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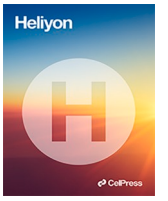

Research article

Changes in flavor quality of raw Liupao tea during the piling process

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ABSTRACT

Purpose: Piling is a critical process for the formation of raw Liupao tea (RLT) flavor quality. However, given the unclear changes in the flavor quality of RLT during the piling process, and the key substances of taste-contributing and aroma components that affecting the flavor quality of RLT was lacking. Herein, the present study aimed at evaluating the changes in flavor quality and its key substances of RLT during the piling process.

Methods: Sensory evaluation was conducted on RLT samples collected at 11 time points during the piling process. The biochemical and aroma components of these samples were detected using UV spectrophotometer, high-performance liquid chromatography (HPLC), and headspace solid-phase microextraction gas chromatography–mass spectrometer (HS-SPME-GC-MS), respectively. Multivariate analysis was performed to investigate the effects of piling time on the flavor quality of RLT.

Key findings: The sensory quality of RLT increased and then decreased during the piling process. The changes in flavor quality of RLT during the piling process could be divided into four stages: 0–6, 9–15, 18–24, and 27–30 h. The flavor quality of RLT changed significantly with the variations in biochemical and volatile compounds. The key contributors to taste changes included significant decreases in (−)-epigallocatechin gallate, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-catechin gallate, and theobromine content and significant increases in gallic acid, gallocatechin, and theaflavin content. Additionally, alterations in the content of 19 characteristic aroma compounds, such as cedrol, methyl salicylate, methyl palmitate, trans-nerolidol, decanal, 6,10-Dimethyl-2-undecanone, neryl alcohol, α-cedrene, and (E, E)-2,4-heptadienal, significantly influenced the aroma of the tea infusion. This study provides insights into the

Abbreviations: (RLT), Raw Liupao tea; (GA), gallic acid; (GC), (−)-gallocatechin; (EGC), (−)-epigallocatechin; (C), (+)-catechin; (GCG), (−)-gallocatechin gallate; (EGCG), epigallocatechin gallate; (CG), (−)-catechin gallate; (ECG), (−)-epicatechin gallate; (EC), (−)-epicatechin; (IRB), institutional review board; (HPLC), high-performance liquid chromatography; (HS-SPME), Headspace solid-phase microextraction; (DVB/CAR/PDMS), Divinylbenzene/Carboxen/Polydimethylsiloxane; (GC-MS), gas chromatography–mass spectrometer; (EI), electronic ionization; (NIST), National Institute of Standards and Technoligy; (HCA), hierarchical cluster analysis; (OPLS-DA), orthogonal partial least squares-discriminant analysis; (VIP), variable importance in projection; (GIBGZARCAS), Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences; (IRB), Institutional review board; (GREB), GIBGZARCAS Research Ethics Board's.

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formation of flavor quality in RLT at different piling stages, which also provide a scientific foundation for optimizing the piling and production processes of RLT.

1. Introduction

Flavor quality serves as a pivotal benchmark for assessing tea quality and is a primary consideration for consumers in their tea purchase decisions [1]. Flavor quality is predominantly determined by flavor substances that influence taste and aroma [2]. For example, the nonvolatile flavor-contributing substances like polyphenols, soluble sugars and free amino acids, as well as aroma-contributing substances such as linalool, linalool oxide II and methyl salicylate were closely related to White peony tea [3], Wuyi rock tea [4], and Liupao tea [5].

Liupao tea, a dark tea with historic significance, is exclusively produced in the Guangxi Province of China [6]. Liupao tea, renowned for its unique flavor of "red, thick, aging, and mellow", has been sold worldwide [6]. Consumers increasingly favor Liupao tea not only for its considerable collection value because of the long historic significance but also for its health benefits [6]. Liupao tea possesses health benefits, including lipid regulation and liver protection [6,7], antioxidation [8], and modulation of gut microbiota [9]. Liupao tea can be produced using traditional or modern processes [10,11]. Liupao tea prepared with the traditional process is also known as raw Liupao tea (RLT). The traditional process includes the following steps: fixing, primary rolling, piling, secondary rolling, and drying [12]. RLT is a commercial product and is also the raw material used for the modern production of Liupao tea [11]. Therefore, the flavor quality of RLT has an important impact on the quality of Liupao tea products and thus the development of the Liupao tea industry.

Piling is a critical process for the formation of RLT flavor quality [13]. During the piling process, there may be three major effects: enzymatic reaction, moist heat effect, and microbial effect [13–15]. To date, abundant literature has reported the RLT flavor quality on the drying process [16] and the tea tree variety [6,17]. Few studies have reported on the piling process. Liu et al. [13] researched on the effect of the piling process on flavor quality of RLT by conducting sensory evaluation and analyzing the aroma compounds in RLT at 2, 12, and 24 h of piling. Their results indicated that the flavor quality was optimal at 24 h. Teng et al. [15] conducted sensory evaluation, biochemical and aroma compounds testing in RLT from 0 to 14 h of piling. Qing et al. [14] conducted sensory evaluation and partial biochemical component detection on tea samples that were collected during 6–16 and 2–12 h of piling, respectively. Their results showed that the sensory quality of RLT first increases and then decreases during the piling process, with the flavor quality being optimum at 12 h. Among those study, there have not systematic study the factor of the change of flavor quality. A systematic study is needed based on a sufficient number of samples taken with the equally speced times and the detection of aroma components and taste components. Besides, the key substances of taste-contributing and aroma components that affecting the flavor quality of RLT was lacking. Given that the changes in flavor quality of RLT during the piling process are not yet clear, the inconsistent quality of RLT poses a substantial impediment to the development of the Liupao tea industry [13]. Thus, it is urgent to systematic study and further optimize the timing and clarify the changes in key compounds in flavor quality of RLT during the piling process. These efforts are essential to produce RLT with consistent flavor quality, and to supply stable raw materials for the production of Liupao tea using modern processing technology.

Our study explores the changes in the flavor quality, biochemical, aroma compounds and the key substances of RLT during the piling process, which provide a scientific basis for optimizing the piling and production processes of RLT.

2. Materials and methods

2.1. Materials and chemicals

The raw materials were fresh tea leaves collected from Guangxi local population species at the one-bud three-leaf stage during the spring season in Guangxi Province, China.

Standards of gallic acid (GA), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechin(C), (−)-gallocatechin gallate (GCG), epigallocatechin gallate (EGCG), (−)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), theophylline, theobromine, and caffeine were all purchased from Shanghai Anpu Cuishi Standard Technical Service Co., Ltd, (Shanghai, China). Ethyl decanoate, rutin, and vitamin C were provided by Runyou Chemical Co., Ltd (Shenzhen, China). Acetonitrile of HPLC grade was obtained from Thermo Fisher Scientific Co., Ltd (Massachusetts, USA). Sodium nitrite, aluminum nitrate, sodium hydroxide, 95 % ethanol, phenol, concentrated sulfuric acid, stannous chloride, ninhydrin, ethyl acetate, and n-butanol were of analytical grade and produced from Shanghai Aladdin Biochemical Technology Co., Ltd.

2.2. Methods

2.2.1. Preparation of RLT samples collected at different piling times

Samples of RLT with different piling time were collected from the RLT production line of Qinyi Liupao tea Professional Cooperative of Cangwu County (wuzhou city, Guangxi, China). The following processes were used for RLT production. Fresh leaves were spread and dried in the air for 8 h and fixed at 220 °C. The leaves were subjected to primary rolling, piling (surrounding temperature as 40 °C, humidity as 90 %), second rolling, and drying (90 ± 5 °C and then 100 ± 5 °C). Samples were collected during piling every 3 h from

0 to 30 h using the five-point sampling method, which is to take equal amounts of tea samples from the surface and middle layers of the tea pile [18].

2.2.2. Sensory evaluation

Sensory evaluation was evaluated by ten experienced professional tea evaluators (six men and four women, aged from 25 to 45 years) with 5–20 years of professional experience in tea studies, according to the standards of China GB/T 23776–2018 and GB/T 14487–2017 respectively, using the password evaluation method. The full score of 100 marks was assigned to individual evaluation, and the weighted score was calculated based on shape (15 %), brew color (10 %), aroma (30 %), taste (35 %), and infused leaf (10 %). Briefly, 3.0 g tea sample was infused with 150 mL boiled water for 5 min. Samples were coded by three-digit numbers, and they were randomly provided to tasters after infused; the brew color, aroma, taste and infused leaves were evaluated after infused, respectively; evaluators were informed about the study's objectives and provided written consent to conduct the sensory analysis in accordance with the GIBGZARCAS Research Ethics Board's (GREB) ethical guidelines. To elucidate the impact of piling time on flavor quality contributed by taste and aroma compounds in RLT, preliminary sensory evaluation was followed by scoring five taste attributes (mellow, thick, bitter, astringent, and strong) and five aroma attributes (floral, sweet, woody, herbal, and aged). The average score of each attribute is used for chart drawing. Scores range from 0 to 10, with higher scores indicating higher taste or aroma intensity, where 0 represents no or imperceptible intensity, and 10 represents extremely high intensity [19]. The appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of this research. All panelists were informed and the informed consent was obtained from all participants for this experiment.

2.2.3. Analysis of biochemical compounds present in the RLT samples

The content of water extract was determined in accordance with the standard of China GB/T 8305–2013 [20]. Tea polyphenol content was determined using the Folin–Ciocalteu method described in the standard of China GB/T 8313–2018 ($\lambda_{max} = 765$ nm) [21]. Total flavonoid content was determined by aluminum chloride colorimetry ($\lambda_{max} = 510$ nm) [22].The content of free amino acids was determined according to the standard of China GB/T 8314–2013 ($\lambda_{max} = 570$ nm) [23]. The content of soluble sugars and tea polysaccharides was determined using the phenol-sulfuric acid method described in the standard of China GB/T 40632–2021 ($\lambda_{max} = 490$ nm) [24]. Thearubigin and theabrownin content was determined according to the agricultural industry standard of China NY/T 3675–2020 ($\lambda_{max} = 380$ nm) [25]. Theaflavin content was determined using spectrophotometry ($\lambda_{max} = 380$ nm) [26]. Absorbance was measured using a T6 UV–Vis spectrophotometer.

The content of GA, eight catechins, and three alkaloids was determined using high-performance liquid chromatography (HPLC) as described in GB/T 8313–2018 [21] with slight modification. The LC-2030C HPLC system (Shimadzu, Japan) was equipped with a ChromCore AQ-C18 column (4.6 mm × 250 mm, 5 μm, NanoChrom, China). The flow rate was 0.8 mL/min, the column oven temperature was 35 °C, the detection wavelength was 278 nm, the mobile phase A was 5 % acetonitrile, and the mobile phase B was 80 % acetonitrile. The gradient elution was as follows: 0–20 min, 100 % A; 20.01–55 min, 100 %–75 % A; and 55.01–65 min, 100 % A. Curves for HPLC is provided in **Appendix S1**.

2.2.4. Analysis of aroma compounds

Qualitative and quantitative analysis of the aroma compounds in RLT by HS-SPME/GC–MS analysis.

2.2.4.1. Sample pretreatment. Headspace solid-phase microextraction (HS-SPME) [5] was used to extract aroma compounds. This method has been appropriately modified. Exactly 3.0 g of pulverized tea sample was placed in a 20 mL headspace vial. Subsequently, 12 mL of boiling water was added and the mixture was thoroughly mixed. Ethyl caprate solution (20 μL, 8.64 mg/L) was added and the vial was sealed and equilibrated at 60 °C for 10 min. A manual 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, Supelco, USA) fiber holder was inserted into the headspace and allowed to adsorb the volatile components for 60 min. The extraction head was immediately inserted into the injection port of a gas chromatography–mass spectrometer (GC-MS) for desorption (250 °C for 15 min) and sample analysis.

2.2.4.2. GC–MS analysis. Aroma compounds were analyzed using an Agilent 7890A-5975C GC-MS system equipped with a DB-5MS column (30 m × 250 μm × 0.25 μm, Agilent, USA) [5]. The temperature program was as follows: initial temperature 40 °C, held for 3 min, increased to 90 °C at 3 °C/min, held for 5 min, increased to 200 °C at 4 °C/min, then to 260 °C at 15 °C/min, and held for 10 min. The solvent delay time was 5 min. The carrier gas was high-purity helium (>99.999 %), the carrier gas flow rate was 1 mL/min, and the injection mode was splitless. The MS conditions were as follows: electronic ionization (EI) mode, ion source temperature 230 °C, ionization energy 70 eV, emission current 34.6 μA, quadrupole temperature 150 °C, interface temperature 280 °C, electron multiplier voltage 350 V, and mass scanning range 35–400 amu. Curves for GC-MS is provided in Appendix S1.

2.2.4.3. Qualitative and quantitative analyses. Qualitation was performed by searching the National Institute of Standards and Technology (NIST, USA) 05.L database (≥80 % match) and PubChem database (https://pubchem.ncbi.nlm.nih.gov). The odor characteristics of aroma compounds were identified according to the literature [1,3,27] and by searching the ChemicalBook database (https://www.chemicalbook.com). Quantification of aroma compounds was performed according to the literature [28], the mass concentrations of the volatile metabolites were calculated with reference to the internal standard method using equation (1):

$$C_i = \frac{C_{is} \times A_i}{A_{is}} \quad (1)$$

where C_i is the mass concentration of any component (μg/L), C_{is} is the mass concentration of the internal standard (μg/L) (ethyl caprate, 8.64 mg/L), A_i is the chromatographic peak area of any component, and A_{is} is the chromatographic peak area of the internal standard.

2.2.5. Statistical analysis

Three replicates were used for each experiment. The results were expressed as mean ± standard deviation. Origin 2021 software (Origin Lab, Massachusetts, USA) was used for data processing and plotting radar and line charts. IBM SPSS Statistics 20 (SPSS, Inc., Chicago, IL, USA) was used for one-way analysis of variance (ANOVA) ($P < 0.05$, significant; $P < 0.01$, highly significant) and Pearson correlation analysis. The R Programming Language (https://www.r-project.org/) was used for hierarchical cluster analysis (HCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA).

Table 1
Sensory description and score results of RLT during the piling process.

Times	Shape		Colour		Aroma		Taste		Infused leaf		Total score
	Description	Score	Description	Score	Description	Score	Description	Score	Description	Score	
0h	Still tight; more uniform; yellow-green	80.40 ± 1.42^{bc}	Yellow-green	79.60 ± 0.84^{f}	Floral; fruity and sweet	81.20 ± 1.31^{d}	Mellow; thick; bitter; astringent	75.00 ± 0.94^{f}	Bright yellow-green; soft	79.50 ± 0.85^{b}	78.58 ± 0.46^{f}
3h	Still tight; more uniform; yellow-green	79.80 ± 1.03^{c}	Yellow-green	82.30 ± 1.70^{ef}	Floral; fruity and sweet	83.90 ± 0.99^{c}	Mellow; thick; bitter; astringent	77.60 ± 0.52^{e}	Bright yellow-green; soft	81.90 ± 1.85a	80.72 ± 0.65^{e}
6h	Still tight; more uniform; green- yellow	81.80 ± 1.93^{bc}	Bright yellow	83.90 ± 3.84^{de}	Floral; fruity with slightly sweet	84.20 ± 0.79^{c}	Mellow; thick; bitter; astringent	78.40 ± 0.52^{e}	Bright green-yellow; soft	82.40 ± 1.07^{a}	81.60 ± 0.41^{e}
9h	Still tight; more uniform; brown-yellow	82.60 ± 1.43^{ab}	Bright yellow	84.40 ± 1.58^{de}	Strong floral and fruity	85.40 ± 1.43^{b}	Mellow; thick; slightly astringent	81.80 ± 0.79^{d}	Bright green-yellow; soft	82.60 ± 0.52^{a}	83.34 ± 0.41^{d}
12h	Still tight; more uniform; brown-yellow	82.80 ± 1.40^{ab}	Bright orange-yellow	86.00 ± 3.12^{cd}	Strong floral and fruity	88.00 ± 1.49^{b}	Mellow; thick; slightly astringent	83.80 ± 2.44^{d}	Bright green-yellow; soft	82.00 ± 1.33^{a}	84.95 ± 0.85^{c}
15h	Still tight; more uniform; yellow-brown	82.40 ± 2.00^{ab}	Bright orange-yellow	88.40 ± 2.27^{bc}	Strong floral and fruity	87.80 ± 1.40^{b}	Mellow; thick; slightly astringent	85.40 ± 0.84^{c}	Slightly dark yellow; soft	81.60 ± 1.71^{ab}	85.59 ± 0.52^{bc}
18h	Still tight; more uniform; yellow-brown	83.60 ± 2.00^{a}	Red-yellow	89.00 ± 2.31^{abc}	Floral; fruity and slightly aged	88.40 ± 1.43^{b}	Barely strong and mellow	88.60 ± 3.37^{b}	Slightly dark yellow; soft	83.60 ± 1.26^{a}	87.33 ± 1.52^{a}
21h	Still tight; more uniform; yellow-brown	83.20 ± 1.81^{a}	Red-yellow	90.40 ± 1.07^{ab}	Floral; fruity and slightly aged	90.60 ± 1.95^{a}	Barely strong and mellow	89.60 ± 1.43^{ab}	Dark yellow-brown; soft	82.20 ± 2.04^{a}	88.28 ± 0.75^{a}
24h	Still tight; more uniform; black brown	83.40 ± 1.07^{a}	Orange-red	90.60 ± 1.07^{ab}	Floral; fruity and slightly aged	88.60 ± 1.26^{b}	Barely strong and mellow	91.00 ± 1.15^{a}	Dark yellow-brown; soft	82.80 ± 1.55^{a}	88.28 ± 0.72^{a}
27h	Still tight; more uniform; black brown	83.40 ± 1.71^{a}	Orange-red	91.20 ± 1.40^{ab}	Floral; fruity and age	88.60 ± 0.52^{b}	Strong and mellow	90.40 ± 2.17^{ab}	Dark yellow-brown; soft	82.20 ± 2.04^{a}	88.07 ± 0.69^{a}
30h	Still tight; more uniform; black brown	83.40 ± 1.71^{a}	Orange-red	91.80 ± 0.79^{a}	Floral; fruity and age; slightly unpleasant	81.80 ± 1.69^{d}	Strong and mellow	91.40 ± 1.07^{ab}	Dark red-brown; soft	81.60 ± 1.43^{ab}	86.38 ± 0.80^{b}

Each tea sample was determined with ten replications. And all data were present by mean value ± SD. Different lowercase letters in the same column indicate a significant difference at the $P < 0.05$ level.

3. Results

3.1. Changes in flavor quality during the piling process

As shown in Table 1, the tea leaves remained tightly rolled and the integrity was unaffected during the piling process. The leaves showed a sequential transition of color in the order of yellow-green, green-yellow, brown-yellow, yellow-brown, and black-brown. The tea infusion showed a corresponding color transition of green-yellow, bright yellow, bright orange-yellow, red-yellow, and orange-red. The aroma in tea infusions was detected in the order of floral, fruity and sweet, strong floral and fruity, floral, fruity and slightly aged, floral, fruity and aged, and slightly unpleasant. The taste was initially mellow, thick, bitter, and astringent, which changed to mellow, thick, and slightly astringent, then barely strong and mellow, and finally strong and mellow. The color of spent tea leaves followed a sequence of bright yellow-green, green-yellow, slightly dark yellow, dark yellow-brown, and dark red-brown. Studies have shown that RLT prepared with different raw materials (tea tree cultivars) and processing duration have different flavor quality. The color of tea infusion can be yellow, orange-yellow, light orange, red-yellow, or orange-red; the taste can be strong and thick, strong and mellow, or brisk and mellow; and the aroma can be floral, honeyed, or floral and aged [6,17]. In the present study, with the increase in piling time,

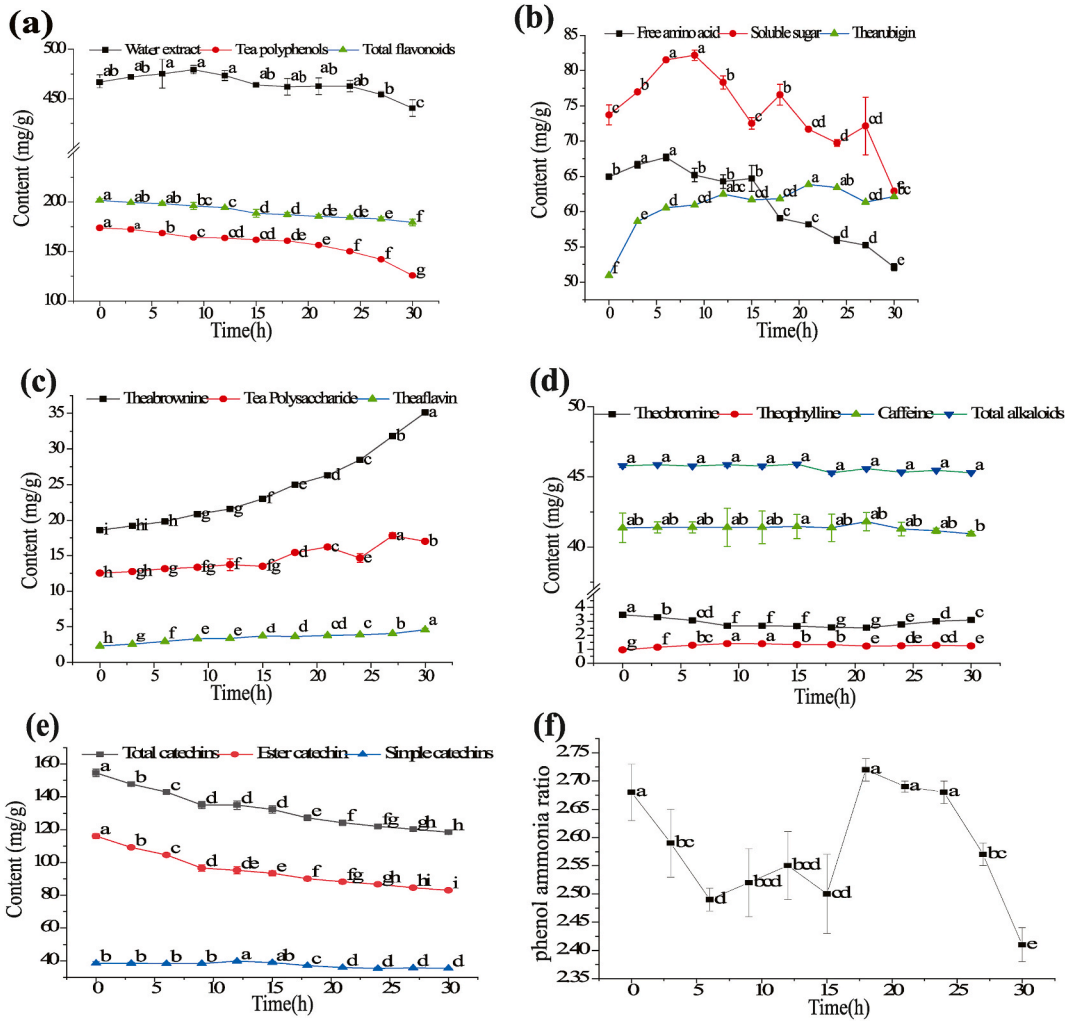

Fig. 1. Content changes of main biochemical components of RLT at different piling times. Errors bars depict ± standard deviation. (a) Changes of water extract, tea polyphenols, and total flavonoids contents during the piling. (b) Changes of free amino acid, soluble sugar and thearubigin contents during the piling. (c) Changes of theabrownine, tea polysaccharide and theaflavin contents during the piling. (d) Changes of theobromine, theophylline, caffeine and total alkaloids contents during the piling. (e) Changes of total catechins, ester catechin and simple catechins contents during the piling. (f) Changes of phenol ammonia ratio during the piling. Different small letters indicate significant differences at the $P < 0.05$ level. Total catechins were summation of (−)-gallocatechin, (−)-epigallocatechin, (+)-catechin, (−)-gallocatechin gallate, epigallocatechin gallate, (−)-catechin gallate, (−)-epicatechin gallate, and (−)-epicatechin contents. Ester catechin were summation of epigallocatechin gallate, (−)-gallocatechin gallate, and (−)-epicatechin gallate contents. Simple catechin were summation of (−)-gallocatechin, (−)-epigallocatechin, catechin, (−)-epicatechin, and (−)-catechin gallate contents. Total alkaloids were summation of theophylline, theobromine, and caffeine contents.

the tea infusion gradually showed orange-red color, strong and mellow taste, and floral and aged aroma. The RLT tea infusion flavor quality with 24 h piling treatment was characterized as orange-red color, barely strong and mellow taste, and floral, fruity and slightly aged aroma. With 27 h piling treatment, the flavor quality improved, and was characterized as orange-red color, strong and mellow taste, and floral, fruity and aged aroma. The reason why the content of compounds with floral and fruity aromas increased and then decreased may be the combined effect of dampness and heat during the piling process and enzymes produced by vigorous microbial metabolism [29]. However, the tea samples collected at 30 h possessed an unpleasant odor. Thus, with the raw materials and processing conditions used in this study, the flavor quality of RLT increased and then decreased during the piling process. In addition, given the 3 h sampling interval, accurately identifying the timing of unpleasant odor occurrence was challenging. Hence, we recommend that the piling time of RLT does not exceed 27 h.

3.2. Compounds influencing the taste of RLT during the piling process

The variations in biochemical compounds during the piling process are shown in Fig. 1a–f and Table 2. As the piling process progresses, water extract, free amino acids, and soluble sugars increased and then decreased ($P < 0.05$). At the end of piling, the water extract, free amino acids, and soluble sugars decreased by 5.62 %, 19.74 %, and 14.67 %, respectively. The content of tea polyphenols and total flavonoid had a significant decrease ($P < 0.05$) of 27.63 % and 11.07 % at the end of piling, respectively. Caffeine did not change significantly throughout the piling process ($P > 0.05$). Theobromine decreased and then increased ($P < 0.05$). Theophylline fluctuated but generally showed a significant increase ($P < 0.05$). The contents of GA, theaflavin, and theabrownin increased significantly ($P < 0.05$). Tea polysaccharides and thearubigin showed fluctuations but significantly increased ($P < 0.05$). Total catechin and ester catechins decreased significantly ($P < 0.05$). Among catechins, EGCG showed the most substantial decrease, followed by ECG, EGC, and CG ($P < 0.05$), with decreases of 33.58 %, 13.46 %, 18.80 %, and 49.28 %, respectively.

HCA was performed on the biochemical compounds of RLT during the piling process, and the results are shown in Fig. 2a. The tea samples collected during 0–6, 9–15, 18–24, and 27–30 h clustered into separate groups. The content of tea polyphenols, ester catechins, and total flavonoid decreased; theobromine and the ratio of polyphenols to amino acids fluctuated and gradually decreased; water extract, soluble sugars, and free amino acids increased and then decreased. These compounds were relatively high at 0, 3, and 6 h of piling; therefore, the tea samples collected before 6 h were classified into one group. C, EC, and caffeine were high at 9, 12, and 15 h; thearubigin and GA were high at 18, 21, and 24 h; theaflavin, theabrownin, and tea polysaccharides were high at 27 and 30 h. These results show significant changes in the content of biochemical compounds during the four stages of piling, which is consistent with the sensory evaluation.

The radar chart illustrates the changes in taste at different piling stages (Fig. 3). The tea infusion of tea samples collected at 0, 3, and 6 h was mellow, thick, bitter, and astringent; the samples collected at 9, 12, and 15 h were mellow, thick, and astringent; the samples collected at 18, 21, 24, 27, and 30 h were mellow and strong, and the intensity of mellow and strong taste gradually increased with

Table 2
Changes in the content of GA and catechin components of RLT during the piling process.

Times	Content (mg/g)								
	GA	GC	EGC	C	EGCG	EC	GCG	ECG	CG
0h	0.767 ± 0.024^{f}	4.32 ± 0.07^{e}	20.90 ± 0.86^{a}	5.21 ± 0.04^{f}	81.69 ± 1.79^{a}	8.10 ± 0.13^{d}	1.83 ± 0.02^{a}	30.45 ± 0.52^{a}	2.07 ± 0.07^{a}
3h	0.855 ± 0.019^{e}	4.46 ± 0.07^{de}	19.81 ± 0.58^{b}	5.82 ± 0.03^{cd}	75.98 ± 0.37^{b}	8.41 ± 0.14^{bc}	1.66 ± 0.02^{b}	29.74 ± 0.21^{ab}	1.82 ± 0.07^{b}
6h	0.877 ± 0.021^{e}	4.58 ± 0.12^{d}	19.27 ± 0.45^{bc}	5.95 ± 0.06^{c}	72.28 ± 0.79^{c}	8.50 ± 0.10^{b}	1.60 ± 0.01^{c}	29.16 ± 0.30^{bc}	1.54 ± 0.06^{c}
9h	0.931 ± 0.025^{d}	5.89 ± 0.14^{abc}	18.37 ± 0.33^{d}	6.12 ± 0.04^{b}	65.11 ± 2.00^{d}	8.05 ± 0.13^{d}	1.62 ± 0.04^{bc}	28.62 ± 0.06^{cd}	1.21 ± 0.05^{d}
12h	0.920 ± 0.012^{d}	5.99 ± 0.21^{ab}	18.72 ± 0.29^{cd}	6.36 ± 0.09^{a}	64.36 ± 1.60^{de}	8.71 ± 0.07^{a}	1.56 ± 0.03^{d}	28.10 ± 0.81^{de}	1.17 ± 0.04^{de}
15h	1.046 ± 0.002^{c}	6.09 ± 0.21^{a}	18.44 ± 0.43^{d}	6.13 ± 0.04^{b}	62.99 ± 1.00^{e}	8.29 ± 0.13^{c}	1.53 ± 0.03^{d}	27.62 ± 0.63^{ef}	1.21 ± 0.03^{d}
18h	1.140 ± 0.019^{a}	5.79 ± 0.07^{bc}	17.32 ± 0.48^{e}	5.92 ± 0.10^{c}	59.93 ± 0.75^{f}	8.10 ± 0.07^{d}	1.46 ± 0.03^{e}	27.42 ± 0.71^{efg}	1.24 ± 0.04^{d}
21h	1.140 ± 0.019^{a}	6.02 ± 0.26^{ab}	16.50 ± 0.23^{ef}	5.74 ± 0.10^{d}	58.41 ± 0.64^{fg}	7.70 ± 0.09^{e}	1.44 ± 0.03^{ef}	27.17 ± 0.58^{efg}	1.20 ± 0.04^{de}
24h	1.140 ± 0.019^{a}	5.85 ± 0.12^{abc}	16.10 ± 0.56^{f}	5.88 ± 0.05^{cd}	57.16 ± 0.40^{gh}	7.55 ± 0.09^{e}	1.40 ± 0.03^{f}	26.89 ± 0.54^{fgh}	1.18 ± 0.04^{de}
27h	1.082 ± 0.007^{b}	5.70 ± 0.02^{c}	16.80 ± 0.23^{ef}	5.58 ± 0.16^{e}	55.58 ± 0.38^{hi}	7.60 ± 0.03^{e}	1.33 ± 0.02^{g}	26.56 ± 0.38^{gh}	1.11 ± 0.03^{ef}
30h	1.036 ± 0.008^{c}	5.64 ± 0.03^{c}	16.97 ± 0.17^{e}	5.52 ± 0.14^{e}	54.26 ± 0.33^{i}	7.35 ± 0.07^{f}	1.31 ± 0.02^{g}	26.35 ± 0.34^{h}	1.05 ± 0.06^{f}

Abbreviations: GA, gallic acid; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; C, (+)-catechin; GCG, (−)-gallocatechin gallate; EC, (−)-epicatechin; EGCG, epigallocatechin gallate; CG, (−)-catechin gallate; ECG, (−)-epicatechin gallate. Each tea sample was determined with three replications. All data were present by mean value ± SD. Different lowercase letters in the same column indicate a significant difference at the $P < 0.05$ level.

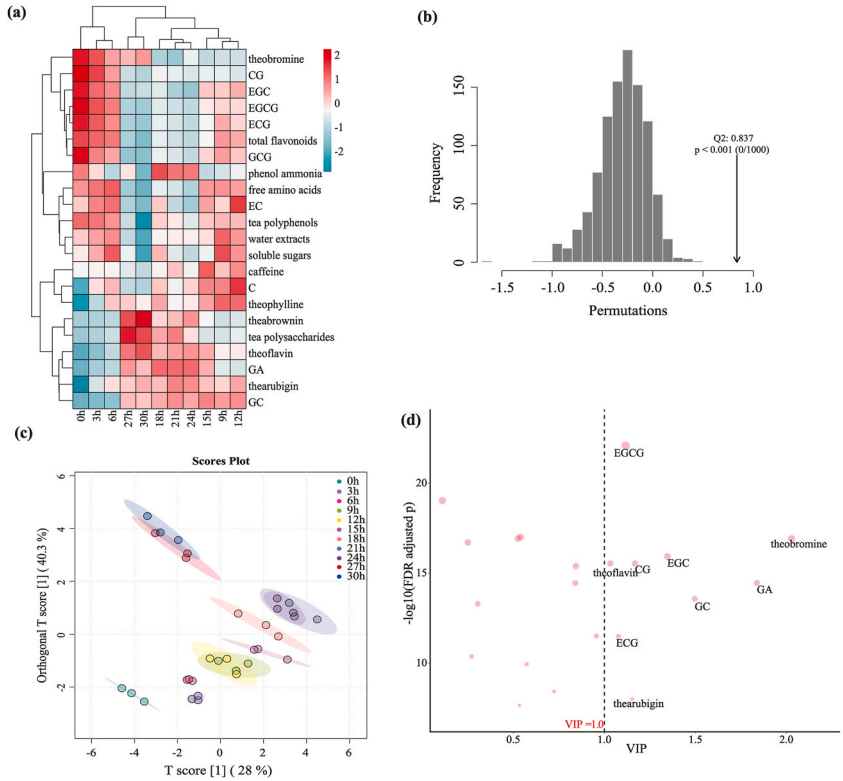

Fig. 2. Orthogonal partial least squares-discriminant analysis (OPLS-DA) and hierarchical cluster analysis (HCA) of biochemical compounds in RLT during the piling process. (a) hierarchical clustering heatmap. Red color indicates high concentration, and blue color indicates low concentration. (b) Scores plot of OPLS-DA. (c) Permutation test. (d) Variable importance in projection. Based on 22 detected compounds, OPLS-DA and HCA could divide RLT samples with different piling time into four groups (0–6, 9–15, 18–24, and 27–30 h). A total of 9 compounds critical for distinguishing the piling stages were identified in samples collected at 11 time points (VIP values > 1).

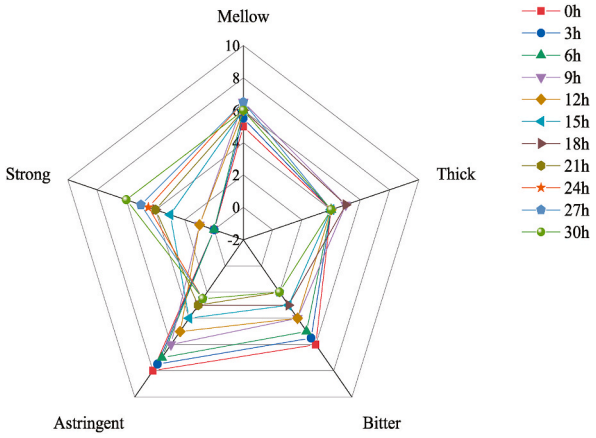

Fig. 3. Radar chart of RLT taste during the piling process. Scores range from 0 to 8, with higher scores indicating higher taste. The taste was initially mellow, thick, bitter, and astringent, which changed to mellow, thick, and slightly astringent, then barely strong and mellow, and finally strong and mellow.

increasing piling time.

To further clarify the key factors affecting the taste, the biochemical compounds of all tea samples were subjected to OPLS-DA. The Q^2 was estimated to be 0.837 ($P < 0.001$) in a permutation test (200 permutations) (Fig. 2b), which indicates high goodness of fit and successful model construction. The results showed that the tea samples with different piling time clustered into four groups (Fig. 2c), indicating different tastes at the four stages of piling. Furthermore, through variable importance in projection (VIP) analysis

(Fig. 2d), the key taste-contributing substances with VIP values > 1 were correlated with taste score [19]. Table 3 illustrates that theaflavin, thearubigin, GA, and GC showed highly significant negative correlations with bitterness and astringency ($P < 0.01$). Theaflavin and GA had highly significant positive correlations with strength ($P < 0.01$). Thearubigin had highly significant positive correlation with mellowness ($P < 0.01$). EGC, EGCG, ECG, and CG showed highly significant positive correlations with bitterness and astringency ($P < 0.01$). Theobromine showed significant positive correlations with thickness, bitterness, and astringency ($P < 0.05$). The bitterness and astringency of tea infusion decreased with significant decreases in EGC, EGCG, ECG, and theobromine, while the strength and mellowness increased with significant increases in theaflavin and thearubigin.

3.3. Compounds influencing the aroma of RLT during the piling process

HS-SPME-GC-MS was performed on tea samples collected at 11 time points during the piling process. The analysis identified 57 aroma compounds (Table 4), including 15 alcohols, 3 aldehydes, 7 ketones, 6 esters, 13 hydrocarbons, 7 heterocycles, and 6 other components.

The categories of aroma compounds are shown in Fig. 4a. These compounds were dominated by alcohols, hydrocarbons, ketones, and esters, which accounted for 88.33–92.53 % of aroma compounds. The relative content of alcohols increased and then decrease, but did not change significantly before and after piling. Alcohols accounted for more than half of aroma compounds. The relative content of hydrocarbons decreased significantly by 48.67 % at the end of piling. The relative content of ketones and esters showed significant increases of 28.58 % and 264.67 %, respectively. The relative content of other aroma compounds decreased by 39.79 %.

The content of compounds with floral and fruity aroma increased and then decreased (such as linalool, dihydrolinalool, and neryl alcohol). Their content was relatively high at 9, 12, and 15 h, ranging from 225.32 to 189.41, 109.81 to 130.44, and 73.23–88.34 μg/L, respectively. This agrees with the strong floral and fruity aroma in sensory evaluation. These compounds accounted for a large fraction of aroma substances in all samples, presumably contributing to the dominance of floral and fruity aroma. The content of α-cedrene with sweet aroma decreased significantly (by 43.34 %) during 0–6 h of piling, which might be the main reason for the diminished sweet aroma in tea infusion. After 15 h of piling, the content of cedrol with pinewood aroma, methyl salicylate with wintergreen oil aroma, germacrene D with woody aroma, and β-ionone and trans-nerolidol with floral aroma increased significantly. Their concentrations reached 39.93, 63.11, 27.21, 66.63, and 31.51 μg/L, respectively, at the end of piling.

The changes in aroma during the piling process are shown in Fig. 4b. The tea samples were dominated by floral and fruity aroma, and the intensity peaked at 15 h. With the increase in piling time, the sweet aroma decreased, while the aged, woody, and herbal aroma increased and peaked at 30 h.

To clarify the key compounds responsible for the differences in aroma during the piling process, the aroma compounds were subjected to OPLS-DA. In a permutation test (200 permutations), the Q^2 value was 0.976 ($P < 0.001$) (Fig. 5a), indicating that the model was well-fitted and successfully constructed. The tea samples of 0–6, 9–15, 18–24, and 27–30 h clustered into separate groups (Fig. 5b), indicating significant differences in the aroma of samples collected at different stages of piling. The aroma of tea infusion was initially floral, fruity and sweet, then strong floral, fruity and finally floral, fruity and aged. A total of 23 volatile compounds critical for distinguishing the piling stages were identified in samples collected at 11 time points (VIP values > 1) (Fig. 5c). These included cedrol, (E)-β-ocimene, methyl salicylate, methyl palmitate, 2-butyl-1-octanol, trans-nerolidol, ocimenol, pentadecane, decanal, 6,10-Dimethyl-2-undecanone, neryl alcohol, α-cedrene, methyl 2-methylpantanoate, (E, E)-2,4-heptadienal, benzaldehyde, and 2-methylpyrazine. In addition, aroma compounds such as β-violetone, linalool, dihydrolinalool, geranylacetone, linalool oxide (I), 1,2,3-trimethoxybenzene, and 1,2,4-trimethoxybenzene also contributed significantly to the changes in aroma during the piling process (VIP values > 0.5). The decrease in compounds with floral and fruity aroma and increase in compounds with woody, herbal, and aged aroma led to the shift from floral aroma to floral, fruity and aged aroma.

Table 5 shows the correlations between aroma and the key different compounds. Sweet aroma had a significant negative correlation with 2-butyl-1-octanol and 6,10-Dimethyl-2-undecanone ($P < 0.05$). Sweet aroma had a highly significant negative correlation with cedrol, methyl salicylate, methyl palmitate, trans-nerolidol, ocimenol, decanal, neryl alcohol, and (E, E)-2,4-heptadienal ($P < 0.01$). There was a significant positive correlation between sweet aroma and α-cedrene ($P < 0.05$). Sweet aroma had a highly significant positive correlation with (*S*)-(+)-5-methyl-1-hexanol, 3-(1-methylethyl)-2-cyclopentene-1-one, decanal, and hexahydro-1,8(2H,5H)-

Table 3
Correlation analysis between key biochemical components and taste of RLT with different piling times.

Component	Mellow	Thick	Bitter	Astringent	Strong
Theaflavin	0.687*	−0.850**	−0.950**	−0.941**	0.932**
Thearubigin	0.795**	−0.710*	−0.768**	−0.735**	0.580
GA	0.648*	−0.747**	−0.930**	−0.942**	0.868**
GC	0.716*	−0.893**	−0.809**	−0.792**	0.640*
Theobromine	−0.640*	0.642*	0.625*	0.610*	−0.398
EGC	−0.765**	−0.330	0.965**	0.945**	−0.889**
EGCG	−0.775**	0.788**	0.975**	0.966**	−0.910**
ECG	−0.715*	0.881**	0.981**	0.981**	−0.942**
CG	−0.846**	0.866**	0.877**	0.854**	−0.742**

Abbreviations: GA, gallic acid; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; EGCG = epigallocatechin gallate; ECG, (−)-epicatechin gallate; CG, (−)-catechin gallate. * * indicates significant correlation at 0.01 level (bilateral), and * indicates significant correlation at 0.05 level (bilateral).

Table 4
Identification results of aroma components in RLT during the piling process.

Compounds	CAS number	Odor Characteristics	0h	3h	6h	9h	12h	15h	18h	21h	24h	27h	30h
			Content (μg/L)										
Alcohols													
Linalool oxide II	60047-17-8	Woody; flowery	11.39 ± 0.26	11.82 ± 0.07	16.22 ± 0.27	13.17 ± 0.34	15.91 ± 0.21	19.88 ± 0.17	17.94 ± 0.10	17.96 ± 0.29	18.47 ± 0.13	20.55 ± 0.22	24.75 ± 0.22
Linalool oxide I	23007-29-6	Woody; flowery	9.33 ± 0.24	8.78 ± 0.17	14.56 ± 0.21	12.35 ± 0.14	14.14 ± 0.23	21.73 ± 0.24	18.1 ± 0.30	20.14 ± 0.48	19.89 ± 0.45	19.77 ± 0.17	29.83 ± 0.17
Linalool	78-70-6	Citrus-like, flowery	213.01 ± 1.56	214.66 ± 1.48	226.44 ± 2.12	225.32 ± 5.32	217.8 ± 2.02	189.41 ± 3.1	207.36 ± 2.89	184.61 ± 2.94	170.48 ± 3.07	143.83 ± 5.12	127.36 ± 2.06
3,7-Dimethyl-6-octen-3-ol	18479-51-1	Woody; citrus; blueberry; weed	98.34 ± 2.41	100.46 ± 1.47	104.78 ± 2.27	109.81 ± 2.27	114.35 ± 1.98	130.44 ± 2.77	120.93 ± 1	109.58 ± 1.13	99.61 ± 2.06	68.78 ± 1.33	65.9 ± 0.71
Phenylethyl alcohol	60-12-8	Rose scent	30.81 ± 0.52	39.43 ± 0.89	27.85 ± 2.03	29.53 ± 1.38	20.32 ± 4.4	24.08 ± 0.8	21 ± 0.63	29.67 ± 1.59	28.36 ± 1.18	26.62 ± 0.42	19.74 ± 0.26
Ocimenol	28977-58-4	Camphoreous	n.d	1.31 ± 0.13	2.88 ± 0.17	4.92 ± 0.17	10.07 ± 0.48	15.22 ± 0.63	15.22 ± 0.63	14.42 ± 0.28	13.71 ± 0.13	13.06 ± 0.27	13.06 ± 0.27
Neryl alcohol	106-25-2	Lemon-like; flowery	37.47 ± 1.76	47.48 ± 0.67	54.22 ± 1.75	73.23 ± 1.68	81.06 ± 4.62	88.34 ± 2.07	87.58 ± 2.25	81.35 ± 1.41	77.44 ± 0.79	85.14 ± 0.77	67.81 ± 1.91
2-Methoxybenzyl alcohol	612-16-8	–	n.d	n.d	n.d	n.d	n.d	2.80 ± 0.13	1.08 ± 0.06	3.41 ± 0.13	2.94 ± 0.51	2.18 ± 0.08	3.89 ± 0.13
Cycloheptanol	502-41-0	–	2.26 ± 0.08	1.95 ± 0.12	1.84 ± 0.08	n.d	n.d	0.98 ± 0.05	n.d	n.d	n.d	n.d	n.d
2-Aminobenzyl alcohol	5344-90-1	–	8.18 ± 0.12	6.09 ± 0.37	4.97 ± 0.30	2.13 ± 0.13	2.71 ± 0.13	0.59 ± 0.08	n.d	n.d	n.d	n.d	n.d
trans-Nerolidol	40716-66-3	Woody; flowery	n.d	1.48 ± 0.13	2.96 ± 0.13	3.97 ± 0.13	3.97 ± 0.13	15.34 ± 0.37	15.34 ± 0.53	20.38 ± 0.42	24.3 ± 0.64	20.61 ± 0.21	31.51 ± 0.63
2-Butyl-1-octanol	3913-02-8	Mushroom	n.d	n.d	n.d	n.d	0.09 ± 0.00	2.13 ± 0.13	1.90 ± 0.04	1.79 ± 0.05	1.73 ± 0.05	1.54 ± 0.05	1.01 ± 0.08
Cedrol	77-53-2	Woody	n.d	n.d	1.29 ± 0.05	1.45 ± 0.05	6.46 ± 0.47	15.46 ± 0.13	11.43 ± 0.13	13.39 ± 0.68	19.82 ± 0.21	23.77 ± 0.13	39.93 ± 0.51
(S)-(+)-5-methyl-1-heptanol	57803-73-3	–	4.65 ± 0.13	1.99 ± 0.10	1.23 ± 0.13	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Phytol	150-86-7	Floral	4.98 ± 0.13	3.28 ± 0.24	2.02 ± 0.16	1.97 ± 0.13	2.22 ± 0.04	2.59 ± 0.08	2.73 ± 0.1	3.43 ± 0.16	2.73 ± 0.13	2.21 ± 0.05	1.2 ± 0.05
Aldehyde													
Benzaldehyde	100-52-7	Bitter almond-like; marzipan-like	15.44 ± 0.60	19.16 ± 0.53	20.58 ± 0.21	10.35 ± 0.21	13.14 ± 0.34	13.35 ± 0.25	15.96 ± 0.32	14.25 ± 0.53	15.64 ± 0.21	20.10 ± 0.35	17.59 ± 0.51
(E,E)-2,4-Heptadienal	881395.00	Fatty; flowery	2.37 ± 0.06	2.875 ± 0.14	4.55 ± 0.14	2.87 ± 0.14	5.00 ± 0.5	5.54 ± 0.17	6.26 ± 0.13	6.15 ± 0.13	6.43 ± 0.13	7.1 ± 0.13	5.45 ± 0.17
Decanal	112-31-2	Fatty; flowery; orange-like	12.08 ± 0.44	10.29 ± 0.13	11.55 ± 0.25	5.65 ± 0.13	5.9 ± 0.35	4.06 ± 0.2	4.45 ± 0.08	4.57 ± 0.19	4.85 ± 0.29	6.26 ± 0.17	4.79 ± 0.06
Ketones													
3,5-Octadien-2-one	38284-27-4	Fruity; green	6.05 ± 0.26	7.30 ± 0.18	7.22 ± 0.23	6.99 ± 0.13	7.97 ± 0.08	7.35 ± 0.13	6.1 ± 0.17	7.87 ± 0.14	8.25 ± 0.10	10.6 ± 0.26	8.81 ± 0.17
3-(1-Methylethyl)-2-cyclopenten-1-one	1619-28-9	–	3.70 ± 0.17	3.45 ± 0.08	3.54 ± 0.20	0.22 ± 0.05	0.22 ± 0.05	n.d	n.d	n.d	n.d	n.d	n.d
4-Acetyl-2,3,4,5,5-pentamethyl-2-cyclopenten-1-one	50506-59-7	–	7.21 ± 0.25	7.80 ± 0.23	8.18 ± 0.16	3.69 ± 0.08	5.37 ± 0.08	4.73 ± 0.26	7.75 ± 0.21	3.61 ± 0.22	4.61 ± 0.30	10.6 ± 0.13	6.88 ± 0.08

(*continued on next page*)

Table 4 (*continued*)

Compounds	CAS number	Odor Characteristics	0h	3h	6h	9h	12h	15h	18h	21h	24h	27h	30h
			Content (μg/L)										
6,10-Dimethyl-5,9-undecadien-2-one	689-67-8	Fresh rose like; green; magnolia fruity	14.54 ± 0.29	11.69 ± 0.64	10.98 ± 1.12	6.49 ± 0.13	5.09 ± 0.71	3.89 ± 0.17	3.08 ± 0.21	4.64 ± 0.17	5.2 ± 0.25	3.63 ± 0.27	4.17 ± 0.63
β-ionone	14901-07-6	Flowery; violet-like	22.88 ± 3.07	28.61 ± 1.31	28.39 ± 0.39	32.86 ± 0.09	28.39 ± 0.54	42.11 ± 0.75	37.97 ± 2.06	45.46 ± 0.75	51.62 ± 1.82	56.57 ± 0.72	66.63 ± 0.17
Hexahydro-1,8(2H,5H)-naphthalenedione	83406-41-1	–	32.42 ± 0.74	29.29 ± 0.78	28.45 ± 1.38	20.9 ± 0.30	20.21 ± 0.18	18.57 ± 0.57	20.8 ± 0.85	14.65 ± 0.43	16.72 ± 0.29	20.44 ± 0.21	18.2 ± 1.48
6,10-Dimethyl-2-undecanone	1604-34-8	Musty	4.68 ± 0.30	4.99 ± 0.13	6.47 ± 0.19	8.26 ± 0.07	11.73 ± 0.17	11.3 ± 0.1	13.7 ± 0.26	10.96 ± 0.13	9.06 ± 0.25	10.88 ± 0.17	12.95 ± 0.27
Esters													
Methyl salicylate	119-36-8	Herbal; mint-like	3.36 ± 0.17	21.47 ± 0.17	31.54 ± 0.25	39.93 ± 0.25	40.65 ± 1.44	46.14 ± 0.77	46.02 ± 1.26	46.05 ± 1.11	48.32 ± 0.6	51.64 ± 1.16	63.11 ± 0.13
Methyl 2-methylpentanoate	2177-77-7	Fruity	5.90 ± 0.21	7.13 ± 0.22	6.71 ± 0.22	8.3 ± 0.22	4.95 ± 0.22	3.1 ± 0.08	2.24 ± 0.05	3.36 ± 0.3	3.41 ± 0.4	4.42 ± 0.17	3.61 ± 0.17
Hexyl 2-(1-naphthyl) acetate	2876-73-5	–	7.07 ± 0.30	7.29 ± 0.09	6.51 ± 0.12	7.07 ± 0.13	5.4 ± 0.13	3.83 ± 0.17	4.56 ± 0.29	4.33 ± 0.34	4.17 ± 0.13	6.32 ± 0.29	5.48 ± 0.29
Didodecyl phthalate	2432-90-8	–	8.60 ± 0.19	9.77 ± 0.41	9.02 ± 0.24	8.6 ± 0.15	9.67 ± 0.17	6.79 ± 0.37	8.02 ± 0.29	7.58 ± 0.29	7.58 ± 0.29	6.4 ± 0.17	4.73 ± 0.17
Methyl hexadecanoate	112-39-0	Waxy	n.d	n.d	5.68 ± 0.17	4.53 ± 0.17	5.45 ± 0.22	4.31 ± 0.26	5.42 ± 0.26	7.44 ± 0.26	7.44 ± 0.26	10.6 ± 0.17	15.77 ± 0.38
Ethyl hexadecanoate	628-97-7	Waxy	4.73 ± 0.13	5.13 ± 0.07	8.34 ± 0.12	12.37 ± 0.67	10.4 ± 0.3	7.89 ± 0.25	6.21 ± 0.25	14.88 ± 0.29	10.96 ± 0.29	15.46 ± 0.17	15.46 ± 0.17
Hydrocarbon													
(E)-β-ocimene	3779-61-1	Sweet herbal flavor	n.d	n.d	7.56 ± 0.34	6.63 ± 0.22	10.18 ± 0.1	7.33 ± 0.17	5.56 ± 0.13	5.82 ± 0.38	5.82 ± 0.38	9.7 ± 0.17	7.19 ± 0.17
Tridecane	629-50-5	–	8.65 ± 0.15	1.73 ± 0.05	1.65 ± 0.13	1.76 ± 0.15	1.31 ± 0.05	0.87 ± 0.05	1.57 ± 0.05	0.7 ± 0.05	0.64 ± 0.05	n.d	n.d
Pentadecane	629-62-9	Waxy	11.07 ± 0.25	6.71 ± 0.30	3.47 ± 0.13	3.47 ± 0.13	3.08 ± 0.13	2.96 ± 0.05	2.07 ± 0.05	3.75 ± 0.13	3.97 ± 0.1	4.75 ± 0.17	3.08 ± 0.17
Tetradecane	629-59-4	Waxy	5.45 ± 0.25	3.71 ± 0.17	2.87 ± 0.17	2.73 ± 0.12	3.11 ± 0.09	2.91 ± 0.26	2.43 ± 0.08	3.3 ± 0.13	3.61 ± 0.22	3.83 ± 0.13	2.21 ± 0.05
4,5-Dimethyl-nonane	17302-23-7	–	2.34 ± 0.09	2.43 ± 0.30	n.d	1.03 ± 0.05	0.89 ± 0.05	0.87 ± 0.05	0.98 ± 0.05	0.81 ± 0.05	0.81 ± 0.05	0.42 ± 0	n.d
(E)-1,3-pentadiene	2004-70-8	–	3.06 ± 0.21	1.03 ± 0.13	2.84 ± 0.15	2.7 ± 0.11	3.13 ± 0.05	3.52 ± 0.17	4.00 ± 0.13	4.45 ± 0.08	3.69 ± 0.17	2.99 ± 0.05	1.43 ± 0.08
α-Cedrene	469-61-4	Sweety	58.47 ± 2.94	55.30 ± 1.74	28.97 ± 0.89	30.05 ± 2.16	26.31 ± 0.51	8.89 ± 0.3	13.37 ± 0.73	17 ± 0.51	22.03 ± 4.78	25.3 ± 0.78	23.01 ± 0.68
Germacrene D	23986-74-5	Woody	4.11 ± 0.52	4.05 ± 0.32	10.43 ± 0.68	14.65 ± 0.13	16.75 ± 1.31	14.71 ± 0.26	17.04 ± 0.44	14.82 ± 0.4	18.73 ± 0.13	22.29 ± 0.79	27.21 ± 0.19
1,3-Dimethyl-naphthalene	575-41-7	–	4.82 ± 0.18	2.72 ± 0.13	0.90 ± 0.14	0.85 ± 0.1	0.82 ± 0.14	1.93 ± 0.15	1.03 ± 0.05	1.03 ± 0.05	2.71 ± 0.05	3.52 ± 0.08	1.2 ± 0.05
Acenaphthene	83-32-9	–	42.57 ± 0.58	38.65 ± 1.98	32.45 ± 1.12	29.65 ± 0.81	22.38 ± 0.34	13.79 ± 0.21	14.01 ± 0.3	15.75 ± 0.42	16.22 ± 0.13	20.1 ± 0.63	11.46 ± 0.13
(+)-Delta-cadinene	483-76-1	Herbal; woody	29.39 ± 1.09	28.55 ± 1.19	18.21 ± 1.26	20.58 ± 0.17	22.38 ± 0.61	12.39 ± 0.46	17.92 ± 0.97	13.03 ± 0.26	13.31 ± 0.26	14.71 ± 0.29	10.88 ± 0.26
2,3,6-Trimethyl-naphthalene	829-26-5	–	11.35 ± 0.50	8.83 ± 0.31	6.76 ± 0.55	6.65 ± 0.26	8.13 ± 0.45	6.74 ± 0.21	7.19 ± 0.29	5.23 ± 0.17	5.79 ± 1.1	5.48 ± 0.68	3.94 ± 0.15
Anthracene	120-12-7	–	18.12 ± 0.29	15.27 ± 0.34	17.42 ± 0.46	13.9 ± 0.84	9.98 ± 0.63	9.12 ± 0.26	6.49 ± 0.34	10.23 ± 0.34	8.84 ± 0.26	10.74 ± 0.77	10.74 ± 0.77

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Table 4 (*continued*)

Compounds	CAS number	Odor Characteristics	0h	3h	6h	9h	12h	15h	18h	21h	24h	27h	30h
			Content (μg/L)										
heterocycles													
1,2- Dimethoxybenzene	91-16-7	Stale	n.d	n.d	n.d	0.34 ± 0.08	0.95 ± 0.05	4.11 ± 0.17	3.89 ± 0.26	5.23 ± 0.13	8.22 ± 0.08	9.79 ± 0.17	11.94 ± 0.26
1,2,3- Rimethoxybenzene	634-36-6	Stale; musty	n.d	n.d	n.d	0.22 ± 0.13	1.2 ± 0.05	3.13 ± 0.57	2.61 ± 0.31	4.45 ± 0.47	6.68 ± 0.17	7.86 ± 0.13	10.71 ± 0.34
1,2,4- Trimethoxybenzene	135-77-3	Stale; musty	n.d	n.d	n.d	0.28 ± 0.05	0.2 ± 0.05	2.63 ± 0.17	2.32 ± 0.1	4.03 ± 0.47	7.75 ± 0.7	4.47 ± 0.17	8.61 ± 0.13
2,3-Dihydro-2-methyl-benzofuran	1746-11-8	–	6.32 ± 0.10	n.d	n.d	2.91 ± 0.1	5.59 ± 0.21	6.71 ± 0.09	3.91 ± 0.21	8.16 ± 0.12	6.57 ± 0.09	5.48 ± 0.34	5.68 ± 0.13
Dibenzofuran	132-64-9	Soil odor	28.30 ± 1.70	20.73 ± 1.69	12.92 ± 2.22	9.17 ± 0.43	5.82 ± 0.63	3.02 ± 0.15	3.86 ± 0.15	3.22 ± 0.17	2.66 ± 0.05	2.04 ± 0.13	n.d
3-butenyl pentyl Ether	34061-78-4	–	2.41 ± 0.06	2.61 ± 0.10	2.05 ± 0.13	2.22 ± 0.04	2.22 ± 0.04	1.34 ± 0.08	1.31 ± 0.05	2.6 ± 0.08	2.02 ± 0.25	1.54 ± 0.05	n.d
Heptylhexylether	7289-40-9	–	2.74 ± 0.13	2.96 ± 0.048	1.12 ± 0.16	1.06 ± 0.13	1.06 ± 0.13	1.99 ± 0.05	2.29 ± 0.13	2.32 ± 0.1	1.82 ± 0.05	1.87 ± 0.05	2.94 ± 0.08
others													
m-Anisidine	536-90-3	–	n. d	n. d	n. d	0.95 ± 0.05	0.95 ± 0.05	4.22 ± 0.17	4.89 ± 0.17	5.98 ± 1.21	4.53 ± 0.22	3.69 ± 0.08	4.89 ± 0.13
(*E*)-5-(pentyloxy)-2-pentene	56052-85-8	Fruity	3.71 ± 0.35	4.03 ± 0.08	2.68 ± 0.08	2.9 ± 0.12	n. d	1.87 ± 0.05	n. d	0.95 ± 0.05	0.75 ± 0.17	n. d	n.d
2-methylpyrazine	109-08-0	–	3.77 ± 0.18	3.96 ± 0.05	3.16 ± 0.13	3.16 ± 0.13	3.16 ± 0.13	2.21 ± 0.05	2.49 ± 0.1	1.79 ± 0.05	2.24 ± 0.21	2.68 ± 0.08	3.63 ± 0.13
2-Hydroxy-3-cyanopyridine	20577-27-9	–	4.36 ± 0.34	4.42 ± 0.21	3.75 ± 0.13	4.5 ± 0.1	5.34 ± 0.92	1.06 ± 0.05	1.87 ± 0.05	1.06 ± 0.05	1.15 ± 0.17	1.23 ± 0.05	1.57 ± 0.05
5-Methyl-isothiazole	693-97-0	–	2.04 ± 0.05	2.09 ± 0.08	1.51 ± 0.17	1.65 ± 0.05	1.65 ± 0.05	2.04 ± 0.05	n. d	1.68 ± 0.08	1.15 ± 0.05	n.d	n.d
Caffeine	58-08-2	–	14.4 ± 1.19	14.18 ± 0.93	19.18 ± 0.29	17.87 ± 0.08	13.73 ± 0.26	6.68 ± 0.13	6.63 ± 0.38	5.54 ± 0.22	4.54 ± 0.15	5.26 ± 0.17	6.93 ± 0.17

Each tea sample was determined with three replications. All data were present by mean value ± SD. nd means not detected.

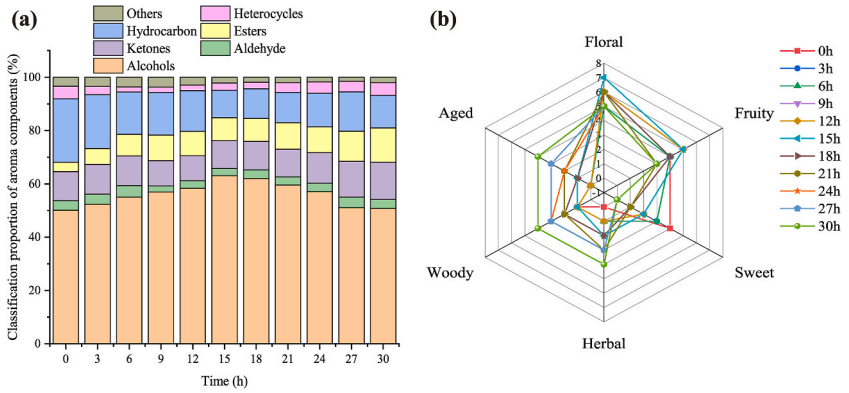

Fig. 4. Relative content of aroma compounds and radar chart of aroma in RLT during the piling process.
(a) Relative content of aroma compounds in RLT during the piling process. (b) Radar chart of RLT aroma during the piling process. Scores range from 0 to 8, with higher scores indicating higher taste or aroma intensity. The aroma of tea infusion was initially floral, fruity and sweet, then strong floral, fruity and finally floral, fruity and aged.

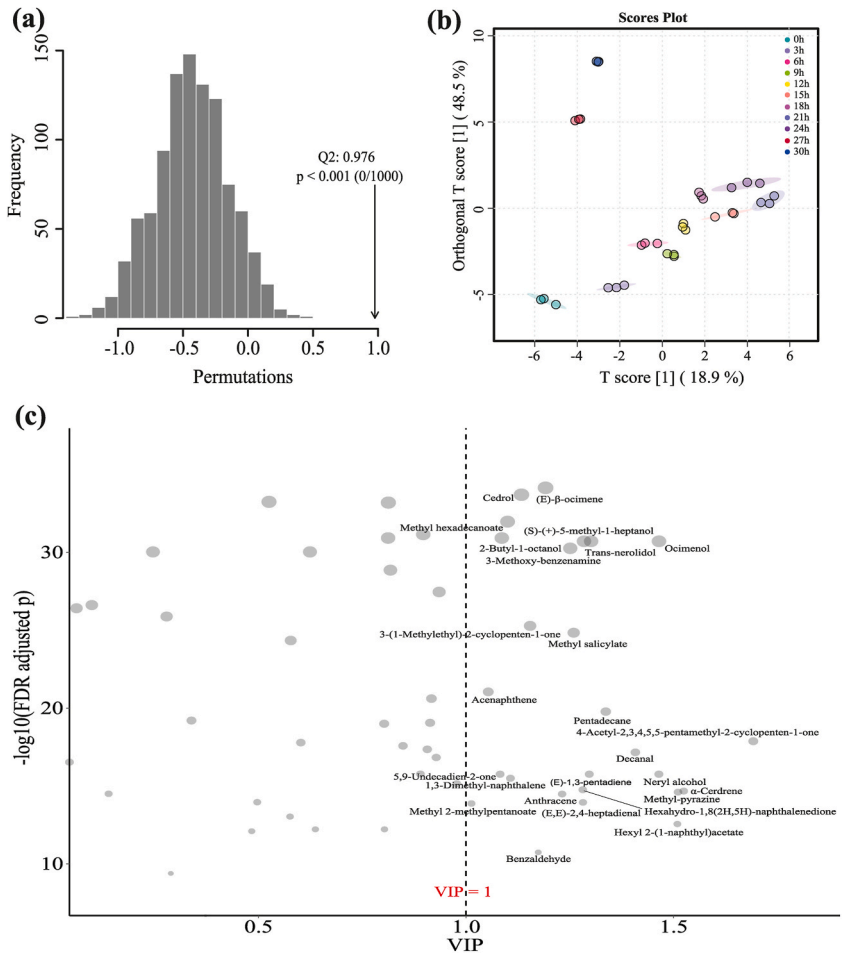

Fig. 5. Orthogonal partial least squares-discriminant analysis (OPLS-DA) of aroma compounds in RLT during the piling process. (a) Scores plot of OPLS-DA. (b) Permutation test. (c) Variable importance in projection. The tea samples of 0–6, 9–15, 18–24, and 27–30 h clustered into separate groups, indicating significant differences in the aroma of samples collected at different stages of piling. A total of 23 volatile compounds critical for distinguishing the piling stages were identified in samples collected at 11 time points (VIP values > 1).

Table 5
Correlation analysis between key volatile components and aroma types.

Components	Floral	Fruity	Sweet	Herbal	Woody	Aged
Cedrol	−0.151	−0.635*	−0.866**	0.923**	0.949**	0.962**
(*E*)-*β*-ocimene	0.225	−0.009	−0.602	0.430	0.337	0.354
Methyl salicylate	0.233	−0.318	−0.906**	0.871**	0.705*	0.753**
(S)-(+)-5-methyl-1-heptanol	−0.429	0.059	0.774**	−0.662*	−0.410	−0.456
Methyl hexadecanoate	−0.122	−0.534	−0.814**	0.872**	0.844**	0.890**
2-Butyl-1-octanol	0.362	−0.411	−0.705*	0.711*	0.522	0.599
trans-Nerolidol	−0.027	−0.680*	−0.909**	0.979**	0.910**	0.946**
Ocimenol	0.449	−0.296	−0.831**	0.793**	0.583	0.665*
m-Anisidine	0.335	−0.494	−0.798**	0.868**	0.661*	0.768**
Pentadecane	−0.518	0.112	0.570	−0.512	−0.226	−0.283
3-(1-Methylethyl)-2-cyclopenten-1-one	−0.497	−0.123	0.827**	−0.694*	−0.518	−0.576
4-Acetyl-2,3,4,5,5-pentamethyl-2-cyclopenten-1-one	−0.371	−0.138	0.118	−0.018	0.076	0.164
Decanal	−0.558	0.086	0.792**	−0.689*	−0.480	−0.534
6,10-Dimethyl-2-undecanone	0.580	−0.027	−0.688*	0.674*	0.452	0.585
(*E*)-1,3-pentadiene	0.557	0.025	−0.173	0.104	−0.106	−0.012
Neryl alcohol	0.614*	0.008	−0.720*	0.566	0.317	0.400
α-Cedrene	−0.552	0.080	0.689*	−0.623*	−0.373	−0.440
Methyl 2-methylpentanoate	−0.359	0.343	0.588	−0.659*	−0.502	−0.596
(*E, E*)-2,4-heptadienal	0.209	−0.445	−0.802**	0.785**	0.598	0.686*
Hexahydro-1,8(2H,5H)-naphthalenedione	−0.394	0.292	0.859**	−0.776**	−0.57	−0.607*
Hexyl 2-(1-naphthyl) acetate	−0.471	0.216	0.599	−0.577	−0.377	−0.397
Benzaldehyde	−0.584	−0.463	0.001	0.184	0.243	0.280
2-Methylpyrazine	−0.466	0.257	0.558	−0.486	−0.231	−0.296

* * indicates significant correlation at 0.01 level (bilateral), and * indicates significant correlation at 0.05 level (bilateral).

naphthalenedione ($P < 0.01$). There was a significant positive correlation between neryl alcohol and floral aroma ($P < 0.05$). Cedrol, methyl salicylate, methyl palmitate, and trans-nerolidol showed highly significant positive correlations with herbal, woody, and aged aroma ($P < 0.01$). The m-anisidine showed highly significant positive correlations with herbal and aged aroma ($P < 0.01$), and a significant positive correlation with woody aroma ($P < 0.05$). There is a strong positive correlation between 2-methylpyrazine, Methyl 2-methylpentanoate and fruity aroma.

4. Discussion

4.1. Effects of key biochemical compounds on taste at different piling stages

Tea taste quality is closely linked to the content and proportion of taste-contributing compounds [3]. Water extract is a general term for the water-soluble substances in tea leaves. High water extract indicates a high content of taste-contributing compounds and high re-steepability [30]. The water extract of tea samples before and after piling were over 40 %, indicating the high quality of RLT as a raw material. Free amino acids are major contributors to the umami and brisk taste of tea infusion and are involved in the formation of tea aroma [31,32]. The content of free amino acids increased and then decreased. The increase may be ascribed to the hydrolysis of soluble proteins in tea leaves in the hot and humid environment at the early stage of piling following fixation. The decrease may be attributed to the consumption of amino acids as a nitrogen source by microorganisms at the late stage of piling, as well as their involvement in the Maillard reaction with reducing sugars [33]. Tea polyphenols are important active components in tea leaves and contribute to the bitterness and astringency of tea infusion [32,34]. Total flavonoid is responsible for the green-yellow color of tea infusion [35] and partly responsible for the bitter and astringent taste of tea infusion. Catechins are important tea polyphenols and contributors to bitter and astringent taste [28]. Catechins can be divided into ester catechins and simple catechins [32], where ester catechins are important contributors to the convergent nature of tea polyphenols in tea infusion. Theobromine is the principal compound responsible for the bitter taste of tea infusion [18]. In this study, the content of bitter and astringent substances such as tea polyphenols, total flavonoid, EGCG, ECG, EGC, and CG decreased significantly during the piling process, and the ratio of polyphenols to amino acids fluctuated and finally decreased. EGCG, ECG, EGC, and CG had a significant positive correlation to bitterness and astringency. Additionally, theobromine showed significant positive correlations with bitterness and astringency, and its content decreased at the end of piling. Consequently, the decreases in polyphenols and theobromine surpassed the consumption of amino acids, leading to a reduction in the bitterness and astringency of tea infusion.

Tea pigments (theaflavin, thearubigin, and theabrownin) are macromolecules formed by the oxidation and polymerization of tea polyphenols (mainly catechins) and their derivatives [36]. These components contribute to the formation of the mellow, thick, strong taste, and red color of Liupao tea infusion [18]. Thearubigin is the major contributor to the red color of tea infusion [36]. Theaflavin increases the brightness and strong taste of black tea infusion [36]. In the present study, theaflavin increased significantly, thearubigin increased with fluctuations, and theabrownin increased significantly during the piling process. This result may be ascribed to the conversion of thearubigin to theabrownin surpassing the formation of thearubigin. In addition, the ester catechin EGCG could be degraded to produce GA and simple catechins during piling [37], which partly explains the significant increase in GA. Sensory evaluation showed that with the increase in piling time, the color of tea infusion gradually changed from green-yellow to orange-red,

and the taste changed from mellow, thick, bitter, and astringent to strong and mellow, which were closely related to the increases in theaflavin and thearubigin. Correlation analysis further revealed that GA, GC, theaflavin, and thearubigin were highly significantly or significantly positively correlated with mellowness and strength.

In summary, the decreases in EGCG, ECG, EGC, CG, and theobromine and the increases in GA, GC, theaflavin, and thearubigin are important factors contributing to the formation of strong and mellow taste during the piling process. The dynamic changes of the biochemical compounds and the taste of RLT during piling can be divided into four stages: 0–6, 9–15, 18–24, and 27–30 h. Therefore, the alterations in biochemical compounds during the piling process change the taste of tea infusion changed from mellow, thick, bitter, and astringent to strong and mellow, and the color shifts from yellowish-green to orange-yellow, red-yellow, and orange-red. The color and taste (strong, mellow, bitter, and astringent) of tea infusion can be used to distinguish RLT at different piling stages.

4.2. Effects of key volatile compounds on aroma at different piling stages

The volatile compounds and their relative content were determined in tea samples collected at 11 time points. The results explain the changes in the aroma of tea samples collected at different piling stages. The 57 aroma compounds were dominated by alcohols, hydrocarbons, esters, and ketones, with alcohols having the highest relative content in all samples, which agrees with the findings of Liu et al. [13]. With the increase in piling time, the relative content of alcohols significantly increased and then decreased. Concurrently, the relative content of compounds with woody, herbal, and aged aroma increased. This trend aligns with previous research on raw dark tea prepared with pile fermentation in a traditional process [29]. The development of aged aroma during the piling process was closely related to the decrease in alcohols with floral and fruity aroma and the increase in compounds with woody, herbal, and aged aroma.

Volatile compounds demonstrate distinctive aromatic characteristics; for instance, alcohols commonly give a floral aroma [4]. In the present study, volatile compounds were correlated with aroma at various stages of piling. The results showed that neryl alcohol had a significant positive correlation with floral aroma [38], and its content increases and then decrease during piling. This might explain the increase and decrease in floral aroma. Moreover, the significant reduction in α-cedrene with sweet aroma could be responsible for the diminishing sweet aroma. In addition, cedrol with pinewood aroma, methyl salicylate with wintergreen oil and herbal aroma, trans-nerolidol with floral aroma, (E, E)-2,4-heptadienal with fatty aroma, 2-butyl-1-octanol with mushroom aroma, methyl palmitate with fruit aroma, and hexahydro-1,8(2H,5H)-naphthalenedione with dry mold odor were generated and accumulated during the piling process. These compounds showed significant positive correlations with aged, woody, and herbal aroma. We speculate that the unique aged aroma might be formed through the synergistic effect of woody, herbal, and aged aroma. However, Ma et al. [39] identified β-ionone as a key compound contributing to the aged aroma of Liupao tea. This discrepancy could be attributed to variations in raw materials and processing methods, resulting in differences in the type and content of aroma compounds, thus affecting the synergistic effect of aroma substances. In addition, the characterization of aroma for four volatile components, namely (S)-(+)-5-methyl-1-hexanol, m-anisidine, 3-(1-methylethyl)-2-cyclopenten-1-one, and hexahydro-1,8(2H,5H)-naphthalenedione, has not been reported. However, these compounds have important roles in the transition of tea infusion aroma, and the underlying mechanisms deserve further investigation. Methoxybenzene compounds such as 1,2,3-trimethoxybenzene are responsible for the aged aroma of aged dark tea; however, their strong mold odor may compromise the aroma quality of tea when present in high concentrations [36]. In our study, the relative content of methoxybenzene compounds with mold odor and hexahydro-1,8(2H,5H)-naphthalenedione with dry mold odor increased with piling time, while the relative content of compounds with floral aroma decreased, which may be responsible for the unpleasant odor in samples collected at 30 h. OPLS-DA showed that the changes in aroma during the piling process could be divided into four stages: 0–6, 9–15, 18–24, and 27–30 h, which is consistent with the hierarchical clustering of biochemical compounds. In summary, the decrease in compounds with sweet aroma and the increase in compounds with floral and fruity aroma promote the transition from floral, fruity and sweet aroma to strong floral, fruity aroma in the early stage of piling. At the late stage of piling, the significant decrease in alcohols with floral and fruity aroma, coupled with the accumulation of compounds with woody, herbal, and aged aroma, facilitate the transition from strong floral and fruity aroma to floral, fruity and aged aroma.

5. Conclusion

During the piling process, the sensory quality of RLT increased first and then decreased, and could be divided into four stages: 0–6, 9–15, 18–24, and 27–30 h. The flavor quality of RLT changed significantly with the variations in biochemical and volatile compounds. At the end of piling, the flavor of tea infusion was characterized by orange-red color, strong and mellow taste, and floral, fruity and aged aroma. EGCG, ECG, EGC, CG, theobromine, GA, GC, theaflavin, and thearubigin were identified as the important compounds affecting the transition from mellow, thick, bitter, and astringent to strong and mellow taste. The shift from floral, fruity and sweet aroma to floral, fruity and aged aroma was closely related to the changes in 19 characteristic aroma compounds, such as cedrol, methyl salicylate, methyl palmitate, trans-nerolidol, decanal, hexahydro-1,8(2H,5H)-naphthalenedione, neryl alcohol, α-cedrene, and (E, E)-2,4-heptadienal. This study provides insights into RLT flavor changes during the piling process and a scientific basis for optimizing the piling and production processes of RLT.

CRediT authorship contribution statement

Huazhen Huo: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Conceptualization. **Aihua Cai:**

Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Yunchang Xie:** Writing – review & editing, Supervision, Conceptualization. **Chunyu Guo:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Data availability

Data included in article/supplementary material/referenced in article.

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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

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