Committee on Food Nutrition

Sugar and Sugar Products

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Summary

Emphasis over the past year has been on editorial effort to bring Chapter 44, Sugar and Sugar Products of the Official Methods of Analysis of AOAC INTERNATIONAL (OMA) to the high standards set for the 17th Edition of the compendium. In addition, 2 method studies were reported and subjected to peer review, respectively, dealing with detection of adulteration of maple syrup by beet or cane sugar and determination of low levels of glucose and fructose in sugar.

The editorial effort could not cover improvements requiring peer review or experimental verification, as needed in some traditional methods based on reducing sugar determinations and in newer methods based on separation, mostly by liquid chromatography (LC), used worldwide to measure sugar product composition and purity. The General Referee will develop a program in this direction.

For the short term, the General Referee recommends the repeal of AOAC Method 920.192 (44.5.11), Winton Lead Number of Maple Syrups. The following methods should be changed to surplus status: 935.63B (44.1.24), Fructose in Sugar and Syrups According to Munson-Walker; 935.64B (44.1.26), Maltose in Sugar and Syrups According to Munson-Walker; 920.183(a) (44.4.09), Sugars (Reducing) in Honey According to Munson-Walker; and 932.15 (44.1.25), Fructose in Sugars and Syrups According to Jackson-Mathews. While the latter method is no longer in use, all other methods above refer to surplus methods and are redundant. Method 920.183(b) (44.4.10), Sugar (Reducing) in Honey According to Constant Volume, Lane-Eynon Method should be retained.

AOAC Method 999.03, Measurement of Total Fructan in Foods, currently in Chapter 44 of the 17th Edition of the OMA should be moved to Chapter 45, Vitamin and Other Nutrients, Subchapter 4, Nutritionally Related Components.

A method utilizing specific natural isotope fractionation and deuterium nuclear magnetic resonance spectroscopy (SNIF-NMR®) to detect beet or cane sugar addition in maple syrup received First Action approval. The method extends existing AOAC Method 995.17 applicable to beet sugar in fruit juices. A question is still open whether stable isotope ratio analysis is an effective means of detection of adulteration of maple syrup or honey when blends of beet and cane sugar or blends of beet sugar and corn syrup are used.

An AOAC/ICUMSA method for trace levels of glucose and fructose in sugar by means of ion exchange chromatography and pulse amperometric detection also received First Action approval. Scope was limited to raw cane sugar, because Horratt values for trace glucose and fructose in refined sugar were deemed too high. Future work is indicated, possibly, to define detection limits of the method and to show how it could be approved also as applicable to refined beet and cane sugars.

Selected Associate Referee Topics

Maple Sap, Maple Syrup, and Maple Products

Associate Referee Germain Brazeau reports on activities at the Canadian Food Inspection Agency about contaminants in maple syrup, such as sorbic acid, lead, iodine, and formaldehyde. Sorbic acid is determined by LC and UV detection at 260 nm. Trace lead is determined by inductively coupled plasma spectroscopy (ICP) of diluted acidified solution after digestion in nitric acid and hydrogen peroxide. Trace iodine is determined as iodide by ion selective electrode, adapted from AOAC Method 992.24, Iodide in Ready-To-Feed Milk-Based Infant Formula (1, 2). Formaldehyde is determined spectrofluorimetrically after reaction with Fluralon P, resulting in 1,4-dihydropyridine. This compound is extracted in isobutanol; the solution emits at 510 nm when excited at 410 nm. Fluorescence of a blank extract is subtracted to correct for interfering materials. This method has been adapted from work by several authors (3–6) and is considered a candidate for a collaborative study by next year. The Associate Referee recommends posting a search for laboratories that are interested in participating.

Recommendations

(1) Repeal Method 920.192 Winton Lead Number of Maple Syrup.
(2) Surplus Methods 935.63B, 932.15, 935.64B, and 920.183(a). Retain Method 920.183(b).
(3) Move Method 999.03 from Chapter 44 to Chapter 45 immediately following AOAC Method 997.08 by the same title.
(4) Cane and Beet Sugar Products: Associate Referee Gillian Eggleston, Southern Regional Research Center, U.S. Department of Agriculture, 1100 Robert E. Lee Blvd, New Orleans, LA 70124, Tel: +1-504-286-4446, Fax: +1-504-286-4367, E-mail: gillian@commservr.srsc.usda.gov, reports on possible ways to improve reproducibility of trace glucose and fructose analysis in refined beet sugar. Proposed approach requires accounting for possible interference of trace residual amino acids and making use of more recently available ion...
chromatography detector. The work may help expanding scope of method below (see 5). Continue study.

(5) AOAC/ICUMSA Method for Glucose and Fructose in Sugar: Associate Referee Kevin Schäffler, Sugar Milling Research Institute, University of Natal, Durban 4041, South Africa, Tel: +27-31-261-6862, Fax: +27-31-261-6866, E-mail: kevin@smlri.org, reports agreement to limit scope of ion chromatographic method followed by pulse amperometric detection for cane sugar (on account of potentially interfering amino acids in beet sugar). So far, however, only raw cane sugar has been approved for First Action. Continued study will verify under what conditions method may be applicable to refined cane and beet sugar and the additional work required. Continue study.

(6) Visual Appearance by Color, Turbidity, and Reflectance: Associate Referee Mary Godshall, Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd, New Orleans, LA 70124-4305, Tel: +1-504-286-4329, Fax: +1-504-282-5387, E-mail: godshall@commserver.srrc.usda.gov, reports no activity. Continue study and review status by end of 2000.

(7) Honey: Associate Referee Peter Martin, RP Services, Orchard Cottage, Crazies Hill, Reading RG10 8LU, United Kingdom, Tel: +44-118-940-2212, Fax: +44-118-940-1235, E-mail: Honeysci@aol.com, did not submit a report. Develop plan for 2001. Continue study.

(8) Maple Sap, Maple Syrup, and Maple Products: Associate Referee Germain Brazeau, Canadian Food Inspection Agency, Bldg 122 CEFT, Ottawa, ON K1A OC6, Canada, Tel: +1-613-759-1218, Fax: +1-613-759-1260, E-mail: gbrazeau@em.agr.can. Call for laboratories to participate in collaborative study of spectrofluorimetric method for formaldehyde in maple syrup. Continue study.

(9) Stable Isotope Ratio Analysis: Associate Referee Réal Paquin, Agriculture Quebec, C2 105, 2700 Rue Einstein, Saint Foy, QC G1P 3W8, Canada, Tel: +1-418-644-5232, Fax: +1-418-643-0131, E-mail: rpaquin@phy.ulaval.ca, worked on the 17th Edition revision and thus did not submit a report. Associate Referee Réal Paquin was of considerable assistance in updating and correcting analytical methods in OMA about this topic in preparation for the 17th Edition. The General Referee is seeking his assistance in updating isotope ratio methods for maple syrup. Continue study.

(10) E-16 Detection of Added Sugar in Maple Syrup by SNIF-NMR®: Associate Referee Yves-Loïc Martin, Eurofins Scientific, Inc., 2394 Rte 130, Dayton, NJ 08810-1519, Tel: +1-732-329-2999, Fax: +1-732-329-1031, E-mail: YvesLoicMartin@Eurofins.com, did not submit a report. The Associate Referee Yves-Loïc Martin has been approved for First Action in 2000. Continue study.

(11) Sugar Alcohols: Associate Referee Jeff Rohrer, Dionex Corp., 1228 Titan Way, PO Box 3603, Sunnyvale, CA 94088-3603, Tel: +1-408-481-4216, Fax: +1-408-737-2470, E-mail: Jeff.Rohrer@dionex.com, reports no activity so far this year. He may want to follow up on a suggestion that combinations of sugar alcohols and intense sweeteners be investigated. Such combinations have been observed in commercial “hypocaloric sweeteners” in Europe. Continue study and develop plan for 2001.

(12) Oligosaccharides: Associate Referee Dierk Martin, Südzucker AG Mannheim/Ochsenfurt, Wormser Straße 11, 67283 Obrigheim/Pfalz, Germany, Tel: +49-6261-635-9277, Fax: +49-6261-635-9331, E-mail: zsekreta@suedzucker.de, reports no activity so far this year. Review status and plans for 2001.

(13) Enzyme and Enzymatic Analysis in Sugar and Sugar Products: Associate Referee Susan Hunyadi Rodgers, Novo Nordisk Biochem, Inc., PO Box 576, Franklinton, NC 27525, Tel: +1-919-494-3252, Fax: +1-919-494-3460, E-mail: SHR@novo.dk, reports no developments of interest so far this year. Evaluate status and relevance of topic by end of 2000.

(14) Weighing, Taring, and Sampling: Associate Referee Michael C. Steele, R. Markey & Sons, Inc., 5 Hanover Sq, 12th Floor, New York, NY 10004-2614, Tel: +1-212-482-8600, Fax: +1-212-344-5838, E-mail: mcsteele@compuserve.com, did not submit a report. Update status of topic and plans for future by end of 2000.


References

(3) De Andrade, J.B., & Bispo, M.S. (1996) American Laboratory August, 56–58

Botanicals and Other Supplements

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Summary

Dietary supplement products remain popular, although growth of the market has slowed considerably from its 1997–1998 pace. Some of the decline in the growth rate is likely due to questions about the quality of supplement products. At a time when there is growing evidence of efficacy of
botanical and other supplements, quality issues are surfacing that make consumers more wary about their purchases (1). Many of these quality issues could be addressed if adequate standards of identity and rugged analytical methods for finished products were available. The intense growth of the industry over the past few years has far outstripped advances in analytical methodology.

The United States Pharmacopoeia (USP) continues to publish botanical monographs. A number of these monographs are official in the USP 24-NF 19 (a listing of the status of the monographs may be found at <www.usp.org>). Standards monographs for Chamomile, Cranberry, Feverfew, powdered Feverfew, Garlic, powdered Garlic, Ginger, powdered Ginger, Ginkgo, Asian Ginseng, and powdered Asian Ginseng have been completed and are Official in USP 24-NF 19 (2).

Monographs have been published in the Pharmaceutical Previews section of Pharmacopeial Forum (PF) as follows: Kava, powdered Kava, powdered Kava extract, semisolid Kava extract, Kava tablets (3), Licorice, powdered Licorice, powdered Licorice extract (4), powdered Garlic extract, Garlic delayed-release tablets (5), Ginger capsules (6), Echinacea angustifolia, powdered E. angustifolia, powdered E. angustifolia extract, E. pallida, powdered E. pallida, powdered E. pallida extract, E. purpurea root, powdered E. purpurea, powdered E. purpurea extract (7), Eleuthero, powdered Eleuthero, powdered Eleuthero extract (8), powdered Milk Thistle extract, Milk Thistle capsules, Milk Thistle tablets (9), Nettle, powdered Nettle, powdered Nettle extract (10), Goldenseal, powdered Goldenseal, powdered Goldenseal extract (11), coenzyme Q10 capsules and tablets (12).

In-process revisions of the chapter on Dissolution and Disintegration (13) as well as monographs on Garlic Fluid extract (14), Asian Ginseng, powdered Asian Ginseng, powdered Asian Ginseng extract (15), St. John’s wort (SJW), powdered SJW extract (16), Saw Palmetto (17), coenzyme Q10 (18), powdered Valerian extract (19), Ginkgo, powdered Ginkgo extract, Ginkgo capsules, Ginkgo tablets (20), Asian Ginseng capsules, Asian Ginseng tablets (21), Kava, powdered Kava, powdered Kava extract, semisolid Kava extract, Kava capsules, and Kava tablets (22) have also been published in the same Forum. The switch of Saw Palmetto in all of its forms from the National Formulary to the USP was also announced in PF (23).


In addition to these organizations, the Institute for Nutraceutical Advancement has an ongoing Methods Validation Program (<http://www.nutraceuticalinstitute.com>) for botanical raw materials testing. Sponsoring organizations (mostly businesses) nominate raw materials for which analytical methods are lacking, and the MVP laboratory develops and validates the method or validates methods submitted by sponsors. The validation process is similar to the AOAC Peer-Verified MethodsSM Program, and some of the methods developed by MVP have been submitted to that program.

A number of methods for determination of active constituents and “marker” compounds in botanicals have been published over the past year. Gurley et al. published a study that used a high-performance liquid chromatographic (HPLC) method to examine conformity to label claims and lot-to-lot variation in ephedrine alkaloid content of Ephedra- (<em>Ephedra</em> spp.) containing dietary supplements (25). Lebot et al. (26) examined morphological, phytochemical, and genetic variation of cultivars of Hawaiian Kava (<em>Piper methysticum</em>), the authors identified 14 different morphotypes and 2 chemotypes (based on results of RP–HPLC analysis for kavalactones). HPLC analysis also disclosed marked variability in kavalactone content for cultivars grown under different conditions. DNA fingerprinting by amplification restriction fragment polymorphism revealed remarkably little genetic variation between the cultivars.

One of the most important recent developments in the botanical supplements field was a series of reports that content of hyperforin in SJW (<em>Hypericum perforatum</em> L.) extract is associated with antidepressant activity in animals (27–29), human volunteers (30, 31), and in vitro (32–35). Orth and Schmidt (36) recently published a paper detailing the stability and stabilization of hyperforin. More recent SJW news are 2 human clinical trials published in the <i>British Medical Journal</i> that demonstrate that SJW is as effective as imipramine in treating mild to moderate depression (37, 38). Piscitelli et al. (39), Breidenbach et al. (40), and John et al. (41) have recently reported potentially harmful interactions between SJW and prescription drugs. Hepatic cytochrome P-450 enzyme systems are postulated to be responsible for these interactions (42, 43). In a study that demonstrates the difficulties involved with focusing on only one or 2 “marker” compounds as indicators of clinical efficacy, Butterweck et al. (44) reported that a fraction from a <i>H. perforatum</i> extract that was rich in flavonoids displayed antidepressant activity in an animal model. Two fractions obtained from a crude extract of the plant were active in the rat forced swimming test, but at different dosages. Preparative HPLC separation of the 2 active fractions found that they were mainly composed of hyperoside, isoquercitrin, miquelianin, and quercitrin (fraction IIa1), and small amounts of hyperoside and astilbin, and a large number of unknown compounds (IIa2). Pure compounds isolated from these fractions (including hyperoside, isoquercitrin, quercitrin, miquelianin, and the aglycons quercetin and astilbin) were tested for activity. All compounds except quercetin, quercitrin, and astilbin were active.

Two papers by Stermitz et al. (45, 46) further illustrate that the focus on single compounds or classes of compounds in setting quality standards for botanicals may not adequately reflect the biological activity of the whole plant. Berberine is known to possess antibacterial activity and is the major alkaloid of a number of plants. Stermitz et al. examined antibacterial activity of a traditional Native American plant, <i>Berberis fremontii</i>, and found that crude plant extracts possessed greater antimicrobial activity than did the pure alkaloid. The
group isolated a flavonolignan (5’-methoxyhydrcarpin) that inhibited the multidrug resistance pump found in the *Staphylococcus aureus* strain used in the study. The pump actively transports cationic substrates such as berberine out of the bacterial cell. Inhibition of the pump allowed the berberine levels to build up to lethal levels in the bacterial cell.

There have been a number of other papers dealing with the General Referee topic published in the past year. Cao and Liu (47) used an HPLC method for determination of rhein, emodin, chrysophanol, physcion, and aloemodin in Chinese rhubarb. Wang et al. (48) used micellar electrokinetic capillary chromatography to measure psoralen and isopsoralen in the Chinese medicinal herb *Psoralea corylifolia* L. A method for preconcentration and rapid HPLC determination of the major terpenes of neem oil was reported (49). Saeki et al. (50) used HPLC to compare saponin levels in commercial samples of *Platycodon grandiflorum* root to each other and with specimens obtained from botanical gardens and collected in the wild. Zhou et al. developed a rapid and sensitive method for the HPLC determination of parthenolide in feverfew (51). Liu et al. (52) evaluated SJW (*H. perforatum*) dietary supplement capsules by RP–LC, LC–PDAD, and LC/MS. Wang et al. (53) used an automated HPLC method with precolumn reductive preconcentration for the determination of ubiquinol and ubiquinone (co-enzyme Q-10) in human plasma. Luippold et al. (54) also examined biological fluids in a SPE/HPLC study of adenosine, S-adenosylhomocysteine and S-adenosylmethionine in biological fluids. Several investigators reported new methods for determination of melatonin. Inuma et al. (55) used pre-column derivatization and reversed-phase HPLC for the analysis, while You et al. (56) combined capillary electrophoresis with amperometric detection for rapid and sensitive determination of this dietary supplement ingredient. Andrisanoa et al. (57) studied the photodegradation of melatonin in commercially available dietary supplement products by HPLC with in-line postcolumn derivatization. The major melatonin degradation product was isolated and characterized (NMR, MS, FTIR), and the degradation kinetics were monitored using the HPLC method described. Pietta et al. (58) compared the antioxidant capacity (TEAC) of *Ginkgo biloba* flavonol and green tea (*Camellia sinensis*) catechin metabolites.

A number of herbal preparations were analyzed for 29 elements by neutron activation analysis (59). None was found to contain levels of toxic elements above those deemed safe by health authorities. Two calcium supplements were analyzed by flow injection (FI)–hydride generation (HG)–electrothermal atomization atomic absorption spectrometry (ETAAS), and the values obtained (0.55 and 0.66 µg/g) agreed with results obtained by previously validated methods. For a 500 mg sample, the limits of detection and quantification were 0.006 and 0.02 µg/g, respectively (60). A study for organochlorine pesticides in medicinal plants sold in Portugal found that 48% of the plants sold in pharmacies and 53% of those sold in medicinal herb stores exceeded maximum permitted residue levels (61). A study designed to evaluate the feed value of raw and cooked velvet bean (*Mucuna pruriens*) in chickens yielded an analytical method for determination of 1,3,4-dihydroxyphenylalanine (1-DOPA) in that plant (62). Hashimoto et al. used HPLC to quantitatively examine a number of Chinese medicinal plants belonging to the genus *Aristolochia* for their content of the nephrotoxic and carcinogenic Aristolochic Acids I and II (63). The authors found these compounds in all members of the genus and some members of the genus *Asarum*. Members of several other species sometimes substituted for *Aristolochia* were found to be devoid of these compounds. Kubec et al. (64) used ion chromatography to concentrate the amino acids of garlic, derivatized them with ethyl chloroformate, and reduced the derivatized S-alk(en)ylicsteine sulfoxides with sodium iodide. The authors then determined the compounds by gas chromatography. In the area of product quality determination, Raffi et al. used electron paramagnetic resonance and thermoluminescence to detect irradiation of herbs, spices, and fruit (65).

**Recommendations**

1. **Ephedra Alkaloids**: Associate Referee Richard A. Myers, 2160 Carolyn Way, Bountiful, UT 84010, Tel: +1-801-975-5132, Fax: +1-801-975-5132, E-mail: rickm@weider.com. Remove Associate Referee. Find and appoint an Associate Referee to design a collaborative study of the LC method for these compounds.

2. **Botanical Microscopy**: Associate Referee Stanley Cichowitz, U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN), HFS 315, 200 C St SW, Washington, DC 20204, Tel: +1-202-205-4480, Fax: +1-202-205-4091, E-mail: snc@fdacf.ssw.dhs.gov, reports that microscopy remains a potentially valuable tool for the identification of powdered botanical material. Unfortunately, although the basic equipment is relatively inexpensive, it remains highly labor intensive and expert driven. New, less labor- and expert-intensive technologies are becoming available, and these need to be compared to microscopy as a means of identifying botanical raw materials. Continue study.

3. **Hydroxycitric Acid in Garcinia cambogia**: Vacant. Find and appoint Associate Referee.

4. **Proanthocyanidins**: Associate Referee Andrew L. Waterhouse, Department of Viticulture and Enology, University of California, Davis, CA 95616, did not submit a report. Continue study.

5. **Ginsenosides (Ginseng Saponins) in Panax Species**: Associate Referees Ebeneezer Asafu-Adjaye, FDA, Testing and Research, Pharmaceutical Science, 4 Research Ct, Rockville, MD 20850, Tel: +1-301-427-1075, and Siu Kay Wong, Hong Kong Government Laboratory, 7/F Honmantin Government Offices, Kowloon, Hong Kong, PRC, Tel: +852-2762-3732, Fax: +852-2714-4083, E-mail: skwong@govlab.gen.gov.hk, report that a collaborative study on the determination of ginsenosides in ginseng has been conducted and the study report received. The report was reviewed and returned to AOAC. Recommend method for First Action, once the Associate Referees have addressed comments. Continue study.
References

(24) American Herbal Pharmacopoeia, Santa Cruz, CA
Infant Formula and Medical Diets

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Summary

Infant formulas are important products in our society. With the Infant Formula Act of 1980, the U.S. Food and Drug Administration has instituted and still utilizes compliance programs for its regulations. The Official Methods of Analysis of AOAC INTERNATIONAL devotes an entire chapter to infant formula methods and medical foods are included in the same chapter. Although the 2 sets of products have similarities, they are quite different. For instance, the ratios of protein to fat to carbohydrate in a medical food may vary considerably depending on a particular medical need. Changes in the ratio or changes in the type of protein, carbohydrate or fat, result in matrix changes in the laboratory sample. These changes may necessitate a new method. Use of encapsulation techniques for nutrients in manufacturing may call for more advanced methods. Methods available now for compositionally more stable infant formulas may not be compatible with a medical food matrix.

Many existing methods for medical foods depend on older technology, currently less favored by analysts because of concerns about safety and environmental impact. Saponification, for example, is a reliable rugged technique; however, it tends to produce high volumes of strong alkaline solvent, possible emulsions when partitioning into organic solvents, tending to generate hazardous waste in significant quantity. Newer rapid methods have been and are being developed; however, they need to be validated. Rapid methods utilizing matrix solid phase dispersion (MSPD) have been developed for vitamins A, E, K, and β-carotene (1–4). Recently, a technique using accelerated solvent extraction in conjunction with MSPD was also developed for vitamin K in medical food (5).

Recommendations

(1) **999.14 Choline in Infant Formula and Milk by Colorimetric Enzymatic Method**: Associate Referees Harvey Indyk, Anchor Products, PO Box 7, Waitoa, New Zealand, Tel: +64-7-889-3989, Fax: +64-7-887-1502, E-mail: indyk@clear.net.nz, and David Woollard, AgriQuality NZ Ltd., PO Box 41, 131 Boundary Rd, Auckland, New Zealand, Tel: +64-9-627-2508, Fax: +64-9-627-9750, E-mail: woollardd@agriquality.co.nz, report that the collaborative study has been completed and adopted as First Action in 1999. Continue study.

(2) **999.15 Vitamin K in Milk and Infant Formulas by LC**: Associate Referees David C. Woollard (see 1) and Harvey Indyk (see 1), report that the collaborative study has been completed and adopted First Action in 1999. Continue study.

(3) **997.05 Taurine in Powdered Milk and Powdered Infant Formulas by LC**: Associate Referees David C. Woollard (see 1) and Harvey Indyk (see 1), report that the collaborative study was adopted First Action in 1997. Recommend for Final Action. Continue study.

(4) **Niacin in Infant Formula**: Associate Referee Denis E. LaCroix, Food Composition Laboratory, Bldg 161, USDA, BARC E, Beltsville, MD, reports that the study Solid-Phase Extraction/Anion-Exchange LC Analysis of Niacin in Infant Formula has been accepted as a validated AOAC Peer-Verified Method #PVM4:1999. Continue study.

(5) **Fat Extraction in Infant Formula Using SFE**: Associate Referee Denis E. LaCroix (see 4) reports that study is complete. The data and protocol has been submitted to AOAC for review as a PVM. Continue study.

(6) **Total Folate, Tri-Enzyme Method**: Associate Referee James Martin, Atlanta Center for Nutrient Analysis, FDA, 60 8th St NE, Atlanta, GA 30309, Fax: +1-404-347-4225, re-
ports that due to workload constraints no progress has been made. Continue study.


(8) Vitamin D in Infant Formulas and Enteral
LC Method: Discontinue Topic.

(9) Folic Acid and Pantothenic Acid: A combined folic and pantothenic acid Associate Referee is not necessary. Change topic title to just Pantothenic Acid. Seeking Associate Referee. Interested scientists or organizations should contact this General Referee or AOAC INTERNATIONAL for additional information.

(10) Repeal Methods 992.06, 992.26 and 992.27 as they have been replaced with better methods in the 17th Edition of OMA.

References

(5) Chase, G.W., & Thompson, B. (2000) J. AOAC Int. 83

Fats and Oils

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Selected Associate Referee Topics

Hydrogenated Fats

Associate Referee M.M. Mossoba and colleagues at FDA, completed work on development and validation of the method for rapid determination of total trans fat content in fats and oils by attenuated total reflection Fourier transform infrared (ATR–FTIR) spectroscopy (1). Collaborative study results demonstrated high accuracy in the range of 1–40% trans, with less than 2% high bias relative to gravimetric values. The method is recommended for determination of unsaturated fatty acids with isolated trans double bonds in fats and oils as well as partially hydrogenated fats, and in lipids isolated from food products containing greater than 1% trans unsaturation. The method does not require weighing or dilution of test material in a solvent and requires about 5 min to perform the ATR–FTIR measurement. The ATR–FTIR method was adopted First Action at the Official Methods Board meeting in May, 2000. It has also been adopted by the American Oil Chemists’ Society (AOCS) as Official Method Cd 14d-99 (2).

Sedman et al. (3) extended the ATR–FTIR method to simultaneous determination of iodine value (IV) and trans content. FTIR-predicted values for IV and trans content of test oil samples were regressed (partial least squares [PLS] regression) against values determined by GC (4) for evaluation of accuracy. Results indicated that the technique was suitable for quality control laboratories requiring a rapid, simple alternative to the GC for acquisition of IV and trans data. Earlier, use of a disposable polyethylene IR card was described (5) as a sample carrier for the quantitative determination of trans content of fats and oils and margarine (oil or melted fat is applied directly to the IR card for analysis by FTIR spectroscopy). The disposable IR card was reported to work well for trans determinations without a heated flow cell or ATR accessory.

Li et al. (6) described the application of PLS to discriminating between different oil and fat products as well as determining IV by FT near-IR spectroscopy. Miyake et al. (7) used high resolution NMR spectroscopy to determine the trans fatty acid content of hydrogenated vegetable oils. Trans fatty acid content was calculated from the number of trans double bond carbons and average molecular weight derived directly from 13C-NMR and 1H-NMR spectra, respectively. Miyake and Yokomizu (8) also developed 13C-NMR methods for determining the composition of cis/trans and positional isomers in hydrogenated vegetable oils. It was suggested that a combination of 13C-NMR and GC should be used for precise determination of cis/trans and positional isomer composition of hydrogenated vegetable oils.

Marine Oils

Associate Referee R.G. Ackman of DalTech at Dalhousie University, reported that a method developed for determining squalene content of olive oil (9), present in the range of 0.3–0.7%, was satisfactory for measuring small amounts (0.03% or less) of squalene in seal oil. The method involves transesterification of the oil with alkali in methanol, hydrogenation of the methyl esters over Adam’s catalyst (PtO2), and GC analysis of the fully saturated methyl esters and squalene present in the methyl esters.

Olive Oil Adulteration

Acuna-Cueva et al. (10) studied the relationship between capillary column GC retention times of sterol trimethylsilyl ethers from olive oil and a set of 60 quantum-chemical, conventional and topological descriptors. Comparison of predicted and observed retention times is a possible technique for corroborating the identity of sterols determined by GC analysis.

Storelli and Gambacorta (11) reported a procedure for determining the identity of extra virgin olive oils based on evaluating the fatty acid and triglyceride fractions of test oils. The procedure allowed detection of small amounts of seed oil including hazelnut oil added to the extra virgin olive oil. Ruiz del Castillo et al. (12) proposed a method for detection of olive oils adulterated with less than 5% hazelnut oil involving reversed-phase (RP) liquid chromatography (LC) coupled to GC (RPLC–GC) for detection of filbertone
(5-methylhept-2-en-4-one). Mariani et al. (13) found that it was possible to detect addition of hazelnut oil to olive oil by evaluating tocopherol content. Olive oil contains more \( \beta \)-tocopherol than \( \gamma \)-tocopherol in comparison to hazelnut oil, while hazelnut oil has a higher content of \( \delta \)-tocopherol than olive oil that contains only trace amounts.

Romani et al. (14) determined the polyphenolic content in Tuscany olive oils by LC with diode array detection after solid–liquid extraction with an Extralut cartridge. Favier et al. (15) investigated the use of CO\(_2\) laser IR optothermal spectroscopy to detect adulteration of extra virgin olive oils with sunflower and safflower oils. Detection limits for these vegetable oils were estimated to be 4.5 and 6%, respectively. Guillen and Cabo (16) evaluated the usefulness of specific FTIR spectral bands for determining the presence of low levels of various seed oils (sunflower, sesame, corn, soybean, etc.) in olive oil, the presence of low concentrations of the oils (5–8%) was detected by observing small changes in certain IR spectral bands.

Sacha et al. (17) applied high-field (600 MHz) \( ^1 \)H-NMR spectroscopy to the analysis of extra virgin olive oil from 4 different Italian regions. The use of multivariate statistical analysis on the normalized intensities of \( ^1 \)H-NMR resonances due to \( \beta \)-sitosterol, \( n \)-alkenals, \( trans \)-2-alkenals and other volatile components allowed classification of oils with respect to region with 96% accuracy. Vlahov et al. (18) used high-resolution \( ^{13} \)C-NMR and statistical methods (PLS, principal components analysis [PCA], principal components regression [PCR], and Neural Nets) to classify extra virgin olive oils by cultivars and by geographical origin. The authors concluded that FT–NMR can be used for rigorous quantitative analysis of olive oils. Sacco et al. (19) used \( ^1 \)H-NMR spectra of phenolic extracts from extra virgin olive oil to classify the oils with respect to cultivar and different locations in the Apulia (Puglia) region of Italy. Olive oils were extracted with methanol and the extracts were washed with hexane to eliminate residual oil to obtain the phenolic extracts for \( ^1 \)H-NMR analysis. In addition, the fatty acid composition of olive oils was determined by GC. Fatty acid composition was used for discrimination of olive variety, while NMR data from the phenolic extracts allowed classification according to geographical origin.

Mavromoustakos et al. (20) focused on the olefinic region of the \( ^{13} \)C-NMR spectrum of virgin olive oil for development of a semiquantitative method to detect adulteration of virgin olive oil with seed oils. The authors observed 12 peaks resonating between 127.5 and 130 ppm (due to oleic and linoleic acid in olive oil) that were altered when authentic virgin olive oil was mixed with seed oil.

**Oxidized Fats**

Xu (21) measured total polar compounds (TPM) in several deep-frying oils (high oleic canola oils, sunflower oil, palm olein oil, and a partially hydrogenated canola oil) used to deep-fry potato chips. Both the AOCS official method (22) and the TPM VERI-FRY\textsuperscript{®} PRO (Libra Technologies, Inc., Metuchen, NJ) quick test were used to measure polar compounds in the frying oils. The quick test was found to be fast, convenient, economical, and reliable. However, quick test measurements at 490 nm rather than 590 nm (as recommended by the company) provided better correlations with TPM determined by the AOCS official method.

Moh et al. (23) described a near infrared (NIR) spectroscopic method for measuring peroxide value (PV) in crude palm oil (CPO). Calibration standards were prepared by oxidizing CPO in a fermenter at 90°C. The method was validated with an independent set of test samples prepared on another day. Bauer-Plank and Steenhorst-Slikker-Veer (24) used RP–HPLC with UV detection for determination of triacylglyceride (TAG) hydroperoxides (HPO) in vegetable oils. They also compared PV determined by iodometric titration and TAG–HPO by quantitative HPLC. Absolute quantitative results could not be obtained by HPLC for TAG–HPO. The authors noted that HPLC–UV should not be considered as an alternative for determination of PV by iodometric titration.

Osada et al. (25) reported the rapid analysis of oxidized cholesterol derivatives (25-hydroxycholesterol, 7-ketocholesterol, etc.) by HPLC combined with diode-array UV and evaporative laser light-scattering detection. The procedure was applied to identification of these oxidation products in food, plasma lipoproteins, and photo-oxidized materials. In addition, identification of the cholesterol oxidation products (COPs) was performed by HPLC–electrospray ionization MS. Tai et al. (26) presented an overview of the analysis, formation, and inhibition of COPs in foods. The authors noted that HPLC failed to resolve several geometrical isomers and double bond-free COPs, such as isomeric 5,6-epoxides and triol, could not be detected by UV. They concluded that the combination of GC and MS is an effective tool for determination of COPs.

**Sterols and Tocopherols (Vitamin E)**

Dutta and Normen (27) developed a method for resolution of all sterols in a complex mixture using low/medium polarity capillary GC. Trimethylsilyl (TMS) ether derivatives were analyzed on a fused silica DB-1701 (14% cyanopropyl-phenyl-methylpolysiloxane) column. Senorans et al. (28) performed analysis of free sterols in edible oils by direct injection of the oils into a GC system with on-line coupling of RP–HPLC and GC. Lechner et al. (29) determined both free and esterified sterols in vegetable oils by coupled on-line normal phase (NP) LC–GC. Choong et al. (30) described a simple, rapid method without derivatization for GC determination of free and total sterols in vegetable fats and oils.

De Greyt et al. (31) compared determination of tocopherols in vegetable oils by GC (using AOCS Official Method Ce 3-74) and by NP–HPLC (using AOCS Official Method Ce 8-89). Improved accuracy and reliability were achieved with the HPLC method. Goffman et al. (32) described an HPLC method for determination of tocopherols in single rape seeds. Tocopherols were extracted with isooctane and the extract was analyzed without additional purification with a 94% isooctane/6% tert-butyl methyl ether mobile phase and fluorescence detection (excitation at 295 nm, emission at 330 nm).
Bonvehi et al. (33) described a liquid chromatographic procedure (μBondapak-C_{18} column, 300 × 3.9 mm id, 10 μm particle size; fluorescence detector, excitation at 296 nm, emission at 330 nm) for determination of tocopherols and tocotrienols in vegetable oils. Solid-phase extraction was used to extract the tocols from the vegetable oils.

**Total, Saturated and Monosaturated Fat in Food Stuffs by Hydrolytic Extraction**

Associate Referee J. Szpylka, General Mills, is continuing investigation of procedures for determining total fat in food. Wei-Zou et al. (34) compared recently developed procedures for determining fat content of foods with the classical gravimetric technique. The authors concluded that acid hydrolysis/mixed ether extraction (followed by GC of the fatty acid methyl esters) is the most appropriate method for meeting labeling requirements for most cereal products.

Any interested scientists or associations performing these analyses are asked to contact this General Referee to become a collaborator or Associate Referee.

**Recommendations**

1. **Total Isolated trans Isomers in Margarines and Shortenings by Infrared Spectroscopy:** Magdi Mossoba, FDA, CFSAN, OPS DCH, 200 C St SW, Washington, DC 20204, Tel: +1-202-205-4938, Fax: +1-202-205-4128, E-mail: mmossoba@bangate.fda.gov. Adopted First Action in May 2000. Continue study. Change Topic Title to 2000.10 Determination of Total Isolated trans Unsaturated Fatty Acids in Fats and Oils by ATR-FTIR.

2. **Marine Oils:** Associate Referee Robert G. Ackman, DalTech, Dalhousie University, Canadian Institute Fisheries Technology, PO Box 1000, 1360 Barrington St, Halifax, NS B3J 2X4, Canada, Tel: +1-902-494-6030, Fax: +1-902-420-0219, E-mail: odoraj@dal.ca. Continue study.

3. **Olive Oil Adulteration:** Associate Referee Enzo Fedeli, Experimental Station for Oils, Via Giuseppe Colombo 79, Milano 20133, Italy, did not submit a report. Continue study.

4. **Oxidized Fats:** Associate Referee Michael Blumenthal, Libra Technologies, Inc., 101 Liberty St, Metuchen, NJ 08840, Tel: +1-732-321-5200, Fax: +1-732-321-5203, E-mail: mmphd@interactive.net, did not submit a report. Continue study.

5. **Sterols and Tocopherols (Vitamin E):** Associate Referee for this subject has resigned. Seeking new Associate Referee. Continue study.

6. **Total, Saturated and Monosaturated Fat in Food Stuffs by Hydrolytic Extraction:** Associate Referee John Szpylka, General Mills, 9000 Plymouth Ave N., Minneapolis, MN 55427, Tel: +1-612-764-3078, Fax: +1-612-764-3078, E-mail: john.szpylka@gennmills.com. Continue study.

7. **Oil in Oilseeds and Foods, Supercritical Fluid Extraction:** Associate Referee Leslie J.D. Myer, LECO Corporation, 3000 Lakeview Ave, St. Joseph, MI 49085-2396, Tel: +1-616-982-5406, Fax: +1-616-982-8987, E-mail: les_myer@leco.com, is continuing investigation of superfluid critical extraction (SFE) for determination of total fat in various matrices. Continue study.

8. **Determination of Fat Content in Baked Cereal Products by SFE:** Associate Referee Leslie J.D. Myer of LECO (see 7), is continuing investigation of SFE for determination of total fat in various matrices. Continue study. General Referee recommends that this topic be moved under the Cereal Products General Referee.

**References**

Dietary Fiber

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AOAC INTERNATIONAL methods for the determination of dietary fiber continue to dominate as the methods most used throughout the world for nutrition labeling of foods. The General Referee published 2 papers this year, (1) “What is Fiber? Current Controversies” in Trends in Food Science & Technology 10, 271–274 (1999) and (2) “When Is Dietary Fiber Considered a Functional Food?” in Biofactors, in press (2000). He was also co-author of a third paper “An Historical Perspective on Defining Dietary Fiber” in Cereal Foods World 44, 367–369 (1999) by J.W. DeVries, L. Prosky, B. Li, & S. Cho. A great deal of time was spent during the year on meetings with American Association of Cereal Chemists (AACC) committee members to determine if a new or revised definition for dietary fiber was called for, taking into consideration the most recent knowledge gathered on the physiological effects of dietary fiber. There was a joint meeting with the International Life Sciences Institute (ILSI), and meetings at the IFT and the AACC annual meetings, and a great deal of E-mail crossed the desks of the committee from national and international experts giving their opinion on the definition. The definition was discussed at the Millennium Vahouny Fiber Conference held in Vienna, VA, in March of this year and was discussed in greater detail at the Dietary Fibre 2000, Joint International Association for Cereal Science and Technology (ICC) and AOAC INTERNATIONAL Conference held in Dublin, Ireland, in May 2000. On June 12, 2000, the definition approved by the AACC Board of Directors was announced by Julie M. Jones, President of the AACC, at a press conference at the IFT meeting in Dallas. The approved definition was

“Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.”

The Joint Food Safety and Standards Group, Food Labeling & Standards Division, United Kingdom’s Ministry of Agriculture Fisheries and Food, announced in August of 1999 that they “now propose adopting AOAC INTERNATIONAL methodology as the UK’s preferred method of analysis for fibre for nutrition labeling purposes.” The recommended reference procedures for analysis of dietary fiber are Official Methods 991.43 and 997.08. All methods being considered for First Action at the present time are for foods that do not quantify as dietary fiber in the standard AOAC methods for dietary fiber, but which are oligosaccharides and polysaccharides that have the physiological actions commonly associated with dietary fiber.

Selected Associate Referee Topics

Polydextrose in Foods—Ion Chromatography Method

Associate Referee Stuart Craig, Danisco, has completed the collaborative study for this method. The Official Methods Board adopted with modifications the Determination of Polydextrose in Foods by Ion Chromatography (E-19) as First Action at the May 2000 meeting in Los Angeles, CA. The method was assigned Official Method number 2000.11. The method will be published as part of the collaborative study and will be included in the first revision to the Official Methods of Analysis of AOAC INTERNATIONAL (2000), 17th Edition.

Eight collaborating laboratories assayed 7 blind duplicate pairs of foods for polydextrose content. The 7 test sample pairs ranged from low (2%) to high (95%) levels. The following foods were prepared with polydextrose mixed into the other ingredients and then baked, cooked, or otherwise prepared: milk chocolate candy, iced tea, sugar cookie, grape jelly, soft jellied candy, and powdered drink mix. Collaborators also received a polydextrose standard to develop a calibration curve (correlation coefficient not less than 0.995). The method detected polydextrose by ion chromatography, after removal of interfering food components (high molecular weight solubles). Repeatability standard deviations (RSDr) ranged from 3.93 to 9.04%; reproducibility standard deviations (RSDb) ranged from 4.48 to 14.06%. The recovery was 94%.

Polydextrose is a 1 kcal/g randomly bonded polysaccharide prepared by vacuum thermal polymerization of glucose, sorbitol, and food acid as catalyst (89:10:1 ratio; 1). The aver-
age degree of polymerization is 12, and the range of molecular weight is 162 to about 20,000, with about 90% between 162 and 5,000 (2, 3). It is an approved food ingredient in over 50 countries and has physiological benefits consistent with dietary fiber (4–13). However, polydextrose (and other similar carbohydrates) are not included in the value obtained by the AOAC assay for total dietary fiber (TDF; 4). This is because polydextrose is not precipitated by an 80% ethanol test sample preparation step.

A number of methods have been developed for measurement of polydextrose in foods (14–18). The first method used acid hydrolysis to break glycosidic bonds, followed by colorimetric determination using phenol and sulfuric acid. This method is difficult, imprecise, and affected by the presence of other carbohydrates. An assay used currently in Japan to quantify polydextrose for subsequent labeling as fiber utilizes HPLC (15). Other HPLC-based methods (16–18) have been published. These HPLC methods work well in relatively simple food systems, but interfering substances (e.g., soluble gums, hydrocolloids, bulking agents, etc.) can confound the chromatogram in some foods. We have further developed the Stumm and Baltes method (16) by improving the test sample preparation step and demonstrating removal of possible interfering ingredients (inulin, maltodextrin, pectin, etc.; 19). This is the first published collaborative study on polydextrose. It has been previously demonstrated (19) that polydextrose gives no significant TDF value by current AOAC TDF methods. Therefore, the value obtained from this method can be added to the TDF value determined by current AOAC TDF methods without concern of double counting.

High-pressure anion exchange chromatography with electrochemical detection (HPAEC–ED) is a sensitive and selective technique for measuring carbohydrates. Stumm and Baltes (16) developed a technique to quantify polydextrose using amyloglucosidase and inulinase to remove soluble interferences, followed by HPAEC–ED. This method used centrifugal ultrafiltration, followed by a more rigorous enzyme treatment (amylglucosidase, isoamylase, fructanase), to remove potential interfering components present in foods.

**Trans Galactooligosaccharides in Food and Food Products, Ion-Exchange Chromatographic Method**

Associate Referee Jaap de Slegte, Borculo Domco Ingredients, The Netherlands, reported that *trans* galactooligosaccharides (TGOS) are fully soluble galactans that can be classified as dietary fiber because they are not digested in the small intestine but are fermented in the colon by the bacterial flora (14). A new analytical method, based on a method published by Quemener et al. (15) has been developed in the laboratory of Borculo Domco Ingredients to assess the concentration of TGOS in food and feed products. In order to validate the method, a collaborative study preceded by a precollaborative trial was conducted in the period from February to May 2000. The goal of the precollaborative trial was to eliminate minor errors from the method and to familiarize the participants with the instructions and the method. Twelve participating laboratories from various European countries (Italy, Germany, France, Switzerland, Finland, and The Netherlands) as well as the United States, received 14 laboratory samples with added TGOS (7 blind duplicates) for analysis. The laboratory samples were (the concentration of added TGOS is in parenthesis): yogurt drink (6%), lemonade syrup (15%), custard (5%), orange juice (4%), pet candy (2%), biscuits (8%), and infant formula (4%). Results were received from 11 laboratories; one failed to produce any results due to staffing changes. At the moment all data is being processed and statistical analysis is being completed to assess the performance of the method. After the calculations are complete, the final report will be written and sent for evaluation to the General Referee, the Statistical Advisor for the Food Nutrition Committee, the members of the Food Nutrition Committee, and finally to the Official Methods Board for approval of the method for First Action.

**Combination of AOAC Method for Total Dietary Fiber with Enzymatic–HPLC Determination of Indigestible Maltoextrin in Foods**

Associate Referees, Kazuhiro Ohkuma, Masutani Chemical Industry Co. Ltd., Japan, and Dennis T. Gordon, North Dakota State University, submitted the following report.

The Associate Referees have developed and validated a total dietary fiber (TDF) method for the measurement of resistant maltodextrin (RMD) in foods that is not fully recoverable by conventional TDF methods such as AOAC 985.29 or 991.43. Because the average molecular weight of RMD is only 2,000 Da, the lower molecular weight soluble dietary fiber components do not precipitate in 78% ethanol, therefore, RMD is not completely quantitated as dietary fiber by the current AOAC methods. The accuracy and precision of the method was evaluated through an AOAC collaborative study. The study involved 10 collaborating laboratories that assayed 12 laboratory samples (6 blind duplicate laboratory samples) containing from 1–95% RMD. The method consisted of 2 parts. In the first part the insoluble dietary fiber (IDF) and the high molecular weight soluble dietary fiber (HMWSDF) were measured using AOAC 985.29. In the second part the low molecular weight soluble dietary fiber (LMWSDF) was determined in the filtrate of AOAC 985.29 by HPLC. The TDF was calculated as the sum of IDF + HMWSDF + LMWSDF. RSD values of the 6 laboratory samples ranged from 1.30–5.45%. RSDv values of the 6 laboratory samples were 2.14–6.79%. The Official Methods Board is now considering the method for First Action.

**Recommendations**

(1) **2000.11 Polydextrose in Foods—Ion Chromatography Method**: Associate Referee Stuart Craig, Danisco Cultor, 440 Saw Mill River Rd, Ardsley, NY 10502, Tel: +1-914-674-6574, Fax: +1-914-674-6575, E-mail: stuart.craig@danisco.com. Adopted First Action in 2000. Continue study.

(2) **Trans galactooligosaccharides in Food and Food Products, Ion-Exchange Chromatographic Method**: Associ-
ate Referee Jaap de Slegte, Borculo Domest Ingredients, Needseweg 23, 7271 AB Borculo, The Netherlands, Tel: +31-545-256789, Fax: +31-545-256625, E-mail: j.deslegte@bdi.nl. Review and evaluate the results of the collaborative study and recommend the method for First Action. Continue study.

(3) Determination of Total Dietary Fiber in Selected Foods Containing Resistant Maltodextrin by HPLC and Enzymatic Gravimetric Method: Associate Referee Kazuhiro Okhuma, Masutani Chemical Industry Co., Ltd., 5-3 Kita-Itami, Itami City, Hyogo, Japan 664-8508, Tel: +81-727-71-2010, Fax: +81-727-72-0766, E-mail: xlm10052@nifty.ne.jp, and Dennis T. Gordon, North Dakota State University, PO Box 5728, Harris Hall, Fargo, ND, Tel: +1-701-231-7712, Fax: +1-701-231-7723, E-mail: dgordon@plains.nodak.edu. Recommend adoption as First Action. Add Dennis T. Gordon as an Associate Referee for this topic. This is a combination of the 2 previous topics called Total Dietary Fiber, Enzymatic HPLC Determination and Quantitative Determination of Maltodextrin, Ion-Exchange Chromatographic Method.

(4) 999.03 Measurement of Total Fructan in Foods, Enzymatic/Spectrophotometric Method: Associate Referee Barry V. Mc Cleary, Megazyme International Ireland, Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland, Tel: +353-1-286-1220, Fax: +353-1-286-1264, E-mail: info@megazyme.com. The method was accepted as First Action by AOAC 2 years ago. The method is described in detail in McCleary, B., Murphy, A., & Mugford, D.C., J. AOAC Int. (2000) 83, 356–364. Recommend approval as Final Action.


References

(13) Polydextrose Food Additive Petition (1978) #9A3441, Pfizer

Water-Soluble Vitamins

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Summary

In the Official Methods of Analysis of AOAC INTERNATIONAL (OMA), progress in the development of water-soluble vitamin methods in foods has been slow because of the inherent difficulty in applying new technologies such as HPLC to low analyte concentrations in complex materials. In the 1960s, a number of good microbiological procedures were developed, validated, and recorded in sub-section 2 of Chapter 45 of OMA (960.46, 952.20, 944.12, 944.13, 945.74, and 940.33). These remain the reference methods for B-vitamins in foods, although there is a case to update them in line with the microbiological procedures for infant formula and medical diets, validated in the early 1990s (OMA, Chapter 50). It should be kept in mind that methods designed for a wide range of food matrixes, called horizontal methods, are often more valuable than matrix-specific ones. However strategically difficult this may seem, an attempt should be made to avoid method replication. The same statements can apply to other matrix–analyte combinations, such as the fat-soluble vitamins, providing common extraction and detection procedures can be developed.

OMA Chapter 45 has many redundant vitamin tests, including the vitamin D rat and chicken bioassays in sub-section 3 and vitamin E colorimetric and GLC tests in sub-chapter 1. The water-soluble vitamin tests are generally based upon manual or semi-automated (AutoAnalyser) procedures. These procedures were developed in cooperation with cereal chemists and have been useful standard methods in the past. However, they are not always reliable because of potential interferences and are in need of replacement based upon
modem technology. Chromatographic separations are now the preferred methods for many water-soluble vitamin methods whenever the target analytes are easily identified and quantitated. Some vitamins such as biotin, vitamin B_{12} and folate are still difficult to measure chemically because of their low concentration or wide diversity of active congeners. In the course of time new techniques will undoubtedly assist in this area particularly with the rapid growth of diode-array detection and LC/MS instrumentation. In the meantime there is a lot of effort being placed into validating folic acid methods by HPLC. ELISA and biosensor techniques will also have an increasing important place in future vitamin-testing laboratories. The General Referee expects rapid growth of these techniques for micronutrient testing, accompanied by increasing miniaturization of current equipment.

Test procedures for niacin and niacinamide (961.14, 975.41, 981.16, and 968.32) are of particular concern to the General Referee because of the use of highly toxic cyanogen bromide. Their replacement therefore represents a priority. An Associate Referee has been found to perform a collaborative study using chromatographic procedures, both HPLC and capillary electrophoresis (CE).

Unfortunately, draft methods for the B-group vitamins (B_{1}, B_{2} and B_{6}) validated by the Committee of European Normalisation (CEN) did not meet the AOAC or ISO 5725 validation criteria. They are high quality procedures but require further validation using the harmonized procedures. This process is underway and attempts are being made to get new Associate Referees through CEN and by advertising in ILM. CEN is also validating a folic acid method using HPLC. This is a particularly popular analyte at the current time, attested to by the many Associate Referees working on new techniques in various committees. This is clearly a case where rationalization of effort is desirable by getting volunteers to co-operate which each other. In other areas, such as vitamin B_{12} (pantothenic acid), fewer advances have been made. An Associate Referee has also recently been selected to replace the surplus AOAC 945.73 (calcium pantothenate in vitamin preparations) and work should be underway early in 2001. If the method can be applied to certain dietary supplements, the scope will be increased.

The vitamin C titration method (967.21) is suitable for many test samples with high ascorbic acid concentrations and low color interference. However, alternative procedures are required for general food testing, particularly unfortified products. Extension of the method to include ascorbic acid and dehydroascorbic acid is also desirable. CEN has a vitamin C project underway and cooperation between the two organizations is being encouraged to produce a joint standard method with AOAC.

**Recommendations**

1. **Detection of Folic Acid in Foods by HPLC**: Associate Referee Shang-Jing (Jean) Pan, Abbott Laboratories, 3300 Stelzer Rd, Columbus, OH 43219, Tel: +1-614-624-7160, Fax: +1-614-624-7270, E-mail: shang-jing.pan@rossnutrition.com, reported that progress had been made on modifying the test sample preparation strategy, LC column, and gradient to improve the performance of the published method (J. AOAC Int. 75, 891–898 [1992]). The modified method is working well for “in-house” test samples. The commencement of the collaborative study has not been announced. Commence the collaborative study as soon as possible. Continue study.

2. **Vitamin C in Foods, HPLC Method**: Associate Referee Alan Brause, Analytical Services of Columbia, Inc., 9151 Rumsey Rd, Ste 190, Columbia, MD 21045, reported that a collaborative study was completed in 1995, which was extended to other food products. Nineteen laboratories participated in the study, using pre-column oxidation to ascorbic acid and UV detection but a full statistical evaluation has not been completed nor a report received by AOAC. Foods with low vitamin C content produced poor data using the described procedure. However, the Associate Referee has recently been in contact stating that a statistical evaluation of the results will be sent to AOAC shortly. Continue study.

3. **Determination of Niacin in Foods by Capillary Electrophoresis and Liquid Chromatography, Acid and Alkaline Extraction**: Associate Referee, V. Craige Trenerry, Australian Government Analytical Laboratories, 51–65 Clarke St, South Melbourne, Victoria 3205, Australia, Tel: +61-3-9685-1716, Fax: +61-3-9685-1788, E-mail: craige.trenerry@agal.gov.au. Prepare the method protocol for AOAC approval at the earliest opportunity. New study.

4. **Determination of Calcium Pantothenate in Multi-Vitamin Premixes by LC**: Associate Referee Gerald A Woollard, Department of Clinical Biochemistry, Auckland Hospital, Park Rd, Auckland, New Zealand, Tel: +64-9-307-4949 ext. 7317, Fax: +64-9-375-4301, E-mail: geraldw@ahsl.co.nz. Prepare the method protocol for AOAC approval at the earliest opportunity. New study.

5. **Thiamine (Vitamin B_{1}) in Foods by HPLC**: Vacant. Seeking new Associate Referee. Any interested scientist or organization is encouraged to contact this General Referee or AOAC INTERNATIONAL for further information.

6. **Riboflavin (Vitamin B_{2}) in Foods by HPLC**: Vacant. Seeking new Associate Referee. Any interested scientist or organization is encouraged to contact this General Referee or AOAC INTERNATIONAL for further information.

7. **Vitamin B_{6} in Foods by HPLC**: Vacant. Seeking new Associate Referee. Any interested scientist or organization is encouraged to contact this General Referee or AOAC INTERNATIONAL for further information.