Apparatus for the Isolation of Trace Volatile Constituents from Foods

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For the purpose of ascertaining the chemical structures of the trace volatile constituents which are responsible for the flavor of foods, successful isolation of such components, without contamination and artifacts, while maintaining the genuine flavor of the original food, is a necessary prerequisite for sophisticated instrumental methods of fractionation and identification. Techniques for the isolation of such flavor constituents from oil, aqueous solutions, and solid masses, both in small quantities and in relatively large amounts, have been successfully developed.

The identification of trace volatile constituents in foods has gained great academic interest in understanding the natural phenomena of flavor and in analyzing the chemical composition of foods. It is also of great practical importance in creating flavor compounds to improve the flavor of foods and to manufacture food analogues. However, in order to obtain useful and meaningful information of this kind, there are two prerequisites to the fractionation and identification steps, namely, selection of representative samples and isolation of the trace volatile constituents from the foods.

Unfortunately, these prerequisites are often neglected, probably because the fractionation and identification steps are more glamorous, using modern, sophisticated instruments, such as the gas chromatograph and the mass spectrometer, while the isolation step is tedious and time consuming.

DISCUSSION

Selection of Food Samples. If our intention is to determine which chemical compounds are responsible for the flavor of a food, then it is absolutely necessary to start with a sample which is recognized by a sensory evaluation panel as truly having a typical genuine flavor of that food. For example, if we want to pursue the flavor of potato chips, we cannot simply walk into a supermarket and pick up a package of potato chips from the shelf. Such a sample might not have a flavor typical of that of good potato chips. In addition, it might have objectionable flavors, such as stale, rancid, reversion, or hydrogenation flavor, which will appear as dangerous artifacts.

Let us use our study of potato chip flavor as an example of the proper selection of samples. We first invited a number of potato chip manufacturers to bring us samples of their best product. These samples were evaluated by a sensory panel, using the profile method, and the sample picked out by the panel as having a well blended, and pleasant, desirable flavor without any burned or harsh off-flavors, was selected for our study. A large batch of these potato chips, over 100 lb, was then prepared, immediately packed in cans under an atmosphere of nitrogen and stored at −10 °C, until they were used for flavor isolation (Deck et al., 1973).

Artifacts Created during the Isolation Process Must Be Avoided. Obviously, the selected sample could not be fed directly into instruments. The volatile compounds responsible for the aroma must first be isolated from the food. To insure that the isolated volatiles do indeed have the genuine flavor of the original food, the volatile compounds must be completely or, at least, nearly completely isolated from the food. Since many components of foods are labile to heat and oxidation, the conditions for the isolation process must be such that no significant chemical reactions are allowed to take place to yield additional volatile compounds as artifacts. In addition, many extractable components of a food are decomposable in the fractionation instrument, with the possible formation of very complex decomposition products, which will be mixed with the true flavor compounds. For example, if lipids, either triglycerides or phosphatides are extracted together with the trace volatile flavor constituents, they will stay in the gas chromatographic injector or column and decompose by heat into various volatile components which will be undistinguishable from the true volatile flavor compounds and thus confuse the results.

In our laboratory, fractionation and identification are not allowed to proceed until an isolate is obtained which is judged by a sensory panel as having an aroma closely reminiscent of the genuine flavor of the original food. Painstaking work is then necessary to prepare an amount of the isolate large enough for systematic fractionation and identification, as well as for the search for the key compounds which have the characteristic flavor of that particular food. Since such compounds, in many instances, are present in only trace amounts, and since such compounds may have unusual structures which require more than mass spectra for their identification, it is desirable to start with as large a sample as possible. As a general rule, we usually isolate the volatile flavor components from 100 lb of the food as a start.

Isolation of Trace Volatile Constituents from Lipid Materials. The process which is commonly used by the edible oil industry to remove odoriferous components from an oil is known in the trade as "deodorization". The apparatus commonly used in the laboratories of oil refineries for this purpose is shown in Figure 1. It is evacuated to a vacuum of 0.05 mm Hg by a high-capacity vacuum pump. The two cold finger traps are cooled by a mixture of dry ice and acetone. Water vapor is generated from the water in the flask on the far left by vacuum and, if necessary, by a heating lamp focused on the surface of the water. The water vapor is led into the bottom of the oil-containing deodorization flask through a gas dispersing tube which has pinholes drilled at equal distance around the circumference of its flanged end. The temperature of the oil is measured by a thermometer inserted into the thermometer well. The oil is usually maintained at 185 °C for 2–4 h, and water corresponding to 5% by weight of the oil is passed through the oil during this time. During

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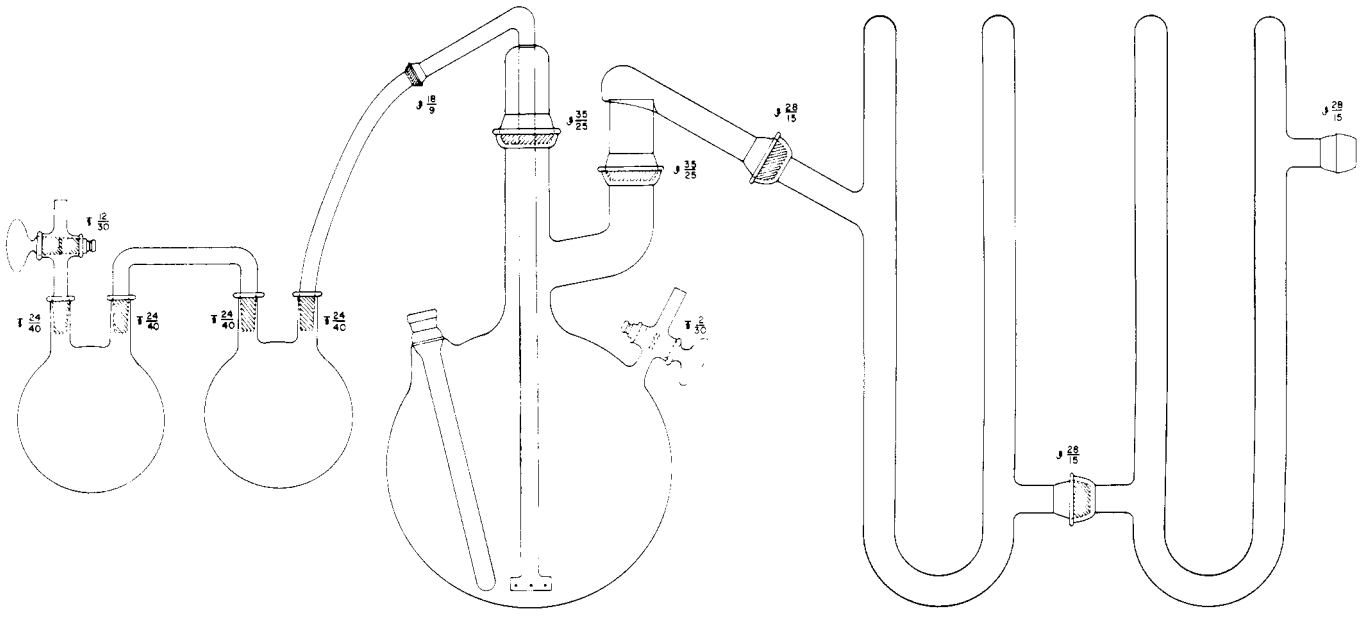

Figure 1. Apparatus commonly used for batch vacuum steam distillation of oils.

deodorization, this water vigorously agitates the oil because its volume is greatly expanded by the high vacuum and the high temperature. The water vapor carrying all the volatile components are collected in the cold traps. The distillate contains not only the volatile flavor compounds originally present in the oil, but also thermal decomposition products of the peroxides and free fatty acids, with a significant amount of triglycerides as contaminants.

Some 20 years ago, the National Soybean Processor's Association wanted to identify the volatile constituents in soybean oil which are responsible for the reversion flavor. As it is known in the trade, reversion flavor is a characteristic, objectionable, beany and grassy flavor developed in soybean oil prior to the inception of rancidity when the peroxide value of the oil is still as low as a few mequiv/kg. A distinguished expert on gas chromatography was asked to approach this problem. The sample submitted to him for the study was the material collected from the cold traps during deodorization of a reverted soybean oil in the apparatus as shown in Figure 1. The sample had an extremely strong objectionable odor, but the characteristic reversion flavor could hardly be detected. This was quite evident because the condensate in the cold traps contained all kinds of volatile decomposition products produced by the oil during the isolation process of 4 h at 185 °C. The odor of such artifacts was far more predominant than the odor of the true reversion flavor. Furthermore, the condensate was also heavily contaminated by triglycerides carried over by the water vapor as well as by free fatty acids.

With such a sample, the expert on gas chromatography, though the foremost in the field, could only produce a massive volume of gas chromatograms which yielded no useful information at all.

When we started to study the reversion of soybean oil, we decided that we must first design and build an apparatus which could isolate the volatile flavor constituents in a reverted soybean oil without creating artifacts. A semicontinuous, countercurrent, vacuum steam distillation apparatus (Chang, 1961) was therefore developed. The apparatus was recently improved as shown in Figure 2.

The apparatus was evacuated to a vacuum of 0.01 mm Hg and the cold finger traps, V and W, were cooled with a mixture of dry ice and acetone. Two coiled traps, X and Y, could be cooled, either with dry ice or liquid nitrogen. The reverted soybean oil in reservoir D was drawn into heat exchanger N by the vacuum in the apparatus, through a needle valve stopcock, H, at a constant flow rate. If solid fat, such as cocoa butter, were to be studied, it could be melted at a constant temperature by the heating mantle, B, while being stirred by the magnetic bar, C. The heat exchanger was circulated with water at 70 °C. In this manner, the oil was continuously heated to this temperature and then dropped into the top plate of the Oldershaw column, O, which contained 15 precision drilled perforated plates, as shown in the insert of the figure.

At the same time, water vapor generated by vigorously boiling water in flask I was allowed to flow upward at a constant flow rate, as indicated by flow meter, L. As the oil continuously flowed downward and water vapor upward, through the Oldershaw column, the water vapor was forced to bubble through the thin layer of the oil on each plate. Consequently, after the water vapor was bubbled through the oil 15 times, and reached the top of the column, it carried with it the volatile flavor constituents of the oil. The current was then passed through an empty trap to remove any contaminated oil carried by the current, and then the water vapor and the volatile flavor compounds were condensed in the cold traps.

During the operation, the oil was immediately cooled down to room temperature after it reached flask P. The total heating time was therefore only $5^1/_2$ min. The vacuum at the bottom of the Oldershaw column was 20 mm Hg and that at the end of the cold traps was 0.05 mm Hg.

The volatile flavor constituents thus isolated from the reverted soybean oil had a genuine characteristic beany and grassy flavor. When the volatile constituents were added back into a freshly deodorized, odorless, and flavorless corn oil, at a concentration of 10 ppm, an experienced sensory evaluation panel identified the mixture as a reverted soybean oil. In addition, the peroxide numbers of the oil before and after the isolation process were identical. Since it is unlikely to form any additional peroxides during the short time of heating under a high vacuum, this appears to be a good indication that no peroxide decomposition did take place during the isolation process.

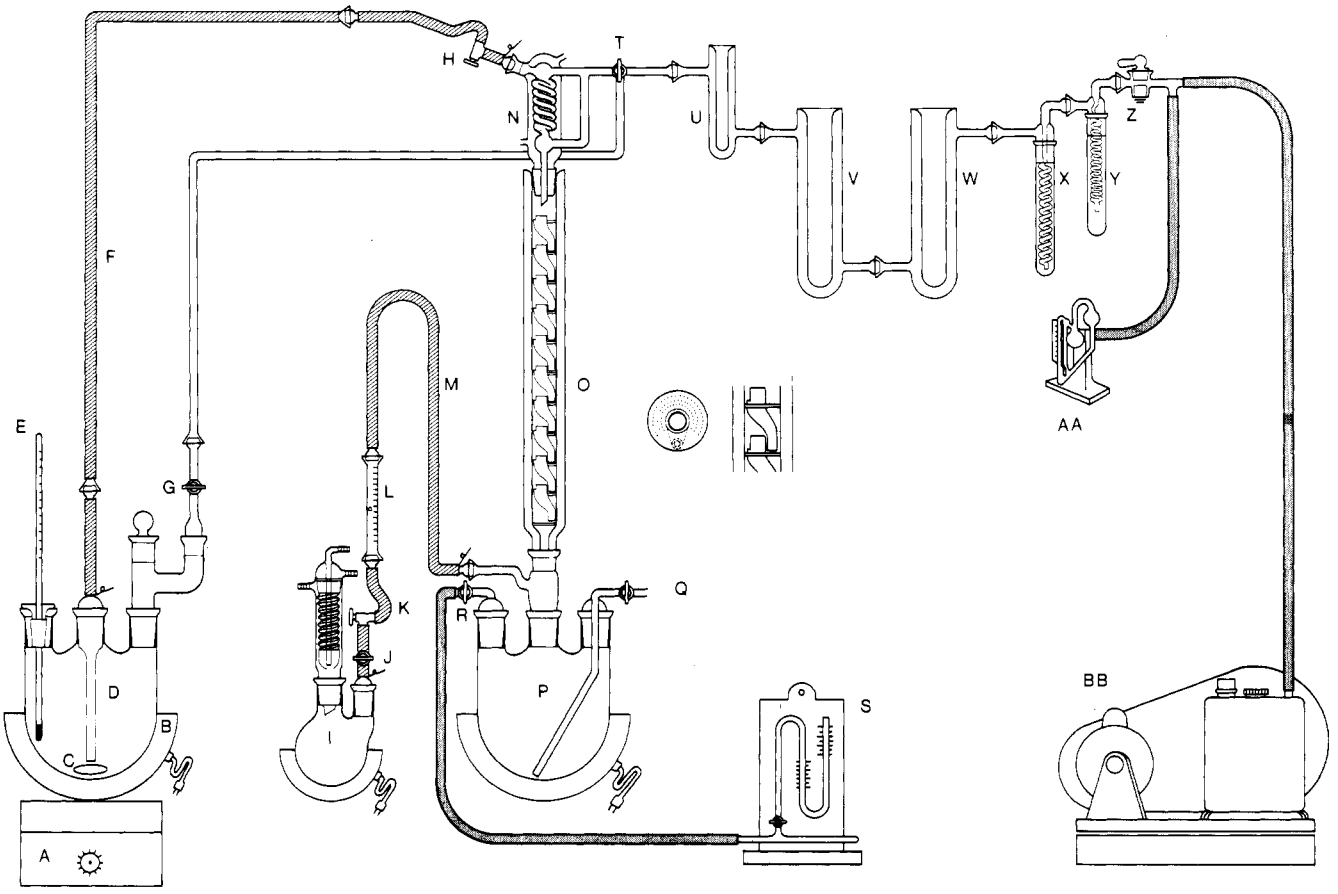

Figure 2. Apparatus for the isolation of trace volatile constituents from oils by semicontinuous countercurrent vacuum steam distillation.

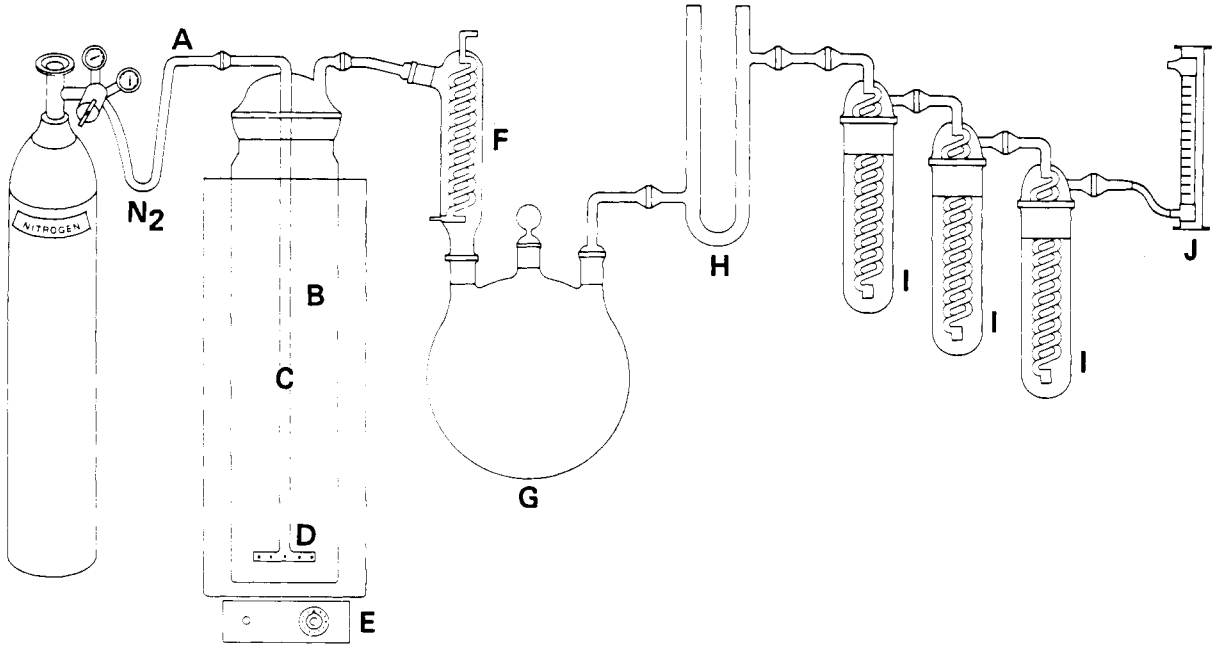

Figure 3. Apparatus for the isolation of trace volatile constituents in headspace gas of foods.

From the volatile constituents isolated by the use of our method, from 65 gal of reverted soybean oil with a peroxide number of 4.3 mequiv/kg, 71 compounds were identified (Smouse and Chang, 1967). Among them, we found that 2-pentylfuran is predominantly responsible for the reversion flavor of soybean oil (Chang et al., 1966). Addition of 2 ppm of 2-pentylfuran to any oil which has been freshly deodorized to an odorless and flavorless state will produce a solution identifiable as a reverted soybean oil by an expert sensory evaluation panel.

Modified Molecular Still for the Isolation of Trace Volatile Constituents from Oils. When the amount of the sample to be processed is relatively small—from 50 to a few hundred milliliters—a modified falling film molecular still reported previously (Chang, 1973) can be used. The apparatus utilizes the principle of vaporization of the flavor compounds from a continuous heated thin film of the oil under a high vacuum. The volatilized flavor compounds

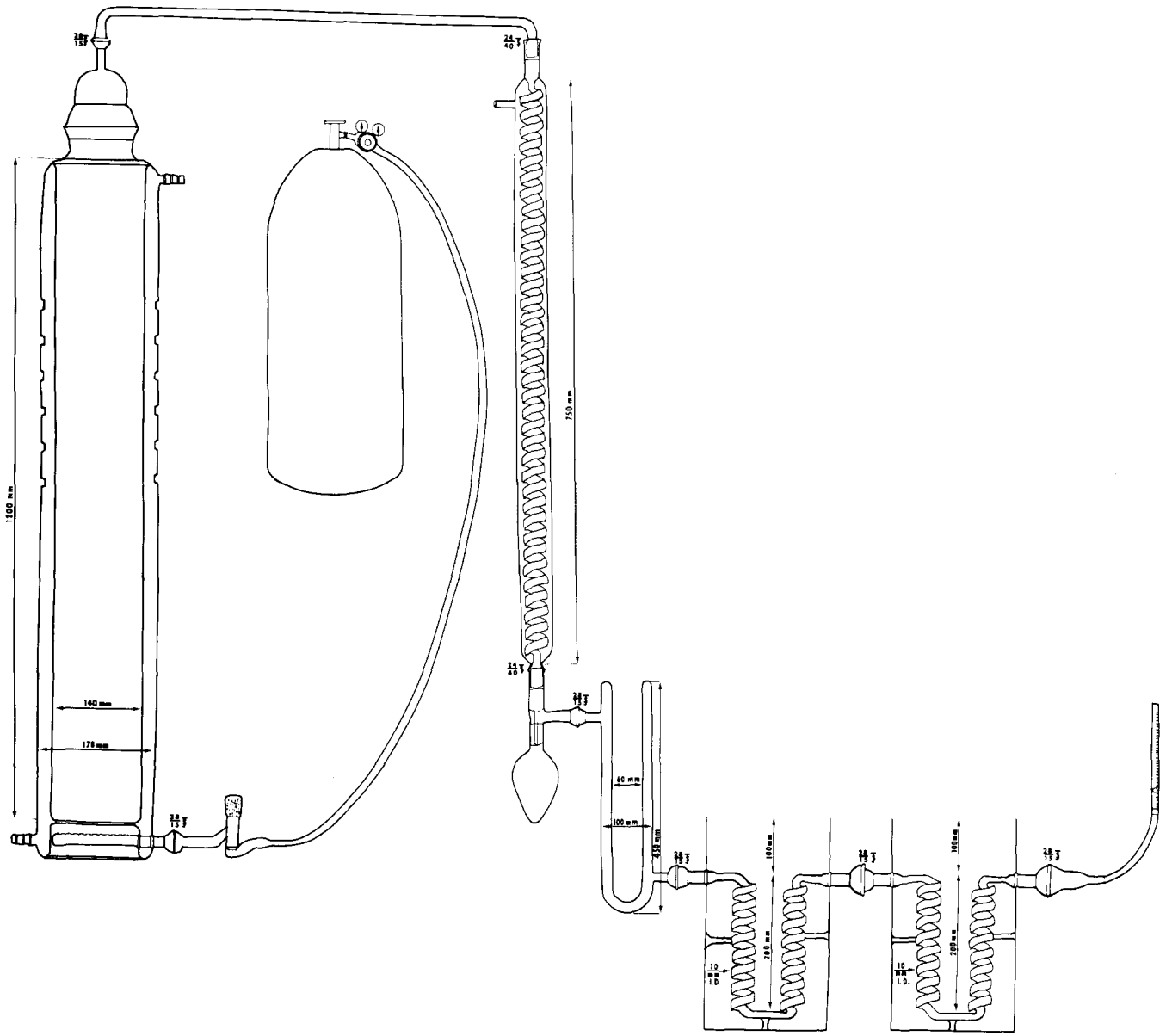

Figure 4. Apparatus for the isolation of trace volatile constituents from relatively large amounts of food.

are then swept by a current of water vapor from the molecular still into cold traps.

Isolation of Volatile Trace Constituents from Aqueous Liquid or Solid Mass. When the food to be studied is in aqueous form, such as apple juice or beer, an apparatus utilizing the principle of flash evaporation and vaporization from a continuous thin heated film under vacuum is used. The apparatus has been reported previously by Herz and Chang (1966). This apparatus can also be used for foods which are a solid mass, such as meat and potato chips. In such cases, the food is first made into a water slurry with the use of a Waring blender before it is put through the isolation apparatus.

We utilized this apparatus successfully in our study of the flavor of cooked beef. Many compounds of great interest (Peterson et al., 1975; Hirai et al., 1973) were identified by infrared and mass spectrometry of the gas chromatographic fractions of the volatile constituents thus isolated (Table I).

Apparatus for the Isolation of Trace Volatile Constituents in Head Space Gas. All the techniques described above utilized the evaporation of the trace volatile flavor constituents from the food under a high vacuum. This greatly increased the amount of volatile flavor compounds collected. However, the application of a vacuum may have altered the relative concentration of the volatile flavor constituents as they appear normally in the space above the food. In other words, the odor of the volatile flavor constituents, as evaporated under high vacuum, may smell somewhat differently than the odor of the food as perceived by the nose when it is being eaten. Therefore, the true aroma of a food must be collected from the headspace of the food as it exists under normal conditions. However, the concentration of the flavor components in the space above the food under normal conditions is extremely low. Consequently, in order to collect a sufficient amount of volatile flavor components for isolation, fractionation, and identification, the amount of food used must be increased. In order to do this, the apparatus, as shown in Figure 3, was designed and built.

A current of nitrogen as measured by a flow meter, J, is continuously passed through the food in the tall glass cylinder, B, which is 66 cm in height and 12 cm in outside diameter. This cylinder is therefore large enough to accommodate $7^1/_2$ lb of peanuts, as an example. The current of nitrogen is led into the bottom of the cylinder and then

Table I. Trace Volatile Constituents of Cooked Beef Flavor Identified Through the Use of the Herz Apparatus

Lactones	
	γ-Caprolactone
	γ-Valerolactone
Furans	
	2-Pentylfuran
	2-Acetylfuran
	5-Methyl-2-acetylfuran
	2-Furaldehyde
	5-Methyl-2-furaldehyde
	5-Trimethylfurfural
	2-Methyltetrahydrofuran-3-one
Nitrogen compounds	
	2,4,5-Trimethyl-3-oxazoline
	2,4,5-Trimethyloxazole
	2-Formylpyrrole
	2-Acetylpyrrole
Sulfur compounds	
	2,5-Dimethyl-1,3,4-trithiolane
	2-Acetylthiazole
	Benzothiazole
	Thiophene-2-carboxaldehyde

distributed through the food by a glass tube, C, which has a large flanged disk bottom with pinholes drilled at equal distance on its circumference, D. The gas current is then passed through a series of traps (H–I), cooled with dry ice and liquid nitrogen, respectively, in order to condense the volatile flavor constituents.

Apparatus for Collection of Trace Volatile Constituents from Large Amounts of Food. It is obvious that in order to obtain sufficient volatile flavor constituents for fractionation and identification, it is desirable to process as large as possible an amount of food. The apparatus was therefore improved and enlarged as shown in Figure 4. It consists of a food-containing column which is 120 cm long and 14 cm in diameter and thus has a volume of 18.5 L, sufficient to accommodate approximately 27 lb of french fries. The food in the column can be maintained at any desired temperature by circulating water or glycerine heated to that temperature in the outer jacket. Helium or nitrogen is led evenly into the bottom of the apparatus through 12 holes of 2 mm diameter, equally spaced on top of a 17 mm o.d. tubing. The gas is then forced through a coarse fritted glass disk to be distributed evenly and allowed to flow upward through the food in the column. Either solid food or liquid materials, like oil, may be used in the column. The pressure of helium or nitrogen will be sufficient to hold the liquid on top of the fritted glass disk.

The gas current passing through the food will carry the trace volatile flavor constituents in the headspace of the food into a cold water condenser, followed by a cold finger trap and, finally, into two highly efficient coiled traps which are each 18 cm in diameter and 30 cm in height. It has two sections of coil each 18 cm in length, made of 1 cm i.d. tubing which are immersed in dry ice and acetone or in liquid nitrogen. These traps are not only highly efficient but are also convenient for washing out the condensate.

Sometimes it is necessary to collect trace volatile flavor components in a manufacturing plant. For example, the collection of the volatile flavor compounds lost during the grinding of freshly roasted coffee beans. For such a purpose, the collection apparatus must be enlarged. Figure 5 shows the assembly of such an apparatus mounted on a table which can be easily rolled into any place to conduct the experiment. A current of air can be drawn through the apparatus by applying a vacuum at the end, or by blowing a current of nitrogen into the closed apparatus and

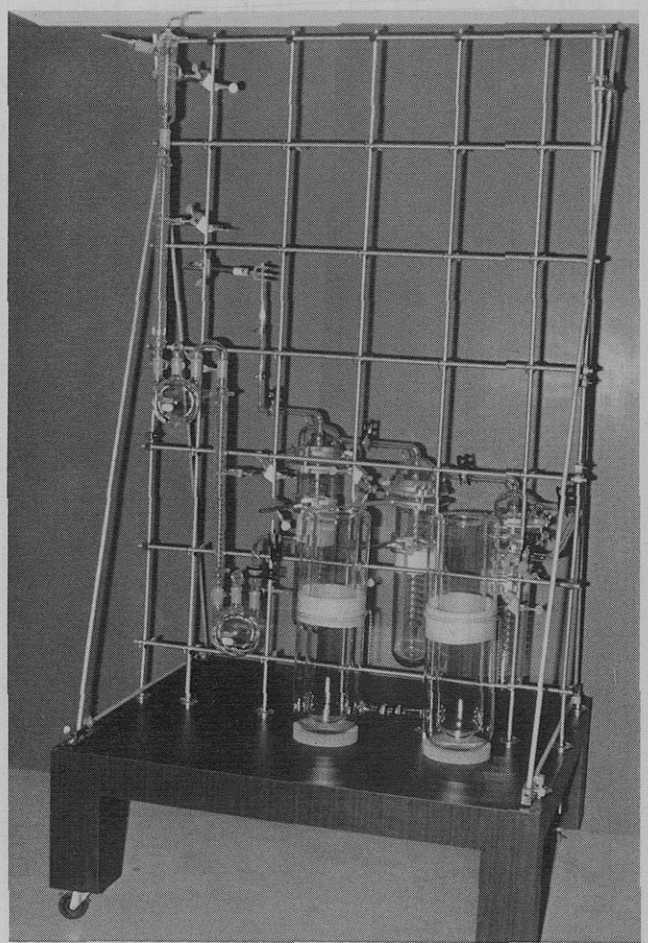

Figure 5. Apparatus for collection of trace volatile constituents in plant operations.

then led into the trap. For example, the grinding of freshly roasted coffee can be easily done in an enclosed machine. A current of nitrogen can be blown into the machine and then led into the apparatus. The cold finger trap is 60 cm high and 17 cm in diameter. The coiled trap is 60 cm long and 12 cm in diameter with coils made of glass tubing, 14 mm in diameter.

The condensate collected in the various traps cooled with dry ice or liquid nitrogen in any of the apparatus just described is usually combined and saturated with sodium chloride. It is then extracted with ethyl ether. After a preliminary extraction in the separatory funnel, the aqueous solution is extracted continuously.

The ethyl ether extracts are then combined, carefully dried with anhydrous sodium sulfate, and concentrated with the use of an Oldershaw column fitted with an adjustable refluxing head to a concentration of 10–50 mL.

The concentrated ethyl ether solution is finally concentrated to a volume suitable for gas chromatographic fractionation by using a spinning-band still. This still has a fractionation ability equivalent to 200 theoretical plates. It is sufficient to remove the solvent without the danger of losing any important flavor compounds. This is assured by frequently smelling the distillate on a perfumer's stick.

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Formation of Flavor Components in Asparagus. 1. Biosynthesis of Sulfur-Containing Acids in Asparagus

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Sulfur-containing acids and esters of white asparagus (*Asparagus officinalis*) were enriched by liquid–liquid extraction with pentane–methylene chloride (2:1), separated by means of LSC on silica gel and preparative GLC and investigated by capillary gas chromatography–mass spectrometry. Besides 1,2-dithiolane-4-carboxylic acid (asparagusic acid), its methyl and ethyl esters, 3-mercaptoisobutyric acid, 3-methylthioisobutyric acid, diisobutyric acid disulfide, and 3-*S*-acetylthiomethacrylic acid were identified as intracellular constituents. The biosynthesis of asparagusic acid was assayed with ^{14}C-labeled precursors and asparagus tissue discs. [^{14}C]-L-Valine was transformed via oxo acid, isobutyrate, methacrylate into 3-methylthioisobutyrate and to a lesser extent into asparagusic acid.

In 1948, Jansen isolated 32 g of a sulfur-containing component from 40 kg of asparagus aroma concentrate. By means of chemical reactions, Jansen determined the structure as 3,3′-dimercaptoisobutyric acid. Jansen suggested that this compound may also be present as a disulfide. Yanagawa et al. (1972) identified three sulfur-containing acids by thin-layer chromatography, mass spectrometry, and NMR spectroscopy. They called 1,2-dithiolane-4-carboxylic acid "asparagusic acid" and 3,3′-dimercaptoisobutyric acid "dihydroasparagusic acid". The third constituent was characterized as *S*-acetyldihydroasparagusic acid. The authors mentioned that all three acids act as growth inhibitors. No comment was made on the flavor characteristics of these components. Investigation of an enzyme-inhibited aroma extract of raw asparagus by means of adsorption chromatography, gas chromatography, and mass spectrometry revealed that asparagusic acid, its methyl and ethyl esters, and seven other sulfur-containing acids are synthesized in the intact plant cells. This is an exceptional case of formation of sulfur-containing flavor components. Normally sulfur compounds in vegetable are formed by enzymatic or chemical splitting of nonvolatile precursors, like *S*-alkylcysteine sulfoxides and glucosinolates during crushing of the plant material. We investigated two possible biosynthetic pathways into which some of the identified acids might fit as intermediates. L-Valine seems to be a possible precursor at least for some of the sulfur-containing acids. Valine might be transformed via isobutyric acid and methacrylic acid into mercaptoisobutyric acid. The transformation of [U-^{14}C]valine into 3-mercaptoisobutyric acid is nicely performed by asparagus tissue discs, but asparagusic acid showed only a small amount of radioactivity.

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MATERIAL AND METHODS

Sample Preparation. White asparagus of the region of Braunschweig, Germany, was prepared for analysis immediately after harvesting: (a) 2.5 kg were homogenized with 2.5 L of methanol in a mixer for 5 min in order to achieve enzyme inhibition; (b) 2.5 kg were homogenized with 2.5 L of phosphate buffer solution of pH 6.8 in a mixer for 5 min. Both of the homogenates were cleared by filtration with a "Hafico" tincture press at 400 atm. The filtrates were acidified to pH 2.5 with HCl and sulfur-containing acids were isolated and enriched by liquid–liquid extraction with pentane–methylene chloride (2:1). After drying over anhydrous Na_2SO_4, the extracts were methylated with CH_2N_2 dissolved in ether and concentrated to defined volumes (e.g., 500 μL).

Adsorption Chromatography. A separation according to polarity of components was carried out by liquid–solid chromatography. Extract (125 μL) was given on cooled columns (200 × 9 mm i.d.), filled with silica gel 60 (Merck 7734) of activity II–III, and seven fractions (40 mL each) with solvents of increasing polarity were eluted with: (I) pentane, (II) pentane (P)/methylene chloride (MC) (9:1), (III) P/MC (2:1), (IV) P/MC (1:2), (V) P/ether (E) (9:1), (VI) P/E (1:1), (VII) ether. Fractions were concentrated to 250 μL and then analyzed by gas chromatography.

Gas Chromatography. Investigations were performed with a Varian Aerograph 2740-1 with two FID, a linear temperature program, and an effluent splitter (10:1), with a Tracor 550 of Techmation, equipped with a FPD for sulfur-selective analysis and with a Perkin-Elmer Multifract F 40 with linear temperature programs and FPD.