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Formation and occurrence of flavor components in Noble muscadine wine

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Flavor development in Noble muscadine wine during fermentation and aging was determined with a combined gas chromatograph–mass spectrometer. The source of 2-phenylethanol, a major aroma component of the wine, is predominantly through biosynthesis during the vinification process, although the alcohol was also found to be present in fresh muscadine grape skin. The involvement of substituted benzene derivatives in the formation of phenyl ethanol during fermentation and aging of wines is proposed. The complexity of the volatile aroma extracts increased with time, especially after fermentable sugars were exhausted. Anaerobic formation of fatty acid esters also commenced after active fermentation had ceased. These compounds constitute major components of the aged muscadine wine. Effluent sniffing indicated that many odorous compounds including phenyl ethanol and methyl succinate contribute significantly to the muscadine wine aroma. Published by Elsevier Science Ltd

INTRODUCTION

The volatile aroma components of muscadine grapes and wines have been extensively studied (Berry *et al.*, 1979; Kepner & Webb, 1956; Welch *et al.*, 1982; Lamikanra, 1986, 1987). In a recent report (Lamikanra, 1987), it was determined that 2-phenylethanol, a compound that has implicated as being a major aroma component of muscadine grapes, is also present in the corresponding wines. This compound appears to contribute to the roselike and fruity character of wines, and occurs in most wines at different concentrations (Amerine & Joslyn, 1970; Nelson *et al.*, 1978; Nykanen, 1986). The muscadine grape appears to be the only grape variety that has been reported to have significant amounts of phenylethanol. Most studies concerning the aroma compounds of muscadine grapes have involved the analysis of trapped volatiles emerging from steam distilled pulverized grapes (Berry *et al.*, 1979; Kepner & Webb, 1956; Welch *et al.*, 1982).

The presence of 2-phenylethanol in both muscadine grapes and wines could imply a direct transfer of this compound from the must to their wines. A quantitative transfer should result in similar concentrations of the alcohol in both must and wine. Conversely, the involvement of biochemical reactions that either produce or degrade 2-phenylethanol during vinification will alter its concentration with time. 2-Phenylethanol, like most higher alcohols, would normally be expected to be synthesized from its corresponding amino acid, phenylalanine (Nykanen, 1986). The relatively low amount of phenylalanine reported for muscadine grapes (Mercy *et al.*, 1981), however, does not appear to support the occurrence of this reaction as the major pathway for the production of 2-phenylethanol in muscadine wines.

This study examines the progress of flavor development in muscadine wine during vinification and the aroma components of the muscadine wine that contribute significantly to the muscadine wine flavor.

MATERIALS AND METHODS

Sample preparation

Replicated fermentations of the Noble muscadine grapes were carried out as previously described (Lamikanra, 1987). Samples (25 ml) taken during the fermentation and aging processes were extracted with pentane and dichloromethane mixture (7:3). Extracts (5 ml) were filtered after drying over anhydrous sodium sulfate, and concentrated by blowing a stream of nitrogen gas over them until the volume was about 1.0 ml.

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Table 1. Identity of volatile constituents

Peak number	Retention time (min)	Compound	Identification
1	15.80	Ethylbenzene	MS
2	17.50	Ethylmethylbenzene	MS
3	18.18	Propylbenzene	MS
4	18.95	Hexanoic acid ethylester	MS
5	20.15	2-Methylpropyl benzene	MS
6	20.65	2,3-Dichloro pyrazine	Standard
7	21.95	2-Phenylethanol	RI, MS ,Standard
8	22.50	Diethyl succinate (butanedioic acid diethylester)	MS
9	22.75	Methyl succinate (butanedioic acid mono-methylester)	MS
10	22.90	Octanoic acid ethyl ester	MS
11	23.50	Benzothiophene	Standard
12	24.17	2-Phenylacetic acid ethylester	MS
13	28.97	4-Methyl benzaldehyde	MS
14	36.82	Hexadecanoic acid ethyl ester	MS

Analysis of volatile compounds

GC/MS analysis of samples were done at the Southern regional Research Center, New Orleans, LA. They were received in 3 ml screw cap vials. In general the vials contained 1 ml of sample. Ten microliters of external standard consisting of 10 ng/μl solution of 2,3-dichloropyrazine and thionaphthalene in hexane were added to each sample. The samples were then concentrated to 50 μl. Samples below 1 ml were reconstituted to 1 ml with methylene chloride and the external standard was added prior to concentrating the sample. One microliter of the concentrated solution was injected into an HP 5988A GC/MS.

The GC was operated in splitless mode utilizing a 50 m, 0.25 μm I.D., DB-5, capillary column. The oven was programmed at an initial temperature of 50°C for 5 min, then ramped to 250°C at 10°C/min and held for 35 min. The mass spectrometer was operated in scan mode from 40 to 400 amu, using 70 eV electrons for ionization.

Sniffer port analysis was performed on an HP 5970 GC equipped with a flame ionization detector. The injection port was operated in splitless mode and the effluent from a 30 m, DB-5, 0.75 μm I.D. capillary column was split evenly between an FID and a heated sniffer port. The injection port was operated in splitless mode and 2–3 microliters of the concentrated sample were injected. The oven was initially held at 35°C for 5 min then ramped at 3°C/min to 250°C and held for an additional 21 min.

RESULTS AND DISCUSSION

Identified compounds and their respective retention times are shown in Table 1. Analysis of juice taken from freshly crushed grapes on a commercial grape crusher indicated the absence of 2-phenylethanol in expressed juice from muscadine grapes (Fig. 1). The alcohol was, however, present in solvent extracts of whole grapes that were blended in a Waring blender. The presence of this compound in blended muscadine grapes most likely results from its presence in the grape skin. Thus, it is not expected that measurable quantities of 2-phenylethanol would be found in fresh muscadine grape juices when crushers that do not substantially break up the grape skin structure are used.

Previous attempts to determine the volatile aroma compounds of muscadine grapes involved the analysis of trapped volatiles evolving from steam distilled grapes (Kepner & Webb, 1956; Berry *et al.*, 1979; Welch *et al.*, 1982). Steam distillation requires that the sample be held at elevated temperatures for extended periods of time. These may result in the formation or degradation of volatiles. An indication that some of the compounds which were previously identified from steam distilled volatiles of muscadine grapes could have resulted from heat application rather than the presence of these compounds in the grapes, is the relatively high content of acetate esters reported to be present in the grapes. Esters are rarely present in appreciable concentration in grapes, except for methyl anthranilate in Concord grapes (Amerine & Joslyn, 1970). Their presence in wines primarily results from reactions that occur during vinification (Nykanen, 1986). Nelson *et al.* (1978) also reported that acetate esters, particularly 2-phenylethyl acetate and isoamyl acetate were distinctly more abundant in thermally vinified wines. In order to determine the possible effect of heat application on the results reported in these studies, the muscadine grape juice was heated to 98°C on a hot plate for 1 and 2 h, respectively, before solvent extraction. Although it was not possible to conclusively ascertain the effect of heat under these conditions, it was noticed that there were significant increases in the concentration levels of substituted furans in the heated samples.

2-Phenylethanol and its acetic acid ethyl ester derivative were detected in musts that maintained contact with the skin for a period of 5 days. Their presence at this stage apparently results from their biosynthesis during the partial fermentation process and possibly, in part, from the direct extraction of 2-phenylethanol from the skins. The biosynthesis of 2-phenylethanol during fermentation is supported by the increase in its concentration with time after the initial 'skin contact' period

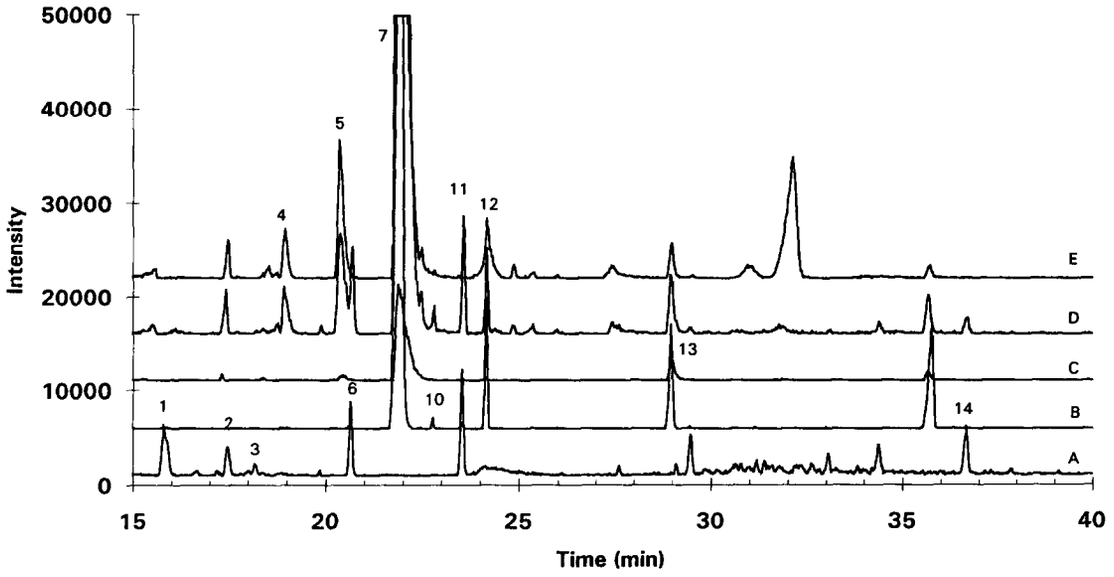

Fig. 1. Chromatograms of extracts from Noble muscadine grape juice (A), its fermenting juice after 10 days (B), and the young wine (C = 5 weeks (when fermentatable sugar was exhausted); D = 7 weeks; and E = 9 weeks after fermentation was initiated). Baselines for each chromatogram have been adjusted by the successive addition of 5000 intensity units.

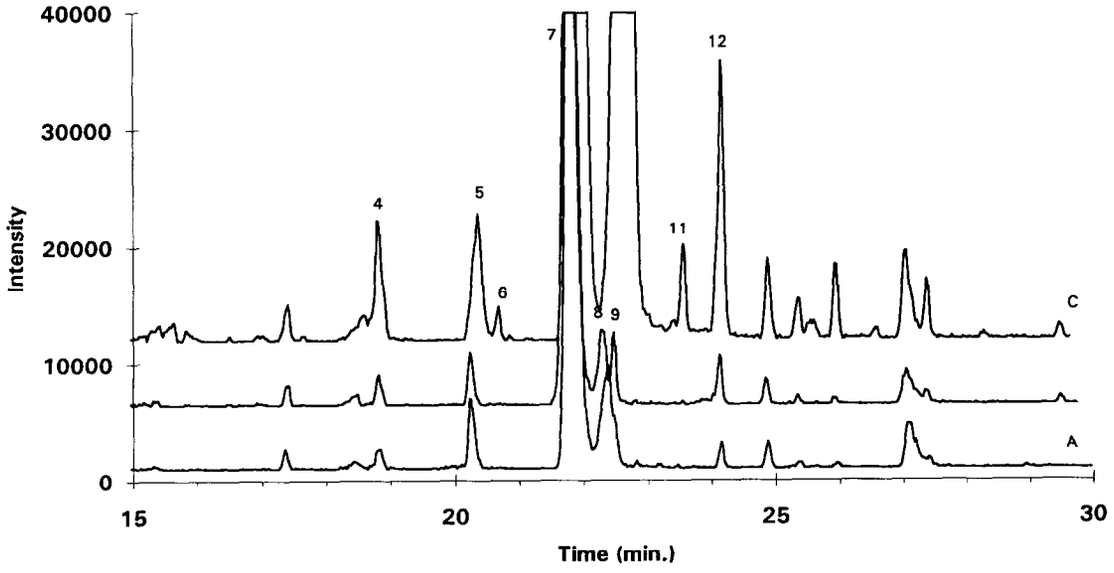

Fig. 2. Effect of aging on aroma compounds of Noble muscadine wine. (A = 26 weeks; B = 36 weeks; and C = 44 weeks after fermentation was initiated.)

(Fig. 1). The increase occurred concurrently with the decrease in ethyl benzene, ethyl methyl benzene, and propyl benzene. The concentration of 2-phenylethanol relative to other volatile constituents of the wine peaked about two weeks after the fermentable sugar was used up (7 weeks after fermentation was initiated), and the formation of butanedioic acid esters as well as other ethyl esters became noticeable. Also noticeable was the development of 4-methyl benzaldehyde during the first 10 days of fermentation. The concentration of this aldehyde, however, subsequently decreased as fermentation progressed. At the first racking (5 weeks after the harvest date), 2-phenylethanol was the predominant compound extracted from the wine.

The complexity of volatile compounds in the wine also increased with age (Fig. 2). Within 2 weeks after racking, several other volatile compounds were detected. The methyl benzaldehyde content continued to decrease with wine age, apparently resulting in the formation of unidentified derivatives that were eluted at 27.1 and 27.4 min, respectively. Effluent sniffing indicated the presence of several odorous compounds, some of which did not correspond to major peaks. It was, however, established that the essence of 2-phenylethanol and butanedioic monomethyl ester and other unidentified compounds contribute significantly to the muscadine wine aroma.

Results of this study support the fact that the source of 2-phenylethanol in muscadine wine is predominantly

through biosynthesis during vinification. Although a very small amount of the alcohol might be contributed to red muscadine wines through direct extraction from the skin during the initial skin contact period with the must, this amount is unlikely to be significant when compared to that produced during the vinification process. In white wines, where the juice is commonly expressed immediately after crushing, and no skin contact period is maintained with the must, the source of 2-phenylethanol in such wines will be solely through biosynthesis. The relative proportion of the alcohol to other volatile compounds is higher than those of most non-muscadine wines (Nykanen & Suomalainen, 1983). The level of 2-phenylethanol in relation to those of the other higher alcohols in muscadine wines is unrelated to the abundance of phenylalanine, a known precursor of the alcohol, relative to other amino acids in the must. Phenylalanine contents of ripe muscadine grapes are lower than those of most amino acids in the grape (Mercy *et al.*, 1981; Kassa, 1994). The concurrent loss of aromatic compounds such as ethyl benzene, ethyl methyl benzene and propyl benzene might, however, indicate their involvement as intermediate compounds in the production of 2-phenylethanol. Such a pathway would imply that phenylalanine is the precursor for 2-phenylethanol in muscadine wines because the deamination and decarboxylation of aromatic amino acids that occur as initial steps in the production of fusel alcohols (Nykanen, 1986) would produce compounds that are analogous. Thus, the high level of 2-phenylethanol in muscadine wines appears to result from conditions and/or compounds present during the vinification process that favor its production in preference to other higher alcohols. This is unique to muscadine grapes, and skin contact with the must during fermentation increases the biosynthetic rate of 2-phenylethanol in muscadine wines (Lamikanra, 1987).

The increase in wine complexity with aging, and the formation of esters of dicarboxylic acids and ethyl esters after active fermentation, are consistent with previous reports on non-muscadine wines (Edwards *et al.*, 1985). Formation of non-volatile wine esters are known to result mainly from anaerobic reactions that occur at the latter stages of fermentation when the fermentable sugar has been exhausted. The strong aroma of muscadine wines might be accounted for by the very high content of 2-phenylethanol and butanedioic acid esters. The presence of butanedioic acid esters is also consistent with reports (Lamikanra, 1993; Lamikanra *et al.*, 1995) that indicate unusually high amounts of succinic acid in muscadine wines, and that the grapes have very high amounts of the acid at fruit set. The significance and role played by this acid and its derivatives, as well as their biosynthetic pathways, need to be further investigated, since their presence in such large quantities appears to be unique to muscadine grape products. The factors that favor the formation of 2-phenylethanol in muscadine wines at a rate higher than those of non-muscadine wines also need to be determined.

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REFERENCES

Amerine, M. A. & Joslyn, M. A. (1970). *Table Wines: The Technology of Their Production*. University of California Press, Berkeley, CA.

Berry, R. E., Coleman, R. L., Wilson, C. W. & Wagner, C. J. (1979). *Tropical Foods: Chemistry and Nutrients*, Vol. 1, eds G. Caralambus & G. E. Inglet, pp. 111–123. Academic Press, New York.

Edwards, T. L., Singleton, V. L. & Boulton, R. (1985). Formation of ethyl esters of tartaric acid during wine aging: chemical and sensory effects. *Am. J. Enol. Vitic.*, **36**, 118–124.

Kassa, A. K. (1995). Changes in free amino acids and some organic compounds with maturity of the Noble cultivar of *Vitis rotundifolia* grape. M.Sc. Thesis, Florida A & M University, Tallahassee, FL.

Kepner, R. E. & Webb, A. D. (1956). Volatile aroma constituents of *Vitis rotundifolia* grapes. *Am. J. Enol. Vitic.*, **1**, 8–18.

Lamikanra, O. (1986). Volatile aroma constituents of Noble muscadine grapes. *Food Chem.*, **19**, 299–306.

Lamikanra, O. (1987). Aroma constituents of muscadine wines. *J. Food Qual.*, **10**, 57–66.

Lamikanra, O. (1993). Changes in organic acid composition during fermentation and aging of muscadine wine. *Am. J. Enol. Vitic.*, **44**, 349.

Lamikanra, O., Inyang, I. D. & Leong, S. (1995). Distribution and effect of grape maturity on organic acid content of red muscadine grapes. *J. Agric. Food Chem.* (in press).

Mercy, J. E., Carroll, D. E. & Young C. T. (1981). Changes in free amino acid and total nitrogen concentrations during maturation of muscadine grapes (*Vitis rotundifolia*). *J. Fd Sci.*, **46**, 543–547.

Nelson, R., Acree, T. E. & Butts, R. M. (1978). Isolation and identification of volatiles from catawba wine. *J. Agric. Fd Chem.*, **26**, 1188–1190.

Nykanen, L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.*, **37**, 84–96.

Nykanen, L. & Suomalainen, H. (1983). *Aroma of Beer, Wine and Distilled Alcoholic Beverages*. Reidel Publishing Co., Boston, MA.

Welch, R. C., Johnson, J. C. & Hanter, G. L. K. (1982). Volatile constituents of muscadine grape (*Vitis rotundifolia*). *J. Agric. Food Chem.*, **30**, 681–684.