Isolation and Concentration of Volatiles in Food Odor Research

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To gain a complete understanding of an odor a three-sided approach of the analysis is necessary— i.e., the identity and total quantity of the individual volatiles in the food, the composition of the vapors over the food, and the physical structure of the food have to be studied. In this paper most of the techniques used for the isolation and concentration of the volatile compounds in food are mentioned or discussed, and the frequency of their application in practical research has been estimated. It is emphasized that more than one procedure for distillation and extraction is to be applied for optimum odor recovery. Care should be taken to prevent artifact formation as much as possible. The best sequence in which the various techniques for the isolation and concentration should be used is considered and the advantages of adsorption and freezing procedures have been stressed.

In food odor research some difference of opinion exists as to the correctness of the approach followed by those studying all volatile compounds that can be isolated from the food—"total volatile analysis"—and by those propagating the direct analysis of the odorous vapors (or actual odor) over the food—"direct vapor analysis" or "head space vapor analysis."

Each of the two approaches has its own objective and its own specific merits and failings. Both are equally limited as neither can, by itself, solve all the problems in odor research. Nor can these problems be solved when both approaches are followed simultaneously and their results joined and considered together. For we will have to realize that there are three basic aspects to food odors, the accurate knowledge of which is essential to an understanding of the whole phenomenon of food odor.

There is first the qualitative and quantitative composition of the odorous volatiles in the food itself. This aspect, the study of which requires the application of "total volatile analysis," forms the basis of all our understanding of the odor of a product.

There is, further, the composition of the odorous vapors over, and in equilibrium with, the food as it is observed by our sense of smell and as it is studied qualitatively, quantitatively, and organoleptically by the investigators favoring "direct vapor analysis." We may call this second aspect the effect of the first.

Finally, however, there is a third aspect: the physical state and distribution of the odorous volatile compounds in the food. This aspect has been little studied as yet (Nawar, 1966; Weurman, 1963). It could be considered the cause of the effectual odor of our product. It relates the composition of the vaporous odors over the product to the overall composition of the volatiles in the product and is completely destroyed and eliminated by the isolation of these volatiles from the food. The basic complex mixture of volatiles in two different food items might be the same, both qualitatively and quantitatively, but the effect might be two different odors. The difference then is caused by the various degrees to which the volatiles are dissolved in the lipoidic or the aqueous phase of the food or by the

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degree to which their effective concentration in either of the two phases is reduced by adsorption onto the solids of the product.

Figure 1 may clarify this point. On the left two different food products have been drawn schematically, and an attempt has been made to bring something of the distribution of the various compounds into the picture. Like most of our food, the two products are basically of an aqueous nature. They contain, apart from water, other volatile material, polar and nonpolar, as well as nonvolatile fat and nonvolatile solutes such as sugar, salts, amino acids, and proteins. In addition, the top product contains solid material that is not dissolved and some of the solutes, volatile as well as nonvolatile, have been adsorbed to its surface. When there is sufficient lipid, as in the second product at the bottom, all nonpolar volatile material is considered to be dissolved in its globules. In the first, however, the amount of fat is too small and some of the nonpolar component material is left in the aqueous phase.
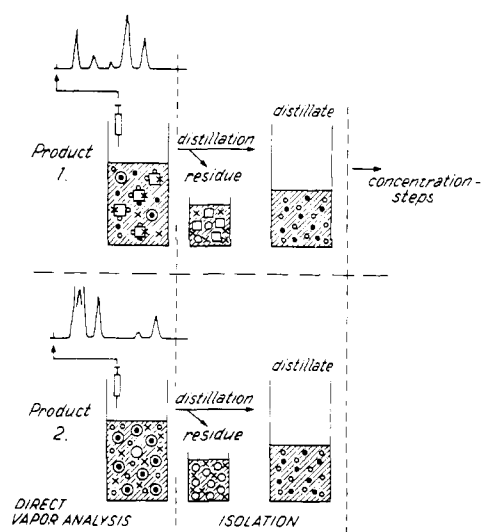

Figure 1. Preparation of sample for analysis I. First step (isolation)

- Volatile apolar solutes   ○ Volatile polar solutes
- ○ Nondissolved lipoid   ☐ Nondissolved solids
- ✕ Nonvolatile solutes   ▨ Water

Although the total amount of volatiles in both instances is the same—as we assume the amounts are of the individual components of the mixture of volatiles even though this could not be shown—the distribution is different. As a consequence the relative vapor pressures of the components and thus their concentrations in the "head space" vary. This is indicated in the two head space chromatograms on the left. So we see that the odors—as an effect caused by the distribution—are different, even when the basis—the total distillates to the right—is the same. This means that for a true understanding of an odor neither the knowledge of "the total volatiles" in the product nor that of the composition of its vapor can be sufficient. Complete knowledge of all three aspects—the basic composition, the odor as the effect, and the distribution of the volatiles in the food as the cause of the effect—is essential.

Only the methods used in "total volatile" analysis, however, are discussed in the following. Earlier publications fully or partly covering the same field are from: Bernhard (1966); Burchfield and Storrs (1962); Burger (1961, 1962); Chang (1966); Drawert (1965); Grosch (1966); Honkanen and Karvonen (1963); Keulemans (1962); Lea et al. (1967); Mehlitz and Gierschner (1963); Meigh (1955); Ryder (1966); Scarisbrick (1955); Schormüller (1962); and Webb (1964).

SAMPLE PREPARATION. ISOLATION OF VOLATILE FROM NONVOLATILE MATERIAL

Often special treatments such as cutting, grinding, mincing, pressing, and centrifuging are applied to the food preceding the isolation and concentration of its volatiles. Precautions, such as working at subzero temperatures under N_2, are essential during those operations to prevent chemical and enzymic side reactions.

Distillation—single-plate, total take-off distillation—is the preferred procedure for the isolation of all volatile material from the product. Extraction, adsorption, etc., are at times applied to the product as a first-step procedure, but then nonvolatile components are isolated along with the volatiles and some form of distillation has to be applied later. In fact, volatility is the only property which all compounds of interest to us have in common and which sets them apart from the rest of the product. And so there is but one true way to isolate these volatiles and that is the one which is based on the use of this property: distillation. All other methods are better judged as concentration procedures and as a rule are best applied following isolation by some form of distillation. There are, however, acceptable exceptions to this rule, particularly in the study of products such as fruit juices, wine, and bread, that contain only small amounts of nonvolatile material soluble in organic solvents.

CONCENTRATION

The main forms of concentration procedures are drawn schematically in Figure 2. The result of a first step isolation (the very dilute aqueous solution at the left) is taken to serve as the starting material for all following procedures. Since there are only two main forms of concentration, freezing and adsorption, the efficiency of which is not reduced with increased dilution, other forms, such as
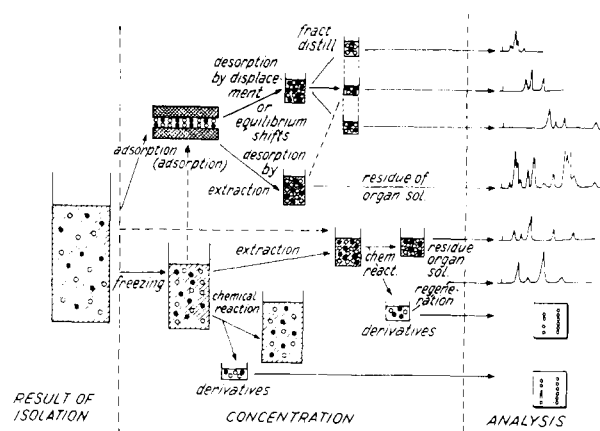

Figure 2. Preparation of sample for analysis II

extraction, cannot be optimal procedures at this first-step concentration stage. There is no point in concentrating a distillate, first by freezing and subsequently by applying adsorption.

Such considerations have led to the compiling of the schemes but they are general schemes with many imperfections. They are not complete, in that no subforms of the procedures have been brought into them. Fractional distillation, when following the isolation process, as well as stripping and restripping—following or replacing isolation—is a subform which can be used to concentrate dilute aqueous solutions. The schemes are not realistic either in that no volatile material is lost or no artifacts are formed. Finally, in practice, the type of product, the quality of the volatiles, or the specificity of the problem we wish to study may lead us to follow another course and to deviate from the sequence of procedures in the schemes, or even to omit some.

However, when we read publications on these practical studies we cannot always feel convinced that the choice of procedures was fully justified or that the decision on the sequence in which they were applied was taken after sufficient deliberation. Can it be that the necessary instrumentation or experience to use it is not always available to the investigators? I further found many odor research laboratories today better equipped with mass spectrometers and other very expensive instruments for identification than with simpler apparatus of advanced design for sophisticated distillation, extraction, and freeze concentration. It must be remembered that errors and faults made in odor research during the early stages of isolation and concentration can never be corrected at any later stage of compound identification.

FREQUENCY OF PROCEDURES USED IN FOOD ODOR RESEARCH

In the schemes only the main types of procedures have been indicated: distillation for the isolation of the components, and freezing, adsorption, extraction, fractional distillation (stripping), and derivative formation—all of them, apart from freezing, more or less selective—for their concentration.

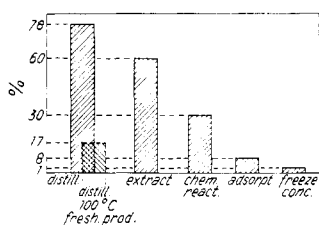It might be of interest to have a few figures on the

Figure 3. Frequency of application of main forms of isolation and concentration procedures

234 studies, 425 applications of procedures

frequency in which these main procedures are used in actual research. To this end some 300 individual articles on food odor studies, published between 1960 and 1967 and picked at random from our reprint collection, were checked. In 235 of these, more or less well defined sequences of isolation and concentration procedures were followed, leading up to the final analysis. A total of 425 procedures was found to have been applied; an average, therefore, of nearly 1.84 methods per study. The frequency distribution over the five main types of isolation and concentration procedures is expressed as a percentage of the total number of studies in Figure 3.

As was clear from the beginning, distillation is the most common method, but the figure is smaller than was anticipated. This means that a number of workers studied products that were already distilled before the investigations were undertaken (distilled spirits, fruit odor concentrates, essential oils, etc.) and that some workers started their final analyses—the actual separation procedures—on mixtures of volatiles which still contained nonvolatile material. If they used gas chromatography—which a number did—they must have found interesting compounds and rather rapidly deteriorating columns. Replaceable precolumns helped to overcome the second drawback in some instances (Schratz and Wahlig, 1965; and many others). On checking these publications, the following aspects emerged: The frequency distribution in Figure 3 shows that isolation by distillation is usually followed by extraction. I believe, however, that this is not the best course to follow in food odor research. Preceding extraction, freeze concentration should be applied, but actually this was done only twice in 235 studies. (Bidmead, 1963; Swoboda, 1966).

Basic and comparative studies on the efficiency of the various forms and subforms of procedures—and of artifact formation during their application—are appallingly rare: nine times in 234. (Edélyi et al., 1967; Heinz et al., 1964; Hobson-Frohock and Lea, 1965; Honkanen and Karvonen, 1963; Kromann et al., 1967; Miers, 1966; Tang and Jennings, 1967; Weurman and de Rooy, 1961; Wucherpfennig and Bretthauer, 1967).

In publications, discussions of why the procedures that are used were chosen are exceptional (16 times in 234) (Bober and Haddaway, 1964; Carson, 1963; Elzakker and van Zutphen, 1961; Hunter and Moshonas, 1966; Kohman, 1952; Lemperle and Mecke, 1965; Mecke et al., 1960;

Mehlitz and Gierschner, 1962; Merritt et al., 1959; Scarpellino and Kosikowski, 1961; Schratz and Wahlig, 1965; Strackenbrock, 1961; Tang and Jennings, 1967; Winter, 1963; Winter et al., 1962; Wiseblatt and Kohn, 1960).

(Steam)-distillation at atmospheric pressure was used much more often in studying the odor of fresh, uncooked, food products than expected (36 times in 234). Can it be correct in so many instances to make this last choice? To answer this question the different subforms of the main type of isolation procedure, distillation, should be considered.

DISTILLATION IN FOOD ODOR RESEARCH

Distillation as an isolation, and, to some extent, concentration procedure may be used in a number of different forms (Table I).

None of the techniques mentioned in Table I are used to separate fractions according to the boiling points of the components. This even holds for multiplate fractional distillation. Actually, at this early stage in odor recovery, fractional distillation is applied for concentration of the odorous volatiles from the water by reflux stripping. A classical example is found in the study of Dimick and Corse (1956, 1957) on the aroma of strawberries, where a 10-fold concentration increase was obtained for the 60-fold concentrate of the volatiles gathered in the preceding isolation step. The method, which should preferably be applied only to solutions previously freed of nonvolatile material, is not used very frequently in the laboratory (Connell, 1964; Heinz et al., 1964; Stevens et al., 1966) possibly because of the difficulty of obtaining the type of column developed by the authors [Shepherd (1957) described a similar column that is easier to construct], but probably also because of the reduced pressure rotary-film, climbing-film, and falling-film evaporators that since have become available. The fast-action climbing and falling-film evaporators or "strippers" are more efficient, easier to handle, and less liable to produce artifacts. Contact times of the solutes with heated surfaces are very short in such arrangements and by repeating passage, concentration increases up to some hundreds of times can be obtained, depending on the product and the degree of completeness of the isolation desired.

Table I.   Forms of Distillation Used in Food Odor Research

Open system, regular single-plate, total take-off distillation:
   at atmospheric pressure
   at reduced pressure (vaccum distillation) (including stripping)
   high vacuum degassing and stripping (of fats or high boiling solvents)
Open system steam distillation (distillation with the introduction of extraneous steam):
   at atmospheric pressure ("steam distillation")
   at reduced pressure ("vacuum steam distillation")
Open system, multiplate, fractional distillation:
   at atmospheric pressure
   at reduced pressure
Open or closed system air or inert gas entrainment
Closed system transfer:
   at atmospheric pressure
   at high vacuum ("low temperature sublimation")

Multiplate fractional distillation on the other hand is very commonly used as a stripping procedure in industrial fruit concentrate manufacture, where very large quantities of material are efficiently dealt with (Bomben et al., 1966, 1967; Büchi, 1962; Eskew, 1957; Mehlitz and Gierschner, 1963; Milleville and Eskew, 1946). Pollard and Beech (1966) discuss industrial thin-layer evaporators and the study of Walker (1962) is of interest for his classification of fruits in relation to the most profitable form of distillation.

By application of the uncommon closed-system transfer method at atmospheric pressure, Owades and Dono (1965) determined the volatile acids in beer directly from the beverage. The method resembles the older Conway procedure for the determination of ammonia and uses dishes of a corresponding type. Distillation is over a short path. The method is basically selective and restricted to applications on a small scale.

A closed-system form of the regular inert gas entrainment procedure, frequently applied in its open form, has been developed by Nawar and Fagerson (1960). A limited amount of nitrogen is continuously pumped through the sample and separately freed from the entrained water (see also Jenard, 1960) and odorous volatiles. Eliminating losses, this method will work well for most of the lower boiling point volatiles but precautions are required where enzymes can continue to act (Drawert et al., 1966; Mehlitz and Gierschner, 1963; Nye and Spoehr, 1943) or life cycle processes in the product are apt to turn to anaerobic dissimilation. The same disadvantages are encountered with other long term, low temperature procedures, such as reduced pressure distillations, the open system entrainment method, or the method of Owades and Dono (1965). Nawar and Fagerson's method has the advantage of only using limited amounts of gas; impurities in the entrainment gases therefore cannot accumulate to cause erroneous results in later analysis.

The "closed-system, high vacuum transfer" method is the technique (Niegisch and Stahl, 1956; Stahl, 1957) of which the applicability in aroma research has been extended so expertly by Merritt and his colleagues (1959, 1963). The potential loss of very low boiling components is minimized and artifact formation, as caused by the application of heat in many other procedures, is eliminated. Quantitative separation, however, of all volatile material from the product appears difficult (Menting and Hoogstad, 1967).

For the isolation of volatile components from fats and oils techniques are used that are particularly adjusted to these products. High vacuum degassing (De Bruyn and Schogt, 1961; Lea and Swoboda, 1962; McGugan and Howsam, 1962) is applied in various forms but is not considered adequate by Angelini et al. (1967) in cases where the more volatile constituents are of specific interest. A system of closed vacuum vapor sampling, followed by an open high vacuum distillation, is preferred by these authors. Forss et al. (1967) compared the efficiency of three different methods for the isolation of small amounts of volatile ketones and alcohols from butter oil. Reduced pressure steam distillation was found the least attractive of the procedures tested and a combined application of high vacuum degassing and cold-finger molecular distillation is favored.

It is not easy during actual research projects to decide which of the main forms to use. "Open system multiplate fractional distillation" is not attractive as a first-step procedure and the choice for the entrainment and transfer procedures is usually restricted to specific problems. In general, however, a choice between the various forms of regular single-plate, total take-off distillation and steam distillation has to be made.

Two main decisions in this respect have to be taken, notably whether or not to reduce the pressure, and thus the temperature, during the operations, and whether or not to make use of steam that is generated outside the system. General rules cannot be given since too much depends on the type of product that is studied and the specific objective of the study. This is easily understood when the investigation of dry products such as spices, cereal grains, dried herbages, or dry fats and oils is contemplated. Only in such instances can undersaturated dry steam at elevated temperatures be used with advantage.

An example is the isolation of the essential oil from leaves of *Mentha piperita* by Lemli (1955). Figure 4 depicts the apparatus used. The steam is overheated in A, conducted through the product, which is heated in the oven, B, to prevent condensation, and then condensed with its charge of volatiles in C. The yield of oil using Lemli's arrangement is almost equal to that obtained by wet distillation in the Clevenger type of apparatus. However, recovery of menthofurane in the oil is four to five times higher in the dry method. During Clevenger distillation, condensed water returns continuously to the distillation flask conveying considerable amounts of menthofurane with it.

The example from Lemli is not a good choice inasmuch as menthofurane is now considered to be an artifact. The disadvantage of the Clevenger way of operation, however,
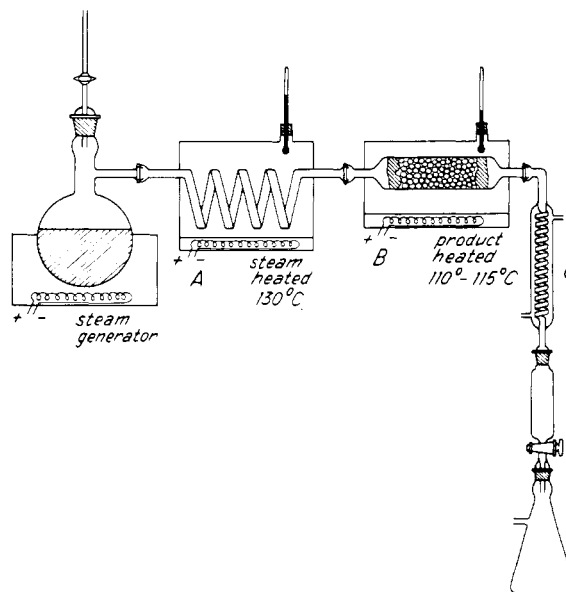

Figure 4. Distillation with dry steam

From: J.A.J.M. Lemli, Dissertation, Groningen University, 1955

as against the methods where the condensate is not returned to the distillation flask, still holds. Examples of raised-pressure wet-steam distillations are given by Silberstein (1954) and Kohman (1952).

It is not always realized that for the isolation of nonpolar volatile material from essentially aqueous systems the problems are basically different from instances when the isolation from fats and oils is our objective. In the first case the water (-vapor) has a positive function in building up the vapor pressure over the system of the nonmiscible components. N_2 for entrainment here cannot fully replace the water and reducing the pressure diminishes efficiency. In the second instance—when the volatile material is in true solution—the function of the water (-vapor) is mainly that of a carrier and can be replaced by N_2, while reducing the pressure now strongly increases the efficiency.

In contrast to the investigation of dry products, the study of the more common aqueous foods and beverages involves a decision between the use of outside steam and/or reduced pressure.

The major advantage of distillation at reduced pressure, with or without the introduction of outside steam and including the vacuum thin film flash evaporation procedures, of course, is the diminished danger of artifact formation. However, the efficiency of the distillation is reduced for nonpolar components that are nonmiscible with water. Larger amounts of water have to be transferred and distillation times tend to become longer. At the same time the temperature of such reduced pressure distillations is near the optimum for enzymic reactions and considerable production of volatiles consequently may take place. Everyday experiences clearly illustrate that the odors of fresh, crushed grapes, strawberries, and onions cannot be isolated in this way, but the danger of enzymic formation of new compounds is as great even where the symptoms are less obvious. Considerable amounts of artifacts are always formed during distillations of regular food products at atmospheric pressure. When the method is applied to fresh products cooked flavor volatiles are isolated instead of the original ones. The investigations on cooked meat flavors by Pippen and Nonaka (1960, 1963) and by Hornstein and Crowe (1964), where the nonenzymic production of volatiles from precursors was studied, should be a loud enough warning in this respect. Similar work has been done by Self et al. (1963) and Swain and Self (1964) on potatoes.

The second decision which has to be made is whether or not to introduce outside steam. The advantages of doing so seem obvious: The process can be continued until all odorous volatiles have been removed; the volume of the mass of product plus water can be kept constant and with it the concentration of salts and the temperature; the prevention of scorching and local overheating is easier; and the transport of vapors is enhanced.

Prevention of overheating and transport of the vapors can also be ensured by sweeping the boiling mass with an inert gas which has the additional advantage that all O_2 can be eliminated from the system before the temperature is raised. In exceptional cases, steam and N_2 are both introduced at the same time. Drawert et al. (1966) and Lemli (1955) strongly emphasize the danger of O_2 being present, and whether steam or N_2 is introduced, full blanks,

following the whole process including subsequent extraction and final separation, should be run, since the introduction of considerable quantities of foreign odorous volatiles together with the steam or sweeping gas may occur. I have never found that this precaution had been taken.

In the foregoing most of the advantages of each (sub-) form of distillation seem to be accompanied by disadvantages.

It will not help us in reaching a conclusion to mention further details and to tell of Honkanen and Karvonen (1966) who replace steam by CO_2 in their distillations of fats and thereby evade the difficulty of later extraction of any aqueous distillate (Figure 5).

Drawert and Rapp (1966) use propane in the same way for the entrainment of volatiles from aqueous alcoholic products. Nor are we helped by mentioning the interesting distillation arrangement of Shipton and Whitfield (1966) for the simultaneous separation of high boiling oils from alcoholic solutions, or the continuous and countercurrent steam distillations of Dutra et al. (1959), or our own technique, for which my colleague Dhont developed the arrangement shown in Figure 6.

The countercurrent procedures have the advantage of shorter heating times but they seem to be somewhat less efficient in the laboratory than in industry.

A separate discussion of the very common procedure in which introduction of steam and pressure reduction are applied together cannot help either. Ahrenst-Larsen and Hansen (1964) published a simple arrangement for such reduced pressure steam distillations, but many others use the technique as an everyday procedure.

No single method of distillation can actually lead to the complete isolation of the unaltered volatiles from all food products. For general applications where all volatile material from our food items are to be isolated with as little artifact formation as possible, at least two, but often possibly three, different distillation procedures have to be used:

For the lower boiling point components, fast action, thin-film, reduced pressure stripping procedures should be applied when the presence of active enzymes is suspected.
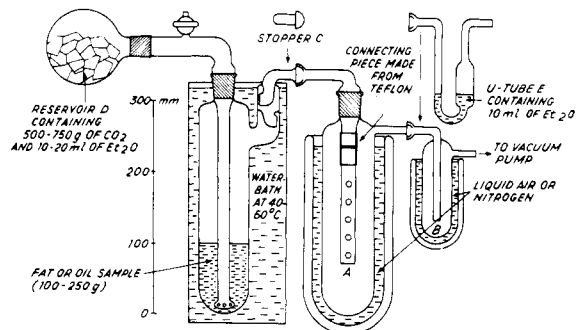
Figure 5. Schematic diagram of carbon dioxide distillation apparatus for removal and collection of flavor compounds from fats and oils

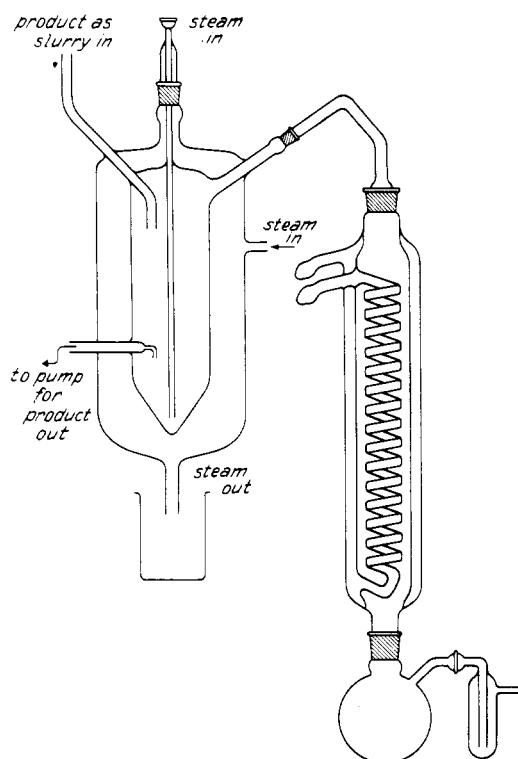From: E. Honkanen and P. Karvonen Acta Chem. Scand 20, 2626 (1966)

Figure 6. Continuous steam distillation

Although the concentration of the odorous volatiles from aqueous distillates can be accomplished in a number of ways (Forss *et al.*, 1967), extraction by nonpolar organic solvents is the most common procedure. For this purpose the laws of liquid-liquid distribution hold but they are not of much help in predicting the best experimental conditions when the complete odor of a product is investigated. The properties of the individual volatiles in the multicomponent mixtures vary to such an extent that it is, as with distillation, unlikely that an ideal solvent or ideal experimental conditions can be found. For some components distribution coefficients will be favorable for the extraction by arrangements with a limited number of theoretical plates; for others, the situation will be unfavorable and long extraction times or large amounts of solvents will be necessary.

Actually, when we try to obtain complete extraction of all components with a single extractant, as is usually done, we apply a semiselective method to a nonselective purpose—and pay for it.

It seems advisable, therefore, not to try to obtain complete extractions by all-in-one-go procedures, but to use successively or parallel a number of extractants, each particularly suitable for a limited number of components in the mixture. The selective character of extraction is then used instead of trying to overcome it. Indeed, we often do so when using isopentane for the extraction of aqueous solutions containing too much ethanol. An additional advantage of using successive extractions would be that components lost by azeotrope formation when the solvent is removed in one instance might be recovered in another.

Many industrial and analytical solvents are at our disposal, all of which need to be purified before being applied. Only a limited number of these are used in odor research. [The Fire Protection Association of London (1965) published a list of some 600 organic solvents which could be tested for our purpose. A few of the ones that I encountered are given in Table II.

It is often thought that very low boiling solvents, such as ethyl chloride (b.p. 12° C.) and isopentane (b.p. 28° C.) should be employed when interest is particularly directed toward the components of low boiling points. This is not correct as a general rule. Such compounds still tend to escape just prior to, or together with, the extractant during its removal. Much more profitable in these instances is the application of high boiling solvents followed by stripping the solutes from the solvent after extraction at reduced pressure (see, however, De Mets and Verzele, 1968).

Saturation with salt of the aqueous solutions that are to be extracted helps the extraction considerably. Usually NaCl or Na_2SO_4 are applied. It is preferable not to use $(NH_4)_2SO_4$ since it may decompose and liberate ammonia. Salts, however, may reduce the selective character of the extraction process and their use therefore should be carefully considered.

Care should be taken when using chlorinated hydrocarbons if mass spectrometry is eventually to be used for identification; the cathode of the ionization chamber of some instruments may become badly contaminated or even permanently damaged.

If crushing and pulping of the food is necessary previous to the stripping process, it should be done in the cold, under N_2, and as shortly before the stripping as possible. When no active enzymes are present, reduced pressure steam distillation at constant volume can be used. Full blanks are necessary when this method is applied.

For the study of the higher boiling components, steam distillation at atmospheric pressure should be used. Such compounds are less likely to be formed as artifacts, with the possible exception of hydrocarbons. If the food product contains macromolecular material, fats, amino acids, sugars, etc., low boiling aldehydes, (methyl-)ketones, acids, acetals, sulfides, amines, H_2S, and NH_3 in the distillate should be disregarded during further analysis, or should be judged with considerable suspicion. A slight reduction of the pressure in the system to boiling points of around 70° C. can be considered, if artifact formation for high boiling components is still feared. Saturation with salts except $(NH_4)_2SO_4$ can be used to compensate for some of the lost efficiency. Oxygen should be eliminated from the system with purified N_2 prior to starting the heating process.

When very low boiling point compounds are considered of specific interest, the use of closed systems becomes necessary. The high vacuum transfer technique of Stahl-Merritt is probably the best.

Whatever distillation procedures are applied, the volatiles from most food products will be obtained in very dilute aqueous solutions. Concentration of these volatiles is necessary preceding the separation of their mixtures into individual components.

Table II. Solvents Used for Extraction in Food Odor Research

Solvent	B.P., ° C.	Misc. with Water[a]	References, etc.
Petroleum ether	30–60	—	
Pentane	36	—	Extracts very little ethanol
Isopentane	28	—	Extracts very little ethanol
Hexane	69	—	
Heptane	98.5	—	
Paraffin oil	210–60	—	Heimann and Strackenbrock, 1963
Tetralin	207	—	De Mets and Verzele, 1968; = tetrahydronaphthalene
Benzene	80	0.1	
Cyclohexane	81	—	
Propane			Drawert et al., 1962; used as gas; condensed together with solutes
Propane/CO₂			
Pentane/dichloromethane			Drawert et al., 1966
Diethyl ether	35	6.9	Artifacts when with peroxides
Diethyl ether/pentane 2:1	33.5		Drawert et al., 1966; azeotropic mixture
Ether/chloroform, 4:1			Schultz and Mohrmann, 1965
Ethyl acetate	77	7.9	Otsuka et al., 1966; partic. phenolics
Isoamyl acetate	142.5	0.16²⁵	Cannot be purified by distillation; see Osterhaus, 1965
Diethylene glycol esters			Bober and Haddaway, 1964
Dinonyl phthalate			Bober and Haddaway, 1964
Polypropylene sebacate			Bober and Haddaway, 1964
Ethanol	78	+	
n-Amyl alcohol	138	2.4	van Gheluwe et al., 1966
Propylene glycol	188	+	Hunter and Moshonas, 1966
Glycerol	290	+	Hunter and Moshonas, 1966
Ethanol/petroleum ether			
Methyl chloride	−24		
Ethyl chloride	12.5	0.57	
Dichloromethane	40.5	2	Wolford et al., 1962
Chloroform	61	0.8	
Carbon tetrachloride	77.5	0.08	Miller et al., 1961
Fluorocarbons	Various	—	Extracts very little ethanol; Stanley et al., 1963
Methyl cellosolve	124	+	= Methyl glycol
Ethyl cellosolve	135	+	= Ethyl glycol
Nitromethane	98–101	9.7	Schmeltz et al., 1967
Dimethyl sulfoxide	66–9 at 10 mm.	+	Decomposes at 100° C.

[a] % (w./v.) at room temperature.

Many types of apparatus used for extraction are very aptly described and discussed by Stage and Gemmeker (1964) who list 191 references on extraction procedures and apparatus. A few of the ones we use may have some feature of interest and are shown in Figures 7 and 8.

Drying agents are used to dehydrate the extracts prior to solvent removal. Nursten and Williams (1966) studied the efficiency of such agents. "Investigations were... carried out on a 10% v./v. solution of ethanol in ether saturated with water, a system simulating the ether extract of a black currant distillate. Calcium sulfate was shown to be much more efficient than sodium sulfate, although the capacity of the sodium salt is much greater, and that is why it is usually applied. Calcium sulfate reduces the water content from 1.28% (the lowest obtainable with sodium sulfate) to 0.62%." The procedure finally adopted by the authors for handling the concentrates of volatiles containing relatively high proportions of alcohol was to remove the bulk of the water first with sodium sulfate and then to finish the drying with calcium sulfate.
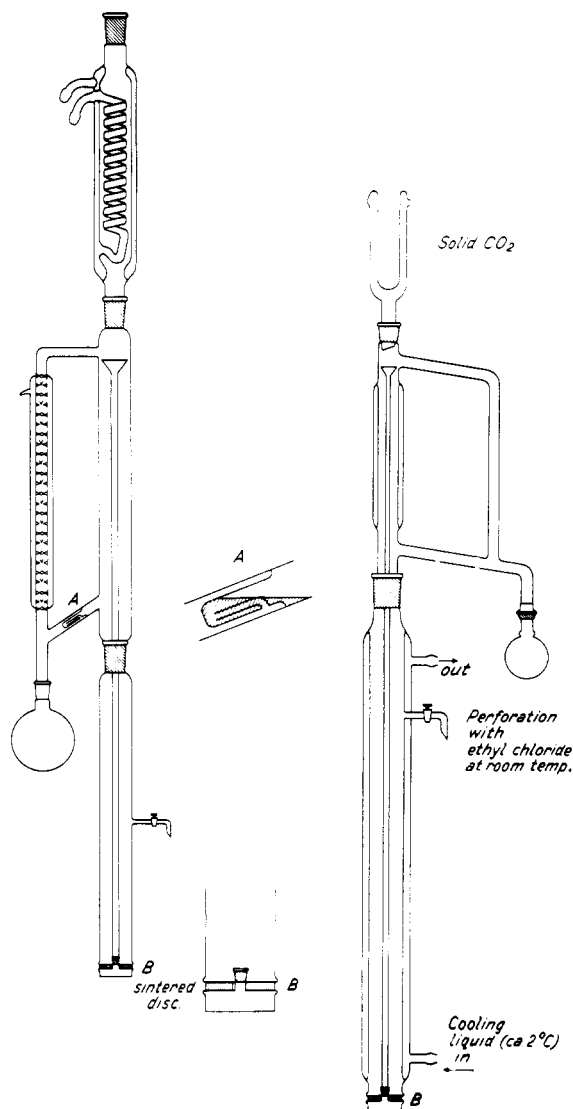

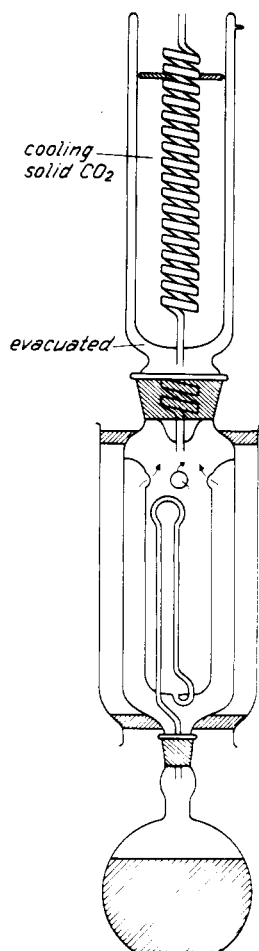Figure 7. Modifications of Kutscher and Steudel perforators

Figure 8. Soxhlet-type apparatus for extraction with methyl chloride

(b.p. 24° C., m.p. 98° C.)

distillation. Since, however, distillation procedures that are applied to food usually result in very dilute aqueous solutions, extraction should not be judged as the concentration procedure of choice at this stage.

Let us consider the case where say 90% of a solute, A, present in a solution at a concentration, C_A, is to be extracted by an organic solvent. When the volumes of the two solvents are equal:

$$K = \frac{C_l}{C_h}$$

in which

K = distribution coefficient for A

C_l = concentration of A in the lighter (top) layer

C_h = concentration of A in the heavier (bottom) layer

The formula represents the first rule of Nernst, which states that for dilute solutions a solute is distributed between two mutually saturated layers in such a way that the ratio of concentrations is constant. The second rule, which tells us that with mixed solutes the components are distributed independently, indicates that we may restrict our considerations to the simplest form (Hietala, 1960).

Figure 11—where K is chosen to be equal to 2—readily shows that it would take 10 times the amount of extractant when a batchwise procedure is applied (or about 10 times the time and the amount of heat in some forms of continuous extraction) than it would have done had the original concentration been 10 times C_A. Correspondingly the amount of impurities (and/or artifacts) in the final concentrate after removing the extractant would be 10 times larger in the first instance than in the second (Hecker, 1955). So concentrating our dilute solutions preceding extraction
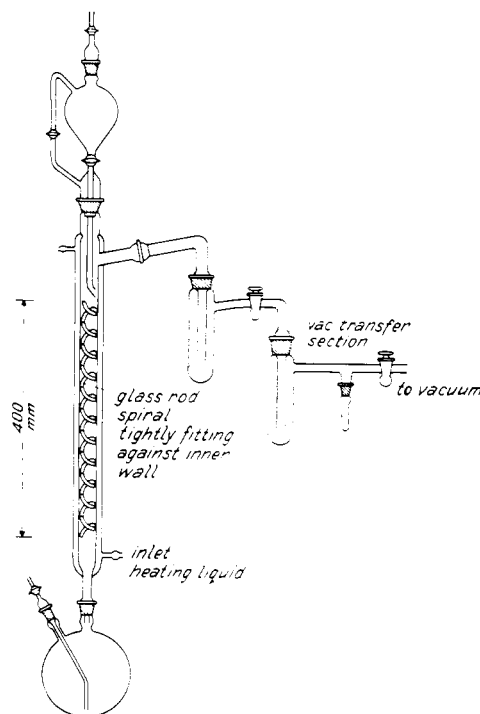
To remove the extractant finally from the odorous mixture in the extract a couple of arrangements and methods are applied. Usually reflux take-off over a Vigreux column of two to four theoretical plates is used, but the use of a simple rotary film evaporator might be better, since it takes less time and heat. The procedure followed by Muller et al. (1964), who first take 80% of the extractant off by thin-film, reduced pressure distillation and then carefully remove the rest by reflux distillation on a Wheeler center rod column, seems to me to be the most sensible. Ahrenst-Larsen and Hansen's (1964) arrangement also seems to work well. Figures 9 and 10 show our own strippers for the isolation of low boiling volatiles from large and small amounts of high boiling solvents (and fats and oils), respectively.

I am not too sure that the arrangement of Radtke et al. (1966) will work better than ours (Figure 9), but their apparatus is easier to construct. Both of them can be improved.

So far extraction has been discussed as if it were the best procedure to follow directly the isolation of the volatiles by

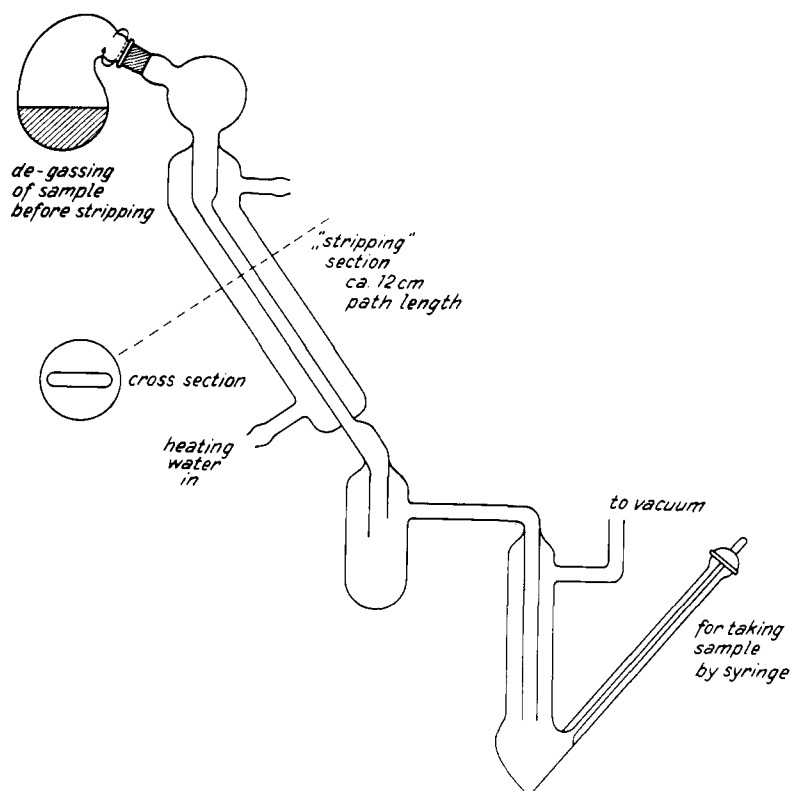Figure 9. Apparatus for stripping of volatiles from high boiling solvents, fats, and oils

Figure 10. Apparatus for stripping of volatiles from high boiling solvents, fats, and oils

is definitely preferable. There is a further reason for this preference. Large amounts of aqueous solutions are difficult to handle during extraction in batchwise, separating-funnel type procedures. One is thus easily inclined to resort to the use of seemingly more sophisticated semicontinuous arrangements such as the perforator type liquid-liquid extractors—where heating is applied and of which variations of the Kutscher-Steudel type instruments are the most commonly used—the column countercurrent extractors with mixing and settling states, the rotating disk contactors, and others (Braus and Miller, 1958; Flath *et al.*, 1967; Jennings, 1965; Werner and Waldichuk, 1962). These are only seemingly more sophisticated, since equilibria are seldom reached as fully as is possible with batchwise procedures. Efficiency of the extractions therefore is reduced and longer extraction times and heating or larger amounts of extractants become necessary.

Therefore, dilute aqueous solutions, as resulting from distillative isolations, should first be preconcentrated by procedures that are not selective and are not unfavorably affected by low concentrations.

Subsequently, extraction could probably be best applied in a few batchwise, separating-funnel type equilibria steps while more than one extractant, each of different character and one of them high boiling, should be used.

Finally, very simple total take-off distillation arrangements without reflux, which prolong heating time too much, should be used for removing the first, major part of the solvent.

The question has now been raised whether methods do exist for concentrating dilute aqueous solutions, without

being selective and adversely affected by low concentrations of the solutes. Freezing and, although to a certain extent selective, adsorption are such methods.

FREEZE CONCENTRATION

One of the earliest applications of freeze concentration was the preparation of some form of apple jack in the old days of England. The prehistoric Celts—as has been suggested by archaeologists—prepared their potent alcoholic drinks from fermented apple juice by letting it freeze in winter. The water was removed as ice, but the increase of the concentration of ethanol in the liquid that was left could not have been more than about two or three times.
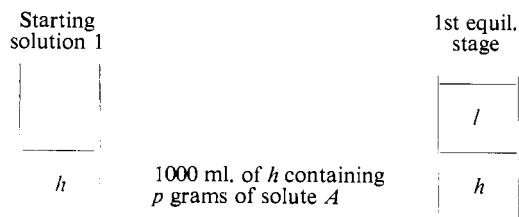
The art of the selective freezing of the water from solutions to concentrate the solutes seems to have been lost almost completely in the world of modern odor research. For although in industry nonfermented fruit juices nowadays are often concentrated in this way, applications in odor research are extremely rare.

Shapiro (1961) was one of the first to propagate the use of freeze concentration in the field of our specific interest. In contrast to still earlier applicants (Gibor, 1961; Haurowitz, 1930; Schildknecht and Mannl, 1957), Shapiro stresses the necessity for the contact layers between the liquid and solid phases to be continuously disturbed. Whether this is attained by shaking the whole freezing system, as is done by Smith and Tasker (1965), or by stirring the solution seems to make no difference so long as the second essential condition is observed, that until the very end of the process, part of the solution remains unfrozen. No experimental data are given by Shapiro but a

$$K = \frac{C_l}{C_h}$$

in which C_l = concentration of solute in lighter phase (l)
C_h = concentration of solute in heavier phase (h)

1. $C_h = p$ grams/1000 ml.

Starting solution 1		1st equil. stage	1000 ml. of l containing	After two equilibrium stages 2000 ml.

1000 ml. of h containing p grams of solute A

l

h

1000 ml. of l containing $\frac{K}{1 + K} \cdot p$ grams of solute A

1000 ml. of h containing $\frac{1}{1 + K} \cdot p$ grams of solute A

After two equilibrium stages 2000 ml. of l contain $\left[\frac{K}{1 + K} + \frac{K}{(1 + K)^2} \right] \cdot p$ grams of solute A, or, if $K = 2$,
$\left(\frac{2}{3} + \frac{2}{9} \right) \cdot p$ grams which is ca. 90% of solute A

2. $C_h = 10 \cdot p$ grams/1000 ml.

100 ml. of h containing p grams of solute A

l

h
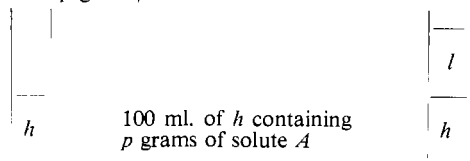
100 ml. of l containing $\frac{K}{1 + K} \cdot p$ grams of solute A

100 ml. of h containing $\frac{1}{1 + K} \cdot p$ grams of solute A

After two equilibrium stages 200 ml. of l contains $\left[\frac{K}{1 + K} + \frac{K}{(1 + K)^2} \right] \cdot p$ grams of solute A, or, if $K = 2$,
$\left(\frac{2}{3} + \frac{2}{9} \right) \cdot p$ grams which is ca. 90% of solute A

When the solvent l is removed from the extracts, the residue contains:

with 1: $\left(\frac{2}{3} + \frac{2}{9} \right) \cdot p$ grams of A minus losses, q grams of impurities of the extractant, and r grams of artifacts

with 2: $\left(\frac{2}{3} + \frac{2}{9} \right) \cdot p$ grams of A minus less losses, $q/10$ gram of impurities of the extractant, and $r/?$ gram of artifacts

Figure 11. Liquid extraction of a solute at two concentrations (1 and 2) in same solvent

number of interesting side phenomena, which may occur during freezing, are discussed.

A more fundamental study of all that happens during the freezing of solutions was made by Schildknecht and Schlegelmilch (1963). The seeding of super cold solutions, the influence of stirring as well as of the rate of cooling, and of the concentration of the starting solution were studied in some detail. Practical data, however, were only given on the purification (not concentration) of a few organic compounds. Their arrangement will not be applicable to batches of much over 200 to 300 ml. In a somewhat modified arrangement, described by Senn (1963), presumably up to 1000 ml. can be dealt with. Many others, of course, used freezing for concentrating nonvolatile compounds in aqueous solutions.

The references given on the freeze concentration of nonvolatile compounds are in no way representative. Schildknecht (1964), in his very instructive book, lists 360 references on zone melting and related investigations, but gives no references to the concentration of volatile compounds in aqueous solutions, though a few of his own experiments in this field are described. The book is a rich source of information on fundamental aspects, definition of notions, and description of apparatus. The aspects in which we are particularly interested are those of the directed normal freezing or progressive freezing, but these are less thoroughly dealt with than are the aspects of actual zone melting. This tendency is even more pronounced in the book on zone melting by Pfann (1958).

I have been able to locate only six publications on the freeze concentration, and ice zone melting, of dilute aqueous solutions of volatile organic compounds. The study of Shapiro (1967) was restricted to experiments with dilute aqueous solutions of acetone and isopropanol. Recoveries,

when five-fold concentration increases were obtained, were 90 and 100%, respectively. It was found necessary to protect the freezing mixture against severe losses by evaporation. The studies by Bidmead (1963), Hale and Cole (1963), and Senn (1963) are of particular interest, since the authors used the procedure in regular food product odor investigations. Senn and Bidmead, using Schildknecht's and Shapiro's techniques, respectively, applied the method to the study of fruit odors. A concentration increase of 10 to 20 times was obtained for samples of 1 and 5 liters. Hale and Cole's study (1963) on the concentration of centrifuged, clear, and odorous bread preferment solutions is interesting in that they did not disturb the layer near to the ice-liquid boundary during freezing. Concentration increases of ca. 10 times the original and recoveries in the concentrate of 80% or slightly more for the carbonyls and of ca. 90% for the acids and ethanol were obtained. Schildknecht (1964) finally mentions the concentration of a few more or less volatile compounds in aqueous solution by ice zone melting (thymochinone, 2,5-dimethyl-1,4-chinone, and vanilline) as does Swoboda (1966) for hexa-2,4-dienal and hexa-2,4-dienoic acid. However, this technique is difficult to use on larger amounts and demands rather elaborate equipment. Huckle (1966) first extracts the aqueous solution of raspberry flavors with benzene and subsequently concentrates the odorous volatiles by zone melting.

APPARATUS

In Figure 12 schematic drawings of various arrangements for freeze concentration are presented. They are all simple and the phase boundary layers are continuously disturbed during freezing in all except the arrangements of Hale and Cole (1963) and of Whitaker (1953). The drawing of the
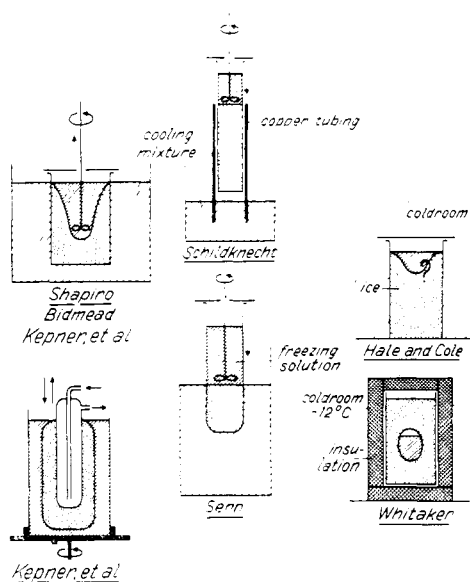
Figure 12. Arrangements for freeze concentration

arrangement used by Kepner *et al.* (1969) is less clear in this respect. The cold-finger on which the ice is deposited and which can be raised from the solution to remove the ice is stationary, while the bath containing the solution to be concentrated rotates during the process. Synthetic solutions can be concentrated up to 40 times or more, as can be done with the other procedures by repeated freezing, but losses due to evaporation could not be well prevented in this arrangement. Further studies by Kepner *et al.* (1969) on synthetic solutions and on distillates of a natural product were performed by following the method of Shapiro and recoveries were measured by gas chromatography. Here the recoveries range from 90 to 100%.

It is further observed in Figure 12 that everywhere layer upon layer of ice is deposited in a specific direction whether it is from the walls of the container inwards, from the wall of the cold-finger outwards, or from the bottom of the tube upwards. Therefore, the main condition for successful freeze concentration made by Schildknecht (1964), that of directed freezing, has been satisfied in all cases.

CAUSE OF LOSSES DURING FREEZING

Since artifact formation during freeze concentration of an aqueous solution is unlikely to occur frequently, the possibility of losing amounts of volatiles during the process seems the only disadvantage that should be considered. Such losses can be caused by occlusion, adsorption, evaporation, channeling, and by specific properties of individual components.

The occlusion of fine droplets of liquid solution in the solidifying ice mass is the only cause that is not selective. It is therefore of not too much concern to us, since it only interferes with the efficiency of the process by reducing the recovery in the concentrate without changing the quantitative ratios of the components. The phenomenon can be detected during freezing by the opaque, turbid appearance of the ice that is formed. Once occlusion has occurred it cannot be corrected except by melting the ice and restarting

the experiment. One can then try to prevent its occurrence by increasing the rate of stirring.

It is unlikely that adsorption accounts for an important part of any losses that may occur. During freezing, countless new layers of ice are continuously deposited onto the ice that has already formed and any adsorbed material would thus be enclosed. Adsorption therefore would take a much heavier toll if it occurred at all. Some material is of course lost by adhesion when removing the ice from the residual concentrated solution and more is lost the more concentrated is the final solution. Washing off the ice, accompanied by some melting, should reduce these losses.

Evaporation is responsible for the major part of the loss of volatiles, and, being selective, tends to alter the quantitative ratio of components and must be reduced as much as possible.

It is difficult to estimate the importance of loss by channeling. Channeling is a term for the inclusion of tiny elongated bubbles of air in the ice mass. As freezing out begins, very clear ice forms on the walls of the container. After some time, when the concentration of the solution has been considerably increased, very fine, silvery channels between 6 and 8 mm. long are formed. The reason for this formation is that the lowering of the temperature of the water in the early stages of the process increases the solubility of air in the water. The water is then being removed until the concentration of air reaches the saturation point. Air bubbles appear on the surface of the ice mass, and at the point of contact they provide considerable insulation to heat transfer. Further formation of ice is prevented for a time, thus causing the development of a channel in the ice of a diameter corresponding to that of the air bubble. The phenomenon may be demonstrated by freezing two samples of freshly distilled water, one of which has been shaken with air. Channels form much earlier and to a greater extent in the sample which had been shaken with air.

The small quantities of air, enclosed in the channels, must contain tiny amounts of the volatile components of the solution corresponding to the composition of the vapor over the solution at the moment the channel is formed. Very rough estimates—taking 10 channels of 0.5-mm. diameter and 5-mm. length per sq. cm. on a total surface area of ice of 2000 sq. cm.—would bring us to a total loss of about 20 ml. of vapor at 0° C. from 1 liter of the original solution. Not serious presumably, but does concentration of vapors take place in the semicapillary channels?

The last factor which must be considered concerns losses that may occur because of specific properties of individual components. It has long been known from investigations on zone refining that a number of compounds accumulate in the solid phase, instead of in the liquid one. Röck (1957) tells us more on these reversed accumulations and Pfann (1958) explains that in fact the solutes which travel in opposition to the moving freezing zone are those which raise the melting point of the solvent. The phenomenon can be predicted to occur from the phase diagrams for the individual compounds. Presumably it is rather rare, and I still feel assured that evaporation is the major cause for the selective loss of volatiles.

Why then is the method of freeze concentration so seldom applied? Possibly because the concentration in-

creases that have been obtained so far by freezing have not been spectacular. Indeed, 20 times is not impressive, but it means that only one twentieth of the impurities of the extractant and only one twentieth of some artifacts accumulate in our final sample for analysis, when extraction is used as the next concentration step.

Kepner et al. (1969) have found it feasible to obtain much higher concentration gains by again freezing the joined concentrates of a number of preceding runs. Usually the amount of ethanol in the original distillate will become the limiting factor on concentrating. If not, the onset of phase separation in the liquid will mark the end of successful freezing.

In any case the concentration gain that can be obtained is determined by the concentration of the starting solution. Contrary to extraction, the more dilute the solution, the smoother the freezing runs, and the more efficient the freeze concentration is. These two procedures ideally support one another when freeze concentration is followed by extraction.

The second reason for freeze concentration being applied so infrequently may be that it is difficult to handle batches larger than 2 or, at most, 5 liters. When some modification of Shapiro's method is applied, high capacity and expensive cryostats have to be used, as the thick layers of ice become effective insulators to the further transfer of heat. Very low cooling temperatures and large amounts of coolant are necessary but they may then easily overshoot the optimum rate of freezing in the early stages of the procedure if a close watch is not kept on temperature regulation. Stirring should be adjusted, the speed being decreased during the process.

It seems advisable therefore to study further the method of Hale and Cole (1963) and to try to improve on it by use of the modification of Whitaker (1953). In his experiments the drums which contain the solution to be frozen are heavily insulated and placed in a cold room. Thus freezing is slowed to such an extent that it is more likely to keep pace with the diffusion of the solutes in the boundary layer. No stirring is necessary and the main difficulty which remains is to decide at which moment to halt the process; complete solidification cannot be allowed to occur if the principle of directed freezing is to be adhered to.

ADSORPTION AND DESORPTION

Adsorption is applied in only 8% of the studies on food odors checked (Figure 3)—18 times in 234 published research papers. Of these applications five referred to the use of polar adsorbents, silica gel being the most common one, by which oxygenated terpene type compounds were separated from the hydrocarbons.

Charcoal is the only other adsorbent used, for it has the great advantage that it is not deactivated by water and has a great capacity for the adsorption of the organic compounds in which we are interested. Even traces of such compounds are picked up from aqueous vapors and liquids.

Like freezing, the efficiency of adsorption is not diminished by increasing dilution but contrary to freezing it is to a certain extent selective and possibly more likely to produce artifacts. This and the other characteristic aspects in the adsorption rules are given by Täufel (1933):

From low concentration solutions relatively larger amounts of the solutes are adsorbed. The time required for reaching adsorption equilibrium strongly depends on the type of charcoal (for coconut charcoal it takes hours compared with minutes for others). In homologous series the higher members are more easily adsorbed. Acids, bases, and alcohols usually are not strongly adsorbed. Adsorption of compounds from solutions decreases for the solvents along the series: water, ethanol, esters, acetone, $CHCl_3$. Compounds that are readily oxidized may become so during adsorption. The less soluble compounds in solutions are more easily adsorbed. Colloids are more strongly adsorbed than crystalloids.

The apparatus used for adsorption in odor research is always simple. It normally consists of a piece of glass tubing, filled with the adsorbent through which the odorous vapors or aqueous distillates run. Since adsorption liberates heat and raised temperature prolongs the equilibration time and reduces the amount of absorbed material, cooling during adsorption is desirable.

Usually desorption, which is as important in odor research as the reverse process, is less easily accomplished and, of course, always demands energy.

The following methods can be, and are, used: desorption by raising the temperature, reducing the pressure, eluting (= extracting), and displacing.

Desorption by reduced pressure, which in itself is not often very effective, is usually combined in a single operation by the desorption that occurs on raising the temperature. Since the heat is to a large extent utilized for breaking the forces of adsorption, rather high temperatures (250° to 300° C.), if applied gradually, can be used without causing heat damage to the compounds that are released.

Preceding desorption, the water adhering to the adsorbent can be removed by heating but preferably by lyophyllization. Strackenbrock (1961, 1962), who used these procedures applied earlier by Turk and Messer (1953), states that no material is lost during drying. The same procedure was followed by Grevers and Doesburg (1962) and by Paillard (1965). Paillard's study is of particular interest for its qualitative and quantitative test on the recovery of 16 alcohols and esters. Heinz et al. (1966) also used freeze-drying to eliminate the very weakly bound water, but subsequently eluted the organics from the coal by extraction with ether, as was done by a number of others (Carson and Wong, 1961; Henze et al., 1954; Steinke and Paulson, 1964). Tang and Jennings (1967) and Jennings and Nursten (1967) finally eluted the adsorbed material very successfully with CS_2.

Desorption by displacement has a number of advantages over the former methods. It may pay to direct specific research to establish fully its possibilities and limitations. The method can probably be developed into a technique for the displacement of complex mixtures from charcoal in such a way that the individual components can be obtained directly for further separation or possibly even identification, without intermediate solvent elimination and GLC.

The choice of the type of charcoal as well as of the displacer and its concentration determines the components that are liberated and the concentration in which they are freed.
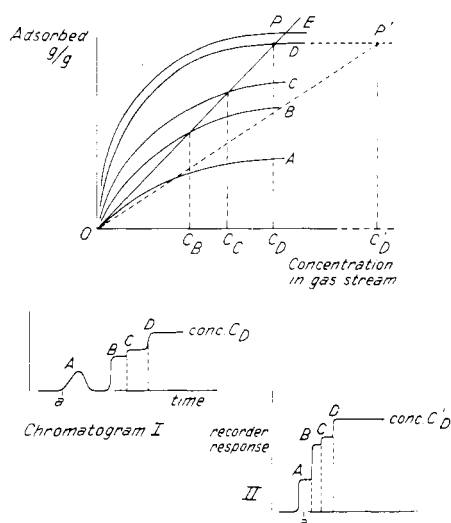
Figure 13. Concentration and isolation by absorption

In Figure 13—taken from Claesson (1946/1947), although it has been slightly altered—the adsorption isotherms for compounds A, B, C, D, and E have been drawn for some specific type of charcoal at a specific temperature. It is suggested that compounds A, B, C, and E are adsorbed on the top of a charcoal column and that the displacer D is mixed in an inert carrier gas at a concentration C_D, which is continuously fed into the column. A detector at the exit of the column will then register a graph of the emerging components.

A straight line is drawn in the figure through the origin and point P, indicating concentration, C_D, of compound D in the carrier gas which causes the corresponding adsorption density for D on the coal. The adsorption isotherm for compound A is not bisected. This means that compound A is not displaced by compound D at the specific concentration of D in the gas, simply because the rate of movement of A along the column is greater than that of D.

Actually, displacement of A by D would occur only when the rate of movement of D is increased by raising its concentration in the carrier gas, and D thereby would overtake A on the column. Such a situation has been marked in the figure by the broken line P'-O, where P' corresponds to a much higher concentration C_D' in the gas.

At concentration C_D again, compound A is simply eluted from the coal by the carrier gas—as can be seen in chromatogram I where A emerges from the column in the form of an elution peak. Compounds B and C on the other hand are displaced by D, and B by C. The vertical broken lines in the chromatograms indicate that the graphs for compounds B and C show displacement peaks and not frontal analysis ones.

At a much higher concentration of D—e.g., C_D'—A as well as B and C is displaced and a chromatogram such as II is registered. Compound E can never be displaced by any concentration of D and very gradual elution at low concentrations in the emerging gas will start taking place only after a long time.

The form of the isotherms depends on the properties of the coal (absorbent) and the more they approach straight

lines and the closer together they are the less displacement occurs and the lower the step heights show in the chromatograms. Once one compound is displaced by another, all of the material of the first emerges from the column in front of the second; basically, as long as no chemisorption takes place, the process gives 100% recovery.

Hassler (1963) gives more general and practical information than Claesson (1946/1947) and two quotations are taken from his book. The first may help to prevent a practical error which possibly is made at times by odor research workers (p. 338): "In an adsorption-desorption process, there are two avenues of loss: One is the material that is lost by not being adsorbed from the original solution; the other is that portion of the adsorbate which is not eluted from the carbon. When larger dosages of carbon are used, less material is lost through not being adsorbed; but the increased amount of carbon results in a greater amount of the adsorbate being retained on the carbon at the elution stage. In each process, there is an optimum dosage of carbon which gives the maximum over-all recovery. This optimum dosage is determined by using various dosages of carbon in the complete adsorption-desorption procedure."

The second quotation may help to overcome the reluctance we often feel toward the use of adsorption in our studies (p. 248): "Chemical changes caused by adsorption on carbon are probably less frequent than might be inferred from the frequent reference to the subject. ... Catalytic oxidation can be avoided by conducting operations in an inert atmosphere. The catalytic activity of the carbon may be controlled by poisoning the active centers before adsorption. As only a small portion of the surface is catalytically active, the poisoning need not greatly reduce the adsorptive power of the carbon."

It is also advisable as a general rule to shorten the time between adsorption and desorption as much as possible to reduce such alterations on the adsorbent that progress with time. To adsorb one day and leave desorption for the next is not an acceptable proposition if the experiments can be arranged otherwise.

QUANTITATIVE STUDIES ON ARTIFACTS AND RECOVERY DURING ADSORPTION AND DESORPTION

Studies on artifact formation on charcoal, as well as on the quantitative aspects of adsorption-desorption processes in odor research, are rare.

The study of Paillard (1965) has been mentioned. No artifacts can be observed in his chromatograms that have been formed during the adsorption and desorption of his test solutions. Recovery after vacuum-heat-desorption for all components was about 70%. So ratios remained virtually unaltered. Carson and Wong (1961) fear that adsorption on charcoal "can lead to decomposition and rearrangement of the volatile components" but their results, when compared with experiments in which no adsorption had been applied, do not confirm this fear for the compounds they were particularly interested in (disulfides and trisulfides). Heinz and Jennings (1966), using charcoal in their studies, also discuss the possibility of artifact formation. Again no actual evidence was encountered and very complex true product odors of pears and apricots were recovered after desorption. The study of Jennings and

Nursten (1967) is of specific interest in that a method is suggested by which the recovery of the adsorption-desorption (elution) procedure could be investigated. The objective of the authors is different from ours at the moment and their figures cannot give an insight to these recovery aspects, since they represent the combined recovery of distillation and adsorption-desorption. No indications, however, of artifact formation could be observed by the authors. Henze *et al.* (1954) also suggest the possibility of artifact formation. This may be understandable in their case, since their arrangement for the study of the emanations of apples in storage demands very long adsorption times (seven months!) and the continuous passage of O_2-containing air (Henze *et al.*, 1953). While these authors consider the possibility that higher boiling point aldehydes are formed from lower boiling ones, Meigh (1957) suggests that artifact formation on charcoal would lead from adsorbed aldehydes to esters.

At our institute a number of adsorption-displacement desorption experiments on model systems were executed (Dhont and Weurman, 1960) in which the behavior of alcohols and esters of lower boiling points were investigated. No elution from a column, containing 0.6 gram of charcoal, of any of the compounds with the exception of methanol was observed by the single passage of N_2 for 3 hours at 100° C. Almost full recovery, on the other hand, of all the individual components was obtained by displacement with dioxan in the vapor state. No artifacts were observed. Dhont (1964) investigated a number of aldehydes in the same way and established that none of them were displaced by dioxan, monochlorobenzene, or monobromobenzene.

James and Phillips (1953) reported on many of the advantages of the technique and, incidentally, also stated that aldehydes "appear to be irreversibly adsorbed" on charcoal. James and Phillips' as well as Dhont's finding that aldehydes are not displaced by the displacers tested may well be correct, but the former authors' suggestion that the compounds were irreversibly adsorbed may be erroneous. For is this not in contradiction with the fact that a number of the investigators that were mentioned earlier have found no difficulty in eluting aldehydes from charcoal with ether or CS_2? James and Phillips (1953) and Griffiths *et al.* (1952) give good descriptions of displacement phenomena and the former authors conclude: ". . . Quantitative analyses can be made with an accuracy of better than 1%. The two basic techniques, adsorption (displacement) and partition (elution), tend to be complementary to one another. The displacement technique produces concentrated zones, ideally suited for quantative analysis and general preparative work". . . .and ". . . The adsorption columns possess self-sharpening properties, which make them useful for the isolation of trace materials. . . ."

This was said some 15 years ago, while now, out of 234 papers on odor research, the tentative use of the displacement technique was only mentioned in one.

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REFERENCES

Ahrenst-Larsen, B., Hansen, H. L., *Wallerstein Lab. Commun.* 27, 41 (1964).
Angelini, P., Forss, D. A., Bazinet, M. L., Merritt, C., *J. Am. Oil Chemists' Soc.* 44, 26 (1967).
Bernhard, R. A., *Advan. Chem. Ser.* 56, 131 (1966).
Bidmead, D. S., "Recent Advances in Food Sciences," Vol. 3, p. 158, Butterworth, London, 1963.
Bober, A., Haddaway, L. W., *J. Gas Chromatog.* 2, 76 (1964).
Bomben, J. L., Kitson, J. A., Morgan, A. I., *Food Technol.* 20, 1219 (1966).
Bomben, J. L., Mannheim, H. C., Morgan, A. I., *Fruchtsaft-Ind.* 12, 44 (1967).
Braus, H., Miller, F. D., *J. Assoc. Offic. Agr. Chemists* 41, 141 (1958).
Büchi, W., Symp. Fruchtaromen, Bern, p. 117, Juris Verlag, Zürich, 1962.
Burchfield, H. P., Storrs, E. E., "Biochemical Applications of Gas Chromatography," Chapters 3, 4, and 5, Academic Press, New York, 1962.
Burger, A. M., *Riechstoffe Aromen* 11, 189–90, 192–5 (1961).
Burger, A. M., *Riechstoffe Aromen* 12, 207 (1962).
Carson, J. F., *Coffee Tea Ind.* 86, 30 (1963).
Carson, J. F., Wong, F. F., *J. Agr. Food Chem.* 9, 140 (1961).
Chang, S. S., "Methodology of Flavor Evaluation," Special Report 66-1, Packaging Institute, New York, 1966.
Claesson, S., *Arkiv Kemi Mineral. Geol.* 23, 55 (1946/47).
Connell, D. W., *Australian J. Chem.* 17, 130 (1964).
Cotner, E. C., Martin, W. H., Mickelson, R., Rutz, W. D., *Am. Milk Rev.* 22, 34 (Nov. 1960).
De Bruyn, J., Schogt, J. C. M., *J. Am. Oil Chemists' Soc.* 38, 40 (1961).
De Mets, M., Verzele, M., *J. Inst. Brewing* 74, 74 (1968).
Dhont, J. H., Central Inst. Nutrition and Food Research T.N.O., Utrecht, Netherlands, private communication (1964).
Dhont, J. H., Weurman, C., *Analyst* 85, 419 (1960).
Dimick, K. P., Corse, J., "Chemistry of Natural Food Flavors," p. 123, Symp., Washington, Quartermaster Food and Container Institute for the Armed Forces, Chicago, 1957.
Dimick, K. P., Corse, J., *Food Technol.* 10, 360 (1956).
Drawert, F., "Analytik der Lebensmittel," Bd. II/1, p. 622, Springer Verlag, Berlin, 1965.
Drawert, F., Heimann, W., Emberger, R., Tressl, R., *Ann. Chemie* 694, 200 (1966).
Drawert, F., Rapp, A., *Uitis* 5, 351 (1966).
Drawert, F., Rapp, A., Bachmann, O., Symp. Fruchtaromen, Bern, p. 235, Juris Verlag, Zürich, 1962.
Dutra, R. C., Jennings, W. G., Tarassuk, N. P., *Food Res.* 24, 688 (1959).
Edélyi, E., Dworschak, E., Vas, K., Lindner, L., Telegdy-Kovats, M., Szöke-Szotyori, K., *Fruchtsaft-Ind.* 12, 54 (1967).
Egli, R., *Glas-Instr. Tech.* 11, 509 (1967).
Egli, R., *Glas-Instr. Tech.* 11, 705 (1967).
Elzakker, A. H. M., van Zutphen, H. J., *Z. Lebensm. Untersuch. Forsch.* 115, 222 (1961).
Eskew, R. K., "Chemistry of Natural Food Flavors," p. 113, Symp., Washington, Quartermaster Food and Container Institute for the Armed Forces, Chicago, 1957.
Fire Protection Association of London, Booklet No. 24, 1965.
Flath, R. A., Black, D. R., Guadagni, D. G., McFadden, W. H., Schultz, T. H., *J. Agr. Food Chem.* 15, 29 (1967).
Forss, D. A., Holloway, G. L., *J. Am. Oil Chemists' Soc.* 44, 572 (1967).
Forss, D. A., Jacobsen, V. M., Ramshaw, E. H., *J. Agr. Food Chem.* 15, 1104 (1967).
Gheluwe, van J. E. A., Belleau, C., Jamieson, A., Buday, A., *Am. Soc. Brewing Chemists Proc.* 1966, p. 49.
Gibor, A., *Science* 133, 193 (1961).
Grevers, G., Doesburg, J. J., Symp. Fruchtaromen, Bern, p. 319, Juris Verlag, Zürich, 1962.
Griffiths, J., James, D., Phillips, C., *Analyst* 77, 897 (1952).
Grosch, W., *Lebensm. Gerichtl. Chemie* 20, 49 (1966).
Hale, W. S., Cole, E. W., *Cereal Chem.* 40, 287 (1963).
Hassler, J. W., "Activated Carbon," Chemical Publishing Co., Inc., New York, 1963.
Haurowitz, F., *Z. Physiol. Chem.* 186, 141 (1930).

Hecker, E., "Verteilungsverfahren im Laboratorium," Verlag Chemie, Weinheim, 1955.
Heimann, W., Strackenbrock, K. H., Z. Lebensm. Untersuch. Forsch. 120, 273 (1963).
Heinz, D. E., Jennings, W. G., J. Food Sci. 31, 69 (1966).
Heinz, D. E., Pangborn, R. M., Jennings, W. G., J. Food Sci. 29, 756 (1964).
Heinz, D. E., Sevenants, M. R., Jennings, W. G., J. Food Sci. 31, 63 (1966).
Henze, R. E., Baker, C. E., Quackenbusch, F. W., J. Agr. Food Chem. 2, 1118 (1954).
Henze, R. E., Baker, C. E., Quackenbusch, F. W., Proc. Am. Soc. Hort. Sci. 61, 237 (1953).
Herz, K. O., Chang, S. S., J. Food Sci. 31, 937 (1966).
Hietala, P., Ann. Acad. Sci. Fennicae Ser. A II. Chemica, 100 (1960).
Hobson-Frohock, A., Lea, C. H., Chem. Ind. 82, 311 (1965).
Honkanen, E., Karvonen, P., Acta Chem. Scand. 17, 1357 (1963).
Honkanen, E., Karvonen, P., Acta Chem. Scand. 20, 2626 (1966).
Hornstein, J., Crowe, P. F., J. Gas Chromatog. 2, 128 (1964).
Huckle, M. T., Chem. Ind. 83, 1490 (1966).
Hunter, G. L. K., Moshonas, M. G., J. Food Sci. 31, 167 (1966).
James, D. H., Phillips, C. S. G., J. Chem. Soc. 1953, p. 1600.
Jenard, H., Brewers Dig. 35, 58 (1960).
Jennings, W. G., Ber. Wiss. Tech. Komm. Intern. Fruchtsaft-Union 6, 277 (1965).
Jennings, W. G., Nursten, H. E., Anal. Chem. 39, 521 (1967).
Kepner, R. E., Straten, S. van, Weurman, C., J. Agr. Food Chem. 17, in press (1969).
Keulemans, A. I. M., Symp. Fruchtaromen, Bern, p. 191, Juris Verlag, Zürich, 1962.
Kobayashi, S., Lee, G. T., Anal. Chem. 36, 2197 (1964).
Kohman, E. F., Food Technol. 6, 288 (1952).
Kromann, R. P., Meyer, J. H., Stielau, W. J., J. Dairy Sci. 50, 73 (1967).
Lea, C. H., Swoboda, P. A. T., J. Sci. Food Agr. 13, 148 (1962).
Lea, C. H., Swoboda, P. A. T., Hobson-Frohock, A., J. Sci. Food Agr. 18, 245 (1967).
Lemli, J. A. J. M., Dissertation, Univ. Groningen, Netherlands, 1955.
Lemperle, E., Mecke, R., Z. Anal. Chem. 212, 18 (1965).
McGugan, W. A., Howsam, S. G., J. Dairy Sci. 45, 495 (1962).
Mecke, R., Schindler, R., De Vries, M., Wein-Wiss. 15, 183 (1960).
Mehlitz, A., Gierschner, K., Ind. Obst-Gemueseverwert. 48, 217 (1963).
Mehlitz, A., Gierschner, K., Symp. Fruchtaromen, Bern, p. 301, Juris Verlag, Zürich, 1962.
Meigh, D. F., J. Sci. Food Agr. 8, 313 (1957).
Meigh, D. F., "Volatile Alcohols, Aldehydes, Ketones, and Esters," in "Modern Methods of Plant Analysis," II, 403, Springer Verlag, Berlin, 1955.
Menting, L. C., Hoogstad, B., J. Food Sci. 32, 87 (1967).
Merritt, C., Bazinet, M. L., Sullivan, J. H., Robertson, D. H., J. Agr. Food Chem. 11, 152 (1963).
Merritt, C., Bresnick, S. R., Bazinet, M. L., Walsh, J. T., Angelini, P., J. Agr. Food Chem. 7, 784 (1959).
Miers, J. C., J. Agr. Food Chem. 14, 419 (1966).
Miller, B. S., Johnson, J. A., Robinson, R. J., Cereal Chem. 38, 507 (1961).
Milleville, H. P., Eskew, R. K., Western Canner Packer 38, 51 (1946).
Muller, C. J., Kepner, R. E., Webb, A. D., J. Food Sci. 29, 569 (1964).
Nawar, W. W., Food Technol. 20, 115 (1966).
Nawar, W. W., Fagerson, I. S., Anal. Chem. 32, 1535 (1960).
Nawar, W. W., Lombard, S. H., Dall, H. E. T., Ganguly, A. S., Whitney, R. McL., J. Dairy Sci. 46, 671 (1963).
Niedzielski, A., Abstracts of Papers, 2nd Intern. Cong. Food Sci. Technol., Warsaw, 264, 1966.
Niegisch, W. D., Stahl, W. H., Food Res. 21, 657 (1956).
Nursten, H. E., Williams, A. A., Chem. Ind. 83, 2188 (1966).
Nye, W., Spoehr, H. A., Arch. Biochem. 2, 23 (1943).
Osterhaus, E., Z. Anal. Chem. 215, 241 (1965).
Otsuka, K., Imai, S., Sanbe, M., Agr. Biol. Chem. 30, 1191 (1966).
Owades, J. L., Dono, J. M., Am. Soc. Brewing Chemists Proc. 1965, p. 157.
Paillard, N., Fruits 20, 189 (1965).
Pavelka, F., Mikrochim. Acta 1964, p. 1121.
Pfann, W. G., Science 135, 1101 (1962).
Pfann, W. G., "Zone Melting," Wiley, New York, 1958.

Pippen, E. L., Nonaka, M., Food Res. 25, 764 (1960).
Pippen, E. L., Nonaka, M., J. Food Sci. 28, 334 (1963).
Pollard, A., Beech, F. W., Process. Biochem. 1, 229 (1966).
Pyne, A. W., Wick, E. L., Food Sci. 30, 192 (1965).
Radtke, R., Mohr, W., Springer, R., Z. Lebensm. Untersuch. Forsch. 129, 349 (1966).
Rhoades, J. W., Millar, J. D., J. Agr. Food Chem. 13, 5 (1965).
Röck, H., "Ausgewählte moderne Trennverfahren," Dietrich Steinkipff, Darmstadt, 38, 1957.
Ryder, W. S., Advan. Chem. Ser. 56, 70 (1966).
Scarisbrick, R., "Volatile Acids" in, "Modern Methods of Plant Analysis," II, 444, Springer Verlag, Berlin, 1955.
Scarpellino, R., Kosikowski, F. V., J. Dairy Sci. 44, 10 (1961).
Schildknecht, H., "Zonenschmelzen," Verlag Chemie, Weinheim, 1964.
Schildknecht, H., Mannl, A., Angew. Chem. 69, 634 (1957).
Schildknecht, H., Schlegelmilch, F., Chem. Ing. Tech. 35, 637 (1963).
Schmeltz, J., Dooley, C. J., Stedman, R. L., Chamberlain, W. J., Phytochemistry 6, 33 (1967).
Schormüller, J., Z. Lebensm. Untersuch. Forsch. 118, 385 (1962)
Schratz, E., Wahlig, T., Planta Med. 13, 218 (1965).
Schultz, O. E., Mohrmann, H. L., Pharmazie 20, 379 (1965).
Self, R., Casey, J. C., Swain, T., Chem. Ind. 80, 863 (1963).
Senn, G., Z. Lebensm. Untersuch. Forsch. 120, 455 (1963).
Shapiro, J., Anal. Chem. 39, 280 (1967).
Shapiro, J., Science 133, 2063 (1961).
Shepherd, B. D., Chem. Ind. 74, 1119 (1957).
Shipton, J., Whitfield, F. B., Chem. Ind. 83, 2124 (1966).
Silberstein, O., Proc. Am. Soc. Hort. Sci. 63, 359 (1954).
Smith, G. H., Tasker, M. P., Anal. Chim. Acta 33, 559 (1965).
Stage, H., Gemmeker, L., Chemiker Z. 88, 517 (1964).
Stahl, W. H., "Chemistry of Natural Food Flavors," p. 58, Symp., Washington, Quartermaster Food and Container Institute for the Armed Forces, Chicago, 1957.
Stanley, W. L., Brekke, J. E., Teranishi, R., U.S. Patent 3,113,-031 (Dec. 3, 1963).
Steinke, R. D., Paulson, M. C., J. Agr. Food Chem. 12, 381 (1964).
Stevens, K. L., Bomben, J., Lee, A., McFadden, W. H., J. Agr. Food Chem. 14, 249 (1966).
Strackenbrock, K. H., Dissertation, Reinischen Fried, Wilhelm University, Bonn, 1961.
Strackenbrock, K. H., Symp. Furchtaromen, Bern, p. 287, Juris Verlag, Zürich, 1962.
Suffis, R., Dean, D. E., Anal. Chem. 34, 480 (1962).
Swain, T., Self, R., European Potato J. 7, 228 (1964).
Swoboda, P. A. T., VIth Intern. Symp. Gas Chromatog., Rome, Bartholomew Press, Dorking, 1966.
Tang, C. S., Jennings, W. G., J. Agr. Food Chem. 15, 24 (1967).
Täufel, K., "Handbuch der Lebensmittelchemie," Springer, Berlin, 1933; II/1, 1967.
Tinner, H., Symp. Fruchtaromen, Bern, p. 377, Juris Verlag, Zürich, 1962.
Turk, A., Messer, P. J., J. Agr. Food Chem. 1, 264 (1953).
Walker, L. H., Symp. Fruchtaromen, Bern, p. 104, Juris Verlag, Zürich, 1962.
Webb, A. D., Qualitas Plant. Mater. Vegetabiles 11, 234 (1964).
Werner, A. E., Waldichuk, M., Anal. Chem. 34, 1674 (1962).
Weurman, C., J. Food Sci. 26, 670 (1961).
Weurman, C., "Recent Advances in Food Science," Vol. 3, Butterworth, London, 1963.
Weurman, C., de Rooy, C., J. Food Sci. 26, 239 (1961).
Whitaker, D. R., Arch. Biochem. Biophys. 43, 253 (1953).
Wilson, T. E., Evans, D. J., Theriot, M. L., Appl. Microbiol. 12, 96 (1964).
Winter, M., Mitt. Gebiete Lebensm. Hyg. 54, 520 (1963).
Winter, M., Palluy, E., Hinder, M., Willhalm, B., Helv. Chim. Acta 45, 2186 (1962).
Wiseblatt, L., Kohn, F. E., Cereal Chem. 37, 55 (1960).
Wolford, R. W., Alberding, G. E., Attaway, J. A., J. Agr. Food Chem. 10, 297 (1962).
Wolfrom, M. L., Binkley, W. W., Bobbio, F. O., El Crisol 7, 35 (1953).
Wong, N. P., Damico, J. N., Saldwin, H., J. Assoc. Offic. Anal. Chemists 50, 8 (1967).
Wucherpfennig, K., Bretthauer, G., Z. Anal. Chem. 228, 342 (1967).