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# Sensory evaluation of the synergism among odorants present in concentrations below their odor threshold in a Chinese jasmine green tea infusion

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The mechanism for the contribution of subthreshold aroma constituents to the overall jasmine tea odor impression was studied on the basis of a sensory evaluation. Binary model aqueous solutions containing the authentic odorants of a jasmine tea infusion, (*E*)-2-hexenyl hexanoate (I), (*Z*)-3-hexenol (II), and indole (III), were each prepared in a concentration below the odor threshold. Each solution had no aroma, but when 4-hexanolide replaced only 5% of each odorant, the odor intensity of each model solution was significantly strengthened. An astringent note and heavy note were recognized for each solution as the commonly perceived characteristics from the sensory evaluation. The concentration of 4-hexanolide added was also at the subthreshold level. The results suggest mutual interaction between odorants I, II, or III and 4-hexanolide. The effect on the overall odor sensation of a jasmine tea infusion by adding 4-hexanolide at a concentration below its odor threshold was also studied. In this case, the intensity of both the sweet and astringent notes was significantly strengthened in comparison with the odor impression of the original jasmine tea infusion. This phenomenon is considered to have been a synergistic effect between subthreshold odor compounds in the jasmine tea infusion. The results of this study clarify for the first time that the subthreshold aroma constituents play an important role in the characteristic flavor of a jasmine tea infusion.

**Keywords:** Hexenyl hexanoate / Jasmine tea / Lactone / Odor synergism / (*Z*)-3-Hexenol

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## 1 Introduction

More than 600 compounds have been identified as constituents of the tea aroma up to the present time [1]. However, the role of subthreshold aroma compounds and the mutual interaction among such odorants in tea have not been clarified. There has been only a little work focused on subthreshold odor compounds and the mutual interaction among food flavor constituents. Hirvi and Honkanen [2] have pointed out that most of the blueberry flavor compounds were present below their odor threshold values and it seemed that synergism played an important role in the overall impression of the odor of blueberry. Kock *et al*. [3] have suggested the importance of the odor synergism between skatole and androstenone from the results of a principal component analysis and correlation analysis of the boar odor constituents. Nawar *et al.* [4] showed that when five subthreshold methyl ketones were presented in a mixture solution, the odor of the mixture was detectable on the basis of sensory evaluation. These reports have suggested the importance of minor compounds on the basis of synergism among the subthreshold odor compounds.

We applied the AEDA method [5] to a Chinese jasmine green tea flavor analysis and reported the odor-active compounds in the previous study [6]. Among these, the mixtures of 4-hexanolide and (*E*)-2-hexenyl hexanoate and of 4-nonanolide and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) were identified as the most odor-active compounds. The respective concentrations of 4-hexanolide, 4-nonanolide, furaneol, and (*E*)-2-hexenyl hexanoate in the jasmine tea infusion were 7.5 ± 3.4 ppb, 16.6 ± 2.7 ppb, 13.3 ± 3.0 ppb, and 9.5 ± 4.0 ppb. The odor threshold levels of first three of these compounds in the literature were, respectively, 1.6 ppm, 0.03 ppm, and 0.1 – 0.2 ppm [7, 8]. These three compounds were therefore contained in the jasmine tea infusion at concentrations below their odor threshold levels. It is particularly interesting that the aroma constituents which were present at such low concentrations were

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**Abbreviations: DOT**, detection odor threshold; **LMS**, labeled magnitude scale; **QDA**, quantitative descriptive analysis; **ROT**, recognition odor threshold

nonetheless very important aroma constituents of the tea infusion. Kumazawa and Masuda [9] have also reported that the high flavor dilution factor (FD-factor) fractions contained qualitatively minor compounds (5-octanolide and 2-aminoacetophenone, and 4-nonanolide, (*Z*)-3-hexenyl (*Z*)-3-hexenoate, β-damascone, and β-damascenone). An investigation of mutual interaction among qualitatively minor compounds is therefore considered to be very important in elucidating the characteristics of the tea aroma.

The mechanism for the contribution of subthreshold aroma constituents to the overall impression of the odor of a jasmine tea infusion is studied by a sensory evaluation in this present work. The mutual interaction among odorants present at concentrations below their odor threshold level is examined by using binary models of aqueous solutions of authentic chemicals. The similarity in chemical structure or olfactory perception of the odorants that interacted with each other is then investigated. The role of subthreshold odorants in the overall odor of the jasmine tea infusion is then identified.

## 2 Materials and methods

### 2.1 Materials and chemicals

Jasmine tea produced in the Fujian province of China in September 2000 was used. It was prepared from green tea (*Camellia sinensis L.*) scented with flowers of *Jasminum sambac*. 4-Hexanolide (99.9%), (*E*)-2-hexenyl hexanoate (97.0%), (*Z*)-3-hexenyl hexanoate (98.5%), indole (99.9%), 2-phenyl ethanol (99.0%), 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene, 99.8%), (*Z*)-3-hexenol (99.8%), hexanoic acid (98.9%), hexanal (98.9%), hexanol (99.1%), (*E*)-2-hexenal (98.7%), 2-hydroxy-3-methyl-4*H*-pyran-4-one (maltol, 99.2%), and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol, 99.9%) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Benzaldehyde (98.9%) was from Kanto Chemical (Tokyo, Japan). (*R*)-(−)-Linalool (96%) and linalool oxide (*trans*-furanoid, 97%) were from T. Hasegawa (Tokyo, Japan). Propylene glycol was purchased from Wako Pure Chemical Industries (Osaka, Japan). (*E*)-2-Hexenyl hexanoate contained as an impurity of hexyl hexanoate at 2.8%. The impurities of (*R*)-(−)-linalool and linalool oxide (*trans*-furanoid) were their optical isomers.

### 2.2 Sample preparation

The individual chemicals dissolved in a small amount of propylene glycol were each diluted to the desired concentration with deodorized water or the jasmine tea infusion. The deodorized water was prepared from purified water that has been boiled, cooled to room temperature, and deodorized with activated charcoal, the charcoal then being filtered off. The jasmine tea infusion was prepared in the way described in our previous study [6]. Hot water (750 mL) was added to 10 g jasmine tea and left to infuse for 3 min. The tea leaves were then filtered off, and the resulting filtrate was used as the jasmine tea infusion. A 20 mL amount of each sample solution was put in a 50 mL screw-capped glass bottle that was labeled with a random three-digit code and allowed to equilibrate for about 1 h at room temperature before being used. The concentration was shown as the weight/weight (ppm or ppb).

### 2.3 Evaluation panels, environmental conditions and experimental design

In order to ensure that the selected panelists had an accurate sense of smell, a screening test was conducted by a sensory evaluation of the T & T olfactometer reagents (Daiichi Yakuhin Sangyo, Tokyo, Japan). Sixteen female university students in the age range of 21 – 31 years passed the test by giving the correct answers for all the samples: 2-phenyl ethanol ($10^{-4.0}$ w/w), methyl cyclopentenolone ($10^{-4.5}$ w/w), isovaleric acid ($10^{-5.0}$ w/w), 4-undecanolide ($10^{-4.5}$ w/w), and skatol ($10^{-5.0}$ w/w). The sensory analysis was conducted in a special testing room that was quiet, comfortable, and provided with an air conditioner and ventilation fan. Each testing area in this room was curtained off for the individual panelists. The randomized complete block design [10] was adopted for the sensory analysis tests, except for the session during which the recognition odor threshold was measured.

### 2.4 Measurement of the detection odor threshold (DOT) of a binary solution

The triangle test [11] was performed to measure the detection odor threshold. Three bottles were presented to each panelist. One bottle contained the sample solution and the other two, a blank, or one bottle contained the blank and the other two, the sample solution. The panelists were told that two of the solutions had the same odor and one was different, and they were asked to identify the odd sample. The sample solutions were binary mixtures of (*E*)-2-hexenyl hexanoate and 4-hexanolide, and (*Z*)-3-hexenyl hexanoate and 4-hexanolide. Five different content ratios (100 : 0, 95 : 5, 90 : 10, 80 : 20, and 0 : 100) were tested at six different total concentrations (10 ppm, 1.0 ppm, $1.0 \times 10^{-1}$ ppm, $1.0 \times 10^{-2}$ ppm, $1.0 \times 10^{-3}$ ppm, and $1.0 \times 10^{-4}$ ppm) (Table 1). The blank samples were deodorized water that contained the same amount of propylene glycol as that in the sample solutions. In one session, five sets of triangle tests on samples with the same total concentration but differing content ratio were evaluated at intervals of 30 s. The

 

**Table 1.** Number of panelists who correctly answered during the triangle tests ($N = 9 \times 2$)

| Concentration (ppm) | Content ratio in water ((*E*)-2-hexenyl hexanoate : 4-hexanolide) | | | | |
|---|---|---|---|---|---|
| | 100 : 0 | 95 : 5 | 90 : 10 | 80 : 20 | 0 : 100 |
| 10 | 18*** | 17*** | 18*** | 16*** | 18*** |
| 1.0 | 16*** | 17*** | 16*** | 17*** | 15*** |
| $1.0 \times 10^{-1}$ | 7 | 12** | 12** | 9 | 9 |
| $1.0 \times 10^{-2}$ | 8 | 4 | 5 | 5 | 5 |
| $1.0 \times 10^{-3}$ | 10 | 3 | 2 | 6 | 6 |
| $1.0 \times 10^{-4}$ | 4 | 6 | 3 | 4 | 4 |
| Concentration (ppm) | Content ratio in water ((*Z*)-3-hexenyl hexanoate : 4-hexanolide) | | | | |
| | 100 : 0 | 95 : 5 | 90 : 10 | 80 : 20 | 0 : 100 |
| 10 | 16*** | 16*** | 16*** | 17*** | 15*** |
| 1.0 | 15*** | 15*** | 16*** | 16*** | 16*** |
| $1.0 \times 10^{-1}$ | 12** | 4 | 8 | 6 | 4 |
| $1.0 \times 10^{-2}$ | 5 | 5 | 6 | 4 | 9 |
| $1.0 \times 10^{-3}$ | 5 | 2 | 7 | 4 | 8 |
| $1.0 \times 10^{-4}$ | 3 | 4 | 6 | 5 | 4 |

Asterisks represent the significance: * $p = 0.14$; ** $p = 0.04$; *** $p \leq 0.01$

order of presentation of the five sets of triangle tests was randomized. Nine panelists had two sessions of these five sets of triangle tests with an interval of at least 24 h between sessions. A total of 18 answers was obtained for each sample solution. The results of the triangle tests were analyzed by the method of Roessler [12]. The minimum concentration at which a sample solution could be significantly distinguished from the blank sample was determined as the detection odor threshold (DOT).

### 2.5 Measurement of the recognition odor threshold (ROT)

ROT was determined by asking whether the panelist could identify the quality of the odor of the sample solution by the staircase procedure. Thirteen panelists participated in this study. The most appropriate word to express the odor quality of each odorant was first determined by several consultations with all the panelists. Three samples at different concentrations were presented for panelist consultation, *e.g.*, 10 ppm, 100 ppm, and 1000 ppm for 4-hexanolide. The odor quality in common with all concentrations of each solution was discussed and the term that best explained the odor quality of the odorant was determined (Fig. 1). A 15-step binary dilution series of each sample solution from 200 ppm (step 1) to 0.012 ppm (step 15) was then prepared (Fig. 1). Each sample was presented twice to a panelist starting at 200 ppm (step 1) at intervals of 30 s. The panelist was instructed to answer whether she could perceive the odor quality of the stimulus or not. For example, the following instruction was used in the case of 4-hexanolide: Can you recognize the "coconut milk-like" odor of the sample? If the panelist could recognize this 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 step 3, step 4, and step 5. If the panelist could not perceive the odor 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 odor quality in both trials for step 4, the perception test for step 5 was repeated. If the panelist failed again to detect the odor quality for step 5, step 4 was tested for a third time. If the odor quality of both trials of the sample could be recognized again for step 4, the concentration of step 4 was determined as the ROT of the odorant for the panelist.

| | ROT in water | | | | | | ROT in jasmine tea infusion |
|---|---|---|---|---|---|---|---|
| Concentration (ppm) | 4-hexanolide | (*E*)-2-hexenyl hexanoate | (*Z*)-3-hexenyl hexanoate | (*Z*)-3-hexenol | hexanol | indole | 4-hexanolide |
| 200.000 | | | | | | | ● |
| 100.000 | ●●●● | | | | | | ●●●●● |
| 50.000 | | | | | | | ●●● |
| 25.000 | ●●● | | | | | ●●●● | ● |
| 12.500 | ●●●●●● | | | | | ●●●● | ●●● |
| 6.250 | | | | | ●●● | | |
| 3.130 | | ●●● | | | ● | ●●● | |
| 1.560 | | ●●● | | ●●● | ●●●● | ●● | |
| 0.781 | | ●●●●●●● | ●● | ●●●●● | ●●●● | | |
| 0.391 | | | ●●●● | ●● | ● | | |
| 0.195 | | | ●●●●●●● | ●●● | | | |
| 0.098 | | | | | | | |
| 0.049 | | | | | | | |
| 0.024 | | | | | | | |
| 0.012 | | | | | | | |
| Odor attribute | coconut milk-like | pungent | green apple-like | green | green | camphoraceous | coconut milk-like |

**Figure 1.** ROT levels plotted for each panelist.

 

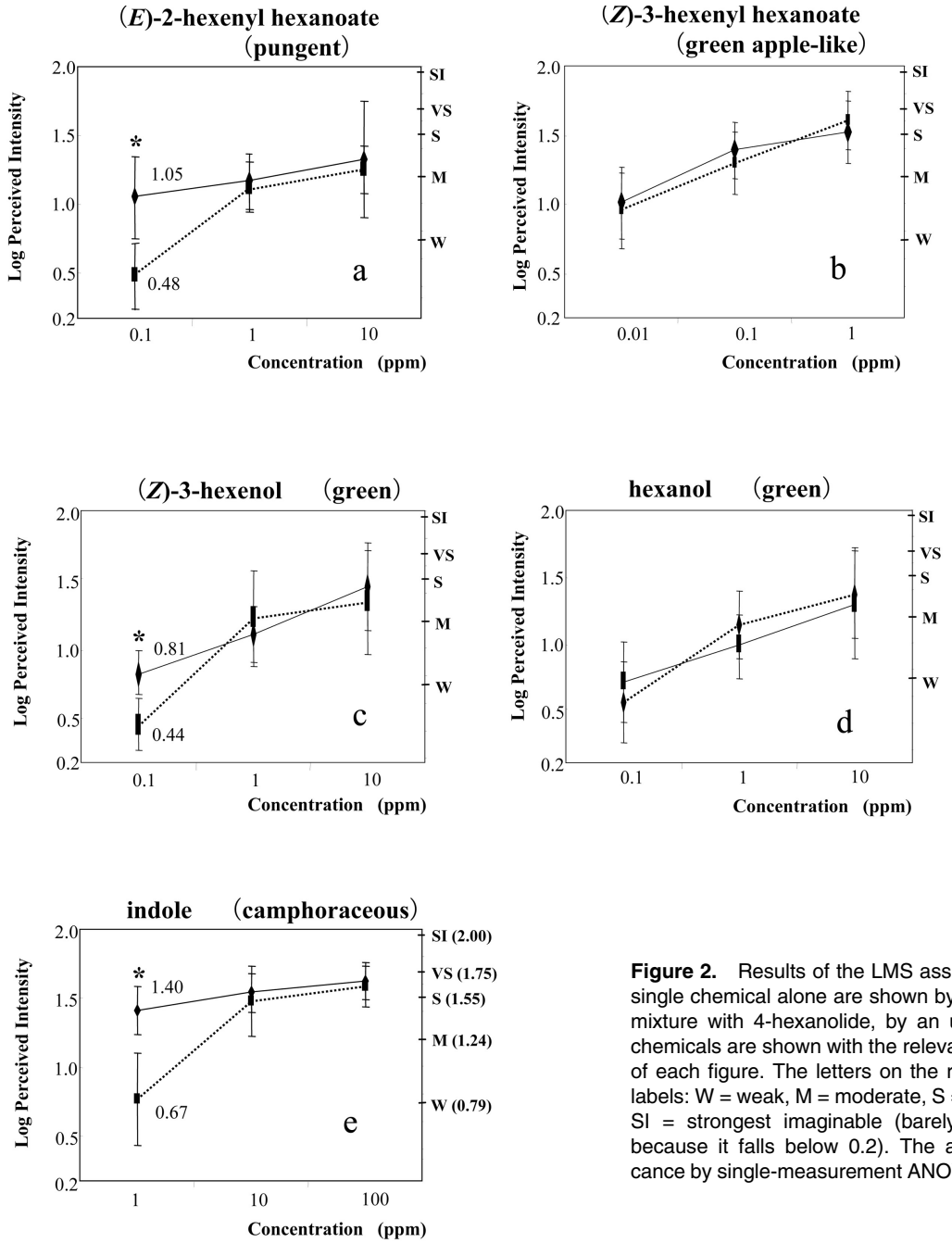


**Figure 2.** Results of the LMS assessment. The plots for the single chemical alone are shown by a broken line, and for the mixture with 4-hexanolide, by an unbroken line. The tested chemicals are shown with the relevant odor attribute at the top of each figure. The letters on the right *y*-axis represent LMS labels: W = weak, M = moderate, S = strong, VS = very strong, SI = strongest imaginable (barely detectable was omitted because it falls below 0.2). The asterisks show the significance by single-measurement ANOVA ($p \leq 0.05$).

### 2.6 Measurement of the odor intensity

The labeled magnitude scale (LMS) [13] was used to measure the strength of the stimulus of each sample. The scale used in this study is shown in Fig. 3. The scale positions of the labels, as a percentage of the full-scale length were as follows: B.D. (barely detectable), 1.4; weak, 6.1; moderate, 17.2; strong, 35.4; very strong, 53.3; strongest imaginable, 100. The interpretation of B.D. was given to the panelists. The LMS was displayed on a computer monitor and responses were made by moving a cursor to the appropriate location on the scale with a mouse. Each panelist evaluated three concentrations of a sample at random in one sitting, that is, the concentration at which none of the panelists could detect the quality of the odorant, the concentration at about the mid ROT level perceived by all the panelists, and the concentration at which all panelists could identify the odor quality of the odorant (Figs. 1 and 2). The interval between presenting each stimulus was 30 s. After at least a 24 h interval, the same panelists tested the same odorant,

 

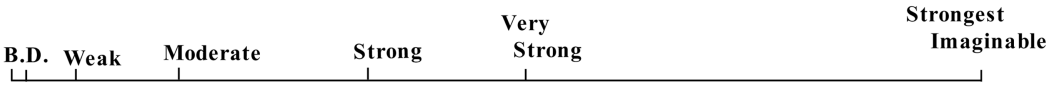


**Figure 3.** LMS devised by Green *et al.* [13] that was used in the present study. B.D. represents "barely detectable".

with 5% of the odorant having been replaced by 4-hexanolide, at the same three concentrations (Fig. 2). Twelve panelists were used to evaluate (*E*)-2-hexenyl hexanoate and thirty for the other compounds. The raw data were normalized by converting to the logarithmic value before statistical analyses by repeated-measures analysis of variance (ANOVA) and single-measure ANOVA.

### 2.7 Determination of the commonly perceived character

A quantitative descriptive analysis (QDA®; Tragon, Redwood City, CA, USA) [14] was performed to identify the commonly perceived character of each odorant as influenced by a subthreshold level of 4-hexanolide. Model solutions of the 14 odorants shown in Table 2 were prepared in a concentration at which all panelists could recognize without doubt the quality of each odorant on the basis of the ROT level for each panelist (Fig. 1). All these model solutions were presented for consultation with thirteen panelists. The terms which expressed the common odor impression for those odorants influenced by 4-hexanolide were first determined. Each panelist selected the most appropriate terms for expressing the common sensation of odor from the 44 words reported by Shimoda *et al*. [15] as being appropriate expressions for food. The panelists were also requested to write down other words with which they could express the common odor of the sample solution, if they thought that the 44 words were not sufficient to describe the odor of the samples. The attributes were finally agreed upon after several consultations among the panelists had been conducted. The 13 panelists then evaluated the model solutions of fourteen odorants (Table 2) according to a line scales (from 0 – absent to 8 – strong) which was displayed on a computer monitor (Compusense five®; Guelph, ON, Canada). The resulting QDA® data were analyzed by Tukey's multiple-comparison test.

### 2.8 Measurement of the odor profile of an odorant or a mixture of odorants

The QDA® method was also performed to measure the odor profile of an odorant or mixture of odorants. The procedure and statistical analyses used were almost the same as those for determining the commonly perceived character just described.

**Table 2.** QDA results for the "astringent" and "heavy" odor attributes from 14 odor-active compounds of the jasmine tea infusion

| Compound | Astringent[a)] | Heavy[a)] |
|---|---|---|
| (*E*)-2-Hexenyl hexanoate | 4.36 a | 3.56 ab |
| Indole | 3.94 a | 3.91 ab |
| 2-Phenyl ethanol | 2.56 abc | 4.14 ab |
| Cyclotene | 2.20 abc | 2.00 abc |
| (*Z*)-3-Hexenyl hexanoate | 1.64 bc | 3.01 abc |
| (*Z*)-3-Hexenol | 1.63 bc | 4.39 a |
| Hexanoic acid | 1.32 bc | 2.80 abc |
| (*R*)-(−)-Linalool | 1.10 bc | 2.12 abc |
| Hexanal | 1.02 bc | 3.43 abc |
| Hexanol | 0.95 bc | 0.44 c |
| Linalool oxide (2*R*-furanoid) | 0.72 c | 1.20 bc |
| (*E*)-2-Hexenal | 0.65 c | 1.23 bc |
| Benzaldehyde | 0.62 c | 2.41 abc |
| Maltol | 0.29 c | 1.16 bc |

a) Average scores from the sensory evaluation (0, absent; 8, strong) by 13 panelists. Different letters (a, b, and c) in a column indicate significant difference by Tukey's multiple-comparison test ($P \leqq 0.05$). The definitions of the odor attributes are given in Table 3.

## 3 Results and discussion

### 3.1 Effect of 4-hexenolide on the DOT of (*E*)-2-hexenyl hexanoate

We reported in the previous study [6] that the most odor-active fraction of jasmine tea aroma was that containing 4-hexanolide and (*E*)-2-hexenyl hexanoate. To clarify the effect of mixing these two odorants, DOT levels of mixtures of (*E*)-2-hexenyl hexanoate and 4-hexanolide in various proportions were measured in an aqueous medium by using the authentic chemicals. In addition to the mixtures of (*E*)-2-hexenyl hexanoate and 4-hexanolide, the aqueous model solutions of (*Z*)-3-hexenyl hexanoate and 4-hexanolide were also studied. (*Z*)-3-Hexenyl hexanoate was not detected as an odor-active compound of the jasmine tea aroma. However, in green tea, oolong tea and black tea, (*Z*)-3-hexenyl hexanoate has been reported as one of the most important aroma constituents exhibiting a green note [1, 16–18]. A preliminary experiment had revealed that the threshold value for a binary solution of (*E*)-2-hexenyl hexanoate and 4-hexanolide in the content ratio of 90 : 10 tended to be lower than that of each chemical alone among the samples tested (binary mixtures of (*E*)-2-hexenyl hexanoate and 4-hexanolide, and of (*Z*)-3-hexenyl hexanoate and 4-hexanolide in content ratios of 100 : 0, 90 : 10, 80 : 20, 70 : 30,

 

60 : 40, 50 : 50, 40 : 60, 30 : 70, 20 : 80, and 0 : 100). Therefore, in this study, the DOT levels of binary mixtures of (*E*)-2-hexenyl hexanoate and 4-hexanolide, and of (*Z*)-3-hexenyl hexanoate and 4-hexanolide in content ratios of 100 : 0, 95 : 5, 90 : 10, 80 : 20, and 0 : 100 (Table 1) were measured more accurately by means of the triangle test with nine panelists. The numbers of panelists who provided correct answers during the triangle tests are shown in Table 1. Those who gave a correct answer gradually decreased as the concentration of each sample was reduced. Since in the case of a (*E*)-2-hexenyl hexanoate alone the minimum concentration at which the panelists could significantly distinguish the stimulus of a sample solution from that of the blank sample was 1.0 ppm, the DOT level of (*E*)-2-hexenyl hexanoate alone is defined as 1.0 ppm. The DOT levels of the binary mixtures of (*E*)-2-hexenyl hexanoate and 4-hexanolide in content ratios of 95 : 5 and 90 : 10 fell to a lower value (0.1 ppm) compared to those for the single chemicals. DOT for the solution of (*Z*)-3-hexenyl hexanoate alone was one order lower in magnitude than that of (*E*)-2-hexenyl hexanoate alone. In contrast to the binary solutions of (*E*)-2-hexenyl hexanoate and 4-hexanolide, DOT for the binary mixtures of (*Z*)-3-hexenyl hexanoate and 4-hexanolide were one order higher than that for the solution of (*Z*)-3-hexenyl hexanoate alone. The results of water resources studies have shown that DOT of a 1 : 1 mixture of *m*-cresol and acetophenone was higher than that of each chemical alone [19, 20]. On the other hand, the possibility of lowering the threshold value when one odorant was mixed with another compound (odor synergism) had only been suggested in the literatures [2 – 4] as already mentioned in the introduction section. The present study directly proves the odor synergism phenomenon in the binary mixture solution for the first time by the results of the sensory evaluation.

### 3.2 Effect of 4-hexanolide on the odor intensity of the other odor-active compounds of jasmine tea

It is important to investigate the change in odor intensity of an odorant when it is mixed with 4-hexanolide, since the odor intensity of (*E*)-2-hexenyl hexanoate was strengthened from subthreshold to suprathreshold level when it was mixed with 4-hexanolide as shown in the preceding section. The 14 odorants shown in Table 2, which were the main odor-active compounds of jasmine tea [6] were used to investigate the changes of odor intensity in an aqueous medium. The purities of the authentic chemicals used were more than 98.5% except (*E*)-2-hexenyl hexanoate containing 2.8% hexyl hexanoate, (*R*)-linalool, and linalool oxide which contain a small quantity of the respective optical isomer as described in Section 2. The ROT levels of (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexenyl hexanoate, (*Z*)-3-hexenol, hexanol, and indole were measured by the staircase procedure by using the samples in a binary dilution series. The results for each panelist are shown in Fig. 1. In addition, the ROT levels of the remaining nine odorants were determined in a similar manner, except for adopting a tenfold dilution series instead of the binary dilution series, though the data are not shown here. On the basis of the data in Fig. 1 and the ROT levels of these remaining nine odorants, the concentration of each compound for the investigation which none of the panelists could detect the odor quality of the odorant was determined as follows: 0.1 ppb, (*R*)-(–)-linalool; 0.1 ppm, (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexenyl hexanoate, (*Z*)-3-hexenol, hexanoic acid, hexanol, and (*E*)-2-hexenol; and 1.0 ppm, indole, 2-phenyl ethanol, cyclotene, hexanal, linalool oxide (2*R*-furanoid), benzaldehyde, and maltol.

Five percent of each odorant was then replaced by 4-hexanolide at these concentrations. The panelists sniffed the odors of these binary solutions. The highest concentration of 4-hexanolide mixed in these binary solutions was 50 ppb in the cases of indole, 2-phenyl ethanol, cyclotene, hexanal, linalool oxide, benzaldehyde, and maltol. The lowest ROT level for 4-hexanolide in aqueous medium among all the panelists was 12.5 ppm as shown in Fig. 1, therefore the concentration of 50 ppb of 4-hexanolide was below its ROT level for all panelists. Among the 14 solutions tested, each odor of the binary solutions of (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexenol and indole with 4-hexanolide was easily recognized apparently by all panelists as "pungent", "green", and "camphoraceous", respectively, each odor character being the same as that of the respective chemical alone. Namely, the odor intensity of these three odorants were lifted up from subthreshold levels to suprathreshold levels by subthreshold 4-hexanolide added. From these results, it was predicted that there would be some mutual interaction between these three odorants and 4-hexanolide in respect of the odor intensity. Therefore, the odor intensity of a binary solution of each of these three odorants and 4-hexanolide was compared to that of a solution of the chemical alone by means of LMS. Three different concentrations were studied: the concentration at which none of the panelists could detect the quality of the odorant, the concentration for the mid ROT level of the all panelists, and the concentration at which all the panelists could identify the odor quality of the odorant (Figs. 1 and 2). In addition to these three compounds, (*Z*)-3-hexenyl hexanoate and hexanol, which respectively are the structural homologues of (*E*)-2-hexenyl hexanoate and (*Z*)-3-hexenol, were studied. The data from the odor intensity test are shown in Fig. 2. The LMS ratings of each chemical alone and its mixtures with 4-hexanolide for (*E*)-2-hexenyl hexanoate (Fig. 2a), (*Z*)-3-hexenol (Fig. 2c) and indole (Fig. 2e) were significantly different ($F(1,11) = 7.93$, $P < 0.05$), ($F(1,12) = 19.7$, $P < 0.001$), and ($F(1, 12) = 6.63$, $P < 0.05$, respectively), by repeated-measure ANOVA. Clear synergism was apparent in the subthreshold concentration of these three odorants.

 

The mean LMS ratings at a concentration below the odor threshold for the odorant alone of (*E*)-2-hexenyl hexanoate (0.1 ppm), (*Z*)-3-hexanol (0.1 ppm), and indole (1 ppm) were all under the "weak intensity" (0.79) of the LMS semantic label: 0.48, 0.44, and 0.67, respectively. When 5% of each odorant was replaced by 4-hexanolide, the ratings significantly rose to above the "weak intensity" of the LMS label: 1.05, 0.81, and 1.40, respectively. On the other hand, at the concentration for the mid ROT level of the all panelists and the concentration at which all the panelists could identify the odor quality of the odorant, the mean LMS ratings between an odorant alone and a binary mixture with 4-hexanolide were not regarded as significantly different. In contrast to (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexanol, and indole, no significant change was apparent in the odor intensity of (*Z*)-3-hexenyl hexanoate (Fig. 2b) and hexanol (Fig. 2d) when they were mixed with 4-hexanolide at any concentration. There was no relationship between the functional groups of an odorant and its mutual interaction with 4-hexanolide.

### 3.3 Extraction of the commonly perceived character

Since the odorants affected by 4-hexanolide had different functional groups in their molecules, another possible link to this synergism was the perceived similarity of the odorants. The perceived similarity among (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexenol, and indole was investigated by the QDA® method with aqueous solutions containing each chemical alone. Sample solutions of the 14 odorants (Table 2) were first prepared in concentrations at which all panelists could easily detect their odor on the basis of the ROT data for the five odorants shown in Fig. 1 and ROT data for the remaining nine odorants that were mentioned in the preceding section. The prepared concentrations were as follows: 100 ppm, indole, 2-phenyl ethanol, cyclotene, (*E*)-2-hexenal, (*Z*)-3-hexenol, hexanoic acid, benzaldehyde, maltol, and hexanol; 10 ppm, (*Z*)-3-hexenyl hexanoate, (*E*)-2-hexenyl hexanoate, hexanal, linalool oxide (2*R*-furanoid); and 1 ppm, (*R*)-(−)-linalool. All these sample solutions were presented for several consultation with all the panelists to discuss whether or not there were any odor impressions that distinguished (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexenol and indole from the other 11 odorants. The terms "astringent" (reminded of an astringent taste) and "heavy" (generates a long-lasting feeling) were agreed upon as the commonly perceived note to these three odorants. Therefore, the fourteen odor-active compounds in jasmine tea in Table 2 were evaluated in respect of the astringent and heavy odor attributes. The results are presented in Table 2. Indole and (*E*)-2-hexenyl hexanoate were evaluated to have a significantly astringent note among the 14 compounds tested by the QDA® method. The panelists detected a more astringent note in (*Z*)-3-hexenol than that in hexanol, although the difference could not be regarded as significant. On the other hand, the heavy note was significantly more intense in (*Z*)-3-hexenol than in hexanol. The panelists detected more of the heavy note in (*E*)-2-hexenyl hexanoate than in (*Z*)-3-hexenyl hexanoate, although the difference was not significant between the two test solutions. The commonly perceived characters of these three odorants of astringent and heavy notes were generally recognized. This is particularly interesting because the astringent and heavy notes in the odor impression were one of the important characteristics of the jasmine tea aroma.

### 3.4 Importance of the subthreshold odorants in jasmine tea

To clarify whether the subthreshold compounds affected the overall odor sensation of jasmine tea, 4-hexanolide was added to the jasmine tea infusion at a concentration below its ROT level as judged by all the panelists. The ROT levels for 4-hexanolide in the jasmine tea infusion are shown in Fig. 1 according to each panelist. In the concentration range of 200 – 12.5 ppm, the panelists could detect the coconut milk-like note of 4-hexanolide. In order to investigate the odor effect of 4-hexanolide, this subthreshold compound was added to the jasmine tea infusion at a concentration of 0.1 ppm (sample II), and the odor quality of sample II was compared to that of the original jasmine tea infusion (sample I) by the QDA® method. Although the original jasmine tea infusion contained 4-hexanolide at a concentration of 7.5 ± 3.4 ppb [6], the concentration of 4-hexanolide in the test sample was below its odor threshold level. The odor attributes, "floral", "woody", "sweet", "bitter", "refreshing", "mild", and "extending", which were used as the terms for evaluating the odor profile of a jasmine tea infusion in our previous study [6] were also used here. The terms "heavy" and "astringent" were also tested in order to detect the effects of added 4-hexanolide. The results are shown in Table 3. The sweet note and astringent note were significantly more intense in the jasmine tea infusion mixed with 4-hexanolide (sample II) than in the original jasmine tea infusion (sample I). The concentrations of (*E*)-2-hexenyl hexanoate, (*Z*)-3-hexenol, and indole in the jasmine tea infusion (9.5 ± 4.0 ppb, 285.0 ± 3.3 ppb, and 306.5 ± 4.5 ppb, respectively) [6] were also at levels below the odor threshold. As already shown in the previous sections, the astringent note and heavy note were the commonly perceived odor qualities of these three odorants, and odor synergism was apparent between each of these three odorants and 4-hexanolide in a solution at a concentration below their odor threshold levels. The clear difference in odor quality between samples I and II is considered to have arisen from the synergistic effect of 4-hexanolide with the other subthreshold odorant, such as (*E*)-2-hexenyl hexanoate, (*Z*)-3-

 

**Table 3.** QDA results for the effects of adding subthreshold 4-hexanolide to the jasmine tea infusion

| Odor attribute | Definition | Jasmine tea infusion alone[a)] (sample I) | Jasmine tea infusion + 4-hexanolide[a)] (sample II) |
|---|---|---|---|
| Floral | Reminded of flower | 5.16a | 5.11a |
| Woody | Wet wood-like | 3.67a | 3.88a |
| Sweet | Honey-like sweet aroma | 3.27a | 4.50b |
| Bitter | Reminded of bitter taste | 3.62a | 3.70a |
| Refreshing | Generates pleasant feeling | 4.29a | 3.74a |
| Mild | Not irritating | 3.94a | 4.51a |
| Extending | Aroma sensation is wide spreading | 4.53a | 4.65a |
| Milky | Reminded of coconut milk | 2.04a | 2.25a |
| Heavy | Generates long-lasting feeling | 3.20a | 4.11a |
| Astringent | Reminded of astringent taste | 3.12a | 4.47b |

a) Average scores from the sensory evaluation (0, absent; 8, strong) by 13 panelists. Different letters (a and b) in a row indicate significant difference by Tukey's multiple-comparison test ($P \leqq 0.05$).

hexanol, and indole odorants found in the jasmine tea infusion. A "honey-like sweet" note was also significantly more intense in sample II than in sample I. This honey-like sweet note did apparently not originate from the 4-hexanolide added. There seems to have been a complex mechanism involved in the honey-like sweet note when subthreshold 4-hexanolide was mixed with the jasmine tea infusion.

Lactone compounds like 4-hexanolide are commonly present in a wide range of teas [1]. (*E*)-2-Hexenyl hexanoate, indole, and (*Z*)-3-hexenol have also been detected as aroma constituents in oolong tea, black tea, and green tea, and not only jasmine tea [1]. The results from the sensory evaluation in this present work directly suggest for the first time the existence and importance of a synergistic effect between the subthreshold aroma compounds in the jasmine tea infusion.

## 4 References


[1] Yamanishi, T., Flavor of green tea. *JARQ* 1978, *12*, 206 – 210.

[2] Hirvi, T., Honkanen, E., The aroma of some hybrids between high-bush blueberry (*Vaccinium corymbosum*, L.) and bog blueberry (*Vaccinium sliginosum*, L.). *Z. Lebensm. Unters. Forsch*. 1983, *176*, 346 – 349.

[3] Kock, H., Heinze, H. P., Potgieter, M. C., Dijksterhuis, B. G., Minnaar, A., Temporal aspects related to the perception of skatole and androstenone, the major boar odour compounds. *Meat Sci.* 2001, *57*, 61 – 70.

[4] Nawar, W. W., Fagerson, S. I., Direct gas chromatographic analysis as an objective method of flavor measurement. *Food Technol.* 1962, *76*, 107 – 109.

[5] Ullrich, F., Grosch, W., Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch*. 1987, *4*, 277 – 282.

[6] Ito, Y., Sugimoto, A., Kakuda, T., Kubota, K., Identification of potent odorants I. Chinese jasmine green tea scented with flowers of *Jasminum sambac. J. Agric. Food Chem*. 2002, *50*, 4878 – 4884.

[7] Belitz, H.-D., Grosch, W., Individual aroma compounds. In: Burghagen, M. M., Hadziyev, D., Hessel, P., Jordan, S., Sprinz, C. (Eds.), *Food Chemistry*, Springer, Berlin 1999, pp. 350 – 351.

[8] Pittet, O. A., Rittersbacher, P., Muralidhara, R., Flavor properties of compounds related to maltol and isomaltol. *J. Agric. Food Chem.* 1970, *18*, 929 – 933.

[9] Kumazawa, K., Masuda, H., Identification of potent odorants in Japanese green tea (sen-cha). *J. Agric. Food Chem.* 1999, *47*, 5169 – 5172.

[10] Poste, M. L., Mackie, A. D., Butler, G., Larmond, E., Experimental design. In: Buckley, T. J. (Ed.), *Laboratory Methods for Sensory Analysis of Food*. Agriculture Canada, Ottawa 1991, pp. 16 – 18.

[11] Poste, M. L., Mackie, A. D., Butler, G., Larmond, E., Triangle test. In: Buckley, T. J. (Ed.), *Laboratory Methods for Sensory Analysis of Food.* Agriculture Canada, Ottawa 1991, pp. 20 – 21.

[12] Roessler, B. E., Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. *J. Food Sci*. 1978, *43*, 940 – 943.

[13] Green, B. G., Shaffer, G. S., Gilmore, M. M., Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. *Chem. Senses* 1993, *18*, 683 – 702.

[14] Stone, H. H., Sidel, J., Oliver, S., Woolsey, A., Singleton, R. C., Sensory evaluation by quantitative descriptive analysis. *Food Technol*. 1974, *28*, 24 – 31.

[15] Shimoda, M., Tsukamoto, Y., Ito, H., Doi, Y., Kameda, W., Osajima, Y., Preparation and evaluation of odor reference solution. *Nippon Shokuhin Kogyo Gakkaishi* 1991, *38*, 371 – 376.

[16] Tokitomo, Y., Ikegami, M., Yamanishi, T., Effects of withering and mass-rolling processes on the formation of aroma components in Pouching-type semi-fermented tea. *Agric. Biol. Chem*. 1984, *48*, 87 – 91.

[17] Renold, W., Näf-Müller, R., Keller, U., Willhalm, B., Ohloff, G., An investigation of the tea aroma part I. New volatile black tea constituents. *Helv. Chim. Acta* 1974, *57*, 143 – 144.

[18] Nose, M., Nakatani, Y., Yamanishi, T., Studies on the flavor of green tea part IX. Identification and composition of intermediate and high boiling constituents in green tea flavor. *Agric. Biol. Chem.* 1971, *35*, 261 – 271.

[19] Baker, A. R., Response parameters including synergism-antagonism in aqueous odor measurement. *Ann. N. Y. Acad. Sci.* 1964, *7*, 495 – 503.

[20] Rosen, A. A., Peter, B. J., Middleton, M. F., Odor thresholds of mixed organic chemicals. *J. WPCF* 1962, *34*, 7 – 13.


 