codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 5 **CX/MAS 09/30/6**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Thirtieth Session
Balatonalmádi, Hungary, 9 - 13 March 2009

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

This document contains the Methods of analysis proposed by the following Committees in Draft Standards under elaboration and Standards that were adopted without the sections on methods of analysis or required further work on that section: Committee on Nutrition and Foods for Special Dietary Uses; Committee on Processed Fruits and Vegetables; FAO/WHO Coordinating Committee for Asia; and Committee on Sugars.

A. Committee on Nutrition and Foods for Special Dietary Uses

Methods of Analysis in the Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants

The 29th Session of the CCMAS endorsed some of the methods of analysis proposed in the above Standard, and the 30th Session of the CCNFSDU proceeded with the consideration of the methods (ALINORM 09/32/26, paras. 17-22). The Committee agreed to request advice from the CCMAS on the criteria for selecting Type II methods from a list of Type III methods because the working group had not found an agreement on such selection criteria. As an interim measure some of the methods were proposed as Type III for endorsement until clarification was received from the CCMAS how to select the appropriate Type II methods; these methods are indicated as III* in the Appendix.

In response to the questions raised by the 28th and 29th Session of the Committee on Methods of Analysis and Sampling it was agreed that:

- Methods using microbioassay had been reviewed
- More updated methods for total carbohydrates (AOAC 986.25, determination by difference) and for fats (AOAC 989.05 and ISO 8381 | IDF 123:2008 or ISO 8262-1 | IDF 124-1:2005) were being recommended;
- Vitamin C was expressed as ascorbic acid and the difference between the proposed methods for Vitamins K, B12 and B6 was provided in the list of methods submitted for endorsement; and
- A method for dietary fibre was not necessary to calculate the total energy as there was insignificant indigestible carbohydrate in infant formula.

See **Table section A** for the complete list of methods of analysis.

B. Committee on Processed Fruits and Vegetables

Standard for Aqueous Coconut Products: Coconut cream and coconut milk (CODEX STAN 240-2003)

When the above-mentioned Standard was finalized (21st CCPFV, 2002), the CCMAS did not endorse the methods for moisture, non-fat solids, total fat and total solids as the methods applied to milk and requested the Committee to provide clarification on whether these methods could also be applied to coconut cream and coconut milk. The standard was adopted by the 26th Session of the Commission (2003) without these methods. Both the 22nd and the 23rd sessions of the Committee agreed to request further comments on these methods based on the request from CCMAS.

The Committee agreed to forward methods of analysis specific for the determination of moisture, non-fat solids, total fat and total solids in coconut cream and coconut milk for endorsement and inclusion in the Standard for Aqueous Coconut Products (ALINORM 09/32/27, para. 83-84 and Appendix VI).

See **Table section B** for the complete list of methods of analysis and sampling plan.

C. FAO/WHO Coordinating Committee for Asia

Determination of capsaicin

The Coordinating Committee recalled that the 28th Session of the CCMAS had agreed to endorse the AOAC method (AOAC 995.03) as Type II and to temporary endorse the methods proposed in Annexes A and B as Type IV, since these methods were not fully validated. The Committee agreed to delete the method in Annex B because it was similar to the AOAC method.

The Coordinating Committee noted that the Republic of Korea had undertaken further validation of the method based on gas chromatography in Annex A and that its result had been published (CRD 11) as requested by the 28th Session of the CCMAS. It was agreed that references to dihydrocapsaicin (DHC) should be removed throughout in Annex A since DHC was not considered a quality factor in this standard.

See **Table section C** for the complete list of methods of analysis.

D. Committee on for Sugars

Standard for Sugars: Method for Determination of Colour in Plantation and Mill White Sugar

The last session of the Committee considered the recommendations of the Committee on Sugars for the methods for determination of colour in plantation and mill white sugar as requested by the Commission.

The Delegation of Brazil proposed that Method GS2/3-9 should be included as an alternative method for determination of colour since the principle of the method was similar to Method GS9/1/2/3-8, was equivalent and was widely used. The Delegation of the EC was of the opinion that the recommendations of the CCS should be supported. The Delegation of the United Kingdom informed the Committee that the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) under the chairmanship of a representative of British Sugar, UK, would be discussing this matter at its next meeting in October 2008 and proposed that the Committee should request an information paper from ICUMSA on its decisions regarding the methods for sugar before further consideration of the methods. In addition, the Delegation of the United Kingdom proposed that the Committee should request that ICUMSA should reconsider the numbering of its methods since the current numbering system was confusing to those not familiar with the analysis of sugar.

In view of the discussion, the Committee agreed to postpone consideration and endorsement of the methods for determination of colour in sugar to its next session pending inputs from ICUMSA.

The reply from ICUMSA is attached as **Table section D** and the Committee is invited to consider how to proceed with the Method for Determination of Colour.

A. COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES

Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants, CODEX STAN 72-1981[1]

Provision	Method	Principle	Notes and Type Proposed
Calories (by calculation)	Method described in CAC/Vol IX-Ed.1, Part III[2]	Calculation method	**Type III** 1. Currently adopted as a Type III method for Special foods. 2. The references in this method (methods of analysis and conversion factors for specific food ingredients) need to be updated.
Total fat	AOAC 989.05 ISO 8381|IDF 123:2008	Gravimetry (Röse-Gottlieb)	**Type I.** This method should apply to milk-based infant formula containing ≤ 5% starch or dextrin
Total fat	ISO 8262-1 |IDF 124-1: 2005	Gravimetry (Weibull-Berntrop)	**Type I**, this method should apply to milk-based infant formula
Fatty acids	AOAC 996.06	Gas chromatography	**Type III*** 1. Validated (but not for infant formulas). . 2. Adopted as Type II for determination of saturated fat for nutrition labelling purposes. 3. Information should be adequate for listing as a reference method (Type II), or if not, a tentative method (Type IV).
Trans fatty acids	AOCS Ce 1h-05	Gas liquid chromatography	**Type III*,** for infant formulae not containing milkfat 1. Method for Determination of *cis*, *trans*, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Vegetable or Non-Ruminant Animal Oils and Fats. 2. Validated (but not for infant formula). Performance statistics were extracted from the collaborative study report and are included with the

[1] ALINORM 09/32/26, Appendix VI

[2] Section 9. Calories by calculation – Section 9.2 Conversion Factors
(a) protein 4 kcal per g
(b) carbohydrate 4 kcal per g
(c) fat 9 kcal per g
(d) monosaccharides 3.75 kcal per g
(e) specific food ingredients See "Energy and Protein Requirements"(FAO Nutrition Meeting Report Series No. 52 or WHO Technical Report Series No. 522)
(f) other specific calorie conversion factors maybe used where the formulation of the food and the nutrient content are known and where such specific conversion factors are physiologically more meaningful than the factors listed above

			method. 3. Adopted as Type II for the purposes of the Guidelines for Nutrition Labelling 4. The method states "The method is not suitable for the analysis of dairy, ruminant, marine, long chain polyunsaturated (PUFA) fats and oils, or products supplemented with conjugated linoleic acid (CLA)." The method should therefore be endorsed for infant formulae not containing milkfat.
Trans fatty acids	AOAC 996.06	Gas chromatography	**Type IV, with optimisation for the determination of TFAs**
Total phospholipids	AOCS Ja7b-91	Gas liquid chromatography	**Type IV with suitable extraction and preparation procedures** 1. The method is applicable to oil-containing lecithins, deoiled lecithins, lecithin fractions; not applicable to lyso-PC and lyso-PE. 2. Validated. Reference Pure Appl. Chem. 64: 447 - 454 (1992). A summary of statistics from the IUPAC phospholipid collaborative study is included with the method. 3. Suitable extraction and preparation procedures applicable to infant formulae are needed in conjunction with this method. The Walstra & de Graaf procedure for the extraction of the fat is suitable. Reference: Walstra, P. & de Graaf, J. J. (1962) *Note on the determination of the phospholipid content of milk products*. Netherlands Milk & Dairy J., 16, 283-287. 4. Recommended as a tentative method (Type IV) since the method is not validated for infant formula.
Total carbohydrates	AOAC 986.25	Determination by difference	**Type II**. Determination by difference, i.e. the remainder after deducting fat, ash and crude protein from total solids.
Moisture/Total Solids	AOAC 934.01 AOAC 925.23, or IDF 12B:1987 ISO 6731:1989	Gravimetry	**Type I** No provision for moisture/total solids, however estimation of moisture content (total solids) is needed for calculation of carbohydrates and calories.
Ash	AOAC 942.05	Gravimetry	**Type I -** No provision for ash, however estimation of ash content is needed for calculation of carbohydrates and calories

Vitamin A[3]	AOAC 992.04 (retinol isomers)	High performance liquid chromatography	**Type III* -** Currently adopted as Type II method for follow-up formula Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)
Vitamin A	AOAC 992.06 (retinol)	High performance liquid chromatography	**Type III*-** Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)
Vitamin A	EN 12823-1:2000 (all-trans-retinol and 13-cis-retinol) Vitamin A (both natural + supplemental ester forms) aggregated and quantified as individual retinol isomers (13, cis and all-trans)	High performance liquid chromatography	**Type III** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15. 2. Collaboratively tested according to ISO 5725, among others an enriched milk powder was included in the validation. In accordance with the EU MAT Certification Study Guidelines, the parameters for margarine (CRM 122) and milk powder (CRM 421) have been defined in an interlaboratory test. The study was organised by the Institute of Food Research, Norwich, United Kingdom. 3. Reference: Finglas, P.M., van den Berg, H. & de Froidmont-Gortz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg.
Vitamin D[4]	AOAC 992.26	HPLC	**Type III,** with limitations on applicability to infant formula containing 488-533 IU/L The minimum requirement for vitamin D in Codex STAN 72 is 280 IU/L D_2 and/or D_3 measured as single component. Method cannot discriminate if both present. Hydroxylated forms not measured.

[3] Note on the form of Vitamin A in Codex Standard 72

Footnote from Codex Stan 72, 3.1 Essential Composition, Vitamin A - *Vitamin A: expressed as retinol equivalents (RE).*

1 μg RE = 3.33 IU Vitamin A = 1 μg all-trans retinol. Retinol contents shall be provided by preformed retinol, while any contents of carotenoids should not be included in the calculation and declaration of vitamin A activity.

Comment: Carotenoids are unequivocally excluded from declaration of vitamin A content.

The requirement that vitamin A content shall be provided by "preformed retinol" implies only naturally present retinol, and excludes the common vitamin A acetate and palmitate supplements. These forms are physiologically active and may be quantified either specifically as intact esters and aggregated with natural retinol, or converted to retinol during analysis. It would seem that the standard should provide for all forms of retinol present in infant formula, whether preformed or derived from supplemental acetate and/or palmitate forms. It does not make sense to exclude vitamin A added for nutrient purposes from this provision and it seems at the least, that "preformed' should be removed from the standard

Vitamin D	EN 12821:2000 (D2 and/or D3 measured as single components. Hydroxylated forms not measured.)	HPLC	**Type III*** 1. Precision data for various foods is in CCNFSDU 29th session CRD 15 2. Validated. Collaboratively tested according to ISO 5725, among others an enriched milk powder was included in the validation. 3. Reference: EN 12821:2000. Foodstuffs - Determination of vitamin D by high performance liquid chromatography - Measurement of cholecalciferol (D3) and ergocalciferol (D2) 4. The parameters on margarine (CRM 122) and milk powder (CRM 421) have been defined in an interlaboratory test, in accordance with the EU MAT Certification Study Guidelines. The study was organized by the Laboratory of the Government Chemist, UK. Reference: Finglas, P.M., van den Berg, H. and de Froidmont-Görtz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg. 5. The parameters on milk, liquid infant, formula, cooking oil, margarine, infant formula and fish oil have been defined in an interlaboratory test according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis. The study was organized by NMKL (Nordic Committee on Food Analysis). Reference: Staffas A, Nyman A. JAOAC Int., 2003, 86:400-406 6. D2 and D3 measured as single component. Method cannot measure the content of vitamin D if both forms are present. Hydroxylated forms not measured. The method is capable to quantitate D2 and D3 in the same sample, it is just not described.
Vitamin D	AOAC 995.05	HPLC	**Type III*** (D_2 or D_3. Method can discriminate if both present. Hydroxylated forms not measured).

[4] Note on the form of Vitamin D in Codex Standard 72 - Footnote from Codex Stan 72, 3.1 Essential Composition, Vitamin D - *Calciferol. 1 µg calciferol = 40 IU vitamin D*

Comment: Calciferol is not specific and conceivably includes all forms of vitamin D. This currently generic descriptor could therefore include the parent forms of vitamin D2 and D3 and the physiologically antirachitic hydroxylated metabolites. For food nutritional labelling requirements it is however implicit that the parent cholecalciferol (vitamin D3) is the target nutrient, given that this is the form commonly added to infant formulas. The current definition does not discriminate ergocalciferol (vitamin D2) which is rarely added to foods.

Vitamin E[5]	AOAC 992.03	HPLC	**Type III*** Measures all-rac-vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual α -congeners.
Vitamin E	EN 12822: 2000 (Measures Vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual tocopherol congeners (α,.β, ..χ, δ).	HPLC	**Type III*** 1. Validated. Precision data for various foods incl. milk powder is in CCNFSDU 29th session CRD 15. Collaboratively tested according to ISO 5725, among others, an enriched milk powder was included in the validation. 2. The parameters on margarine (CRM 122) and milk powder (CRM 421) of different methods for the determination of Vitamin E (α-tocopherol) have been defined in an international comparison study organised by the European Commissions Standard, Measurement and Testing program. Reference: Finglas, P.M., van den Berg, H. and de Froidmont-Gortz, I., 1997. The certification of the mass fractions of vitamins in three reference materials: margarine (CRM 122), milk powder (CRM 421), and lyophilized Brussels sprouts (CRM 431). EUR-Report 18039, Commission of the European Union, Luxembourg. 3. In accordance with ISO 5725 : 1986 [19], the validation data on milk powder and oat powder have been defined in an inter-laboratory test. The test was conducted by the Max von Pettenkofer-Institute of the Federal Health Office, Food Chemistry Department, Berlin, Germany. Reference: Untersuchung von Lebensmitteln - Bestimmung von Tocopherolen und Tocotrienolen in dietätischen Lebensmitteln L 49.00-5 September 1998 (Food Analysis - Determination of tocopherols and tocotrienols in dietetic foodstuffs L 49.00-5 September 1998) in: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG: Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedargsgegenständen/Bundesgesundheitsamt (In: Collection of official methods under article 35 of the German Federal Foods Act, Methods of sampling and analysis of foods, tobacco products, cosmetics

[5] Note on the form of Vitamin E in Codex Standard 72 - Footnote from Codex Stan 72, 3.1 Essential Composition, Vitamin E

1 mg α-TE (alpha-tocopherol equivalent) = 1 mg d-α-tocopherol

Vitamin E content shall be at least 0.5 mg α-TE per g PUFA, using the following factors of equivalence to adapt the minimal vitamin E content to the number of fatty acid double bonds in the formula: 0.5 mg -TE/g linoleic acid (18:2 n-6); 0.75 α-TE/g α-linolenic acid (18:3 n-3); 1.0 mg α-TE/g arachidonic acid (20:4 n-6); 1.25 mg α-TE/g eicosapentaenoic acid (20:5 n-3); 1.5 mg α-TE/g docosahexaenoic acid (22:6 n-3).

Comment: The standard does not provide conversion factors to determine tocopherol equivalents derived from the multiple vitamin E congeners potentially present in an infant formula. Neither the congeners (α, β, γ, δ), their tocotrienol equivalents or the supplemental α -tocopheryl acetate form

			and commodity goods/Federal Health Office), Loseblattausgabe September 1998, Bd. 1 (Loose leaf edition as of 1998-09, Vol.1) Berlin, Köln: Beuth Verlag GmbH 4. Measures Vitamin E (both natural + supplemental ester forms) aggregated and quantified as individual tocopherol congeners (α, β, γ, δ).
Vitamin K[6]	AOAC 992.27 (trans-K_1).	HPLC.	**Type III***
Vitamin K	AOAC 999.15	HPLC with C30 column to separate the cis- and the trans- K vitamins	**Type III* -** Proposed by CCNFSDU 28. CCMAS 28 asked for clarification of the differences from AOAC 992.27. Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column. Can also discriminate and measure dihydro-K_1 and menaquinones). Consideration needs to be given to i) ability to discriminate the cis and trans-forms of K1 which can be accomplished with a C30 column, ii) whether the menaquinones (K2) be included. AOAC 999.15 is a more specific fluorescence method than AOAC 999.27 and has a better sample preparation with enzyme instead of a labor-intensive multistep procedure. AOAC 995.15 & EN 14148 are based on a joint AOAC/EN collaborative study. The main weakness with this procedure is that both cis- and trans- K1 (total K1) are determined. The cis-form is inactive. To overcome this problem, the C18 HPLC column must be replaced by a C30 HPLC column which separates the two vitamers.
Vitamin K	EN 14148:2003 (vitamin K_1) (Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column.)	High performance liquid chromatography	**Type III*** 1. Precision data for various foods including a range of infant formulae is in CCNFSDU 29th session CRD 15. 2. Validated. The precision data have been defined in an international collaborative study: 3. Reference: Indyk, H. E. and Woollard, D. C.: Vitamin K in Milk and Infant Formulas by Liquid Chromatography: Collaborative study. J. AOAC intern. 83, 2000, 121-130. 4. Measures either aggregated cis + trans K_1 or can measure individual cis

[6] Note on the form of Vitamin K in Codex Standard 72. - The standard provides no qualification on the definition of forms of vitamin K.
Comment: Vitamin K present in infant formulas can include cis and/or trans K1, dihydro-K1, and the menaquinone series, and a more rigorous definition may be required.

			and trans forms depending on LC column.
Thiamin[7]	AOAC 942.23 (Measures all vitamin B_1 forms and aggregates as thiamine)	Fluorimetry	**Type III or IV -** Currently adopted as Type II method for Special foods. 1. Validated on many food matrixes, but not infant formula or similar food matrixes. 2. The method has been used traditionally 3. The method is not applicable in presence of materials that adsorb thiamin or which contain extraneous materials which affect thiochrome. 4. Measures all vitamin B_1 forms and aggregates as thiamine. Subject to significant spectral interference.
Thiamin	AOAC 986.27	Fluorimetry	**Type III *-** (Measures all vitamin B_1 forms as thiamine)
Thiamin	EN 14122:2003 (Measures all vitamin B_1 forms (natural and added free, bound and phosphorylated) following extraction and conversion to thiamine)	High performance liquid chromatography with pre- or post column derivatization to thiochrom	**Type III*** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15 2. Collaboratively tested according to ISO 5725, among others, an enriched milk powder was included in the validation. In accordance with the EU SMT Certification Study guidelines, the data given for CRM 121 (wholemeal flour), CRM 421 (milk powder), CRM 485 (mixed vegetables) and CRM 487 (pig's liver) have been defined in an interlaboratory test. The Institute of Food Research, Norwich, UK on behalf of the EU Community Bureau of Reference, conducted the study. Reference: Finglas, P. M., Scott, K. J., Witthoft, C. M., van den Berg, H. and de Froidmont-Gortz, I.: The certification of the mass fractions of vitamins in four reference materials: Wholemeal flour (CRM 121), milk powder (CRM 421), lyophilised mixed vegetables (CRM 485) and lyophilised pig's liver (CRM 487). EUR-report 18320, Office for Official Publications of the European Communities, Luxembourg, 1999. 3. The data given for tube feeding solution, baby food, powdered milk, meal with fruits, yeast and cereal, chocolate powder and food supplement have been defined in a French interlaboratory test.. Reference: Arella, F.,

[7] Note on the form of Thiamin in Codex Standard 72. - The standard provides no qualification on the definition of forms of thiamine.
Comment: Several endogenous phosphorylated forms exist in infant formulas, although vitamin B1 is usually dominated by the supplement thiamine hydrochloride. In this case, units of expression (free base vs hydrochloride salt) need to be defined.

			Lahély, S., Bourguignon, J. B. and Hasselmann, C.: Liquid chromatographic determination of vitamin B1 and B2 in foods. A collaborative study. Food Chem. 56, 1996, 81-86. 4. Measures all vitamin B_1 forms (natural and added free, bound and phosphorylated) following extraction and conversion to thiamine.
Riboflavin[8]	AOAC 985.31	Fluorimetry	**Type III* -** . Measures free and bound forms. Uncertain whether phosphorylated forms captured. Subject to significant spectral interference.
Riboflavin	EN 14152:2003 (Measures natural and supplemental forms, free, bound and phosphorylated (FMN and FAD) aggregated and measured as riboflavin.)	High performance liquid chromatography	**Type III*** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15. 2. Collaboratively tested according to ISO 5725, an enriched milk powder was included in the validation. The parameters on CRM 421 (milk powder) and CRM 487 (pig liver) of different methods for the determination of riboflavin (Vitamin B2) have been defined in an international comparison study organised by the European Commissions Standard, Measurement and Testing programme. Reference: Finglas, P. M., Scott, K. J., Witthoft, C. M., van den Berg, H. & de Froidmont-Gortz, I.: The certification of the mass fractions of vitamins in four reference materials: Wholemeal flour (CRM 121), milk powder (CRM 421), lyophilised mixed vegetables (CRM 485) and lyophilised pig's liver (CRM 487). EU Report 18320, Office for Official Publications of the European Communities, Luxembourg, 1999. 3. Both natural and supplemental forms, free, bound and phosphorylated (FMN and FAD) aggregated and measured as riboflavin.

[8] Note on the form of Riboflavin in Codex Standard 72 - The standard provides no qualification on the form of riboflavin.
Comment: Several endogenous phosphorylated forms exist in infant formulas, eg free and/or bound riboflavin, FMN, FAD etc. Vitamin B2 is generally enhanced through supplementation with either free riboflavin or FMN.

Niacin[9]	AOAC 985.34 (niacin (preformed) and nicotinamide)	Microbioassay and turbidimetry	**Type III** 1. CCMAS recommended review and replacement with a more modern method. 2. Validated 3. AOAC 985.34 Niacin and Niacinamide (Nicotinic Acid and Nicotinamide) in Ready-to-Feed Milk-Based Infant Formula; Microbiological-turbidimetric method. First Action 1985; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.19. 4. Reference: JAOAC 68: 514 - 522 (1985). 5. The method is applicable to baby foods (meat based), beverages, juices, cereal products, cheese, dairy products, fruits and potato products. 6. Free and bound forms aggregated and measured as nicotinic acid.
Niacin	prEN 15652:2007 (Free and bound and phosphorylated forms measured either as aggregate of nicotinic acid + nicotinamide, or as individual forms)	High performance liquid chromatography	**Type III* when published as EN method** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15 2. Collaboratively tested according to ISO 5725, among others, an enriched milk powder was included in the validation.The precision data for the determination of niacin were established according to ISO 5725-2 in 2002 by an international collaborative study organised by AéRIAL (CRT: Centre de Ressources technologiques) and the CGd'UMA (Commission Générale d'Unification des Méthodes d'Analyses) according to ISO 5725-2 in 1999 by a French collaborative study organized by CGd'UMA, 3. Reference: • To be published: Bergantzlé M., Validation study on the determination of niacin by HPLC in several matrices; • Lahély S., Bergantzlé M., Hasselmann, C.: Fluorimetric determination of niacin in foods by highperformance liquid chromatography with post-column derivatization Food chem., 65, 129-133 (1999) 4. Free and bound and phosphorylated forms measured either as aggregate of nicotinic acid + nicotinamide, or as individual forms

[9] Note on the form of Niacin in Codex Standard 72. - *Niacin refers to preformed niacin.*
Comment: Niacin is the generic descriptor for two vitamers, nicotinic acid and nicotinamide. However terminology differs between the USA and Europe for this vitamin and this standard needs to be unambiguous. Other forms also exist, eg NAD, NADH etc. It is therefore unclear what is meant by "preformed niacin".

Vitamin B_6[10]	AOAC 985.32 (Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine and measures as pyridoxine.)	Microbioassay	**Type III** 1. 2. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods, and asked for clarification of the differences from AOAC 961.15. 3. Validated AOAC Method 985.32. (Pyridoxine, Pyridoxal, Pyridoxamine) in Ready-to Feed Milk-Based Infant Formula Microbiological Method. First Action 1985; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.18. Reference: JAOAC 68: 514 - 522 (1985). 4. Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine and measures as pyridoxine.
Vitamin B_6	AOAC 2004.07 (Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.)	High performance liquid chromatography	**Type III*** 1. Validated. The method is applicable to the determination of vitamin B6 in milk- and soy based liquid infant formula at 0 -1mg/100g. Reference: JAOAC Int. 88: 30 - 37 (2005) Results of the interlaboratory study for vitamin B6 in reconstituted infant formula (milk- and soy-based) are included with the method. Measures free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.
Vitamin B_6	EN 14166:2008 (Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine (including phosphorylated forms) and	Microbioassay	**Type III** 1. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 2. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15 Foodstuffs - Determination of vitamin B6 by microbiological assay The following data were obtained in an interlaboratory trial held in 1996 between participating European laboratories. Reference: The certification of the mass fractions of vitamins in four reference materials: wholemeal flour

[10] Note on the form of Vitamin B_6 in Codex Standard 72. - The standard provides no qualification on the form of vitamin B6.
Comment: This means all forms are potentially included, i.e. pyridoxine, pyridoxal, pyridoxamine and the related phosphorylated forms. Vitamin B6 is generally enhanced through supplementation with pyridoxine, and could be expressed as either the free base or hydrochloride salt. Methods for vitamin B6 can therefore measure and report single or aggregate forms.

	measures as pyridoxine.)		(CRM 121), milk powder (CRM 421), lyophilised mixed vegetables (CRM 485) and lyophilised pigs liver (CRM 487). Finglas, P.M., Scott, K.J., Witthoft, C., van den Berg, H. & Froidmont-Görtz, I. (1999); EUR-report 18320, Office for Official Publications of the European Communities, Luxembourg. 3. Aggregates free and bound pyridoxal, pyridoxine and pyridoxamine (including phosphorylated forms) and measures as pyridoxine.
Vitamin B_6	EN 14663:2005 (includes glycosylated forms) (Free and bound phosphorylated and glycosylated forms measured as the individual forms pyridoxal, pyridoxine and pyridoxamine.)	High performance liquid chromatography	**Type III** 1. Validated. Precision data for various foods (semolina with milk, powder; potato puree, powder; vegetables with ham (baby food); multi vitamin drink) is in CCNFSDU 29th session CRD 15 The precision data for the determination of vitamin B6 were established in an interlaboratory test according to ISO 5725 carried out by the former BgVV (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, German Federal Institute for Consumer protection and veterinary medicine). Reference: Bognár, A.: Bestimmung von Vitamin B6 in Lebensmitteln mit Hilfe der Hochdruckflüssig- Chromatographie (HPLC). Z Lebensm Unters Forsch A, 1985, 181: 200 – 205 2. Free and bound phosphorylated and glycosylated forms measured as the individual forms pyridoxal, pyridoxine and pyridoxamine.
Vitamin B_6	EN 14164:2008 (Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.)	High performance liquid chromatography	**Type III*** 1. Precision data for the determination of vitamin B6 in baby food, biscuit, cereal, yeast, tube-feeding solution, chocolate powder and powdered milk were established in an interlaboratory test according to ISO 5725 carried out by DGCCRF (Direction Génerale de la Concurrence, de la Consommation et de le Repression des Fraudes). Reference: Bergaentzlé M., Arella F., Bourguignon J.B., Hasselmann C., Determination of vitamin B6 in foods by HPLC: a collaborative study. Food Chem (1995), 52, 81-86 2. The precision data for the determination of vitamin B6 in reconstituted infant formula were established in an interlaboratory test according to AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis. Reference: Mann D.L., Ware G.W., Bonnin E. Liquid Chromatographic analysis of vitamin B6 in reconstituted infant formula: Collaborative Study. JAOAC (2005), 88,1:30-37 3. Free and bound phosphorylated forms (pyridoxal, pyridoxine and pyridoxamine) converted and measured as pyridoxine.

Vitamin B_{12}[11]	AOAC 986.23 (Measures total vitamin B_{12} as cyanocobalamin	Turbidimetric Method	**Type III*** 1. CCMAS asked for clarification of the differences from AOAC 952.20. A great difference between AOAC 952.20 and AOAC 986.23 methods is the sample matrix; the first is applicable in vitamin preparations, but not in infant formulae. 2. Validated AOAC Method 986.23 Cobalamin (Vitamin B12 Activity) in Milk-Based Infant Formula. Turbidimetric method (microbiological). First Action 1986; Final Action 1988. Official Methods of AOAC Int. (18th ed., 2005): 50.1.20. Reference: JAOAC 69: 777 - 785 (1986). 3. Measures total vitamin B_{12} as cyanocobalamin.
Pantothenic acid[12]	AOAC 992.07 (Measures total pantothenate (free pantothenic acid + CoA- + ACP-bound) and measured as D-pantothenic acid (or calcium D-pantothenate).)	Microbioassay	**Type III.** In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which has been used traditionally should currently be endorsed as Type III and recommended as **Type IV** when another method can be recommended as Type II or III 1. The method was listed for use with infant formula in CODEX STAN 234-1999, rev. 2006. 2. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 3. Validated. Results of the interlaboratory study supporting acceptance of the method (milk-based liquid, ready-to-feed) are presented in the method. Reference: J. AOAC Int. 76: 399 - 413 (1993). 4. Measures total pantothenate (free pantothenic acid + CoA- + ACP-bound) and measured as D-pantothenic acid (or calcium D-pantothenate).
Folic acid[13]	AOAC 992.05 (Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid.)	Microbioassay	**Type III** In line with the CCMAS 28 request to review methods using microbioassay as a principle, the suggestion is this method which has been used traditionally should currently be endorsed as Type III and recommended as **Type IV** when another method can be recommended as Type II or III. 1. CCMAS 28 states in general, methods using microbioassay as a principle should be reviewed in order to replace them with more modern methods. 2. Validated. Results of the interlaboratory study supporting acceptance of the method (milk-based, ready-to-feed) are listed in the method.

[11] Note on the form of Vitamin B_{12} in Codex Standard 72.: The standard provides no qualification on the form of vitamin B_{12}.
Comment: This means all forms are potentially included. However cyanocobalamin is the form used in food supplementation and most extraction conditions employed will convert multiple endogenous forms to a single cyano form.
[12] Note on the form of Pantothenic acid in Codex Standard 72.: The standard provides no qualification on the form of pantothenic acid.
Comment: This means all forms are potentially included eg the free calcium pantothenate supplement and that derived from Coenzyme A. It is important to define units of expression either as pantothenic acid or the calcium salt.

			3. Measures free folic acid + free, unbound natural folates, aggregated and measured as folic acid.
Folic acid	EN 14131:2003 (Total folate (free + bound), aggregated and measured as folic acid.)	Microbioassay	**Type III** In line with the CCMAS 28 request to review methods using microbioassay as a principle, this method which has been used traditionally should currently be endorsed as Type III and recommended as **Type IV** when another method can be recommended as type II or III 1. 2. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15 The precision of the method was established by interlaboratory tests conducted within the European Union's Standards, Measurement and Testing (EU SMT) programme, and carried out in accordance with ISO 5725. Reference: Finglas, P.M., et al., The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM 121), milk powder (CRM 421), lyophilized mixed vegetables (CRM 485) & lyophilized pig's liver (CRM 487). B1, B6 & folate in CRM 121; B1, B2, B6, B12 & folate in CRMs 421 & 487, and B1, B6, folate & carotenoids in CRM 485. 1999, Luxembourg: Office for Official Publications of the European Communities. 3. Equivalent to AOAC 992.05. Note that these methods quantify total folate, including folates of natural source and not folic acid alone, which is used as source for fortification. 4. Measures total folate (free + bound), aggregated and measured as folic acid.
Folic acid	J AOAC Int. 2000:83; 1141-1148 (Measures free folic acid + proportion of free, natural folate)	Optical Biosensor Immunoassay	**Not recommended as Type III as it is not established as official methodology.** In line with the CCMAS 28 request to review methods using microbioassay as a principle,this method which is recently introduced and currently under AOAC collaborative study should be endorsed as **Type IV** 1. Reference: Indyk HE, Evans EA, et al. J AOAC Intl. 2000:83:1141-1148, Determination of Biotin and Folate in Infant Formula and Milk by Optical Biosensor-Based Immunoassay. http://www.atyponlink.com/AOAC/doi/abs/10.5555/jaoi.2000.83.5.1141 2. Measures free folic acid + proportion of free, natural folate.
Folic acid	J Chromatogr. A., 928, 77-90, 2001	High performance liquid	**Not recommended as Type III as it is not established as official methodology.** In line with the CCMAS 28 request to review methods using microbioassay as a principle, this method

[13] Note on the form of Folic acid in Codex Standard 72.: The standard is specific for folic acid.
Comment: Currently the provision is specific for folic acid which implies that only the free supplemental form should be quantified during analysis, and expressed as ug (despite DFE gaining common usage). However, such a test method would exclude all natural forms present in milk, and therefore invalidate currently recommended microbiological assay methods.

	(Measures total folates after conversion to, and measurement as 5-Me-H4PteGlu)		
Vitamin C[14]	AOAC 985.33 (measures ascorbic acid (AA))	2,6-dichloroindophenol titrimetry	**Type III*** CCMAS asked for clarification on how vitamin C was expressed. Determines only L(+) ascorbic acid and not the total amount for which the amount of dehydroascorbic acid has to be included. This method is specific for reduced ascorbic acid only.
Vitamin C	EN 14130:2003 (Measures ascorbic acid + dehydroascorbic acid).	High performance liquid chromatography	**Type III*** 1. Validated. Precision data for various foods is in CCNFSDU 29th session CRD 15. Validated Collaboratively tested according to ISO 5725, an enriched milk powder was included in the validation. The precision parameters for orange juice, liquid soup, powder milk, freeze-dried soup, breakfast cereals and fruits baby food have been defined in a collaborative study 2. Reference: Arella F., Deborde J.L., Bourguignon J.B., Hasselmann C., (1997), Ann. Fals. Exp. Chim., 90, N°940:217-233. 3. Measures total L-ascorbate (Ascorbic acid + dehydroascorbic acid).

[14] Note on the form of Vitamin C in Codex Standard 72.
"expressed as ascorbic acid"
Comment: Further clarification of form(s) of vitamin C is required, eg ascorbic acid (AA), oxidised dehydroascorbic acid (DHA), or total ascorbate (AA + DHA), since both forms are physiologically active. However, the enantiomeric D-forms are not antiscorbutic and need to be discriminated.

Biotin[15]	EN 15607:2008 (d-biotin) (Measures total D-biotin (free + D-biocytin)	High performance liquid chromatography	**Type III*** 1. Validated. Precision data for various foods including infant milk powder is in CCNFSDU 29th session CRD 15. Collaboratively tested according to ISO 5725, among others, an enriched infant milk powder was included in the validation. The data were obtained in an interlaboratory study organized by CGd'UMA (Commission Générale d'Unification des Méthodes d'Analyses) in 2000. It was organized in accordance with ISO 5725-2. Reference: Arella, F., Deborde, J.L., Bourguignon, J.B., Bergaentlze, M., Ndaw, S., Hasselmann, C.: Liquid chromatographic determination of biotin in foods. A collaborative study. Ann. Fals. Exp. Chim., 93, 951,193-200 (2000) 2. Measures total D-biotin (free + D-biocytin)
Iron	AOAC 985.35	Atomic absorption spectrophotometry	**Type II -** The method is applicable to the determination of Ca, Mg, Fe, Zn, Cu, Mn, Na, and K. Currently listed for copper determination in edible casein products and whey powders (Type II) Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as AOAC 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method.
Iron	AOAC 984.27	ICP emission spectroscopy	**Type III**
Calcium	ISO 8070 | IDF 119: 2007	Flame atomic absorption spectrophotometry	**Type II** - Current Codex method for special foods, and adopted by CAC 31 for infant formula, Type II, for determination of Na and K.
Calcium	AOAC 985.35	Atomic absorption spectroscopy	**Type III** Validated. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as AOAC 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method. References: JAOAC 68: 514 - 522 (1985), J. AOAC Int. 80: 834 - 844 (1997).

[15] Note on the form of Biotin in Codex Standard 72
The standard provides no qualification on the form of biotin.
Comment: Free d-biotin is generally used as a supplement. However, endogenous biotin is mostly present as a protein-bound form, which may be liberated as bioactive d-biocytin. Attention needs to be given to which forms are to be quantified.

Calcium	AOAC 984.27	ICP emission spectroscopy	**Type III -** Current Codex method (Type III) for Special foods.
Phosphorus	AOAC 986.24	Spectrophotometry (molybdovanadate)	**Type II -** Current Codex method for special foods. .
Phosphorus	AOAC 984.27	ICP emission spectroscopy	**Type III** Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, and Zinc in Infant Formula. In this method, a test portion is digested in HNO_3 / $HClO_4$ and elements are determined by ICP emission spectroscopy.
Magnesium	ISO 8070 | IDF 119: 2007	Flame atomic absorption spectrophotometry	**Type II** Current Codex method for special foods and infant formula, Type II, for determination of Na and K. Reference of the collaborative study: International Dairy Journal, Volume 18, Issue 9, September 2008, Pages 899-904, Determination of sodium, potassium, calcium and magnesium content in milk products by flame atomic absorption spectrometry (FAAS): A joint ISO/IDF collaborative study, Laurent Noël, Michael Carl, Christelle Vastel and Thierry Guérin
Magnesium	AOAC 985.35	Atomic absorption spectroscopy	**Type III** Validated for infant formula. Interlaboratory study matrices include enteral product, ready-to-feed soy formula, soy powder and whey powder (same matrices as AOAC 986.24 Phosphorus). The results of the interlaboratory study supporting acceptance of the method are presented in the method.
Magnesium	AOAC 984.27	ICP emission spectroscopy	**Type III**
Chloride	AOAC 986.26	Potentiometry	**Type II**
Manganese	AOAC 985.35	Atomic absorption spectrophotometry	**Type II**
Manganese	AOAC 984.27	ICP emission spectroscopy	**Type III**
Iodine	AOAC 992.24	Ion-selective potentiometry	**Type II, for milk-based formula -** Current Codex method for milk-based follow-up formula Validated. The method is applicable to ready-to-feed milk-based infant formula containing 75-150 microgram/L iodide. The results of the interlaboratory study supporting acceptance

			of the method (ready-to-feed milk-based infant formula) are stated in the method.
Selenium	AOAC 996.17	Continuous hydride generation atomic absorption spectrometry (HGAAS)	**Type IV** Validated (not with infant formula). Interlaboratory study included samples with selenium levels from 0.25 to 5,450 micrograms/g. Accuracy of method was substantiated by in-house analyses of NIST SRMs (1657 Wheat Flour; 1577a Bovine Liver; 1643c Trace Elements in Water). The results of the interlaboratory study supporting acceptance of the method are listed in the method.
Selenium	EN 14627	Hydride generation atomic absorption spectrometry (HGAAS)	**Type IV** - Foodstuffs. Determination of trace elements. Determination of total arsenic and selenium by hydride generation atomic absorption spectrometry (HGAAS) after pressure digestion - Not validated for infant formulas
Selenium	AOAC 2006.03	ICP emission spectroscopy	**Type IV** - Validated (not with infant formula). Interlaboratory study included samples with selenium levels from 0.25 to 257 micrograms/g. The results of the interlaboratory study supporting acceptance of the method are included in the method.
Copper	AOAC 985.35	Atomic absorption spectroscopy	**Type II**
Copper	AOAC 984.27	ICP emission spectroscopy	**Type III**
Zinc	AOAC 985.35	Atomic absorption spectroscopy	**Type II** (see above for reference to this method)
Zinc	AOAC 984.27	ICP emission spectroscopy	**Type III** - Validated for infant formula.
Choline[16]	AOAC 999.14	Enzymatic Colorimetric Method	**Type II, with limitations on applicability due to choline and ascorbate concentration.** Validated. The method is applicable to the determination of choline in milk and infant formula containing 45-175 mg solids/100 g. The method does not apply to powdered infant formula/milk containing more than 100 mg vitamin C/100 g solids because of ascorbate suppression of color development. The results of the interlaboratory study supporting acceptance of the method are included in the method.
Chromium (Section B of	EN 14082	AAS after dry ashing	**Type IV** Foodstuffs. Determination of lead, cadmium, zinc, copper, iron, and chromium by AAS after

[16] Note on the form of Choline in CODEX STAN 72. - The standard provides no qualification on the form of choline.
Comment: Free choline is one of a number of salts used as supplement. However a number of bound forms are also present in infant formulas including added lecithin and endogenous components of milk phospholipid. Units of expression also require definition (eg as choline hydroxide).

STAN 72 only)			dry ashing. Infant formula was not included in the validation.
Chromium (Section B of STAN 72 only)	EN 14083	Graphite furnace AAS after pressure digestion	**Type IV.** Foodstuffs. Determination of lead, cadmium, chromium and molybdenum by GF-AAS after pressure digestion. Infant formula was not included in the validation.
Chromium (Section B of STAN 72 only)	AOAC 2006.03	ICP emission spectroscopy	**Type IV** Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizers (Microwave Digestion and Inductively Coupled Plasma-Optimal Emission Spectrometry). Infant formula was not included in the validation.
Molybdenum (Section B of STAN 72 only)	EN 14083	Graphite furnace AAS after pressure digestion	**Type IV** Foodstuffs. Determination of lead, cadmium, chromium and molybdenum by GF-AAS after pressure digestion. Infant formula was not included in the validation.
Molybdenum (Section B of STAN 72 only)	AOAC 2006.03	ICP emission spectroscopy	**Type IV** Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizers (Microwave Digestion and Inductively Coupled Plasma-Optimal Emission Spectrometry).

B. CODEX COMMITTEE ON PROCESSED FRUITS AND VEGETABLES[17]

Draft Standard for Certain Canned Vegetables (At Step 8)
Draft Standard for Jams and Jellies (at Step 8)
Standard for Aqueous Coconut Products: Coconut Cream and Coconut Milk (CODEX STAN 240-2003)

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Jams and jellies	Filll of Containers	CAC/RM 46-1972	Weighing	Type I – Codex General Method for processed fruits and vegetables
Jams and Jellies	Soluble solids	ISO 2173:2003 AOAC 932.12	Refractometry	Type I – Codex General Method for processed fruits and vegetables
Jams and Jellies	Calcium	AOAC 968.31	Complexometry/ Titrimetry	Revocation recommended in jams and jellies by CCPFV as no provisions exist

[17] ALINORM 09/32/27, Appendices II, III and VI

Jams and Jellies	Mineral impurities (sand)	AOAC 971.33	Gravimetry	Revocation recommended in jams and jellies by CCPFV as no provisions exist
Certain Canned Vegetables	Mineral impurities (sand)	AOAC 971.33	Gravimetry	Type I – method currently used for canned strawberries and jams and jellies Current method for canned palmito: ISO 762:1982 (confirmed 1992)
Canned Green beans	Tough strings	CAC/RM 39-1970	Stretching	Type I – confirmation of existing method
Canned Green peas	Types of peas, distinguishing	CAC/RM 48-1972	Visual inspection	Type I – confirmation of existing method
Canned Green Peas	Proper fill (in lieu of drained weight)	CAC/RM 45-1972	Pouring and measuring	Revocation recommended as it no longer applies to peas and CAC/RM 46-1972 already covers the product
Canned Green Peas	Solids, alcohol insoluble	AOAC 938.10	Gravimetry including sieving	Revocation recommended as no provisions exist
Canned Green Peas	Calcium	AOAC 968.31	Complexometry/ Titrimetry	Revocation recommended as no provisions exist – currently Type II as General Method for Processed Fruits and Vegetables
Canned mature processed peas	Solids, total	AOAC 964.22	Gravimetry (vacuum oven)	Recommended for revocation by CCPFV as no provisions exist
Aqueous Coconut Products	Total Fats	Bligh-Dyer	Gravimetry	Chloroform-methanol Extraction method for foods in general – Type IV
Aqueous Coconut Products	Total Fats	AOAC 963.15	Gravimetry	Soxhlet Extraction method for foods/cacao beans and its products – Type I
Aqueous Coconut Products	Total Fats	AOAC 983.23	Gravimetry	Chloroform-methanol Extraction method for foods in general – Type I
Aqueous Coconut Products	Total Fats	ISO 1211:1999	Gravimetry Alkaline hydrolysis method Röse-Gottlieb method	
Aqueous Coconut Products	Total solids	ISO 6731:1989	Gravimetry Drying in hot air oven at 102 ±2°C	

Aqueous Coconut Products	Non-fat solids	ISO 1211:1999 and ISO 6731:1989	Gravimetry Alkaline hydrolysis method Röse-Gottlieb method Gravimetry Drying in hot air oven at 102 ± 2°C Calculation: Subtracting total fats from total solids	
Aqueous Coconut Products	Moisture	ISO 6731:1989	Gravimetry Drying in hot air oven at 102 ± 2°C Calculation: Subtracting total solids from 100	

Proposed Sampling Plans

(Draft Standard for Certain Canned Vegetables and Draft Standard for Jams and Jellies)

SAMPLING PLANS

The appropriate inspection level is selected as follows:

Inspection level I - Normal Sampling

Inspection level II - Disputes, (Codex referee purposes sample size), enforcement or need for better lot estimate

SAMPLING PLAN 1

(INSPECTION LEVEL I, AQL = 6.5)

NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)		
Lot Size (N)	**Sample Size (n)**	**Acceptance Number (c)**
4,800 or less	6	1
4,801 - 24,000	13	2
24,001 - 48,000	21	3
48,001 - 84,000	29	4
84,001 - 144,000	38	5
144,001 - 240,000	48	6
more than 240,000	60	7
NET WEIGHT IS GREATER THAN 1 KG (2.2 LB) BUT NOT MORE THAN 4.5 KG (10 LB)		
Lot Size (N)	**Sample Size (n)**	**Acceptance Number (c)**
2,400 or less	6	1
2,401 - 15,000	13	2
15,001 - 24,000	21	3
24,001 - 42,000	29	4
42,001 - 72,000	38	5
72,001 - 120,000	48	6
more than 120,000	60	7
NET WEIGHT GREATER THAN 4.5 KG (10 LB)		
Lot Size (N)	**Sample Size (n)**	**Acceptance Number (c)**
600 or less	6	1
601 - 2,000	13	2
2,001 - 7,200	21	3
7,201 - 15,000	29	4
15,001 - 24,000	38	5
24,001 - 42,000	48	6
more than 42,000	60	7

SAMPLING PLAN 2

(Inspection Level II, AQL = 6.5)

NET WEIGHT IS EQUAL TO OR LESS THAN 1 KG (2.2 LB)		
Lot Size (N)	**Sample Size (n)**	**Acceptance Number (c)**
4,800 or less	13	2
4,801 - 24,000	21	3
24,001 - 48,000	29	4
48,001 - 84,000	38	5
84,001 - 144,000	48	6
144,001 - 240,000	60	7
more than 240,000	72	8
NET WEIGHT IS GREATER THAN 1 KG (2.2 LB) BUT NOT MORE THAN 4.5 KG (10 LB)		
Lot Size (N)	**Sample Size (n)**	**Acceptance Number (c)**
2,400 or less	13	2
2,401 - 15,000	21	3
15,001 - 24,000	29	4
24,001 - 42,000	38	5
42,001 - 72,000	48	6
72,001 - 120,000	60	7
more than 120,000	72	8
NET WEIGHT GREATER THAN 4.5 KG (10 LB)		
Lot Size (N)	**Sample Size (n)**	**Acceptance Number (c)**
600 or less	13	2
601 - 2,000	21	3
2,001 - 7,200	29	4
7,201 - 15,000	38	5
15,001 - 24,000	48	6
24,001 - 42,000	60	7
more than 42,000	72	8

C. FAO/WHO COORDINATING COMMITTEE FOR ASIA

1. Draft Standard for Gochujang (At Step 8) [18]

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Gochujang	Capsaicin	According to the method described in the Annex to the Standard (see below)	Gas chromatography	AOAC 995.03 endorsed by CCMAS 29 as Type II. CCASIA agreed to retain the Annex A method and to delete the Anenx B method because it was similar to the AOAC method

Determination of capsaicin in *Gochujang* using Gas Chromatography (GC) detection

1. SCOPE

This method is suitable for the determination of capsaicin in *Gochujang* using chromatographic detection. The method uses squalene as an internal standard. The concentration of capsaicin is expressed as ppm.

2. PRINCIPLE

To extract capsaicin, the mixture is blended to a homogeneous consistency. Capsaicin in *Gochujang* is extracted with 100% methanol, followed by methanol – hexane fractionation to remove hydrophilic and hydrophobic interfering substances by a separating funnel. Capsaicin in methanol layer is extracted with dichloromethane (DCM) and the saturated NaCl, concentrated by a rotary evaporator. A portion of the concentrated sample extract is then taken and completely solved with DCM containing squalene as an internal standard for analysis using gas chromatographic detection.

3. REAGENT AND MATERIALS

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade and water of at least grade 3 as defined in ISO 3696.

3.1 Reagents

3.1.1 Capsaicin (99 + %, $C_{18}H_{27}NO_3$, Fw 305.42, CAS 404-86-4)

3.1.2 Squalene (CAS 111-02-4)

3.1.3 Hexane

[18] ALINORM 09/32/15, Appendix II

3.1.4 Methanol

3.1.5 Methanol + Water (80 + 20)

3.1.6 Dichloromethane

3.1.7 Sodium chloride

3.1.8 Sodium sulfate

3.2. Preparation of standard solution

3.2.1 Capsaicin Stock solution (A)

Weigh approximately 100 mg of capsaicin, making up to 100 ml in a volumetric flask with DCM to give solution (A) of approximate 1000 μg/ml.

3.2.2 Capsaicin working solution (B)

Prepare 100 ml intermediate solution B by dilution of 10 ml solution A (3.2.1) with 100 ml of DCM to exactly 100 μg/ml in DCM.

3.2.3 Squalene internal standard working solution (C)

Weigh approximately 100 mg squalene and make up to 250 ml in a volumetric flask with DCM to give a solution (C) of approximately 400 μg/ml in DCM.

3.3 Calibration solutions of capsaicin

Dispense volumes of the 100 μg/ml solution (B, 3.2.2) into 50 ml round flask, dried up and add 2 ml of internal standard working solution (C, 3.2.3) to give 10.0, 50.0, 100.0, 300.0, 500.0 μg/ml capsaicin.

4. APPARATUS

4.1 Gas chromatograph with flame ionization detector (FID)

The following conditions have been found to be suitable:

4.1.1 Injector / Detector temperature : 320°C / 350°C

4.1.2 Oven temperature program: 220°C for 1 minute, ramp at 5°C/min to 250°C, hold for 13 minutes and raise to 280°C holding 5 min by 20°C/min. Helium carrier gas at 1.5 ml/minute

4.1.3 Make split injection of 1.0uL with split ratio 1:5

4.2 GC column, 30 m x 0.32 μm, 0.25 μm film thickness, HP-1 or equivalent

4.3 Analytical balance, capable of weighing to 4 decimal places

4.4 Shaker, capable of attaining 2,000 rpm

4.5 Centrifuge, capable of attaining 3,500 rpm

4.6 Filter paper (Waterman No. 2 or equivalent)

5. LABORATORY SAMPLES

On receipt, samples are given a unique sample number. *Gochujang* sample is stored at below 4°C. All other samples are stored at room temperature in an air tight container prior to analysis.

6. PROCEDURE

6.1 Laboratory sample

Samples should be minced or grated to a homogeneous mixture. All samples should be stored in the air-tight container and at room temperature prior to analysis. All samples should be mixed thoroughly to a homogeneous mixture before analysis.

6.2 Test sample

6.2.1. Thoroughly mix the sample. Weigh, to the nearest 0.01 g, and 10 g portion of *Gochujang* into a centrifuge bottle (250 ml, Nalgene).

6.2.2 Add 50 ml of methanol and shaking for 2 hours, extracting capsaicin.

6.2.3 Filter the extract with Watman No. 2 filter paper into a 250 ml flask (Ext-A).

6.2.4 Add additional 30 ml of methanol to residue and shaking for 1 hour, extracting capsaicin (Ext-B).

6.2.5 Repeat step 6.2.3 to 6.2.4 (Ext-C)

6.2.6 Combine Ext-A, Ext-B and Ext-C in 250 ml round bottom flask, concentrating up to approximately 5 ml.

6.2.7 Solve the concentrate with 20 ml of 80% methanol and 20 ml of hexane.

6.2.8 Transfer the solution into a 250 ml separating funnel.

6.2.9 Shake and separate into two layers, methanol layer (M1-layer, upper) and hexane layer (H1-layer, lower)

6.2.10 Reserve H1-layer in 100ml flask and transfer M1-layer (6.2.9) into a separating funnel and add additional 20 ml of hexane.

6.2.11 Repeat step 6.2.9 to 6.2.10 (M2-layer and H2-layer)

6.2.12 Repeat step 6.2.9 to 6.2.10 (M3-layer and H3-layer)

6.2.13 Combine H1-layer, H2-layer and H3-layer (HC-layer) in the 250ml separating funnel, adding 20 ml 80% methanol, shaking and separating into two layers, methanol layer (M'1-lower layer) and hexane layer (H'1-upper layer).

6.2.14 Reserve M'1-layer in the new 250 ml flask.

6.2.15 Add 20 ml of 80% methanol into the separating funnel containing HC-layer, shaking and separating into two layers (M'2-layer and H'2-layer)

6.2.16 Combine the all M-layer in the new separating funnel (250 ml), adding 20 ml of saturated NaCl and 20 ml of DCM.

6.2.17 Shake and separate into two layers (D1-layer and WM1-layer) in the 250 ml separating funnel.

6.2.18 Transfer D1-layer into the new 250 ml round flask.

6.2.19 Add additional 20 ml DCM into the separating funnel (6.2.16), shaking and separating into two layers (D2-layer and WM1-layer)

6.2.20 Repeat step 6.2.16 (D3-layer and WM1-layer)

6.2.21 Combine D1-layer, D2-layer and D3-layer into the 250 round flask, concentrating it (C-D)

6.2.22 Transfer the concentrate (C-D, 6.2.21) into a 100 ml round flask, solving it completely with DCM.

6.2.23 Mount approximate 3 g of sodium sulfate on the filter paper and dehydrate C-D by passing through sodium sulfate

6.2.24 Collect the dehydrated C-D layer in 50 ml round flask and concentrate to dryness by the rotary evaporator

6.2.25 Solve the concentrate with 2 ml of DCM containing squalene as the internal standard solution (C, 3.2.3)

6.2.26 Analyze the sample solution by GC

7. CALCULATION – INTERNAL STANDARD METHOD

7.1 Measure the area of the capsaicin and squalene peaks.

7.2 Calculate the ratio of the capsaicin and squalene peak areas.

7.3 Construct a calibration graph for the standards by plotting the peak area ratio against the weight in microgram of capsaicin in the vial.

7.4 Calculate the slope of the calibration line.

7.5 Divide the peak area ratio of the unknowns by the value of the slope to give the weight of capsaicin per vial for the unknown samples.

8. FINAL PRESENTATION OF RESULTS

Results are expressed as ppm and quoted to 2 significant digits.

REFERENCES

1. W. Hawer and J. Ha et al. : Effective separation and quantitative analysis of major heat principles in red pepper by capillary GC, Food Chemistry, 49, pp.99-103, 1994.

2. J. Jung and S. Kang : A new method for analysis of capsaicinoids content in microcapsule, Korean J. Food Sci. Technol., Vol.32, No. 1, pp.42-49, 2000.

3. C.A. Reilly et al. : Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products, J. of Forensic Science, Vol.43, No. 3, pp.502-509, 2001.

4. Ha et al. : Gas Chromatography Analysis of Capsaicin in Gochujang, Journal of AOAC International Vol. 91. No. 2.2008.

Appendix I.

Table 1. Summary of repeatability test for trial proper samples (ppm)

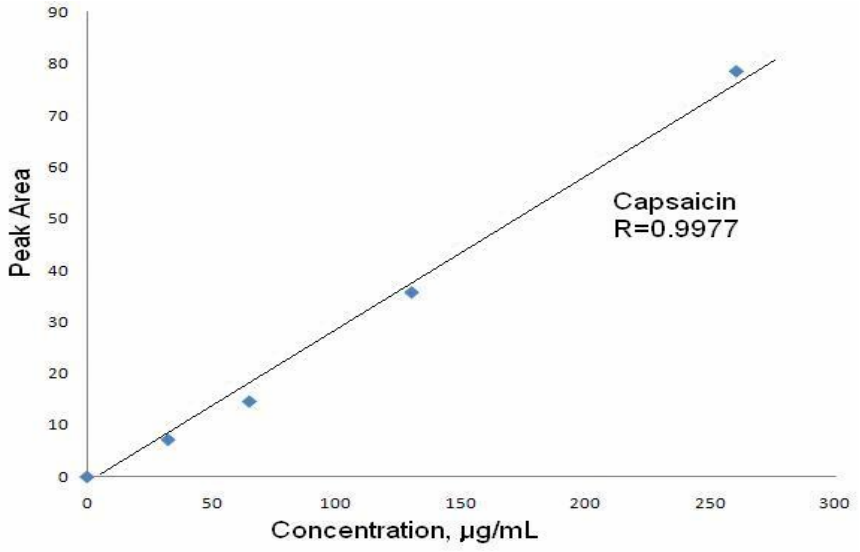
Test No.	*Gochujang - K*
1	64.7
2	69.0
3	70.6
4	71.8
5	70.5
Mean	69.3
RSD,%	3.99

Table 2. Summary of recovery test for trial proper samples (%)

Test No.	*Gochujang – K*
1	80.47
2	77.29
3	87.97
4	91.00
5	95.18
Mean	86.38
RSD,%	8.56

Appendix II

Fig.1. Calibration curve of capsaicin by GC method.



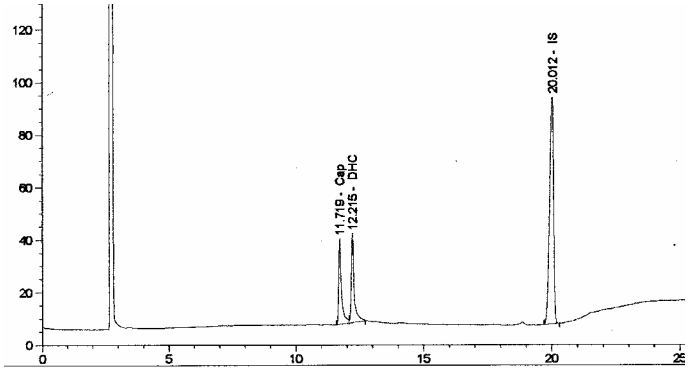


Fig. 2. GC chromatogram of capsaicin standards.

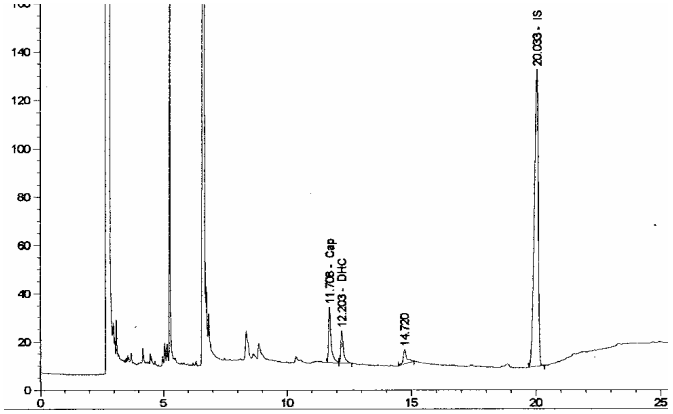


Fig. 3. GC chromatogram of capsaicin in *Gochujang*.

2. Proposed Draft Standard for Fermented Soybean Paste (At Step 5/8)[19]

COMMODITY	PROVISION	METHOD	PRINCIPLE	Notes and Type proposed
Fermented Soybean Paste	Total Nitrogen	AOAC 984.13	Kjeldahl	
Fermented Soybean Paste	Amino Nitrogen	AOAC 920.154 B	Volumetry	Method for amino nitrogen in meat, with the conditions specified below
Fermented Soybean Paste	Moisture	AOAC 934.01	Gravimetry	At a drying temperature of 70ºC or lower.

9.2 Determination of Amino Nitrogen

According to AOAC 920.154 B (*Sorensen* Method) on the following conditions:

Preparation of test samples : Weigh 2 g of sample into a 250 ml beaker and mix the sample with 100 ml of cold (15°C) NH_3-free H_2O and then stir the mixture for 60 min. Next, decant the mixture through a quantitative filter and collect the filtrate in a 100 ml volumetric flask.

Endpoint - A pH meter shall be used to determine the endpoint instead of optical verification of colours.

3. Proposed Draft Standard for Edible Sago Flour (At Step 5) [20]

Sago Flour	Moisture Content	ISO 712:1998	Gravimetry	
Sago Flour	Ash (inorganic extraneous matter)	ISO 2171: 1980	Gravimetry	
Sago Flour	Acidity	AOAC.2005.939.05C	Titrimetry	
Sago Flour	Crude Fibre	ISO 6541:1981	Gravimetry	Modified Sharrer method
Sago Flour	Starch	AOAC.2005.920.44.		

[19] ALINORM 09/32/15, Appendix IV
[20] ALINORM 09/32/15, Appendix V

D. COMMITTEE ON SUGARS

Standard for Sugars: Method for Determination of Colour in Plantation and Mill White Sugar : see attached Letter from ICUMSA

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Dr Roger Wood
Codex Committee on Methods of Analysis and Sampling

16 January 2009

Dear Roger

Paragraph twelve of the report of the twenty-ninth session of the Codex Committee on the Methods of Analysis and Sampling, requested ICUMSA to provide information on its decision regarding the status of Method GS2/3-9. This followed the Brazilian delegation request to maintain its use in Codex. This matter was discussed during the Executive Committee Meeting of ICUMSA at its 26th Session and I am writing to inform you that this method, together with Method GS9/1/2/3-8, remain within the ICUMSA Methods Book but with different status assigned. Method GS2/3-9 has Accepted Status and Method GS9/1/2/3-8 Official Status. Based on this, ICUMSA recommends that users adopt Method GS9/1/2/3-8 where possible but accepts that Method GS2/3-9 still has utility and will give similar results to GS9/1/2/3-8 over its scope of application (up to 600 IU).

Also the report asks ICUMSA to reconsider its method numbering system as it can appear confusing to users. This was also discussed by the Executive Committee and will be investigated to see if a better system can be devised. In the meantime a matrix will be published at the front of the ICUMSA Methods Book to help users who are unfamiliar with the numbering system.

I hope this helps. Please let me know if you require further information.

Yours sincerely

Geoff Parkin
President, ICUMSA