CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 2 **CRD08**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON FATS AND OILS
Twenty-Seventh Session
Virtual, 18 - 26 October 2021
MATTERS REFERRED BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER SUBSIDIARY BODIES

(Comments from EU, Norway and GOED)

EU

Mixed Competence.

Member States Vote

Part 4.4 Referral to CCFO: For consideration and reply by CCFO

The European Union and its Member States (EUMS) welcome that AOCS and ISO are collaborating to produce identical methods for synthetic antioxidants to replace AOCS Ce-6-86 by 2023. This puts the acceptance of the method for the determination of antioxidants on a broader basis. The extent to which the method AOAC 983.15 will then still be required cannot be assessed at the present time.

The EUMS consider that it would not have an impact for trade when endorsing the AOCS methods for fatty acid composition for Type II.

Method ISO 935:1988 and AOCS Cc12-59 are different to some extent but the used titre assembly or cooling apparatus are comparable, differences of the methods apply only to details in the procedure. Therefore, the EUMS do not expect any impact for trade upon retyping. In addition, the application of this method is probably not wide-spread today, so that no influence on the market is to be expected for this reason.

The EUMS are of the opinion that the Crismer value is not relevant today anymore because other methods to proof identity of oils are available. In some cases, this method might be applied in the oleochemical industry. The Halphen test could have some importance in other countries for the fast quantitative detection of cottonseed oil in other oils. No major equipment is required, so that it cannot be ruled out that the method is still used in some countries.

Method ISO 18609:2001 uses hexane to solve the unsaponifiable matter instead of diethyl ester used by method ISO 3596:2001 or AOCS Ca 6b-53. For health reasons, diethyl ether is preferable to hexane because hexane is metabolized in the body to 2,5-hexanedione, this causes nerve damage and is excreted in the urine. Therefore, today other solvents increasingly replace hexane. Thus, and in addition to the fact that the method produces systematically underestimated results, the EUMS would recommend to reject method ISO 18609:2001.

Norway

Norwegian comment to CX/FO 21/27/2, B Matters for action, para 16, Annex I, para 47, 2nd bullet

We would like to provide our comment on agenda item 2, CX/FO 21/27/2, B Matters for action, para 16, Annex I, para 47, 2nd bullet regarding the request from CCMAS to consider the criteria approach for the methods for determination of total arsenic in fats and oils (all) and inorganic arsenic in fish oils. We kindly ask CCFO to consider our proposals below:

We support establishing method performance criteria, as it gives laboratories more flexibility in the choice of method, and the applicability and the performance of the selected methods (examples of applicable methods) are considered more carefully through validation documentation. We suggest criteria for arsenic and inorganic arsenic based on the Maximum Level (ML) for edible fats and oils given for arsenic at page 45 of CXS 193-1995.

For edible fats and oils, the Maximum Level (ML) is 0.1 mg/kg. For fish oils covered by CXS 329-2017, the ML

is for fish oils (As-in). Countries or importers may decide to use their own screening when applying the ML for As-in in fish oils by analyzing total arsenic (As-tot) in fish oils. If the As-tot concentration is below the ML for As-in, no further testing is required, and the sample is determined to be compliant with the ML. If the As-tot concentration is above the ML for As-in, follow-up testing shall be conducted to determine if the As-in concentration is above the ML.

We suggest the following performance criteria for arsenic and inorganic arsenic given in Table 1 and 2 respectively:

Table 1: Method performance criteria for arsenic

Commodity: Edible fats and oils **Provision: Arsenic** **ML (mg/kg): 0.1 mg/kg**						
Min. Appl. Range (mg/kg)	**LOD (mg/kg)**	**LOQ (mg/kg)**	**Precision (%) No more than**	**Recovery (%)**	**Examples of applicable* methods**	**Principle**
0.032–0.17	0.01	0.02	44	80-110	AOAC 963.21 and AOAC 942.17	Kjeldahl flask digestion and Colorimetry (molybdenum blue)
					AOAC 963.21 and AOAC 952.13	Kjeldahl flask digestion and Colorimetry (diethyldithiocarbamate)
					AOAC 986.15	Atomic absorption spectrophotometry
					NMKL 186 / AOAC 2013.06 / EN 15763	ICP-MS

* The applicability of the methods has to be verified by the standard developing organisations

Table 2: Method performance criteria for inorganic arsenic

Commodity: Fish oil **Provision: Inorganic arsenic** **ML (mg/kg): 0.1 mg/kg**						
Min. Appl. Range (mg/kg)	**LOD (mg/kg)**	**LOQ (mg/kg)**	**Precision (%) No more than**	**Recovery (%)**	**Examples of applicable methods***	**Principle**
0.032–0.17	0.01	0.02	44	80-110	EN 15517	Hydride generation atomic absorption spectrometry (HGAAS) after acid extraction
					EN 16802	Anion-exchange HPLC-ICP-MS

* The applicability of the methods has to be verified by the standard developing organisations

GOED

Matters referred by CCMAS, B. Matters for Action / CCMAS 41, Review of Methods of Analysis in CXS 234-1999: Fats and Oils Workable Package, point 49.

GOED, the Global Organization for EPA and DHA Omega-3s, represents the worldwide EPA and DHA omega-3 industry, with a mission to increase consumption of EPA and DHA omega-3s around the world. The membership is built on a quality standard unparalleled in the market and members must comply with quality and ethics guidelines that ensure members produce quality products that consumers can trust. Our 170+ members represent the entire supply chain of EPA and DHA omega-3s, from fisheries and crude oil suppliers to refiners, concentrators and finished product brands.

GOED requests the below information be uploaded on the Codex website as a Conference Room Document (CRD), in relation to *CX/FO 21/27/02* for Agenda Item 2 on the Review of Methods of Analysis in CXS 234-1999: Fats and Oils Workable Package.

1. Methods for the quantification of omega-3 fatty acids in Fish oils

We refer to agenda item point 2, point 49, as forwarded from CCMAS.

For the category “Fish oils,” a number of methods for the determination of “Fatty acid composition” are listed. In our opinion, suitable methods for the quantification of the omega-3 fatty acids, EPA, DHA and the Total Omega-3 Fatty Acids in fish oils should be added (in addition to AOCS Method Ce 1i-07 which is already provided in CXS234). These are:

- European Pharmacopoeia method 2.4.29 “Composition of Fatty Acids in Oils rich in Omega-3 Acids”
- United States Pharmacopeia method USP401 “Fats and Fixed Oils.”

Whereas we support elevating method AOCS Ce 1i-07 to a Type II method status, both mentioned pharmacopeial methods are considered equally suitable for the quantification of EPA, DHA and Total Omega-3 Fatty Acids in fish oils (composed of triglycerides, as well as omega-3 ethyl ester concentrates prepared from fish oils). These methods are widely employed in the industry globally, and used on par with the AOCS Ce 1i-07 method in the Laboratory Proficiency Program that the American Oil Chemists’ Society (AOCS) organizes annually for laboratories to quantify EPA, DHA and Total Omega-3 Fatty Acids in fish oils and omega-3 concentrates. Both pharmacopeial methods could be considered a Type II method, and method validation details are retained by the respective pharmacopeial organizations.

As additional information to CCFO, GOED provides the following information and suggestions regarding methods applicable to the commodity Fish oil:

2. Moisture and volatile matter in Fish oils

Comment – The method ISO 662 “Moisture and volatile matter” (listed in CX/FO 21/27/2 - Appendix II – Method “ISO 662”) for the determination of moisture and volatile matter in fish oils is a very old method and is not suitable for newer type of fish oils that are commercialized today. Such oils today consist of refined fish oils and concentrates of EPA and DHA that are very sensitive to oxidation, and for sure will rapidly oxidize (if not handled under an inert atmosphere) under the conditions specified in this method which involving drying at 105°C. Such refined and concentrated fish oils will gain weight due to a very fast oxidation instead of losing weight as expected by the loss of moisture and volatile matter, and this method is therefore not universally useful for fish oil anymore. It is possible that at the time the method was developed and adopted by Codex its suitability was limited to crude fish oils, for which ISO 662 remains in use today.

For this reason, GOED recommends the inclusion of suitable methods for the commodity “Fish Oils” for the determination of water/moisture content that are based on Karl Fischer titration, notably AOCS Official Method Ca 2e-84 (“Moisture, Karl Fischer Method”), European Pharmacopoeia method 2.5.12 (“Water: Semi-Micro Determination”), and the United States Pharmacopeia method 921 (“Water Determination”).

In addition, CCMAS may want to consider addressing the suitability of ISO 662 for “Fats and Oil (all)”. It is probably more correct to limit the recommended use of this method only for specific named oils, for example:

The recommendation to use ISO 662 for the determination of “Moisture and volatile matter” should be maintained for “Named Vegetable Oils.”

The recommendation to use ISO 662 for the determination of “Moisture and volatile matter” should be

maintained for “Olive Oils and Olive Pomace Oils.”

3. Arsenic, under the category “Fats and Oils (all)”

Codex has adapted the following requirement for arsenic[1] in edible oils, in CXS 193-1995 (General Standard for Contaminants and Toxins in Food and Feed, see page 45); “If the As-tot concentration is below the maximum levels (ML) for As-in, no further testing is required, and the sample is determined to be compliant with the ML. If the As-tot concentration is above the ML for As-in, follow-up testing shall be conducted to determine if the As-in concentration is above the ML.”

For fish oils covered by CXS 329-2017, the ML is for (As-in). Hence, we suggest including a recommended method for the analysis of inorganic arsenic (As-in) that is suitable for fish oils (including krill oil) in CX/FO 21/27/2 - Appendix II:

- Analysis of foodstuffs - Determination of inorganic arsenic in algae - Atomic absorption spectrometry-hydride technique (HGASS) after acid extraction (adoption of the standard of the same name, DIN EN 15517, September 2008 edition) - DIN EN 15517

	Commodity	Provision	Method	Principle	Type
Current	Fats and Oils (all)	Arsenic	AOAC942.17	Colorimetry (molybdenum blue)	III
Revised	Fats and Oils (all)	Arsenic	AOAC 963.21 and AOAC 942.17	Kjeldahl flask digestion and colorimetry (molybdenum blue)	III
Current	Fats and Oils (all)	Arsenic	AOAC952.13	Colorimetry (diethyldithiocarbamate)	II
Revised	Fats and Oils (all)	Arsenic	AOAC 963.21 and AOAC 952.13	Kjeldahl flask digestion and colorimetry (diethyldithiocarmabate)	II
Revised	Fats and Oils (all)	Arsenic	AOAC 986.15	Atomic absorption spectrophotometry	III
Proposed (additional)	Fats and Oils (all)	Arsenic, inorganic	DIN EN 15517	Atomic absorption spectrometry-hydride technique (HGASS)	II/III

4. Phospholipids, under the category Fish oils

GOED supports the inclusion of method USP-FCC 12 2S (krill oil phospholipids) in Appendix II of CX/FO 21/27/2 for the commodity fish oils, provision “phospholipids”, as a Type I method.

Krill oils are marine oils rich in the omega-3 fatty acids EPA and DHA, which also fall within the scope of the Codex Standard for Fish Oil (CXS 329-2017). CXS 329-2017 applies to fish oils for human consumption, with the term fish oils referring to oils derived from fish and shellfish, including krill oil. As of today, krill oil is the only phospholipid-rich oil included under named fish oils. Currently, CODEX STAN 234-1999 recommends analyzing phospholipids by the method described in the USP FCC 10 2S Krill oil monograph, under specific test “Phospholipids,” with reference to Nuclear Magnetic Resonance (NMR) Spectroscopy, described in Appendix II. The phospholipid method in the Krill oil monograph provides detailed method instructions and includes sample preparations, reference standards, recommended proton resonance frequency and resolution, instructions for data collection of the H and P spectrum, analysis of six major phospholipid types, calculation of total phospholipids, etc.

While the NMR spectroscopy description under USP-FCC 11 1S could be understood as a reference to a

[1] Definition of Arsenic: total (As-tot) when not otherwise mentioned; inorganic arsenic (As-in); or other specification.

general method principle description in FCC, it is not specific for phospholipids in krill oil (or other marine oils). We are concerned that by removing the reference to the USP FCC 10 2S Krill oil monograph, the Codex recommended method for phospholipids in marine oils will lose specificity and open the possibility for the use of non-qualified NMR-based methods and a larger variation in test results.

Proficiency Testing of 31P NMR Method for Phospholipid Analysis in Krill Oil has been published in *J Am Oil Chem Soc*[2]. From personal communication with Bernd Diehl, the 31P NMR Method for Phospholipid Analysis in Krill Oil in USP FCC 10 2S is based on the same principle as the method published in *J Am Oil Chem Soc*. The method described in the USP FCC 10 2S Krill oil monograph is the only officially available method that is validated for determining Phospholipid content in krill oils. Because of this, we suggest keeping the 31P NMR Method for Phospholipid Analysis method, as a type I, Defining Method.

By changing from a type I to a type IV method, as discussed in CCMAS41, the committee would furthermore open up phospholipid analysis to other phospholipid quantification methods that are less specific and not able to differentiate fraudulent products, which is a major problem in several Asian markets. Many other phospholipid quantification methods are indirect and do not differentiate if phospholipid originates from krill or from soya lecithin, and even presence of salts, giving false positive results.

For these reasons, GOED supports the inclusion of method USP-FCC 12 2S (krill oil phospholipids) in Appendix II of CX/FO 21/27/2 for the commodity fish oils, provision “phospholipids”, as Type I method.

Thank you for your consideration,

[2] Zailer, Monakhova, Diehl. 31P NMR Method for Phospholipid Analysis in Krill Oil: Proficiency Testing—A Step toward Becoming an Official Method. J Am Oil Chem Soc. 2018. DOI 10.1002/aocs.12153