

Potent Aroma Compounds of Two Red Wine Vinegars

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Gas chromatography olfactometry (GCO) was used to determine key aroma compounds of two red wine vinegars. Sensory analysis was performed to choose the best neutralization agent of acetic acid (NaOH or MgO) and to test representativeness of four extracts obtained by different methods (dichloromethane extraction, XAD-2, mixture of XAD-2 and XAD-7, and Extrelut resins extraction). Neutralization with NaOH followed by dichloromethane extraction was selected to extract volatile compounds of vinegars. Key odorant compounds were determined by GCO based on detection frequency with 13 people. In the two red wine vinegars, 13 odors were perceived by at least 70% of the panelists, and 8 compounds among the 13 were identified: acetic acid, 3-methylbutyric acid, 2-phenyl-1-ethanol, 2,3-butanedione, butyric acid, 2-methylbutyric acid, mixture of 2- and 3-methyl-1-butanol, and two newly identified compounds in vinegar, 3-hydroxy-2-pentanone and 3-(methylthio)-1-propanal. Quantification of all the volatile compounds was performed by GC-FID, and 10 other compounds were identified for the first time in wine vinegar.

Keywords: *Aroma; red wine vinegar; gas chromatography olfactometry; detection frequency; quantification*

INTRODUCTION

Wine vinegar is used as condiment and also as ingredient in a lot of dressing sauces. The flavor of wine vinegar depends on the constituents formed during the fermentation of the wine and during the stock or the aging. To increase the aromatic quality of wine vinegar and to propose new products to the consumers, manufacturers have to choose the best raw material as well as the best process of acetification. A preliminary step to reach this objective could be the determination of volatile compounds that have an impact on the odor of the vinegar and to quantify them during the process. Volatile compounds of vinegar have been investigated by different authors (Aurand et al., 1966; Khan et al., 1972; Blanch et al., 1992; Gerbi et al., 1992). However, there is no information on the impact of these compounds for wine vinegar aroma. Even if acetic acid is responsible for the typical vinegar note, other volatile compounds should have a non-negligible flavor impact for the overall vinegar aroma.

Gas chromatography olfactometry (GCO) is a powerful tool for the identification of key flavor compounds in aroma extracts (Mistry et al., 1997). Different olfactometry techniques have been developed: dilution methods such as CHARM analysis (Acree et al., 1984) and aroma extract dilution analysis (AEDA) (Ulrich and Grosh, 1987); and intensity methods such as OSME (McDaniel et al., 1990; Miranda-Lopez et al., 1992). Abbott et al. (1993a) and Guichard et al. (1995) observed points of uncertainty concerning between- and within-individual reproducibility for these two techniques.

Recently, a new method based on detection frequency was proposed by Linssen et al. (1993) and Pollien et al. (1997). Priser (1997) compared the three types of techniques (CHARM, OSME, and detection frequency) on champagne wines and concluded that the key compounds contributing mainly to the flavor were identical whatever the method considered. However, detection frequency method seems to be a better way to determine key compounds in a minimum of time because it does not require a trained panel. Only one injection by panelist is needed, and the great number of panelists limits the problem of anosmia.

Etievant et al. (1994) pointed out the necessity of obtaining an extract representative of the product. Due to its high concentration in vinegar and high volatility, acetic acid is the main odor compound in vinegar extracts, and it is difficult to identify other volatiles present in trace amounts. It is thus necessary to eliminate acetic acid before extraction. To neutralize acetic acid, Jones and Greenshield (1969) used sodium hydroxide solution, but saponification of the esters might occur. Acids could also be neutralized with solid magnesium oxide (Khan et al., 1972). More recently, Gerbi et al. (1992) proposed ammonia to have a more precise pH control compared with magnesium oxide. We decided to compare neutralization of acetic acid with sodium hydroxide and magnesium oxide. Ammonia was discarded due to its undesirable odor.

Several techniques have already been applied to wine vinegar aroma extraction. Direct injection of vinegar was performed by Jones and Greenshields (1969), Khan et al. (1966), Mecca and Vecchio (1977), Cabezudo et al. (1978), and Troncoso-Gonzales and Guzman-Chozas (1987). This technique is limited to the compounds present in high concentration, such as alcohols and esters. Determination of vinegar flavor compounds by headspace analysis (Aurand et al., 1966; Ferrer-Gime-

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nez and Clotet-Ballus, 1979) was also limited by the lack of sensitivity (~10 compounds identified). Solvent extractions were performed by Suomalainen and Kangasperko (1963), Kahn et al. (1972), and Gerbi et al. (1992) with a mixture of solvents, diethyl ether/pentane (2:1), or pentane/dichloromethane (7:3). A lot of compounds have been identified in different chemical classes: alcohols, esters, acids, carbonyl compounds, lactones, and ethers. Simultaneous steam distillation–solvent extraction was tested by Blanch et al. (1992) on conventional and sherry wine vinegars. This technique allows extraction of the same classes of compounds as solvent extraction but could lead to the formation of artifacts due to the heating of the sample at 120 °C. Gerbi et al. (1992) used Extrelut resin on wine vinegar. Compared with solvent extraction, the aroma profile is the same but the concentrations of some compounds (alcohols, acids, and esters) are higher. Thus, we decided to test the following extraction techniques: liquid–liquid with dichloromethane, Extrelut resin, XAD-2 resin, and a mixture of XAD-2 and XAD-7 resins.

The purpose of our work is to identify odorant compounds of two different red wine vinegars. The effect of neutralization on the odor of the vinegar was tested using two agents of neutralization: NaOH and MgO. Four extraction techniques were performed on neutralized vinegars. The technique giving the most representative extract was selected by sensory analysis. GCO was then applied on the most representative extract using detection frequency treatment. Quantification and identification of the volatile compounds were made by GC-FID and GC/MS, GC/IR, and chemical ionization, respectively.

MATERIALS AND METHODS

Vinegars. Two red wine vinegars were analyzed: vinegar A (7° acetic, pH 3.00) was a commercial brand and vinegar B (8° acetic, pH 3.06) was used as an ingredient in food products.

Analytical Reagents. Sodium hydroxide was obtained from J. T. Baker (Deventer, Holland) and solid magnesium oxide from Prolabo (Paris, France). Dichloromethane (97% pure) (SDS, Villeurbanne, France) was distilled. Water was purified with a Milli-Q system (Millipore S.A., Saint-Quentin, France). XAD-2 was purchased from Prolabo and XAD-7 from Fluka (Buchs, Switzerland). The resins were washed separately in a Soxhlet apparatus with methanol for 24 h and then with diethyl ether for 24 h (Guichard et al., 1993). The resins were dried for 60 min at room temperature and then for 24 h at 60 °C. Extrelut resin, a wide-pore kieselguhr with a high pore volume, was obtained from Merck (Clevenot, France). Resin was purified according to the method described by Dirninger et al. (1993).

Neutralization of Vinegar. *Chemical Part.* A scheme of the experimentation is given in Figure 1. An aliquot of vinegar B (initial pH 3.06) was neutralized to pH 6 with sodium hydroxide (vinegar NaOH) or solid magnesium oxide (vinegar MgO). From the initial acidity of the vinegar (80 g of acetic acid/L), the quantity of acetic acid neutralized was estimated and added to the neutralized vinegars. The pH was then adjusted to its initial value with HCl (33%). These vinegars were labeled vinegar NaOH^o and vinegar MgO^o.

Sensory Part. A panel of 20 assessors was constituted. Two triangular tests were performed to compare (a) vinegar NaOH^o and initial vinegar and (b) vinegar MgO^o and initial vinegar. Both triangular tests were performed in the same session and were duplicated in another session. The order of tests was equilibrated for each session: half of the panel began the sessions with test a, the other half with test b. To be tested, initial vinegar and neutralized vinegars were diluted to contain only 1% acetic acid (Gonzales-Vinas and Salvador, 1996).

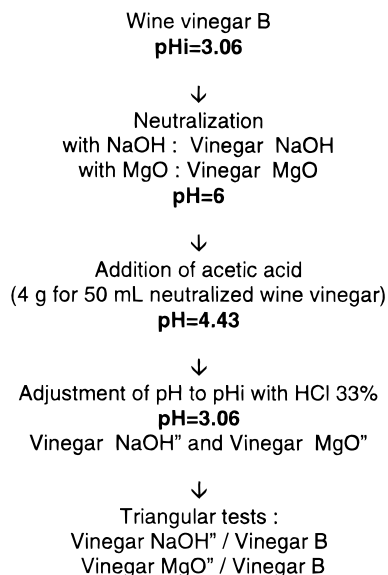


Figure 1. Scheme of the experimentation on neutralization of vinegar.

Presentation of the samples was made in black coded glasses (15 mL) with red light and was randomized over all the subjects.

Extraction Techniques. *Dichloromethane Extraction.* An aliquot of 200 mL of neutralized vinegar and 50 mL of dichloromethane were mixed in a flask cooled with melting crushed ice for 30 min. The vinegar/dichloromethane emulsion formed during stirring was broken with a Teflon bar in the separatory funnel. The same operation was made twice with 30 mL of CH₂Cl₂. Extract was dried over anhydrous sulfate and stored at −18 °C.

XAD-2 Resin Extraction. The volatile constituents were extracted following the procedure of Hawthorne et al. (1987). Neutralized vinegar, 45 mL, 2 g of XAD-2, 13.5 g of NaCl, and 2 mL of 1 N HCl were magnetically stirred for 90 min at 200 rpm in a flask and then poured into a glass column (35 cm long and 1.5 cm i.d.) plugged with glass wool. Complete transfer of the resin was achieved by rinsing out the bottle with saturated NaCl solution (3 × 10 mL). Excess of salt was removed with 2 × 10 mL of bidistilled water. Volatile compounds were eluted from XAD-2 resin with dichloromethane (5 × 2 mL) with 2 min contact time between each solvent addition. Extract was dried over anhydrous sulfate and stored at −18 °C. A total of 180 mL of neutralized vinegar was analyzed in four extractions.

Mixture of XAD-2 and XAD-7 Resins Extraction. The volatile compounds of 180 mL of neutralized vinegar were extracted with the same procedure as XAD-2 resin extraction using a mixture of 2 g of XAD-2 and 2 g of XAD-7.

Extrelut Extraction. This extraction was based on the method proposed by Gerbi et al. (1992). A column (45 cm long and 2.5 cm i.d.) plugged with glass wool was filled with 25 g of Extrelut resin. Vinegar (45 mL) was percolated through the resin until complete adsorption (~30 min). Volatiles were eluted continuously with 125 mL of dichloromethane. Dichloromethane extract was dried over anhydrous sulfate and stored at −18 °C. A total of 180 mL of neutralized vinegar was analyzed in four extractions.

For each resin, the four extracts were pooled together. The extracts were then concentrated to 1 mL with a Kuderna-Danish apparatus at 60 °C and then to 100 μL under nitrogen flow.

Representativeness of the Extracts. *Panel.* The panel was composed of 11 assessors of our laboratory (7 females and 4 males).

Sample Preparation and Presentation. As vinegar is mainly composed of water, extracts were reincorporated into water for the odor comparison with neutralized vinegar. To have an equivalent odor intensity of the extracts reincorporated in

water and of the neutralized vinegar, the extracts were diluted in water in the following proportion: neutralized vinegar, 1:11; liquid-liquid extract, 100 μL in 400 mL of water; resins extracts, 100 μL in 200 mL of water. At these concentrations dichloromethane, which has an undesirable odor and an anesthetic effect, was not smelled. Concentration and dilution of the extracts were done the day before the session and samples stored at 4 °C overnight.

Similarity Test. The reference (neutralized vinegar) and the four samples (15 mL of reference or samples in black coded glasses covered with a Petri box) were presented to the panel. The order of sample presentation was randomized over all the subjects. Panelists were asked to smell the odor of the reference first and then the odors of the four samples. They had to place each of the four extracts on the same unstructured 10 cm scale anchored with "near to the reference" at the left and "far from the reference" at the right. Similarity scaling was measured between the left anchor of the scale and the position of the sample on the scale. Results were analyzed with a two-way analysis of variance (similarity scaling = sample + subject + ϵ) with the Statistical Analysis System software (SAS Institute, Inc., Cary, NC). A Student-Newmans-Keuls test was used to perform a multiple comparison of means. Values were also converted into ranks.

GCO. *GC Conditions.* For GCO analysis, the factor of concentration of the extracts (1/200) was chosen to detect between 25 and 35 odorants. Injection of 2 μL of the concentrated extract was performed on a Hewlett-Packard 5890 Series II chromatograph equipped with an on-column injector (J&W Scientific Inc., Folsom, CA) and a DB-FFAP fused capillary column (30 m \times 0.32 mm i.d., film thickness = 0.25 μm) (J&W Scientific Inc.) directly connected to both flame ionization detector and sniffing port. The hydrogen carrier gas velocity was 50 cm s^{-1} . The injector and detector temperatures were maintained at 250 °C, and the oven temperature was held at 40 °C for 2 min and then programmed to 240 °C at 5 °C min^{-1} . Humidified air was added in the sniffing port at 100 mL min^{-1} . Homemade software COCONUT (R. Almanza and P. Mielle, INRA) was used for data acquisition. A solution of hydrocarbons (C_{10} – C_{26}) was injected daily in the same conditions to calculate retention indices.

Odor Detection Frequency. A panel of 13 judges was selected among people already experienced in GCO. Assessors were asked to smell the effluent of the column (45 min analysis) and to press the space bar on a computer keyboard during the whole sensory impression. They were also asked to give a verbal description of each perceived odor, even if they did not recognize the odor (descriptor = unknown). The start time and end time of the odor-active region measured in seconds were collected directly with the COCONUT software. Data were processed with CHRISKOV software (R. Almanza, INRA): linear retention indices corresponding to the start and the end of an odor area were computed and visualized on the chromatogram. Thus, an odor-active region was defined by a verbal description, a starting linear retention index and an end linear retention index. For the two extracts, data from the 13 panelists were first analyzed separately and were then cumulated. The detection frequency of odor having the same retention time and a similar description were calculated.

Identification. *Mass Spectrometry and Chemical Ionization.* Identifications were performed on a Hewlett-Packard 5890 Series II chromatograph coupled to a Nermag R 10-10 C mass spectrometer. Column and oven temperatures were the same as mentioned above. The helium linear velocity was 35 cm s^{-1} . Detector temperature was 260 °C, and source detector was 150 °C. Solvent extracts of the two vinegars were concentrated to 500 μL before splitless injection. Compounds were identified by comparison of spectra with those of the Wiley 138 library. Chemical ionization spectra, using ammonia as reagent gas with a source pressure of 0.3 Torr, were obtained at 70 eV with a source temperature of 90 °C and instrument scanning from 60 to 300 amu in 0.7 s.

Transform Infrared Spectrometry. GC-FTIR spectra were collected with a Bio-Rad Digilab FTS 60A spectrometer. This was connected by means of a Digilab Tracer direct-deposition

Table 1. Similarity of the Odors of the Four Extracts to Vinegar Reference: Scaling and Rank^a

type of extract	similarity scaling, cm (95% confidence interval)	rank			
		first	second	third	fourth
liquid-liquid	1.97a (0.97)	7	4	0	0
XAD-2 resin	4.12b (1.73)	3	4	3	1
XAD2-XAD7 resins	9.11c (1.58)	0	1	0	10
Extrelut resin	5.96b (0.79)	1	2	8	0

^a Eleven panelists, unstructured scale; scalings with the same letter were not significantly different at a level of 5%.

interface to a Hewlett-Packard HP 5890 Series II chromatograph that was equipped with a splitless-split injector. GC separation was performed on the same column and at the same oven temperature as mass spectrometry. Helium was used as carrier gas at a velocity of 20 cm s^{-1} . The spectrometer was controlled and data were acquired with an SPC 3200 data system. The spectral resolution was 8 cm^{-1} , and real-time spectra were obtained by co-addition of four scans (Semon et al., 1998).

Identifications were confirmed for most components by comparison of retention indices of pure compounds analyzed under identical conditions.

Quantification. Relative concentrations of the volatile compounds were determined for the two vinegars. The internal standard, methyl hexanoate, was added to neutralized vinegar at a concentration of 451 $\mu\text{g}/200\text{ mL}$.

RESULTS AND DISCUSSION

Neutralization of Acetic Acid. Two agents of neutralization were tested: MgO and NaOH. Neutralization by MgO is slow, and the exact pH is difficult to obtain. On the contrary, the control of pH with NaOH is easier. Neutralization induces a modification of the odor of the vinegar due to salification of acetic acid and other acids. Khan et al. (1972) suggested that NaOH could also lead to saponification of the esters. Therefore, sensory tests were performed to determine if neutralization has an impact on odor-active compounds other than acids. To limit salification of other acids in addition to acetic acid, the pH of the vinegar was adjusted to 6 instead of 7. Triangular tests showed that there was no difference between odors of vinegar NaOH' and those of initial vinegar at a level of 5% (12 correct responses of 24) and that there was a significant difference (5% level) between odors of vinegar MgO' and those of initial vinegar (15 correct responses of 24). If saponification of esters occurred with NaOH, it seems that these esters are not important for the flavor of our vinegars. Therefore, NaOH was chosen for this study.

Representativeness of the Extracts. Two extraction techniques based on hydrophobicity of volatile compounds were tested: resins and liquid-liquid extractions. Extraction by adsorption on resin was recommended by several authors on different liquid products such as white wine with Extrelut resin (Dirninger et al., 1993) or beer with XAD resins (Hawthorne et al., 1987; Abbott et al., 1993b). Two types of resin were therefore tested: Extrelut as a hydrophilic resin and XAD-2 as a hydrophobic resin. A mixture of XAD-2 and XAD-7 resins was also examined to extract compounds of different polarities. Extractions with resins were compared with solvent extraction. Dichloromethane was used as solvent for the four techniques. Panelists were asked to score the similarity between the odor of the four extracts and the odor of the neutralized vinegar. Results (Table 1) indicate that among the four extracts tested, dichloromethane extract is the most representa-

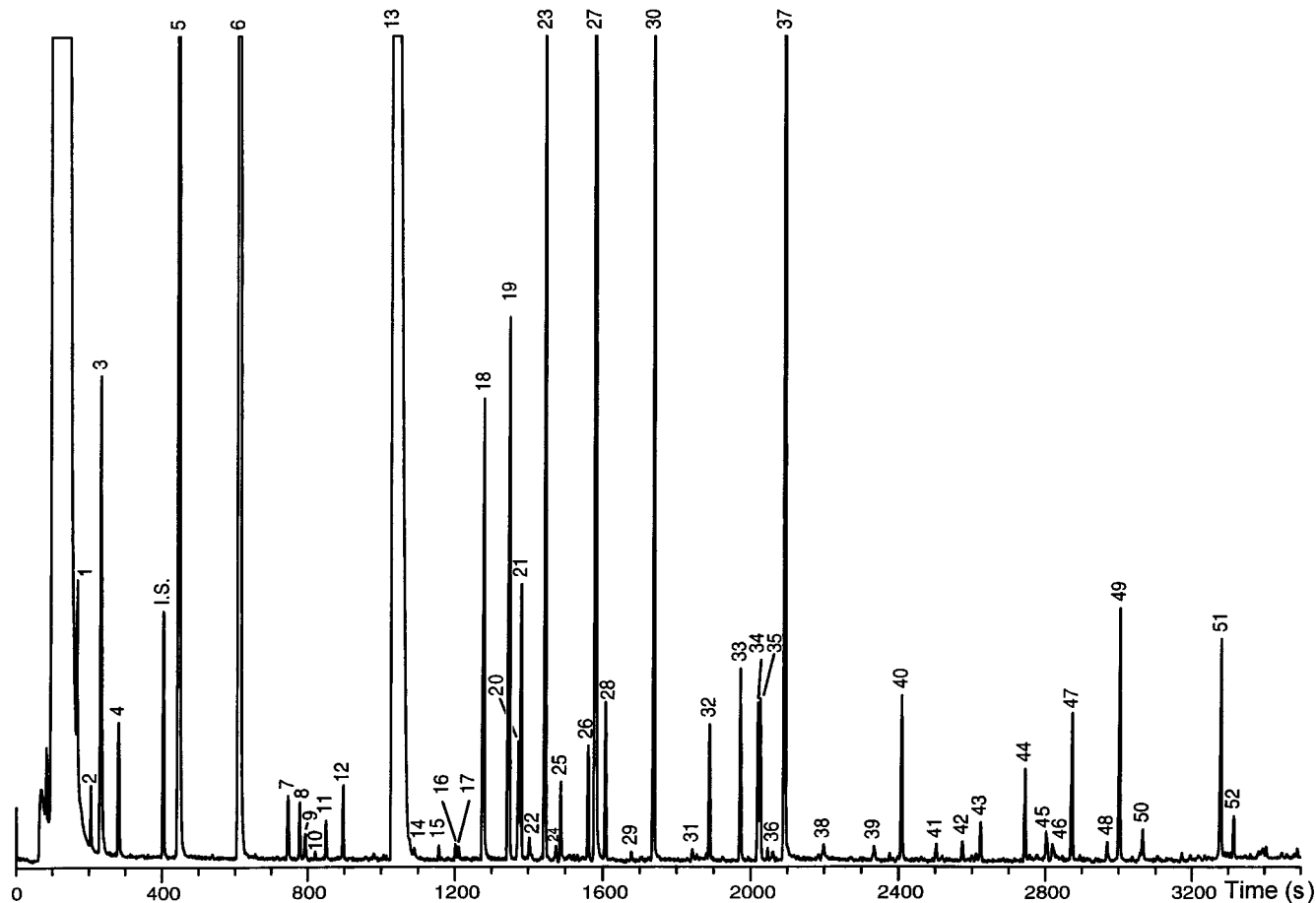


Figure 2. Chromatogram of the dichloromethane extract of vinegar B neutralized with NaOH (see conditions in the text). Peak identification: 1, unknown; 2, unknown; 3, 2-methyl-1-propanol; 4, isoamyl acetate; 5, mixture of 2-methyl-1-butanol and 3-methyl-1-butanol; 6, 3-hydroxy-2-butanone; 7, 3-hydroxy-2-pentanone; 8, ethyl 3-hydroxypropanoate; 9, 2-hydroxy-3-pentanone; 10, unknown; 11, 3-ethoxy-1-propanol; 12, 3-acetoxy-2-butanone; 13, acetic acid; 14, furan-2-carboxaldehyde; 15, unknown; 16, ethyl 3-hydroxybutanoate; 17, benzaldehyde; 18, 2,3-butanediol; 19, 2-methylpropanoic acid + 2,3-butanediol monoacetate; 20, unknown; 21, 2,3-butanediol monoacetate; 22, unknown; 23, 2-(3*H*)-dihydrofuranone + butanoic acid; 24, unknown; 25, unknown; 26, 1,3-propanediol diacetate; 27, 3-methylbutanoic acid; 28, diethyl succinate; 29, 3-(methylthio)-1-propanol; 30, 1,3-propanediol monoacetate; 31, unknown; 32, 2-phenylethyl acetate; 33, hexanoic acid + unknown; 34, benzyl alcohol; 35, unknown; 36, unknown; 37, phenylethanol; 38, heptanoic acid; 39, 4-ethylguaiaicol + unknown; 40, octanoic acid; 41, unknown; 42, unknown; 43, 4-ethylphenol; 44, 1,2,3-propanetriol monoacetate; 45, unknown; 46, 1,2,3-propanetriol monoacetate; 47, unknown; 48, unknown; 49, unknown; 50, unknown; 51, benzeneacetic acid; 52, unknown; internal standard, methyl hexanoate.

tive. It is ranked by 7 of 11 panelists at the first place, and the similarity scaling is low and significantly different (5% level) from those obtained with the other methods. The same result was obtained by Abbott et al. (1993c) on wine. Thus, we have finally chosen to first neutralize vinegar to pH 6 with NaOH and then to extract volatile compounds with dichloromethane.

Quantification and Identification. Figure 2 shows the chromatogram obtained by injection of the dichloromethane extract, and Table 2 lists the 52 quantified compounds. Among them, 36 are identified and 12 had never been previously reported in wine vinegar. These new compounds are principally esters and ketones. Acetic acid esters, 2,3-butanediol monoacetate, 1,2,3-propanetriol monoacetate, and 1,2,3-propanetriol diacetate are probably formed during the aging by reaction of acetic acid respectively with 2,3-butanediol and glycerol. For 1,3-propanediol monoacetate and diacetate, it can be noticed that 1,3-propanediol was never mentioned in red wine, but it was identified in cider vinegar by Kaway et al. (1991). Three new ketones were identified: 3-hydroxy-2-pentanone, 2-hydroxy-3-pentanone, and 3-acetoxybutan-2-one. De Revel and Bertrand (1994) showed that 3-hydroxy-2-pentanone and 2-hydroxy-3-

pentanone could result from the reduction of 2,3-pentanedione by *Saccharomyces cerevisiae* in wine and that the level of these substances in wine was low (<10 mg/L). Another ketone, 3-acetoxybutan-2-one, was identified by Schreier (1980) in Burgundy Pinot noir red wines. The other compounds, 4-ethylguaiaicol, 4-ethylphenol, 3-(methylthio)-1-propanol, and benzeneacetic acid, had already been identified in wine. The concentrations of the majority of the volatile compounds, such as acids, were higher in vinegar B. These results can be explained by the difference between the total acidities of the two vinegars. The major volatile compounds quantified in these two red wine vinegars are 2- and 3-methyl-1-butanol, 2-hydroxy-3-butanone, acetic acid, 3-methylbutanoic acid, and 2-phenylethanol. It is in agreement with the quantifications of Gerbi et al. (1992) and Blanch et al. (1992).

GCO. The odors smelled by fewer than four judges, considered as odor noise, were discarded. The 30 remaining odors are presented in Table 3. Among them, 26 are found in the two vinegars. Only 3 odors are detected by all of the panelists (no. 13, 27, and 37) in the two vinegars, and 11 are smelled by 60% of the assessors (no. A, B, D, 5, 7, E, 19, 23, 32, K, and Q). In

Table 2. Concentrations of the Volatile Compounds in the Two Red Wine Vinegars (Milligrams per Liter Methyl Hexanoate Equivalent): Means of Three Replicates^a

peak	retention index	identification	nature of the identification	vinegar A	vinegar B	earlier identified in wine vinegar
1	1014	2-methylpropyl acetate	MS	1.23 (0.01)	1.05 (0.05)	1, 2, 3
2	1049	unknown		0.06 (0.01)	0.42 (0.04)	
3	1078	2-methyl-1-propanol	MS, GC	8.02 (0.68)	6.18 (0.23)	1, 2, 3, 5, 6, 7, 8, 9
4	1116	isoamyl acetate	MS, GC	2.37 (0.06)	1.55 (0.04)	1, 2, 3, 6, 7, 8, 9
5	1207	2- and 3-methyl-1-butanol	MS, GC, CI	29.58 (1.38)	24.75 (1.09)	1, 2, 3, 5, 6, 7, 8
6	1301	3-hydroxy-2-butanone	MS, CI	S	S	1, 4, 7, 8, 9
7	1338	3-hydroxy-2-pentanone	MS, GC, CI, IR	0.55 (0.00)	0.69 (0.05)	
8	1345	ethyl 2-hydroxypropanoate	MS, GC, CI, IR	0.64 (0.01)	0.59 (0.05)	7, 8, 9
9	1355	2-hydroxy-3-pentanone	GC, CI, IR	0.26 (0.01)	0.31 (0.04)	
10	1359	unknown	MW = 146	0.08 (0.02)	0.13 (0.02)	
11	1376	3-ethoxy-1-propanol	MS, GC, CI	0.25 (0.02)	0.29 (0.28)	8
12	1389	3-acetoxy-2-butanone	MS, CI	0.49 (0.14)	0.74 (0.05)	
13	1426	acetic acid + 3-(methylthio)-1-propanal	MS, GC, CI, IR	63.53 (1.90)	121.60 (2.96)	7, 8
14	1460	2-furancarboxaldehyde	MS, GC	0.19 (0.03)	0.14 (0.07)	7
15	1498	unknown		0.10 (0.02)	0.14 (0.01)	
16	1519	ethyl 3-hydroxybutanoate	MS, GC, CI, IR	0.19 (0.02)	0.19 (0.01)	7
17	1523	benzaldehyde	MS, GC, CI, IR	0.17 (0.02)	0.15 (0.03)	3, 4, 7, 8, 9
18	1547	2,3-butanediol	MS, IR	2.72 (0.24)	5.99 (0.68)	7, 8, 9
		+ propanoic acid	MS, GC			7, 9
19	1573	2-methylpropanoic acid + 2,3-butanediol monoacetate	MS, GC	2.26 (0.15)	7.08 (0.27)	7, 8, 9
			MS, CI, IR			
20	1580	unknown		0.65 (0.08)	1.27 (0.03)	
21	1587	2,3-butanediol monoacetate	MS, CI, IR	1.15 (0.11)	2.42 (0.11)	
22	1600	unknown		0.19 (0.04)	0.37 (0.21)	
23	1637	γ -butyrolactone + butanoic acid	MS, GC, CI, IR	9.89 (0.33)	10.29 (0.53)	3, 7, 8
			MS, GC			7, 8, 9
24	1640	unknown		0.14 (0.07)	0.23 (0.03)	
25	1649	unknown		0.34 (0.01)	0.77 (0.10)	
26	1660	1,3-propanediol diacetate	MS, IR, CI	0.49 (0.08)	1.14 (0.06)	
27	1667	3-methylbutanoic acid	MS, GC, IR	17.95 (0.00)	25.99 (1.36)	7, 8
28	1684	diethyl succinate	MS, IR	1.32 (0.01)	1.51 (0.09)	3, 7, 8, 9
29	1719	3-(methylthio)-1-propanol	MS, GC	0.12 (0.02)	0.11 (0.03)	7
30	1754	1,3-propanediol monoacetate	MS, IR, CI	3.36 (0.00)	9.36 (0.55)	
31	1794	unknown		0.45 (0.77)	0.11 (0.20)	
32	1820	2-phenylethyl acetate	MS, GC, IR, CI	1.11 (0.09)	1.36 (0.06)	3, 7, 8
33	1849	hexanoic acid + unknown	MS, GC, IR	1.73 (0.18)	1.91 (0.14)	7, 8, 9
34	1883	benzyl alcohol	MS, IR	1.93 (0.01)	1.47 (0.07)	7, 8, 9
35	1889	unknown	MW = 129	0.92 (0.02)	1.62 (0.08)	
36	1899	unknown		0.24 (0.31)	0.13 (0.01)	
37	1925	2-phenylethanol	MS, GC, CI, IR	24.41 (0.18)	26.44 (1.42)	3, 7, 8, 9
38	1956	heptanoic acid + unknown	MS	0.23 (0.05)	0.19 (0.15)	7
39	2039	4-ethylguaiacol	MS, GC	0.24 (0.31)	0.25 (0.04)	
40	2065	octanoic acid	MS, GC, IR	1.94 (0.05)	1.67 (0.07)	7, 8
41	2142	unknown		0.11 (0.03)	0.27 (0.03)	
42	2155	unknown		0.26 (0.07)	0.32 (0.03)	
43	2185	4-ethylphenol	MS, GC	0.32 (0.10)	0.49 (0.01)	
44	2250	1,2,3-propanetriol monoacetate	MS, CI, IR	0.40 (0.01)	1.01 (0.19)	
45	2282	unknown		0.38 (0.09)	0.49 (0.05)	
46	2299	1,2,3 propanetriol diacetate	MS, CI, IR	0.21 (0.1)	0.30 (0.10)	
47	2355	unknown	MW = 130	1.29 (0.00)	1.71 (0.14)	
48	2392	unknown	MW = 130	0.26 (0.02)	0.35 (0.07)	
49	2411	unknown	MW = 146	1.55 (0.11)	2.68 (0.28)	
50	2458	unknown		0.50 (0.15)	0.53 (0.02)	
51	2573	benzeneacetic acid	MS	1.49 (0.08)	2.80 (0.30)	
52	2585	unknown	MW = 163	0.56 (0.06)	1.44 (0.02)	

^a Abbreviations: MS, mass spectrometry; GC, injection of the pure compound; CI, chemical ionization; IR, infrared spectroscopy; S, saturated. Literature cited: 1, Suomaleinen and Kangasperko, 1963; 2, Aurand et al., 1966; 3, Khan et al., 1972; 4, Olmedo et al., 1973; 5, Cabezero et al., 1978; 6, Troncozo-Gonzalez and Guzman-Chozas, 1987; 7, Blanch et al., 1992; 8, Gerbi et al., 1992; 9, Rizzo, 1991. Standard deviations are given in parentheses.

wine vinegar, flavor compounds have different origins, the wine, the alcoholic and/or the acetic fermentation, and the chemical reactions during the aging. Three classes of compounds seem to be important for vinegar aroma: acids, alcohols, and esters.

Acids. Among the nine acids identified in the two vinegars, five were smelled by the panelists. All of the panelists detected acetic acid (no. 13) and described it as vinegar. Neutralization of acetic acid is incomplete at pH 6, and thus the remaining concentration was still above its detection threshold in vinegar. This confirms that acetic acid is the most important aromatic com-

pound in vinegar. Other acids such as 2-methylpropanoic acid (no. 19), butyric acid (no. 23), and 3-methylbutyric acid (no. 27) have also high detection frequencies. They are characterized by cheesy, rancid notes. Propanoic acid was smelled by only a few people, who described it as having a pleasant odor (no. 18). The odor description of this acid is in agreement with its concentration in vinegar: below 100 mg/L, propanoic acid has a fruity odor, and above 100 mg/L, a cheesy odor (Arctander, 1969). Acids are already present in wine, but their contribution for wine aroma is not clearly demonstrated (Etievant, 1991). Some of these acids can

Table 3. Detection Frequency of the Odors of Wine Vinegars A and B Detected and Described by the Sniffing Panel

odor	retention index	descriptors	tentative identification	detection frequency	
				vinegar A	vinegar B
A		fruity, strawberry	ester?	9	10
B		butter, caramel	2,3-butanedione	12	12
C	1030	strawberry, apple, raspberry	ester?	7	3
D	1068	fruity, wine, plastic	ester?	9	11
3	1078	bleach, chocolate, fusty	2-methyl-1-propanol	6	2
4	1115	fruity, sweet	isoamyl acetate	9	5
5	1207	feet, bleach, fusty	2- and 3-methyl-1-butanol	10	11
7	1338	roasty, toasty, popcorn	3-hydroxy-2-pentanone	13	11
13	1426	vinegar, acetic acid	acetic acid	13	13
E	1455	potato, crushed potato	3-(methylthio)-1-propanal	10	11
F	1536	fruity, floral	unknown	0	6
18	1547	fruity, floral, acid	propanoic acid	5	2
19	1573	feet, gruyere cheese, fusty	2-methylpropanoic acid	10	9
23	1637	cheese, butter, rancid, feet	butyric acid	11	12
27	1667	ripened cheese, feet, rancid	3-methylbutyric acid	13	13
29	1719	dried bread, potato, hay	3-(methylthio)-1-propanol	4	3
G	1730	fruity, floral, vegetable	benzyl acetate	6	5
32	1820	fruity, floral, rose	2-phenylethyl acetate	8	8
H	1830	rose, fruity, floral	unknown	7	4
I	1849	sour, spicy, potato	unknown	6	0
J	1860	spicy, sour, burnt	unknown	4	4
K	1875	camphor, medicinal	unknown	12	9
37	1925	fruity, floral, rose	2-phenyl-1-ethanol	13	13
L	1956	fruity, floral, honey, wax	unknown	6	3
39	2039	spicy, coconut,	4-ethylguaiaicol	6	2
M	2052	caramel, spicy, clove	unknown	5	6
N	2107	caramel, beer	unknown	4	2
O	2172	fruity, burnt caramel, beer	unknown	4	0
P	2219	coffee, burnt wood, spicy	unknown	0	4
Q	2250	spicy, coffee, curry	unknown	11	10

^a Odor numbers are those used in Table 2 for quantification. Letters are used when there is no identified compound except for E and G, odors that were identified by GC/MS but were too small to be quantified, and B, which was eluted with the solvent.

also be formed by oxidation of linear alcohols such as 1-propanol and 2-butanol by *Acetobacter* (Jones and Greenshield, 1969).

Alcohols. Together with esters, alcohols are the main compounds characteristic of a type of vinegar. Only five alcohols were detected by the panelists. Among them, 2-phenyl-1-ethanol (no. 37) was perceived by all of the panelists in the two vinegars. It has already been identified as an impact flavor compound in wines (Etievant, 1991). Its formation could also occur during sugar fermentation by yeast. Odor 5 (bleach, feet odor) is associated with two coeluted alcohols: 2-methyl-1-butanol and 3-methyl-1-butanol. Their concentrations in vinegar are high [25–29 mg/L standard equivalent in our vinegar and in a range of 10–100 mg/L in other wine vinegars (Blanch et al., 1992)]. As mentioned by Etievant (1991), these two alcohols have similar odors but different perception thresholds in wine. Thus, it is not possible to determine which one is responsible for the odor. It can be noticed that the ratio 2-methyl-1-butanol/3-methyl-1-butanol is often used to identify genuine vinegars and also to study the acetification process (Nieto et al., 1993). Three other alcohols were detected by the panelists: 2-methyl-1-propanol (no. 3) with bleach, chocolate notes, 3-(methylthio)-1-propanol (no. 29) characterized by a dried bread or potato odor, and 4-ethylguaiaicol with spicy, coconut notes. As are 2- and 3-methyl-1-butanol, 2-methyl-1-propanol is consumed during the fermentation process (Cabezudo et al., 1977) and thus is an indicator of the acetification. Moreover, 3-(methylthio)-1-propanol was identified in different red wines and was characterized by a strong cooked potato note (Baumes et al., 1986).

Esters. In vinegar, most of the esters result from the fermentation of alcohols or by reaction of acids on

alcohols during aging. They are often responsible for fruity, floral notes. Thus, odors A, C, and D could be due to esters, but the corresponding peaks were too small to be identified by mass spectrometry. Ethyl butanoate could be responsible for odor C: it was identified by Aubry (1999) in Pinot noir wine at the same retention time with a strawberry note. Among the three identified esters (no. 4, G, and 32), 2-phenylethyl acetate was detected by 62% of the assessors in the two vinegars, benzyl acetate by 46%, and isoamyl acetate (no. 4) was better perceived in vinegar A. Ethyl acetate, which is known to be present in vinegar, was not detected by the judges. Ethyl acetate is the most important ester in vinegar and results from the process of acetification. It has an ether-like odor reminiscent of pineapple and is responsible for a sour-like odor of vinegar. Ethyl acetate is coeluted with dichloromethane. As dichloromethane has an anesthetic effect, assessors began their sniffing 1 min after injection, explaining why ethyl acetate was not smelled.

Some odors detected by 70% of the assessors correspond to compounds that belong to other chemical classes or are unidentified. 2,3-Butanedione (no. B) was smelled by >90% of the panelists in the two vinegars with a characteristic buttery-like odor. It is formed by oxidation of 3-hydroxy-2-butanone but is also present in wine at concentrations between 0.2 and 4.1 ppm (Etievant, 1991). On the contrary, 3-hydroxy-2-butanone, which is present in vinegar at a higher concentration (175–600 mg/L; Olmedo et al., 1973), was smelled only in vinegar A by three assessors. Among the new compounds identified in vinegar but already identified in wine, 3-hydroxy-2-pentanone (no. 7, roasty, toasty notes) was smelled by at least 80% of the panelists and 4-ethylguaiaicol (no. 39) was described as

spicy. Potato odor (no. E) was associated with 3-(methylthio)-1-propanal. Four odors were smelled in only one vinegar: no. I (unknown) and no. O (unknown) in vinegar A, no. F (unknown) and no. P (unknown) in vinegar B. As these compounds are not identified, it is difficult to conclude if the differences observed come from the wine used for the fabrication of the vinegar or from the acetic fermentation. The compounds responsible for odors A, D, K, and Q have not yet been identified.

Conclusion. The volatile compounds contributing to two red wine vinegar aromas were determined by GCO. Among the 30 odors detected, 26 odors were common to the two analyzed vinegars. These compounds are mainly produced during the acetic fermentation, such as acetates or acids, but compounds present in the initial wine, such as 3-hydroxy-2-pentanone, 4-ethylguaiacol, and 3-(methylthio)-1-propanol, also contribute to the aroma of wine vinegar. Moreover, the 12 volatile compounds identified for the first time in red wine vinegar indicate the importance of the aroma composition of the initial wine.

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