Reports

Volatile Saturated Aliphatic Aldehydes in Rancid Fat

A great deal of attention has been given in recent years to the isolation and identification (*1*) of aldehydes, ketones, and dicarbonyls in food and natural products. These compounds are recognized as influencing quality and flavor. Little is known concerning the carbonyl compounds present in rancid fats. Klose (*2*) found hexanal to be the major volatile carbonyl and probably the major carbonyl compound in oxidized turkey fat. A complete qualitative determination of volatile saturated aldehydes in rancid tissue fats has not been previously described. This report deals with the identification of volatile saturated aliphatic aldehydes present in rancid (*3*) pork fat.

Carbonyls volatilized by steam for 22 min from 10.0 g of mildly rendered (< 100°C) fat were collected in an excess of 2,4-dinitrophenylhydrazine in 2*N* HCl. After standing overnight, the cloudy solution of 2,4-dinitrophenylhydrazones (DNPH) was extracted with carbon tetrachloride and then benzene. Chromatography on 20-percent hydrated alumina (*4*) yielded monocarbonyl and dicarbonyl DNPH fractions from the carbon tetrachloride and a monocarbonyl DNPH fraction from the benzene extract. The monocarbonyls from the carbon tetrachloride extract were separated into from two to four apparently different classes (*5*) [depending on peroxide values (1 to 86), temperature of storage, and whether the sample was cooked] on untreated Whatman No. 3 filter paper by ascending development with a petroleum ether fraction boiling at 37° to 40°C. Description of the method of separation into classes, and studies of the variations in proportions of the classes with oxidation and processing will be reported elsewhere (*6*). These class fractions, on the basis of their absorption maxima in carbon tetrachloride of 343 to 346, 349 to 352, 360 to 365, and 370 to 380 mμ, appeared to be DNPH's of saturated aldehydes, saturated ketones or a mixture, 2-enals and 2,4-dienals, respectively. The dicarbonyl DNPH class had a maximum absorption at 370 to 390 mμ (*6, 7*).

Propanal and hexanal DNPH's have been separated and identified from the fraction with an absorption maximum at 343 to 346 mμ. The compounds were separated on Whatman No. 3 paper impregnated with 20 percent propylene glycol by ascending development with 96 percent Skellysolve C and 4 percent methanol, a rapid, new method for separating C_1 to C_{14} DNPH's of saturated aliphatic aldehydes (*8*). Similarly, methanal and ethanal DNPH's were separated and identified in the monocarbonyl fraction from the benzene extract. This fraction was also found to contain acetone and methyl ethyl ketone DNPH's. Ethanal DNPH was isolated in two polymorphic forms. These were identified by comparison with two forms from a low-melting preparation of authentic ethanal DNPH (*8*).

Identification was made by comparison of R_F values with those of authentic derivatives, nonseparation on filter paper when mixed with authentic derivatives, maxima in carbon tetrachloride, fading rate in alcoholic alkali (*8*) of major maxima at 430 to 434 mμ, and a disappearing secondary maximum at 520 mμ which distinguishes saturated aldehyde DNPH's from saturated ketone and 2-enal DNPH's (*9*).

Screening of the other monocarbonyl DNPH fractions gave no indication of other saturated carbonyls. In the samples of rancid fat examined thus far, saturated carbonyl compounds with a carbon chain greater than six do not appear to be present. The chances of such compounds being mixed with other carbonyl classes seem remote since authentic higher-molecular-weight saturated compounds added to the monocarbonyl fraction separated only in the saturated class. Therefore, the separation of monocarbonyls into classes (*6*) is remarkably precise. The second monocarbonyl fraction with maximum at 349 to 352 mμ, which was at first thought to be a saturated ketone or a mixture of classes, is evidently a separate class. Six components were found in this class; these appeared to be C_6 to C_{11} carbonyls. Their spectral properties did not agree with any of the classes studied by Jones *et al.* (*9*); the absence of secondary maxima indicated unsaturation, but the major maxima were intermediate between saturated carbonyl and 2-enal DNPH's. It is tentatively suggested that they may be unconjugated unsaturated compounds such as have been detected in milk fat (*10*). The 2-enal class had five components and the 2,4-dienal class had three components that were apparently C_6 to C_{12} compounds.

In a sample of unheated rancid pork fat (peroxide 86), the saturated aldehydes had the following proportions; 93.6 percent hexanal, 5.8 percent propanal, 0.4 percent ethanal, and 0.2 percent methanal. The saturated aldehyde class is the major one in uncooked rancid tissue fat. Heating at 165°C, which approximates the temperature of cooking, produces large increases in total carbonyls (*11*), monocarbonyls, and the proportion of the conjugated unsaturated classes (*6*). Ethanal, propanal, and hexanal were identified as saturated compounds lost during cooking. The predominance of saturated carbonyls in unheated fat is therefore considerably modified when the fat is cooked. Methanal was not detected in cooked fat tissue.

A. M. Gaddis
Rex Ellis

Eastern Utilization Research and Development Division, Agricultural Research Service, Beltsville, Maryland

References and Notes

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2. A. A. Klose, unpublished data (1952).
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5. J. Brekke and G. J. McKinney, *ibid.* 27, 238 (1950).
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All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in Science 125, 16 (4 Jan. 1957).

Additional Trace Element Analyses of Standard Granite G-1 and Standard Diabase W-1

More and more investigators have been reporting on their analyses of various trace elements in the standard granite G-1 and standard diabase W-1 which have been described by Fairbairn *et al.* (*1*) and Ahrens (*2*) and distributed by the U.S. Geological Survey. The meth-

Table 1. Determination of some trace element concentrations in standard granite G-1 and standard diabase W-1 compared with the data of Smales (*5*). The limit of detection for Pb, Sn, Ni, Co is about 2 parts per million.

Element	Concentration (ppm)			
	W-1		G-1	
	This report	Smales	This report	Smales
Cr	105		22	
Pb	n.d.*		48	
Sn	n.d.*		n.d.*	
Zr	125		180	
Ni	110	73	n.d.*	1.0
Co	41	49	n.d.*	2.1

* The element was sought but not detected at the level of sensitivity stated above.

ods of analysis include emission-spectrographic, chemical, x-ray fluorescence, isotope-dilution, and neutron-activation techniques. Of these, the last two generally are considered to be less susceptible to systematic errors caused by matrix effects or contamination.

This paper reports preliminary results obtained for several trace elements by an emission-spectrographic technique which utilizes the buffering action of calcium carbonate. The results on standard granite G-1 and standard diabase W-1 are presented in Table 1 and are meant to add to the accumulating data on these rocks. I make no spectacular claims for accuracy for the elements listed. I feel that the technique employed may be satisfactory in eliminating the effects of varying matrices. This technique has proved to be successful in the analysis of the trace alkaline-earths which are particularly sensitive to matrix composition (*3*). The method has the additional advantage that it can be extended to a wide range of geologic materials ranging in composition from pure silicates through calcareous shales to limestones.

Standards were made by mixing the metal oxides of the elements to be sought in varying amounts in an albite base. The silicate was then mixed with an equal amount of analytical grade calcium carbonate (*4*) which was free of the elements investigated. Correction was made for the high lead content of the albite (90 parts per million). Otherwise, the albite was also free of the elements to be sought.

The procedure of analysis is briefly the following: Five milligrams of each sample are weighed accurately to within 3 percent on a Roller-Smith torsion balance and arced to completion at 16 amp (d-c arc) in deep-cratered electrodes to avoid spattering. Kodak SA No. 1 plates are used. A set of standards is run in triplicate on each plate, and working curves are constructed directly by calculating the intensity of the line of the element sought (with proper background corrections). No internal standard is used. The precision in most cases can be expressed as a coefficient of variation of 10 for a wide range of concentrations. The coefficient of variation increases, however, with decreasing concentration to about 25 for the range around 10 parts per million and lower. This is a common feature of emission-spectrographic analysis.

Comparisons are made in Table 1 for Ni and Co between the values obtained by the technique described above and those of Smales (*5*), who used the method of neutron-activation analysis. It is seen that agreement is not complete for these few samples. It is probably true that neutron activation yields more accurate results, but it is important to have all the variously determined data available. The accuracy of a technique cannot be established by comparisons of one or two samples. Turekian, Gast, and Kulp (*3*) use a method of assessing the accuracy of an emission-spectrographic technique for strontium determination when compared with isotope-dilution analyses. They had seven different rocks to compare.

The reasons for discrepancies in the values reported for standard granite G-1 and standard diabase W-1 among the various investigators and techniques can conveniently be broken down in the following manner: (i) errors in the analyses of G-1 and W-1 due to systematic errors (due to matrix effects) inherent in the scheme of analysis and requiring radical revision of the method to insure accurate results; (ii) errors which are due to ephemeral mistakes such as faulty standard preparation, and so forth, which may give poor results for the one or two samples analyzed. If many samples were compared, then certainly these mistakes would be discovered and rectified; (iii) the possibility of the inhomogeneity of the standard rock powders distributed. Certain trace elements have associations with particular minerals; hence any variation in the relative amounts of the latter will be reflected in the former; (iv) the "accurate" techniques of isotope dilution and neutron activation are also susceptible to systematic errors though of a different kind from those to which the emission-spectrographic or x-ray techniques are susceptible.

In light of the above situation, two suggestions can be made regarding the reporting of comparison analyses. First, the analyst using the emission-spectrographic or x-ray fluorescence technique should exercise caution in his claims for accuracy when he is dealing with complex materials such as rocks. Such claims as those of Hower and Fancher (*6*) for accuracy cannot go unchallenged where there are marked discrepancies between their values and those of other reputable analysts using matrix-sensitive techniques. In addition, there are serious discrepancies with available neutron-activation values that have been reported by Smales (*5*).

Second, it is obvious that comparisons to test accuracy and permit interlaboratory standardization must be made on more than two samples. In the cases of standard granite G-1 and standard diabase W-1, only one figure may be available for comparison, as in the case of Pb, Co, and Ni, because the other rock is below the limit of detection for the emission-spectrograph and x-ray fluorescence methods. Hence no real judgment can be made of the validity of a technique of analysis even with the available comparisons with neutron activation or isotope dilution values.

KARL K. TUREKIAN

Department of Geology,
Yale University,
New Haven, Connecticut

References and Notes

1. H. W. Fairbairn *et al.*, *U.S. Geol. Survey Bull.* *980* (1951).
2. L. H. Ahrens, *Quantitative Spectrochemical Analysis of Silicates* (Addison-Wesley, Cambridge, Mass., 1955).
3. K. K. Turekian *et al.*, *Spectrochim. Acta* 9, 40 (1957).
4. Unfortunately, analytical grade calcium carbonate is not free of strontium and barium. If these elements are also sought, a system of purification that I described in a previous article (*3*) must be used.
5. A. A. Smales, *Geochim. et Cosmochim. Acta* 8, 300 (1955).
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Potentiation by Ouabain of Contractile Response of Myocardium to Glucose

Ouabain increases the rate of oxidation of C^{14}-labeled glucose to CO_2 by dog heart slices respiring in Krebs-Ringer phosphate medium, but is without effect on the oxidation of pyruvate (*1*). The significance of this observation with respect to the positive inotropic action of the drug is unknown, for no studies with contracting cardiac preparations have been reported. We have investigated the effect of ouabain on the contractile response of isolated rat ventricle strips to glucose and pyruvate and have obtained results which bear upon the afore-mentioned observations (*2*). Glucose is relatively ineffective by comparison with pyruvate as an energy source for rat myocardium in phosphate medium (*3*), and an increased rate of oxidation of the former might be expected to increase the ability of this substrate to support contractile activity.

Strips prepared from the right ven-