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Evaluation of the synergism among volatile compounds in Oolong tea infusion by odour threshold with sensory analysis and E-nose

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ABSTRACT

Twenty-four kinds of representative aroma compounds in Oolong tea were selected to evaluate the interactions by the variation in the threshold values of these compounds before, and after, they were mixed. Result demonstrated that the ratios of the referenced threshold values of heptanal, β-damascenone, and methional to their determined thresholds were larger, namely, 5, 3.85, and 2.5, respectively. On the other hand, the mixed compounds with similar structure and aroma mainly presented a synergistic effect and additive action. Moreover, a masking effect was found among compounds with different structures. (E)-2-hexenal was added to tea infusion at a concentration below its threshold level to investigate whether, or not, the sub-threshold compounds affected the overall odour sensation of Oolong tea by sensory analysis and electronic nose (E-nose). The result indicated that the aroma of the tea infusion with added (E)-2-hexenal had changed before, and after, they were mixed.

1. Introduction

Flavour experts are always asked certain questions about aroma. For example, whether the aroma of a mixture is the simple addition of those of its component parts? Can we predict the aroma characteristics of a mixture according to the aroma of its components parts? Whether compounds with little individual aroma activity can significantly affect the aroma of a mixture? Whether, or not, there are interactions among aroma compounds? To answer these questions, scholars have conducted several research projects in recent years.

For instance, by using the variation in the threshold value of a single aroma compound and that in the mixture as the indicator, researchers have classified the interaction among aroma compounds into four types: no-effect, masking effect, additive action, and synergistic effect. (Saison, De Schutter, Uyttenhove, Delvaux, & Delvaux, 2009) These studies focused on the interaction between unsaturated olefine aldehydes and ester compounds. The results showed that there were obvious additive or synergistic effects among unsaturated olefine aldehydes with similar structures (homologues), while little interaction was found among compounds with different structures and aroma. Researchers investigated the effect of adding aroma compounds with sub-threshold concentrations on those with supra-threshold concentrations. (Miyazawa, Gallagher, Preti, & Wise, 2008) The results indicated that aroma compounds with different dilution multiples exhibit different influences on those with high thresholds: for acetic acid, the concentration of aroma compounds after dilution was inversely proportional to the interaction, that was, the smaller the concentration, the larger the interaction on 2-hydroxy-3-methyl-2-cyclopentene-1-one (ML), furan-2-ylmethanethiol (FM), and 3-methyl-3-sulfanylbutyl acetate (ASC). As for butyric acid, no obvious difference can be found in the interaction of ML, FM, and ASC by changing the dilution.

Moreover, some investigations focused on the interaction between 4-hexanolide and aroma compounds ((E)-2-hexenyl hexanoate, ()-3-hexenol, indole) with sub-threshold concentrations, which showed that the intensities of (E)-2-hexenyl hexanoate, ()-3-hexenol, indole were enhanced by adding 4-hexanolide. That was, there was a synergistic effect between 4-hexanolide and these compounds. These aroma compounds, with their low thresholds, make a significant contribution to the aroma of jasmine tea. (Ito & Kubota, 2005) By designing three binary systems and blank triangle tests, the influence of a wood compound with sub-threshold and concentrations on fruit aroma compounds with supra-threshold concentrations was investigated. (Atanasova et al., 2005) The results indicated that the wood compound

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improved the intensity of fruit aroma compounds. The aforementioned literature showed that the aroma of a mixture was not equal to the simple sum of those of its components. Therefore, it was difficult to predict the aroma of a certain system according to the proportions and concentrations of compounds added. Interactions such as the promoting effect and the restrictive function were indeed found among aroma compounds. In particular, adding seemingly insignificant amounts of compounds with sub-threshold concentrations can sometimes exert a remarkable influence on the overall aroma. This phenomenon was contradictory to classical aroma chemical theory which considers that only those aroma compounds with supra-threshold concentrations can contribute to the aroma of a system. (Atanasova et al., 2005)

Oolong tea, as a special tea in China, is mainly produced in Guangdong, Fujian, and Taiwan. Though it merely accounts for 5–7% of the total yield of tea in China, it is called "the Champagne of Teas" owing to its distinct aroma and mellow taste. (Liu, Jin, Hao, Lin, & Su, 2013; Zhang, Xiao, Du, & Zhu, 2012; Zhong, Lu, Lin, Tan, & Guo, 2009) However, there was little research on the aroma of Oolong tea, and the interactions among its sub-threshold aroma compounds were still unclear. Therefore, by studying Oolong tea, this research primarily illustrated the interactions among some aroma compounds based on the experimental data obtained, hoping to provide a theoretical basis for improving the quality of Oolong tea. Therefore, the aims of this study were (i) to determine the selected odour thresholds in Oolong tea, and (ii) to investigate the possible interactions between the sub- or odourants of Oolong tea.

2. Materials and methods

2.1. Chemicals

Authentic standards were obtained from the following sources. 2-Methylpropanal, 3-methylbutanal, 2-methylbutanal, propanal, hexanal, heptanal, (E)-2-pentenal, (E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal, phenyl acetaldehyde, (E,E)-2,4-heptadienal, (E,E)-2,4-hexadienal were purchased from Alfa Aesar Corporation (Tianjin, China). Butyl acetate, ethyl acetate, 3-methylbutyl acetate, acetic acid, hexanoic acid, heptanoic acid and octanoic acid were purchased from Shanghai Pu-Jie Fragrance . Ltd (Shanghai, China). Methional, 2,5-dimethylpyrazine, β-ionon and β-damascenone were provided by Sigma-Aldrich (. Louis, MO). All of the chemical standards used above were of food grade.

2.2. Tea sample and preparation

The method of sample preparation was drawn from previous research. (Zhu et al., 2015) Lightly fermented Oolong tea (Tieguanyin) was analysed in the present study. The tea sample was purchased from a local market in Shanghai, China. The sample was kept in the supplied packaging (aluminium foil material) and stored in a refrigerator (at 4 °C) until analysed. Twenty grams of Oolong tea were weighed and placed in a pre-warmed tea pot and 1000 mL of distilled water (at 80 °C) was added. The tea was brewed for 5 min and swirled 10 times clockwise while brewing. Then, the tea was poured through a porcelain strainer into a pre-warmed porcelain bowl. These sample infusions were stored in a refrigerator (at 4 °C).

2.3. Measurement of odour threshold

The measurement of odour threshold was based on ASTM (E679-04) (2011) and previous research with minor modification. (Saison et al., 2009) odour threshold was determined by asking whether, or not, the panelist could identify the quality of the odour of the sample solution by the staircase procedure. Thirteen panelists participated in this study. The most appropriate word used to express the odour quality of each odorant was first determined by several consultations with all of the panelists. The panelists were informed of the nature of the additive and a standard solution was presented at the entrance to the tasting room. A series of test samples was prepared by dispersing the substance whose threshold was to be determined in the medium of interest. Panelists were presented with six three-alternate-forced choice (3-AFC) tests spaced by a factor of 2.0. The panel members started at the highest concentration step, which should be two concentration steps above the estimated threshold. If the panelist could recognise odour in both trials on the same sample, the sample at the next lower concentration (step 2) was similarly tested, and the same test procedure was repeated for steps 3–5. If the panelist could not perceive the odour quality in even in one trial for step 5, the sample solution was changed to the one with the next higher concentration (step 4). If the panelist could perceive the odour quality in both trials for step 4, the perception test for step 5 was repeated. If the panelist failed again to detect the odour quality for step 5, step 4 was tested a third time. The best-estimate threshold (BET) was calculated as the geometric mean of the last missed concentration and the next (adjacent) higher concentration. The panelist indicates which of the three samples was different from the other two. A choice must be made, even if no difference was noted, so that all data can be utilised. Individual best-estimate values of threshold were derived from the pattern of correct/incorrect responses produced respectively by each panelist. Mixture thresholds were derived by geometrical averaging of the individual best-estimate thresholds. All the experiments were performed in triplicate.

According to this method, the interaction relationship between compounds could be categorised into four types: the first was "no-effect" which meant that the threshold of the mixture of compounds equalled the single threshold values of each compound; secondly, a masking effect suggested that the threshold of the mixture of compounds was greater than 100% of the threshold of the single compound; the third was additive action which showed that the threshold of the mixture was 50% of that of the single compound; while the last was a synergistic effect which implied that the threshold of the mixture was less than 50% of the individual compound thresholds.

2.4. Sensory evaluation of tea infusions

The tea infusion was evaluated by a well-trained panel of ten members (five males and five females, age: 20–45). Before the quantitative descriptive analysis, 10 mL of tea infusion was placed in a 50 mL-plastic cup covered with a Teflon cover and was subjected to a panelist in laboratory without peculiar smell at 25 °C. Then, the panelists had profoundly discussed aroma compositions of the tea infusion through three preliminary sessions (each spent 2 h), until all of them had agreed to the degree of aroma. Subsequently, the organoleptic characteristics descriptive were quantified using five sensory attributes (roast, sweet, green and grassy, sulphur and floral), to evaluate aroma tea infusion. These five aroma terms were defined as following aromas: 2-methylpyrazine for "roast" note, maltol for "sweet" note, hexanal for "green and grassy" note, dipropyl disulfide for "sulphur" note, phenylethyl alcohol for "floral" note. The complete blocks were estimated for original tea infusion (sample 1) and original tea infusion added with (E)-2-hexenal (sample 2) in triplicate for each treatment at random. The mean value of sample 1 and sample 2 were presented by the triplicate means score, which were rated

on the following scale from 0 (undetected), 3 (weak), 5 (moderate), 10 (strong). The sensory evaluations were performed in triplicate.

2.5. Electronic nose

FOX 4000 nose from Alpha-MOS (Toulouse, France) coupled with 18 metal oxide sensors (LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTL, LY2/gCT, T30/1, P10/1, P10/2, P40/1, T70/2, PA/2, P30/1, P40/2, P30/2, T40/2, T40/1, TA/2) fitted with a headspace auto sampler HS100 was used. (Wu, Wang, Tao, & Ni, 2016) The E-nose also contained a transistor used for amplifying or switching electronic signals. Molecules entering the sensor area were charged either positively or negatively, which then had a direct effect on the electric field.

The electronic nose was used to tentatively estimate the aroma profile similarity between original tea infusion and the tea infusion with (E)-2-hexenal added. The procedures were as follows: 1 g of those solutions were prepared in a 10 mL glass vial and capped with a Teflon rubber cap. Those two vials were orderly placed in the automatic sampler in the headspace system. Each vial was incubated at 25 °C for 20 min under agitation (500 rpm). Headspace (2500 mL) carried by air (150 mL/min) was injected into the E-nose. Sensor resistance was measured during 120 s at the rate of one acquisition every 1 s. The injected volume was 250 μL. The parameters were optimized in details and each analysis was repeated three times.

2.6. Statistical analysis

The odour thresholds of individual and mixture were submitted to standard deviation (SD) analysis. The quantitative descriptive sensory analysis were submitted to variance analysis (ANOVA). Duncan's multiple comparison tests were applied to determine significant differences of sensory attributes. All the analysis was carried out employing the XLSTAT ver.7.5 (Addinsoft, New York, NY, USA).

3. Results and discussion

3.1. Threshold determinations of aroma compounds in Oolong tea infusion

According to previous studies on Oolong tea conducted by the authors, twenty-four kinds of representative aroma compounds were selected to evaluate the interactions among them. Based on the differences in functional groups, these compounds could be separated into three categories: aldehydes, acids, and esters, while the former could be further divided into saturated aliphatic aldehydes, single unsaturated olefinic aldehydes, and double unsaturated olefinic aldehydes. Since the interactions in this experiment were investigated on the basis of the variation in the threshold values of these compounds before and after they were mixed, the threshold values had to be accurate. However, since the threshold values found in the literature were evaluated using different systems, they could not serve as a fair computational basis for comparison. Therefore, to exclude the effect of measurement/evaluation system on the threshold values of the compounds, this experiment determined the threshold values of these compounds using an addition method with practical tea infusion as the system. (Brown, Clapperton, Meilgaard, & Moll, 1978) Moreover, the aroma characteristics of the compounds were described. Table 1 listed the determined and referenced threshold values of these compounds together with an aroma description thereof.

As shown in Table 1, there were significant differences between the referenced and determined threshold values. None of the ratios of these two values was consistent. Thereinto, the ratios of the referenced threshold values of heptanal, β-damascenone, and methional to their determined thresholds were shown to be larger, namely, 5, 3.85, and 2.5, respectively. β-Damascenone, which was considered as apple, rose, and honey odours, exhibited an extremely low odour perception threshold (0.0013 μg/kg) in water. The value was approximately eight times greater than that investigated in our experiment. The matrix might be responsible for the difference of those values. According to previous research, β-damascenone demonstrated a significant influence on tea aroma and was likely to enhance the fruity aroma of tea infusion. (Zhu et al., 2015) Methional, with its distinctive roast potato odour, was an important volatile sulphur compound in Oolong tea, which degraded from a non-volatile sulphur-containing amino acid, such as methionine. (Herbst-Johnstone, Nicolau, & Kilmartin, 2011) The threshold of methional was 0.5 μg/kg in our investigation, which was higher than that (0.25 μg/kg) found in a previous study. (Zhu et al., 2015)

From the Table 1, acid compounds, such as acetic acid, hexanoic acid, heptanoic acid, and octanoic acid presented sour, sweat, and cheese notes. The thresholds of those compounds were generally larger than those of other compounds, 22,000 μg/kg, 1840 μg/kg, 3000 μg/kg, and 1900 μg/kg, respectively. Solubility in water, which was closely related to the volatility of a compound, was probably the main cause of large threshold values. The investigation was conducted according to the findings of other authors, who indicated that hydrophobic compounds had a strong volatility when in aqueous solution. (Saison et al., 2009) Furthermore, the determined thresholds of those compounds were 2100 μg/kg, 3602 μg/kg, 133.33 μg/kg, and 158.33 μg/kg, respectively. Beside hexanoic acid, the ratios of those compounds were small, being 0.1, 0.04, and 0.08, respectively. This difference was most likely caused by the different systems and threshold determinations for compounds. This research was performed using a tea infusion, while most of the referenced research quoted values based on a pure water system. These two systems differed in that many volatile, and non-volatile, compounds were contained in the tea infusion, which inevitably interacted with those compounds added.

As reported in the literature, there were large amounts of non-volatile compounds in the tea infusion, including caffeine, carbohydrates, tea polyphenols, and amino acids. These compounds influence the release of aroma substances in the tea infusion not only through physical action, but through direct, or indirect, chemical reactions. (Saucier, Guerra, Pianet, Laguerre, & Glories, 1997) For instance, amino acids could catalyse carbonyl compounds to form enols and there were condensation reactions between catechins and aldehyde compounds. All of these indicated that the non-volatile compounds in tea could influence the aroma compounds in a tea infusion and finally affected the threshold values of these aroma compounds.

In addition, to determine threshold values, the tea infusion endogenously contains the compounds added, which could also influence the threshold values. According to the literature, the threshold value of 2,3-butanedione was highly dependent on the endogenous concentration of the system. When the endogenous concentration was 30–300 ppb, the threshold value of 2,3-butanedione was about 70–150 ppb, while when the endogenous concentration was zero, the threshold value was 14–61 ppb. (Kluba et al., 1993; Meilgaard, Reid, & Wyborski, 1982) Therefore, it could be concluded that the presence of endogenous compounds in a tea infusion might also have caused the differences in threshold values.

Table 1
Odour thresholds of twenty-four selected compounds from experiment and literature with aroma description.

No	Compound	Aroma description[A]	Threshold (Literature[B], ppb)	Threshold (Determined[C], ppb)	SD[D]	Ratio[E] (Determined/Literature)
1	propanal	solvent, pungent	81	47.65	5.24	0.59
2	2-methylpropanal	pungent, malt, green	0.7	0.35	0.04	0.50
3	3-methylbutanal	cocoa, almond	0.2	0.33	0.05	1.67
4	2-methylbutanal	malt, cocoa	1	0.2	0.02	0.20
5	hexanal	grass, tallow, fat	4.5	0.38	0.04	0.08
6	heptanal	fat, citrus, rancid	550	2750	45.00	5.00
7	(E)-2-pentenal	grass, tomato	310	17.22	1.89	0.06
8	(E)-2-hexenal	green, leaf	82	58.57	7.61	0.71
9	(E)-2-heptenal	soap, fat	13	7.22	0.79	0.56
10	(E)-2-octenal	green, nut, fat	3	6	0.73	2.00
11	(E,E)-2,4-hexadienal	green	94.8	23.7	2.79	0.25
12	(E,E)-2,4-heptadienal	nut, fat	56	11.91	1.85	0.21
13	phenyl acetaldehyde	hawthorne, honey, sweet	4	2.5	0.27	0.63
14	ethyl acetate	pineapple	6200	2790	62.00	0.45
15	butyl acetate	pear	58	77.33	7.12	1.33
16	3-methylbutyl acetate	banana	2	0.28	0.03	0.14
17	acetic acid	sour	22000	2100	36.00	0.10
18	hexanoic acid	sweat, cheese	1840	3602	94.00	1.96
19	heptanoic acid	sour	3000	133.33	14.67	0.04
20	octanoic acid	sweat, cheese	1900	158.33	20.58	0.08
21	methional	cooked potato	0.2	0.5	0.06	2.50
22	2,5-dimethylpyrazine	cocoa, roasted nut	20	35	3.15	1.75
23	β-ionone	violet, flower, raspberry	7	3	0.28	0.43
24	β-damascenone	Apple, rose, honey	0.0013	0.01	0.00	7.69

[A] The aroma description of compounds in tea infusion.
[B] The thresholds of compounds referred to literature (Van Gemert, 2003).
[C] The thresholds of compounds determined in experiment.
[D] The standard deviation of thresholds of compounds investigated in experiment.
[E] The ratio of determined threshold to literature.

3.2. Interactions between aroma compounds in Oolong tea infusion

The threshold values of compounds could indirectly reflect the influence of the compounds on the aroma so that we could consider that the entire aroma of compounds was the addition of the aroma of each single compound (at least approximately). However, doing so neglected the interactions between compounds, which varied from those seen in normal conditions. Therefore, with threshold values as a reference, this research compared the variation of threshold values of compounds before, and after, being mixed so as to evaluate the relationship among compounds.

Based on the literature, for convenience of calculation, compounds were added into the tea infusion in proportion to their individual thresholds. (Saison et al., 2009) The ratio (%) of the determined threshold value of a compound in the mixture to the original threshold of the compound was used to represent the degree of practical interaction. In this experiment, nineteen compound combinations were tested, in which eleven were combinations of compounds with similar structure or aroma while eight were composed of compounds with irrelevant structures.

According to Table 2, when compounds with similar structure and aroma were mixed, except for (E,E)-2,4-hexadienal and (E,E)-2,4-heptadienal, other mixtures had a synergistic effect and additive action. In contrast, the masking effect was mainly found in mixtures composed of compounds with large structural differences between them. 2-Methylbutanal and 3-methylbutanal were isomers: 2-methylbutanal presented coffee, fruit, and chocolate-like aroma, and its threshold was 0.2; while with a threshold of 0.33, 3-methylbutanal also showed coffee and cacao aroma. Therefore, 2-methylbutanal and 3-methylbutanal had similar properties. After they were mixed, the mixture gave off a pleasing smell of coffee and chocolate with a threshold of 44% of the single compound thresholds, thus exerting a strong synergistic effect. In the practical blending of coffee or chocolate aroma, flavorists always used these two in the proper proportion so as to make a more mellow, delicate, coffee aroma. By adding 2-methylpropanal to the mixture of 2-methylbutanal and 3-methylbutanal, additive action could also be found. According to the literature, these three compounds showed strong additive action in the mixture of grape wine, which was consistent with the findings of the current work. However, compared to binary mixtures, perceptual suppression was observed frequently in mixtures with a high number of compounds. (Bult, Schifferstein, Roozen, Voragen, & Kroeze, 2001)

Similar situations were listed in Table 2: hexanal and heptanal, (E)-2-heptenal and (E)-2-hexenal, ethyl acetate and butyl acetate were homologues, and were differentiated only in the methylene structure and had strong synergistic effects after being mixed. In addition, although hexanal and (E)-2-hexenal, (E,E)-2,4-heptadienal and (E)-2-heptenal, had dissimilar structures, they presented similar aroma, thus also showing a synergistic effect. Therefore, compounds with similar structures or aroma seemed to present either a synergistic effect or additive action; however, this did not mean that all homologues could present a synergistic effect after mixing. For instance, although (E,E)-2,4-hexadienal and (E,E)-2,4-heptadienal, acetic acid and hexanoic acid had similar thresholds and aroma, they produced a masking effect after being mixed. The reason for this remained unclear and was deemed to warrant further research.

Moreover, a masking effect, or weak additive action, was found among compounds with different structures. The ratios of (E,E)-2,4-hexadienal and phenyl acetaldehyde, (E)-2-hexenal and phenyl acetaldehyde were 67% and 54%, respectively, all of which presented weak additive actions. As listed in Table 2, a masking effect was evident between ethyl acetate and acetic acid, hexanoic acid and ethyl acetate, 2-methylbutanal and butyl acetate, and (E)-2-hexenal and ethyl acetates. Although ester compounds were esterified from acid compounds and were therefore closely related (in theory), a masking effect was found between them. This indicated that ester compounds could mask the aroma of other compounds. (Saison et al., 2009)

Table 2
Threshold values of mixtures of compounds in the ratio of their individual thresholds (TH) with aroma description.

Similar aroma and structure	TH of mixture (%)[A]	SD[B]	Aroma description[C]	Distinct aroma and structure	TH of mixture (%)	SD	Aroma description
3-methylbutanal 2-methylbutanal	44	3.4	malt, cocoa, almond	(E,E)-2,4-hexadienal phenyl acetaldehyde	67	5.9	honey, green
hexanal heptanal	37	4.2	grass, tallow, fat, rancid	phenyl acetaldehyde (E)-2-hexenal	54	6.3	honey, green, sweet
(E)-2-heptenal (E)-2-hexenal	49	5.2	green, nut, fat	ethyl acetate acetic acid	103	11.3	pineapple, sour
(E,E)-2,4-hexadienal	168	12.8	green, fat	hexanoic acid	244	14.3	sweat, cheese, pineapple
(E,E)-2,4-heptadienal				ethyl acetate			
2-methylpropanal			pungent, malt, green, cocoa	2-methylbutanal	149	12.3	malt, cocoa, pear
3-methylbutanal 2-methylbutanal	75	6.3		butyl acetate			
				(E)-2-hexenal	169	10.2	green, leaf, pineapple
hexanal (E)-2-hexenal	48	3.9	green, leaf, fat	ethyl acetate			
				(E)-2-hexenal	69	7.2	green, leaf, sour
(E,E)-2,4-heptadienal (E)-2-heptenal	45	5.3	nut, fat, soap	acetic acid			
				hexanoic acid	94	8.3	sweat, cheese, green
ethyl acetate butyl acetate	43	3.5	ftuit	(E)-2-hexenal			
				2,5-dimethylpyrazine	138	9.2	green, roasted nut
butyl acetate 3-methylbutyl acetate	36	3.8	fruit, pear, banana	(E)-2-hexenal			
				methional	169	10.3	cooked potato, green
ethyl acetate butyl acetate 3-methylbutyl acetate	68	7.2	fruit, pear	(E)-2-hexenal			
				β-ionone (E)-2-hexenal	143	11.4	violet, flower, green
acetic acid hexanoic acid	97	8.9	sour, sweat, cheese				
				β-damascenone (E)-2-hexenal	48	5.4	apple, honey, green

[A] Threshold values of mixtures of compounds in the ratio of their individual thresholds.
[B] The standard deviation of thresholds of mixtures compounds.
[C] The aroma description of mixture compounds.

The ratio of (E)-2-hexenal to acetic acid was 69%, while the ratio of (E)-2-hexenal to hexanoic acid was 94%. Acetic acid and hexanoic acid were homologues, which merely differed in their four methylenes. Even so, these two molecules exhibited different abilities in enhancing the aroma intensity of other compounds, which agreed with reports in the literature. (Miyazawa et al., 2008) This research investigated the enhancement effect of acetic acid and butanoic acid on the aroma of ML, FM, and ASC. The results revealed that acetic acid showed a better aroma-enhancing effect than butanoic acid.

Consequently, the knowledge of the odour threshold of a volatile compound in mixture might not be sufficient to indicate its effect on the whole aroma. Thus, it would be necessary to take into account these perceptual interactions to estimate the contribution of the sub- and compounds to the total odour intensity and quality in a mixture. (Atanasova et al., 2005)

3.3. Contribution of (E)-2-hexenal to the aroma of Oolong tea

To clarify whether, or not, sub-threshold compounds affected the overall odour sensation of Oolong tea, (E)-2-hexenal was added to tea infusion (sample 2) at a concentration (0.03 ppm) below its threshold level (Table 1). The odour quality of sample 2 was compared to that of the original tea infusion (sample 1) by sensory analysis. According to the previous research, five sensory attributes were commonly selected to describe the overall aroma by the panelists: "roast", "sweet", "green and grassy", "sulphur", and "floral". (Culleré, Cacho, & Ferreira, 2007)

Although the panelists expressed their perceptions against different scoring criteria, due to their differences in age, background, and olfactory sensitivity, no significant interaction between panel member and replication was found, indicating that all of the panelists were reproducible with regard to the scoring of all attributes in triplicate (data not shown). As shown in Fig. 1, the intensities of roast and sulphur aromas in the tea sample after the addition of (E)-2-hexenal were reduced compared with those in the original

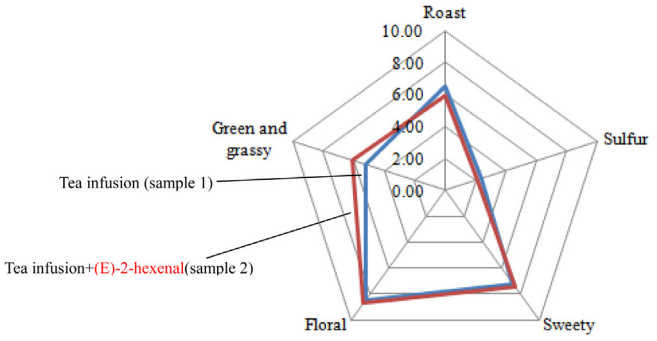

Fig. 1. Sensory evaluation of tea infusions adding (E)-2-hexenal to the Oolong tea infusion.

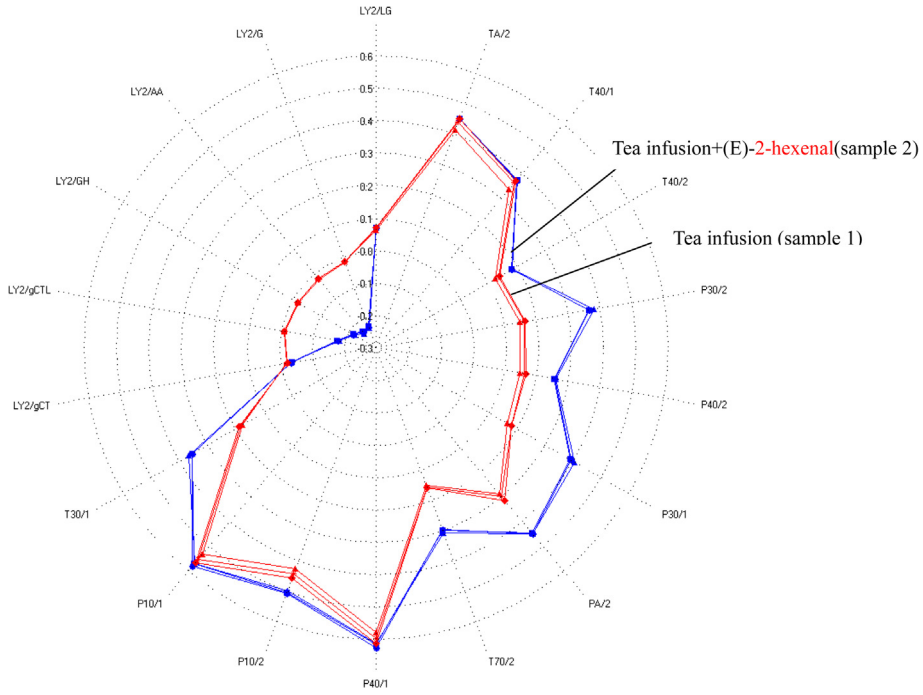

Fig. 2. Aroma profile of tea infusions adding (E)-2-hexenal to the Oolong tea infusion by E-nose.

tea sample. Based on previous research, the roast aroma was related to 2,5-dimethylpyrazine, furfurylthiol and furfural, while sulphur stemmed from methanethiol, dimethyl disulfide and methional. (Culleré et al., 2007) It could also be seen from Table 2 that (E)-2-hexenal masked 2,5-dimethylpyrazine and methional, which explained why the intensities of roast and sulphur aromas were decreased to a certain extent. In contrast, the intensity of the "sweet" aroma in the tea sample was enhanced compared with that of the original tea sample. According to the literature, "sweet" aroma presented a positive correlation with 6-methyl-5-hepten-2-one (not shown in Table 1), as well as β-damascenone. (Zhu et al., 2015) Although 6-methyl-5-hepten-2-one was considered a fruity aroma, it could greatly enhance the perception of sweetness in tea infusions. (Qin et al., 2013) As shown in Table 1, β-damascenone was considered to have been the characteristic sweet aroma. As shown in Fig. 1, these two tea infusions presented great differences in the intensity of "green and grassy" aromas, which was positively related to hexanal, (E)-2-pentenal, heptanal, (E)-2-hexenal, and (E)-2-heptenal. According to previous research, aldehyde compounds with six to ten carbons were associated with being perceived as grass, green plants, citrus, and fatty notes. This green aroma was sometimes referred to as a fresh note, and the chemicals responsible were predominantly C6 aldehydes, preferably unsaturated. (Zhu et al., 2015) As seen from Table 2, strong synergistic effects occurred between (E)-2-hexenal, (E)-2-heptenal, and hexanal when (E)-2-hexenal was added. Therefore, the intensity of "green and grassy" notes was enhanced compared to the original infusion. According to our previous findings, a "floral" aroma was highly correlated to hotrienol, geraniol, phenylethyl alcohol, and β-ionone. (Zhu et al., 2015) It was important to note that there were masking effects between (E)-2-hexenal and β-ionone, but the "floral" aroma intensity was enhanced. This was most likely induced by the synergistic effects between (E)-2-hexenal and hotrienol, as well as between geraniol and phenylethyl alcohol. This also indicated that there were complicated interactions among different compounds so that we could not determine the variation of the aroma of a system simply based on the relationship between two compounds. On the contrary, various types of factors needed to be taken into consideration. Although onerous, research into the interactions among aroma compounds proved to be significant when studying aroma.

3.4. Electronic nose analysis of aroma of tea infusion

The electronic nose, as a better method of analysing aroma, was similar to the human nose. Differing from general chemical analysis instruments such as gas chromatograph (GC) and high performance liquid chromatograph (HPLC), the electronic nose method did not need to separate volatiles. Therefore, it could obtain all of the information pertinent to volatile compounds in samples rather than qualitative and quantitative results of one or several compounds in the samples detected. In addition, compared with sensory analysis, the electronic nose method could more precisely and objectively measure aroma at faster speeds. During E-nose analysis of original tea infusion (sample 1) and the tea infusion with (E)-2-hexenal added (sample 2), a radar graph (Fig. 2) was automatically generated by the built-in software of the E-nose system. As could be seen, there were obvious differences in most of the 18 sensors between the profiles of the fingerprints of these two infusions. According to Fig. 2, the response values of sensors LY2/LG, TA/2, T40/1, T40/2, P30/2, P40/2, P30/1, 1/2, T70/2, P40/1, P10/2, P10/1, and T30/1 for both samples were above zero: in contrast, the response values of the other sensors (LY2/G, LY2/AA, LY2/GH, LY2/Gctl, and LY2/gCT) were negative. Among those sensors, P40/1, P10/1, P10/2, TA/2, and T40/1 presented relative higher response values than those in other sensors for sample 2. Similarly, sample 1 showed high response signals in sensors P40/1, P10/1, P10/2, TA/2, T40/1, P30/2, P30/1, and PA/2. Furthermore, these two tea infusions presented significant differences in the response values in sensors P30/2, P30/1, P40/2, PA/2, T70/2, T30/1, and LY2. The response values of sample 2 were larger than those of sample 1 on sensors P30/2, P30/1, P40/2, PA/2, T70/2, and T30/1, while the opposite was found with the LY2 sensor. Thus, sensors P30/2, P30/1, P40/2, PA/2, T70/2, T30/1, and LY2 signifi-

cantly contributed to the flavour discrimination of those two samples. This indicated that the aroma of the tea infusion with added (E)-2-hexenal had changed, which agreed with the results obtained through previous sensory analysis.

4. Conclusion

The phenomenon of interactions among different types of aroma compounds were evaluated in this experiment. The result indicated that the compounds with similar structure and aroma might be prone to present synergistic effect or additive action. On the contrary, the compounds with different structures usually demonstrated masking action. Moreover, the effect on the overall odour sensation of tea infusion by adding ((E)-2-hexenal) at a concentration below its odour threshold was also studied by sensory analysis and E-nose. The result suggested that the aroma of the tea infusion had changed before, and after, they were mixed. The findings further illustrated that the sub-threshold aroma compound might play an important role in the aroma of Oolong tea infusion.

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