# Effect of cultivation conditions on odor character and chemical profile of shiso (*perilla frutescens*) flavor

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**Abstract**
Flavor profiling and sensory evaluations were carried out on shiso leaves cultivated under various conditions. The growth stage, leaf position and cultivation environment (in a glasshouse or in a growth chamber) affected the odor character and volatiles profile significantly, while fertilizer type (chemical or organic) had no effect. Linalool, neral, geranial, t-shisool, eugenol, 1-octen-3-ol and benzaldehyde are regarded as key chemicals that form the odor characteristics in addition to the major component, perillaldehyde.

**Key Words**
Odor characteristics, multivariate analysis, Shiso, head space gas (HSG), volatiles profile.

## Introduction

We have investigated the relationship between chemical components, odor character, and preference on shiso, a popular herb in Japan (Tanaka *et al.* 2008). Recently, cultivation methods, such as fertilizing style, have attracted increasing attention with regard to the quality of farm products. Little is known about the effect of fertilizer properties, soil condition, growth stage, and the other cultivation factors on the variation pattern of sensory character, chemical profile, and their relationship in response to cultivation method used with shiso must be investigated. In this study, solid phase micro extraction gas chromatography/mass spectrometry (SPME -GC/MS) based profiling and sensory evaluations were carried out on the identical samples. This was a pilot experiment intended to help us determine which factors affected odor characters and the volatiles profile of shiso. We hope the information gathered will help to clarify the relationship between sensory qualities and flavor profiles as they relate to cultivation method.

## Methods

*Shiso sample*

A local variety (A) and a breeding line (H) of Ibaraki prefecture were cultivated in planters in a glasshouse or growth chamber. Sampling was carried out at mid- and late- vegetative stage. Standard leaves (6 to 7cm length, suitable for shipping), top leaves that had not yet developed (smaller than 2cm) and lower leaves that had developed more than 4 weeks before were sampled. The experimental design is shown in Table1.

**Table 1. Experimental design for cultivation plots.**

| Plot name | Basal application | Additional application | Cultivation environment |
|---|---|---|---|
| Chemical fertilizer ( C) | $(NH_4)_2SO_4$, KCl, $NaH_2PO_4$ | $(NH_4)_2SO_4$ | Glasshouse (20 ℃<) |
| Organic fertilizer (O) | Certificated organic | non | Glasshouse (20 ℃<) |
| Growth Chamber (G) | $(NH_4)_2SO_4$, KCl, $NaH_2PO_4$ | $(NH_4)_2SO_4$ | Growth chamber (25 ℃) |

*Sample preparation*

Finely cut shiso leaves (3g) were shaken with 10% NaCl (30 mL). They were centrifuged, and the supernatants were stored at –30°C after dividing them into small portions.

*SPME-GC/MS analysis*

The supernatant above (1 mL) and internal standard ($^{13}$C-benzylalchol (0.04%, 10□L)) were combined in a 20 mL glass vial for SPME (Supelco Corp., Bellefonte.PA). After 5 minutes of incubation, volatile components in the head space of the vial were extracted with Stableflex fiber (2 cm, 50/30□m, DVB/carboxen/PDMS) at 35°C for 10 minutes without agitation. The fiber was inserted into the inlet port (250°C) of a GC/MS system (Agilent 5973N) to desorb the volatiles and introduce them to the column under splitless mode. A DB-Wax (60m (length), 0.25mm(i.d.), 0.25□m(df) ) column was employed for analysis.



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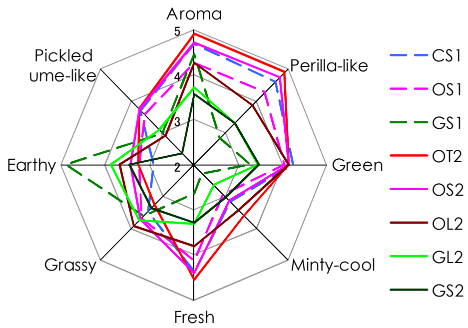
*Sensory evaluation*

Smelling-strips were soaked in shiso samples after thawing and evaluated by trained 17 panels. Quantitative descriptive analysis (QDA) with a 7-point category scale for estimating odor strength was carried out with 5 replications. The words for sensory attributes evaluation were aromatic, fresh, perilla-like, pickled ume-like, green, grassy, earthy, minty-cool.

## Results

*Variations in odor character with sensory evaluation*

The effects of leaf position and cultivation environment were significant on variety A (Figure 1). Though the balance of odor properties was similar between the undeveloped top leaves and the standard leaves, the top leaves presented a stronger odor than did the standard leaves. The lower leaves emitted a grassy, earthy and weakly aromatic odor. Though the effect of growth stage was not uniform for each attribute, a significantly more aromatic and perilla-like odor was presented at the late vegetative stage. The effect of fertilizer type, organic or chemical, was not detected.




| Plots | Cultivation environment | Fertlizer type | Leaf position | Growth stage |
|---|---|---|---|---|
| CS1 | glasshouse | Chemical | Standard | mid-vegetative |
| OS1 | glasshouse | Organic | Standard | mid-vegetative |
| GS1 | growth chamber | Chemical | Standard | mid-vegetative |
| OT2 | glasshouse | Organic | Top | late-vegetative |
| OS2 | glasshouse | Organic | Standard | late-vegetative |
| OL2 | glasshouse | Organic | Lower | late-vegetative |
| GL2 | growth chamber | Chemical | Lower | late-vegetative |
| GS2 | growth chamber | Chemical | Standard | late-vegetative |

**Figure 1. Sensory attributes of Variety A.**

A clustering analysis was carried out (Figure 2). The difference in cultivation environment, that is glasshouse or growth chamber, resulted in large clusters forming. The sensory attributes were also divided into a cluster containing the features perilla-like, fresh, minty-cool, green and pickled ume-like, a cluster containing the features grassy and earthy. The attributes in the same cluster presented high correlations with each other, especially fresh and perilla-like (not shown).

*Profiles of head space volatiles*

Table2 shows the relationship of notable flavor components and words used in the sensory evaluation. Perillaldehyde, neral, geranial, eucalyptol, methyl salicylate gave high positive correlation with the attributes of aroma, fresh, perilla-like, green and minty-cool. Neral, germacrene D, bergamotene, 1-octen-3-ol, eucalyptol and on the like were negatively correlated with green or earthy attributes. The level variation of benzaldehyde, α-terpineol and *t*-shisool was larger than that of the remaining components due to the fertilizer type. At the late-vegetative stage, the volatile profiles showed that top note chemicals decreased and sesquiterpenes increased. Multivariate analyses were conducted on profiles of shiso leaves volatiles. The major clusters were separated by cultivation environment, followed by leaf position and growth stage. Fertilizer type was not significant for the volatiles profile. These results agreed with the results of sensory evaluation.

## Discussion

Thirty eight chemicals were picked up that correlated to sensory attribution. Among them, linalool, neral, geranial, t-shisool, eugenol, 1-octen-3-ol and benzaldehyde potentially affect the odor characteristics of shiso. Considering that these are potent odorants, these chemicals appear to be key components determining the shiso odor character. These results were very similar to those of our previous report (Tanaka *et al.* 2008). The grassy attribute didn't correlate positively with any chemicals and the earthy attribute was correlated with only two chemicals. This indicated that nothing can form the attributes of grassy and earthy independently except the interaction of multiple components. This indicates that not only the major component, perillaldehyde, but also complicated chemicals that correlate to odor attribute positively and negatively determine the odor characters in harmony.

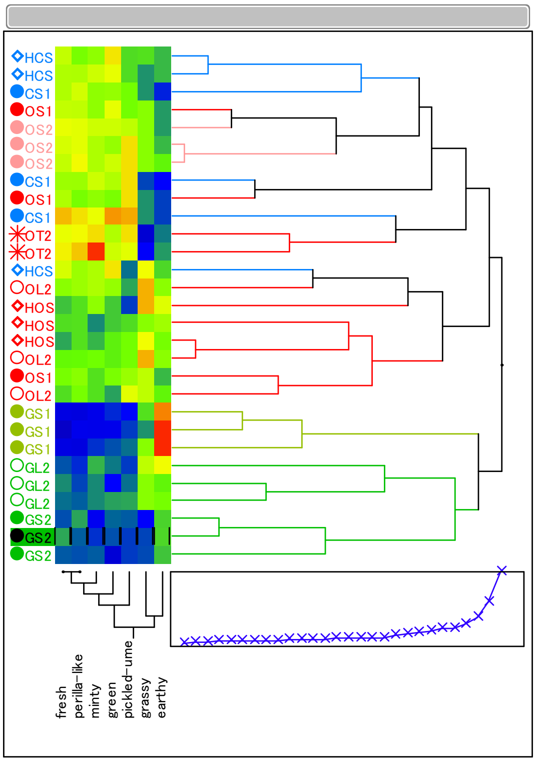



**Figure 2. Hierarchical clustering of cultivation method with sensory attributes**
**Plot names are the same as in Figure 1 for Variety A. HCS and HOS are Variety H, and the conditions correspond to CS1 and OS1 of Figure 1, respectively.**

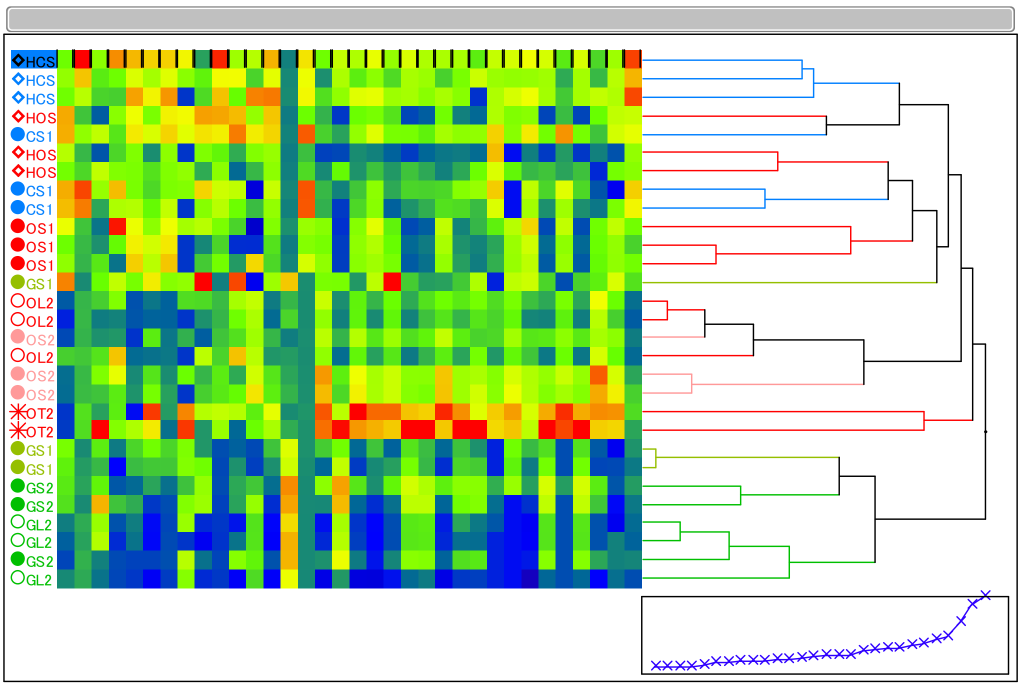



**Figure 3. Hierarchical clustering of cultivation method with volatiles profile.**
**Plot names are the same as in Figure 2.**

**Table 2. Simple correaltion between chemicals and odor attributes.**

| | KI | Chemicals Name *: tentatively | Attribute aroma | fresh | perilla | ume | green | grassy | earthy | minty | Tendency Stage | Fertilizer | Position |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 916 | *2-Ethylfuran | -0.17751 | -.428* | -.394* | -0.268 | -0.241 | 0.047 | 0.289 | -.538** | mid>late | C>O | |
| 2 | 980 | 1-Penten-3-one | 0.424* | 0.492** | 00.369* | 0.253 | 00.634** | -0.274 | -0.448 | 0.412* | | C>O | |
| 3 | 1030 | 1S-.alpha.-Pinene | -0.365 | -0.272 | -0.256 | -0.329 | -0.413* | 0.092 | 0.278 | -0.076 | | | |
| 4 | 1090 | Hexanal | 0.446* | 0.335 | 0.412* | 0.273 | 0.421* | 0.149 | -0.134 | 0.245 | | | |
| 5 | 1159 | .beta.-Myrcene | 0.240 | 0.172 | 0.232 | 0.208 | 0.001 | -0.334 | -0.021 | 0.399* | | | |
| 6 | 1180 | MW136 | 0.389* | 0.441* | 0.411* | 0.160 | 0.509** | -0.191 | -0.354 | 0.452* | | | |
| 7 | 1226 | Eucalyptol | 0.646** | 0.434* | 0.470* | 0.393* | 0.434* | -0.622** | -0.267 | 0.450* | | | T>S>L |
| 8 | 1253 | gamma-Terpinene | 0.303 | 0.322 | 0.287 | 0.143 | 0.431* | -0.147 | -0.287 | 0.182 | | | |
| 9 | 1310 | 1-Octen-3-one | 0.430* | 0.147 | 0.189 | 0.022 | 0.318 | -0.172 | -0.106 | 0.071 | | | |
| 10 | 1389 | 3-Hexen-1-ol, (Z)- | -0.271 | -0.414* | -0.387* | -0.317 | -0.332 | 0.196 | 0.393* | -0.403* | | | L>S>T |
| 11 | 1394 | 3-Octanol | 0.579** | 0.689** | 0.662** | 0.403* | 0.730** | -0.342 | -0.544** | 0.612** | | C>O | T>S>L |
| 12 | 1408 | 2-Hexen-1-ol,(E)- | 0.494** | 0.260 | 0.319 | 0.192 | 0.313 | -0.049 | -0.111 | 0.166 | | | |
| 13 | 1413 | Benzene, 1,4-bis(1-methylethyl) | 0.450* | 0.236 | 0.236 | 0.197 | 0.390* | -0.258 | -0.247 | 0.094 | | C>O | S>T,L |
| 14 | 1450 | 1-Octen-3-ol | 0.646** | 0.535** | 0.567** | 0.326 | 0.599** | -0.439* | -0.364 | 0.518** | | | T>S>L |
| 15 | 1472 | *1-Hexanol, 2-ethyl- | -0.311 | -0.372* | -0.420* | -0.181 | -0.413* | -0.344 | 0.197 | -0.285 | | | |
| 16 | 1548 | Benzaldehyde | 0.346 | 0.492** | 0.407* | 0.291 | 0.600** | -0.289 | -0.574** | 0.346 | | C>O | |
| 17 | 1548 | Linalool | 0.433* | 0.334 | 0.445* | 0.441* | 0.166 | -0.263 | -0.094 | 0.491** | mid<late | C<O | T>S>L |
| 18 | 1579 | beta-Cubebene | 0.167 | 0.155 | 0.171 | 0.070 | 0.015 | -0.392* | -0.008 | 0.428* | | | T>S>L |
| 19 | 1638 | * Dihydrocarvone | 0.232 | -0.277 | -0.133 | -0.032 | -0.313 | -0.141 | 0.468* | -0.186 | | | |
| 20 | 1644 | * Limonene,1,2-epoxide | 0.201 | -0.304 | -0.162 | -0.057 | -0.336 | -0.111 | 0.491** | -0.209 | | | |
| 21 | 1674 | (E)-beta.-Farnesene | 0.238 | 0.349 | 0.335 | 0.154 | 0.245 | -0.326 | -0.244 | 0.531** | | | |
| 22 | 1700 | Neral | 0.625** | 0.686** | 0.751** | 0.667** | 0.559** | -0.532** | -0.481** | 0.758** | | | T>S>L |
| 23 | 1712 | alpha-Terpineol | 0.466* | 0.273 | 0.374* | 0.272 | 0.142 | -0.338 | 0.024 | 0.495** | mid<late | | T>S>L |
| 24 | 1714 | * 4-(5-methyl-2-furanyl)-2-Butanone | 0.274 | -0.362 | -0.285 | -0.150 | -0.319 | -0.495** | 0.493** | -0.212 | | | T>S,L |
| 25 | 1736 | alpha-trans-Bergamotene | 0.305 | 0.269 | 0.293 | 0.139 | 0.151 | -0.444* | -0.079 | 0.526** | | | T>S,L |
| 26 | 1748 | * GermacreneD | 0.258 | 0.272 | 0.294 | 0.179 | 0.135 | -0.437* | -0.119 | 0.537** | | | T>S,L |
| 27 | 1753 | Geranial | 0.615** | 0.676** | 0.743** | 0.629** | 0.539** | -0.497** | -0.462* | 0.761** | | | T>S,L |
| 28 | 1762 | alpha-Farnesene | 0.586** | 0.434* | 0.478** | 0.292 | 0.391* | -0.503** | -0.177 | 0.628** | | | T>S,L |
| 29 | 1765 | * gamma-elemene | 0.505** | 0.286 | 0.335 | 0.347 | 0.325 | -0.622** | -0.142 | 0.441* | | | T>S,L |
| 30 | 1812 | Methyl salycilate | 0.583** | 0.695** | 0.754** | 0.527** | 0.673** | -0.354 | -0.575** | 0.772** | | | T>S,L |
| 31 | 1833 | Perilla aldehyde | 0.738** | 0.653** | 0.711** | 0.568** | 0.626** | -0.522** | -0.451* | 0.664** | | | T>S>L |
| 32 | 1888 | Nerol | 0.493** | 0.370* | 0.440* | 0.307 | 0.286 | -0.383* | -0.138 | 0.505** | | C<O | T>S,L |
| 33 | 1902 | MW204 | 0.263 | 0.254 | 0.272 | 0.121 | 0.126 | -0.397* | -0.062 | 0.509** | mid<late | | T>S,L |
| 34 | 1906 | MW 180 | 0.604** | 0.690** | 0.758** | 0.736** | 0.525** | -0.569** | -0.609** | 0.760** | | | T>S,L |
| 35 | 1906 | Phenol, p-tert-butyl- | 0.264 | 0.254 | 0.273 | 0.118 | 0.128 | -0.395* | -0.062 | 0.509** | mid<late | | T>S,L |
| 36 | 1906 | 3-Cyclohexene-1-ethanol, beta-4- | 0.666** | 0.562** | 0.641** | 0.544** | 0.516** | -0.439* | -0.332 | 0.611** | | | T>S,L |
| 37 | 1930 | t-Shisool | 0.439* | 0.468* | 0.562** | 0.495** | 0.312 | -0.205 | -0.270 | 0.573** | mid<late | C<O | T>S,L |
| 38 | 2118 | Eugenol | 0.456* | 0.516** | 0.479** | 0.209 | 0.609** | -0.308 | -0.427* | 0.494** | | | |

The effect of fertilizer type was not clear, so the other conditions of the cultivation environment and growing stage were larger factors, affecting the odor character and volatile profile of shiso. In this experiment, the growth of shiso was not uniformly between the plots as a corollary to the difference of cultivation method. Another experiment to analyse both factors of cultivation condition and growth difference is required. Further study of the relationship between preference test, sensory evaluation for characterizing and GC/MS profiling will be carried out to improve the flavor quality of shiso with breeding and cultivation techniques. Such a method would be a model for study on high quality agricultural production.

## References


Tanaka F (2008) Flavor characterization of shiso (*Perilla frutescens*) using GC/MS based profiling and sensory odor evaluation. Proceedings of the 5th International Conference on Plant Metabolomics, Yokohama, p210-211, http://prime.psc.riken.jp/icpm2008/index.html