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Impacts of Extraction Methods on Volatile Constituents of Longan Flower

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ABSTRACT

This study investigated the impacts of three extraction methods on the composition of the flavor isolates of fresh longan (*Euphoria longana* Lam.) flower. These extraction methods included Lickens-Nickerson (L-N) steam distillation solvent extraction, direct solvent extraction (DSE), and headspace purge and trap (HS) methods. Volatile compounds were then analyzed qualitatively and quantitatively by GC and GC-MS. A total of 51 volatile compounds were identified from fresh longan flower in this study. The major volatile compounds were trans-caryophyllene, linalool oxide and α -humulene by L-N, linalool oxide, 2-phenylethanol and epoxylinolool by DSE, and trans-ocimene, linalool oxide, and linalool by HS, respectively.

Key words: aroma, Lickens-Nickerson, direct solvent extraction, headspace purge and trap, longan flower

INTRODUCTION

Longan (*Euphoria longana* Lam.) fruit is one of the most plentiful summer fruits in Taiwan and is consumed throughout Asia. This fruit is a favorite among connoisseurs and a traditional Chinese blood tonic. In Chinese medicine the flesh of longan is used as a stomachic, febrifuge, vermifuge, and also as an antidote for poison⁽¹⁾. In Asia, longan fruit is sold on the fresh market, but can also be canned or dried.

The longan flower is small, inconspicuous, and yellow brown in color. In Taiwan, longan is flowering during the period from February to May, with the fruit maturing from July to September. In Chinese herb markets longan flower is sold as traditional medicine⁽¹⁾. In addition, longan flower is a good source of honey. Its aroma is complex. The volatile components of longan flower, recovered by various aroma extraction and concentration methods are different. Therefore, it is important to choose a suitable extraction procedure that may qualitatively and quantitatively extract the original aroma of longan flowers. Blanch *et al.*⁽²⁾ stated that several methods have been developed to analyze the volatile components. Each of them presents some advantages and disadvantages. Usually, it is necessary to combine different methods for the complete extraction of all the

volatile compounds contained in a sample⁽³⁾.

The headspace technique is becoming more and more popular. In this method, the volatile compounds are swept along with a carrier gas and then condensed in a cold trap⁽⁴⁻⁷⁾. In this study, the composition of the volatile components in the flavor isolates of fresh longan flower by the simultaneous hydrodistillation-extraction (Lickens-Nickerson apparatus) method, direct solvent extraction (DSE) method, and headspace purge and trap (HS) method were compared.

MATERIALS AND METHODS

I. Materials

Fresh longan flowers were obtained from the same tree in a farm at Sutou near Changhua County, Taiwan. HPLC grade dichloromethane was used (Sigma, USA). The gases used were: nitrogen as the carrier gas for the corresponding extraction procedure, to obtain an inert atmosphere when necessary and as the make-up gas for the flame ionization detector (FID), helium as the carrier gas for the GC-mass spectrometry (GC-MS), and air and hydrogen for the FID. All gases were from Terng Shyang Gas Co. (Changhua, Taiwan). Chemical standards were purchased from Sigma (St. Louis, MO, USA).

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II. Methods

(I) Likens-Nickerson Procedure (LN)

The method proposed by Pino *et al.*⁽⁸⁾ was employed with minor modification. Longan flowers (450 g) were placed in a 5-L round-bottom flask with distilled water (1500 mL), and then simultaneously distilled and extracted for 2 hr in a Likens-Nickerson apparatus with 25 mL of redistilled dichloromethane. The volatile concentrate was dried over anhydrous sodium sulfate and concentrated to 0.6 mL on a Kuderna-Danish evaporator (Seng Long Co., Taichung, Taiwan) at 40°C for 1 hr and then to 0.2 mL with a gentle nitrogen stream.

(II) Direct Solvent Extraction

The procedures for DSE were modified from Lee *et al.*⁽⁹⁾. One hundred and fifty grams of longan flower was put in a 1-L flask with 500 mL of petroleum ether, and stirred at 500 rpm at 25°C for 24 hr. After filtering and removing the flower, the solvent was concentrated to 0.6 mL using a Kuderna-Danish evaporator, added with 10 mL of anhydrous ethanol to remove the wax and pigments from the longan flower, dried over anhydrous sodium sulfate, and then concentrated to 0.2 mL with a gentle nitrogen stream.

(III) Headspace Purge and Trap

This method was modified from Wartelle *et al.*⁽¹⁰⁾. Three hundred grams of longan flower were put in a 1-L flask (in 50°C water bath) and purged using an S.I.S. Model TD-3 purge-and-trap system (Scientific Instrument Services, Ringoes, NJ, USA). Each sample was purged with dry nitrogen at a rate of 20 mL/min for 4 hr onto a stainless steel thermal desorption tube (11 cm × 3 mm I.D.) that had been packed with adsorbent (25 mg of Tenax TA and 25 mg of Carbotrap) and stoppered at both ends with glass wool. After the adsorption was completed, the desorption tube was thermal-desorbed on an S.I.S. TD-3 desorption unit directly connected to the injector of the GC-MS. Thermal desorption was conducted at 250°C for 10 min with helium at a flow-rate of 20 mL/min. The GC column was immersed in liquid nitrogen to avoid the tailing of volatile components in gas chromatography.

(IV) Chromatography

A gas chromatograph (Hewlett-Packard 5890, Avondale, PA, USA) equipped with an FID was used for quantification of the volatile components extracted from the samples according to three techniques described above. One microliter of the sample extract was injected in the splitless mode using a 60 m DB-1 (J&W Scientific, Folsom, CA, USA) capillary column of 0.32 mm I.D. and 1 µm film thickness. The injector temperature was

kept at 250°C and the FID at 280°C. The carrier gas was nitrogen at a flow rate of 3 mL/min. The oven temperature was elevated from 40°C to 240°C in a gradient of 2.5°C/min and held at 240°C for 60 min. An internal standard (4-heptanone, 5.5 mg) was used for the quantitative analysis of LN and DSE extraction methods. However, no internal standard was used in HS. The composition of the aroma compounds from longan flowers in HS was calculated by the peak area % for each volatile compound (peak area of each compound / total peak area of all compounds × 100%). Isolated peaks were identified using mass spectral data, RI, and the odor description by gas chromatograph olfactometry. A Hewlett-Packard 5973 mass detector fitted with a 5890 GC was used. The ionization of the samples was achieved at 70 eV. A Hewlett-Packard Chemstation equipped with Wiley 275 library was used for the components identification⁽⁶⁾.

RESULTS AND DISCUSSION

The volatile constituents of fresh longan flowers extracted by three methods are shown in Table 1. A total of 51 constituents were identified in fresh longan flowers from three extraction methods: 25 by LN, 43 by DSE, 20 by HS. Classifications by function-groups, such as ketone, hydrocarbon, aldehyde, alcohol, ester and acid, of volatile compounds in fresh longan flower are also shown in Table 1. The yield of volatile compound from DSE (total 502 ppm) is higher than that from LN (140 ppm). This might be due to the heat conduction in the LN method since the high temperature leads to a big loss of aromatic components. Concerning the volatile compounds from the extraction methods, alcohols comprise the most abundant volatiles in both DSE and HS, 61% and 57%, respectively. The major volatile components of fresh longan flowers by DSE were 2-phenylethanol (26%; floral, honey-like odor), epoxylinool isomers 1 and 2 (total 17%; honey, sweet odor) and linalool oxide isomers 1 and 2 (total 12%; floral, woody odor). Transocimene (19%; fresh longan-like odor) and linalool (20%; floral odor) were the major constituents found by HS. Hydrocarbons comprised the largest group of volatiles in LN (50%). The major constituents in the LN isolate were found to be trans-caryophyllene (27%; honey-like, sweet, woody odor), linalool oxide isomers 1 and 2 (total 27%; floral, woody odor) and α -humulene (14%; woody odor). Matich *et al.*⁽¹¹⁾ mentioned that linalool oxide may be an artifact of the steam distillation process. Linalool oxide was found to be a major constituent in both LN and DSE methods. Only a tiny amount (<1%) of linalool was found via LN. Linalool was not found in DSE, while it was a major component in HS (up to 20%, Table 1). In addition, higher amounts of alcohol and ester, including epoxylinool, 2-phenylethanol and ethyl 9-octadecenoate (17%, 26%, and 4%, respectively), were obtained by DSE than those by LN. However, the contents of hydro-

Table 1. Comparisons of the yield of volatile compounds isolated from flowers of *Longan* by three extraction method.

No. ^a	Compound ^b	CAS No.	RI ^c	Odour description ^d	LN ^e		DSE ^e		HS ^e	
					Conc. (ppm)	Comp. (%)	Conc. (ppm)	Comp. (%)	Conc. (ppm)	Comp. (%)
Ketone										
2	3-buten-2-one	78-94-4	621	sweet	0.15	0.11	0.66	0.13	n.d. ^f	n.d.
6	4-methyl-2-pentanone	108-10-1	732	floral, honey-like	2.01	1.47	10.79	2.15	n.d.	n.d.
33	β -damascenone	23726-93-4	1360	sweet, floral, honey-like	0.53	0.39	n.d.	n.d.	n.d.	n.d.
Hydrocarbon										
14	α -terpinene	99-86-5	1013	citrus-lemomy	0.2	0.15	n.d.	n.d.	n.d.	n.d.
15	limonene	138-86-3	1031	citrus, fruity, lemony	0.73	0.54	n.d.	n.d.	1.57	1.57
16	trans-ocimene	502-99-8	1052	fresh Longan-like	1.02	0.75	2.42	0.48	18.9	18.9
23	p-1,3,8-menthatriene	21195-59-5	1114	roasty	0.3	0.22	n.d.	n.d.	2.42	2.42
28	2-methyl naphthalene	91-57-6	1273	sweet, floral, woody	n.d.	n.d.	7.01	1.4	n.d.	n.d.
30	1-methyl naphthalene	90-12-0	1278	woody, floral	n.d.	n.d.	7.53	1.5	n.d.	n.d.
32	δ -elemene	20307-84-0	1333	sweet, laver-like	2.8	2.05	10.66	2.12	n.d.	n.d.
34	α -copaene	3856-25-5	1374	floral, honey	4.46	3.27	6.76	1.35	n.d.	n.d.
35	β -cubebene	13744-15-5	1385	radish-like	n.d.	n.d.	5.89	1.17	n.d.	n.d.
36	β -elemene	515-13-9	1388	sweet	2.72	1.99	7.62	1.52	n.d.	n.d.
37	junipene	475-20-7	1401	floral	n.d.	n.d.	0.94	0.19	n.d.	n.d.
38	trans-caryophyllene	87-44-5	1415	honey-like, sweet, woody	35.8	26.24	37.67	7.5	6.54	6.54
39	α -humulene	6573-98-6	1448	woody	18.65	13.67	14.55	2.9	2.24	2.24
40	β -bisabolene	495-61-4	1495	woody	n.d.	n.d.	0.52	0.1	n.d.	n.d.
41	δ -cadinene	483-76-1	1510	dry-woody	n.d.	n.d.	5.52	1.1	0.55	0.55
Aldehyde										
7	hexanal	66-25-1	776	grassy	1.53	1.12	3.55	0.71	1.06	1.06
11	heptanal	111-71-7	878	oil-fatty, rancid odor	7.15	5.24	8.15	1.62	4.69	4.69
12	benzaldehyde	100-52-7	929	fruity, sweet	1.41	1.03	4.25	0.85	1.14	1.14
20	nonanal	124-19-6	1080	fatty	n.d.	n.d.	16.55	3.3	n.d.	n.d.
Alcohol										

Table 1. continued

No. ^a	Compound ^b	CAS No.	RI ^c	Odour description ^d	LN ^e		DSE ^e		HS ^e	
					Conc. (ppm)	Comp. (%)	Conc. (ppm)	Comp. (%)	Conc. (%)	Comp. (%)
3	2-methyl-3-buten-2-ol	115-18-4	640	sweet	3.43	2.51	n.d.	n.d.	n.d.	n.d.
4	2-pentanol	6032-29-7	676	fruity, alcohol-like	n.d.	n.d.	2.35	0.47	n.d.	n.d.
5	2-methyl-1-butanol	137-32-6	718	sweet, honey-like	n.d.	n.d.	n.d.	n.d.	n.d.	2.73
8	3-methyl-2-buten-1-ol	556-82-1	779	honey-like	1.76	1.29	1.78	0.35	n.d.	n.d.
10	furfuryl alcohol	98-00-0	830	sweet, caramellic	n.d.	n.d.	2.12	0.42	n.d.	n.d.
13	phenylmethanol	100-51-6	1006	floral, sweet	n.d.	n.d.	1.5	0.3	2.56	n.d.
18	linalool oxide (isomer 1)	5989-33-3	1070	floral, woody	9.22	6.76	13.86	2.76	12.27	n.d.
19	linalool oxide (isomer 2)	5989-33-3	1075	floral, woody	26.29	19.27	46.39	9.24	10.89	n.d.
21	linalool	78-70-6	1080	floral	0.42	0.31	n.d.	n.d.	20.19	n.d.
22	2-phenylethanol	60-12-8	1090	floral, honey-like	2.82	2.07	129.67	25.82	5.63	n.d.
25	epoxylinolol (isomer 1)	14049-11-7	1190	honey, sweet	6.42	4.71	47.23	9.4	0.97	n.d.
26	epoxylinolol (isomer 2)	14049-11-7	1195	honey, sweet	n.d.	n.d.	36.96	7.36	n.d.	n.d.
42	spathulenol	77171-55-2	1550	honey	n.d.	n.d.	5.03	1	n.d.	n.d.
43	viridiflorol	552-02-3	1578	woody, floral	n.d.	n.d.	10.13	2.02	n.d.	n.d.
44	t-murolol	19912-62-0	1641	honey	n.d.	n.d.	4.91	0.98	n.d.	n.d.
45	torreyol	19435-97-3	1650	honey	n.d.	n.d.	2.18	0.43	n.d.	n.d.
46	farnesol	4602-84-0	1745	honey, sweet, floral	2.64	1.94	1.18	0.23	1.89	n.d.
Ester										
9	butyl acetate	123-86-4	798	fermented, fruity	0.1	0.07	4.87	0.97	0.29	n.d.
27	2-phenylethyl formate	103-45-7	1225	mushroom-like	n.d.	n.d.	2	0.4	n.d.	n.d.
31	methyl 2-aminobenzoate	134-20-3	1305	honey, sweet	n.d.	n.d.	1.88	0.37	n.d.	n.d.
47	ethyl tetradecanoate	124-06-1	1778	mild oil-etheral odor	n.d.	n.d.	0.82	0.16	n.d.	n.d.
48	ethyl pentadecanoate	41114-00-5	1868	honey-like, sweet	n.d.	n.d.	1.52	0.3	n.d.	n.d.
49	methyl hexadecanoate	112-39-0	1911	floral	n.d.	n.d.	5.41	1.08	n.d.	n.d.
50	ethyl hexadecanoate	628-97-7	1980	fatty, waxy	0.55	0.40	2.89	0.58	n.d.	n.d.
51	ethyl 9-octadecenoate	111-62-6	2142	honey, sweet, oily	n.d.	n.d.	18.51	3.69	n.d.	n.d.

Table 1. continued

No. ^a	Compound ^b	CAS No.	RI ^c	Odour description ^d	LN ^e		DSE ^e		HS ^e	
					Conc. (ppm)	Comp. (%)	Conc. (ppm)	Comp. (%)	Conc. (ppm)	Comp. (%)
1	acetic acid	64-19-7	602	sour	n.d.	n.d.	2.23	0.44	2.05	2.05
17	heptanoic acid	111-14-8	1067	sour-sweet-like, fatty odor	n.d.	n.d.	1.33	0.26	n.d.	n.d.
24	benzoic acid	65-85-0	1143	urine-like	n.d.	n.d.	1.05	0.21	1.42	1.42
29	nonanoic acid	112-05-0	1275	fatty-waxy	3.32	2.43	3.39	0.67	n.d.	n.d.
	Total				140	100	502	100	100	100

^a The numbering refers to elution order.

^b The compound was identified by GC-MS, RI, and odour description.

^c Retention index relative to C₅-C₂₅ n-alkanes on DB-1 capillary column.

^d Odour description by using Gas Chromatograph-Olfactometry (GCO).

^e The values (ppm) represent averages of three determinations. L-N: Likens-Nickerson steam distillation solvent extraction method; DSE: direct solvent extraction method; and HS: head space purge and trap method.

^f Not detected.

carbon and aldehyde compounds such as trans-caryophyllene, α -humulene and heptanal in LN were higher than those via DSE. In HS extraction, the compounds extracted from fresh longan flowers are mainly linalool derivatives including linalool and linalool oxide that are presumably metabolites of phenylalanine and tyrosine. Similarly, Matich *et al.*⁽¹¹⁾ in his investigation on the hardy kiwi (*Actinidia arguta*) flower mentioned that only small amount of linalool was found in solvent extracts, while linalool was a major component in the headspace. They also mentioned that headspace sampling was helpful to identify the aroma-contributing compounds, while solvent extraction was helpful to identify the less-volatile flavor and possible biosynthetic precursors of some of the volatile compounds.

I. Direct Solvent Extraction (DSE)

The DSE is a simple and low cost method for the aroma extraction of longan flower. However, it has disadvantages including the use of organic solvent and being time-consuming. Matich *et al.*⁽¹¹⁾ also mentioned that the solvent extracts display a bias towards higher molecular weight, non-volatile and wax-soluble fatty acid esters and hydrocarbons (C15-C20). In our studies the major volatile components of fresh longan flower obtained by DSE are esters, hydrocarbons and alcohols. The major aromatic compounds are: linalool oxide, 2-phenylethanol, epoxy-linalool, trans-caryophyllene, nonanal and ethyl 9-octadecenoate. These results indicated that the major volatile compounds are of higher molecular weight, wax soluble fatty acid esters and hydrocarbons. This is comparable to the findings of Matich *et al.*⁽¹¹⁾. Conversely, linalool is an important aroma compound in longan flower⁽¹²⁾, but it was not detected by DSE. These results revealed that we are unable to obtain all of the aromatic compounds of fresh longan flower by DSE and require other extraction methods to analyze the original aromatic components of fresh longan flowers.

II. Likens-Nickerson (LN) Extraction

The LN extraction method has been employed in many aroma studies^(8,13-16). The simultaneous hydro-distillation solvent-extraction yielded clear and colorless oil from fresh longan flower. However, there are several disadvantages to this method, including the loss or degradation of the existing components and the formation of some new aromatic compounds^(6,7). As shown in Table 1, this method has good extraction efficiency for all the components with different volatilities. The major volatile components of fresh longan flower by LN are hydrocarbons and alcohols. The main aroma compounds include trans-caryophyllene, linalool oxide and α -humulene. In particular, trans-caryophyllene existed in all of the longan products (including flower, fresh fruit, dried-fruit and honey) and was analyzed in high concentrations^(12,17),

indicating that it contributes a lot to the flavor of longan products. However, various hydrocarbon compounds in low concentration were found by LN; the result might be due to the thermal degradation of aromatic compounds at high temperature leading to the formation of some new volatile compounds.

III. Headspace (HS) Purge and Trap

This is a method of choice for the quantification of the volatile compounds in fresh longan flower because the sample preparation and the procedure of analysis are simple. Moreover, the cost is low. Since no organic solvent is used, the concern of solvent toxicity is nil.

As illustrated in Table 1, the major volatile compounds of fresh longan flower by HS are alcohols and hydrocarbons, such as linalool oxide, linalool and transocimene. The relative contents of linalool and transocimene are higher by HS than those determined by DSE and LN. These volatile compounds are important components of fresh longan flower. These results might be due to the better efficiency of HS purge and trap method in the extraction of highly volatile components. Furthermore, the volatile compounds, trans-caryophyllene and linalool oxide, are also found by LN, DSE and HS. The above results showed that LN, DSE and HS are all suitable for extracting trans-caryophyllene and linalool oxide. Our previous investigation revealed that the volatile linalool oxide was an important flavor compound in characterizing the aroma of fresh longan flower⁽¹²⁾.

CONCLUSIONS

From the results of this study, we concluded that different volatile compositions could be obtained by a variety of sampling and extractions. The LN extraction had to via high temperature distillation process, which might cause the loss and degradation of some volatile compounds and produce the new volatile compounds absent in fresh longan flower. DSE identified the less-volatile or more matrix-soluble flavors or aroma compounds and possible biosynthetic precursors of some volatile compounds. However, HS sampling might be suitable in identifying the compounds that contributed to the aroma of fresh longan flower. Therefore, we have to apply various extraction methods to recover the original aroma of fresh longan flower. Furthermore, it was found in this study that transocimene (fresh longan-like odor), linalool (floral odor), linalool oxide (floral, woody odor) and benzene ethanol (floral, honey-like odor) were the most contributing volatile components in fresh longan flower.

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