

Concentration of Volatile Compounds from Dilute Aqueous Solutions

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Two methods, developed for flavor studies, for the concentration of volatile compounds from dilute aqueous solutions without the use of organic solvents are described. One method is applicable to a large range of volumes, in this work from 5 to 5000 ml., and involves distillation at 0 to 20 torr through a vertical condenser held at 0° C. until a small volume ($1/1000$ to $1/50$ of the original volume) is collected in a liquid nitrogen trap. This distillate frequently contains most of the volatile compounds. The second method, used to obtain a concentrate

of the lower boiling compounds almost free of water, involves high-vacuum sublimation of the aqueous solution through a trap at approximately -55° C. into a liquid nitrogen trap. This sublimation technique is used mainly to concentrate distillates obtained by the first method and is applicable to compounds that are normally lost by solvent extraction techniques. The following compounds were studied: the C_{1-10} *n*-alkan-1-ols, the C_{3-12} *n*-alkan-2-ones, and the C_{3-10} ethyl *n*-alkanoates.

The success of any flavor investigation depends largely on the methods used to remove or concentrate the flavor compounds from their substrate for subsequent characterization. The requirement of any isolation technique is maximum yield of representative material without heat damage or contamination. Three techniques for isolation of flavors from dilute aqueous solutions are in common use—solvent extraction, steam distillation, and head space analysis. The first suffers from artifacts due to the concentration of any solvent impurity and from loss of low boiling compounds, while the last frequently over-emphasizes the importance of the more volatile components. Reymond *et al.* (1966) favored steam distillation to provide a more diversified characterization of the flavor, but found it necessary to use head space analysis for the most volatile compounds.

Isolation of food flavors by steam distillation provides relatively large volumes of an aqueous extract containing compounds at a level of 10 p.p.m. or less. To separate and analyze such solutions by a combination of gas chromatography and spectroscopy, a concentration factor of 100 to 1000 is required. Several authors—e.g., Weurman (1961)—have attempted to solve this problem by evaporation through a cyclone still to give a 10-fold concentration for one pass. Similarly, Bidmead and Welti (1960) obtained a 100-fold concentration using a fractionating column operated for 1 hour under total reflux and at 160 torr followed by a take-off of one-thirtieth of the total reflux. Others—e.g., Rhoades and Millar (1965)—have removed the more volatile compounds with a stream of an inert gas; the volatiles were collected in a cold trap and extracted with solvent.

As none of these techniques easily gives the required results, attempts were made to achieve the necessary concentration by refluxing the solution through a cold condenser into a liquid nitrogen trap.

In addition, the technique of Stahl (1957) and Merritt *et al.* (1959), who used sublimation under high vacuum to separate the more volatile components from the main water fraction, has been extended to obtain microliter quantities of water containing a considerable percentage of the volatile components.

The application of both of these techniques to the concentration of *n*-alkan-1-ols from butter has been described briefly (Stark and Forss, 1966). Since then, Whitfield and Shipton (1966) have reported similar experiments on the recovery of *n*-alkan-1-ols in their work on the flavor of frozen peas. The present paper gives full experimental details and an evaluation of the techniques when used in model systems to concentrate homologous series of alcohols, methyl ketones, and ethyl esters.

MATERIALS AND METHODS

Stock solutions of C_{1-10} *n*-alkan-1-ols, C_{3-12} *n*-alkan-2-ones, and C_{3-10} ethyl *n*-alkanoates were prepared containing approximately 0.1 μ l. of each component per ml. of distilled water. These solutions were diluted with distilled water immediately prior to use. The final concentrates (distillates) were diluted with water to correspond to the volume of the stock solution originally used (1 to 10 ml.). Aliquots were analyzed by gas chromatography with a 5% Carbowax 20M on acid-washed, 60- to 70-mesh Chromosorb G column (10 foot \times $3/16$ -inch O.D.) programmed from 60° to 160° C. at 2°, 4°, or 8° C. per minute using a hydrogen flame ionization detector.

In quantitative gas chromatography of aqueous solutions, maintenance of the same conditions for all samples is essential. For example, the distillates should be reconstituted to the volume of the stock solution added, and the same volume—e.g., 10 μ l.—should be used for the analysis. Percentage yields were determined by comparing peak heights in these chromatograms with those obtained from the stock solution under identical conditions.

EXPERIMENTAL AND RESULTS

Concentration under Reflux. The apparatus (Figure 1) consists of a 5-liter flask connected to a mechanical pump through a double-surface (Davies) condenser cooled to 0° C. and traps cooled in liquid nitrogen. Stock solution containing 2 to 40 μ l. of mixtures of the test compounds was diluted to 2 liters in the 5-liter flask, magnetically stirred, cooled to 5° C., and evacuated slowly to 6 ± 2 torr with the liquid nitrogen level well above the side arm of trap *G*. Stopcock *J* was then closed, the liquid nitrogen lowered to below the sidearm of trap *G*, and the solution warmed with magnetic stirring until liquid water returning to the 5-liter flask was clearly visible in the bottom one-third of the con-

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denser. The temperature of the solution at which this occurred varied according to the pressure—e.g., 6° C. at 6 torr and 20° C. at 15 torr. A total of about 40 ml. of distillate was collected in 2 to 4 hours. The 40 ml. could be reduced to a smaller volume—e.g., 4 ml.—using the same technique with trap *G* as the distillation flask and trap *H* (or trap *I* for volumes less than 1 ml.) as the receiving vessel.

Two-liter quantities of solution need to be degassed

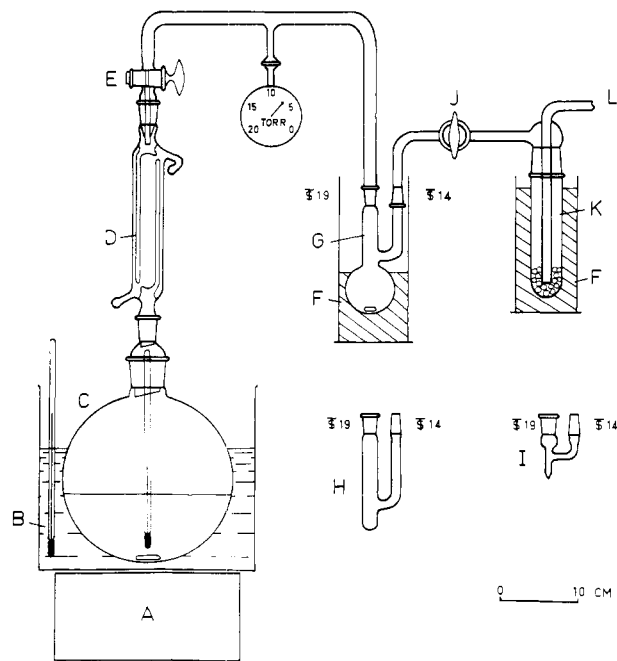


Figure 1. Apparatus for concentration under reflux

A. Magnetic stirrer and heater. B. Water bath. C. 5-liter flask with $\text{\textcircled{F}} 34$ neck. D. Double-surface (Davies) condenser with $\text{\textcircled{F}} 24$ joints. E. Stopcock, 2 mm. F. Liquid nitrogen vessels. G. Collecting flask, volume 100 ml. H. Collecting trap for volumes 2 to 10 ml. I. Collecting trap for volumes below 1 ml. J. Stopcock, 4 mm. K. Trap containing glass beads. L. Connection to mechanical pump, pressure 10^{-2} torr

slowly and evenly to avoid entrainment of the distillate into the second trap *K* and into the pump. This could be achieved by just opening stopcock *E* and adjusting it continuously, as the pressure decreased, to prevent violent boiling. It was simpler and more satisfactory to evacuate through a glass capillary tube (5 cm. \times 0.5-mm. bore) connected below *E*.

Experiments were carried out to determine the recoveries in each of the two stages, 2 liters to 40 ml. and 40 ml. to 4 ml. Trap *H* was used to collect the distillate (approximately 5 ml.) from 2 liters of solution in one stage.

Table I shows typical recoveries of alcohols, ketones, and esters in single-stage distillations of 2-liter solutions degassed through a capillary. In this case, a concentration factor of 400 was achieved. The recovery of alcohols, ketones, and esters was best for the compounds of intermediate chain length, the most volatile compounds showing a greater tendency to pass through traps and the least volatile compounds tending to remain in the solution or to condense in the manifolds. The latter effect can be minimized by heating the manifolds and by reducing the pressure—e.g., 10^{-2} torr—at the end of the distillation with *E* closed. If two successive 5-ml. fractions were collected, the approximate over-all mean recoveries of compounds were 80% in the first trap, 10% in the second trap, and 5% in the third or bead trap (Table I). The single-stage distillation was more rapid and much simpler than the two-stage, and yields were similar.

There was no correlation between the concentrations of compounds (2 to 40 $\mu\text{l.}$ in 2 liters) in the solution and the per cent recoveries.

Small scale experiments (40 ml. of solution and distillates of 0.1 to 1.0 ml.) showed recoveries of the lower boiling compounds comparable with those obtained from the 2 liters of solution, but the recoveries of the highest boiling compounds were somewhat lower.

Variations in apparatus and technique were evaluated, such as the use of single wall condensers, smaller-bore condensers, two or three condensers in series, condenser packed

Table I. Percentage Recoveries of Organic Compounds from 2 Liters of Water by One-Stage Distillation

Carbon Number	Alcohols, 10 $\mu\text{l.}^a$			Ketones		Esters				
	1st Distillate	2nd Distillate	Total	Expt. 1 2 $\mu\text{l.}^a$	Expt. 2 10 $\mu\text{l.}^a$	Expt. 2, 20 $\mu\text{l.}^a$				
						Expt. 1 ^b 2 $\mu\text{l.}^a$	1st Distillate	2nd Distillate	Bead Trap	Total
12	52	71
11	67	70
10	38	16	54	75	75	70	45	17	5	67
9	49	17	66	91	85	78	60	16	6	82
8	63	13	76	94	97	84	67	12	7	86
7	83	11	94	97	89	83	85	8	7	100
6	91	11	102	95	84	84	86	6	8	100
5	84	7	91	98	84	91	90	5	8	103
4	87	12	99	89	83	98	85	4	7	96
3	75	8	83	99	78	100	86	4	8	98
2 1	71	7	78							
Volume of distillate, ml.	4.9	5.0	9.9	6.4	5.2	5.2	6.0	7.8	0.2	14.0

^a Volume of compounds added.

^b No capillary below *E* (Figure 1).

with Fenske helices, increased boil-up rate with partial flooding of the condenser, or saturating of the solution with NaCl. None of these modifications provided any increase in efficiency or recovery and, indeed, several were markedly inferior—thus, smaller diameter condensers invariably allowed some physical transfer of liquid plugs.

Sublimation Technique. Five milliliters of aqueous solutions containing 1 to 5 μ l. of the alcohol, ketone, or ester mixtures were placed in flask *A* (Figure 2) and frozen in liquid nitrogen with stopcock *D* closed. The apparatus was then evacuated through *D* to a pressure of less than 10^{-4} torr by means of an oil diffusion pump. Stopcock *D* was closed, the solution was allowed to melt to eliminate dissolved gases, was then refrozen and re-evacuated. This

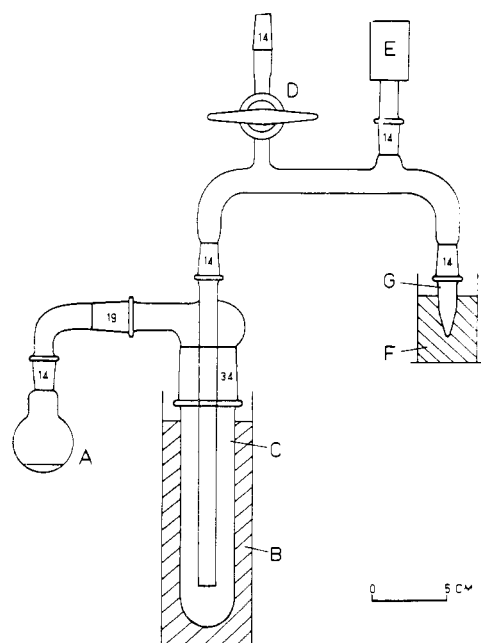


Figure 2. Apparatus for concentration by sublimation

A. 25-ml. flask. *B.* Dry ice and acetone bath. *C.* Trap at -55° C. *D.* Stopcock (6 mm.) to diffusion pump. *E.* Pirani gage. *F.* Liquid nitrogen vessel. *G.* Trap for distillate (less than 100 μ l.)

Numbers indicate $\overline{\text{T}}$ sizes

freezing and thawing was repeated until a residual gas pressure less than 10^{-4} torr was obtained (usually 2 cycles). Trap *C* was cooled in a mixture of dry ice and acetone (or ethanol) to a desired temperature in the range -70° to -30° C. and trap *G* was cooled in liquid nitrogen. The solution in *A* was allowed to warm up and sublime through trap *C* into trap *G*. Most of the water remained in trap *C*, and the more volatile organic compounds distilled into trap *G*. With the model systems, 5 ml. of water were added to trap *G* (a longer trap was used), and it and the contents of trap *C* were analyzed by gas chromatography.

Table II shows typical recoveries with the dry ice and acetone (trap *C*) at -55° C. The amount of water and also the range of compounds collected in the liquid nitrogen trap varied with the temperature of the dry ice-acetone mixture. A temperature of -55° C. yielded less than 100 μ l. of water with compounds boiling well above water in good yield—e.g., the C_{8-9} ethyl esters. In flavor studies, these distillates were analyzed directly by combined gas chromatography and mass spectrometry. When lower boiling compounds were being studied, lower temperatures—e.g., -65° C.—were used, and very little water distilled into the liquid nitrogen trap.

DISCUSSION

Concentrations of 400-fold may be achieved by distillation through a vertical condenser at 0° C. with yields of better than 80% for *n*-alkan-1-ols, *n*-alkan-2-ones, and ethyl *n*-alkanoates boiling below 175° C. Yields of the higher boiling compounds studied generally were better than 40%. In flavor studies, no great advantage is to be gained by collecting a second equal volume of distillate (5 ml. in the distillation of 2-liter solutions) unless a subsequent 200-fold concentration of the combined distillates is contemplated—i.e., an over-all 40,000-fold concentration and a final volume of 50 μ l. For quantitative isolation studies, the procedures most commonly used to obtain the aqueous solution containing the volatile compounds—e.g., steam distillation of butter oil—are usually less efficient than the concentration methods described.

To obtain the lower boiling compounds in a volume of less than 100 μ l., vacuum sublimation is a simpler secondary concentration step than repeated reflux concentration—Merritt *et al.* (1959) sublimed frozen solutions at tempera-

Table II. Percentage Recoveries of Organic Compounds by Sublimation through a Cold Trap at -55° C.

Carbon Number	Alcohols ^a	Ketones ^a	Esters ^a
12	...	4 (247° C.)	...
11	...	6 (228° C.)	...
10	0	7 (211° C.)	32 (208° C.)
9	0	20 (195° C.)	60 (189° C.)
8	10 (195° C.)	80 (173° C.)	80 (168° C.)
7	12 (177° C.)	100 (151° C.)	100 (145° C.)
6	43 (158° C.)	100 (128° C.)	100 (122° C.) [0.037 torr]
5	86 (138° C.) [<0.001 torr]	100 (102° C.) [0.011 torr]	100 (99° C.) [0.083 torr]
4	88 (118° C.) [0.004 torr]	100 (80° C.) [0.55 torr]	100 (77° C.) [0.35 torr]
3	97 (97° C.) [0.016 torr]	100 (56° C.) [1.5 torr]	100 (54° C.) [1.75 torr]
2	96 (78° C.) [0.10 torr]		
1	96 (65° C.) [0.38 torr]		

^a Boiling points in parentheses. Vapor pressure at -55° C. in brackets, calculated from the data of Stull (1947).

tures from -80° to -196° C. In our experience, the method worked most satisfactorily when the frozen solid had an open porous structure with a large surface area, which was achieved by the introduction of the additional trap at -55° C. In the present technique, the frozen solution is allowed to sublime at ambient temperature or in a water bath at 50° C. through traps at -55° and -196° C.; it is quicker and will give a very good concentration even for compounds with boiling points well above that of water (Table II).

When removing flavors from natural products, it is often unnecessary to isolate a steam distillate before concentration; the flavor concentrate may be produced directly by refluxing the product—e.g., buttermilk—or a suspension in water—e.g., casein or by combining the steam distillation with the concentration—e.g., butter oil (Forss and Holloway, 1967). Subsequent vacuum sublimation gives the more volatile flavor compounds in less than 100 μ l. of

water. This solution is well suited to analysis and compound identification by continuous gas chromatography and mass spectrometry.

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