-octen-3-one. In contrast, glycine had practically no effect on the aroma release except for 1-octen-3-one and geranial. Together with the data obtained with 2% Longjing tea nonvolatile fraction, the decrease of 1-octen-3-one and geranial was the result of the combined effect of both polyphenols and glycine.

These results indicate a major role of tea catechins in the interaction with tea aroma compounds. However, the interaction mechanism at the molecular level remained not well understood. Nevertheless, several hypotheses can be suggested to explain partially the decrease of carbonyls release such as polymerization with tea catechins in a similar way as previously reported for the polymerization of catechin and epicatechin induced by acetaldehyde (33, 34). Also, the π – π stacking, which might occur between π -electrons of tea polyphenols and pyrazines or carbonyls (via the C=O double bond), might

explain the interaction causing a decreasing release of these aroma compounds. Such a π – π stacking was reported in the complex formation between polyphenols and aromatic aroma compounds (35–42). Finally, the polyphenol might be hydroxy-alkylated in the presence of carbonyl compound as recently reported by Totlani and Peterson (43).

Model Study with 1-Octen-3-one and Glycine. Glycine affected significantly only the release of 1-octen-3-one and geranial and not other carbonyl compounds. To better understand this result, a model study was carried out with 1-octen-3-one and glycine at the ratio of 1:1000. Aqueous solutions of 1-octen-3-one with and without glycine, both containing octanol as internal standard, were heated for 1 h at 50 °C to mimic the conditions used to determine the aroma release in tea model systems and then extracted with dichloromethane. GC analysis of the organic extracts showed a 99.5% decrease of 1-octen-3-one relative to the internal standard when heated in the presence of glycine. It is worth noting that smaller decreases of 33.7 and 31.6% were found with the reactions of 1-octen-3-one and glycine at ratios of 1:10 and 1:1, respectively. Such a decrease could be accounted for by an irreversible interaction of 1-octen-3-one and glycine. Therefore, the hydro-soluble components of the reaction mixture were lyophilized and analyzed by LC-MS. Besides glycine, a new compound was detected with a mass of 202 by positive electrospray. Using tandem MS/MS, a fragmentation pattern with masses of 202, 88, 60, and 42 was found as shown in **Figure 8**. In addition, hydrogen/deuterium exchange experiments revealed a 3 mass unit shift with the protonated molecular ion, indicating two mobile hydrogen atoms in the molecule. According to the MS data, the formation of a new molecule, namely, *N*-1-(3-oxo-octyl)glycine (**Figure 9**), can be suggested.

To corroborate this suggestion and to get a deeper insight at the molecular level, NMR spectroscopy using a mixture of pure 1-octen-3-one with glycine was investigated at the ratio of 1:1000 to stay approximately in the same ratio compared to our model described in **Figure 7**. The ^1H NMR spectrum of

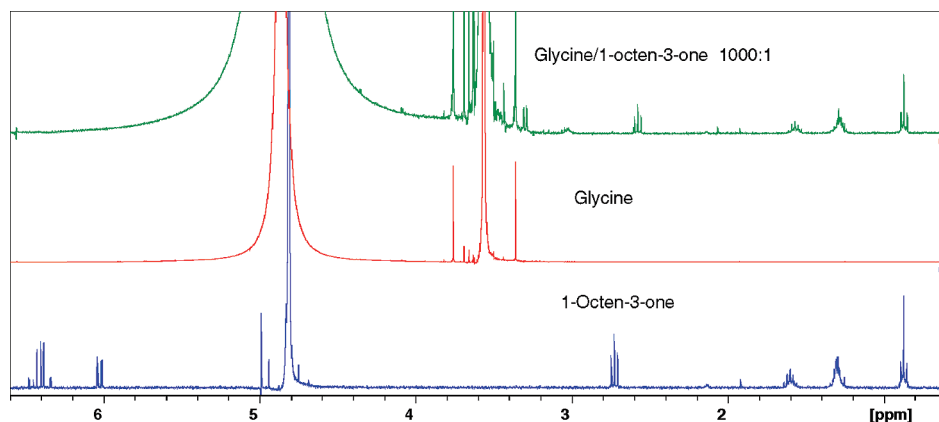


Figure 10. ^1H NMR spectrum of a mixture of glycine and 1-octen-3-one at the ratio of 1000:1 and the individual reactants.

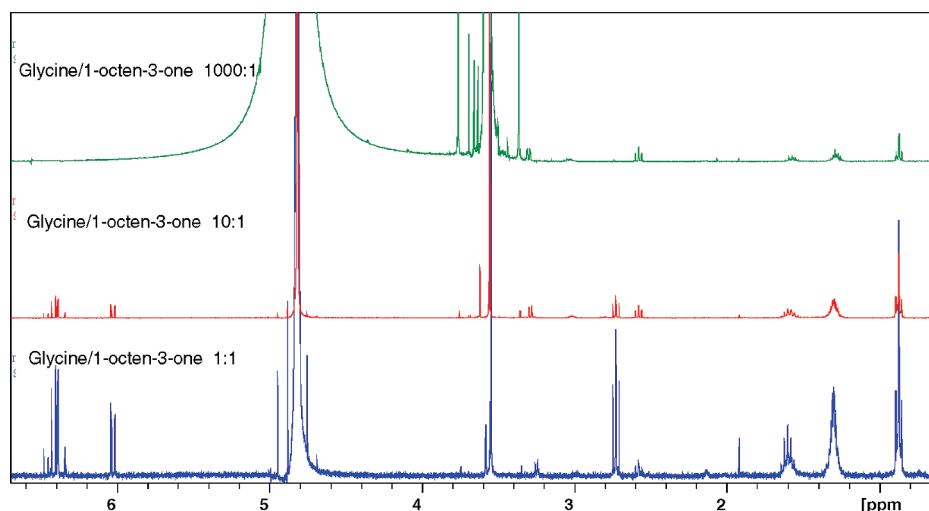


Figure 11. ^1H NMR spectrum of a mixture of glycine and 1-octen-3-one at the ratios of 1000:1, 10:1, and 1:1.

the mixture of glycine/1-octen-3-one (Figure 10) showed that the signals at δ 6–6.5 of the ABC system of the three vinylic protons of 1-octen-3-one disappeared and those in the region δ 3–3.8 appeared, which would correspond to NMR signals of the complex system AA'BB' from the ethylene $\text{CH}_2\text{--CH}_2$ partially overlapped by the glycine side bands. In addition, a CH_2 singlet signal appeared at δ 3.64, which is downfield compared to the CH_2 singlet of glycine (δ 3.55). Moreover, a triplet appeared at δ 2.60, whereas the triplet at δ 2.74 of CH_2 at C-4 of 1-octen-3-one disappeared. On the basis of this spectroscopic information, the suggestion for the formation of a new product, that is, *N*-1-(3-oxo-octyl)glycine (Figure 9), can be tentatively identified. It is worth noting that the formation of the new product was dependent on the excess of glycine. It was complete, partial, and almost nonexistent at glycine/1-octen-3-one ratios of 1000:1, 10:1, and 1:1, respectively, as shown by the NMR spectra in Figure 11. This compound resulted from the Michael addition (i.e., 1,4-addition) of the glycine free amino group to the double bond of 1-octen-3-one. It is known that the 1,2-addition yielding an imine is kinetically more favored than the 1,4-addition. However, the first reaction is reversible and the latter (i.e., 1,4-addition) is not. Accordingly, the equilibrium was shifted in the long term toward the formation of the 1,4-addition product, that is, *N*-1-(3-oxo-octyl)glycine (Figure 9). This compound has not yet been described in the literature (no CAS Registry Number was available).

The results obtained in this work indicate that a temporal change occurred in the headspace release of tea aroma in the Longjing tea infusion and in a model system mimicking

Longjing tea infusion. Nonvolatile components present in the green tea infusion decreased strongly the release of most carbonyl aroma compounds and, in particular, of 1-octen-3-one. The effects were smaller for aroma compounds of other chemical nature, that is, pyrazines, esters, and alcohols. The main contribution to the decreasing release of tea aroma compounds was from nonvolatile tea catechins. Only α,β -unsaturated carbonyls, that is, 1-octen-3-one and geranial, were affected by glycine, a nonvolatile free amino acid occurring in green tea infusion, in addition to tea catechins. Thanks to the investigations using a model mixture, glycine was demonstrated to react with 1-octen-3-one via a 1,4-addition to generate *N*-1-(3-oxo-octyl)glycine, which can explain the effect of glycine on the release of 1-octen-3-one at the molecular level. Geranial may react with amino acids in an analogous manner. Upon aging of both Longjing tea infusion and the model system, a new volatile compound, 2-butyl-2-octenal, was found, which increased with time. It is worth noting that results on both the release effect of glycine on 1-octen-3-one and the formation of 2-butyl-2-octenal in green tea were demonstrated at the molecular level for the first time in this work.

The decrease of the release of the aroma compounds in the headspace of Longjing green tea infusion upon aging would certainly affect the aroma profile of Longjing green tea infusion, which is frequently kept at around 50 °C and consumed during the whole day in China. Moreover, the increasing formation of 2-butyl-2-octenal upon aging would also influence the aroma profile of Longjing green tea infusion, as 2-butyl-2-octenal was found in this work to elicit citrus, grassy, and fruity flavor notes.

Therefore, investigations on the isolation of other enals and their sensory properties are necessary to fully characterize the impact of the enals affecting the flavor quality change of green tea infusion upon aging, which deserves further work.

On the basis of our findings on the formation of 2-butyl-2-octenal in tea leaves, this molecule can be used as a freshness marker of commercial Longjing green tea and likely other commercial green teas.

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