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Analytical Methods

Discrimination of teas with different degrees of fermentation by SPME–GC analysis of the characteristic volatile flavour compounds

Li-Fei Wang [a], Joo-Yeon Lee [b], Jin-Oh Chung [a], Joo-Hyun Baik [a], Sung So [a], Seung-Kook Park [b,*]

[a] *Tea Research Group, Amore-Pacific Corporation R&D Center, Yongin-si, Gyeonggi-do 446-729, Republic of Korea*
[b] *Department of Food Science and Biotechnology, Kyung Hee University, Seocheon-Dong, Giheung-Gu, Yongin-Si, Gyeonggi-do 446-701, Republic of Korea*

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Abstract

As tea is traded all over the world, it is necessary for both customs officers and business investigators to develop an easy and reliable method to discriminate teas from each other. A total of 56 kinds of various green, Oolong, and black teas were collected from different countries and markets, and their catechin contents and volatile flavour compounds (VFC) were compared by analyses, using HPLC and solid-phase microextraction–gas chromatograph (SPME–GC). It was found that neither total catechin nor individual catechin contents in green and Oolong teas were significantly different among the samples investigated, but the fermentation processes altered the profiles of tea VFC. Because many of the individual VFC did not change in response to the fermentation levels, several VFC in combination might be more reliable than a single compound to identify broader ranges of teas. A total concentration of five VFC, *trans*-2-hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate, and indole, was shown to be able to discriminate clearly unfermented and fermented teas, while that of *trans*-2-hexenal and methyl salicylate together supplied an index to differentiate semi- and fully-fermented teas. In addition, the SPME–GC analysis was also able to distinguish real jasmine teas from fake jasmine teas based on the disappearance of some grassy/green odorants.

Keywords: Tea discrimination; Fermentation; Volatile flavour compounds (VFC); SPME–GC; Catechin contents; HPLC

1. Introduction

In response to the promotion of the health benefits of tea consumption, teas are not only becoming popular beverages, but their pharmaceutical and industrial applications are also in development. As tea is traded all over the world, some trade disputes in regard to the types of teas occur now and then. For example, many tea-producing countries or areas, such as Japan, Taiwan, and Korea, tax imported teas differently, based on the fermentation degree (e.g., Korean customs has a more than 500% tariff rate on unfermented green teas, but only 40% on fermented Oolong and black teas). However, there has been no standard method internationally recognized for tea classification. At present, Korean customs classifies teas according to "infusion colour", that may be a more exact standard than that of many countries which only rely on the appearance of the dry tea leaves, that often does not discriminate between green teas and the lightly fermented Oolong teas.

Another example is the simplification of semi-fermentation processing called "increasing the greenness of Oolong teas". This is recently becoming more common among some Oolong tea manufacturers to skip several time-consuming and labour-intensive steps normally used in traditional processing. The result is that Oolong teas actually resemble the green teas in flavour. Due to the complex processing steps and the limited supply, Oolong teas usually have a higher unit price than green and black teas in the

* Corresponding author. Tel.: +82 31 201 2655; fax: +82 31 204 8116.
E-mail address: winepark@hotmail.com (S.-K. Park).

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international tea market. However, no method other than sensory evaluation, which requires experienced tea experts, is available to assist tea buyers in avoiding the improperly fermented Oolong teas. Therefore, it would be useful for both customs houses and tea companies if reliable and objective tea discrimination methods were developed. This would help to ensure the fairness on the tea trade worldwide.

Generally, teas are classified based on the "processing methods" of which "degrees of fermentation" are among one of the most important characteristics. According to the various levels of fermentation, teas can be divided into three major categories: unfermented green teas, semi-fermented Oolong teas, and fully-fermented black teas, which account for 24%, 1%, and 75% of the total world tea production, respectively (Wan, 2004). Because "fermentation" in modern tea science is mainly defined as the oxidation of catechins, the contents of catechins or their oxidation products, theaflavins and thearubigins, may be the first consideration for setting discrimination standards. Up to the present, HPLC analysis of catechins is practiced in almost all tea laboratories (Bronner & Beecher, 1998; Coto, Yoshida, Kiso, & Nagashima, 1996), but for the quantification of theaflavins and thearubigins, the spectrophotometric method is still practically the major method of analysis (Angayarkanni, Palaniswamy, Murugesan, & Swaminathan, 2002; Yao et al., 2006). However, besides its unreliability and non-specificity (Peterson et al., 2004), the spectrophotometric method may not be appropriate for the practical implementation of bulky samples due to the essential time-consuming multistage extractions. Although the HPLC method has been developed to quantify four of the theaflavins (Lee & Ong, 2000; Su, Leung, Huang, & Chen, 2003), its actual application may still depend on the universality of the commercial authentic compounds. Therefore, discrimination of teas by the contents of theaflavins or thearubigins would be inappropriate at the present time.

The original purpose of tea fermentation was to enhance the flavours of teas. Flavour, described as taste and aroma, is the most important element for tea evaluation (Wang, Park, Chung, Baik, & Park, 2004). In previous research, more attention was paid to the organic components contributing to tea taste, such as polyphenols, caffeine, and amino acids, compared to the VFC that make up tea aroma. The expensive traditional assays for VFC in terms of time and labour might be one of the major reasons they have not been assessed (Dutta, Hines, Gardner, Kashwan, & Bhuyan, 2003). In addition, the multiplicity of VFC detected in teas, amounting to hundreds, complicates the results (Robinson & Owuor, 1992). Due to the development of solid-phase microextraction (SPME) (Baptista, da P Tavares, & Carvalho, 1998), isolation of VFC from samples is a much more simple process in comparison with the simultaneous distillation and extraction (SDE) (Ravichandran & Parthiban, 1998; Togari, Kobayashi, & Aishima, 1995). Since gas chromatography–olfactometry (GC–O) is possible, research now can be focused on the important odour active VFC (van Ruth, 2001). Thus aroma analysis of various teas for characterization by SPME–GC/GC–O can be justified (Guillot et al., 2006).

2. Materials and methods

2.1. Tea samples

Thirty-four green teas, numbered from 1 to 34, products of Korea, China and Japan; fourteen Oolong teas, numbered from 35 to 48, collected from Taiwan and China; and eight black teas, numbered from 49 to 56, obtained from Taiwan, China, India, England and Sri Lanka, were used in this study. Different categories of teas, including products graded from high to low, were analyzed for both catechin content and aroma composition. In addition, six jasmine teas sent from the Korean customs office were compared by SPME–GC analysis for quality investigation.

2.2. Catechin analysis

2.2.1. Extraction

Since in our previous experiments it had been observed that the efficiency of catechin infusion was greatly influenced by the condition of the tea (it may take more time for catechins to infuse out from the tightly pressed teas than the broken or smashed leaves), all teas were firstly ground (IKA-Analytical Mill, Rose Scientific, Alberta, Canada; grinding time, 15 s), then 2 g of the tea powder were stirred with 80 ml boiling water in a 90 °C water bath for 5 min. The tea infusions were filtered through 0.45 μm syringe filters (Target®-Nylon 13 mm, National Scientific. Rockwood, TN, USA) before HPLC analysis.

2.2.2. HPLC analysis

For HPLC analysis, the method described by Coto et al. (1996) was used with the exception of the column (Phenomenex Luna 5 μ C_{18}, 150 × 4.6 mm). The total catechin content was calculated from the sum of seven flavanols, i.e., gallocatechin (GC), epigallocatechin (EGC), catechin, epicatechin (EC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG; not present in all of the tea samples), and epicatechin gallate (ECG). Catechin gallate (CG), the epimer of ECG, may exist in the sterilized tea beverages (Wang, Kim, & Lee, 2000), but was not detectable in the dry tea products. Quantification of catechins was performed by comparing their peak areas of the HPLC chromatograms with those of authentic compounds (Sigma Chemical, St. Louis, Mo, USA.). Every sample was assayed in duplicate.

2.3. Analysis of aroma compositions

2.3.1. Sample preparation

Four grams of tea leaves were stirred in 80 ml boiling water for 5 min in a 250 ml beaker which was covered by

watch glass. To avoid the distinctive smell from filter paper, 5 ml of the tea infusions were directly pipetted into a 10 ml SPME vial without filtration, and 1.5 g of NaCl were added for near saturation.

2.3.2. SPME and GC analysis

An SPME fiber (75 μm Carboxen-PDMS; Supelco, Inc., Bellefonte, PA, USA) was exposed to the sample headspace in a 50 °C oven while the extract was continuously stirred for 30 min. The VFC were desorbed by inserting the SPME fiber into a GC injector (injector temperature 230 °C) in splitless mode connected with a fused-silica GC column (DB-1, 30 m, 0.53 mm ID, 1.5 μm film thickness) (J&W Scientific, Folsom, CA, USA) for 15 min. The initial temperature of the GC was set at 40 °C for 4 min, then the oven temperature was increased at a rate of 5 °C/min until reaching 230 °C which was maintained for another 3 min. The detector temperature was set at 250 °C.

2.3.3. GC–MS analysis

For GC–MS analysis, a GC (HP 6890) coupled with a mass spectrometer (HP 5973, Hewlett-Packard, Palo Alto, CA, USA) was used, but with a different column (HP-1, 30 m, 0.32 mm ID and 0.25 μm film thickness). The GC operating conditions (temperature and time) were the same as described above. The mass spectrometer was operated in the electron-ionization (EI) mode at an ionization voltage of 70 eV. Runs of hydrocarbon mixture (ASTM D5307, 4-8182; Supelco, Bellefonte, PA, USA) were performed under the same GC conditions described previously, and the RI calculated were referred to those of the previously(X) published Kovat indices.

2.3.4. Quantification of VFC

Since the tea extracts had not been filtered in advance, tea particles (especially the steamed green teas and CTC black teas) were present in the samples. Previously, 1 μl of 0.03% ethyl heptanoate had been added to the samples as internal standard, but the presence of tea particles caused adsorption of the internal standard varying degrees (tea leaf itself could also absorb odorant). Accordingly, external standardization was performed for the quantification of individual VFC with 200 μg/l (ppb) ethyl heptanoate in triplication under the same SPME–GC conditions as described above.

2.4. Determination of key odorants with GC–O

The GC–O system consisted of a Hewlett-Packard HP 5890 series II Plus gas chromatograph equipped with an injection port and a humidified odour port. The column and GC oven conditions were the same as those described previously. Because the column outlet was not splitted, we acquired the FID signal first then redirected the column outlet into the sniffing port for GC–O. Both the detector and the connecting line to the port were held at about 200 °C. Humidified oxygen (40 ml/min) was supplied to the GC effluent at the sniffing port. The make-up gas for the detector and port was nitrogen at 30 ml/min. Two assessors separately sniffed the aromas of the eluted components, described them in their own words and rated the intensity of the odours perceived. The descriptors were recorded alongside the retention time of the VFC (Solina, Baumgartner, Johnson, & Whitfield, 2005).

3. Results and discussion

3.1. HPLC analysis of catechins

In theory, fermentation processes cause the oxidation of catechins, so the teas fermented for less time are supposed to contain more catechins. However, that concept can be applied only when all of the teas originate from the same species of tea trees grown under the same conditions. The nature of fresh tea leaves, before processing, also plays a key role in determining the catechin content of the final products (Wu, 1997). Because catechins negatively impact on the sensory qualities of green teas, the species with less catechins are usually selected for green tea manufacturing (Wu, 1997). Also, the techniques of fermentation processing might have been developed to trim the undesirable flavours of tea leaves high in catechins. As shown in Fig. 1, even though as expected, the fully fermented black teas contained the least amount of catechins among these three categories of teas ($p < 0.05$), whereas the total catechin contents were actually not significantly different between green and Oolong teas ($p > 0.05$). Thus, total catechin content is not an effective characteristic for tea discrimination.

It was also difficult to classify teas of various fermentation levels according to their individual catechin composition. As shown in Fig. 2a, green teas seemed to contain higher ECG, but lower EGC than Oolong teas. However,

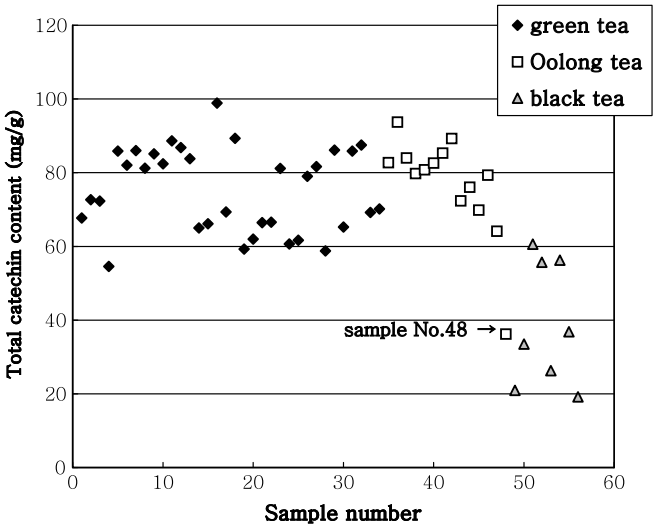

Fig. 1. Total catechin content (mg/g) in 56 teas of various fermentation degrees. Sample no. 48 is "Oriental beauty", the most heavily fermented Oolong tea made from young shoots that are originally low in catechin content.

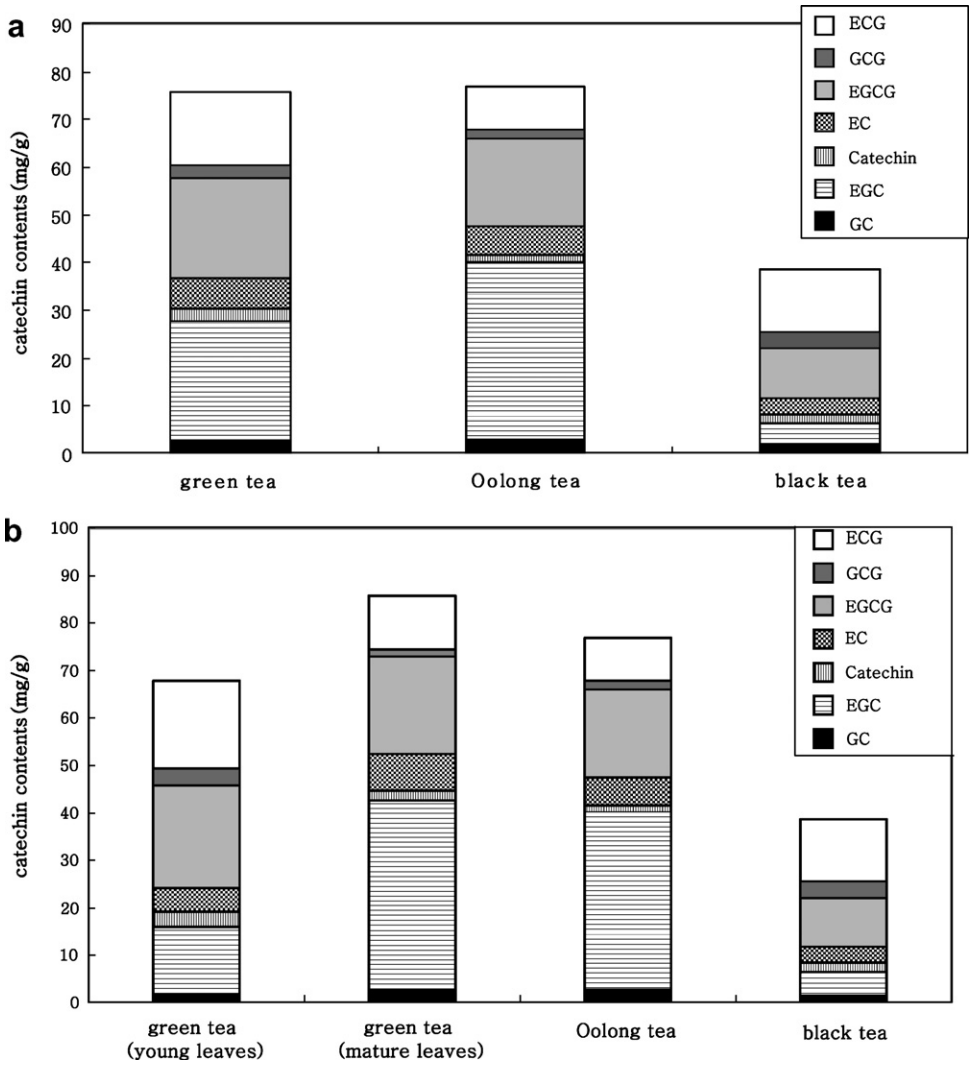

Fig. 2. Catechin compositions of green, Oolong, and black teas. In (a), the data of green, Oolong, and black teas are the averages of 34, 14, and 8 samples, respectively. In (b), the data of green teas are further divided based on their raw materials. The data of young leaf green teas (higher-grade) are the average of 19 samples, while those of mature leaf green teas (lower-grade) are calculated from the results of 15 samples.

within green teas themselves, a lot of differences can arise between the high- and low-grade ones (Fig. 2b). We found, as previously reported by Lin, Tsai, Tsay, and Lin (2003), EGCG is not always the dominant catechin in teas, and the EGC content can be even 2–3 times higher than that of EGCG in many cases. In the high-grade green teas that were mostly made from hand-harvested soft buds and/or the first young leaves, EGCG was generally the dominant catechin. However, in the lower-grade ones made from the more mature fresh leaves resulting from coarse plucking, the EGC content was much higher than that of EGCG. It has been reported that the amount of galloylcatechins (EGCG and ECG) falls, but that of EGC and EC rises as tea leaves age (Caffin, Dayarcy, Yao, & Rintoul, 2004). Therefore compared to the green teas made from buds/young leaves, those with large leaves may contain more EGC than EGCG ($p < 0.05$), higher EC ($p < 0.05$), but lower ECG ($p < 0.05$). On the other hand, Oolong teas, no matter whether high or low-grade, usually use mature large leaves as the raw materials. Most of them, after being semi-fermented, still have a catechin composition similar to those of the green teas made from mature leaves and cannot be well distinguished. In black teas that are also made from the mature leaves, less EGC than EGCG ($p < 0.05$) was preserved after heavy fermentation. It can be attributed to EGC being less stable (The long-term stability of tea catechins has been shown in the order of EC > ECG > EGCG > EGC (Su et al., 2003)).

3.2. SPME–GC–MS/GC–O data

The GC–O experiments attributed most of the grassy or green odours of green teas to some of the VFC appearing on the front portion (especially those between 5 and 12 min) of GC chromatograms, while many of the VFC detected thereafter were important for the fruity/floral or other fermented aromas. Fig. 3 shows typical GC

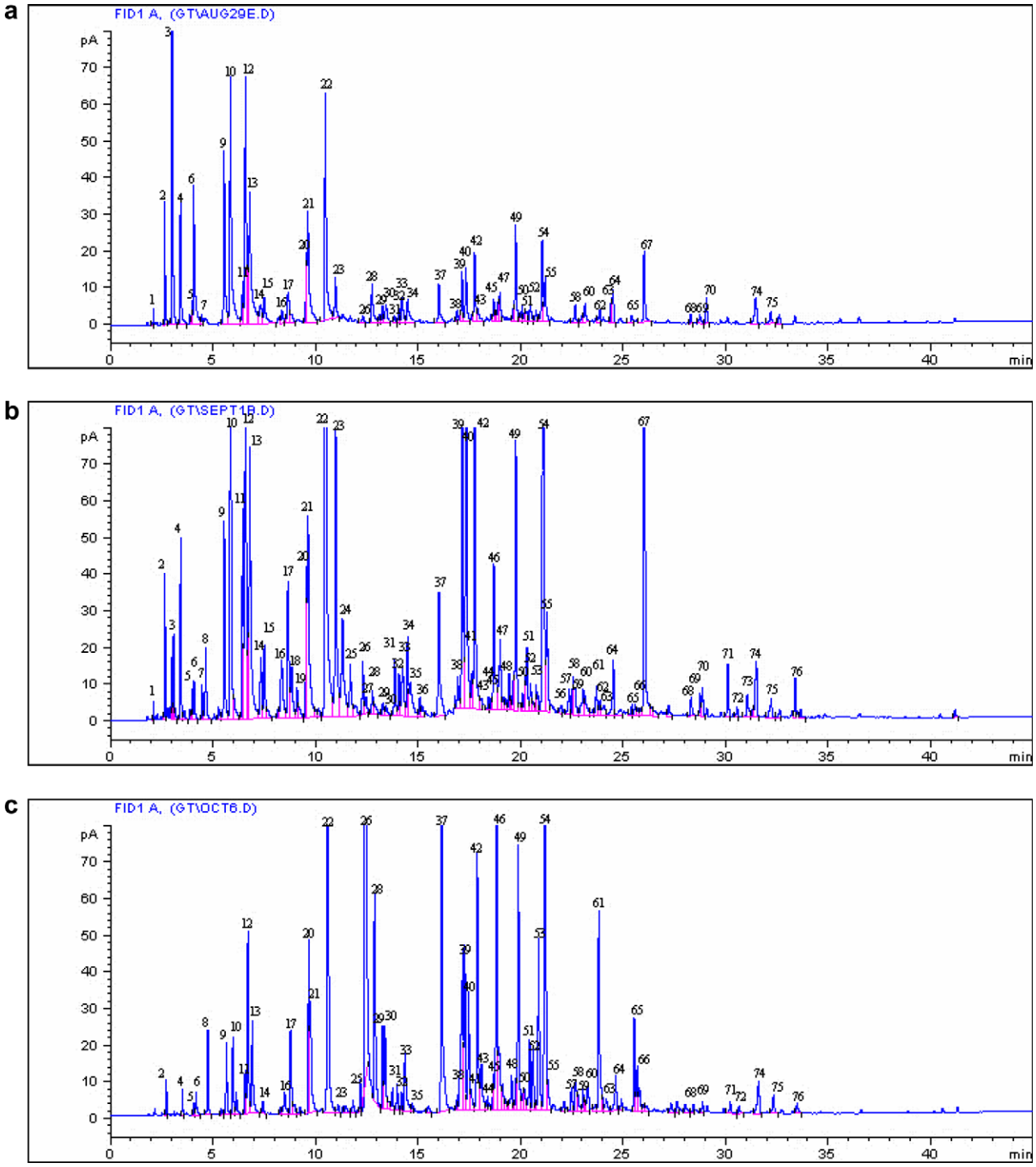

Fig. 3. GC chromatograms of VFC in various teas listed according to their fermentation degrees (a) Ouksu green tea (a tama-ryokucha processed by both steaming and roasting procedures), (b) Dong-Ding Oolong tea (medium fermented Oolong tea), and (c) English breakfast black tea. (For identity of peaks, see Table 1.)

chromatograms from three representative tea samples with different fermentation degrees. It was found that fermentation processing can, but not necessarily, cause the loss of grassy or green flavours, whereas formation of the fruity/floral and other fermented characters increases. Generally, Oolong teas with fermentation degrees between those of green and black teas contain both the green- and most of the black-tea characteristic flavours. Therefore, more complicated patterns of VFC can be observed in the semi-fermented Oolong teas than in the unfermented green or the fully fermented black teas. In order to identify and describe as many VFC peaks as possible these chromatograms, "Dong-Ding Oolong" with a medium-degree of fermentation was selected as the example, as shown in Table 1.

Table 1
Identification and odour description of VFC in Dong-Ding Oolong tea (Fig. 3b)

Peak no.	Compound name	RI	Odour description[a]
1–8	These compounds were not identified by GC–MS, and also no Kovat's index data available.	387–603	No characteristic smells detected
9	3-Methyl butanal	637	Malt. bitter almond, chocolate
10	2-Methyl butanal	648	Green grass, malt, almond
11	1-Penten-3-one	667	Harsh, pungent
12	1-Penten-3-ol	671	Green vegetable, butter
13	Pentanal	677	Woody, vanilla, nutty
14	2-Ethyl furan	692	Burnt, sweet, coffee
15	2-Methyl-2-butenal	697	Fruity green
16	*trans*-2-Pentenal	723	Pungent, green, apple, tomato
17	3-Ethyl-2-methyl-1-pentene	732	
18	n.i.	737	
19	n.i.	745	
20	Toluene	757	No detectable smell
21	2-Penten-1-ol	759	Fresh green, metallic
22	*n*-Hexanal	781	Green, grassy, metallic
23	1-Ethyl–H-pyrrole	793	
24	Furfural	800	Baked bread, almond
25	2-Methyl-2-pentenal	812	Grassy, green
26	*trans*-2-Hexenal	830	Fragrant, sweet, fruity (apple)
27	Furfuryl alcohol	835	Cooked sugar (low)
28	*cis*-3-Hexenol	844	Fresh, fruity green
29	n.i.	858	
30	n.i.	862	
31	1,4-Dimethylbenzene	873	Sweet aromatic
32	3-Methyl acetate-1-butanal	879	
33	2-Heptanone	883	Spicy, cinnamon
34	*cis*-4-Heptenal	889	Fatty, oily, steamed potato
35	*n*-Heptanal	892	Fatty, oily, nutty, green
36	2,5-Dimethyl pyrazine	905	Roasted nuts, bread
37	Benzaldehyde	934	Fragrant, sweet, almond
38	1,2,3-Trimethyl benzonitrile	961	
39	6-Methyl-5-hepten-2-one	967	Herbaceous, pungent, oily
40	*trans*, *trans*-2,4-Heptadienal	973	Fatty, nutty, hay, fishy
41	2-Ethyl-5-methylpyrazine	981	Sweet, roasted, nutty
42	*cis*, *trans*-2,4-Heptadienal	984	Fatty, nutty
43	γ-Terpinene	993	Citrus
44	Decane	1004	
45	Benzyl alcohol	1007	Mild sweet, roasted
46	Phenylacetaldehyde	1012	Floral, lilac
47	1-Ethyl-2-formylpyrrole	1022	
48	α-Isophorone	1037	Sweet green, tobacco
49	3,5-Octadien-2-one	1048	Fruity, hay, oxidized
50	1,3-Cyclooctadiene	1065	
51	*cis*-Linalool oxide	1070	Sweet floral, green, fruity
52	6-Methyl-3,5-heptadien-2-one	1078	Woody, spicy, cinnamon
53	*trans*-Linalool oxide	1089	Sweet floral, citrus, fruity
54	Linalool	1089	Floral
55	Benzeneacetonitrile	1094	
56	1-(2-Aminophenyl)-ethanone	1118	
57	Benzyl acetate	1131	Floral, fruity, sweet, fresh
58	3,5-Diethyl-2-methylpyrazine	1138	Roasted, nutty, vegetable
59	4-Ethylbenzaldehyde	1155	Bitter and sweet, almond
60	*n*-Fufurylpyrrole	1158	Vegetable, onion green
61	Methyl salicylate	1176	Sweet, spicy, minty
62	Safranal	1182	Herbaceous (saffron)
63	3,4-Dimethylbenzaldehyde	1188	Fragrant, bitter almond
64	β-Cyclocitral	1202	Mild green, minty, fruity
65	Geraniol	1220	Floral (rose), sweet
66	Citral	1223	Citrus, lemon
67	Indole	1232	Nutty, floral
68	Tridecane	1274	
69	*trans*-β-Damascenone	1284	Rose-like
70	*cis*-Jasmone	1288	Floral (jasmine)
71	α-Ionone	1407	Woody, berry, floral, nutty
72	Geranyl acetone	1417	Floral (rose), fresh fruity
73	2,5-Cyclohexadiene-1, 4-one	1428	
74	β-Ionone	1438	Woody, floral (rose, violet)
75	Cadinene	1460	Spicy woody
76	Nerolidol	1483	Floral (rose), apple, green

The same peak numbers in Fig. 3a and c also represent the same compounds as (b). n.i.: compound not identified.

[a] Odour description was the results of GC-O test on tea samples made up with the database of authentic compounds developed in our laboratory.

A total of more than 70 VFC have been detected, but only a relatively small proportion of them showed significant differences in composition between the unfermented and semi-/fully fermented teas. Fermentation did not make all of the aroma compounds change in the same direction. For example, there was not much indole with a pungent smell in green teas, but its level increased quickly by more than 10 times at the beginning of fermentation (as observed in the chromatogram of lightly fermented Pauchong Oolong, figure not shown) then gradually decreased when the fermentation had been continued. Finally, in the most heavily fermented Oolong tea "Oriental beauty" and all black teas, there was almost no detectable amount of indole. Conversely, methyl salicylate with a sweet and spicy odour appears only in the teas that have at least a medium-degree fermentation, but cannot be detected in the unfermented and lightly fermented teas. Therefore, it may not be correct to use only one compound as an index for the degree of fermentation. Instead, several compounds

considered together may offer a more buffered index that can be applied to a broader range of teas.

Thirteen VFC, including *n*-hexanal, *trans*-2-hexenal, benzaldehyde, 6-methyl-5-hepten-2-one, *trans, trans*-2,4-heptadienal, *cis, trans*-2,4-heptadienal, phenylacetaldehyde, 3,5-octadien-2-one, linalool, benzeneacetonitrile, methyl salicylate, geraniol, and indole, were observed to increase after fermentation. Except benzeneacetonitrile, all the other twelve VFC have also been mentioned in previous reports on tea aroma (Baptista et al., 1998; Togari et al., 1995). When all of the teas were compared for their total concentrations of these thirteen VFC, the unfermented and fermented teas can be clearly divided by the line of total concentration of 100 μg/l (Fig. 4).

Of course, it would be more advantageous for practical application if these thirteen compounds could be reduced in number. As mentioned previously, methyl salicylate and indole are the two important compounds that have to be taken into account simultaneously to cover all the degrees of fermentation found in commercial teas. In addition, *trans*-2-hexenal has been discussed in previous publications as an efficient compound for fermentation discrimination (Ravichandran & Parthiban, 1998; Togari et al., 1995), so it was also continued to be included, even though our analyses showed that it only appeared significantly in the heavily fermented teas. Benzaldehyde alone may not be effective enough to differentiate green teas from the lightly fermented Oolong teas, but it increased steadily and correlated well with fermentation degrees, so it is included in our analysis. We found that the three compounds, methyl-5-hepten-2-one, *trans*, *trans*-2,4-heptadienal, and *cis*, *trans*-2,4-heptadienal, often changed in step, but the changes of methyl-5-hepten-2-one were more readily detectable in the chromatograms, so it was used as the representative of these three VFC. On the other hand, linalool and geraniol cannot be relied on for separating green teas from the fermented teas, because a few green teas of high quality contained these two compounds in high levels as fermented teas tend to do. Phenylacetaldehyde and 3,5-octadien-2-one will also not be further considered for the purpose of discriminating between different degrees of fermentation, because their concentrations and the fermentation levels did not correlate well (In many cases, there was more phenylacetaldehyde and 3,5-octadien-2-one in Oolong than in black teas.). As a result, five VFC are selected for the discrimination between degrees of tea fermentation, and their total concentrations in various teas are compared in Fig. 5a. Because there is no problem in classifying the black teas with high levels of these five VFC totaling more than 100 μg/l, those points for samples 51, 52, 53, 55 and 56 were removed, and the figure of total concentrations below 100 mg/l was extended for clarity (Fig. 5b). As can be seen from Fig. 5b, a total concentration of these five VFC of 20 μg/l and below separates all of the unfermented tea samples from those fermented.

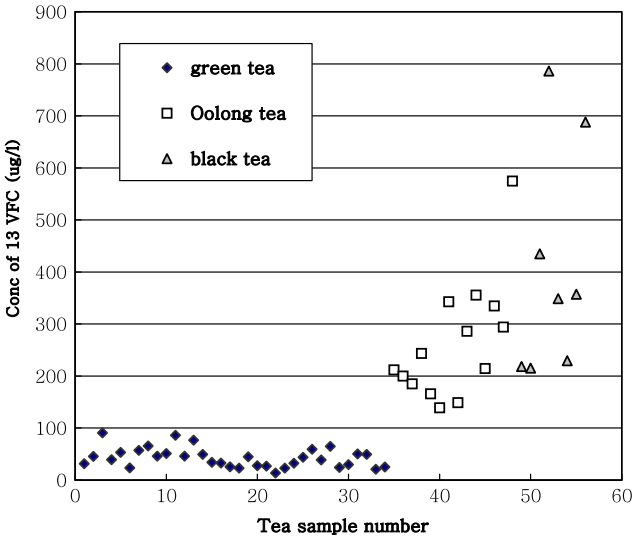

Fig. 4. The total concentrations (μg/l) of thirteen VFC in 56 teas of various fermentation levels. (The 13 VFC include *n*-hexanal, *trans*-2-hexenal, benzaldehyde, 6-methyl-5-hepten-2-one, *trans*, *trans*-2,4-heptadienal, *cis*, *trans*-2,4-heptadienal, phenylacetaldehyde, 3,5-octadien-2-one, linalool, benzeneacetonitrile, methyl salicylate, geraniol, and indole.)

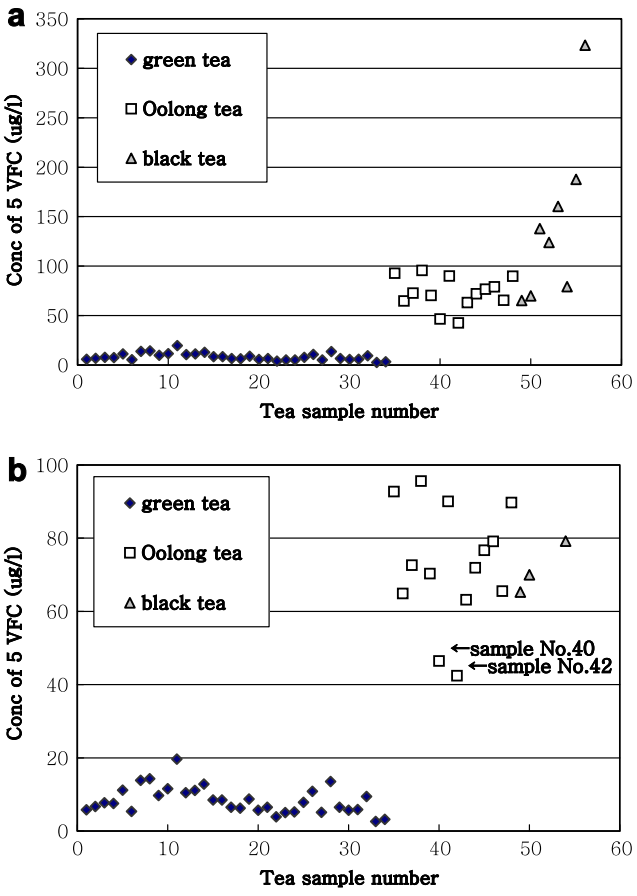

Fig. 5. The total concentrations (μg/l) of five VFC in 56 teas of various fermentation degrees. (The five VFC are *trans*-2-hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate, and indole.) (The portion of total concentrations below 100 μg/l in (a) is extended and shown in (b), and samples no. 40 and 42 are the Oolong tea samples improperly fermented and improperly stored, respectively.)

However, the separation between two groups becomes even clearer when the samples no. 40 and 42 are excluded. Sample 40 was probably an example of improperly fermented Oolong tea, as can be inferred from its infused tea leaves, which did not have enough red colour on the edges. Sample 42 was a product purchased from a traditional market in China. In some of the traditional tea markets, loose teas of middle and low quality (not branded products) are often not tightly sealed in packages, but exposed to light at room temperature for display to customers. Some flavour is lost and the quality changes in such situations. As a whole, the two sample t-test showed that green teas were significantly lower in the five VFC described above compared to Oolong and black teas ($p < 0.05$), but there was no significant difference between Oolong and black teas ($p > 0.05$). Cluster analysis also accurately divided the 56 tea samples into two groups, the unfermented and fermented, based on the total concentrations of these five VFC (Fig. 6).

To differentiate Oolong from black teas, the total contents of *trans*-2-hexenal and methyl salicylate supplied a valuable index ($p < 0.05$), except for the case of sample 48 (Fig. 7). Sample 48 "Oriental beauty" was the most heavily fermented Oolong tea produced only in Taiwan. Its raw material came from the young shoots with white floss that had been infested by *Jacobiasca formosana* (Paoli) (a type of green fly). The injured tea leaves were said to give a "honey-like" flavour after fermentation. Because this tea originated from young leaves and was also processed by heavy fermentation, its total catechin content was obviously lower than the other Oolong teas (Fig. 1). In view of the levels of *trans*-2-hexenal and methyl salicylate, it was also more similar to black teas (Fig. 8). Since its levels of 1-penten-3-ol and pentenal were higher than many other black teas (the figure not shown), "Oriental beauty" might be distinguished from other ordinary black teas based on its heavier green smell due to the high level of

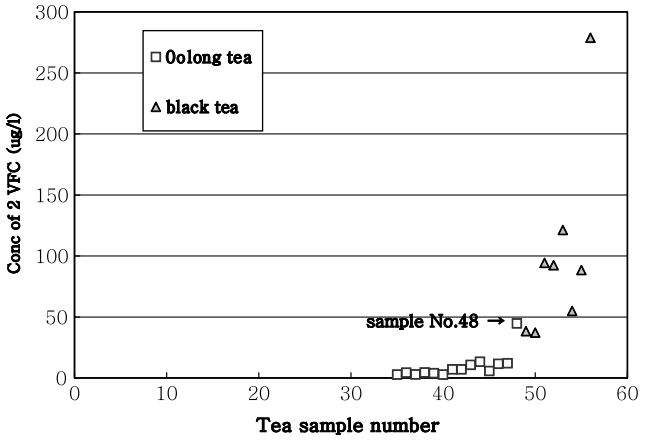

Fig. 7. Differentiation of Oolong and black teas by the concentrations of *trans*-2-hexenal and methyl salicylate. (Sample no. 48 is the most heavily fermented Oolong tea, "Oriental beauty".)

1-penten-3-ol. However, further experiments with more samples are necessary to be able to draw any further conclusions.

For a long time, people noticed that tea leaves not only disperse aroma, but also absorb odorants. By taking advantage of the odour-absorbing characteristic of dry tea leaves, another category of teas, flower teas, were developed. Even though all green, Oolong and black teas can be used to make flower teas, degree of fermentation is usually not considered for flower teas in tea science. They are in an independent category apart from green, Oolong, and black teas. Many kinds of flower teas, such as rose black tea or osmanthus Oolong tea, can be found in the market, but the most popular one may be jasmine tea made from green teas and jasmine flowers. In the processing of flower-tea leaf blending, the refined green teas are blended with jasmine blossoms to absorb aroma from the flowers. For the higher-grade jasmine teas, this step is usually repeated more than three times by removing the used flowers, drying

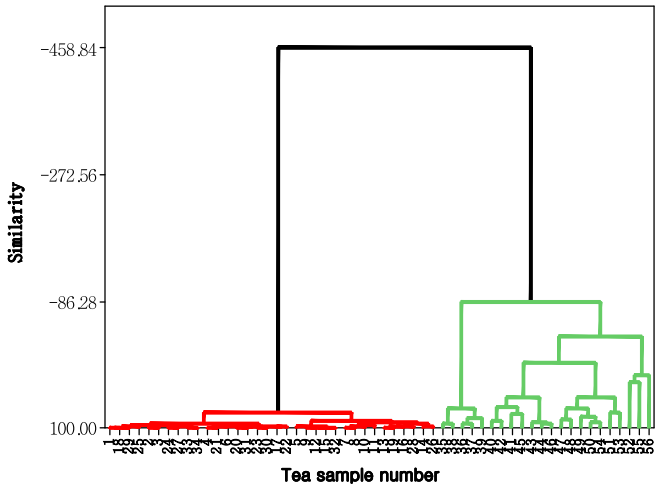

Fig. 6. Clustering of 56 tea samples based on the total concentrations of five VFC: *trans*-2-hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate, and indole. (Tea samples 1–34 are unfermented green teas, and tea samples 35–56 are fermented Oolong and black teas.)

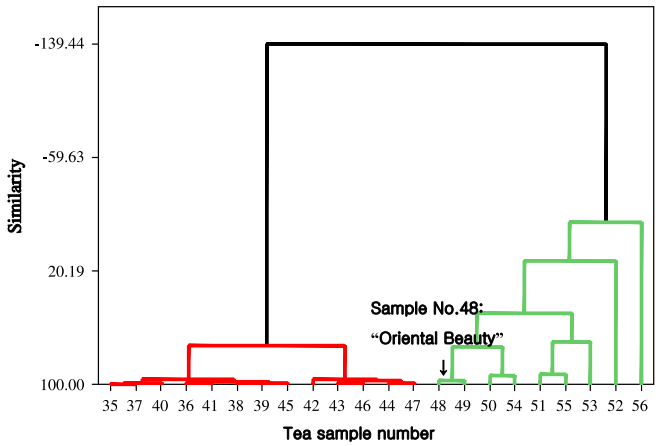

Fig. 8. Clustering of 22 fermented teas based on *trans*-2-hexenal and methyl salicylate. (Tea samples 35–48 are semi-fermented Oolong teas among which sample 48 "Oriental beauty" is the most heavily fermented. Tea samples 49–56 are fully fermented black teas.)

the tea leaves (moisture content in tea leaves may be increased to 12–16% or even higher during flower-tea blending. This would reduce the aroma absorbing ability of tea leaves if they had not been sufficiently dried for the next blending.), and mixing again with the fresh flowers. For the best blending result, the atmospheric temperature must be over 30 °C. This is one of the reasons why jasmine teas are mainly produced in tropical or subtropical areas. The temperature in the tea-flower piles can also rise up to 40–48 °C due to the high metabolic rate of blossoming flowers. Therefore, in such a high moisture and temperature condition, a post-fermentation reaction may occur, resulting from the auto-oxidation of tea polyphenols. Fig. 9 shows the similar VFC composition of four jasmine tea samples analyzed. All of them contained high amounts of benzaldehyde, 6-methyl-5-hepten-2-one, methyl salicylate, and indole with average concentrations of 136, 103, 153 and 172 μg/l, respectively, but no *trans*-2-hexenal was detected. It was very interesting to find that many of the VFC, the peaks in front of *cis*-3-hexenol, were lost during the flower-tea blending processing. The explanation could be that, when the tea leaves and fresh jasmine flowers are in contact with each other, not only is the flower aroma of jasmines absorbed by tea leaves, but some of the grassy/green odorants of tea leaves are also absorbed by the flowers. This suggests techniques to selectively reduce some unpleasant odours but preserve desirable odours.

Of course, not all the high quantities of those four VFC might result from fermentation. The aroma of jasmine itself cannot be ignored, and some interactions between tea leaves and flowers during blending must also be taken into account. However, it can be inferred that these four VFC might not be the major contributors to jasmine tea aroma, because some samples not high in these four VFC also smelled of jasmine (Fig. 10a and b). The sample of Fig. 10a was a tea bag product in which artificial aroma chemicals existing as white and yellow granules were visible. In another case, a tea manufacturer tried to extract the aroma compounds from jasmine flowers and then spread them on tea leaves to simplify the processing procedure. The dry leaves of this kind of jasmine tea usually only smell good, but don't give enough flavour to their infusions, because the flower aroma has not penetrated the tea leaves sufficiently deeply. In China, these flower teas are considered "fake", and the sample of Fig. 10b was an example of this type of "fake" jasmine tea. We observed that linalool and benzyl acetate were two of the important components characterizing jasmine aroma, because in all the six jasmine teas analyzed, regardless of being real or fake, were high in these two compounds. Even though it is not easy to produce a harmonious flavour when adding artificial aromas, it is definitely possible to produce jasmine teas without using the real flowers and labour-intensive processing. Here SPME–GC analysis is recommended as a good method for discriminating between real jasmine teas and fake ones. That is, the tea leaves which have not been processed by "flower blending" maintain the VFC detectable in front of *cis*-3-hexenol on the GC chromatograms. Even if a few of the peaks in front of *cis*-3-hexenol are still present after flower blending, their total peak area on the GC chromatograms will not exceed 1% of the total peak area of the 13 major jasmine tea aroma compounds, including *cis*-3-hexenol, benzaldehyde, 6-methyl-5-hepten-2-one, *cis*-3-hexenyl acetate, benzyl alcohol, methyl benzoate, linalool, benzyl

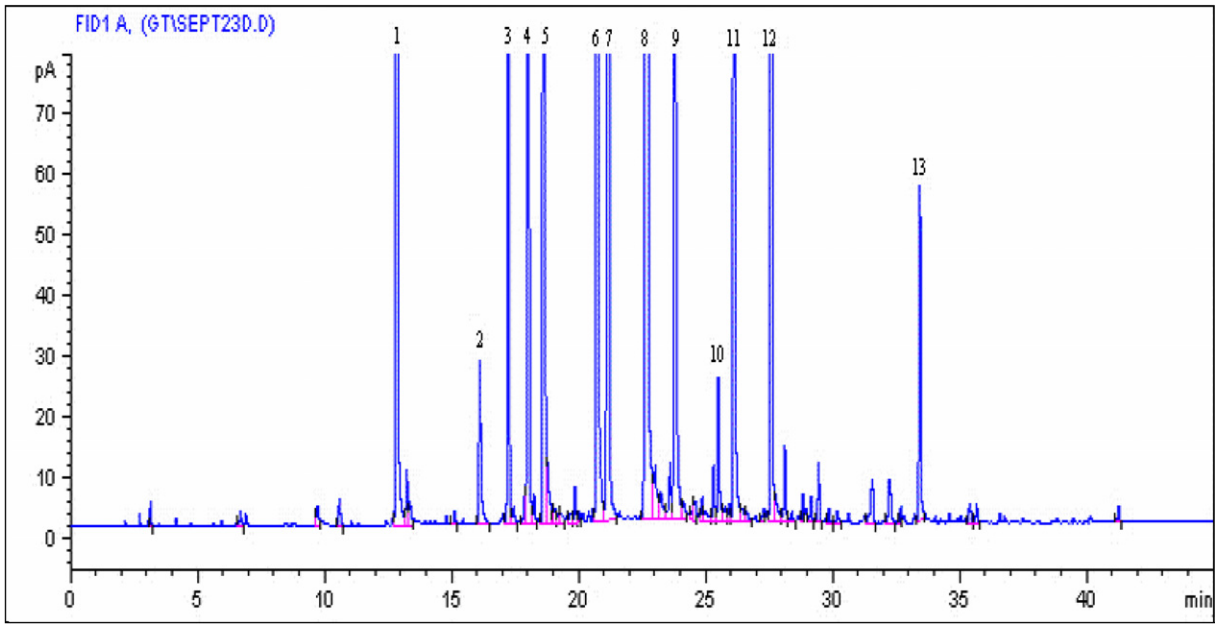

Fig. 9. The characteristic GC chromatogram of real jasmine tea VFC. Peak identification: (1) *cis*-3-hexenol; (2) benzaldehyde; (3) 6-methyl-5-hepten-2-one; (4) *cis*-3-hexenyl acetate; (5) benzyl alcohol; (6) methyl benzoate; (7) linalool; (8) benzyl acetate; (9) methyl salicylate; (10) geraniol; (11) indole; (12) methyl-2-aminobenzoate; (13) *cis*-3-hexenyl benzoate.

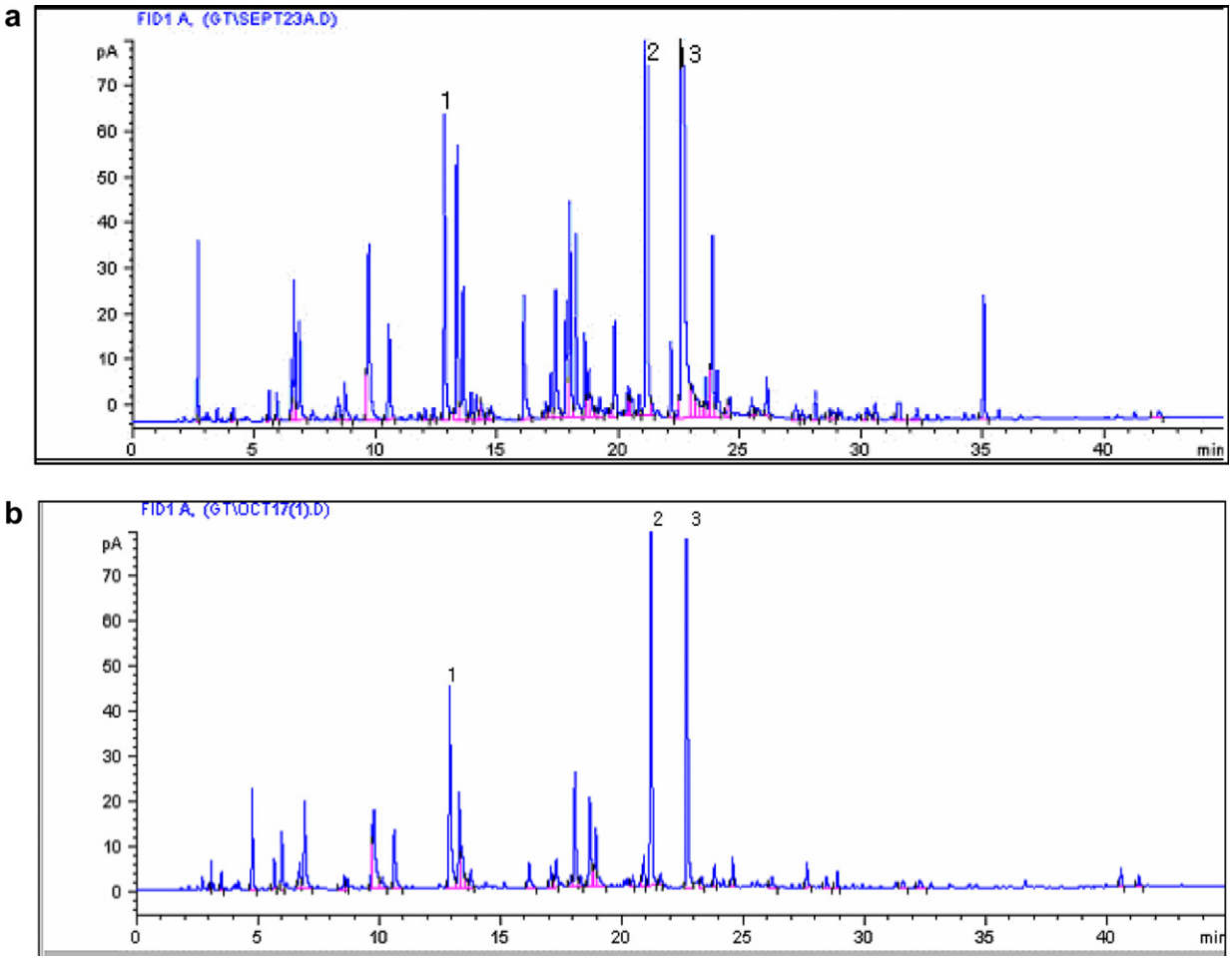

Fig. 10. Two examples of the aroma GC chromatograms detected in fake jasmine teas. Peak identification: (1) *cis*-3-Hexenol; (2) linalool; (3) benzyl acetate.

acetate, methyl salicylate, geraniol, indole, methyl 2-amino-benzoate, and *cis*-3-hexenyl benzoate (Fig. 9).

4. Conclusions

Neither the total catechin contents nor the individual catechin compositions can accurately separate green teas from Oolong teas. On the other hand, the SPME–GC analysis provides a convenient and reproducible method for tea discrimination. The total concentration of five VFC, i.e., *trans*-2-hexenal, benzaldehyde, methyl-5-hepten-2-one, methyl salicylate and indole, has been shown to distinguish unfermented teas from fermented ones, while that of *trans*-2-hexenal and methyl salicylate may classify the semi- and fully-fermented teas. Also, the maintenance of the VFC detectable in front of *cis*-3-hexenol on the GC chromatograms allows "fake" jasmine teas to be separated from real jasmine teas.

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