Some Observations on the AOAC Method for the Analysis of Brominated Oils[1]

R.R. LOWRY and **I.J. TINSLEY**, Dept. of Agricultural Chemistry and Environmental Health Sciences Center, Oregon State University, Corvallis, OR 97331

ABSTRACT

Increased sensitivity and improved reproducibility in the analysis by gas liquid chromatography of brominated oils has been obtained by re-esterification following debromination. The variety of reactions that can occur at various temperatures and their effects on the procedure are discussed. In addition to defining optimal procedures, it was established that reagents that are more easily and safely handled than metallic sodium give equivalent results.

INTRODUCTION

Considerable interest on the part of regulatory agencies, brominated oil producers and soft drink bottlers stems from the uncertain status of these oils in regard to human health. Methods were developed by Conacher et al. (1) to monitor concentration and define their effect. The method uses the formation of a derivative and is now accepted as an official first action by the Association of Official Analytical Chemists (AOAC) (2).

Using this procedure with known standards, it became apparent that the reproducibility was very dependent on the handling of individual samples. Efforts to define the causes of this variation led to the suggested changes in procedure described here, which improve reproducibility and increase sensitivity.

EXPERIMENTAL PROCEDURES

Materials

All reagents are reagent-grade unless otherwise specified and all solvents, except methanol and diethyl ether which were reagent-grade, were distilled in an all-glass apparatus. Brominated fatty acid methyl esters of 99+% purity were obtained from Nu-Chek-Prep of Elysian, MN, and the Dri-Na^R was from J.T. Baker Chemical Co., Phillipsburg, NJ. The sodium-lead alloy reacts slowly in storage; consequently, after removal of material, the bottle should be gassed with nitrogen, sealed with tape and placed in a desiccator for storage. Shelf-life is estimated to be 1 year after opening. The amount of alloy necessary to give a 1.0% solution was derived from the lot analysis on the bottle and was added to the required volume of methanol. After the sodium was dissolved (caution: hydrogen evolved!) the mixture was filtered through paper to remove the residual lead.

GLC Analysis

The analyses were done using a 0.03" by 50 ft open tubular stainless steel column coated with Apiezon L as previously described (3), installed in a Hewlett-Packard Model 700 gas chromatograph equipped with a hydrogen flame detector. The Apiezon L was purified by a modification of an unpublished procedure (T. Mon, personal communication). A ca. 10% solution of the grease in hexane was filtered through folded filter paper. The filtrate was applied to an activated alumina column and eluted with additional hexane to give a light lemon-yellow material representing 55% by wt of the original material. This material is considered to be essentially equivalent to JXR, SE-30, or SP 2100 (4) and can be easily stripped from used capillary columns to permit recoating (3). Soft drinks analyzed were obtained from local sources and were extracted by the AOAC method (2).

Re-esterification after Reaction

From some preliminary reaction time studies, it was obvious that the amount of derivative obtained decreased with increasing reaction time as shown by curve A (Fig. 1). The replicate samples were heated with sodium methoxide (NaOMe) for the time intervals indicated and analyzed by gas liquid chromatography (GLC). A duplicate series was further treated with 5% HCl (gas) in dry methanol, a reagent used to form methyl esters from free fatty acids; the increased recovery is apparent (curve B). A similar duplicate series also was run, using methyl stearate, to verify that ester hydrolysis was involved; they are shown as curve C, without re-esterification; and D, with re-esterification.

Analysis of Samples

Samples of MDBS, MTBS and commercially available orange soft drinks were analyzed by the AOAC method (2) with the following modifications: (a) Dri-Na^R was used as the sodium source; (b) re-esterification was done; (c) GLC conditions were as described. The results obtained from the standards are shown in Table I and the corres-

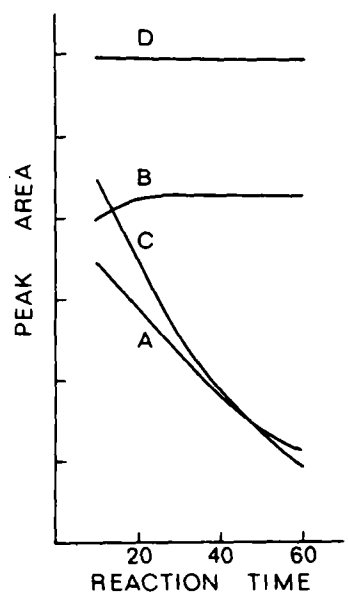

FIG. 1. Curves representing the peak areas (in cm^2) obtained for the residual methyl esters found at reaction times of 10, 20, 30, 40, 50 and 60 min at 80 C with NaOMe. A: Residual methyl esters obtained directly from MDBS; B: the same after remethylation with acid catalyst; C: methyl esters remaining from methyl stearate; D: the same after remethylation with acid catalyst.

[1]Technical paper no. 5831, Oregon Agricultural Experiment Station.

ponding response factors are in Table II along with values reported previously by Conacher (2). Both the magnitude and variability of the response factor is improved. Table III gives a comparison of the original method and the modified method described herein when used for soft drink analysis. Peak areas were obtained both by a hand measuring technique and by weighing.

TABLE I

Percentage Recovery of Standards by the Two Methods

	μg Added	% AOAC	AOAC % remethylated
DBS	5.988	71.5	95.5
	9.980	70.2	92.6
	19.96	64.4	89.3
TBS	6.006	55.6	67.6
	10.01	55.4	73.4
	20.02	49.6	66.8

TABLE II

Response Factors

	Col. study[a]	AOAC	AOAC remethylated
DBS	1.62 ± 0.10	1.46 ± 0.06	1.08 ± 0.02
TBS	2.12 ± 0.10	1.88 ± 0.10	1.45 ± 0.06

[a]Averages of values reported in ref. 2, collaborative study.

TABLE III

Values Obtained for Soft Drinks

	AOAC	AOAC remethylated
Peaks weighed		
BVO/sample (mg)	4.932 ± 0.79	6.524 ± 0.53
Deviation (%)	16.1	8.1
Values of remethylated (%)	75.7	100
Peaks measured		
BVO/sample (mg)	3.644 ± 0.63	7.032 ± 0.53
Deviation (%)	17.2	7.5
Values of remethylated (%)	51.8	100
n	8	9

No response factor used in above data.

All samples were 280 mL each at room temperature.

RESULTS AND DISCUSSION

Competing Reactions

Several possible reactions may occur, including the following: $RCHBrCHBrR' + R''ONa \rightarrow$

I. $RCHOR''CHBrR' + NaBr$

II. $RCHOR''CHOR''R' + 2NaBr$

III. $RCH = CBrR' + NaBr + R''OH$

IV. $RC \equiv CR' + 2NaBr + 2R''OH$

Reactions I and II are the well known Williamson synthesis for making ethers, usually done at a temperature between 70 and 100 C. The third (dehydrohalogenation) is postulated from nuclear magnetic resonance (NMR) spectra (Fig. 2) and mass spectroscopy done as part of this study. The 9 and 10 monobromo methyl octadecenoates were obtained by reaction at room temperature for 65 hr using

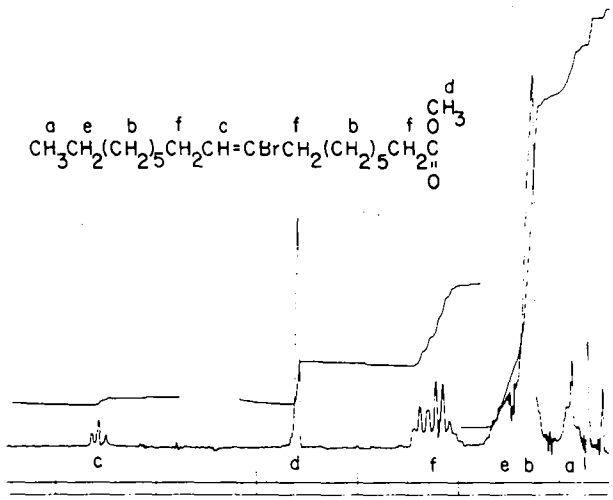

FIG. 2. NMR spectra, integration trace and structure of compounds obtained. Letter assignments above the protons identify origin of resonance shifts. Identified as a mixture of the isomers, methyl 9 and 10, monobromo-oleate with the 9 isomer shown.

MDBS as the starting material. This product gave a single peak with GLC and, upon hydrogenation with Adam's catalyst, a single peak corresponding to methyl stearate was obtained using an EGS column.

The fourth reaction has been described previously (5) and was done at 150 C using potassium hydroxide in place of the alkali methoxide. Running the reaction in our laboratory with either MDBS or MTBS at higher temperatures (100-120 C) than specified by Conacher (58-60 C, determined in our laboratory; refs. 1 and 2) gave additional peaks on an Apiezon L column, including ones with shorter retention times, suggesting cleavage of the carbon chain. The nature of the end-products seems to be quite temperature-dependent, suggesting that large variations in results between laboratories or personnel could easily be due to that factor alone.

The reaction products formed at 80 C from the tetrabromo compound could be separated into 2 fractions by silver nitrate thin layer chromatography suggesting that at least one of the derivatives was unsaturated. Mass spectral analysis showed that one of the 3 peaks obtained by GLC from MTBS had 2 components, one of which contains a single bromine. Further identification work was not performed on the products from MTBS.

A second, competing reaction is that of saponification of the ester bond. Alkaline reaction conditions induce the splitting of any esters present, despite the fact that such reagents are commonly used for transesterification. Sensitivity was increased several-fold and consistent results are no longer dependent on precise repetition of temperature, heating times and reagent concentration. Figure 1 shows that considerable saponification does occur; the level can vary considerably between sets and reagent batches. After a 30-min reaction, only 55.0 and 43.5% of the MDBS and methyl stearate, respectively, remained in the ester form and after 60 min, this had dropped to 25.7 and 15.6% (curves A and C). In Table III, using values obtained from weighing the peak areas, 24.3% saponification has taken place with BVO samples in 1 hr. Differences in these values for MDBS, methyl stearate and BVO may be related to structural differences in the materials. Other sources for the variation in the amount of saponification that occurs is not apparent. Since it can be readily standardized by remethylation, this would seem to be a reasonable and prudent step to add to the procedure.

Sources of Sodium Metal

The use of metallic sodium presents certain hazards, particularly with lab personnel who have not been thoroughly trained in its use. In addition, it is not convenient to handle and can lead to hydrocarbon contamination from the protective "oils" or solvents in which sodium metal is usually stored. Therefore, 2 more easily handled forms of sodium were evaluated—sodium methoxide powder and Dri-Na^R, a sodium-lead alloy. With a series of MDBS standards, no significant differences could be detected by GLC using the 3 sources of sodium. Areas obtained by GLC, retention times, the shape of the major peak, and occurrences of minor peaks appeared identical. There appears to be no reason that the 3 sources cannot be used interchangeably and the Dri-Na^R is certainly both the most convenient and safest to use.

Suggested Modifications to the Procedure

Re-esterification of the ester bond would reduce the opportunity for significant losses and decrease the variance between replicate samples; therefore, this step should be added to the procedure. Since both variation in temperatures and localized overheating occurs with the use of mantles, the substitution of controlled-temperature heating blocks is suggested. Third, because of the ease of handling and increased safety, the replacement of metallic sodium with the sodium-lead alloy is recommended.

ACKNOWLEDGMENTS

B.A. Jones provided technical assistance.

REFERENCES

1. Conacher, H.B.S., R.K. Chadha and M.R. Sahasrabudhe, JAOCS 46:558 (1969).
2. Conacher, H.B.S., J. Assoc. Off. Anal. Chem. 56:602 (1973).
3. Lowry, R.R., and I.J. Tinsley, JAOCS 52:298 (1975).
4. Supelco, Inc. Catalog #10, Supelco, Inc., Bellefonte, PA, pp. 40-42.
5. "Organic Syntheses," Collective Vol. 3, John Wiley & Sons, New York, NY, 1955, p. 785.

[Received March 17, 1980]

Comparative Study of Methods of Determining Oil Content of Sunflower Seed

J.A. ROBERTSON and **W.R. WINDHAM**, USDA, SEA, AR, R.B. Russell Agricultural Research Center, Athens, GA 30613

ABSTRACT

An extraction-gravimetric method (AOCS Official Method Ai 3-75) was compared with 2 instrumental techniques, near-infrared reflectance (NIR) spectroscopy and wide-line nuclear magnetic resonance (NMR), for the determination of the oil content of oilseed-type hybrid sunflower seed. Eight sunflower seed samples of varying oil contents, replicated 5 times, were analyzed by the 3 procedures. The overall mean oil contents and standard deviations for the 8 samples were: AOCS method, 44.5% ± 0.33%; NMR, 44.8% ± 0.27%; and NIR, 44.2% ± 0.81%. Analysis of variance of the means of the 3 methods of analysis indicated no difference ($p>0.05$) in oil content due to the method. However, there was a difference ($p<0.001$) in total oil content due to replicated analyses of the same sample with the NIR method. With the AOCS and NMR methods, no effect ($p>0.05$) of replicated analyses of the same sample was found. The NMR method was more precise and reproducible than the other 2 methods. Although the NIR mean oil contents were not significantly different from the means of the other 2 methods, the coefficient of variations for all samples were consistently higher for the NIR analyses than for the AOCS and NMR analyses.

INTRODUCTION

The standard method for the determination of oil content of oilseeds since about 1880s has been the direct solvent extraction method. All international and most domestic trading of oilseeds are based on this technique and as such is accepted as the "Reference Method" of analysis. The extraction method usually used is a slow and time-consuming process and involves use of flammable solvents. Moreover, it leads to the destruction of the sample, which can be an inconvenience, in particular for the plant breeder. These serious drawbacks resulted in the development of wide-line nuclear magnetic resonance (NMR) and near-infrared reflectance (NIR) spectroscopy techniques.

Wide-line NMR is a term used to describe low resolution nuclear magnetic resonance. The NMR technique measures total hydrogen associated with the oil and water in seed (the only liquid constituents) independent of the hydrogen associated with the non-oil matrix (1). If the measurement is made on dry seed, the response of the apparatus is directly proportional to the quantity of oil present in the seed (2). Accurate estimates of oil content of oilseeds, however, can be made when moisture contents are below 4% (3).

In 1960, Conway (4) first used NMR to analyze whole seed for oil content. Since the process is nondestructive and feasible even on single seeds, geneticists and plant breeders have used the technique extensively (5-7). NMR provides a rapid, accurate means of measuring oil content of oilseeds (3,8) and has been found to be more reproducible and statistically more reliable than AOCS and other extraction methods (2,3,9).

Robertson and Morrison (8) reported that NMR gave accurate estimates of the oil contents of sunflower seed, but they found the NMR response varied, depending on the linoleic acid content. Wide-line NMR now is being used in the domestic trading of sunflower seed.

The establishment of NIR as a viable procedure for the estimation of protein in simple commodities was first reported in 1973 (10). NIR has since become firmly established as a simple, rapid and effective analytical tool for the simultaneous prediction of oil, protein and moisture content of grains and oilseeds (11-13). However, the technique also is destructive and has not been applied with