Freeze Concentration of Volatile Components in Dilute Aqueous Solutions

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Two methods for freeze concentration of dilute aqueous solutions of volatile organic compounds were investigated. Recoveries by Method A (cold finger arrangement) were highly variable (65 to 100%) because of selective losses by evaporation which could not be readily prevented. Recoveries by Method B (Shapiro-type arrangement), where evaporation losses could be minimized, were 90% or better for all components without appreciable ratio changes in the final concentrates. Concentration factors of five- to better than 40-fold, depending on the concentrations of the volatile components in the starting solutions, were attainable without appreciable losses by trapping in the ice phase. The utility of freeze concentration as a preliminary concentration step in the investigation of food aroma distillates is indicated.

Shapiro (1961, 1967) has described a method of concentrating dilute aqueous solutions of nonvolatile and volatile compounds by selective freezing out of the water. He obtained recoveries of the order of 90 to 100% after fivefold concentrations of aqueous solutions of sodium chloride, 2-propanol, and acetone. Kobayashi and Lee (1964) and Smith and Tasker (1965) concentrated solutions of inorganic salts and dyes using the techniques of Shapiro (1961) and obtained recoveries of nearly 100% at concentration factors of 10 to 20. Baker (1965, 1967) used freeze concentration to identify trace organics in industrial waste water. Schildknecht and Schlegelmilch (1963) in a more fundamental study investigated the effects of speed of stirring, rate of cooling, and initial concentrations of the components on freeze concentration as applied to the purification of several organic compounds. The use of the freezing-out technique for the concentration of heat-labile or volatile components in the laboratory was reported by Hale and Cole (1963) in studies on bread preferment solutions, by Wilson et al. (1964) for concentration of solutions of biological materials, and by Bidmead (1963) and Senn (1963) in investigations of fruit juices. Huckle (1966) concentrated benzene extracts of fruit juices by means of a specially designed zone melting apparatus.

In spite of these efforts, freeze concentration has not been used very often in the laboratory in aroma research. Usually the volatile components in distillates from fruits and other food products are isolated by extracting them with low-boiling organic solvents, followed by careful removal of the solvent by any of a variety of distillation techniques. The extraction process entails certain potential disadvantages, such as accumulation in the aroma concentrate of higher boiling impurities from the solvent, loss of low-boiling components, changes in ratios of concentrations, and artifact formation. When concentrating of the volatiles is attempted by distilla-

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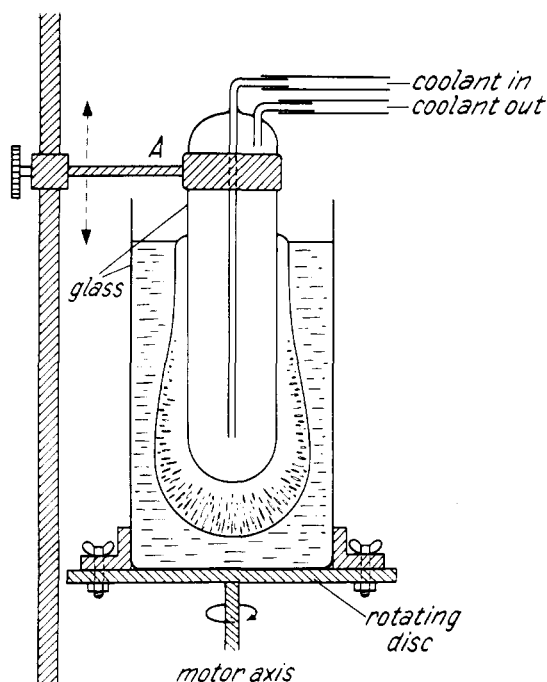[1] Present address, Department of Chemistry, University of California, Davis, Calif. 95616

Table I. Composition of Synthetic Aqueous Solutions

Components, μl./L.	Solutions			
	A	B	C	D
Ethyl formate	2	6	20	
Ethyl butyrate	2	6	20	
Ethyl caproate	5	15	50	18
3-Pentanone	4	12	40	
4-Heptanone	5	15	50	
3-Heptanone	5	15	50	
3-Octanone	8	24	80	
Ethanol	120	360	1000	
1-Propanol				240
1-Butanol	120	360		
3-Methylbutan-1-ol				360
1-Butanal				8
1-Hexanal				12

tion, changes in ratios cannot be prevented even if artifact formation can be eliminated by reducing the pressure (Forss et al., 1967). These disadvantages are often neglected in practice. Direct vapor analyses of volatile components by gas chromatographic head space techniques can overcome most of the above disadvantages and give chromatograms from which the actual concentrations or concentration ratios of aroma components in distillates may be calculated in many instances (Kepner et al., 1964). However, the sensitivities of the detection systems used are often not sufficient to permit detection of the components present in trace amounts. A 20- to 50-fold concentration of a distillate, accomplished by a method which does not involve the above listed difficulties, would greatly facilitate such analyses.

This report presents the results of investigations on freeze concentration as such a useful step in the analysis or isolation of volatile components in aqueous distillates in aroma research.

EXPERIMENTAL

Solutions. Pure organic compounds were used to prepare four test solutions containing varying amounts of the volatile components in distilled water (Table I). The desired amounts

Figure 1.   Freeze concentration apparatus

Method   A. Cold finger arrangement

Figure 2.   Freeze concentration apparatus

Method B.   Shapiro type arrangement

of the organic compounds were added to the water by means of syringes calibrated in microliters. Under the gas chromatographic conditions used, the solutions produced chromatograms with all peaks separated and all peak heights of the same order of magnitude at the same sensitivity for a given solution.

Freezing Procedures. METHOD A (cold finger arrangement). The apparatus is shown schematically in Figure 1. In the experiments described here, the cold finger condenser (o.d. 4.5 cm.) was suspended in a 3-liter Dewar flask (i.d. 14 cm.) with the bottom of the condenser about 2 cm. above the bottom of the Dewar flask. Two liters of solution, precooled to 4° C., were placed in the flask, which was located centrally on the disk and rotated at 80 r.p.m. while coolant at −15° C. was circulated through the condenser. During the freezing process, about 5.5 to 6 hours, the condenser was raised an additional 4 to 5 cm. above the bottom of the flask. At the end of the concentration period the condenser with its ice layer was raised above the solution and the surface of the ice quickly rinsed with a small amount of cold distilled water which was allowed to run back into the concentrated solution. The ice layer was easily removed from the condenser for analysis by passing warm water through the condenser.

METHOD B (Shapiro arrangement). A 3-liter cylindrical, stainless steel container was cooled either by placing it in the cabinet of a freezer or by pumping coolant at −25° C. through a semicircular pipe wrapped around the container (Figure 2). A mechanical stirrer was positioned such that the 2.5-cm. blade was about 1 cm. above the bottom of the container. A 2.5-liter portion of solution, precooled to 4° C., was placed in the precooled container, which was then covered with a polythene sheet. The level of the solution inside the container was 1 cm. above the level of the coolant outside. The stirrer was rotated slowly until a thin layer of ice had formed on the wall of the container. During the subsequent freezing period the stirrer was rotated as rapidly as possible without causing splashing or beating air into the solution, and was gradually
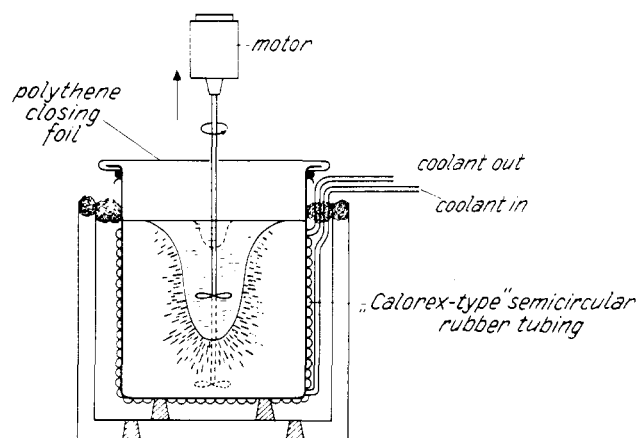
slowed down and raised as freezing progressed to prevent the blades coming in contact with the ice. The solution became concentrated into a conical hole at the end of the freezing period and the concentrate was pipetted out for analysis. The ice was not rinsed before it was melted for analysis of entrapped volatiles.

Gas Chromatographic Analysis. The starting test solutions, concentrates, and melted ice fractions were analyzed gas chromatographically, using head space techniques (Kepner *et al.*, 1964). The concentrates and melted ice fractions were diluted with distilled water to the original volume of the starting solution before analysis. A standard solution of 2-heptanone was added to each solution analyzed to act as an internal standard. Thirty milliliters of each solution in a 100-ml. serum flask was equilibrated for 45 minutes at 36° C. A 5-ml. gas sample was removed and injected into a 3.75 meter × 4 mm. i.d. column containing 15% LAC-IR-296 on acid-washed Chromosorb W, 60- to 80-mesh, column temperature 70° C., flame ionization detection with N_2, H_2, and O_2 flow rates of 30, 30, and 100 ml. per minute, respectively.

RESULTS AND DISCUSSION

The compositions of the synthetic mixtures used in this investigation are listed in Table I. With the exception of l-butanol, solutions A, B, and C contain the same components with the concentrations increased approximately threefold (B over A, C over B). The composition range in the solutions was designed to facilitate investigation of the effect of concentration on the crystallization process and trapping of the solutes in the ice phase. Shapiro (1961) has stressed the necessity of continuously disturbing the contact layers between the liquid and solid phases and Schildknecht (1964) the concept of "directed" freezing as important considerations for successful freeze concentration. These conditions are satisfied by the two techniques described here.

As artifact formation will, in general, be at a minimum under the conditions of freeze concentration, the major problem that must be considered in the process is possible loss of the volatile components. The main methods by which losses may occur are occlusion, adsorption, evaporation, and "channeling" in the ice layer. Of these, only occlusion of drops of the liquid solution is not selective and would, therefore, affect the over-all efficiency of the process but not the ratio of components in the final concentrate. The occurrence of occlusion is usually indicated by the formation of opaque ice during

Table II. Recoveries of Volatiles in Concentrates

Method A. Cold Finger Arrangement

Components	Solution A[a] (3 Experiments), Concentrated up to 4-Fold		Solution B[a] (3 Experiments), Concentrated up to 4-Fold	
	Av., %	Stand. dev.[b]	Av., %	Stand. dev.[b]
Ethyl formate	68	3	64	2
Ethyl butyrate	78	5	74	4
Ethyl caproate	73	1	74	4
3-Pentanone	88	2	86	2
4-Heptanone	83	9	82	3
3-Heptanone	85	2	84	4
3-Octanone	84	7	83	1
Ethanol	99	6	98	4
1-Butanol	100	3	95	5

Method B. Shapiro Arrangement

Components	Solution B[c] (5 Experiments), Concentrated up to 15-Fold		Solution C[c] (2 Experiments), Concentrated up to 6-Fold	
	Av., %	Stand. dev.[b]	Av., %	Stand. dev.[b]
Ethyl formate	98	8	94	6
Ethyl butyrate	92	5	93	8
Ethyl caproate	96	7	100	2
3-Pentanone	95	2	90	3
4-Heptanone	96	4	94	5
3-Heptanone	96	5	95	1
3-Octanone	95	6	100	1
Ethanol	97	5	90	4
1-Butanol	96	4		

Components	Solution D[c] (5 Experiments), Concentrated up to 20-Fold	
	Av., %	Stand. dev.[b]
Ethyl caproate	91	7
1-Propanol	92	9
3-Methylbutan-1-ol	92	9
1-Butanal	93	9
1-Hexanal	90	8

[a] Concentration in ice during all experiments, max. 1%.
[b] Standard deviation according to Bauer (1960).
[c] Concentration in ice during all experiments max 4%.

Table III. Evaporation Losses in Freeze Concentration Using Cold Finger Arrangement

Components	Normal Freeze Concn., % Recovery	Rotated at 0° C. without Freezing, % Recovery
Ethyl formate	63	59
Ethyl butyrate	77	75
Ethyl caproate	74	78
3-Pentanone	86	85
3-Heptanone	84	85
3-Octanone	83	85
Ethanol	99	103

the ice when the solution becomes saturated with air as a result of the concentration process. Ice then freezes around these bubbles, with the resultant formation of air channels which may be 6 to 8 mm. in length or even longer. When concentrating freshly distilled water or fresh distillates of aroma components, the formation of channels occurs only in the final stages of the freezing process. The loss of volatiles in these channels is a selective process, in that it is dependent on the vapor pressures of the components in equilibrium with the solution at that time, but it can probably be neglected because of the small total volume of the channels.

Freezing at a faster rate by decreasing the temperature of the circulating coolant and/or the rate of stirring from the optimum conditions listed above gave an opaque ice layer which contained 10 to 20% or sometimes more of the volatiles, apparently by occlusion of the solution. A selective adsorption or other selective trapping of volatiles in the ice was not indicated by the experimental results. The recoveries of volatiles by the cold finger method of freeze concentration varied considerably (Table II). The alcohols gave recoveries of nearly 100%, whereas only about 70% of the esters and 85% of the ketones remained in the concentrates. That these losses were due primarily to evaporation was shown by comparing the concentrations of the volatiles in a typical freeze concentration run with the concentrations in the solution after it was rotated in the cold finger apparatus for 6 hours while the solution was held at 0° C. but without freezing. The results, shown in Table III, demonstrate almost identical losses under the two sets of conditions. Shapiro (1967) likewise observed large losses by evaporation during freeze concentrations of aqueous solutions of iodine.

In the experiments by Method B (Shapiro arrangement, Figure 2), 2500-ml. aliquots of solutions B, C, and D (Table I) were concentrated up to 15-, 6-, and 20-fold, respectively, as indicated in Table II. The recoveries of the volatile components in the concentrates were better than 90% in all cases and approached 100% in many instances. Because of the polythene cover used, losses by evaporation were small, as shown by the essentially equal recoveries of esters, ketones, and alcohols. Analysis of the ice phase showed a maximum of 4% of the total volatiles remaining in this fraction. The larger losses in the ice fraction by this method were probably due as much to increased trapping of volatiles in the ice as it formed as to the fact that after the concentrate was pipetted out at the end of the freezing process the surface of the ice cone could not be conveniently rinsed thoroughly. The rates of freezing and stirring again influenced the structure of the ice layer in a manner similar to that discussed for the cold finger experiments.

Figure 3 shows the gas chromatographic analyses for a typical freeze concentration run with a synthetic aqueous
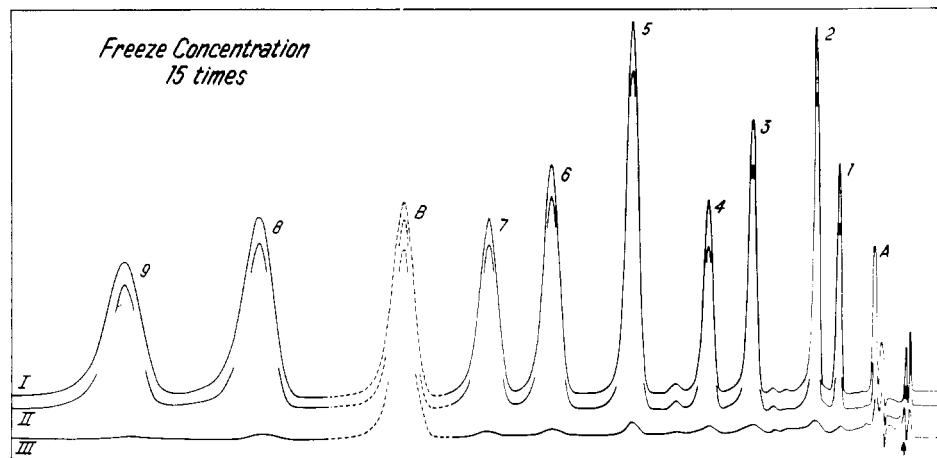
the freezing process. The other methods of loss of volatiles, if they occurred, would change the ratios of the components in the final concentrates.

In the experiments using the cold finger apparatus (Figure 1) the best results were obtained by circulating coolant at −15° C. and rotating the disk at 80 r.p.m. Under these conditions it was possible to concentrate 2000 ml. of solution to 40 to 50 ml. in a 9-hour period without trapping the volatiles in the ice. For comparison of the techniques, solutions A and B (Table I) were concentrated approximately fourfold in continuous runs of 5.5 to 6 hours' duration. The recoveries of the volatile components are given in Table II. The ice layer formed on the cold finger condenser was transparent, indicating no occlusion of liquid droplets, and contained only about 1% of the original amounts of volatile constituents as detected in the melted ice by head space analyses. A phenomenon which occurs to a varied extent with this procedure, and seems to depend on the amount of air dissolved in the solution, is the formation of very fine air channels in the freezing ice layers on the cold finger condenser. The channels are initiated by minute air bubbles which adhere to the surface of

Figure 3.   Head space analyses of freeze concentration fractions obtained by Method B from solution B

Gas chromatographic conditions listed in text
    I.   Original solution
II,III. Concentrate and melted ice fractions, respectively, after dilution back to original volume
                            A. Air peak
                            B. Standard (2-heptanone)
                              1. Ethyl formate
                              2. Ethanol
                              3. 3-Pentanone
                              4. Ethyl butyrate
                              5. 1-Butanol
                              6. 4-Heptanone
                              7. 3-Heptanone
                              8. Ethyl caproate
                              9. 3-Octanone
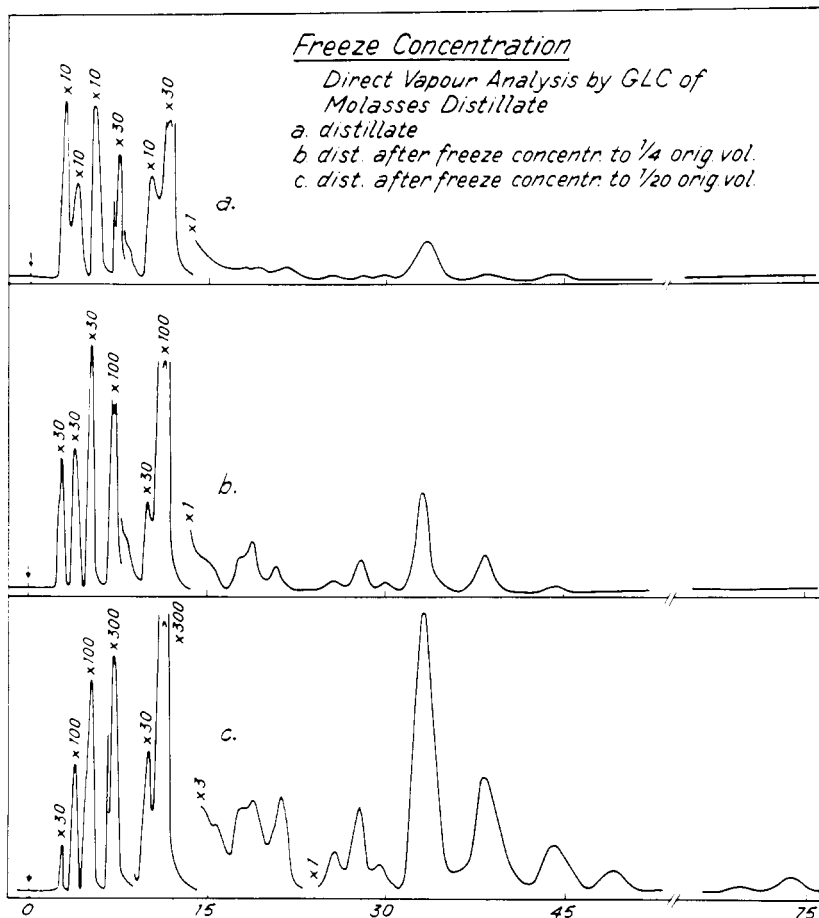

Figure 4.   Freeze concentration of a molasses distillate

solution using the Shapiro-type equipment of Figure 2. Peak B represents the added internal standard and peaks 1 to 9 represent the components of the synthetic mixture. Curve I is the head space analysis of the starting solution, curve II the analysis of the solution after concentration from 2250 ml. to 150 ml. and dilution back to the original volume, and curve III the analysis of the melted ice phase diluted back to the original volume. A careful comparison of curve II with curve I shows that the ratios of the components after the 15-fold concentration and dilution back to the original volume for analysis are essentially the same as the starting solution. The small losses of the different components in the ice phase are demonstrated by curve III.

Both types of equipment can be used successfully to concentrate dilute aqueous solutions of volatiles. The concentration factors which could be attained in one 5- to 6-hour freezing period were four- to fivefold with the cold finger equipment and up to 20-fold by Method B. In each case as the ice layer becomes thicker the rate of freezing slows down in the latter stages of the process when the solution becomes more concentrated. The cold finger method has the advantage that the structure of the ice can be observed more easily during the freezing process and the ice phase can be removed from the condenser periodically, if desired. By combining concentrates and removing the ice phase periodically, concentration factors of over 40-fold were obtained by Method A.

However, ratio changes due to evaporation losses could not be conveniently prevented under the conditions used. Prevention of evaporation losses was much easier using Method B. and changes in ratios of components during the concentration process were minimal. Freeze concentration under the conditions described is limited primarily by the increasing concentrations of the dissolved compounds. When the solution becomes too concentrated, the ice layer tends to be opaque and large amounts of the dissolved components are trapped in the ice phase. This result was observed when solution C

was concentrated more than the sixfold indicated in Table II, because of the high concentration of ethanol in the starting solution. Freeze concentration, in contrast to solvent extractions, is more efficient and smoother running the more dilute the starting solution. In food aroma research freeze concentration of dilute aqueous distillates affords definite advantages as a primary concentration step to be followed by direct head space analyses or further concentration by another procedure, such as solvent extraction, depending on the objectives of the investigation. A practical illustration of the use of freeze concentration in aroma research (Weurman. 1966) is presented in Figure 4, which shows the head space analysis of the volatile components from a molasses distillate at different levels of concentration.

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