Analysis of Volatile Compounds in Pumpkin with 'Taro-like' Aroma Using Solid Phase Micro-extraction and Gas Chromatography-Mass Spectrometry Combined with Chemometrics

Junxing Li [1,2], Yujuan Zhong [1,2], Wenwen Wang [3], Haibin Wu [1,2], Jianning Luo [1], Hao Gong [1], Hexun Huang [1], Joerg Riener [4]

[1]Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China
[2]Guangdong Key Laboratory for New Technology Research of Vegetables, Guangzhou, China
[3]Agilent Technologies (China) Co. Ltd, Beijing, China
[4]Agilent Technologies Waldbronn, Germany

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Introduction

Pumpkin is one of the most popular vegetables around the world. Pumpkin showing 'Taro-like' aroma is an ideal material for the study of pumpkin aromatic trait. However, there is limited information available on its volatile compounds profiles, in particular, the key aroma compounds causing the typical aroma of pumpkin. Solid phase micro-extraction coupled with gas-chromatography-mass spectrometry (GC-MS) is proved to be an effective way to extract and analyze volatile compounds. In addition, chemometrics has advantages in extracting relevant information and discovering patterns in the large series of data. Therefore, chemometrics methods combined with HS-SPME/GC-MS could be an effective and convenient tool for comprehensive analysis of volatiles in pumpkin with 'Taro-like' aroma.

Experimental

Samples

Three experimental groups, including three replicates, one pumpkin sample with 'Taro-like' aroma (YJ) and two pumpkin samples without the special aroma (EY and 278), were used in this study. All plants were grown in the field of the Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China. All samples (mature fruits about 45 days after pollination) were plucked. Moreover, the content changes of key aromatic compounds at the different development stage of YJ fruit were analyzed. Fruits were sampled in three stages: unpollinated, 25 days after pollination and 45 days after pollination. All samples were freeze-dried and powdered prior to analysis.

SPME Conditions

1 g of sample powder was accurately weighed into a 20 mL vial, followed by adding 1 μL 3-nonanone (0.04 μg/μL) into the vial as internal standard. The vial was sealed immediately and equilibrated at 70 ℃ for 2 min using water bath. Afterward, the SPME fiber was exposed to the sample head space for 35 min at 70 ℃. Finally, the SPME fiber was desorbed for 4.5 min by maintaining the GC-MS injection port at 270 ℃.

Instrument Conditions

GC system: Agilent 7890B; MS system: Agilent 7000D;

Column: DB-5MS (60 m×0.32 mm×0.25 μm);

Column temperature: 50 ℃ hold 3 min ,

at 5 ℃ /min to 250 ℃ hold 5 min;

Carrier gas: Helium; Flow rate: 1.0 mL/min;

Injection port temperature: 270 ℃;

Ion source: EI; Ionization voltage: 70 eV;

Ion source temperature: 280 ℃;

Scan mode: full scan, 35-500 m/z.

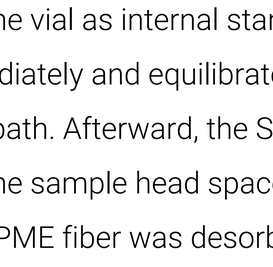

Figure 1. 7000D GC-MS/MS system

Results and Discussion

Identification of Volatiles in YJ Sample

The total ion chromatograms of YJ sample was shown in Figure 2. The volatile components of the mature fruit of YJ were collected and analyzed. A total of 31 volatiles, including 12 aldehydes, 6 ketones, 5 heterocyclic compounds, 4 alcohols, 1 ester, 1 lactone, 1 benzene and 1 alkene, were obtained by comparing mass spectra and retention index (RI) with that in NIST 14 Library and available standards (Table 1). Moreover, the content of trans-2-hexenal was up to 0.0182 mg/kg dry weight, followed by 2-acetyl-1-pyrroline, and the relative content was 0.0133 mg/kg dry weight.

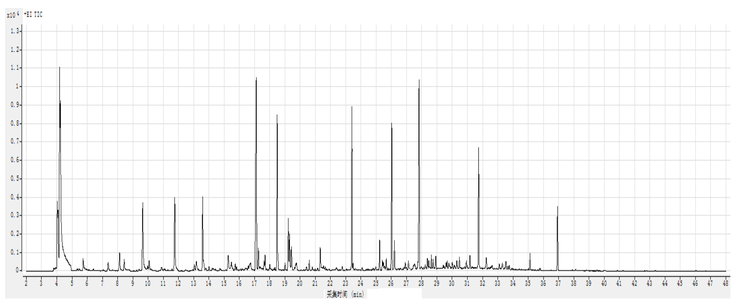

Figure 2. The total ion chromatogram of YJ sample

Table 1. The identified volatiles in YJ fruit

Compounds	RI - sample	RI - NIST Library	Relative contents* (×10⁻²mg/kg dry weight)
Toluene	764	763	0. 13±0. 03
Hexanal	799	800	0. 36±0. 03
(E)-2-Hexenal	850	854	1. 82±0. 51
1-Hexanol	865	868	0. 18±0. 02
Methional	906	907	0. 06±0. 05
2-Acetyl-1-pyrroline	920	922	1. 33±0. 13
Benzaldehyde	964	962	0. 23±0. 03
1-Octen-3-ol	978	980	0. 08±0. 03
(E)-5-(pentyloxy)-2-Pentene	983	-	0. 05±0. 01
2-pentyl-Furan	989	993	0. 06±0. 02
Octanal	1002	1003	0. 03±0. 01
(E,E)-2,4-Heptadienal,	1012	1012	0. 03±0. 02
Benzyl alcohol	1035	1036	0. 16±0. 05
3,5,5-trimethyl-3-Cyclohexen-1-one	1043	1044	0. 07±0. 03
Benzeneacetaldehyde	1046	1045	0. 07±0. 01
2-Acetylpyrrole	1064	1064	0. 06±0. 02
2-Pyrrolidinone	1072	1076	0. 18±0. 07
(E)-6-Nonenal	1102	1101	0. 12±0. 05
Nonanal	1104	1104	0. 24±0. 10
2,6-dimethyl-Cyclohexanol	1114	1112	0. 08±0. 04
Isophorone	1125	1124	0. 03±0. 01
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1147	1144	0. 09±0. 03
(E,Z)-2,6-Nonadienal	1154	1156	0. 65±0. 19
(E)-2-Nonenal	1161	1162	0. 32±0. 05
Decanal	1206	1206	0. 05±0. 02
Caprolactam	1259	1253	0. 06±0. 004
α-Ionone	1427	1426	0. 08±0. 03
(E)-6,10-dimethyl-5,9-Undecadien-2-one	1448	1453	0. 05±0. 01
Dimethyl phthalate	1450	1455	0. 04±0. 03
β-Ionone	1484	1486	0. 12±0. 05
(R)-5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-Benzofuranone	1541	1538	0. 08±0. 04

Data Filtering and Chemometric Analysis

Raw data of three samples acquired by GC-MS system was first deconvolved by Masshunter Qualitative software and then exported as .cef documents for further analysis. The .cef documents were imported into MPP for data mining and chemometric analysis. To exclude entities with poor repeatability and validity, frequency filtration (frequency > 60%) and coefficient of variability (CV < 30%) were applied as screening parameters. The remained entities were analyzed using PCA (Principal Component Analysis). Between YJ and EY, the PC1 and PC2 explaining 58.24% and 19.19% of the variables (Figure 3). And the first two principal components had explained 78.78% of the total variance between YJ and 278 (Figure 3). The PCA analysis of volatiles can distinguish the pumpkin resources quite well, indicating the significant difference of volatiles between the pumpkin with 'Taro-like' aroma and pumpkin without the special aroma.

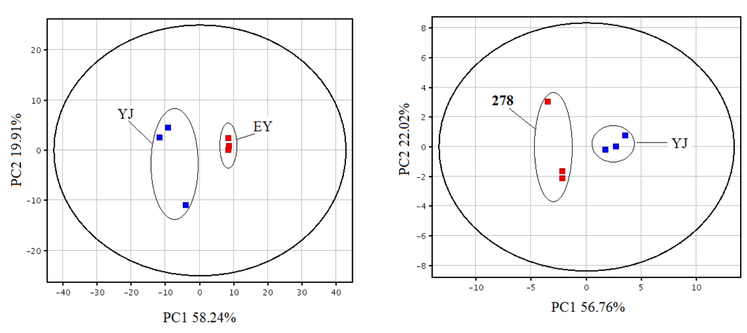

Figure 3. Principle component analysis (PCA) of YJ, EY and 278

Results and Discussion

Identification of Differential Volatiles

Analysis of variance (ANOVA) was performed to retain entities that displayed significant differences among groups. Eventually, 15 and 10 different compounds obtained using statistical analyses, of which 10 and 6 compounds could be identified by comparing mass spectra and retention index (RI) with that in NIST 14 Library and available standards between YJ and EY, YJ and 278, respectively. Compared with EY, six volatiles, including 2-acetyl-1-pyrroline, 2-acetylpyrrole, (E)--6-nonenal, 2,6-dimethyl-cyclohexanol, α-Ionone and β-Ionone, were only detected in YJ fruit. While compared with 278, only two compounds, including 2-acetyl-1-pyrroline and benzaldehyde, were detected in YJ fruit. 2-Acetyl-1-pyrroline (2-AP) was the unique volatile compound in YJ fruit compared with the two sets of control samples.

The Content Changes of 2-AP

Finally, the variation trend of 2-AP during different development stages of fruit, including unpollinated fruit, fruit at 25 and 45 days after pollination (Figure 4). Figure 5 shows unpollinated fruit and fruit obtained at the 25th day after pollination had a similar amount of 2-AP, while the 2-AP content deceased significantly in fruit at late maturity stage. This study has laid the foundation for future studies on 'Taro-like' aroma trait in pumpkin.

Figure 4. Fruit from different development stages of YJ

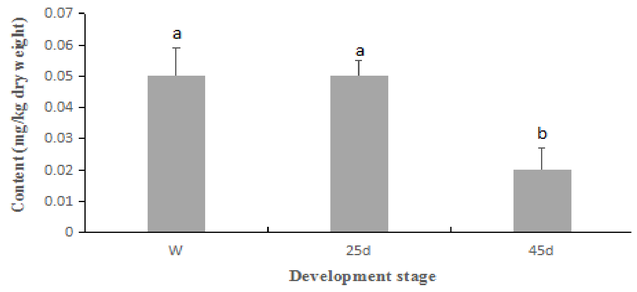

Figure 5. The abundance of 2-AP in the different development stage of YJ fruit

Conclusions

- SPME combined with GC-MS analysis for profiling of volatile metabolites in pumpkin fruit has been developed.
- A total of 31 volatile flavor compounds were identified in mature fruit of YJ pumpkin with 'Taro-like' aroma using GC-MS.
- Differential volatile metabolites were identified to distinguish three samples with/without 'Taro-like' aroma.
- 2-Acetyl-1-pyrroline (2-AP) was the unique volatile compound in YJ fruit compared with the two sets of control samples, which indicating 2-AP is the key aromatic compound for 'Taro-like' aroma.
- The relative content of 2-AP showed higher level in the early development stages, and then significantly decreased at late maturity stage.
- This findings significantly extend our novel insight into the 'Taro-like' aroma of pumpkin based on the levels of volatile metabolites.