COMISIÓN DEL CODEX ALIMENTARIUS **S**

Organización de las Naciones Unidas para la Alimentación y la Agricultura

Organización Mundial de la Salud

Viale delle Terme di Caracalla, 00153 Roma, Italia - Tel: (+39) 06 57051 - Correo electrónico: codex@fao.org - www.**codex**alimentarius.org

Tema 3 del programa **CX/MAS 23/42/3**

Mayo de 2023

PROGRAMA CONJUNTO FAO/OMS SOBRE NORMAS ALIMENTARIAS

COMITÉ DEL CODEX SOBRE MÉTODOS DE ANÁLISIS Y TOMA DE MUESTRAS

Cuadragésima segunda reunión

Budapest (Hungría)

13 - 16 de junio de 2023 con la aprobación del informe el 20 de junio de 2023 (de manera virtual)

RATIFICACIÓN DE LAS DISPOSICIONES SOBRE MÉTODOS DE ANÁLISIS Y PLANES DE MUESTREO EN LAS NORMAS DEL CODEX

1. El presente documento contiene los métodos de análisis (apéndices I, II, III, IV y V) propuestos por los siguientes comités:

- Comité del Codex sobre Nutrición y Alimentos para Regímenes Especiales
    - Modificar los *Métodos de análisis y de muestreo recomendados* (CXS 234-1999) para incluir la nueva entrada "producto para niños pequeños" con los "preparados complementarios".
    - Métodos para la vitamina B12; los aminoácidos totales (excluidos la taurina y el triptófano), y el triptófano en la *Norma para preparados para lactantes y preparados para usos medicinales especiales destinados a los lactantes* (CXS 72-1981).
- Comité del Codex sobre Especias y Hierbas Culinarias
    - Métodos de análisis de las disposiciones de la *Norma para las raíces secas, rizomas y bulbos: jengibre seco o deshidratado* (CXS 343-2021), la *Norma para partes florales secas: clavos de olor* (CXS 344-2021) y la *Norma para la albahaca seca* (CXS 345-2021) (normas adoptadas por la Comisión del Codex Alimentarius (en adelante, la Comisión), en su 44.º período de sesiones (2021), en el trámite 8, REP21/CAC44, párr. 38 y Apéndice III).
    - Métodos de análisis de las disposiciones de la *Norma para partes florales secas: azafrán* (CXS 351-2021) y la *Norma para semillas secas: nuez moscada* (CXS 352-2022) (normas adoptadas por la Comisión, en su 45.º período de sesiones (2022) en el trámite 8, REP22/CAC45, párr. 94(i)-(ii) y Apéndice II).
    - Métodos de análisis de las disposiciones de la *Norma para el chile y el pimentón secos o deshidratados* (CXS 353-2022) (norma adoptada por la Comisión, en su 45.º período de sesiones, en el trámite 5/8, REP22/CAC45 párr. 94(iii) y Apéndice II).
    - Métodos de análisis de las disposiciones del Proyecto de norma para el cardamomo pequeño seco y el Proyecto de norma para las especias derivadas de frutos y bayas secos (Parte A: pimienta de Jamaica, baya de enebro y anís estrellado) (normas adoptadas por la Comisión, en su 45.º período de sesiones, en el trámite 5, REP22/CAC párr. 97 y Apéndice III, y enviadas a la Comisión, con miras a su adopción en el trámite 8, en su 46.º período de sesiones, (2023)).
- Comité Coordinador FAO/OMS para África
    - Métodos de análisis de las disposiciones de la *Norma para la carne seca* (CXS 350-2022)) (adoptada por la Comisión, en su 45.º período de sesiones, en el trámite 8, REP22/CAC45, párr. 149 y Apéndice II).
- Comité Coordinador FAO/OMS para Asia
    - Métodos de análisis y muestreo de las disposiciones del Proyecto de norma regional para los productos de soja fermentados con *Bacillus* spp. y el Proyecto de norma regional para productos de arroz cocido envuelto en hojas de plantas (enviados a la Comisión con miras a su adopción en el trámite 5/8, en su 46.º período de sesiones).
- Comité Coordinador FAO/OMS para América del Norte y el Pacífico Sudoccidental
    - Métodos de análisis de las disposiciones del Proyecto de norma regional para el zumo (jugo) fermentado de fruto de noni (enviados a la Comisión con miras a su adopción en el trámite 8, en su 46.º período de sesiones).

- Revisión del Procedimiento normalizado de actuación para la identificación de kavalactonas y flavokavinas en los productos a base de kava fresco y seco mediante cromatografía en capa fina de alto rendimiento de la *Norma regional para productos a base de kava que se utilizan como bebida mezclados con agua* (CXS 336R-2020) (adoptada por la Comisión, en su 43.º período de sesiones (2020) en el trámite 5/8).

COMITÉ DEL CODEX SOBRE NUTRICIÓN Y ALIMENTOS PARA REGÍMENES ESPECIALES (CCNFSDU) (43.ª reunión, 2023)

***Métodos de análisis de las disposiciones de la Norma para preparados complementarios* (CXS 156-1987)[1]**

2. El CCNFSDU, en su 43.ª reunión, acordó informar al CCMAS para que incluyera una nueva entrada titulada "producto para niños pequeños" dentro de la sección "preparados complementarios" del documento CXS 234.

Métodos para la vitamina B12; los aminoácidos totales (excluidos la taurina y el triptófano), y el triptófano en la Norma para preparados para lactantes y preparados para usos medicinales especiales destinados a los lactantes (CXS 72-1981)[2]

3. El CCNFSDU, en su 43.ª reunión, acordó remitir los métodos de análisis para la vitamina B12, los aminoácidos totales (excluidos la taurina y el triptófano) y el triptófano al CCMAS para su ratificación e inclusión en el documento CXS 234. El CCNFSDU también acordó que debería insertarse una nota en CXS 234 para aclarar que las disposiciones son métodos para el análisis de aminoácidos totales (excluidos la taurina y el triptófano) y el triptófano, es decir, para su uso de acuerdo con la Sección 3.1.3a notas 3 y 4 a pie de página del documento CXS 72.

4. **Se invita** al CCMAS **a ratificar** los métodos del Apéndice I.

COMITÉ DEL CODEX SOBRE ESPECIAS Y HIERBAS CULINARIAS (CCSCH) (reuniones quinta[3] y sexta[4])

Métodos de análisis de las disposiciones de la Norma para raíces secas, rizomas y bulbos: jengibre seco o deshidratado (CXS 343-2021), Norma para partes florales secas: clavos de olor (CXS 344-2021), Norma para la albahaca seca (CXS 345-2021), Norma para partes florales secas: azafrán (CXS 351-2021), Norma para semillas secas: nuez moscada (CXS 352-2022), Norma para el chile y el pimentón secos o deshidratados (CXS 353-2022), Proyecto de norma para el cardamomo pequeño seco, Proyecto de norma para las especias derivadas de frutos y bayas secos (Parte A: pimienta de Jamaica, baya de enebro y anís estrellado)

5. **Se invita** al CCMAS **a ratificar** los métodos de análisis del Apéndice II.

COMITÉ COORDINADOR FAO/OMS PARA ÁFRICA (CCAFRICA) (24.ª reunión)[5]

Métodos de análisis de las disposiciones de la Norma para la carne seca (CXS 350-2022)

6. **Se invita** al CCMAS **a ratificar** los métodos de análisis del Apéndice III.

COMITÉ COORDINADOR FAO/OMS PARA ASIA (CCASIA) (22.ª reunión)[6]

Métodos de análisis y muestreo de las disposiciones del Proyecto de norma regional para los productos de soja fermentados con Bacillus spp. y el Proyecto de norma regional para productos de arroz cocido envuelto en hojas de plantas

7. **Se invita** al CCMAS **a ratificar** los métodos de análisis del Apéndice IV.

COMITÉ COORDINADOR FAO/OMS PARA AMÉRICA DEL NORTE Y EL PACÍFICO SUDOCCIDENTAL (CCNASWP) (16.ª reunión)[7]

Métodos de análisis de las disposiciones del Proyecto de norma regional para el zumo (jugo) fermentado de fruto de noni

8. El CCNASWP, en su 15.ª reunión (2019), señaló que se deberían utilizar los métodos pertinentes de análisis y muestreo que figuraban en los *Métodos de análisis y de muestreo recomendados* (CXS 234-

1 REP23/NFSDU43, párr. 51
2 REP23/NFSDU43, párrs. 122, 123 y Apéndice VI
3 REP21/SCH05 párrs. 36, 65, 81, 115, 149 y apéndices II, III, IV, V, VI
4 REP22/SCH06 párrs. 39, 59, 80, 107, 121(i) y apéndices III, IV, V, VI, VII, Parte A
5 REP22/AFRICA24, párr. 40 (i) y Apéndice III
6 REP23/ASIA22, párr. 50 (ii) 83 (ii) y apéndices V, VII
7 REP23/NASWP16, párr. 28 (iii) 73 (i) y apéndices III, VII Part A

1999). Asimismo, el Comité acordó remitir las disposiciones relativas a los métodos de análisis y muestreo al CCMAS para su ratificación.[8]

9. El CCMAS, en su 41.ª reunión (2021), no ratificó[9]:

- los métodos AOAC 983.17/EN 12143/IFUMA 8/ISO 2173, ya que la conveniencia de ampliar los métodos al zumo (jugo) de noni requería una evaluación adicional por parte del CCMAS; y tomó nota de la oferta de IFU de realizar un pequeño estudio individual o entre laboratorios para determinar su idoneidad para el zumo (jugo) de noni;
- los métodos para la identificación de la escopoletina y del ácido deacetilasperulosídico, observando que era necesario introducir cambios en los métodos para dar una indicación clara del modo de separación por extracción en fase sólida necesario, y acordó solicitar al CCNASWP que aportara aclaraciones al respecto.

10. El CCNASWP, en su 16.ª reunión (2023), estudió la solicitud formulada por el CCMAS, en su 41.ª reunión, y revisó los métodos de análisis, incluidos los anexos A (identificación de la escopoletina) y B (identificación del ácido deacetilasperulosídico) y acordó[10] encargar al Coordinador Regional del CCNASWP que trabajara con los miembros de la región de América del Norte y el Pacífico Sudoccidental para resolver las cuestiones pendientes relacionadas con los métodos de análisis y muestreo (en concreto, la especificación del cartucho de extracción en fase sólida y el método de cromatografía líquida de alto rendimiento para identificar la escopoletina y el ácido deacetilasperulosídico) con el fin de remitir los métodos de análisis y los anexos A y B al CCMAS con miras a su ratificación en su 42.ª reunión.

11. Se puede consultar más información sobre los métodos de análisis actualizados para la identificación de la escopoletina y el ácido deacetilasperulosídico en el documento de sala CRD05 presentado por el Coordinador Regional del CCNASWP en colaboración con los miembros de la región de América del Norte y el Pacífico Sudoccidental, que está disponible en la página web de la 42.ª reunión del CCMAS[11].

12. **Se invita** al CCMAS **a ratificar** los métodos de análisis del Apéndice V del siguiente modo:

i. Ratificar el método de extracción en fase sólida/cromatografía en capa fina del Anexo A (actualizado) como Tipo IV para la identificación de escopoletina en el zumo (jugo) fermentado de fruto de noni.

ii. Ratificar el método de cromatografía en capa fina que figura en el Anexo B (actualizado) como Tipo IV para la identificación del ácido deacetilasperulosídico en el zumo (jugo) de fruto de noni fermentado.

En los casos en que en el Manual de procedimiento se indique una clasificación de Tipo IV que refleja un método introducido recientemente pero para el que aún no se han determinado los criterios necesarios para su aceptación por el CCMAS.

Nota 1: Las modificaciones al Anexo A (en comparación con el Apéndice II del documento CX/MAS 21/41/3) están resaltadas en rojo e incluyen instrucciones adicionales para el tipo de cartucho de extracción en fase sólida y los pasos en el procedimiento de dicha extracción. Otras mejoras sugeridas son el uso de un estándar de escopoletina de 0,10 mg/ml en la cromatografía en capa fina de escopoletina en lugar de 1,0 mg/ml, ya que esta última concentración es excesivamente superior a la de la muestra típica de zumo (jugo) de noni.

Nota 2: Las modificaciones al Anexo B (en comparación con el Apéndice II del documento CX/MAS 21/41/3) están resaltadas en rojo e incluyen la eliminación de las instrucciones de extracción en fase sólida y una dilución simple de las muestras de zumo (jugo) de noni con metanol. También, la adición de instrucciones para la preparación de la solución de para-anisaldehído para la identificación del ácido deacetilasperulosídico (Anexo B). Nótese que la preparación de la solución de p-anisaldehído incluida utiliza menos ácido sulfúrico (un 4 %) en comparación con el porcentaje especificado originalmente por West y Deng (es decir, un 10 %); consideramos que el exceso de ácido planteó un problema de calentamiento e inocuidad para la preparación y el uso. La solución de p-anisaldehído resultante no sufrió ningún defecto en su capacidad para visualizar los analitos deseados.

iii. La verificación/validación de un solo laboratorio para la identificación de escopoletina mediante extracción en fase sólida/cromatografía en capa fina y de ácido desacetilasperulosídico mediante

8 REP20/NASWP15, párrs. 80, 83(ii) y Apéndice II
9 REP21/MAS41, párrs. 13-14.
10 REP23/NASWP16, párr. 73(ii) y Apéndice VII, Parte B
11 https://www.fao.org/fao-who-codexalimentarius/meetings/detail/es/?meeting=CCMAS&session=42

cromatografía en capa fina en zumo (jugo) de fruto de noni fermentado en el Anexo C.

iv. Las metodologías de cromatografía en capa fina recomendadas anteriormente para facilitar el avance del Proyecto de norma regional para el zumo (jugo) fermentado de fruto de noni al trámite 8, señalando que en el futuro se dispondrá de un método de cromatografía líquida de alto rendimiento con detector de arreglo de diodos (DAD por sus siglas en inglés).

Revisión del Procedimiento normalizado de actuación para la identificación de kavalactonas y flavokavinas en los productos a base de kava fresco y seco mediante cromatografía en capa fina de alto rendimiento de la Norma regional para productos a base de kava que se utilizan como bebida mezclados con agua (CXS 336R-2020)

13. El CCNASWP, en su 15.ª reunión (2019), acordó[12] remitir las disposiciones sobre métodos de análisis y muestreo al CCMAS para su ratificación.

14. El CCMAS, en su 41.ª reunión (2021), señaló que la revisión de las referencias no produjo un procedimiento claro para determinar la kavalactona o las flavokavinas, y que parecía que había diferentes secciones dentro de cada referencia que debían seguirse y que la referencia de 2016 podría no ser necesaria para las flavokavinas.

15. El CCMAS, en su 41.ª reunión, acordó solicitar al CCNASWP que estudiara la posibilidad de elaborar un único método por pasos o Procedimiento normalizado de actuación que recogiera los pasos necesarios para cada disposición en un documento fácil de seguir[13].

16. El CCNASWP, en su 16.ª reunión (2023), examinó la petición formulada por el CCMAS, en su 41.ª reunión, y revisó el Procedimiento normalizado de actuación en consecuencia. El Comité acordó además remitir al CCMAS la revisión del procedimiento normalizado de actuación para la identificación de kavalactonas y flavokavinas en productos de kava frescos y desecados mediante cromatografía en capa líquida de alto rendimiento con miras a su ratificación [14].

17. **Se invita** al CCMAS **a ratificar** la revisión del Procedimiento normalizado de actuación que figura en el Apéndice V.

12 REP20/NASWP15, párr. 96(iii)
13 REP21/MAS41, párrs. 15-17
14 REP23/NASWP16, párr. 28(iii), Apéndice III

APÉNDICES
SOLO EN IDIOMA ORIGINAL

APPENDIX I

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES (CCNFSDU43)

Methods of analysis for provisions in the Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CXS 72-1981)

All additions are shown in **bold underlined** font.

Commodity	Provision	Method	Principle	Type
Infant Formula	**Vitamin B12**	**AOAC 2014.02**	**LC-UV**	**III**
	Total amino acids (excluding taurine and tryptophan) **For use according to Section 3.1.3 (a) footnotes 3 and 4 of CXS 72-1981**	**AOAC 2018.06 / ISO 4214 | IDF 254 /AACC 07-50.01**	**UHPLC-UV**	**II**
	Tryptophan **For use according to Section 3.1.3 (a) footnotes 3 and 4 of CXS 72-1981**	**AOAC 2017.03**	**HPLC**	**II**

APPENDIX II

CODEX COMMITTEE ON SPICES AND CULINARY HERBS (CCSCH5)

Methods of analysis for provisions in the Standard for Dried Roots, Rhizomes and Bulbs – Dried or Dehydrated Ginger (CXS 343-2021)

Parameter	Method	Principle	Type[1]
Moisture	ISO 939	Distillation	I
Total Ash on dry basis	ISO 939 and ISO 928	Distillation and Gravimetry	I
Acid Insoluble Ash on dry basis	ISO 939 and ISO 930	Distillation and Gravimetry	I
Volatile Oil on dry basis	ISO 939 and ISO 6571	Distillation followed by Volumetry	I
Extraneous Matter	ISO 927	Visual Examination followed by Gravimetry	I
Foreign Matter	ISO 927	Visual Examination followed by Gravimetry	I
Insect Damage	Method V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual) MPM: V-8. Spices	Visual Examination	IV
Whole dead insect	ISO 927	Visual examination	I
Mammalian/ Other Excreta	MPM V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual) MPM: V-8. Spices (For whole)	Visual Examination followed by Gravimetry	IV
Mould visible	Method V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual) MPM: V-8. Spices	Visual examination	IV
Live Insect	ISO 927 AOAC 960.51	Visual Examination Visual Examination	IV IV
Calcium (as oxide) on dry basis	ISO 1003, Annex A	Chemical reaction followed by gravimetry	IV
SO_2	AOAC 963.20	Colorimeter	II

[1] According to the definition of “types of method of analysis” as per Codex Procedural Manual Section II

Methods of analysis for provisions in the Standard for Dried Floral Parts-Cloves (CXS 344-2021)

Parameter	Method	Principle	Type[1]
Moisture	ASTA 2.0	Distillation	I
Volatile oil	ISO 6571	Distillation Volumetry	I
Total ash (dry basis)	ISO 928	Gravimetry	I
Acid Insoluble Ash	ISO 930	Gravimetry	I
Extraneous matter	ISO 927	Visual Gravimetry	I
Foreign matter	ISO 927	Visual Gravimetry	I
Insect damage	ISO 927 Method V-8 Spices, Condiments, Flavors and Crude Drugs	Visual Examination Visual Examination	IV IV
Insects/Excreta/Insect fragments	ISO 927	Visual Examination	IV
Crude fibre	ISO 5498	Gravimetry	I
Mould visible	Method V-8 Spices, Condiments, Flavours and Crude Drugs	Visual Examination	IV
Live insect	ISO 927	Visual Examination	IV
Mammalian or/and Other excreta	Method V-8 Spices, Condiments, Flavours and Crude Drugs	Visual Examination	IV

[1] According to the definition of “types of method of analysis” as per Codex Procedural Manual Section II

*Latest edition or version of the approved method should be used

Methods of analysis for provisions in the Standard for Dried Leaves - Dried Basil (CXS 345-2021)

Parameter	Method	Principle	Type
Moisture	ISO 939	Distillation	I
Total Ash	ISO 928	Gravimetry	I
Acid Insoluble Ash	ISO 928 and ISO 930	Gravimetry	I
Volatile Oil	ISO 6571	Distillation Volumetry	I
Extraneous Matter	ISO 927	Visual Examination followed by Volumetry	I
Foreign Matter	ISO 927	Visual Examination followed by Volumetry	I
Insect Damage	Method V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual, FDA Technical Bulletin Number 5)	Visual Examination	IV
Insects/Excreta/Insect Fragments	Method appropriate for particular spice from AOAC Chapter 16, subchapter 14	Visual Examination	IV
Mould damage	Method V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual, FDA Technical Bulletin Number 5)	Visual examination (for whole)	IV
Mammalian Excreta, And Other Excreta	Method V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual, USFDA, Technical Bulletin V.39 B) (For whole)	Visual Examination	I

* Latest edition or version of the approved method should be used.

[2] According to the definition of “types of method of analysis” as per Codex Procedural Manual Section II.

CODEX COMMITTEE ON SPICES AND CULINARY HERBS (CCSCH6)

Methods of analysis for provisions in the Standard for Dried Floral Parts – Saffron (CXS 351-2021)

Provision	Method	Principle	Type
Moisture	ISO 3632-2	Gravimetry	I
Total Ash	ISO 3632-2 and ISO 928	Gravimetry	I
Acid Insoluble Ash	ISO 3632-2 and ISO 930	Gravimetry	I
Soluble extract in cold water	ISO 3632-2 and ISO 941	Extraction	I
Taste strength (expressed as picrocrocin) $A_{1cm}^{1\%}$ 257 nm	ISO 3632-2	Absorbance	IV
Aroma strength (expressed as safranal) $A_{1cm}^{1\%}$ 330 nm	ISO 3632-2	Absorbance	IV
Coloring strength (expressed as crocin) $A_{1cm}^{1\%}$ 440 nm	ISO 3632-2	Absorbance	IV
Extraneous Matter	ISO 3632-2	Visual Examination followed by Gravimetry	I
Foreign Matter	ISO 3632-2	Visual Examination followed by Gravimetry	I
Insect Damage	ISO 927	Visual Examination followed by Gravimetry	I
Whole dead Insects /Insect Fragments	ISO 927	Visual Examination	I
Visible mould	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macro analytical Procedure Manual, FDA Technical Bulletin Number 5) http://www.fda.gov/Food/FoodScienceResearch/Laoratory Methods/ucm084394.htm#v-32	Visual Examination followed by Gravimetry	I
Mammalian Excreta	Macro analytical Procedure Manual, USFDA, Technical Bulletin V.39 B (For whole)	Visual Examination followed by Gravimetry	I
Other Excreta	AOAC 993.27 (For Ground)	Enzymatic Detection Method	IV
Rodent filth	ISO 927	Visual Examination	I

1 Latest edition or version of the approved method should be used

2 The methods of analysis will be included in CXS 234-1999 after endorsement by CCMAS and the following text replace the Table

"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."

Methods of analysis for provisions in the Standard for Dried Seeds - Nutmeg (CXS 352-2022)

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.

Provision	Method[1]	Principle	Type
Moisture content	ISO 939	Distillation	I
Total ash	ISO 939 and ISO 928	Distillation Gravimetry	I
Acid-insoluble ash	ISO 939 and ISO 930	Distillation Gravimetry	I
Water-insoluble ash	ISO 939 and ISO 929	Distillation Gravimetry	I
Volatile oil content	ISO 939 and ISO 6571	Distillation Distillation	I
Extraneous matter	ISO 927	Visual examination followed by gravimetry	I
Foreign matter	ISO 927	Visual examination followed by gravimetry	I
Visible mould	ISO 927	Visual examination followed by gravimetry	I
Insect defiled/infested	MPM V-8 Spices, Condiments, Flavours and Crude Drugs A. General methods for spices herbs and botanicals (V 32)	Visual Examination followed by gravimetry	I
Dead insect, insect fragments, rodent contamination	ISO 927	Visual examination	I
Live insect	ISO 927	Visual examination	I
Mammalian and or other excreta	Macroanalytical Procedure Manual (MPM) USFDA technical bulletin V.41	Visual examination followed by gravimetry	I
Piece of mace	ISO 927	Visual examination followed by gravimetry	I

The methods of analysis will be included in CXS 234-1999 after endorsement by CCMAS

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.

[1] Latest edition or version of the approved methods should be used.

Methods of analysis for the provisions off size, when sized and broken/damaged among the whole to be developed.

Methods of analysis for provisions in the Standard for Dried or Dehydrated Chilli Pepper and Paprika (CXS 353-2022)

Provision	Method[1]	Principles	Type[2]
Moisture	ISO 939	Distillation	I
Total Ash	ISO 939 and ISO 928	Distillation Gravimetry	I
Acid-insoluble ash	ISO 939 and ISO 930	Distillation Gravimetry	I
Pungency Scoville Heat units	ASTA 21.3	Chromatography	IV
	ISO 3513	Sensory evaluation	I
Colour value	ISO 7541	Spectrophotometry	IV
Mammalian excreta	ISO 927	Visual examination followed by Gravimetry (whole)	I
Mould damage	MPM V-8 Spices, Condiments, Flavours and Crude Drugs A. General methods for spices herbs and botanicals (V 32)	Visual Examination (for whole)	I
	AOAC 945.94	Visual Examination (for Ground)	I
Insect Damage	MPM V-8 Spices, Condiments, Flavours and Crude Drugs A. General methods for spices herbs and botanicals (V 32)	Visual Examination followed by Gravimetry	I
Extraneous matter[3]	ISO 927	Visual Examination followed by Gravimetry	I
Foreign matter[4]	ISO 927	Visual Examination followed by Gravimetry	I
Live insect	ISO 927 / AOAC 960.51	Visual Examination	I
Insect filth	ISO 927	Visual Examination	I
Insect fragments	ISO 927	Visual examination counting	I
Rodent hair	AOAC 978.22 (Ground chilli) AOAC 977.25 B (Ground paprika)	Microscopic examination Microscopic examination	I I

[1]Latest edition or version of the approved method should be used.

[2]According to the definition of "types of method of analysis" as per Codex Procedural Manual Section II

[3] Vegetative matter associated with the plant from which the product originates but not accepted as part of the final product.

[4] Any visible/detectable objectionable foreign matter or material not usually associated with the natural components of the spice plant, such as sticks, stones, burlap bagging, metal, etc.

The methods of analysis will be included in CXS 234-1999 after endorsement by CCMAS and the following text shall replace the Table

"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

Methods of analysis for provisions in the draft Standard for Dried Small Cardamom

Provision	Method[1]	Principle	Type [2]
Moisture	ISO 939	Distillation	I
Total Ash	ISO 939 and ISO 928	Distillation and Gravimetry	I
Acid Insoluble Ash	ISO 939 and ISO 930	Distillation and Gravimetry	I
Volatile Oil	ISO 939 and ISO 6571	Distillation followed by Volumetry	I
Extraneous Matter	ISO 927	Visual Examination followed by Gravimetry	I
Foreign Matter	ISO 927	Visual Examination followed by Gravimetry	I
Insect defiled/infested	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual) MPM: V-8. Spices	Visual Examination followed by Gravimetry	I
Immature and shrivelled capsules	ISO 927	Visual Examination followed by Gravimetry	I
Mammalian or/and other excreta	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual) MPM: V-8. Spices	Visual Examination followed by Gravimetry	I
Mould visible	Method V-8 Spices, Condiments, Flavors and Crude Drugs (Macroanalytical Procedure Manual) MPM: V-8. Spices	Visual Examination followed by Gravimetry	I
Empty and malformed capsules	IS 1907:1984	Visual Examination followed by Gravimetry	I
Whole insect Live/dead	ISO 927	Visual examination followed by Gravimetry	I
Light seeds	ISO 927	Visual examination followed by Gravimetry	I

1 Latest edition or version of the approved method should be used

2 According to the definition of “types of method of analysis” as per Codex Procedural Manual Section II

***** The methods of analysis will be included in CXS 234-1999 after endorsement by CCMAS and the following text replace the Table

“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”

Methods of analysis for provisions in the draft Standard for Spices Derived From Dried Fruits and Berries (Part A - Allspice, Juniper berry And Star anise)

Sl. No	Spices	Provision	Method[1,2]	Principles	Type
1	Dried Allspice Dried Juniper Berries Dried Star Anise	Moisture	ISO 939	Distillation	I
		Total ash	ISO 939 and ISO 928	Distillation followed by gravimetry.	I
		Acid- insoluble	ISO 939 and ISO 930	Distillation followed by gravimetry.	I
		Volatile oils	ISO 939 and ISO 6571	Distillation followed by gravimetry.	I
		Extraneous matter	ISO 927	Visual examination followed by gravimetry	I
		Foreign matter	ISO 927	Visual examination followed by gravimetry	I
		Mould visible	ISO 927	Visual examination followed by gravimetry	I
		Mammalian excreta	MPM V-8 Spices, Condiments, Flavors and Crude Drugs A. General methods for spices herbs and botanicals (V 32) *https://www.fda.gov/food/laboratory- methods-food/mpm-v-8-spices-condiments- flavors-and- crude-drugs* (Applicable to whole form of the spices)	Visual examination followed by gravimetry	I
		Whole dead insect	ISO 927	Visual examination	I
			AOAC 969.44	Flotation method	IV
		Insect fragments	ISO 927	Visual examination counting	I
			AOAC 975.49	Flotation method	IV

Sl. No	Spices	Provision	Method[1,2]	Principles	Type
		Insect damage	MPM V-8 Spices, Condiments, Flavours and Crude Drugs General methods for spices herbs and botanicals (V 32) (Applicable to whole form of the spices)	Visual examination followed by gravimetry or counting	I
		Mould damage	MPM V-8 Spices, Condiments, Flavours and Crude Drugs General methods for spices herbs and botanicals (V 32) (Applicable to whole form of the spices)	Visual examination followed by gravimetry or counting	I
2	Allspice (whole, cracked/ pieces)	Filth (list all the filth here-for example - mammalian excreta)	AOAC 965.40	Flotation	I
	Allspice (Ground/ powdered)	Light filth (list all the filth here-for example- mammalian excreta)	AOAC 981.21	Flotation	I
3	Juniper Berries, Star Anise, (cut/broken, ground/ powdered)	Light filth (list all the filth here-for example- mammalian excreta)	AOAC 975.49	Flotation	I

[1] Latest edition or version of the approved method should be used

[2] The methods of analysis will be included in CXS 234-1999 after endorsement by CCMAS and the following text replace the Table

"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."

APPENDIX III

FAO/WHO COORDINATING COMMITTEE FOR AFRICA (CCAFRICA24)

Methods of analysis and sampling for provisions in the Regional Standard for Dried Meat (CXS 350-2022)

1. METHODS OF ANALYSIS AND SAMPLING

8.1 Methods of Analysis[15]

Provision	Method	Principles	Type
Moisture Content	AOAC 950.46B	Gravimetry	I
Total Fat	ISO 1443	Gravimetry	I
Nitrogen*	ISO 937*	Titrimetry	II
Chloride as Sodium Chloride (≥ 1.0%)	ISO 1841-1	Volhard method	III
Chloride as Sodium Chloride (≥ 0.25%)	ISO 1841-2	Potentiometry	II
Ash	ISO 936	Gravimetry	I
Water Activity	ISO 18787	Electrometry	II
*nitrogen-to-protein conversion factor = 6.25			

8.2 Sampling

Sampling shall be in accordance with the *General Guidelines on Sampling* (CXG 50-2004).

[15] After adoption, the table containing the Methods of Analysis will be removed and replaced with the following Text, as per the requirements of the Procedural Manual:
"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."

APPENDIX IV

FAO/WHO COORDINATING COMMITTEE FOR ASIA (CCASIA22)

Methods of analysis and sampling for provisions in the draft Regional Standard for Soybean Products Fermented with Bacillus Species

9. METHODS OF ANALYSIS AND SAMPLING[16]

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. Determination of Moisture Content

Natto: According to AOAC 925.09.(Type I Gravimetry (vacuum oven))

Cheonggukjang: According to AOAC 934.01. (Type I Gravimetry)

Thua Nao: According to AOAC 925.09. (Type I Gravimetry (vacuum oven))

9.2. Determination of Protein Content

Natto: According to AOAC 988.05. (Type I Titrimetry, Kjeldahl digestion)

(Nitrogen factor 5.71)

Cheonggukjang: According to AOAC 988.05. (Type I Titrimetry, Kjeldahl digestion)

(Nitrogen factor 5.71)

Thua Nao: According to AOAC 988.05. (Type I Titrimetry, Kjeldahl digestion)

(Nitrogen factor 5.71)

9.3. Determination of Lipid Content

Natto: According to AOAC 963.15. (Type I Gravimetry (Soxhlet Extraction))

(Quantity of sample:4g)

Cheonggukjang: According to AOAC 963.15. (Type I Gravimetry (Soxhlet Extraction))

(Quantity of sample:5g)

[16] The analytical methods will be removed when the standard is adopted by CAC and included in CXS 234-1999.

ANNEX I

Sampling Plans (AQL=6.5)

Sampling plan 1 – Normal sampling

Lot size (N)	Sample size (n)	Acceptance number (c)
4,800 or less	6	1
4,801-24,000	13	2
24,001-48,000	21	3
48,001-84,000	29	4
84,001-144,000	38	5
144,001-240,000	48	6
More than 240,000	60	7

Sampling plan 2 – Dispute, enforcement or need for better lot estimate

Lot size (N)	Sample size (n)	Acceptance number (c)
4,800 or less	13	2
4,801-24,000	21	3
24,001-48,000	29	4
48,001-84,000	38	5
84,001-144,000	48	6
144,001-240,000	60	7
More than 240,000	72	8

Methods of analysis and sampling for provisions in the draft Regional Standard for Cooked Rice Wrapped in Plant Leaves

10. METHODS OF ANALYSIS AND SAMPLING[17]

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. Determination of Peroxide Value

10.1.1. Extraction of Oils from the Product

10.1.1.1. Apparatus

(a) Rotary evaporator

(b) Water bath

10.1.1.2. Extraction

Remove the product package and plant leaves, etc., take out the edible part of the representative sample, crush it and put it in a homogenizer or glass mortar, and grind it continuously to make the sample fully mashed and mixed well, and then put it in the wide-mouth bottle, and add 2 to 3 times the sample volume of petroleum ether (boiling range: 30°C-60°C). After fully mixing, stopper the bottle and leave for more than 12 hours. Filter all the solution with a funnel filled with anhydrous sodium sulphate into a round-bottom flask. Rinse the residue in the wide-mouth bottle with petroleum ether. Repeat the filtration once with a new anhydrous sodium sulphate funnel, if the filtrate is not clear enough. Evaporate the petroleum ether in the round-bottom flask under reduced pressure on a rotary evaporator at below 40°C, and the residue is the test sample. A sufficient number of representative samples should be selected to ensure that not less than 8 grams of the test sample can be obtained. The test sample should be tested as soon as possible.

10.1.2. Determination

According to ISO 3960 or AOCS Cd 8b-90 (03) (Type I Titrimetry (Colorimetric)).

[17] The analytical methods will be removed when the standard is adopted by CAC and included in CXS 234-1999.

APPENDIX V

FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND SOUTHWEST PACIFIC (CCNASWP16)

Methods of analysis and sampling for provisions in the draft Regional Standard for Fermented Noni Fruit 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 2016.12	Headspace GC-FID	IV	
Identification of scopoletin	Annex A*	Solid phase extraction and 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

Noni fruit juice is filtered through a 0.45 µm membrane filter and then purified by solid-phase extraction (SPE) with Waters OASIS® HLB 6cc 200 mg extraction cartridges (or similar solid-phase extraction cartridge), after first equilibrating with methanol (5 mL) followed by deionized water (5 mL). The filtered juice samples (3 mL) are then loaded onto the equilibrated cartridge and washed with 5% methanol (MeOH) in deionized water (5 mL). The cartridges are allowed to dry under flow of air for 5 mins and then, eluted with MeOH (3mL). The MeOH eluate is retained for TLC analysis. The SPE flow rates of equilibration, wash and elution solvents through the cartridge is approximately 1 drop per second.

2. PREPARATION OF REFERENCE STANDARD

2.1 A reference standard is prepared by dissolving 0.1 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. After spotting the plates are 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.

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

Noni fruit juice is filtered through a 0.45 µm membrane filter and diluted 1:1 with MeOH ~~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.]~~.

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. PREPARATION OF p-ANISALDEHYDE SOLUTION

Anisaldehyde solution was prepared by dissolving 2g of p-anisaldehyde in 96 mL of ethanol with stirring. The solution was then acidified through dropwise addition of concentrated sulfuric acid (4 mL).

4. IDENTIFICATION

4.1 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. After spotting samples are again dried at 110°C or through application of heat via a heat gun for a period of 8-10 seconds. The TLC plates are developed with a mobile phase of dichloromethane: methanol: water (13:6:1, v/v/v). Upon completion of elution, the plate is air dried and developed by spraying with 2% anisaldehyde / 4% sulfuric acid in ethanol (EtOH) solution and then heat in oven at 110 °C for 1-5 minutes to reveal and maximise the blue colour. Identify deacetylasperulosidic in samples by comparing spot Rf values and colour with reference standard solution on same TLC plate.

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

ANNEX C

SINGLE LABORATORY VERIFICATION / VALIDATION FOR IDENTIFICATION OF SCOPOLETIN AND DEACETYASPERULOSIDIC ACID IN FERMENTED NONI JUICE

The performance characteristics validation for an 'Identification test' is usually limited to 'Selectivity'. Where the capability of an analytical procedure to identify an analyte can be confirmed by obtaining positive results comparable to a known reference material with samples containing the analyte, along with negative results from samples which do not contain the analyte. In addition, the identification test can be applied to materials structurally similar to or closely related to the analyte to confirm that an undesired positive response is not obtained. Specificity/selectivity can be verified by demonstrating that the measured result of an analyte is comparable to the measured result of a second, well characterized analytical procedure (e.g., an orthogonal procedure).

Thus,

a) the colour response with the TLC visualization technique with standards, and a relative response for increasing standard concentration tested was confirmed,

b) the coloured TLC spot with samples with a Rf similar to the standard was confirmed for different Fermented Noni juices from a range of pacific island locations (supplied by Scientific Research Organisation of Samoa (SROS)-Apia),

c) various juices observed mixed in commercial Noni products were tested along with a Noni Juice by TLC to confirm a negative result for other juices.

d) an orthogonal HPLC technique based on Choi et al (2022)[11] was used to measure concentrations or absence of the identity analytes for selected samples, and PDA spectra along with Rt used to confirm HPLC peak identity.

For Scopoletin Identification

a) Colour response under UV@365nm and relative intensity/response for Scopoletin TLC standards at 0.001, 0.01, 0.1 and 1.0 mg/mL in MeOH. We thus suggest that a 0.10 mg/mL Scopoletin standard may be more appropriate in the Scopoletin TLC identification.

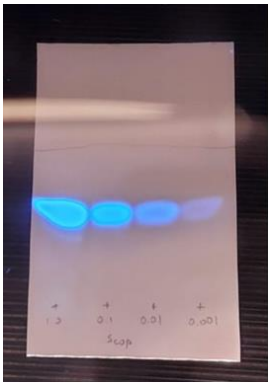

Figure 1 TLC for Scopoletin standards at 1.0, 0.1, 0.01 and 0.001 mg/mL in MeOH at 365nm.

b) Colour response under UV@365nm and R_f relative to standard Scopoletin for various Pacific Island samples.

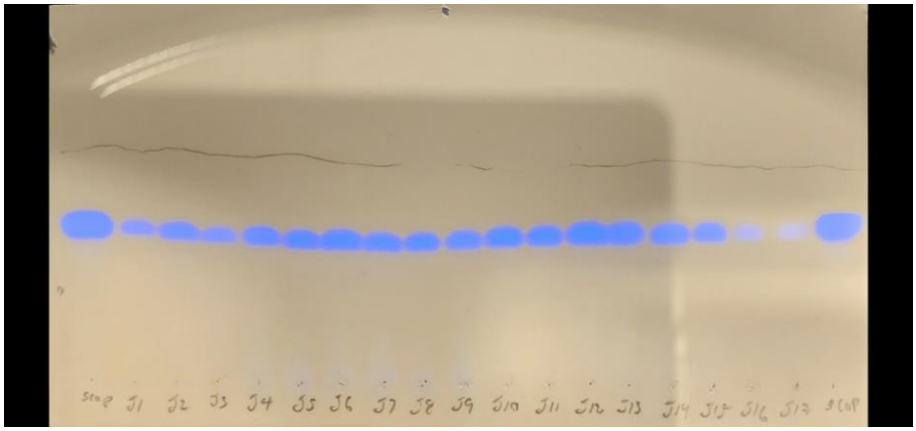

Figure 2. Scopoletin standard plus SPE extraction & TLC with UV@365nm visualization of fermented Noni juice samples, with left to right, standard; fermented Noni juice samples J1-17; standard.

Standard and Pacific Island samples J18-J19.

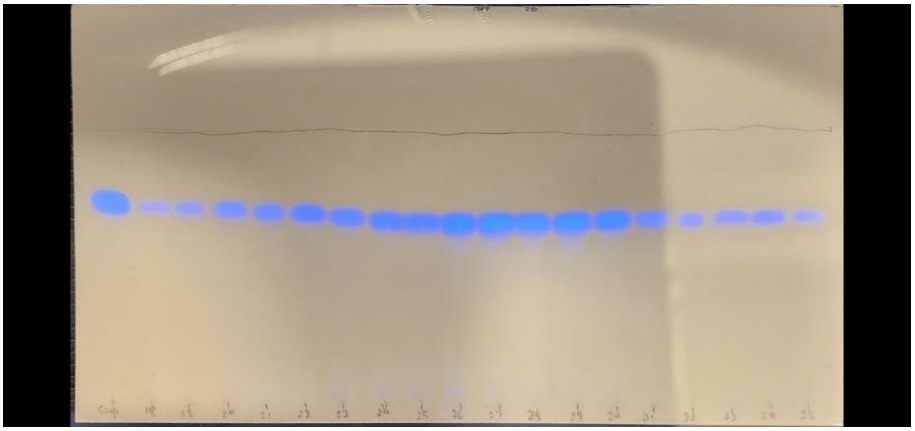

Figure 3. Scopoletin standard plus SPE extraction & TLC with UV@365nm visualization of fermented Noni juice samples, with left to right, standard; fermented Noni juice samples 18-35.

c) Following is the Scopoletin TLC Identification test applied to various juices observed mixed in commercial Noni products, including commercial pineapple juice, apple and blackcurrant juice, grape juice, pear juice, and coconut juice.

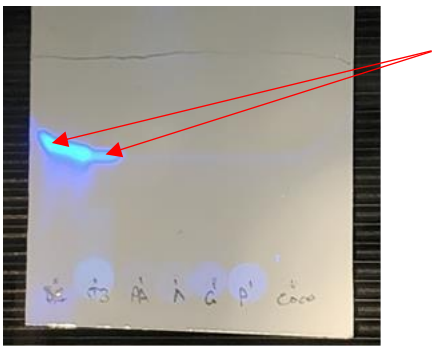

Figure 4. Scopoletin ID TLC for a Noni juice plus various other juices; from left to right, Scopoletin (0.1mg/mL), Noni Juice#3(J3), Pineapple juice (PA), Apple and Blackcurrant juice(A), Grape juice(G), Pear juice(P), and Coconut juice (Coco). Scopoletin band for standard and Noni Juice#3 indicated by red arrows, where the absence of similar band for the other samples gives a negative Scopoletin Identification.

a) An orthogonal HPLC technique based on Choi et al (2022)[11] used to confirm 'presence' or 'absence' of the identity analytes for selected samples, and PDA spectra along with peak at Rt=22.8min(approx.) used to confirm HPLC peak identity.

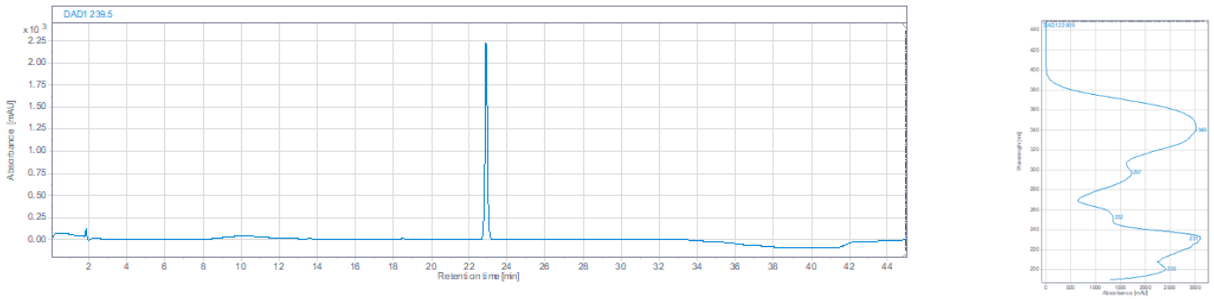

Figure 5. Scopoletin standard, HPLC-DAD chromatogram, 10µL injection, @ 239.5nm and peak UV spectra.

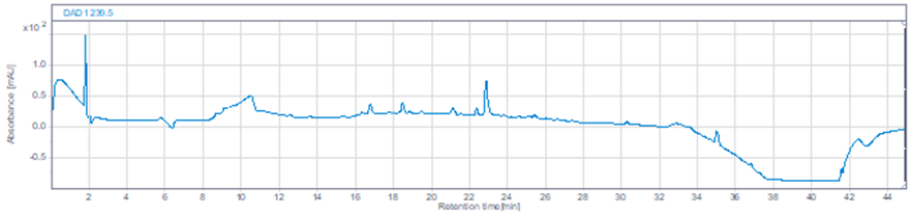

Figure 6. Juice#3, filtered, HLB-SPE 100% MeOH elution solution and injected 10µL on HPLC-DAD @ 239.5nm

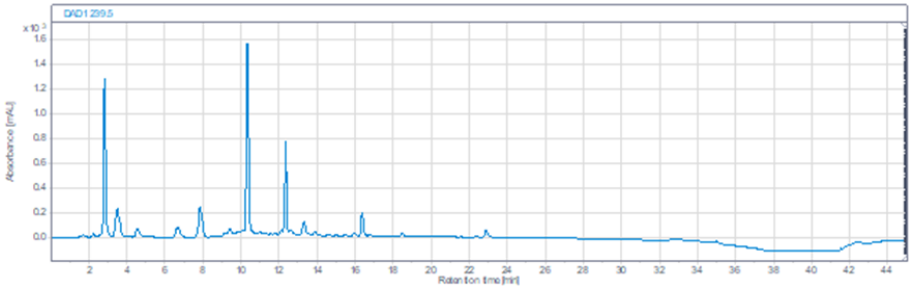

Figure 7. Juice#3, filtered 0.45um, no SPE, and direct injected 10µL on HPLC-DAD @ 239.5nm.

See section f) for HPLC-DAD conditions.

For Deacetylasperulosidic acid Identification

a) Colour response with 2% anisaldehyde / 10% sulfuric acid-ethanol (EtOH) solution then heating for visualisation, and relative intensity/response at 1.0, 0.5, 0.25 and 0.1 mg/mL Deacetylasperulosidic acid.

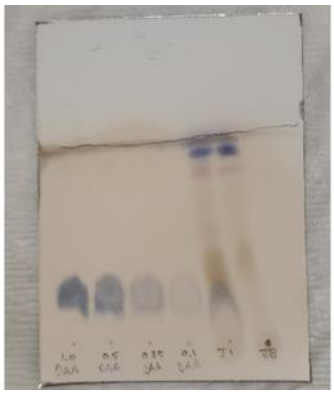

Figure 8. TLC standard solutions spots 1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL; Juice 1; Juice 8.

b) Colour response with 2% anisaldehyde / 10% sulfuric acid-ethanol (EtOH) solution then heating for visualisation, and R_f relative to standard Deacetylasperulosidic acid for various Pacific Island samples.

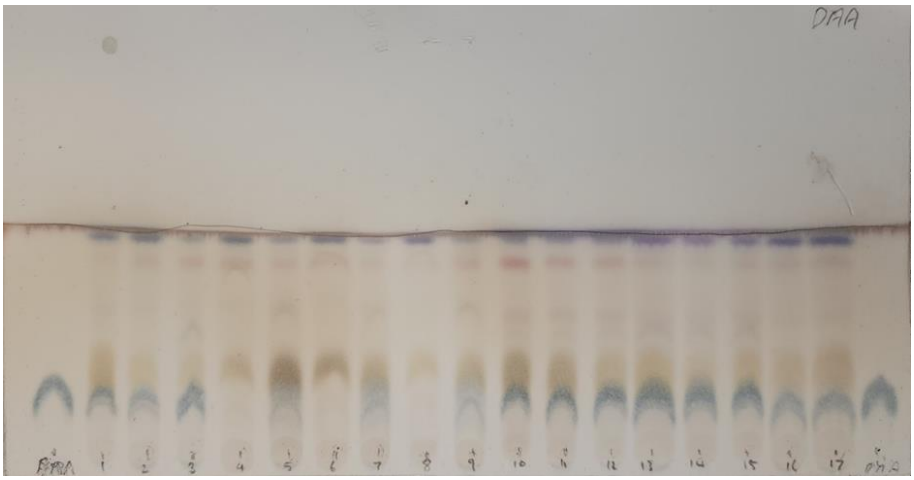

Figure 9. From left to right, DAA standard; fermented Noni juice samples 1-17; DAA standard; with TLC visualised with 2% anisaldehyde / 4% sulfuric acid-ethanol (EtOH) solution then heating.

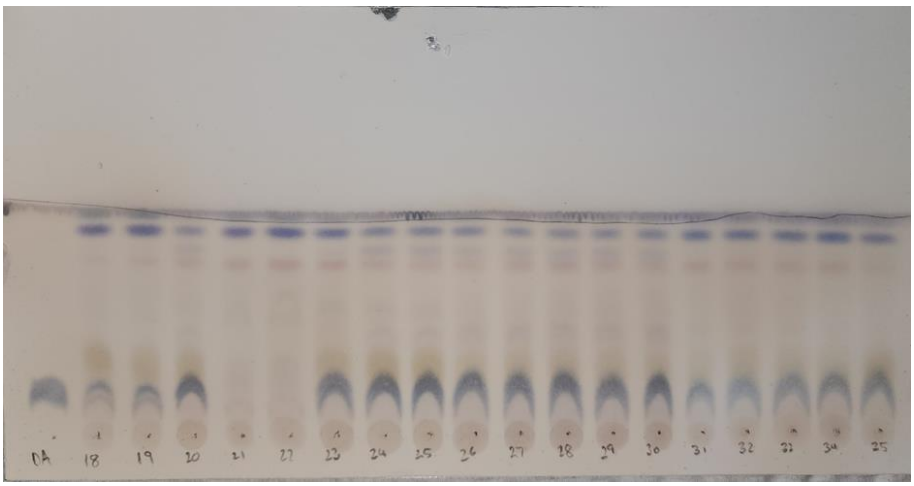

Figure 10. From left to right, DAA standard, fermented Noni juice samples 18-35; with TLC visualisation with 2% anisaldehyde / 4% sulfuric acid-ethanol (EtOH) solution then heating.

Note: Samples sourced from SROS-Apia for purpose of verification of TLC method for Scopoletin and DAA. Samples may have been subjected to adverse conditions during transport or pre-sampling prior to shipment to Australia. No conclusion can be inferred for Juices, 4, 6, 8, 21, 22 other than HPLC-DAD and TLC are in alignment in the absence or scarcity of DAA analyte. Further investigation would be required on non-compliant sample to determine the reason behind these atypical or non-compliant findings.

c) Following is the Deacetylasperulosidic acid TLC Identification test applied to various juices observed mixed in commercial Noni products, including commercial pineapple juice, apple and blackcurrant juice, grape juice, pear juice, and coconut juice.

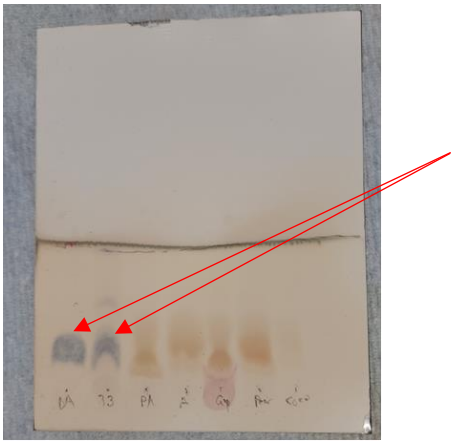

Figure 11. Deacetylasperulosidic acid ID TLC for a Noni juice plus various other fruit juices; from left to right, Deacetylasperulosidic acid (0.5mg/mL DA), Noni Juice#3(J3), Pineapple juice (PA), Apple and Blackcurrant juice(A), Grape juice (Gp), Pear juice (Pear), and coconut juice (Co). Deacetylasperulosidic acid blue band indicated by red arrow in standard and Juice#3, where the absence of similar blue bands for the other samples gives a 'negative' identification.

d) An orthogonal HPLC technique based on Choi et al (2022)[11] used to confirm 'presence' or 'absence' of the identity analytes for selected samples, and PDA spectra along with Rt used to confirm HPLC peak identity.

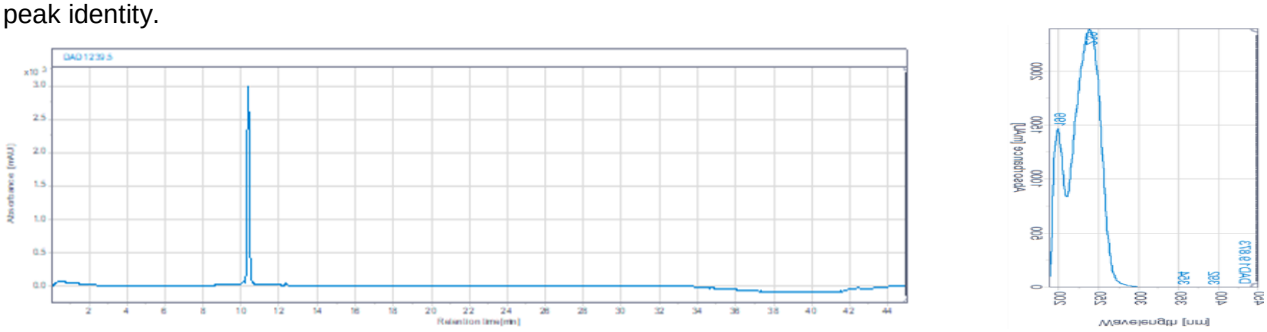

Figure 12. Deacetylasperulosidic acid 2 mg/mL; HPLC-DAD chromatogram, 10µL injection, @ 239.5nm; and peak UV spectra.

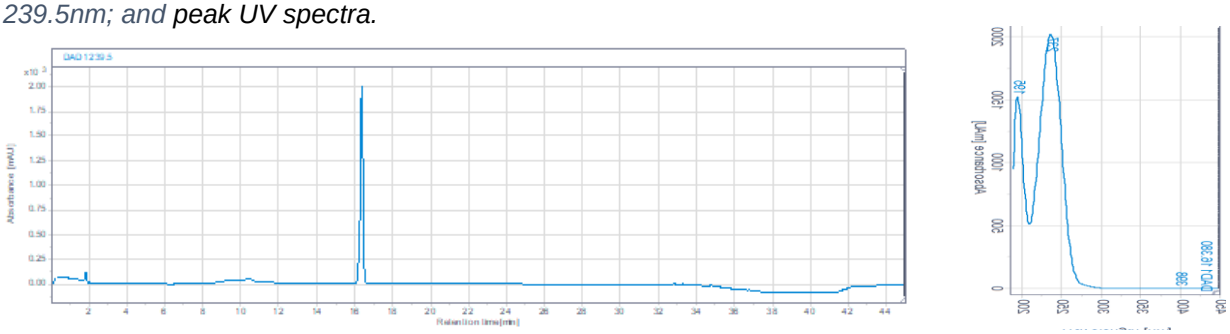

Figure 13. Asperulosidic acid; HPLC-DAD chromatogram, 10µL injection, @ 239.5nm; and peak UV spectra.

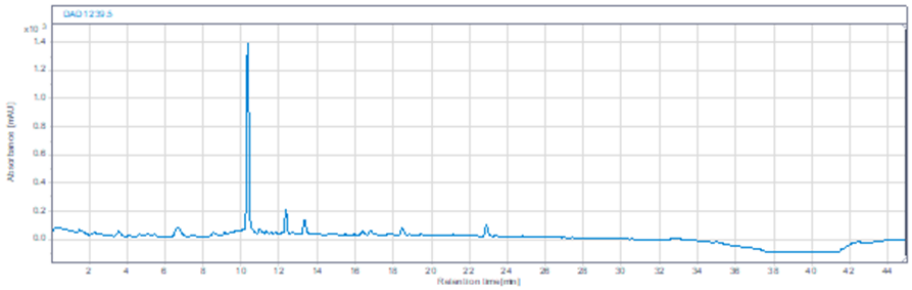

Figure 14. Juice 1, 0.45um filtered & no SPE, direct injected 10µL on HPLC-DAD @ 239.5nm

See section f) for HPLC-DAD conditions.

e) HPLC confirmation of the Deacetylasperulosidic acid ID by TLC for selected Pacific Island Noni Juice samples where 'negative' and 'positive' DAA IDs were observed.

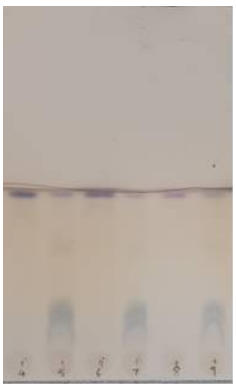

Figure 15 Cropped section of Fig 10, highlighting Deacetylasperulosidic acid ID by TLC for a selection of various Pacific Island Noni Juice samples where 'negative' and 'positive' DAA IDs were observed.

The juices in the following chromatograms were 0.45µm filtered and injected directly onto the HPLC-DAD with 10µL injection. The specific pattern to note is that according to the TLC, juices 4, 6 and 8 show a 'negative' DAA identification; while juice 5, 7, 9 show a 'positive' DAA identification. As observed in the following the HPLC-DAD chromatograms confirm the TLC results, with 'presence' or 'absence' of a sharp DAA peak at approximately 9.9 min, with 10uL injection, using 239.5 nm wavelength detection. Note, all these juices have a peak at R_t=22.8mins, thus positive ID for Scopoletin. Note the 10x reduction in absorbance scale for the negative results for DAA.

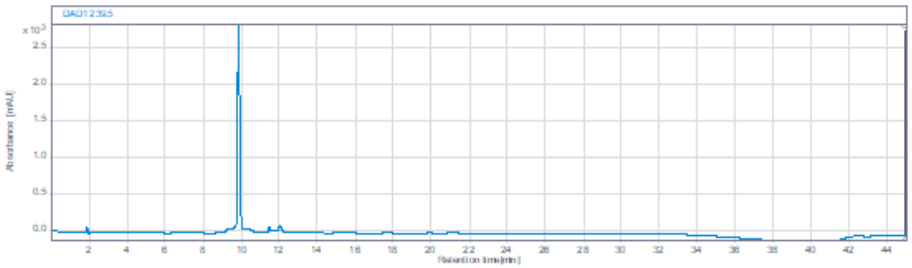

Figure 16. HPLC of DAA standard 2mg/mL with peak at 9.9mins.

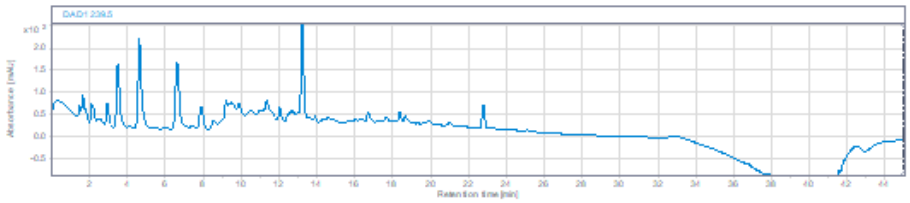

Figure 17. HPLC injection of Pacific Island juice#4, confirming 'negative' result for DAA.

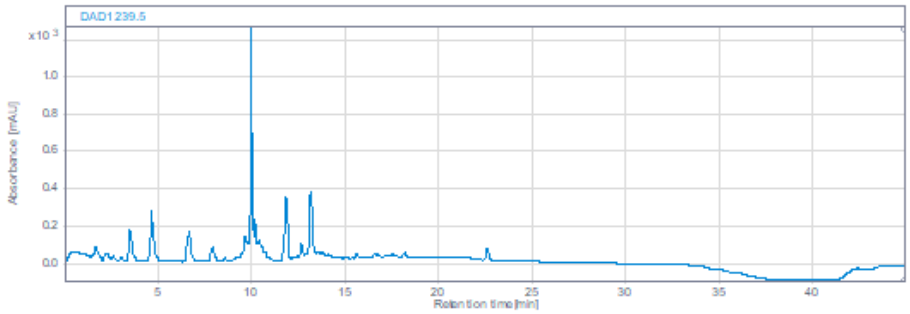

Figure 18. HPLC injection of Pacific Island juice#5, confirming 'positive' result for DAA.

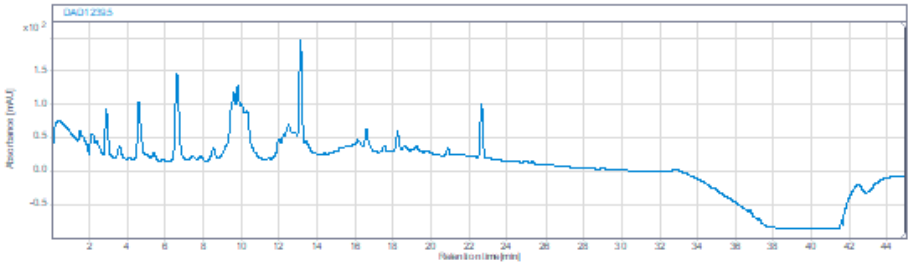

Figure 19. HPLC injection of Pacific Island juice#6, confirming 'negative' result for DAA.

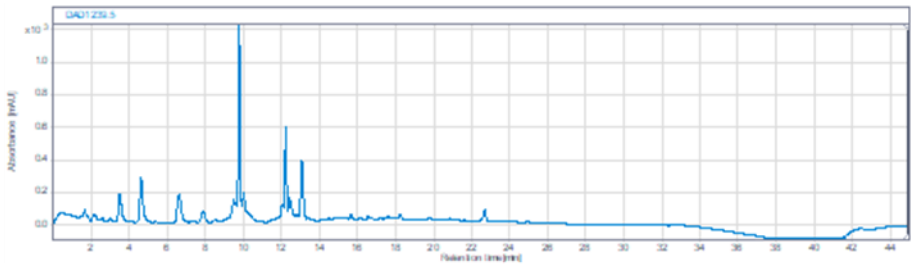

Figure 20. HPLC injection of Pacific Island juice#7, confirming 'positive' result for DAA.

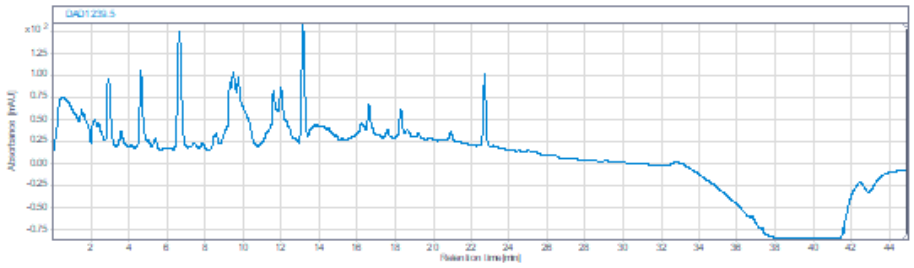

Figure 21. HPLC injection of Pacific Island juice#8, confirming 'negative' result for DAA.

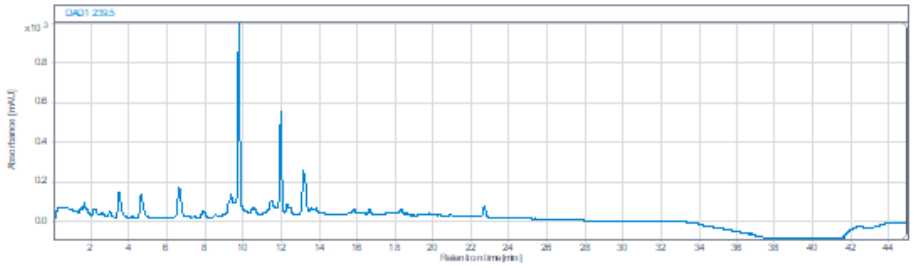

Figure 22. HPLC injection of Pacific Island juice#9, confirming 'positive' result for DAA.

f) Conditions for HPLC-DAD

HPLC-DAD was performed on an Agilent 1260 Infinity HPLC

Instrument: Agilent 1260 Infinity

Gradient:

Time(min)	0.1% Formic acid in deionised water	Acetonitrile
0	100	0
5	100	0
30	65	35
35	0	100
39	0	100
40	100	0
45	100	0

Run time: 45 minutes

Wavelengths monitored: 239.5 nm (Deacetylasperulosidic Acid); 344 nm (Scopoletin),

Peak width: >0.2 min (4s response time) 1.25Hz

Injection volume: 10µL

Mobile phase flow rate: 1.0 mLs/minute

Column temperature: 25 ^{o}C

Column: Agilent, Zorbax Eclipse Plus C18. 5um, 4.6 x 150 mm, (PN:959993-902, SN:USUXB20707, LN:B20104)

Guard Column: Agilent, Zorbax Eclipse Plus C18 2.1 x5, 1.8 micron (PN:821725-901, SN: USEDP03464)

Standard Operating Procedure for the Identification of Kavalactones and Flavokavains in Fresh and Dried Kava Products by High Performance Thin Layer Chromatography in the Regional Standard for Kava Products for Use as a Beverage when Mixed with Water (CXS 336R-2020)

1.0 Introduction

Piper methysticum G. Forst. (Piperaceae) rhizomes and roots are peeled, grinded, macerated in cold water, and pressed through a cloth strainer to prepare kava, a non-alcoholic beverage. The composition and quality of kava can be highly variable, depending on the age of the plant, the variety, and the part used to prepare the beverage: roots, rhizomes, or basal stems. The six major kavalactones (KLs: yangonin = Y, dihydrokavain = DHK, desmethoxyyangonin = DMY, kavain = K, dihydromethysticin = DHM and methysticin = M) are responsible for the physiological effect and are usually quantified with HPLC. There is a second group of molecules is flavokavins (FKs: A, B, C). The chemical composition of the kava extract is strongly influenced by the extraction solvent and extraction technique. This procedure is based on analytical procedure using High Performance Thin Layer Chromatography (HPTLC). The HPTLC is a validated procedure for 174 varieties of kava.

Scope: Identification of Kavolactones and Flavokavins by High Performance Thin Layer Chromatography

2.0 Materials and methodology

2.1 Preparation of Samples

- Wash by hand under cold running water the kava roots and peeled rhizomes.
- Cut into small pieces the kava organs with a knife.
- Sun-dry the kava pieces for 3 days (similar to traditional practises).
- Ground the dried kava matter into powder using a Forplex F00 1218 hammer mill to achieve <2 mm particle size and pack into labelled zip-log plastic bags.
- Further ground the kava powder to very fine kava flour texture using a coffee grinder.
- Weigh the kava flour sample then dry in an oven at 60°C for 6 hours.

2.2 Preparation of Reference Standard

- Make available Six kavalactone and three flavokavain standards of analytical grade possibly available from Sigma-Aldrich including standards of:

  Six kavalactones:

  - methysticin (M),
  - dihydromethysticin (DHM),
  - kavain (KAV),
  - dihydrokavain (DHK),
  - yangonin (Y),
  - desmethoyyangonin (DMY),

  Three flavokavain:

  - flavokavain A (FKA),
  - flavokavain B (FKB) and
  - flavokavain C (FKC).

- Accurately weigh 1.0mg individually the pure kava standard powder into 1ml acetone
- store in dark at 4°C if analysed later.

  Checking Purity of Standards:

- Conduct peak purity tests for the kava standards using the UV Vis spectrophotometer and compare the UV spectra.

2.3 Sample extraction

- Weigh 10g of kava powder,
- Transfer to a clean 50ml polypropylene centrifuge tube and add 30ml acetone.
- Sonicate the tubes in a water bath for 30min
- Transfer to a centrifuge instrument and set at4500 rpm for 10min.
- Transfer the supernatant to a 9mm wide opening screw thread vial of 2ml amber glass.
- Store vials in refrigerator at 4°C in dark till required for analysis.

2.4 Identification by High Performance Thin Layer Chromatography (HPTLC)

2.4.1 Chemicals and reagents for HPTLC analysis

- Analytical grade solvent (acetone, dioxane, hexane and methanol).
- Silica gel 60 F254 plates (dimension; 20 x 10cm) using Camag HPTLC system with an automatic TLC sampler (ATS 4) coupled to an automatic developing chamber (ADC 2) and a visualizer as well as a TLC Scanner 4 controlled with winCATS software.

2.4.2 Check standards and prepare Sample Run

- Prepare standards and sample solutions at bands(length of 8 mm, 250 nL/s delivery speed, track distance 8.0 mm and distance from the edge of 15 mm).
- Conduct standard linearity curve check by using the HPTLC plates. Apply different stock solutions (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 µL) of the six KLs and three FKs scan at 240nm (for M, DHM, K, DHK) and scan at 355nm (for Y, DMY, FKA, FKB, FKC).
- Add 10 mL mobile phaseto develop the plates using hexane:dioxane (8:2 v/v) with a migration distance of 80 mm at room temperature after 30 s of pre-drying and no tank saturation.
- Visual documentation of the plates is carried out at 254 nm and 366 nm.
- Scan the plates in reflectance mode at 240 nm (for M, DHM, K and DHK) and at 355 nm (for Y, DMY, FKA, FKB, FKC) with D2 and W lamp slit dimension 8.00 mm × 0.20 mm, scanning speed 20 mm/s, and data resolution 100 µm/step.
- Identify the Peak area measurements (in area units, AU).
- Ensure that the total analytical time is 50 min for 20 samples and 10 mL of mobile phase (corresponding to 2.5 min and 0.5 mL per sample).

3.0 References

Lebot, V., Michalet, S., Legendre, L. (2019). Kavalctone and Flavokavins Profile Conttribute to Quality Assessment of Kava (Piper methysticum G. Forst), the Traditional Beverage of the Pacific. *Beverages*. 2019, 1-14.