Tomato Aroma Components: Identification of Glycoside Hydrolysis Volatiles

Ron G. Buttery,* Gary Takeoka, Roy Teranishi, and Louisa C. Ling

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

A glycoside fraction, amounting to 250 ppm, was isolated from fresh tomatoes by using procedures previously established for grapes and fruits. Hydrolysis of the glycoside fraction was carried out at pH 2.5, 3.0, 4.1, and 5.0 to give the volatile aglycons, which were isolated by steam distillation continuous extraction. Major volatiles identified at all pH values included 3-methylbutyric acid, β-damascenone, phenylacetaldehyde, 2-phenylethanol, linalool, hotrienol, α-terpineol, linalool oxides, 4-vinylguaiacol, and 4-vinylphenol. Others also found in smaller amounts included benzaldehyde, dehydro-β-ionol (tentative), dihydroactinidiolide, and more than 15 other apparent C_{13} norisoprenoids that could not be identified. Hydrolysis at pH 2.5 and 3 gave major amounts of 4-(2',3',6'-trimethylphenyl)-3-buten-2-one (tentative), which was not present in the products from hydrolysis at pH 4.1 and 5.

INTRODUCTION

Glycosides have been found to be important precursors of volatiles in grapes and wine [e.g., Williams et al. (1989), Sefton et al. (1989), and Gunata et al. (1985)] and other fruits [e.g., Schwab and Schreier (1988) and Winterhalter and Schreier (1987)]. It has been pointed out by the grape and other fruit researchers that both the enzymes (e.g., during fermentation) and the heat used during processing (under the normal low pH of fruits) can result in the hydrolysis of the glycosides and the release of flavor compounds. Many of the glycosidically bound volatiles in grapes and other fruits have been found by the above researchers to be C_{13} norisoprenoid and other ionone-related compounds. Previous studies on tomato volatiles had identified C_{13} norisoprenoids including geranylacetone (Buttery et al., 1968), β-damascenone (Buttery et al., 1990a), and β-ionone and its epoxide (Viani et al., 1969). Some of the authors had suspected (Buttery et al., 1990a,b) that these and other tomato paste volatiles might result from glycoside hydrolysis in a way similar to that found in grapes and fruits. The present work was begun to learn more about volatile-producing tomato glycosides. Some previous studies on nonvolatile aglycon lipid and steroid producing tomato glycosides had been carried out (Galliard et al., 1977). Results of preliminary studies on isolation of tomato glycosides and their hydrolysis to 3-methylbutyric acid, linalool, α-terpineol, and β-damascenone have been previously presented (Buttery et al., 1989).

EXPERIMENTAL PROCEDURES

Materials. The tomatoes used were a processing line FM 785 grown in the Sacramento Valley, CA, during the 1989 season. The fresh ripe tomatoes were stored at room temperature and used within a few days.

Amberlite XAD-2 (20-60 mesh) resin was washed with water by decantation to remove fine material and then cleaned by treatment in a Soxhlet apparatus with pentane, ethyl acetate, and methanol (refluxing each for 8 h). The cleaned resin was then stored under methanol.

Isolation of Tomato Glycosides. This followed closely methods that had been developed for the isolation of glycosides from grapes (Gunata et al., 1985). Fresh tomatoes (300 g) were blended for 2 min and then filtered immediately through a 0.5 in. thick layer of Celite 545 (prewashed) to give a clear, slightly yellow, filtrate. These operations were carried out as quickly as possible (<1 h) with the tomato blend cooled (0-10 °C). This filtrate was then placed on a column of Amberlite XAD-2 resin, 190 mm long by 45 mm i.d., which had been prewashed with water (500 mL). After the filtrate had passed onto the resin, the column was eluted with water (1500 mL, to remove sugars, etc.). The column was then eluted with pentane (500 mL). Both the water and pentane eluents were discarded. The tomato glycoside fraction was then eluted with methanol (500 mL). The methanol was removed under reduced pressure at 40 °C. The light brownish solid that remained was scraped out of the flask and dried under vacuum in a desiccator over P_2O_5 for 24 h. It was then stored at -20 °C.

Acid Hydrolysis of Tomato Glycosides. For hydrolysis at pH 5 a solution of NaH_2PO_4 (10 g) in water (500 mL) was prepared and boiled in an open flask for 30 min to remove volatiles and then cooled. Tomato glycosides (300 mg) were dissolved in 100 mL of water, and the resulting solution was extracted with diethyl ether (2 × 50 mL). This ether wash was discarded. The aqueous glycoside solution was then added to the above NaH_2PO_4 solution in a 500-mL flask. By use of a Likens-Nickerson steam distillation continuous extraction (SDE) head with diethyl ether as solvent the mixture was refluxed for 3 h at atmospheric pressure. The resulting ether extract was then dried over anhydrous sodium sulfate and concentrated to ca. 100 μL by using a warm water bath.

Hydrolysis was also carried out at pH 2.5, 3.0, and 4.1 in a similar way with fresh samples of tomato glycosides and by adjusting the pH of the NaH_2PO_4 solution with 3 N phosphoric acid.

Exhaustive hydrolysis at pH 4.1 was carried out by using essentially the same procedure as above but refluxing (with SDE) for 67 h. The same mixture of glycosides was then acidified to pH 1.5 with 10% sulfuric acid and refluxed for a further 24 h. The ether extracts were dried and concentrated as for the above samples.

Enzyme Hydrolysis of Tomato Glycosides. Tomato glycosides (80 mg) were dissolved in water (100 mL). β-Glucosidase (25 mg) was added to the solution and the mixture stirred for 2 days at 35 °C. Volatiles were isolated from the mixture by sweeping with air (3 L/min) for 2 h and trapping the volatiles on Tenax. Hydrolysis was also carried out in a similar way except a pectinase (90 mg; Rohapect C, Rohm Tech., Inc.) was used. Volatiles were extracted from the Tenax with ether and concentrated in the usual way.

Capillary Gas-Liquid Chromatography-Mass Spectrometry (GC-MS). This was carried out by using a commercial 60 m long by 0.25 mm i.d. fused silica capillary wall coated with DB-1 in a HP 5890 gas chromatograph that was directly coupled to a HP 5970 quadrupole mass spectrometer. The GLC oven was kept at 30 °C for the first 25 min after injection (1/
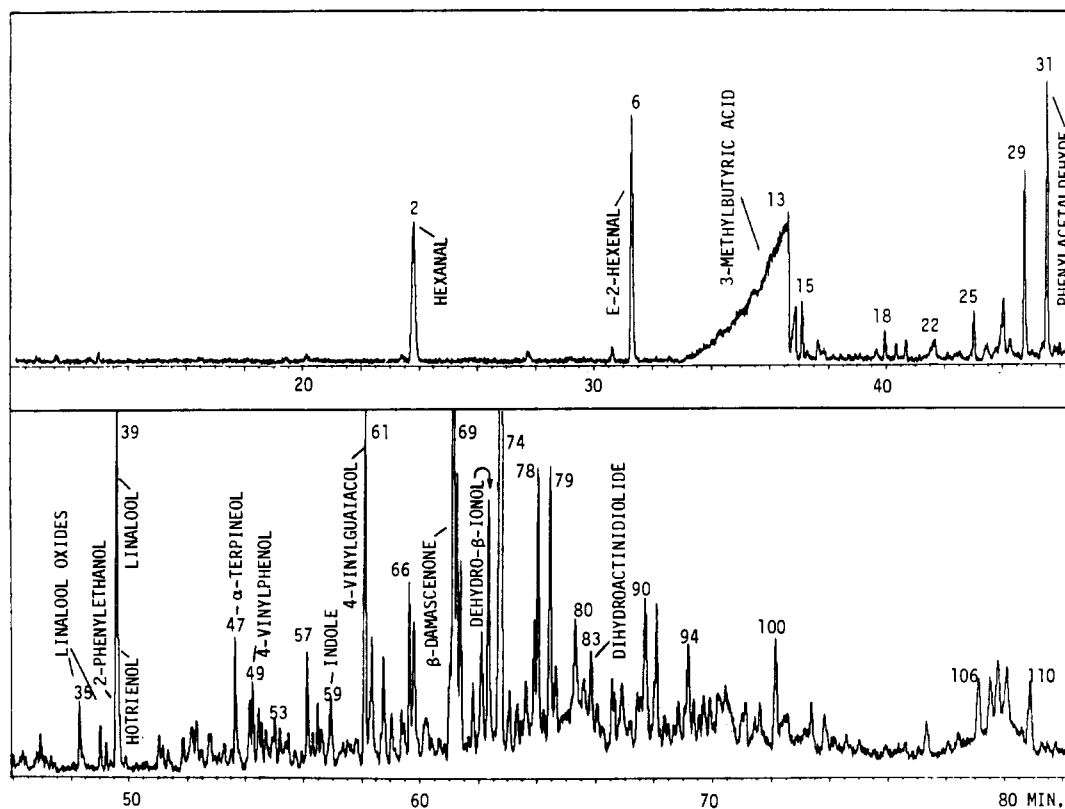
Tomato Aroma Components

J. Agric. Food Chem., Vol. 38, No. 11, 1990 **2051**

Figure 1. Capillary GLC total ion chromatogram of the volatiles isolated (SDE) from hydrolysis of tomato glycosides at pH 4.1.

20 split injector at 170 °C) and then programmed at 4 °C/min until 200 °C and held at that temperature for 30 min.

Authentic Samples. These were obtained from reliable commercial sources or synthesized by established methods. A sample of dihydroactinidiolide was kindly supplied by Dr. Matthias Guntert of Haarmann and Reimer GmbH, West Germany. Compounds were purified by GLC separation and their identities checked by spectral means (MS or IR).

RESULTS AND DISCUSSION

Fresh ripe tomatoes were blended, and the blended material was filtered through Celite 545 to remove fibrous material. The glycoside fraction was then isolated by using a column of Amberlite XAD-2 resin and appropriate eluting solvents following closely the well-established methods for grapes and other fruits (Gunata et al., 1985; Winterhalter and Schreier, 1987). The dried glycoside fraction amounted to 250 ppm (average of three isolations) of the whole tomato. An infrared absorption spectrum (in KBr) carried out on the tomato glycoside fraction showed strong absorption maxima at 3370, 2930, 1637, and 1074 cm^{-1}, moderate absorption maxima at 1513, 1384, 1262, and 1163 cm^{-1}, weak maxima at 746, 700, and 600 cm^{-1}. Some of these absorption maxima (3370 and 1074 cm^{-1}) are consistent with the sugar part of the glycosides. Others at 1513, 746, and 700 cm^{-1} are reasonable for the presence of benzyl groups.

A ^{1}H NMR spectrum (in D$_2$O) showed complex peaks in the area δ 3–4.5 with major maxima at δ 3.4, 3.75, 3.85, 3.95, and 4.4, consistent with a sugar derivative. The spectrum also showed complex aliphatic peaks in the area δ 0.8–2.5, some of which (0.89 and 0.99) indicate possible (CH$_3$)$_2$CHCH$_2$ groups, and complex bands in the area δ 6.8–7.8, some being reasonable for aromatic compounds such as the benzyl group (δ 7.3 peak).

Glycoside Hydrolysis at pH 2.5–5. The acid hydrolysis of glycosides in fruits has usually been carried out at ca. pH 3–3.5, close to the normal pH values of the fruits.

Tomatoes, however, are not quite as acid, and the normal tomato has a pH value of ca. 4–4.6. In the present study hydrolysis of the tomato glycosides was carried out at several pH values from 2.5 to 5.0. The hydrolysis was carried out by adding a measured amount of the tomato glycoside fraction to a dilute solution of NaH$_2$PO$_4$ for pH 5 and for lower pH values together with an adjusted amount of H$_3$PO$_4$. By use of a Likens-Nickerson simultaneous steam distillation continuous extraction (SDE) head at atmospheric pressure with ether as solvent the mixtures were boiled for 3 h at 100 °C. The volatile aglycons were isolated as formed into the ether, which on completion of the hydrolysis was dried and concentrated. The hydrolysis of the glycosides at 100 °C seemed reasonable in view of the fact that tomato processing usually involves similar temperatures.

Figure 1 shows a GLC total ion chromatogram of the volatiles isolated at pH 4.1. Table I lists the compounds identified by GLC-MS with approximate values for the concentration of these compounds as parts per million (ppm) of the glycoside fraction (determined from flame ionization detection peak areas) for the pH values 3, 4.1, and 5. The data in Table I are for the 3-h isolation period. It was found that if the hydrolysis/SDE was continued for longer periods, higher yields of aglycons were obtained. Figure 2 shows the structures of some of the compounds listed in Table I. It can be seen that the major compound produced is 3-methylbutyric acid (together with a small percentage of 2-methylbutyric acid). Schwab and Schreier (1988) have found that 2-methylbutyric acid occurs in the bound form as a glycoside in apples. Other major compounds identified include β-damascenone, phenylacetaldehyde, 2-phenylethanol, linalool, hotrienol, α-terpineol, linalool oxides, 4-vinylguaiacol, 4-vinylphenol, hexanal, and (*E*)-2-hexenal. A major component found with hydrolysis only at the more acid pH values 2.5 and 3 (and not at pH 4.1 and 5) was (*E*)-4-(2′,3′,6′-trimethylphenyl)-

Table I. GLC–MS Identification of Tomato Glycoside Volatiles Hydrolyzed at pH 3.0, 4.1, and 5.0 (Isolated by SDE) and Concentrations Found in Parts per Million of the Total Tomato Glycosides

compd[a]	major mass spec ions[b]	KI[c]	concn in glycoside, ppm		
			pH 3	pH 4.1	pH 5
hexanal	44, 56, 72, 82	772	460	260	290
(E)-2-hexenal	41, 55, 69, 83, 98	844	340	240	240
3-methylbutyric acid	60, 41, 87	830	3100	1100	1200
benzaldehyde	77, 105, 51, 39, 63	918	34	8	20
(E)-2-heptenal	41, 55, 83, 70, 97, 112	927	19	10	16
6-methyl-5-hepten-2-one	43, 69, 55, 108, 126, 83	961	37	20	25
phenylacetaldehyde	91, 120, 65, 39, 51, 77	1006	140	80	130
linalool oxide A	59, 43, 94, 68, 111, 83	1056	100	5	14
linalool B	59, 43, 94, 68, 111, 83	1070	90	6	13
(E)-6-methyl-3,5-heptadien-2-one	109, 43, 81, 53, 124, 65	1074	18	7	12
2-phenylethanol	91, 122, 65, 39, 51, 77	1081			
linalool	93, 71, 41, 55, 80, 121	1083	150	80	85
hotrienol (I)	43, 71, 82, 67, 55, 91	1085			
α-terpineol	59, 93, 121, 81, 136, 43	1170	90	18	27
4-vinylphenol	120, 39, 91, 65, 51, 79	1190	54	10	55
2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9(1)-triene (IV) (tent.)	159, 174, 131, 144, 105, 91	1200	80	6	10
indole	117, 90, 63, 39, 51	1250	40	10	25
4-vinylguaiacol	150, 135, 39, 77, 51, 107	1280	150	42	84
β-damascenone (II)	69, 121, 41, 105, 190, 91	1360	340	82	120
dehydro-β-ionol (VI) (tent.)	119, 43, 192, 159, 91, 105	1400	10	4	5
dihydroactinidiolide (III)	111, 137, 43, 180, 67, 124	1480	<5	7	8
(E)-4-(2′,3′,6′-trimethylphenyl)-3-buten-2-one (V) (tent.)	173, 129, 115, 145, 188, 158	1475	1600	<3	<1

[a] Mass spectrum and GLC Kovats index consistent with that of authentic sample except for those labeled "tent." (tentative), where no authentic sample was available but mass spectrum was consistent with published spectra. [b] One major ion each 14 mass units. Most intense ions first. Molecular ion in italics if shown. [c] Kovats index found on DB1 fused silica capillary.
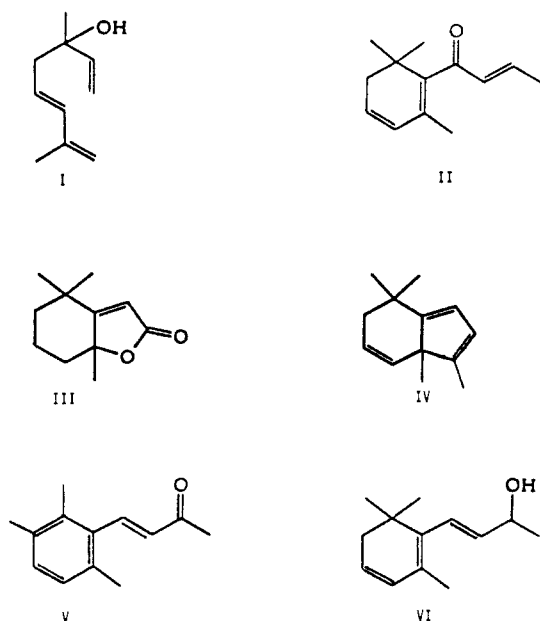
Figure 2. Structures of some of the volatile tomato glycoside hydrolysis products identified.

3-buten-2-one (identified by comparison with published spectra; Thomas et al., 1969). Accompanying this compound were three other unknowns with very similar mass spectra. One is probably the Z form, and the others may represent different arrangements on the benzene ring. The Z and E forms of this compound had been found previously in grape juice by Williams et al. (1982). A number of additional compounds were also identified (MS and GLC RT consistent with authentic samples) in trace amounts which were not resolved enough from other components to make quantitative analysis possible. These included eugenol, benzoic acid, geranial, and (E)-6-methyl-3,5-heptadien-2-one.

Aldehydes and Ketones. Hexanal and (E)-2-hexenal are probably not bound as glycosides in the intact tomato. It seems more likely that after the tomato is blended, there is a high concentration of these alde-

hydes which react with the sugars to form acetals at the normal slight acidity of the tomato. This is probably also true with 6-methyl-5-hepten-2-one in the blended tomato. Such reactions of aldehydes with sugars are well-known [cf. Wolfrom and Thompson (1957)].

Phenylacetaldehyde and benzaldehyde, on the other hand, probably occur as bound glycosides in the intact tomato. We attempted to use saturated CaCl₂ solution during the blending of the tomatoes to stop enzyme formation of hexanal and (E)-2-hexenal and other aliphatic aldehydes. However, the high viscosity and density of the mixture caused problems in filtration and Amberlite XAD-2 chromatography.

Unstable Aglycons. Previous work on grape and fruit glycosides [cf. Williams et al. (1989)] has shown that many of the original aglycons are unstable and that under conditions similar to those used in this study they decompose or rearrange to more stable compounds. Such conditions do occur in the normal processing of tomatoes. Probably the most important of this group is β-damascenone. Sefton et al. (1989) recently reported the identification of the acetylene C₁₃ norisoprenoid megastigm-5-en-7-yne-3,9-diol as the most likely precursor of β-damascenone in grape juice glycosides. This precursor was not found in the tomato glycoside volatiles, but milder isolation conditions (than those used here) are probably necessary. The phenols 4-vinylphenol and 4-vinylguaiacol probably result from decarboxylation of coumaric and ferulic acids, respectively [cf. Tressl et al. (1977)], which themselves are bound as glycosides and released during the hydrolysis.

Exhaustive Hydrolysis. To estimate the maximum amount of volatile aglycons present in the tomato glycosides, hydrolysis was also carried out for a very long period (67 h) at pH 4.1 and later at strongly acid conditions pH 1.5 for an additional 24 h. The maximum amount of volatile aglycons obtained under these conditions amounted to 5.8% of the tomato glycosides. The maximum amount of 3-methylbutyric acid formed 1.3% of the glycosides and 2-phenylethanol 0.5%. This would indicate that the bulk

Tomato Aroma Components

J. Agric. Food Chem., Vol. 38, No. 11, 1990 **2053**

of the glycoside fraction consists of glycosides of nonvolatile aglycons, which is as might be expected.

Unidentified Volatiles. There are a large number of compounds that could not be identified. These include peaks 70, 71, 74, and 77–79 and more than 10 other compounds eluting between 58 and 76 min in Figure 1. All of these compounds seem to be ionone related. Peak 74 is a relatively major component with a molecular weight of 224 and major ions at 122, 43, 165, 147, 109, and 91. Peak 66 has a mass spectrum (major ions at 107, 79, 91, 122, 149, and 55) similar to that reported by Kaiser and Lamparsky (1978) for 2,7-epoxymegastigma-4,8-diene but with some distinct differences in relative ion intensities.

Open-Chain Norisoprenoids. The major norisoprenoids in fresh tomato volatiles are open-chain compounds such as 6-methyl-5-hepten-2-one, geranylacetone, and pseudoionone, which are reasonable fragments of the main tomato pigments such as lycopene and related carotenoids. However, the cyclic norisoprenoids seem to predominate in the volatiles from glycoside hydrolysis, and no geranylacetone or pseudoionone was detected. The mechanisms for the formation of open-chain and cyclic norisoprenoids in tomatoes seem to be different.

Enzyme Hydrolysis. Preliminary studies were also carried out by using enzymes to hydrolyze tomato glycosides. The enzymes used were a β-glucosidase and (separately) a pectinase. Hydrolysis was carried out for 2 days at 35 °C for both enzymes. The yield of volatiles was smaller and showed many fewer volatiles than acid hydrolysis. The volatiles obtained were similar for both enzyme systems and consisted of benzaldehyde, phenylacetaldehyde, 2-phenylethanol, 3-methylbutanol, and (*E*)-2-hexenal.

ACKNOWLEDGMENT

LITERATURE CITED

Buttery, R. G.; Seifert, R. M. Volatile Tomato Constituents: Identification of 2,6-Dimethylundeca-2,6-dien-10-one. *J. Agric. Food Chem.* **1968**, *16*, 1053.

Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C. Identification of Additional Tomato Volatiles. Presented at the 189th National Meeting of the American Chemical Society, Miami Beach, FL, Sept 1989, paper 55.

Buttery, R. G.; Teranishi, R.; Ling, L. C.; Turnbaugh, J. G. Quantitative and Sensory Studies on Tomato Paste Volatiles. *J. Agric. Food Chem.* **1990a**, *38*, 336–340.

Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C. Identification of Additional Tomato Paste Volatiles. *J. Agric. Food Chem.* **1990b**, *38*, 792–795.

Galliard, T.; Matthew, J. A.; Wright, A. J.; Fishwick, M. J. Enzymatic Breakdown of Lipids to Volatile and Non-Volatile Carbonyl Fragments in Disrupted Tomato. *J. Sci. Food Agric.* **1977**, *28*, 863–868.

Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. Extraction and Determination of Free and Glycosidicaly Bound Fractions of Some Grape Aroma Components. *J. Chromatogr.* **1985**, *331*, 83–90.

Schwab, W.; Schreier, P. Simultaneous Enzyme Catalysis Extraction: A Versatile Technique for the Study of Flavor Precursors. *J. Agric. Food Chem.* **1988**, *36*, 1238–1242.

Sefton, M. A.; Skouroumounis, G. K.; Massy-Westropp, R. A.; Williams, P. J. Norisoprenoids in Vitis vinifera White Wine Grapes. *Aust. J. Chem.* **1989**, *42*, 2071–2084.

Strauss, C. R.; Gooley, P. R.; Wilson, B.; Williams, P. J. Applications of Droplet Countercurrent Chromatography. *J. Agric. Food Chem.* **1987**, *35*, 519–524.

Thomas, A. F.; Willhalm, B.; Muller, R. The Mass Spectra of Doubly Unsaturated Carbonyl Compounds. *Org. Mass Spectrom.* **1969**, *2*, 223–239.

Tressl, R.; Bahri, D.; Holzer, M.; Kossa, T. Formation of Flavor Components of Cooked Asparagus. *J. Agric. Food Chem.* **1977**, *25*, 459–463.

Vianni, R.; Bricout, J.; Marion, J. P.; Muggler-Chaven, F.; Reymond, D.; Egli, R. H. *Helv. Chim. Acta* **1969**, *52*, 887–891.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. Use of C_{18} Reversed Phase LC For Isolation of Monoterpene Glycosides and Nor-Isoprenoid Precursors from Grape Juice and Wines. *J. Chromatogr.* **1982**, *235*, 471–480.

Williams, P. J.; Sefton, M. A.; Wilson, B. Nonvolatile Conjugates of Secondary Metabolites as Precursors of Varietal Grape Flavor Components. *Flavor Chemistry, Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989; pp 35–48.

Winterhalter, P.; Schreier, P. Influence of Sample Preparation on the Composition of Quince Flavor. *J. Agric. Food Chem.* **1987**, *35*, 335–337.

Wolfrom, M. L.; Thompson, A. Glycosides, Simple Acetals, Thioacetals. In *The Carbohydrates, Chemistry, Biochemistry, Physiology*; Pigman, W., Ed.; Academic Press: New York, 1957; pp 229–236.