CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda item 7 **CRD1 Rev.**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND THE SOUTH WEST PACIFIC

Sixteenth Session

Nadi, Fiji

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REVISED DRAFT REGIONAL STANDARD FOR FERMENTED NONI FRUIT JUICE

(Prepared by the Electronic Working Group chaired by Tonga and co-chaired by Samoa)

1. SCOPE

This standard applies to fermented noni fruit juice, as defined in Section 2 below, which is used as a food or food ingredient. This standard does not apply to non-fermented noni fruit juice, other noni products from fruit, leaves, bark or flowers, or noni products for medicinal purposes.

2. DESCRIPTION

2.1. Product Definition

The fermented noni fruit juice is the juice product that is derived from the fermenting of fresh fruits of noni plants[1], *Morinda citrifolia* L. variety *citrifolia*[2] of the Rubiaceae family.

2.2 Noni Fruits

Fresh, firm and mature to ripe noni fruits, with greenish-yellow to white colour, are harvested, washed and left to dry. Optionally, the fruits may be crushed to a pulp (excluding seeds). Fruits that are over-ripe, fallen,green, bruised and/or damaged, or containing foreign materials such as sticks, stem, leaves, bark and root material should be rejected and not be used in the production of fermented noni fruit juice.

2.3 Fermentation of Noni Fruit Juice

Whole fruits or fruit pulp are fermented ~~spontaneously~~ naturally or by starter culture. Juice is extracted or collected from the fermented fruit and filtered.~~from the fermented products.~~ The ~~resultant 100%~~ fermented noni fruit juice is pasteurized or otherwise treated to eliminate pathogens of public health significance.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 Ingredients

The fermented noni fruit juice as defined in section 2.

3.2 Fermented noni fruit juice

a)	Brix value (soluble solids)		5.5° minimum
b)	pH	3.5-3.9	
c)	Ethanol		less than 0.5% v/v
d)	Deacetylasperulosidic acid	Present	
e)	Scopoletin		Present[3]

[1] Common names of noni are great morinda, beach mulberry, Indian mulberry, ach, mengkudu, nono, nonu, noni and cheesefruit.

[2] Two types of large fruits with oval leaves and small fruits with elongated leaves (Wagner, Herbst and Sohmer, 1990, "*The Manual of the Flowering Plants of Hawaii"* (Copyright 1990, Bishop Museum, Honolulu).

[3] *Scopoletin is present naturally in fermented noni fruit juice. Some reports have shown potential toxicity of scopoletin. Therefore, the scopoletin levels should be kept as low as technologically feasible until a safe level~~is~~ is established by JECFA.*

3.3 Definition of defects

To the extent possible, fermented noni fruit juice shall be free from objectionable matter (e.g. noni leaves, seed fragments, fruit skin fragments, stems, insects, etc) and according to Good Manufacturing Practice.

4. FOOD ADDITIVES

No additives are permitted in the product as defined by the scope.

5. CONTAMINANTS

The products covered by this standard shall comply with the Maximum Levels for contaminants that are specified for the product in the *General Standard for Contaminants and Toxins in Food and Feed* (CXS 193-1985); and the Maximum Residue Limits for pesticides established by the Codex Alimentarius Commission.

6. HYGIENE

It is recommended that the products covered by the provisions of this standard be prepared and handled in accordance with appropriate sections of the *General Principles of Food Hygiene* (CXC 1-1969), and other relevant Codex texts such as Codes of Hygienic Practice and Codes of Practice.

The product should also comply with any microbiological criteria established in accordance with the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997).

7. PACKAGING

The fermented noni fruit juice products must be packed in containers that safeguard its hygienic, and organoleptic quality. The materials used for packaging must be new (for the purposes of this standard, this includes recycled material of food-grade quality.) The containers shall meet the quality, hygiene, ventilation and resistance characteristics to ensure suitable handling, shipping and preserving of the fermented noni fruit juice. Packages must be free of all foreign matter and smell.

8. WEIGHTS AND MEASURES

8.1 Fill of the container

8.1.1 Minimum fill

The container should be well filled with the product and the product shall occupy not less than 90% of the water capacity of the container. The water capacity of the container is the volume of distilled water at 20°C which the sealed container will hold when completely filled.

9. LABELLING

The products shall be labelled in accordance with the *General Standard for the Labelling of Prepackaged Food* (CXS 1-1985).

9.1 Name of the product

The name of the food product shall be “Fermented Noni Fruit Juice”. The term “noni fruit juice” may be replaced by a term which has customarily been used to describe the product in the country in which the product is intended to be sold (e.g., “nonu juice” or “nono juice”).

10. METHODS OF ANALYSIS AND SAMPLING

For checking the compliance with this standard, the methods of analysis and sampling contained in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) relevant to the provisions in this standard, shall be used.

10.1 Methods of Analysis

Provision	Method	Principle	Type	Notes
Brix value (Soluble solids)	AOAC 983.17 EN 12143 IFUMA 8 ISO 2173	Refractometry	I	Adopted for fruit juices and nectars
pH value	NMKL 179 / AOAC 981.12	Potentiometry	II	Adopted for fruit juices and nectars

Ethanol	IFUMA 52 AOAC 2017.07	Enzymatic determination	IV	
	AOAC ~~Method~~2016.12	Headspace GC-FID	IV	
Identification of scopoletin	Annex A*	Thin layer chromatography (TLC) [or,] [High-performance liquid chromatography (HPLC)]	IV	
Identification of deacetylasperulosidic acid	Annex B*	Thin layer chromatography (TLC) [or,] [High-performance liquid chromatography (HPLC)]	IV	

** In compliance with the general criteria for testing laboratories laid down in ISO/IEC Guide 17025:2017*

ANNEX A

IDENTIFICATION OF SCOPOLETIN

1. PREPARATION OF SAMPLES

Noni fruit 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 (specify type of cartridge in terms of solid phase) is first equilibrated with water (volume/water to be specified mLs~~mls~~), followed by methanol ([volume/methanol to be specified] mLs~~mls~~). The samples are then loaded onto the cartridge and washed with 5% ([volume/methanol to be specified]mLs~~mls~~) methanol (MeOH) in water, followed by 100% ([volume/methanol to be specified]mLs~~mls~~) MeOH. The MeOH eluate is retained for TLC analysis.]

2. PREPARATION OF REFERENCE STANDARD

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

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

3.2 [HIGH PERFORMANCE LIQUID CHROMATOGRAPHY]

[Preparation of samples for HPLC identification test]

[For the HPLC analysis of analytes, 1 mL of noni fruit juice is mixed ~~mixed~~ with 1 mL of MeOH, vortex for 1 min, and prepared into a concentration of 0.5 mL/mL solution. All samples are~~were~~ filtered through a nylon microfilter (0.45 µm pore size) before HPLC analysis.]

[Chromatographic system and HPLC identification test]

[~~Details to be provided~~[The chromatography separation and identification should be done on any HPLC system that consist of:

a. Sample injector – The sample can be manually injected but it is desirable to have an autosampler.

b. Column oven – An oven with a temperature range of 30ºC – 80ºC.

c. Pump – A pump system with sufficient pressure to push the sample and eluting solvents through the column. The pump system should be capable to elute the sample using isocratic or gradient mode.

d. Detector – A photodiode array (PAD) or UV detector that provides an appropriate UV wavelength.

e. Column – C18 column with dimensions of 4.6 x 250 mm, 5.0µm or 4.6 x 150, 5.0µm

f. Eluent – A liquid solvent consists of distil water and an organic solvent.

g. Operation and processing software

h. Autosampler – those that can inject 20µL volume of sample or/and standard

HPLC Analysis Conditions:

Column	C18 (4.6mm x 250 mm., 5.0µm or 4.6mm x 150mm., 5µm)
Temperature	30ºC- 40ºC
Eluent (Mobile phase)	Methanol : Water (30 : 70 v/v)
Elution mode	Isocratic

Flow rate	1ml/min
Sample volume	20µl
Detector (PAD/UV) wavelength	300nm

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[HPLC identification test – acceptance criteria]

[~~Details to be provided~~[a. Linear regression coefficient (R^2) for calibration curve should be > 0.98

b. Retention time and peak shape of the peak due to scopoletin in sample chromatogram should be similar to that of the peak seen in the standard chromatogram. If the peak differs in shape, perform peak purity on the peak to ensure that the response is due to histamine using diode array detector.

c. If a QC sample was extracted and analysed, calculate the result and compare against previous results and accept/reject criteria if available.

d. Calculated recoveries shall be 85% - 110%

e. All samples shall be analysed in triplicates and reported their averages. In the event a replicate is an outlier, report the average of the other two replicates otherwise repeat the analysis.

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REFERENCES

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

~~1.~~**2.** Sun-Il Choi, Hee-Yeon Kwon, Im-Joung La, Yeon-Hui Jo, Xionggao Han, Xiao Men, Se-Jeong Lee, Yong-Deok Kim, Geum-Su Seong, and Ok-Hwan Lee. Development and Validation of an Analytical Method for Deacetylasperulosidic Acid, Asperulosidic Acid, Scopolin, Asperuloside and Scopoletin in Fermented Morinda citrifolia L. (Noni). Separations 2021, 8, 80.

~~2.~~**3.** 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.~~**4.** 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.~~**5.** 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.~~**6.** 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

Noni fruit 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 (specify type of cartridges in terms of solid phase) is first equilibrated with water ([volume/water to be specified] mLs~~mls~~), followed by methanol ([volume/methanol to be specified] mLs~~mls~~). The samples are then loaded onto the cartridge and washed with 5% MeOH ([volume/methanol to be specified] mLs~~mls~~) in water, followed by 100% MeOH ([volume/methanol to be specified] mLs~~mls~~). The MeOH eluate is retained for TLC analysis.]

2. PREPARATION OF REFERENCE STANDARD

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

2.2 Alternately, certified *Morinda citrifolia* reference plant material may be prepared in the same manner asthe samples to be analyzed. The certified *Morinda citrifolia* reference material should be from the samepart 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 mobile phase of dichloromethane: methanol: water (13:6:1, v/v/v). Spray developed platewith 2% anisaldehyde / 10% sulfuric acid-ethanol (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.

3.2 [HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)]

[Preparation of samples for HPLC identification test]

[One gram of the fresh fruit juice diluted with 5 mL of H_2O-MeOH (1:1), and mixed thoroughly; the solution collected into a 5 mL volumetric flask, mixed thoroughly and then filtered through a 0.2 µm PTFE filter for HPLC analysis.]

[Chromatographic system and HPLC identification test]

[~~Details to be provided~~[The chromatography separation and identification should be done on any HPLC system that consist of:

a. Sample injector – The sample can be manually injected but it is desirable to have an autosampler.

b. Column oven – An oven with a temperature range of 30°C – 80°C.

c. Pump – A pump system with sufficient pressure to push the sample and eluting solvents through the column. The pump system should be capable to elute the sample using isocratic or gradient mode.

d. Detector – A photodiode array (PAD) or UV detector that provides an appropriate UV wavelength.

e. Column – C18 column with dimensions of 4.6 x 250 mm, 5.0µm or 4.6 x 150, 5.0µm

f. Eluent – A liquid solvent consists of distil water and an organic solvent.

g. Operation and processing software

h. Autosampler – those that can inject 20µL volume of sample or/and standard

HPLC Analysis Conditions:

Column	C18 (4.6mm x 250 mm., 5.0µm or 4.6mm x 150mm., 5µm)
Temperature	30°C- 40°C
Eluent (Mobile phase)	Methanol : Water (30 : 70 v/v)
Elution mode	Isocratic

Flow rate	1ml/min
Sample volume	20µl
Detector (PAD/UV) wavelength	300nm

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[HPLC identification test – acceptance criteria]

[~~Details to be provided~~[a. Linear regression coefficient (R^2) for calibration curve should be > 0.98

b. Retention time and peak shape of the peak due to deacetylasperulosidic acid in sample chromatogram should be similar to that of the peak seen in the standard chromatogram. If the peak differs in shape, perform peak purity on the peak to ensure that the response is due to histamine using diode array detector.

c. If a QC sample was extracted and analysed, calculate the result and compare against previous results and accept/reject criteria if available.

d. Calculated recoveries shall be 85% - 110%

e. All samples shall be analysed in triplicates and reported their averages. In the event a replicate is an outlier, report the average of the other two replicates otherwise repeat the analysis.

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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.

Sun-Il Choi, Hee-Yeon Kwon, Im-Joung La, Yeon-Hui Jo, Xionggao Han, Xiao Men, Se-Jeong Lee, Yong-Deok Kim, Geum-Su Seong, and Ok-Hwan Lee. Development and Validation of an Analytical Method for Deacetylasperulosidic Acid, Asperulosidic Acid, Scopolin, Asperuloside and Scopoletin in Fermented Morinda citrifolia L. (Noni). Separations 2021, 8, 80

~~1.~~**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.

~~2.~~**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