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The application of untargeted metabolomics coupled with chemometrics for the analysis of agitation effects on the sensory profiles of matcha tea

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ABSTRACT

In the study, the effects of agitating parameters (different agitating rates and time) on the aroma and taste profiles of matcha tea were systematically investigated by the combination of untargeted metabolomics and chemometrics. The aroma profiles of matcha tea agitated at low rates (500 rpm) and for 30 s were more richness than that agitated with other parameters by sensory analysis and gas chromatography-ion mobility spectrometry. The key aroma compounds contributed to the sensory differences of matcha tea agitated at different rates and time were analyzed by gas chromatography-mass spectrometry and partial least square-discriminate analysis (PLS-DA), which were further verified by the triangle test. Thereinto, 2,4-decadienal associated with the sweet, brown and seaweed aroma significantly affected the aroma profiles of matcha tea with different agitating rates and time. The levels of bitterness and astringency were also higher in matcha tea with low agitating rates and time by sensory evaluation, which were attributed to the variations of phenolic compounds. Flavonol glycosides, gallic acid and (−)-gallocatechin were determined the key compound to the taste differences of matcha tea with different agitating parameters by the analysis of PLS-DA based on the results of high performance liquid chromatography and the sensory verification. And flavonol glycosides were mainly contributed to the bitterness and astringency, and gallic acid and (−)-gallocatechin influenced the umami and sweetness of matcha tea. Consequently, agitation has the potential to affect the sensory profiles of matcha tea by changing aroma and taste substances.

1. Introduction

Matcha is a special steamed green tea powder with the particle size less than 1–10 μm and usually used as a food ingredient or beverage due to the distinctive flavor profiles and health benefits (Huang et al., 2022; Ye et al., 2024), where the global matcha market size is expected to culminate in $4.48 billion by 2027 (https://www.alliedmarketresearch.com/matcha-tea-market-A09945). The distinctive sweet and roasted aroma and the high level of umami taste of matcha are completely different from ordinary green tea, which are caused by the unique cultivation and processing conditions (Baba et al., 2017; Huang et al., 2024). Therefore, the strict selection of tea plant species (Baba et al., 2017), sun-shaded time and frequency (Tan et al., 2019), the way of fixation, baking (Shi et al., 2022) and milling (Huang et al., 2022) are required to adjust the ratio of odor-active volatile compounds, polyphenols, amino acids, carbohydrates, organic acids, and alkaloids for improving the sensory profiles of matcha, especially for the matcha consumed as a beverage (Rezaeian and Zimmermann, 2022).

Agitation is a necessary step when matcha is consumed as a tea beverage, where tea powder needs to be mixed with water by agitation to achieve the best flavor. In traditional Japanese ceremony called '*sadō*', agitation is an important process to distinguished matcha tea made by experts and beginners (Goto et al., 2017; Kanazawa et al., 2015a). This is because agitation has the potential to impact the sensory attributes of matcha tea by facilitating the diffusion, dissolution and oxidation of flavor compounds (Ahmed et al., 2019; Das and Eun, 2018; Das et al., 2020; Grenville et al., 2015; Huang et al., 2023; Weterings et al., 2022). Firstly, agitation can change the solubility and transport coefficients of flavor compounds in liquid and gas phases (Ahmed et al., 2019; Das and Eun, 2018; Weterings et al., 2022). Aroma substances are

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gradually transferred from the liquid phase to the gas phase due to the increase of agitating rates and time (Weterings et al., 2022). The diffusion and dissolution rates of taste substances in matcha tea can be enhanced by agitation. Secondly, agitation can increase the amount of dissolved oxygen in matcha tea system. A large amount of air is dissolved in the liquid with the increase of agitating rates and time (Kerdouss et al., 2006), leading to an increase in oxidation degree of flavor substances. Phenolic compounds associated with the bitterness and astringency of matcha tea can be converted into smaller molecular flavor compounds when exposed to oxygen (Lin et al., 2008; Wu et al., 2022).

Overall, agitation has the possibility to be a process to further optimize the sensory profiles of matcha tea. At present, most studies only focused on the influence of agitation on the rheological properties of W/O emulsion or cream paste foods (Gilbert and Turgeon, 2021; Guenard-Lampron et al., 2020; Márquez et al., 2005; Meixner et al., 2023; Renan et al., 2009), but ignored the effect of agitation on the chemical properties of foods. The research on flavor substances affected by agitating was limited to furan and furan derivative in coffee (Rahn et al., 2019), but not all flavor substances and the corresponding sensory changes. And the studies about the agitation of matcha tea only focused on the differences in the movements of agitating matcha tea (such as the trajectory, rates and time of agitation) by different makers (Goto et al., 2017; Kanazawa et al., 2015a), or the impact of agitation on formation and distribution of foam in matcha tea (Kanazawa et al., 2015b). The influence of agitation on the sensory profiles and flavor substances of matcha tea has not been explored. Thus, this study systematically investigated the effects of two agitating parameters (agitating rates and time) on the aroma and taste profiles of matcha tea, and the resulting impact on the release of aroma and taste substances from matcha tea. Finally, chemometrics methods were introduced to analyze the key aroma and taste substances affecting the sensory performance of matcha tea with three agitating rates and time.

2. Materials and methods

2.1. Chemicals

Hexanal, 2-octanol, acetonitrile, formic acid, phenolphthalein and total antioxidant capacity assay kit (DPPH method) were purchased from Macklin Biochem (Shanghai, China); caramel flavors were purchased from Quintescents (Guangzhou, China); alcohol, alum, monosodium glutamate, sucrose, citric acid, sodium hydroxide and sodium carbonate were purchased from Sinopharm Chemical Reagent (Shanghai, China); n-alkanes mixed standard (C7-C40), Folin-Ciocalteu reagent, amino acid assay kit (Ninhydrin colorimetry), gallic acid (GA) and catechins were purchased from Shanghai Yuanye Co., Ltd. (Shanghai, China); n-ketones (C4-C9) was purchased from Hanon Advanced Technology Co., Ltd; total carbohydrate assay kit were purchased from Beijing Solarbio Co., Ltd. (Beijing, China); caffeine and flavonol glycosides were purchased from GlpBio Co., Ltd. (Montclair, USA). Ultrapure water was used for all experiments from an AWL-6000-M ultrapure water machine (Aquapro, FL, USA).

2.2. Matcha tea samples and preparation

Two types of matcha samples made with different raw material and processes were used to explore the regularity and generalities of the effect of agitation on the aroma and taste profile of matcha tea, including Yabunaki (YB) collected from Henan Shimo mocha Co., Ltd (Henan, China) and Gyokuro (GK) collected from Jinhua feicui Tea Industry Co., Ltd (Zhejiang, China). The particle sizes of the two matcha samples were about 6.5 μm and 10.4 μm, respectively. And the collected samples were stored in the refrigerator at −20 °C until the experiment. Matcha tea was prepared by mixing 1 g of matcha powder with 100 mL of ultrapure water (75 °C) in a 440 mL tea bowl. Then the mixture was agitated using an electric agitator (Beizhu, Jiaxing, China) at low (500 rpm), middle (800 rpm) and high (1100 rpm) rates for 30 s, 1 min and 2 min, respectively. A constant temperature water bath device was used in the agitating process, so that the temperature of matcha tea stayed about 60 °C when agitation was completed. The size of the bowl and agitator is shown in Fig. 1.

2.3. Human sensory panel test

All the sensory test was performed in a standard tasting room in compliance with the requirements of ISO 8589:2007 (ISO, 2007). Panelists (9 females and 5 males; from 20 to 35 years old) were informed about the background and objectives of this study following the screening test (Daniel Estevez-Lopez, Garcia-Gomez, Lourdes Vazquez-Oderiz, Munoz Ferreiro, & Angeles Romero-Rodriguez, 2021; ISO, 2012). The two types of matcha tea with different agitating parameters were divideded into 30 mL servings in 50 mL cups labeled with a randomly three-digit number and provided to panelists one by one in a random order at room temperature (25 ± 1 °C). Firstly, the aroma of matcha tea was evaluated by panelists after swirling the cup several times (Koch, Muller, Joubert, van der Rijst and Naes, 2012). Secondly, the taste were assessed by sipping a mouthful of matcha tea and retaining it in the mouth for 7–8 s. Thirdly, 'astringency' was recorded within 3–4 s after spitting the tea (Zhang et al., 2016). In the final session, a total of 10 descriptive terms were determined, and the specific definitions and reference standards are showed in Table 1. Then, the intensity of attributes in matcha tea was evaluated by panelists using a 15-cm continuous line scale (0 = none, 15 = extreme).

The verification of the contribution of key flavor substances to the sensory differences of matcha tea with different agitating parameters were based on the triangle test. Firstly, the key aroma compounds to the sensory differences of matcha tea with different agitating rates were verified, and the specific steps were as follows:

Step 1. The standards of key aroma compounds were added to the matcha tea agitated at high rates when the agitating time was 30 s, and then it as the test object was poured randomly into one or two of three cups. Subsequently, the matcha tea agitated at low rates for the same time was poured into the remaining two cups or one cup. The above three cups of samples as one test group were provided to participants in a random order with covers at room temperature (25 ± 1 °C). Samples were divided into 20 mL in 50 mL cups marked with three random numbers.

Step 2. Panelists were instructed to smell the aroma for 7–8 s after swirling the cup several times and write down the number of the sample that was different from others. Completing the evaluation of one sample, participants were required to get fresh air for 30 s.

Step 3. The answers for one test round were checked. If the number of correct answers in a test group was below 10, this test group was marked "right". Then, the matcha tea agitated at low and middle rates was compared with that agitated at high rates for other agitating time. If the number of the "right" test group was greater than or equal to 4, the result of verification was true.

Secondly, the key aroma compounds to the sensory differences of matcha tea with different agitating time were verified. The matcha tea agitated for 30 s and 1 min was compared with that agitated for 2 min at low, middle and high rates by repeating Step 1–3. And the standards of key aroma compounds were added to the matcha tea agitated for 2 min and used as the test object.

Finally, the key taste compounds to the sensory differences of matcha tea with different agitating rates and time were verified using the same steps as the key aroma compounds, except for Step 2. Instead, panelists with a nose clip were instructed to sip a mouthful of matcha tea, retain it in the mouth for 7–8 s, then feel the touch of the oral cavity for 3–4 s after spitting the tea, and write down the number of the sample that was

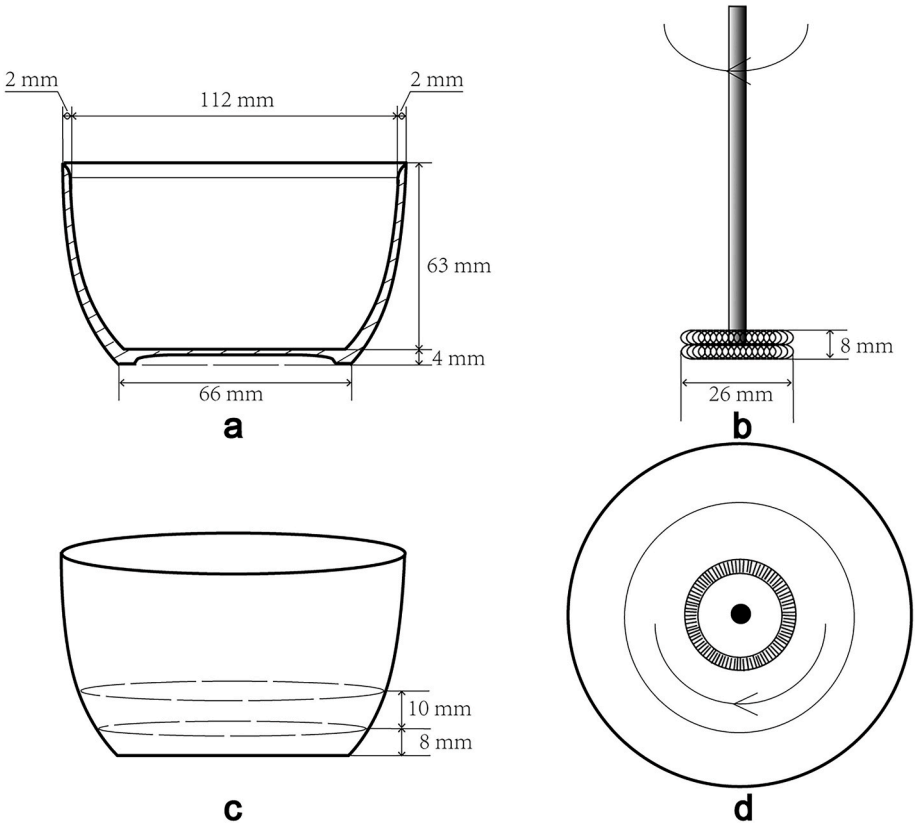

Fig. 1. The sizes and position of tea bowl and agitator. The sizes of tea bowl (a) and agitator (b); the position of agitator in the bowl (8 mm from the bottom) and the height of matcha pulp level (18 mm) (c); the position and rotation direction of agitator in the bowl (d).

different from others. Completing the evaluation of one sample, panelists were required to gargle with ultrapure water and have some unsalted biscuits to relieve taste bud stress for 15 min. The matcha tea with different agitating rates and time was firstly carried out the triangle test as the reference before the comparison of the matcha tea with the standards of key flavor compounds. And the composition and concentration of key flavor compounds were descried in detail in Section 3.2 and 3.3.

2.4. Physicochemical properties analysis of matcha tea

The stability of matcha tea was assessed by multiple light scattering instrument (TURBISCAN Lab ^{expert}, Formulaction, Toulouse, France) to ensure the uniformity of collected samples for the analysis of aroma and taste components. Matcha tea samples in a cylindrical glass cell were periodically scanned from the bottom to the top by a pulsed near-infrared LED at a wavelength of 880 nm for 1 h at intervals of 30 s at 25 °C. During the initial 60-s interval, global turbiscan stability indexes (GTSI) of all samples was below 0.5 (Fig. 2), indicating a high uniformity of the samples collected during this period. Therefore, the sampling of subsequent detection was conducted within the first 60 s after agitating the matcha tea.

The total antioxidant capacity (TAC) assays of matcha tea were carried out by WFJ 7200 UV–Vis Spectrophotometers (Unico, Shanghai, China). A 2 g matcha tea was incubated in an ice water bath for 10 min, and then centrifuged at 4 °C and 3500 rpm for 10 min. The extracted supernatant was filtered through a 0.45-μm syringe filter (mixed cellulose, Navigator, Tianjin, China) for analysis. The TAC was detected by the kit and recorded at the absorbance of 515 nm. The calibration curve was $y = 1.4144x - 0.0081$ (x was the concentration of Trolox (μmol/mL) and y was the absorbance difference of spectrum).

2.5. Aroma analysis of matcha tea

2.5.1. Aroma profiles analysis of matcha tea

The aroma profiles of matcha tea was analyzed by gas chromatography-ion mobility spectrometry (GC-IMS) (Flavourspec, G. A.S, Dortmund, Germany) equipped with a MXT-5 capillary column (15 m × 0.53 mm × 1 μm, Restek, Beijing, China). Five grams of matcha tea in a 20 mL headspace vial were incubated at 60 °C for 5 min at 500 rpm. Then, 500 μL of the headspace gas was injected into the inlet (65 °C) by a gastight syringe (60 °C). The column and drift tube were maintained at 80 °C and 45 °C, respectively. The carrier gas (N_2, 99.99% pure) flow ramp was as follows: 2 mL/min, 0–2 min; 2–10 mL/min, 2–10 min; 10–100 mL/min, 10–20 min; 100–150 mL/min, 20–30 min. The specific detection information for all signal peaks detected by GC-IMS is presented in Table 2.

2.5.2. Aroma substances analysis of matcha tea

The aroma substances of matcha tea was analyzed by gas chromatography-mass spectrometry (GC-MS) (7890A-5975C, Agilent, CA, USA) equipped with a HP-INNOWAX column (60.0 m × 0.25 mm × 0.25 μm, Agilent, USA) according to the reference method (Tan et al., 2019) with modifications. Five grams of matcha tea and 10 μL of 2-octanol (0.512 mg/L, as internal standard) in a 20 mL headspace vial were kept in a 60 °C water bath for 5 min at 500 rpm. Next, a Supelco 85-μm Carboxen/Polydimethylsiloxane fiber was inserted into the vial for the 30 min of extraction. The desorption was done at the GC injector (230 °C) for 5 min. The flow rate of carrier gas (He, purity >99.999%): 1 mL/min. Temperature program was as follow: 50 °C for 5 min; 5 °C/min to 160 °C and held at 160 °C for 5 min; 10 °C/min to 240 °C and held at 240 °C for 20 min. The qualitative and quantitative information of compounds detected by GC-MS is showed in Table 3.

Table 1
Definitions and reference standards of sensory descriptive terms for matcha tea.

Descriptive terms	Definitions	Reference standards (0 = none, 15 = extreme)
Seaweed	Aroma associated with moist dirt, shellfish, fresh ocean fish and sea-tangle, etc.	Dried laver = 8.0 (aroma) Add 1 g of dried laver to 100 mL of water (25 °C) for 5 min
Brown	Aroma associated with cooked character, like bake bread with butter, toasted, caramel, and almost burnt aromatic	Caramel flavors = 7.0 (aroma) Add 0.1 g of caramel flavors to 100 mL of water (25 °C) for 10 min
Sweet	Sweet fragrance associated with the impression of sugar, honey, fruit or flowers	Honey (COFCO, Beijing, China) = 7.0 (aroma) Add 4 g of honey to 100 mL of water (55 °C) for 2 min
Woody	The musty, dark and dry aroma associated with barrels, dry wood, trunk	Cedar oil (LIUPIN, Shanghai, China) = 6.0 (aroma) Add 1 g of cedar oil to 100 mL of water (25 °C) for 5 min
Green	The slightly pungent aroma and flavor associated with green beans, leaf, and newly cut grass	1 mg/L Hexanal = 3.0 (aroma) Add 1 mg of hexanal to 1 L of water (25 °C) for 5 min
Astringency	A feeling of a puckering, drying, and tingling sensation on the surface of tongue and mouth	0.5% Alum solution = 4 0.7% Alum solution = 8 1.0% Alum solution = 12
Bitterness	A basic taste factor of which caffeine in water is typical	0.01% Caffeine solution = 4 0.02% Caffeine solution = 8 0.04% Caffeine solution = 12
Umami	A chemical feeling factor of which monosodium glutamate (MSG) is typical	0.014% MSG solution = 4 0.028% MSG solution = 8 0.050% MSG solution = 12
Sweetness	A basic taste factor of which sucrose is typical	0.40% Sucrose solution = 4 1.00% Sucrose solution = 8 2.00% Sucrose solution = 12
Sourness	A basic taste factor of which citric acid is typical	0.020% Citric acid = 4 0.035% Citric acid = 8 0.050% Citric acid = 12

2.6. Taste analysis of matcha tea

2.6.1. Taste profiles analysis of matcha tea

The taste profiles analysis was constituted by the total carbohydrate (TC), total free amino acid (TFAA), total phenols content (TPC) and total acid (TA) assays of matcha tea. The TC, TFAA and TPC were performed by UV–Vis Spectrophotometers, and the pretreatment procedure of the sample was in accordance with the method described for TAC in Section 2.4. The TC and TFAA determination was conducted using the kits. The TPC detection was measured according to the method of CNOS GB/T 8313-2018 (GB/T, 2018). The TA was determined by acid base titration based on the method of CNOS GB 12456-2021 (GB, 2021). The specific detection parameters are presented in Table 4.

2.6.2. Taste substances analysis of matcha tea

The taste substances analysis of matcha tea were performed by high performance liquid chromatography (HPLC) (1525–2489, Waters, MA, USA) equipped with a C18 column (250 mm × 4.6 mm, Agilent, CA, USA). The sample pretreatment was consistent with Section 2.6.1. The detection was carried out at 278 nm for caffeine, GA and catechins, and 360 nm for flavonol glycosides, respectively. Mobile phase A was 0.1 % formic acid in water, and mobile phase B was acetonitrile. Flow rate was 1 mL/min. Temperature was 35 °C, and injection volume was 10 μL. Gradient elution was as followed: 5% B, 0–2 min; 5–13% B, 2–8 min; 13–28% B, 8–22 min; 28–95% B, 22–25 min; 95-5% B, 25–30 min. The information for standards detected by HPLC is showed in Table 5.

2.7. Data analysis

All experiments were conducted in triplicate except for sensory evaluation. The significant differences between the groups were determined by one-way ANOVA analysis based on Duncan's multiple range test at $p \leq 0.05$ using the SPSS 21.0 (IBM SPSS Inc., IL, USA). Principal component analysis (PCA) and partial least square-discriminate analysis (PLS-DA) were carried out based on the normalization by auto scaling (mean-centered and divided by the standard deviation of each variable) of raw data using SIMCA-P™ 14.1 (UMetrics AB, Umea, Sweden). Other data analysis was using Excel 2016 (Microsoft, WA, USA).

3. Results and discussion

3.1. Effect of agitating parameters on sensory performance

The results for the sensory profiles of matcha tea agitated with different parameters are shown in Fig. 3. The individual aroma score of seaweed, brown, sweet, woody and green comprised the total aroma score of matcha tea. In YB samples, the total aroma score gradually reduced due to the increase of agitating rates and time, from 23.9 ± 0.8 at low agitating rates for 30 s to 9.6 ± 1.5 at high agitating rates for 2 min (Fig. 3a). Thereinto, seaweed, woody and green aroma accounted for a relatively high proportion of the total aroma score of YB matcha tea, which were affected significantly by higher agitating rates and time. In GK matcha tea, the highest value of total aroma score was 16.9 ± 1.8 when agitating at middle rates for 30 s, and the lowest value was 11.4 ± 1.1 when agitating at high rates for 2 min (Fig. 3b). This indicated that the effect of agitation on the aroma score of YB samples was greater than that of GK samples, but an obvious reduction in the total aroma score of the two matcha tea could be observed with the increase of agitating rates and time.

The total taste score of matcha tea was consisted of the individual aroma score of astringency, bitterness, umami, sweetness and sourness. In YB samples, the total taste score showed a decreased trend with the increase of agitating rates and time in general, reducing from 22.1 ± 2.1 at low agitating rates for 30 s to 13.0 ± 1.2 at high agitating rates for 2 min (Fig. 3c). Bitterness and astringency always dominated the total taste score of YB matcha tea, despite the scores of umami and sweetness as the increase of agitating rates and time. However, umami and sweetness gradually occupied the main taste score of GK samples due to the increase of agitating rates and time (Fig. 3d). The sourness score was higher in GK samples than in YB samples, but the agitating effect on sourness of matcha tea not showed a prominent regularity.

PCA was used to analyze the distribution of sensory scores of matcha tea with different agitating parameters. In Fig. 3e, it was distinctly observed that YB matcha tea agitated at three rates were located in different regions on the biplot of scores and loadings plots with the first two principal components (PCs) accounting for 88.6% of the cumulative percentage of total variation. Aroma, bitterness and astringency contributed to the score of YB samples agitated at low rates in positive PC 1, and sweetness and umami affected the scores of YB samples agitated at high rates in negative PC1. YB samples agitated at middle rates was located near the origin of the PC1 axis, which was also the location of sourness. YB samples with different agitating time could not be gathered in one region due to the influence of agitating rates. However, at the same agitating rate, YB samples were sequentially aligned along the PC1 axis from right to left with the increase of agitating time. This suggests that the impact of agitating rates on the sensory performance of YB matcha tea was more significant compared to that of agitating time.

In Fig. 3f, the area of GK matcha tea agitated at different rates were partially overlapped on the biplot with the first two PCs accounting for 83% of the cumulative percentage of total variation. Due to the

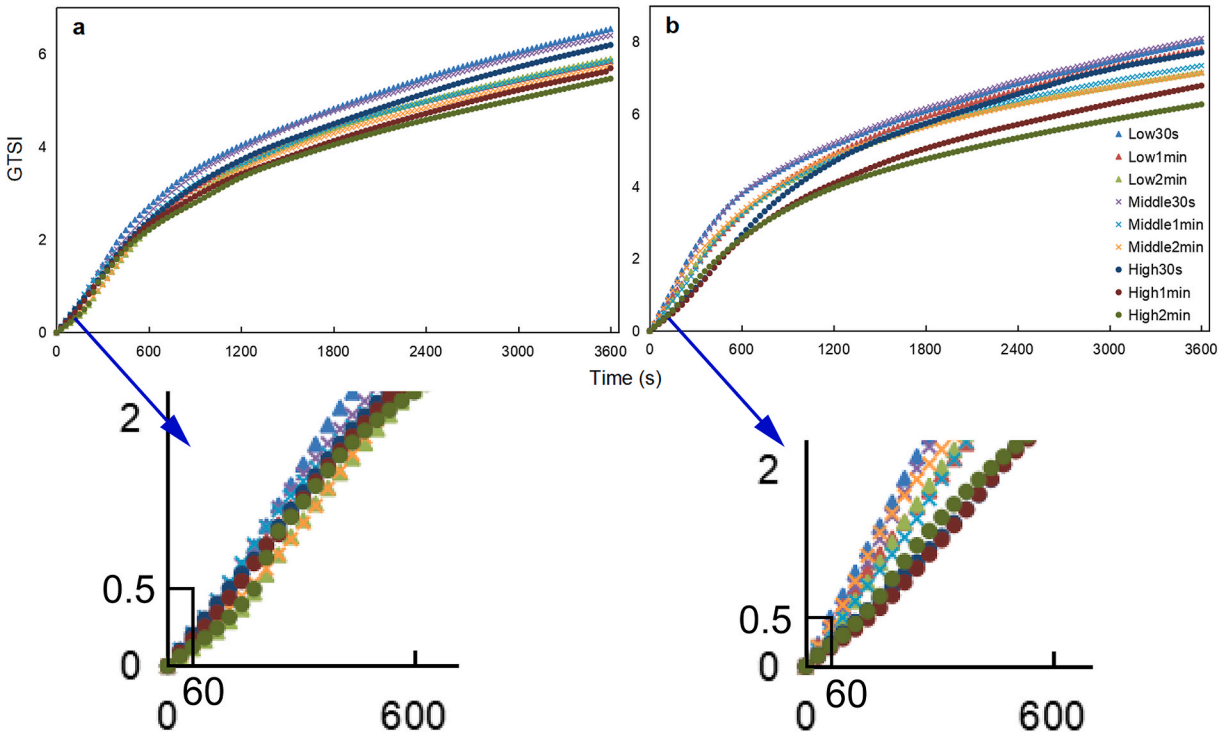

Fig. 2. Stability of the matcha tea with different agitating ways. Global turbiscan stability indexes of YB samples (a) and GK samples (b).

contribution of aroma, bitterness and astringency, GK samples agitated for 30 s was obviously distinguished from that agitated for other time on the PC1 axis. Woody aroma, bitterness and astringency contributed to the score of GK samples agitated for 1 min in negative PC 2, and seaweed, brown, sweet, green, sweetness, umami and sourness affected the scores of GK samples agitated for 2 min in positive PC2. However, the distribution of GK samples with different agitating rates existed a large overlap due to the influence of agitating time. This suggests that the impact of agitating time on the sensory performance of GK matcha tea was more significant compared to that of agitating rates. Although the impact of agitating rates and time on the sensory profile of matcha tea depends on the sample type, the aroma and taste differences of matcha tea with different agitating parameters were distinct. Furthermore, aroma, bitterness and astringency contributed a lot to the matcha tea agitated at low rates and for 30 s, and the matcha tea agitated at high rates and for 2 min contained more umami and sweetness.

3.2. Effect of agitating parameters on aroma

3.2.1. Aroma profiles

In Fig. 4a, 51 signal peaks were identified by GC-IMS in YB samples, and it was evident that over 50% of the signal peaks (the red frame) was higher in matcha tea with low agitating rates and time, which included 14 qualitative signal peaks as shown in Table 7. Among them, sulfur compounds (such as butyl sulfide, 1-propene-3-methylthio, dipropenyldisulfide and diethyl disulfide) were associated with the seaweed aroma (Baba et al., 2017), and methylpyrazine and 2-acetylpyrrole was correlated to the brown aroma (Liu et al., 2012; https://www.femaflavor.org/flavor-library), which were consistent with the result of the sensory performance (Fig. 3e). Moreover, 3-pentanone, linalool oxide, 2,3-butanediol, propanoic acid and ethyl isovalerate were related to the fruity and floral aroma (http://www.flavornet.org/flavornet.html; https://www.femaflavor.org/flavor-library), which had a similarity with the sweet, green and woody aroma to a certain extent. A total of 44 signal peaks were also recognized in GK samples (Fig. 4b), but only 13 signal peaks (the green frame) were clearly observed to have higher intensity in matcha tea when agitating at low rates and time. This finding was also consistent with the sensory performance, as indicated by the higher aroma scores observed in GK samples agitated for 30 s (Fig. 3f).

PCA was developed based on the signal peaks of GC-IMS to further analyze the aroma profiles of matcha tea with different agitating parameters. In Fig. 4c, the YB samples agitated for 30 s were differentiated from those agitated for 1 min and 2 min via PC1 on the biplot of PCA. The first three PCs explained 81.3% of the total variation in the GC-IMS data. A total of 34 signal peaks contributed to the scoring in positive PC1, and the YB matcha tea agitated for 30 s were also located in the region. The YB samples agitated with different rates showed an evident differentiation via PC2. The YB samples with middle and high agitating rates mainly contributed to the scoring in positive PC2, and the samples with middle and low agitating rates were located in the negative PC2 axis. In Fig. 4d, the GK samples agitated at low rates were clearly differentiated from those agitated at middle and high rates on the biplot with PC 1 and 2 accounting for 43.1% and 25% of the variation respectively. And 24 signal peaks contributed to the scoring of the GK matcha tea agitated at low rates in negative PC1. The GK samples agitating at low and middle rates for 30 s were observed to distinguish from others on this biplot, but the samples agitating at high rates for 30 s were difficult to differentiated from those agitated for 1 min and 2 min. Therefore, the aroma profiles of matcha tea agitated at low rates and agitated for 30 s showed a distinct difference from those agitated at other parameters across the types of matcha tea.

3.2.2. Aroma substances

The constraints of the GC-IMS detecting and analytical condition results in the limited numbers and qualitative analysis of signal peaks (Li et al., 2020). Thus, GC-MS was used to analyze the aroma substances of matcha tea with different agitating parameters. A total of 136 aroma compounds were detected in matcha tea as shown in Table 3. PLS-DA, as a supervised classification method, is usually used to establish the prediction model for visualizing the distributions of samples with different groups and assess the importance of each variable to grouping by

Table 2
Information for all signal peaks detected by GC-IMS.

Number	Signal peaks	CAS	Molecular weight	RI[a]	Rt[b] (s)	Dt[c] (ms)	Comment
1	Dimethyl sulfide	75-18-3	62.1	518.40	85.35	0.94	
2	1-Propene-3-methylthio	10152-76-8	88.2	689.30	157.83	1.06	
3	Propanoic acid	79-09-4	74.1	685.70	155.68	1.13	Monomer
4	3-Pentanone	96-22-0	86.1	703.90	166.75	1.10	Monomer
5	2-Methylpropanoic acid	79-31-2	88.1	740.70	191.74	1.18	Monomer
6	2-Methylpropanoic acid	79-31-2	88.1	742.10	192.82	1.41	Dimer
7	2-Hexanone	591-78-6	100.2	785.80	228.16	1.19	
8	2-Furanmethanol	98-00-0	98.1	849.20	292.42	1.11	Monomer
9	2-Furanmethanol	98-00-0	98.1	845.90	288.62	1.36	Dimer
10	(Z)-3-hexen-1-ol	928-96-1	100.2	864.70	310.81	1.25	Monomer
11	Pentanoic acid	109-52-4	102.1	892.40	346.95	1.26	Monomer
12	Pentanoic acid	109-52-4	102.1	891.50	345.68	1.56	Dimer
13	Benzaldehyde	100-52-7	106.1	952.30	440.78	1.18	Monomer
14	Benzaldehyde	100-52-7	106.1	951.30	438.88	1.52	Dimer
15	2-Ethyl-5-methylpyrazine	13360-64-0	122.2	1000.40	534.97	1.15	Monomer
16	Hexanoic acid	142-62-1	116.2	1004.10	542.89	1.33	Monomer
17	Hexanoic acid	142-62-1	116.2	1003.30	541.30	1.70	Dimer
18	2-Acetylpyrrole	1072-83-9	109.1	1058.90	678.16	1.15	
19	Ethyl heptanoate	106-30-9	158.2	1085.80	756.48	1.40	
20	(Z)-3-nonen-1-ol	10340-23-5	142.2	1151.60	989.07	1.48	
21	Heptanoic acid	111-14-8	130.2	1076.10	727.21	1.40	
22	Butyl sulfide	544-40-1	146.3	1086.60	758.86	1.34	
23	(Z)-3-hexen-1-ol	928-96-1	100.2	863.90	309.78	1.51	Dimer
24	3-Pentanone	96-22-0	86.1	702.60	165.90	1.34	Dimer
25	Propanoic acid	79-09-4	74.1	687.60	156.79	1.25	Dimer
26	2-Ethyl-5-methylpyrazine	13360-64-0	122.2	1002.10	538.63	1.66	Dimer
27	Linalool oxide	60047-17-8	170.3	1077.60	731.74	1.25	
28	2,3-Butanediol	513-85-9	90.1	781.80	224.68	1.37	
29	Dipropenyldisulfide	2179-57-9	146.3	1082.40	746.09	1.20	
30	Diethyl disulfide	110-81-6	122.2	926.90	398.02	1.16	
31	Ethyl isovalerate	108-64-5	130.2	929.80	402.69	1.26	
32	Methylpyrazine	109-08-0	94.1	834.50	275.95	1.11	Monomer
33	Methylpyrazine	109-08-0	94.1	834.50	275.95	1.36	Dimer
34	Area 1	unidentified	0	616.30	120.53	0.92	
35	Area 2	unidentified	0	772.80	216.92	0.95	
36	Area 3	unidentified	0	742.50	193.11	1.31	
37	Area 4	unidentified	0	754.80	202.39	1.17	
38	Area 5	unidentified	0	752.60	200.73	1.40	
39	Area 6	unidentified	0	774.00	217.95	1.08	
40	Area 7	unidentified	0	784.40	226.89	1.42	
41	Area 8	unidentified	0	818.70	259.34	1.19	
42	Area 9	unidentified	0	774.00	217.95	1.31	
43	Area 10	unidentified	0	785.10	227.55	1.38	
44	Area 11	unidentified	0	875.00	323.77	0.94	
45	Area 12	unidentified	0	1091.00	772.81	1.19	
46	Area 13	unidentified	0	1083.80	750.37	1.23	
47	Area 14	unidentified	0	1046.60	644.89	1.18	
48	Area 15	unidentified	0	1054.90	667.21	1.26	
49	Area 16	unidentified	0	907.30	368.08	1.04	
50	Area 17	unidentified	0	760.30	206.72	1.05	
51	Area 18	unidentified	0	736.50	188.74	1.04	

[a] The retention index values of compounds on MXT-5 capillary column.
[b] The retention time (s) of compounds on MXT-5 capillary column.
[c] The drift time (ms) of compounds in the drift tube.

variable importance for the projection (VIP) values (Tian et al., 2021). Therefore, PLS-DA was used to observe the distribution of matcha tea with different agitating parameters and the contribution degree of compounds based on the GC-MS results.

In Fig. 5a, YB samples with three agitating rates were distinctly separated on the biplot of PLS-DA model. Three latent components were selected to build the PLS-DA model due to the significant effect on the prediction ability parameter (Q^2) by 7-fold cross validation. And the goodness of fit (R^2) and Q^2 are the evaluation criteria of the PLS-DA model, where the Q^2 value exceeds 0.4 and the R^2 value approaches 1, suggesting that the model is acceptable (Li et al., 2020). The R^2 and Q^2 of the PLS-DA model developed based on the GC-MS data of YB samples were 0.901 and 0.812 respectively, indicating a high robustness and predictive accuracy of this model. The location distance of YB samples agitated at low and middle rates on the biplot was closer, and all the aroma compounds were also highly concentrated in this region. Among these, over 50% aroma compounds contributed to scoring in negative PC2, and YB samples agitated at low rates were also located in this region, which were also consistent with the sensory performance (Fig. 3e). And 51 aroma compounds with VIP greater than 1 were marked on the biplot for further analysis of the aroma compounds significantly affected by agitating rates.

In Fig. 5b, GK samples with three agitating rates were not completely separated on the biplot of PLS-DA model. And the R^2 and Q^2 of the PLS-DA model established by two latent components were 0.6 and 0.374 respectively, indicating GK samples with different agitating rates could not be accurately distinguished based on aroma compounds. However, the contribution degree of aroma compounds to the samples with different agitating rates can be observed by the biplot. A total of 53 aroma compounds with VIP greater than 1 were labeled in the biplot, and 26 of these compounds also appeared in compounds with VIP greater than 1 in YB samples, as shown in Table 6. The odor activity

Table 3
Qualitative and quantitative information of compounds detected by GC-MS.

Code	RT[a] (min)	Compounds	CAS	Henry's law constants[b] [H, mol/(m3Pa)]	RI[c]	LRI[d]	Quantitative Ions (m/z)
1	7.303	Acetone	67-64-1	2.7×10^{-1}	677.0	792	43
2	10.022	2-Ethylfuran	3208-16-0	–	892.5	950	81
3	10.740	Pentanal	110-62-3	6.6×10^{-2}	949.5	968	44
4	11.945	1-Penten-3-one	1629-58-9	–	1019.6	1019	55
5	12.578	Toluene	108-88-3	1.6×10^{-3}	1041.6	1038	91
6	13.363	Butyl acetate	123-86-4	3.8×10^{-2}	1068.7	1081	43
7	13.778	Hexanal	66-25-1	4.5×10^{-2}	1083.1	1073	56
8	15.115	Ethylbenzene	100-41-4	1.4×10^{-3}	1127.9	1128	91
9	15.456	(E)-2-Pentenal	1576-87-0	–	1139.0	1127	55
10	15.617	1,3-Dimethylbenzene	108-38-3	1.4×10^{-3}	1144.3	1134	91
11	15.752	1-Butanol	71-36-3	1.2	1148.7	1138	56
12	15.882	p-Acetylphenol	99-93-4	–	1153.0	–	121
13	15.895	Beta-pinene	127-91-3	2.1×10^{-4}	1153.4	1159	93
14	16.055	Beta-Myrcene	123-35-3	4.0×10^{-4}	1158.7	1161	93
15	16.123	1-Penten-3-ol	616-25-1	–	1160.9	1166	57
16	16.947	Heptanal	111-71-7	3.4×10^{-2}	1192.9	1184	70
17	16.974	D-Limonene	5989-27-5	3.8×10^{-4}	1210.8	1213	68
18	17.689	beta-Phellandrene	555-10-2	1.8×10^{-4}	1212.0	1211	93
19	17.736	2,4-Limonene	13898-73-2	–	1213.5	–	93
20	18.297	(E)-2-Hexenal	6728-26-3	1.4×10^{-1}	1231.5	1241	55
21	18.453	2-Pentylfuran	3777-69-3	–	1236.5	1249	138
22	18.580	Ethyl hexanoate	123-66-0	1.9×10^{-2}	1240.5	1227	88
23	18.659	(Z)-Beta-ocimene	3338-55-4	4.0×10^{-4}	1243.1	1234	93
24	18.936	(Z)-4-Heptenal	6728-31-0	8.8×10^{-2}	1251.9	1240	41
25	18.963	1-Pentanol	71-41-0	8.6×10^{-1}	1252.8	1265	55
26	19.000	(E)-Beta-Ocimene	3779-61-1	3.0×10^{-4}	1254.0	1244	93
27	19.081	3-Methyl-3-butenol	763-32-6	–	1256.6	1240	56
28	19.534	Hexyl acetate	142-92-7	2.0×10^{-2}	1271.1	1264	43
29	19.583	Styrene	100-42-5	3.6×10^{-3}	1272.7	1262	104
30	19.809	Methylpyrazine	109-08-0	4.5	1279.9	1266	94
31	19.929	p-Cymene	99-87-6	9.6×10^{-4}	1283.7	1290	119
32	20.257	2-Octanone	111-13-7	5.0×10^{-2}	1294.2	1295	58
33	20.410	Octanal	124-13-0	2.0×10^{-2}	1299.1	1298	41
34	20.698	(E)-2-(2-Pentenyl)furan	70424-14-5	–	1309.0	–	79
35	20.714	1-Octen-3-one	4312-99-6	–	1309.6	1308	70
36	21.090	(Z)-2-Pentenol	1576-95-0	–	1322.5	1332	57
37	21.162	(Z)-4-Hexen-1-yl acetate	42125-17-7	–	1325.0	–	67
38	21.334	Vinyl hexanoate	3050-69-9	–	1330.9	–	43
39	21.431	2,2,6-Trimethylcyclohexanone	2408-37-9	–	1334.3	1319	82
40	21.488	2,5-Dimethylpyrazine	123-32-0	7.1	1336.2	1351	108
41	21.526	(E)-2-Heptenal	18829-55-5	1.3	1337.5	1321	41
42	21.815	6-Methyl-5-hepten-2-one	110-93-0	1.2×10^{-1}	1347.5	1357	43
43	21.989	1-Hexanol	111-27-3	7.6×10^{-1}	1353.5	1364	56
44	22.067	(E)-2-Nonen-1-ol	31502-14-4	–	1356.2	–	57
45	23.000	(Z)-3-Hexen-1-ol	928-96-1	–	1388.3	1367	67
46	23.441	Nonanal	124-19-6	1.1×10^{-2}	1403.7	1413	57
47	23.488	p-Hydroxybenzaldehyde	123-08-0	1.9×10^{4}	1405.5	–	121
48	23.716	2,5-Dimethylfuran	625-86-5	–	1413.9	–	96
49	23.954	Isophorone	78-59-1	1.5	1422.7	1568	82
50	24.219	4-Hydroxy-2-methylacetophenone	875-59-2	–	1432.5	–	135
51	24.545	(E)-2-Octenal	2548-87-0	1.3×10^{-1}	1444.6	1429	70
52	24.563	3-Furaldehyde	498-60-2	–	1445.2	1459	95
53	24.683	1-Octen-3-ol	3391-86-4	1.9×10^{-1}	1445.0	1460	57
54	24.865	2-Ethyl-3,6-dimethylpyrazine	13360-65-1	–	1456.4	1467	135
55	25.030	Acetic acid	64-19-7	4.0×10^{1}	1462.5	1469	43
56	25.168	1,6,6-Trimethylcyclohexene-3-one	17299-41-1	–	1467.6	–	95
57	25.168	(E)-3-Hexenyl butyrate	16491-36-4	–	1467.6	1455	67
58	25.311	2-Ethyl-3,5-dimethylpyrazine	13925-07-0	2.9	1472.9	1473	135
59	25.518	Octyl acetate	112-14-1	8.6×10^{-3}	1480.5	–	43
60	25.580	Furfural	98-01-1	2.6	1482.8	1492	96
61	25.718	3,5,5-Trimethyl-2-hexene	26456-76-8	–	1487.9	–	57
62	25.760	2-Ethylhexanol	104-76-7	4.6×10^{-1}	1489.5	1491	57
63	26.288	Decanal	112-31-2	6.3×10^{-3}	1509.3	1498	57
64	26.290	(E,E)-2,4-Heptadienal	4313-03-5	–	1509.4	1498	81
65	26.872	3,5-Octadien-2-one	38284-27-4	–	1531.6	1564	95
66	27.254	Linalool	78-70-6	2.0×10^{-1}	1546.2	1559	71
67	27.281	Geranyl butyrate	106-29-6	–	1547.2	–	93
68	27.291	(E)-2-Nonenal	18829-56-6	5.8×10^{-2}	1547.6	1533	70
69	27.331	Benzaldehyde	100-52-7	4.0×10^{-1}	1549.1	1560	106
70	27.519	1-Octanol	111-87-5	3.4×10^{-1}	1556.3	1569	56
71	28.171	(−)-Menthol	2216-51-5	6.6×10^{-1}	1581.2	1612	123
72	28.459	Dimethyl Sulfoxide	67-68-5	9.8×10^{2}	1592.1	1573	63
73	28.523	5-Methylfurfural	620-02-0	4.7	1594.6	1604	110
74	28.778	(E,Z)-2,6-Nonadienal	557-48-2	8.2×10^{-2}	1603.8	1590	41

(*continued on next page*)

Table 3 (*continued*)

Code	RT[a] (min)	Compounds	CAS	Henry's law constants[b] [H, mol/(m3Pa)]	RI[c]	LRI[d]	Quantitative Ions (m/z)
75	28.954	2-Acetyl-5-methylfuran	1193-79-9	–	1609.7	–	109
76	29.053	10-Methyl-1-undecene	22370-55-4	–	1613.0	–	56
77	29.112	Maltol	118-71-8	7.3×10^{2}	1615.0	1980	126
78	29.173	(E)-2-Octenol	18409-17-1	–	1617.0	1597	57
79	29.475	Dioxitol	111-90-0	4.4×10^{2}	1627.1	1612	45
80	29.728	Tea pyrrole	2167-14-8	–	1635.5	1610	108
81	30.010	Beta-cyclocitral	432-25-7	–	1644.9	–	137
82	30.173	p-Methylbenzaldehyde	104-87-0	5.3×10^{-1}	1650.4	1639	119
83	30.394	1-Nonanol	143-08-8	2.7×10^{-1}	1657.7	1648	56
84	30.479	(Z)-3-Hexenyl hexanoate	31501-11-8	–	1660.6	1708	82
85	30.717	Benzeneacetaldehyde	122-78-1	1.6	1668.5	1646	91
86	30.756	(Z)-Beta-Farnesene	28973-97-9	–	1669.8	–	69
87	30.765	Safranal	116-26-7	–	1670.1	1625	107
88	30.990	Acetophenone	98-86-2	1.1	1672.9	1689	105
89	31.539	2-Allyloxyethanol	111-45-5	–	1695.9	–	58
90	31.836	Bourgeonal	18127-01-0	1.1	1705.9	–	175
91	31.991	Alpha-terpineol	98-55-5	4.4	1711.2	1720	59
92	33.000	Beta-Bisabolene	495-61-4	–	1745.4	–	93
93	33.548	2-Phenylpropionaldehyde	93-53-8	–	1764.1	–	105
94	33.568	2,4-Dimethylbenzaldehyde	15764-16-6	–	1764.8	1726	133
95	33.645	Octyl methanoate	112-32-3	1.7×10^{-3}	1767.4	–	55
96	33.757	2-Undecanol	2463-77-6	–	1771.2	–	70
97	33.947	1,1,6-Trimethyl-1,2-dihydronaphthalin	30364-38-6	–	1777.6	1753	157
98	34.040	Azulene	275-51-4	1.5×10^{-1}	1780.8	–	128
99	34.386	Alpha-curcumene	644-30-4	–	1792.6	–	119
100	34.845	Methyl salicylate	119-36-8	1.6	1810.4	1784	120
101	34.993	2-Butyrylfuran	4208-57-5	–	1816.9	–	95
102	35.399	2,4-Decadienal	2363-88-4	–	1834.7	1811	81
103	35.826	Paeonol	552-41-0	–	1853.3	–	151
104	35.882	Geraniol	106-24-1	3.1	1855.7	1858	69
105	35.940	Hexanoic acid	142-62-1	9.9	1858.3	1838	60
106	35.951	Dihydro-beta-ionone	17283-81-7	–	1858.7	–	121
107	36.056	(−)-Calamenene	483-77-2	–	1863.3	1839	159
108	36.253	(E)-Geranylacetone	3796-70-1	–	1871.9	1868	43
109	36.394	Alpha-ionone	127-41-3	–	1878.0	1858	121
110	36.539	Butyl butanoate	109-21-7	1.7×10^{-2}	1884.4	–	71
111	36.671	2-Methylpropanoate	74381-40-1	–	1890.2	–	71
112	36.896	Benzyl alcohol	100-51-6	1.7×10^{1}	1900.0	1870	79
113	37.638	Phenylethyl alcohol	60-12-8	1.7×10^{1}	1939.5	1948	91
114	38.039	Fenchane	6248-88-0	–	1960.8	–	81
115	38.203	Beta-ionone	14901-07-6	–	1969.5	1976	177
116	38.626	Diethylene glycol	111-46-6	3.3×10^{4}	1992.1	–	45
117	38.876	Cyclamen aldehyde	103-95-7	2.3×10^{-1}	2006.4	–	190
118	38.890	Beta-damascenone	23726-93-4	3.2×10^{-1}	2007.2	1827	121
119	39.041	(−)-Citronellene	10281-56-8	–	2016.7	–	82
120	39.206	Beta-ionone epoxide	23267-57-4	–	2027.2	2033	123
121	39.311	3,4-Dehydro-Beta-ionone	1203-08-3	–	2033.8	–	175
122	39.581	(±)-trans-Nerolidol	40716-66-3	–	2050.8	2054	69
123	40.425	2-Butyldecahydronaphthalene	6305-52-8	–	2104.3	–	137
124	40.515	p-Cresol	106-44-5	1.3×10^{1}	2110.4	2093	107
125	40.943	Phytone	502-69-2	–	2139.2	–	43
126	41.022	5-Hydroxyvanillin	3934-87-0	–	2144.6	–	167
127	41.310	Leaf acid ester	25152-85-6	–	2164.0	–	105
128	41.542	Nonanoic acid	112-05-0	6.1	2179.8	2157	60
129	41.806	Olivetol	500-66-3	–	2197.4	–	124
130	42.491	Pinocamphone	15358-88-0	–	2242.9	–	152
131	43.765	2,4-Di-t-butylphenol	96-76-4	–	2327.3	2314	191
132	45.572	Dihydroactinidiolide	15356-74-8	–	2448.2	2372	111
133	46.361	Benzoic acid	65-85-0	2.9×10^{2}	2500.7	2432	105
134	46.380	Diethyl disulfide	110-81-6	3.7×10^{-3}	2502.0	–	122
135	47.055	Indole	120-72-9	1.9×10^{1}	2547.0	2449	117
136	54.178	Caffeine	58-08-2	9.0×10^{5}	3021.1	3140	194

[a] The retention time (min) of compounds.
[b] Henry's law constants (25 °C) come from http://www.henrys-law.org/.
[c] The retention index values of compounds.
[d] The retention index on HP-Innowax reported in literatures.

value (OAV) of a compound is the ratio of the concentration to the corresponding threshold, and the OAV exceeds 1, suggesting that the compound is important to shape the overall aroma profile of the sample (Shi et al., 2022). The OAV values of decanal, p-methylbenzaldehyde, 2, 4-decadienal, geraniol, beta-ionone and beta-damascenone were greater than 1 in Table 6. Among them, p-methylbenzaldehyde, geraniol, beta-ionone and beta-damascenone were related to the sweet, woody and green aroma, and decanal and 2,4-decadienal were associated with the sweet, brown and seaweed aroma (Tan et al., 2019), which significantly affected the sensory performance of matcha tea with different agitating rates.

In Fig. 5c, YB samples agitated for 30 s were distinctly differentiated from that agitated for 1 min and 2 min on the biplot of PLS-DA model, but YB samples agitated for 1 min and 2 min were completely

Table 4
The detection parameters of total taste substance of matcha tea.

Indicators	Absorbance (nm)	Calibration curves	x	y	R^{2}[a]	Linear range
Total carbohydrate (TC)	540	y = 0.6573x - 0.0438	The concentration of glucose (mg/mL)	The absorbance difference of spectrum	0.9989	0.1–1 mg/mL
Total free amino acid (TFAA)	570	y = 0.0433x + 0.0087	The concentration of amino acid standard solution (μg/mL)	The absorbance difference of spectrum	0.9986	0.4–2 μg/mL
Total phenols content (TPC)	765	y = 0.0066x - 0.0048	The concentration of gallic acid (μg/mL)	The absorbance difference of spectrum	0.9991	10–50 μg/mL

[a] The regression coefficients of the standard curves.

Table 5
Information for standards detected by HPLC.

Compounds[a]	CAS	Purity	MW	Calibration curves (mg/L)[b]	R^{2}[c]	Linear range (mg/L)
Caffeine	58-08-02	HPLC ≥98%	194.19	y = 19196x + 1000000	0.9947	145.6–1300
Gallic acid (GA)	149-91-7	HPLC ≥98%	170.12	y = 4255.8x + 99659	0.9910	8.39–37
(−)-Gallocatechin (GC)	3371-27-5	HPLC ≥98%	306.27	y = 1064.8x + 8902.3	0.9926	12.05–83
(−)-Epigallocatechin (EGC)	970-74-1	HPLC ≥98%	306.27	y = 1236.5x - 57599	0.9977	79.23–262
(+)-Catechin (C)	154-23-4	HPLC ≥98%	290.27	y = 12925x + 10400	0.9992	6.41–23.55
(−)-Epicatechin (EC)	490-46-0	HPLC ≥98%	306.27	y = 5212.4x + 118732	0.9983	24.86–137
(−)-Epigallocatechin-3-gallate (EGCG)	989-51-5	HPLC ≥98%	458.37	y = 11698x + 288047	0.9963	306.94–1015
(−)-Gallocatechin gallate (GCG)	4233-96-9	HPLC ≥98%	458.37	y = 79201x - 14836	0.9982	1.6–6.6
(−)-Epicatechin-3-gallate (ECG)	1257-08-5	HPLC ≥98%	442.37	y = 14353x + 44185	0.9979	31.6–104.5
(−)-Catechin gallate (CG)	130405-40-2	HPLC ≥98%	442.37	y = 10101x + 4485.8	0.9989	5.14–34
Myricetin-3-o-galactoside (Myr-gal)	15648-86-9	HPLC ≥98%	480.38	y = 14845x - 6634.9	0.9994	1.31–32.8
Myricetin-3-o-rhamnoside (Myr-rha)	17912-87-7	HPLC ≥98%	464.38	y = 30183x - 7616.5	0.9996	1.58–15.76
Quercetin-3-o-galactoside (Que-gal)	482-36-0	HPLC ≥98%	464.38	y = 36429x - 8422.4	0.9999	0.78–7.84
Quercetin-3-o-glucoside (Que-glu)	21637-25-2	HPLC ≥98%	464.38	y = 37383x - 4567.6	0.9998	0.74–7.4
Kaempferol-3-o-rutinoside (Kae-rut)	17650-84-9	HPLC ≥98%	594.52	y = 26725x - 823.6	0.9999	0.73–7.32
Kaempferol-3-o-β-D-glucoside (Kae-glu)	480-10-4	HPLC ≥98%	448.38	y = 35370x - 3066.8	0.9999	0.37–7.36

[a] The standards come from Shanghai Yuan Ye Bio-technology Co., Ltd (Shanghai, China).
[b] y, The peak area of the compound; x, Concentration (mg/L).
[c] The regression coefficients of the standard curves.

indistinguishable. The R^2 and Q^2 of the PLS-DA model established by four latent components were 0.795 and 0.459 respectively, indicating the robustness and predictive accuracy of this model were acceptable. Over 40% of aroma compounds were contributed to the differentiation, which were distributed in the region of YB samples agitated for 30 s. And the 39 aroma compounds significantly affected by agitating time (VIP >1) were marked on the biplot.

In Fig. 5d, GK samples with three agitating time could be distinguished on the biplot of PLS-DA model. The R^2 of 0.790 and the Q^2 of 0.538 indicated a good fit and stability of the PLS-DA model established based on three latent components. A majority of the aroma compounds were found to be highly concentrated in the region of matcha tea agitated for 1 min and 30 s. Combined with Tables 3 and it was evident that the content of compounds with high Henry's law constants was higher in matcha tea agitated for 1min. Henry's law constants is the molar partition coefficient of aroma compounds between the liquid and gas phase, and the aroma compound with higher Henry's law constants means the better solubility in the liquid phase (Weterings et al., 2022). The aroma compounds with high Henry's law constants might not have completed the gas-liquid interface breakthrough until 2 min of agitating, resulting in their gathering in the system within 1 min of agitating. A total of 49 aroma compounds with VIP >1 were circled in the biplot, but only 10 compounds were also in the data set with VIP greater than 1 in the PLS-DA model based on GC-MS results of YB samples with different stirring times (Table 7). According to the aroma description and OAV in Table 7, beta-phellandrene associated with the green and woody aroma, ethyl hexanoate and styrene related to the sweet, green and woody aroma, 3,5-octadien-2-one and 2,4-decadienal correlated with the sweet, brown and seaweed aroma (Baba et al., 2017) were important to the aroma sensory attributes of matcha tea with different agitating time. Notably, 2,4-decadienal was the only aroma compound significantly impacted the overall aroma profile of matcha tea with different agitating rates and time.

3.2.3. *Verification of key aroma substances*

The contribution of key aroma substances to the discrimination of matcha tea with different agitating parameters was verified based on PLS-DA models. A total of 26 compounds with VIP >1 in common between YB and GK samples were used to reconstruct the PLS-DA model to distinguish matcha tea with different agitating rates (Fig. 6a and b). The R^2 and Q^2 of the PLS-DA model established based on the 26 compounds of YB samples were 0.810 and 0.788, respectively, which were slightly below those of the original model (Fig. 6a). However, the R^2 and Q^2 of the model built by the 26 compounds of GK samples were significantly higher than those of the original model, which were 0.649 and 0.533, respectively (Fig. 6b). And there were 10 compounds with VIP >1 in common between YB and GK samples to reconstruct the PLS-DA model to distinguish matcha tea with different agitating time (Fig. 6c and d). The Q^2 of the models established based on the 10 compounds of YB and GK samples was higher than that of the original models, although the R^2

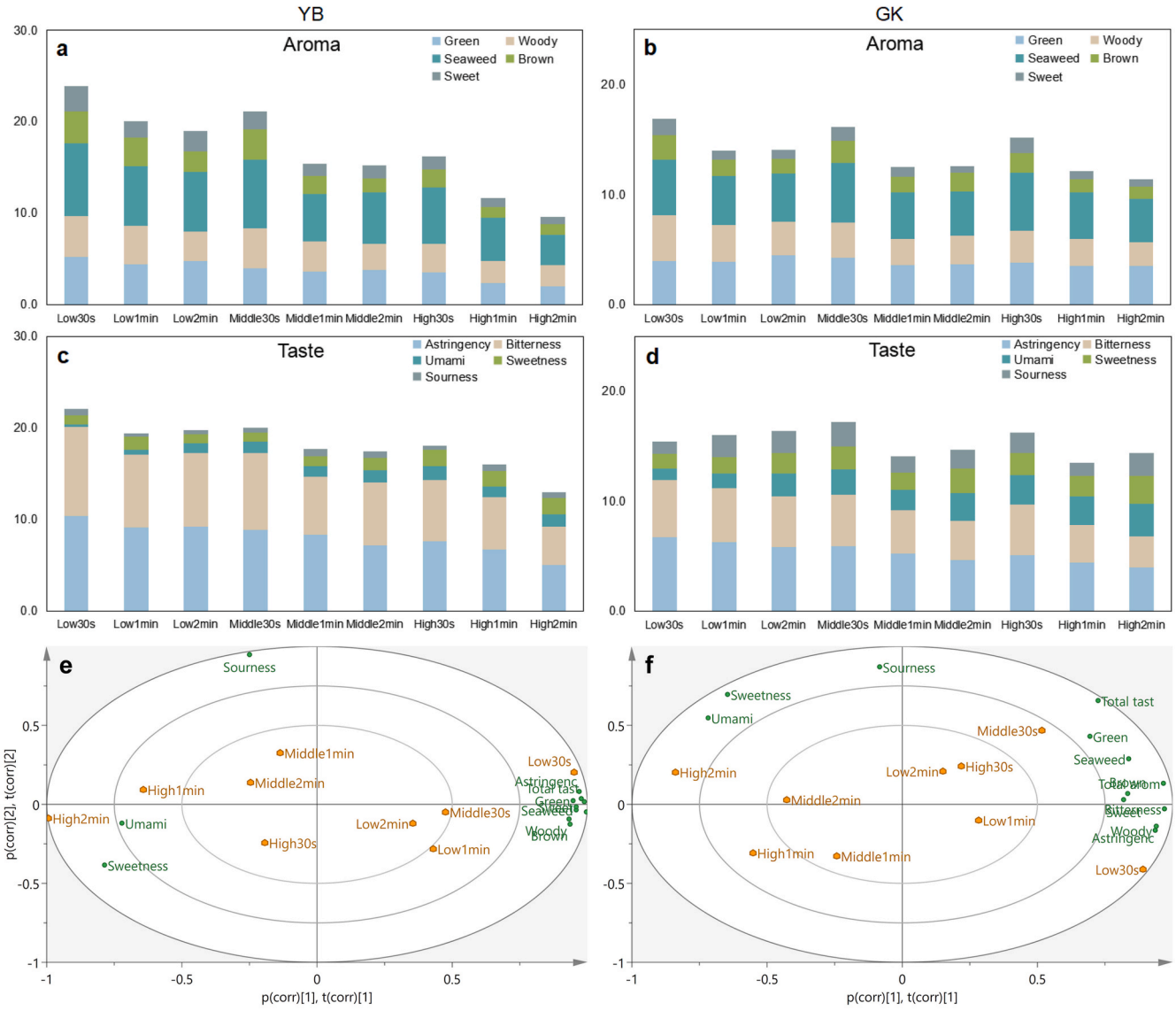

Fig. 3. The sensory performance of matcha tea with different agitating parameters. The aroma score of YB samples (a) and GK samples (b) with different agitating parameters; the taste score of YB samples (c) and GK samples (d) with different agitating parameters; the biplot plot consisted of the scores plot and the loadings plot of PCA developed based on the sensory scores of YB samples (e) and GK samples (f) with different agitating parameters.

was slightly lower. This suggests that the aroma compounds with VIP >1 in common between YB and GK samples had the ability to better distinguish matcha tea with different agitating parameters.

Furthermore, the contribution of key aroma compounds to the sensory differences of matcha tea with different agitating rates and time were verified based on the triangle test. The aroma of YB and GK samples agitated at high rates was compared with that agitated at low and middle rates at the same agitating time in Table 8, because the level of the 26 compounds in YB and GK samples agitated at high rates was lower than that agitated at low and middle rates. At the same agitating time, the matcha tea with different agitating rates could be distinguished by panelists except for GK samples agitated at middle and high rates for 2 min. And the number of correct answers gradually was reduced as the increase of agitating time, indicating that the influence of agitating rates on the aroma of matcha tea decreased when agitating time increased. When the standards of compounds with VIP >1 in common between YB and GK samples were added to the matcha tea agitated at high rates, the number of correct answers was reduced significantly. And the number of "right" groups was consistently higher than 4, although the standards added were reduced to decanal, p-methylbenzaldehyde, 2,4-decadienal, geraniol, beta-ionone and beta-damascenone (with OAV >1 in Table 6). This suggested that decanal, p-methylbenzaldehyde, 2,4-decadienal, geraniol, beta-ionone and beta-damascenone were the key compounds to the aroma differences of matcha tea with different agitating rates.

The level of 10 compounds with VIP >1 in common to distinguish agitating time in matcha tea agitated for 2 min was lower than that agitated for 30 s and 1 min at the same agitating rate, so the significance of aroma differences of matcha tea agitated for 2 min and agitated for 30 s and 1 min were tested (Table 8). The number of "right" groups was lower than 4, although the aroma differences of YB and GK samples agitated for 1 min and 2 min were not significant at middle, high and low rates, respectively. This suggests that there were aroma differences of YB and GK samples with different agitating time at the same agitating rate, especially for matcha tea agitated for 30 s and 2 min. However, matcha tea agitated for different time could not distinguished at all based on aroma, when the standards of compounds with VIP >1 were added to the matcha tea agitated for 2 min. Although the standards were reduced to beta-phellandrene, ethyl hexanoate, styrene, 3,5-octadien-2-one and 2,4-decadienal (see Table 7 for OAV >1), the number of "right" groups also exceeded 4. The above results verified that beta-phellandrene, ethyl

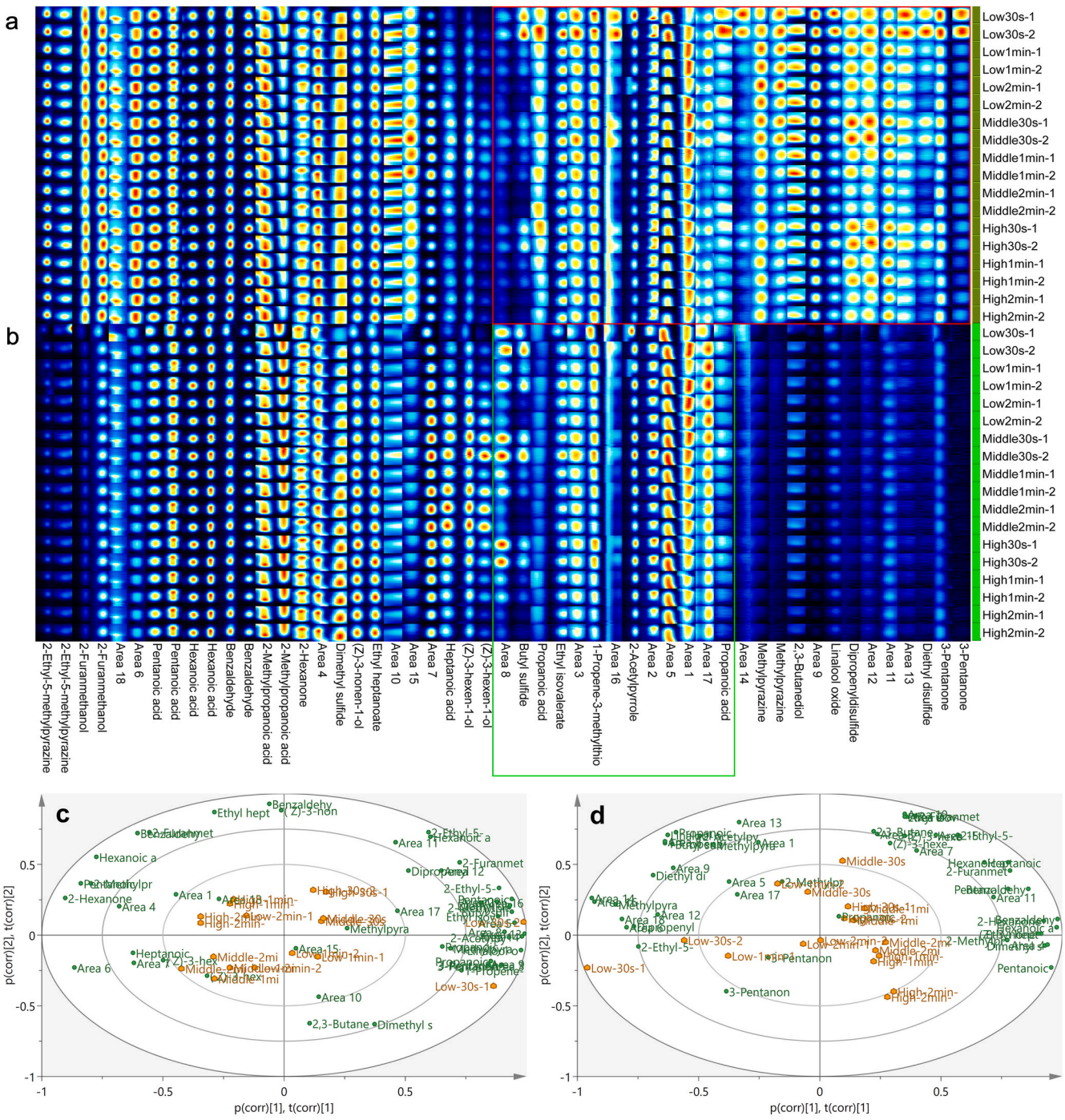

Fig. 4. The aroma profiles of matcha tea with different agitating parameters by GC-IMS analysis. Gallery plot of YB samples (a) and GK samples (b); the biplot plot consisted of the scores plot and the loadings plot of PCA developed based on the GC-IMS results of YB samples (c) and GK samples (d) with different agitating parameters.

hexanoate, styrene, 3,5-octadien-2-one and 2,4-decadienal were the key aroma compounds to the sensory differences of matcha tea with different agitating time.

3.3. Effect of agitating parameters on taste

3.3.1. Taste profiles

In Fig. 7, the total amount of taste substances in matcha tea with different parameters were analyzed. In Fig. 7a, agitating rates had no significant effect on the TC of YB samples when agitated for 30 s, but the TC of YB samples agitated at high rates was the lowest when the agitating time was 1 and 2 min. And there was no significant difference in the TC of YB samples with three agitating time when agitated at low and high rates. However, these phenomena were different in GK samples in Fig. 7b. The TC of GK samples agitated at low rates for 30 s was the lowest, and there was no significant difference in the TC of GK samples with three agitating rates when agitated for 1 min. And agitating time had no significant effect on the TC of GK samples when agitated at middle rates. This suggests that the impact of agitation on the TC of matcha tea varied with each sample.

In Fig. 7c, the TFAA of YB samples with middle agitating rates was the highest at the same agitating time, and there was significant

Table 6
Information of key aroma compounds (VIP >1) in matcha tea that were notably influenced by the rate of agitating.

Aroma compounds	Producers	Purity	Description of aroma[a]	Thresholds of aroma[b]	OAV[c] (YB)	OAV[d] (GK)
Acetone	SCR	≥99.7%	Pungent	0.382	1.4573	0.5248
(E)-2-Pentenal	Macklin	95%	Strawberry, fruit, tomato	0.98	0.6489	0.9612
(Z)-4-Hexen-1-yl acetate	–	–	–	–	–	–
Decanal	Macklin	97%	Floral, fried, orange peel, penetrating, tallow	0.003	85.8126	67.9919
(−)-Menthol	Macklin	99.5%	Mint, cool	2.28	0.0102	0.01233
Dioxitol	Macklin	99%	–	–	–	–
p-Methylbenzaldehyde	Macklin	≥99%	Fruity	0.0012	13.8606	12.6848
Acetophenone	Macklin	≥99.5%	Floral, sweet	0.065	0.7790	0.7136
Octyl methanoate	Macklin	≥98%	Floral	–	–	–
Azulene	Macklin	99%	–	–	–	–
2,4-Decadienal	Macklin	90%	Coriander, deep fried, fat, oil, oxidized	0.0003	29.7622	137.7193
Geraniol	Macklin	98%	Sweet, floral, rose, geranium	0.0011	23.8090	43.6592
Hexanoic acid	Aladdin	99%	Sour, rancid	0.89	0.0514	0.0206
(E)-Geranylacetone	Macklin	≥98%	Fruity	0.681	0.2475	0.6613
Phenylethyl alcohol	Macklin	≥99%	Fruity, honey, lilac, rose, wine	0.56423	0.0471	0.0517
Fenchane	–	–	–	–	–	–
Beta-ionone	Macklin	97%	Violet, floral, fruity, woody	0.0035	653.6766	877.8977
Diethylene glycol	Macklin	>99%	–	240	0.0004	0.0001
Beta-damascenone	IsoReag	95%	Rose, floral, sweet, honey	0.000006	1421.1272	925.7867
(−)-Citronellene	–	–	–	–	–	–
Beta-ionone epoxide	–	–	Fruity, woody	–	–	–
(±)-trans-Nerolidol	Macklin	95%	Woody, floral, wax	0.25	0.2461	0.1787
2-Butyldecahydronaphthalene	–	–	–	–	–	–
Nonanoic acid	Macklin	98%	Fat, green, sour	8.8	0.0049	0.0015
Olivetol	Macklin	98%	–	–	–	–
Caffeine	GlpBio	≥98%	–	–	–	–

[a] Description of aroma compounds, taken from https://www.femaflavor.org/flavor-library and http://www.flavornet.org.
[b] Thresholds of aroma compounds in water, taken from Gemert (2011).
[c] Odor activity values of aroma compounds in YB matcha tea agitated at a low rate for 30 s.
[d] Odor activity values of aroma compounds in GK matcha tea agitated at a low rate for 30 s.

Table 7
Information of key aroma compounds (VIP >1) in matcha tea that were notably influenced by the time of agitating.

Aroma compounds	Producers	Purity	Description of aroma[a]	Thresholds of aroma[b]	OAV[c] (YB)	OAV[d] (GK)
1-Butanol	Macklin	99.5%	Fruity	0.4592	0.1443	0.0073
Beta-pinene	Macklin	≥95%	Pine, polish, woody	0.14	0.1983	0.3128
Beta-phellandrene	Meryer	95%	Mint, terpentine	0.036	15.5917	20.1682
Ethyl hexanoate	Macklin	99%	Apple peel, brandy, fruit gum, overripe fruit, pineapple	0.0022	653.0725	408.2344
Styrene	Aladdin	99%	Balsamic, gasoline	0.0036	28.1872	16.9798
3,5-Octadien-2-one	Bidepharm	98%	Fruity, fat, mushroom	0.15	1.2078	3.1243
Azulene	Macklin	99%	–	–	–	–
2,4-Decadienal	Macklin	90%	Coriander, deep fried, fat, oil, oxidized	0.0003	29.7622	137.7193
(−)-Calamenene	–	–	Herb, spice	–	–	–
(E)-Geranylacetone	Macklin	≥98%	Fruity	0.681	0.2475	0.6613

[a] Description of aroma compounds, taken from https://www.femaflavor.org/flavor-library and http://www.flavornet.org.
[b] Thresholds of aroma compounds in water, taken from Gemert (2011).
[c] Odor activity values of aroma compounds in YB matcha tea agitated at a low rate for 30 s.
[d] Odor activity values of aroma compounds in GK matcha tea agitated at a low rate for 30 s.

difference between the TFAA of YB samples with middle and other agitating rates when agitated for 30 s and 1 min. The TFAA of YB samples with low agitating rates was the lowest for 30 s, but that with high agitating rates was the lowest for other agitating time. As for the low agitating rates, the TFAA of YB samples agitated for 2 min was highest. However, the TFAA of YB samples agitated for 1 min and 30 s was highest at middle and high agitating rates, respectively. In Fig. 7d, the TFAA of GK samples with middle agitating rates was also the highest at the same agitating time, and there was significant difference between the TFAA of GK samples with middle and other agitating rates when agitated for 1 min and 2 min. Moreover, the increase of agitating time led to the growth of TFAA in GK samples agitated at low and middle rates, while the opposite was observed at high agitating rates, indicating the occurrence of Maillard reaction combined with the TC analysis. The high agitating rates subjected matcha tea to higher shear stress, potentially leading to a reduction of the activation energy required for the Maillard reaction (Yu et al., 2016).

In Fig. 7e, the TA of YB samples agitated at high rates was the lowest at the same agitating time, although there was no significant difference in the TA of matcha tea when agitated at middle and high rates for 2 min. As for the low and high agitating rates, the TA of YB samples agitated for 30 s was the lowest. However, the TA of YB samples agitated for 2 min was the lowest at middle agitating rates. In Fig. 7f, agitating rates had no significant effect on the TA of GK samples when agitated for 2 min, but when the agitating time was 30 s and 1 min, the highest TA values were observed in GK samples agitated at middle and low rates, respectively. As for the middle and high agitating rates, the TA of GK samples agitated for 1 min was the lowest. Whereas, the lowest TA values were observed in GK samples agitated for 30 s at low rates. The above results reveals that only when agitated for 1 min did the matcha tea samples agitated at a middle rate have the highest TFAA, and there was no distinct rule in the TC and TA of the two matcha tea with different agitating parameters.

In Fig. 7g, the TPC of YB samples was negatively related to agitating time and rates in general. However, there was no significant difference in the TPC of YB matcha tea agitated for 1 and 2 min at low agitating rates. And the TPC of YB samples agitated for 2 min did not decrease

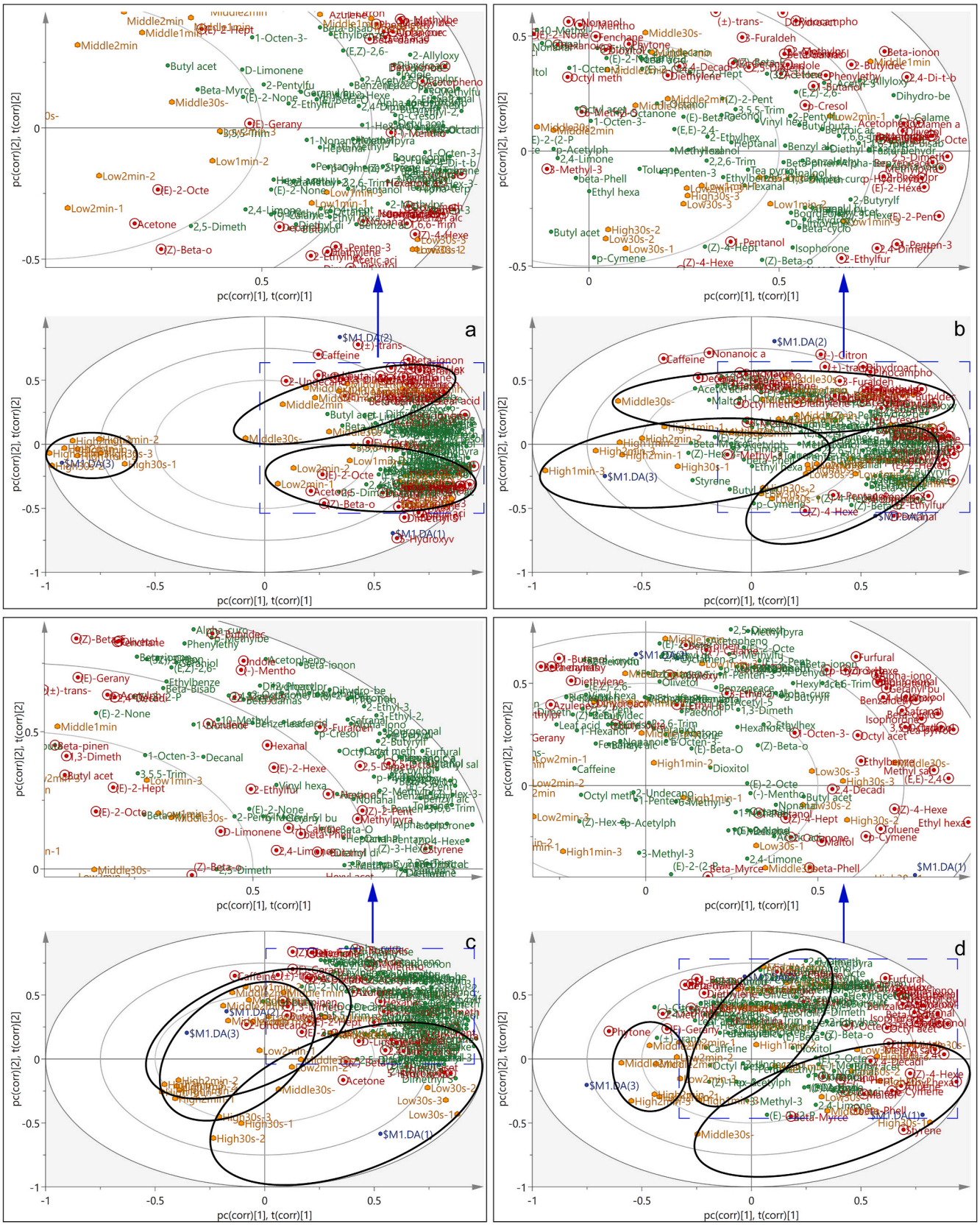

Fig. 5. PLS-DA analysis of the matcha tea with different agitating parameters based on the results of GC-MS. The biplot plot consisted of the scores plot and the loadings plot of the PLS-DA models developed based on the GC-MS results of YB samples (a) and GK samples (b) with different rates; the biplot plot consisted of the scores plot and the loadings plot of the PLS-DA models developed based on the GC-MS results of YB samples (c) and GK samples (d) with different time.

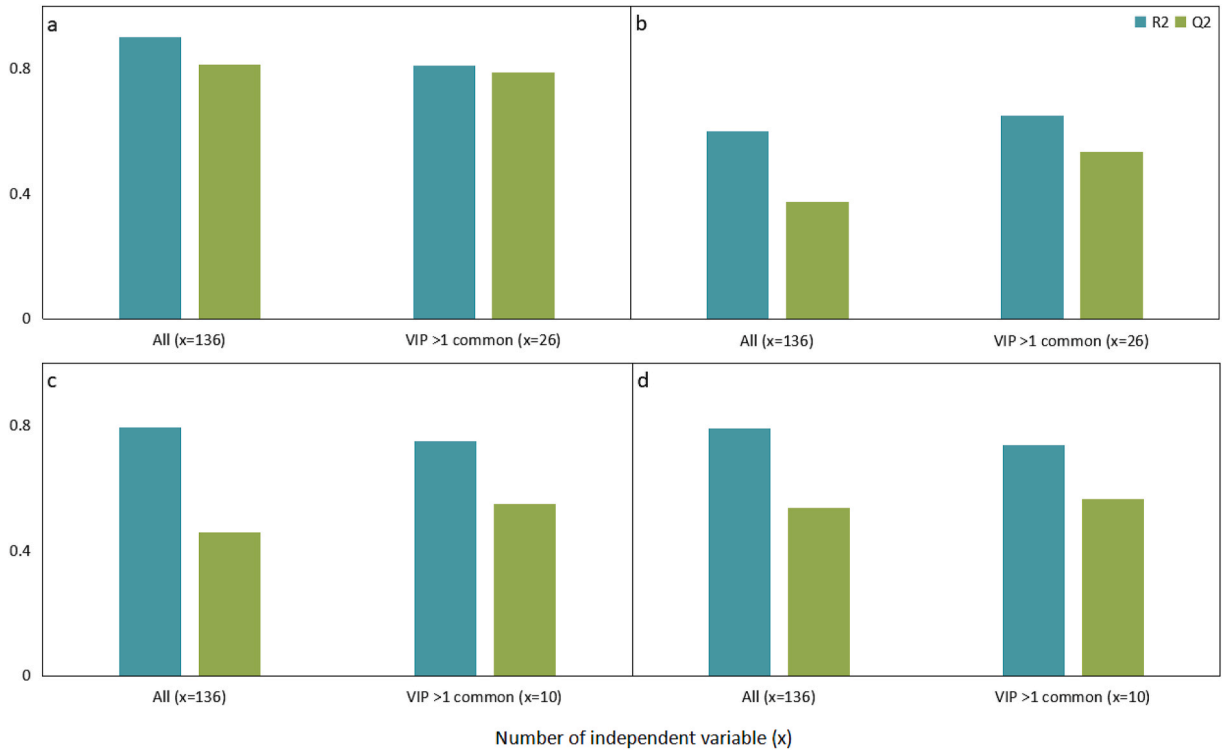

Fig. 6. The comparison of the R^2 and Q^2 of the PLS-DA model established based on all aroma compounds and that reconstructed based on the aroma compounds with VIP >1 in common between YB and GK samples. (a) The R^2 and Q^2 of the PLS-DA model established based on all aroma compounds of YB samples agitated at different rates and that reconstructed based on the 26 compounds with VIP >1 in common between YB and GK samples. (b) The R^2 and Q^2 of the PLS-DA model established based on all aroma compounds of GK samples agitated at different rates and that reconstructed based on the 26 compounds with VIP >1 in common between YB and GK samples. (c) The R^2 and Q^2 of the PLS-DA model established based on all aroma compounds of YB samples agitated for different time and that reconstructed based on the 10 compounds with VIP >1 in common between YB and GK samples. (d) The R^2 and Q^2 of the PLS-DA model established based on all aroma compounds of GK samples agitated for different time and that reconstructed based on the 10 compounds with VIP >1 in common between YB and GK samples.

Table 8
The number of panelists who chose the correct answer and the number of "right" groups in the triangle test for verification of the key aroma compounds.

Number of correct answers		Without standards		With standards (VIP >1)		With standards (OAV >1)	
		YB	GK	YB[a]	GK[a]	YB[b]	GK[b]
30 s	Low-high	13	13	6	7	7	4
	Middle-high	12	11	10	4	7	8
1 min	Low-high	13	12	7	5	8	5
	Middle-high	12	12	4	6	7	6
2 min	Low-high	10	12	4	9	7	9
	Middle-high	11	9	5	8	4	7
Number of "right" groups		**0**	**1**	**5**	**6**	**6**	**6**
Low	30s-2min	11	12	4	9	4	8
	1min–2min	11	9	6	8	8	6
Middle	30s-2min	11	13	8	5	10	10
	1min–2min	9	12	4	8	5	6
High	30s-2min	10	11	6	5	7	5
	1min–2min	9	13	8	6	4	7
Number of "right" groups		**2**	**1**	**6**	**6**	**5**	**5**

[a] The standards were the compounds with the information of producers in Tables 6 and 7, and the concentration of the standard was the concentration difference of the corresponding compound in matcha tea with different agitating rates and time.

[b] The compounds with the information of producers and OAV >1 in Tables 6 and 7

significantly as the agitating rate increased from middle to high rates. In Fig. 7h, the TPC of GK samples agitated at low rates decreased significantly as the agitating time increased from 30 s to 2 min. And the trend of TPC for GK samples agitated for 2 min was completely negatively correlated with the agitating rate. However, there was no significant difference in the TPC of GK matcha tea agitated for 30 s and 1 min at middle and high agitating rates. This suggests that the TPC of different matcha tea samples was not affected by agitation to the same extent, but it generally decreased with the increase of agitating rates and time. This was because the oxidation degree of matcha tea was enhanced with the increase of agitating rates and time, where the TAC of YB and GK samples showed a similar trend to the TPC as shown in Fig. 8. It can be clearly seen that the effect of agitating rates on the TAC of YB samples at the same agitating time was not significant (Fig. 8a), but the TAC of GK samples decreased significantly with the increase of agitating rates when the agitating time was 30 s (Fig. 8b). And the increase of agitating time from 30 s to 2 min caused a significant decrease in the TAC of the two matcha tea agitated at the same rate. Therefore, the effect of agitating rates on the oxidation degree of matcha tea depended on the sample, and the increase of agitating time to 2 min significantly increased the oxidation degree of matcha tea. And the TPC trend of matcha tea with different agitating parameters was consistent with that of taste scores (Fig. 3c and d), suggesting that the taste scores of matcha tea with different agitating rates and time might be correlated with the phenolic compounds. Therefore, the specific phenolic compounds were further analyzed to explore the substances affecting the taste profiles of matcha tea by agitation.

3.3.2. *Taste substances*

In Fig. 9, the specific phenolic compounds of matcha tea with different agitating parameters were further analyzed by PLS-DA. In Fig. 9a, YB samples with three agitating rates were not completely separated by the changes in phenolic compounds on the biplot of PLS-

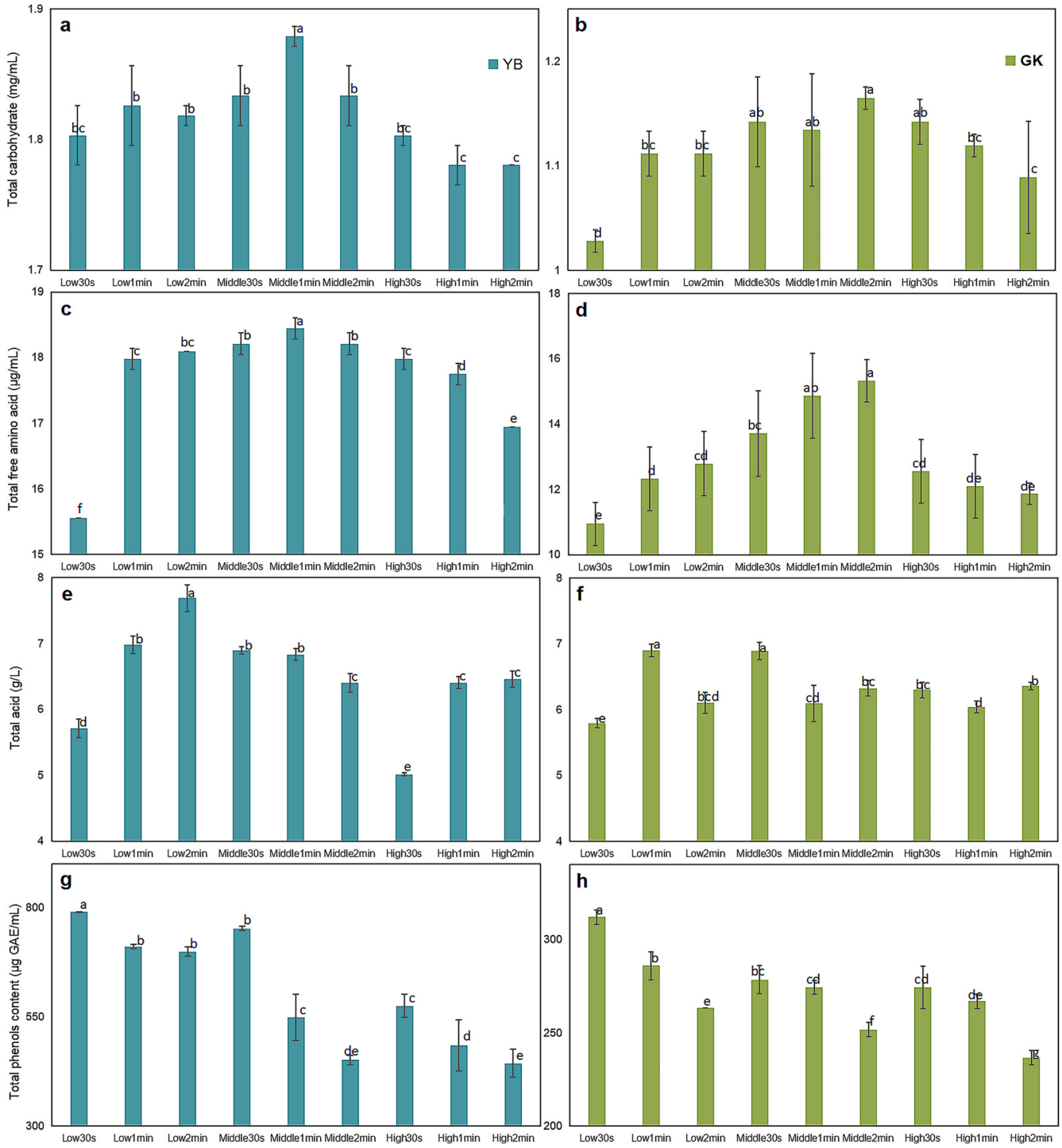

Fig. 7. Taste profiles of matcha tea with different agitating parameters. The total carbohydrate contents of YB samples (a) and GK samples (b); the total free amino acid contents of YB samples (c) and GK samples (d); the total phenols content of YB samples (e) and GK samples (f); the total acid of YB samples (g) and GK samples (h). (Error lines were based on Standard Deviation; 'a', 'b', 'c', 'd', 'e' referred to the significant differences between the groups were determined by one-way ANOVA analysis based on Duncan's multiple range test at $p \leq 0.05$.)

DA model, although the model with R^2 of 0.625 and Q^2 of 0.462 was acceptable. Flavonol glycosides were mainly concentrated in YB matcha tea agitated at low rates, whereas caffeine and the majority of catechins were primarily located in the region of matcha tea agitated at middle rates. This observation may be attributed to the accelerated cleavage of flavonol glycosides by high-rate agitation, which was similar to the hydrolysis of glycosidic bonds by acids and enzymes (Kim et al., 1991). Catechins might have a greater solubility in matcha tea with middle agitating rates but would reduce by hydrolysis reaction with the increase of agitating rates (Han and Shahidi, 2023), resulting in the release of GA in matcha tea agitated at high rates (Fig. 9a). Flavonol glycosides, (−)-epigallocatechin (EGC) and GA were regarded as the key phenolic compounds affected the taste profiles of YB matcha tea agitated at different rates due to their VIP values greater than 1.

In Fig. 9b, it was obviously a separation for GK samples agitated at different rates on the PLS-DA biplot. The robustness and predictive

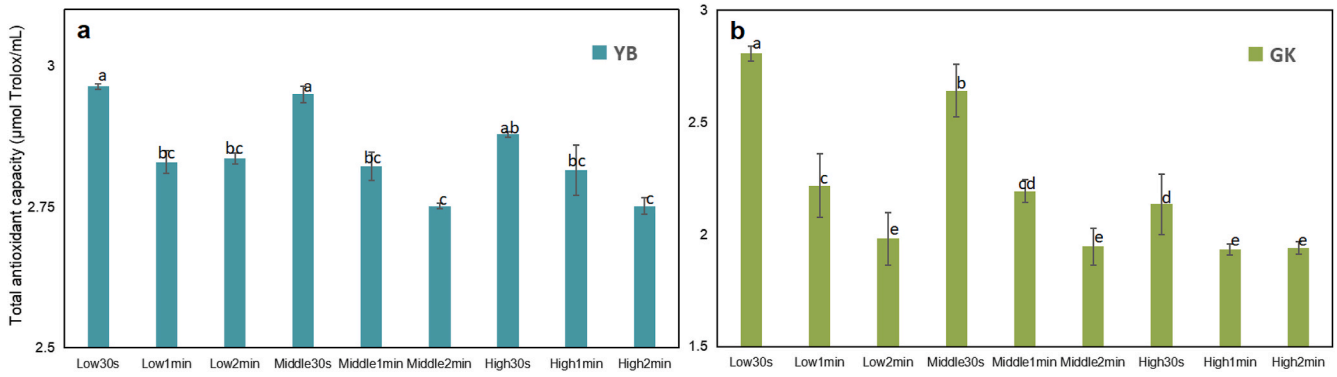

Fig. 8. Total antioxidant capacity of YB samples (a) and GK samples (b) with different agitating ways. (Error lines were based on Standard Deviation; 'a', 'b', 'c', 'd', 'e' referred to the significant differences between the groups were determined by one-way ANOVA analysis based on Duncan's multiple range test at $p \leq 0.05$).

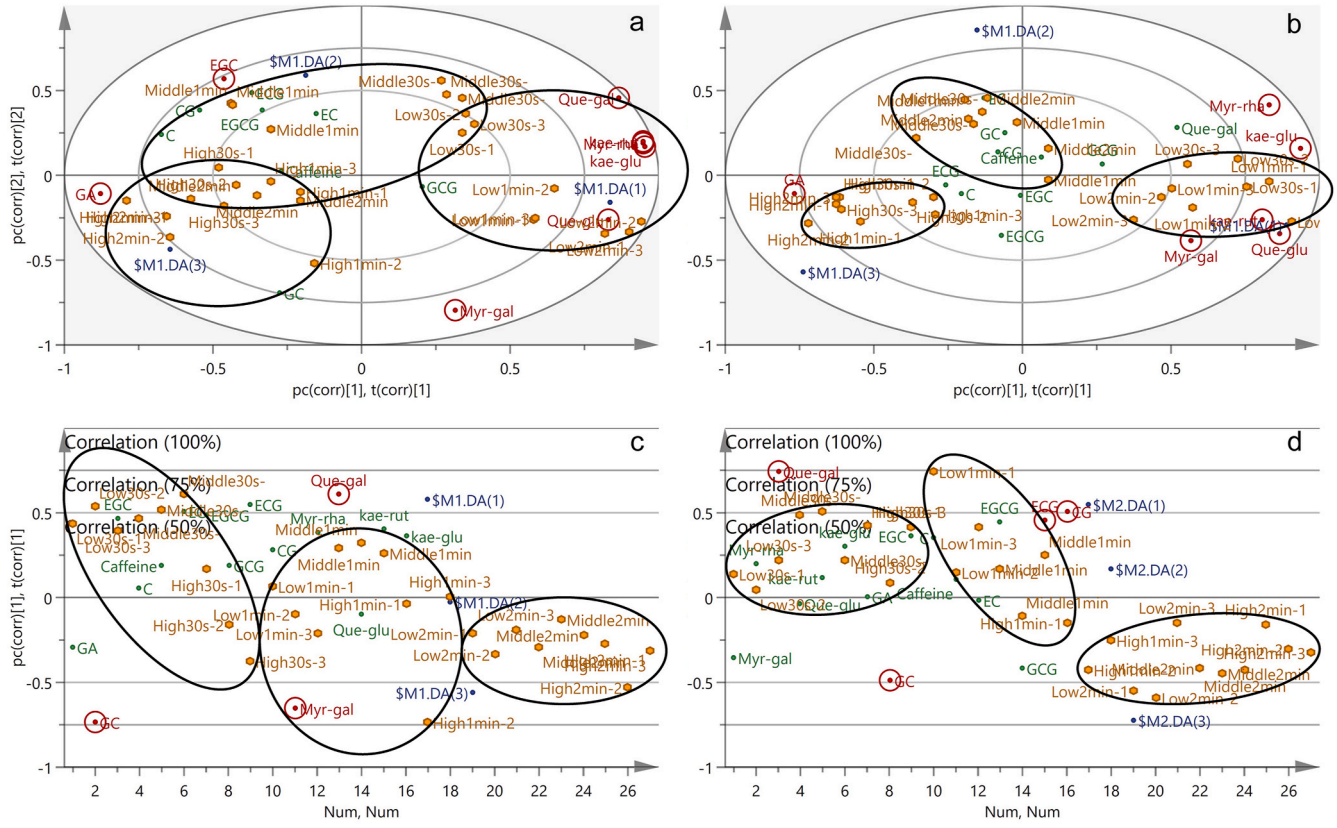

Fig. 9. PLS-DA analysis of the matcha tea with different agitating parameters based on the phenolic compounds. The biplot plot consisted of the scores plot and the loadings plot of the PLS-DA models developed based on the phenolic compounds of YB samples (a) and GK samples (b) with different rates; the biplot plot consisted of the scores plot and the loadings plot of the PLS-DA models developed based on the phenolic compounds of YB samples (c) and GK samples (d) with different time.

accuracy of the PLS-DA model with two latent variables established based on the phenolic compounds of GK samples were good due to the R^2 of 0.837 and the Q^2 of 0.56. It was observed that myricetin-3-o-galactoside (Myr-gal), myricetin-3-o-rhamnoside (Myr-rha), quercetin-3-o-glucoside (Que-glu), kaempferol-3-o-rutinoside (Kae-rut), kaempferol-3-o-β-D-glucoside (Kae-glu), and GA were the phenolic compounds with VIP >1 in this PLS-DA model, which also appeared in compounds with VIP greater than 1 in the PLS-DA model established based on the phenolic compounds of YB samples agitated at different rates. According to the results of sensory analysis (Fig. 3e and f), Myr-gal, Myr-rha, Que-glu, Kae-rut, and Kae-glu were identified as the primary factors influencing the bitterness and astringency of matcha tea with low agitating rates. Although flavonol glycosides are mainly responsible for the astringency of tea, it can also enhance the bitterness of caffeine (Ye et al., 2022). And GA was found to predominantly contribute to the umami and sweetness of matcha tea with high agitating rates, which was also supported in the research of Ye et al. (2022).

In Fig. 9c, YB samples with three agitating time could not be differentiated by variations in phenolic compounds on the biplot of PLS-DA model with the first latent component. And the R^2 and Q^2 of the PLS-DA model were 0.216 and 0.068 respectively. This suggests that the effect of agitating time on the levels of phenolic compounds in matcha tea was not regular. The majority of phenolic compounds were found to be more abundant in the positive Y-axis, which confirmed that high levels of bitterness and astringency in matcha tea agitated at middle and high rates for 30 s (Fig. 3e). By calculating the VIP values of all phenolic

compounds in the model, quercetin-3-o-galactoside (Que-gal), Myr-gal and (−)-gallocatechin (GC) with VIP >1 were identified as the important compounds for the taste of YB samples with different agitating time.

In Fig. 9d, GK samples agitated for different time were also unable to be distinguished by PLS-DA model established based on phenolic compounds. The R^2 and Q^2 of the model with one latent component were 0.287 and 0.134 respectively, indicating the poor stability and predictive ability of the model. The VIP values of que-gal, (−)-epicatechin-3-gallate (ECG), (−)-catechin gallate (CG) and GC exceeded 1, which were circled in the biplot. Among them, Que-gal and GC were also the compounds with VIP >1 in the PLS-DA model established based on the phenolic compounds of YB samples with different agitating time. Based on the results of the sensory analysis (Fig. 3e and f), Que-gal mainly impacted the bitterness and astringency of matcha tea agitated for 30 s. And GC was observed to primarily influence the umami and sweetness of matcha tea agitated for 2 min. The higher levels of GC in matcha tea agitated for a long time may be attributed to the epimerization of EGC. Long-time agitation may enhance the energy of matcha tea system, resulting in a conversion of catechins from epi-structure to non-epi-structure (Ananingsih et al., 2013; Wang et al., 2008).

3.3.3. *Verification of key taste substances*

The importance of key taste substances on the distinction of matcha tea with different agitating rates and time was demonstrated by PLS-DA analysis. There were 6 phenolic compounds (Myr-gal, Myr-rha, Que-glu, Kae-rut, Kae-glu and GA) with VIP >1 in common between YB and GK samples as the input dataset of PLS-DA model to distinguish matcha tea with different agitating rates (Fig. 10a and b). The R^2 and Q^2 of the models established based on the 6 compounds of YB and GK samples was higher than that of the original models, which were 0.723 and 0.604 for YB samples (Figs. 10a), 0.859 and 0.798 for GK samples (Fig. 10b), respectively. A total of 2 compounds (Que-gal and GC) with VIP >1 in common between YB and GK samples were used as the input dataset of PLS-DA model to distinguish matcha tea with different agitating time (Fig. 10c and d). The R^2 and Q^2 of the PLS-DA model established based on the 2 compounds of YB samples were 0.176 and 0.149, respectively, and the R^2 was slightly below that of the original model (Fig. 10c). In addition, the R^2 and Q^2 of the model built by the 2 compounds of GK samples were higher than those of the original model, which were 0.294 and 0.241, respectively (Fig. 10d). This suggests that flavonol glycosides, GA and GC had the ability to better distinguish matcha tea with different agitating rates and time.

The effect of key phenolic compounds on the taste differences of matcha tea with different agitating rates and time was further verified by the triangle test of sensory evaluation. Since the content of the 6 compounds in YB and GK samples agitated at high rates was lower than that agitated at low and middle rates at the same agitating time, the samples agitated at high rates was used as the test object to identify the taste differences with the samples agitated at low and middle rates (Table 9). Except for YB and GK samples agitated at middle and high rates for 2 min and 30 s, the taste differences of matcha tea with different agitating rates were evident at the same agitating time. This indicates that agitating rates affected the taste of matcha tea significantly when agitating time was 1 min, and the taste differences of matcha tea agitated at low and high rates were significant at all agitating time. However, the number of correct answers was reduced apparently when Myr-gal, Myr-rha, Que-glu, Kae-rut, Kae-glu and GA were added to the matcha tea agitated at high rates. And the number of "right" groups was

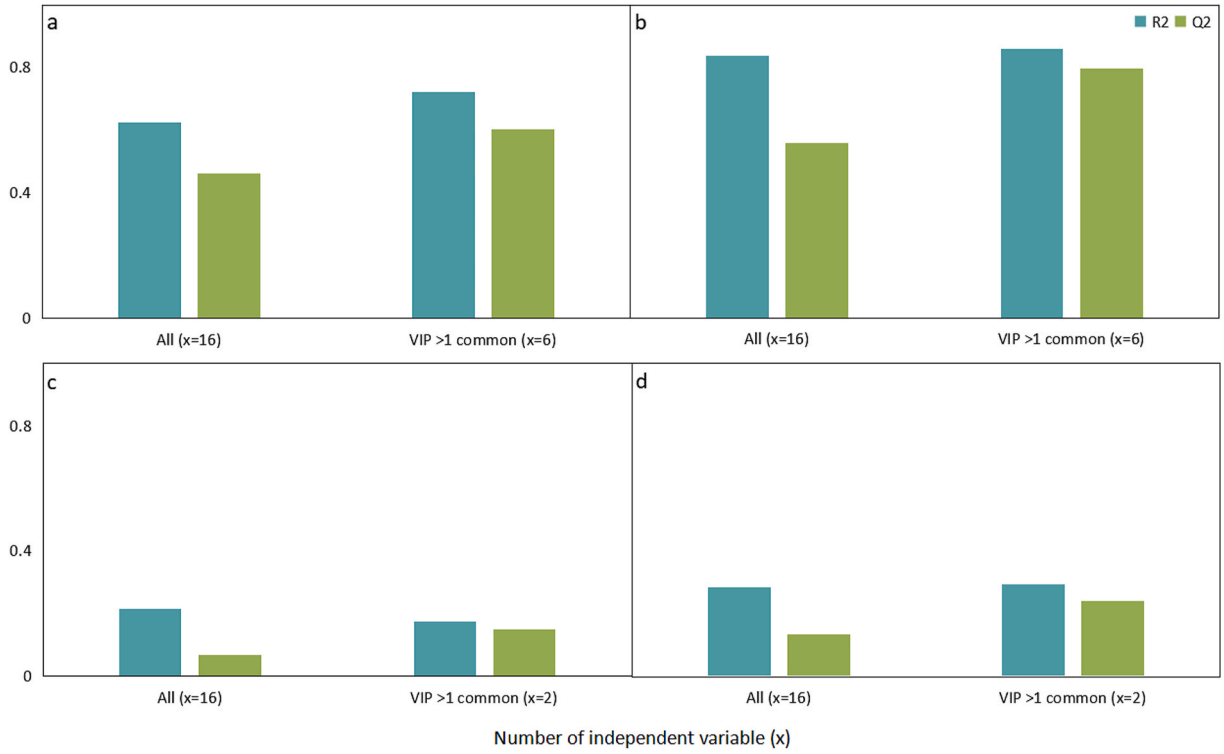

Fig. 10. The comparison of the R^2 and Q^2 of the PLS-DA model established based on all phenolic compounds and that reconstructed based on the phenolic compounds with VIP >1 in common between YB and GK samples. (a) The R^2 and Q^2 of the PLS-DA model established based on all phenolic compounds of YB samples agitated at different rates and that reconstructed based on the 6 compounds with VIP >1 in common between YB and GK samples. (b) The R^2 and Q^2 of the PLS-DA model established based on all phenolic compounds of GK samples agitated at different rates and that reconstructed based on the 6 compounds with VIP >1 in common between YB and GK samples. (c) The R^2 and Q^2 of the PLS-DA model established based on all phenolic compounds of YB samples agitated for different time and that reconstructed based on the 2 compounds with VIP >1 in common between YB and GK samples. (d) The R^2 and Q^2 of the PLS-DA model established based on all phenolic compounds of GK samples agitated for different time and that reconstructed based on the 2 compounds with VIP >1 in common between YB and GK samples.

Table 9
The number of panelists who chose the correct answer and the number of "right" groups in the triangle test for verification of the key taste compounds.

Number of correct answers		Without standards		With standards (VIP >1)	
		YB	GK	YB[a]	GK[a]
30 s	Low-high	12	10	7	8
	Middle-high	10	9	8	6
1 min	Low-high	12	13	7	10
	Middle-high	12	10	9	7
2 min	Low-high	10	11	6	6
	Middle-high	9	10	7	8
Number of "right" groups		**1**	**1**	**6**	**5**
Low	30s-2min	13	12	6	9
	1min–2min	12	13	4	6
Middle	30s-2min	13	11	5	8
	1min–2min	13	10	4	5
High	30s-2min	11	11	10	9
	1min–2min	10	9	6	6
Number of "right" groups		**0**	**1**	**5**	**6**

[a] The standards were myricetin-3-o-galactoside (Myr-gal), myricetin-3-o-rhamnoside (Myr-rha), quercetin-3-o-glucoside (Que-glu), kaempferol-3-o-rutinoside (Kae-rut), kaempferol-3-o-β-D-glucoside (Kae-glu), gallic acid (GA), quercetin-3-o-galactoside (Que-gal), and (−)-gallocatechin (GC), and the concentration of the standard was the concentration difference of the corresponding compound in matcha tea with different agitating rates and time.

higher than 4, indicating that the 6 compounds were the key compounds to the taste differences of matcha tea agitated at different rates.

The significance of taste differences of matcha tea agitated for 2 min and agitated for 30 s and 1 min were tested (Table 9), because the content of Que-gal and GC in matcha tea agitated for 2 min was lower than that agitated for 30 s and 1 min at the same agitating rate. There were significant differences in the taste of YB and GK samples with different agitating time at the same agitating rate, expect for matcha tea agitated for 1 min and 2 min at high rates. In addition, the number of correct answers gradually was reduced when agitating rates increased, indicating that the effect of agitating time on the taste of matcha tea decreased as the increase of agitating rates. When Que-gal and GC were added to the matcha tea agitated for 2 min, the taste differences of matcha tea agitated for different time could not be identified by panelists, expect for matcha tea agitated for 30 s and 2 min at high rates. And the number of "right" groups exceeded 4, which verified that Que-gal and GC were the key phenolic compounds to the taste differences of matcha tea with different agitating time.

4. Conclusion

In this study, the effect of agitating rates and time on the sensory profiles and corresponding flavor components of matcha tea was obviously. The aroma profiles of matcha tea agitated at low rates (500 rpm) and for 30 s was most abundant, and the influence of agitating rates on the aroma of matcha tea decreased gradually when agitating time increased. However, not all aroma substance met the regular, which was related to the Henry's law constants of compounds. Decanal, p-methylbenzaldehyde, 2,4-decadienal, geraniol, beta-ionone and beta-damascenone were the key compounds to the aroma differences of matcha tea agitated at different rates. And the aroma differences of matcha tea with different agitating time were mainly attributed to beta-phellandrene, ethyl hexanoate, styrene, 3,5-octadien-2-one and 2,4-decadienal. Thereinto, 2,4-decadienal associated with the sweet, brown and seaweed aroma significantly influenced the aroma profiles of matcha tea with different agitating rates and time.

The effect of agitating time on the taste of matcha tea decreased as the increase of agitating rates. The bitterness and astringency were stronger in matcha tea with low agitating rates and time, and the sweetness and umami of matcha tea with high agitating rates and time were higher, which attributed to the change of phenolic compounds. Myr-gal, Myr-rha, Que-glu, Kae-rut, Kae-glu and GA were key to the taste differences of matcha tea with different agitating rates. And Que-gal and GC predominantly contributed to the taste differences of matcha tea agitated for different time. Among them, flavonol glycosides were mainly responsible for the bitter and astringent taste of matcha tea. GA and GC were predominantly associated with the umami and sweetness of matcha tea.

This study revealed the effects of agitation on the sensory profiles of matcha tea, which are obviously affected by both aroma and taste substances. The phenomenon and mechanism of matcha tea sensory quality affected by agitation can provide reference for the formulation of standardized agitating parameters in beverage market. In forthcoming research, the agitating parameters can be set more finely, and the influence of different agitating tools on the sensory profiles of matcha tea also needs to be further explored. The selection of matcha tea samples can be more targeted based on cultivars, regions of production, and manufacturing methods, which will facilitate the development of more precise and applicable agitation guidelines.

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Ethical statements

Ethical approval for the involvement of human subjects in this study was granted by the Medical Ethics Committee in School of Medicine of Zhejiang University, Reference number 2019-046, dtd 3/4/2019. All subjects signed a consent form to participate in the sensory and consumer tests.

CRediT authorship contribution statement

Siying Li: Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **Hehe Tian:** Formal analysis, Investigation, Resources, Software. **Guilong Zhu:** Methodology, Investigation, Validation. **Zhenbo Wei:** Funding acquisition, Supervision, 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.

Data availability

Data will be made available on request.

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