

# Contribution of Free and Glycosidically Bound Volatile Compounds to the Aroma of Muscadine Grape Juice

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## ABSTRACT

Free and glycosidically bound volatile compounds were isolated and identified from muscadine grape juice. Most abundant in free and bound form was the muscadine character-impact aroma compound 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol). Other aroma compounds, such as *o*-aminoacetophenone and 2-phenylethanol were found in free and bound forms. A high level of 2-phenylethanol was in the glycoside form and many bound monoterpenes were identified. *p*-Vinylguaiacol, a potential off-flavor in muscadine juice was found at a low level in bound form. Enzymatic hydrolysis of glycoside precursors could lead to release of volatile compounds with both potentially positive and deleterious effects on the overall aroma.

**Key Words:** muscadine grape, aroma, glycosidically bound, Furaneol, *o*-aminoacetophenone

## INTRODUCTION

GLYCOSIDES ARE IMPORTANT FLAVOR PRECURSORS IN MANY FRUITS and vegetables, particularly, grapes. Although such glycoside precursors are odorless, aroma compounds (aglycons) are released after glycoside linkage hydrolysis. Glycosidically bound aroma compounds have been reported in grape (Gunata et al., 1985), raspberry (Pabst et al., 1991), pineapple (Wu et al., 1991), celery (Tang et al., 1990), and tomato (Buttery et al., 1990). Volatile aroma compounds of muscadine grapes have been identified (Welch et al., 1982; Horvat and Senter, 1974; Lamikanra, 1987; Lamikanra et al., 1996; Baek et al., 1997). However, there is no report on the glycosidically bound aroma compounds.

In our previous study, aroma-active compounds in muscadine grape juice were evaluated using aroma extract dilution analysis (Baek et al., 1997). We found that 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol) and *o*-aminoacetophenone were primarily responsible for the characteristic candy-like and foxy aroma notes, respectively, of muscadine grape juice. Furaneol glycoside has been isolated from strawberry (Mayerl et al., 1989) and from other fruits and vegetables (Buttery et al., 1990; Pabst et al., 1991; Wu et al., 1991).

Our objective was to identify and quantify free and glycosidically bound aroma compounds in muscadine grape juice.

## MATERIALS & METHODS

### Samples

Muscadine grapes (*Vitis rotundifolia* Michx. cv. Carlos) were harvested at Crystal Springs, MS (CS-1 and CS-2) and Hammond, LA

(CS-LA). CS-1 and CS-2 were harvested at the same ripening stage on two harvest dates (August 26, 1994 and September 1, 1994). After harvesting, grapes were frozen at -18°C until juice extraction.

### Reagents

*p*-Nitrophenyl  $\beta$ -D-glucopyranoside (pNp-Glu), *p*-nitrophenyl  $\alpha$ -L-arabinopyranoside (pNp-Arap), *p*-nitrophenyl  $\alpha$ -L-arabinofuranoside (pNp-Araf), *p*-nitrophenyl  $\alpha$ -L-rhamnopyranoside (pNp-Rha), *p*-nitrophenol, and N-heptyl  $\beta$ -D-glucopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO). Amberlite XAD-2 (20-60 mesh) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Pectinase AR 2000 (pectinase from *Aspergillus niger*) was obtained from Gist-Brocades Food Ingredients, Inc. (King of Prussia, PA).

### Juice preparation

After being spray washed using a rotary rod washer (model C, Dixie Cannery Equipment, Athens, GA), grapes were thawed at 25°C. The grapes were crushed using a hand crusher, and pressed (17,340 kPa) in a hydraulic press (model 91-228, NAPA Lifting Equipment, St. Louis, MO). The juice was filtered through cheesecloth (Fisher Scientific, NJ), and then centrifuged at  $2,000 \times g$  for 30 min. The juice was stored at 4°C (overnight) prior to extraction of volatiles and isolation of glucosides.

### Extraction of free volatile compounds

Volatile compounds were extracted using a liquid-liquid continuous extractor (1-L, cat. no. LG-6996-100, Lab Glass, Vineland, NJ), which had been previously silylated with dimethyldichlorosilane (cat. no. 18090, Alltech, Deerfield, IL). Muscadine juice (500 mL, or 250 mL for CS-LA) plus deodorized distilled water (650 mL, or 900 mL for CS-LA) and 1-heptanol (246.6 g, internal standard, the same amount for CS-LA) were placed in the liquid-liquid continuous extractor. Dichloromethane (125 mL) was used as extracting solvent. Extractions were carried out for 12h at 25°C. After extraction, water was removed by freezing, and then the extract was dried over 3g of anhydrous sodium sulfate. Extracts were concentrated to 500  $\mu$ L (or 250  $\mu$ L for CS-LA) under a gentle stream of nitrogen. All extractions were performed in duplicate.

### Isolation of muscadine grape glycosides

Amberlite XAD-2 (20-60 mesh) was cleaned as described by Buttery et al. (1990) and the resin was packed into a glass column (2 cm i.d.  $\times$  45 cm length) to a height of 10 cm. Isolation procedure followed the method developed by Gunata et al. (1985). The resin was washed with 100 mL of pentane, 100 mL of methanol, and then 100 mL of deodorized distilled water (DDW). Muscadine juice (250 mL, or 125 mL for CS-LA) containing N-heptyl- $\beta$ -D-glucoside (1 mg, internal standard; I.S.) was passed through the column at a flow rate of 10 mL/min. The resin was washed with 250 mL of DDW to remove water soluble compounds such as sugars and acids, and then with 250 mL of pentane to remove free volatiles. The glycoside fraction was eluted from the resin with 250 mL methanol and stored at -20°C. Methanol was removed using a Rotavapor (Büchi, Switzerland) under reduced pressure at 45°C. For a column blank, DDW (250 mL) plus I.S. (1 mg) were treated as above.

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**Enzymatic hydrolysis of muscadine grape glycosides**

Muscadine grape glycosides were dissolved in 50 mL of 0.05M sodium citrate buffer (pH 4.65). The solution was extracted with 100 mL of pentane to remove any traces of free volatiles. The solution was divided into two fractions, that is, 25 mL of the solution was placed in a screw capped test tube (25 mm i.d.  $\times$  150 mm length) and another 25 mL into another test tube for an enzyme blank. Pectinase AR 2000 (60 mg, Gist-Brocades Food Ingredients, Inc., King of Prussia, PA) was added to the solution in one test tube, while inactivated AR 2000 (95°C for 30 min) was added to the enzyme blank. Oxygen was removed under a nitrogen stream to prevent oxidation during reaction. Toluene (100  $\mu$ L) was added to retard microbial growth. The tubes were sealed with PTFE-lined screw caps. The solutions were left to react for 2 days at 40°C. After reaction, 20 mL of dichloromethane was added to the solution to extract volatiles. Test tubes were centrifuged at 850  $\times$  g for 30 min to separate dichloromethane and water layers, and dichloromethane was collected and the extraction step repeated. Water was removed by freezing, and the extract was dried over 3g of anhydrous sodium sulfate. Extracts were concentrated to 100  $\mu$ L before analysis.

**Enzyme assays.**  $\beta$ -D-Glucosidase activity was measured as follows; 0.2 mL of AR 2000 solution was added to 0.2 mL of a 4 mM solution of pNP-Glu in 0.05M citrate buffer (pH 4.7). Reaction was carried out for 20 min at 40°C. Sodium carbonate (1.2 mL of 1M solution) was added to stop the reaction and absorbance was measured at 400 nm. Enzyme assay for  $\alpha$ -L-arabinofuranosidase,  $\alpha$ -L-arabinopyranosidase, and  $\alpha$ -rhamnosidase activities were the same as above except that pNP-Araf, pNP-Arap, and pNP-Rha served as substrates, respectively. AR 2000 solution was diluted with 0.05M citrate buffer (pH 4.7) to give a final assay absorbance between 0.01 and 1.00. A standard curve was prepared using p-nitrophenol. One unit of activity was defined as one nmole of p-nitrophenol liberated/min/mg of enzyme.

**Gas chromatography-Mass spectrometry (GC-MS)**

GC-MS system consisted of an HP 5890 GC/HP 5972 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). Each extract (1  $\mu$ L) was injected (splitless mode; injector temperature 200°C; 60 sec valve delay) into a fused silica capillary column (DB-WAX, 60 m length  $\times$  0.25 mm i.d.  $\times$  0.25 (m film thickness; J & W Scientific, Folsom, CA). Helium was used as carrier gas at a linear velocity of 25.0 cm/sec. Oven temperature was programmed from 40°C to 200°C at a rate of 3°C/min, with initial and final hold times of 5 and 60 min, respectively. MSD conditions were as follows: capillary direct interface temperature, 200°C; ion source temperature, 300°C; ionization energy, 70 eV; mass range, 33–350 a.m.u.; electron multiplier voltage was 200V above Atune value; scan rate, 2.2 scans/sec. Duplicate analyses were performed for each LLE extract.

**Compound identification**

Compound identifications were based on comparison of GC retention indices and mass spectra of unknowns with those of authentic standard compounds. Tentative identifications were based on matching mass spectra of unknowns with those in a published mass spectral database (Wiley 138, John Wiley and Sons, Inc., 1990).

**Quantification**

MS response factors for each compound relative to the internal standard were used to quantify positively identified compounds. Corrected concentrations of glycosidically bound volatiles were obtained after subtracting enzyme and column blanks from samples.

**RESULTS & DISCUSSION**

GLYCOSIDICALLY BOUND MONOTERPENES IN GRAPES (*Vitis vinifera*) are generally linked with disaccharidic moieties, such as 6-O-(-L-rhamnopyranosyl)- $\beta$ -D-glucopyranosides and 6-O-(-L-arabinofuranosyl)- $\beta$ -D-glucopyranosides, and to a lesser extent with  $\beta$ -D-glucopyranosides (Williams et al., 1982a, 1983).

$\alpha$ -L-Arabinofuranosidase or  $\alpha$ -L-rhamnosidase initially hydrolyze those disaccharides to release  $\beta$ -D-glucosides, which are subject to cleavage by  $\beta$ -D-glucosidase to release terpenols (Gunata et al., 1988). Therefore, those three enzyme activities are essential for the release of bound terpenyl aglycons. Pectinase AR2000 has several glycosidase activities (Table 1). AR 2000 possessed all glycosidase activities required for the release of bound monoterpenyl aglycons. Acid hydrolysis could also be applied to release the glycosidically bound aroma compounds, but rearrangement of their aglycons may occur (Williams et al., 1982b).

In our previous study, predominant aroma-active compounds in muscadine grape juice were reported (Baek et al., 1997). It was concluded that 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol), with candy-like aroma, was the most intense aroma compound. The other aroma-active compounds, such as *o*-aminoacetophenone (foxy), 2-phenylethanol (rosy), 2,3-butanedione (buttery), ethyl butanoate (fruity), and ethyl 2-methylbutanoate (green apple-like), were considered important in the aroma of muscadine grape juice. The distribution of free and glycosidically bound forms of these aroma-active compounds in muscadine juice was determined (Table 2). Free form of Furaneol was present at a concentration of 15.4, 9.6, and 12.1 ppm in CS-1, CS-2, and CS-LA, respectively. Higher levels of this compound were present as glycoside precursors (18.8, 51.1, and 14.8 ppm in CS-1, CS-2, and CS-LA, respectively). A glycoside of Furaneol was first identified in strawberry (Mayerl et al., 1989) and bound Furaneol has been isolated from many fruits and vegetables (Wu et al. 1991; Buttery et al. 1994). *o*-Aminoacetophenone and 2-phenylethanol were present in both free and glycosidically bound forms, whereas 2,3-butanedione, ethyl butanoate, and ethyl 2-methylbutanoate were in free form only. *o*-Aminoacetophenone was present in free form at 19, 10, and 14 ppb in CS-1, CS-2, and CS-LA, respectively, and in glycosidically bound form at 24, 39, and 12 ppb, respectively. Furaneol and *o*-aminoacetophenone (candy and foxy aroma notes, respectively) are responsible for the characteristic aroma of American (labrusca) type grapes (Shure and Acree, 1994). High levels of glycosidically bound Furaneol and *o*-aminoacetophenone indicate that the labrusca note may be enhanced by enzymatic hydrolysis of the glycoside linkage to provide free forms of the compound. High levels of both free and glycosidically bound 2-phenylethanol were present. The free form ranged from 0.78 ppm (CS-LA) to 0.93 ppm (CS-2), and glycosidically bound from 3.9 ppm (CS-LA) to 7.8 ppm (CS-2). 2-Phenylethanol has been reported to be important in the flavor of Noble muscadine wine (Lamikanra, 1987; Lamikanra et al., 1996).

Quantitative levels of free and glycosidically bound forms of additional aroma compounds were found in muscadine grape juice (Table 2). Most alcohols were present in both free and glycosidically bound form. 1-Hexanol, (*E*)-2-hexen-1-ol, and 2,3-butanediol were present at high levels in free form. However, much higher levels of alcohols were in the bound form. The contribution of these alcohols to overall aroma is probably low because they generally have high aroma detection thresholds.

*p*-Vinylguaiacol was found mostly in glycosidically bound form. This compound was considered to be an off-flavor in orange juice (Tatum et al., 1975) and can be formed by thermal degradation of ferulic acid (Fiddler et al., 1967; Naim et al., 1988). The free form ranged from 20 ppb to 35 ppb and the bound form from 978 ppb to 1,844 ppb. Considering the low aroma threshold value of *p*-vinylguaiacol (10 ppb) and its aroma property (curry-like), we hypothesized that this compound

**Table 1. Glycosidase activities in pectinase AR 2000**

Type of glycosidase activity	Activity (nmole $\cdot$ min <sup>-1</sup> $\cdot$ mg <sup>-1</sup> ) <sup>a</sup>
$\alpha$ -L-Arabinofuranosidase	1200
$\alpha$ -L-Arabinopyranosidase	47
$\alpha$ -L-Rhamnosidase	17
$\beta$ -D-Glucosidase	380

<sup>a</sup>1 unit equals nmole of p-nitrophenol liberated/min/mg pectinase.

**Table 2—Free and glycosidically bound volatile flavor compounds in muscadine grape**

		Concentration (ppb) <sup>a</sup>					
		CS-1		CS-2		CS-LA	
		F <sup>c</sup>	B <sup>d</sup>	F	B	F	B
	Ri <sup>b</sup>						
<b>Acids</b>							
acetic acid	1451	140 ± 59	20 ± 15	280 ± 140	160 ± 120	57 ± 57	25 ± 19
3-methyl butanoic acid	1672	— <sup>e</sup>	—	—	—	—	—
hexanoic acid	1852	31 ± 5	—	45 ± 21	—	22 ± 27	—
<b>Alcohols</b>							
2-butanol	1024	3 ± 1	23 ± 2	3 ± <0.1	17 ± 7	2 ± 1	16 ± 4
2-methyl-1-propanol	1092	18 ± 3	13 ± 1	22 ± 5	29 ± 9	22 ± 4	18 ± 1
1-butanol	1145	79 ± 12	120 ± 4	77 ± 5	250 ± 80	38 ± 7	72 ± 5
3-methyl-1-butanol	1210	94 ± 9	140 ± 4	100 ± 9	270 ± 75	83 ± 13	130 ± 6
1-pentanol	1253	25 ± 3	21 ± 2	27 ± 3	41 ± 11	21 ± 4	14 ± <0.1
2-methyl-1-pentanol	1302	7 ± <0.1	nd <sup>f</sup>	7 ± 1	nd	13 ± 2	nd
(Z)-2-penten-1-ol	1322	21 ± 2	nd	24 ± 3	nd	22 ± 1	nd
1-hexanol	1359	430 ± 33	1500 ± 8	460 ± 13	2800 ± 640	460 ± 21	600 ± 690
(E)-3-hexen-1-ol	1366	7 ± 1	62 ± 2	10 ± 2	79 ± 27	7 ± 5	30 ± 2
(Z)-3-hexen-1-ol	1387	41 ± 3	71 ± 1	43 ± 5	140 ± 31	40 ± 5	70 ± 2
(E)-2-hexen-1-ol	1409	140 ± 10	37 ± 1	120 ± 7	86 ± 19	180 ± 19	37 ± 1
(Z)-2-hexen-1-ol	1417	7 ± 1	4 ± <0.1	9 ± 1	8 ± 2	8 ± 1	3 ± <0.1
2,3-butanediol	1545	110 ± 37	1 ± <0.1	130 ± 37	3 ± 2	39 ± 1	2 ± <0.1
1-octanol	1561	21 ± 2	1300 ± 32	25 ± 5	2300 ± 490	17 ± 2	1000 ± 38
1,3-butanediol	1747	55 ± 19	nd	4 ± 4	nd	18 ± 2	nd
(E,Z)-2,6-nonadien-1-ol	1771	6 ± 1	nd	6 ± 1	nd	2 ± 2	nd
α-methyl benzenemethanol <sup>g</sup>	1817	3 ± <0.1	130 ± 6	5 ± 1	100 ± 83	1 ± 2	61 ± 6
benzenemethanol	1880	49 ± 6	820 ± 2	61 ± 11	1800 ± 500	25 ± 10	870 ± 22
2,6-bis (1,1-dimethylethyl)-4-methylphenol <sup>g</sup>	1910	15 ± 2	nd	18 ± 3	nd	25 ± 3	nd
2-phenylethanol	1922	870 ± 78	3900 ± 63	930 ± 38	7800 ± 2200	780 ± 32	3900 ± 130
1,4-butanediol <sup>g</sup>	1927	29 ± 6	nd	31 ± 4	nd	25 ± 4	nd
benzenepropanol <sup>g</sup>	2053	9 ± 2	83 ± 2	11 ± 2	96 ± 46	5 ± 6	29 ± 6
4-methylphenol <sup>g</sup>	2084	1 ± <0.1	190 ± 22	1 ± <0.1	310 ± 110	1 ± <0.1	140 ± 77
p-vinylguaiacol	2198	32 ± 1	980 ± 58	35 ± 7	1800 ± 750	20 ± 23	1200 ± 46
n-heptane-1,7-diol <sup>g</sup>	2253	22 ± 3	37 ± 7	25 ± 6	83 ± 71	10 ± 1	21 ± 7
4-methoxy benzenemethanol <sup>g</sup>	2284	2 ± 2	100 ± 3	5 ± 3	140 ± 100	3 ± 1	110 ± 6
1,9-nonanediol <sup>g</sup>	2466	24 ± 3	28 ± 2	26 ± 5	65 ± 21	10 ± 7	9 ± 5
4-methoxy benzenepropanol <sup>g</sup>	2471	8 ± 1	120 ± 4	9 ± 2	180 ± 67	3 ± 3	30 ± 2
1-phenyl-1,2-ethanediol <sup>g</sup>	2554	14 ± 3	56 ± 7	19 ± 4	110 ± 27	5 ± 6	49 ± 2
<b>Aldehydes</b>							
hexanal	1079	19 ± 2	6 ± 2	11 ± 2	9 ± 3	28 ± 5	14 ± 4
(E)-2-hexenal	1217	46 ± 3	2 ± <0.1	21 ± 2	5 ± 2	66 ± 9	7 ± 1
benzaldehyde	1528	19 ± 2	nd	8 ± 9	36 ± 51	14 ± 2	nd
phenylacetaldehyde	1645	11 ± 2	46 ± 2	5 ± 4	66 ± 17	14 ± <0.1	44 ± 16
4-hydroxy-3-methoxy benzaldehyde (vanillin) <sup>g</sup>	2561	7 ± 3	nd	9 ± 2	tr <sup>g</sup>	27 ± 8	tr
<b>Esters</b>							
ethyl acetate	845	51 ± 11	nd	74 ± 14	nd	29 ± 8	nd
ethyl butanoate	1034	28 ± 3	nd	40 ± 5	nd	14 ± 5	nd
ethyl 2-methylbutanoate	1051	3 ± 1	nd	5 ± 1	nd	0.4 ± <0.1	nd
butyl acetate	1071	49 ± 4	nd	56 ± 6	nd	35 ± 6	nd
ethyl 2-butenate	1162	29 ± 2	tr	36 ± 4	tr	33 ± 5	tr
ethyl hexanoate	1234	20 ± 1	nd	28 ± 4	nd	16 ± 2	nd
hexyl acetate	1275	5 ± <0.1	nd	6 ± 1	nd	2 ± 2	nd
ethyl 2-hexenoate	1346	6 ± 1	tr	11 ± 2	tr	3 ± <0.1	tr
ethyl octanoate	1435	2 ± <0.1	nd	3 ± 1	nd	1 ± <0.1	nd
methyl 3-hydroxy butanoate	1485	53 ± 7	72 ± 4	62 ± 6	130 ± 28	40 ± 2	49 ± 3
ethyl 3-hydroxy butanoate	1524	530 ± 46	1200 ± 12	550 ± 39	1800 ± 440	390 ± 9	530 ± 8
ethyl 3-hydroxyhexanoate	1684	17 ± 2	18 ± 12	23 ± 6	49 ± 14	9 ± 4	9 ± 10
benzyl acetate	1731	2 ± 1	nd	5 ± 2	nd	1 ± <0.1	nd
methyl 2-hydroxy benzoate	1782	nd	58 ± 1	nd	81 ± 56	nd	56 ± <0.1
ethyl benzene acetate	1789	2 ± <0.1	nd	4 ± 1	nd	2 ± 2	nd
2-phenyl ethyl acetate	1820	9 ± 1	nd	8 ± 2	nd	2 ± 2	nd
<b>Furans and Furanones</b>							
2-pentylfuran	1230	3 ± <0.1	3 ± <0.1	4 ± 1	4 ± 2	1 ± 2	tr
dihydro 2(3H)-furanone <sup>g</sup>	1634	30 ± 21 ± <0.1	36 ± 11	2 ± <0.1	60 ± 8	tr	nd
5-ethyldihydro-2(3H)-furanone <sup>g</sup>	1708	26 ± 4	7 ± <0.1	32 ± 6	18 ± 4	20 ± 4	8 ± <0.1
dihydro-4-methyl-2(3H)-furanone <sup>g</sup>	1815	5 ± <0.1	nd	5 ± 1	nd	2 ± 2	nd
2,5-dimethyl-4-hydroxy-3(2H)-furanone	2045	15000 ± 2200	19000 ± 1200	9600 ± 810	51000 ± 20400	12000 ± 680	15000 ± 6400
<b>Ketones</b>							
2,3-butanedione	982	15 ± 3	nd	23 ± 2	nd	12 ± 8	nd
2-heptanone	1181	1 ± 1	nd	6 ± 1	nd	1 ± <0.1	nd
3-hydroxy-2-butanone	1290	860 ± 140	tr	840 ± 13	tr	570 ± 11	tr
acetophenone	1654	7 ± 1	7 ± 1	8 ± 2	12 ± 1	2 ± 2	tr
o-aminoacetophenone	2223	19 ± 2	24 ± 1	10 ± 4	39 ± 47	14 ± 1	12 ± 8
<b>Terpenes</b>							
linalool oxide isomer <sup>g</sup>							
linalool oxide isomer <sup>g</sup>	1479	nd	9 ± <0.1	nd	2 ± 3	nd	2 ± <0.1
linalool <sup>g</sup>	1549	tr	10 ± 1	tr	12 ± 2	tr	5 ± 1
α-terpineol <sup>g</sup>	1703	8 ± 1	58 ± 1	2 ± <0.1	120 ± 32	3 ± <0.1	80 ± 4
β-cyclogeraniol <sup>g</sup>	1726	tr	15 ± <0.1	tr	20 ± 15	tr	13 ± <0.1
citral <sup>g</sup>	1737	tr	6 ± <0.1	tr	11 ± 3	tr	4 ± 4
(E)-p-menth-2-en-7-ol <sup>g</sup>	1765	tr	12 ± 8	tr	27 ± 6	tr	14 ± 1
(E)-geraniol	1851	5 ± 1	400 ± 13	8 ± 1	570 ± 150	2 ± 2	170 ± 33
3,7-dimethyloct-1-ene-3,7-diol <sup>g</sup>	1979	65 ± 8	110 ± 18	78 ± 14	250 ± 130	76 ± 9	140 ± 30
3,7-dimethyloctane-1,7-diol <sup>g</sup>	2206	71 ± 7	790 ± 14	87 ± 16	1400 ± 560	52 ± 11	880 ± 31

<sup>a</sup>Compound tentatively identified based on mass spectrum. <sup>b</sup>Average ± standard deviation (n=4). <sup>c</sup>Retention index on DB-Wax column. <sup>d</sup>Free form. <sup>e</sup>Bound form. <sup>f</sup>Not available due to peak co-elution. <sup>g</sup>Not detected. <sup>h</sup>Trace amount.

would contribute unfavorably to overall aroma quality of muscadine grape juice after enzymatic hydrolysis of glycoside precursor(s). Glycosidically bound *p*-vinylguaiacol has been reported in tomatoes (Buttery et al., 1990) and raspberries (Pabst et al., 1991).

Monoterpenes are important in the aroma of *Vitis vinifera* grapes and wines (Strauss et al., 1986). In muscadines, free (*E*)-geraniol contributed to the overall aroma (Baek et al., 1997). Free monoterpene compounds were present in trace or low concentration in muscadine juice. However, considerable amounts of glycosidically bound monoterpenes were present. Many terpenols were detected after enzymatic hydrolysis. Linalool,  $\beta$ -cyclogeraniol, (*E*)-citral, (*E*)-*p*-menth-2-en-7-ol, and ( $\alpha$ -terpineol were present in trace amounts in the free fraction. The other terpenes were present mostly in bound forms. Levels of bound terpenols have been reported to be much higher than free terpenols in many cultivars of grapes (Gunata et al., 1985). (*E*)-Geraniol, nerol, and linalool were the most abundant terpenols in *Vitis vinifera* grapes (Gunata et al., 1985, 1988; Williams et al., 1982a). In muscadine, only ( $\alpha$ -terpineol and (*E*)-geraniol were detected in considerable amounts in free form. (*E*)-geraniol was the most abundant among bound terpenols and nerol was not detected. High levels of bound (*E*)-geraniol (170–570 ppb) were far above its aroma threshold of 4.1 ppb (Pino et al., 1986). Therefore this compound would probably contribute to the aroma of muscadine after enzymatic hydrolysis of its glycoside precursor. The increase of these terpenols is thought to enhance or modify the aroma of muscadine grape-based products. These terpenols are known to be important in wine aroma (Ribéreau-Gayon et al., 1975). Linalool oxide, reported to be important in the flavor of Oolong tea (Moon et al., 1994), was present only in its glycosidically bound form. Monoterpene polyols are odorless but may be precursors for monoterpenols (Vasserot et al., 1995). These compounds (e.g. 3,7-dimethyloct-1-en-3,7-diol and 3,7-dimethyl-1,7-octanediol) were present at considerably higher levels than the other terpenes. Bound forms of polyols were more abundant than free polyols, which have been reported in muscat grapes (Williams et al., 1980).

Most esters were present only in free form except for hydroxy esters. The hydroxy esters were found at higher levels in bound forms than in free forms. Ethyl 3-hydroxy butanoate was the most abundant among bound hydroxy esters. This aroma-active compound with its burnt marshmallow and muscadine-like character has been identified in muscadine juice (Baek et al., 1997). Glycosidically bound hydroxy esters also have been found in pineapple (Wu et al., 1991).

## CONCLUSIONS

2,5-DIMETHYL-4-HYDROXY-3(2H)-FURANONE, *o*-aminoacetophenone, and 2-phenylethanol, characteristic aroma-active compounds of muscadine grape juice, were present in both free and glycosidically bound forms. Other important aroma-active compounds such as 2,3-butanedione, ethyl butanoate, and ethyl 2-methylbutanoate were present only in free form. Many monoterpenes were present only in glycosidically bound form. *p*-Vinylguaiacol, a potential off-flavor in muscadine juice, was present mostly in the glycosidically bound form.

These results suggests that controlled application of glycosidase may enhance the characteristic aroma of muscadine grape juice.

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