Glycosidically Bound Aroma Compounds in Ginger (*Zingiber officinale* Roscoe)

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Free and glycosidically bound aromas from ginger juice were isolated and separated by an Amberlite XAD-2 column. Aroma compounds from a bound fraction were released by almond β-glucosidase hydrolysis. By use of octanol as the internal standard, volatile components of free and bound fractions were analyzed by GC and GC–MS. Glycosidically bound aliphatic alcohols, monoterpene alcohols, acids, and aldehydes were reported in ginger for the first time.

INTRODUCTION

Volatile ginger oil has been the subject of many research studies (Lawrence, 1984; Chen, 1986; Chen and Ho, 1988). A recent report by Lawrence (1984) listed the identification of 114 volatile components from ginger. Moyler (1984) has compared the advantages of liquid carbon dioxide extraction over conventional methods such as solvent extraction or steam distillation by showing reconstructed GC chromatograms which clearly displayed the differences. Chen and Ho (1989) compared the capillary GC analyses of volatile compounds derived from steam distillation of ginger with those extracted by liquid carbon dioxide. It is interesting to note that the amount of most monoterpene alcohols and sesquiterpene alcohols in distilled oil was higher than those extracted by liquid carbon dioxide. It was suggested that the differences were due to the thermal degradative effect of steam distillation upon the nonvolatile glycosides of monoterpene alcohols and/or sesquiterpene alcohols.

In the past few years the analysis of flavor precursors and intermediates, especially glycosides in fruits, has received increasing interest and attention (Strauss et al., 1986; Schwab and Schreier, 1988; Mayerl et al., 1989). The present study confirmed the existence of glycosidically bound aroma compounds in ginger.

EXPERIMENTAL PROCEDURES

Reagents. The solvents (*n*-pentane, diethyl ether, and methanol) were of HPLC grade from Fisher Scientific Co. (Springfield, NJ). Diethyl ether and *n*-pentane were redistilled prior to use. A standard of *n*-paraffins (C_5–C_{26}) was obtained from Alltech Associates (Deerfield, IL). Amberlite XAD-2 (20–60 mesh) nonionic polymeric adsorbent, α-terpineol, linalool, 2-undecanone, and *n*-octanol were obtained from Aldrich Chemical Co. (Milwaukee, WI). Citral (geranial and neral) was obtained from Fritzsche Dodge & Olcott Inc. (New York). Citronellol and geraniol were obtained from H & R GmbH (Holzminden, West Germany). Almond β-glucosidase was obtained from Sigma Chemical Co. (St. Louis, MO).

Fresh mature ginger (*Zingiber officinale* Roscoe) rhizomes were purchased from a local market.

Separation of Free and Bound Aroma Compounds. Rhizomes (950 g) of ginger were washed, sliced, and blended in a high shear blender with 600 mL of distilled water. About 800 mL of clear ginger juice was obtained by vacuum filtration through a bed of Celite 545 (J. T. Baker Chemical Co., Phillipsburg, NJ). The clear ginger juice obtained was then passed through a solvent-washed (Gunata et al., 1985) Amberlite

XAD-2 column (1 cm i.d. × 50 cm) with a flow rate of 2.0 mL/min. The column was then rinsed with 100 mL of distilled water to eliminate sugars, acids, and other water-soluble compounds. The fraction containing free aromas was eluted by 900 mL of pentane/ether (P/E; 1:1) at a flow rate of 2.0 mL/min. The glycosidically bound fraction was subsequently eluted by 900 mL of methanol. The methanol eluate was then concentrated to dryness by a stream of nitrogen. The dried material was then dissolved in 150 mL of 0.2 M citric–phosphate buffer solution (pH 5). The buffered mixture was washed twice with 120 mL of P/E (1:1) to remove possibly existing free aromas. The P/E extracts were combined with the previously obtained free fraction eluate. The combined P/E was dried over anhydrous sodium sulfate and then concentrated to a final volume of 1 mL with nitrogen. The glycosidically bound compounds dissolved in the buffer solution were hydrolyzed by almond β-glucosidase (150 mg, 5.3 units/mg) at 37 °C for 72 h. The liberated aglycons were extracted with 120 mL of P/E (1:1) three times. The extract was dried over anhydrous sodium sulfate and concentrated to a final volume of 0.5 mL with a stream of nitrogen.

Octanol (0.95 mg/mL ether) was added as the internal standard to both free and bound fractions before concentration.

GC and GC–MS Analyses of Volatile Compounds. A Hewlett-Packard 5890 gas chromatograph equipped with a fused silica column [60 M × 0.25 mm i.d.; d_f = 0.25 μm; Durawax 4 (90% dimethyl polysiloxane and 10% polyethylene glycol); J & W Scientific] and a flame ionization detector was used to analyze the volatile components in free and bound fractions. The operating conditions were as follows: injector and detector temperature, 250 °C; helium carrier flow rate, 0.8 mL/min; temperature program, 50–225 °C at 2 °C/min and held at 225 °C for 80 min. Quantitative determinations were carried out by a Hewlett-Packard 3396A integrator without considering response factors. Linear retention indices were calculated against *n*-paraffin standards (C_5–C_{26}; Alltech Associates) as references (Majlat et al., 1974). GC–MS analysis was accomplished by using a Varian 3600 gas chromatograph coupled directly to a Finnigan 4500 mass spectrometer. The column and temperature program were the same as described above. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 140 °C and then recorded on an Incos data system.

RESULTS AND DISCUSSION

Shown in Table I are the free and glycosidically bound aroma compounds identified in ginger juice. The identification was accomplished by comparing GC retention indices and/or MS spectra either with those of authentic compounds or with published data (Heller and Milne, 1980; Jennings and Shibamoto, 1980; Swigar and Silverstein,

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Table I. Free and Glycosidically Bound Aroma Compounds from Ginger

compound	I_k (Durawax 4)	μg/kg (ppb) free	bound	ID
α-pinene	8	69	—[a]	b
2-pentanol	985	4801	14	b, c
camphene	1011	51	–	b
myrcene	1081	104	–	b
β-phellandrene	1129	67	–	b
2-heptanol	1202	65	1036	b, c
1-hexanol	1229	–	17	b, c
rose oxide	1275	–	12	b
2-octanol	1283	–	10	b, c
acetic acid	1309	368	–	b, c
linalool oxide	1330	84	7	b
6-methyl-5-hepten-2-ol	1338	–	29	b
linalool oxide	1361	–	8	b
nerol oxide	1363	–	6	b
2-nonanol	1393	340	–	b, c
benzaldehyde	1397	–	10	b
linalool	1422	180	42	b, c
n-octanol	1429	I.S.	I.S.	
α-ylangene	1440	99	–	b
4-terpineol	1478	99	–	b
2-undecanone	1491	140	–	b, c
neral	1554	488	50	b, c
α-terpineol	1563	1808	172	b
borneol	1565	546	–	b
p-mentha-1,5-dien-8-ol	1587	–	9	b
2-hydroxy-1,8-cineole	1597	–	8	d
geranial	1601	706	75	b
citronellol	1629	2315	141	b
zingiberene	1634	8880	–	
bisabolene	1642	2296	–	b
nerol	1658	3759	121	b
ar-curcumene	1671	2609	–	b
β-sesquiphellandrene	1679	3485	–	b
geraniol	1706	21736	781	b
2-hydroxy-1,8-cineole	1722	–	179	b
p-mentha-1,5-dien-7-ol	1825	–	20	b
p-mentha-2,8-dien-1-ol	1834	–	17	b
p-mentha-1,8-dien-7-ol	1850	–	63	b
nerolidol	1907	410	–	b
1,8-terpine hydrate	1940	1808	–	d
zingiberenol	1978	794	–	b
cis-selinen-4-ol	1990	167	–	b
β-eudesmol	2097	485	–	b
cis-geranic acid	2122	–	38	b
2,6-dimethyl-3,7-octa-diene-1,6-diol	2136	–	287	b
trans-geranic acid	2162	5564	–	b
2,6-dimethyl-2,6-octa-diene-1,8-diol	2403	–	306	b
2,6-dimethyl-2,6-octa-diene-1,8-diol	2436	–	660	b

[a] Not detected. [b] Mass spectral data from the following sources: Heller and Milne (1980); Jennings and Shibamoto (1980); TNO (1980); Swigar and Silverstein (1981). [c] GC analyses of authentic compounds. [d] Tentative identification with mass spectral data matched the following source: Chen (1986) or structure proposed based upon mass spectra only.

1981; Ten Noever de Brauw, 1983; Chen, 1986). The quantitation of each compound was conducted by using n-octanol as the internal standard without considering GC–FID response factors. The free aroma fraction had a "spice", "ginger-like", "floral" aroma, while the glycosidically bound fraction had no odor. Only after enzymic hydrolysis did the bound fraction have the characteristic spicy, fatty green, floral, and ginger-like aroma.

Except for geranic acids and 2-pentanol, all the free volatile compounds shown in Table I were reported previously in ginger (Lawrence, 1984; Chen, 1986; Chen and Ho, 1988). Among them, geraniol was the most abundant. Neral, geranial, α-terpineol, β-sesquiphellandrene, and ar-curcumene were reported to be the most

important compounds contributing to the overall characteristic ginger aroma (Bednarczyk and Kramer, 1971; Bednarczyk et al., 1975). All these compounds were detected in the present study. However, many of the previously reported sesquiterpenes and sesquiterpene alcohols in ginger (Lawrence, 1984; Chen, 1986; Chen and Ho, 1988) were not found in this study. The differences of samples and methodology used may be responsible for the discrepancies.

As shown in Table I, the glycosidically bound aromas isolated from ginger were mainly alcohols. It is very surprising that 2-heptanol is the most abundant bound aroma component in ginger. In addition, it is unusual that small amounts of aliphatic alcohols such as 2-pentanol, hexanol, and 2-octanol were also present in glycosidic forms. The role of these glycosidically bound aliphatic alcohols in ginger is unknown. Glycosidic terpene alcohols, such as geraniol, nerol, and citronellol, have been found in rose petals (Francis and Allcock, 1969). The above-mentioned volatiles, linalool, α-terpineol, benzyl alcohol, and 2-phenylethanol, were also reported in grapes and apricots (Salles et al., 1988).

It is very interesting that the ratios of the amount of glycosidic to free linalool, nerol, geraniol, and α-terpineol are less than 1 in ginger, while they are greater than 1 in grapes, apricots, and mangos (Salles et al., 1988). On the other hand, linalool, nerol, geraniol, and α-terpineol in passion fruit are not present in free form but rather in bound glucosidic form (Engel and Tressl, 1983). It is also interesting that many aroma compounds, such as 2,6-dimethyl-3,7-octadiene-1,6-diol, 2,6-dimethyl-2,6-octadiene-1,8-diol, 2-hydroxy-1,8-cineole, p-mentha-1,5-dien-8-ol, p-mentha-1,5-dien-7-ol, p-mentha-2,8-dien-1-ol, p-mentha-1,8-dien-7-ol, and p-8-cymenol, were identified only in the glycosidically bound fraction in the present study. Glycosidic 2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyl-2,7-octadiene-1,6-diol have been found in papayas and grapes (Williams et al., 1989; Schwab et al., 1989). Although some sesquiterpene alcohols are present in their free forms, no glycosidic sesquiterpene alcohols were observed in ginger. It is possible that the glycosidic sesquiterpene alcohols were present in trace amounts which were beyond the extraction limit of the isolation methods we used. Glycosidically bound cis-geranic acid and trans-geranic acid in Table I are reported in ginger for the first time. Glycosidic geranic acid and neric acid have been reported in the herb Melissa officinalis (van den Dries and Svendsen, 1989). These acids may bind to sugar in the form of an ester. The free acids can be released either by the action of an enzyme or by heat in an acidic environment. Meanwhile, glycosidically bound aldehydes such as neral, geranial, and benzaldehyde were observed in ginger for the first time. Glycosidic benzaldehyde was also reported in bitter almond oil, peaches, apricots (Windholz, 1983), and grapes (Williams et al., 1989). Aldehydes may bind to sugar in the form of acetal and/or hemiacetal, which are labile in acidic environment. The glycosidic benzaldehyde found in bitter almond oil is called amygdalin. It is composed of two molecules of D-glucose and one molecule of l-mandelonitrile. When released by enzymes or hot diluted mineral acids, it yields benzaldehyde, HCN, and two molecules of glucose (Windholz, 1983). Among the three glycosidically bound terpene alcohol oxides listed in Table I, linalool oxide and nerol oxide have also been reported in bound form in apricots and grapes (Salles et al., 1988). Salles et al. (1988) proposed that free linalool oxide can be produced through three different ways: (1) hydrolysis of glycosid-

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ically bound linalool oxide; (2) hydrolysis of 3,7-dimethyloct-1-ene-3,6,7-triol in free or bound forms; and (3) oxidation of linalool during the extraction and concentration steps.

Chen and Ho (1988) reported that the amount of monoterpene and sesquiterpene alcohols in distilled oil is more than that of liquid carbon dioxide extracted from ginger. They suggested that the differences were due to the thermal degradative effect of steam distillation upon the nonvolatile glycosides of monoterpene and/or sesquiterpene alcohols. It is well-known that many volatiles in plants exist as nonvolatiles before they are released by either enzymic or chemical reactions during growth and food processing. In the present study, the detection of glycosidically bound aliphatic alcohols, monoterpene alcohols, and acids can further support their explanation.

ACKNOWLEDGMENT

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