codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 5a) **CX/MAS 07/28/6-Add.1**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Twenty-eighth Session
Budapest, Hungary, 5 – 9 March 2007

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

This document contains the Methods of analysis proposed by the following Committees in Draft Standards and Proposed Draft Standards under elaboration or as update of current methods.

This document includes the replies of individual Committees to the questions from the last session(s) of CCMAS concerning specific methods of analysis under the relevant sections.

A. Codex Committee on Fats and Oils

B. Codex Committee on Nutrition and Foods for Special Dietary Uses

A. CODEX COMMITTEE ON FATS AND OILS[1]

1. Draft Standard for Fat Spreads and Blended Spreads (At Step 8)

The methods for fat spreads and blended spreads were endorsed by the 25th Session of the CCMAS (2005), with the exception of the following methods.

COMMODITY	PROVISION	METHOD	PRINCIPLE	NOTE	TYPE	STATUS
Fat Spreads and Blended Spreads	Moisture	ISO 3727-1/IDF 80-1: 2001; or AOAC 920.116	Gravimetry	CCFO to clarify the applicability to fat spreads (method used for butter)	I	TE
	Solids-non-fat content	ISO 3727-2/IDF 80-2: 2001	Gravimetry		I	TE
	Calculation of the fat content	ISO 3727-3/IDF 80-3: 2003	Calculation		I	TE

In reply to the question from CCMAS on the applicability of these methods to fat spreads, the 20th CCFO (2007) noted that ISO/IDF had validated a method for the determination of fat in a range of butters and fat spreads, including low fat content samples, and observed satisfactory results (ISO FDIS 17189 – 2003, IDF 194-2003, Butter, Edible oil Emulsions and Spreadable Fats, Determination of Fat Content (Reference Method)) which used a similar principle to the method temporarily endorsed by CMAS. The Committee therefore agreed that, in the absence of other evidence, the methods currently "temporarily endorsed" should be forwarded to CCMAS for endorsement as Type I methods (ALINORM 07/30/17, paras. 59-60).

2. Draft Amendment to the Standard for Named Vegetable Oils: Rice Bran Oil (at Step 6)

COMMODITY	PROVISION	METHOD	PRINCIPLE	Type Proposed
Named Vegetable Oils: rice bran oil	gamma oryzanols	see description below	spectrophotometry	IV

Method of Analysis for Gamma Oryzanols

1. Definition

This method is used to determine gamma oryzanol content (%) in oils from spectrophotometer absorption measurements at the wavelength of maximum absorption near 315nm.

2. Scope

Applicable to crude rice bran oil.

[1] ALINORM 07/30/17, Appendices II, V and VIII

3. Apparatus

3.1. Spectrophotometer - for measuring extinction in the ultraviolet between 310 and 320 nm.

3.2. Rectangular quartz cuvettes - having an optical light path of 1 cm.

3.3. Volumetric flask - 25mL.

3.4. Filter paper - Whatman no.2, or equivalent.

4. Reagents

4.1. n-Heptane - Spectrophotometrically pure.

5. Procedure

5.1. Before using, the spectrophotometer should be properly adjusted to a zero reading filling both the sample cuvette and the reference cuvette with n-Heptane.

5.2. Filter the oil sample through filter paper at ambient temperature.

5.3. Weigh accurately approximately 0.02g of the sample so prepared into a 25mL volumetric flask, make up to the mark with n-Heptane.

5.4. Fill a cuvette with the solution obtained and measure the extinction at the wavelength of maximum absorption near 315nm, using the same solvent as a reference.

5.5. The extinction values recorded must lie within the range 0.3-0.6. If not, the measurements must be repeated using more concentrated or more diluted solutions as appropriate.

6. Calculation

Calculate gamma oryzanol content as follows:

$$\text{Gamma oryzanol content, \%} = 25 \times (1 / W) \times A \times (1 / E)$$

Where -

W = mass of sample, g

A = extinction (absorbance) of the solution

E = specific extinction $E^{1\%}_{1cm} = 359$

3. Update of existing methods for fats and oils

The changes (highlighted) concern the references to existing methods. The principle and type are unchanged.

COMMODITY	PROVISION	METHOD	PRINCIPLE	Type
Fats and Oils (all)	Arsenic	AOAC 952.13 (Codex general method) ~~IUPAC 3.136~~	Colorimetry (diethyldithiocarbamate)	II
Fats and oils	Butylhydroxyanisole, butylhydroxytoluene, tert-butylhydroquinone, & propyl gallate	AOAC 983.15; **or AOCS Ce-6-86**	Liquid chromatography	II

Fats and Oils (all)	Insoluble impurities	~~IUPAC 2.604~~ ISO 663:2000	Gravimetry	I
Fats and Oils (all)	Lead	AOAC 994.02 ~~IUPAC 2.623 (or 2632?)~~ ISO 12193:1994 (Codex general method) **or AOCS Ca 18c-91 (03)**	Atomic absorption spectrophotometry (direct graphite furnace)	II
Fats and Oils (all)	Matter volatile at 105°C	~~IUPAC 2.601~~ ISO 662:1998	Gravimetry (open-drying)	I
Fats and Oils (all)	Soap content	BS 684 Section 2.5; **or AOCS Cc 17-95 (97)**	Gravimetry	I
Fats and oils not covered by individual standards	Acid Value	~~IUPAC 2.201~~ ISO 660:1996; **or AOCS Cd 3d-63 (03)**	Titrimetry	I
Fats and oils not covered by individual standards	Copper and Iron	AOAC 990.05 ISO 8294:1994; **or AOCS Ca 18b-91 (03)** ~~IUPAC 2.631~~ (Codex general method)	Atomic absorption Spectrophotometry (direct graphite furnace)	II
Fats and oils not covered by individual standards	Peroxide value	~~IUPAC 2.501 (as amended)~~ AOCS Cd 8b-90 ISO 3961:1998	Titrimetry using *iso*-octane	I
Named Animal Fats	Acidity	~~IUPAC 2.201~~ ISO 660:1996 **amended 2003; or AOCS Cd 3d-63 (03)**	Titrimetry	I
Named Animal Fats	GLC ranges of fatty acid composition	~~IUPAC 2.301, 2.302 and 2.304 or~~ ISO 5508: 1995 and ISO 5509: 2000 **or AOCS Ce 2-66 (97) and Ce 1e-91 (01) or Ce 1f-96 (02)**	Gas chromatography of methyl esters	II
Named Animal Fats	Copper and Iron	AOAC 990.05 ISO 8294:1994; **or AOCS Ca 18b-91 (03)** ~~IUPAC 2.631~~ (Codex general method)	Atomic absorption Spectrophotometry (direct graphite furnace)	II
Named Animal Fats	Iodine value (IV)	~~IUPAC 2.205/1~~, ISO 3961: 1996; or AOAC 993.20; or AOCS Cd 1d-1992 (97)	Wijs-Titrimetry	I

Named Animal Fats	Peroxide value	~~IUPAC 2.501 (as amended)~~ AOCS Cd 8b-90 (97) ISO 3961:1998	Titrimetry using *iso*-octane	I
Named Animal Fats	Relative density	~~IUPAC 2.101 with the appropriate conversion factor~~ **Note**: Needs to be replaced with ISO/AOCS method for apparent density	Pycnometry	II
Named Animal Fats	Refractive index	~~IUPAC 2.102~~ ISO 6320:1995; **or AOCS Cc 7-25 (02)**	Refractometry	II
Named Animal Fats	Saponification value	~~IUPAC 2.202~~ ISO 3657:1988; **or AOCS Cd 3-25 (03)**	Titrimetry	I
Named Animal Fats	Unsaponifiable matter	~~IUPAC 2.401 (part 1-5)~~ ~~ISO 3596-1:1996 and Amendment 1 1997~~ ~~ISO 3596-2:1988 and Amendment 1 1999~~ **ISO 3596:2002 or ISO 18609: 2000; or AOCS Ca 6b-53 (01)**	Titrimetry after extraction with diethyl ether	I
Named Animal Fats	Titre	~~IUPAC 2.121~~ ISO 935:1988; **or AOCS Cc 12-59 (97)**	Thermometry	I

B. CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES[2]

Draft Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (at Step 8)

The list of methods proposed by the CCNFSDU includes several methods that were previously endorsed (listed with the Type). When other methods are proposed, they are highlighted and the current method is mentioned for ease of reference in italics.

Commodity	**Provision**	**Method**	**Principle**	**Type**
Infant formula	Dietary fibre, total	AOAC 991.43	Gravimetry (enzymatic digestion)	I
	Iodine (milk based formula)	AOAC 992.24	Ion-selective potentiometry	II
	Pantothenic acid	AOAC 992.07	Microbioassay	II
	Vitamin A (retinol isomers)	AOAC 992.04	Liquid chromatography	II

[2] ALINORM 07/30/26, Appendix II

	Vitamin A (retinol)	AOAC 992.06	Liquid chromatography	II
	Vitamin K	AOAC 992.27	Liquid chromatography	II
	Vitamin K	**AOAC 999.15**	Liquid chromatography	
	Vitamin D (D_3, milk based infant formula)	AOAC 992.26	Liquid chromatography	II
	Vitamin E (milk based infant formula)	AOAC 992.03	Liquid chromatography	II
	Vitamin B_{12}	**AOAC 986.23**	Turbidimetry	
	Vitamin B_{12}	*current standard:AOAC 952.20*	*Microbioassay*	*II*
	Vitamin B_6	**AOAC 985.32**	Microbioassay	
	Vitamin B_6	*current standard:AOAC 961.15*	*Microbioassay*	*II*
	Vitamin C	**AOAC 985.33**	2,6dichloroindophenol titrimetry	
	Vitamin C	*current standard: AOAC 967.21*	*Colorimetry (dichloroindophenol)*	*III*
	Choline	AOAC 999.14		
	Calcium	AOAC 984.27	ICP emission spectrometry	III
	Chloride	**AOAC 986.26** (chloride in milk based infant formula)	Potentiometry	
	Chloride	*current standard:AOAC 971.27 (Codex general method/canned vegetables)*	*Potentiometry*	*II*
	Fatty Acids	**AOAC 996.06**	Gas liquid chromatography	
	Fill of containers	CAC/RM 46-1972	Weighing	I
	Folic acid	**AOAC 992.05.**	Microbioassay	
	Folic acid	*current standard: AOAC 944.12*	*Microbioassay*	*II*
	Linoleic acid	AOAC 992.25	Gas chromatography	
	Niacin and nicotinamide	AOAC 985.34	Microbioassay and turbidimetry	
	Phosphorus	AOAC 986.24	Colorimetry (molybdovanadate)	II
	Protein efficiency ratio (PER)	AOAC 960.48	Rat bioassay	I
	Riboflavin	AOAC 985.31 (refers to AOAC 970.65)	*Fluorometry*	
	Riboflavin	*current standard: AOAC 970.65*	*Fluorometry*	*II*

	Selenium	AOAC 986.15 (Codex general method)	Atomic absorption spectrophotometry	II
	Sodium and potassium	ISO 8070 IDF 119A	Flame emission spectrophotometry	II
	Sodium and potassium	AOAC 984.27	ICP emission spectrometry	III
	Thiamine	AOAC 986.27 (thiamine in milk based infant formula)	Fluorometry	
	Thiamine	*current standard: AOAC 942.23 (thiamine in foods)*	*Fluorometry*	*II*
	Total dietary fibre	AOAC 985.29	Gravimetry (enzymatic digestion)	I
	Carbohydrates	method described in CAC/VOL IX Ed 1, Part III - see attachment		
	Crude protein	method described in CAC/VOL IX Ed 1, Part III (see text below)		
	Fat	CAC/RM 55-1976 - see attachment		

Determination of Crude Protein

6.1 According to the Kjeldahl method to determine the total nitrogen content (N) of the formula followed by calculation of crude protein content as follows:

N x factor = crude protein

6.2 Factors to be used

Wheat Protein: 5.70 - Soya Protein: 6.25 - Milk Derived Protein: 6.38

Mixed proteins:

(a) Where the food is comprised of more than, e.g. 90% (dry weight) of either wheat, soya or milk derived protein, the protein factor shall be 5.70, 6.25 and 6.38, respectively.

(b) Where the food is comprised of a known major amount (e.g. 80%) of dry weight of either wheat, soya or milk derived protein, the factor for that protein shall be the appropriate factor as shown above. Where the remaining protein is of an unknown mixture of these proteins, the factor 6.25 shall be applied to the remaining amount of protein.

(c) Where the amount each of wheat, soya or milk derived protein in the food is known, the protein factor used shall be that derived proportionally using the above factors or, using the applicable conversion factors given in “Energy and Protein Requirements”(FAO Nutrition Meetings Report Series, No. 52 or WHO Technical Report Series No. 522).

Results are expressed as g crude protein per 100 g of the food as sold and per 100 kcal and/or per 100 kJ (For calculation of calories, see Method 9 below).

9.9.2 When the product contains less than 15% protein and the quality is less than 70% that of casein, directions on the label shall state "Milk or formula but no water shall be used for dilution or mixing" or an equivalent statement.

9.9.3 When the product contains more than 15% protein, the instructions for dilution on the label shall state that water, milk or formula may be used for dilution or mixing, in accordance with medical advice or the legislation of the country in which the food is sold.

10. METHODS OF ANALYSIS AND SAMPLING

See Part III of this Section.

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PART III

METHODS OF ANALYSIS FOR FOODS FOR INFANTS AND CHILDREN

The methods of analysis referred to hereunder apply to the Codex Standards for Infant Formula, Cereal-based Foods for Infants and Children, and Canned Baby Foods.

1. Determination of Carbohydrate by Difference 1/

For the purposes of Section 10.3(a) of the Recommended International Standard for Infant Formula, Section 9.3(a) of the Recommended International Standard for Cereal-based Foods for Infants and Children, and for the calculation of Calories (see para 9.2(b) below), "carbohydrate by difference" is determined from the results of the determination of fat (Method 2), ash (Method 4), crude protein (Method 6), loss on drying (Method 8) and crude fibre (Method 5).

Results are expressed as g carbohydrate (by difference) per 100 g of the food as sold.

2. Determination of Fat

2.1 Definition of Fat

"Fat" includes all mono-, di- and triglycerides, together with other extractable substances such as phospholipids.

2.2 Method 1 - For All Infant Foods (CAC/RM 55-1976)

2.2.1 Scope and Field of Application

This method specifies the determination of the total fat content of foods for infants and children 2/. It has been based on the ISO International Standard No. 1443 - Meat and Meat Products - Determination of Total Fat Content, First Ed., 1973-04-15.

1/ This method is applicable where it is known that there is little or no "unavailable" carbohydrates other than crude fibre. (See also para. 89 of ALINORM 81/26).
2/ The fat obtained cannot be used for the determination of the characteristics of the fat.

2.2.2 Definition of Total Fat

Total fat of foods for infants and children: the fat extracted under the operating conditions described (see also para 2.1 Definition of Fat).

The total fat content is expressed as a percentage by mass.

2.2.3 Principle

Boiling of the test portion with dilute hydrochloric acid to free the occluded and bound lipid fractions, filtration of the resulting mass, drying, and extraction with n-hexane or light petroleum of the fat retained on the filter.

2.2.4 Reagents

All reagents shall be of a recognized analytical quality. Water shall be distilled water or water of at least equivalent purity.

2.2.4.1 Dry extraction solvent, n-hexane or, alternatively, light petroleum distilling between 30 and 60°C. For either solvent, the residue on complete evaporation shall not exceed 0.001 g per 100 ml.

2.2.4.2 Hydrochloric acid, approximately 4N solution. Dilute 100 ml of concentrated hydrochloric acid (ρ_{20} = 1.19 g/ml) with 200 ml of water and mix.

2.2.4.3 Blue litmus paper.

2.2.4.4 Boiling chips.

2.2.5 Apparatus

Usual laboratory equipment not otherwise specified, and the following items:

2.2.5.1 Conical flask, capacity 250 ml, fitted with a clock glass or condenser.

2.2.5.2 Clock glass or Petri dish, diameter not less than 80 mm.

2.2.5.3 Extraction thimble, made of defatted filter paper, glass, alumina or Teflon, contributing negligible to the blank.

2.2.5.4 Cotton wool, defatted.

2.2.5.5 Extraction apparatus, continuous or semi-continuous, for example, the Soxhlet type, with an extraction flask of about 150 ml capacity. Cooler fitted with drying tube or cotton wool.

2.2.5.6 Sand bath or water bath, electrically heated or similar suitable apparatus.

2.2.5.7 Drying oven, electrically heated, capable of being controlled at 102 ± 2°C.

2.2.5.8 Analytical balance.

2.2.5.9 Fluted filter paper, qualitative, of medium speed, e.g. S. en S., Selecta No. 595 1/2 Ø 15 cm. Normal filter paper, e.g. S. en S. 595.

2.2.5.10 Gas burner or thermostat controlled electric hot plate.

2.2.5.11 Crucible tongs or flat tipped pincette.

2.2.6 Procedure

2.2.6.1 Preparation of the Sample

Render the sample uniform by shaking or mixing. Keep it in a completely filled air tight container and store in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as possible, but in any case within 24 hours with paste-like samples.

2.2.6.2 Test Portion

Weigh 3 to 10 g - corresponding to ca. 3 g of dry matter - of the mixed sample to the nearest 0.001 g into the 250 ml conical flask (2.2.5.1).

2.2.6.3 Determination

Dry the flask of the extraction apparatus (2.2.5.5) containing some boiling chips, for 1 hour at 103 ± 2°C in the drying oven (2.2.5.7). Allow the flask to cool to room temperature and weigh to the nearest 0.0001 g. Add to the test portion 50 ml of the hydrochloric acid (2.2.4.2) and cover the conical flask (2.2.5.1) with a small watch glass or a "cold finger", or connect with reflux condenser. Heat the conical flask on an asbestos wire gauze by means of a gas burner or electric hot plate until the contents begin to boil. Continue boiling over a small flame or electric hot plate for 1 hour and shake occasionally. Keep volume of liquid nearly constant by adding water if necessary. Add 150 ml of hot water at the end and if desired ca. 1 g of diatomaceous earth (e.g. Hyflo-supercel) or approximately 100 cm^2 of defatted filter paper torn to small pieces.

Moisten the (fluted) filter paper (2.2.5.9) - use if desired a double filter - held in a glass funnel with water, and transfer immediately the hot contents from the flask to the filter. Wash the flask and the watch glass thoroughly three times with hot water, transfer the washings quantitatively on the filter and dry in the oven. Wash the filter with hot water until the washings do not affect the colour of the blue litmus paper. Put the filter paper on the clock glass or Petri dish (2.2.5.2) and dry for 1 hour in the oven at 102 ± 2°C. Allow to cool. (When filter paper has been added, dry overnight at 60°C). Roll up the filter paper and insert it into the extraction

thimble (2.2.5.3). Remove any traces of fat from the clock glass or the Petri dish, using cotton wool (2.2.5.4) moistened with the extraction solvent (2.2.4.1), and also transfer the cotton wool to the thimble. Place the thimble in the extraction apparatus. The filter paper shall be handled with tongs that can be rinsed or with a pincette. Pour the extraction solvent into the dried flasks of the extraction apparatus. Wash the inside of the conical flask used for the disintegration with hydrochloric acid, and the covering watch glass, cold finger or condenser with a portion of the extraction solvent and add it to the extraction flask. The total solvent quantity shall be one and a half to two times the capacity of the extraction tube of the apparatus. Fit the flask to the extraction apparatus. Heat the extraction flask for 4 hours on the sand bath, water bath or other apparatus (2.2.5.6) in such a way that the filter is extracted with at least 420 ml of petroleum ether.

After extraction, take the flask containing the liquid from the extraction apparatus and distil off the solvent, using, for example, the sand bath or water bath. Evaporate the last traces of the solvent on the water bath, using an air current, if desired.

Dry the extraction flask for 1 hour in the drying oven at 102 ± 2°C and, after allowing to cool to room temperature, weigh to the nearest 0.0001 g. Repeat these operations until the results of two successive weighings do not differ more than 1 mg. Verify the completion of the extraction by taking a second extraction flask and extracting for a further period of 1 hour with a fresh portion of the solvent. The increase in mass shall not exceed 2 mg; otherwise continue extraction for one or more hour periods in the same manner until completed. Add weight of fat recovered by this procedure to that of the fat from the first extraction. Carry out two determinations on the same prepared sample and two blank determinations on 10 ml of distilled water.

2.2.7 Expression of Results

2.2.7.1 Method of Calculation and Formula

The total fat content of the sample, expressed as a percentage by mass, is equal to:

$$(M_2 - M_1) - (B_2 - B_1) \times \frac{100}{M_0}$$

where

M_0 = mass, in grammes, of the test portion

M_1 = mass, in grammes, of the extraction flask with boiling chips

M_2 = mass, in grammes, of the flask and boiling chips with the fat, after drying

B_1 = mass, in grammes, of the flask used in the blank

B_2 = mass, in grammes, of the flask with the extract in the blank

Take as the result the arithmetic mean of the two determinations, if the requirement of 2.2.7.2 is satisfied.

Report the result rounded to two decimal places.

2.2.7.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not be greater than 0.2 g of total fat per 100 g of sample of dry products and not greater than 0.05 g per 100 g of liquid or paste-like samples or 0.2 g per 100 g of dry matter.

2.2.8 Test Report

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified, or regarded as optional, as well as any circumstances that may have influenced the result. The report shall include all details required for complete identification of the sample.

2.3 Method 2 - For Infant Foods containing Sugar and/or Dextrin Maltose but not containing Starch, Meat or Vegetable Products (Alternative Method)

According to Method B-2 (1967), Code of Principles concerning Milk and Milk Products, International Standards and Standard Methods of Sampling and Analysis for Milk Products, Seventh Ed., CAC/M/1-1973; Determination of the Fat Content of Dried Milk.

Note: (a) The addition of 5 ml ethanol before the second extraction, as in the Mojonnier tester procedure, somewhat improves the reproducibility of results, especially with weighings greater than 3 g of products containing much saccharose.

(b) It is not mandatory to carry out the third extraction in the case of products with fat content less than 1%.

3. Determination of Linoleate

Method to be elaborated (see para 90, ALINORM 76/23).

4. Determination of Ash

According to the method of the AOAC (Official Methods of Analysis of the AOAC, 1970, 7.010 Ash) 1/.

1/ Temporarily endorsed (see para 106, ALINORM 76/23).