Measurement of Fat Autoxidation and Browning Aldehydes in Food Vapors by Direct Vapor Injection Gas-Liquid Chromatography

RON G. BUTTERY[1] and ROY TERANISHI

Western Regional Research Laboratory, Albany, Calif.

Gas-liquid chromatography by direct injection of food vapors is shown to be applicable to the quantitative determination of volatile compounds in the vapor above food. The concentration of hexanal and other fatty acid autoxidation products in the vapor above reconstituted dehydrated potato can be readily determined. The method is also suitable for measuring the concentration of browning products, 2-methylpropanal and 2- and 3-methylbutanal, in the vapor. It can be extended to measure the concentration of these compounds in the product itself. Reproducibility is satisfactory; values obtained show a standard deviation of less than 10% of the average. The method has the advantage that the concentrations of several different compounds arising from more than one type of food deterioration can be followed with the one simple, rapid, and specific analysis.

THE FLAVOR of food is a complex sensation involving taste and odor. Odor has been considered the most important sensory component of flavor (3). The human evaluation of odor involves some type of quantitative and qualitative analysis of the volatiles in the vapor above the food by the olfactory senses. Routine organoleptic appraisal of food aroma is time-consuming and not highly reliable. Therefore, an instrumental means of evaluation would be desirable. Various approaches have been used to develop such a method, including the use of gas-liquid chromatography (GLC) (2, 4, 5, 15, 18, 19) and other methods (9). GLC using the direct injection of food vapors would seem to be one of the most fundamental and promising approaches.

The term "aromagram" has been adopted generally at the Western Regional Research Laboratory to mean a gas chromatogram obtained by the direct injection of a sample of the vapors over a food or other odorous material into a suitable gas-liquid chromatography apparatus.

The present work describes a study of the quantitative aspects of the aromagram method and its use to follow the development of oxidative rancidity and browning.

Experimental

Materials. Dehydrated Idaho Russet Burbank potato granules, prepared by the conventional add-back process, were obtained from a manufacturer in Idaho. For the autoxidation study, dehydrated potato granules (5 to 7% moisture, 300 p.p.m. SO_2) were sealed into 6-ounce cans (104 grams of granules per can). The cans were then filled with an oxygen atmosphere. Storage was at 24° C., and cans were removed from storage at intervals for each analysis. For the accelerated browning study, dehydrated potato granules in bulk form (9.3% moisture, 300 p.p.m. SO_2) were stored in an air atmosphere in an oven maintained at 65° C. Samples were removed at intervals for analysis.

Carrots were freeze-dried (2% moisture) and stored in cans in air at 100° F. and in nitrogen at 32° F. Authentic samples of aldehydes were obtained from reliable commercial sources or synthesized and purified just prior to use by gas-liquid chromatography.

Water used for making solutions of benzene and aldehydes was distilled, and at least one fourth by weight boiled away to remove any organic volatiles.

Gas-Liquid Chromatography. Detectors of the McWilliam and Dewar dual-flame type (16) were used for GLC, with modifications as described previously (4, 5, 18).

The columns were 5 feet long by 0.25-inch o.d. and 0.21-inch i.d. stainless steel, and packed with 30% Apiezon M on 60- to 80-mesh firebrick. The columns were heated in an oven consisting of an aluminum cylinder, 12 inches in diameter by 7 inches long, heated by nichrome wire wound evenly around the cylinder and electrically insulated from it by a sheet of asbestos paper. The oven was heat insulated with a 2-inch layer of glass fiber.

The capillary columns were 1000 feet long by 0.034-inch i.d., nylon, and coated with General Electric Silicone fluid SF-96 (100). Two identical capillary columns were used in the instrument in conjunction with dual-flame detection.

Nitrogen carrier gas was saturated with water by passing it through a strong-walled, borosilicate glass vessel containing a Fiberglas cloth wick. The saturation of the carrier gas with water (16) is very important to the method, particularly with temperature programming. It prevents tailing and also, apparently, prevents any residual adsorption in the GLC apparatus and column, for there is no noticeable residue from one injection coming through with the next.

The GLC conditions for the packed column were exactly as described previously (5). The nylon capillary columns were temperature programmed (nonlinearly) from 35° to 86° C. The capillary column carrier nitrogen flow was 20 ml. per minute controlled by a flow regulator. Hydrogen flow rate to each detector was 20 ml. per minute. Air flow rate was 1000 ml. per minute.

The sensitivity limit (twice the noise level) of the GLC apparatus, with the 0.25-inch o.d. column packed with 30% Apiezon M on 60- to 80-mesh firebrick at 115° C., was 4×10^{-12} grams per second for benzene (as dilute solutions in water). This is similar to that obtained by Condon, Scholly, and Averill (6), who reported a figure of 3×10^{-12} grams per second. Other workers have obtained similar values. The amount of drift was less than four times the noise level per hour.

The unit was used at a range varying from the limit mentioned to a sensitivity 160 times less. The 0.25-inch o.d. apparatus has been used daily for $1^1/_2$ years close to its limit without any special maintenance besides changing batteries and gas cylinders. The original column is still being used and has shown very little change in characteristics.

Sampling Procedures. POTATO GRANULES. A 15.0-gram sample was added to 150 ml. of preboiled distilled water at the boiling point (100° C.) in a 250-ml. Erlenmeyer flask. The flask was immediately covered with aluminum foil and the mixture gently swirled for exactly 60 seconds. The temperature of the mixture was then 93° C. At this point, a hypodermic syringe (at room temperature) with needle removed was pushed through the aluminum foil and a 10.0-ml. sample of the vapor taken with the end of the syringe about 1 inch above the surface of the mixture. The hypodermic needle was then replaced and the sample injected into the GLC unit.

FREEZE-DRIED CARROTS. The procedure was similar to that described for potato granules, except that a 10.0-gram sample was added to 100 ml. of water at the boiling point.

To prevent any possible memory effect, all syringes and flasks were used only once and then cleaned. Cleaning usually involved washing in the normal way with detergent, rinsing very thoroughly with water, and drying in an oven at 100° C. at least overnight. Apparatus was removed from the oven and allowed to cool to room temperature just prior to use.

Results and Discussion

Proper choice of detector and columns is particularly important for direct GLC analysis of food vapors (*1, 2, 4, 5, 15–17*). Most work of this type has been done with packed columns. Little advantage has been found in the use of conventional 0.01-inch i.d. capillary columns, because the vapor samples are relatively large and usually too dilute to make a split-stream injector feasible. Averill (*1*) has used a 0.02-inch i.d. capillary with a split-stream injector using a 1 to 10 ratio. E. M. Fredericks of the Shell Development Co. suggested 0.034-inch i.d. nylon capillary columns (1000 feet long) might be useful to the authors, because they can take a large flow rate and therefore do not necessarily require split-stream injection (*20*). One drawback of the nylon columns, however, is that the background noise level is higher than with the packed columns. This is due, apparently, to the relatively high vapor pressure of the nylon material.

One of the biggest problems with the method of direct injection of food vapors

is choosing a completely satisfactory stationary phase. The stationary phase should give good resolution with oxygenated compounds, have a low vapor pressure, and not be affected by the air and water injected with the sample. No stationary phase tried fulfills all of these requirements. Unfortunately, with most of the polar stationary phases, a low vapor pressure is usually accompanied by a high viscosity. A low-viscosity stationary phase is essential with capillary columns. The only stationary phases that combine a low vapor pressure with a low viscosity at relatively low temperatures are the nonpolar ones, such as Apiezon M and silicone oils, such as General Electric SF-96 (100). Apiezon M grease melts to a low-viscosity liquid at 60° to 70° C. and is satisfactory 20° or more above this temperature. The silicone oil, G.E. SF-96 (100), has a fairly low viscosity even at room temperature and is the most satisfactory stationary phase tried for the nylon capillary columns, which must be kept below 100° C. because of the nylon vapor pressure.

Aromagram Reproducibility. If direct injection GLC of food vapors is to be used in routine control work, it is particularly important that the method be reproducible from one month to the next. Reproducibility with gas chromatography equipment requires that the N_2 carrier gas flow, the temperature of the column, and the detector factors —e.g., H_2 flow rate, air flow rate, battery supply voltages—always be kept as close as possible to the same values. It is necessary, also, that the unit be used only for vapor samples. For the present work, the pressure of the carrier gas immediately before the column read on a wide-scale meter (to ±1%) gave a more accurate indication of flow than did ball-type flow meters also employed. Temperature was maintained within ±0.5° C. by manual adjustment of an autotransformer or by a thermistor temperature control unit.

Reproducibility of instrument performance can be checked by injecting a standard solution of an organic compound in pure water. This is one of the easiest and more accurate ways of injecting a known weight of organic compound into an apparatus equipped with flame ionization detection. This method was orginally suggested by McWilliam and Dewar (*16*). For work here, solutions containing 8 μl. of benzene in 1 liter of water were used. Benzene has a solubility of 700 p.p.m. in water and is very stable. The stability of very dilute solutions of other compounds, such as alcohols, aldehydes, and esters, is not known. Usually 8 μl. of the benzene solution were injected at each time. This amount contains 5.4×10^{-8} grams of benzene. The relationship of the weight of benzene to the area of

the chart was then determined. The flame ionization detector is linear over a wide range (*6, 16*).

Sampling Method. In using the aromagram method in previous and the present work, the sample is drawn up in a glass hypodermic syringe. There is some question when dealing with very small concentrations of whether, in a quantitative analysis, the adsorption of compounds on the glass wall of the hypodermic syringe can be neglected. A better material than glass for the syringes would probably be Teflon, which adsorbs very little of the usual organic compounds.

A simple experiment demonstrated the degree of hexanal adsorption on a 10-ml. glass syringe (at room temperature). A 1-ml. glass syringe was used to draw 1.0 ml. of the vapor above a solution of 10 parts of hexanal in 10^6 parts of water. The sample was injected directly into the unit. The area under the hexanal peak was then measured. The procedure was repeated three times, and the average area calculated. The 1-ml. glass syringe was then used to draw up 1 ml. of the vapor and to inject it into a 10-ml. glass syringe (needle removed) opened to the 10.0-ml. mark. The needle of the 10-ml. syringe was then replaced and the vapor injected into the GLC unit. The area of the new hexanal peak was measured. This procedure was also repeated three times, and the results were averaged. The difference between the average area found when the 1-ml. syringe was used and that when the 10-ml. syringe was used was less than 2%, showing that the adsorption of hexanal by the 10-ml. glass syringe was negligible for most purposes. Adsorption of similar aldehydes also would probably be negligible for this method.

Two conditions must be kept constant for reproducible analyses—the temperature of the food mixture at the time the sample is drawn into the syringe, and the ratio of water to sample, if water is added. These requirements follow from the normal vapor-solution equilibrium laws (Raoult's and Henry's laws). In the present work, the sample was drawn into the syringe with the needle removed, then the needle (25 gauge) was fixed to the syringe immediately. In this way, the sample could be drawn quickly. The syringes were used at room temperature; besides being more convenient, this gave more reproducible results than heated syringes.

Reproducibility of the method was tested with a uniform batch of dehydrated potato granules. The area under the peak corresponding to the retention time of hexanal was measured. The analysis was repeated nine times. The standard deviation was ±8% of the average area. The results for peaks other than hexanal were similar. This degree of error might be important in
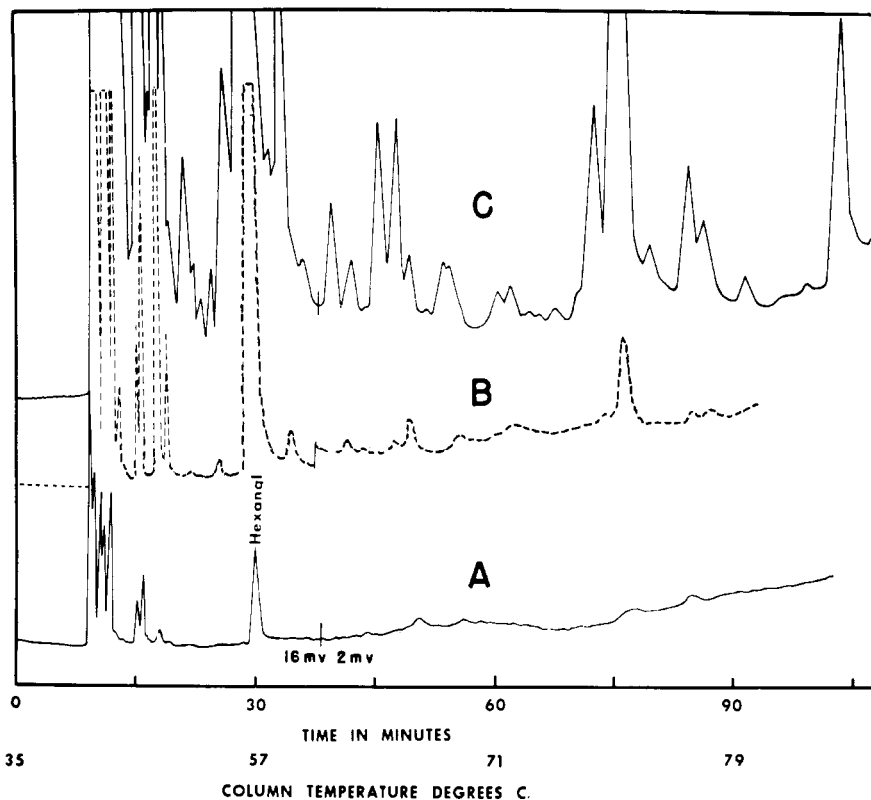
Figure 1. GLC curves obtained by direct injection of 10 ml. of the vapor above reconstituted samples of dehydrated potato granules stored for different periods in oxygen

(GLC conditions using nylon capillary columns are described in text)
(A) fresh potato granules; (B) stored in oxygen atmosphere for 4 months; (C) stored in oxygen atmosphere for 2 years
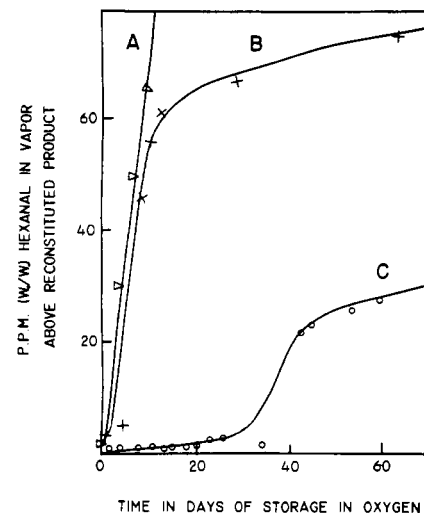

Figure 2. Effect of storage time on hexanal concentration in vapors above reconstituted dehydrated potato granules containing different levels of antioxidants

(Granules stored in oxygen at room temperature)
(A) no BHA and no BHT; (B) 0.1 p.p.m. BHA and 0.1 p.p.m. BHT; (C) 0.5 p.p.m. BHA and 1.3 p.p.m. BHT
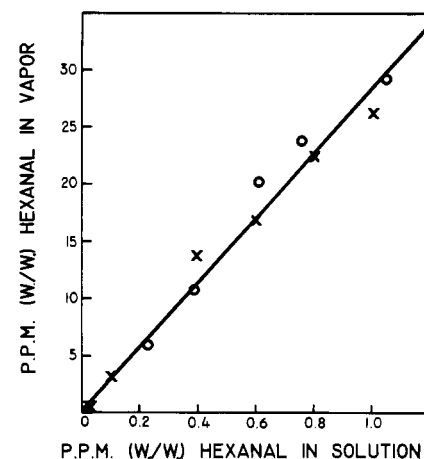

Figure 3. Relationship of the concentration of hexanal in the vapor phase to its concentration in solution in water and in solution in reconstituted potato granules at 93° C.

0 = reconstituted dehydrated potato; X = water

some cases, but it must be remembered that the amounts of compound being measured are extremely small, in the range of 10^{-10} to 10^{-6} gram. A higher degree of precision is probably not necessary in flavor investigations because it is doubtful whether the human olfactory sense is nearly as precise.

Fatty Acid Autoxidation. The autoxidation products of linoleate and linolenate have been reported (8, 12, 13), and a fairly strongly supported mechanism has been established (7). A major volatile product from the autoxidation of linoleate is hexanal. The concentration of hexanal in the vapor above a food product can be measured easily. Aromagrams of the vapor above samples of dehydrated potato granules stored in oxygen for different periods (Figure 1) show an increase with time of storage in several peaks, of which the biggest is n-hexanal.

Rates of increase in hexanal concentration in the vapor above the product can be used to compare susceptibility to autoxidation of dehydrated potato granules with different levels of antioxidant (Figure 2). Although the increase in hexanal was used for this purpose, the authors do not imply that hexanal is mainly responsible for the characteristic odor of rancidity. The

rancid odor probably is not attributable to any one compound but is, rather, a function of several compounds produced by fatty acid autoxidation. Other autoxidation products could be followed in a similar way.

From the concentration of hexanal in the vapor above the autoxidized product, the concentration in the product can be calculated from an experimentally obtained curve. Such a curve for reconstituted, freshly dehydrated potato granules (Figure 3) was constructed by adding known amounts of hexanal in a dilute water solution to the reconstituted granules immediately prior to sampling (at 93° C.). The fresh granules alone showed a hexanal concentration in the vapor of 0.5 p.p.m. For comparison, a plot of the relation of hexanal concentration in water, at 93° C., to its concentration in the atmosphere above the water is shown. For foods that are mostly aqueous, the vapor-solution equilibrium probably lies fairly close to that for dilute solutions of the compound in water.

Autoxidation of unsaturated fatty acids can be followed in a similar way in other products.

Figure 4 compares aromagrams of dehydrated carrots stored in air at 100° F. and in nitrogen at 32° F. Similar

curves were obtained for other products, including puffed white bulgur and walnuts.

Browning. Browning (Strecker degradation) aldehydes are formed from the reaction of amino acids with dicarbonyl sugar browning products (11, 14). With dehydrated potato products there is a marked increase in the aldehydes, 2-methylpropanal and 2- and 3-methylbutanals, when the product is scorched or browned. These would originate from valine, leucine, and isoleucine—
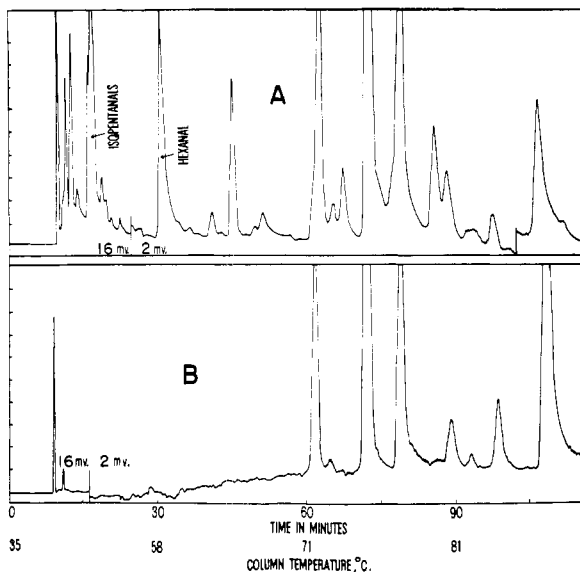
Figure 4. Aromagrams of reconstituted dehydrated carrots that had been stored under different conditions

(GLC conditions using nylon capillary columns are described in text)
(A) dehydrated carrots stored 5 months in air at 38° C., (B) dehydrated carrots stored 5 months in vacuum at 0° C.
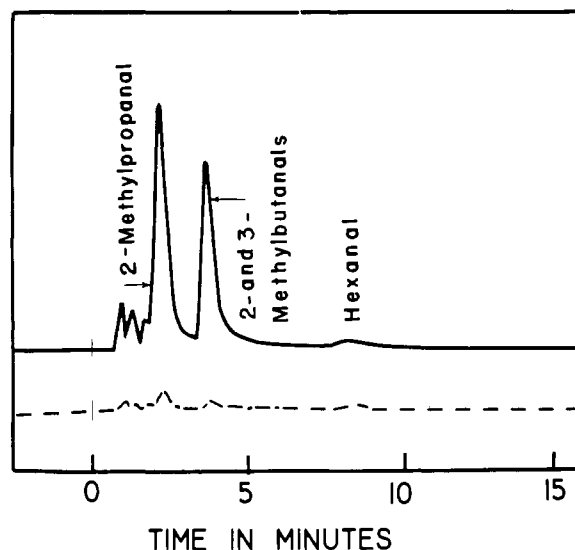

Figure 5. Aromagrams of reconstituted fresh potato granules (- - -) and potato granules that had been stored 48 hours at 60° C. to accelerate browning (———)

(GLC conditions using 0.25-inch o.d., packed columns are described in text)

TIME AT 65° C. STORAGE IN HOURS
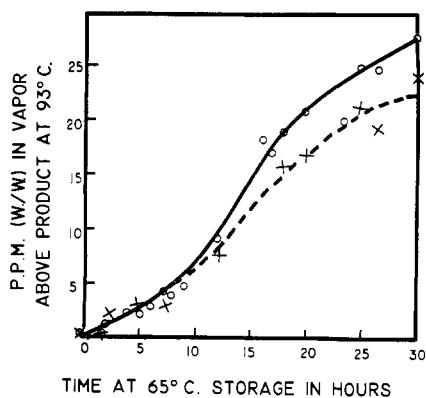
Figure 6. Effect of storage of potato granules at 65° C. on the concentration of 2-methylpropanal and of 2- and 3- methylbutanals (as a single peak) in the vapors above the reconstituted potato (at 93° C.)

X = 2-methylpropanal; O = 2- and 3-methyl-butanals

three amino acids occurring in high proportion in the potato (*10*). Aromagrams of the vapor above reconstituted, freshly dehydrated potato granules and of vapor above similar granules stored at 60° C. for 48 hours to accelerate browning reveal a marked increase in peaks corresponding to the retention times of 2-methylpropanal and 2- and 3-methylbutanals as a result of the storage (Figure 5). The concentration of these aldehydes in the vapor above the reconstituted product (at 93° C., 15-gram sample in 150 ml. of water) increases with time of

storage of the granules at 65° C. (Figure 6). The branched aldehydes are potent flavoring compounds and probably contribute considerably to browning flavor in potato products.

Organoleptic appraisal of dilute solutions of these aldehydes showed that 2-methylpropanal and 3-methylbutanal could be detected in concentrations of less than 1 part in 10^9 parts of water by weight.

Acknowledgment

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