

APPENDIX II

PROPOSED DRAFT REGIONAL STANDARD FOR FERMENTED NONI FRUIT JUICE
(For Adoption at Step 5)

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 juice of noni fruit or 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 plants¹, *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 fruits, green, bruised and or damaged fruit, or foreign material such as sticks, stem, leaves, bark and root material should be rejected and not used in the production of fermented noni fruit juice.

2.3 Fermentation of Noni Fruit Juice

Whole fruits or fruit pulp are fermented spontaneously or by starter culture. Juice is extracted 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 | 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 |

3.3 Definition of defects

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

3.4 Food Additives

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

4. 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.

¹ Common names of noni are great morinda, beach mulberry, Indian mulberry, ach, mengkudu, nono, nonu, noni and cheese fruit.

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 established by JECFA.

5. 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* (CAC/RCP 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).

6. PACKAGING

The fermented noni fruit juice products must be packed in containers that safeguard its hygienic, ~~nutritional~~ 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 juice. Packages must be free of all foreign matter and smell.

7. WEIGHTS AND MEASURES

7.1 Fill of the container

7.1.1 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.

8. LABELLING

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

8.1 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”).

9. METHODS OF SAMPLING AND ANALYSIS

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.

9.1 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	

* 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

- 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 R_f 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 R_f 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.