



JOINT FAO/WHO FOOD STANDARDS PROGRAMME

FAO/WHO COORDINATING COMMITTEE FOR North America and the South West Pacific

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DRAFT REGIONAL STANDARD FOR FERMENTED NONI FRUIT JUICE

(at Step 7)

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

Codex Members and Observers wishing to submit comments, at Step 6, on this draft (Appendix I) should do so as instructed in CL 2022/83/OCS-NASWP available on the Codex webpage/Circular Letters 2022

1. Background

Draft Standard

1.1. In June 2019, the 15th Session of the FAO/WHO Regional Coordinating Committee for North America and South West Pacific (CCNASWP15) agreed to:

- forward the proposed draft regional standard for fermented noni fruit juice (draft Standard) to CAC43 for adoption at Step 5;
- forward the relevant draft provisions for endorsement to the respective committees as follows:
 - Codex Committee on Food Additives (CCFA)
 - Codex Committee on Food Labelling (CCFL)
 - Codex Committee on Methods of Analysis and Sampling (CCMAS)
- request Joint FAO/WHO Expert Committee on Food (JECFA) to retain scopoletin on the priority list and to call upon Member countries to generate and submit data to support the conduct of the safety evaluation;
- convene an EWG, to be chaired by Tonga and co-chaired by Samoa and working in English only, to further advance the draft regional standard taking into account the discussions at CCNASWP15 for consideration at CCNASWP16.

1.2. The food additive and food labelling provisions in the draft standard were endorsed respectively at CCFA52 (September, 2021)¹ and at CCFL46 (October, 2021)².

1.3. CCMAS41 (May, 2021)³ did not endorse:

- The AOAC 983.17/ EN 12143/ IFUMA 8/ ISO 2173 as the appropriateness of extending the methods to fermented noni fruit juice needed further evaluation by CCMAS; but noted the offer of the international fruit and vegetable juice association (IFU) to do a small single or inter-laboratory study to determine its fitness for purpose in fermented noni fruit juice;
- The methods for the identification of scopoletin and deacetylasperulosidic acid (D-Acid), but noted that changes needed to be made to the methods to give a clear indication of the solid phase extraction

¹ REP21/FA, paragraphs 85 (i) a)

² REP21/FL, paragraph 16

³ REP21/MAS, paragraphs 13-14

separation mode needed and agreed to request CCNASWP to provide clarification. CCMAS41 agreed to inform CCNASWP accordingly.

1.4. In advising CCNASWP delegates, who attended CCMAS41, advised informally that the lack of endorsement was not because the information provided was incorrect, rather that clarification was needed to provide the confidence in the method to enable CCMAS to endorse it.

1.5. CAC43 (December 2021) adopted the draft regional standard for fermented noni fruit juice at Step 5.

Progress on submitting data to JECFA for safety evaluation

1.6. CCNASWP15 agreed to request the Codex Committee on Contaminants in Food (CCCF) to retain scopoletin on the priority list and to call upon Codex Members to generate and submit data to support the conduct of the safety evaluation by JECFA.

1.7. CCNASWP15 also requested FAO and WHO to organize a new call for data for the safety evaluation of scopoletin. FAO reminded that a full dataset including exposure and toxicity was required.

1.8. CCCF14 (2021)⁴ agreed to keep scopoletin in the priority list awaiting feedback from CCNASWP16 (2023) on the provision of necessary data and studies for JECFA to perform the safety evaluation of scopoletin and to encourage Codex Members to generate and submit data to GEMS/Food to support the safety evaluation by JECFA.

1.9. CCCF15 did not discuss scopoletin awaiting the outcomes of the discussion at CCNASWP16 (2023) on the regional standard for fermented noni fruit juice based on the outcomes of a consultant's report on the findings of the toxicological data review of scopoletin.

1.10. In response to the call for data, Samoa led a workstream to generate data to support the submission to JECFA on the conduct of the safety evaluation. As well as gathering, testing and analysing Samoa's data, they also encouraged other Member countries to send their fermented noni fruit juice samples to the Scientific Research Organisation of Samoa (SROS) for testing and analysis. Further PHAMA-Plus⁵ provided funding to support the transportation of the samples to Samoa⁶.

2. EWG process

2.1. Invitations to join the EWG for the draft standard were disseminated in December 2021 with a deadline for response by 1 February 2022. 11 Member countries responded⁷.

2.2. The revised draft standard (at step 6), incorporating the discussion at CCNASWP16, was posted on the e-Forum Platform on 3 March 2022 with comments due by 11 April 2022. Five countries responded⁸.

2.3. Following compilation of comments and incorporation into a second draft, the revised draft was posted on the e-Forum Platform on 7 July 2022 with comments due by 1 August 2022. Three countries responded (Australia, Canada, and New Zealand).

2.4. Compilation of comments and incorporation into a third draft followed for submission to the Codex Secretariat for circulation (via circular letter) to all Members and Observers of CCNASWP for comments ahead of CCNASWP16.

3. Issues considered

3.1. The Draft Regional standard for Fermented Noni Fruit Juice was adopted at Step 5 with square brackets remaining in six places, all of which were contained in the Annex A (Identification of Scopoletin) and Annex B (Identification of D-Acid) and as such relate to the Methods of Analysis section. The changes made to the draft Standard subsequent to CCNASWP15 (including as a result of consultation and feedback from CCMAS) are set out (section by section) as follows:

- a) *Section 3.2 (Ingredients) and 10.1 (Methods of analysis)* –The provision of Brix Value has been updated to include '(soluble solids)' because it was suggested in CCMAS41 that this provision be modified to "Brix Values (Soluble solids)", as adopted provision for "Adopted for fruit juices and nectars" is "Soluble solids".

⁴ REP21/CF, paragraph 231 (ii), Appendix VIII

⁵ PHAMA-Plus provides practical and targeted assistance to help Pacific island countries manage regulatory aspects associated with exporting primary and value-added products.

⁶ At the time of writing Samoa had received product samples from Cook Island, Niue, Tonga, Solomon Is, Vanuatu and was anticipating samples would be received from Fiji and Papua New Guinea.

⁷ The countries included Australia, Canada, Cook Islands, Fiji, New Zealand, Papua New Guinea (PNG), Samoa, Solomons, Tonga, United States of America and Vanuatu.

⁸ The countries included Australia, Canada, New Zealand, Tonga, and the United States of America.

- b) *Section 10.1 (Table) Updated Methods* – The methods were updated for Brix Value (Soluble solids) to include EN12143, IFUMA 8, and ISO 2143, for pH value to include AOAC 981.12 and for Ethanol to include AOAC 981.12 and AOAC method 2016.12 because these methods listed have been endorsed by CCMAS41 (2021) in REP21/MAS – Appendix II and included in CXS234-1999 respectively.
- c) *Section 10.1 (Table) Updated Principles* – The Principle for Ethanol has been updated to include 'Headspace GC-FID' for use with the updated method 2016.12 because since proposed method for Ethanol has not been validated for fermented noni fruit juice or previously endorsed by CCMAS, "Headspace GC-FID" is considered as one of the options included as Type IV methods to be considered until fermented noni fruit juice specific validation is undertaken for this standard.
- d) As information required for the Annexes on Thin Layer Chromatography (TLC) method was not forthcoming on (due in part to the fact that those in the EWG were not familiar with this method), the High-performance liquid chromatography (HPLC) method has been proposed by member countries. It is considered to be quite satisfactory due to the fact that it is fast, reproducible, reliable and less prone to operator bias (and possibly an alternative to TLC if it can be determined that the TLC method is no longer in use). Accordingly the addition of the HPLC method has been reflected in the Principle for Identifying both Scopoletin and D-Acid.
- e) *Section 10.1 (Table) Updated Type* – The Type of method for Ethanol has been updated from a type II to, two type IV methods, to reflect the new method and the fact that neither of the methods proposed have been established specifically for fermented noni fruit juice.
- f) Given the use of the HPLC method has not yet been established for fermented noni fruit juice a substantial piece of work could be required before CCMAS are likely to have full confidence in the use of this method for fermented noni fruit juice. It is proposed therefore that both the TLC and the HPLC methods are retained at least until efforts are made to complete the level of specification in TLC or HPLC, required for CCMAS to have a full level of comfort in the method.
- g) *Section 1 of both Annex A and B - Preparation of samples.* Both CCMAS and CCNASWP sought clarification to the preparation of the samples. This included specifying the type of cartridge in terms of solid phase and the measure for equilibration for both water and methanol. This information has not yet been provided. Accordingly the text remains in square brackets.

However, paragraphs one (Section 1.1) and three (Section 1.3) were removed from Section 1 (Annex A and B) as the information contained was deemed unnecessary because clarification on the scope of the standard showed that it focused solely on the fermented noni fruit "juice" only whereas Section 1.1 focused on "fruit" and Section 1.3 focused on "noni dried powder" is outside the scope of the standard.
- h) *Section 3.1 of both Annex A and B - Correcting the methodology for thin layer chromatography (TLC) analysis* The text '*lower solution*' was removed from the square bracket section relating to the mobile phase to avoid confusion as "mobile phase" provides a more common understanding of the concept. The square brackets have been removed accordingly.
- i) *Section 3.2 of both Annex A and B – Updating the methodology for HPLC.* A new section on HPLC has been added in light of comments above, noting details are to be provided on the Chromatographic system and HPLC identification test and the HPLC identification test – acceptance criteria, as identified by the square brackets remaining in this section.

4. Recommendations

CCNASWP16 is invited to:

- a) Note that the Draft Regional Standard for Fermented Noni Fruit Juice (draft Standard) provides that scopoletin levels should be kept as low as technologically feasible until a safe level is established by JECFA.
- b) Note that CCNASWP has been requested by CCMAS to provide clarification on the methods for the identification of scopoletin and deacetylasperulosidic acid in terms of a clear indication of the solid phase extraction separation mode. This information has not yet been provided. Once available, CCMAS will be informed accordingly, and the draft Standard (or adopted standard, as relevant) should be updated accordingly.
- c) Note that the process of collecting, testing and analysing samples of fermented noni fruit juice has begun. Once all interested member countries in the South West Pacific (SWP) region have contributed to the data package, it will be submitted to JECFA for assessment. The resulting assessment is intended to inform the draft Standard (or adopted standard, as relevant). CCCF will be informed accordingly.
- d) Consider recommending that the Draft Regional Standard for Fermented Noni Fruit Juice be adopted by CAC46 at Step 8 (as presented in Appendix I).

This version includes:

- Changes recommended by the eWG up to June 2019;
- Comments from the eWG in response to three rounds of consultation on the e-Forum Platform.

DRAFT REGIONAL STANDARD FOR FERMENTED NONI FRUIT JUICE

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 plants⁹, *Morinda citrifolia* L. variety *citrifolia*¹⁰ 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 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 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 ¹¹

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-

⁹ Common names of noni are great morinda, beach mulberry, Indian mulberry, ach, mengkudu, nono, nonu, noni and cheese fruit.

¹⁰ 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).

¹¹ 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.

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 AOAC2017.07	Enzymatic determination	IV	
	AOAC Method2016.12	Headspace GC-FID	IV	
Identification of scopoletin	Annex A*	Thin layer chromatography (TLC) or, High-performance liquid	IV	

		chromatography (HPLC)		
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

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 (mls), followed by methanol (mls). The samples are then loaded onto the cartridge and washed with 5% (mls) methanol (MeOH), followed by 100% (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 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 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.

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 mixed with 1 mL of MeOH, vortex for 1 min, and prepared into a concentration of 0.5 mL/mL solution. All samples were filtered through a nylon microfilter (0.45 µm pore size) before HPLC analysis.

Chromatographic system and HPLC identification test

[Details to be provided]

HPLC identification test – acceptance criteria

[Details to be provided]

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.

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 (mls), followed by methanol (mls). The samples are then loaded onto the cartridge and washed with 5% MeOH (mls), followed by 100% MeOH (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 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 mobile phase of dichloromethane: methanol: water (13:6:1, v/v/v). Spray developed plate with 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 R_f 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 H₂O-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]

HPLC identification test – acceptance criteria

[Details to be provided]

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.
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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