COMMUNICATIONS

Reproducibility of Headspace Analysis of Wines

Wine headspace volatiles, collected by displacement onto a Tenax GC adsorbent trap by a modification of the procedure described by Noble (1978), were analyzed quantitatively by gas chromatography (GC). Triplicate analyses were made using two displacement end points, and the reproducibility of the two procedures was evaluated by calculation of the coefficients of variation (CV) of the peak areas. Of the 48 peaks quantified, 60% had a CV less than 0.30, with 21–25% having CV less than 0.10. Small peaks consistently showed the poorest reproducibility. CV for the larger peaks, ranging from 0.010–0.077, were similar to those reported by Stenroos et al. (1976) in the GC analysis of beer volatile extracts. Variation in the displacement end point did not significantly affect the reproducibility of the procedure; however, both sets of data represented a significant improvement in reproducibility over the results reported previously by Noble (1978).

In wine aromas, over 150 compounds have been identified and are common to most *Vitis vinifera* varieties, as summarized by Webb and Muller (1972). Consequently, to investigate characteristic aromas of varietal wines, such as those of Cabernet Sauvignon grapes, reproducible quantitative analysis of the headspace (HS) volatiles is necessary in addition to their identification.

To provide mild enrichment of the HS volatiles, adsorbent porous polymer traps have been used for the analysis of many aromas including those of beer (Jennings et al., 1972) and wine (Flath et al., 1972; Noble, 1978). In comparison to the nonequilibrium sparging techniques used in most HS studies, sampling of essentially equilibrium concentrations of HS volatiles has been done by displacement with a fluid (von Sydow et al., 1970) and motor driven syringe (Bertuccioli and Montedoro, 1974).

Although reproducibility is essential in analysis of HS for comparison of volatile profiles, only two investigations included its estimation. In triplicate HS analyses of coffees, made from distilled, hard, and soft water, respectively, 27, 43, and 48% of the 44 peaks quantified had coefficients of variation (CV) less than 0.10 (Tassan and Russell, 1974). Poorer reproducibility was found in wine HS analyses (Coope, 1977). Of the 47 peaks quantified, of which only five were major components, only 13% had coefficients of variation less than 0.10 (n = 5).

In this paper, a technique for the collection of headspace volatiles by displacement is described which is a modified version of that used by Coope (1977) and Noble (1978). The effect of variation in termination of the displacement on reproducibility is discussed.

EXPERIMENTAL SECTION

Headspace Collection System. Headspace volatiles were collected from wine by displacement using the system shown Figure 1, which was modified from that used by Coope (1977). Here flask B used for equilibration of wine volatiles had a ground glass joint, with no grease, rather than a Teflon lid. After 200 mL of wine was equilibrated at 20 °C for 20 min in flask B, a sweep flow of 20 mL/min nitrogen was used to control the flow of 500 mL of displacing wine from the upper container A. Volatiles in the displaced HS were adsorbed on 200 mg of Tenax GC (C).

At one of two points described below, the Tenax trap was removed from flask B, and ethanol and water eluted by nitrogen at 50 mL/min for 20 min. The trap was then connected to the switching box, described by Coope (1977) and Noble (1978). Volatiles, desorbed by heating the trap to 130 °C, were flushed from the trap for 25 min with nitrogen at 50 mL/min into a concentrator coil immersed in dry ice–ethanol. The sample was then flashed onto the gas chromatograph (GC) column by switching the valves and heating the concentrator coil with a heat gun at 250 °C for about 45 s.

To evaluate the effect of variation in termination of displacement, three runs were made in which the Tenax GC trap was removed at the appearance of the first sparging bubble of nitrogen in flask B (set A). Triplicate runs were also made in which the trap was removed when the displacement wine was completely drained from the top flask (A), prior to the appearance of a sparging bubble (set B).

Gas Chromatography. Volatiles were analyzed by a Hewlett-Packard 7620A research gas chromatograph with the injection port modified to permit a direct connection from the spiral trap to the column (via 0.75 mm i.d. ss. tubing heated to 100 °C). A 152.5 m × 0.75 mm i.d. coiled s.s. capillary column coated with solutions of 10% SF-96 (50) and 0.5% Ipegal C0880 in 1:1 acetone/ether was used. With a nitrogen carrier flow rate of 7.0 mL/min, the column had about 150 000 theoretical plates. Nitrogen make-up flow rate was 23 mL/min. The flame ionization detector temperature was 200 °C with a hydrogen flow rate of 25 mL/min and air flow of 500 mL/min. The runs were temperature programmed from 40 to 52 °C at 0.5 °C/min, from 52 to 140 °C at 1.0 °C/min, and from 140 to 160 °C at 0.5 °C/min. Peak areas were integrated using an Autolab Minigrator.

Retention times across all peaks were extremely consistent with coefficients of variation of retention times (CVT) across the six runs ranging from 0 to 0.022, of which 63% of the 48 peaks common to all chromatograms had CVT less than 0.003. Accordingly, the same peak numbers were assigned to all separated peaks with similar retention times across the six runs. To determine reproducibility of the two sets, coefficients of variation were calculated for each peak within a triplicate set using integrator values for peak areas.

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Table I. Means and Coefficients of Variations for Peak Areas[a]

	set A		set B	
peak no.	mean	CV	mean	CV
1	8.26	0.275	7.06	0.142
3	6.37	0.058	9.24	0.385
4	312.30	0.077	314.66	0.106
8	1.88	0.613	2.51	0.529
9	0.55	0.692	1.92	1.167
21	212.95	0.066	205.50	0.099
24	64.39	0.440	73.98	0.308
25	43.12	0.419	52.84	0.411
27	2.16	0.111	1.80	0.139
40/42	172.70	0.134	191.63	0.071
47	217.70	0.071	271.20	0.088
48	14.62	0.408	26.47	0.292
52	248.32	0.052	244.30	0.103
63	3.31	0.057	2.64	0.695
65	2.35	0.188	4.45	0.418
67	4.02	0.119	3.90	0.287
68	16.77	0.203	15.20	0.086
69	6.85	0.125	6.60	0.507
71	3.88	0.341	4.23	0.207
72	24.96	0.103	21.84	0.296
76	203.04	0.010	204.40	0.028
79	26.40	0.116	26.98	0.197
80	26.48	0.132	37.88	0.707
84	6.30	0.177	6.59	0.309
85	14.31	0.160	12.72	0.341
87	12.43	0.316	17.20	0.329
88	1.54	0.989	4.93	0.811
91	7.48	0.267	7.64	0.483
93	0.65	0.682	5.08	0.485
99	3.30	0.796	7.76	0.434
100	8.26	0.181	11.92	0.168
103	87.82	0.060	79.89	0.148
105	8.10	0.052	11.02	0.209
108	8.92	0.145	8.43	0.014
109	3.17	0.191	5.32	0.443
112	3.22	0.528	4.08	0.299
115	5.59	0.187	3.69	0.570
129	160.50	0.006	151.00	0.041
138	7.30	0.416	5.22	0.226
142	18.91	0.198	20.01	0.114
144	1.89	0.241	1.99	0.186
147	95.57	0.050	98.27	0.085
148	75.82	0.138	65.18	0.172
153	12.91	1.567	9.05	1.502
157	1.53	0.583	0.49	0.721
158	22.59	0.152	27.16	0.189
163	70.73	0.138	59.11	0.089
165	146.30	0.022	140.00	0.053

[a] $n = 3$.

RESULTS AND DISCUSSION

Peak area means and their coefficients of variation are shown in Table I for both sets A and B. The relative distributions of the reproducibility of sets A and B, together with that of Coope (1977), are shown in Table II. Larger errors are associated with smaller peaks most frequently. In set A, the five largest peaks, 4, 21, 47, 52, and 76, have CV ranges of 0.01 to 0.077, which is comparable to the excellent reproducibility found by Stenroos et al. (1976) for large peaks in beer volatile extracts. In contrast, the five smallest peaks, 8, 9, 90, 93, and 157, have poor reproducibility with CV from 0.583–0.989. The major source of error probably lies in variation in the points at which integration begins and ends. Bottle to bottle variation or "ghost" bleed may be responsible for the enormous error associated with peak 153 in both sets.

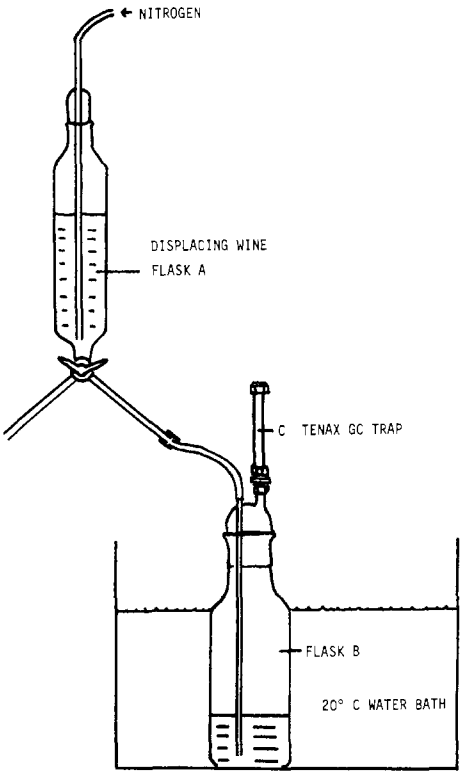

Figure 1. Headspace displacement system.

There was no significant difference in reproducibility distribution between sets A and B ($\chi^2 = 4.17$, 3 df) suggesting that minor variations in displacement volume are unimportant. Comparing the reproducibility of sets A and B with that of Coope, both sets represent a significant improvement in reproducibility over Coope's procedure ($\chi^2 = 29.80$, $p < 0.001$, 3 df and $\chi^2 = 13.89$, $p < 0.01$, 3 df, respectively). The improvement in reproducibility can most probably be attributed to the improved glassware used here and increased precision in execution of the entire procedure.

Other then obvious integration errors, other causes of variation include adsorption effects of GC columns and Tenax GC as described by Berezkin (1974) and Huber et al. (1973) which cannot be evaluated by our methods.

Further improvement in the reproducibility of wine HS analyses could be obtained by collection of a larger HS volume. However, the high concentrations of a few major components, such as peaks 4, 21, 47, 52, and 76 [higher alcohols (fusel oils) and ethyl esters], are a limiting factor. Selection of optimum parameters or a different adsorbent for selective elution of the higher alcohols, during the

Table II. Distribution of Peak Area Coefficients of Variation

		percentage of peaks in each category		
class of reproducibility	range of CV	set A, 48 peaks	set B, 48 peaks	Coope,[a] 47 peaks
very high	CV ≤ 0.1	25	21	13
high	0.1 < CV ≤ 0.3	45	37	19
medium	0.3 < CV ≤ 0.5	12	22	34
low	CV > 0.5	17	20	34

[a] Coope (1977).

development step, and retention of volatiles of interest may provide a solution.

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Ann C. Noble*[1]
Aki A. Murakami[2]
George F. Coope III[2]

[1]Department of Viticulture and Enology
University of California
Davis, California 95616
[2]Miller Brewing Company
Milwaukee, Wisconsin

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Thermodynamic Measurements on the Interaction of Porcine Trypsin with Single- and Two-Chain Trypsin Inhibitors from Corn Seeds

The reactions of the single- and two-chain forms of corn trypsin inhibitor with porcine trypsin in a cacodylate buffer at pH 6.5, 20 °C, both occur with 1:1 stoichiometry, have association equilibrium constants on the order of 10^7 M^{-1}, and indistinguishable enthalpy changes lying near 3.6 kcal/mol. The reactions are therefore driven by positive entropy changes. It is suggested that conversion of this inhibitor from its single- to two-chain form involves more than a simple hydrolysis of a peptide bond.

Complex formation between trypsin and proteinaceous trypsin inhibitors is of considerable interest because of the specificity and strength of these interactions. In the substantial body of investigation on trypsin inhibitors relatively little attention has been given to thermodynamics, even though a thorough grasp of the energetics will be essential to fully understand the nature of the binding reactions. Calorimetric studies have been reported on the interactions of bovine trypsin with the Kunitz and Bowman-Birk inhibitors isolated from soybeans (Turner et al., 1975; Barnhill and Trowbridge, 1975). Here we report the results from a study of the association reactions of porcine trypsin with the single- and two-chain forms of corn trypsin inhibitor, a protein that is quite different structurally from both of the soybean inhibitors (Swartz et al., 1977).

EXPERIMENTAL PROCEDURES

Single- and two-chain forms of trypsin inhibitor were purified from seeds of *opaque-2* corn by salt extraction of ground seeds, affinity chromatography on trypsin-Sepharose, and ion-exchange chromatography on DEAE-cellulose. Inhibitor concentrations were determined using $E_{280nm}^{1\%} = 20$ (Swartz et al., 1977).

Equilibrium constants for the reactions of trypsin inhibitors with porcine trypsin (Sigma; Trypsin Type IX) were estimated as described by Bieth (1974), using *p*-nitrophenyl *p'*-guanidinobenzoate (Chase and Shaw, 1967) to measure concentrations of unbound trypsin. In a spectrophotometer cell, we combined 25 μL of trypsin solution (ca. 5–8 mg/mL in 1 mM HCl) with various amounts of inhibitor solution (ca. 1 mg/mL) that had been dialyzed against 0.001 M $CaCl_2$/0.25 M NaCl/0.05 M sodium cacodylate buffer (pH 6.5). The volume of inhibitor solution added ranged from 25 to 350 μL. Buffer was then added to obtain a precisely known final volume (always ca. 1 mL). The cell was placed in a 20 °C water bath for 10 min. *p*-Nitrophenyl *p'*-guanidinobenzoate (10 μL of a 0.01 M solution in dimethylformamide) was then added quickly, and after the contents of the cell were mixed, 50 μL of 1 M Tris base was added to bring the pH to 8.3. The absorbance at 410 nm was monitored immediately by use of a Beckman Model 25 spectrophotometer. From the initial burst in absorbance we subtracted the small absorbance observed when the enzyme was omitted. In each equilibrium constant determination, the concentration of free trypsin was determined in duplicate or triplicate at each of five to seven inhibitor concentrations. A total of six such experiments was performed with the single-chain inhibitor and one with the two-chain inhibitor.

Plots of inhibitor concentration against $(1 - a)$, where a is the proportion of enzyme that is not bound to inhibitor, indicated that under our conditions the reactions of the inhibitors and trypsin fit case "b" according to Bieth (1974). We therefore obtained the dissociation constant associated with each experiment by plotting $I^o/(1 - a)$ against $1/a$, where I^o is the total inhibitor concentration in each reaction mixture. The slope of each least-squares line gave a value for the appropriate dissociation constant (Bieth, 1974). A value of 12 500 was used as the molecular weight of both the single- and the two-chain forms of the inhibitor (Swartz et al., 1977).

In our calorimetric measurements we used a batch microcalorimeter similar to that described by Kitzinger and Benzinger (1963). In a typical experiment the two bicompartmented microcalorimeter vessels were loaded as follows: the reaction vessel had 5 mL of a buffer solution containing trypsin (ca. 5 mg/mL) in one compartment and 5 mL of the same buffer solution containing trypsin inhibitor (ca. 1 mg/mL) in the other compartment; the blank vessel had 5 mL of buffer solution in each of the two

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