

# Method development update

## Chromatography Committee

*The following summary was prepared from the report of the Chromatography Committee Chairman John Callahan from Colgate-Palmolive.*

The Chromatography Committee met on Tuesday, May 19, 1987, in New Orleans. The meeting was well attended, with 11 members and seven guests, and there appeared to be considerable interest in the new activities of the committee.

Progress is being made on the collaborative study for HPLC analysis of triglycerides being

TABLE 1

Sample Weight and Catalyst Mixture Specified in Standard and Amended Kjeldahl Method

	Kjeldahl Method AOCS Aa 5-38 (Rev. 1973)	Modified Kjeldahl Method
Sample weight:	1.7032 g	1.7032 g
Catalyst mixture:	0.65 g Mercury (0.7 g Mercuric oxide) 15 g K <sub>2</sub> SO <sub>4</sub> 25 ml H <sub>2</sub> SO <sub>4</sub>	0.7 g Copper Sulfate 15 g K <sub>2</sub> SO <sub>4</sub> 25 ml H <sub>2</sub> SO <sub>4</sub>

*We're looking forward to the housewarming!*

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TABLE 2

Cottonseed Analysis: Comparison of Copper Catalyst and Mercury Catalyst

	N	$\bar{x}$ , % Ammonia <sup>a</sup>	SD	RSD, %
Mercury Catalyst				
Sample 1	13	4.05	0.06	1.57
2	13	4.63	0.08	1.73
3	13	4.59	0.03	0.74
4	13	4.40	0.06	1.27
5	13	4.37	0.07	1.55
Copper Catalyst (1 hr digestion)				
Sample 1	13	3.99	0.07	1.82
2	13	4.61	0.09	1.88
3	13	4.54	0.13	2.86
4	13	4.34	0.09	2.18
5	13	4.32	0.09	2.04
Copper Catalyst (1.5 hr digestion)				
Sample 1	13	4.02	0.10	2.40
2	13	4.63	0.08	1.70
3	13	4.55	0.05	1.18
4	13	4.37	0.04	0.93
5	13	4.36	0.06	1.29

<sup>a</sup>Calculated to dry-weight basis from % ammonia and % moisture data submitted by collaborators.

TABLE 3

Cottonseed Meal Analysis: Comparison of Copper Catalyst and Mercury Catalyst

	N	$\bar{x}$ , % Ammonia <sup>a</sup>	SD	RSD, %
Mercury Catalyst				
Sample 1	13	8.62	0.09	1.08
2	13	9.05	0.12	1.36
3	13	8.59	0.10	1.15
4	13	9.04	0.07	0.82
5	13	8.56	0.08	0.98
Copper Catalyst (1 hr digestion)				
Sample 1	13	8.56	0.10	1.13
2	13	9.00	0.19	2.10
3	13	8.56	0.11	1.34
4	13	8.99	0.09	0.96
5	13	8.53	0.11	1.28
Copper Catalyst (1.5 hr digestion)				
Sample 1	13	8.59	0.08	0.95
2	13	9.03	0.12	1.34
3	13	8.58	0.10	1.18
4	13	9.01	0.08	0.91
5	13	8.55	0.08	0.98

<sup>a</sup>Calculated to dry-weight basis from % ammonia and % moisture data submitted by collaborators.

coordinated by V.K.S. Shukla of Aarhus Oliefabrik. Shukla's published method [fette, Seifen, Anstrichmittel 85:274, (1983)], which utilizes non-aqueous reversed-phase HPLC on a C18 bonded column with acetonitrile/THF mobile phase and UV detection at 220 nm, will be used in the collaborative study. Palm oil, cocoa butter, rapeseed oil, soybean oil and coconut oil will be included in the study. Shukla has approximately 15 collaborators from both the U.S. and Europe. Samples should be sent to collaborators by September 1, with a December 1 deadline for submitting results.

Phil Bross of Procter & Gamble has agreed to coordinate a collaborative study on HPLC analysis of tocopherols. Bross's method, presented at the 1986 AOCS meeting in Hawaii, will be used in the study. The method is normal phase HPLC on an amino bonded silica column using hexane/isopropanol gradient elution and UV detection at 295 nm. The method uses an internal standard (pentamethyl-6 chromanol), which currently is not commercially available. Bross has agreed to supply sufficient amounts of this compound for the study and to pursue the possibility of having it made available commercially. The study will include only oil samples and not deodorizer distillates. It was suggested that we look at four to five samples but so far only palm oil and soybean oil have been decided upon. The tentative timetable for this study is to have a single test sample sent to collaborators early in the fall, a final protocol submitted for approval by late this year and a full study under way in January 1988.

John McKinney of Ranchers Cotton Oil, Fresno, California, has agreed to coordinate a collaborative study on the analysis of free gossypol in unprocessed cottonseed products.

Those present at the meeting felt that the committee should participate in a joint AOCS-AOAC collaborative for *cis,cis*-polyunsaturated and *trans* fatty acids being coordinated by Dick DePalma of Procter & Gamble. The scope of the pre-collaborative study was to

TABLE 4

Peanut Analysis: Comparison of Copper Catalyst and Mercury Catalyst

		N	$\bar{x}$ , % Ammonia <sup>a</sup>	SD	RSD, %
Mercury Catalyst					
Sample	1	6	6.13	0.08	1.32
	2	6	6.10	0.10	1.70
	3	6	6.11	0.08	1.31
	4	6	6.07	0.08	1.27
	5	6	5.98	0.07	1.17
Copper Catalyst (1 hr digestion)					
Sample	1	6	6.08	0.10	1.59
	2	6	6.07	0.08	1.33
	3	6	6.06	0.06	0.94
	4	6	6.04	0.10	1.66
	5	6	5.93	0.10	1.72
Copper Catalyst (1.5 hr digestion)					
Sample	1	6	6.11	0.08	1.22
	2	6	6.07	0.09	1.55
	3	6	6.06	0.09	1.45
	4	6	6.04	0.09	1.51
	5	6	5.93	0.04	0.59

<sup>a</sup>Calculated to dry-weight basis from % ammonia and % moisture data submitted by collaborators.

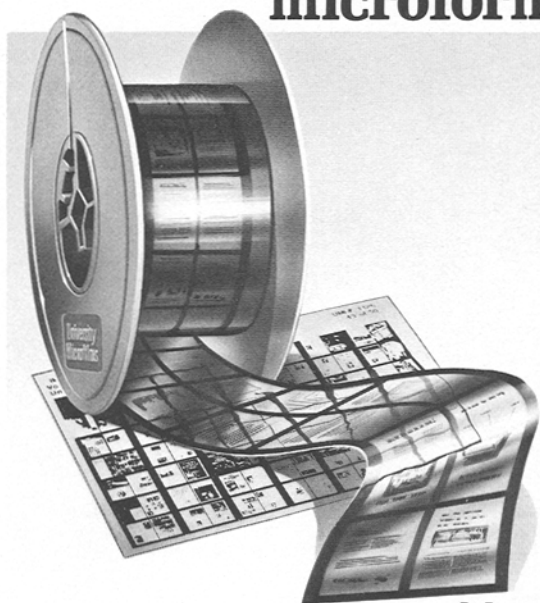
measure C18 monoene *trans* (elai-date) in vegetable oils but was expanded to include *cis,cis* PUFA (linoleate and linolenate).

There also was strong support at the meeting for participating in the upcoming collaborative on fish oils being initiated by Robert Ackman and the AOAC. According to David Firestone, chairman of the Uniform Methods Committee (UMC), the study will be based on the method published in the April 1987 *JAOCS* and will look at fish oil composition in general. Collaborators will be needed.

Four IUPAC methods have been rewritten and submitted to the UMC for consideration as Recommended Practices. Those methods are:

- 1.171 Determination of Total Hexane Content in Extraction Meals
- 1.172 Determination of Free Hexane in Extraction Meals
- 2.607 Determination of Hexane Residues in Oils
- 2.310 Determination of Butyric Acid

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TABLE 5

Cottonseed Analysis: Duplicate Results Reported by Six Laboratories

Sample	Lab	% Ammonia, moisture-free basis <sup>a</sup>					
		A. HgO		B. CuSO <sub>4</sub> , 1 hr		C. CuSO <sub>4</sub> , 1.5 hr	
1	1	4.03, 4.09	4.03, 4.00	4.07, 4.02			
	4	3.99, 3.94	4.04, 4.04	3.94, 3.92			
	6	4.03, 3.99	4.03, 4.07	4.07, 4.13			
	7	4.11, 4.01	3.95, 3.95	3.97, 3.99			
	9	4.00, 4.06	3.89, 3.87	3.93, 3.93			
11	4.05, 4.05	3.96, 3.96	4.02, 4.02				
2	1	4.66, 4.67	4.70, 4.64	4.62, 4.65			
	4	4.76, 4.76	4.74, 4.78	4.80, 4.84			
	6	4.59, 4.59	4.53, 4.49	4.55, 4.51			
	7	4.64, 4.71	4.60, 4.67	4.64, 4.63			
	9	4.57, 4.51	4.50, 4.48	4.55, 4.57			
11	4.64, 4.64	4.59, 4.64	4.60, 4.64				
3	1	4.57, 4.60	4.62, 4.58	4.51, 4.47			
	4	4.58, 4.52	4.49, 4.49	4.54, 4.50			
	6	4.56, 4.56	4.64, 4.70	4.59, 4.59			
	7	4.58, 4.62	4.52, 4.58	4.62, 4.62			
	9	4.56, 4.54	4.45, 4.47	4.55, 4.51			
11	4.59, 4.60	4.50, 4.59	4.50, 4.54				
4	1	4.40, 4.43	4.38, 4.40	4.40, 4.40			
	4	4.41, 4.47	4.41, 4.41	4.37, 4.41			
	6	4.25, 4.31	4.39, 4.29	4.41, 4.31			
	7	4.47, 4.43	4.36, 4.43	4.43, 4.41			
	9	4.37, 4.36	4.34, 4.36	4.43, 4.41			
11	4.44, 4.45	4.43, 4.45	4.37, 4.40				
5	1	4.40, 4.45	4.38, 4.41	4.35, 4.31			
	4	4.52, 4.54	4.39, 4.39	4.48, 4.52			
	6	4.31, 4.28	4.27, 4.31	4.35, 4.33			
	7	4.46, 4.43	4.33, 4.41	4.40, 4.37			
	9	4.33, 4.35	4.29, 4.29	4.33, 4.36			
11	4.38, 4.29	4.35, 4.35	4.39, 4.38				

<sup>a</sup>Calculated from % ammonia and % moisture data submitted by collaborators.

San Francisco. The AOCS Technical Director will be the task force coordinator.

#### Collaborative studies

The following collaborative studies have been proposed and are in the discussion stage: Wax in Sunflower Oil, Evaluation of the Tecator TKN Method for Crude Protein in Oilseed Meal, Evaluation of a Specific Ion Electrode for Sodium in Foods (mayonnaise, peanut butter, margarine, salad dressings, etc.). Interested persons should contact the AOCS Technical Director.

#### Comparison of the mercuric oxide and copper sulfate catalyzed methods for crude protein by TKN

The following report is based on a collaborative study organized by John Williams of U.S. Department of Agriculture's Cotton Division in conjunction with the Smalley Program. The report was drawn up by David Firestone of the U.S. Food and Drug Administration, Williams, and David Berner, AOCS Technical Director.

The need to reduce pollution from the Kjeldahl method for crude protein (total nitrogen) has long been recognized. Various catalysts or mixtures of catalysts have been investigated and copper sulfate has become a popular alternative to mercury. Rexroad and Cathey (1) demonstrated that low levels of CuSO<sub>4</sub> can be used as a substitute for mercury in the Kjeldahl method. Kane (2) conducted an interlaboratory comparison of copper vs. mercury as the digestion catalyst in the manual Kjeldahl determination of crude protein in animal feed constituents including cottonseed meal and soybean meal, and the copper catalyst method was adopted by the AOAC official first action (3).

The AOCS Seed and Meal Analysis Committee recently carried out preliminary studies of the Kjeldahl method (Aa 5-38) with CuSO<sub>4</sub> catalyst. Five laboratories participated in the 1984-85 study, six laboratories in the 1985-86 study. Good agreement was obtained between results with the mercury catalyst and the new copper

Based on meeting attendance and the interest shown in new projects, Callahan is very optimistic that the Chromatography Committee can be active and productive.

#### Recently approved methods

The Uniform Method Committee (UMC) has approved the Kel-Foss Automatic TKN method for determining crude protein in soybean meal. The method is restricted to soybean meal until a collaborative study including other oilseed meals can be organized. Another TKN method, the copper sulfate cata-

lyzed procedure for crude protein in oilseed meals, also has been approved. These and other methods will be published in December 1987 in the *Additions and Revisions to Methods*.

#### Joint task force

As a result of a suggestion by Lars Appleqvist, a joint AACC-AOAC-AOCS Task Force is being organized to assess the feasibility of harmonizing the oil in oilseed and crude protein (TKN) methods of the three organizations. The task force was organized at the recent AOAC International Meeting in

TABLE 6  
Cottonseed Meal Analysis: Duplicate Results Reported by Six Laboratories

Sample	Lab	% Ammonia, moisture-free basis <sup>a</sup>					
		A. HgO		B. CuSO <sub>4</sub> , 1 hr		C. CuSO <sub>4</sub> , 1.5 hr	
1	1	8.62, 8.59	8.64, 8.71	8.64, 8.68			
	4	8.66, 8.63	8.60, 8.60	8.67, 8.58			
	6	8.39, 8.41	8.54, 8.50	8.34, 8.37			
	7	8.61, 8.56	8.50, 8.41	8.61, 8.51			
	9	8.61, 8.61	8.56, 8.54	8.61, 8.58			
	11	8.75, 8.53	8.42, 8.59	8.61, 8.70			
2	1	9.08, 9.02	9.02, 9.06	9.07, 9.08			
	4	9.22, 9.24	9.30, 9.24	9.23, 9.23			
	6	8.82, 8.89	8.86, 8.90	8.82, 8.86			
	7	9.09, 9.00	8.94, 9.00	9.04, 8.99			
	9	8.93, 8.95	8.93, 8.97	8.97, 8.95			
	11	9.09, 9.11	9.15, 9.15	9.15, 9.09			
3	1	8.72, 8.68	8.67, 8.59	8.56, 8.59			
	4	8.47, 8.55	8.44, 8.51	8.43, 8.43			
	6	8.58, 8.62	8.51, 8.44	8.42, 8.51			
	7	8.56, 8.58	8.51, 8.51	8.59, 8.54			
	9	8.58, 8.56	8.49, 8.52	8.58, 8.54			
	11	8.60, 8.67	8.54, 8.74	8.65, 8.73			
4	1	9.10, 9.07	9.01, 9.08	9.10, 9.13			
	4	9.02, 9.12	9.07, 9.13	9.07, 9.07			
	6	8.90, 8.92	8.88, 8.90	8.83, 8.79			
	7	9.04, 9.02	9.02, 9.03	8.99, 9.02			
	9	8.95, 8.97	8.95, 8.93	8.98, 8.99			
	11	8.99, 9.06	8.88, 8.98	9.06, 8.95			
5	1	8.55, 8.52	8.58, 8.61	8.61, 8.58			
	4	8.61, 8.61	8.64, 8.64	8.60, 8.65			
	6	8.54, 8.47	8.54, 8.45	8.56, 8.47			
	7	8.51, 8.49	8.45, 8.41	8.47, 8.45			
	9	8.54, 8.55	8.52, 8.54	8.56, 8.54			
	11	8.56, 8.56	8.71, 8.58	8.61, 8.61			

<sup>a</sup>Calculated from % ammonia and % moisture data submitted by collaborators.

TABLE 7  
Cottonseed Analysis: Precision of Results Reported by Six Laboratories<sup>a</sup>

Sample	1	2	3	4	5
Mercury Catalyst;					
$\bar{x}$ , % Ammonia	4.03	4.65	4.57	4.40	4.40
SD	1.05	0.58	0.51	0.65	0.75
RSD, %	1.18	1.68	0.62	1.58	2.06
Copper Catalyst,					
1 hr digestion;					
$\bar{x}$ , % Ammonia	3.98	4.61	4.55	4.39	4.35
SD	0.39	0.76	0.83	0.83	0.63
RSD, %	1.65	2.26	1.76	1.04	1.17
Copper Catalyst,					
1.5 hr digestion;					
$\bar{x}$ , % Ammonia	4.00	4.63	4.54	4.40	4.38
SD	0.60	0.49	0.51	0.76	0.49
RSD, %	1.73	2.21	1.14	0.76	1.48

<sup>a</sup>See Table 5 for individual results reported by each laboratory.

catalyst (various AOCS Check Meal Series samples including soybean, peanut, cottonseed, cottonseed meal, safflower, rapeseed and protein concentrate were used in these studies). The only difference noted was that several laboratories required longer (20–30 minutes additional) digestion times. Accordingly, a collaborative study was conducted in early 1987 to compare the use of the mercury and copper catalyst (Table 1) for determination of total nitrogen by AOCS Method Aa 5–38. Five samples each of cottonseed, cottonseed meal and peanuts were sent to 15 participating laboratories with instructions to examine each of the samples in duplicate using the standard method (mercury catalyst) and the method revised to permit use of the copper catalyst with a reflux time of 1 hour and 1.5 hours.

Results were received from all 15 laboratories. Two laboratories used catalysts other than mercury or mercury oxide (copper sulfate or copper sulfide; selenium was substituted for the mercury catalyst) and their results were not included in the comparison of mercury vs. copper catalyst. Summaries of the results from the remaining laboratories [average values of mean ( $\bar{x}$ ), standard deviation (SD) and % relative standard deviation (RSD, %); 6 laboratories for the peanut samples] are shown in Tables 2–4. The precision data were calculated from averages submitted by the 13 laboratories. Seven laboratories submitted average results only, rather than the individual results of the duplicate analyses that were provided by the other six laboratories.

The results of duplicate analysis of the cottonseed and cottonseed meal samples from the six laboratories are recorded in Tables 5 and 6. An insufficient number of duplicate results were submitted for the peanut samples. Precision data for the cottonseed and cottonseed meal (six laboratories) are shown in Tables 7 and 8. Results obtained with copper sulfate catalyst were in good agreement with those obtained with the mercury catalyst. Means were slightly higher with the mercury catalyst; however,

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means with the copper sulfate catalyst (1.5 hours digestion) were close behind. Relative standard deviations were generally well below 2% and there was no statistically significant difference between the mercury and copper catalyst procedures. The Uniform Methods Committee approved adoption of the copper catalyst method with

1.5 hour digestion as an official AOCS method.

### Acknowledgment

Statistical data were provided by R.H. Albert. Kjeldahl catalysts mix was provided by Pope Kjeldahl Mixtures Inc., Dallas, Texas. The following laboratories participated in the collaborative study: A & L

Plains Agricultural Labs, Lubbock, Texas; Anderson Clayton & Co., Abilene, Texas; Barrow-Agee Laboratories Inc., Memphis, Tennessee; Fox Testing Lab Inc., Lubbock, Texas; Hahn Laboratories, Columbia, South Carolina; Mid-Continent Laboratories Inc., Greenwood, Mississippi; Mid-Continent Laboratories Inc., Jackson, Mississippi; Mid-Continent Laboratories Inc., Memphis, Tennessee; Morris Testing Labs, Macon, Georgia; Pattisons Laboratories, Harlingen, Texas; Plains Coop. Lab., Lubbock, Texas; Pope Testing Labs Inc., Dallas, Texas; Ranchers Cotton Oil, Fresno, California; Southern Cotton Oil Co., Memphis, Tennessee; Woodson Tenent Laboratories, N. Little Rock, Arkansas.

TABLE 8

Cottonseed Meal Analysis: Precision of Results Reported by Six Laboratories<sup>a</sup>

Sample	1	2	3	4	5
Mercury Catalyst;					
$\bar{x}$ , % Ammonia	8.58	9.04	8.60	9.01	8.54
SD	0.78	0.43	0.42	0.42	0.27
RSD, %	1.20	1.47	0.81	0.80	0.51
Copper Catalyst, 1 hr digestion;					
$\bar{x}$ , % Ammonia	8.55	9.04	8.54	8.99	8.56
SD	0.71	0.35	0.81	0.45	0.56
RSD, %	1.04	1.61	1.05	0.95	1.08
Copper Catalyst, 1.5 hr digestion;					
$\bar{x}$ , % Ammonia	8.58	9.04	8.55	9.00	8.56
SD	0.58	0.29	0.47	0.40	0.37
RSD, %	1.39	1.50	1.12	1.19	0.79

<sup>a</sup>See Table 6 for individual results reported by each laboratory.

### References

1. Rexroad, P.R., and R.D. Cathey, *J. Assoc. Off. Anal. Chem.* 59:1213 (1976).
2. Kane, P.F., *Ibid.* 67:869 (1984).
3. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th Edition, Association of Official Analytical Chemists, Arlington, VA, 1984, Method 7.033-7.037.

## Publications

### Book reviews

**Phytic Acid: Chemistry and Applications**, edited by Ernst Graf (Pilatus Press, 703 109th Ave. NW, Minneapolis, MN 55422, 1986, 344 pp., US \$54.95, elsewhere \$64.95).

This rather comprehensive paperback volume on phytic acid and its applications contains 20 chapters compiled by various authors. In general, the book is well illustrated although in a few instances, the printing is below standard. An adequate index is provided.

The book deals with the following topics: an overview discussing the possible beneficial effects of phytic acid; phytate metabolism in plants; fine structure of phytate-rich particles in plants; phytate and mineral bioavailability; analytical methods for phytate; phytases; commercial aspects of phytic acid; synthesis

and applications of immobilized phytic acid; radioactively labeled phytic acid and applications; biological properties of phytate-containing radiopharmaceuticals; influence of calcium on trace metal-phytate interactions; phytate and the epidemiology of heart disease, renal calculi and colon cancer; influence of phytic acid on starch digestibility and blood glucose response; inositol phosphates as modulators of oxygen affinity in hemoglobin (this aspect of phytic acid chemistry being the one with which biochemists are probably most familiar); incorporation of phytic acid into erythrocytes and its medical use; short- and long-term physiological effects of improved oxygen transport by red blood cells containing inositol hexaphosphate; cement-forming properties of phytic acid; interactions of inositol phosphates with

mineralized tissues; phytic acid and aflatoxin metabolism; and the use of phytic acid in the stripping volt-ammeteric determination of rare earth metals.

The antinutritional properties of phytic acid, i.e., formation of insoluble complexes with essential metal ions, have been the subject of much research and controversy for many years. Although this book does little toward resolving this controversy, it is timely in view of renewed interest in this important area of research. Each chapter has a reference list at its conclusion that provides an invaluable entry into the literature. This book should prove of interest to readers of *JAACS*. It is recommended reading, particularly for those contemplating research in this area.

W.J. Evans  
USDA, SRRC  
New Orleans, LA 70179