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Characterisation of the volatile compounds profile of Chinese pan-fried green tea in comparison with baked green tea, steamed green tea, and sun-dried green tea using approaches of molecular sensory science

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ABSTRACT

Pan-fried green tea (PGT) is an easily acceptable tea drink for general consumers. In this study, volatile profiles and characteristic aroma of 22 representative Chinese PGT samples were extracted using stir bar sorptive extraction (SBSE) and analysed by gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O) analysis, and odour activity value (OAV) calculations. In total, 88 volatile compounds were identified. Alcohols (45%), esters (19%), and ketones (16%) were the dominant volatiles, and geraniol (484.8 μg/kg) was the most abundant volatile component in PGT, followed by trans-β-ionone and linalool. In addition, the differences of aroma characteristics among PGT and other three types of green tea, namely baked green tea, steamed green tea, and sun-dried green tea, were also observed using partial least squares discriminant analysis (PLS-DA) and heatmap analysis, and it was found that β-myrcene, methyl salicylate, (*E*)-nerolidol, geraniol, methyl jasmonate were generally present at higher content in PGT. This is the first comprehensive report describing the volatile profiles of Chinese PGT, and the findings from this study can advance our understanding of PGT aroma quality, and provide important theoretical basis for processing and quality control of green tea products.

1. Introduction

Green tea is a type of unfermented tea, produced mainly in China, Japan, Korea, and Vietnam, and is popular with an increasing number of consumers because of its charming flavour and health benefits (Guo et al., 2021; Xing et al., 2019). Generally, green tea products can be subdivided into pan-fried green tea (PGT), baked green tea (BGT), steamed green tea (SGT), and sun-dried green tea (SDGT) according to different manufacturing technologies in China (Fig. 1). Among the numerous green tea products, the production and consumption of PGT is the largest in the world, particularly in China. PGT has a long history as a traditional drink, which is a promising and easily acceptable tea drink for general consumers due to its flavour quality (Zhu et al., 2021b), such as the soothing aroma and mellow taste, and is becoming increasingly popular and highly desired by tea consumers worldwide (Yang et al., 2020). There is now a wide variety of PGT products available on the market. For example, the most famous premium green tea in China, including Xihu Longjing tea (Zhu et al., 2016), Biluochun tea (Wang et al., 2016), and Xinyang Maojian tea (Sun et al., 2014), which are renowned internationally because of their superior flavour quality, all fall within the scope of PGT products.

Aroma is one of the most important determinants of tea quality, and the essence of tea aroma is composed of diverse volatile compounds at various concentrations (Zhu et al., 2016). To date, more than 260 odour compounds have been identified in green teas (Wan, 2003). Although there have been several reports regarding PGT volatiles, they have only focused on a single type of PGT sample (Sun et al., 2014; Wang et al., 2020; Zhu et al., 2016). In fact, the aroma profile and characteristic aroma of PGT have not been systematically investigated and remain unknown. In addition, the differences in aroma characteristics between PGT and the other three types of green tea are poorly understood. These gaps in knowledge seriously hamper further understanding of the PGT aroma chemistry.

It is well known that PGT products are generally produced through a

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particular processing technology, particularly the longstanding pan-frying manufacturing process. Therefore, we speculated that the different PGT products may have similar volatile profiles and characteristic aromas. The means of molecular sensory science can systemically elucidate the characteristic aroma of foods, which is a comprehensive approach including gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O) (Amanpour et al., 2015), aroma extract dilution analysis (AEDA) (Kang and Baek 2014), and odour activity values (OAV) etc., and has been successfully applied in aroma studies of tea and other foods (An et al., 2019; Xu et al., 2021b). Notably, some advanced extraction techniques have been gradually applied in the analysis of tea volatiles. Stir bar sorptive extraction (SBSE) as an efficient pre-treatment method has been successfully applied in the tea volatiles extraction where recovery is high because of the larger phase volume on the stir bar (Ochiai et al., 2018; Wang et al., 2020).

Therefore, the aim of this study was to clarify the aroma profile and characteristic aroma of Chinese PGT using molecular sensory science approaches, including SBSE-GC-MS, GC-O, and OAV, and to reveal the differences in volatile profiles among the four types of green teas. The results obtained from this study will provide scientific theory for the scientific assessment of aroma quality and improvement of the processing technology of green tea products.

2. Materials and methods

2.1. Tea samples

Twenty-two representative Chinese pan-fried green tea samples (PGT1-PGT22) with a high-quality grade (special grade and first grade), including Biluochun tea, Longjing tea, Xinyang Maojian tea, Gougunao tea, and Yuhua tea, were collected and selected from the local tea markets. In addition, three baked green tea samples (BGT1-BGT3), five steamed green tea samples (SGT1-SGT5), and five sun-dried green tea samples (SDGT1-SDGT5) were selected to compare the volatiles with PGT samples, which were representative samples that could represent the aroma quality characteristics of their own types. Detailed information is provided in Table S1.

2.2. Chemicals

n-Alkanes (C8–C40) was purchased from J&K Scientific Ltd. (Beijing, China). Anhydrous sodium chloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). Purified water was purchased from Wahaha Group Co., Ltd. (Hangzhou, China). Authentic aroma standards were purchased from J&K Scientific Co., Ltd., Macklin Biochemical Co., Ltd. (Shanghai, China), and Accela ChemBio Co., Ltd. (Shanghai, China), including hexanol (≥99%), 2,6-dimethyl-5-heptenal (97%), β-damascenone (mixture of isomers), nerol (97%), linalool oxide (pyranoid) (mixture of isomers), β-cyclocitral (98%), indole (99%), coumarin (99%), δ-cadinene (95%), trans-β-ocimene (98%), hexanal (97%), heptanal (98%), linalool (98%), geraniol (98%), α-ionone (≥90%), trans-β-ionone (98%), 3,5-octadien-2-one (mixture of isomers), methyl jasmonate (95%), dodecanal (95%), 3-nonanone (≥97%), and decanoic acid ethyl ester (≥99%).

2.3. SBSE procedure

In detail, 600 mg of each ground sample together with 500 mg of NaCl were weighed and placed in a 20 mL headspace bottle. Then, 10 mL of boiling water was used for brewing. After equilibrating for 1 min, a polydimethylsiloxane (PDMS) twister (Gerstel, Germany; 10 mm length, 1.0 mm thickness, 24 μL capacity) was immersed to extract volatile components, which lasted for 30 min at 80 °C and 1200 rpm on a stirrer (SP200-2 T; Miu Instruments Co. LTD, Hangzhou, China; multiposition hotplate stirrer). Twisters were rinsed with purified water, wiped by nonwovens after extraction, and then transferred to a vial waiting for thermal desorption tubes for subsequent GC-MS analysis. All samples were analysed in triplicate.

2.4. Thermal desorption

Thermal desorption of the twisters was performed for subsequent GC-MS analysis. The parameters of the thermal desorption unit (TDU) were as follows: the initial temperature of 30 °C was held for 1 min, then increased by 100 °C/min to 240 °C and held for 5 min. The cooling injection system (Gerstel CIS-4 PTV injector) was maintained at −100 °C for 1 min with liquid nitrogen (99.99%), and then ramped to 280 °C and held for 3 min at a rate of 12 °C/s.

2.5. GC-MS analysis

GC-MS analysis was performed using an Agilent 7890 B GC system coupled with an Agilent 5977 B MSD mass spectrometer (Agilent, Santa Clara, CA, USA). An Agilent HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used to isolate volatile compounds. The oven temperature ramp was as follows: the initial temperature was held for 2 min at 50 °C, increased to 170 °C (held for 5 min) at a rate of 4 °C/min, then ramped to 265 °C held for 5 min at 10 °C/min. High-purity helium (>99.99%) was used as a carrier, and the flow rate was set to 1.6 mL/min in solvent vent mode. The mass spectrometer parameters were as follows: electron ionisation mode, EI. The ion-source temperature was

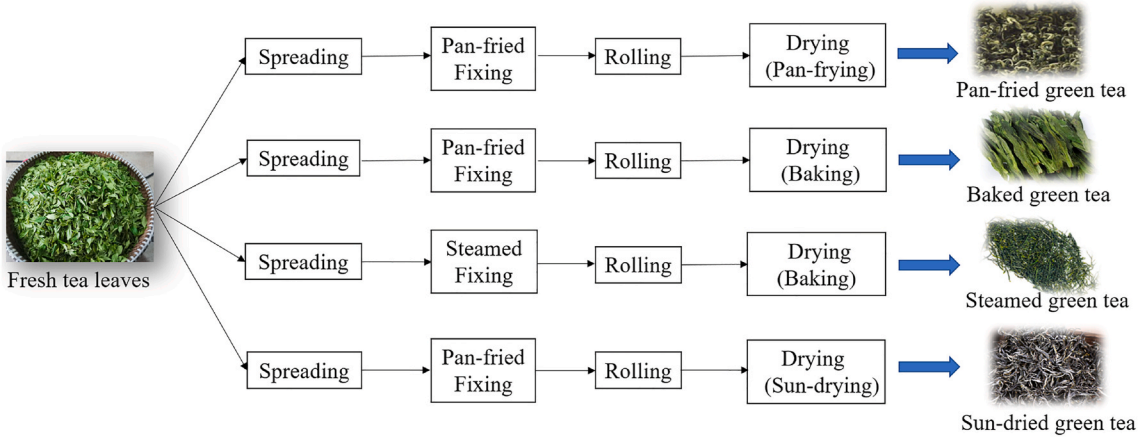

Fig. 1. Flow diagram of manufacture processing of four types of green teas. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

220 °C. The electronic energy was 70 eV. The interface temperature was set at 280 °C. The mass scan range was 30–600 AMU.

2.6. Identification and quantification of the volatile compounds in PGT

The identification of compounds was based on the database library NIST 2014. For identification of compounds, the retention index (RI) values of the compounds obtained from the experiment were compared with the theoretical values of the *n*-alkane (C8–C40) series. In general, the compounds can be confirmed if the difference between the two values is less than 20.

Quantitative analysis of all aroma compounds was mainly based on their abundance in the GC-MS analysis. The relative content of a compound was expressed as the ratio of its abundance to the total abundance. The key aroma-active compounds of PGT identified by GC-O were further accurately quantitated using authentic standard compounds. Quantitative experiments were conducted based on a tea blank matrix to reduce the influence of the matrix. The blank matrix preparation steps were as follows: 40 g of tea was brewed with boiling water until there was no obvious aroma. The brewed tea leaves were transferred to a round-bottomed flask for rotation and rotated until the tea sample had no related compounds detected by GC-MS. In this study, 10 μL of each concentration standard solution was added to the blank matrix. Finally, standard curves based on the peak area of each concentration were established to calculate the concentration of the compound to be quantified.

2.7. GC-O-MS analysis

In this study, PGT1-PGT22 was mixed in equal proportions to make a standard mixed sample for GC-O analysis. The GC procedure in GC-O analysis was consistent with the GC-MS analysis. The GC-O-MS system comprises an olfactory detection port (ODP2; Gerstel GmbH & Co. KG, Germany), and an MS system. Volatile compounds were transferred to two systems with a 1:1 split ratio after separation by column. High-purity nitrogen (99.99%) was used as the carrier gas in the sniffing process. The temperatures of the ODP injector and transfer line were set at 230 °C and 260 °C, respectively.

The six panellists (three men and three women, aged from 25 to 40 years) of GC-O analysis belonged to the Tea Research Institute, Chinese Academy of Agricultural Sciences. They had worked in the field of tea science for at least six years, and all had more than four years of professional sensory experience. Before the formal experiment, each assessor was well-trained in the odour characteristics of the chemical standards for up to 90 h in order to get familiar with the different odour characteristics and odour intensities by using a series of standard solutions with different concentrations (Wang et al., 2020). In the subsequent GC-O experiment, assessors evaluated the odour characteristics and aroma intensities (AIs) of the volatile compounds at the sniffing port. The AIs were recorded as 1–4 from weak to strong. At the same time, the retention time of corresponding compounds was recorded by the computer. The odourants sniffed by at least three assessors with similar aroma descriptions at the same retention time were selected, and further identified by retention indices and aroma standards. The AIs of key aroma-active compounds were the average from the assessors.

2.8. OAV calculation

The OAV was calculated to characterise some key aroma-active compounds. The OAV was calculated according to equation (1).

$$OAV(i) = C(i)/OT(i) \quad (1)$$

where OAV(i) is the OAV of compound(i), C(i) is the content of compound(i) calculated by standard compounds, and OT(i) is the odour threshold of compound (i).

2.9. Statistical analysis

The data were expressed as the mean of all samples in this type of green tea after calculating the average of the three biological replicates of each sample. The total ion chromatogram (TIC) was performed using Origin 2018 software (OriginLab Corporation, Northampton, MA, USA). Histograms were obtained using GraphPad Prism 8 (GraphPad Software Corporation, La Jolla, CA, USA). Venn was obtained from Jvenn (http://jvenn.toulouse.inra.fr/app/example.html). Partial least squares discriminant analysis (PLS-DA) was performed using SIMCA 14.1 (Umetrics Corporation, Umeå, Sweden). Heatmap analysis was performed by statistical multivariate analyses on MetaboAnalyst5.0 (https://www.metaboanalyst.ca).

3. Results and discussion

3.1. Characterisation and comparison of volatile compounds in PGT by SBSE-GC-MS

3.1.1. Identification of volatile compounds in PGT

In the present study, 88 volatile compounds were identified in 22 PGT samples by SBSE-GC-MS (Table S2). These compounds were grouped into 10 categories according to their chemical structural characteristics: esters (20), alcohols (13), ketones (12), alkanes (11), aldehydes (10), alkenes (8), aromatics (6), oxygen heterocyclics (5), acids (2), and nitrogen heterocyclic compounds (1) (Fig. 2A). In contrast, the predominant volatiles in the PGT samples were alcohols (45.08%), followed by esters and ketones accounting for 19.20% and 16.43% of all volatiles, respectively. Furthermore, aromatic compounds (1.86%), alkenes (1.81%), and nitrogen heterocyclics (0.61%) were present at lower levels.

In addition, it was found that a total of 51 compounds out of 88 compounds jointly existed in all PGT samples. As shown in Fig. 2B, among these common volatiles, alcohols (46.50%), esters (21.71%), and ketones (15.54%) were also present in high proportions, whereas aromatic compounds (1.98%), alkenes (1.44%), and acids (0.98%) were minor volatile components. It is noteworthy that the proportion of various categories in 51 common compounds was similar to that of all volatiles, suggesting that alcohols, esters, and ketones might be the primary contributors to the PGT aroma quality.

Alcohols compounds showed the highest amounts in PGT samples, in accordance with a previous report by Wang et al. (2020). As shown in Table S2, geraniol was the most abundant compound with a relative content of 23.78%, followed by linalool (5.22%), phytol (4.85%), and phenylethyl alcohol (3.84%). Geraniol is frequently found in green tea and is present in Longjing tea at a high level (Wang et al., 2020). Second, linalool is a requisite volatile component in the formation of various tea aromas, especially in high-quality Chinese green tea (Flaig et al., 2020). Geraniol and linalool are the typical enol aroma compounds in tea, both of which showed a large decline with shoot maturity, corresponding with the samples in our study being premium tea made from tender fresh leaves (Xu et al., 2021a). Moreover, phytol was found at high relative concentrations, which is in agreement with the previous examination of Xihu Longjing tea (Zhu et al., 2016). Phenylethyl alcohol was the major aromatic alcohol odourant in all the extracts, and exogenous β-glucosidase treatment was an effective pathway to increase its content (Ni et al., 2021). The other compounds with high relative contents in the alcohol group were (*E*)-nerolidol (2.37%) and 3,7-dimethyl-1,5,7-octatrien-3-ol (2.31%). It is noteworthy that pan-frying affects the composition ratio of volatiles, especially the content of enols (e.g., geraniol, linalool, and (*E*)-nerolidol) and aromatic alcohols (e.g., phenylethyl alcohol) are markedly increased. (Lin et al., 2020). Some alcohol compounds with a higher relative content in our samples might be partly ascribed to this pathway.

Ester compounds usually exhibit a sweet, floral, and fruity aroma. In this study, cis-3-hexenyl hexanoate (3.45%), methyl salicylate (3.18%),

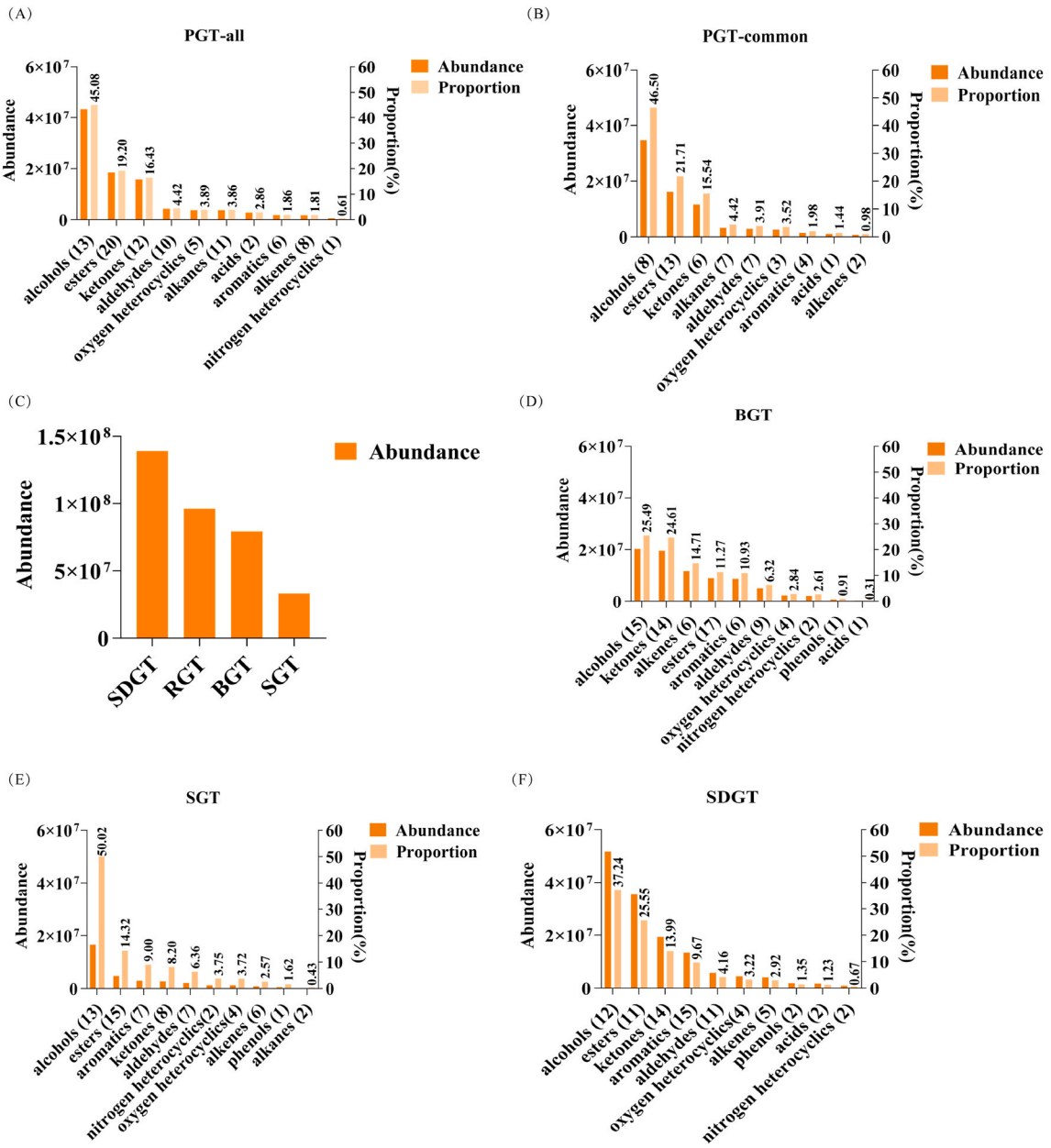

Fig. 2. Classification and proportion of volatile components. **(A)** All volatiles in pan-fried green tea (PGT). **(B)** Common volatiles in roasted green tea. **(C)** Comparation of four green tea types. **(D)** Volatiles in baked green tea (BGT). **(E)** Volatiles in steamed green tea (SGT). **(F)** Volatiles in sun-dried green tea (SDGT). . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and dihydroactinidiolide (2.30%) were the dominant esters. Of which methyl salicylate with fresh and minty odour is a glycosidically bound non-alcoholic volatile compound, and has been found in various teas (Ho et al., 2015). Furthermore, ketones were also recognised as one of the most abundant components of Biluochun tea (Wang et al., 2016), which is consistent with our findings. In this trial, the ketone odourants with high relative contents were trans-β-ionone (5.78%) and cis-jasmone (4.39%). Among these, trans-β-ionone, which is the most abundant ketone compound, is the aromatic precursor of dihydroactinidiolide in green tea (Ho et al., 2015). As reported above, the current research on the volatiles of PGT has focused more on Longjing tea. We found that cis-jasmone was also detected in Xihu Longjing tea at high relative concentrations (Zhu et al., 2016).

In this study, pyrazine, as one of Maillard reaction products, was not detected. The main reason for this maybe lies in the fact that tea polyphenols rich in green tea are characterized as powerful carbonyl compound scavengers to inhibit an advanced Maillard reaction pathway (Song et al., 2002), so these compounds are usually present at trace levels (from pg/mL to ng/mL) (Ochiai et al., 2013). The detection sensitivity of gas chromatography-triple quadrupole tandem mass spectrometry used in a previous study was much higher than that of GC-MS used in this study. On the other hand, the HS-SPME applied in the previous study might be more sensitive to pyrazines (Chen et al., 2021). It was worth noting that the advanced SBSE technique was gradually applied in extraction of pyrazines compounds, such as fractionated (Fr-) SBSE (a conventional multi-SBSE extraction, followed by a solvent assisted SBSE extraction) (Ochiai et al., 2020). Therefore, the detection of pyrazine by enhanced SBSE method deserves our further investigation.

3.1.2. Comparison of volatile profiles between PGT and the other three types of green tea

As shown in Fig. 2C, the total abundance of volatiles in SDGT was the highest, while that of SGT was the lowest. Furthermore, the volatile compositions of the four types of green tea varied (Fig. 2D–F & Table S3). Among these, BGT refers to a type of green tea that was dried by baking, and the primary compound categories were alcohols (25.49%), ketones (24.61%), alkenes (14.71%), esters (11.27%), and aromatic compounds (10.93%). The characteristic processing technology of SGT is the steam fixing, in which the endogenous enzyme activity is inactivated by steam, and its dominant volatiles were alcohols (50.02%), esters (14.32%), aromatic compounds (9.00%) and ketones (8.20%). It was observed that the relative content of alcohols in PGT and SGT was higher, nearly one-half of the total volatile components. This is in good agreement with a previous study (Sun et al., 2014). SDGT is a type of green tea that utilised sunlight to drop water content to less than 10%, and alcohols (37.24%), esters (25.55%), and ketones (13.99%) were the major categories. These tests revealed that some differences in content and composition were present among the different types of green tea products. Notably, alcohols, ketones, and esters were the common categories in the four types of green tea, but more apparent differences existed in proportions.

Moreover, to further understand the volatile characteristics of PGT, 32 common volatiles in four types of green tea were selected using a Venn diagram (Fig. S1) to perform PLS-DA. As shown in Fig. 3A, all samples could be divided into four sections by PLS-DA, although there was a slight crossover between BGT and PGT. The factor ($R^2Y = 0.782$, $Q^2(cum) = 0.574$) of PLS-DA indicated that the model had favourable description and predictability of the data, and $R^2 = 0.307$, $Q^2 = -0.475$ ($n = 200$) of the validated model proved that the PLS-DA model was not overfitting (Fig. 3B). Meanwhile, the differences in the common volatile compounds in the four types of green teas were also observed by heatmap analysis (Fig. 3C). By contrast, PGT contained a higher abundance of β-myrcene, methyl salicylate, (*E*)-nerolidol, geraniol, and methyl jasmonate. This further proves that pan-frying could increase the content of enols in tea. In addition, eight volatiles were present in larger amounts in BGT, including trans-linalool oxide (furanoid), α-ionone, hexanal, 9,12-octadecadienoic acid, methyl ester, γ-terpinene, β-cyclocitral, 6,10-dimethyl-5,9-undecadien-2-one, and trans-β-ionone. While octanal, 1,3-dimethylbenzene, phytol, safranal, 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate, nonanal, trans-linalool oxide (pyranoid), cis-jasmone, decanal, and linolenic acid, and methyl ester were detected in higher amounts in SGT. The remaining compounds in 32 common compounds were dominant in SDGT when compared with PGT, BGT, and SGT.

3.2. Qualitative and quantitative analysis of key aroma-active compounds in PGT

3.2.1. Key aroma-active compounds in PGT identified by GC-O analysis

GC-O is a powerful tool for discriminating against the significance of one component in the volatile extracts, which combines the human olfactometer with the GC-MS technique (Song and Liu, 2018). It is a technology that has important applications in molecular sensory science. Generally, the odourants identified by GC-O could be considered as key aroma-active compounds. Tea aroma is generally composed of diverse volatile compounds at various concentrations. However, only the key aroma-active compounds direct sensory perception and contribute to the aroma. Therefore, the key aroma-active compounds play the crucial roles in formation of tea aroma quality. As listed in Table 1, a total of 23 key aroma-active compounds with the AIs ranged from 2.00 to 3.20 were detected in the PGT samples. trans-β-ionone

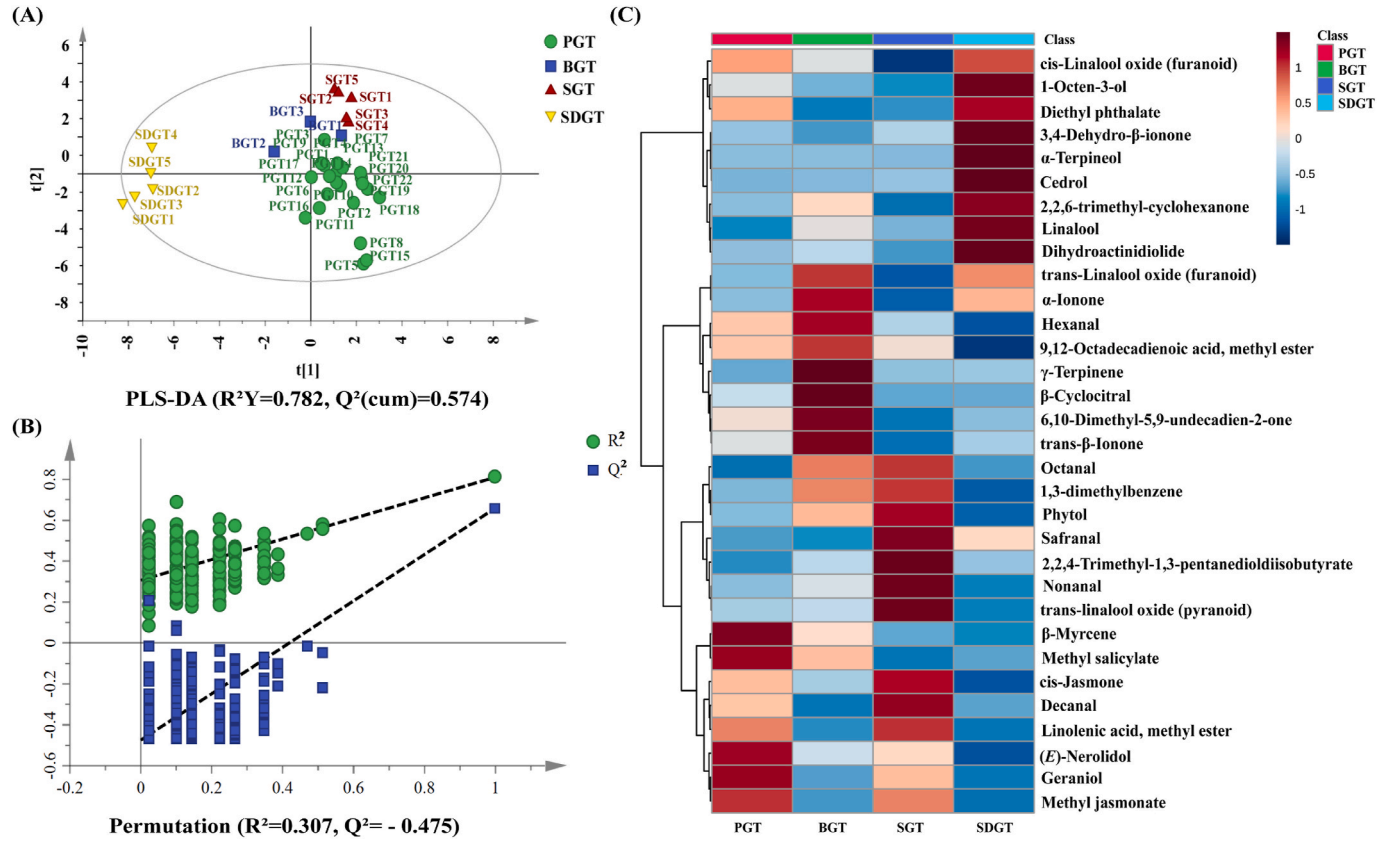

Fig. 3. (A) Score plot of partial least squares discriminant analysis (PLS-DA). **(B)** Validation of PLS-DA model with 200 permutation tests. **(C)** Heatmap of 32 common volatile compounds in four green teas. (Notes: PGT, pan-fried green tea; BGT, baked green tea; SGT, steamed green tea; SDGT, sun-dried green tea). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Key aroma-active compounds of pan-fried green tea by GC-O analysis and OAV values.

No.	Compounds	CAS	Odour descriptions [a]	Odour types [b]	AIs [c]	Content (μg/kg)	OT (μg/kg) [d]	OAV	Identification methods [e]
1	trans-β-Ionone	79-77-6	floral fruity violet coconut	Floral	3.20	109.7 ± 11.1	0.007	15671	MS, RI, O, Std
2	α-Ionone	127-41-3	sweet floral fruity creamy	Floral	3.00	0.9 ± 0.3	0.4	2	MS, RI, O, Std
3	Linalool	78-70-6	floral sweet fresh	Floral	2.83	150.3 ± 6.6	1.5	100	MS, RI, O, Std
4	Geraniol	106-24-1	citrus fruity floral rose woody	Floral	2.80	484.8 ± 32.2	7.5	65	MS, RI, O, Std
5	Nerol	106-25-2	sweet fruity floral	Floral	2.67	18.2 ± 0.5	300	< 1	MS, RI, O, Std
6	3-Nonanone	925-78-0	caramel jasmine	Floral	2.67	7.4 ± 0.2	30	< 1	MS, RI, O, Std
7	Indole	120-72-9	floral	Floral	2.50	148.0 ± 15.7	140	1	MS, RI, O, Std
8	Methyl jasmonate	1211-29-6	sweet floral	Floral	2.00	199.0 ± 9.6	70	3	MS, RI, O, Std
9	α-Cadinol	481-34-5	spicy bitter sour floral celery	Herbal woody	3.00	413.0 ± 19.3	–	–	MS, RI, O
10	δ-Cadinene	483-76-1	fresh herbal sweet floral	Herbal woody	2.83	68.1 ± 9.3	1.5	45	MS, RI, O, Std
11	1-Hexanol	111-27-3	fruity sweet nutty	Herbal woody	2.60	34.8 ± 3.7	500	< 1	MS, RI, O, Std
12	trans-Linalool oxide (pyranoid)	39028-58-5	fresh caramel lemon	Herbal woody	2.40	427.7 ± 22.2	300	1	MS, RI, O, Std
13	trans-β-Ocimene	3779-61-1	sweet floral	Herbal woody	2.20	2.8 ± 0.3	0.0187	147	MS, RI, O, Std
14	β-Damascenone	23726-93-4	sweet floral fruity sugar	Fruity	2.80	15.7 ± 0.1	0.0014	11236	MS, RI, O, Std
15	α-Cyclocitral	432-24-6	citrus fresh alcoholic floral	Fruity	2.50	147.5 ± 10.9	5	30	MS, RI, O
16	3,5-Octadien-2-one	38284-27-4	fresh earthy cucumber	Fruity	2.00	55.6 ± 4.1	300	< 1	MS, RI, O, Std
17	Heptanal	111-71-7	herbal fatty pungent	Green	2.67	19.2 ± 2.5	3	6	MS, RI, O, Std
18	Hexanal	66-25-1	fresh green woody bamboo	Green	2.50	19.4 ± 3.4	4.5	4	MS, RI, O, Std
19	5-Ethyl-6-methyl-3(*E*)-hepten-2-one	57283-79-1	green herbal sweet floral	Green	2.50	18.1 ± 0.8	–	–	MS, RI, O
20	2,6-Dimethyl-5-heptenal	106-72-9	sweet floral fruity	Sweet	3.00	11.1 ± 0.2	3530	< 1	MS, RI, O, Std
21	Coumarin	91-64-5	floral coconut	Sweet	3.20	247.5 ± 29.6	50	5	MS, RI, O, Std
22	1-Octen-3-one	4312-99-6	pungent green rusty	Earthy	3.00	7.5 ± 0.4	0.005	1498	MS, RI, O
23	Dodecanal	112-54-9	soapy green	Aldehydic	3.00	13.7 ± 0.7	1.07	13	MS, RI, O, Std

Note: a, Description of the smell sniffed by GC-O analysis.
[b] odour types were obtained from website (http://www.thegoodscentscompany.com/search2.html).
[c] Aroma intensities.
[d] Odour threshold, reference from (Feng et al., 2021; Van Gemert, 2013; Yang et al., 2021).
[e] MS, identification based on the NIST 2014 mass spectral database; RI, retention index; O, olfactometry; Std, the compounds were identified using authentic standard compounds.

(3.20) and coumarin (3.20) possessed the highest AIs, followed by 1-octen-3-one (3.00), dodecanal (3.00), α-ionone (3.00), 2,6-dimethyl-5-heptenal (3.00), and α-cadinol (3.00). trans-β-ionone exhibits a floral and coconut-like odour and is a key aroma-active compound in chestnut-like aroma green teas (Zhu et al., 2018). Meanwhile, trans--β-ionone is a link between aroma and taste. It is a crucial aroma component that can influence the taste perception of black tea infusions by enhancing sweetness (Yu et al., 2021). Coumarin is associated with sweet and flower-like aroma, and drying temperature influences its content in tea (Yang et al., 2009). Among these, α-cadinol had a higher content of about 413.0 μg/kg, which imparted floral and fresh odour to green tea.

Additionally, δ-cadinene (2.83), linalool (2.83), geraniol (2.80), and β-damascenone (2.80) also had relatively higher AI values. Although linalool mainly presented floral notes, enantiomers (*R*-(−)-linalool and *S*-(+)-linalool) showed slightly different scents, and their configuration was greatly affected by the processing technology (Zhu et al., 2021a). Moreover, Table 1 showed that the aroma characteristics of key aroma-active compounds in PGT were first floral, followed by fruity, green, herbal, and woody, and minor in sweet and other notes.

3.2.2. *Key aroma-active compounds identified by OAV calculation*

The OAV has been frequently utilised to evaluate the contribution of an odourant to the overall tea aroma (Wang et al., 2019). The volatiles with OAV ≥1 were generally designated as the key aroma-active compounds, and considered to markedly contribute to the aroma characteristics. The compounds with higher OAV were more important for the formation of aroma quality. To further verify the key aroma-active compounds identified by GC-O, we calculated the OAVs of these compounds. As listed in Table 1, 16 odourants with OAVs greater than 1 were identified. First, trans-β-ionone (OAV = 15671), β-damascenone (OAV = 11236), 1-octen-3-one (OAV = 1498), trans--β-ocimene (OAV = 147), and linalool (OAV = 100) were the volatiles with OAVs greater than 100. trans-β-ionone is essential for the formation of tea aroma because of its extremely low threshold (0.007 μg/kg) (Sun and Chen, 2017) (Van Gemert, 2013). It was worth noting that trans--β-ionone had both the highest OAV and AI, indicating that it was positively associated with PGT aroma quality. β-Damascenone, which has a low odour threshold of 0.0014 μg/kg, has a fruity odour and is believed to enhance the fruity aroma of tea (Zhu et al., 2015). The contents of β-damascenone, 1-octen-3-one, and trans-β-ocimene were lower than those of the majority of key aroma-active compounds. Nonetheless, these compounds still possessed higher OAVs owing to their low threshold. Some compounds had both high aroma intensities and high OAVs, indicating that a positive relationship was present in OAV calculations and GC-O analysis.

Besides, geraniol (OAV = 65), δ-cadinene (OAV = 45), α-cyclocitral (OAV = 30), and dodecanal (OAV = 13) had OAVs ranging from 10 to 100. Except for two odourants without odour thresholds recorded, there were five odourants with OAV<1 but could be identified by GC-O analysis (Yang et al., 2021). This was probably related to the different thresholds of these odourants in water and air (Zhu et al., 2015). In total,

there was acceptable agreement between the GC-O analysis and OAV calculations, indicating that the results of the GC-O analysis were reliable.

3.2.3. Comparison of key aroma-active compounds between PGT and other types of green tea

In the other three types of green tea, 27, 30, and 27 compounds were identified as the key aroma-active compounds in BGT, SGT, and SDGT, respectively (Table S4). As shown in the Venn diagram (Fig. 4), a total of 10 aroma-active compounds (shown in the black frame) were common components in PGT, BGT, SGT, and SDGT. Among these, linalool, 1-octen-3-one, and trans-β-ionone were present at higher AIs in the four types of green tea.

In addition to the common compounds, PGT contained two additional key aroma-active compounds that were not detected in the other types of green tea, namely 1-hexanol and α-cyclocitral. Among the identified key aroma-active compounds, cis-linalool oxide (furanoid), nonanal, (*Z*)-3-hexen-1-ol, acetate, methyl geranate, 2-undecanone, and 3-octen-2-one were found in BGT, but not in PGT, SGT, and SDGT. These were mainly responsible for the fresh, sweet, and caramel-like aroma of the BGT samples. cis-jasmone had the highest AI in the BGT. The compound with the highest AI in SGT was trans-β-ionone (3.67), which was similar to that of PGT. Some compounds were unique key aroma-active compounds of SGT, including (*Z*)-3-hexenyl isovalerate, theaspirane, biphenyl, jasmine lactone, β-cubebene, limonene, and cis-3-hexenyl butyratecis-3-hexenyl butyrate. Moreover, 1-octen-3-one (3.50) had the highest AI in SDGT, and several compounds were only sniffed in SDGT, including (*E*)-nerolidol, decanal, and (*E*)-3,7-dimethyl-2,6-octadienal, etc.

3.3. The possible formation pathways of key aroma-active compounds in PGT

According to diverse metabolic pathways of volatile compounds, they can be divided into fatty acid derivatives, volatile terpenes and volatile phenylpropanoids/benzenoids, carotenoid derivatives, glycosidically-bound volatiles (Yang et al., 2013). In addition, Maillard reaction is an important pathway to development of aroma in tea processing. The manufacturing process of PGT production generally consists of four stages: spreading, fixation, rolling, and drying. PGT is dried by pan-frying which significantly distinguishes it from others, and pan-frying as the characteristic manufacturing process impacts the chemical profile and aroma of pan-fried green tea (Zhu et al., 2021b). The special flavour quality of the PGT is mainly formed in this process, during which moisture in tea leaves is volatilised, and aroma precursors are efficiently transformed (Lin et al., 2020). In addition, fixing is another vital manufacturing process for the PGT. The enzymatic activity still exists before the leaf temperature rises to the denaturation temperature in the preliminary stage of fixing. Enzymatic action and oxidative cleavage were accelerated to a certain degree, accompanied by protein hydrolysis into amino acids and starch hydrolysis into sugar (Wan, 2003). These reactions will provide precursor substances for the

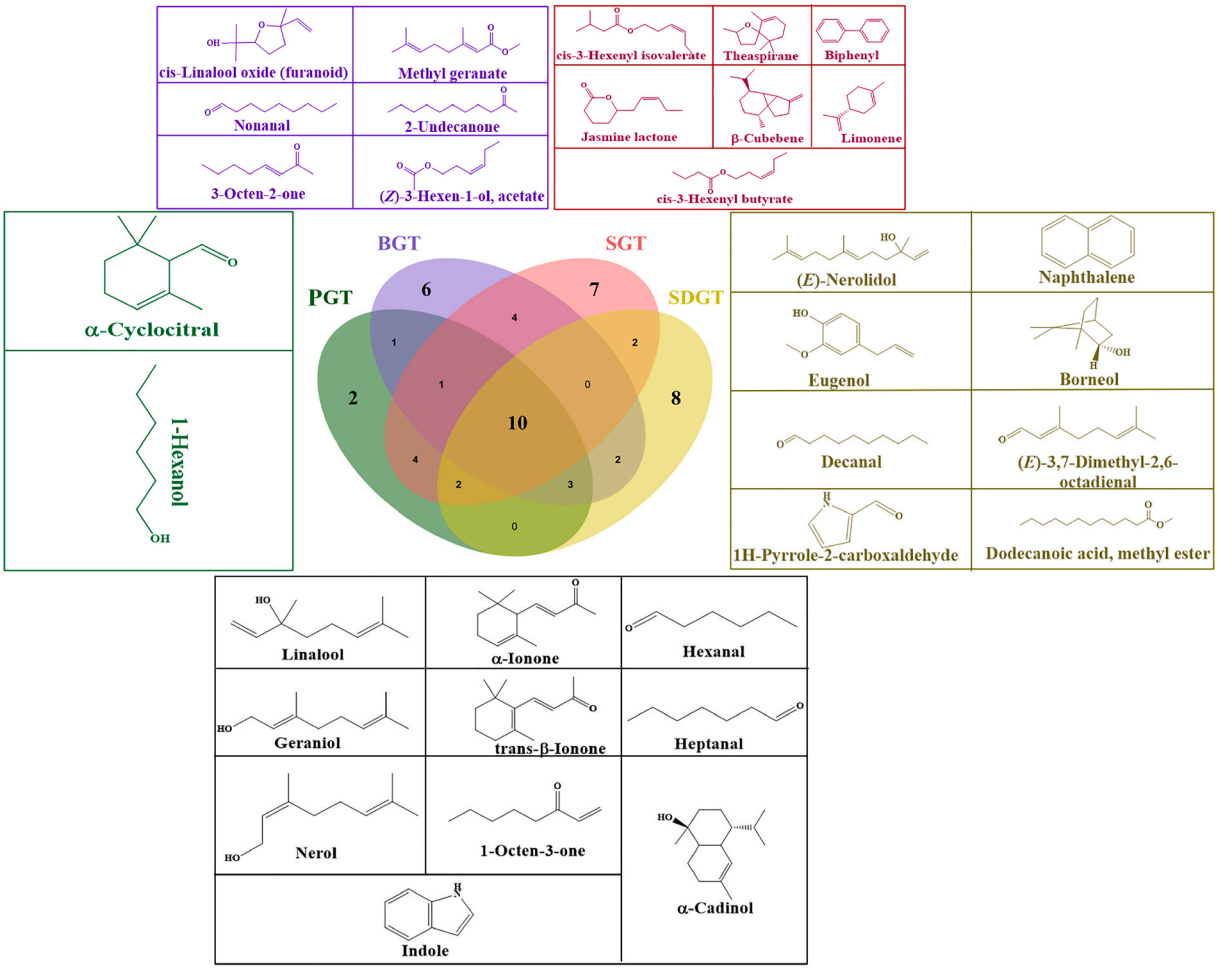

Fig. 4. Venn diagrams of the key aroma-active compounds of four types of green tea products along with structural formulas of common and unique compounds. (Notes: PGT, pan-fried green tea; BGT, baked green tea; SGT, steamed green tea; SDGT, sun-dried green tea). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

formation of aromatic compounds. As a result, the aroma quality of PGT was obviously different from that of the other three types.

Previous researches have shown that heptanal originates from the thermal degradation of oleic acid and palmitoleic acid, while hexanal originated from linoleic acid (Ho et al., 2015; Yang et al., 2013) (Fig. 5). Previous studies on aroma dynamic characteristics in drying processing technology have indicated that heptanal plays an indispensable role in distinguishing between the three fragrance types caused by different drying times (Yang et al., 2021). In addition, because the enzymatic action accelerated during the preliminary stage of fixing, a certain amount of hexanal, heptanal, and methyl jasmonate may be derived from the oxidation of fatty acids.

Volatile terpenoids are important for enhancing the fragrance of foods, such as linalool and geraniol, an important class of monoterpenoid compounds. These compounds have the same precursor, namely geranyl pyrophosphate (GPP) (Yang et al., 2013). It is noteworthy that cyclisation, dehydration, and isomerisation of terpene compounds often appear due to thermal reactions in the pan-frying stage, and geraniol and linalool might be transformed mutually under these conditions. In addition, nerol, as the isomer of geraniol, may be mutually transformed. Furthermore, glycosides play an important role in the aroma of green tea, which is closely related to the formation of volatile terpenoids. Some researchers considered that linalool oxides were not derived from the oxidisation of linalool instead of their glycoside forms (Nishikitani et al., 1999; Wang et al., 2000), but some studies also suggest that linalool can be converted to the corresponding oxides (Zeng et al., 2021). Considering the effect of high temperature in the fixing stage of PGT on enzyme activity, glycoside hydrolysis might be a vital pathway of linalool oxides. As shown in Fig. 5, glycosidic precursors of volatiles in PGT are mainly present as "β" configuration, confirming that different glycosides might have a synergistic effect when they are converted into aromatic compounds (Zhang et al., 2020).

Carotenoids are the important precursors of some ketones. Non-enzymatic degradation of carotenoids is possibly a pivotal pathway in the formation of green tea aroma. α-Carotene is a key component related to the aroma of tea and is a vital precursor of α-ionone and β-ionone. β-Ionone can also be transformed from β-carotene. As mentioned earlier, an increase in endogenous carotene content would enhance the volatile compound index to nearly twice the original value (Ravichandran, 2002). In addition, there may be two pathways to generate β-damascenone in green tea. One is the oxidation of neoxanthin, a carotenoids component (Yang et al., 2013). Another is the degradation pathway of glycosides due to the high temperature during the process of drying and fixing (Kinoshita et al., 2010). Further research is required to verify the role of these compounds in the overall aroma of PGT. In order to understand the formation mechanisms of PGT volatiles in depth, some possible formation pathways of the key aroma-active compounds based on previous research were summarised and further proposed. Furthermore, Maillard reaction is an important source of food flavour, and it often occur in pan-frying process of PGT. Indole, as a key aroma-active compound in PGT, can be derived via Amadori products about L-tryptophan under the pyrolysis conditions (Ho et al., 2015).

4. Conclusions

Herein, the volatile properties of Chinese pan-fried green teas were comprehensively analysed for the first time using molecular sensory science approaches. We identified 88 volatile compounds from 22 representative PGT samples and identified 16 potent odourants in pan-fried green tea. Moreover, differences in odourants among pan-fried

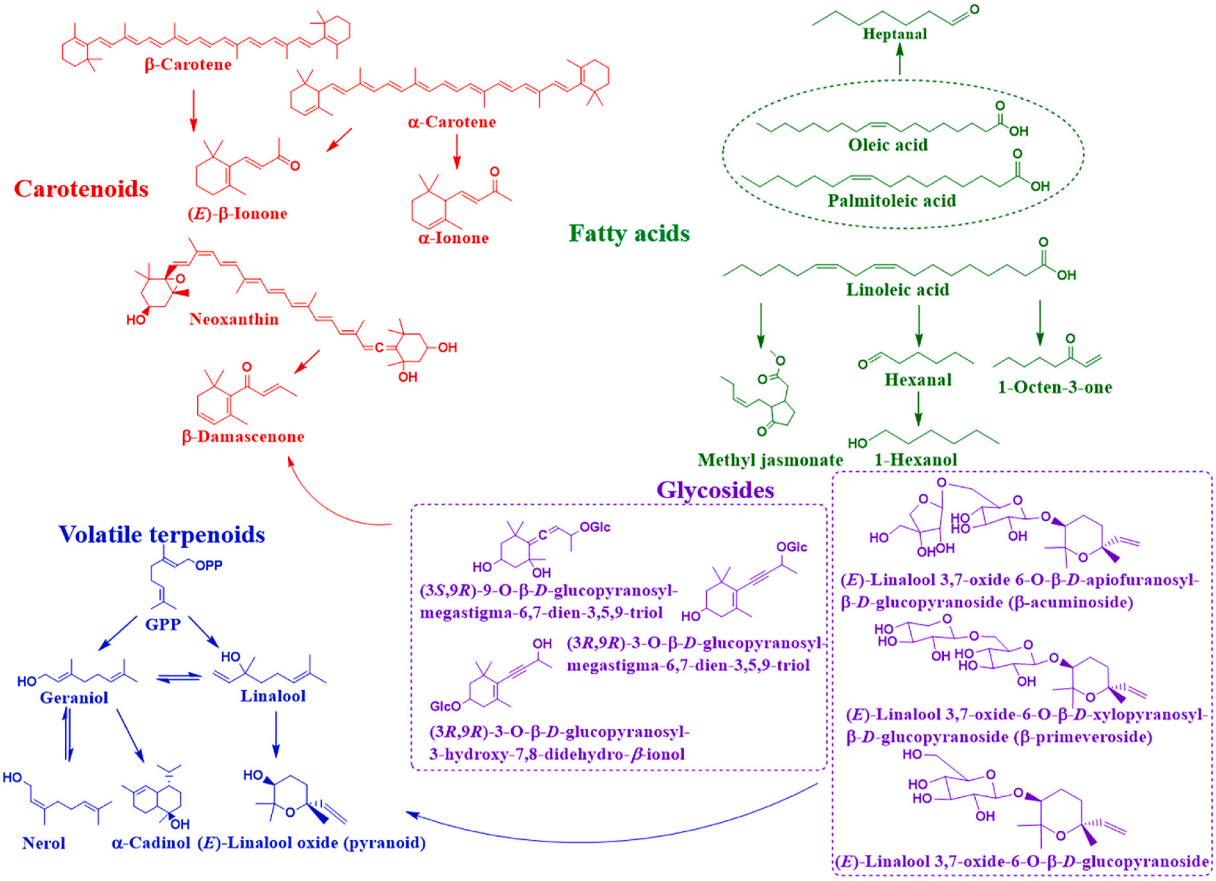

Fig. 5. Possible formation pathways of some key aroma-active compounds in pan-fried green tea. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

green teas, steamed green teas, baked green teas, and sun-dried green teas were also revealed. The results clearly indicate that alcohols, esters, and ketones are the dominant volatiles in PGT. Geraniol is the most abundant volatile component, and trans-β-ionone with floral notes has both the highest aroma intensity value and OAV. In addition, differentiation of the four kinds of green tea was clearly evident from the characteristic aroma. PGT could be distinguished from the other three types of green tea via PLS-DA, and the content levels of common volatiles in all four kinds of green tea could be clearly reflected by heatmap analysis. The findings from this study may provide novel information on the aroma chemistry and quality control of PGT, and further studies on the reciprocal effects of these key aroma-active compounds and the objective evaluation technology of PGT aroma quality are necessary and urgently warranted.

Ethical statement

The content of the sensory evaluation experiment in this study has been informed consent of the candidates and has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study was approved by the Academic Committee of Tea Research Institute, Chinese Academy of Agricultural Sciences.

CRediT authorship contribution statement

Yali Shi: Investigation, Methodology, Software, Writing – original draft. **Yin Zhu:** Investigation, Methodology, Software. **Wanjun Ma:** Methodology, Software. **Zhi Lin:** Project administration, Writing – review & editing. **Haipeng Lv:** Investigation, Methodology, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.06.012.

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