Isolation of Volatile Components from a Model System

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A description is given of a modification of the Likens and Nickerson apparatus for isolating volatiles from foods, beverages, and other agricultural products by simultaneous steam distillation and extraction (SDE) with an organic solvent. The new apparatus was evaluated by experiments with dilute aqueous solutions of a model mixture of 12 volatile compounds, representative of those found in fruit essences. Analysis of the recovered material was done by quantitative gas chromatography. Most of the components showed nearly quantitative recovery after 1 h (some, better than 90% in 10 min), but ethyl 3-hydroxyhexanoate required 4 h to reach 90% recovery. Hexane was shown to be an excellent extracting solvent for most of the components except low molecular weight, water-soluble compounds (ethanol and ethyl acetate, which form azeotropes with hexane). For these compounds, diethyl ether gave considerably better results. Although most of the components appeared to be chemically stable when the initial solution in the still pot was acidic, down to pH 3.4, linalool and citronellal showed instability in the lower part of this pH range. This was corrected by raising the pH to 6.5. SDE at reduced pressure (100 mm of Hg), and thus at a lower temperature, also favored stability, but recovery of the hydroxy ester was decreased drastically.

One of the principal methods for separating the volatile substances from foods, beverages, and other agricultural products is that of steam distillation, frequently followed by extraction with an organic solvent. A simple and effective means for performing these two operations simultaneously was published by Likens and Nickerson (1964) with a drawing of their distillation–extraction head. Advantages were that the desired substances were concentrated thousands-of-fold, from the ppb range in aqueous media, in a single operation of 1 h. A relatively very small quantity of organic solvent was used, thus minimizing the possibility of artifact introduction from this source. The work of these authors was on hop oil, beer, and related substances.

A similar isolation method, but with more complicated apparatus, which provides for cooling of the steam condensate before it makes contact with the extracting solvent and dispersing of the latter with a sinter was described by Williams (1969).

In recent years the Likens and Nickerson (1964) apparatus has been used in a number of laboratories. To mention a few examples, Buttery et al. (1968) isolated the volatile oil from carrots, not only at atmospheric pressure but also at reduced pressure (60–80 mm of Hg), thus obtaining flavoring substances related to both cooked and raw carrots. Other workers avoided thermally induced changes in the original sample by extracting both volatile and nonvolatile matter with an organic solvent in the cold and subsequently isolating the volatile fraction with the Likens and Nickerson apparatus. Maarse and Kepner (1970) used a small-scale modified apparatus in a study of the essential oil of the needles of Douglas fir (*Pseudotsuga menziesii*). Essential oils were isolated directly from the leaves of California bay (*Umbellularia californica*) (Buttery et al., 1974) and of vinegar weed (*Trichostema lanceolatum*) (Schultz et al., 1976). Effective use of the method for isolating volatiles from lipids can be seen in the work of Flath et al. (1973) on olive oil. MacLeod and Cave (1975) used a modified apparatus, which included a double-surface water condenser with coolant surrounding the separation area also, in their study of the volatile components of eggs.

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The present paper gives a detailed description of a modification of the Likens and Nickerson (1964) apparatus, designed by one of the authors (R.A.F.), and a report on experiments with model systems, undertaken to determine the degree of recovery of representative compounds under various conditions. The name "SDE" (simultaneous distillation and extraction) is proposed for this isolation method and the apparatus.

APPARATUS

The modified distillation–extraction head is shown in Figure 1. As with the earlier apparatus, a relatively large flask, for the sample (with added water if necessary), is coupled at the lower right joint, and a smaller vessel for the organic solvent (less dense than water) and extract is attached at the left. As SDE proceeds, the two liquid phases of the condensate continually return to their respective flasks; an interface between the two phases forms in the separatory tube (central tube below the condenser) a little below the lower end of the solvent-return arm.

Special features of this new design are as follows. First, the condenser surface is large (cooling water rises through an annular space and then descends through a helical coil). This permits rapid distillation without overloading the condenser, and rapid distillation not only saves time but shortens the duration of heat exposure for sensitive substances. Second, the separatory tube is jacketed, in a similar manner to that used by MacLeod and Cave (1975), so that when operation is at reduced pressure with ice water as coolant, the organic solvent does not tend to revaporize. Third, there is a mixing chamber for the vapors at the top of the condenser, with the steam riser entering this chamber tangentially at the rear and the solvent vapor tube at the front. The rationale is that the mixing of the vapors before condensation should give more intimate contact of the substances involved and thus better extraction.

MATERIALS AND METHODS

Model Mixtures. Two mixtures of compounds representative of fruit essence constituents were prepared (Tables I and II). For each mixture, approximately 8 g of each compound (only 4 g of the hydroxy ester, due to the limited amount available) was accurately weighed into a brown bottle, 0.1% of Antioxidant 330 (Ethyl Corporation, Baton Rouge, La.) was added, and the mixture was kept at -34 °C.

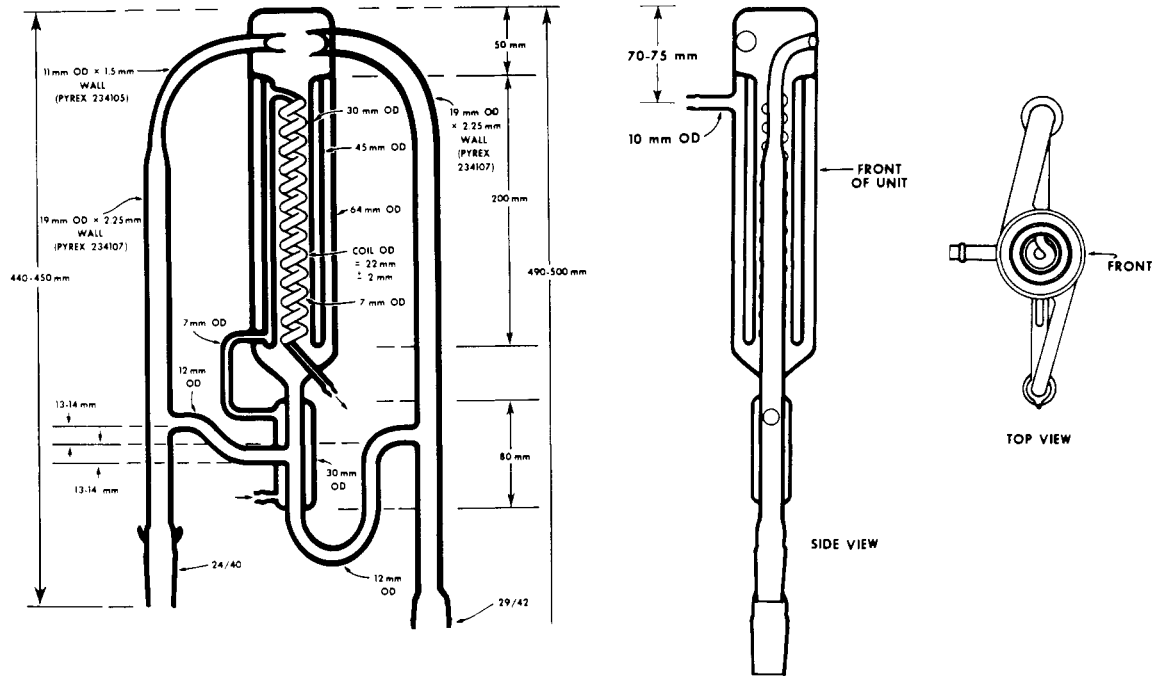

Figure 1. Simultaneous steam distillation–extraction (SDE) head.

Table I. Recovery of Components from the First Model Mixture with Various Times of SDE[a] (Recovery as Percent of Initial Amount)

	Time of SDE, min			
	10	30	60	120
Ethyl butyrate	93	96	95	96
Ethyl hexanoate	97	101	100	100
Ethyl octanoate	99	101	101	100
Ethanol	0	0	0	0
1-Hexanol	77	97	99	99
Linalool	93	100	99	100
Carvone	65	95	99	100
Limonene	96	99	99	98

[a] At pH 5.0 and atmospheric pressure; initial concentration of each component was 210 ppm (w/v); solvent, 125 mL of hexane.

SDE Procedure. Five milliliters of model mixture was pipetted into 2.5 L of commercial purified water, buffered at the desired pH with citrate (phosphate for pH 7.8) at 0.05 M, in a 5-L flask. Except where noted otherwise, the pH was 5.0 (although this is not the best pH for stability of one of the components). In a few of the runs, 5.00 mL of diluted model mixture, in ethanol, was used. The 5-L flask was joined to the right-hand riser of the SDE head, and a small flask containing 125 mL of high-purity hexane (or other solvent, or lesser amount, where noted) and 12 mg of Antioxidant 330 was connected to the other riser. The U-tube was filled with water to the lower end of the solvent-return arm. Distillation from both flasks was carried out for an arbitrary period of time, the solvent starting first. Zero time was taken to be when condensed water started dropping into the solvent in the separatory tube and final time when heat to the water was turned off. Distillation of the solvent was continued a few minutes until the water stopped boiling, in order to keep the extract dry. Most of the solvent was then removed from the extract by distillation with a 40-cm Fenske column packed

Table II. Recovery of Components by SDE from the Second Model Mixture at a Concentration of 165 ppm (w/v) for Each Compound (Recovery as Percent of Initial Amount)

Time of SDE:	1 h									4 h
Pressure:	Atmospheric pressure								100 mm	Atm
Vol of Solvent:	125 mL							10 mL[a]	125 mL	125 mL
Solvent:	Hexane					Pentane	Ether	Hexane	Hexane	Hexane
pH:	3.4	5.0	6.5	7.8	5.0[b]	5.0	5.0	5.0	5.0	5.0
Ethyl acetate	0	0	0	0	0	59	89	19	0	0
Ethyl butyrate	98	99	99	91	99	101	97	84	100	98
Ethyl hexanoate	100	101	101	95	101	102	99	97	103	99
Ethyl Octanoate	99	99	100	95	100	102	100	99	100	99
Ethyl 3-hydroxy hexanoate	41	41	41	19	42	44	49	30	6	90
Ethanol	0	0	0	0	0	0	58	0	0	0
1-Hexanol	101	101	103	98	100	102	100	96	98	100
Linalool	73	99	100	96	99	99	97	97	99	98
Octanal	102	102	103	98	102	103	101	99	103	101
Citronellal	59	78	98	94	81	81	79	77	95	80
Carvone	98	97	98	95	98	99	97	97	92	99

[a] For this run, additional hexane (~13 mL) was added through the vent to fill the solvent overflow arm before the distillation was started, and the extract was not concentrated after SDE. [b] 1.0 mL of acetic acid (glacial), titrated in solution to pH 5 with sodium hydroxide, also was present in this run in addition to the usual citrate buffer at 0.05 M.

with glass helices and a water bath.

Quantitative GC Analysis. The amount of each component of the model mixture present in the concentrated extract was determined with a Hewlett Packard gas chromatograph, Model 5831A, using its internal standard method. The column (Mon et al., 1967) was a 500-ft, 0.03-in. i.d. stainless steel open tubular column, coated with Tween 20 (Atlas Chemical Industries, Inc., Wilmington, Del.) with 5% Igepal CO-880 (General Analine and Film Corp., New York, N.Y.). Column temperature started at 60 °C for 10 min and then was programmed at 1.0 °C/min to 175 °C. Injector and FID temperatures were 140 and 210 °C, respectively.

Analysis of the concentrated extract was done by accurately weighing in about 400 mg of dodecane, the chosen internal standard, and injecting a 0.10-μL sample into the gas chromatograph. A standardized sample of model mixture, with added hexane, was used for calibrating the instrument with response factors. All standardized extracts were analyzed in duplicate, and the mean value for percent recovery was calculated for each component.

The standard deviation of individual results of duplicate analyses from their means was ±0.86% (based on 100% recovery). (This value was calculated from all analyses of the second model mixture and its extracts, all from undiluted model mixture, with results for all components pooled together, although there was more variability within duplicates for ethanol and ethyl acetate than for the higher-boiling components). Accuracy of the mean recoveries was not as good as would be expected from the low standard deviation, due to a number of small errors in handling the materials before the GC analysis.

RESULTS AND DISCUSSION

The degree of recovery of individual components from the model mixture under various conditions is shown in Tables I and II. Recoveries for various times of SDE with hexane as extractant were determined first. Several of the components, including the monofunctional aliphatic esters (ethyl butyrate and heavier), showed better than 90% recovery in 10 min. Distillation would be expected to be slowed by the presence of a hydroxy group, or other substituent or structural feature, leading to an increase in the ratio of solubility-in-water to azeotropic composition. Thus in 10 min, 1-hexanol showed only 77% and carvone only 65% recovery, but linalool surprisingly showed 93%. Most of the components showed recoveries near 100% in 1 h. (This time was used in most of the subsequent runs.) However, only 41% of the hydroxy ester, ethyl 3-hydroxyhexanoate, was found after 1 h and a time of 4 h was required for 90% recovery. No appreciable loss for any of the components was observed from continuing the SDE beyond 1 h to 2 or 4 h, in contrast with the decrease for some compounds reported by Likens and Nickerson (1964).

In all of the runs with hexane, neither ethyl acetate nor ethanol was detected in the concentrated extract. These compounds form azeotropes with hexane, and any which might have remained in the extract flask during SDE was lost when the solvent was removed with the Fenske column.

When pentane was used as solvent (Table II), the recovery of ethyl acetate was 59%. Apparently pentane and ethyl acetate do not form an azeotrope, or if they do, the concentration of ethyl acetate in it is quite low. (No azeotropic data for this system was found in the literature; Horsley and co-workers (1952); Horsley and Tamplin (1962).) When diethyl ether was used as solvent, 89% of the ethyl acetate and 58% of the ethanol were recovered.

Table III. Recovery of Components by SDE from the First Model Mixture at Various Degrees of Dilution[a,b] (Recovery as Percent of Initial Amount)

	Time of SDE, h				
	1				3
	Concn of each component, ppm (w/v)				
	210	21[c]	2.1[c]	0.21[c]	0.21[c]
Ethyl butyrate	95	98	95	93	93
Ethyl hexanoate	100	104	100	96	95
Ethyl octanoate	101	98	90	89	87
Ethanol	0			~0.01	
1-Hexanol	99	98	91	86	94
Linalool	99	101	97	95	95
Carvone	99	97	90	83	92
Limonene	99	93	80	85	84

[a] At pH 5.0 and atmospheric pressure, with 125 mL of hexane. [b] The analytical data for this table, except the first column of figures, did not show the usual good consistency. [c] The initial concentration of ethanol in the aqueous medium in the large still pot was about 1500 ppm (w/v).

The relatively low recovery for ethanol was due to the only slightly favorable liquid–liquid partition coefficient.

A "concentrated extract" with about the same solvent content (approximately 65%) as the extracts discussed above can be obtained without concentrating after the SDE run, by using only 10 mL of solvent initially in the solvent flask instead of the usual 125 mL. (Likens and Nickerson (1964) used only 5 mL of solvent and added more during the run to compensate for evaporation loss.) When this procedure was used (Table II), several of the components showed practically as good recovery as they did with the larger amount of solvent. However, ethyl butyrate, ethyl 3-hydroxyhexanoate, and 1-hexanol were lower. These three components probably have less favorable liquid–liquid partition coefficients than the other components (except ethyl acetate and ethanol) and therefore suffered from the necessarily slower solvent distillation rate. However, ethyl acetate showed up, at 19%, since it was not subjected to hexane removal with the Fenske column.

Effect of Initial Degree of Dilution. In the experiments discussed so far, the initial concentration of each component in the aqueous mixture was in the order of 200 ppm, which is within the range of concentration of principal constituents likely to occur when essential oils from leaves or other plant materials are isolated by SDE. In many cases, though, the constituents of interest are present at much lower concentrations. Recoveries of the components of the first model mixture at dilutions 10-, 100-, and 1000-fold greater than usual are shown in Table III. There appeared to be a gradual falling off of recovery, so that when the initial concentrations were 0.21 ppm (w/v), recoveries were in the range of 83 to 96%. However, the quantitative GC method as used by us was less consistent with these extracts than it was with the extracts obtained when initial component concentration was near 200 ppm. Thus, all we can conclude is that there was no great lowering of percent recovery when initial concentration was lowered, and perhaps for most of the components there was no lowering at all.

Theoretically, the time required for quantitative recovery of a given compound which is only slightly soluble in water and does not associate or react chemically should be the same regardless of concentration, within the very dilute range. This prediction is developed as follows. As pointed out by Buttery et al. (1969), the air–water partition coefficient of a volatile solute is the same at any concentration in the dilute range where Henry's law applies.

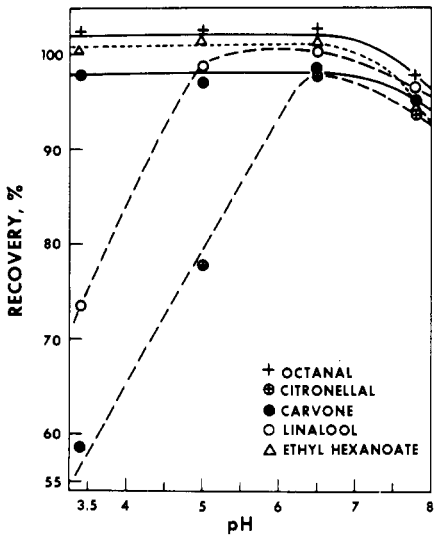

Figure 2. Effect of pH on recovery of some of the components by SDE.

They determined air–water coefficients at 25 °C experimentally and found no appreciable change over a 1000-fold increase in concentration up to saturation (5000 ppm for hexanal and 4000 ppm for 2-heptanone). If we assume that other slightly soluble compounds behave similarly, not only at 25 °C but also at 100 °C, and that the vapor and boiling liquid in the still pot are as close to equilibrium at one concentration as another, then the percent rate of distillation should be the same. If we further assume that partitioning of the solute between hexane and water in the SDE head approaches equilibrium as closely at one concentration as another, then the time required for quantitative recovery of the solute should be the same.

This last assumption may not be as reliable as the others except for even more dilute solutions which would give homogenous condensates in the absence of extracting solvent. For example, a solute present near or above saturation in the aqueous mixture in the still pot (as was limonene at 210 ppm) would normally appear partly as an "oil" phase in the condensate. When it is subsequently (perhaps only very slightly later) contacted by the solvent, dissolving of the "oil" would be expected to be faster than extraction of the solute from the aqueous phase. If this same solute is initially present at greater dilution, there would be no "oil" phase and extraction would all be at the lower rate. In the present SDE head, which provides for mixing of the vapors before condensation, this effect should be minimized.

Chemical Stability of Components. For all of the SDE runs discussed above, the pH of the aqueous mixture was 5.0. The only component for which there was definite evidence of instability at this pH was citronellal. Recovery data for runs with the pH at other levels is shown in Table II and Figure 2. At pH 3.4, both linalool and citronellal showed instability, but there was no evidence of ester hydrolysis. All of the compounds present showed good stability when steam distilled at pH 6.5, citronellal showing 98% recovery, but most of the components were less stable at pH 7.8. Likens and Nickerson (1964) showed an optimum pH range of 5.8 to 6.6 for a number of compounds, including methyl esters of aliphatic acids, with lower recoveries below, as well as above, this range.

The advantage of better chemical stability given by operating the SDE at reduced pressure was demonstrated in a run at 100 mm of Hg (vapor temperature, 52 °C), with the pH at 5.0 (Table II). The citronellal recovery was 95%, apparently not quite as good as by raising the pH to 6.5. However, the recovery of carvone dropped to 92%, and only 6% of the ethyl 3-hydroxyhexanoate was found. It would appear that the volatility of this hydroxy ester in aqueous solution changes with temperature at a considerably greater rate than the corresponding rate for the other solutes present.

CONCLUSIONS

It can be seen from the results presented that no single set of operating conditions is the best for all applications, although it may be predicted that nearly quantitative recovery of all components of the particular model mixtures of this study could be achieved by an SDE run at pH 6.5, at atmospheric pressure, with 125 mL of diethyl ether, for 8 h. Frequently it is desired to exclude ethanol from the extract. In this case, and when other very water-soluble compounds are of no interest, hexane may be the most convenient solvent. For investigating the volatiles from uncooked food or other plant material of unknown composition, one could make two SDE runs: first, at reduced pressure, to avoid losing possible labile constituents and to prevent development of additional compounds from cooking the carbohydrates, proteins, etc.; and second, at atmospheric pressure, for an extended time, to recover possible constituents which show steam distillation behavior similar to that of the hydroxy ester of this study. After the general nature of volatilities and stabilities of the constituents is found, a single and likely shorter SDE procedure might be chosen to get additional extract, if needed, for further study.

The new SDE apparatus has already been used for the isolation of volatiles from various agricultural products in current studies by the authors and other researchers at this laboratory. Performance has been very satisfactory. Results of these studies will appear in forthcoming publications.

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