GLOBAL ORGANIZATION FOR EPA AND DHA OMEGA-3S

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**TO**: Dutch Secretariat for CX/MAS and Secretariat, Codex Alimentarius Commission

**FROM**: Global Organization for EPA and DHA Omega-3s (GOED)

**RE**: Agenda Item 4.2 - Review of Methods of Analysis in CXS234: Fats and Oils Workable Package

**DATE**: May 10, 2021

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/MAS 21/41/5* for Agenda Item 4.2 on the Review of Methods of Analysis in CXS234: Fats and Oils Workable Package.

**Review of Methods**

Whereas GOED has previously reviewed individual methods that are listed in the document "Review of Methods of Analysis in CXS234: Fats and Oils Workable Package," upon request by AOCS, GOED has now reviewed the complete document.

Based on our review, GOED has the following comments and suggestions:

**1. Moisture and volatile matter in Fish oils**

Appendix I, Part A – Method "ISO 662" for the determination of "Moisture and volatile matter," is not suitable for fish oils. The drying at 105ºC. will lead to a very fast oxidation of fish oils, with a concomitant increase in weight (instead of a loss in weight due to removal of water). Whereas the method is likely suitable for most vegetable oils, some amendments are suggested to be made:

- Page 4 – Suitability of ISO 662 for "Fats and Oil (all)" should be removed.

- Page 9 – The recommendation to use ISO 662 for the determination of "Moisture and volatile matter" should be maintained for "Named Vegetable Oils."
- Page 12 – The recommendation to use ISO 662 for the determination of "Moisture and volatile matter" should be maintained for "Olive Oils and Olive Pomace Oils."

GOED recommends the inclusion of suitable methods for the determination of water/moisture content in Fish oils 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").

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

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). 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 used on par with the AOCS Ce 1i-07 method in the Laboratory Proficiency Program that AOCS organizes annually for laboratories to measure EPA, DHA and Total Omega-3 Fatty Acids. Both pharmacopeial methods could be considered a Type II method, and method validation details are retained by the respective pharmacopeial organizations.

## 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."

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

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):

- 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 colorime- try (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 colorime-try (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. Additional tests for the category Fish oils

In general, for the category "Fish oils" some additional useful tests could be included:

- Unsaponifiable Matter. Suitable methods: European Pharmacopoeia 2.5.7; USP/NF 401 "Unsaponifiable Matter"; AOCS Ca 6b-53.
- Cold Test. Suitable methods: European Pharmacopoeia method "Stearin" (for cod-liver oil); AOCS Cc 11-53.
- Density. Suitable methods: AOCS Cc 10c-95; AOCS To 1a-64.

## 5. ISO methods for Fish oils

Already in 1997-1998, IFOMA, the predecessor of IFFO (https://www.iffo.com/), made an evaluation to harmonize its approved methods for Fish Oil and Fishmeal with ISO methods considered suitable for fish oils (and fishmeal). The committee may want to evaluate including the following suitable methods in its document listing:

- Unsaponifiable Matter. Method: ISO 3596-1 (1988)
- Specific Gravity of Fish Oil: Method: ISO 6883 (2017).
- Insoluble impurities in Fish Oil. Method: ISO 663 (2017)
- Color of Fish Oil – Method: ISO/CD 15305 (Lovibond Color Method - 1998); AOCS Td 1a-64 (Gardner Color Method)
- Sampling: Method: ISO 5555 (2001)

## 6. Phospholipids, under the category Fish oils

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 (CODEX CXS 329-2017). CODEX 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 IIC. 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 nuclear magnetic resonance spectroscopy description under USP-FCC 11 1S could be understood as a reference to a 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

[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

oils. Because of this, we suggest keeping the 31P NMR Method for Phospholipid Analysis method, as a type I, Defining Method.

| | Commodity | Provision | Method | Principle | Type |
|---|---|---|---|---|---|
| **Current** | Fish oils | Phospholipids | USP-FCC 10 2S (Krill oil):<br><br>Phospholipids Nuclear Magnetic Resonance, Appendix IIC | NMR Spectroscopy | I |
| **Revised** | Fish oils | Phospholipids<br><br>*Canada: USP does not publish validation data, refer to JAOCS article | USP-FCC 11 1S | Nuclear Magnetic Resonance<br><br>Spectroscopy | IV |
| **Proposed**<br><br>**(instead of revised)** | Fish oils | Phospholipids | USP-FCC 11 1S (Krill oil):<br><br>Phospholipids Nuclear Magnetic Resonance, Appendix IIC | Nuclear Magnetic Resonance<br><br>Spectroscopy | I |

By changing from a type I to a type IV method, 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.

Thank you for your consideration,

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