CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Agenda Item 4.1 **CRD08 REV**

ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

42nd Session
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REVIEW OF METHODS OF ANALYSIS IN CXS 234: FATS AND OILS WORKABLE PACKAGE: COMMENTS

Comments and changes in reply to CX/MAS 23/42/4

(Electronic Working Group chaired by The Netherlands)

Background

1. This document is a reply to comments and changes suggested through the Codex Online Commenting System as summarized in CX/MAS 23/42/4. We would like to thank all contributors for their efforts.
2. In Appendix I, changes made as a result from CX/MAS 23/42/4 are displayed in **Red.**
3. Appendix I, Section A contains matters agreed by CCFO27. Major changes suggested to this section most probable need to be agreed upon by CCFO28.
4. Any background information concerning the deletion or re-typing of methods in Appendix I can be found here:
    a. Section A: REP/MAS41
    b. Sections B and C: CL 2022/60/OCS-MAS
5. This CRD will be used in the PWG on Endorsement on Monday June 12th 2023. As this document is subjected to changes, an updated CRD will be made available for the plenary session of CCMAS42.

General Comments

6. Currently, there is no check on the usage of certain terminologies between the workable packages. We advise to harmonize the terminologies used in CXS 234-1999 after revision of a workable package has been completed.
7. Examples of comments that concern this matter:
    a. ‘Liquid chromatography’, suggestion to add the description of the detector;
    b. Synthetic Phenolic antioxidants (PG, THBP, TBHQ, NDGA, BHA, BHT, OG, DG);
    c. Usage of abbreviations, ie. HPLC, HPLC-UV-DAD, etc.
    d. Addition of the respective Codex standard next to the commodity and provision pair.
8. We agree for Olive oils and Olive Pomace oils to follow the suggestion of Canada: ‘IOC, ISO and AOCS methods were identical at one time by design and agreement. The revision cycles of the three organizations are vastly different, with IOC being quicker to make improvements, and the other two updating the methods when alerted to the need for updating, or during a revision cycle, which means that AOCS and ISO may not have the most recent methods. As a result, for this activity, Canada is recommending that the IOC methods be held as Type II and that the other methods be retained as Type III, where they are not identified as Type I.

Revision numbers are not necessary as analysts are required to use the latest published version of analytical methods.

9. We would like to point out the matter raised by the EU that it is uncommon that a Type IV method exists next to a Type I method. This is now the case for:

a. a. Named animal fats – Titre. We suggest to implement a footnote as soon as the typing of both methods is agreed.

b. b. Named vegetable oils – Unsaponifiable matter. Here a footnote was suggested to clarify the presence of the Type IV method.

APPENDIX I

Section A – Matters agreed by CCFO27

Commodity	Provision	Method	Principle	Type	*Comment*
Fats and oils	~~Butylhydroxyanisole, butylhydroxytoluene, tert-butylhydroquinone, & propyl gallate~~	~~AOAC 983.15; or AOCS Ce 6-86~~	Liquid chromatography	~~II~~	
Fats and oils	**Synthetic phenolic antioxidants**	~~**AOCS Ce 6-86**~~ **AOCS Ce 6a-2021**	Liquid chromatography	II	
Fats and oils	**Synthetic phenolic antioxidants**	AOAC 983.15	Liquid chromatography	**III**	
Fish oils	Fatty acid composition	~~*AOCS Ce 1a-13*~~	~~Capillary GLC~~	~~III~~	
Fish oils	Fatty acid composition	~~*AOCS Ce 2-66*~~	~~Preparation of methyl esters by fatty acids~~	~~III~~	
Fish oils	Fatty acid composition	~~*AOCS Ce 2b-11*~~	~~Alkali hydrolysis~~	~~III~~	
Fish oils	Fatty acid composition	~~*AOCS Ce 2b-11 and AOCS Ce 1j-07*~~	~~Gas Chromatography of methyl esters~~	~~III~~	
Fish oils	Fatty acid composition	~~*AOCS Ce 1j-07*~~	~~Capillary GLC~~	~~III~~	
Fish oils	Fatty acid composition	~~*ISO 12966-2*~~	~~Gas chromatography~~	~~III~~	
Fish oils	Fatty acid composition	~~*ISO 5508*~~	~~Gas chromatography~~	~~III~~	
Fish oils	Fatty acid composition	***AOCS Ce 2-66 and AOCS Ce 1i-07***	**Gas Chromatography of methyl esters**	~~III~~ **II**	
~~Fish oils~~	~~Fatty acid composition~~	~~**AOCS Ce 2-66 and AOCS Ce 1a-13**~~	~~**Gas Chromatography of methyl esters**~~	~~**Remove**~~	
Fish oils	Fatty acid composition	**AOCS ~~Ce 2b-11~~ Ce 2c-66 and AOCS Ce 1i-07 / AOCS Ce 1j-07**	**Gas Chromatography of methyl esters**	III	*Ce 2b-11 is for finished food and microencapsulated foods. Ce 2c-66 is is applicable to common fats, oils, and fatty acids with the exception of milk fats.*

Fish oils	Fatty acid composition	**ISO 12966-2 and ISO 12966-4**	**Gas Chromatography of methyl esters**	III	
~~Fish oils~~	~~Fatty acid composition~~	~~AOCS Ce 1b 89~~	~~GLC~~ **~~Gas Chromatography of methyl esters~~**	~~**III / Remove**~~	*Method surplussed by AOCS*
Named Animal Fats	~~GLC ranges of fatty acid composition~~	~~ISO 5508 and ISO 12966-2; or AOCS Ce 2-66 and Ce 1e-91 or Ce 1f-96~~	Gas chromatography of methyl esters	~~II~~	
Named Animal Fats	Fatty acid composition	~~ISO 12966-2 and ISO 12966-4 / AOCS Ce 2-66 and Ce 1f-96 1j-07~~	Gas Chromatography of methyl esters	~~II~~	
Named Animal Fats	Fatty acid composition	**AOCS Ce 2-66 and Ce 1j-07**	Gas Chromatography of methyl esters	II	
~~Named Animal Fats~~	~~Fatty acid composition~~	**~~AOCS Ce 2-66 and~~** ~~**Ce 1f-96**~~	~~Gas Chromatography of methyl esters~~	~~II~~ **~~III~~**	*Method surplussed by AOCS*
Named Animal Fats	Fatty acid composition	**ISO 12966-2 and ISO 12966-4**	Gas Chromatography of methyl esters	~~II~~ **III**	
Named Animal Fats	Titre	~~ISO 935; or AOCS Cc 12-59~~	Thermometry	~~I~~	
Named Animal Fats	Titre	**ISO 935**	Thermometry **(not applied to Titre below 30°C)**	I	*Suggested type IV*
Named Animal Fats	Titre	**AOCS Cc 12-59**	Thermometry	~~I~~ **IV/Removal**	*Type IV next to Type I / Suggest to be Type I Refer to original review data below.*
Named Vegetable Oils	Crismer value	AOCS Cb 4-35 and AOCS Ca 5a-40	Calculation from individual fatty acid composition (gas chromatography of methyl esters) and turbidity	I	
Named Vegetable Oils	Halphen test	AOCS Cb 1-25	Colorimetry	I	

Named Vegetable Oils	Unsaponifiable matter	~~ISO 3596; or ISO 18609; or AOCS Ca 6b-53~~	~~Gravimetry~~	~~I~~
Named Vegetable Oils	Unsaponifiable matter	**ISO 3596 / AOCS Ca 6b-53**	**Diethyl ether extraction and** gravimetry, drying at 103 °C and titrimetry (colorimetry) [**and correction for free fatty acids titrimetry (colorimetry)]**[b]	I
Named Vegetable Oils	Unsaponifiable matter	**ISO 18609**[a]	**Hexane extraction and** Gravimetry, drying at 103 °C and titrimetry (colorimetry) [**and correction for free fatty acids titrimetry (colorimetry)]**[b]	~~I~~ **IV / Removal**

a. Results obtained from ISO 18609 are systematically lower. In case of limitations due to climate or regulations that prohibit the use of diethyl ether, ISO 18609 can be used instead of the Type I method.

b. The technique according to ISO is gravimetric. The correction by titration and colorimetry is only when it is necessary to correct for free fatty acids.

Review information for Named animal fats – Titre – ISO 935 and AOCS Cc 12-59

1. Fitness for Purpose: Answer yes or no; if no please comment
    a. Does the method test for the provision? Enter answer: YES
    b. Is the method applicable for the commodity? Enter answer: YES
    c. Is there a CODEX standard for the provision for said commodity? If NO, this the provision listed or identified somewhere else? Enter answer[1]: YES
    d. Is the method performance applicable to the Codex specifications listed for that provision/commodity (e.g. LOQ, applicable range)? Enter answer: YES

    Standard locations:

    STAN 19-1981 for fats and oils all or not covered by individual standards
    STAN 33-1981 for olive oils and olive pomace oils
    STAN 210-1999 for named vegetable oils
    STAN 211-1999 for named animal fats
    STAN 329-2017 for fish oils

    e. Is the test method sound? (See Eurochem guide[2]) Enter answer: YES
    a. Is the method properly validated or verified? (Eurochem).
    Enter answer: NO, none of the two methods include validation data. However, both are old, widely accepted legacy methods.

2. Confirm that, if more than one method is listed, the methods are truly identical. Choose one of the following per Codex definitions. Please comment if definition has changed. If there is only one method, skip to question 3.

    Identical
    Complementary

    Enter Answer: The two methods are not identical, nor complementary. Both methods share the same principle and are fairly similar, but they have critical differences. Critically, ISO 935:1988 describes a single apparatus setting that allows measuring titre values above 30°C. AOCS Cc 12-59 requires the assembling of two different apparatuses: one for titre values below 35°C, and one above for titre values above 35°C. There are also other differences in the sample preparation. As example, ISO 935:1988 (section 9.1) requires neutralizing the free fatty acids with sulfuric acid solution (generally 50 mL) until reaching neutral pH, verified with methyl orange, then wash 3 times with 150 mL of hot sodium chloride solution. Differently, Method AOCS Cc 12-59 requires addition of 50 mL of sulfuric acid solution without checking final pH (presumably turning acid, 1(c)), then wash the free fatty acids by adding an unspecified amount of water and boiling (1(d)), and repeat last step until the

[1] Information Document Sections that apply: Section 3.2.i All proposed method of analysis must have a direct pertinence to the Codex Standard to which they are directed; Section 3.9.i.a “an attribute in a Codex standard with a limit/range of values or a characteristic (authenticity)

[2] B. Magnusson and U. Ornemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org

washing water is neutral (1(e)). Also, in ISO 935:1988 purified free fatty acids are dried by mixing with 5 grams of anhydrous sodium sulfate (last step of 9.1). In AOCS Cc 12-59 the free fatty acids are dried by heating at 130°C for an unspecified length of time (1(g)).

If available and/or possible, give a brief summary table of the validation for each attribute for in the appendix of the US information document[3]:

Attribute – XXX	**Method A**	**Method B**
Matrices, samples used in collaborative study	None	None
Concentration range of matrices validated	None	None
Repeatability (RSD_r or s_r)	None	None
Reproducibility (RSD_R or s_R)	None	None
Recovery range from SLV/MLT	None	None
Accuracy (certified materials)	None	None
Limit of Quantification	None	None
Codex Stan 211-1999, titre of:		
Lard	32-45°C	32-45°C
Rendered pork fat	32-45°C	32-45°C
Premier jus	42.5-47°C	42.5-47°C
Tallow	40-49°C	40-49°C

Note: SLV refers to Single Laboratory Validation. MLT refers to Multi-Laboratory Testing studies (i.e., collaborative studies).

3. Confirm that the typing of the method is correct (See **Appendix 4**).

   Answer yes or no; if no please indicate correct type and comment.

Enter answer: Method ISO 935:1988 should remain type I, while AOCS Cc 12-59 should become type IV. As described above, the two methods are not identical, and none includes validation data. Method ISO 935:1988 (applicable for titre values above 30°C) describes a single procedure for determining the titre of all commodities reported in CXS 211-1999 (32-49°C); AOCS Cc 12-59 instead requires using one apparatus for titre values above 35°C, and another one for titre values below 35°C. In some instances AOCS Cc 12-59 does not indicate precise amounts of reagent/solvents (example, water for washing free fatty acids) or timings (example, how long the free fatty acids are dried at 130°C). As result, ISO 935:1988 should be preferred over AOCS Cc 12-59.

[3] Annex I of information document http://www.fao.org/fileadmin/user_upload/codexalimentarius/committee/docs/INF_CCMAS_END_e.pdf

Section B – Fish oil - Vitamin A and Vitamin D review

Commodity	Provision	Method	Principle	Type	Comment
Fish Oil	**Vitamin A[a]**	European Parharmcopeia Monograph on Cod Liver Oil (Type A), monograph 01/2005:1192, with LC end-point 2.2.29	~~LC~~ **Liquid Chromatography**	III	
Fish Oil	**Vitamin A[a]**	EN 12823-1 ~~(Determination of vitamin A by high performance liquid chromatograph – Part 1: Measurement of all-E-retinol and 13- Z-retinol)~~	~~LC~~ **Liquid Chromatography**	~~III~~ **II**	
Fish Oil	Vitamin D[b]	NMKL 167 ~~/~~ EN 12821 ~~(Determination of vitamin D by high performance liquid chromatography – Measurement of cholecalciferol (D3) or ergocalciferol (D2))~~	~~LC~~ **Liquid Chromatograpy**	~~III~~ **II**	
					EN 12821 and NMKL 167 are identical.

[a] The respective standard on fish oils CXS 329-2017 states that Vitamin A is expressed as 'Retinol equivalents'(RE) where RE takes into account the fact that different vitamers of vitamin A differ in activity. ISO/TR 23304:2021 "Food products – Guidance on how to express vitamins and their vitamers" may give clarity on this matter, for example for the relevant activities of the all-E-retinol levels and 13-Z-retinol levels.

[b] The provision accounts for Vitamin D2 and D3.

Section C – Review of Olive oil and Olive Pomace Oils methods

Provision	Method	Principle	Type	*Comment*
Absorbency in ultra-violet	~~COI/T.20/Doc. No. 19; or ISO 3656; or AOCS Ch 5-91~~	~~Absorption in ultra violet~~	~~II~~	
Absorbance in ultra-violet	**COI/T.20/Doc. No. 19 ~~/ ISO 3656~~**	**Spectrophotometry**	II	
Absorbance in ultra-violet	**AOCS Ch 5-91**	**Spectrophotometry**	~~II~~ **III**	
Absorbance in ultra-violet	**ISO 3656**	**Spectrophotometry**	**~~II~~ III**	
Difference between the actual and theoretical ECN 42 triglyceride content	COI/T.20/Doc. No. 20 **and COI/T.20/Doc. No. 33/Rev.1** ~~or AOCS Ce 5b-89~~	~~Analysis of triglycerides of HPLC and calculation~~ **Calculation from triglycerides by HPLC and Fatty Acid Methyl Esters by Gas Chromatography**	I	
Erythrodiol and uvaol	**COI/T.20/Doc.no. 26**	**Gas chromatography**	**II**	*Doc.no.30 is not in use any more*
Fatty acid composition	**COI/T.20/Doc. no. 33**	**Gas chromatography of methyl esters**	**II**	
Halogenated solvents - traces	**COI/T.20/Doc. no. 8**	**Gas chromatography**	**II**	
Lead	AOAC 994.02~~; or~~ **/** ISO 12193~~; or~~ **/** AOCS Ca 18c-91	~~AAS~~ **Atomic absorption spectrophotometry (direct graphite furnace)**	II	*Performance criteria instead?*
Organoleptic characteristics	COI/T.20/Doc. No. 15	~~Panel test~~ **Sensory analysis by a panel**	I	
Refractive index	~~ISO 3960; or AOCS Cd 8b-90~~ **ISO 6320 / AOCS Cc 7-25**	Refractometry	I	*
Relative density	ISO 6883, with the appropriate conversion factor~~; or~~ **/** AOCS Cc 10c-95	Pycnometry	I	
Saponification value	ISO 3657~~; or~~ **/** AOCS Cd 3-25	~~Titrimetry~~ **(Colorimetric)**	I	
~~Sterol composition and total sterols~~ **~~4α-desmethylsterol and total sterol content~~ Desmethylsterol (% of total sterols)**	COI/T.20/Doc. No. ~~30~~ **26**~~; or~~ **/** ISO 12228-2~~; or~~ **/** AOCS Ch 6-91	**Thin-layer chromatography and** Gas Chromatography	II	

Stigmastadiene~~s~~ **content**	Col/T.20/Doc. No. 11~~; or / ISO 15788-1; or / AOCS Cd 26-96~~	**Preparative column chromatography and** Gas chromatography	II	*COI document was updated, ISO and AOCS no changes*
Stigmastadiene~~s~~ **content**	**ISO 15788-1; or / AOCS Cd 26-96**	**Preparative column chromatography and** Gas chromatography	**III**	
Stigmastadiene~~s~~ **content**	ISO 15788-2	~~HPLC~~ **Liquid Chromatography**	III	
***Trans* fatty acids content**	~~COI/T.20/Doc. No 17/Rev.1~~ **COI/T.20/Doc. No 33/Rev.1 /** ~~ISO15304~~	**Gas Chromatography of methyl esters**	II	*Were ISO and ACOS methods updated?*
Trans fatty acids content	**ISO 12966-4 / AOCS Ch2a-94**	**Gas Chromatography of methyl esters**	**III**	
Unsaponifiable matter	~~ISO 3596; or ISO 18609; or AOCS Ca 6b-53~~	~~Gravimetry~~	~~I~~	
Unsaponifiable matter	**ISO 3596 / AOCS Ca 6b-53**	**Gravimetry, drying at 103 ºC and titrimetry (colorimetry)**	I	
Unsaponifiable matter	**ISO 18609**	**Gravimetry, drying at 103 ºC and titrimetry (colorimetry)**	~~I~~ **IV**	*Type IV next to type I suggests removal of this line*
Wax content	COI/T.20/Doc. no. ~~18~~ **28**~~; or~~ **/** **AOCS Ch 8-02**	Gas chromatography	II	
Wax content	**AOCS Ch 8-02**	**Gas chromatography**	**III**	*COI document was updated, AOCS no changes*