CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Agenda Item 3 **CX/MAS 20/41/3**

January 2020

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

41st Session
Budapest, Hungary, 11 - 15 May 2020

ENDORSEMENT OF METHODS OF ANALYSIS AND SAMPLING PLANS FOR PROVISIONS IN CODEX STANDARDS

1. This document contains the methods of analysis (Appendix I, II, III and IV) proposed by the following Committees:

- FAO/WHO Coordinating Committee for Africa (methods of analysis for provisions in the proposed draft standard for dried meat)
- FAO/WHO Coordinating Committee for North America and South West Pacific (Methods of analysis for provisions in the proposed draft regional standard for fermented noni fruit juice and the proposed draft regional standard for kava products for use as a beverage when mixed with water)
- FAO/WHO Coordinating Committee for Near East (Methods of analysis for provisions in the draft regional standard for mixed zaatar)

  and

- Codex Committee on Nutrition and Foods for Special Dietary Uses (methods of analysis for provisions in the standard for infant formula and fomulas for special medical purposes intended for infants)

FAO/WHO COORDINATING COMMITTEE FOR AFRICA (CCAFRICA23)[1]

Methods of analysis for provisions in the proposed draft standard for dried meat

2. The Committee **is invited to endorse** the methods of analysis in Appendix I.

FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND SOUTH WEST PACIFIC (CCNASWP15)[2]

Methods of analysis for provisions in the proposed draft regional standard for fermented noni fruit juice and the proposed draft regional standard for kava products for use as a beverage when mixed with water

3. The Committee **is invited to endorse** the methods of analysis in Appendix II.

FAO/WHO COORDINATING COMMITTEE FOR NEAR EAST (CCNE10)[3]

Methods of analysis for provisions in the proposed draft regional standard for mixed zaatar

4. The Committee **is invited to endorse** the methods of analysis in Appendix III.

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES (CCNFSDU41)

Methods of analysis for provisions in the Standard for infant formula and formulas for special medical purposes intended for infants (CXS 72-1981)[4]

[1] REP20/AFRICA, para 102 ii) and Appendix V
[2] REP20/NASWP, paras 83 (ii), 96 (iii) and Appendix II, III
[3] REP20/NE, para 87 (ii) and Appendix IV
[4] REP20/NFSDU, para 197

5. The Committee agreed to submit the methods for thiamine, riboflavin, niacin, vitamin B_6, choline, carnitine, beta-carotene, lycopene, fructans and biotin to CCMAS for review and endorsement and inclusion in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) and request CCMAS to re-type the existing Type II methods for aforementioned nutrients as Type III in CXS 234-1999; and inform CCMAS that it could include AOAC 2011.14 / ISO 15151 | IDF 229 for calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sodium and zinc as Type III in CXS 234-1999.

6. The Committee **is invited to endorse** the methods of analysis and consider the re-typing of existing methods in Appendix IV.

APPENDIX I

FAO/WHO COORDINATING COMMITTEE FOR AFRICA (CCAFRICA23)

Methods of analysis for provisions in the proposed draft standard for dried meat

10. METHODS OF ANALYSIS AND SAMPLING

Method	Title	PRINCIPLES	TYPE
AOAC 988.05	Determination of Moisture Content	Gravimetry	I
ISO 1443 (AOAC 960. 39)	Determination of Crude Fat	Gravimetry	I
AOAC 928.08	Determination of Crude Protein	Kjeldhal	II
ISO 937	Determination of Crude Protein	Titrimetry	II
ISO 1841-1 and ISO 1841-2	Determination of Edible Salt	Potentiometric / Volhard method	II
AOAC 940.26	Determination of Ash Content	Gravimetry	I
ISO 18787	Determination of Water Activity	Potentiometric	II

APPENDIX II

FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND SOUTH WEST PACIFIC (CCNASWP15)

Methods of analysis for provisions in the proposed draft regional standard for fermented noni fruit juice

10. METHODS OF ANALYSIS AND SAMPLING

10.1 Methods of Analysis

Provision	Method	Principle	Type	Notes
Brix value	AOAC 983.17	Refractometry	I	Adopted for fruit juices and nectars
pH value	NMKL 179	Potentiometry	II	Adopted for fruit juices and nectars
Ethanol	IFUMA 52	Enzymatic determination	II	Adopted for fruit juices and nectars
Identification of scopoletin	Annex A*	Thin layer chromatography	IV	
Identification of deacetylasperulosidic acid	Annex B*	Thin layer chromatography	IV	

ANNEX A

IDENTIFICATION OF SCOPOLETIN

1. PREPARATION OF SAMPLES

1.1 Noni fruit is mashed. Two grams of mashed fruit is extracted twice with 125 milliliters methanol. The methanol extract is concentrated by evaporation of the solvent under vacuum. The extract is then re-dissolved in a small quantity of methanol, such as 10 milliliters.

1.2 Noni juice is filtered through a 0.45 µm membrane filter and then purified by solid-phase extraction (SPE) with Waters OASISS® extraction cartridges, or similar solid-phase extraction cartridge. [SPE cartridges is first equilibrated with water, followed by methanol. The samples are then loaded onto the cartridge and washed with 5% MeOH, followed by 100% MeOH. The MeOH eluate is retained for TLC analysis.]

1.3 One gram of noni fruit powder is extracted with 5 milliliters of methanol. The methanol extract is filtered and evaporated to dryness under vacuum at 50°C. The extract is dissolved into one milliliter of methanol.

2. PREPARATION OF REFERENCE STANDARD

2.1 A reference standard is prepared by dissolving 1 mg scopoletin in 1 milliliter of methanol.

2.2 Alternately, certified *Morinda citrifolia* reference plant material may be prepared in the same manner as the samples to be analyzed. The certified *Morinda citrifolia* reference material should be from the same part of the plant as the samples to be analyzed.

3. IDENTIFICATION

3.1 THIN LAYER CHROMATOGRAPHY

Spot 5 microliters of sample solutions and reference standard solution on a silica gel [60 F254] thin layer chromatography (TLC) plate, previously dried at 110 °C for 15 minutes in a drying oven. [Develop the plate with a lower solution mobile phase of dichloromethane:methanol (19:1, v/v).] View bright fluorescent blue colours on developed plate under UV lamp, 365 nm. Identify scopoletin in samples by comparing Rf values and colours to the standard.

REFERENCES

1. Deng S, West BJ, Jensen J. A Quantitative Comparison of Phytochemical Components in Global Noni Fruits and Their Commercial Products. Food Chemistry 2010, 122 (1): 267-270.

2. Potterat O, et al. Identification of TLC markers and quantification by HPLC-MS of various constituents in noni fruit powder and commercial noni-derived products. Journal of Agricultural and Food Chemistry 2007, 55(18):7489–7494.

3. Basar S, Westendorf J. Identification of (2E, 4Z, 7Z)-Decatrienoic Acid in Noni Fruit and Its Use in Quality Screening of Commercial Noni Products. Food Analytical Methods 2011, 4(1):57-65. DOI: 10.1007/s12161-010-9125-9.

4. Chan-Blanco Y, et al. The ripening and aging of noni fruits (*Morinda citrifolia* L.): microbiological flora and antioxidant compounds. Journal of the Science of Food and Agriculture 2007, 87:1710 – 1716.

5. West BJ, Deng S. Thin layer chromatography methods for rapid identity testing of *Morinda citrifolia* L. (noni) fruit and leaf. Advance Journal of Food Science and Technology 2010, 2(5):298-302.

ANNEX B

IDENTIFICATION OF DEACETYLASPERULOSIDIC ACID

1. PREPARATION OF SAMPLES

1.1 Noni fruit is mashed. Two grams of mashed fruit is extracted twice with 125 milliliters methanol. The methanol extract is concentrated by evaporation of the solvent under vacuum. The extract is then re-dissolved in a small quantity of methanol, such as 10 milliliters.

1.2 Noni juice is filtered through a 0.45 µm membrane filter and then purified by solid-phase extraction (SPE) with Waters OASISS® extraction cartridges, or similar solid-phase extraction cartridge. [SPE cartridges is first equilibrated with water, followed by methanol. The samples are then loaded onto the cartridge and washed with 5% MeOH, followed by 100% MeOH. The MeOH eluate is retained for TLC analysis.]

1.3 One gram of noni fruit powder is extracted with 5 milliliters of methanol. The methanol extract is filtered and evaporated to dryness under vacuum at 50°C. The extract is dissolved into one milliliter of methanol.

2. PREPARATION OF REFERENCE STANDARD

2.1 A reference standard is prepared by dissolving 1 mg deacetylasperulosidic acid in 1 milliliter of methanol.

2.2 Alternately, certified *Morinda citrifolia* reference plant material may be prepared in the same manner as the samples to be analyzed. The certified *Morinda citrifolia* reference material should be from the same part of the plant as the samples to be analyzed.

3. IDENTIFICATION

3.1 THIN LAYER CHROMATOGRAPHY

Spot 5 microliters of sample solutions and reference standard solution on a silica gel [60 F254] thin layer chromatography (TLC) plate, previously dried at 110 °C for 15 minutes in a drying oven. [Develop the plate with a lower solution mobile phase of dichloromethane: methanol: water (13:6:1, v/v/v).] Spray developed plate with 2% anisaldehyde, 10% sulfuric acid-EtOH solution then heat in oven at 110 °C for 1 minute to reveal blue colour. Identify deacetylasperulosidic in samples by comparing Rf values and colours to the standard.

REFERENCES

1. Potterat O, et al. Identification of TLC markers and quantification by HPLC-MS of various constituents in noni fruit powder and commercial noni-derived products. Journal of Agricultural and Food Chemistry 2007, 55(18):7489–7494.

2. Deng S, et al. Determination and comparative analysis of major iridoids in different parts and cultivation sources of *Morinda citrifolia*. Phytochemical Analysis 2011, 22(1):26-30.

3. West BJ, Deng S. Thin layer chromatography methods for rapid identity testing of *Morinda citrifolia* L. (noni) fruit and leaf. Advance Journal of Food Science and Technology 2010, 2(5):298-302.

Methods of analysis for provisions in the proposed draft regional standard for kava products for use as a beverage when mixed with water

8. METHODS OF ANALYSIS AND SAMPLING

Provision	Method	Principle	Type
Noble kava varieties	Lebot V, Legendre L (2016), Comparison of kava (Piper methysticum Forst.) varieties by UV absorbance of acetonic extracts and high-performance thin-layer chromatography. Journal of Food Composition and Analysis 48:25-33. http://dx.doi.org/10.1016/j.jfca.2016.01.009 and Lebot V, Michalet S, Legendre L. (2019). Kavalactones and flavokavins profiles contribute to quality assessment of kava (Piper methysticumG.Forst.), the traditional beverage of the Pacific. Beverages 2019, 5, 34; https://doi.org/10.3390/beverages5020034	High performance thin layer chromatography and/or UV absorbance of acetonic extracts measured at 440 nm (less or equal to 0.9)	IV
Moisture	*The Fiji Kava Standard 2017*. Section 8.1	Gravimetry	I
[Flavokavins	Lebot V, Legendre L (2016), Comparison of kava (Piper methysticumForst.) varieties by UV absorbance of acetonic extracts and high-performance thin-layer chromatography. Journal of Food Composition and Analysis 48:25-33. http://dx.doi.org/10.1016/j.jfca.2016.01.009 and Lebot V, Michalet S, Legendre L. (2019). Kavalactones and flavokavins profiles contribute to quality assessment of kava (Piper methysticumG.Forst.), the traditional beverage of the Pacific. Beverages 2019, 5, 34; https://doi.org/10.3390/beverages5020034	High performance thin layer chromatography and/or UV absorbance of acetonic extracts measured at 440 nm (less or equal to 0.9)]	IV

APPENDIX III

FAO/WHO COORDINATING COMMITTEE FOR NEAR EAST (CCNE10)

Methods of analysis for provisions in the proposed draft regional standard for mixed zaatar

8. METHODS OF ANALYSIS AND SAMPLING

Provision	Method	Principle	Type*
Sodium chloride	AOAC 960.29	Titrimetry (Mohr: determination of chloride, expressed as sodium chloride)	
Moisture	AOAC 925.10	Gravimetry, drying at 130°C	
Acid-insoluble ash	AOAC 941.12	Gravimetry, Furnace, 550°C (for the HCl insoluble ignited residue)	
Extraneous Matter	ISO 927	Visual Examination, followed by Volumetry	I
Foreign Matter	ISO 927	Visual Examination, followed by Volumetry	I
Insects/Excreta/Insect Fragments	Method appropriate for particular spice from AOAC Chapter 16, subchapter 14 [ISPM 08 Determination of Pest Status in an area]	Visual Examination	IV
Mould damage	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual, FDA, Technical Bulletin Number 5)	Visual examination (for whole)	IV
Excreta Mammalian, Excreta Other	Macroanalytical Procedure Manual, USFDA, Technical Bulletin V.39 B (For whole) AOAC 993.27 (For Ground)	Visual Examination Enzymatic Detection Method	IV IV

APPENDIX IV

COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES (CCNFSDU41)

Methods of analysis for infant formula

Commodity	Provision	Method	Principle	Proposed Type
Infant Formula	Thiamine	**AOAC 2015.14 / ISO DIS 21470**	**Enzymatic digestion and LC-MS/MS**	**II**
		EN 14122	HPLC with pre- or post-column derivatization to thiochrom	~~II~~ **III**
		AOAC 986.27	Fluorimetry	III
	Riboflavin	**AOAC 2015.14 / ISO DIS 21470**	**Enzymatic digestion and LC-MS/MS**	**II**
		EN 14152	HPLC	~~II~~ **III**
		AOAC 985.31	Fluorimetry	III
	Niacin	**AOAC 2015.14 / ISO DIS 21470**	**Enzymatic digestion and LC-MS/MS**	**II**
		EN 15652	HPLC	~~II~~ **III**
		AOAC 985.34	Microbioassay and turbidimetry	III
	Vitamin B_6	**AOAC 2015.14 / ISO DIS 21470**	**Enzymatic digestion and LC-MS/MS**	**II**
		AOAC 2004.07 / EN 14164	HPLC	~~II~~ **III**
		AOAC 985.32	Microbioassay	III
		EN 14166	Microbioassay	III
	Choline	**AOAC 2015.10 / ISO DIS 21468**	**LC-MS/MS**	**II**
		AOAC 999.14	Enzymatic Colorimetric Method with limitations on applicability due to choline and ascorbate concentration	~~II~~ **III**
	Carnitine	**AOAC 2015.10 / ISO DIS 21468**	**LC-MS/MS**	**II**
	Fructans	**AOAC 2016.14 / ISO DIS 22579 | IDF 241**	**Enzymatic digestion with HPAEC-PAD**	**II**
	Beta Carotene	**AOAC 2016.13 / ISO DIS 23443**	**UHPLC**	**II**
	Lycopene	**AOAC 2016.13 / ISO DIS 23443**	**UHPLC**	**II**
	Biotin	AOAC 2016.02 **/ ISO 23305**	HPLC-UV	II
		EN 15607	HPLC-fluorescence	III