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Free and glycosidically bound aroma compounds in lychee (*Litchi chinensis* Sonn.)

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Abstract

Free and glycosidically-bound volatile compounds were isolated and separated from fresh clear lychee juice using an Amberlite XAD-2 column. The volatile compounds from the bound fraction were released by hydrolysis with almond β-glucosidase. Volatile components of both free and bound fractions were then determined by GC and GC–MS, and they showed similar volatile profiles. Totally, 25 compounds were identified in both fractions, including one ester, 14 alcohols, two aldehydes, four acids, two ketones and two terpenes. In the free fraction (2907 mg kg^{-1}), the major volatile compounds found were acetoin (30.1%), geraniol (15.6%), 3-methyl-2-buten-1-ol (15.3%), octanoic acid (7.28%), 2-phenylethanol (4.91%), *cis*-ocimene (4.32%), and butyric acid (3.40%). In the bound fraction (1576 mg kg^{-1}), the latent major volatile compounds found were geraniol (73.7%) and geranial (7.95%). In aroma evaluation, the free volatile fraction showed a fresh-fruity, lychee-like aroma whereas the bound fraction was odourless. The characteristic lychee-like aroma was noted in the bound fraction after enzymatic hydrolysis. On combination of the free and hydrolysed bound fractions, a strongly fruity, lychee-like aroma was perceived.
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1. Introduction

Odourless glycosides are able to release volatile compounds (aglycones) by enzymatic or acid hydrolysis and can be considered as aroma precursors in plant materials, especially fruits and vegetables. Glycosides with a β-glycosidic linkage are more common than those with an α-glycosidic linkage (Nirmala, Nenon, & Narayanan, 1992). Therefore, the β-glucosidase from bitter almonds is commonly used for hydrolysis of most β-glycosides to enhance the aroma. Free and glycosidically-bound volatile components have been reported in fruits such as grape (Gunata, Bayonove, Baumes, & Cordonnier, 1985; Strauss, Gooley, Wilson, & Williams, 1987; Voirin, Baumes, Gunata, Bitteur, Bayonove, & Tapiero, 1992; Voirin, Baumes, Sapis, & Bayonove, 1992), passion fruits (Engel & Tressl, 1983), papaya (Heidlas, Lehr, Idstein, & Schreier, 1984; Schwab, Mahr, & Schreier, 1989), raspberry (Pabst, Baron, Etievant, & Schreier, 1991), mango (Sakho, Chassagne, & Crouzet, 1997), pineapple (Wu, Kuo, Hartman, Rosen, & Ho, 1991; Ho, Sheen, Kuo, Hartman, & Rosen, 1990), peach (Ho et al., 1990), tomatoes (Krammer et al., 1994), and strawberry (Roscher, Herderich, Steffen, Schreier, & Schwab, 1996). The aromatic components of fruits are found to be present, either in the free form, or bound to sugar in the form of glycosides, which could be considered as a source of latent and potential aroma.

Lychee (*Litchi chinensis* Sonn.) fruit, with bright red or dark red skin and slightly transparent, white pulp, is one of the most popular fruits of summer in Taiwan. Reports on volatile components in lychee were limited (Kuo, Chen, & Wu, 1984, Ong & Acree, 1998; Wu, 1970). Kuo et al. (1984) used the headspace adsorption method and silica gel fractionation to analyse volatile components in fresh lychee. Ong and Acree (1998) used both Freon 113 and ethyl acetate to extract lychee volatiles and characterised the odour-active compounds using gas chromatography/olfactory (GC/O) and gas chromatography–mass spectrometry (GC–MS). However, there

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is no report on glycosidically-bound aroma compounds. Therefore, our objective was to isolate and separate free and glycosidically-bound aroma compounds in lychee juice using an Amberlite XAD-2 column. The volatile compounds from the bound fraction were released by hydrolysis with almond β-glucosidase. Volatile components of both free and bound fractions were then determined by GC and GC–MS.

2. Materials and methods

2.1. Fruit material

Fresh lychee grown in Taichung, Taiwan, was purchased from the local market.

2.2. Reagents

The GR grade solvents (*n*-pentane and diethyl ether) were obtained from Merck Co. (Darmstadt, Germany) and were redistilled before use according to the method of Gunata et al. (1985). Methanol and acetonitrile were of HPLC grade, obtained from BDH Laboratory Supplies (Poole, England). Standard *n*-paraffins (C_6–C_{25}) were purchased from Alltech Associates (Deerfield, IL). Almond β-glucosidase was obtained from Sigma Chemical Co. (St. Louis, MO). Pure standards were purchased from Aldrich Chemicals Co. (Milwaukee, WI), Oxford Chemicals Co. (Hartlepool, England) and Bedoukian Research Inc. (Danbury, CT).

2.3. Fractionation of free and bound fractions of the aroma

Fresh clear lychee juice was prepared from skinned, deseeded whole fruit pulp. Lychee flesh (1.0 kg) was blended with 500 ml of saturated NaCl solution at 4 °C in a Waring blender for 30 s. The clear juice was obtained by centrifugation at 3000×*g* and 4 °C for 20 min, and filtration through a bed of Celite. The lychee residue was twice blended each with 100 ml of saturated NaCl solution at 4 °C for 30 s and centrifuged and filtered as described above. The combined clear juice was then subjected to adsorption chromatography in a glass column (30×2 cm) containing solvent-washed Amberlite XAD-2 as adsorbent. Subsequently, the column was rinsed with 300 ml of deionised water to eliminate sugars, acids and other water-soluble compounds.

The free fraction of the aroma adsorbed on the column was eluted with 200 ml of *n*-pentane/diethyl ether solvent (P/E, 1/1, v/v) at a flow rate of 1–1.5 ml min^{-1}. After 0.5 ml of 100 ppm of cyclohexyl *n*-butyrate in P/E solvent was added as internal standard, the eluted P/E solvent was dried over anhydrous sodium sulfate (Merck) and then concentrated to a final volume of 100 μl using a Vigreux column at 40 °C. The concentrated extract thus obtained was the free fraction of the lychee aroma and directly injected for GC and GC–MS analyses.

After the free fraction was eluted, the bound fraction of the aroma adsorbed on the column was eluted with 200 ml of methanol. The eluted solvent was then dried over anhydrous sodium sulfate, filtered and concentrated to a final volume of 1 ml under vacuum at 40 °C. Subsequently, the glycosidically-bound fraction was dissolved in 100 ml of 0.1 M citrate-phosphate buffer solution (pH 5.0) and the solution was washed twice, each with 40 ml P/E, to eliminate possible traces of the free fraction. The separated P/E solvent was dried over anhydrous sodium sulfate, combined with the eluted P/E solvent and concentrated as described above.

Subsequently, 2 ml of the enzyme solution (almond β-glucosidase, 25 mg/ml in 0.1 M citrate-phosphate buffer, pH 5.0) was added to the washed solution containing the bound fraction and the mixture was vortexed. The flask containing the mixture was sealed and placed in a water-bath at 37 °C for 72 h. The liberated aglycones were then extracted three times, each with 30 ml of P/E solvent. After adding 0.5 ml of 100 ppm cyclohexyl *n*-butyrate in P/E solvent as internal standard, the solution was then dried over anhydrous sodium sulfate, filtered and concentrated to give a final volume of 100 μl using a Vigreux column at 40 °C. The concentrated extract thus obtained was the bound fraction of the lychee aroma and directly injected for GC and GC–MS analyses. The experiment was conducted in triplicate for both free and bound fractions.

2.4. Gas chromatography and gas chromatography-mass spectrometry

A Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a fused silica capillary column (60 m×0.25 mm i.d.; 0.25 μm film thickness, DB-Wax; J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used to analyse the volatile compounds in both free and bound fractions. The operating conditions were as follows: injection temperature, 250 °C; detector temperature, 250 °C; nitrogen carrier flow rate, 1.2 ml min^{-1}; temperature programme, 40–210 °C at 2 °C min^{-1} and held at 210 °C for 40 min. A split ratio of 80:1 was used. Linear retention indices were calculated against *n*-paraffin standards (C_6–C_{25}, Alltech) according to Schomberg and Dielmann (1973).

GC–MS analysis was accomplished using a Hewlett-Packard 5890 Series II gas chromatograph coupled directly to a Hewlett-Packard 5972A MSD mass spectrometer. Identical column and temperature programmes were carried out as those in GC analysis. The temperature of transfer line was 265 °C. The helium carrier flow rate was 1.0 ml min^{-1}. Splitless injections

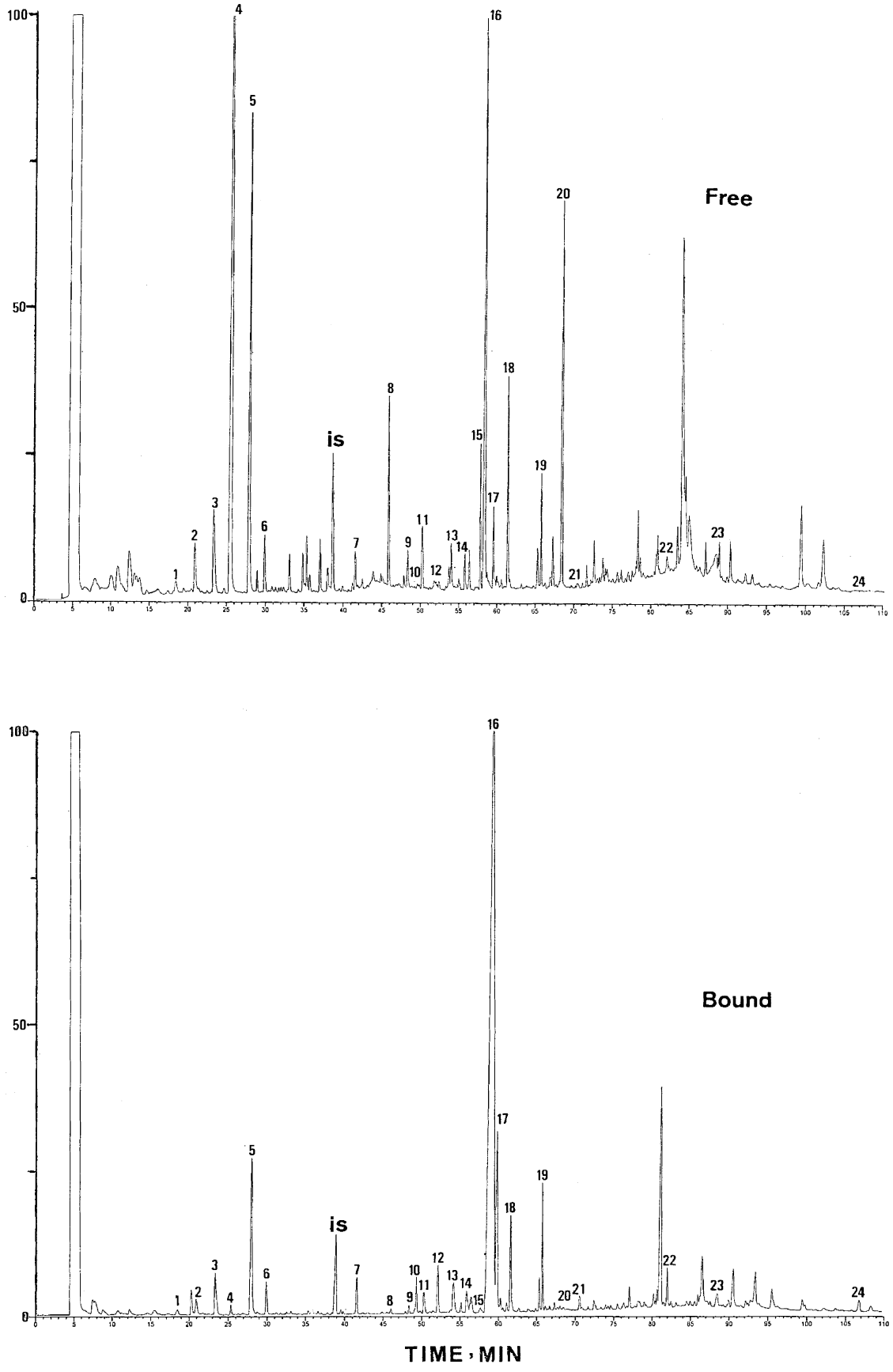

Fig. 1. Gas chromatograms of free and glycosidically-bound volatile compounds in clear lychee juice.

were employed with a purge delay time of 1 min and the mass spectra were obtained with electron multiplier voltage and electron ionization energy of 1353 V and 70 eV, respectively. The mass spectra were compared with those of the Wiley computer library. In addition, some of the compounds were further identified with commercially available authentic compounds. The amount of each compound was determined using an internal standard method and calculated by each peak area in the gas chromatogram.

3. Results and discussion

Both free and glycosidically-bound fractions of lychee aroma were subjected to gas chromatographic analysis, and their volatile components are shown in Fig. 1. Generally, the two fractions exhibited similar volatile profiles, except for some quantitative differences. These volatile compounds were primarily identified by their Kovats indices, using C_6–C_{25} *n*-alkanes, analysed by GC–MS and further confirmed by authentic compounds. Totally, 25 compounds (24 peaks, two compounds in peak 14) were identified in both fractions (Table 1), including one ester, 14 alcohols, two aldehydes, four acids, two ketones and two terpenes. The total amounts of volatile compounds in the free and bound fractions were 2907 and 1576 mg kg^{-1}, respectively. The amount of volatile compounds in the bound fraction was half the amount of volatile compounds in the free fraction, indicating that the aroma would be enriched by 50% as the volatile compounds in the bound fraction were released by enzymatic hydrolysis. However, the aroma characteristic would be changed to more floral and lemon-like (Arctander, 1969) due to high amounts of geraniol (1162.18 mg kg^{-1}) and geranial (125 mg kg^{-1}) in the bound fraction.

In the free fraction of lychee aroma, the major volatile compounds (>98 mg kg^{-1}) found were acetoin (30.1%), geraniol (15.6%), 3-methyl-2-buten-1-ol (15.3%), octanoic acid (7.28%), 2-phenylethanol (4.91%), *cis*-ocimene (4.32%), and butyric acid (3.40%). The amounts of these seven compounds accounted for 80.9% of the aroma in the free fraction. According to Arctander (1969), acetoin shows an intensely creamy, fatty, buttery odour and is perceptible at concentrations near 1 ppm, down to 0.2 ppm according to individual sensitivity. Therefore, acetoin might be a major characteristic compound in lychee aroma. Geraniol has a floral odour, whereas the aroma of 2-phenylethanol is sweet and honey-like at

Table 1
Free and glycosidically-bound volatile compounds in lychee juice

Peak No[a]	Compound	Kovats index (DB-Wax)	Amount[b] (mg kg^{-1} pulp)				
			Free	(%)	Bound	(%)	ID[c]
1	Myrcene	1156	23.5±9.11	0.81	5.14±0.84	0.33	GC, MS
2	Isoamyl alcohol	1195	80.2±17.6	2.76	1.76±1.40	0.11	GC, MS
3	*cis*-Ocimene	1239	126±26.1	4.32	10.11±1.6	0.64	GC, MS
4	Acetoin	1271	874±80.3	30.1	0.09±0.01	0.01	GC, MS
5	3-Methyl-2-buten-1-ol	1313	445±80.3	15.3	24±2.70	1.52	GC, MS
6	1-Hexanol	1344	57±12.8	1.98	4.89±0.43	0.31	GC, MS
7	Linalool	1533	25±2.60	0.88	0.35±0.04	0.02	GC, MS
8	Butyric acid	1604	98±1.06	3.40	1.31±0.77	0.08	GC, MS
9	3-Methylbutyric acid	1647	29±0.39	1.01	1.54±0.60	0.10	GC, MS
10	Neral	1667	1.50±0.12	0.05	60.4±21.32	3.83	GC, MS
11	Terpinyl acetate	1683	45.9±7.35	1.58	11.2±7.20	0.71	GC, MS
12	Geranial	1716	14.4±0.37	0.49	125±41.52	7.95	GC, MS
13	Citronellol	1754	34.6±3.30	1.19	30.6±6.10	1.94	GC, MS
14	Nerol + styralyl alcohol	1785	27.8±4.59	0.96	17.6±1.89	1.12	GC, MS
15	Hexanoic acid	1823	65.8±7.98	2.26	0.91±0.49	0.06	GC, MS
16	Geraniol	1859	454±5.67	15.61	1162±9.61	73.7	GC, MS
17	Benzyl alcohol	1866	52.7±3.75	1.82	2.93±1.47	0.19	GC, MS
18	2-Phenylethanol	1901	143±9.04	4.91	54.2±7.14	3.44	GC, MS
19	Phenol	1987	48.1±18.93	1.65	28.2±11.55	1.79	GC, MS
20	Octanoic acid	2045	211±5.86	7.28	1.47±0.25	0.09	GC, MS
21	*p*-Isopropylbenzyl alcohol	2090	5.55±2.31	0.19	6.95±0.50	0.44	MS
22	Farnesol	2351	3.01±0.43	0.10	1.59±0.80	0.10	MS
23	Drimenol	>2500	38±5.26	1.33	13.6±3.13	0.86	MS
24	Vanillylacetone	>2500	<1.0	<0.03	10.0±0.84	0.63	MS
	Total		2907		1576		

[a] The peak numbers correspond to Fig. 1.
[b] Each value is expressed as mean±standard deviation ($n=3$).
[c] ID: identification; GC, compared with retention times of authentic compounds; MS, identified by mass spectra.

concentrations lower than 40 ppm (Arctander, 1969). *cis*-Ocimene was described by a sensory panel as a warm-herbaceous, very diffusive odour of poor tenacity (Arctander, 1969). However, it is the odour compound characteristic of lychee in aroma evaluation. In addition, octanoic acid shows an oily-musty odour and, in dilution, it was more fruity and pleasant (Arctander, 1969). Butyric acid is known to have a powerful, penetrating, diffusive sour odour, reminiscent of rancid butter (Arctander, 1969). However, 3-methyl-2-buten-1-ol has a sharp, green-oily note (Arctander, 1969), and these seven major volatile compounds in combination give rise to the overall aroma of lychee.

In the bound fraction of lychee aroma, the latent major volatile compounds (>98 mg kg^{-1}) found were geraniol (73.7%) and geranial (7.95%). The amounts of these two compounds accounted for 81% of the latent aroma in the bound fraction. As mentioned earlier, after enzymatic hydrolysis, these two compounds would give rise to more floral and lemon-like aroma. In the bound fraction, some compounds, such as neral, geranial, *p*-isopropylbenzyl alcohol and vanillylacetone, were present in amounts higher than those in the free fraction.

Six-carbon compounds, which were generally formed from lipid oxidation by enzymes or heat, were not detected in either free or bound fractions of lychee aroma. Apparently, during sample preparation, the enzyme systems were effectively inhibited and, also, no heat was introduced. Some of these volatile compounds, found in both free and bound fractions were previously reported in lychee (Kuo et al., 1984). However, six compounds were newly identified in lychee, including acetoin, 3-methyl-2-buten-1-ol, benzyl alcohol, phenol, *p*-isopropylbenzyl alcohol and farnesol, accounting for 49.2 and 4.05% of the aroma in the free and bound fractions, respectively. The distinct profiles of volatile compounds found in this study and by Kuo et al. (1984) might have resulted from the different methods used for aroma isolation. This study used XAD-2 resin to extract the free and glycosidically-bound volatile fractions from lychee juice whereas Kuo et al. (1984) employed Tenax-GC to trap the volatiles in the headspace of lychee juice based on the volatility of compounds. Therefore, the aroma profile in Table 1 seems to embody all the volatile compounds in lychee juice.

Interestingly, many alcohols, such as isoamyl alcohol, 3-methyl-2-buten-1-ol, linalool, citronellol, geraniol, benzyl alcohol, 2-phenylethanol, phenol, *p*-isopropylbenzyl alcohol and farnesol, were found in both free and bound fractions. Among those, geraniol, linalool and benzyl alcohol, have also been determined in grapes and bound as arabinoglucosides (Reyne, Calmes, & Crouzet, 1992). However, phenol was reported in the free form and did not occur in bound form in pineapple (Wu et al., 1991). Evidently, geraniol was found to be the predominant compound in the free or bound fractions, and contributed significantly to the characteristic lychee aroma. This result differed from the findings of Kuo et al. (1984) that geranial was the most abundant compound, followed by geraniol in the fraction III concentrate. In GC/O analysis, geraniol was also determined to contribute notably to the aroma of lychee (Ong & Acree, 1998). In addition, 2-phenylethanol was found in both free and bound fractions (143 and 54.2 mg kg^{-1}, respectively). However, glycosidic 2-phenylethanol has been found in mango (Reyne et al., 1992), muscadine grape (Baek & Cadwallader, 1999), and apricot (Salles, Jallageas, Fournier, Tabet, & Crouzet, 1991).

In aroma evaluation, the free volatile fraction showed a fresh-fruity, lychee-like aroma whereas the bound fraction was odourless. However, the characteristic lychee-like aroma was noted in the bound fraction after enzymatic hydrolysis. Upon the combination of the free and hydrolysed bound fractions, strongly fruity, lychee-like aroma was perceived. These results suggest that controlled application of glycosidase in lychee processing may enhance the characteristic aroma of lychee juice.

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