Research Article

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Changes in volatile compounds upon aging and drying in oolong tea production

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Abstract

BACKGROUND: Long-term storage (aging) with periodic drying of fresh oolong tea gives rise to so-called old oolong tea. Alteration of aroma compounds is expected when a fresh oolong tea is converted into an old one, as the two teas smell drastically different. The aim of this study was to compare the volatile compounds in fresh and old oolong teas.

RESULTS: Significant differences were observed between the volatile compounds in fresh and old oolong teas. This observation suggested that long straight chains of alcohols and acids were putatively decomposed while shorter-chain acids, their amide derivatives and many nitrogen-containing compounds were generated during the tea conversion processes. The overall patterns of volatile compounds observed in five different preparations of old oolong tea were fundamentally identical. This consensus pattern was different from that observed in oolong tea either stored for more than 10 years without drying or prepared at relatively low temperatures and short baking time.

CONCLUSION: Characteristic aroma nitrogen-containing compounds, including *N*-ethylsuccinimide, 2-acetylpyrrole, 2-formylpyrrole and 3-pyridinol, were consistently found in the examined old oolong teas. These compounds might be regarded as typical constituents at least for a certain kind of old oolong tea.

Keywords: aging; aroma; drying process; old oolong tea; volatile compounds

INTRODUCTION

Various types of tea are mainly classified into green tea (unfermented), oolong tea (partially fermented) and black tea (fully fermented) according to the degree of fermentation during their preparation, where the term 'fermentation' refers to natural browning reactions induced by oxidative enzymes in the cells of tea leaves.[1] Oolong tea is the most favourite choice for Taiwanese owing to the special taste and odour generated during the versatile processes for its preparation. In the preparation of oolong tea, young green shoots are freshly harvested and allowed to undergo a semi-fermentation process.[2] The fermentation degree of oolong tea ranges from 20 to 80% depending on the demand of customers.

Oolong tea tends to absorb substantial moisture from the air after a certain period of storage and thus needs to be refined by drying in a specialised oven. Periodic drying plays an important role in maintaining and improving the quality of oolong tea after long-term storage. Different drying protocols have been developed to produce variable types of old oolong tea heated at 80–140 °C for 8–72 h each time with storage intervals of 1–12 months. The heating temperature, duration and frequency may change in different times of drying for the same preparation. In general, tea is frequently heated at relatively low temperatures for a short baking time in the early stages of preparation. Traditionally, old oolong tea is the name given to those oolong teas that have been stored for more than 5 years and refined at least annually, batch after batch of approximately 5 kg of tea each time, at various desired temperatures. Empirically, the longer oolong tea is stored and further oxidised gradually, the better it is in terms of taste and beneficial effects to human health.[3]

Almost all preparations of old oolong tea possess some common characteristics, e.g. their infusions look dark red or black and taste slightly sour, together with the special sensation known for oolong tea. In our previous study, two distinct characteristics were observed between old and new oolong teas by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis.[3–6] One was the substantial accumulation of gallic acid in old oolong tea, presumably released from (−)-epigallocatechin gallate (EGCG) during the drying process. The substantial accumulation of gallic acid was in agreement with the decrease in pH value of tea infusion from 5.5 to 5 after conversion by periodic drying and might at least partly lead to the slightly sour taste commonly

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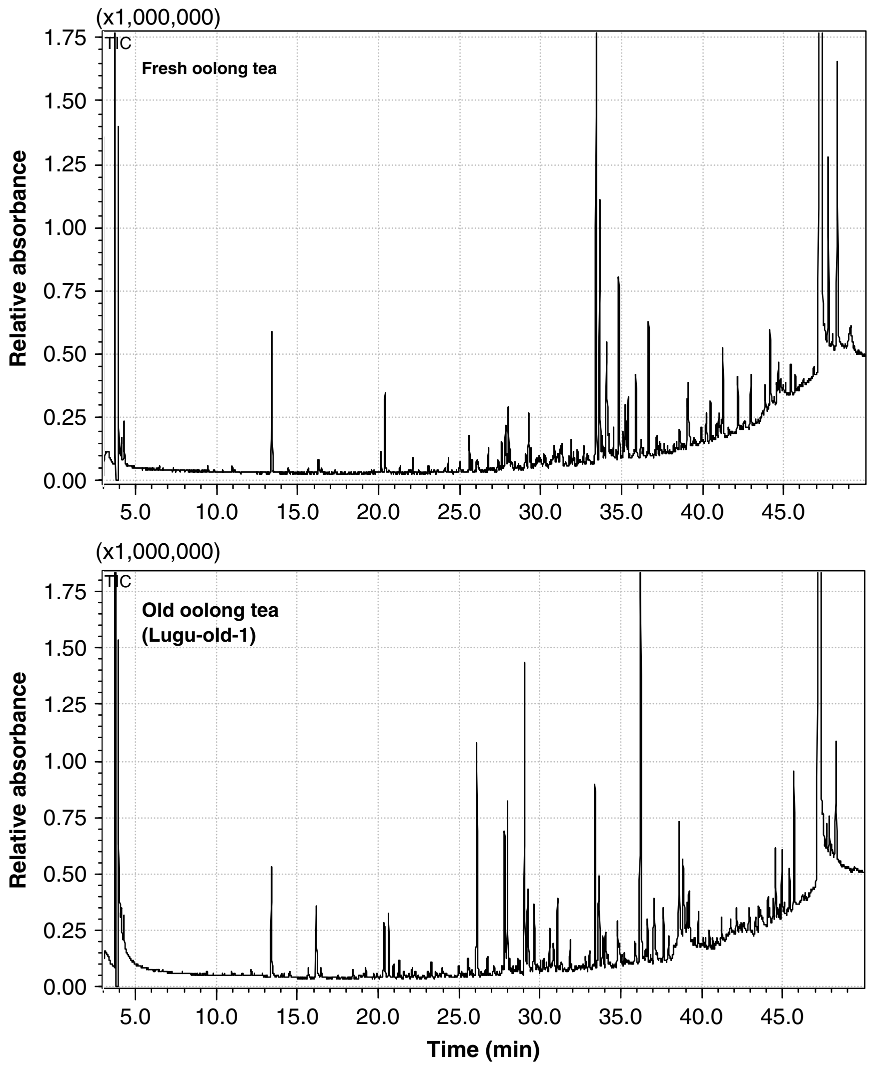

Figure 1. GC/MS chromatograms of volatile compounds in fresh and old oolong teas.

experienced for old oolong tea. The other characteristic was the unique occurrence of three flavonols, myricetin, quercetin and kaempferol, in old oolong tea. These three flavonols presumably resulted from deglycosylation of their glycoside derivatives in fresh oolong tea under the heating condition of periodic drying.

Besides the alteration of phenolic compounds detected by LC/MS/MS analysis in the previous study, alteration of aroma compounds is expected when a fresh oolong tea is converted into an old one under the tedious processes, since the smells of fresh and old oolong teas are distinctly different. Generally, the aroma composition of tea infusion is a significant impact factor in the evaluation of tea quality.[7] In the present study we aimed to compare the volatile compounds in fresh and old oolong teas by aroma extract dilution analysis with the aid of gas chromatography/mass spectrometry (GC/MS) and therefore to deduce the alteration of volatile compounds during the tea conversion processes. To evaluate the effects of long-term storage (aging) and mild drying on the alteration of volatile compounds, an oolong tea stored for more than 10 years without any drying and a semi-old oolong tea prepared at relatively low temperatures and short baking time were also analysed and compared.

MATERIALS AND METHODS

Chemicals and materials

All chemicals were purchased from E. Merck Co. (Merck KGaA, Darmstadt, Germany) unless stated otherwise. Water was purified using a Millipore clear water purification system (Millipore Direct-Q, Billerica, MA, USA). Fresh oolong tea (spring 2009) was prepared with young green shoots of tea (*Camellia sinensis* L., Chin-shin oolong) plants harvested from Lugu village, Nantou County, Taiwan following the traditional semi-fermentation process with a final fermentation degree of approximately 80%. Four preparations of old oolong tea from the same cultivar, termed Lugu-old-1 (autumn 2004), Lugu-old-2 (spring 2005), Lugu-old-3 (spring 2006) and Lugu-old-4 (autumn 2006), were provided by the same supplier of fresh oolong tea using continual refinement processes by drying at 120–140 °C for 10 h every 3–4 months for more than 3 years. The drier was composed of two parts, a charcoal heater made of stainless steel (90 cm in diameter and 60 cm in height) and a tea container with a steel net at the bottom (80 cm in diameter) encircled with dry bamboo sheet (40 cm in height). A preparation of old oolong tea termed Ali-old (spring 2006) was provided by a different supplier using a similar drying process with the same cultivar of oolong tea grown in Mountain Ali, Chayi County, Taiwan.

Table 1. Volatile constituents identified in aroma concentrate of fresh oolong tea and old oolong tea (Lugu-old-1)

Peak no.	Retention time (min)	Compound	Fresh oolong tea	Old oolong tea
1	12.16	2,5-Dimethylpyrazine	−	+
2	13.41	4-Hydroxy-4-methyl-2-pentanone	++	++
3	16.21	Acetic acid	+	++
4	18.47	Propanoic acid	−	+
5	20.14	3,7-Dimethyl-1,5,7-octatrien-3-ol	+	−
6	20.36	1,2-Ethanediol	++	++
7	20.64	*N*-Ethylacetamide	−	++
8	20.96	*N*-Ethylformamide	−	+
9	21.30	3-Furylmethanol	−	+
10	22.11	5-Ethyldihydro-2(3*H*)-furanone	+	+
11	24.29	2-(2-Butoxyethoxy)ethanol	+	−
12	25.59	Hexanoic acid	+	+
13	26.05	Benzyl alcohol	−	+
14	26.10	*N*-Ethylsuccinimide	−	+++
15	26.77	Phenylethyl alcohol	+	+
16	27.59	(3*E*)-2,6-Dimethyl-3,7-octadiene-2,6-diol	+	−
17	27.83	2-Methyl-3-hydroxypyrone	+	+++
18	28.00	2-Acetylpyrrole	+	+++
19	28.08	(*E*)-3-Hexenoic acid	+	+
20	29.05	2-Formylpyrrole	−	++++
21	29.26	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	+	++
22	29.65	2-Methylcyclohexane-1,3-dione	−	++
23	30.60	2-Formyl-1-methylpyrrole	−	+
24	31.08	Methyl 1,5-dimethyl-2-pyrrolecarboxylate	−	++
25	33.41	(2*E*)-2-Hexenyl hexanoate	++++	+++
26	33.61	2,6-Dimethoxyphenol	+++	−
27	33.63	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	−	++
28	33.66	Methyl 6-oxo-1,4,5,6-tetrahydro-3-pyridinecarboxylate	−	+
29	34.07	2-Methyl-3,5-dihydroxypyrone	++	+
30	34.80	4,4,7a-Trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4*H*)-one	+++	+
31	36.20	3-Pyridinol	−	++++
32	36.65	2*H*-1-Benzopyran-2-one	+++	+
33	37.62	5-(Hydroxymethyl)-2-furaldehyde	−	++
34	38.60	4-Hydroxy-3-methoxybenzaldehyde	−	++
35	39.09	5-Hydroxy-4,6-dimethyl-3-pyridine-methanol	++	++
36	39.19	Methyl 5-oxo-2-pyrrolidinecarboxylate	−	++

Intensities: ++++, $>1.2 \times 10^6$; +++, $>6 \times 10^5$; ++, $>3 \times 10^5$; +, $<3 \times 10^5$; −, undetectable.

A preparation of stored oolong tea, termed Lugu-stored (spring 1997), purchased from a local shop was prepared for more than 10 years by the same process as that for the preparation of fresh oolong tea without any follow-up drying process. A preparation of semi-old oolong tea, termed Ali-semi-old (spring 2008), was provided by another supplier using the same cultivar of oolong tea grown in Mountain Ali followed by continuous refinement of drying processes at 100–120 °C for 6 h every month for 6 months.

Preparation of tea infusions

Deionised hot water (100 °C, 4 L) was added to oolong tea (200 g) and left to brew for 3 min. The leaves were then removed using a coarse filter paper. The filtrate (3 L) was immediately cooled to 30 °C in tap water and extracted by ether with the assistance of a separation funnel. The ether layer was dried over anhydrous sodium sulfate and the solvent was removed by a rotary evaporator to 5 mL in volume at atmospheric pressure. The volatile compounds were concentrated under a nitrogen stream to 30 µL just before injection for GC/MS analysis.

GC/MS analysis and mass spectral identification

Tea infusions were analysed using a Shimadzu GC-2010 (Shimadzu, Chiyoda-ku, Tokyo, Japan) gas chromatograph/mass spectrometer equipped with a quadrupole mass analyser. Samples of 1 µL were injected onto the column. Analytical conditions were as follows: 30 m × 0.25 mm i.d., 0.25 µm BP-20 (forte, SGE, Ringwood, Victoria, Australia) column; carrier gas, helium at 29 mL min^{-1}. The column temperature was programmed from 50 to 260 °C at a rate of 5 °C min^{-1} in all runs. The injector temperature was 170 °C. The column flow rate was 1 mL min^{-1} and the split ratio was 1 : 20. The mass spectrometer was used under the following conditions: ionisation voltage, 70 eV; ion source temperature, 170 °C; interface temperature, 170 °C. Mass spectral identification was achieved by comparing spectra with the commercial mass spectral databases NIST and LIBTX. Components were tentatively

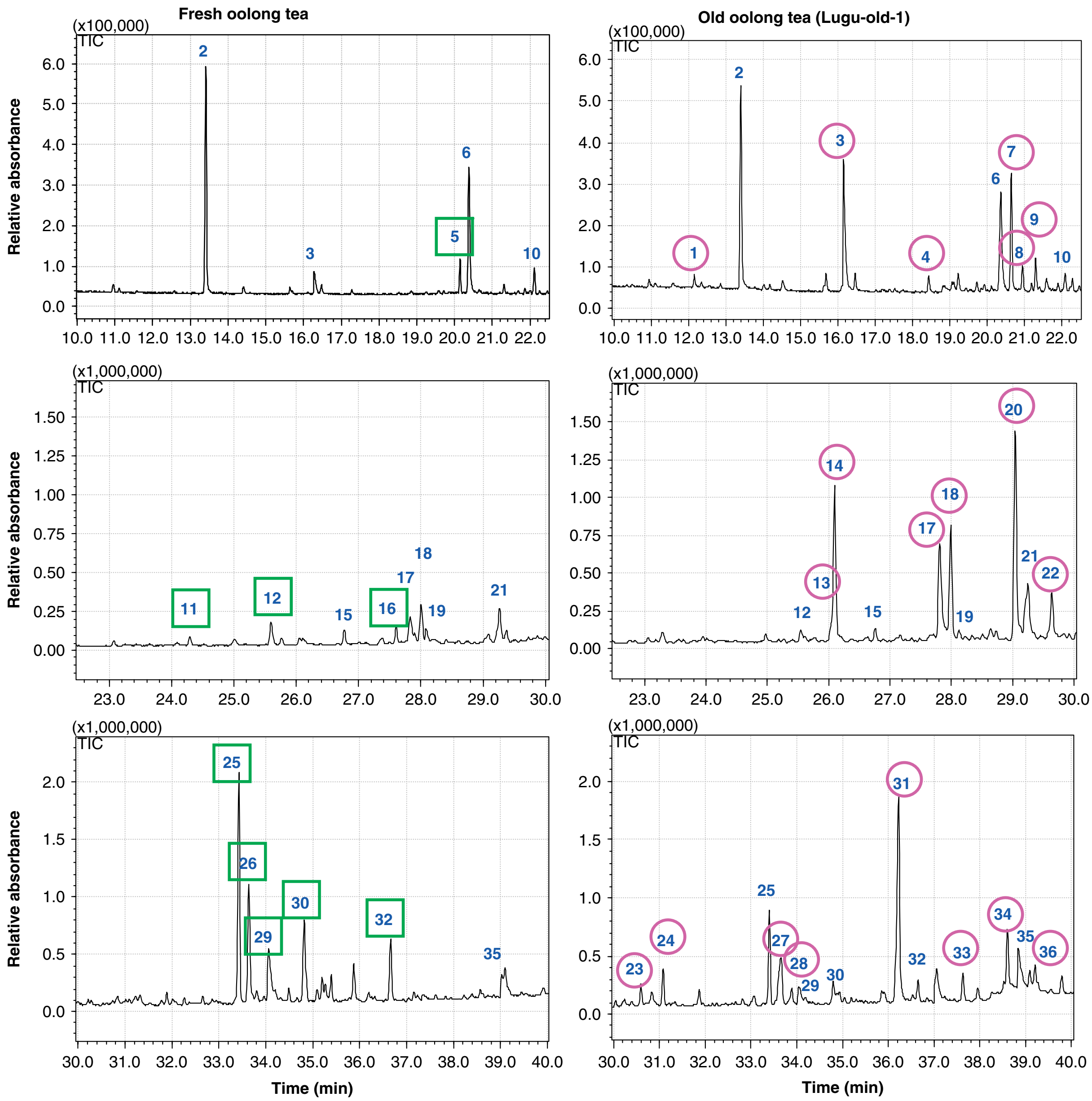

Figure 2. Expanded GC/MS chromatograms of volatile compounds in fresh and old oolong teas. Squares and circles indicate unique compounds or compounds with significantly higher contents in fresh and old oolong teas respectively. The names of tentatively identified compounds as well as their retention times are given in Table 1.

identified by agreement of their retention times and mass spectra with published data and, if available, with those of authentic compounds.[8]

RESULTS AND DISCUSSION

Analysis and comparison of volatile compounds in fresh and old oolong teas

Aroma constituents of various tea products are interesting research topics with potential commercial applications and have been continually investigated in human history. The first scientific studies were performed more than 70 years ago, leading to the identification of linalool, geraniol and (*Z*)-3-hexenol in the volatile fractions of tea leaf and beverage.[9] In this study, GC/MS chromatograms of the volatile compounds from a preparation of fresh oolong tea and a preparation of old oolong tea (Lugu-old-1) were compared (Fig. 1). Significantly different patterns of volatile compounds were observed for the fresh oolong tea and the old one. A total of 36 compounds were tentatively identified by agreement of their mass spectra (Table 1), and these components were mostly identified as the aroma compounds of tea and other vegetable food sources in previous studies.[7,10,11]

A detailed comparison between the volatile compounds of the fresh oolong tea and those of the old oolong tea is shown in Fig. 2. Among the 36 tentatively identified peaks, nine compounds found in the fresh oolong tea were undetectable or significantly less abundant in the old oolong tea (indicated by squares in the left panels of Fig. 2). In contrast, 20 principles found in the old oolong tea were undetectable or significantly less abundant in the fresh oolong tea (indicated by circles in the right panels of

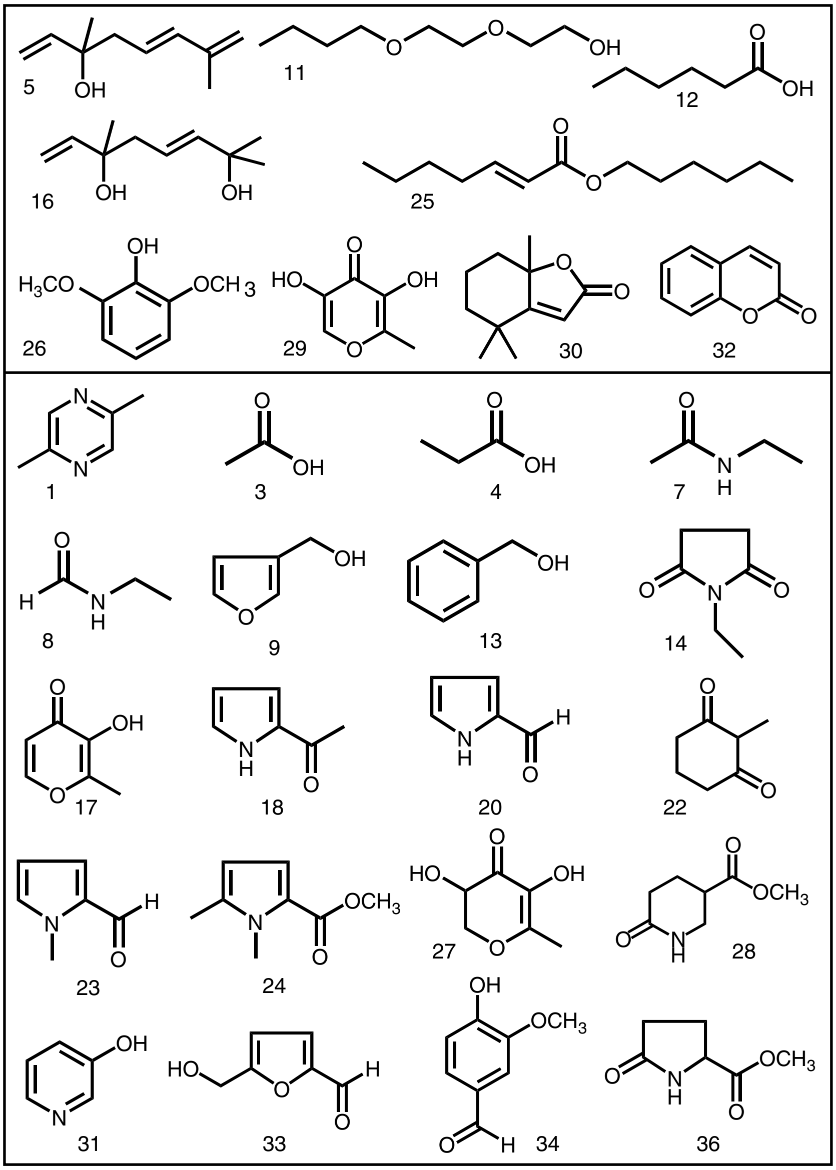

Figure 3. Chemical structures of those compounds with significantly different contents in fresh and old oolong teas as indicated in Fig. 2. The names of these compounds are given in Table 1.

Fig. 2). The chemical structures of these nine compounds and 20 principles showing significantly different amounts between the fresh and old oolong tea infusions are depicted in Fig. 3. The comparison suggested that long straight chains of alcohols and acids as well as some phenolic compounds (upper panel in Fig. 3) were putatively decomposed while shorter-chain acids and their amide derivatives (peaks 3, 4, 7 and 8) as well as many nitrogen-containing compounds such as pyrrole and pyridine derivatives (lower panel in Fig. 3) were presumably generated during the process of converting a fresh oolong tea into an old one. The putative changes in volatile compounds detected in the tea conversion process, including the elimination of long straight chains of alcohols and acids (having a strong herbal smell) and the generation of nitrogen-containing compounds (having a gentle baking smell), might explain how the conversion process could affect the odour impression in fresh and old oolong teas.

Judging from the relative contents of volatile compounds in the fresh oolong tea and the old one as well as their chemical structures as shown in Figs 1–3, we propose that 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (peak 27) might be converted from 2-methyl-3,5-dihydroxypyrone (peak 29) and that 4-hydroxy-3-methoxybenzaldehyde (peak 34) was possibly derived from 2,6-dimethoxyphenol (peak 26) during the drying processes. In addition, propanoic acid and acetic acid (peaks 4 and 3 respectively) probably resulted from the decomposition of hexanoic acid and (2*E*)-2-hexenyl hexanoate (peaks 12 and 25 respectively).

Aroma constituents of old oolong tea

According to the current study, aroma constituents in the old oolong tea are mainly composed of nitrogen-containing heterocycles, including *N*-ethylsuccinimide, 2-acetylpyrrole, 2-formylpyrrole and 3-pyridinol (peaks 14, 18, 20 and 31 respectively), which resulted in the baking smells. Of course, it is comprehensible that some minor unidentified constituents may also contribute to

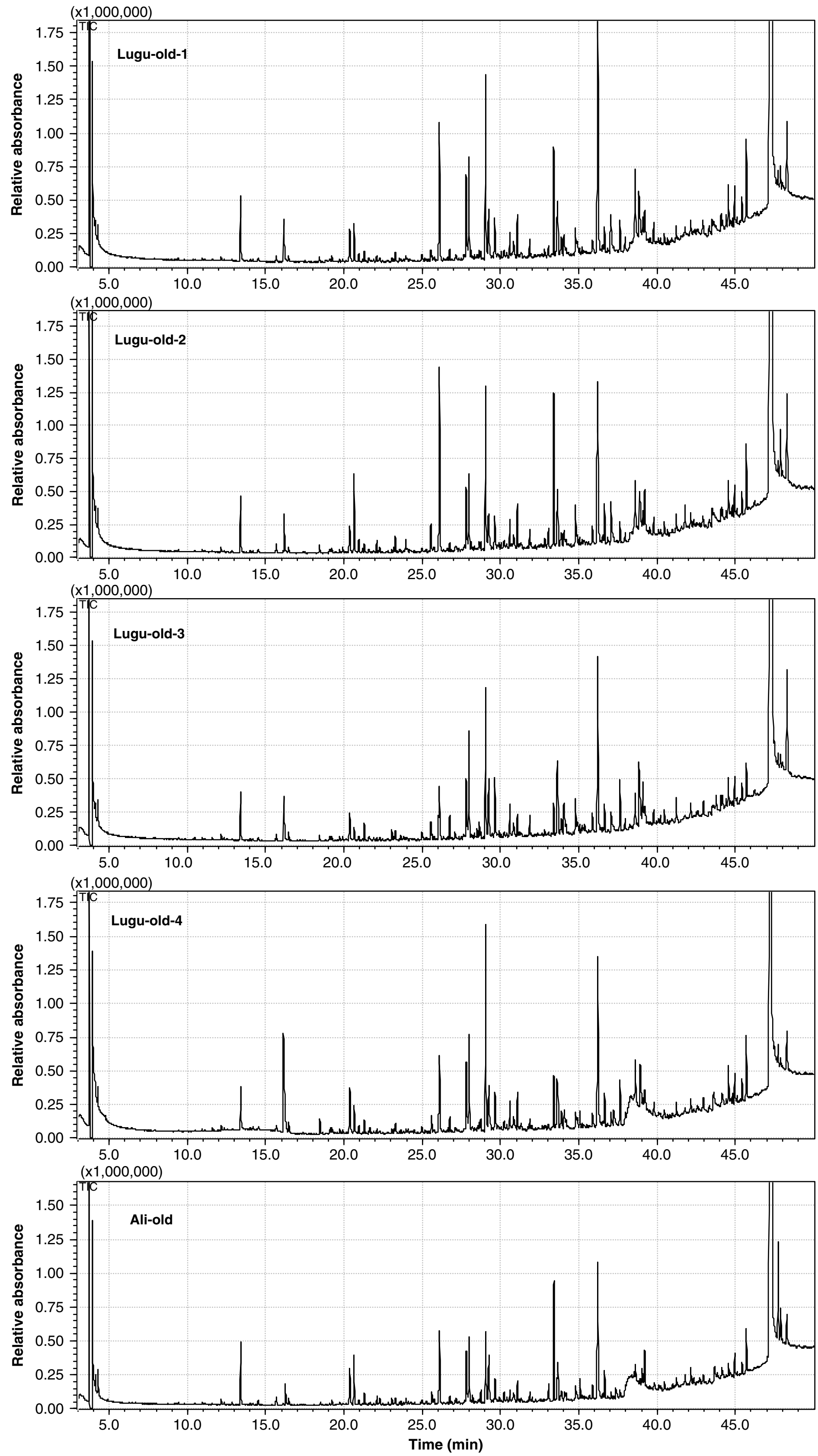

Figure 4. GC/MS total ion chromatograms of volatile compounds in five preparations of old oolong tea.

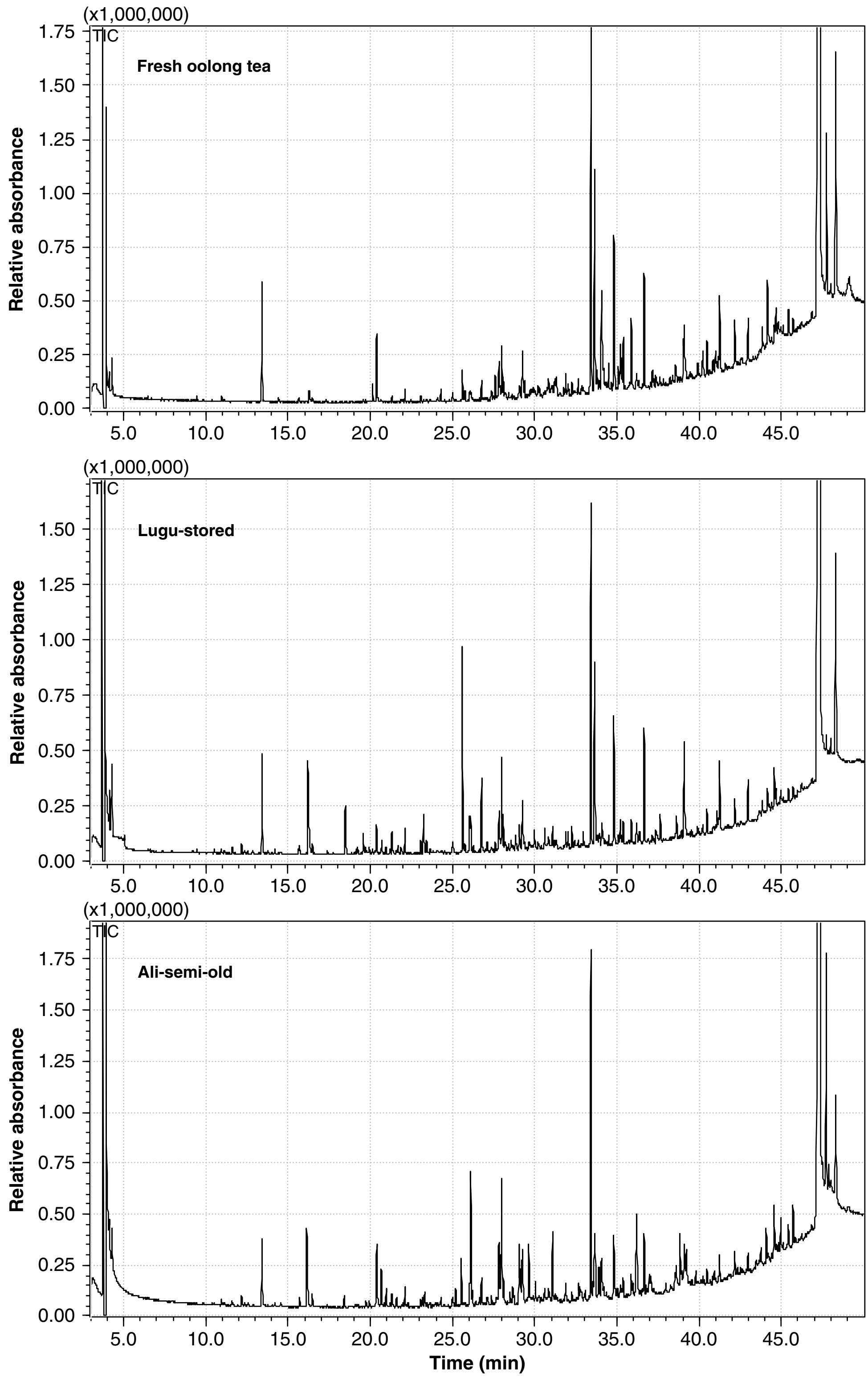

Figure 5. GC/MS total ion chromatograms of volatile compounds in oolong tea stored for 10 years without any drying (Lugu-stored) or treated with a mild drying process (Ali-semi-old).

the resultant smell of old oolong tea. *N*-Ethylsuccinimide is also found in black tea and is speculated to be derived from theanine by a Strecker degradation during tea fermentation.[12] Similar compounds such as *N*-ethylacetamide and *N*-ethylformamide are also putatively originated from amino acids present in tea preparation.[13] These findings further substantiate the importance of amino acids as precursors of tea volatiles.[14] 2-Acetylpyrrole and 2-formylpyrrole are the two most abundant and widely occurring pyrroles in foods.[15] 2-Acetylpyrrole has a caramel-like odour while 2-formylpyrrole has a sweetcorn-like odour. The mechanism for

Figure 6. Tea leaves and infusions of four different oolong teas. Tea leaves are shown before and after tea preparation.

the formation of pyrroles is assumed to be a consequence of the participation of proline and hydroxyproline in the Strecker degradation as originally proposed Hodge *et al.*[16] and lately supported by Tressl *et al.*[17]

Comparison of volatile compounds in different preparations of old oolong tea

To examine if the alteration of volatile compounds during the process of converting a fresh oolong tea into an old one is characteristic, volatile compounds from four other old tea preparations (Lugu-old-2, Lugu-old-3, Lugu-old-4 and Ali-old) were analysed by GC/MS and their total ion chromatograms were compared with that of Lugu-old-1 (Fig. 4). The results showed that the overall patterns of volatile compounds in these five preparations of old oolong tea were fundamentally identical, though the intensities of some flavour compounds varied among the preparations. Evidently, the conversion of odorants during drying processes could be consistently reproduced by different operators and by using different fresh oolong tea preparations. The major aroma compounds such as *N*-ethylsuccinimide, 2-acetylpyrrole, 2-formylpyrrole and 3-pyridinol found in the consensus pattern might be regarded as typical constituents at least for a certain kind of old oolong tea prepared under defined conditions of periodic drying processes.

Volatile compounds in oolong tea stored for more than 10 years or treated with mild drying processes

To examine the effects of long-term storage (aging) and mild drying processes on the aroma composition of oolong tea, volatile compounds in a preparation of oolong tea (Lugu-stored) stored for more than 10 years without any follow-up drying process and those in a preparation of semi-old oolong tea (Ali-semi-old) prepared by mild drying processes (relatively low temperatures and short baking time in comparison with the conversion processes of old oolong tea) were subjected to GC/MS analysis. The results showed that volatile compounds in both preparations were similar to those in the fresh preparation of oolong tea, though their intensities fluctuated in these three samples (Fig. 5). Consequently, the overall pattern of volatile compounds in either Lugu-stored or Ali-semi-old preparation was analogous to that in fresh oolong tea and thus different from that in old oolong tea. According to this observation, the drying process seemed to be more important than the aging process in terms of generating unique volatile compounds in old oolong tea, which could not be produced by either long-term storage without any drying or mild drying processes.

Two main factors have been emphasised in the preparation of old oolong tea: one is the drying (heating) process and the other is the aging conversion. Some suppliers mainly focus on refining the drying conditions while others believe in the indispensability of long-term storage for undefined aging reactions. In general, old oolong tea prepared by a combination of drying and aging processes has a better taste than that prepared by either drying or aging conversion. Assuming the presence of indispensable aging reactions in the preparation of old oolong tea, it will be motivating to see if these aging reactions could be technically replaced by chemical reactions in certain conditions, such that high quality of old oolong tea could be chemically converted from fresh oolong tea in a much shorter period of time, such as a few days or a couple of months.

Tea leaves and infusions of different oolong tea preparations

Tea infusions of fresh oolong tea, Lugu-stored, Ali-semi-old and Lugu-old-1 as well as their leaves before and after tea preparation were compared (Fig. 6). After storage for more than 10 years, Lugu-stored was no longer fresh yellow/green as seen for the fresh oolong tea. The infusion colour of Lugu-stored (dark yellow) was slightly darker than that of fresh oolong tea (light yellow). However, the tea leaves of Lugu-stored could fully expand to their original sizes like those of fresh oolong tea when they absorbed hot water in regular tea making. Mild drying processes led to a black colour of Ali-semi-old, and its tea leaves could no longer fully expand to their original sizes after tea making in a manner similar to old oolong tea. However, the infusion colour of Ali-semi-old was light yellow/red in comparison with the dark red (black) infusion of old oolong tea.

CONCLUSION

In this study, significant differences were observed in the volatile compounds in fresh and old oolong teas. Four major aroma nitrogen-containing heterocycles, *N*-ethylsuccinimide, 2-acetylpyrrole, 2-formylpyrrole and 3-pyridinol, were consistently found in the examined old oolong teas. These compounds were presumably ring-cyclised through condensation reactions during the tea conversion processes and could not be produced by either long-term storage or mild and short drying processes. Therefore they might be regarded as typical constituents at least for a certain kind of old oolong tea.

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