Determination of Volatile Organic Compounds in Macadamia Flower by Simultaneous Distillation Extraction and Static Headspace

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Simultaneous distillation extraction and static headspace techniques were compared for their effectiveness in the extraction of volatile flavour compounds from macadamia flower samples. Experimental results showed static headspace lacked the sensitivity needed to evaluate certain flavour volatiles, such as esters. Considerable improvements in the extraction ability of the simultaneous distillation extraction over the static headspace method were noted. About 63 different compounds were identified including acids, alcohols, aldehydes, ketones, esters, hydrocarbons and other a few compounds, which can offer some reference for application of the Macadamia flower in large scale.

Key Words: Macadamia flower, GC-MS, Simultaneous distillation extraction, Static headspace.

INTRODUCTION

Macadamia is a genus of flowering plants in the family *Proteaceae*. Macadamia was introduced into the China in recent years. The tree is originally discovered growing in hard, well-drained soil along the Yunnan, Hainan and Guangxi[1,2].

Macadamia nuts have a unique delicate flavour, crunchy texture and high oil content. The kernels are rich in mono-unsaturated fatty acids[3-5] and may reduce serum cholesterol when included in a healthy diet[6,7]. Frequent consumption of nuts has been linked to a lowered risk of cardiovascular disease. Phytochemicals, especially phenolics, in nuts may be considered to be the major bioactive compounds for health benefits. Nine types of tree nuts and peanuts commonly available in the United States were evaluated for total phenolic and flavonoid contents, antioxidant and antiproliferative activities[8].

However, the flower of Macadamia was not researched in the last years. In this sense, simultaneous distillation extraction and static headspace were compared for their effectiveness in the extraction of volatile flavour compounds from Macadamia flower samples. The GC-MS method used for analyzing the volatile organic compounds in the flowers of Macadamia firstly in this paper.

EXPERIMENTAL

Ethanol (99.5 % purity), dichloromethane (99.5 % purity) and acetone (99.5 % purity) were purchased from Shanghai.

Macadamia flower samples: In 2010 Macadamia flowers was obtained from Xishuangbanna of Yunnan Province. Macadamia flowers were transported with stems in water immediately after harvest to Kunming. Whole flowers (2.5-5 g) that were 50-75 % open and in good quality. The closed system was allowed to equilibrate for 3 days at room temp. The liquid phase obtained was then dried using a Buchi rotary evaporator (R-215). Traps were stored at 15 ℃ before GC-MS analysis (storage was less than 2 weeks).

Sampling conditions of static headspace: Approximately 500 mg of Macadamia flower were weighed into a 10 mL headspace vial and the vials were sealed with silicone rubber PTFE caps. Samples were homogenized for 1 min, using a vortex. Samples were equilibrated at 60 ℃ for 15 min in a 2t® vial heater, then 500 μL were removed by a static headspace sampler, sampling time was 30 s. The gas volume sample was then injected in the GC-MS system.

Sampling conditions of simultaneous distillation extraction: Simultaneous distillation extraction was carried out in a micro version apparatus. Dichloromethane was used as solvent, For the extraction, 10 g of Macadamia flower and 250 mL distilled water were placed in a 500-mL flask, 30 mL dichloromethane was placed in a 50-mL flask, stream distillation was stopped after 2 h, while the solvent extraction was continued for a further 15 min. The extract was concentrated to 1.0 mL at 55 ℃ by Buchi rotary evaporator (R-215, Switzerland).
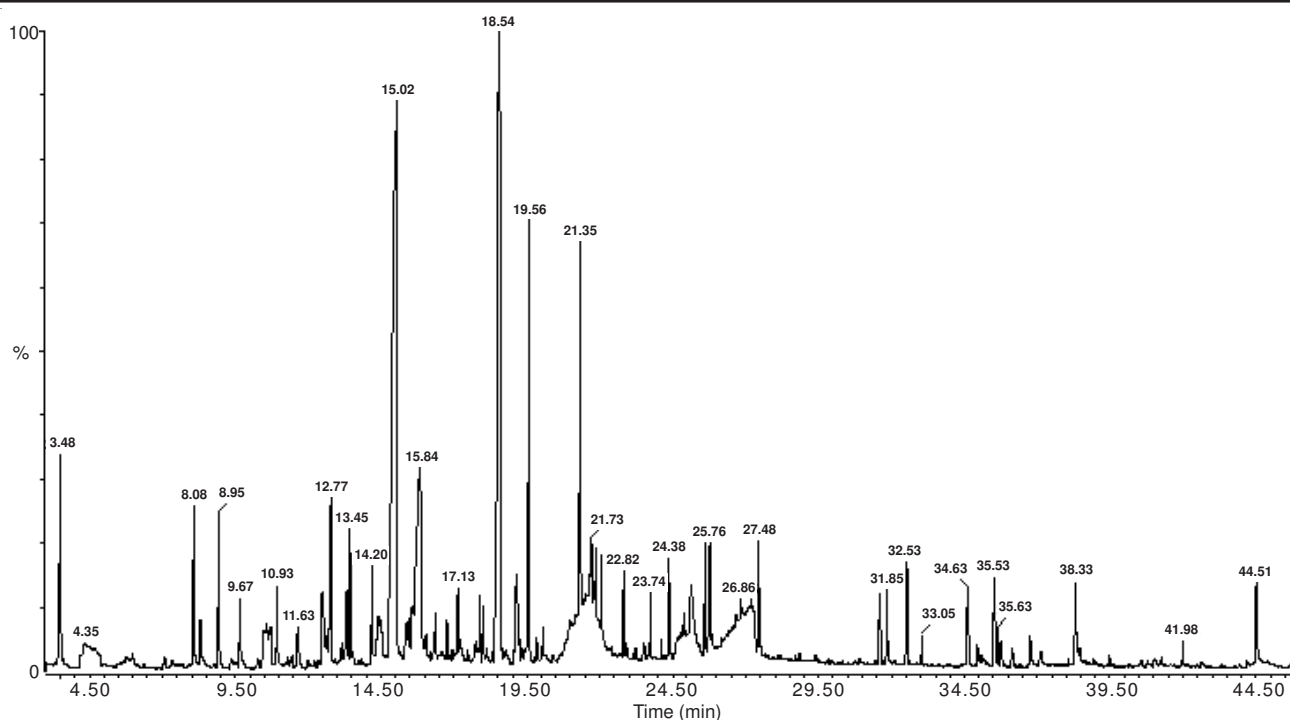
Fig. 1. GC-MS Chromatograms of volatile compounds by SDE

Condition of GC-MS: The GC-MS system was from autosystem turbomass PerkinElmer, USA. The GC/MS analysis was carried out by using a PerkinElmer Clarus 600 gas chromatograph coupled to a 600 T Mass spectrometer. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}; the gas chromatograph was operated in splitless mode. Chromatographic separation was performed on a 30 m·0.25 mm, df 0.25 µm HP-5MS capillary column (Agilent Technologies) and a 60 m·0.32 mm, df 0.18 µm HP-VOC capillary column (Agilent Technologies). The following GC oven temperature program was applied: 50 ℃ for 2 min, 3 ℃ min^{-1} to 100 ℃, 20 ℃ min^{-1} to 250 ℃, 250 ℃ hold for 4 min. Transfer line and source were maintained at the temperature of 280 ℃ and 230 ℃ respectively. The mass spectrometer was operated by recording the current of the ions from 3 to 54 min.

Initially, full scan EI data were acquired to determine appropriate masses under the following conditions: ionization energy: 70 eV, full scan data were recorded from 28 to 550 m/z, scan time: 0.2 scan/s. All the analyses were performed setting the electron multiplier voltage at 1200 V. Signal acquisition and elaboration were performed using the PerkinElmer Chemstation.

RESULTS AND DISCUSSION

Compounds are extracted by simultaneous distillation extraction: The simultaneous distillation extraction extracts displayed 63 compounds (Fig. 1), including: 6 aldehydes, 5 acids, 15 alcohols, 10 esters, 1 ether, 3 glycosides, 1 amine, 4 alkanes and others compounds (Table-1). A feature of the data in Tables-1 is the amounts of compounds identified by the simultaneous distillation extraction. 5-Hydroxymethyl furfural (5-HMF) was present in solvent extracts (at 19.40 % of total). 5-hydroxymethyl furfural, a common major product of the Maillard reaction, is formed in many foods from reaction of reducing sugars and amino acids[9,10]. Cuzzoni *et al.*[11] reviewed the different available data regarding this compound. In an analytical screening of nearly 500 food samples, relatively high 5-hydroxymethyl furfural concentrations (exceeding 1 g/kg) have been reported for specific products such as dried fruits, caramel products and juice made from dried plums. Formation of 5-hydroxymethyl furfural in foods varies with processing and storage conditions and is especially dependent on temperature and pH. This has been confirmed in model experiments with fruit juices and concentrates as well as in heat-treated milk[12-14]. 5-Hydroxymethyl furfural has also been identified in chewing tobacco, cigarette smoke and wood smoke[15-17]. A contribution of 5-hydroxymethyl furfural to flavour soy sauce has even been described[18,19].

Compounds were detected by Static Headspace connected to GC-MS: The 27 compounds were detected by the static headspace connected to GC-MS (Fig. 2), including: 10 aldehydes, 1 acids, 2 alcohols, 1 esters, 1 ether, 2 alkanes and others compounds (Table-2). The detected compounds were small molecule, because of their low boiling point, it is appropriate to be static headspace detection.

Comparison of simultaneous distillation extraction and static headspace: The compounds collected by the two sampling methods also reflected their boiling point, physical and chemical properties in the sample matrix. For example, Acetaldehyde, *n*-valeraldehyde, hexanal, *n*-heptaldehyde and nonanal were detected in the headspace of the flower samples and not in the simultaneous distillation extraction GC-MS (Tables 1 and 2). Because of the polar nature of these aldehydes their air/water partition coefficients might be lower than their air/wax partition coefficients. Headspace sampling identified, which compounds contributed to the aroma of these flowers. Simultaneous distillation extraction identified the less-volatile or more matrix-soluble flavour or aroma compounds

TABLE-1
COMPOUNDS ARE IDENTIFIED WITH SDE GC-MS

Peak no.	t_R (min)	Compound Name	REV	CAS	Peak area (%)
1	3.484	Allylamine	764	107-11-9	2.8
2	4.354	Furfural	890	1998-1-1	3.33
3	8.075	L-Threonine	686	72-19-5	2.09
4	8.946	Bicyclo[4.2.0]octa-1,3,5-triene-7,8-dione	728	6383-11-5	1.62
5	9.371	Benzyl alcohol	923	100-51-6	0.28
6	9.666	Phenylacetaldehyde	990	122-78-1	0.77
7	10.581	2,4-Dihydro-2,4,5-trimethyl-3H-pyrazol-3-one	779	17826-82-3	1.61
8	10.726	2,5-Furandicarboxaldehyde	931	823-82-5	0.87
9	10.927	α,5-Trimethyl- 5-ethenyltetrahydro-α-*trans*-2-furanmethanol	994	34995-77-2	0.89
10	11.312	Linalool	815	78-70-6	0.1
11	11.447	Lauric aldehyde	932	112-54-9	0.16
12	11.632	2-Phenylethanol	945	1960-12-8	0.69
13	12.612	2,3-Benzopyrrole	772	120-72-9	0.23
14	12.772	1-Ethylsulfinylethene	645	32568-51-7	1.74
15	13.142	*Iso*-pregol	790	89-79-2	0.16
16	13.312	2,2,6-Trimethyl-6-vinyltetrahydropyran-3-ol	838	14049-11-7	0.75
17	13.448	*cis*-5-Ethenyltetrahydro-α, α-5-trimethyl-2-furanmethanol	825	5989-33-3	1.21
18	13.538	3,8-Dioxatricyclo[3.2.1.02,4]octan-6-ol	817	120201-29-8	0.12
19	14.198	Pentadecane	947	629-62-9	0.94
20	14.428	*n*-Heptyl acrylate	721	2499-58-3	0.97
21	14.518	*n*-Hexyl acetate	671	142-92-7	0.53
22	15.023	5-Hydroxymethyl furfural	768	67-47-0	19.4
23	15.348	Ethyl phenylacetate	889	101-97-3	0.38
24	15.443	Methyl phenethyl ether	865	3558-60-9	0.75
25	15.843	D(-)-Tartaric acid diethyl ester	770	13811-71-7	9.27
26	16.049	α-ethylidene-Benzeneacetaldehyde	973	4411-89-6	0.29
27	16.359	Hexyl *iso*-butyrate	749	2349-7-7	0.66
28	16.949	Pentadecane	960	629-62-9	0.1
29	17.134	5-Ethenyltetrahydro-α, α, 5-trimethyl-, *trans*-2-furanmethanol	787	34995-77-2	0.99
30	17.244	*n*-Nonanol	759	143-08-8	0.4
31	17.889	1-Octen-3-olbutyrate	773	16491-54-6	0.69
32	18.014	(Z)-1,1-Dimethoxy-9-octadecene	653	15677-71-1	0.64
33	18.134	Oleic aldehyde dimethyl acetal	702	10032-13-0	0.14
34	18.329	Octadecane	882	593-45-3	0.08
35	18.545	*p*-Hydroxybenzaldehyde	990	123-08-0	13.75
36	19.235	α,5-Trimethyl-5-ethenyltetrahydro-α *trans*-2-furanmethanol	879	34995-77-2	0.3
37	19.35	Tetradecanol	961	112-72-1	0.09
38	19.46	Capric acid	879	334-48-5	0.17
39	19.855	Benzeneethanol	917	5381-92-0	0.23
40	21.351	4-Hydroxybenzyl cyanide	956	14191-95-8	4.66
41	21.726	3-Phenylpropene-3-ol	716	4393-06-0	1.1
42	21.881	Capric acid	628	334-48-5	0.77
43	22.061	4,6-di-*tert*-butyl-*o*-cresol	950	616-55-7	0.69
44	22.126	α-D-glucopyranoside	797	97-30-3	0.04
45	22.821	Triethyl phosphate	831	78-40-0	0.85
46	24.102	Glycine	799	6556-12-3	0.16
47	24.722	Ethyl 2,3-epoxybutyrate	812	19780-35-9	0.41
48	24.892	Methyl β-D-Glucopyranoside	837	709-50-2	0.94
49	25.162	Methyl β-L-arabinopyranoside	856	1825-00-9	2.3
50	25.602	Phenyl-1,2,3,4-thiatriazol,5-Phenyl-1,2,3,4-thiatriazole	631	34733-85-2	1.18
51	26.688	C-Methylinositol	751	472-95-7	2.83
52	26.863	1-C-methyl-, scyllo-inositol	800	564-92-1	1.81
53	27.183	2-C-Methyl-myo-inositol	887	472-96-8	3.68
54	27.478	Ethyl 3,4-dihydroxybenzoate	966	3943-89-3	1.25
55	31.605	*Iso*-vegetable alcohol	901	505-32-8	0.69
56	31.855	Palmitic acid	966	1957-10-3	0.86
57	32.525	Ethyl ester	922	628-97-7	0.95
58	34.626	Phytol	893	150-86-7	0.76
59	35.531	(E)-4-Nonen-1-ol	960	16695-34-4	0.83
60	36.146	Ethyl stearate	918	111-61-5	0.19
61	38.332	(1-Methyl-2-phenylpropyl)benzene	912	4613-11-0	1.35
62	38.462	Heptadecanoic acid	668	506-12-7	0.18
63	44.515	Myristyl alcohol	988	6624-79-9	1.32

and possible biosynthetic precursors of some of the volatile compounds.

Conclusion

About 63 different compounds were identified including acids, alcohols, aldehydes, ketones, esters, hydrocarbon and

other a few compounds, which can offer some reference for application of the Macadamia flower in large scale. HS GC-MS analysis of volatile compounds is a widely used technique. However, for many analyses, the HS GC-MS method lacked the sensitivity needed to perform adequately. Simultaneous distillation extraction had the ability to perform these analyses
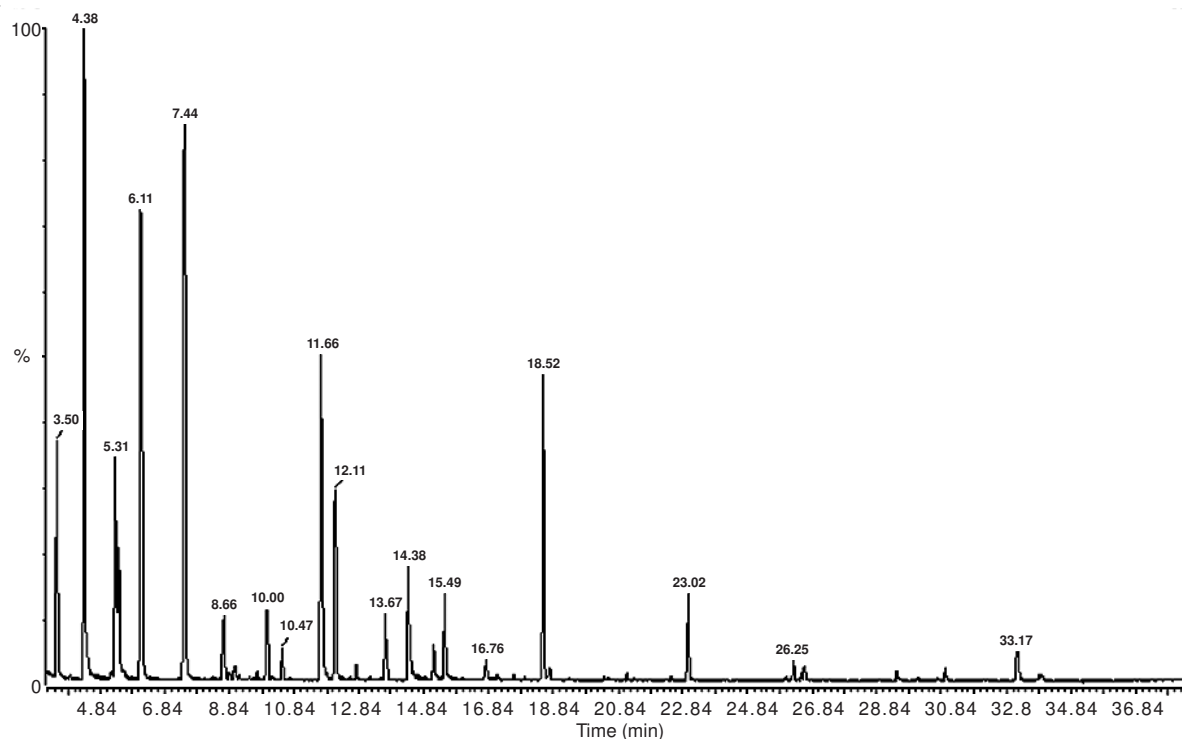

Fig. 2. GC-MS Chromatograms of volatile compounds by HS GC-MS

TABLE-2
COMPOUNDS ARE IDENTIFIED WITH HS GC-MS

Peak no.	t_R (min)	Compound name	REV	CAS	Peak area (%)
1	3.505	Acetaldehyde	999	75-07-0	5.86
2	4.355	Nitrosomethane	933	865-40-7	16.52
3	5.186	*n*-Pentane	809	109-66-0	0.14
4	5.311	*n*-Butane	912	106-97-8	5.82
5	5.416	Acetic acid	633	372-09-8	3.37
6	6.106	Dimethyl sulfide	997	75-18-3	12.45
7	7.442	*Iso*-butylaldehyde	988	78-84-2	15.86
8	8.657	Vinyl acetate	944	108-05-4	1.82
9	8.997	2-Butanone	860	78-93-3	0.34
10	10.003	Chloroform	979	67-66-3	1.86
11	10.468	*iso*-Butyronitrile	968	78-82-0	0.85
12	11.663	*iso*-Valeraldehyde	990	590-86-3	8.85
13	12.114	Methyl ethyl acetaldehyde	980	96-17-3	5.17
14	12.754	(Z)-2-penten-1-o	903	1576-95-0	0.42
15	13.674	*n*-Valeraldehyde	975	110-62-3	1.76
16	14.364	3-Hydroxy-2-butanone	939	513-86-0	3.18
17	15.155	2,3-Dimethylbutanedinitrile	942	16411-13-5	0.98
18	15.495	3-methyl-Butanenitrile	965	625-28-5	2.09
19	16.755	1-Pentanol	835	71-41-0	0.54
20	18.521	Hexanal	980	66-25-1	7.37
21	18.736	*Trans*-3-Octene	883	14919-01-8	0.25
22	23.023	*n*-Heptaldehyde	982	111-71-7	2.11
23	26.255	Benzaldehyde	974	100-52-7	0.51
24	26.61	2-Amylfuran	935	3777-69-3	0.28
25	29.476	Phenylacetaldehyde	914	122-78-1	0.24
26	30.961	Nonanal	924	124-19-6	0.30
27	33.172	Benzyl cyanide	971	140-29-4	1.05

where HS GC-MS fell short. Comparison of simultaneous distillation extraction and HS showed that simultaneous distillation extraction determinations of flavour compounds were, on average, more sensitive under the conditions employed in this study. The increased sensitivity allowed fast, accurate determinations of flavour compounds and easy performance of analyses. Consequently, simultaneous distillation extraction was suitable for simple, rapid while HS GC-MS was used for proper quantitative analysis.

REFERENCES

1. G.H. Li and H. Yue, *Chin. J. Trop. Crops*, **30**, 730 (2009).
2. H. Yue Hai and L.L. Chen, *J. Agric. Sci.*, **21**, 1395 (2008).
3. H. Koaze, D.S. Ndaka, P.N. Karanja, K. Ishibashi and N. Baba, *Food Sci. Technol. Res.*, **8**, 32 (2002).
4. C.G. Cavaletto, in eds.: S. Nagy and P.E. Shaw, Macadamia Nuts, Tropical and Subtropical Fruits: Composition, Properties and Uses, Westport, CT.: AVI Publishing, Inc., pp. 542-561 (1980).
5. C.G. Cavaletto, in ed.: H.T. Chan, Macadamia nuts, Handbook of Tropical Foods. New York: Marcel Dekker, Inc., pp. 361-397 (1983).
6. C. Cavaletto, A. Dela Cruz, E. Ross, and H.Y. Yamamoto, *Food Technol.*, **20**, 108 (1966).
7. J.D. Curb, G. Wergowske, J.C. Dobbs, R.D. Abbott and B.J. Huang, *Arch. Int. Med.*, **160**, 1154 (2000).
8. M.L. Garg, R.J. Blake and R. Wills, *J. Nut.*, **133**, 1060 (2003).
9. J. Yang and R.H. Liu, *Food Sci. Technol.*, **42**, 1 (2009).
10. M.J. Antal and W.S.L. Mok, *Carbohyd. Res.*, **199**, 109 (1990).
11. M.T. Cuzzoni, G. Stoppini, G. Gazzani and P. Mazza, *Food Chem. Toxicol.*, **26**, 815 (1988).
12. C. Janzowski, V. Glaab, E. Samimi, J. Schlatter, G. Eisenbrand, *Food Chem. Toxicol.*, **38**, 801 (2000).
13. D.B. Gomis, M.D.G. Alvarez, L.S. Naredo and J.J.M. Alonso, *Chromatographia*, **32**, 45 (1991).
14. A. Kern, *Ber. Wissen.-Techn. Komm.*, **5**, 203 (1964).
15. F.J. Morales, C. Romero and S. Jimenez-Pèrez, *Chromatographia,* **33**, 45 (1992).
16. I.T. Baldwin, L. Staszak-Kozinski and R. Davidson, *J. Chem. Ecol.*, **20**, 2345 (1994).
17. D.K. Black, *Chem. Ind.*, **32**, 1380 (1966).
18. C.C. Chou and S.S.Q. Hee, *J. Agric. Food Chem.*, **42**, 2225 (1994).
19. K. Kiahara, *Nippon-Shoyu-Kenyusho-Zasshi*, **21**, 127 (1995).