Report of the Fat Analysis Committee, 1962

THE 1961 report of the Fat Analysis Committee contained a summary of the work of the various committees then in operation. In 1961–1962 the subcommittees on Hydroxyl Value, Dilatometric Methods, Consistency, and the Determination of Soap in Refined Oils have been discontinued. The following information on the activities of the subcommittees has been supplied by the subcommittee chairmen.

Subcommittee on Revision of AOCS Tables, M. F. Lauro, Chairman

A complete revision of Section I in the AOCS Methods Book has been accomplished. The revised copy has been submitted to the Uniform Methods Committee for their approval and disposition. The Chairman of this subcommittee comments as follows: "With respect to these tables, there is still much to be desired, in filling some blank spaces and in narrowing the range of values somewhat. I have drawn from recent sources of literature and from our own analyses of commercial fats and waxes in order to obtain values representing more closely these of the market. For this reason also, the common name of the commodity is given rather than the botanical as a good many of the oils are blends. Their wide diversity make it impossible to hew to the line too closely. However, the tables do furnish a very useful service especially to the trade.

"In view of improved methods of analysis by instrumentation, from spectrograph to gas chromatograph, perhaps these values are of limited service in the determination of purity. I can forsee in the near future a filing of 'thumb prints' to identify each fat or wax, the characteristic graphs representing in detail the composition either in terms of fatty acids or what is more important in glycerides as they actually exist. In the conventional analysis, sometimes about the only value worth while is the iodine number as for example in soya, corn and sunflower oils, any difference diminishing in admixtures, whereas recourse can be had to structural content or configuration for a precise determination. It is suggested therefore that a subcommittee be appointed to start collecting such graphs. Meantime, this committee should be enlarged to collect further data as time goes on, if I am to remain chairman."

Subcommittee on Free Fatty Acids, W. O. Lundberg, Chairman

This subcommittee reports as follows: "The Subcommittee on Free Fatty Acids carried on some fairly extensive studies several years ago, and made recommendations to the Uniform Methods Committee concerning methods for the determination of free fatty acids. At that time, Mr. Mehlenbacher indicated there was no immediate need for further work by this Subcommittee, and therefore it has been inactive during the past few years. I am not aware that any problems have arisen which make it advisable to reactivate the Committee at this time, and therefore we have no plans for the immediate future. However, if you know of a need for further work by our group, please let me know."

There is still a need in our methods book for an electrometric method for the determination of free fatty acids in dark stocks. This Committee should prepare, if possible, such a method for the Uniform Methods Committee's consideration.

Subcommittee on Drying Oils, K. E. Holt, Chairman

"1. *Tentative Method Ka-3-58, Color.* The use of liquid colors as primary standards for method Ka-3-58 have not proven satisfactory and working in conjunction with ASTM D-1, Sub IX, Color Group, a series of glass discs have been developed and approved as primary color standards. These discs are being manufactured by Lovibond Company and have been checked and approved by Comm. members. It is the intent that the glass discs will be shown in the method as primary standards with the liquids as secondary standards. The method will be rewritten as soon as discs are commercially available.

"2. *Official Method Ka-6-59.* Viscosity of Transparent Liquids by Bubble Time Method. Discussions and collaborative laboratory work are in progress with the Gardner Laboratories and Cannon Instrument Company for the manufacture and sale of comparator viscosity and standards marked in bubble seconds as per table given in Ka-6-59. The light and heavy series have been produced by the Gardner Laboratory and approved by members of the Subcommittee. The Gardner Laboratory is currently producing prototypes of the very heavy series and has informed me they will submit them within the next few days for approval. The Cannon Instrument Company have submitted only the heavy series and these have been approved. The precision on all of the tubes submitted has been within 1% of the actual timed viscosity which is considerably better than we had originally expected.

"The very light series, that is 0.32 bubble seconds and below have proven more difficult to produce. The timing of bubble seconds on this series proved impossible and a method utilizing a movie camera was devised in the ADM Laboratory for measurement of bubble rise in this series. We are preparing the master standards for the Gardner and Cannon Laboratories using this technique. The Gardner Laboratory's time table calls for marketing of the entire numerical series sometime in June. No time table has been furnished by the Cannon Instrument Company.

"3. *Tentative Method Ka-8-58, Saponification Value.* The desire of AOCS to eliminate the use of ethyl alcohol in their methods whenever possible has prompted us to review the Saponification Value Method and study possible substitutions. Initial work in Subcommittee laboratories has shown that isopropyl alcohol may be a satisfactory substitution. A collaborative study designed to prove out these initial findings is being planned for the near future.

"4. A Dry Time Method is needed in the Drying Oil Analysis Methods. ASTM D-1 has a special Committee making a survey of all Dry Time Methods now utilized in the industry and we believe that any Dry Time Method for Section K should await the completion of this study.

"All other methods currently listed in Section K appear to be in satisfactory order."

Subcommittee on FAC Colors, E. W. Blank, Chairman

"Labline, Inc. of Chicago, Ill. is actively engaged in developing a three aperture colorimeter for use

with permanent F.A.C. glass color standards. The colorimeter should be completed shortly.

"The Subcommittee has agreed on a suitable arrangement of the glass color standards for use with the colorimeter. Prototype standards should be available soon.

"Upon submission of a completed model of the colorimeter together with a set of standards, including manufacturing tolerances on the later, the instrument will be circulated to the Subcommittee members for their approval. It is anticipated that the Subcommittee may be able to submit a close-out report in time for AOCS action at the Fall meeting."

Subcommittee on Commercial Fatty Acids, R. H. Dreyer, Chairman

The Committee for the Analysis of Commercial Fatty Acids has been inactive during the past year. This Committee has been asked to do some cooperative work with A.S.T.M. on those methods requiring the use of ethyl alcohol. The work will be pointed toward the replacement of ethyl alcohol by isopropyl alcohol which requires no permit for use in the laboratory. Additional work in the future will be planned as required.

Subcommittee on Metals in Fats and Oils, C. L. Hoffpauir, Chairman

"Due to the press of other activities, the work of the Metals in Fats and Oils Subcommittee has been in abeyance, and hence there is no progress to report."

Subcommittee on Determination of Saturated Fatty Acids, D. F. Kuemmel, Chairman

"I would hereby like to officially request that the subcommittee for the Determination of Saturated Fatty Acids be disbanded. The primary reason for terminating the activities of the subcommittee is the success of gas chromatography in determining saturated acids. Harold Boyd of General Mills and Hans Wolff of Staley Mfg. Co. are in agreement with me on discontinuing this subcommittee. I have not heard from Messrs. Luckmann and Buswell, but interpret their silence as not being interested in continuing the project."

The request of this committee for termination will be accepted.

Subcommittee on Gas Chromatographic Methods, E. M. Sallee, Chairman

"The initial goal of the subcommittee was a procedure suitable for the common commercial fatty acids. A procedure was written on the basis of previous committee experience and adopted at the Spring Meeting in St. Louis. Since preparation of methyl esters is a separate procedure being studied by the Spectroscopy Committee, it was agreed that samples for cooperative testing would be methyl esters. A known mixture and methyl esters of coconut, tallow, and vegetable fatty acids were distributed in August. Results were received from 20 collaborators and statistical analysis carried out on the data from 15 (5 labs reported late).

"On the basis of the cooperative test results the subcommittee recommended the inclusion of a tentative method in the AOCS Book of Methods."

Subcommittee on Determination of Hydrocarbons, R. L. Gregory, Chairman

This is a joint committee with the A.O.A.C. The approach has been to seek a relatively simple general method. Initially, column chromatography employing silica gel and activated alumina for separating hydrocarbons from a solution of the previously isolated unsaponifiable was investigated. This has been simplified to direct application of a solution of fat to an alumina column without prior isolation of unsaponifiable. The subcommittee has most recently evaluated the latter technique on high and low grade tallow with and without added mineral oil (at 3 selected levels). The recoveries and precision to this point are wholly satisfactory. Remaining considerations include studying recovery of hydrocarbons from lard and vegetable oils under conditions similar to those used previously for tallow."

Subcommittee on Oxygen Absorption, R. L. Gregory, Chairman

The Chairmanship of this subcommittee has been assumed in recent months from B. N. Rockwood, who began a study of 3 methods (1) a modified ASTM oxygen bomb (2) oxygen absorption by the Eckey procedure, and (3) the active oxygen method AOCS Cd12-57. These techniques were applied to lard, hydrogenated vegetable oil, edible tallow, and inedible tallow, all with and without antioxidants. The past chairman reported difficulty in that the interval between preparation and analysis of samples was an uncontrolled variable.

"In the interim since the last collaborative study, I'm sure that all interested parties have continued to pursue their individual research approaches to this problem. For this reason it was felt that a review of the current status of methodology and approach in the various participating laboratories would be the best basis for reactivating this committee research.

"In Swift's analytical research division, we have continued to pursue most actively the modified ASTM oxygen bomb in the same manner as investigated by the committee, but have also investigated possible means of shortening the elapsed time required for a determination using the same equipment and conditions. This, we believe, can be accomplished using an appropriate copper catalyst. Our work along these lines has been very promising and the time requirement can be reduced as much as desired. I have suggested to the membership that a modification using copper catalyst be included in this year's collaborative study.

"The level of catalyst serves as a variable control of reaction time with the remaining factors fixed. This approach could lead to the adoption of an official method which would also be applicable to control laboratories for routine examination of product. This seems infinitely desirable in that a standardized method would then be available to the industry for trading considerations. This technique could be studied in comparison with those already selected on animal and vegetable fats with and without antioxidants as previously. The committee has been acquainted with this proposal and suggestions have been solicited."

Subcommittee on Analysis of Feed Grade Fats, F. W. Quackenbush, Chairman

"The work of this Committee has been delayed somewhat because of the uncertainty of the analyt-

ical methods needed for a proper evaluation of feed grade fats. Initially, the intent was to provide methodology which would safeguard the use of fats free from toxic substances such as the chick edema factor when used in feeds. Tentative definitions have been drafted by the Association of American Feed Control Officials to include four specifications: free fatty acids, total fatty acids, total unsaponifiable, and total insoluble matter. The work of the Committee so far has established methodology suitable to both AOCS and A.O.A.C. on the first two of the above items. Methodology is not satisfactory for total fatty acids and not fully satisfactory for insoluble matter. Present work involves improvement in methodology of these two procedures and this will be the basis for discussion at the New Orleans meeting.

"The mission of this Committee will not be fully accomplished even if these four procedures are completely worked out to the satisfaction of both Societies. We do not have, and there is not on the horizon, a suitable procedure which will detect the presence of a small amount of toxic materials in fats. Such substances do show up in the hydrocarbon fraction and we are much interested in the progress that is being made by the Committee on Determination of Hydrocarbons. The Committee will also discuss again the feasibility of any further developments in methodology to detect toxic substances. It is not expected that any recommendations for method changes will be forthcoming at the present time."

Subcommittee on Analysis of Fatty Nitrogen Compounds, G. G. Wilson, Chairman

"The original AOCS Subcommittee was merged with a similar subcommittee of A.S.T.M. to form the present AOCS-A.S.T.M. Committee on Fatty Nitrogens.

"Following procedures have been approved by the Committee and forwarded to AOCS Uniform Methods Committees, and A.S.T.M. D-1 for adoption:

Fatty Amines—APHA Color, Gardner Color, % Water—Karl Fischer Method, Total Amine Value—Potentiometric Referee Method, Total Amine Value—Indicator Alternate Method, Primary—Secondary—Tertiary Amine Values—Potentiometric Referee Method, Primary—Secondary—Tertiary Amine Values—Indicator Alternate Method, I. V.

Fatty Quaternary Ammonium Chlorides—APHA Color, Gardner Color, % Water,—Karl Fischer Method, Acid Value and Amine Value, % Ash, Flash Point, I. V., Average Mol. Wt., % Non-Volalites, pH.

"We now have the following Task Groups at work:

Task Group 1—Fatty Aminoamides and Fatty Imidazolines
Task Group 2—Dimethyl Fatty Amines
Task Group 3—Fatty Diamines
Task Group 4—Fatty Amines

"We expect all of these groups to have new methods ready by next spring."

Subcommittee on Analysis of Antioxidants, E. R. Sherwin, Chairman

"During the past ten months, we have been working on a program to determine the interlaboratory error in the proposed methods for the quantitative analysis of propyl gallate or NDGA in lard. The laboratories collaborating in this program are the New York District of the FDA, Swift and Company Research Laboratories in Chicago, the FDA Laboratory in Washington, D.C., the Kansas City District Laboratory of the FDA, the Food Chemistry Section of the Canadian Department of National Health and Welfare in Ottawa, and the Research Center of General Foods Cooporation in Tarrytown, N. Y. Each of these laboratories, in addition to the Food Laboratory of Eastman Chemical Products, Inc., were supplied with a set of 24 samples of antioxidant-treated lard, each sample to be analyzed for propyl gallate or NDGA content in accordance with the analytical method being evaluated. All the laboratories completed their analytical work and reported the results, but some apparent discrepancies in the data led us to ask each laboratory to check calculations. The analytical results are in the hands of our statisticians, here at Eastman, and we hope to have their report on the test method in the near future. The report from the statisticians will enable us to decide our future course of action in the evaluation of the analytical method for propyl gallate and NDA in fat."

Subcommittee on Lecithin, T. C. Smith, Chairman

"For most part, the activities of the Lecithin Subcommittee have been confined to some private investigational work in connection with the determination of moisture by the distillation method for control purposes in our Lecithin processing plants.

"Our work has led us to the conclusion that the sample size (50 to 100 gms) as specified in AOCS Tentative Method Ja 2-46, is too large for realistic moisture results. It is assumed that the bp is proportional to the concentration. While we do not have any data at the moment to substantiate this assumption, we do have evidence of decomposition and higher apparent moisture values as the concentration of the lecithin in toluene is increased; therefore, to minimize the amount of decomposition and the resulting increase in moisture due to possible splitting off of water from sugars present, we reduced the sample size to 25 g for all types of lecithin.

"We have experimentally substituted benzol for toluene and found that the former (benzol) gave consistently lower moisture results on all grades of lecithin tested, ranging from 7.7 to 37.8% lower. At one time Lisle Brown (retired) suggested the use of benzol instead of toluene for distillation moisture determinations because of the difference in the bp of the two solvents and further stated that there was a pronounced difference in the amount of decomposition of the lecithin as evidenced by the degree of darkening of the lecithin in toluene. Our experience has confirmed the findings of Mr. Brown, however we believe that the sample size should be considered as a contributing influence on decomposition.

"The Lecithin Subcommittee, under the chairmanship of Herb Iveson, conducted several collaborative studies (1955–56) in the use of the Karl Fischer Method vs. the Toluene Distillation Method. At this time Staley included results obtained with benzol along with those they obtained by Karl Fischer and the toluene distillation method.

"The collaborative work reported left much to be desired. The reason for this is because of the different versions of the Karl Fischer method. viz.; electrometric and visual endpoint. The latter may have been responsible for discrepancies on dark material.

"The Society should have a color method for grading lecithin. The N.S.P.A., in their year book, give

lecithin specifications, including color to be read on 5% lecithin in water—white mineral oil against the 1953 Gardner color scale. At the present time the Central Soya laboratories are using the Gardner-Hellige varnish disc for reading the color of undiluted lecithin. We feel that the latter allows for much closer matching of colors than the 1953 Gardner color scale because of the difficulty in getting a match between the diluted lecithin and the standard.

"I feel that both moisture and color have been neglected because of a lack of interest and being contented to use house methods and/or modifications of existing methods of technical societies.

"The above subject matter can be discussed at the New Orleans meeting."

General Discussion

In addition to the work carried out by the regular Subcommittees, additional work has been carried out by the committees generally. This work will be discussed under the following headings.

1. *Testing a new supply of AOCS activated bleaching earth.* In collaboration with the Bleaching Committee of the AOCS, and the technical committee of the N.S.P.A., arrangements have been made to test a new lot of activated bleaching earth for suitability as a standard for the bleaching of both edible and inedible oils. This test program is now in progress. The actual instructions to the collaborators in the checking of the inedible oil samples has been graciously carried out by the Smalley Committee, under the Chairmanship of the Subcommittee on Tallows and Grease, J. R. Harrison. The collaborators in this test program were those laboratories who participated in the 1962 Smalley Test Program.
2. *Sampling and analysis of commercial fats and oils.* Procedure D of AOCS method C1-47 is for "Continuous Flow Method for sampling tank or tank cars during loading or unloading." This procedure has been unsatisfactory over the years because of inability to control the amount of sample taken. Under the conditions of the procedure frequently considerably in excess of 50 gal of oil are obtained. The Fat Analysis Committee is studying ways and means of controlling the amount of oil taken under this procedure and will, upon completion of its work, make recommendations to the Uniform Methods Committee for the necessary changes.
3. *AOCS procedure for the determination of neutral oil Ca 9f-57.* The Fat Analysis Committee has been informed by the Uniform Methods Committee and by the N.S.P.A. that consideration is being given to the adoption of the neutral oil determination as a replacement for the cup loss in trading soy bean oil. While the procedure in question has been a part of the AOCS methods since 1957, certain recommendations for changes in the method are being studied and can be made immediately to the Uniform Methods Committee if it becomes necessary to do so. The revised procedure is being checked by the Smalley Committee on 1962's Smalley samples. Recommendations for changes will be delayed until the information from this committee is available.
4. *Carbonyl Oxygen.* The Uniform Methods Committee has suggested the need for a procedure for the determination of carbonyl oxygen. Suitable procedures are available in the literature. At the committee meeting in 1962, these available procedures will be discussed and action will be taken to make the best procedure available to the society.
5. *Additional work.* Such additional work by the committee will be undertaken in 1962 as is required by request of the Uniform Methods Committee or the suggestion of AOCS members individually, or as may be deemed advisable by the chairman of the Fat Analysis Committee or by its members.

R. C. STILLMAN, Chairman,
Fat Analysis Committee

Autoxidation of Fatty Materials in Emulsions. I. Pro-oxidant Effect of Histidine and Trace Metals on the Oxidation of Linoleate Esters[1]

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Abstract

Aqueous emulsions of methyl or ethyl linoleate (sodium dodecylsulfate as emulsifier) together with such added components as l-histidine, metal chlorides, buffers, and acid or alkali, were oxidized in the dark with shaking in an oxygen atmosphere. Under optimum conditions (pH 6.5), the linoleate peroxide content, after 2 hr autoxidation at 20C, was increased more than 3-fold by the addition of 1 ppm of ferrous or ferric ions, approximately 20-fold by a 0.01 M concentration of histidine and more than 60-fold by the addition of both histidine and ionic iron. The pro-oxidative effect of other transition metal ions (Cu^{++}, Co^{++}, Cr^{+++}, Mn^{++}, and Ni^{++}) also was investigated. None of these ions had a significant effect alone. Combined with 0.01 M histidine, only Mn^{++} increased peroxidation over that when when histidine alone was added.

The pro-oxidative action of histidine was retarded approximately 60% by 0.1 N acetate buffer and completely repressed by 0.05 M phosphate, nonionic emulsifiers, and low and high pH. The threshold concentration of histidine necessary for pro-oxidative action was greater than 0.0001 M.

[1] Presented in part at the AOCS meeting, St. Louis, Mo., 1961.
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