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Identification of Volatile Flavour Components of *Tuber melanosporum* Using Simultaneous Distillation-Extraction

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Abstract

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Black truffles are famous for their unique flavours. Headspace solid-phase microextraction and the electronic nose have been used to analyse their flavours in some investigations. In a previous work, the volatile flavour compounds in black truffles harvested in China were extracted using simultaneous distillation-extraction (SDE) and analysed using gas chromatography-mass spectrometry (GC-MS). Extraction conditions were optimised in that previous study and are now applied in the present work. The temperature of the solvent flask was maintained at 70°C using a water bath and the samples were placed in boiling water; extraction time was 3 hours. Fifty-seven volatile flavour compounds were tentatively identified, including seven alcohols, two acids, six esters, 12 aldehydes, 14 ketones, two phenols, six pyrazines, six sulphur compounds and three other components. Aldehydes and ketones were present at the highest levels.

Keywords: gas chromatography-mass spectrometry; Black truffle; SDE

Black truffles (*Tuber melanosporum)* are a kind of edible fungus that grow in the ground. They are generally globular in shape with a wrinkled external surface, and they range in colour between dark brown and black. Consumption by animals results in the dispersal of black truffle spores in nature. Black truffles are more appreciated for their flavour than for their nutritional value (Costa *et al.* 2015). The composition of the involved aromas is very complex, and different metabolic pathways participate in their production, as has been clarified by studying the genome of *Tuber melanosporum* Vittad. (Berna 2010).

Because flavour is the most important characteristic of this fungus, several studies have reported the chemical constituents considered to be responsible for the typical aroma. Compounds such as 2-methylbutanal and 3-methylbutanal seem to be common constituents of all truffle aromas (Costa *et al.* 2015). Bis(methylthio)methane among sulphur compounds and hexanal, 2-methylbutanal and 3-methylbutanal among aldehydes, have been reported as the predominant compounds in the truffle volatile fraction, with qualitative fluctuations depending upon variables such as truffle type and geographical origin (Fiecchi 1967; Gioacchini 2008).

The use of a simultaneous distillation-extraction (SDE) method to analyse the composition of volatile compounds in black truffles has never before been reported in the literature. The choice of pre-treatment method greatly influences the composition of volatile compounds that are extracted. In order to obtain a comprehensive understanding of the aromatic

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components of black truffles harvested in China and to provide a certain theoretical basis for the study of the flavour of black truffles harvested in China, this paper extracted the volatile compounds of black truffles using an SDE method.

MATERIAL AND METHODS

Samples and reagents. The truffles used in this work were collected from Yuxi Yunnan (China); all were blast-dried until water content was less than 10%. 1,2-Dichlorobenzene (internal standard) was obtained from Dr. Ehrenstorfer (Germany). *n*-Alkane standard (C_7–C_{30}) was purchased from Supelco (USA). *n*-Hexane and sodium sulphate anhydrous were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All reagents were of analytical grade.

Simultaneous distillation-extraction. The SDE apparatus was similar in design to that of Likens and Nickerson (1964), and was purchased from Beijing Glass Instrument Factory (China). Immediately before analysis, 20 g of truffle were cut from mature truffles that were dried in slices. The sample, together with 200 ml deionised water, was placed into a 500-ml flask which was attached to one arm of the SDE apparatus. A 250-ml round-bottom flask with 100 ml of *n*-hexane as extraction solvent was linked to the other arm of the SDE apparatus. Before the experiment, the SDE system was purged for 5 min under a stream of *n*-hexane vaporisation. The sample flask was heated by an electric heating jacket to keep the deionised water boiling, and the temperature of the solvent flask was maintained by a water bath at 70 ± 1°C. After the two arms started to reflux, each extraction was carried out for 3 hours. After cooling to ambient temperature, the extract was collected and dried over anhydrous sodium sulphate overnight. This was followed by filtration and then concentration down to 2 ml using a rotary evaporator. The SDE extractions were performed in triplicate.

Gas chromatography-mass spectrometry (GC-MS) analysis. A 7890 Gas Chromatograph system coupled with a 5975C mass-selective detector (MS) (Agilent Technologies, USA) equipped with a HP-INNOWax capillary column (60 mm × 0.25 mm ID; 0.25 μm film thickness) was used to separate and identify the extracted compounds. The temperature of the injector was 250°C. The oven temperature was 40°C for 3 min, then rose to 150°C at 5°C/min and was held for 1 minutes. After that, the temperature rose to 220°C at 10°C/min and was held for 2 minutes. The carrier gas (He) flow rate was 1 ml/min. The electron impact (EI) ion energy was 70 eV, and the temperature of the ion source was 230°C; the chromatograms were obtained by recording the total ion currents in the range of 29–300 *m/z*.

Identification of volatile components. Tentative identification of the volatile compounds was based on comparing mass spectra and retention indices (RI) with the Wiley7n.l (Hewlett Packard, USA) and Nist05a.l databases and previously reported RI. Some compounds were identified by the injection of the authentic compounds into the GC–MS system, while the RI of the compounds was calculated using an *n*-alkane (C_7–C_{30}) series under the same conditions according to the equation of Van den Dool and Kratz (Dool & Kratz 1963). The peak areas of the identified components were calculated using retention times of longer than 6 min (the end of the solvent peak).

RESULTS AND DISCUSSION

Black truffles extracts were made using the SDE conditions determined in a previous work performed in our laboratory and analysed by GC–MS. Mass spectrometry data and comparison with relevant databases (Cong 1987; Zhu 1987) resulted in the tentative identification of 57 volatile compounds in the black truffle species that are listed in Table 1 along with a determination of their relative concentrations (as normalised areas).

The 57 volatile flavour compounds listed in Table 1 include alcohols (7), acids (2), esters (6), aldehydes (12), ketones (14), phenols (2), pyrazines (6), sulphur compounds (6), and other compounds (3). Among the aromatic compounds were 4.76% acids, 5.36% esters, 32.02% aldehydes, 27.57% ketones, 3.84% sulphur compounds, 8.35% alcohols, 5.91% pyrazines, 6.42% ethers, and 5.71% phenols. Sulphur compounds are the key characteristic components of truffle aroma (D'Auria *et al.* 2013). Several papers have reported the chemical constituents of black truffles using headspace solid-phase microextraction; low boiling point compounds accounted for a large proportion of the compounds identified, but in this paper, many higher boiling point compounds were discovered.

The content of γ-nonanolactone was highest out of all the volatile flavour compounds, with a relative content of 27.98%. Some eight carbon compounds

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Table 1. Tentatively identified volatile flavour compounds in black truffle

No.	Compounds	Aroma[a]	Retention index	Concentration (mg/l)	Threshold (mg/l)	OAV
Aldehydes						
1	2-furancarboxaldehyde	sweet, woody, bready, nutty	845	1.96	0.25	7.840
2	heptanal	fresh aldehydic fatty	952	3.56	0.14	25.429
3	benzaldehyde	sweet, oily, almond	1021	3.50	0.5	7.000
4	benzeneacetaldehyde	honey, sweet, floral	1092	2.37	0.0017	1394.118
5	1-ethyl-1H-pyrrole-2-carbaldehyde	burnt roasted smoky	1096	0.67		
6	decanal	aldehydic waxy orange	1267	0.12	0.094	1.277
7	(2E,4E)-hepta-2,4-dienal	fatty, green oily	1276	5.35	0.057	93.860
8	4-methoxy-benzaldehyde	sweet powdery mimosa	1320	0.15	0.0002	750.000
9	(2Z)-2-phenyl-2-butenal	sweet narcissus cortex beany	1339	2.87	1.7	1.688
10	γ-nonanolactone	coconut creamy waxy	1434	27.98	0.00008	349750.000
11	5-methyl-2-phenyl-2-hexenal	bitter cocoa, honey, aldehydic	1562	1.03		
12	5,9-dimethyl-4,8-decadienal	citrus aldehydic marine floral ozone	1652	1.38		
Benzenes						
13	ethylbenzene	aromatic	893	2.79	0.018	155.000
14	1,3-dimethyl-benzene	plastic	907	6.30	0.52	12.115
Sulphocompounds						
15	3-(methylsulfanyl)propanal	potato, vegetative	956	1.00		
16	tetrahydrothiophen-3-one	garlic, meaty	1010	0.70	0.0012	583.333
17	thialdine	roasted meaty beefy	1264	0.73	5.51	0.132
18	3-methylthio-1-propanol	sulfurous onion sweet soup vegetable	1833	0.76	0.015	50.667
Ketones						
19	2-furyl methyl ketone	sweet, nutty	964	5.73		
20	1-octen-3-one	herbal mushroom earthy	1038	2.92	0.0012	2433.333
21	cycloheptanone	mint smell	1060	1.05	0.75	1.400
22	(3E)-3-octen-2-one	creamy, earthy	1089	4.00	0.02	200.000
23	9-decen-2-one	fruity pear	1145	0.89		
24	3-nonen-2-one	fruity berry	1196	0.97	0.03	32.333
25	2,3-hexanedione	butter oily fatty, creamy phenolic	1222	0.90	0.06	15.000
26	4-(2-furanyl)-3-buten-2-one	sweet spicy warm balsam	1249	0.81		
27	2-decanone	orange, floral	1254	0.45	0.11	4.091
28	3-decen-2-one	fatty green fruity apple	1301	1.00	0.03	33.333
29	2-undecanone	waxy fruity creamy	1360	9.34	0.03	311.333
30	cis-γ-dodec-6-enolactone	fatty, waxy, creamy	1731	7.51		
31	2-pentadecanone	fatty spicy floral	1766	2.48	0.165	15.030
32	pinocamphone	spicy	1952	4.20		
33	neryl acetone	fatty metallic	2098	4.54	0.049	92.653
Pyrazines						
34	2-ethyl-6-methyl-pyrazine	roasted hazelnut	1055	1.22	1.2	1.017
35	2,3-dimethyl-5-ethylpyrazine	burnt popcorn ,roasted cocoa	1138	1.90	0.9	2.111
36	3-ethyl-2,5-dimethyl-pyrazine	potato cocoa roasted nutty	1129	2.17	1.4	1.550
37	2-acetyl-1,4,5,6-tetrahydropyridine	creamy bready	1200	0.33		
38	2-butyl-3-methylpyrazine	anise licorice	1456	0.37	0.17	2.176
39	2-butyl-3,5-dimethyl-pyrazine	sweet earthy	1522	3.42	0.18	19.000

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Table 1 to be continued

No.	Compounds	Aroma[a]	Retention index	Concentration (mg/l)	Threshold (mg/l)	OAV
Others						
40	2-pentylfuran	green, waxy, with musty	1050	8.91	0.27	33.000
41	Indole	animal floral moth	1367	0.98	0.000033	29696.970
42	3,4-dimethylanisole	anisum,fruit	1464	0.32		
Alcohols						
43	2-ethyl-3-hexen-1-ol	green leafy	1030	0.12		
44	1-octen-3-ol	mushroom, earthy, fungal	1042	2.94	0.06	49.000
45	2-ethyl-1-hexanol	sweet fatty fruity	1083	1.11	0.74	1.500
46	2-methylpropanol	musty phenolic	1115	2.00	0.02	100.000
47	citronellol	floral, rose, sweet,	1509	0.17	0.045	3.778
48	farnesyl alcohol	mild fresh sweet linden	1644	6.00	0.1	60.000
49	cyclohexanol	minty cooling, herbaceous peppermint nuance	1748	0.95	0.64	1.484
Acids						
50	linoleic acid	faint fatty	2126	7.56		
51	oleic acid	fatty, vegetable oil with lard	2361	0.09		
Esters						
52	ethyl benzenecarboxylate	musty sweet wintergreen	1232	0.83	0.006	138.333
53	allyl 2-ethyl butyrate	nut fruit peach-pit cherry-pit	1635	0.72	0.67	1.075
54	benzoic acid, cyclohexyl ester	mild balsamic floral herbal	1837	0.57		
55	butyl citrate	herbal plastic	2163	2.93	1.2	2.442
56	tributyl acetylcitrate	very faint herbal wine sweet	2207	2.72	1.4	1.943
57	glyceryl monooleate	bland fatty waxy	2392	0.75		

[a]aroma searching from The Good Scents Company Information System

contribute to the characteristics of mushroom flavour, mainly the typical mushroom soil fragrance, including 1-octen-3-one and 1-octen-3-ol (Maga 1981; Tsai *et al.* 2009).

There is no complete agreement in the literature about the compounds responsible for the truffle aroma. For instance, Talou *et al.* (1987) described 2-methylbutanol and dimethyl sulfide to be responsible for sulfurous and pungent notes and determined that both had great importance for the perception of the final aroma. Mauriello *et al.* (2004) also found dimethyl sulfide in nine truffle species using SPME methods, and many aldehydes were found in both of the studies cited above. These findings might be due to different extraction methods and the different origins of the materials. In this study, 3-methylpropanol, as well as some sulfur compounds and aldehydes were the predominant compounds, in agreement with other studies. The composition of aromatic components may also be affected by location. For example, it is important to emphasise the lack of 2,3-butanedione, a compound previously found in some black truffles of Italian origin but not described in French ones (Paloma *et al.* 2003). Further, the use of a solvent delay time in the chromatographic analysis of SDE extracts was longer than in SPME; this could lead to the loss of volatile components.

The fact that 2,3-butanedione could not be found in the black truffles of Chinese origin may also be associated with the higher extraction temperatures. Higher temperatures hinder adsorption in the solvent, because they elicit a decrease in the partition coefficient between volatile compounds and solvents (Verzera *et al.* 2004). The content of hydrocarbons, esters and acids content increases with temperature. The higher temperature and the longer extraction time in SDE enhances the extraction of these kinds of compounds, while the most volatile compounds and those with low molecular weights were clearly better extracted using SPME. The evaporation step during SDE may increase the loss of the most volatile compounds (Barra *et al.* 2007; Montserrat *et al.* 2011). SPME extraction is affected not only by the thickness and polarity of the fibre but also by other

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parameters such as temperature, time and sample amount (Kataoka & Pawliszyn 2000). The study of Majcher (2009) showed that the SPME extraction technique was not suitable for the isolation of high molecular weight compounds or those with a strong affinity to the matrix.

CONCLUSION

In this study, the volatile components of black truffles harvested in China were isolated using SDE pre-treatment methods and analysed using GC/MS. A total of 57 volatile flavour compounds were identified, predominantly alcohols, ketones and aldehydes. Due to the long duration and high temperatures of the extraction, compounds with low boiling points or those that evaporate easily were detected at lower levels. Therefore, the SDE method is suitable for the extraction of high boiling point compounds.

References

Barra A., Baldovini N., Loiseau A.M., Albino L., Lesecq C., Lizzani C.L. (2007): Chemical analysis of French beans (*Phaseolus vulgaris* L.) by headspace solid phase microextraction (HS-SPME) and simultaneous distillation/extraction (SDE). Food Chemistry, 101: 1279–1284.

Berna A. (2010): Metal oxide sensors for electronic noses and their application to food analysis. Sensors, 10: 3882–3910.

Cong P.Z. (1987): Application of Mass Spectrometry in Natural Organic Chemistry. Beijing, Science and Technology Publishing House. (in Chinese)

Costa R., Fanali C., Pennazza G., Tedone L., Dugo L., Santonico M., Sciarrone D. (2015): Screening of volatile compounds composition of white truffle during storage by GC-(FID/MS) and gas sensor array analyses. LWT-Food Science and Technology, 60: 905–913.

D'Auria M., Racioppi R., Rana G. L. (2013): Volatile organic compounds of *Schenella pityophilus*. Natural Product Research, 27: 41–44.

Fiecchi A., Kienle M., Scala A., Cabella P. (1967): Bis-methylthiomethane, an odorous substance from white truffle, pico. Tetrahedron Letters, 8: 1681–1682.

Gioacchini A., Menotta M., Guescini M., Saltarelli R., Ceccaroli P., Amicucci A. (2008): Geographical traceability of Italian white truffle (*Tuber magnatum* Pico) by the analysis of volatile organic compounds. Rapid Communications in Mass Spectrometry, 22: 3147–3153.

Kataoka H., Lord H.L., Pawliszyn J. (2000): Application of solid phase microextraction in food analysis. Journal of Chromatography A, 880: 35–62.

Likens S., Nickerson G. (1964): Detection of certain hop oil constituents in brewing products. Proceedings of the American Society of Brewing Chemists, 5: 5–13.

Maga J.A. (1981): Mushroom flavor. Journal of Agricultural and Food Chemistry, 29: 1–4.

Majcher M., Jelen H.H. (2009): Comparison of suitability of SPME, SAFE and SDE methods for isolation of flavor compounds from extruded potato snacks. Journal of Food Composition and Analysis, 22: 606–612.

Mauriello G., Marino R., D'Auria M., Cerone G., Rana G. L. (2004): Determination of volatile organic compounds from truffles via SPME-GC-MS. Journal of Chromatographic Science, 42: 299–305.

Montserrat R.A., Liliana V., Stefania V. (2011): Characterisation of volatile composition of white salsify by headspace solid-phase microextraction (HS-SPME) and simultaneous distillation–extraction (SDE) coupled to GC–MS. Food Chemistry, 129: 557–564.

Paloma D., Elena I., Francisco J.S., Guillermo R. (2003): Truffle aroma characterization by headspace solid-phase microextraction. Journal of Chromatography A, 1017: 207–214.

Talou T., Delmas M., Gaset A. (1987): Principal constituents of black truffle (*Tuber melanosporum*) aroma. Journal of Agricultural and Food Chemistry, 35: 774–777.

Tsai S.Y., Huang S.J., Lou S.H. (2009): Flavour components and antioxidant properties of several cultivated mushrooms. Food Chemistry, 113: 578–584.

van den Dool H., Kratz PD. (1963): A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. Journal of Chromatography A, 11: 463–471.

Verzera A., Ziino M., Condurso C., Romeo V., Zappalà M. (2004): Solid-phase microextraction and gas chromatography-mass spectrometry for rapid characterisation of semi-hard cheeses. Analytical and Bioanalytical Chemistry, 380: 930–936.

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