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Methodology

Collaborative studies

Several of the AOCS technical committees have proposed collaborative studies for 1987. Other committees have studies in the planning stages.

All of these studies will need interested participants and laboratories having the necessary equipment, if special equipment is required. Equipment and instrument manufacturers have been most helpful in identifying equipment owners who would be willing to participate in collaborative studies. This simplifies the task of organizing the study. In those cases where availability, time and logistics permit, manufacturers may agree to loan equipment to the laboratories for the time it takes to complete the analysis. This facilitates the collaborative study.

For 1987, the following collaborative studies have been proposed:

- erucic acid (capillary gas chromatography)
- triglycerides (high performance liquid chromatography)
- tocopherols (high performance liquid chromatography)
- tocopherols (capillary gas chromatography)
- cold test
- dienoic acids
- oil content of rapeseed
- oil content of safflower seed
- standardized bleaching method

Anyone interested in participating in these collaborative studies is asked to contact the AOCS technical director as soon as possible.

There are other potential studies still in the initial planning stages. For example, a quality control method is needed to determine the purity of hexane used in the extraction of oilseeds. The gas chromatography committee currently is reviewing methods, and any suggestions for a useful method would be appreciated. Also, it has been suggested that an official method for automated total Kjeldahl nitrogen (TKN) analysis would be of benefit to laboratories performing a considerable number of TKN analyses. While one such method has been adopted by the Association of Official Analytical Chemists (AOAC) for the analysis of nitrogen in feeds, its utility in analyzing a variety of oilseed meals needs to be verified. Communications are under way with an equipment manufacturer. There is a possibility of organizing a joint AOCS-AOAC collaborative study to determine if the automated TKN method is applicable to a variety of oilseed meals.

Dave Berner
AOCS Technical Director

AOAC report

Former AOCS President David Firestone serves as general referee on fats and oils for the Association of Official Analytical Chemists (AOAC). The following is Firestone's report to the AOAC 1986 annual meeting. The report includes a summary of actions by the International Union for Pure and Applied Chemistry (IUPAC) Commission on Fats and Oils, which met in September in Vienna, Austria. Firestone is senior research chemist with the Food and Drug Administration's Division of Chemical Technology in Washington.

Antioxidants

Associate referee B.D. Page is continuing to investigate procedures to confirm the presence of antioxidants detected by the LC

TABLE 1

Repeatability Data[a] of Chromatograms Obtained With or Without Premixing of 30% Acetonitrile in Water Solvent

	Not premixed		Premixed	
Antioxidant	Peak area[b]	Peak height[c]	Peak area[b]	Peak height[c]
PG	0.355	0.542	0.309	0.563
THBP	0.222	—[d]	0.306	—[d]
TBHQ	0.278	0.791	0.370	0.772
NDGA	0.526	0.552	0.499	2.05
BHA	0.154	0.981	0.076 (0.0038)[e]	3.76
Ionox	0.237	0.659	0.345	3.48
BHT	0.221	0.576	0.183	1.97

[a]C.V., %; n = 4.
[b]By integrator.
[c]By hand measurement.
[d]Off scale—not determined.
[e]Integrator split BHA isomers. Unbracketed value, sum of isomers; bracketed value, 3-BHA only.

method (1). A diode array detector was used with the AOAC procedure to check UV maxima of peaks in several oils versus reference antioxidants. In one instance, the diode array detector was used to check suspected TBHQ in a safflower oil sample. It was demonstrated that TBHQ was not present since the UV maximum of the sample peak was different from that of a TBHQ standard. A number of different types of oils will be examined to further evaluate the new detector.

An investigator recently questioned whether chromatographic performance of the LC method for antioxidants in fats and oils (2) might be diminished due to production of a thermal gradient if the 30% acetonitrile in water mobile phase was not premixed (the eluting solvent is a gradient consisting of 30% acetonitrile in water to 100% acetonitrile). Accordingly, the associate referee compared chromatograms produced with and without premixing of the 30% acetonitrile in water. Chromatograms were generated by a dual-pump system. No significant difference in the chromatograms was observed with premixing versus not premixing (with premixing, the first peak, PG, eluted about six seconds earlier). Repeatability of the chromatograms with or without premixing is given in Table 1. A single pump system was not available to determine whether the temperature drop of the solvent would not be moderated by the connecting hardware to produce different chromatography than that obtained with the dual-pump system.

Emulsifiers

Associate referee H. Bruschweiler developed a procedure to analyze emulsifiers and lecithin in margarine. Preliminary separation on a silica column was applied. Triglycerides were eluted with hexane/diethylether (9:1), the polar fraction containing mono- and diglycerides with chloroform/methanol (2:1) and lecithin was eluted with methanol. The polar fraction contining mono- and diglycerides was silylated for GC analysis. The lecithin fraction was hydrolyzed and silylated and the silyl derivatives of α- and β-glycerol phosphates obtained were used for quantitation by GC. MS identification of peaks present in the polar fraction is useful because of the large number of components present in this fraction.

The associate referee has prepared a report for publication describing the results of collaborative study of the GC method for determination of components of ester-emulsifiers after hydrolysis and silylation. The collaborative study included GC determination of hydrolysis products of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, sorbitan monostearate and sorbitan monostearate in coconut oil. The procedure, also evaluated in the associate referee's laboratory, was found to be applicable to analysis of other emulsifiers (either in concentrates or as components in fats and oils) such as lactic acid monoglycerides, glycerol monooleates, sucrose esters of fatty acids, polyglycerol stearate and sorbitan tristearate. Identification and determination of the trimethylsilyl derivatives can be carried out with capillary as well as with packed columns.

A final report is under preparation of the capillary GC method for determination of mono- and diglycerides in concentrates and in fats and oils. The associate referee reported that recovery of mono- and diglycerides from concentrates or from fats and oils (1985 collaborative study) was in the range of 96–103%.

Hydrogenated fats

Associate referee R.A. DePalma has initiated an interlaboratory study of a capillary GC method for determination of C-18 monoene *trans* levels in hydrogenated fats and oils. Samples under study include mixtures of methyl elaidate and methyl oleate, commercial salad oil methyl esters and commercial salad oil that requires conversion to methyl esters before analysis. A 60 m × 0.25 mm fused silica capillary column coated with SP-2340 (cyano-silicone stationary phase) is required. If the results of the study are satisfactory, this method will undergo full collaborative study.

Lower fatty acids

There was no associate referee activity on this topic during the past year. Bannon et al. (3) reported that accurate analysis of fatty acid methyl esters prepared from fats containing short chain fatty acids required optimizing the total chromatographic system using a carefully prepared methyl ester primary standard and applying as correction

factors the theoretical flame ionization detector responses of Ackman and Sipos (4). The authors noted that errors result from failure to methylate quantitatively, subsequent saponification of the esters when alkaline methylating agents are used, incomplete transfer of the short chain esters from the aqueous to the organic layer, losses of short chain esters during work up or storage, discrimination during GC analysis, inadequate resolution of the short chain esters during GC, inaccurate integration of peak areas and failure to use accurate response factors. The authors outlined an optimized methylation procedure suitable for fats with fatty acids containing four or more carbon atoms. The optimum procedure rendered unnecessary the use of methyl pentanoate as an internal standard for accurate quantitation of methyl butyrate. Bannon (5) reported earlier a modified procedure for methylation of fats and oils with boron trifluoride-methanol, which was shown to maximize extraction of the esters into isooctane solution and improve quantitative accuracy and precision for esters down to and including methyl caproate.

Marine oils

Associate referee R.G. Ackman has initiated a preliminary interlaboratory evaluation of the efficiency and suitability of capillary GC for analysis of marine oil fatty acid methyl esters. Gelatin capsules of cod liver oil were sent to 14 participating laboratories with instructions to methylate a test portion with boron trifluoride-methanol reagent and examine the methyl esters using a flexible fused silica capillary column 25 m or more in length and 0.25–0.35 mm ID with bonded Carbowax-20 M (or equivalent polyglycol) liquid phase (e.g., Supelcowax-10 30 m × 0.25 mm, or 0.32 mm, column with 0.25 μm-thick coating). Comments from the collaborators suggested that a quantitative standard simulating the composition of fish oil should be included in a full collaborative study.

Olive oil adulteration

Associate referee E. Fedeli carried out a study of the triglyceride-free apolar fraction (including hydrocarbons, linear alcohol esters, sterol esters and fatty acid methyl and ethyl esters) isolated from virgin, refined and husk (pits and pomace) olive oils by silicic acid column chromatography (6). Capillary GC was used to analyze the apolar fraction eluted from the silicic acid column. Capillary GC and IR were used to prove the identity of the various components in the apolar fraction isolated by TLC. Determination of as little as 10% of husk oil in pressed (virgin) olive oil was demonstrated by statistical analysis of linear alcohol (wax) ester data from capillary GC analysis (husk oil contained 236–756 mg wax esters/100 g oil versus 8–31 mg/100 g in virgin olive oils). The associate referee also has been involved in developing methodology for determining triglyceride composition of olive oils and other oils and fats.

Oxidized fats

Associate referee A.E. Waltking has resigned. Michael M. Blumenthal has been appointed associate referee to replace Waltking. Sotirhos et al. (7) developed an HPLC method for analysis of oxidative and polymerized decomposition products in heated oils. Oil samples are passed through a silica Sep-Pak followed by elution of the Sep-Pak with solvents of increasing polarity to isolate polar oxidative and polymerized decomposition products, which are analyzed by normal phase silica column HPLC. The International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats and Derivatives carried out an initial interlaboratory study of a method for determining triglyceride polymers in fats and oils by HPLC (8). Separations are carried out on a column packed with 5-μm styrene-divinylbenzene size exclusion gel (differential refractometer detector). Three test samples and a check sample were analyzed by 10 laboratories (polymer contents, 2–22%; reproducibility CV, 7–30%). A second interlaboratory study will be carried out in 1987.

Pork fat in other fats

There was no associate referee activity on this topic during the past year. A report by the associate referee on detection of lard in

hydrogenated fats was published recently (9).

Sterols and tocopherols

Associate referee R.J. Reina participated in a 1986 interlaboratory study of an HPLC method for determining tocopherols, conducted by the IUPAC Commission on Oils, Fats and Derivatives (10). Four vegetable oil samples were distributed with tocopherol and tocotrienol levels covering the range likely to be encountered in vegetable oils (one of the samples was a refined palm oil specifically included for tocotrienols). In addition, two reference samples of blended oils were provided to assist in identification of the tocopherols. Participants also were supplied with copies of chromatograms indicating the HPLC separation of individual tocopherols and tocotrienols present in the reference samples. Results were received from 19 laboratories. Reproducibility CVs varied from 14–25% for vegetable oils containing 69–225 μg/g of various tocopherols. A reproducibility CV of 31% was obtained for β-tocopherol and 7% for γ-tocopherol in a soybean oil containing 17 and 508 μg/g, respectively, of these tocopherols. The collaborative results are under review by the Commission on Oils, Fats and Derivatives.

Bross (11) reported an improved HPLC analysis of tocopherols that affords separation of α-, β- and γ-tocopherols as well as the corresponding tocotrienols, requires no sample cleanup or sample preparation other than dilution and is applicable to fresh or oxidized oils and fats. Analysis is carried out on a 25 cm × 4.6 mm Supelcosil LC-NH_2 (amino bonded silica) 5 μm particle diameter analytical column with a 3 cm × 2.1 mm Peliguard LC-NH_2 40 μm particle diameter guard column. Limit of detection was reported to be ca. 8 ppm with a 1-cm path length UV absorption detector at 295 nm.

Commission on Oils, Fats and Derivatives, Applied Chemistry Division, IUPAC

The commission met on Sept. 2–4, 1986, in Vienna, Austria. The commission discussed 19 projects and topics, including methods for determining polycyclic aromatic mineral oil residues, phospholipids by HPLC, commercial lecithin products, polyenoic acids in food fats, tocopherols by HPLC, toxic metals in oils and fats, triglyceride composition by HPLC, polymerized triglycerides by reversed phase HPLC, polymerized triglycerides by gel permeation HPLC, *trans* unsaturation in margarines by GC and antioxidants by HPLC.

A capillary GC method for determining n-3 and n-6 fatty acids in food fats was subjected to collaborative study (12). Duplicate (blind coded) samples of partially hydrogenated low erucic rapeseed oil, partially hydrogenated soybean oil and partially hydrogenated soybean oil mixed with partially hydrogenated herring oil (a total of 10 samples) were sent to 16 laboratories. The method specified use of a 25–50-m fused silica or glass capillary column (0.20–0.35-mm ID) with a stationary phase of moderate polarity (Carbowax 20 M, Durabond 225, FFAP, Silar 5CP, Supelcowax 2-4080, CP SIL 88 or other suitable phase). Unfortunately, no GC performance specifications were given. One collaborator noted that separation of components in the test portions depended upon the polarity of the GC stationary phase. Good results required restricting the polarity of the stationary phase to a specific, small range. The collaborator also remarked that a reference sample and labeled chromatogram (noting all important peaks) were needed. CP SIL 88 was preferred by another collaborator over Silar 5CP. A third collaborator observed that *cis* and *trans* isomers of 18:1(n-9) acids were adequately resolved with a 30-m SP-2330 glass capillary column. Reproducibility CVs for n-3 acids in the test portions were 3.5% (3% n-3 acids), 16% (1.0% n-3 acids), 56% (0.24% n-3 acids), 106% (0.06% n-3 acids) and 185% (0.03% n-3 acids). Reproducibility CVs for n-6 acids in the test portions were 4% (42% n-6 acids), 9% (18% n-6 acids), 17% (16% n-6 acids), 32% (1.6% n-6 acids) and 44% (3.5% n-6 acids).

Results of collaborative study of the IUPAC method for determining erythrodiol in vegetable oil (and the method itself) were published in Pure and Applied Chemistry (13). Methods for determining (a) mono- and diglycerides; (b) hexane in solvent extracted oilseed meals; (c) iron, copper and nickel in vegetable oils by AAS; and (d) butyric acid in fats containing butterfat (and including statistical parameters as well as results of collaborative study) have been prepared in IUPAC format prior to submission to Pure and Applied Chemistry for publication.

The commission has prepared a report on precision clauses for inclusion in standard methods. The report includes definitions of repeatability and reproducibility values and presents several examples of statistical reports for standard methods.

Recommendation

Continue study on all topics.

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