Full Length Research Paper

Study on flavour volatiles of γ-aminobutyric acid (GABA) green tea

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The volatile components of γ-aminobutyric acid (GABA) tea produced by two different kinds of technological process separately namely: vacuum and water immersion were studied. It was shown by the sensory evaluation that the color of the soup and the extracted leaves of GABA tea were similar to that of the oolong tea, and the smell was similar to the aroma of cooked sweet red dates mixed with some of sour. In GABA tea, the contents of 2,6-bis(1,1-dimethylethyl)-4-methyl phenol, methyl myristate, methyl laurate and methyl palmitate were higher than that of the normal green tea. The characteristic aroma constituents of the GABA tea made by vacuum treatment concluded methyl myristate, hexadecane, methyl laurate and methyl palmitate, while to the GABA tea made by water immersion, they were 2,6-bis (1,1-dimethylethyl) -4-methylphenol and 1-octanol.

Key words: γ-Aminobutyric acid (GABA), GABA tea, volatile flavour compounds.

INTRODUCTION

Tea is the most widely consumed non-alcoholic beverage in the world. Recent studies showed that green tea not only contributes to reducing the risk of cardiovascular disease and some forms of cancer but also offers other benefits such as better oral hygiene, lower hypertension, reduction in body weight, antibacterial and antiviral activity, and antifibrotic effects (Yang et al., 2000; Lin and Lin-Shiau, 2006; Cabrera et al., 2006).

γ-Aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system and is known to mediate presynaptic inhibition of primary afferent fibers in the motor system and may also be involved in postsynaptic forms of motor neuron   inhibition

(Curtis and Lacey, 1994). Amino acid neurotransmitters are critical for the function of the central nervous system and play an important role in brain function and neurological disease (Olney, 1990; Gulati and Santon, 1960; Watanabe et al., 2002; Mine et al., 2010; Wong et al., 2003). GABA tea is a kind of natural tea product that contains high levels of GABA, and its GABA content, which amounts to the 20 to 30 times of the ordinary green tea, is at least 1.5 mg/g (Tsushida and Murai, 1987; Sawai et al., 2001; Wang et al., 2006). It not only has the sanitarian functions of ordinary tea, but also has lots of pharmacological effects of γ-aminobutyric acid. In recent years, as a natural health beverage, GABA tea with significant lowering blood pressure function and other many health care efficacies became a research hot spot and favored by consumers, particularly patients with high blood pressure (Abe et al., 1995; Abdou et al., 2006; Ho et al., 2009; Cheng and Tsai, 2009). At present, the researches mainly focus on GABA enrichment techniques, the functions and mechanisms of GABA tea, production and processing equipment research and standard setting et al. Very little work has been done on the aroma composition of GABA tea.

In tea, volatile organic components are present in   very

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minute quantities, that is, 0.01% of the total dry weight, but these have high impact on the flavour of the products due to their low threshold value and resulting high odour units (Yao et al., 2005; Shimoda et al., 1995; Baptista et al., 1998; Kenji and Hideki, 2002).

As a result of the special treatment given to fresh leaves of GABA tea in the early processing stage, the finished product has a unique sense of aroma in the sensory quality, and it remains controversial on the unusual smell. This study took GABA tea through vacuum processing and water immersion processing respectively as the object to investigate the aroma compositions. By comparing and contrasting, this study analysed the GABA tea volatile flavour components and its special aroma compositions, and compared the aroma and the sensory quality on different treatment GABA tea, and provided theoretical basis for the improvement in GABA tea quality.

MATERIALS AND METHODS

Fresh tea leaves of Jinxuan clone were obtained from the tea garden of South China Agricultural University. They consisted mainly of two leaves and a bud. The main equipments are AB204-N type analytical balance (Mettler Toledo instrument Co., LTD.), Agilent 1200 high pressure liquid chromatography (HPLC) (Agilent Technologies Co., LTD.), SDE distillation apparatus (Custom-tailor), TRACE2000 GC-MS instrument (The United States Finnigan company) and the main reagents are γ-aminobutyric acid (Sigma Chemical Co.), L -glutamic acid (Sigma Chemical Co.), ether (AR) (Jiangsu qiangsheng chemical Co., LTD. in Chinese), Ethyl caprate (Sigma Chemical Co.), Anhydrous sodium sulphate (AR) and (Tianjin fuchen chemical factory in Chinese).

Tea sample preparation

The control tea sample was manufactured in a traditional way rolling after harvesting fresh leaves, drying at 110°C spreading out and drying at 80°C until the enough dry. The vacuum processed samples preparation was from fresh leaves, vacuum seal for 3 h, cutting off sealing bag to spread out for 2 h, vacuum seal for 3 h again, taking out leaves to manufacture according to the control sample tea immediately. While the water immersion processed samples preparation was requested to put fresh leaves into tap water at ambient temperature dipping 3 h twice, spreading out for 2 h between the two immersions, then taking out leaves to remove blade surface water and manufacturing according to the control tea sample.

GABA content determination

Referring to methods described previously (Khuhawar and Rajper, 2003; Zhang and Bown, 1997; Mengerink et al., 2002; Syu et al., 2008), the GABA content is determined by OPA pillars derivative HPLC. More than three replicate samples were taken for analysis, the values were averaged, and the standard deviation (SD) was obtained for statistical data analysis.

Sensory quality evaluation

Sensory quality evaluation was carried out on the methodology of evaluation of tea (GBT23776-2009).

Extraction of volatile organic components by simultaneous distillation extraction (SDE)

20 g of tea placed in a round-bottom flask of 2 L capacity was dissolved in 450 ml of boiling deionized water, using electric heating cap to maintain tea micro boiling. 50 ml of ether was taken into a 250 ml extraction flask along with 0.1 ml ethyl caprate (concentration for 200 ppm) as internal standard. Picking up the round-bottom flask was on one side of the SDE apparatus, and the extraction flask was on the other side. Meanwhile ether maintained boiling through dipping the bottom of the flask into the 45°C water bath, the process of condensation backflow extraction was continued for 1 h. When the extraction liquid cooled, 5 g anhydrous sodium sulphate was added to the liquid. Through one night placed in the refrigerator, the extract was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under vacuum in a rotary evaporator to 2 ml. This concentrate was used for GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The concentrated extracts from the different extraction tea samples above were analysed on a Finnigan TRACE 2000 GC-MS system. The environmental temperature was 25°C and humidity was 60%. A HP-1 column (30 m × 0.25 mm) was used with helium as a carrier gas. The GC over temperature was programmed to hold at 50°C for 1 min and then to increase to 120°C at 3°C /min holding for 2 min, and then to increase to 180°C at 5°C/min holding for 10 min. The injector temperature was 230°C. Column flow rate was 1.0 ml/min. The MS used was electron impact (EI) ion source and scanned at 70 eV over 35 to 335 amu.

Identification of compounds was first attempted using mass spectral libraries Willey and NIST. Corroboration of the identification was then sought by matching the mass spectra of compounds with those present in our own library built up from using some authentic flavour compounds and in the literature. The relative content of each component (component peak area and internal standard peak area ratio) was obtained by using peak area normalization quantitative and finally by matching the retention time and retention indices of the compounds to determine part components further.

Statistical analysis

Data in this paper were analysed using the software of SAS (Version 9.1, SAS Institute Inc, USA).

RESULTS

Sensory quality evaluation

It was shown by the sensory evaluation that compared to the control tea sample, the color of the soup and the extracted leaves of GABA tea were similar to that of the oolong tea (Table 1). Processed tea samples have a unique sense of aroma that can be distinguished from other teas easily. The smell was similar to the aroma of cooked sweet red dates, but at the same time it was mixed with some of sour, thus leading to some consumers finding difficult to accept the smell of GABA tea.

GABA content detection

From the Table 2, GABA content of two   processed   tea

Table 1. Sensory evaluation of sample teas.

Sample	Appearance	Soup colour	Aroma	Taste	Material
Vacuum	Green with red, tippy	Light yellow, bright	Red dates flavour, sweet aroma	Fresh and sweet	yellowish green with red edge
Water immersion	Yellowish green with red, tippy	Golden yellow, bright	Red dates flavour	Fresh and sweet	yellowish green with red edge
Control	Green, tippy	Yellow green, bright	Pure	Fresh and heavy	bright green

Table 2. Contents of GABA of sample teas (mg/100g).

Sample	Vacuum	Water immersion	Control
GABA	185.02±0.25	178.15±0.18	12.21±0.06

Value (mg/100 g) = mean ± SD (n ≥ 3).

samples was higher than 1.5 mg/g, the lowest content of GABA tea (Tsushida and Murai, 1987) and that of vacuum processed tea sample was slightly higher than that of water immersion processed tea sample.

Volatile flavour compounds analysis

The main volatile flavour compounds analysis of tea samples

A total of 40 compounds were detected in vacuum processed tea sample (Table 3), including 14 kinds of aliphatic hydrocarbons, four kinds of esters, three kinds of alcohols, two kinds of aromatic hydrocarbons, one kind of phenol, one kind of sulfocompound and four kinds of other unknown compounds. Compounds whose content was in the top 10 and percentage of the total 10 compounds are listed in Table 4. Table 4 shows that the main volatile compounds, which comprised 84.33% of the total composition, were 2,6-bis(1,1-dimethylethyl)-4-methylphenol, methyl

myristate, hexadecane, methyl laurate, methyl hexadecanoate and 1-octanol in the GABA tea made by vacuum treatment.

In water immersion processed tea sample, there were 30 compounds detected, including 15 kinds of aliphatic hydrocarbons, four kinds of esters, two kinds of alcohols, four kinds of aromatic hydrocarbons, one kind of phenol, alkene and quinone respectively, and two kinds of other unknown compounds. In Table 4, the main volatile compounds in the GABA tea made by water immersion treatment were 2,6-bis(1,1-dimethylethyl)-4-methylphenol, 1-octanol, methyl myristate, methyl laurate, methyl hexadecanoate, hexadecane and heptadecane, which comprised 92.75% of the total composition.

There were 37 compounds detected in control sample green tea, including 20 kinds of aliphatic hydrocarbons, four kinds of esters, two kinds of alcohols, two kinds of aromatic hydrocarbons, two kinds of phenols, one kind of aldehyde, quinone and sulfocompound separately, and three kinds of other unknown compounds. From the Table 4, we found that the major volatile compounds of control green tea were 2,6-bis(1,1-dimethylethyl)-

4-methylphenol, methyl myristate, 1-octanol, heptadecane, hexadecane, methyl hexade-canoate (methyl palmitate), methyl laurate and eicosane, and these compounds comprised 84.97% of the total composition.

Eight kinds of compounds were mutual among the main volatile compounds of three tea samples. The most of the eight kinds of compounds had a lower content in control sample green tea than the GABA tea. Methyl myristate, hexadecane, methyl laurate and methyl hexadecanoate were present in relatively high amounts in vacuum processed tea sample, while 2,6-bis(1,1-dimethylethyl)-4-methylphenol and 1-octanol were detected in relatively high amounts in water immersion processed tea sample.

The comparison of volatile flavour components of sample teas

It was clear from the Table 5 that aliphatic hydrocarbons, esters and phenol were present in higher amount in the volatile compounds of three

Table 3. The aromatic constituents and their relative content of sample teas (%).

Peak	Retain time	Compound	Formula	Relative content（%）		
				Vacuum	Water immersion	Control
1	6.23	1,2-Dimethylbenzene	C_8H_{10}	0.16	/	0.16
2	7.48	Unknown matter	C_6H_8O	/	/	0.02
3	7.52	Ethyl disulfide	$C_4H_{10}S_2$	0.05	/	0.04
4	11.02	Decane	$C_{10}H_{22}$	0.03	/	/
5	14.09	1-Octanol	$C_8H_{18}O$	7.98	13.37	9.78
6	15.45	Undecane	$C_{11}H_{24}$	0.12	/	0.08
7	16.06	Unknown matter	$C_{10}H_{16}O$	0.07	/	/
8	16.75	Unknown matter	$C_{11}H_{20}$	0.06	/	/
9	18.16	3-Methyldecane	$C_{11}H_{24}$	0.03	/	/
10	18.41	2-Methylundecane	$C_{12}H_{26}$	0.06	/	0.04
11	18.69	3-Methylundecane	$C_{12}H_{26}$	0.08	/	/
12	20.08	Dodecane	$C_{12}H_{26}$	0.28	/	0.19
13	20.65	2,6-Dimethylundecene	$C_{13}H_{28}$	0.11	/	0.08
14	22.99	2-Methyl dodecane	$C_{13}H_{28}$	0.14	/	0.08
15	23.34	3-Methylnonane	$C_{10}H_{22}$	0.26	/	/
16	23.42	2-Hexyloctanol	$C_{14}H_{30}O$	0.16	/	/
17	24.22	Unknown matter	$C_{11}H_{10}$	0.08	/	/
18	24.58	Tridecane	$C_{13}H_{28}$	1.35	1.17	0.85
19	25.68	Unknown matter	$C_{14}H_{30}$	/	/	0.14
20	26.75	Heptylcyclohexane	$C_{13}H_{26}$	0.07	/	/
21	27.80	2-methyltridecane	$C_{14}H_{30}$	0.43	/	0.38
22	29.35	Tetradecane	$C_{14}H_{30}$	3.06	2.60	2.23
23	29.77	Aldehyde peach	$C_{14}H_{28}O$	/	/	0.31
24	31.15	Hexadecanol	$C_{16}H_{34}O$	/	0.6	/
25	31.19	2-Hexyl-1-decanol	$C_{16}H_{34}O$	0.67	/	/
26	31.51	2,6-Di-tert-butyl-p-benzoquinone	$C_{14}H_{20}O_2$	/	0.61	0.49
27	31.60	2,6,10,14-Tetramethyl heptadecane	$C_{21}H_{44}$	0.56	0.91	0.54
28	31.74	2-Methyltetradecane	$C_{15}H_{32}$	0.85	0.69	0.39
29	31.96	3-Methyltetradecane	$C_{15}H_{32}$	0.28	0.26	/
30	32.61	Phytane	$C_{20}H_{42}$	/	/	1.38
31	32.99	2,6-Di-tert-butyl-4-methylpheno	$C_{15}H_{24}O$	22.38	30.15	22.78
32	33.15	2,4-Bibutylphenol	$C_{14}H_{22}O$	/	/	1.40
33	33.67	Methyl Laurate	$C_{13}H_{26}O_2$	10.77	8.71	6.75
34	34.53	Unknown matter	C19H31F7O2	/	0.83	/

Table 3. The Contd.

35	34.88	2-Methylpentadecane	$C_{16}H_{34}$	1.55	0.76	/
36	34.99	3-Methyltetradecane	$C_{15}H_{32}$	/	/	0.61
37	35.90	Hexadecane	$C_{16}H_{34}$	11.81	7.50	7.81
38	37.26	Octadecane	$C_{18}H_{38}$	/	/	3.96
39	37.36	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	/	/	0.41
40	37.58	3-Methylpentadecane	$C_{16}H_{34}$	/	1.20	/
41	37.62	2-Methylpentadecane	$C_{17}H_{36}$	1.46	/	/
42	37.93	1-Nonadecene	$C_{19}H_{38}$	/	0.24	/
43	37.99	Pentadecanol	$C_{15}H_{32}O$	/	/	0.23
44	38.50	Heptadecane	$C_{17}H_{36}$	6.55	6.00	8.59
45	38.59	2,6,10,14-Tetramethylpentadecane	$C_{19}H_{40}$	/	1.36	0.29
46	39.11	Methyl myristate	$C_{15}H_{30}O_2$	15.70	13.03	10.24
47	40.24	2-Methylheptadecane	$C_{18}H_{38}$	0.8	0.64	1.22
48	40.66	Phenanthrene	$C_{14}H_{10}$	/	0.45	/
49	40.72	Anthraquinone	$C_{14}H_{10}$	0.39	/	0.83
50	43.42	Diisobutyl phthalate	$C_{16}H_{22}O_4$	0.28	0.52	0.45
51	44.57	Unknown matter	$C_{16}H_{32}O$	7.19	6	6.39
52	45.03	2-Methylanthraquinone	$C_{15}H_{12}$	/	0.35	/
53	45.07	Eicosane	$C_{20}H_{42}$	2.24	/	5.85
54	45.51	Nonadecane	$C_{19}H_{40}$	1.33	1.53	1.13
55	45.83	1-Methylphenanthrene	$C_{15}H_{12}$	/	0.44	/
56	46.09	4-Methylphenanthrene	$C_{15}H_{12}$	/	0.15	/
57	46.73	Hexadecanoate	$C_{17}H_{34}O_2$	8.5	7.99	6.78

"/" :This component was not found.

tea samples by classification, and followed by alcohols and aromatic hydrocarbons.

Esters content of vacuum processed tea sample and water immersion processed tea sample were 11.03 and 6.03% higher than that of control tea sample, respectively. In vacuum processed tea sample esters content was 5% higher than water immersion processed tea sample, while phenols and alcohols content of vacuum processed tea sample was 12.77 and 5.16% lower, respectively

than that of water immersion processed tea sample.

The characteristic aroma constituents of sample teas

In this research, the characteristics aroma constituents mean that they were present only in one tea sample and none in other teas, or they

were at some level of abundance in one tea sample but existing very little in other teas. Compared to the volatile compounds of three tea samples, the characteristics aroma constituents were followed separately:

Vacuum processing tea: 2-hexyloctanol (0.16%), heptylcyclohexane (0.07%), 2-hexyl-1-decanol (0.67%).

Water immersion processing tea: hexadecanol (0.6%), 1-nonadecene (0.24%), phenanthrene

Table 4. The main aromatic constituents and their relative content of sample teas (%).

Main volatile compound	Relative content (%)		
	Vacuum	Water immersion	Control
2,6-Di-tert-butyl-4-methylpheno	22.38	30.15	22.78
Methyl myristate	15.70	13.03	10.24
Hexadecane	11.81	7.50	7.81
Methyl Laurate	10.77	8.71	6.75
Hexadecanoate	8.5	7.99	6.78
1-Octanol	7.98	13.37	9.78
Heptadecane	6.55	6.00	8.59
Tetradecane	3.06	2.60	2.23
Eicosane	2.24	/	5.85
Octadecane	/	/	3.96

Table 5. The aromatic components of sample teas (%).

Component	Aliphatic hydrocarbon	Ester	Phenol	Alcohol	Aromatic hydrocarbon	Sulfocompound	Quinon	Aldehyde	Alkene
Vacuum	35.45	35.25	22.38	8.81	0.55	0.05	/	/	/
Water immersion	28.13	30.25	30.15	13.97	1.39	/	0.61	/	0.24
Control	36.11	24.22	24.18	10.01	0.99	0.04	0.49	0.31	/

(0.45%), 2-methylanthraquinone (0.35%), 1-methylphenanthrene (0.44%), 4-methylphen-anthrene (0.15%).

Control tea: aldehyde peach (0.31%), phytane (1.38%), 2,4-bibutylphenol (1.40%), pentadecanol (0.23%) (Figure 1).

Fatty acid ester aroma composition analysis

In Oolong tea, some studies showed that fatty acid esters played the very important part of flavor of finished product but could not be detected in fresh leaves (Dongyan et al., 2003; Kawakami et al., 1995). In GABA tea, relevant studies mentioned that the smell of fatty acid esters with sweet and sour presenting during vacuum treatment to GABA tea became weakened in drying, but it can still be distinguished from ordinary tea easily (Jiang, 2009). So the characteristic aroma of the GABA tea was closely related with fatty acid ester. This study lists the fatty acid ester compositions of the main volatile compounds of three tea samples and compared their content (Table 6).

Each content or the total content of the three main fatty acid ester of two processed tea samples were significantly higher than control tea sample, and the total content was higher 11.2 and 5.96%, respectively. The results coincide with the literature that the characteristic aroma of the GABA tea was closely related with fatty acid ester.

DISCUSSION

In this paper, the fatty acid ester aroma com-position analysis showed that the characteristic aroma of the GABA tea and fatty acid ester were closely related. Methyl myristate, which is a flavor
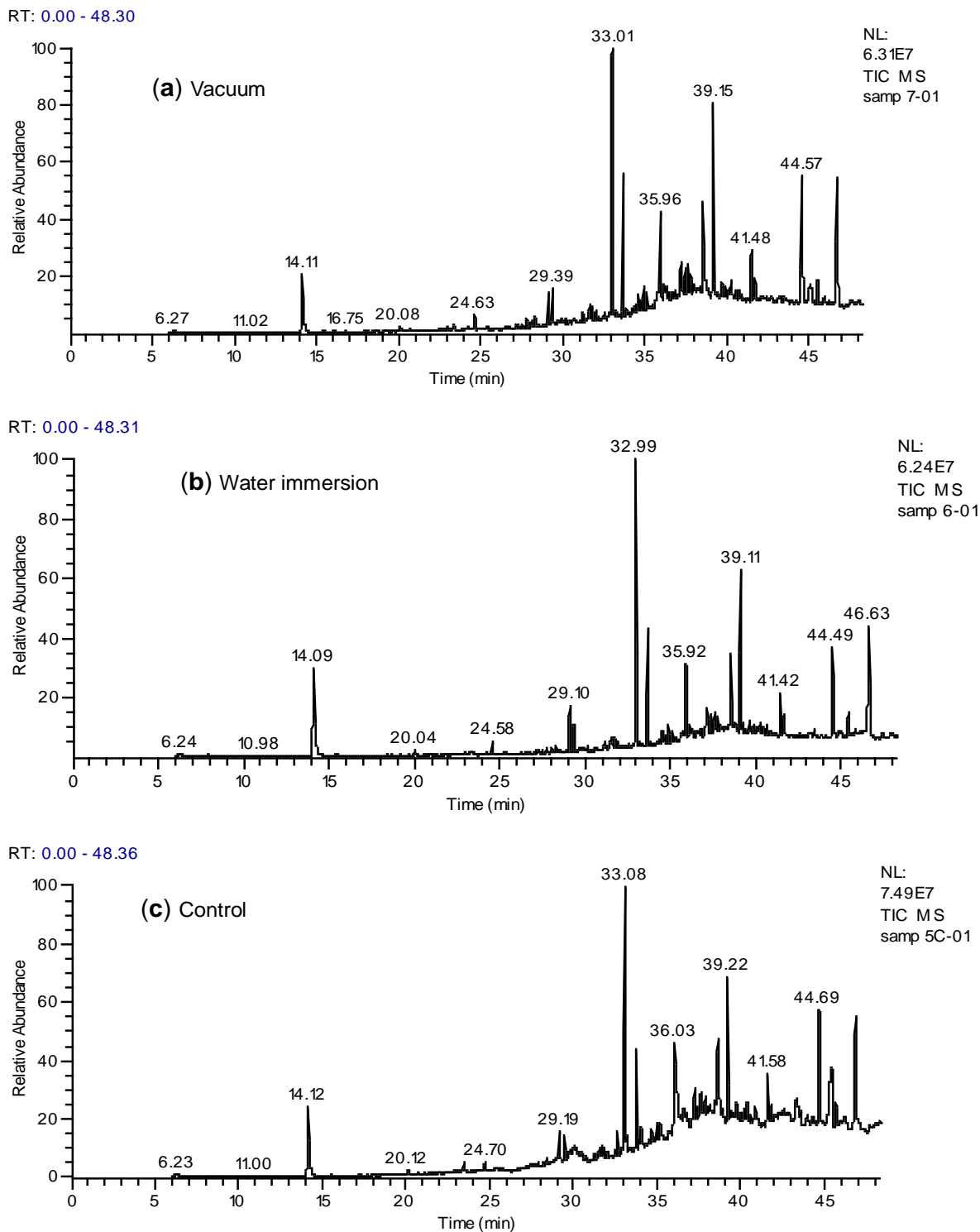
Figure 1. The relative abundance of aromatic constituents of sample teas In: (a) vacuum, (b) water immersion and (c) control.

material often used to allocate honey and coconut milk, and methyl laurate, which is aroma similar to bouquet and flowery, have a certain correlation with the characteristic aroma of the GABA tea. In GABA tea, the contents of 2,6-bis(1,1-dimethylethyl)-4-methyl phenol, methyl myristate, methyl laurate and methyl palmitate were higher than that of the control green tea and these constituents determined the peculiar scent of GABA tea.

Table 6. The relative content of aliphatic ester of sample teas (%).

Component	Methyl myristate	Methyl laurate	Hexadecanoate	Total content of fatty acid ester
Vacuum	15.7	10.77	8.5	34.97
Water immersion	13.03	8.71	7.99	29.73
Control	10.24	6.75	6.78	23.77

Methyl myristate, hexadecane, methyl laurate and methyl palmitate were present in relatively higher amounts in vacuum processed tea samples than water immersion processed tea sample. 2,6-bis(1,1-dimethylethyl)-4-methylphenol and 1-octanol (aroma like rose and orange sweet) were detected in obviously higher amounts in water immersion processed tea sample. This explained that the aroma of GABA tea produced by different treatment is unlike.

Moreover, in this experiment the aroma components were extracted by simultaneous distillation extraction (SDE) method which can obtain high levels of aroma compositions with a small amount of samples and simplified operation. Although, there were much difference in sensory aspect between obtained volatile oil and the characteristic smell of tea. In addition, the extremely low intensities of the chromatographic peaks did not correspond well to the relative abundance of aromatic constituents of tea samples. Therefore, the absolute concentrations of the volatile GABA tea components still need to be determined.

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