

Determination of the Impact of the Metabolites of Sorbic Acid on the Odor of a Spoiled Red Wine

Mary G. Chisholm* and James M. Samuels

The Pennsylvania State University at Erie, The Behrend College, Erie, Pennsylvania 16563

Gas chromatography-olfactometry (GCO) was used to produce an odor chromatogram of a spoiled red wine resulting from treatment with potassium sorbate. A model solution was used to determine the retention indices and odor intensities of the metabolites of sorbic acid resulting from bacterial spoilage. By using the odor chromatogram of the model to identify the sorbic acid metabolites in the wine, the impact of these compounds on the wine could be determined. 2-Ethoxyhexa-3,5-diene (geranium), 1-ethoxyhexa-2,4-diene (mint/garlic), and ethyl sorbate (honey/apple) were found to have the greatest impact on the wine odor.

INTRODUCTION

Sorbic acid [(*E,E*)-2,4-hexadienoic acid] is widely used as a yeast inhibitor for the stabilization of table wines containing residual sugar. Lactic acid bacteria are not inhibited by sorbic acid at levels below 300 mg/L (Edinger and Splittstoesser, 1986a), the legal limit for wines made in the United States, and some strains are unaffected at levels of 1000 mg/L. Sulfur dioxide is an effective inhibitor of lactic acid bacteria at concentrations as low as 10 mg/L free SO₂, at a pH of 3.5 or less (Liu and Gallander, 1983). Used together, sorbate, added as potassium sorbate, and sulfur dioxide can prevent secondary fermentations and control the growth of lactic acid bacteria in sweet table wines at levels as low as 80 mg/L sorbate and 30 mg/L sulfur dioxide (Ough and Ingraham, 1960). At high yeast levels and higher pH, larger quantities of sorbic acid and sulfur dioxide are required for control of yeast and microbial growth. Current practices vary widely but seem to fall in the range 200-250 mg/L sorbate and 10-30 mg/L free sulfur dioxide.

Both sorbic acid and sulfur dioxide lead to undesirable sensory properties in wines. The threshold of free sulfur dioxide varies with the pH of the wine and can be detected above a concentration of 15-40 mg/L (Amerine and Roessler, 1983). The reported values for the odor detection threshold of sorbic acid vary. Ough and Ingraham (1960) report a value of 135 mg/L and found that some members of their trained panel could detect sorbic acid at concentrations of 50 mg/L in a white Riesling. Auerbach (1959), Terceļ and Adamic (1965), and Postel and Drawert (1970) all determined the threshold of sorbic acid to be between 240 and 500 mg/L. Tromp and Agenbach (1981) found that at concentrations of 200 mg/L a trained panel could not distinguish the treated wine from the control. They determined the threshold of sorbic acid to be 300-400 mg/L. Two untrained panels could not distinguish between untreated and treated wine samples containing 200 mg/L sorbic acid (Jakob, 1973; Lück and Nue, 1965).

Wines treated with sorbic acid can develop an odor resembling crushed geranium leaves. This odor appears only when conditions in the wine permit the growth of lactic acid bacteria (Burkhardt, 1973; Radler, 1976; Würdig et al., 1975), which causes the bacterial reduction of sorbic acid to sorbyl alcohol [(*E,E*)-2,4-hexadien-1-ol] (Edinger and Splittstoesser, 1986b; Würdig et al., 1975), the precursor of the compound with a geranium odor. The compound responsible for the geranium odor was identified by two groups as 2-ethoxyhexa-3,5-diene (Crowell and Guymon, 1975; von Rymon-Lipinski et al., 1975), which is one

of several metabolites formed spontaneously in wine from 2,4-hexadien-1-ol.

If bacterial reduction of sorbic acid does not occur, because the growth of lactic acid bacteria has been controlled by added sulfur dioxide, then sorbic acid will eventually react with ethanol to produce ethyl sorbate (ethyl 3,5-hexadienoate) (De Rosa et al., 1983). This ester is reported to possess an objectionable pineapple/celery odor with a threshold of 0.3 mg/L in 10% ethanol-water. Ethyl sorbate is produced as a linear function of time: an initial concentration of 200 mg/L sorbic acid gave 1.65 mg/L ethyl sorbate after 12 months and 2.5 mg/L after 18 months, suggesting that after 10 weeks it is present above the threshold level of 0.3 mg/L.

In wines where bacterial spoilage has occurred, ethyl sorbate will also be formed since it is a natural metabolite in wines containing sorbic acid (Crowell and Guymon, 1975; von Rymon-Lipinski et al., 1975). The bacterial reduction to give 2,4-hexadien-1-ol will therefore compete with the formation of ethyl sorbate, and the product ratio will depend upon the rate at which lactic acid bacteria are formed in the wine. This depends primarily upon sulfur dioxide and pH levels, but as soon as 2,4-hexadien-1-ol is formed, the reaction to give 2-ethoxyhexa-3,5-diene is instantaneous. In addition, there are other odor-active products of this reaction: 3,5-hexadien-2-ol and 1-ethoxyhexa-2,4-diene. Crowell and Guymon (1975) suggested that the ethers possessed the most intense and objectionable odors, but they did not determine the threshold of any of the sorbic acid metabolites or describe their odors.

The objective of this study was to determine the relative intensities of the odors of a red wine that had undergone bacterial spoilage, using gas chromatography-olfactometry (GCO). This technique enables descriptors for each compound with odor activity to be defined, and by determination of their relative intensities, the impact of each on the overall wine odor could be deduced. GCO provides odor measurements because the eluting molecules reach the olfactory receptors directly through the nose. Aroma, which is experienced retronasally, can only be inferred from these studies and is measured using standard sensory analysis (Etiévant and Issanchou, 1987).

By using gas chromatography-mass spectrometry (GC-MS), the compounds with the greatest odor intensity could then be quantified and identified. From these determinations, the impact of the metabolites of sorbic acid on the odor of the wine can be assessed.

EXPERIMENTAL PROCEDURES

Materials. A 1988 nouveau red wine, made from 75% Gamay Beaujolais and 25% Chambourcin grapes, was obtained from a

commercial winery in the Lake Erie region of Pennsylvania. At bottling, 1.5% residual sugar and 50 mg/L free sulfur dioxide were added, and the wine was stabilized by adding 400 mg/L potassium sorbate. The wine was analyzed 16 months after it was made, when a strong odor of geranium leaves was apparent, indicating that lactic bacteria spoilage had occurred, despite the level of sulfur dioxide used. This was believed to be the result of botrytis in the grapes, which consumed significant amounts of the added sulfur dioxide, allowing bacterial spoilage to occur.

Chemicals. Analytical grade Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) and ethyl acetate were redistilled before use. Potassium sorbate and all other chemicals used were obtained from reliable commercial suppliers.

Sample Preparation. Wine samples were prepared for GCO by simple liquid-liquid extraction. The wine was extracted with Freon by stirring at 60 rpm for 30 min at 25 °C. The Freon layer was separated, dried with anhydrous magnesium sulfate, and reduced to an 81-fold concentrate on a rotary evaporator at 35 °C and 0.5 Pa. A series of 3-fold dilutions was prepared for GCO from the concentrated extract, with each sample in the series being one-third the concentration of the previous one. A total of eight samples made up a series, the last having been diluted by a factor of 729 and the first being at a 243-fold concentration, obtained by injecting a 3- μ L sample of the 81-fold extract onto the column. All other column samples were 1 μ L. Additional volatiles were extracted from the same wine sample by repeating the extraction using ethyl acetate and preparing a similar series of diluted samples. All samples were stored at 5 °C in amber bottles until used.

Model Solution. A solution of 2,4-hexadien-1-ol in a wine medium was prepared to determine the relative intensities of the odors of the metabolites of sorbic acid and the retention indices at which they eluted. Sorbic acid was reduced chemically, to avoid isomer mixtures, instead of bacteriologically, using lithium aluminum hydride. Sorbic acid (0.5 g) was reduced to give 0.4 g of 2,4-hexadien-1-ol, confirmed by infrared spectroscopy. The alcohol was dissolved in 15 mL of 95% ethanol and then mixed with 60 mL of 0.1 M hydrochloric acid. This solution was stoppered and stirred for 60 h at 25 °C. The mixture was extracted with 2 \times 10 mL of ethyl acetate, and the combined fractions were dried over magnesium sulfate. The product ratio was taken from the normalized data derived from the integration of GC peaks without consideration of GC-FID response factors. A set of samples, diluted as described above, was prepared for GCO.

Gas Chromatography (GC). The most concentrated solution from the model reaction was analyzed using a Hewlett-Packard gas chromatograph, Model 5890, set up for high-resolution gas chromatography (HRGC). The operating conditions were as follows: column, fused silica, methyl silicone, SPB-1, supplied by Supelco, Bellefonte, PA, 15 m \times 0.32 mm; film thickness, 0.25 μ m; temperature program, isothermal at 35 °C for 3 min and then raised at 6 °C/min to 250 °C; injector temperature, 150 °C; flame ionization detector (FID) temperature, 225 °C; helium carrier gas linear velocity, 25 cm/s; injection mode, splitless.

Retention indices (RI) were measured using a series of *n*-alkanes, C₇-C₁₈, chromatographed under identical operating conditions as the above sample. Retention data from the hydrocarbons were converted to retention indices (Kováts, 1965), which were used to characterize the products from the model solution.

Gas Chromatography-Olfactometry (GCO)-Charm Analysis. Sensory data were collected for the wine extracts and the model solution using a Hewlett-Packard 5840 gas chromatograph, modified for sniffing of the column effluents (Acree et al., 1976). Each set of samples was coded and sniffed blind in random order, as described by Acree et al. (1984). When the sniffing was complete, each set was checked to ensure that at least one sample contained no odors and that the dilution was sufficient to ensure that each compound was below its odor detection threshold. Odor detection thresholds were measured in nanograms per stimulus by determining the amount of an odor-active compound present in the most dilute sample that caused a response from the sniffer. Odor detection thresholds were measured in nanograms per liter by calculating the amount of each compound found by GCO and determining its concentration in the sniff air from the recorded duration of the odor and the flow rate of the sniff air.

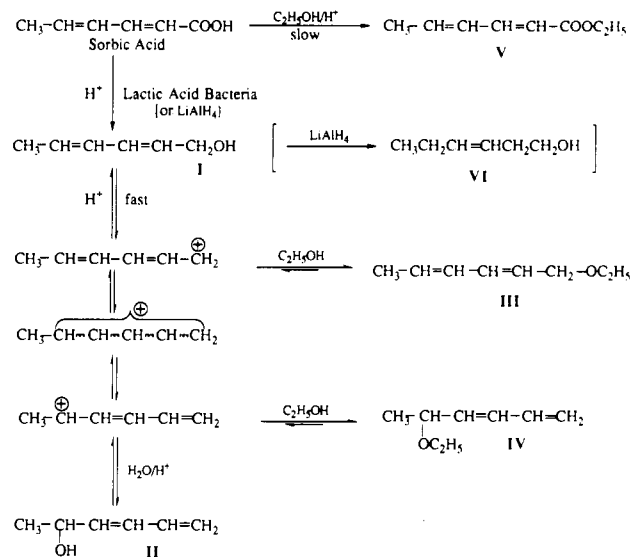


Figure 1. Fate of sorbic acid in wine. The reactions occurring in the model solution are shown in parentheses. I, 2,4-hexadien-1-ol (sorbic alcohol); II, 3,5-hexadien-2-ol; III, 1-ethoxy-2,4-hexadiene; IV, 2-ethoxy-3,5-hexadiene; V, ethyl sorbate; VI, *cis*-3-hexen-1-ol.

The standard hydrocarbon series was run as described above, so that the odors could also be characterized by their retention indices.

The operating conditions for GCO and HRGC were as described for the Hewlett-Packard 5890.

Gas Chromatography-Mass Spectrometry (GC-MS). The mass spectra of compounds found in the model solution were obtained using a Finnigan Inco 50B ion trap mass spectrometer interfaced with a Hewlett-Packard 5890B gas chromatograph. Spectra were collected in the electron impact (EI) mode at 70 eV, using a SPB-1 column, with a source temperature of 225 °C and a filament emission current of 1 mA. The temperature program was isothermal at 60 °C for 2 min and then raised at 20 °C/min to 250 °C. Spectra were recorded on a Data General DG 10 Inco operating system. Identification was achieved by using the NIST library of mass spectra and by comparison to published spectra (McLafferty and Stauffer, 1989).

The operating conditions for the wine extracts were identical except that the rate of heating was 6 °C/min.

RESULTS AND DISCUSSION

Model Solution. Four major products are formed spontaneously when 2,4-hexadien-1-ol is placed in an acidic environment (Crowell and Guymon, 1975). In addition, a small amount of *cis*-3-hexen-1-ol together with trace amounts of ethyl sorbate was found. A reaction scheme for the formation of these metabolites is shown in Figure 1. The product ratios, together with their retention indices, odor descriptors, and odor detection thresholds, are shown in Table I. Mass spectra for products I-IV are shown in Figure 2. The odor and FID chromatograms of the model solution are shown in Figure 3. 2-Ethoxy-3,5-hexadiene was found to possess over 99% of the total Charm (Acree et al., 1984) of the model solution, indicating that it will impact more on the odor of a spoiled wine than the other metabolites of sorbic acid. The yields of ethyl sorbate and *cis*-3-hexenol were too low under the reaction conditions used to be detected in the odor chromatogram. The odor detection threshold of ethyl sorbate was determined by diluting a sample of known concentration until it could no longer be detected by GCO.

Wine Odor. The odor chromatograms of the Freon and ethyl acetate extracts of the spoiled nouveau wine are shown in Figure 4. The compounds of highest odor intensity are listed in Table II together with the metabolites of sorbic acid. 2-Ethoxy-3,5-hexadiene and 1-ethoxy-2,4-

Table I. Products I-VI from Model Reaction

| no. | compound | % formed | odor descriptor | Kovats index (SPB-1) | threshold, ng/stimulus |
|-----|------------------------|-----------------|-----------------|----------------------|------------------------|
| I | 2,4-hexadien-1-ol | 11.8 | fresh grass | 882 | 787 |
| II | 3,5-hexadien-2-ol | 30.1 | green leaves | 786 | 6020 |
| III | 1-ethoxy-2,4-hexadiene | 15.6 | mint/garlic | 936 | 115 |
| IV | 2-ethoxy-3,5-hexadiene | 38.8 | geranium leaves | 846 | 0.13 |
| V | ethyl sorbate | tr ^a | honey/apple | 1075 | |
| VI | cis-3-hexen-1-ol | 2.6 | straw | 854 | |

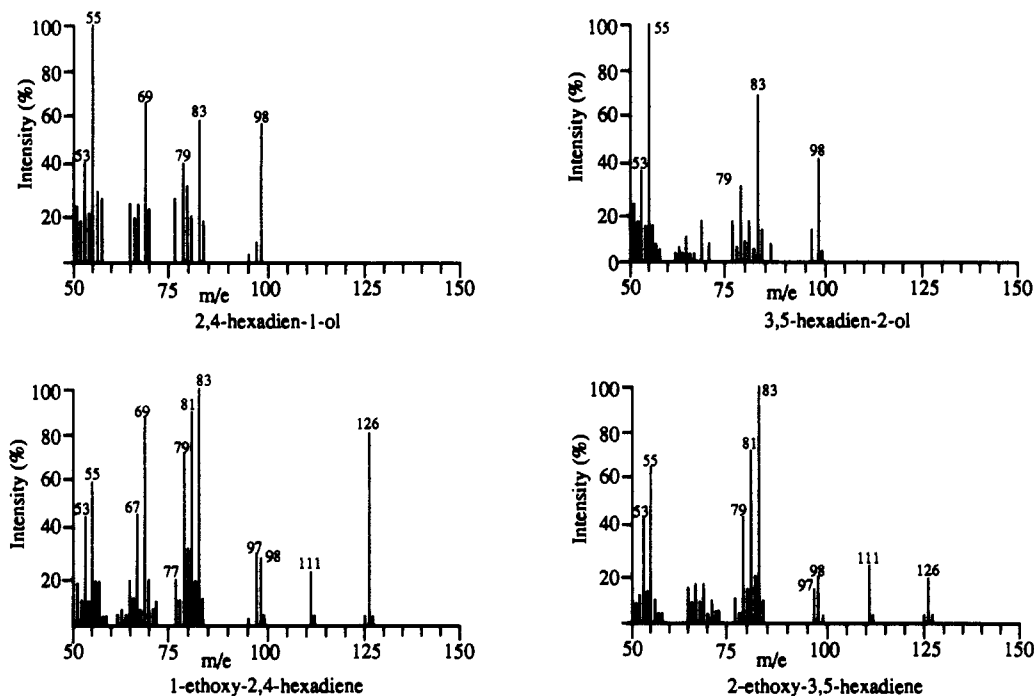
^a Trace.

Figure 2. Mass spectra of the products from the model reaction.

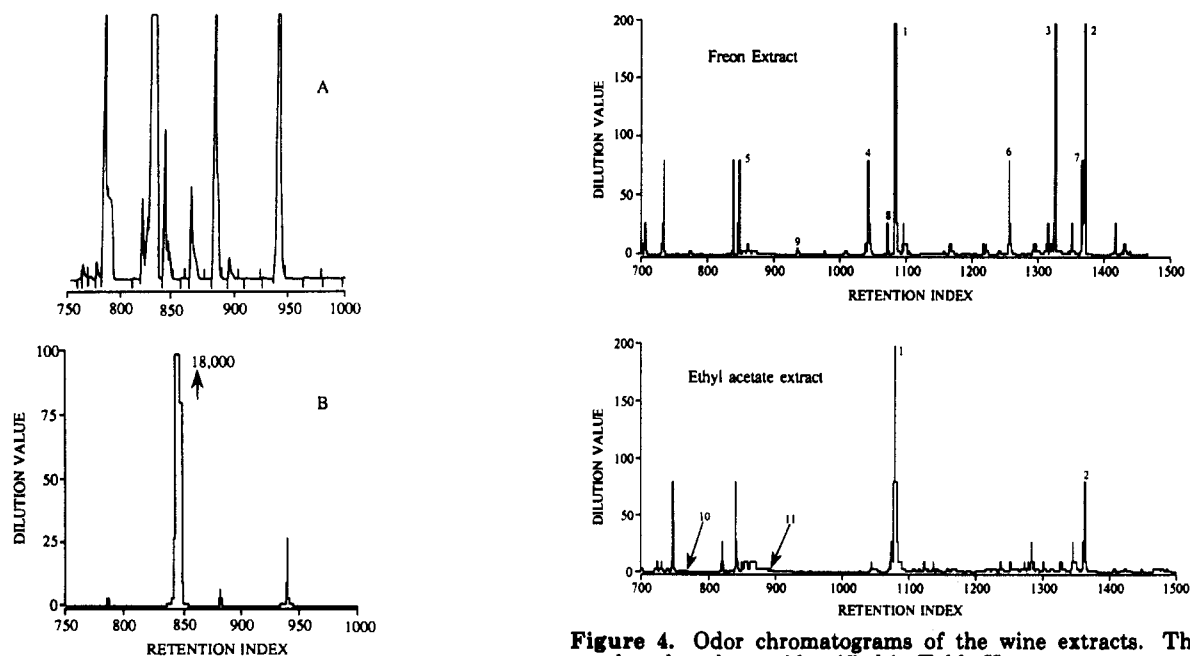


Figure 3. FID (A) and odor (B) chromatograms of the products from the model reaction.

hexadiene are extracted completely by Freon, whereas 3,5-hexadien-2-ol and 2,4-hexadien-1-ol are not extracted by Freon and appear in the ethyl acetate extract. The odor chromatograms show that the contribution to the complete wine aroma from these compounds is significant in the case of 2-ethoxy-3,5-hexadiene and negligible in the case of the other compounds. The contribution from ethyl sor-

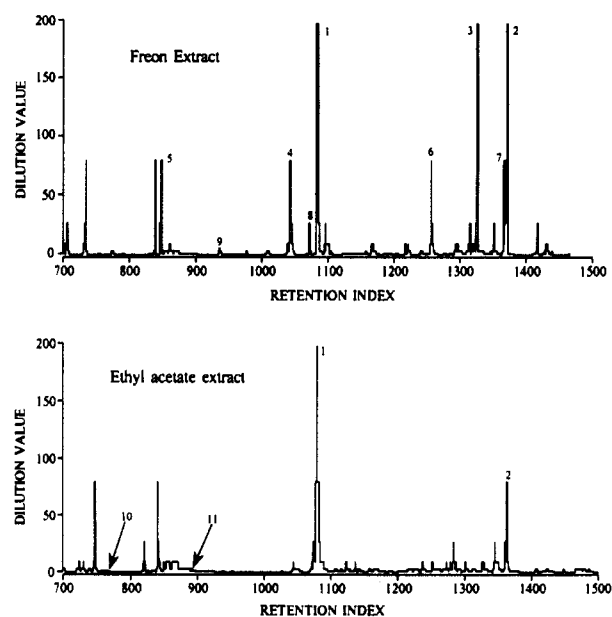


Figure 4. Odor chromatograms of the wine extracts. The numbered peaks are identified in Table II.

bate, not present in the model, but formed over a period of time in the wine, is also significant and shows predominantly in the Freon extract.

Very low concentrations of these compounds were detected by GCO, although they did not show in the mass spectral data. The alcohols were detected by the sniffer once in the most concentrated extract, and 1-ethoxy-2,4-hexadiene was detected in two dilutions. By tagging these

Table II. Major Odor-Active Compounds Found in Freon Extract

| peak no. | compound | Kovats index (SPB-1) | odor descriptor | % Charm |
|-------------------|------------------------|----------------------|-----------------|-----------------|
| 1 ^b | 2-phenylethanol | 1083 | rose | 45.0 |
| 2 ^b | β -damascenone | 1368 | cooked apple | 16.1 |
| 3 | ? | 1323 | musty | 11.8 |
| 4 | ? | 1043 | urine | 4.1 |
| 5 ^c | 2-ethoxy-3,5-hexadiene | 850 | geranium leaves | 3.3 |
| 6 | ? | 1256 | floral | 2.8 |
| 7 ^b | vanillin | 1350 | chocolate | 1.7 |
| 8 ^b | ethyl sorbate | 1071 | honey/apple | 1.0 |
| 9 ^c | 1-ethoxy-2,4-hexadiene | 940 | mint | 0.3 |
| 10 ^{a,c} | 3,5-hexadien-2-ol | 775 | green leaves | tr ^d |
| 11 ^{a,c} | 2,4-hexadien-1-ol | 891 | grass | tr |

^a Found in ethyl acetate extract. ^b Identification by MS(EI) of the wine extract, RI, and aroma at the sniff port. ^c Identification by MS(EI) of the model solution, RI, and aroma at the sniff port. Compounds 1-7 are the most intense aroma compounds found in the freon extract (see Figure 4). Compounds 5 and 8-11 are metabolites of sorbic acid. ^d Trace.

compounds in the model solution, it was possible to identify them in the wine from their retention index and odor descriptor, as they are present in above-threshold levels but below FID or MS detection limits.

Table II shows that 2-phenylethanol and β -damascenone were found to be the most dominant odors in the spoiled wine. These odors dominate the aroma of many wines, and β -damascenone is generally considered to be a desirable aroma compound (Winterhalter et al., 1990). It can also be seen that several of the primary odors found in the wine sample were described with words like musty and urine. Of the metabolites of sorbic acid, only 2-ethoxy-3,5-hexadiene and ethyl sorbate made any measureable impact on the wine odor. 2-Ethoxy-3,5-hexadiene, with an odor detection threshold of less than 1 ng/L, will always be present in above-threshold amounts if bacterial spoilage has occurred. Ethyl sorbate will eventually be formed whether bacterial spoilage has occurred or not. Its odor detection threshold was found to be 0.56 mg/L, which is similar to the value reported by De Rosa et al. (1983). This is low enough that it will be detectable in wines where sorbic acid has been added (~0.37 g/L as potassium sorbate), but its pineapple/celery aroma, although reported as responsible for an adverse flavor effect (De Rosa et al., 1983), was found in this study to possess an apple/honey tone.

CONCLUSION

Odor intensity measurements show that if sorbic acid is used as a yeast inhibitor for the stabilization of wines containing residual sugar, then it is imperative that bacterial spoilage be prevented. 2-Ethoxy-3,5-hexadiene, with its offensive geranium-like odor, has an odor detection threshold that is low enough to ruin a wine if it is formed in even trace amounts. The other metabolites resulting from bacterial spoilage have higher odor detection thresholds, and although they are formed in quantities similar to that of 2-ethoxy-3,5-hexadiene, they have a minimal effect on the odor of the wine and have much less offensive odors as shown by GCO. Ethyl sorbate will be formed in above-threshold amounts whenever sorbic acid is used. However, its ester-like odor may produce an aroma that is more acceptable in a wine than that of 2-ethoxy-3,5-hexadiene.

ACKNOWLEDGMENT

We thank Terry E. Acree for help in interpreting the Charm chromatograms and Lord Corp., Chemical Products

Group, 2000 West Grandview Blvd., Erie, PA 16509, for mass spectra determinations.

LITERATURE CITED

- Acree, T. E.; Butts, R. M.; Lee, C. Y. Sniffer to determine the odor of gas chromatographic effluents. *Anal. Chem.* **1976**, *48*, 1821-1822.
- Acree, T. E.; Barnard, J.; Cunningham, D. G. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.* **1984**, *14*, 273-286.
- Amerine, M. A.; Roessler, R. B. *Wines, Their Sensory Evaluation*; Freeman: New York, 1983; pp 36-37.
- Auerbach, R. C. Sorbic acid as a preservative agent in wine. *Wines Vines* **1959**, *40*, 26-28.
- Burkhardt, R. Occasional occurrence of unpleasant odor and flavor of musts (sweet reserve) and wines after treatment with potassium sorbate. *Ges. Dtsch. Chem. Fachgruppe Lebensm. Gericht. Chem. Mitteilungsbl.* **1973**, *27*, 259-261.
- Crowell, E. A.; Guymon, J. F. Wine constituents arising from sorbic acid addition, and identification of 2-ethoxyhexa-3,5-diene as source of geranium-like off-odor. *Am. J. Enol. Vitic.* **1975**, *26*, 97-102.
- De Rosa, T.; Margheri, G.; Moret, I.; Scarponi, G.; Versini, G. Sorbic acid as a preservative in sparkling wine. Its efficacy and adverse flavor effect associated with ethyl sorbate formation. *Am. J. Enol. Vitic.* **1983**, *34*, 98-102.
- Edinger, W. D.; Splittstoesser, D. F. Sorbate tolerance by lactic acid bacteria associated with grapes and wine. *J. Food Sci.* **1986a**, *51*, 1077-1078.
- Edinger, W. D.; Splittstoesser, D. F. Production by lactic acid bacteria of sorbic alcohol, the precursor of the geranium odor compound. *Am. J. Enol. Vitic.* **1986b**, *37*, 34-38.
- Etiévant, P. X.; Issanchou, S. Wine Taste. *Recherche* **1987**, *18*, 1344-1353.
- Jakob, L. The use of sorbic acid for the preservation of table wine. *Allg. Dtsch. Weinfachztg.* **1973**, *109*, 360-361.
- Kováts, E. Gas chromatographic characterization of organic substances in the retention index system. *Adv. Chromatogr.* **1965**, *1*, 229-247.
- Liu, J. W. R.; Gallander, J. F. Effect of pH and sulfur dioxide on the rate of malolactic fermentation in red table wines. *Am. J. Enol. Vitic.* **1983**, *34*, 44-46.
- Lück, E.; Nue, H. Prevention of secondary fermentation of sweet wines with sorbic acid. *Z. Lebensm. Unters. Forsch.* **1965**, *126*, 325-335.
- McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*; Wiley: New York, 1989.
- Ough, C. S.; Ingraham, J. L. Use of sorbic acid and sulfur dioxide in sweet table wines. *Am. J. Enol. Vitic.* **1960**, *11*, 117-122.
- Postel, W.; Drawert, F. Sensory threshold value of sorbic acid in German white wines. *Z. Lebensm. Unters. Forsch.* **1970**, *144*, 245-252.
- Radler, F. Degradation of sorbic acid by bacteria. *Bull. O.I.V.* **1976**, *49*, 629-635.
- Terceij, D.; Adamic, J. The biological stabilization of sweet wines with sorbic acid and diethylpyrocarbonate. *Rebe Wein* **1965**, *15*, 279-290.
- Tromp, A.; Agenbach, W. A. Sorbic acid as a wine preservative—its efficacy and organoleptic threshold. *S. Afr. J. Enol. Vitic.* **1981**, *2*, 1-5.
- von Rymon-Lipinski, G. W.; Lück, E.; Oeser, H.; Lömker, F. Formation and causes of the "geranium off-odor". *Mitt. Klosterneuburg* **1975**, *25*, 387-394.
- Winterhalter, P.; Sefton, M. A.; Williams, P. J. Volatile C₁₃-norisoprenoid compounds in Riesling wine are generated from multiple precursors. *Am. J. Enol. Vitic.* **1990**, *41*, 277-283.
- Würdig, G.; Schlotter, H. A.; Klein, E. Origin of geranium aroma in wines treated with sorbic acid. *Connaiss. Vigne Vin* **1975**, *9*, 43-55.

Received for review September 19, 1991. Accepted January 8, 1992.

Registry No. Ph(CH₂)₂OH, 60-12-8; CH₂=CHCH=CHCH(OEt)CH₃, 56752-55-7; (E,E)-CH₃CH=CHCH=CHCO₂Et, 2396-84-1; CH₃CH=CHCH=CHCH₂OEt, 56752-54-6; CH₂=CHCH=CHCH(OH)CH₃, 3280-51-1; CH₃CH=CHCH=CHCH₂OH, 111-28-4; (E,E)-CH₃CH=CHCH=CHCO₂H-K, 24634-61-5; β -damascenone, 23726-93-4; vanillin, 121-33-5.