

# Comparison of Amberlite XAD-2/Freon 11 Extraction with Liquid/Liquid Extraction for the Determination of Wine Flavor Components

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An extraction method using Amberlite XAD-2 sorbent with Freon 11 to extract wine volatiles was evaluated and compared with Freon 11 liquid/liquid extraction. The experiments were performed on a synthetic model solution containing 40 typical wine volatiles. Relative recoveries by XAD-2 extraction were measured and compared with those of liquid/liquid extraction. Results indicated that XAD-2 extraction using Freon 11 as the eluent is comparable to liquid/liquid extraction for most volatiles of interest in wine. Both sample preparation methods exhibit a high relative recovery for longer chain alcohols, esters, aldehydes, ketones, and monoterpenes. However, they show a low extraction efficiency for low molecular weight alcohols and organic acids. This was further confirmed by comparing differences in the measured concentrations of volatiles in a Riesling wine.

**Keywords:** *Wine volatiles; Amberlite XAD-2 extraction; liquid/liquid extraction; capillary gas chromatography*

## INTRODUCTION

Aroma substances are important in wine as they make a major contribution to the quality of the final product. Several hundred chemically different flavor compounds such as alcohols, esters, organic acids, aldehydes, ketones, and monoterpenes have been found in wines (Rapp, 1988; Webster et al., 1993). It is the combined contribution of these compounds that forms the character of a wine. Since many viticulture and enology factors greatly influence the types and concentrations of flavor compounds (Macaulay and Morris, 1993), the ability to determine each individual component would provide an approach to optimize the operational conditions (i.e., canopy management of the vine, harvest parameters, juice preparation and fermentation techniques, use of yeasts, lactic acid bacteria and enzymes, and wine aging).

Several analytical methods have been developed for the extraction and determination of wine flavor compounds. These include purge and trap (i.e., dynamic headspace analysis) (Garcia-Jares et al., 1995), liquid/liquid extraction (Marais, 1986), XAD-2 resin extraction (Gunata et al., 1985), supercritical fluid extraction (Blanch et al., 1995), and solid phase microextraction (Yang et al., 1995), followed by chromatographic determinations. Each sample preparation procedure is subject to its particular drawbacks, although offering specific advantages under certain circumstances.

Continuous liquid/liquid extraction is a widely used sample preparation method for the determination of wine volatiles. Although it is a time-consuming technique, the extract produced contains a wide spectrum of volatile components (Marais, 1986). A number of solvents have been used for the enrichment of aroma substances. Among them, trichlorofluoromethane (Freon 11) has been found to be well suited for extracting volatiles from a matrix with a high alcohol content such

as wine (Park and Noble, 1993). Although liquid/liquid extraction with Freon 11 has proven to be an efficient method to isolate specific classes of volatiles such as monoterpenes (Park et al., 1991; Marais, 1986), systematic evaluation of this extraction method for wine volatiles in terms of relative recoveries has not been reported.

Another common sample preparation method in wine analysis is the utilization of a polymeric resin such as Amberlite XAD-2. This procedure includes adsorption of the flavor compounds on the resin followed by elution with appropriate solvents. The approach has the advantage that free and glycosidically bound flavor compounds can be isolated and separated in one sample preparation step (Williams, 1993). The bound fraction is eluted using ethyl acetate after the free fraction has been eluted with pentane (Gunata et al., 1985). However, pentane usually yields low recoveries of the free flavor compounds due to their limited solubility.

In the current studies, a solvent with an extractive capacity higher than that of pentane (i.e., Freon 11) was used in combination with Amberlite XAD-2 resin to extract wine volatiles. The experiments were performed on a synthetic model solution containing 40 typical wine volatiles. The recoveries relative to an internal standard were measured using XAD-2/Freon 11 extraction and compared to those determined by liquid/liquid extraction. Both extraction methods were then applied to a Riesling wine to compare differences in the measured concentrations of the aroma components.

## EXPERIMENTAL PROCEDURES

**Materials.** Trichlorofluoromethane (Freon 11) was purchased from DuPont Inc. (Wilmington, DE) and redistilled before use. Amberlite XAD-2 resin obtained from Supelco, Inc. (Bellefonte, PA), was sequentially washed with methanol, acetonitrile, and diethyl ether as described by Wilgilius et al. (1987). Flavor chemicals, which ranged from 95% to 99% purity, were purchased from either Aldrich Chemical Co. (Milwaukee, WI), Fluka Chemical Co. (Ronkonkoma, NY), ICN Pharmaceuticals Inc. (Plainview, NY), or Dragoco Inc. (To-

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towa, NJ). All reagents were of HPLC grade from Aldrich and used without further purification. Ultrapure water (18 kM $\Omega$ ) was obtained from a Millipore (Bedford, MA) Milli-Q reagent system. A model wine solution was prepared according to the method of Wildenradt et al. (1974). It was composed of potassium bitartrate at 1.15 g/L and tartaric acid at 0.7 g/L (pH 3.1) in 11.5% (v/v) aqueous ethanol. The stock solution used to evaluate the extraction recovery was made by dissolving 6 mg of each of the flavor compounds listed in Table 2 in 100 mL of the model wine solution (i.e., 60 ppm). The synthetic working standard solution was prepared by diluting 1 mL of the stock solution with 250 mL of the model wine solution, yielding a final concentration of 0.24 ppm for each component. The same flavor compounds were dissolved in acetone at 60 ppm for the chromatographic calibration. The Riesling wine was made in 1992 from grapes grown at the OARDC Grape Research Branch at Kingsville using standard wine-making practices.

**Sample Preparation.** (a) *Liquid/Liquid Extraction.* Continuous liquid/liquid extraction was carried out on a downward displacement liquid/liquid extraction system similar to that used by Marais (1986). To reduce the degree of emulsification at the Freon 11/water interface, samples were first saturated with sodium chloride and cooled to approximately 0 °C prior to extraction. Subsequently, 1 mL of the internal standard (i.e., 2-octanol at 60 ppm) was added to 250 mL of the working standard solution or wine, and the samples were extracted continuously with approximately 100 mL of distilled Freon 11 for 24 h. The Freon 11 extract was dried over anhydrous sodium sulfate and concentrated to 0.2 mL at 32 °C by means of a 50 cm Vigreux column. The final volume was adjusted to 1 mL with acetone. Extracts were stored at -20 °C until chromatographic analysis.

(b) *XAD-2 Extraction.* The XAD-2 extraction was carried out on a water jacketed glass column (30 × 1 cm i.d.) from ACE Glass Inc. (Vineland, NJ). The column was packed with approximately 12 cm of washed Amberlite XAD-2 resin and sequentially conditioned with methanol (4 × 20 mL), diethyl ether (4 × 20 mL), and distilled water (2 × 50 mL). A sample of 50 mL of the working standard solution or wine containing 0.2 mL of the internal standard (i.e., 2-octanol at 60 ppm) was applied to the column, which was then washed with 50 mL of water to eliminate water-soluble compounds such as sugars. While ice water was circulated through the water jacket, 50 mL of Freon 11 eluent was collected. Any trace of water that coeluted with Freon 11 was removed by freezing the eluent at -12 °C and drying over anhydrous sodium sulfate. The dried eluent was concentrated to 0.1 mL using the apparatus similar to that with liquid/liquid extraction. The final volume was diluted to 0.2 mL with acetone. All extractions were repeated at least three times to determine the reproducibility of the extraction procedures.

**Gas Chromatography.** Gas chromatography was carried out on a Hewlett-Packard (Avondale, PA) Model 5890 gas chromatograph equipped with a split/splitless capillary injection port and flame ionization detector. Separations were performed on a DB-Wax polyethylene glycol capillary column (60 m × 0.25 mm i.d., 0.25  $\mu$ m film thickness) from J&W Scientific (Folsom, CA). The operating conditions were the following: carrier gas flow (He), 1.6 mL/min; makeup gas (N<sub>2</sub>), 30 mL/min; hydrogen flow rate, 30 mL/min; air flow rate, 300 mL/min; injection mode, split; split ratio, 100:1; injected volume, 0.4  $\mu$ L for liquid/liquid extract and 2  $\mu$ L for XAD-2 eluent; injector temperature, 200 °C; detector temperature, 250 °C. The oven temperature was held at 40 °C for 3 min, then raised to 210 °C at 4 °C/min, and held at 210 °C for 15 min. A HP 3365 series II Chemstation was used for acquiring and processing the data. Measured retention times and peak areas represented at least duplicate injections. Identities of the volatile compounds were confirmed by comparing retention times and mass spectra with those of synthetic standards. Quantification was accomplished using the internal standard method with 2-octanol as the internal standard. Detection limit was estimated by considering the minimum detectable amount of solute for which the peak area was 5 times higher than the background signal.

**Gas Chromatography–Mass Spectrometry.** Gas chromatography–mass spectrometry was performed on a HP 5970 GC–MS equipped with chemical ionization and quadrupole mass analyzer. The GC conditions were the same as described previously except for the injection. A splitless technique was used with the split valve closed for 0.7 min. Mass spectra were taken over the *m/z* range 30–300, utilizing an ionizing voltage of 70 eV.

## RESULTS AND DISCUSSION

The 40 synthetic aroma compounds used for the recovery study represent the major classes of flavor constituents commonly found in wines (Webster et al., 1993; Versini et al., 1994). They include the alcohols, aldehydes, ketones, esters, acids and monoterpenes listed in Table 1. A synthetic working standard solution was prepared by dissolving each component in a model wine solution containing 0.19% tartaric acid and 11.5% ethanol at pH 3.1. Both liquid/liquid and XAD-2 extraction procedures were applied using Freon 11 as solvent. Figure 1 represents the chromatograms of the extracts. Listed in Table 1 are recoveries of the aroma compounds relative to the internal standard 2-octanol.

Although not shown in Figure 1, the chromatographic profile of the working standard solution is similar to that of the extract from the liquid/liquid extraction except for alcohols and acids. Liquid/liquid extraction with Freon 11 exhibits a low extraction capacity for alcohols with a low molecular weight (column 4, Table 1). Relative recoveries increase as the hydrocarbon portion of the molecule lengthens (i.e., average recoveries increase from 4% for isobutanol to 78% for 1-hexanol). These results are consistent with other studies using liquid/liquid extraction with Freon 11 (Rapp et al., 1980). A similar extraction behavior was observed for the organic acids. No butyric acid is recovered at the concentration level examined (column 4, Table 2). Average relative recoveries increase as a function of the increased hydrophobic properties of the molecule (i.e., 75% recovery was observed for octanoic acid and 126% for decanoic acid). The extraction efficiency for esters, ketones, aldehydes, and monoterpenes is greater than 70% with the exception of ethyl lactate at 38% (column 4, Table 1). The liquid/liquid extraction with Freon 11 is particularly suitable for the monoterpenes as reflected by more than 80% relative recoveries.

The Freon 11 eluent from the Amberlite XAD-2 extraction exhibits a different concentration profile as shown in Figure 1b. The differences arise from a greater decrease in the relative recoveries of organic acids and components with a low boiling point. No organic acids were recovered with XAD-2/Freon 11 extraction with the exception of decanoic acid, which showed 29% recovery (column 6, Table 1). This is probably due to the column's being washed with water before the collection of the Freon 11 eluent. The components with a low boiling point, as represented by a short retention time in Figure 1b, show smaller relative recoveries than those with a higher boiling point. However, for most volatiles of interest in wine, the XAD-2 extraction with Freon 11 shows a comparable extraction behavior. As in the case of liquid/liquid extraction, the short-chain alcohols are extracted less effectively than longer chain alcohols (column 6, Table 1). The relative recoveries for the longer chain alcohols, aldehydes, and ketones are larger than those determined by liquid/liquid extraction with Freon 11. Most monoterpenes show comparable recovery results. Although the average recoveries of esters decrease more

**Table 1. Relative Recoveries (RR) and Coefficients of Variation (CV) for Liquid/Liquid and Amberlite XAD-2 Extraction of Flavor Compounds from Synthetic Model Solution**

peak no.	compound	RT (min)	liquid/liquid extraction		XAD-2 extraction	
			% RR	% CV	% RR	% CV
1	ethyl butanoate	12.00	85.08	1.61	53.70	3.53
2	hexanal	13.62	84.11	0.58	75.65	2.03
3	isobutanol	14.04	3.95	17.97	7.49	6.19
4	isoamyl acetate	15.16	93.17	0.77	64.81	2.24
5	1-butanol	16.07	9.01	3.15	13.45	2.62
6	limonene	18.12	73.70	2.28	45.58	0.93
7	amyl alcohols	18.45	46.08	14.66	59.05	3.77
8	ethyl hexanoate	19.47	89.00	1.79	69.38	0.38
9	hexyl acetate	20.96	92.89	0.64	69.61	0.53
10	ethyl lactate	23.71	37.87	17.72	18.70	9.14
11	1-hexanol	24.05	77.93	1.05	91.91	0.32
12	<i>cis</i> -3-hexen-1-ol	25.25	81.09	4.30	97.52	0.96
13	2-octanol	26.43	int <sup>a</sup>	int	int	int
14	ethyl octanoate	27.00	73.01	0.97	58.26	3.61
15	linalool oxide 1	27.52	79.49	1.29	77.98	4.66
16	nerol oxide	28.45	92.35	0.58	84.61	1.74
17	linalool oxide 2	28.53	78.53	3.72	81.34	5.36
18	benzaldehyde	30.50	93.90	0.49	102.59	3.98
19	linalool	30.84	101.50	0.50	97.12	1.75
20	linalyl acetate	31.27	78.98	1.53	66.28	5.36
21	terpinen-4-ol	33.05	113.19	0.77	105.50	2.65
22	butyric acid	33.68	nd <sup>b</sup>	nd	nd	nd
23	ethyl decanoate	33.90	94.42	4.75	81.83	7.67
24	acetophenone	34.76	90.07	0.26	99.74	1.44
25	diethyl succinate	35.18	88.93	0.14	84.17	4.15
26	citral 1	35.58	90.38	0.84	90.10	2.90
27	$\alpha$ -terpineol	36.03	107.17	3.95	105.03	3.54
28	$\gamma$ -caprolactone	36.53	74.66	7.83	90.31	1.13
29	citral 2	37.12	89.68	2.02	84.12	3.56
30	citronellol	37.89	85.25	0.53	86.00	3.91
31	nerol	39.03	97.65	2.74	99.32	3.93
32	2-phenethyl alcohol	39.55	82.15	5.83	102.70	7.32
33	phenethyl acetate	39.72	100.26	1.07	98.80	2.71
34	geraniol/hexanoic acid	40.35	nd	nd	nd	nd
35	benzyl alcohol	41.46	53.75	6.15	71.63	4.65
36	$\beta$ -ionone	43.43	101.23	5.18	86.30	4.54
37	octanoic acid	46.36	75.15	0.80	nd	nd
38	methyl cinnamate	47.27	144.61	0.03	178.86	2.73
39	4-ethylphenol	49.81	78.36	3.95	48.34	7.64
40	decanoic acid	53.19	136.94	0.03	28.49	6.81
41	$\gamma$ -decanolactone	58.93	103.16	2.94	112.85	1.76

<sup>a</sup> int, internal standard. <sup>b</sup> nd, not detected.

**Table 2. Mean Concentration (Milligrams per Liter) of Flavor Compounds in Riesling Wine Determined by Liquid/Liquid and XAD-2 Extraction**

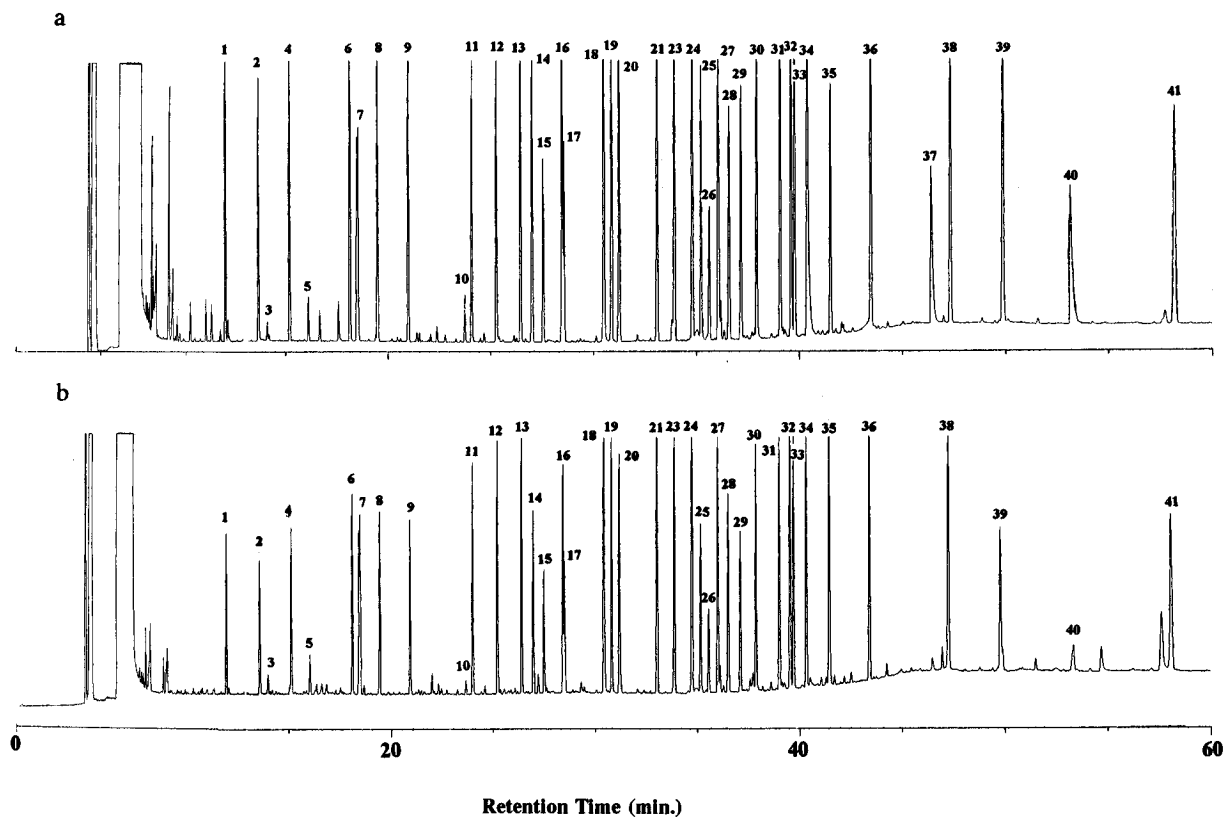
compound	liquid/liquid	XAD-2	compound	liquid/liquid	XAD-2
ethyl butanoate	0.20	0.14	linalool	0.21	0.18
isobutanol	0.60	0.44	butyric acid	0.32	0.13
isoamyl acetate	1.04	0.83	ethyl decanoate	0.34	0.38
1-butanol	0.06	0.06	diethyl succinate	0.23	0.23
amyl alcohols	30.48	32.86	$\alpha$ -terpineol	0.07	0.06
ethyl hexanoate	0.88	0.79	citronellol	0.05	0.06
hexyl acetate	0.17	0.13	phenethyl acetate	0.15	0.13
ethyl lactate	2.28	0.74	hexanoic acid	2.77	0.92
1-hexanol	1.03	1.13	benzyl alcohol	0.02	0.04
<i>cis</i> -3-hexen-1-ol	0.02	0.03	octanoic acid	10.23	4.22
2-octanol	int	int	4-ethylphenol	0.03	0.07
ethyl octanoate	0.99	0.90	decanoic acid	4.88	2.08

than 10% with XAD-2 extraction, the extraction efficiency is still more than 60% for most esters (column 6, Table 1).

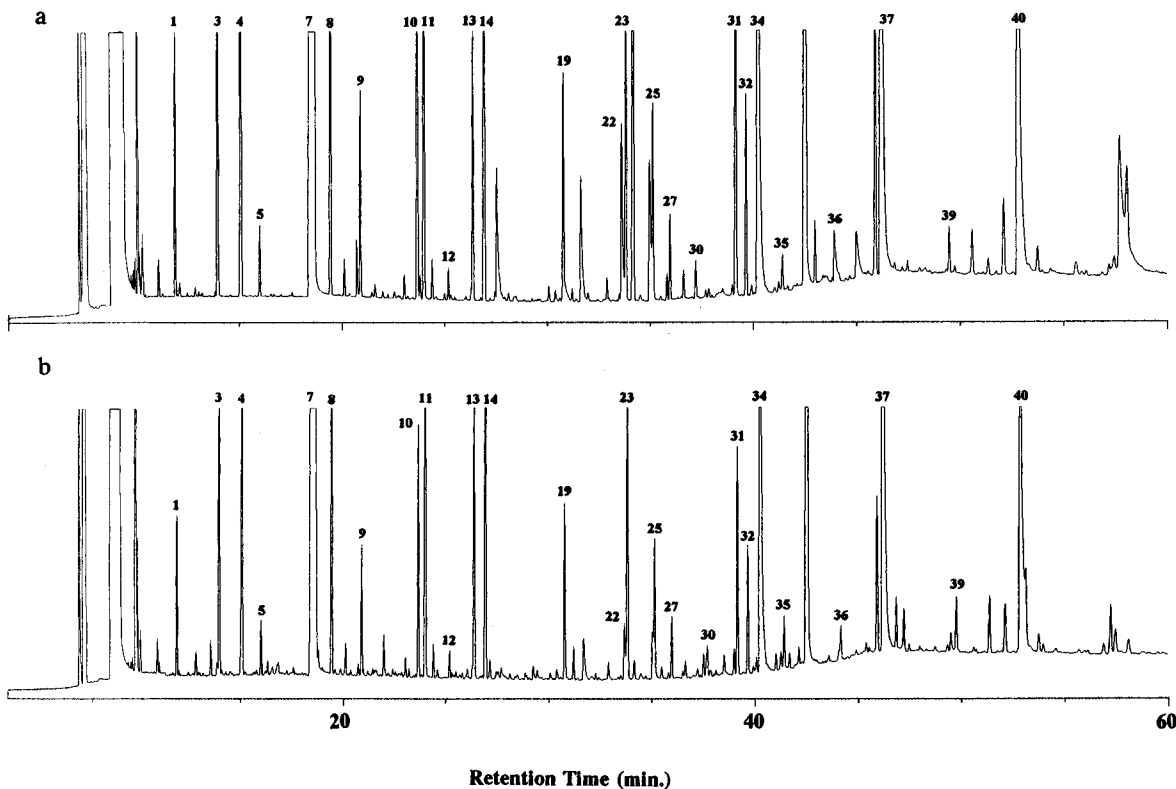
The XAD-2/Freon 11 extraction procedure exhibits an experimental precision similar to that of the liquid/liquid extraction for most components (Table 1). The coefficients of variation (CV) for the XAD-2/Freon 11 extraction are in the range of 1–7%. These are comparable to those reported by Webster et al. (1993), who used Amberlite XAD-2/pentane for wine volatile analysis. Liquid/liquid extraction shows a high CV value for isobutanol (18%), the amyl alcohols (15%), and ethyl

lactate (18%). A higher sensitivity was observed for most flavor compounds tested with liquid/liquid extraction. Under the current experimental conditions a detection limits of 6–14 ppb with liquid/liquid extraction and 30–63 ppb with XAD-2 extraction were estimated for longer chain alcohols, esters, aldehydes, ketones, and monoterpenes. A lower detection limit could be obtained by concentrating the extract to a smaller volume, decreasing the injector split ratio, or using splitless injection techniques.

The extracts of a Riesling wine obtained from both extraction methods contain a similar spectrum of vola-



**Figure 1.** Chromatograms of Freon 11 extract of the synthetic model standard solution: (a) liquid/liquid extraction; (b) XAD-2 extraction. Refer to Table 2 for peak identification.



**Figure 2.** Chromatograms of Freon 11 extract of a Riesling wine: (a) liquid/liquid extraction; (b) XAD-2 extraction. Refer to Table 2 for peak identification.

tile components but with a different concentration profile as shown in Figure 2. The differences are consistent with those obtained with the synthetic working standard solution. Smaller amounts of organic acids were recovered with XAD-2 extraction as reflected by a reduction in the concentrations of more than 50% (Table

2). Since only hexanoic acid was identified by GC-MS in peak 34 of Figure 2, the peak area was utilized to calculate the concentration of hexanoic acid, although geraniol would chromatographically coelute with hexanoic acid under the current separation conditions if it was present in the wine. High amounts of short-chain

alcohols and organic acids were determined by using both extraction methods (Table 2). However, they do not represent the actual concentrations in the wine due to the low recoveries. As in the case of the working standard solution, XAD-2 extraction exhibits a comparable concentration profile for alcohols, esters, aldehydes, ketones, and monoterpenes (Table 2). Since Freon 11 does not extract glycosidically bound components in wines (Williams et al., 1982), the use of XAD-2 resin with Freon 11 may provide a possibility to isolate and separate free and bound flavor components by one sample preparation step.

## CONCLUSION

Although there are differences in the measured concentrations, Amberlite XAD-2 extraction with Freon 11 is a comparative extraction method with liquid/liquid extraction for most volatiles of interest in wine. Both sample preparation methods show acceptable extraction recoveries for the longer chain alcohols, esters, aldehydes, ketones, and monoterpenes. However, they exhibit a low extraction efficiency for low molecular weight alcohols and organic acids.

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