Report of the Instrumental Techniques Committee[1,2] AOCS, 1970-1971

INTRODUCTION

The Instrumental Techniques Committee met twice during the past year. The first meeting was held Monday, September 28, 1970, in Meeting Room 8 of the Conrad-Hilton, Chicago, Illinois, during the 44th Fall Meeting of the Society held jointly with the International Society for Fat Research—The First ISF-AOCS World Congress. The second meeting of the Committee was held on Monday, May 3, 1971, in the Walnut Room of the Shamrock-Hilton Hotel, Houston, Texas, during the 62nd Annual Meeting.

SPECTROSCOPY SUBCOMMITTEE

The Spectroscopy Subcommittee discussed plans to initiate collaborative investigations of two proposals, both designed to modify and extend the scope of AOCS Official Method Cd 14-61. The first proposal would afford where applicable a more rapid determination of isolated *trans* isomers in fatty acids, esters and triglycerides than possible with the Official Method Cd 14-61, and would permit the determination to be made without recourse to external standards. The proposed alternate method has been published (1). Reports of earlier collaborative work have indicated that, while permitting a more rapid analysis, the proposed method may not be as precise as AOCS Method Cd 14-61, principally because different instruments, even of the same make and model, usually give somewhat different absorptivities for the same sample. Furthermore it may be considerably limited in scope (2). The Committee has decided (3) that since the proposed method is less precise and is limited in scope when compared to the Official Method, it cannot be recommended to replace Cd 14-61. However its speed and simplicity could make it a very valuable alternate method where applicable and when highest precision is not an essential requirement.

The second proposal is designed to modify Method Cd 14-61 to permit more accurate measurement of very low concentrations of isolated *trans* content. The method to be investigated in collaborative effort has also been published (4). Successful completion of collaborative study of these two procedures would lead to a recommendation to the Uniform Methods Committee of either additional alternative methods to Cd 14-61 or alternate procedures to be incorporated into a revised Cd 14-61 to permit the rapid technique where desirable and to afford an alternate procedure for measurement of very low concentrations of isolated *trans* isomers. Collaborative testing of the proposed alternative methods or procedures is planned to begin shortly.

NMR SPECTROSCOPY SUBCOMMITTEE

The Committee's newest Subcommittee has been completing organization plans and considering proposals for initial collaborative investigation. Announcement was made that Mr. A.J. Haighton from Unilever Research, Vlaardingen, Netherlands, has accepted chairmanship of a Task Group to study a method for the determination of Solid Fat Index by Nuclear Magnetic Resonance. Collaborative studies of the NMR wide line spectroscopy procedure will include a comparison of results with those obtained by the AOCS Official SFI Dilatometric Method Cd 10-57. Also if qualified, experienced individuals can be found to establish a Task Group, the procedure will be compared in collaborative investigation with a procedure to determine Solid Fat Index by Differential Scanning Colorimetry.

A second proposal to be investigated by this Subcommittee is the determination of oil content of oilseeds by wide line NMR. The Subcommittee is actively seeking a chairman for a Task Group to initiate collaborative investigations of the NMR technique. There has been considerable interest in collaborative investigations of the instrumental method, hopefully to terminate in a recommendation for an Official Method in the AOCS "Book of Official Methods."

ATOMIC ABSORPTION SUBCOMMITTEE

A second collaborative test involving eight collaborating laboratories has been completed as part of a test to develop and evaluate a method for the determination of trace metals in vegetable oils and animal fats by atomic absorption spectrophotometry. In this study, using a method essentially as previously published (5), two soybean oil samples were analyzed for chromium, copper, iron, nickel and sodium content. The two samples were analyzed on two consecutive days by the direct atomic absorption method with the samples suitably diluted with methyl isobutyl ketone (MIBK). The samples were prepared by adding given quantities of NBS organometallic salts to soybean oil which had been previously doubly molecularly distilled. Standards at three concentrations were prepared in a similar manner.

The collaborators were requested to prepared suitable dilutions of the samples and standards and make the requested measurements.

Results were reported by eight of the ten collaborative laboratories participating:

TABLE I

Statistical Data on Collaborative Results: Chromium Determinations

	Sample A		Sample B	
Collaborator[a]	Day 1	Day 2	Day 1	Day 2
2	2.50 ppm	2.58 ppm	8.61 ppm	8.75 ppm
3	2.60	2.60	8.70	8.80
4	2.50	2.50	8.70	8.80
5	2.60	2.50	8.20	8.10
6	2.60	2.60	8.38	8.38
7	2.50	2.50	8.00	8.05
9	1.60[b]	2.35	6.65[b]	8.50
10	2.50	2.75	8.50	8.35
Mean	2.543	2.548	8.441	8.466
Standard deviation	0.0535	0.1155	0.2654	0.3007
Relative standard deviation	2.10	4.53	3.14	3.55
Average relative standard deviation	3.33			

[a] No results were received from Collaborators 1 and 8.
[b] Values were rejected on the basis of the outlier test.

TABLE II

Statistical Data on Collaborative Results: Copper Determinations

	Sample A		Sample B	
Collaborator	Day 1	Day 2	Day 1	Day 2
2	3.54 ppm	3.48 ppm	9.70 ppm	9.50 ppm
3	3.50	3.60	9.60	9.60
4	3.50	3.50	9.50	9.50
5	3.50	3.60	9.30	10.00
6	3.37	3.35	9.37	9.35
7	3.40	3.35	9.65	9.45
9	3.50	3.55	9.70	9.60
10	3.75	3.75	9.40	9.50
Mean	3.508	3.522	9.528	9.562
Standard deviation	0.1137	0.1344	0.1574	0.1940
Relative standard deviation	3.24	3.81	1.65	2.03
Average relative standard deviation	2.68			

---

[1] Report of collaborative work from Government, Industrial, and Academic Laboratories by members of ARS, USDA, Southern and Eastern Marketing and Nutrition Divisions; Anderson, Clayton and Company, Food Division; A.E. Staley Mfg. Co.; and The Pillsbury Company.

[2] A review of the Instrumental Techniques Committee since its foundation was erroneously titled "Report of the Instrumental Techniques Committee, AOCS 1970–1971," in JAOCS 48:140A–141A (1971). The following, and not the earlier article, is the annual report of the Committee.

TABLE III

Statistical Data on Collaborative Results: Iron Determination

Collaborator	Sample A		Sample B	
	Day 1	Day 2	Day 1	Day 2
2	2.00	2.07	8.08	7.96
3	2.10	2.00	7.90	8.00
4	1.90	1.90	7.90	7.80
5	2.00	2.00	7.70	7.70
6	2.00	2.15	8.10	8.12
7	2.05	2.45	8.75	8.59
9	2.05	2.10	8.00	8.15
10	0.95[a]	2.15	6.95	7.75
Mean	2.014	2.102	7.922	8.009
Standard deviation	0.627	0.1640	0.4990	0.2875
Relative standard deviation	3.11	7.80	6.30	3.59
Average relative standard deviation	5.19			

[a] Value was rejected on the basis of the outlier test.

TABLE IV

Statistical Data on Collaborative Results: Nickel Determinations

Collaborator	Sample A		Sample B	
	Day 1	Day 2	Day 1	Day 2
2	2.68	2.59	9.17	9.13
3	2.90	3.20	8.90	9.20
4	3.00	3.00	9.30	9.30
5	2.80	3.10	8.40	8.90
6	3.13	3.20	9.35	9.36
7	3.75	3.00	9.00	9.00
9	3.20	3.25	9.30	9.25
10	3.50	3.75	8.90	8.50
Mean	3.120	3.136	9.040	0.080
Standard deviation	0.3599	0.3239	0.3159	0.2797
Relative standard deviation	11.53	10.33	3.49	3.08
Average relative standard deviation	7.11			

TABLE V

Statistical Data on Collaborative Results: Sodium Determinations

Collaborator	Sample A		Sample B	
	Day 1	Day 2	Day 1	Day 2
2	0.74 ppm	0.74 ppm	2.20 ppm	2.25 ppm
3	0.70	0.71	2.20	2.10
4	0.70	0.70	2.30	2.40
5	0.70	0.60	2.60	2.50
6	0.73	0.73	2.31	2.18
7	0.75	0.75	2.20	2.21
9	0.76	0.75	2.41	2.42
10	0.90	0.80	2.40	2.15
Mean	0.748	0.722	2.328	2.276
Standard deviation	0.0661	0.0580	0.1395	0.1451
Relative standard deviation	8.84	8.03	5.99	6.37
Average relative standard deviation	7.31			

TABLE VI

Summary of Results

Metal	Added	Found		Added	Found	
		Day 1	Day 2		Day 1	Day 2
Cr, ppm	2.50	2.543	2.548	8.50	8.441	8.466
Cu	3.50	3.508	3.522	9.50	9.528	9.562
Fe	2.00	2.014	2.102	8.00	7.922	8.009
Ni	3.00	3.120	3.136	9.00	9.040	9.080
Na	0.60	0.748	0.722	1.70	2.328	2.276

In all cases except for sodium the mean values reported by the collaborators are acceptably close to the concentrations of the metals added to the samples. As indicated in the above summary the sodium concentrations found by the collaborators were consistently higher than the amount intentionally added. The ubiquitous nature of sodium was inadvertently added to the samples. Nonetheless the collaborative values for sodium were found to be acceptably precise for these low concentrations.

Generally the precision was greater for Sample B than for Sample A, although two exceptions were noted: The first day results for chromium and iron in Sample A are more precise than would be predicted from the relative standard deviations of the remaining determinations for these metals. Since an outlier was dropped from both of these determinations, the improved precision is easily rationalized.

A convenient preparation of metal free vegetable oil remains to be a major requirement if the direct analysis approach is to be widely used. Molecular distillation is not an entirely satisfactory approach because of the expense associated with this technique, and because of the difficulty in removing all traces of metals, especially sodium, by this approach.

It was reported by one collaborator that after setting the zero absorption with MIBK, positive absorption values were obtained for the blank oil for Ni and Fe and a negative signal was observed for Na. These findings, which are not uncommon, point up the need for zeroing the instrument with the blank oil solution. The same collaborator also pointed out a potential hazard in analyzing oil solutions. If the burner is not flushed with MIBK between samples, unburned oil will condense inside the burner which may cause the flame to flash back.

Another collaborator found rather dramatic shifts between days in the calibration curves for chromium, iron and nickel. These variations arise from the difficulty in obtaining a constant ratio between the acetylene and MIBK flows on a day to day basis, resulting in a change in the stoichiometry of the flame. Accordingly this variation can be minimized by closely controlling the acetylene flow.

Ranking the analyses in order of decreasing precision yields the following relationship: copper, chromium, iron, nickel and sodium.

Based on the collaborative results herein, the direct atomic absorption analysis of these metals in vegetable oil samples suitably diluted with methyl isobutyl ketone is judged to be an acceptable procedure. The Committee agreed that the procedure should be written in suitable format for the Book of Tentative and Official Methods of the AOCS and submitted to the Uniform Methods Committee with recommendation for its adoption as a Tentative Method of the Society.

The Direct Method will however lack adequate sensitiv-

(Continued on page 422A)

• *Report of Instrumental Techniques Committee* . .

(Continued from page 413A)

ity for determinations of metals at very low concentrations. For such analyses a suitable preconcentration procedure will have to be developed. This fact had been confirmed in earlier collaborative investigations. The Subcommittee will continue with an investigation of methods most suitable to achieve preconcentration of the metals present in low concentration with the objective of achieving a satisfactory procedure which will permit accurate and rapid determination of very small traces of metals in such vegetable oils and animal fats.

GAS LIQUID CHROMATOGRAPHY SUBCOMMITTEE

With a second revision of AOCS Official Method Ce 1–62 (Corrected 1964, Revised 1970) to extend the scope by including the use of flame detectors, adopted by the Uniform Methods Committee (3), the Subcommittee has embarked upon further improvement of the method for the determination of fatty acid composition of fats and oils by gas liquid chromatography. A Task Group has been established with Mr. John L. Iverson, Chairman, to start work on a collaborative investigation of a procedure involving programmed temperature analysis. Successful completion of this work will involve either further revision of Method Ce 1–62, or recommendation for adoption of a second, alternate method since the programmed temperature analysis may be an alternate method not intended to completely replace the present method.

The Subcommittee has also announced the establishment of two additional Task Groups. With Dr. Jairo E. Pena as Chairman, a Task Group will study methods for the determination of pesticides in fats, oils and other lipids by means of gas liquid chromatography. Collaborative work, planned to start shortly, will investigate procedures which will include the pesticides normally found in this class of compounds, but with a careful check on certain others not normally found but which may be present because of accidental contamination. A procedure proposed and studied by the Food & Drug Administration has been recommended by the new Chairman for initial investigation.

A third Task Group has been established under Francis E. Luddy, Chairman, to study procedures for the quantitative analysis of free sterols in fats and oils. A literature study of methods is being made. The most promising will be recommended for collaborative study, with the ultimate object of selecting a specific method for recommendation for adoption as an official method of the Society.

The gas liquid analysis of sterols in vegetable oils and animal fats has been receiving attention in connection with the establishment of specifications for identification of fats and oils by gas liquid chromatography. Qualitative gas liquid chromatographic patterns of the sterol distribution would be established, similar to the specification of fatty acid composition for identification of fats and oils by gas liquid chromatography which have been published (6). At the request of the Codex Committee on Fats and Oils of the Joint Food and Agricultural Organization of the United Nations and the World Health Organization (FAO/WHO) Codex Alimentarious Commission, the Society, through the Instrumental Techniques Committee and the Gas Chromatography Subcommittee, established gas liquid chromatographic patterns to permit the qualitative identification of eleven vegetable oils and animal fats (6). These standards received considerable attention at meetings of the Codex Committee on Fats and Oils sponsored by the Joint FAO/WHO Codex Alimentarius Commission, and the proposal to use them as an official method for identification of specific oils and fats was given partial support.

Subsequent study of the proposal by Codex Committee delegates resulted in the recommendation that values found in commercial samples of all the fats and oils obtained from all producing and importing countries be included in the specifications. However extension of the specifications to include an almost endless list of varieties and species of each vegetable oil or animal fat will so increase the range of fatty acid content that unique identification of specific fat or oil would be impossible. The U.S. Delegate to the Codex Committee on Methods of Analysis and Sampling, agreeing that "the unlimited revision of the proposed values will render these criteria practically useless," proposed that these data be given with some frequency distribution of the fatty acids characteristic of each vegetable oil or animal fat. However examination of the available data reveals that this suggestion is perhaps impractical. First we do not have any data regarding the specific species and/or varieties of the eleven vegetable oil and animal fats which would cover all of these fats and oils obtained from all producing and importing countries. Secondly the data from which the published specifications were compiled (6) vary from good quantitative data to semiquantitative results and, in a large number of cases, to no quantitative data at all. Thus the frequency distribution of the specific fatty acids in individual vegetable oil and animal fats is not available in any sort of statistical manner. To obtain such data even for only eleven oils and fats, particularly if all possible sources, species, varieties, etc., are to be considered, would be a research program of considerable magnitude.

An alternate proposal to the use of frequency distribution patterns of the individual fatty acids in the selected oils and fats has been the determination of qualitative patterns based on the identification of the sterols in each oil or fat by means of gas liquid chromatography. In cases where the fatty acid distribution does not afford a unique identification of the specific unknown vegetable oil or animal fat, a combination of the fatty acid and sterol distribution patterns, even if confined merely to qualitative patterns, might suffice for a positive identification.

Unfortunately there are problems in the implementation of the sterol specification approach. Unlike the fatty acid distribution in various oils and fats, very little has been published regarding the sterol content of either vegetable oils or animal fats by gas liquid chromatography; the amount published is insufficient for an adequate file of data to prepare satisfactory specifications. A survey of the fat and oil industry to elicit unpublished data which could be used by the Committee, and supplementation of these data with specific experiments to obtain data necessary to complete even minimum specifications would be required. Unfortunately funds for such a survey or such research do not appear to be available. As the Society is committed to helping the Codex Committee on Fats and Oil to a possible and practical extent, the Committee through the Subcommittee on Gas Chromatography should attempt limited investigation of the feasibility of augmenting the fatty acid specifications with similar sterol specifications.

R.T. O'Connor, Chairman
R.R. Allen, Subcommittee Chairman
W.A. Bosin, Subcommittee Chairman
K.M. Brobst, Subcommittee Chairman
S.F. Herb, Subcommittee Chairman

REFERENCES

1. Allen, R.R., JAOCS 46: 552–553 (1969).
2. O'Connor, R.T., R.R. Allen, K.M. Brobst, J.R. Chipault, S.F. Herb and C.W. Hoerr, Ibid. 46: 602A, 604A (1969).
3. O'Connor. R.T., R.R. Allen, K.M. Brobst, J.R. Chipault, S.F. Herb and C.W. Hoerr, Ibid. 47: 552A, 554A, 556A (1970).
4. Haung, A., and D. Firestone, JAOCS 54: 47–51 (1971).
5. Piccolo, B., and R.T. O'Connor, Ibid. 45: 789–792 (1968).
6. O'Connor, R.T., and S.F. Herb, JAOCS 47: 186A, 195A, 197A (1970).

◆