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Measuring Flavor Changes with Vapor Sampling and GLC Analysis[1]

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ABSTRACT

A review of headspace gas chromatographic analysis including its use in qualitative and quantitative analysis, and some sources of errors and limitations of this method is presented. Special emphasis is given to combining headspace gas sampling with salting-out procedures to enrich vapors, steam distillation coupled with headspace gas chromatographic analysis, and subtractive techniques for identification.

Headspace gas chromatographic (GLC) analysis is well covered in books by Hachenberg and Schmidt (*10*) and Charalambous (*6*) and a review paper by Drozd and Novak (*8*). Hachenberg reviewed Analytical Applications of Headspace Analysis in Part 1 of his book and Schmidt discussed Physico-Chemical Applications of this method in Part 2. Charalambous edited the book *Analysis of Foods and Beverages: Headspace Techniques,* a compilation of papers presented at a symposium on headspace gas chromatographic analysis by the Flavor Subdivision of the Agricultural and Food Chemistry Division of the American Chemical Society.

Methods and techniques for analyzing a wide variety of food aromas are presented by authorities throughout the world. Although most of the contributors have emphasized practical methodology and techniques for specific food products, there are chapters on "Uses and Abuses" and "Quantitative Headspace Analysis", that are of a more general nature. The review paper by Drozd and Novak (*8*) in 1979 describes the principal developments of this technique over the past two decades. They discussed some theoretical aspects of headspace gas analysis from a mathematical point of view, such as distribution of solute in the gas-condensed phase system, stripping of solute components with a stream of gas, and quantitation procedures in head space gas analysis. A major portion of their review, however, is devoted to applications of headspace gas sampling with emphasis on problems in quantitation.

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It becomes obvious as one searches the literature on headspace sampling and GLC analysis that this technique is widely used in virtually all areas that require analysis of volatile materials. Most papers, however, involve analysis of flavors. In fact, flavor chemists have led in development of most of the sophisticated methods.

This manuscript presents an overview of some of the practical aspects and applications of headspace sampling and GLC analysis. It will describe some advantages as well as limitations of the method. Some applications of the subtractive technique as a useful identification and complementary analytical tool will be reviewed. The author feels it is important to emphasize the value of combining headspace gas sampling, salting-out procedures and subtractive techniques in identifying and quantifying chemical compounds in a complex mixture at and below the ppm level. The use of subtractive techniques for identification is not included in previous review articles on headspace gas sampling.

HEADSPACE GAS SAMPLING

As others have pointed out (*6*), the term "*headspace*" gas sampling or analysis is frequently misused. It implies that vapors over a sample are in equilibrium with the sample material being analyzed. In most instances where headspace gas analysis is reported, this is not so. Usually the concentration of most volatile materials in the headspace area associated with flavors is so low that it would be necessary to use a very large volume of vapors to produce a response to GLC analysis. Even with large volumes, trace components would not be perceptible; moreover the large volume of vapor is incompatible with GLC analysis and would result in broad, poorly resolved peaks. Consequently, analysts have generally concentrated the sample or the sample vapors before analyzing the "headspace" gas. When the material is concentrated before GLC analysis, the so-called headspace profile does not accurately reflect the quantitative distribution of volatile materials in true headspace. In spite of this limitation, there are innumerable applications of the technique in qualitative and quantitative analyses. Flavor chemists, however, must be aware that when a concentration proce-

dure is involved, whether salting-out, distillation, cryogenic or pre-column trapping, there is a distortion of the concentration distribution of the volatile materials.

QUALITATIVE ANALYSIS

Many of the earliest reports of headspace gas analysis were of a qualitative nature. Most of these papers were by flavor chemists. Some of the first chromatographic analysis of flavors were from vapors drawn directly over the food (*5,16,22*). However, with the large volume of gas sample required, it was necessary to use packed columns. Resolution was not good and only volatile materials in relatively high concentration were recorded. Other researchers attempted to increase sensitivity by concentrating the volatile substances (*18*) or enriching the vapors by salting-out techniques (*2,14*). More recently, concentration procedures have involved steam distillation, cryogenic traps, use of solid absorbant traps and on-column trapping. These methods are reviewed by Drozd and Novak (*8*).

It is important to reiterate that chromatograms from concentrated vapors should be viewed with reservations. Regardless of the concentration method, a distortion of the true headspace profile results. Jennings and Filsoof (*12*) compared several sampling procedures in the analysis of a mixture of ten compounds that had a wide range of volatile materials and functional groups. These methods included direct injection of the liquid; headspace sampling of the liquid, headspace over a 100 ppm aqueous solution, headspace sampling of a 100 ppm solution with added NaCl, concentrating on Tenax and Porapak Q, and a distillation-extraction procedure. They concluded that no single sample system can be regarded as uniformly satisfactory.

Although much information can be gleaned from chromatograms representing aromas, they should be evaluated with caution. Particularly useful headspace profiles are those which reflect changes that occur with different processing (*20*) and storage conditions (*13,15*). These types of changes are generally easy to interpret.

QUANTITATIVE ANALYSIS

As instruments, detectors, columns, and isolation techniques for GLC analysis improved, semi-quantitative and quantitative analyses of trace levels of organic materials were reported. These methods were not usually designed to analyze the complete flavor essence; rather they measured quantitatively specific volatile compounds within defined matrices. Direct headspace gas sampling over salt-saturated aqueous solutions, including a number of biological fluids, allowed for GLC analysis of relatively low boiling volatile materials at below the ppm level (*3,14,19*). It is readily apparent from those studies that it is necessary to prepare standard curves for the volatile material in question in the biological fluids being analyzed. Solvent effects of lipids and interaction with other components will affect the equilibrium between the condensed and vapor phases. This can be illustrated by differences of slopes of standard curves of various volatile chemicals added to different biological fluids or waters (*14,19*).

Concentration of the volatile materials by steam distillation (*4*), cryogenic traps (*9*), pre-column sorbents (*7*), a trap and purge sampler system (*21,23*), or on-column trapping (*11,17*) increases the sensitivity of the headspace gas chromatographic method considerably. In addition to distorting aroma profiles, there are two principal disadvantages to the concentration methods. First, the additional steps in the concentration procedure are often very time consuming and may eliminate it as simple routine analysis. Second, precautions must be taken that artifacts are not produced.

SOURCES OF ERRORS AND LIMITATIONS OF GAS CHROMATOGRAPHIC HEADSPACE ANALYSIS

Wyllie et al. authored the first chapter in the book, *Analysis of Foods and Beverages: Headspace Techniques (6)* and described variations among several sampling techniques in measuring odor profiles of mixtures of known materials as well as foods. They pointed out some of the disadvantages and abuses of sampling equilibrated headspace vapors over a sample and concluded that more than one sampling procedure is necessary to obtain a complete picture of the volatile materials present. Hachenberg and Schmidt (*10*) also pointed out various sources of errors in using headspace gas sampling for subsequent GLC analysis. They gave particular attention to the necessity of adequate equilibration of the sample with its vapors and to absorption of materials on the injection septum. Weurman (*24*) suggested that to gain a complete understanding of an odor, one must identify and quantify the sum total of the individual volatiles substances in the food, determine the vapors over the food, and understand the physical structure of the food.

Great care must be exercised in interpreting the odor profiles of chromatograms. It is important to consider the type of equilibrium in question, whether the system is a static closed one or a dynamic one where the concentration in the condensed phase will change in the course of sampling; the time required for equilibrium vapor pressure to be established; solvent effect and solute interaction with sample constituents (lipids, proteins, etc.); temperature of the sample; possible degradation or alteration of the materials of interest; and production of volatile materials from decomposition or alteration of sample constituents. When these factors are considered and analytical methods are designed to give specific information, headspace gas sampling for GLC analysis is a very useful technique.

HEADSPACE GAS (HSG) SAMPLING WITH SALTING-OUT PROCEDURES

The following discussion is based on some personal experiences with HSG analysis and some techniques that our research group has found to be useful. Most of our GLC analytical work has been on a 10 ft × 1/8 in. column packed with 80-100 mesh firebrick or Chromosorb P coated with 20% carbowax 20 M. The instrument used was a 500 series Varian-Aerograph gas chromatograph (GC) with a flame ionization detector.

One of the most important considerations in making analysis of trace materials is to accentuate the analytical capability of the GC by fine-tuning it to operate with good baseline stability at near the maximum sensitivity of the instrument. Obviously, it is difficult to do this; any problems in the system (electronic, gas fluctuation, contamination in the column, injector or detector, and sampling system) will be magnified as one works at the maximum sensitivity. However, establishing those conditions is essential in making direct routine headspace analyses at the ppm level.

For quantitative work, it is essential that one prepares solutions accurately in the ppm and ppb range at the time the sample is being analyzed. A technique we have found quite useful and simple is capillary weighing. We prepare stock solutions of 100 ppm concentrations of low boiling volatile materials in short-drawn capillary tubes. The volatile materials can be weighed to 0.1 mg in these drawn-capillary tubes and weights adjusted by absorbing excess from the capillary by touching the tip with the corner of a blotting paper (Kimswipe). Thus 10.0 mg of the medium/100 ml give 100 ppm. These solutions then are kept refrigerated. Whereas the 100 ppm solutions are relatively stable at refrigeration temperature, the 1.0 ppm and lower concentrations solutions must be made fresh weekly.

It is apparent for those working in ppm and ppb concentrations that glassware must be scrupulously clean and oven-dried, distilled H_2O must be boiled and then kept in closed flasks, and the room must be relatively free of odors and volatile chemicals.

Particular attention must be given to the syringe and needle. Residual sample material is often retained, particularly on the syringe needle. Immediately after making an injection, one must insert the gas tight syringe into a water-aspirator-suction system to air sweep the glass syringe and needle for 15 min between sampling.

Memory peaks are not limited to residual material in the syringe. When a high concentration of volatile material is analyzed, it is likely that a significant amount of that material will appear on the next chromatogram. This is particularly true if aqueous vapor sampling is used. To eliminate this, it may be necessary to inject vapors of pure water between sample analyses.

DISTILLATION OF AQUEOUS MATERIALS

Volatile materials with boiling points near 200°C can be steam-distilled in a simple distillation apparatus such as the Kemmerer-Hallet type micro-Kjeldahl distillation unit (*4*). Collecting the distillate in a tube immersed in a simple ice bath will result in a several fold increase in volatile substances. It also will free the volatile materials from interaction or solvent effects of lipids and proteins. Using this rapid steam distillation procedure followed by headspace sampling of the distillate allows the rapid direct analysis of volatile materials at the ppb level.

This procedure provides those working with off-flavors with the means to confirm that the volatile materials reported from the analysis do contribute to the flavor in question. If the distillate of an off-flavor food is added to a material that does not have the off-flavor in proportion to that removed and the off-flavor is produced, the analyst knows that the distillate contains that off-flavor.

SUBTRACTIVE TECHNIQUES

Elimination of volatile materials from headspace vapors by using selective chemical reagents that are specific for particular functional groups is a very useful identification tool. This technique is particularly well suited to headspace gas chromatographic analysis. For example, volatile carbonyl compounds when reacted with hydroxylamine form relatively non-volatile oximes. Hence, a before and after analysis of samples with carbonyl compounds in a complex mixture of volatile materials will allow the analyst to pinpoint those carbonyl compounds. Usually when the functional group is known, it is possible to identify the compound by retention time. Similarly, reagents are available to eliminate peaks from esters, sulfides and mercaptans, alcohols, acids, and bases (*1*).

An additional use of this technique that has not been reported is combining this procedure with mass spectrometry (MS) for confirming identification in complex mixtures. Not only confirmation but simply reducing the total number of peaks by selectively eliminating large numbers of peaks should simplify the MS interpretations and identification.

CONCLUSION

Headspace gas chromatographic analysis is an extremely useful tool for a wide range of applications. It is necessary, however, to use caution in interpreting results of these analyses. When some form of enrichment of the vapor is used, there will be an alteration of the concentration of various components in the sample material analyzed. Thus those components will not likely represent concentrations that are present over the food in its natural state. When the quantitative analysis are made by this method of individual components, it is necessary that standard curves be prepared in the specific matrix being analyzed. Interaction of volatile components such as lipids, proteins, acids, bases, etc., will affect the concen-

tration of vapors markedly in the headspace area. When these factors are properly considered, headspace gas chromatographic analysis is a rapid, simple, reliable analytical tool with extensive applications.

REFERENCES

1. Bassette, R., S. Ozeris, and C. H. Whitnah. 1962. Gas chromatographic analysis of head space gas of dilute aqueous solutions. Anal. Cham. 34:1540-1543.
2. Bassette, R., S. Ozeris, and C. H. Whitnah. 1963. Direct chromatographic analysis of milk. J. Food Sci. 28:84-90.
3. Bassette, R., and G. Ward. 1969. Vapor sampling and gas liquid chromatography of some materials in biological solutions. Microchem. J. 14:471-477.
4. Bassette, R., and G. Ward. 1975. Measuring parts per billion of volatile materials in milk. J. Dairy Sci. 58:428-429.
5. Buttery, R. G., and R. Teranishi. 1961. Gas liquid chromatography of aroma of vegetables and fruit. Direct injection of aqueous vapors. Anal. Chem. 33:1439-1441.
6. Charalambous, G. (ed.). 1978. Analysis of foods and beverages. Headspace techniques. Acad. Press., N.Y.
7. Dirinck, P., L. Schreyen, and N. Schamp. 1977. Aroma quality evaluation of tomatoes, apples and strawberries. J. Agric. Food Chem. 25:759-762.
8. Drozd, J., J. Novak. 1979. Headspace gas analysis by gas chromatography. J. Chromatogr. 165:141-165.
9. Gottauf, M. Z. 1966. Verbessert quantitative gas-chromatographische Spuren-Analyses flüchtiger organischer Verbindungen in Wasser. Z. Anal. Chem. 218:175-184.
10. Hachenberg, H., and A. P. Schmidt. 1977. Gas chromatographic headspace analysis. Heyden, London, N.Y., Rheine.
11. Heatherbell, D. A., R. E. Wrolstad, and L. M. Libbey. 1971. Isolation, concentration and analysis of carrot volatiles using on-column trapping and gas-liquid chromatography-mass spectrometry. J. Agric. Food Chem. 19:1069-1073.
12. Jenning, W. G., and M. Filsoof. 1977. Comparison of sample preparation techniques for gas chromatographic analysis. J. Agric. Food Chem. 25:440-445.
13. Jeon, I. J., and R. Bassette. 1983. Analysis of n-pentanal and n-hexanal as indices of potato chip shelf life. J. Food Qual. (In press.)
14. Kepner, R. E., H. Maarse, and J. Strating. 1964. Gas chromatographic head space techniques for the quantitative determination of volatile components in multicomponent aqueous solutions. Anal. Chem. 36:77-82.
15. Loney, B. E., R. Bassette, and T. J. Claydon. 1968. Chemical and flavor changes in sterile concentrated milk during storage. J. Dairy Sci. 57:1770-1775.
16. Mackay, D. A. M., D. A. Lang, and M. Burdick. 1961. Objective measurement of odor. Ionization detection of food volatiles. Anal. Chem. 33:1369-1374.
17. Morgan, M. E., and E. A. Day. 1965. Simple on-column trapping procedures for gas chromatographic analysis of flavor volatiles. J. Dairy Sci. 48:1382-1384.
18. Nawar, W. W., and I. S. Fergerson. 1960. Technique for collection of food volatiles for gas chromatographic analysis. Anal. Chem. 32:1534.
19. Ozeris, S., and R. Bassette. 1963. Quantitative study of gas chromatographic analysis of head space gas of dilute aqueous solutions. Anal. Chem. 35:1091.
20. Patte, H. E., E. O. Beasley, and J. A. Singleton. 1965. Isolation and identification of volatile components from high-temperature-cured off-flavored peanuts. J. Food Sci. 30:388-392.
21. Phillips, R. J. 1982. Routine analysis of volatile components in foods and beverages. Amer. Lab. 14:(5):110-118.
22. Teranishi, R., R. G. Buttery, and R. E. Lundin. 1962. Gas chromatography. Direct vapor analyses of food products with programmed temperature control of dual columns with dual flame ionization detectors. Anal. Chem. 34:1033-1035.
23. Wellnitz-Ruen, W., G. A. Reineccius, and E. L. Thomas. 1982. Analysis of the fruity off-flavor in milk using headspace concentration capillary gas chromatography. J. Agric. Food Chem. 30:512-514.
24. Weurman, C. 1969. Isolation and concentration of volatiles in food odor research. J. Agric. Food Chem. 17:370-384.