Food Chemistry 271 (2019) 211–215

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Volatile fingerprints and biomarkers of three representative kiwifruit cultivars obtained by headspace solid-phase microextraction gas chromatography mass spectrometry and chemometrics

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ARTICLE INFO

Keywords:
Kiwifruit
Volatile fingerprint
Volatile biomarker
HS-SPME-GC-MS
Multivariate analysis

ABSTRACT

Volatile aroma of kiwifruit is a mixture of complicated and time-dependent compounds, and thereby the study of these compounds required distinguished analytical techniques as well as robust data analysis techniques. In this work, we report on the volatile fingerprints and biomarkers of three representative kiwifruit cultivars with commercial importance using headspace solid-phase microextraction gas chromatography-mass spectrometric (HS-SPME-GC-MS) coupled with multivariate analysis. As a result, 95 volatiles have been analyzed from the fingerprints, and ultimately six of which were identified as volatile biomarkers of the kiwifruit cultivars studied, which are formic acid octyl ester, 2-Methylbicyclo[4.3.0]non-1(6)-ene, 1-ethoxy-2,4-hexadiene, and 2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene for Jintao (*A. chinensis*), and 1-methoxy-2-methyl-benzene and (E,E)-2,4-heptadienal for Cuiyu (*A. deliciosa*), respectively. Since the samples of each cultivar were in various maturities, these compounds could be taken as the maturity-independent volatile biomarkers for the kiwifruit cultivars, which would be valuable for marker-assisted flavour breeding in the kiwifruit production.

1. Introduction

Kiwifruit (the genus *Actinidia*) which contains more than 70 species, is native to China, and has now attracted growing international commercial interest, especially in East Asia and New Zealand. The excellent flavour and nutritional qualities, e.g., high content of vitamin C and mineral substances, are the main reasons for its wide acceptance by consumers and its growing value in the fruit market (Ferguson & Stanley, 2003). However, among the kiwifruit species, only a few species are of appreciated flavour and great commercial importance, mainly *Actinidia deliciosa* and *Actinidia Chinensis* (Belrose Inc, 2011). Therefore, the understanding of the flavour and the preferences driving consumer choice of the existing kiwifruit cultivars may play an important role in the breeding of new cultivars with higher market values, which is essential for kiwifruit production and consumption.

The volatile aroma is one of the most important factors influencing the kiwifruit flavour and the consumer acceptance, together with sweetness and acidity (Marsh, Friel, Gunson, Lund, & MacRae, 2006). To understand the volatile aroma of different kiwifruit cultivars aimed at breeding of new cultivars, two steps should be taken successively (Garcia, Quek, Stevenson, & Winz, 2012). One is to determine the volatile compounds in the kiwifruit and reveal the differences of volatile compounds among species, and the other is to interpret the specific compounds responsible for the aroma and to obtain aroma target for breeding. Currently, many methods have been developed for the determination of volatile compounds in kiwifruit. Since the high performance on separation and identification of volatiles in complex samples, gas chromatography-mass spectrometry (GC–MS) has been used as a dominant technique for the analysis of volatile compounds in kiwifruit (Biniecka & Caroli, 2011; Garcia, Quek, Stevenson, & Winz, 2011; Wang, MacRae, Wohlers, & Marsh, 2011, Zhang, Zhang, Zhong, & Guo, 2016). However, before the instrumental analysis, sample preparation should be taken for concentrating the volatile compounds from the complicated matrices. Generally, the extraction techniques were mostly based on the solubility or volatility of the volatile compounds, e.g., solvent extraction (Jordan, Margaria, Shaw, & Goodner, 2005), distillation (Takeoka, Guntert, Flath, Wurz, & Jennings, 1986; Young, Paterson, & Burns, 1983) and headspace techniques (Dijksterhuis, & Piggott, 2001; Friel, Wang, Taylor, & Macrae, 2007). Since the volatile compounds could respond differently to different techniques, there is

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https://doi.org/10.1016/j.foodchem.2018.07.169
Received 30 March 2018; Received in revised form 22 July 2018; Accepted 25 July 2018
Available online 25 July 2018

no single extraction technique to obtain a whole picture of all the volatile compounds. Although solvent extraction and distillation can result in a wide range of composition information, they are unreliable in providing a picture of volatiles that is responsible for real sensation of the aroma. In this context, headspace techniques are of growing importance. Compared to the low sensitivity in static headspace and complex apparatus in dynamic headspace, headspace solid phase microextraction (HS-SPME) is widely used as a high sensitivity and damage-free method for the concentrating of volatile compounds reflecting the real sensory quality of the food stuffs (Gunther, Matich, Marsh, & Nicolau, 2011; Wan, Stevenson, Chen, & Melton, 1999). Benefiting from the development of the analytical techniques, more than hundred volatile compounds have been identified from various kiwifruit cultivars. However, the differences of volatile compounds among different cultivars and the volatile biomarkers for a specific cultivar are still difficult to be revealed, because the volatile compounds contain in living organisms, and their composition in kiwifruit is influenced by several factors regarding the maturity of the fruit, e.g., the levels of soluble solids, the firmness of the fruit body. As a consequence, individual detection of the kiwifruit volatile of different cultivars may lead to failure in obtaining maturity-independent differences and biomarkers, and thereby multiple detections of each cultivar and the supporting multivariate analysis techniques are desired.

Chemometrics is widely applied to extract useful information from chemical systems to solve complicated problem with multivariate, e.g., discriminative analysis and omics analysis in the field of food and drug research (Cheng, Seal, Macrae, & Wang, 2011; Guo, Yuan, Dou, & Yue, 2017; Sarbu et al., 2012; Zhang & Guo, 2017). For the complicated and time-dependent volatile compounds in kiwifruit, it is suitable to use chemometrics for the identification of independent differences and biomarkers among cultivars. However, to obtain reliable results and avoid the false positives in biomarker identification, it is crucial to have adequate sample size that is particularly problematic in cases when the number of variables greatly exceeds the number of samples (Broadhurst & Kell, 2006; Sugimoto, Kawakami, Robert, Soga, & Tomita, 2012). In this work, we collected samples of various maturities of three representative kiwifruit cultivars of commercial importance. The main focuses were on the analysis volatile fingerprints of the kiwifruit samples using HS-SPME-GC-MS and the identification of the independent differences and biomarkers among different cultivars using chemometric analysis, which will open a more full-scale look at the volatile aroma profile of kiwifruit and provide guidance for marker-assisted breeding aimed at improving the flavour of kiwifruit cultivars.

2. Experimental

2.1. Chemicals and materials

Sodium chloride used in the experiment is of analytical grade and purchased from commercial sources without further purification. Samples of three different kiwifruit cultivars, i.e., Cuiyu (*Actinidia deliciosa*), Jintao (*Actinidia chinensis*) and Tuguomaohua (*Actinidia eriantha*), were provided by National kiwifruit Germplasm Repository of China, Wuhan botanical garden, Chinese Academy of Sciences. Ten samples with different maturities were collected for each cultivar, and the levels of soluble solid and the firmness were measured to characterize the samples, which were listed in Table 1. The samples were stored in room temperature for 5–7 days, and then prepared for the analysis.

2.2. Apparatus and operations conditions

The SPME is carried out using a manual device with holder and fiber from Supelco (Belefonte, PA). The fiber used for extracting volatiles is divinlbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS). The GC–MS analysis of the volatile compounds from kiwifruit is employed using a GC system (Agilent 7890A, US) equipped with HP-5 capillary column, and MS system (Agilent 7000C, US). GC separation of the volatiles is conducted under carrier gas of helium which was at a flow rate of 1 mL/min. The column temperature program of GC was initially set at 30 °C for 8 min, and gradually increased to 150 °C at 5 °C/min, then kept there for 3 min. For GC–MS detection, electron ionization (EI) system for acquiring mass spectra were over the mass range of 25 – 500 amu, and the ionization energy used is at 70 eV.

Table 1
Sample information of the three kiwifruit cultivars.

Sample No.	Cuiyu (*A. deliciosa*)		Jintao (*A. chinensis*)		Tuguomaohua (*A. eriantha*)	
	Soluble solid, %	Firmness, kg/cm^2	Soluble solid, %	Firmness, kg/cm^2	Soluble solid, %	Firmness, kg/cm^2
1	17.3	4.02	12.0	4.02	16.1	0.67
2	17.1	4.88	12.8	4.88	14.6	1.31
3	17.7	3.07	12.5	3.07	12.1	1.21
4	15.8	6.45	11.9	6.45	12.4	1.02
5	15.7	8.34	13.5	8.34	13.8	0.87
6	17.3	5.33	14.1	6.33	12.4	1.57
7	17.4	4.07	13.5	4.07	14.4	0.93
8	15.1	6.70	13.7	6.7	13.7	1.43
9	16.6	5.44	13.4	5.44	12.3	0.98
10	16.7	5.83	13.2	5.83	12.8	1.28

2.3. Procedures for sample preparation

5.0 g of pulverized fruit tissue and 1.0 g sodium chloride were placed into a headspace sample vial (2 mL) containing a magnetic rotor. After the sample vial was sealed with a PTFE/silicone septum and an aluminum cap, it was placed into a water bath at 40 °C with magnetic stirring that the rotor can rotate in the fruit tissue. Meanwhile, a manual SPME device with holder and embedded fiber pierced through the septum and reached the headspace above the sample. After the fiber was stretched out and exposed to headspace volatiles for 30 min, it was then withdrawn and the whole holder was transferred to the GC injection port for desorption for 5 min at a temperature of 250 °C. In the meantime, the GC–MS system was triggered for the separation and detection of the desorbed volatiles.

2.4. Multivariate data analysis

The multivariate data analysis was performed on software SIMCA (version P11, Umetrics, Sweden). Principal component analysis (PCA) was used as an unsupervised pattern for statistical procedure that converts a set of observations of possible correlated variables into a set of values of linearly uncorrelated variables (principal components) using orthogonal transformation. Partial least square discriminant analysis (PLS-DA) was used as a regression method between response and independent variable for supervised clustering, which is to determine the features that best describe the differences between the groups.

3. Results and discussion

3.1. Volatile profiles of the three cultivars

Under the above sample preparation procedures of the HS-SPME analysis, the column temperature program of GC has been optimized in advance for the separation of the volatile compounds from kiwifruit. As a result, the volatile compounds of 30 samples of three kiwifruit cultivars, i.e., Cuiyu (*A. deliciosa*), Jintao (*A. chinensis*) and Tuguomaohua (*A. eriantha*), have been analyzed using HS-SPME-GC-MS. The chromatograms of three samples of the three cultivars (No. 1 in Table 1)