Methods development update

Automated color in oils

The following article on the automated determination of color in oils was prepared by Mike Erickson of Interstate Foods Corp. Erickson currently is a member of the Commercial Fats and Oils Technical Committee and project coordinator for the Automated Color in Oils study.

Automated determination of color in oils has been the target of skepticism for some time. While automated colorimeter manufacturers are quick to point out how well results from their instruments correlate with results obtained by the traditional manual methods, "in-house" users in actual practice typically fail to substantiate the correlation. For example, results generated by a limited collaborative study conducted in September 1986 showed refined, unbleached cottonseed oil to range from 2.2 red to 4.6 red on split samples analyzed both manually and automatically. From 14 laboratories, the results obtained using automated colorimeters were consistently higher, except for one result in which the automated color was reported to be 2.2 red *lower* than the manual color.

These inconsistencies are in direct conflict with results published in a report generated by one automated colorimeter manufacturer, in which "darker" oils (oils greater that 4.0 red) showed comparative differences in red color of only a few tenths between automated and manual results. It should be noted that refined, bleached cottonseed oil did show reasonably good correlation when split samples used in the limited collaborative study were analyzed by both manual and automated methods.

In an effort to resolve the inconsistencies noted in the limited collaborative study and in response to the need for AOCS continually to investigate new and emerging technologies pertaining to fats and oils analysis, there will be a two-phase collaborative study tentatively scheduled to begin in January 1988. The first phase of the study already has been approved by the Uniform Methods Committee. In the first phase, a total of 18 samples will be analyzed in the collaborative effort. Samples will be issued through two separate mailings. A self-addressed, stamped envelope, along with a reporting form indicating the reporting deadline, will be included with each set of samples.

Active participation in this study will help AOCS to establish criteria governing the applicability of automated colorimeters. The potential is for developing, at a minimum, an AOCS Recommended Practice to be used conditionally in routine color analysis.

Phase 2 will attempt to establish a spectrophotometric method for use as an "official" or "referee" method when it becomes necessary to resolve disputes between laboratories. Phase 2 currently is in the planning stages. Suggestions would be appreciated.

Any laboratory wishing to participate in Phase 1 of the collaborative study on automated color in oils is asked to contact

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IUPAC report

The following report on the recent IUPAC meetings was prepared by David Firestone of the U.S. Food and Drug Administration in Washington, DC. Firestone is chairman of the AOCS Uniform Methods Committee.

The IUPAC Commission on Oils, Fats and Derivatives, meeting earlier this year in Münster, West Germany, and Boston, Massachusetts, discussed 16 projects and topics, including methods for determination of polycyclic aromatic hydrocarbons (PAHs), mineral oil residues, color in lecithins, acetone insoluable materials in lecithins, polyenic (n-3 and n-6) fatty acids in food fats, tocopherols by HPLC, determination of heavy metals by graphite furnace atomic absorption spectrophotometry (AAS), triglycerides according to their equivalent chain number (ECN) by RP-HPLC, phospholipids by HPLC, polymerized triglycerides by gel permeation (GP) HPLC, antioxidants by HPLC, *trans* unsaturation in fats and oils, fatty acid composition of unhydrogenated and hydrogenated animal fats and marine oils, mono- and diglycerides by HPLC, extraction of fats from foods, and guidelines for carrying out and evaluating the results of collaborative studies.

Study was proposed of two methods for PAHs, one a rapid method for benzo (a) pyrene (BaP) in fats and oils, the other a method for total PAHs in fats and oils. The method for BaP involves passage of a petroleum ether solution of the sample through a column of neutral aluminum oxide followed by additional cleanup by RP-HPLC (fluorimetric detection) and optional confirmation by GC/MS. The method for total PAHs involves complexation-extraction with caffeine, silica gel column purification, and RP-HPLC separation and quantitation with a diode array (UV) detector.

A single method is not capable of determining all the substances that might be considered mineral oil residues. Conventional GC would be applicable to the middle fraction of petroleum products, generally termed mineral oil. Headspace GC would be suitable for analysis of the light fraction, and HPLC could be considered for the heavy fraction. Study of an alumina column-capillary column GC method was proposed for determination of the middle fraction.

Capillary column GC was evaluated for determination of n-3 and n-6 polyunsaturated fatty acids in vegetable oils with different degrees of hydrogenation. Five samples of soybean oil were submitted to collaborators for analysis by capillary column GC (most participants used

CP Sil 88 columns). Resolution of individual fatty acids presented a problem with highly hydrogenated samples. Variations in reported results also were attributed to difficulties in identifying individual components. It was decided that a revised method should be drafted in which interpretation of the GC chromatogram is standardized, and that a capillary column GC method hould be proposed for determining the complete fatty acid composition (including *trans* components) of unhydrogenated and hydrogenated animal fats and marine oils and blends of marine and vegetable oils.

A collaborative study was carried out to check the applicability of the IUPAC HPLC method for tocopherols to products such as margarine which contain added tocopherol esters (added α-tocopherol acetate). Four samples (two pairs of blind duplicates) were sent to 10 collaborating laboratories. One of the pairs contained 10 μg/g α-tocopherol and a quantity of added α-tocopherol acetate equivalent to 85 μg/g α-tocopherol. A significant number of laboratories reported poor results for the samples with added α-tocopherol acetate, apparently due to incomplete saponification prior to HPLC analysis. The method was deemed suitable for free tocopherols but requires revision to define the saponification time, temperature and degree of vigorous shaking required to achieve complete saponification of tocopherol esters.

A method was proposed for determination of lead in fats and oils by graphite furnace AAS. Use of a matrix modifier (lecithin) is required to prevent loss of lead at the ashing temperature. A collaborative study will be carried out beginning in late 1987.

Also, a collaborative study was completed on a RP-HPLC method for determination of ECNs of triglycerides. Soybean, almond and sunflowerseed oils, and mixtures of almond and sunflowerseed oil were examined by 18 laboratories. The results were satisfactory, except for ECN 50 (present at 1-2% level). It was proposed that a 1987-88 study of the method include analysis of palm, rapeseed and olive oils. Determination of low levels of trilinolein would allow detection of small amounts of undeclared seed oils in olive oil (olive oil contains only a very low or undetectable level of trilinolein in the triglycerides). It also was suggested that the method could be extended to determination of mono- and diglycerides.

A method for determination of polymerized triglycerides by GP-HPLC was subjected to collaborative study. The method was unsatisfactory for levels less than 5%. A new collaborative study was planned for levels of 1-5% following validation of a revised procedure.

Excellent results were obtained in an interlaboratory study of AOAC method 20.009-20.013 for determination of antioxidants in oils and fats, and the method was adopted by the commission. New topics for consideration included methodology for extracting lipids from foods and methods for detecting refining and determining minor components of fats and oils.

The commission issued a set of guidelines for design, conduct and interpretation of collaborative studies representing the main recommendations agreed upon at a joint ISO/IUPAC/AOAC Harmonization Workshop held in May 1987 in Geneva. The guidelines state that the minimum number of materials to be used in most collaborative studies is five and the minimum number of participating laboratories is eight (with an absolute minimum of five laboratories). The best estimates of repeatability are to be obtained using a split-level design (single values from each of two closely related materials); a combination of split levels and blind duplicates in the same study; or alternatively, blind duplicates. Analysis of known (parallel) duplicates should be discouraged. Precision estimates should be calculated with no outliers removed and with outliers removed, using the Cochran and Grubbs outlier tests. The Grubbs test should only be applied to laboratory means. Outlier removal should be halted when more than 22% (i.e., greater than two of nine laboratories) would be removed as a result of sequential application of the outlier tests.

Methods for emulsifiers, thiobarbituric acid, TLC of phospholipids and metals (iron, copper and nickel) in vegetable oils by AAS were prepared for publication in **Pure and Applied Chemistry**. The 7th revised and enlarged edition of the Commission's **Standard Methods for the Analysis of Oils, Fats and Derivatives** (Blackwell Scientific Publications, Boston) was published in 1987.

The commission's new chairperson and vice chairperson are Joyce Beare-Rogers and A. Dieffenbacher, respectively. D. Pocklington continues as secretary. The commission's next meeting will be held Aug. 16-18, 1988, at the University of St. Andrews, St. Andrews, Scotland.

1987 Additions and Revisions to Methods

The 1987 additions and revisions to Methods number 56. Of these, 39 are revised methods and 17 are new methods. Because of the large number of additions and revisions, the usual mailing date of December 15 cannot be met. The projected mailing date now is between February 15 and March 1, 1988.

Considerable appreciation is owed to the technical committee for time spent in writing and approving these methods, to the Uniform Methods Committee for expediting the final approval of these methods and to Lee Gross of AOCS Production Department for facilitating the typesetting and printing of the methods.