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Research Article

Differentiation of Eight Commercial Mushrooms by Electronic Nose and Gas Chromatography-Mass Spectrometry

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Volatile profiles of eight mushrooms were characterized by gas chromatography-mass spectrometry and electronic nose analysis. Volatile compounds including 11 alcohols, 11 ketones, 15 aldehydes, 3 sulfur compounds and alkenes, 8 terpenes, 7 acid and esters, 5 heterocyclic compounds, 20 aromatic compounds, and 4 other compounds were identified. The overall aroma properties of the mushrooms were analyzed by the electronic nose. Results indicated that the e-nose sensors have the ability to accurately respond to different mushrooms with similar fingerprint chromatograms. The relationship between the GC-MS data and e-nose responses of different mushrooms was modeled by principal component analysis and partial least squares regression. This combination for the volatile analysis with chemometric methods can be applied to distinguish different mushrooms successfully. Furthermore, it is concluded that the volatile composition of commercial mushrooms could benefit a finger spectrum by e-nose to identify the species of edible fungi.

1. Introduction

Mushrooms are fleshy and fruiting bodies containing a wide range of edible fungi, such as *Lentinus edodes* (shiitake), *Pleurotus abalonus*, *Agrocybe aegirit*, *Hericium erinaceus*, *Pleurotus eryngii*. Because of their attractive tastes, flavors, and nutritional characteristics, mushrooms are commonly used as food ingredients and also as one of the fundamental components in traditional Chinese medicines [1]. Generally, the specific pleasant odors of mushroom species and their products are described as almond-like or anise-like odors, floral or herb odors, or fruity odors [2]. For instance, the fruity flavor is typical of some species such as *Armillaria mellea*, *Ceriporiopsis subvermispora*, and *Dichomitus squalens* [3]. The fragrant flavor is achieved from *Pleurotus sapidus* and *Stereum sanguinolentum*, whereas the pleasant and anise are considered as characteristic flavors of *Phaeolus schweinitzii* and *Gloeophyllum odoratum* [2].

Although the quality of mushrooms is highly associated with numerous factors including aroma, taste, color, and texture, the aroma of the mushroom plays a major role in sensory attributes and consumer acceptance [1]. Because the unique mushroom flavors correspond to species, this could be employed to discriminate different mushroom species [4]. As one of the main compounds accounting for the unique mushroomy flavor, 1-octen-3-ol was first discovered in *Tricholoma matsutake* [5], and subsequently a series of C8 aliphatic components was reported to be responsible for the mushroom flavor, such as 3-octanol and 3-octanone [6]. Gas chromatography-mass spectrometry (GC-MS), gas chromatograph-flame ionization detector (GC-FID), and headspace-gas chromatograph (HS-GC) analysis have been widely applied in the analysis of mushroom volatile components [7]. Currently, approximately 150 different volatile compounds have been identified in mushrooms and classified into several categories such as alcohols, aldehydes, alkanes, aromatics, sulphur compounds, lower terpenes, and others [8]. Malheiro et al. [9] demonstrated the potential of using volatile components to discriminate six mushroom species, using GC-MS combined with e-nose.

Sensory evaluation is a common method in the flavor analysis of foods. However, there are a number of disadvantages in sensory evaluation including high cost of training panelists, panelist subjectivity, incapacity of online monitoring, and time-consuming. As an alternative approach, electronic nose (e-nose) combining with GC-MS is an innovative and emerging technology for odor analysis with the powerful capability in qualitative and quantitative determination of trace volatile components in food samples [20]. This method exhibits great advantages such as rapid detection, high objectivity, high sensitivity (suitable for tiny amount of samples), long-term routine application, simplicity, and ease of use. Feng et al. [21] analyzed the volatile compounds of *Mesona Blumes* gum/rice extrudates using GC-MS and e-nose, and the results showed that this was able to effectively distinguish *Mesona Blumes* gum/rice (MBG) extrudates at different MBG content. Wang et al. [20] also demonstrated that the e-nose sensors combining with GC-MS were capable of clearly and rapidly distinguishing the flavor differences among synthetic milk, natural milk, and the enzyme-induced milk. Furthermore, this method has been also utilized in the identification of the geographical origin of propolis [22].

Traditionally, e-nose can be just used as a discrimination tool to differentiate various samples. However, it still suffers from detailed information regarding the difference between the discriminated samples. It is well known that sensors on e-nose could have different stimuli to different chemical compounds, which might be used as a typical approach to correlate the chemical compounds and sensors of e-nose. Therefore, it can be used as a finger spectrum to characterize the concrete chemical compound. However, little information has been reported in odor analysis of mushrooms by GC-MS and e-nose. The major objectives of this work were to (1) study the feasibility of electronic nose sensors for discriminating the different mushrooms; (2) investigate the volatile compositions of mushrooms using GC-MS analysis; (3) conduct the correlation analysis between aroma compounds and electronic nose responses for the interpretation of sensor properties using multivariate analysis of principal component analysis (PCA) and partial least squares regression (PLSR).

2. Materials and Methods

2.1. Materials. The eight dried commercial edible mushrooms of *Pleurotus abalonus*, *Agrocybe aegirit*, *Hericium erinaceus*, *Grifola frondosa*, *Coprinus comatus*, *Boletus edulis*, *Lentinula edodes*, *Pleurotus eryngii* were purchased from a supermarket of Tesco, Shanghai, China. The species of the mushrooms were identified by the manufacturers and labeled in the package bags. After arrival, the samples were redried at an 80°C oven for 4 h to achieve same moisture content. The dried mushrooms were crushed in a disintegrator (Dianjiu Traditional Medicine Machinery Manufacture Co. Ltd., Shanghai, China) and the powders were packaged in PVC bags and kept in a dry and dark place at −18°C for further use.

Standard compounds of 1-octen-3-ol, nerolidol, spathulenol, cedrenol, 3-octanone, 2-octanone, isovaleraldehyde, hexanal, octanal, dimethyl trisulfide, furan, 2-pentyl-, 2-ethyl-3,6-dimethylpyrazine, benzaldehyde, phenyl acetaldehyde, anethole, benzothiazole, ortho cresol, naphthalene, 2,6-dimethyl-, 1,1′-biphenyl, 4-methyl-, α-cubebene, cyperene, α-copaene, methyl cinnamate, nonanoic acid, and β-ionone were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical reagent grade and were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. Ultrapure water was obtained from Watsons, Shanghai, China.

2.2. Preparation of Mushroom Extracts. The mushroom powders were sieved through 80 mesh griddles and about 25 g was transferred into a 2 L round bottom flask. Solvent of deionized water was added in the flask at a solid-liquid ratio of 1 : 10 and steam distilled for 2.5 h. After cooling to ambient temperature, the distillation extract was collected and equal volume of anhydrous ethyl ether was added for extracting the flavor compounds. The extract was dried over anhydrous sodium sulphate, maintained at freeze temperature of −18°C to remove water (as ice crystals), and then concentrated to 1 mL prior to further analysis.

2.3. Volatile Compound Analysis

2.3.1. GC-MS Analysis. GC-MS analysis was conducted using an Agilent 7890N gas chromatography-5975 mass selective detector (GC-MS) (Agilent Technologies Inc., Palo Alto, CA), equipped with a HP-INNOWAX column (60 m × 0.25 mm × 0.25 μm). The carrier gas was used as helium at a constant flow rate of 1.0 mL/min. The injector port was heated to 250°C, using the splitless injection mode. The initial oven temperature was maintained at 40°C for 3 min, then raised to 150°C at a rate of 5°C/min and held for 1 min, and finally raised to 220°C at a rate of 10°C/min and maintained for 2 min. The temperatures of injector and detector were 250°C and 220°C, respectively. The mass spectra were captured in the electron impact (EI) ionization mode, with an ionization voltage of 70 eV and a scanning range of m/z 40–400. Other parameters included the ion source of 230°C and mass spectrometry interface of 280°C. Each measurement was performed in triplicate and repeated three times.

2.3.2. Identification and Quantification of Volatile Compounds. The identification of volatile compounds was based on computer matching with the mass spectra in the NIST 05, WILEY and ADAMS libraries, as well as by comparison of the mass spectra and retention indices (RI) according to those reported in the literatures [1, 10–19, 23]. In addition, a home-made library in the Shanghai Institute of Technology, based on the analysis of reference oils and commercially available standards, was also used for the identification and quantification.

2.4. Electronic Nose Analysis of Volatile Compounds

2.4.1. The Preparation of the Sample for Electronic Nose. For e-nose analysis, the mushroom powders were sieved through

40 mesh griddles. About 0.2 g powders was put into a 10 mL vial and kept in a chamber at controlled temperature (37°C) and humidity (50%) [24] for further use.

2.4.2. Electronic Nose Detection. An e-nose of AlphaMOS FOX 4000 (AlphaMOS, Toulouse, France) was applied to study the volatile compounds. The device was composed of eighteen metal-oxide sensors with a headspace autosampler HS100, e-nose unit, and e-nose software. 18 different metal oxide sensors could be divided into three chambers [25], which were three types of sensors, that is, six "LY" type sensors, five "T" type sensors, and seven "P" type sensors. The response characteristics of the gas sensors varied depending on their types [26]. Types P and T sensors are based on tin dioxide (SnO_2) but have different sensor geometries. LY sensors are chromium–titanium oxides (Cr_2–xTixO_3–y) and tungsten oxide (WO_3) sensors [25]. Various types of sensors were used in instruments to ensure sufficient sensitivity and selectivity.

Each sample vial was heated to 50°C and then agitated at 500 rpm for 900 s immediately prior to injection. The sample headspace volume of 2.5 mL was drawn from the vial at 500 μL/s, using a syringe maintained at 60°C. The sample was injected into the e-nose at a speed of 500 μL/s and delivered to the sensors with a purified air carrier gas ($O_2 + N_2 > 99.95\%$, $O_2 = 20 \pm 1\%$, $H_2O < 5$ ppm, $CO_2 < 5$ ppm, $C_nH_m < 5$ ppm) at a flow rate of 150 mL/min. Sensor resistances were recorded for 120 s, and 600 s of delay was used to allow the sensor to return to baseline values before the next injection.

2.5. Statistical Analysis. The GC-MS profiles of mushroom were analyzed by PCA, and PLS2 was used to explain correlations among GC-MS and e-nose data sets. Partial least squares regression (PLSR) [25] were performed with GC-MS and e-nose data. For determining the predictability of e-nose sensors from GC-MS data, PLS1 regression was performed with GC-MS data as the X-variable and e-nose data as Y-variable. Regression coefficients were analyzed by jack-knifing.

All variables were centered and standardized to make each variable has a unit variance and zero mean before applying PLS analysis. All PLSR models were validated using full cross-validation. Statistical analysis was performed by using the Unscrambler v.9.7 (CAMO ASA, Oslo, Norway).

3. Results and Discussion

3.1. Volatile Compounds in Different Mushrooms. The volatile compounds in the mushrooms were extracted by steam distillation and then analyzed by GC-MS. Table 1 lists the tentatively identified 88 compounds in which 31 compounds were identified using the Wiley MS Library Database and 51 were identified by comparison of the retention time and the MS spectrum of the pure chemical standards. These include 11 alcohols, 11 ketones, 15 aldehydes, 3 sulfur compounds and alkenes, 8 terpenes, 8 acid and esters, 5 heterocyclic compounds, 20 aromatic compounds, and 4 other compounds.

Alcohols have been considered as the main odorants of the mushroomy aroma (Cho et al. [1, 8]). In the present work, the alcohols with the high concentrations were detected in species of *P. abalonus*, *L. edodes*, and *P. eryngii*, followed by *H. erinaceus* (Figure 1). Among the alcoholic compounds, 1-octen-3-ol has the highest concentration in *L. edodes* (Table 1), whereas 3-octanol owns the highest concentration in *P. abalonus* (~11.6%). It has been reported that the C8 aliphatic compounds, including 1-octen-3-ol, 3-octanol, 3-octen-2-one, and 2-octenal, 3-octanone, are the major contributors to the characteristic flavor of mushroom of *Tricholoma matsutake* [27]. These C8 compounds are mainly formed by the oxidation of linoleic or linolenic acids in the presence of enzymes of lipoxygenase and hydroperoxide lyase [28].

The results also indicated that *P. abalonus*, *A. aegirit*, *and C. comatus* contained the highest level of ketones (Figure 1), accounting for ~26.0%, 19.2%, and 18.1% of the total volatile compounds in these species, respectively. Ketones of 3-octanone and 2-undecanone were identified in all of the tested species and *P. abalonus* consisted of the highest level compared to others (Table 1). The characteristic component of 3-octanone is a common herb aroma, and 2-undecanone is considered to be the main compound responsible for fruity flavor [29]. It is well-recognized that some odor-active ketone substances, such as β-ionone and trans-geranyl acetone, belong to oxidative by-products or degradation products derived from carotenoids (and therefore called norisoprenoids) and have been identified in mushrooms [30]. Ketones of β-ionone and β-dihydro-ionone are also important flavor compounds in some port wines [31]. In this work, geranyl acetone is the highest content in the mushrooms of *A. aegirit* and *C. comatus*, with the aroma description of green and magnolia, implying that this compound could be a flavor marker of these mushroom species.

Aldehydes were the third most representative chemicals in the tested mushrooms, with 15 compounds being identified. About 35.8% of the total flavor compounds in *A. aegirit* and about 48.2% in *H. erinaceus* were aldehydes (Figure 1). Among the identified aldehydes, hexanal (5.7%), nonanal (7.2%), 6-nonenal (4.2%), and (2E, 4E)-2, 4-decadienal (12.2%) had the highest concentration in the species of *P. eryngii*, *C. comatus*, *A. aegirit*, and *H. erinaceus*, respectively. In addition, octanal and (E)-2-octenal had the highest concentrations in *H. erinaceus* and *P. eryngii* (Table 1). Of interest, no aldehyde was detected in the mushroom of *L. edodes*. A homologous series of n-aldehydes from C-5 to C-10 and simple unsaturated aldehydes from C-7 to C-10 were observed in the samples (Table 1). These compounds could be derived from the products of degradation or oxidation of the lipid in mushrooms [32]. (E)-2-Heptenal, 2-octenal, and (2E, 4E)-2, 4-decadienal was observed in all species except in *L. edodes*. It was suggested that the aldehydes of 5-methyl-2-phenyl-2-hexenal, benzaldehyde, and phenyl acetaldehyde were generated from the Maillard reaction pathway [33]. Chen and Wu [16] also demonstrated the presence of 5-methyl-2-phenyl-2-hexenal in mushroom of *Agaricus subrufescens*. Volatile compounds of aldehydes generally displayed coarse and heavy aromas of raw fish [34]. Different

Table 1: Volatile profile of eight commercial mushroom species expressed in normalized chromatographic peak area.

No.[a]	Compounds	RI[b]	RI_{lit}[c]	*P. Abalones*[d]	*A. aegirit*	*H. erinaceus*	*G. frondosa*	*C. comatus*	*A. edulis*	*L. edodes*	*P. eryngii*	Odour description[e]	ID[f]
	Alcohols												
1	3-Octanol	1381	1388	11.617	nd[g]	nd	nd	nd	nd	0.092	nd	moss, nut, mushroom	BC
2	2-Octanol	1403	1332	0.241	nd	nd	nd	nd	nd	nd	nd	mushroom, fat	BC
3	1-Octen-3-ol	1436	1458	7.093	1.106	9.436	0.83	1.106	2.957	14.764	10.021	mushroom	BC
4	1-Octanol	1531	1553	nd	nd	nd	nd	0.483	nd	nd	nd	chemical, metal, burnt	BC
5	2-Undecanol	1702	1719	1.357	0.651	nd	nd	0.936	0.247	nd	0.364	mandarin	BC
6	Nerolidol	2021	2009	0.502	0.727	2.462	1.043	nd	1.892	0.241	0.628	wood, flower, wax	ABC
7	(6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	2006	—	nd	nd	nd	nd	1.548	nd	nd	nd	—	BC
8	Spathulenol	2111	2129	nd	nd	nd	0.262	nd	nd	nd	nd	herb, fruit	ABC
9	Cedrenol	2134	2113	0.178	1.468	1.181	nd	nd	nd	0.341	1.804	fruit	ABC
10	α-Cadinol	2218	2191	nd	nd	nd	0.116	nd	nd	0.131	nd	herb, wood	ABC
11	β-Eudesmol	2222	2246	nd	nd	nd	nd	nd	nd	0.185	nd	—	ABC
	Ketones												
12	3-Octanone	1244	1244	11.192	0.266	0.097	nd	0.163	0.153	0.315	0.469	herb, butter	ABC
13	2-Octanone	1279	1285	nd	0.398	nd	0.58	0.192	0.094	nd	0.687	soap, gasoline	ABC
14	1-Octen-3-one	1289	1313	0.477	6.64	nd	1.319	nd	nd	nd	nd	mushroom, metal	BC
15	2,3-Octanedione	1305	1328	nd	nd	nd	nd	nd	nd	nd	2.743	milk	BC
16	2-Nonanone	1371	1388	nd	nd	nd	0.293	0.582	nd	nd	0.576	hot milk, soap, green	ABC
17	2-Decanone	1483	1494	0.822	0.186	0.232	0.396	nd	0.838	nd	0.707		BC
18	3-Nonen-2-one	1503	1493	0.418	0.609	nd	0.276	1.318	0.814	nd	0.276	pungent, mushroom	BC
19	2-Undecanone	1589	1543	3.327	2.458	1.916	0.326	2.185	1.29	0.074	0.848	orange, fresh, green	ABC
20	3-Decen-2-one	1610	1603	2.171	0.363	nd	0.152	0.533	1.882	nd	nd	—	BC
21	Geranyl acetone	1848	1840	1.221	3.605	nd	nd	3.827	nd	0.328	nd	magnolia, green	ABC
	Aldehydes												
22	Isovaleraldehyde	913	650	0.225	1.549	nd	nd	nd	nd	nd	1.204	—	ABC
23	3-Methylbutanal	961	910	nd	nd	0.457	nd	1.179	nd	nd	0.488	malt	ABC

Table 1: Continued.

No.[a]	Compounds	RI[b]	RI_{lit}[c]	*P. Abalones* [d]	*A. aegirit*	*H. erinaceus*	*G. frondosa*	*C. comatus*	*A. edulis*	*L. edodes*	*P. eryngii*	Odour description[e]	ID[f]
24	Hexanal	1068	1084	nd	nd	nd	nd	nd	nd	nd	5.68	grass, tallow, fat	ABC
25	Octanal	1283	1280	nd	0.384	1.405	0.458	1.293	0.294	nd	1.373	fat, soap, green	ABC
26	(E)-2-Heptenal	1316	1243	0.616	0.669	0.473	0.528	0.552	0.077	nd	nd	soap, fat, almond	ABC
27	Nonanal	1376	1385	nd	5.265	5.723	1.238	7.192	1.464	nd	3.328	fat, citrus, green	ABC
28	2-Octenal	1423	1437	3.534	2.054	2.369	1.186	2.053	2.305	nd	0.516	green	BC
29	Decanal	1487	1484	0.181	1.502	nd	nd	1.392	nd	nd	nd	soap, orange peel, tallow	ABC
30	(Z)-6-Nonenal	1529		2.679	4.219	3.642	nd	1.467	nd	nd	nd	—	BC
31	(E)-2-Decenal	1641	1611	1.409	nd	nd	nd	nd	nd	nd	nd	tallow, orange	ABC
32	(2Z)-2-Butyl-2-octenal	1666	—	nd	2.108	0.56	nd	nd	nd	nd	nd	—	BC
33	(2E,4E)-2,4-Nonadienal	1698	1695	0.67	0.573	0.952	nd	0.332	nd	nd	0.28	fat, wax, green	BC
34	2-Dodecenal	1738	1807	nd	nd	1.118	nd	nd	nd	nd	nd	green, fat, sweet	BC
35	(2E,4E)-2,4-Decadienal	1810	1827	6.265	7.805	12.196	1.778	2.349	0.162	nd	1.193	fried, fat	ABC
36	5-Methyl-2-phenyl-2-hexenal	2076	2070	0.492	0.919	0.959	nd	0.383	nd	nd	nd	—	ABC
	Sulfocompounds												
37	Dimethyl trisulfide	1372	1377	0.04	nd	nd	nd	nd	nd	0.092	0.522	—	ABC
38	2,3,5-Trithiahexane	1653	1632	nd	nd	nd	nd	nd	nd	1.212	0.818	sulfur, fish, cabbage	BC
39	1,2,4-Trithiolane	1757	1816	1.203	0.416	2.11	nd	1.326	nd	22.417	0.199	—	BC
	Heterocyclic compound												
40	Furan, 2-pentyl-	1203	1240	0.117	0.305	nd	nd	0.096	nd	nd	0.109	green bean, butter	ABC
41	2-Ethyl-3,6-dimethylpyrazine	1441	1449	0.113	nd	nd	nd	nd	0.164	nd	nd	potato, roast	ABC
42	1-Isoamyl-2-formyl pyrrole	1793	—	nd	nd	nd	1.12	nd	4.94	nd	nd	—	BC
43	1-(2-Methylbutyl)-2-formyl pyrrole	1794	—	nd	0.253	nd	0.633	nd	1.989	nd	nd	—	BC
44	2(3H)-Furanone, dihydro-5-pentyl-	2038	—	0.499	nd	nd	nd	nd	nd	nd	0.351	—	ABC
45	2-N-Propyl-5-(2-methylpropyl)thiophene	2133	—	nd	nd	nd	nd	nd	1.67	nd	nd	—	BC
	Aromatic compounds		—										
46	Benzaldehyde	1521	1495	0.822	2.601	1.182	1.692	2.681	7.989	nd	1.502	almond, sugar	ABC

Table 1: Continued.

No.[a]	Compounds	RI[b]	RI_{lit}[c]	*P. Abalones*[d]	*A. aegirit*	*H. erinaceus*	*G. frondosa*	*C. comatus*	*A. edulis*	*L. edodes*	*P. eryngii*	Odour description[e]	ID[f]
47	Benzaldehyde, 4-methyl-	1621	1753	nd	0.122	nd	0.272	0.146	nd	0.05	1.058	sweet	ABC
48	1-Phenylethanone	1642	—	nd	nd	nd	nd	nd	2.727	nd	nd	must, flower, almond	ABC
49	Phenyl acetaldehyde	1645	1659	1.273	9.358	0.529	1.973	2.317	nd	nd	0.971	—	ABC
50	Methyl N-hydroxy benzene carboxamide	1726	—	1.592	nd	3.399	nd	nd	nd	nd	nd	—	BC
51	Benzeneacetic acid, methyl ester	1746	—	nd	nd	nd	nd	nd	10.672	nd	nd	—	ABC
52	Benzenamine, 3-methyl-	1798	—	0.204	nd	0.278	nd	0.139	nd	nd	0.204	—	BC
53	Anethole	1825	1281	1.234	3.37	1.766	1.901	1.992	0.761	0.204	0.255	—	ABC
54	Naphthalene, 2-methyl-	1862	—	0.535	1.252	0.142	0.196	0.594	0.636	0.253	0.415	—	ABC
55	butylated hydroxytoluene	1901	1898	0.547	0.429	0.363	0.441	nd	0.46	0.373	1.02	—	ABC
56	2-Phenyl-2-butenal	1933	—	0.457	nd	0.111	nd	0.199	nd	nd	nd	—	ABC
57	N-Ethylpropionanilide	1938	—	nd	nd	nd	0.648	nd	1.3	nd	nd	—	BC
58	Benzothiazole	1967	1902	nd	0.242	0.375	0.608	nd	nd	0.246	1.666	gasoline, rubber	ABC
59	Ortho-Cresol	1988	2017	nd	0.249	nd	0.477	0.419	nd	nd	0.281	phenol	ABC
60	1,1′-Biphenyl	1996	—	0.514	0.994	0.374	nd	0.395	0.32	nd	0.152	—	BC
61	Naphthalene, 2,6-dimethyl-	2015	2038	0.489	1.485	nd	nd	0.814	0.12	nd	0.291	grass	ABC
62	1,1′-Biphenyl, 4-methyl-	2105	2117	0.157	0.193	nd	nd	0.124	nd	nd	nd	—	ABC
63	Biphenylene	2223	—	0.137	1.605	nd	nd	nd	nd	nd	0.068	—	BC
64	1-Hydroxy-2,4-di-tert-butylbenzene	2270	—	nd	nd	nd	nd	1.509	nd	nd	nd	—	BC
65	2,4-Di-tert-butylphenol	2287	—	2.932	0.852	1.494	1.795	nd	0.87	0.508	1.918	—	BC
	Terpenes												
66	α-Cubebene	1447	1463	nd	nd	nd	nd	nd	nd	0.023	0.083	herb, wax	ABC
67	β-Cubebene	1526	1546	nd	nd	nd	nd	0.333	nd	nd	nd	citrus, fruit	BC
68	γ-Muurolene	1591	1681	nd	nd	nd	0.144	nd	nd	nd	nd	herb, spice	BC
69	δ-Cadinene	1620	1749	nd	nd	nd	nd	0.416	nd	nd	nd	medicine, wood	BC

Table 1: Continued.

No.[a]	Compounds	RI[b]	RI_{lit}[c]	*P. Abalones*[d]	*A. aegirit*	*H. erinaceus*	*G. frondosa*	*C. comatus*	*A. edulis*	*L. edodes*	*P. eryngii*	Odour description[e]	ID[f]
70	Cedran-8-ol	2092	—	nd	nd	nd	0.112	2.12	nd	nd	nd	—	BC
71	Cyperene	2114	—	nd	nd	nd	nd	nd	0.975	nd	nd	—	ABC
72	α-Copaene	2182	1488	nd	nd	nd	0.906	0.452	nd	0.363	nd	wood, spice	ABC
73	Acenaphthylene	2205	—	nd	nd	nd	nd	0.291	0.633	nd	nd	—	ABC
	Alkenes												
74	(3E)-3-Ethyl-2-methyl-1,3-hexadiene	1411	1415	0.316	0.376	0.427	0.126	0.25	0.128	nd	0.526	—	BC
75	1-Cyclooctene	1598	—	nd	0.47	nd	nd	nd	nd	nd	nd	—	BC
76	1-Decene	1935	2041	nd	nd	nd	nd	1.134	0.311	0.153	nd	alkane	BC
	Acid and esters												
77	Octyl formate	1529	1481	nd	nd	1.79	0.898	nd	0.213	0.402	0.513	fruity, rose	ABC
78	Fumaric acid, butyl cis-non-3-enyl ester	1628	—	2.091	nd	nd	nd	nd	nd	nd	nd	—	BC
79	Methyl cinnamate	2082	2056	0.782	0.605	nd	nd	nd	nd	nd	nd	strawberry	ABC
80	Nonanoic acid	2125	2202	nd	nd	nd	nd	0.155	nd	nd	nd	green, fat	ABC
81	n-Hexadecanoic acid	2173	1973	0.317	nd	nd	nd	nd	nd	nd	nd	—	ABC
82	Diethyl phthalate	2353	2204	nd	nd	nd	0.054	2.228	nd	0.39	nd	—	ABC
83	Hexanedioic acid, bis(2-ethylhexyl) ester	2507	—	nd	nd	nd	0.802	nd	5.916	nd	nd	—	BC
	Others												
84	DL-Menthol	1633	1626	nd	0.403	nd	0.464	0.178	nd	0.033	nd	peppermint	ABC
85	Azulene	1734	—	nd	nd	nd	nd	2.056	nd	nd	nd	—	BC
86	β-Dihydro-ionone	1819	—	nd	0.225	0.318	nd	1.506	nd	0.608	nd	—	BC
87	β-Ionone	1926	1912	0.178	nd	0.339	nd	2.271	nd	0.787	nd	flower, raspberry	ABC
88	Fluorene	2353	—	0.152	0.962	0.12	nd	0.364	0.193	0.069	0.06	—	ABC

[a]No.: numbers of the compounds observed in GC-MS profiles.

[b]The retention index as determined on HP-INNOWAX column (30 m × 0.25 mm × 0.25 mm) using homologous series of C8–C30 alkanes.

[c]Relative retention index taken from http://www.flavornet.com/, and/or (Cho et al. 2008 [1]; Alasalvar et al. 2005 [10]; Pennazza et al. 2013 [11]; Andreani et al. 2012 [12]; Pino 2010 [13]; Grosshauser and Schieberle 2013 [14]; Chen et al. 2009 [15]; Chen and Wu 1984 [16]; Yang et al. 1998 [17]; Chevance and Farmer 1999 [18]; Sun et al. 2014 [19]).

[d]Relative peak areas of volatile components in the eight mushrooms on HP-INNOWAX column, respectively.

[e]Odour description from http://www.flavornet.com/.

[f]Identification proposal is indicated by the following: A, by comparison of the retention time, mass spectrum, and retention index of authentic standard; B, identification by comparing EI mass spectrum with the mass spectral database Wiley and NIST 05; and C, by comparison of RI with those reported from literatures.

[g]Not detected.

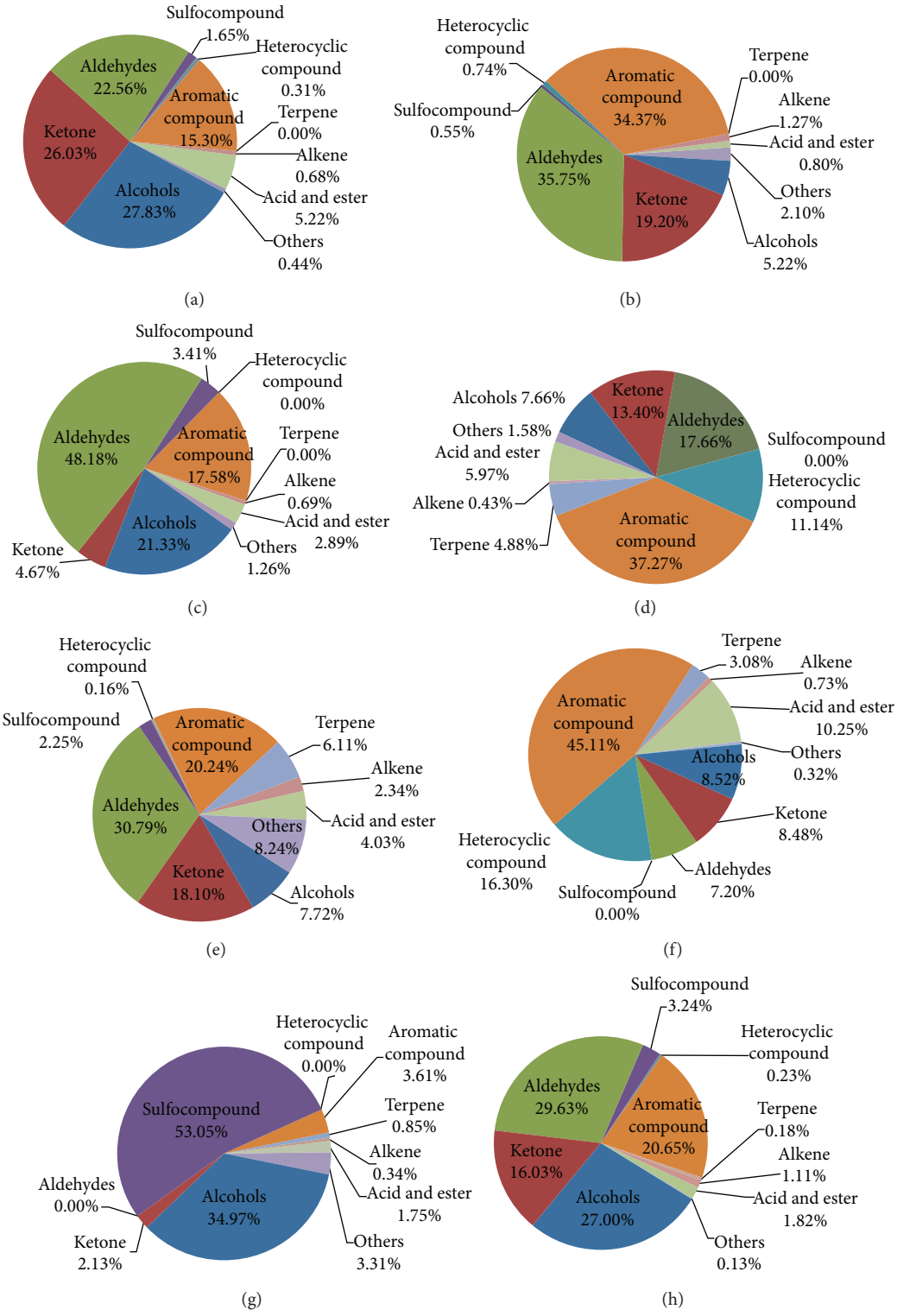

Figure 1: Percentage composition of the main groups of volatile compounds in different mushrooms. (a) *Pleurotus abalonus*; (b) *Agrocybe aegirit*; (c) *Hericium erinaceus*; (d) *Grifola frondosa*; (e) *Coprinus comatus*; (f) *Boletus edulis*; (g) *Lentinula edodes*; (h) *Pleurotus eryngii*.

types and levels of aldehydes in different mushrooms might also be used to discriminate the mushroom species. It was noted that the dried commercial mushrooms underwent the drying process and generated some compounds such as 1-octen-3-ol. The redrying process aims to offer better store samples, and steam distillation is used to extract the volatile compounds from mushroom. It is well-known that the mushroom flavor can be enhanced after cooking or heating treatment, because of the increases in the concentrations of some compounds such as 1-octen-3-ol [35]. Therefore, the "artifacts of volatile compounds" produced from Maillard reaction and lipid oxidation also are recognized as the intrinsic flavor compounds of mushroom.

Particularly, sulfur compounds were detected mainly in the flavor extract of *L. edodes*. The volatile compounds in *L. edodes* showed a singular intense aroma of 1, 2, 3, 5, 6-pentathiepane (also named lenthionine) and a few sulfur containing degradation products of S-alkyl cysteine sulfoxide. It was also illustrated that dimethyl trisulfide, dimethyl disulfide, 1, 2, 4-trithiolane, and 1, 2, 4, 6-tetrathiepane were identified as further sulfurous metabolites in *L. edodes* [36]. In this study, 1, 2, 4-trithiolane was determined as the most abundant component (22.4%) followed by 1-octen-3-ol (14.8%) in *L. edodes* (Table 1).

Pyrazines, furan, and pyrroles generally provide desirable popcorn and nutty aroma to foods, obtained from Maillard reaction [37]. These three classes of chemicals were previously found in mushrooms [38] and vegetables [2] with the sensory properties of bell pepper, and peas, pungent and earthy. In the present work, 1-isoamyl-2-formyl pyrrole and 1-(2-methylbutyl)-2-formyl pyrrole were determined with high concentration (1.99%–4.94%) in *B. edulis* (Table 1).

Of the components identified, aromatic compounds were one of the important groups in all mushrooms, for example, *P. abalonus* (15.3%), *A. aegirit* (34.4%), *H. erinaceus* (17.6%), *G. frondosa* (37.3%), *C. comatus* (20.2), *L. edodes* (3.6), *B. edulis* (45.1%), and *P. eryngii* (20.7%). Benzaldehyde, phenyl acetaldehyde, anethole and benzeneacetic acid, and methyl ester were the most abundant components (Table 1). The existence of such a large amount of aromatic components may be the cause of "almond-like" aroma during blending of these mushrooms [16]. The high content of these compounds and their similarities in structure indicate that the aromatic compounds may have a common origin. The formation of benzaldehyde and benzyl alcohol could be increased to a significant extent if benzoic acid was blended with fresh mushrooms, suggesting that the occurrence of enzymes could be responsible for the reduction of benzoic acid or its derivative into benzaldehyde and others [16].

The small amounts of terpenes were identified in 8 mushroom species (Table 1). Eight terpene-like compounds were detected and cedran-8-ol was the most abundant terpene (2.12%) of the total volatiles identified in *C. comatus*. Terpenes are often found in essential oils and contain characteristic odors [39], such as γ-muurolene, and α-cubebene and β-cubebene were reported in the *P. betulinus* with the odors, which were described as wood, spice, herb, and fruit flavor [40].

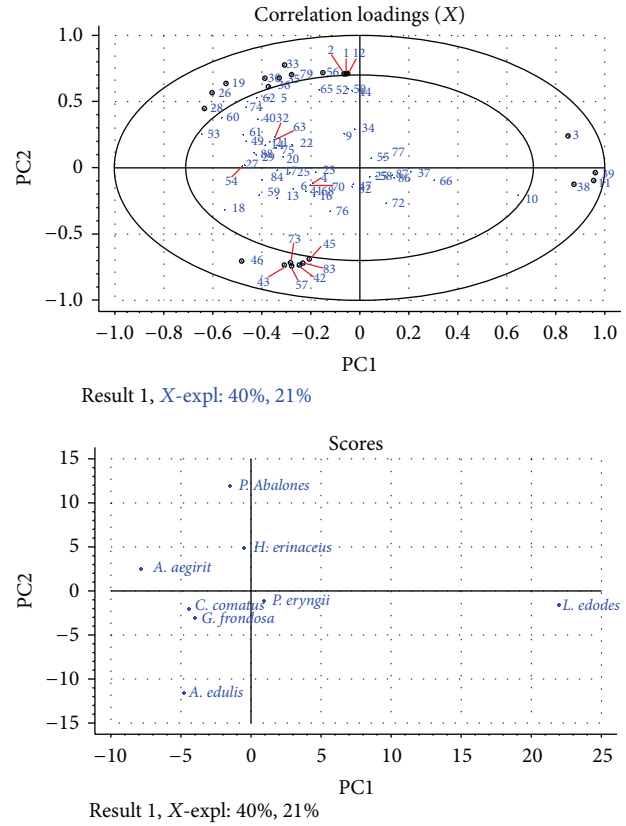

FIGURE 2: Principal components diagram of the 8 mushroom'volatile compounds (variables). Ellipses represent $r^2 = 0.5$ and 1.0, respectively.

Three alkenes, for example, (3E)-3-ethyl-2-methyl-1, 3-hexadiene, 1-cyclooctene, and 1-decene, were determined in the eight mushroom samples, but only 1-decene had a relative higher concentration in quantity (1.13%, Table 1). Almost all alkenes could be derived from lipid degradation [41].

Table 1 also showed that 7 compounds were found in the group of acids and esters, including octyl formate, methyl cinnamate, and nonanoic acid. The acids and esters have been reported to be the major odors of fruit and grass flavors, such as octyl formate rich in blackberry with strong odors of orange fruit and rose [42]. Methyl cinnamate was reported to be abundant in the volatile compounds of pine-mushroom [1], and it was noted that this compound could prevent from the attack of the mycophagous collembolan to mushrooms [43].

An azulene-type compound was identified in *C. comatus*. To our best knowledge, this compound has only been isolated previously in the mushroom of *Lactarius salmonicolor* [44]. DL-Menthol with strong inhibitory activity against fungi of Trichoderma [45] was also detected in the tested mushrooms. This implied that some mushrooms have natural biofungicide activity.

Principal component analysis (PCA) of mushroom volatile compounds is as follows: to discriminate different mushroom species according to the GC-MS identified compounds, a PCA was performed in Figure 2, indicating

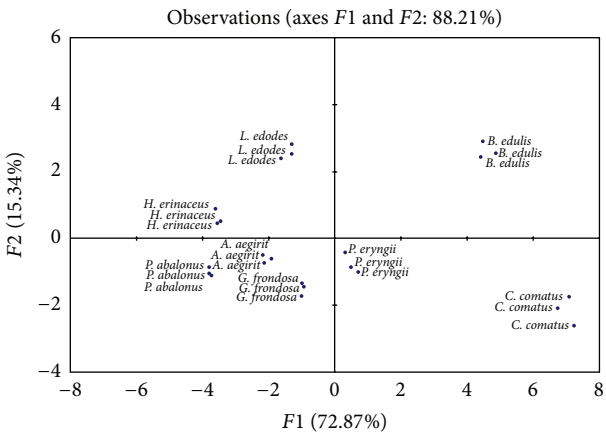

Figure 3: Plot of the first two principal components of the PCA model built with the electronic nose data related to the eight mushroom samples.

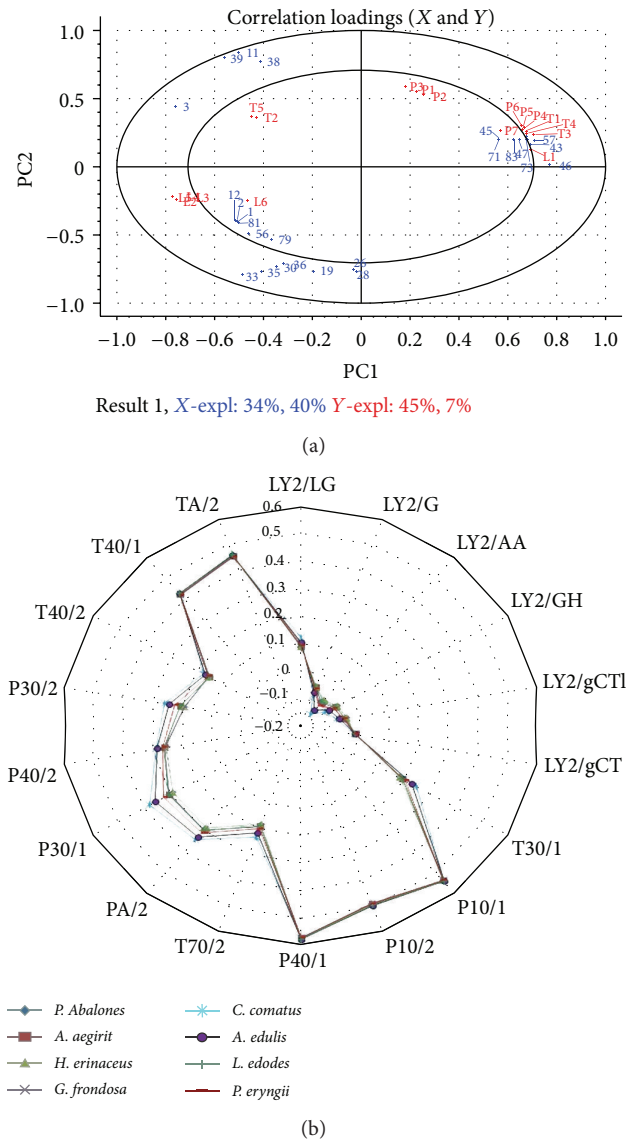

Figure 4: (a) Factor loading of PLS components 1 and 2 on GC-MS data and 18 electronic nose gas sensors (L1–6 denotes LY2/LG, LY2/G, LY2/AA, LY2/GH, LY2/gCTl, and LY2/gCT, respectively; T1–5 denotes T30/1, T40/1, T40/2, T70/2, and TA/2, respectively; P1–7 denotes P10/1, P10/2, P40/1, PA/2, P30/1, P40/2, and P30/2, respectively). (b) Spider plot of the electronic nose sensors to the eight mushrooms.

the projection of the GC-MS data for all samples. It was shown the differences among them. PCA provided a separation of the samples with 41% and 20% of the variation that accounted for PC1 and PC2, respectively. The contribution rate of the accumulative total of variance of the 2 factors in PCA is 61% representing that two PCs can explain 61% of the whole mushroom volatiles. The eight mushroom species were distributed according to their respective major compounds, where 3-octanol (1), 3-octanone (12), 2-octanol (2), 1-octen-3-ol (3), 1, 2, 4-trithiolane (39), octanal (28), 1-octen-3-one (14), anethole (53), 6-nonenal (30), and phenyl acetaldehyde (49) had the higher power of discrimination. The first principal component (Figure 2) clearly separates *P. abalonus* from others due to the high content of 3-octanol (1) and 3-octanone (12). Even between *Pleurotus* species of *P. abalones* and *P. eryngii*, *P. eryngii* can be distinguished from others due to its high contents in hexanal (24) and 1-octen-3-ol (3).

According to the abovementioned volatile profile, PCA revealed that *L. edodes* have higher content of 1-octen-3-ol (3) and 1, 2, 4-trithiolane (39), while *A. aegirit* have higher content of anethole (53). Meanwhile, phenyl acetaldehyde (49) and *C. comatus* have higher content of nonanal (27). Species of *H. erinaceus* was distinguished based on its 2, 4-decadienal (35) content, which was the most important differentiator compound among its volatile compounds. Another species of *B. edulis* was also separated based on higher contents in benzaldehyde (46), benzeneacetic acid, methyl ester (51), and 1-isoamyl-2-formyl pyrrole (42) (Figure 2).

3.2. Discrimination of Different Mushrooms by Electronic Nose (E-Nose). For better visualization of data, PCA was performed to identify the patterns of correlation with individual composition variables in the discrimination among different mushroom samples by using signals corresponding to three repeated exposures of each sample in Figure 3. The clearly different distributing results of different mushroom samples in PCA analysis in Figure 3 confirmed that the e-nose sensors have the ability to accurately respond to different mushrooms with similar fingerprint chromatograms. In addition, the differences between groups have been visualized by PCA plots more clearly (Figure 3).

As shown in Figure 3, each group was clearly distinguished from the other groups by using PCA analysis. There was a main separation among different mushroom samples and all the mushroom samples were separated into eight groups. Even the same species such as *P. abalonus* and *P. eryngii* can be separated clearly. The score plot for the first two principal components (PC1 and PC2) is shown in Figure 3. The score plot reveals the separation along PC1 accounting for 72.87% of the total data variance in the sample set, while

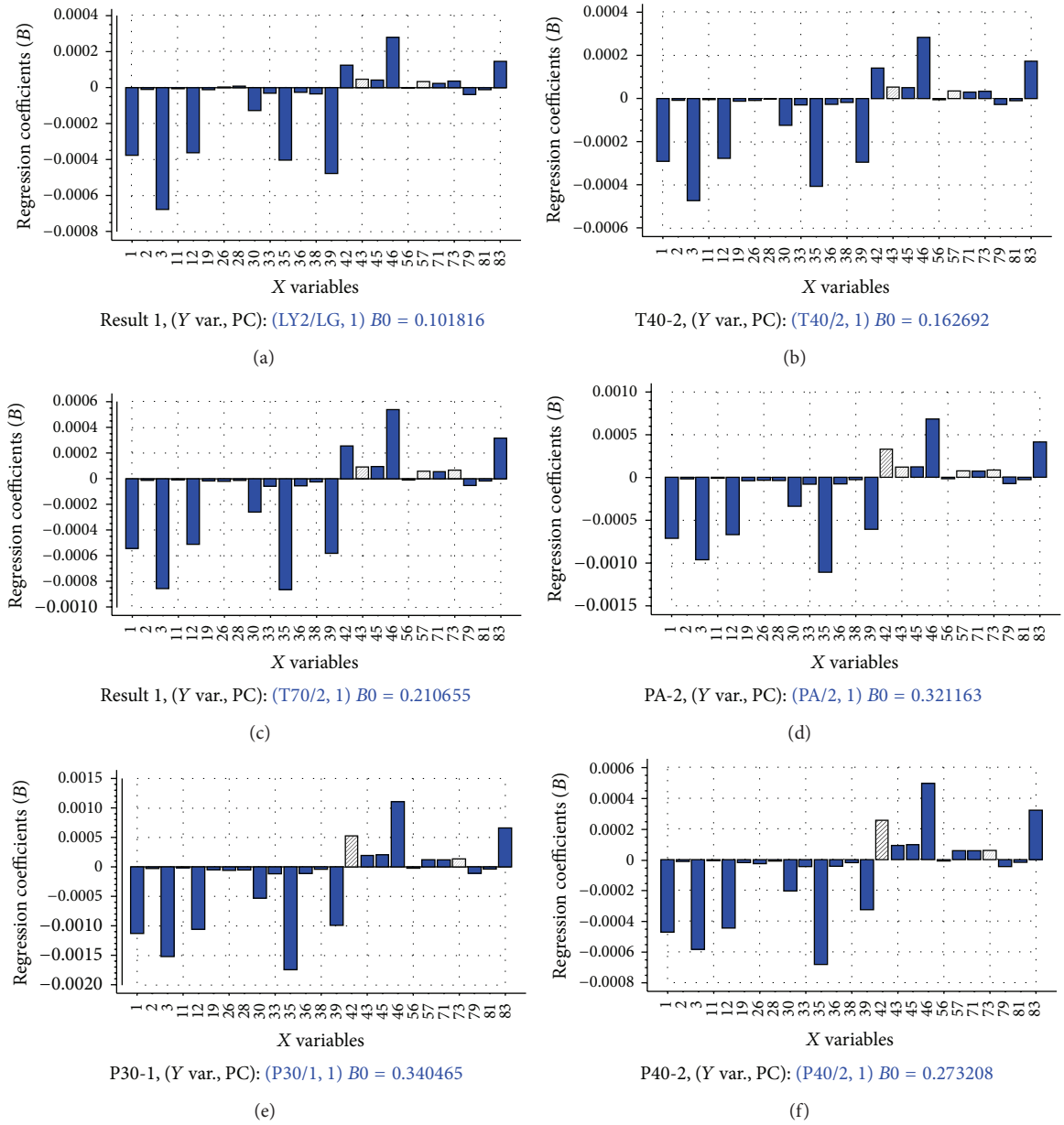

FIGURE 5: Estimated regression coefficients and significance indications (streaked bars) from PLS1 predication models for section of gas sensors LY2/LG (a), T40/2 (b), T70/2 (c), PA/2 (d), P30/1 (e), and P40/2 (f) from GC-MS data.

separation along PC2 accounted for 15.34% of the variation in the sample set.

The results indicated that eight mushroom samples can be distinguished on the basis of different odors by e-nose with PCA method. Therefore, according to the obtained results, the e-nose can be used as a useful tool for quickly distinguishing the mushrooms, taking into account the concentration of volatile compounds. The findings from analyses carried on mushroom samples were in agreement with the results obtained by means of GC-MS as they both separated mushroom samples successfully. Hence, e-nose could be used as an identification tool for mushroom.

3.3. Relationship between GC-MS Profiles and E-Nose Analysis. To study relationships between GC-MS data and e-nose responses, two data sets were analyzed by PLS. The GC-MS data were selected from all GC-MS profiles, according to the results in Figure 2, which located in the ring. Figure 4(a) shows two factors loading plot for GC-MS data (X-matrix) and 18 sensors (Y-matrix). Four sensors clusters located in three quadrants and 25 GC-MS peaks are placed in three locations. The derived PLSR model included two significant PCs successfully explaining 74% of the cross validated variance. Figure 4(b) indicates a typical response of the e-nose sensors for the measurement of the eight mushroom samples.

Each curve represented the maximum response value of the mushroom volatiles on the sensors during parsing time. Although they had a roughly identical trend on the 18 sensors, there were significant differences between eight mushrooms on sensors, for instance, P30/2, P40/2, P30/1, and PA/2.

To further investigate the compounds with the greater contribution to e-nose sensors, PLS1 regression analysis was carried out. Figure 5 shows the results from the PLS1 regression analysis for the contribution of GC-MS profiles to the sensors response data LY2/LG, T40/2, T70/2, PA/2, P30/1, and P40/2. Streaky bars demonstrated significant GC-MS compounds. In general, enormous volatiles were negatively related to selected sensors by means of their regression coefficients in PLS regression modeling. Merely two compounds, 1-(2-methylbutyl)-2-formyl pyrrole (43) and N-ethylpropionanilide (57), appeared to possess significant sensitivity and explained 78% and 70% of the variation in LY2/LG (A) and T40/2 (B), respectively. Three compounds, 1-(2-methylbutyl)-2-formyl pyrrole (43), N-ethylpropionanilide (57), and acenaphthylene (73), illustrated significant sensitivity and explained 68% of variation in T70/2 (C). Four compounds, 1-isoamyl-2-formyl pyrrole (42), 1-(2-methylbutyl)-2-formyl pyrrole (43), N-ethylpropionanilide (57), and acenaphthylene (73), suggested significant sensitivity and explained 64% of variation in PA/2 (D). Two compounds, 1-isoamyl-2-formyl pyrrole (42) and acenaphthylene (73), had significant sensitivity and explained 64% and 62% of the variation in P30/1 (E) and P40/2 (F), respectively. The above results were similar to the conclusions in literature [46–50]. The object substances for LY/LG were oxynitride and sulfide, and P30/1 was hydrocarbons and ammonia, and PA/2 was ammonia and amine compounds, and so forth.

4. Conclusions

In this study, it was the first time that the volatile compounds of 8 different edible mushroom species were characterized by using both GC-MS and e-nose. Based on the GC-MS analysis, a total of 88 volatile compounds were identified and differences in the composition of volatile components from eight mushrooms were observed. It was feasible to classify the mushroom samples into eight groups by using GC-MS and e-nose. Elementary results confirmed the usefulness of GC-MS and electronic nose for classification purpose of mushroom. This combination for the volatile analysis with chemometric methods can be applied to distinguish different mushrooms successfully. Furthermore, this study results about the volatile composition of commercial mushrooms could help to set up a finger spectrum by e-nose to identify the species of edible fungi.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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