do not measure DHA may substantially underestimate vitamin C. However, since the relative contribution of DHA to vitamin C activity is not constant, the error due to nonestimation of DHA will not remain constant for any produce.

Registry No. DHA, 490-83-5; ascorbic acid, 50-81-7.

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Received for review July 1, 1983. Revised manuscript received January 19, 1984. Accepted March 2, 1984.

Quantitative Study of Volatiles in a Model System by a Headspace Technique

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A test solution was prepared with 16 pure compounds belonging to various chemical classes and representative of those found in fruits and fermented beverages aroma. These volatiles were extracted during 24 h by nitrogen entrainment associated with continuous Freon 11 liquid extraction in an apparatus similar to the one described by Rapp and Knipser. Analysis of the recovered material was done by quantitative gas chromatography. Reproducibility of the method is good; coefficients of variation are below 15%. Polar compounds such as alcohols, 4-ethylphenol, and lactones are not quantitatively recovered (2–10%) as they are badly entrained with nitrogen. Unstripped compounds are recovered by back-extraction of the remaining test solution. Apolar compounds such as terpene hydrocarbons are completely entrained with nitrogen but are partially lost during the extraction procedure. Losses are below 20%.

In the recent years, N_2 entrainment associated with trapping on a porous polymer has been widely used to study the aroma of wines and fruits (Jennings et al., 1972; Noble, 1978; Williams and Strauss, 1977; Murray, 1977). Minor components often critically important to aroma have been detected by this method while they could not have been revealed by an equilibrium method such as liquid and piston displacement (Williams et al., 1978).

The porous polymers have the common advantage of possessing minimal retention for ethanol as well as water, but some specific drawbacks have been detected for each of them. Tenax has been extensively used to study wines volatiles (Bertuccioli and Viani, 1976; Noble, 1978; Noble et al., 1980). Because of its high thermal stability, heavy compounds were quickly desorbed but its low specific area could create a fast saturation of the trap (Butler and Burke, 1976; Brown and Purnell, 1979). Porapack and Chromosorbs have higher sampling capacity, but high boiling compound recoveries are incomplete due to their low thermal stability (Williams et al., 1978). These different selectivities of porous resins induce distortion in the composition of collected headspace volatiles. A last common drawback is the necessity of conditioning the trap before and after each sample collection.

To avoid the disadvantages associated with the use of an adsorbent, Rapp and Knipser (1980) have proposed a method that combines N_2 entrainment and volatile trapping by Freon 11 continuous extraction. Williams (1982) advocated this method to collect volatiles of fruit or alcoholic beverages, but this procedure has been questioned by Novak (1981, 1982). However, recently Simpson and Miller (1983) and Guichard (1984), respectively, applied this technique with success to the study of Riesling wine and raspberry.

Rapp and Knipser evaluated reproducibility of their method for terpenoid components and others volatiles of interest for Moriot Muscat wine aroma. In this work we have tested this method with a model system of pure compounds belonging to various chemical classes of interest for fruit or fermented beverage aroma. Recovery of the pure compounds and the losses associated with this method were investigated by quantitative estimations of the constituents in the aroma extract and in the remaining test solution. The reproducibility of the techniques was determined for each compound.

MATERIALS AND METHODS

Model Solution. The test solution was made by adding 1 ppm (v/v) of each pure compound to a 10% aqueous ethanol solution. Water was purified on XAD_2 resin as described by James et al. (1981).

Extraction Procedure. The apparatus as proposed by Rapp and Knipser to collect volatiles was slightly modified: the tip of the collecting funnel was fitted with a fritted disc (porosity 2), which creates a dispersion of solvent in the sample.

Eight-hundred milliliters of test solution was placed in a flask kept in a water bath at 25 °C. Volatiles were entrained by purified nitrogen swept at the rate of 50

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0021-8561/84/1432-0838$01.50/0

Table I. Recovery of Components from the Model Solution in the Freon Extract and in the Remaining Model Solution (Recovery as Percent of the Initial Amount)[a]

compounds	recoveries in the Freon extract		recoveries in the back-extractions of the unstripped volatiles		total recoveries	
	$\bar{x} \times 100$ (A)	$\sigma/\bar{x} \times 100$	Freon 11 (B)	CH_2Cl_2 (C)	A + B	A + C
4-methyl-1-pentanol	2.5	12.6	38.3	78.0	40.8	80.5
1-hexanol	2.6	14.8	44.9	89.2	47.5	91.8
2-heptanone	31.5	9.7	68.0	65.3	99.5	96.8
α-pinene	80.9	8.7			80.9	80.9
β-pinene	85.4	7.3			85.4	85.4
ethyl hexanoate	74.9	7.8	19.1	20.3	94.0	95.2
γ-hexalactone			7.8	94.7	7.8	94.7
linalool	9.4	9.5	90.5	77.0	99.9	86.4
4-ethylphenol			37.7	101.6	37.7	101.6
nerol	2.1	11.6	97.8	79.8	99.9	81.9
2-phenylethyl acetate	2.1	11.2	92.2	84.3	94.3	86.4
γ-nonalactone			90.5	91.2	90.5	91.2
damascenone	7.5	8.5	90.9	74.9	98.4	81.4
humulene	83.8	1.7			83.8	83.8
ethyl cinnamate	1.6	3.5	95.2	85.2	96.8	86.8
β-ionone	7.1	5.3	76.2	60.5	83.8	67.6

[a] $\bar{x}$: mean of four extractions. $\sigma/\bar{x}$: coefficient of variation.

mL/min. The entrained volatiles were trapped in 10% aqueous ethanol (250 mL). This solution was continuously extracted with distilled Freon 11 (100 mL) for a period of 24 h. The condenser (coil condenser, Quickfit 778-54; 50 cm) was chilled at −30 °C with aqueous ethyl alcohol. All operations were carried out in a temperature-regulated laboratory at 16–17 °C.

Gas Chromatography. The quantitative analysis of aroma compounds was carried out by a glass capillary GC system on a chromatograph, Girdel 300, equipped with a flame ionization detector. The capillary column was a SE-52 (40-m length and 0.4-mm i.d.). The temperature was programmed from 40 to 180 °C at 2 °C min^{-1}. The carrier gas was helium at the rate of 2.5 mL min^{-1}. Peak areas were integrated with a Minigrator (Spectra Physics).

Quantification and Reproducibility. For the quantification, 1 mg of dodecane was added in the extracts before concentration. The 100-mL extracts were successively concentrated down to 1 mL with two Dufton-type columns of different sizes. These were made by coiling nickel wire around a glass rod, which was then tightly fritted into a narrow glass tube. The biggest column used to concentrate the sample from 100 mL to about 5 mL was made with 1 mm thick wire, a 6.5 mm o.d. rod, and a 19/26 Quickfit ground joint. The narrower column for further concentration is nearly the same as those described by Loyaux et al. (1981), a 3.9 mm o.d. rod and a 10/19 Quickfit ground joint. Both columns have been experimented with a 200 mg L^{-1} solution of 2-pentanone and 2-hexanone. No loss has been observed during the concentration step from 100 mL to 1 mL.

Each extract was analyzed in triplicate by GC. For each analysis, the ratio V/D (V and D, respectively represent volatile area and dodecane area) of each compound was evaluated. The reproducibility of the GC analysis was about 97%. The percentage recovery (A) of each volatile (mean of triplicate GC analyses) by the extraction procedure was calculated as

$$A = \frac{(V/D)_{\text{extract}}}{(V/D)_{\text{initial test solution}}} \times 100 \qquad (1)$$

Four extractions of the solution were carried out. The means ($\bar{x}$) and coefficients of variation ($\sigma/\bar{x}$) were evaluated for the percentage recoveries of each volatile. The linearity of detector response was tested for each compound with three solutions containing 100 μL L^{-1} dodecane and respectively 100, 50, and 25 μL L^{-1} of each pure compound.

In spite of the low temperature of the condenser, we have noticed that Freon 11 was not completely recondensed. The losses were estimated at about 15% (v/v). Possible losses of volatile compounds were evaluated as follows: (1) The model solution, which has been already stripped with nitrogen for 24 h, was split in two equal volumes that were respectively extracted with Freon 11 (3 × 40 mL) and dichloromethane (3 × 40 mL). The amount of volatiles thus recovered was determined (B and C). (2) The aqueous ethanol solution remaining in the extractor was back-extracted with Freon 11 (3 × 20 mL). No volatile was detected in this extract. The losses (L), in percent of each compound, can be calculated as

$$L = 100 - [A + \max(B, C)] \qquad (2)$$

The better recovery of both back-extractions [max(B, C)] was considered for the evaluation of the losses for each compound.

RESULTS AND DISCUSSION

Results are shown in Table I. It appears that the Rapp and Knipser method seems to be effective for apolar compounds such as terpene hydrocarbons, whatever their boiling point, but polar compounds are not quantitatively recovered: alcohols are only recovered to an extend of 2–10% while 4-ethylphenol, γ-hexalactone, and γ-nonalactone were not detected in the extract. This result agrees with study of Tsugita et al. (1979), who have not observed any recovery for similar compounds by nitrogen entrainment. For medium-polar compounds such as esters and ketones, recovery increases when the boiling point decreases.

As no volatile was detected in the aqueous ethyl alcohol solution when back-extracted by Freon 11, this shows that the entrained volatiles that have been trapped were progressively extracted by Freon 11.

After back-extraction by Freon 11, the total recovery (A + B) exceeds 90% for medium-polar compounds such as esters and ketones. Terpene hydrocarbons are completely entrained with nitrogen, but their recovery doses not exceed 85%, which means that these apolar compounds are probably not completely trapped in the aqueous ethyl alcohol solution and lost by entrainment. As Freon 11 does not completely extract alcohols, 4-ethylphenol, and γ-

hexalactone, (A + B) remains lower than 50% but (A + C) is above 90%.

For medium and highly polar compounds, losses are negligeable, which agrees with the results of Rapp and Knipser (1980). For less polar compounds such as terpene hydrocarbons, not studied by these authors, losses can be up to 20%.

The reproducibility of the method is good. Coefficients of variation are below 15%. In comparison with the results of Williams et al. (1978) for synthetic mixtures, it is better than with a purging technique but worse than with piston displacement.

By this method ethyl alcohol is not extracted and other alcohols are weakly extracted. Therefore, this method is of great interest to study the aroma of fermented beverages as there is no saturation of the extract by the alcohols formed during fermentation. It means that the time of extraction can be increased to recover a greater amount of esters and high-boiling compounds. No saturation has been noticed in the trapping step due to solvent rectifying. Owing to the good reproducibility of this method, low variations of volatiles among different samples can easily be revealed even if they are not quantitatively recovered.

ACKNOWLEDGMENT

We are grateful to Dominique Langlois of this laboratory, who made the apparatus.

Registry No. Freon 11, 75-69-4; 4-methyl-1-pentanol, 626-89-1; 1-hexanol, 111-27-3; 2-heptanone, 110-43-0; α-pinene, 80-56-8; β-pinene, 127-91-3; ethyl hexanoate, 123-66-0; γ-hexalactone, 695-06-7; linalool, 78-70-6; 4-ethylphenol, 123-07-9; nerol, 106-25-2; 2-phenylethyl acetate, 103-45-7; γ-nonalactone, 104-61-0; damascenone, 23726-93-4; humulene, 6753-98-6; ethyl cinnamate, 103-36-6; β-ionone, 79-77-6.

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Received for review January 10, 1984. Accepted April 3, 1984.

Hydrolysis of Wheat Straw Hemicelluloses and Heteroxylan (Larchwood) by Human Colon *Bacteroides ovatus* B4-11 Enzymes

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An intracellular enzyme fraction that catalyzes the degradation of wheat straw hemicelluloses and heteroxylan (larchwood) was isolated from *Bacteroides ovatus* B4-11. The intracellular enzyme(s) that degraded hemicelluloses and heteroxylan was (were) present at low levels in B4-11 grown on glucose or xylose. The hydrolytic activity was markedly increased when B4-11 was grown in the presence of hemicelluloses or heteroxylan. The percent hydrolysis calculated from the reducing sugar data was substantially higher than that calculated from HPLC data. The intracellular enzyme(s) of B4-11 grown on crude hemicellulose hydrolyzed and released about 13, 22, 4, 19, and 2% of the total sugars, respectively, from heteroxylan, crude hemicellulose, hemicellulose A, hemicellulose B, and wheat straw in 18 h. Hemicellulose A induced enzymes released about 5% of the total sugars from hemicellulose A in 18 h, while hemicellulose B induced enzymes liberated about 8% of the total sugars from hemicellulose B. Heteroxylan-induced enzymes released about 5% of total sugars from heteroxylan in 18 h. Xylose was the predominant sugar in addition to substantial amounts of oligomers in the hydrolysates.

Hemicellulose ranks second to cellulose in abundance in agricultural waste residues such as wheat straw and oat hulls. The hemicellulose content in plants changes with growth and maturity (Wilkie, 1979). Hemicelluloses are heteropolysaccharides and made up of at least two to four different types of sugar residues depending upon the source (Aspinall, 1970; Dekker, 1979; Wilkie, 1979). For example, hemicellulose A of wheat straw consists of three neutral sugar residues (xylose, arabinose, and glucose) and glucuronic acids, whereas hemicellulose B has four neutral sugar residues (xylose, arabinose, glucose, and galactose) and glucuronic acids (Bishop, 1953; Aspinall and Meek, 1956; Reddy et al., 1983).

Digestion of polysaccharides by rumen bacteria has been investigated in some detail (Dehority, 1973; Dekker, 1976; Chesson, 1981; Brice and Morrison, 1982; Williams and Withers, 1982a,b), but relatively little is known about hemicellulose degradation by human colon bacteria. *Bacteroides* sp. account for approximately 20% of the normal human fecal flora (Moore and Holdeman, 1974; Holdeman et al., 1976) and are known to ferment a wide

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0021-8561/84/1432-0840$01.50/0