Characterization of the effect of cis-3-hexen-1-ol on green tea aroma

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cis-3-Hexen-1-ol has been regarded as the main source of green aroma (or green odor) in green tea. However, no clear findings on the composition of green aroma components in tea and the effect of cis-3-hexen-1-ol on other aroma components have been reported. In this study, the main green aroma components in green tea were characterized, especially the role of cis-3-hexen-1-ol in green aroma was analyzed and how it affected other aroma components in green tea was studied. Based on the GC–MS detection, odor activity value evaluation, and monomer sniffing, 12 green components were identified. Through the chemometric analysis, cis-3-hexen-1-ol was proven as the most influential component of green aroma. Moreover, through the electronic nose analysis of different concentrations of cis-3-hexen-1-ol with 25 other aroma components in green tea, we showed that the effect of cis-3-hexen-1-ol plays a profound effect on the overall aroma based on the experiments of reconstitution solution and natural tea samples. GC–MS and CG-FID confirmed that the concentration range of the differential threshold of green odor and green aroma of cis-3-hexen-1-ol was 0.04–0.52 mg kg⁻¹.

Abbreviations
VOCs  Volatile organic compounds
OAV  Odor activity value
HS-SPME  Head-space solid-phase microextraction
GC–MS  Gas chromatography-mass spectrometry
GC-FID  Gas chromatography–flame ionization detector
E-nose  Electronic nose
OT  Odor threshold

cis-3-Hexen-1-ol is one of the most important VOCs in plants. It was widely found in fresh leaves of tea as an important allelochemicals responding to mechanical injury. Many studies have been carried out to reveal its allelopathic effects on plants, pests, and natural enemies from previous reports. cis-3-Hexen-1-ol is also a significantly important active-component which contributes to the “green, grass and fresh” odor in vegetable food, such as grapes, passion fruits, and Toona sinensis. Though tea is one of the largest consumption drink in human being’s life, no previous studies have been devoted to study the contribution of cis-3-hexen-1-ol on the favorite aroma of tea. On the other hand, cis-3-hexen-1-ol could also plays a determining role as the “raw grass” odor of finished green tea due to its over strong and sharp green aroma. Therefore, these observations highlight the importance to systematically study the effects of cis-3-hexen-1-ol on the overall aroma of finished tea products. Due to the strong feedback of cis-3-hexen-1-ol in the odor system, it could be also important to study the effect of cis-3-hexen-1-ol in the mixture with other odor components in the green tea.

As mentioned before, “raw grass” odor is thought to have a negative effect on the aroma of green tea, thus cis-3-hexen-1-ol of green tea should be controlled in a very low range (descriptive evaluation considers that it should be as low as unrecognizable). In manufacturing process, most of cis-3-hexen-1-ol is volatilized and/or converted into other aroma components (e.g. trans-3-hexen-1-ol, jasmonate) due to high temperature in sterilization and drying. The volatilization of low-boiling aroma components, like 1-pentanol (fruity), citronellol (floral) and linalool (floral), weakens silky and refreshing flavor of tea. Meanwhile, Maillard reaction might occur...
In manufacturing process, in which the overall aroma of tea will be altered into roast and chestnut-like aroma. Whether cis-3-hexen-1-ol plays the most important role in providing green odor of tea and how it affects the tea aroma are still unclear and further investigations are needed.

In recent years, some studies about the influences of cis-3-hexen-1-ol on the aroma have been reported. However, most of the researches were devoted to detection, qualitative and quantitative analysis, and rare studies focused on the effects of interactions of different components on the tea odor. To our best knowledge, no study about the components of green aroma of tea and how cis-3-hexen-1-ol affects the overall aroma of tea have been reported. Further investigations are needed to clarify that whether cis-3-hexen-1-ol at different concentrations synergize or mask other aroma components in the mixed system of finished tea, and how it fluctuates from raw green odor (high concentration) to green aroma (low concentration).

In this study, the identification of green aroma (odor)-producing components and dominant components of green tea were studied by four steps: (1) qualitative and quantitative analysis of VOCs of green tea in different processing stages by HS-SPME-GC–MS/GC-FID; (2) evaluation about the conversion process of green tea from green odor to green aroma by sensory evaluation and E-nose; (3) evaluation, screening and ranking of the aroma active components of finished tea by the OAV and aroma monomer sniffing; (4) correlation analysis and stepwise linear regression. In addition, effect pattern and ability of aroma active components of finished tea by reconstitution experiment; (2) evaluation of the effects of different concentrations of cis-3-hexen-1-ol on other aroma components in mono-system by mixing different concentrations of cis-3-hexen-1-ol with each aroma component respectively; (3) evaluation, screening and ranking of the aroma processing stages by HS-SPME-GC–MS/GC-FID; (2) evaluation about the conversion process of green tea by sensory evaluation and E-nose; (3) evaluation, screening and ranking of the aroma active components of finished tea by the OAV and aroma monomer sniffing; (4) correlation analysis and stepwise linear regression. In addition, effect pattern and ability of aroma active components of finished tea by reconstitution experiment; (2) evaluation of the effects of different concentrations of cis-3-hexen-1-ol on other aroma components in mono-system by mixing different concentrations of cis-3-hexen-1-ol with each aroma component respectively; (3) evaluation of the effects of cis-3-hexen-1-ol on aroma profile in tea system by addition of gradient concentration of cis-3-hexen-1-ol to finished tea without green odor. Herein, the effects of cis-3-hexen-1-ol on the aroma of green tea were characterized, and a new strategy for studying the effects of specific aroma component on the whole aroma profile in food was presented.

Method and material

Materials. Tea samples and process parameters. The raw materials (fresh tea buds) were taken from 5-year-old tea trees (Camellia sinensis) which cultivar was Fuxuan No. 9, the standard for picking was whole bud from the same batch, and tea garden located in Mingshan District (N30° 17′, E103° 21′).

Above raw materials were processed immediately in the production line of “Green Bud Tea” in a tea factory in Mingshan District, and the samples were taken at five processing stages: spreading (sample A), fixing (sample B), carding (sample C), pressing flat (sample D) and drying (finished tea) (sample E). Sampling 250 g at each processing stage was put into a refrigerator at –40 °C for freezing storage (in March 2018).

Specific process parameters were as follows:

- Spreading (light withering), which step that put tea buds in withering trough and withered lightly for 10 h (the thickness was 8 cm and the temperature was 17 ± 2 °C).
- Fixing, which step that inactivated enzyme activity of tea buds using infrared plate-type fixing machine, the set temperature was 105 °C (10 min), and measured temperature was 69.30 ± 0.39 °C. After fixing, tea buds were cooled for 10 min to 20 ± 2 °C.
- Carding, which step that the tea buds were stir-fried at low temperature to volatilize water and developed aroma while straightening out the shape by a carding machine (Supplementary Annex S1), the set temperature was 100 °C (45 min), frequency was 107 r min⁻¹, and measured temperature was 46.27 ± 0.28 °C.
- Pressing flat, which step that added pressure bars (the length, diameter and mass of each bar were 60 cm, 3.2 cm and 1.1 kg respectively) (Fig. 1) in the above carding machine to flat tea buds, the set temperature was 68 °C (10 min), frequency was 50 r min⁻¹, and measured temperature was 26.30 ± 0.22 °C.
- Drying, which step that the tea buds were dried at low temperature for a long time with the above carding machine, and the finished tea (water content less than 5%, m/m) was finally obtained, the set temperature was 66 °C (260 min), frequency was 80 r min⁻¹, and measured temperature was 40.90 ± 0.67 °C.
Chemicals and reagents. cis-3-Hexen-1-ol and ether standard (purity > 99.97%), which were purchased from Sigma-Aldrich (Shanghai, China), were used for GC-FID quantitative analysis, and GC–MS qualitative analysis with recovery ratio correction; C4–C20 normal paraffin standards (purity > 99.97%, used for RI calculation) was purchased from Sigma-Aldrich (Shanghai, China); hexanal, myrcene, 1-penten-3-ol, 2-hexenal (mixture of cis and trans isomers, molar ratio about 1:1), 1-pentanol, trans-2-heptenal, 1-hexanol, (E)-3-hexen-1-ol, cis-3-hexen-1-ol, 1-octen-3-ol, furanoid-linalool oxide (mixture of cis and trans isomers, molar ratio about 1:1), pyran-linalool oxide (mixture of cis and trans isomers, molar ratio about 1:1), benzaldehyde, 1-ethyl-1H-pyrrole-2-carboxaldehyde, linalool, phenylacetaldehyde, citral, dodecanal, methyl salicylate, citronellol, nerol, geraniol, jasmine, 1-tetradecylaldehyde, above 24 aroma components (purity > 98%) which were purchased from Rhawn reagent (Shanghai, China), were used for aroma profile test and reconstitution experiment.

Equipment. HS-SPME was used to extract the aroma compounds. The SPME fibre was 50/30 μm DVB/CAR/PDMS which length was 1 cm (Sigma-Aldrich, Shanghai, China), and the fiber penetration depth into the headspace was 2 cm. The size of the extraction bottle was 15 mL.

GC–MS was used for qualitative analysis, which was a Shimadzu GC-2014 (Kyoto, Japan) fitted with FID-2014 flame ionization detector.

E-nose was used to assist sensory evaluation, which was a FOX 4000 from Alpha-MOS (Toulouse, France) including 18 different metal oxide semiconductor gas sensors, combined with a headspace auto-sampler.

Methods. Measurements conditions of HS-SPME and GC–MS. HS-SPME conditions and extracting method. The SPME fibre was 50/30 μm DVB/CAR/PDMS which length was 1 cm (Sigma-Aldrich, Shanghai, China), and the fiber penetration depth into the headspace was 2 cm. The size of the extraction bottle was 15 mL.

When extracting aroma substances, a tea sample (5.0 g) was placed in an extraction bottle, which was equilibrated in a thermostatic water bath at 10 min at 80 °C and then sampled for 30 min in the head space. After that, SPME fibre was then withdrawn and directly introduced to the GC–MS, and the process was repeated 3 times.

GC–MS conditions. Capillary–column chromatography was DB-W AX (30 m × 250 μm × 0.25 μm) (Agilent), the sampling was manual, no shunt was used, the sample was at a constant temperature, and the temperature of the injection port and GC–MS direct interface were both 250 °C, helium was used as a carrier gas at a flow rate of 1.67 mL min⁻¹. Temperature programming: the column temperature was 45–220 °C; the starting column temperature was 40 °C, which was held for 2 min, increased to 180 °C at a rate of 3 °C min⁻¹, held for 3 min, then increased to 220 °C at a rate of 10 °C min⁻¹, and held for 3 min.

The ion source temperature was 220 °C, its electron ionization energy was 70 eV, the quadrupole mass filter was operated at 150 °C, and the scanning quality range was set at 20–700 amu.

Qualitative analysis of aroma compounds. Qualitative analysis: the NIST Standard spectral library (https://www.nist.gov/) and retention indices (RIs) from other literature were used to match ion mass spectra, then completed qualitative analysis of aroma components:

\[ \text{RI} = 100n + 100 \times \frac{RTx - RTn}{RTn + 1 - RTn} \]

where RI, were retention indices of analyte, n, the number of carbon atoms of n-alkanes before the analyte, RTx, the retention time of the analyte, RTn, the retention time of n-alkanes before the analyte, RTn+1, the retention time of n-alkanes after the analyte.

Measurements conditions of GC-FID. HS-SPME conditions and extracting method. Same as “HS-SPME conditions and extracting method”.

GC-FID conditions. Capillary–column chromatography was AT-SE-54 (30 m × 250 μm × 0.33 μm), and the detector was hydrogen flame ionization detector (FID). The sampling was manual, no shunt was used, the sample was at a constant temperature, and the temperature of the injection port and the detector were both 230 °C, nitrogen was used as a carrier gas at a flow rate of 1.00 mL min⁻¹. Temperature programming: the column temperature was 40–230 °C; the starting column temperature was 40 °C, which was held for 2 min, increased to 110 °C at a rate of 2 °C min⁻¹, held for 2 min, then increased to 130 °C at a rate of 7 °C min⁻¹, and held for 2 min, finally, increased to 230 °C at a rate of 5 °C min⁻¹, and held for 8 min.

Standard curve experiment of cis-3-hexen-1-ol. Using ether as solvent, the gradient concentration standard solutions of cis-3-hexen-1-ol (V/V%) were 0.1% (1.692 mg mL⁻¹), 0.2% (3.384 mg mL⁻¹), 0.3% (8.46 mg mL⁻¹), 0.4% (13.536 mg mL⁻¹) and 0.5% (16.92 mg mL⁻¹), respectively. The above standard solution was detected by GC-FID according to “HS-SPME conditions and extracting method” and “GC-FID conditions” method, then the standard curve of cis-3-hexen-1-ol was obtained. The experiment was repeated 3 times.

Qualitative and quantitative determination of cis-3-hexen-1-ol in tea samples. Tea samples were obtained according to “Tea samples and process parameters”, and the peak area of cis-3-hexen-1-ol in tea samples was determined by “HS-SPME conditions and extracting method” and “GC-FID conditions”, qualitative determination of chlorophyll in tea samples is determined by peak time of standard samples, the content of cis-3-hexen-
Preparation of the reconstitution solution. The aroma reconstitution solution was prepared according to the method established by our group. The chosen aroma monomers were floral (reference aroma monomer was 3,7-dimethyl-1,6-octadien-3-ol), fruity ((Z)-3,7-dimethyl-2-hexen-1-ol), green (cis-3-hexen-1-ol), roasted (turfural), and herbal ((Z)-3-methyl-2(2-pentenyl)-2-cyclopenten-1-one), respectively. 7-point scoring method was selected for evaluation, which were 0 (odorless), 0.5, 1, 1.5, 2, 2.5, and 3 (very strong).

Quantitative determination of other aroma components in tea samples. The content of other aroma compounds was calculated by peak area ratio of GC–MS, based on cis-3-hexen-1-ol content measured by 2.2.2.4 method.

\[
W_i = W_s \cdot \frac{A_i}{A_s}
\]

Which \(W_s\) was the analyte concentration in mg kg\(^{-1}\), \(W_i\) the concentration of measured cis-3-hexen-1-ol content in mg kg\(^{-1}\), \(A_i\) the analyte area, and \(A_s\) the peak area of cis-3-hexen-1-ol.

Effects of different concentrations of cis-3-hexen-1-ol on the strength of aroma monomers in tea samples. Based on the types and contents of aroma compounds detected in finished tea (sample E) which was detected from GC–MS, the double concentrations of each monomer in aqueous solution (mg kg\(^{-1}\)) were prepared respectively (except cis-3-hexen-1-ol). Then the concentration of cis-3-hexen-1-o in sample E was treated as 100%, then 20%, 100%, 200%, 400%, 1,000%, and 2,000% cis-3-hexen-1-ol aqueous solutions (mg kg\(^{-1}\)) were prepared respectively.

Next, different concentrations of cis-3-hexen-1-ol solution were mixed with aroma monomer solution at 1:1 (10 mL/10 mL), thus different concentrations of cis-3-hexen-1-ol (10%, 50%, 100%, 200%, 500%, and 1,000%) mixed with other aroma monomers were obtained.

Finally, the above solution was sniffed and graded (7-point scoring method) by a six-person (3 male with 3 female) evaluation team which trained in systematic sniffing (specific training methods refer to the previous reports of our group, including two parts, strength training of aroma monomer and recognition training of mixed solution). Using distilled water (0%) as CK, room temperature 23 ± 1 °C, all experiments were repeated 3 times. The influence of cis-3-hexen-1-ol on aroma monomers could be judged by the strength change of aroma monomers.

Effect of different concentrations of cis-3-hexen-1-ol on the overall aroma profile of finished tea and reconstitution solution. The concentration of cis-3-hexen-1-ol in sample E was treated as 100%, each 5 g finished tea (sample E) was treated as a strength training group, and added 0% (CK, the total cis-3-hexen-1-ol in the sample was 100%), 10% (total was 110%), 50% (total was 150%), 100% (total was 200%), 200% (total was 300%), 500% (total was 600%) and 1,000% (total was 1,100%) cis-3-hexen-1-ol respectively, in addition, the same treatment was carried out on the reconstitution solution to verify the consistency of the change of the overall aroma caused by cis-3-hexen-1-ol in natural tea and reconstituted solution.

Then aroma profile analysis was carried out according to the method established by our group. The chosen descriptors were floral (reference aroma monomer was 3,7-dimethyl-1,6-octadien-3-ol), fruity (Z)-3,7-dimethyl-2,6-octadien-1-ol, green (cis-3-hexen-1-ol), roasted (turfural), herbal ((Z)-3-methyl-2(2-pentenyl)-2-cyclopenten-1-one), respectively. 7-point scoring method was selected for evaluation, which were 0 (odorless), 0.5, 1, 1.5, 2, 2.5, and 3 (very strong).

Data processing method. Excel 2016 were used to analyse the data and draw figures, and the SPSS 25 was used to correlate analysis and stepwise linear regression, the Photoshop CS6 were used to draw the tea processing equipment, and chemDraw 18 were used to draw chemical structures.

Ethics requirements. In the sensory evaluation part of this study, trained evaluators were used to conduct necessary sensory evaluation on tea samples. Except for the above parts, this study does not include other work with human or animal. The study was approved by the local ethics committee in China, and all methods were carried out in accordance with relevant guidelines and regulations, informed consent was obtained from all subjects before their participation in the study.

Results and analysis
Detection result of GC-FID. Firstly, the content of cis-3-hexen-1-ol in 5 tea samples was determined by GC-FID standard curve. The standard curve was shown as Fig. 1a, \(R^2 = 0.9895\), it is considered that the results of linear fitting were well, then based on the above standard curve and the results of GC-FID detection of tea samples, the content of cis-3-hexen-1-0 in tea samples can be calculated.

From Fig. 1b, the cis-3-hexen-1-ol content of sample A was the highest (7.27 mg kg\(^{-1}\)), and then decreased with the processing until sample E reached the lowest (0.04 mg kg\(^{-1}\)). This result was consistent with previous studies, that is, the content of cis-3-hexen-1-ol formed by lipoxygenase pathway in fresh leaves will continue to decline during the processing of green tea. And there were at least 3 reasons for the decrease, first, it will gradually
volatilize with the heating in the processing; and then, it will isomerize at high temperature and convert to trans-3-hexen-1-ol; in addition, it may be cyclized and converted into jasmone. Thus, the concentration of cis-3-hexen-1-ol in different tea samples was obtained. Combined with the subsequent GC–MS detection data, the relative concentration of each aroma component in 5 tea samples could be calculated.

Detection results of GC–MS and calculation results of OAV. The aroma compounds of different tea samples were determined by HS-SPME–GC–MS, and qualitative analysis was carried out by NIST Standard spectral library (https://www.nist.gov/) and Retention Indices (RIs), this was shown in Supplementary Annex S3 (relative peak area percentage was calculated by peak area normalization method). Then the other aroma compounds of tea samples were quantified by the content of cis-3-hexen-1-ol determined above, meanwhile, the aroma characteristics of monomers were determined by FEMA GRAS Database (https://www.femaflavour.org/) and sensory experiments, OAV was calculated by odor threshold (OT) reviewed in Leffingwell & Associates Threshold Value Database (https://www.leffingwell.com/) and other literature. The results were shown in Table 1.

Table 1 showed that 38 VOCs were detected and qualitatively identified in tea samples at different processing stages, and the relative content of other compounds could be calculated according to the peak area ratio because the content of cis-3-hexen-1-ol in each tea sample was known.

OAV evaluation results (Table 1) showed that among the 38 VOCs identified by GC–MS, 32 were aroma components. For sample E, there were 25 aroma components of OAV > 1 (group 1), of which the largest was (Z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one (5,000.00), the smallest was 1-Penten-3-ol (1.02), 7 aroma components of OAV < 1 (group 2), 6 odorless or non-aroma components (group 3). Among the 32 aroma components, 12 aromas contributed to green aroma were identified by the sensory evaluation group, according to the descending order of the OAV in sample E, they were geraniol (OAV = 192.00), hexanal (OAV = 116.00), (Z)-2-hexenal (59.00), (E)-2-hexenal (57.83), (E)-3-hexen-1-ol (39.14), citronellol (25.67), 1-hexanol (3.63), 1-penten-3-ol (1.02), cis-3-hexen-1-ol (0.20), 2-ethyl-1-hexanol (0.01), (Z)-2-penten-1-ol (0.00) and (E)-2-hexen-1-ol (0.00), respectively.
| No. | aroma components | Concentration (mg kg⁻¹)¹ | OAV¹ | Aroma discription² | Reference Sniffing tests COG³ |
|-----|-----------------|--------------------------|------|-------------------|--------------------------------|
| 33  | (Z)-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one | 0.17 0.13 0.11 0.19 1.55 | 0.0003 | 559.03 424.14 352.73 625.14 5,000.00 | Floral Herb, aging, flower 0 |
| 20  | 3,7-Dimethyl-1,6-octadien-3-ol | 6.91 3.51 2.31 1.03 2.15 | 0.0015 | 4,604.85 2,342.07 1,539.57 687.65 1,435.56 | Coriander, floral, lavender, lemon, rose Floral 0 |
| 24  | Dodecanal | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 400.26 509.03 501.92 679.13 | Citrus, fat, lily Fat, oil, gardenia 0 |
| 16  | Linalool oxide (pyranoid) (III and IV) | 2.13 1.80 2.79 0.92 1.88 | 0.0060 | 354.92 299.48 465.81 153.44 313.33 | Leafy, earthy, sweet, floral, creamy Flower 0 |
| 15  | 1-Octen-3-ol | 0.19 0.45 0.84 1.21 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 7   | 1-Pentanol | 0.09 2.97 2.84 3.06 1.32 | 0.0050 | 18.72 593.38 568.94 611.21 264.00 | Fruity Fruity, sweet 0 |
| 17  | Linalool oxide (furanoid) (I and II) | 5.83 9.00 0.75 0.96 1.44 | 0.0075 | 777.96 119.59 100.01 127.87 92.00 | Geranium, lemon peel, passion fruit, peach, rose Sweet, fruit, flower, lemon, green 1 |
| 28  | Geraniol | 0.19 0.45 0.84 1.21 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 1   | Hexanal | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 400.26 509.03 501.92 679.13 | Citrus, fat, lily Fat, oil, gardenia 0 |
| 23  | 3,7-Dimethyl-(Z)-2,6-Octadienal | 1.03 0.41 0.39 1.00 1.87 | 0.0300 | 34.19 13.55 13.07 33.25 62.44 | Lemon Lemon, sweet, enzymatic fermentation 0 |
| 5   | (Z)-2-Hexenal | 3.05 3.16 0.43 1.53 2.36 | 0.0400 | 76.35 78.98 10.66 38.15 59.00 | Fresh fruit, green Fresh fruit, green, sweet 1 |
| 4   | (E)-2-Hexenal | 1.84 1.78 1.64 1.39 2.31 | 0.0400 | 46.12 44.38 41.08 34.63 57.83 | Fresh fruit, green Fresh fruit, green, sweet 1 |
| 22  | Benzeneacetaldheyde | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 25  | Methyl salicylate | 1.45 1.09 0.77 0.84 3.13 | 0.0600 | 24.25 18.21 12.86 13.99 52.22 | Almond, caramel, pepper-mint, sharp Holly oil, mint, sweet, sharp 0 |
| 2   | β-Myrcene | 0.55 0.09 0.42 0.43 0.67 | 0.0140 | 39.00 6.21 30.23 30.75 47.62 | Balsamic, fruit, geranium, herb, must Balsamic, fruit, herb, must 0 |
| 11  | (E)-3-Hexen-1-ol | 0.29 3.13 3.94 3.30 2.74 | 0.0700 | 4.12 44.78 56.29 47.13 39.14 | Green Green 1 |
| 21  | 1-Ethyl-1H-pyrole-2-carboxaldehyde | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 26  | Citronellol | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 19  | Benzaldehyde | 0.12 0.24 0.78 1.68 4.25 | 0.3000 | 0.42 0.81 2.61 5.60 14.18 | Bitter almond, burnt sugar, cherry, malt, toasted pepper Bitter almond, cherry 0 |
| 35  | Tetradecanal | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 10  | 1-Hexanol | 2.73 0.25 0.08 0.51 1.39 | 0.2500 | 10.92 1.01 0.31 2.05 3.63 | Banana, flower, grass, herb Green, flower, unripe banana 1 |
| 27  | (Z)-3,7-Dimethyl-2,6-octadien-1-ol | 0.18 0.39 0.02 0.20 0.62 | 0.4000 | 0.45 0.98 0.04 0.51 1.55 | Floral, fruit Sweet, fruit, flower, lemon 0 |
| 37  | 1-Hexadecanol | 0.00 0.43 0.54 0.54 0.73 | 0.0011 | 95.56 223.45 420.17 605.25 290.00 | Cucumber, earth, fat, floral, mushroom 0 |
| 3   | 1-Penten-3-ol | 0.15 0.08 1.10 2.30 3.05 | 3.0000 | 0.05 0.03 0.37 0.77 1.02 | Butter, fish, green, oxidized, wet earth Wet earth, fishy-like, green 1 |
| 32  | Phenylethyl alcohol | 0.98 0.08 1.10 2.30 3.05 | 3.0000 | 0.05 0.03 0.37 0.77 1.02 | Butter, fish, green, oxidized, wet earth Wet earth, fishy-like, green 1 |

Continued
In addition, compared with sample A and sample E, among the 32 aroma components, the content of 16 aroma components increased, which were (Z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, 1-octen-3-ol, 1-pentanol, (E)-2-heptenal, hexanal, 3,7-dimethyl-(Z)-2,6-octadienal, (E)-2-hexenal, methyl salicylate, β-myrcene, (E)-3-hexen-1-ol, benzaldehyde, (Z)-3-hexen-1-ol, benzaldehyde, 2-ethyl-1-hexanol, respectively, and the number of aroma components containing fruit aroma was the largest (9); and there were 9 aroma components with decreasing content, which were 3,7-dimethyl-1,6-octadien-3-ol, linalool oxide (pyranoid) (III and IIII), geraniol, (Z)-2-hexenal, 1-hexanol, (Z)-3-hexen-1-ol, 3-methyl-1-butanol, (Z)-2-penten-1-ol and (E)-2-hexen-1-ol respectively, and among them, the majority contained green (6); and there were 7 new aroma components generated in course of processing, which were Dodecanal, Linalool oxide (furanoid) (I and II), benzeneacetaldehyde, 1-ethyl-1H-pyrrole-2-carboxaldehyde, citronellol, tetradecanal, 1-hexadecanol, respectively, including 4 floral components and 3 sweet components.

In summary, the changes of aroma components and their contents in green tea samples during processing have been identified. However, E-nose test and flavor profile test were still needed to determine the changes of the overall aroma profile of green tea samples, and conducting association analysis with both of them, then whether

| No. | aroma components | Concn (mg kg⁻¹) | OT (ppm) | OAV | Aroma discription |
|-----|-----------------|-----------------|----------|-----|------------------|
| 12  | cis-3-Hexen-1-ol | 7.27            | 2.25     | 1.69| 0.52             | 0.04 | 0.2000 | 36.35 | 11.25 | 8.45 | 2.60 | 0.20 | Grass, green fruit, green leaf, herb, unripe banana |
| 18  | 2-Ethyl-1-hexanol | 0.15            | 0.29     | 0.36| 0.37             | 1.79 | 270.0000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | Green, rose |
| 6   | 3-Methyl-1-butanol | 0.14            | 0.03     | –   | –                | –   | 0.2500  | 0.56 | 0.12 | 0.00 | 0.00 | 0.00 | Burnt, cocoa, floral, malt |
| 9   | (Z)-2-Penten-1-ol | 0.17            | 0.66     | 0.46| –                | –   | 0.7200  | 0.23 | 0.92 | 0.64 | 0.00 | 0.00 | Banana, green, rubber |
| 13  | (E)-2-Hexen-1-ol | 1.42            | 0.80     | 0.43| –                | –   | 0.6000  | 2.37 | 1.33 | 0.72 | 0.00 | 0.00 | Blue cheese, vegetable |
| 38  | 1-Nonadecanol    | –               | –        | –   | 2.45             | 0.48 | Null    | Null | Null | Null | Null | Null | Odorless |
| 29  | Propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethyl-pentyl propyl ester | 0.33 | 1.32 | 1.83 | 3.03 | 5.21 | Null | Null | Null | Null | Null | Null | Null |
| 30  | Propanoic acid, 2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl) propyl ester | 0.25 | 1.45 | 1.62 | 2.21 | 3.73 | Null | Null | Null | Null | Null | Null | Null |
| 31  | Propanoic acid, 2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester | 0.28 | 0.69 | 1.30 | 2.95 | 4.07 | Null | Null | Null | Null | Null | Null | Null |
| 34  | 2,6-Bis(1,1-dimethylhexyl)-4(1-methylpropyl)phenol | 0.44 | 0.82 | 1.01 | 3.08 | 5.10 | Null | Null | Null | Null | Null | Null | Null |
| 36  | 4-Octadeylmorpholine | –               | –        | –   | 1.37             | 0.51 | Null    | Null | Null | Null | Null | Null | Suspected pesticides |

### Table 1

Content of aroma components, OAV and aroma description of green tea samples detected and calculated by GC–MS. *Here the number was the same as Supplementary Annex S3, but the sorting was in descending order according to the aroma components’ OAV of sample E. The relative content of each aroma component was calculated by peak area ratio (except cis-3-hexen-1-ol), ‘–’ means undetected, and A represented sample A (spreading), B represented sample B (fixing), C, sample C (carding), D, sample D (pressing flat), E, sample E (drying). The OT of all aroma components came from Leffingwell and Associates data base (https://www.leffingwell.com), and Compilations of Odour Threshold Values in Air, Water and other Media AND Compilations of Flavour Threshold Values in Water and other Media (2011 Editions) — by L.J., van Gemert (https://www.thresholdcompilation.com), except for (Z)-3-methyl-2-(2-pentenyl)-2-cyclopen ten-1-one, it was determined by our group, Null means not found or odorless. Reference means these descriptor was retrieved from FEMA GRAS data base (https://www.femaflavor.org), except for linalool oxide, sniffing test, means the data came from the sniffing test. COG, contribution of green aroma, which was determined by aroma monomer sniffing test, contributions were 1 and no contribution was 0, ‘–’ means no testing. f1,f2 Data sources were Wang et al. *In the table, the aroma components distinguished by the black line were group 1 (OAV > 1 in sample E), group 2 (OAV < 1 in sample E), group 3 (non aroma components), respectively.

In addition, compared with sample A and sample E, among the 32 aroma components, the content of 16 aroma components increased, which were (Z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, 1-ocoten-3-ol, 1-pentanol, (E)-2-heptenal, hexanal, 3,7-dimethyl-(Z)-2,6-octadienal, (E)-2-hexenal, methyl salicylate, β-myrcene, (E)-3-hexen-1-ol, benzaldehyde, (Z)-3,7-dimethyl-2,6-octadien-1-ol, 1-penten-3-ol, phenylethyl alcohol, furfural and 2-ethyl-1-hexanol, respectively, and the number of aroma components containing fruit aroma was the largest (9); and there were 9 aroma components with decreasing content, which were 3,7-dimethyl-1,6-octadien-3-ol, linalool oxide (pyranoid) (III and IIII), geraniol, (Z)-2-hexenal, 1-hexanol, (Z)-3-hexen-1-ol, 3-methyl-1-butanol, (Z)-2-penten-1-ol and (E)-2-hexen-1-ol respectively, and among them, the majority contained green (6); and there were 7 new aroma components generated in course of processing, which were Dodecanal, Linalool oxide (furanoid) (I and II), benzenacetaldehyde, 1-ethyl-1H-pyrole-2-carboxaldehyde, citronellol, tetradecanal, 1-hexadecanol, respectively, including 4 floral components and 3 sweet components.

In summary, the changes of aroma components and their contents in green tea samples during processing have been identified. However, E-nose test and flavor profile test were still needed to determine the changes of the overall aroma profile of green tea samples, and conducting association analysis with both of them, then whether
the aroma profile of green tea samples is consistent with the changes of aroma components could be judged, as well as whether the most important aroma component affecting green aroma is cis-3-hexen-1-ol or not.

**Results of aroma profile, E-nose experiments and chemometrics analysis.** The results of aroma profile experiment were shown in Fig. 2a. In terms of aroma characteristics of tea samples, the green (score = 2.75) and fruit (1.50) of spreading tea (sample A) were the strongest, the roasted (2.25) of fixing tea (sample B) was the strongest, and the sweet (2.25) and flower (2.08) of drying tea (sample E) were the strongest, this indicated that the aroma characteristics of tea samples changed dramatically during the processing, and the green odor is converted into green aroma between sample D and sample E, at this time, the content of cis-3-hexen-1-ol decreased from 0.52 to 0.04 mg kg\(^{-1}\), which indicated that when cis-3-hexen-1-ol was near this content, the green odor disappeared and the green aroma appeared.

In terms of the dynamic change of aroma profile, green aroma gradually decreased (2.75 → 0.67) in the whole processing process, and the score was less than 1 at the time of pressing flat (sample D, 0.92) and later, floral (0.92 → 2.08) and fruity (0.75 → 1.17) aroma increased gradually from the beginning of fixing, and herbal aroma fluctuated the least during the processing (0.33 → 0.58).

In addition, it should be noted that the difference of aroma profile between sample A and sample E was consistent with the difference of aroma activity components, that is the increase of the content of aroma components that contribute to the floral (4 aroma components), fruity (9 aroma components) and sweet (3 aroma components) aroma, and the decrease of the content of aroma components that contribute to the green (6 aroma components), and it had been analyzed in detail in “Detection results of GC–MS and calculation results of OAV”.

To summarize, the aroma characteristics of tea samples changed dramatically during the processing, and the main change was that the aroma of green and roasted declined, while that of floral, fruity, fresh, herbal and sweet rose. Moreover, the inflexion point of green odor transforming green aroma occurred after pressing flat. However, there were still some deficiencies in the qualitative analysis of tea aroma changes based on the aroma profile test, it is necessary to use E-nose to assist the test and try to characterize the change of its overall characteristics from another perspective.

The results of the E-nose test were shown in Fig. 2b. Compared with carding tea (sample C, sensing value\(\text{average} = 0.2714\)) and drying tea (sample E, sensing value\(\text{average} = 0.2570\)), pressing flat tea (sample D, sensing value\(\text{average} = 0.1598\)) had a sharp decline in the sensing value of E-nose sensor, which was the lowest among all samples, and this was consistent with the results of aroma profile test, that is, the aroma components have changed significantly in pressing, the green odor disappeared and the green aroma appeared. In addition, the change rule of tea aroma in processing was evaluated by E-nose's radar map, which showed that it was irregular and repeated fluctuation, specifically, the 12 sensors of T series and P series all showed sample C > sample E > sample B > sample A > sample D, which those dramatic and repeated changes were consistent with the results of aroma profile test.

In addition, it can be seen from the overall E-nose radar profile of sample C and sample E that they had a high similarity, but in the aforementioned aroma profile test, they were quite different, this is because the sensing value of E-nose was an electrical signal generated based on the content of the chemical group (or ion) corresponding to the VOCs adsorbed by the sensor array (relative to the calibration reagent), it could be seen from Supplementary Annex S2 that E-nose sensors used in this test were all broad-spectrum sensors, they cannot specifically identify key aroma components such as cis-3-hexen-1-ol, but only chemical groups (or ions) such as fluoride (LY2/LG, P40/1, T40/1), chlorine (T30/1, P10/1, P40/1, P40/2, T40/2), therefore, it could only characterize the difference of chemical groups (or ions) of VOCs in different samples, so as to determine the difference of overall aroma, but not the source of the difference or quantitative analysis.

In fact, based on the above-mentioned E-nose detection principle, some common characteristics of tea could also be found. For example, the sensing values of all tea samples in the sensing area of LY2 (LY2/LG, LY2/G, LY2/AA, LY2/Gh, LY2/gCTL, LY2/gCTL) were lower (~ 0.0244 to 0.0616), this was related to the detection of air pollutants such as fluoride, chloride, oxynitride, sulfide, ammonia, hydrogen sulfide by the above sensors, and almost no such substances exist in natural tea samples, the similar cases also included the relatively low sensing values of tea samples in T40/2 (chloride) and T70/2 (toluene, xylene, carbon monoxide), obviously, there were very few corresponding substances in natural tea samples. Interestingly, the above two results were consistent with the E-nose detection of oolong tea by Zuobing Xiao et al.\(^{17}\), which also proved that it may be a universal attribute of tea.

Overall, through E-nose detection, it could be seen that the aroma of green tea changed dramatically in the whole processing, and the change was the largest before and after pressing flat (sample D), which was consistent with the results of aroma profile test, but it cannot be confirmed that these changes were caused by cis-3-hexen-1-ol or green aroma. Therefore, further chemometrics analysis was needed for the quantitative evaluation of aroma components.

The chemometrics analysis was divided into two steps. First, the correlation analysis was carried out to evaluate the correlation between OAV of 32 aroma activity components (group 1 and group 2 in Table 1) detected in tea samples and the green score in the aroma profile (Pearson correlation coefficient, two-tailed test), the results were shown in Table 2. It could be seen that OAV of 7 aroma components is significantly (or extremely significantly) related to the green score, among which 5 components were significantly positively related, namely 3,7-dimethyl-1,6-octadien-3-ol, geraniol, (E)-3-hexen-1-ol, 3-methyl-1-butanol and (E)-2-hexen-1-ol, and among the 5 components mentioned above, geraniol, (E)-3-hexen-1-ol and (E)-2-hexen-1-ol had been confirmed by the evaluation team to contribute to green aroma in the aroma description test (Table 1), therefore these 3 components could be considered as the direct contribution components of green aroma in green tea; then, the other 2 significantly related components were 3,7-dimethyl-1,6-octadien-3-ol (floral) and 3-methyl-1-butanol (burn, cocoa, malt), which suggested that they might have synergistic effect on green tea aroma; in addition, dodecanal
(E)-3-hexen-1-ol), $R^2 = 0.995$, $P = 0.000 < 0.05$, this showed that the equation had metrological significance; in addition, two independent variables in the equation can pass the significance test ($P < 0.05$) and the variance inflation factor (VIF) test ($VIF < 10$), which showed that the equation fits well and has no multicollinearity, and directly contributed to the aroma of green tea.

Effect of cis-3-hexen-1-ol. The independent variable was the change of OAV of 32 aroma components from sample A to sample E, and the corresponding variable was the aroma profile score of green aroma. The correlation coefficient was Pearson coefficient; "*" means $P < 0.05$, "**" means $P < 0.01$.

**Table 2.** Correlation analysis results of OAV of each aroma contribution components and green score of aroma profile. The independent variable was the change of OAV of 32 aroma components from sample A to sample E, and the corresponding variable was the aroma profile score of green aroma. The correlation coefficient was Pearson coefficient; "*" means $P < 0.05$, "**" means $P < 0.01$.

| Aroma contribution components | Pearson correlationa | Significance | Aroma contribution components | Pearson correlation | Significance |
|-------------------------------|----------------------|-------------|-------------------------------|---------------------|-------------|
| (Z)-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one | −0.501 | 0.390 | (E)-3-Hexen-1-ol | −0.796 | 0.107 |
| 3,7-dimethyl-1,6-Octadien-3-ol | 0.924* | 0.025 | 1-Ethyl-1H-pyrrole-2-carboaldehyde | −0.507 | 0.383 |
| Dodecanol | −0.963** | 0.008 | Citronellol | −0.654 | 0.232 |
| Linalool oxide (pyranoid) (III and IV) | 0.379 | 0.529 | Benzaldehyde | −0.688 | 0.199 |
| 1-Octen-3-ol | −0.639 | 0.246 | Tetradecanal | −0.605 | 0.280 |
| 1-Pentanol | −0.642 | 0.243 | 1-Hexanol | 0.789 | 0.112 |
| Linalool oxide (furandion) (I and II) | −0.704 | 0.184 | (Z)-3,7-dimethyl-2,6-octadien-1-ol | −0.495 | 0.397 |
| Geraniol | 0.887* | 0.045 | 1-Hexadecan | −0.600 | 0.285 |
| (E)-2-Heptenal | −0.976** | 0.004 | 1-Penten-3-ol | −0.738 | 0.154 |
| Hexanal | 0.076 | 0.904 | Phenylethyl alcohol | −0.277 | 0.652 |
| 3,7-Dimethyl-(Z)-2,6-octadienal | −0.268 | 0.663 | Furfural | −0.800 | 0.104 |
| (Z)-2-Hexenal | 0.321 | 0.598 | cis-3-Hexen-1-ol | 0.988** | 0.002 |
| (E)-2-Hexenal | −0.104 | 0.868 | 2-Ethyl-1-hexanol | −0.661 | 0.225 |
| Benzeneacetaldehyde | −0.507 | 0.383 | 3-Methyl-1-Butanol | 0.940* | 0.018 |
| Methyl salicylate | −0.283 | 0.645 | (Z)-2-Penten-1-ol | 0.142 | 0.820 |
| β-Mycene | 0.049 | 0.938 | (E)-2-Hexen-1-ol | 0.930* | 0.022 |

It could be seen that the effect of cis-3-hexen-1-ol on 25 aroma activity components could be divided into 4 groups: group I (increase), which included 5 aroma activity components, namely 3,7-dimethyl-1,6-octadien-3-ol, dodecanol, 1-pentanol, tetradecanal and 1-hexanol; group II (decrease), including 5 aroma active components, namely geraniol, (E)-2-heptenal, hexanal, (Z)-3,7-dimethyl-2,6-octadien-1-ol and 1-penten-3-ol; group III (fluctuation), including 5 aroma activity components, namely (Z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, 3,7-dimethyl-(Z)-2,6-octadienal, (E)-2-heptenal, benzeneacetaldehyde and citronellol; group IV (keep stable), including 4 aroma activity components, namely (Z)-2-hexenal, methyl salicylate, (fat, oil, gardenia) and (E)-2-heptenal (almond, fat, fruit), which had significant negative correlation, suggested that they may have inhibitory effect on green tea aroma, but this needs further experimental verification.

Finally, stepwise linear regression analysis was carried out. OAV of 12 aroma components considered to have green aroma contribution in 5 tea samples (sample A to sample E) was taken as independent variable, and green score was taken as corresponding variable to carry out stepwise linear regression equation fitting, since the above 12 aroma components were assumed to be all possible sources of green aroma in tea samples, no constant was set during fitting, and the fitting equation was $S_{\text{green}} = 0.073 \times X_1 + 0.014 \times X_2 \ (X_1 = \text{OAV} (\text{cis}-3\text{-hexen}-1\text{-ol}), \ X_2 = \text{OAV} ((\text{E})-3\text{-hexen}-1\text{-ol}), R^2 = 0.995, P = 0.000 < 0.05$), this showed that the equation had metrological significance; in addition, two independent variables in the equation can pass the significance test ($P < 0.05$) and the variance inflation factor (VIF) test ($VIF < 10$), which showed that the equation fits well and has no multicollinearity, and the results showed that the OAV changes of cis-3-hexen-1-ol and (E)-3-hexen-1-ol were linearly correlated with the change of green aroma score, among which the coefficient of OAV (cis-3-hexen-1-ol) was higher (0.073), therefore cis-3-hexen-1-ol was the most influential component among the 12 aroma components contributing for green aroma.

In summary, 12 aroma components that contribute to green tea aroma were identified by sniffing of aroma monomer, among which 3 components were positively correlated with green aroma score through correlation analysis, and they were the direct contribution components of green aroma of green tea, finally, through stepwise linear regression analysis, the fitting equation of green aroma score was obtained, which was $S_{\text{green}} = 0.073 \times X_1 + 0.014 \times X_2 \ (X_1 = \text{OAV} (\text{cis}-3\text{-hexen}-1\text{-ol}), \ X_2 = \text{OAV} ((\text{E})-3\text{-hexen}-1\text{-ol})), R^2 = 0.995, P = 0.000 < 0.05$, this showed that the equation fits well and has no multicollinearity, and the results showed that the OAV changes of cis-3-hexen-1-ol and (E)-3-hexen-1-ol were linearly correlated with the change of green aroma score, among which the coefficient of OAV (cis-3-hexen-1-ol) was higher (0.073), thus it was proved that cis-3-hexen-1-ol is the most direct contribution to the aroma of green tea.

**Effect of cis-3-hexen-1-ol on the aroma intensity of other aroma components in aroma monomer system.** In order to find out how cis-3-hexen-1-ol affected the overall aroma of tea, it was necessary to study the influence of cis-3-hexen-1-ol on 25 aroma activity components in the aroma monomer system, therefore, based on the aroma components and content in sample E, mixed the different concentrations of cis-3-hexen-1-ol with each aroma component in pairs, and sniffed the changes of aroma intensity. Saw Fig. 3 for the statistical results.

It could be seen that the effect of cis-3-hexen-1-ol on 25 aroma activity components could be divided into 4 groups, group I (increase), which included 5 aroma activity components, namely 3,7-dimethyl-1,6-octadien-3-ol, dodecanol, 1-pentanol, tetradecanal and 1-hexanol; group II (decrease), including 5 aroma active components, namely geraniol, (E)-2-heptenal, hexanal, (Z)-3,7-dimethyl-2,6-octadien-1-ol and 1-penten-3-ol; group III (fluctuation), including 5 aroma activity components, namely (Z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, 3,7-dimethyl-(Z)-2,6-octadienal, (E)-2-heptenal, benzeneacetaldehyde and citronellol; group IV (keep stable), including 4 aroma activity components, namely (Z)-2-hexenal, methyl salicylate,
1-ethyl-1H-pyrrole-2-carboxaldehyde and benzaldehyde. This showed that cis-3-hexen-1-ol could enhance group I aroma, inhibit group II aroma and interfere with group III aroma to achieve the effect on green tea aroma, however, the aroma of group IV was not affected by cis-3-hexen-1-ol.

In addition, it should be noted that the score of 6 aroma components was always less than 0.5, which means that they probably were odourless at the concentration of sample E, so it was impossible to judge the effect of cis-3-hexen-1-ol on them, however, this was not consistent with the results based on OAV evaluation, which may be related to the volatility of aroma threshold, that is, the thresholds of the same aroma (using the same solvent) determined by different sniffing groups may be different, so further correction may be needed to determine whether they were affected. At the same time, 4 of the 5 aroma components enhanced by cis-3-hexen-1-ol contributed to the floral or fruity aroma (3,7-dimethyl-1,6-octadien-3-ol, 1-pentanol, tetradecanal, 1-hexanol),

**Figure 3.** Effect of (Z)-3-hexen-1-ol on the aroma intensity of other aroma components in aroma monomer system. (a) The concentration of each aroma component was set to the concentration detected in sample E (Table 1); (b) the content of (Z)-3-hexen-1-ol in sample E was 100% (0.04 mg kg⁻¹), thus "10%" was 0.004 mg kg⁻¹, "50%" was 0.02 mg kg⁻¹, "100%" was 0.04 mg kg⁻¹, "200%" was 0.08 mg kg⁻¹, "500%" was 0.2 mg kg⁻¹, "1,000%" was 0.4 mg kg⁻¹; (c) "Increase" means that the score of aroma intensity was significantly higher than CK at "1,000%", "decrease" means that the score was significantly lower than CK at "1,000%", "fluctuate" means that the score changed significantly during the process, but the difference between "1,000%" and CK was not significant, "keep stable" means that the difference of all concentrations is not significant; "unable to judge" means the score of the aroma component at all concentrations was less than 0.5, so the evaluation team can't accurately judge the change trend.
which suggested that green aroma may have synergistic effect with the expression of floral and fruity aroma characteristics, and it could also explain the reason sample A had a high score of floral (1.83) and fruity score (1.50) (Fig. 2a). In addition, cis-3-hexen-1-ol did not have the general enhancement effect on other green aroma components as expected, in fact, among the 8 components that contributed to green, there were 3 decreased, which were geraniol, hexanal and 1-penten-3-ol respectively, 3 fluctuated, which were (E)-2-hexenal, benzeneacetaldehyde and citronellol respectively, and 1 couldn’t judge which was trans-3-hexen-1-ol, only 1 increased which was 1-hexanol, it is speculated that the reason was that cis-3-hexen-1-ol has obvious grass and sharp characteristics, so it is easy to cause other aroma components to change or be inhibited, another similar component was methylis salicylas, which also had similar change or inhibitory effect on other aroma, however, this needs further experimental verification.

**Effect of cis-3-hexen-1-ol on the overall aroma of green tea in mixed systems.** The influence of cis-3-hexen-1-ol on the aroma components has been studied in the monomer system test mentioned above. However, in the "natural mixed system" of tea, the influence of cis-3-hexen-1-ol may be different, so it needed to be verified in tea and the corresponding aroma reconstitution solution.

First, according to the composition and content of 25 aroma components (Table 2, sample E, group1) detected from sample E, the reconstituted aroma was reconstituted with redistilled water as a solvent (results were shown in Fig. 4a). It could be seen that compared with the sample E, the green (1.17) and fruity (1.42) aroma of the reconstitution solution were higher, while the sweet (2.08) and roasted (0.83) aroma were insufficient, this showed that although the overall aroma characteristics of sample E could be reappear to a certain extent according to the evaluation of aroma profile score, there were still significant differences between both of them, and the possible reasons will be further elaborated in the discussion.

Secondly, cis-3-hexen-1-ol with concentration of 10%, 50%, 100%, 200%, 500% and 1,000% (based on cis-3-hexen-1-ol content in sample E) was added into sample E and reconstituted solution respectively, and then the aroma profiles were evaluated (Fig. 4b).

The results showed that in terms of the overall change trend of aroma, the reconstitution solution and sample E showed the same trend, with the increased of cis-3-hexen-1-ol concentration, green (grass) was increasing, herbal was decreasing, floral and fruity was fluctuating, this showed that the influence of different concentrations of cis-3-hexen-1-ol on the overall aroma of green tea in mixed systems. In terms of the overall change range of aroma score, the total change range of aroma profile score of the reconstitution solution (29.56%) was larger than that of sample E (23.58%), which indicated that the influence of different concentrations of cis-3-hexen-1-ol on the reconstitution solution was greater than that of sample E, suggesting that there may be some substances in tea samples that can inhibit the aroma of cis-3-hexen-1-ol, or keep the original aroma characteristics, so that the aroma characteristics of sample E were more difficult to affect. In addition, the reconstitution solution showed green odor at 200% exogenous cis-3-hexen-1-ol (0.08 mg L⁻¹), while sample E appeared at 1,000% (0.44 mg kg⁻¹, 0.40 mg kg⁻¹ exogenous + 0.04 mg kg⁻¹ endogenous), which was consistent with the result of the disappearance of green odor between sample D (concentration of cis-3-hexen-1-ol was 0.52 mg kg⁻¹) and sample E (concentration was 0.04 mg kg⁻¹), it’s further proved that the threshold range of cis-3-hexen-1-ol was 0.04–0.52 mg kg⁻¹ when the green odor changes into green aroma in tea, but the difference between the reconstitution solution and sample E also showed that the performance of green odor was not only determined by the concentration of (exogenous) cis-3-hexen-1-ol in tea samples, but also may influenced by some unknown “inhibitors” of green odor. In addition, regarding the difference between green aroma and green odor, the evaluation team believed that it was related to the “grass smelling” of cis-3-hexen-1-ol, which is consistent with previous research results, McRae Jeremy F’s research²⁰ suggested that the grass smelling of cis-3-hexen-1-ol may caused different odor receptor reactions with green aroma, which may lead to unpleasant green odor.

Finally, tried to match the test results of the "natural mixed system" (Fig. 4b2) with the test results of the aroma monomer system (Fig. 4). The increase of green and fresh aroma may be related to the increase of the content of cis-3-hexen-1-ol and the synergistic enhancement of 1-hexanol (green, flower, unripe banana), while the decrease of sweet and the fluctuation of floral and fruity aroma may be related to the masked of geraniol (sweet, fruit, flower, lemon, green) and (z)-3,7-dimethyl-2,6-octadien-1-ol (sweet, fruit, flower, Lemon), and the weakening of herbal aroma may be affected by (z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, however, the change of roasted aroma seemed unable to be explained by the aroma components in Fig. 4, which needed further experiment.

In summary, the effects of cis-3-hexen-1-ol on other aroma components and on the overall aroma of green tea were explored through aroma monomer system test and mixed system test, the results of the former test showed that there were 4 main modes of cis-3-hexen-1-ol on other aroma components, namely increase, decrease, fluctuate and keep stable; the results of the latter experiment showed that cis-3-hexen-1-ol mainly enhanced green and fresh, masked herbal and roasted, interfered and even masked sweet, floral and fruity to achieve the effect on the aroma of green tea, in addition, the performance of other aroma is consistent with that of aroma monomer test except roasted.
Discussion

Molecular structure basis of green aroma components in tea. 12 aroma components which contributed to green aroma were identified from the natural aroma components of tea, and compared with their molecular structure, it could be found that there was a certain consistency. Therefore, the structural basis of the green aroma components of tea was discussed.

According to Amoore’s “Stereochemical Theory of Odor”\(^{21}\), the aroma characteristics are affected by the functional groups and Stereochemical structure of the components. On this basis, it could be found that most of the structural units of the 12 components detected in this experiment had unsaturated carbon–carbon double bonds, and there were saturated or unsaturated oxygen atoms 1 or 3, and the carbon chains are all 8 carbon (or less) short chains. In addition, carbon–carbon double bond, hydroxyl group, carboxyl group and other functional
groups were often considered as aroma generating groups in the study of aroma, and when there were two functional groups with acceptable hydroxyl groups in the molecular structure in a relatively close position, the aroma intensity was stronger.

Based on the Inelastic Electron Tunneling Spectroscopy (IETS) technology, Turin proposed a new theory of aroma vibration. It is believed that the vibration energy of aroma components could be recognized by the olfactory signal receiver of human body, after that, he used IETS to analyze the components considered to have bitter almond aroma, and found that hydrogen cyanide and benzaldehyde had characteristic peaks around 920 cm$^{-1}$ and 2,000 cm$^{-1}$, respectively. In terms of green aroma components, such as (E)-3-hexen-1-ol and (E)-2-hexen-1-ol, they were reported to have characteristic peaks only at 2,000 cm$^{-1}$, but not at 920 cm$^{-1}$. Therefore, it is possible that the IETS characteristic peak of green aroma is 2,000 cm$^{-1}$, but it needs to be further verified by experiments.

In summary, based on the Stereochemical Theory of Odor, it is speculated that the components of green aroma in tea are short carbon chain structure of 8 carbon (or less), and there are unsaturated carbon–carbon double bond and 1,3-position saturated (or unsaturated) oxygen atom; based on the new aroma vibration theory, it is speculated that the components of green aroma in tea are the components with characteristic peak at 2,000 cm$^{-1}$ in IETS scanning.

The difference between the results of reconstitution solution and tea sample test. In this experiment, there were some differences in aroma profile between the reconstitution solution and the tea sample. We speculate that this may be related to the reconstituted substrate composition and the potential contributing components which OAV < 1.

In the existing research, the aroma reconstitution of tea was often carried out with pure water as the substrate, which is because the tea drinking way is to brew with boiling water. However, the results of this experiment and the reported recombination experiments showed that there were some differences between the reconstitution solution and the tea samples, which may come from not only the qualitative and quantitative detection errors, but also the interference of non-volatile substances in tea. Previous studies have shown that the aromatic strength of linalool (3,7-dimethyl-1,6-octadien-3-ol) may be inhibited by the esters in the substrate, while the esters in tea may exceed 8% of the dry weight, which may lead to the inhibition of the aroma of 3,7-dimethyl-1,6-octadien-3-ol, so that the flower aroma score in the reconstitution solution was lower than that of the tea sample. And some soluble sugars may enhance the perception of sweet aroma, Isogai found that sucrose could significantly enhance the perception of sweet aroma, while the sucrose content in tea can generally reach 10$^2$–10$^3$ mg kg$^{-1}$, which may lead to the weakening of sweet aroma in the reconstitution solution.

In addition, the selection of aroma components in the reconstitution solution was based on OAV, however, olfactory stimulation depends on the content and proportion of aroma components in the inhaled air, and the latter is affected by the volatilization rate and saturation of aroma substances from the substrate, which depends on the overall Saturated Vapor Pressure of the solution. Previous studies have shown that the composition and content of aroma components in aqueous solution will affect the saturated vapor pressure of the solution, and according to Raoult’s law, the higher the content of solute is, the higher the saturated vapor pressure is, the more volatile the solution is. However, the measurement of odor threshold of aroma monomer is carried out under the condition of single solute, and its saturated vapor pressure is not the same as that of the mixed solution. Therefore, the odor threshold measured by aroma monomer may be different from the mixed state of tea samples, and the odorless monomer components and nonvolatile components may also affect the volatile ability of aroma components.

Overall, the difference of aroma profile between the reconstitution solution and the tea sample may be due to the interference of non-volatile substances and odorless volatile substances in the tea sample, thus the reconstitution of tea aroma should consider the preparation of more complex substrate with higher reduction degree and the selection of some volatile components with OAV < 1.

Conclusion

There were 32 VOCs that contribute to the aroma of green tea, 12 of which contributed to the aroma characteristics of green, they were geraniol, hexanal, (Z)-2-hexenal, (E)-2-hexenal, (E)-3-hexen-1-ol, 1-hexanol, 1-penten-3-ol, cis-3-hexen-1-ol, 2-ethyl-1-hexanol, (Z)-2-penten-1-ol and (E)-2-hexen-1-ol, respectively. Among the above 12 components, 3 were positively correlated with the green score of aroma profile, they were geraniol, cis-3-hexen-1-ol and (E)-2-hexen-1-ol, respectively. Further fitting stepwise linear regression equation, was $S$ (green) = 0.073 × OAV (cis-3-hexen-1-ol) + 0.014 × OAV (E)-2-hexen-1-ol). Therefore, cis-3-hexen-1-ol played the leading role in green aroma of green tea.

There were four types of effect with cis-3-hexen-1-ol on other 19 aroma in green tea, the first was increase effect (5 aroma components), including 3,7-dimethyl-1,6-octadien-3-ol, dodecanal, 1-pentanol, tetradecanal and 1-hexanol; the second was decrease effect (5 aroma components), including geraniol, (E)-2-heptenal, hexanal, (Z)-3,7-dimethyl-2,6-octadien-1-ol and 1-penten-3-ol; the third was fluctuate effect (5 aroma components), including (Z)-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, 3,7-dimethyl-(Z)-2,6-octadienol, (E)-2-hexenal, benzeneacetaldehyde and citronellol; the forth was keep stable effect (4 aroma components), including (Z)-2-hexenal, methyl salicylate, 1-ethyl-1H-pyrrrole-2-carboxaldehyde and benzaldehyde. At the same time, the above changes could partly explain the effect of cis-3-hexen-1-ol on the overall aroma of green tea, that is to say, with the increase of exogenous cis-3-hexen-1-ol concentration, green and fresh of green tea increased, herbal, roasted and sweet decreased, floral and fruit fluctuated.

When green tea changed from green odor to green aroma, the concentration of endogenous cis-3-hexen-1-ol varied from 0.04 to 0.52 mg kg$^{-1}$; in addition, the critical concentration of conversion between green odor
and green aroma was 0.08 mg L⁻¹ in the aroma reconstitution solution and 0.44 mg kg⁻¹ (0.40 mg kg⁻¹ exogenous + 0.04 mg kg⁻¹ endogenous) in the tea samples.

Data availability
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References

1. Cofer, T. M., Seidl-Adams, I. & Tumlinson, J. H. From acetoin to (Z)-3-hexen-1-ol: The diversity of volatile organic compounds that induce plant responses. J. Agric. Food Chem. 66(43), 11197–11208 (2018).
2. Saijo, R. & Takeo, T. Increase of cis-3-hexen-1-ol content in tea leaves following mechanical injury. Phytochemistry 14(1), 181–182 (1975).
3. Cofer, T., Seidl-Adams, I. & Tumlinson, J. H. From acetoin to (Z)-3-hexen-1-ol: The diversity of volatile organic compounds that induce plant responses. J. Agric. Food Chem. 20(20), 10 (2018).
4. Malheiro, R., Casal, S., Cunha, S. C., Baptista, P. & Pereira, J. A. Identification of leaf volatiles from olive (Olea europaea) and their possible role in the ovipositional preferences of olive fly, Bactrocera oleae (Rossi) (Diptera: Tephritidae). Phytochemistry 121, 11–19 (2016).
5. Keeler, A. Defensive function of herbivore-induced plant volatile emissions in nature. Science 291(5511), 2141–2144 (2001).
6. Fan, W., Xu, Y., Jiang, W. & Li, J. Identification and quantification of impact aroma compounds in 4 nonfloral vitis vinifera varieties grapes. J. Food Sci. 75(1), S81–S88 (2010).
7. Janzantti, N. S. & Monteiro, M. Changes in the aroma of organic passion fruit (passiflora edulis f. flavicarpa del.) during ripeness. J. Food Sci. Technol. 59(2), 612–620 (2014).
8. Yang, W., Cadwallader, K. R., Liu, Y., Huang, M. & Sun, B. Characterization of typical potent odorants in raw and cooked toona sinensis (a juss.) m. roem. by instrumental-sensory analysis techniques. Food Chem. 282(JUN1), 153–163 (2019).
9. Harper, S. H. & Smith, R. J. D. Stereochemical studies of olefinic compounds. Part iv. The configuration of ‘leaf alcohol’ and a further synthesis of cis-jasmine. J. Chem. Soc. 15, 12 (1955).
10. Zhou, J. C., Chen, F. Y., Wang, L. Y. & Xiao, Z. B. Evaluation of the synergism among volatile compounds in oolong tea during ripening. Food Chem. 148(48C), 388–395 (2014).
11. Zhu, Y. et al. Identification of key odorants responsible for chestnut-like aroma quality of green teas. Food Res. Int. 108(JUN1), 74–82 (2018).
12. Wang, Y. et al. Changes in global aroma profiles of cabernet sauvignon in response to cluster thinning. Food Res. Int. 122(AUG1), 56–65 (2019).
13. Xin, H., Wu, B., Zhang, H., Wang, C. & Li, S. Characterization of volatile compounds in flowers from four groups of sweet osmanthus (osmanthus fragrans) cultivars. Can. J. Plant Sci. 93(5), 923–931 (2013).
14. Kang, S. et al. Identification and quantification of key odorants in the world’s four most famous black teas. Food Res. Int. 121(JUL1), 73–83 (2019).
15. Forero, D. P., Orrego, C. E., Peterson, D. G. & Osorio, C. Chemical and sensory comparison of fresh and dried lulo (solanum quitoense lam) fruit aroma. Food Chem. 169(feb15), 85–91 (2015).
16. Qin, Z., Pang, X., Chen, D., Chen, H. & Wu, J. Evaluation of Chinese tea by the electronic nose and gas chromatography–mass spectrometry: Correlation with sensory properties and classification according to grade level. Food Res. Int. 52(2), 864–874 (2013).
17. Zhu, J. C., Chen, F., Wang, L. Y., Niu, Y. W. & Xiao, Z. B. Evaluation of the synergism among volatile compounds in oolong tea infusion by odour threshold with sensory analysis and e-nose. Food Chem. 221, 1484–1490 (2017).
18. Nie, C. N. et al. Comparison of different aroma-active compounds of sichuan dark brick tea (Camellia sinensis) and sichuan fuhuan brick tea using gas chromatography–mass spectrometry (GC–MS) and aroma descriptive profile tests. Eur. Food Res. Technol. 245(9), 1963–1979 (2019).
19. Rietjens, I. M. C. M., Cohen, S. M., Eisenbrand, G., Fukushima, S. & Taylor, S. V. Fema gras assessment of natural flavor complexes: Cinnamomum and myroxylon-derived flavoring ingredients. Food Chem. Toxicol. 135, 110949 (2019).
20. Mcrae, J. F. Genetic variation in the odorant receptor or2j3 is associated with the ability to detect the “grassy” smelling odor, cis-3-hexen-1-ol. Chem. Senses 37(7), 585–593 (2012).
21. Amoore, J. E., Johnston, J. W. & Rubin, M. The stereochemical theory of odor. J. Theor. Biol. 20(6), 773–791 (1966).
22. Czerney, M., Brueckner, R., Kirchhoff, E. & Schmitt, R. The influence of molecular structure on odor qualities and odor detection thresholds of volatile alkylated phenols. Chem. Senses 36(6), 539–553 (2011).
23. Schoenauer, S., Buergy, A., Kreissl, J. & Schieberle, P. Structure/odor activity studies on aromatic mercaptans and their cyclohexane analogues synthesized by changing the structural motifs of naturally occurring phenyl alk(en)ethanols. J. Agric. Food Chem. 20, 20 (2019).
24. Keskik, M., Takahashi, Y. K., Kei, I. & Shin, N. Odor maps in the dorsal and lateral surfaces of the rat olfactory bulb. Eur. Food Res. Technol. 245(4604), 1345–1351 (1983).
25. Lu, T. A spectroscopic mechanism for primary olfactory reception. Chem. Senses 6(5), 773–791 (1996).
26. Turin, L. A method for the calculation of odor character from molecular structure. J. Theor. Biol. 216(3), 367–385 (2002).
27. Hoffenden, L. J. W., Ylayyan, V. A. & Fortin, J. Investigation of vibrational theory of olfaction with variously labelled benzaldehydes. Food Chem. 73(1), 67–72 (2001).
28. Liao, M., Qi, S., Wei, G., Yang, X. & Schieberle, P. Characterization of the key odorants in a high-grade Chinese green tea beverage (Camellia sinensis; Jinghuan cha) by means of the sensomics approach and elucidation of odorant changes in tea leaves caused by the tea manufacturing process. J. Agric. Food Chem. 68(18), 5168–5179 (2020).
29. Miettinen, S. M., HyvNen, L. & Tuorila, H. Timing of intensity perception of a polar vs nonpolar aroma compound in the presence of added vegetable fat in milk. J. Agric. Food Chem. 51(18), 5437–5443 (2003).
30. Isogai, T. & Wise, P. M. The effects of odor quality and temporal asymmetry on modulation of taste intensity by retrolandoral odorants. Chem. Senses 11(7), 557–566 (2016).
31. Zinkernagel, T. Prediction of partitioning between complex organic mixtures and water: Application of polynomial nonlinear free energy relationships. Environ. Sci. Technol. 40(2), 536–545 (2006).
32. Wiens, M. A. The influence of sugar concentrations on the vapor pressure of food odor volatiles in aqueous solutions. J. Food Sci. 33(1), 1–2 (2006).
33. Wang, D., Ando, K., Morita, K., Kubota, K. & Kobayashi, A. Optical isomers of linalool and linalool oxides in tea aroma. J. Agric. Chem. Soc. Jpn. 58(11), 2050–2053 (1994).
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Author contributions
C.N. was responsible for the sorting of the experimental data and proofreading and wrote the manuscript. Y.G. was responsible for the design, implementation of the tests and collection of raw data. J.B. revised the manuscript critically for important intellectual content. H.L., X.Z., C.W., and S.L. were responsible for some experiments. X.D. provided design ideas, ensured the smooth progress of the tests and revised the outline and the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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