



PROGRAMME MIXTE FAO/OMS SUR LES NORMES ALIMENTAIRES
COMITÉ DU CODEX SUR LES MÉTHODES D'ANALYSE ET D'ÉCHANTILLONNAGE

Quarante-deuxième session

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**CONFIRMATION DES DISPOSITIONS RELATIVES AUX MÉTHODES D'ANALYSE ET PLANS
D'ÉCHANTILLONNAGE FIGURANT DANS LES NORMES CODEX**

1. Le présent document décrit les méthodes d'analyse (Annexes I, II, III, IV et V) proposées par les Comités suivants:
 - Comité du Codex sur la nutrition et les aliments diététiques ou de régime
 - Modifier les *Méthodes d'analyse et d'échantillonnage recommandées* (CXS 234-1999) afin d'inclure une nouvelle entrée «produit pour enfants en bas âge» à «préparations de suite».
 - Méthodes pour la vitamine B12; les acides aminés totaux (à l'exception de la taurine et du tryptophane), et le tryptophane dans la *Norme pour les préparations destinées aux nourrissons et les préparations données à des fins médicales spéciales aux nourrissons* (CXS 72-1981).
 - Comité du Codex sur les épices et les herbes culinaires
 - Méthodes d'analyse pour les dispositions dans les *Norme pour les racines, les rhizomes et les bulbes séchés: gingembre séché ou déshydraté* (CXS 343-2021), *Norme pour les parties florales séchées: clous de girofle* (CXS 344-2021) et la *Norme pour le basilic séché* (CXS 345-2021) (normes adoptées par la Commission du Codex Alimentarius, à sa 44^e session (2021) à l'étape 8, REP21/CAC44, paragraphe 38 et annexe III).
 - Méthodes d'analyse pour les dispositions dans les *Norme pour les parties florales séchées – safran* (CXS 351-2021) et *Norme pour les graines séchées – noix de muscade* (CXS 352-2022) (normes adoptées par la Commission, à sa 45^e session (2022) à l'étape 8, REP22/CAC45, paragraphe 94(i)-(ii) et annexe II).
 - Méthodes d'analyse pour les dispositions dans la *Norme pour le piment et le paprika séchés ou déshydratés* (CXS 353-2022) (norme adoptée par la Commission, à sa 45^e session à l'étape 5/8, REP22/CAC45 paragraphe 94(iii) et annexe II).
 - Méthodes d'analyse pour les dispositions dans le projet de norme pour la petite cardamome séchée, le projet de norme pour les épices issues de fruits et de baies séchés (partie A – piment de la Jamaïque, baies de genévrier, anis étoilé) (normes adoptées par la Commission, à sa 45^e session à l'étape 5, REP22/CAC paragraphe 97 et annexe III, présentées à la Commission, à sa 46^e session (2023) pour adoption à l'étape 8).
 - Comité FAO/OMS de coordination pour l'Afrique
 - Méthodes d'analyse pour les dispositions dans la *Norme pour la viande séchée* (CXS 350-2022) (adoptée par la Commission, à sa 45^e session, à l'étape 8, REP22/CAC45, paragraphe 149 et annexe II).
 - Comité FAO/OMS de coordination pour l'Asie
 - Méthodes d'analyse et d'échantillonnage pour les dispositions dans le projet de norme régionale pour les produits à base de soja fermenté sous l'action de *Bacillus* spp et le projet de norme régionale pour le riz cuit enveloppé dans des feuilles (présentés pour adoption à l'étape 5/8 par la Commission, à sa 46^e session).
 - Comité FAO/OMS de coordination pour l'Amérique du Nord et le Pacifique Sud-Ouest
 - Méthodes d'analyse pour les dispositions dans le projet de norme régionale pour le jus de noni fermenté (présenté pour adoption à l'étape 8 par la Commission, à sa 46^e session).

- La révision de la procédure opérationnelle standard pour l'identification des kavalactones et des flavokavaiïnes dans les produits du kava frais et séchés, par chromatographie sur couche mince à haute performance dans la *Norme régionale pour les produits du kava à usage de boisson lorsqu'ils sont mélangés à de l'eau* (CXS 336R-2020) (adoptée par la 43^e session de la Commission (2020) à l'étape 5/8).

COMITÉ DU CODEX SUR LA NUTRITION ET LES ALIMENTS DIÉTÉTIQUES OU DE RÉGIME (43^e session du CCNFSDU, 2023)

Méthodes d'analyse pour les dispositions de la Norme les préparations de suite (CXS 156-1987)¹

2. À sa 43^e session, le CCNFSDU est convenu d'informer le CCMAS d'inclure une nouvelle entrée «produit pour enfants en bas âge» dans la section «préparations de suite» de la norme CXS 234.

Méthodes pour la vitamine B12; les acides aminés totaux (à l'exception de la taurine et du tryptophane), et le tryptophane dans la Norme pour les préparations destinées aux nourrissons et les préparations données à des fins médicales spéciales aux nourrissons (CXS 72-1981)²

3. À sa 43^e session, le CCNFSDU est convenu de transmettre les méthodes d'analyse pour la vitamine B12, les acides aminés totaux (à l'exception de la taurine et du tryptophane), et le tryptophane au CCMAS pour la confirmation et l'inclusion au document CXS 234. Le CCNFSDU a également convenu d'ajouter une note au document CXS 234 afin de préciser que les dispositions sont des méthodes d'essai pour les acides aminés totaux (à l'exception de la taurine et du tryptophane) et le tryptophane, notamment pour l'emploi conforme à la Section 3.1.3a, notes de bas de page 3 et 4 de la norme CXS 72.
4. Le CCMAS **est invité à confirmer** les méthodes dans l'annexe I.

COMITÉ DU CODEX SUR LES ÉPICES ET LES HERBES CULINAIRES (5^e session du CCSCH³, 6^e session du CCSCH⁴)

Méthodes d'analyse pour les dispositions dans la Norme pour les racines, les rhizomes et les bulbes séchés: gingembre séché ou déshydraté (CXS 343-2021), Norme pour les parties florales séchées: clous de girofle (CXS 344-2021), Norme pour le basilic séché (CXS 345-2021), Norme pour les parties florales séchées – safran (CXS 351-2021), Norme pour les graines séchées – noix de muscade (CXS 352-2022), Norme pour le piment et le paprika séchés ou déshydratés (CXS 353-2022), Projet de norme pour la petite cardamome séchée, projet de norme pour les épices issues de fruits et de baies séchés (partie A – piment de la Jamaïque, baies de genévrier, anis étoilé)

5. Le CCMAS **est invité à confirmer** les méthodes d'analyse dans l'annexe II.

COMITÉ FAO/OMS DE COORDINATION POUR L'AFRIQUE (24^e session du CCAFRICA)⁵

Méthodes d'analyse pour les dispositions dans la Norme pour la viande séchée (CXS 350-2022)

6. Le CCMAS **est invité à confirmer** les méthodes d'analyse dans l'annexe III.

COMITÉ FAO/OMS DE COORDINATION POUR L'ASIE (22^e session du CCASIA)⁶

Méthodes d'analyse et d'échantillonnage pour les dispositions dans le Projet de norme régionale pour les produits à base de soja fermenté sous l'action de *Bacillus* spp, Projet de norme régionale pour le riz cuit enveloppé dans des feuilles

7. Le CCMAS **est invité à confirmer** les méthodes d'analyse dans l'annexe IV.

COMITÉ FAO/OMS DE COORDINATION POUR L'AMÉRIQUE DU NORD ET LE PACIFIQUE SUD-OUEST (16^e session du CCNASWP)⁷

Méthodes d'analyse pour les dispositions dans le Projet de norme régionale pour le jus de noni fermenté

8. À sa 15^e session (2019), le CCNASWP a fait observer qu'il convenait d'appliquer les méthodes pertinentes de la norme *Méthodes d'analyse et d'échantillonnage recommandées* (CXS 234-1999). Le Comité a également convenu d'envoyer les dispositions pour les méthodes d'analyse et d'échantillonnage

¹ REP23/NFSDU43, paragraphe 51

² REP23/NFSDU43, paragraphes 122, 123 et annexe VI

³ REP21/SCH05 paragraphes 36, 65, 81, 115, 149 et annexes II, III, IV, V, VI

⁴ REP22/SCH06 paragraphes 39, 59, 80, 107, 121(i) et annexes III, IV, V, VI, VII Partie A

⁵ REP22/AFRICA24, paragraphe 40 (i) et annexe III

⁶ REP23/ASIA22, paragraphe 50 (ii) 83 (ii) et annexe V, VII

⁷ REP23/NASWP16, paragraphe 28 (iii) 73 (i) and annexe III, VII Partie A

au CCMAS pour confirmation.⁸

9. À sa 41^e session (2021), le CCMAS n'a pas confirmé:⁹

- les méthodes AOAC 983.17 / EN 12143 / IFUMA 8 / ISO 2173, estimant qu'il lui fallait évaluer plus avant la pertinence d'étendre ces méthodes au jus de noni; et il a pris note de la proposition de l'unité chargée de la facilitation de la mise en œuvre de réaliser une petite étude faisant appel à un ou plusieurs laboratoires afin de déterminer si ces méthodes sont adaptées au jus de noni;
- les méthodes d'identification de la scopolétine et de l'acide diacétyl-aspérulosidique, relevant qu'il fallait y apporter des modifications afin d'indiquer clairement la méthode de séparation permettant d'extraire la phase solide, et il est convenu de demander au CCNASWP d'éclaircir ce point. .

10. À sa 16^e session (2023), le CCNASWP a examiné la demande du CCMAS, à sa 41^e session, et a révisé les méthodes d'analyse y compris l'annexe A (identification de la scopolétine) et l'annexe B (identification de l'acide diacétyl-aspérulosidique) et est convenu¹⁰ de charger le Coordonnateur régional du CCNASWP de coopérer avec les pays membres de la région du NASWP afin de résoudre les questions restées en suspens concernant les méthodes d'analyse et d'échantillonnage (en particulier la spécification relative à la cartouche d'extraction en phase solide et la méthode HPLC permettant d'identifier la scopolétine et l'acide diacétyl-aspérulosidique) de sorte que les méthodes d'analyse et les annexes A et B puissent être transmises au CCMAS pour confirmation à sa 42^e session.

11. De plus amples informations sur la mise à jour des méthodes d'analyse pour l'identification de la scopolétine et l'acide diacétyl-aspérulosidique se trouvent dans le document de séance CRD05 présenté par le coordonnateur régional du CCNASWP en collaboration avec les membres de la région du NASWP qui est disponible sur la page web de la quarante-deuxième session du CCMAS¹¹.

12. Le CCMAS **est invité à confirmer** les méthodes d'analyse dans l'annexe V comme suit:

- i. Confirmer la méthode par extraction en phase solide- chromatographie sur couche mince (CCM) se trouvant à l'annexe A (mise à jour) en tant que type IV pour l'identification de la scopolétine dans le jus de noni fermenté.
- ii. Confirmer la méthode par chromatographie sur couche mince (CCM) se trouvant à l'annexe B (mise à jour) en tant que type IV pour l'identification de l'acide diacétyl-aspérulosidique dans le jus de noni fermenté.

Dans les cas où dans le Manuel de procédure est indiqué une classification de type IV qui reflète une méthode récemment introduite mais pour laquelle les critères nécessaires pour son acceptation par le CCMAS n'ont pas encore été déterminés.

Note 1: la modification apportée à l'annexe A (comparé au document CX/MAS 21/41/3, annexe II) ont mises en évidence en rouge et comprennent des instructions additionnelles pour le type de cartouche par extraction en phase solide et les étapes de transformation de ladite extraction. D'autres améliorations suggérées sont l'utilisation d'un standard de 0,10 mg/ml dans la scopolétine CCM de scopolétine au lieu de 1,0 mg/ml, étant donné que cette dernière concentration est excessivement supérieure à l'échantillon typique de jus de noni fermenté.

Note 2: les modifications apportées à l'annexe B (comparé au document CX/MAS 21/41/3, annexe II) sont mises en évidence en rouge et comprennent l'élimination des instructions d'extraction en phase solide et une simple dilution des échantillons de jus de noni fermenté avec du méthanol. De plus, l'addition d'instructions pour la préparation de la solution de para-anisaldéhyde pour l'identification de l'acide diacétyl-aspérulosidique (Annexe B). Il est à noter que la préparation de la solution de para-anisaldéhyde utilise moins d'acide sulfurique (4%) par rapport au pourcentage spécifié à l'origine par West et Deng (à savoir 10%); nous considérons que l'excès d'acide a causé un problème de réchauffement et sécurité pour la préparation et l'utilisation. La solution de para-anisaldéhyde ainsi obtenue n'a subi aucun défaut dans sa capacité à visualiser les analytes souhaités.

- iii. La vérification/validation par un seul laboratoire de l'identification de la scopolétine par la méthode par extraction en phase solide- chromatographie sur couche mince (CCM) et de l'acide diacétyl-aspérulosidique par la CCM dans le jus de noni fermenté dans l'annexe C.
- iv. Les méthodologies CCM recommandées ci-dessus pour faciliter la progression du projet de norme

⁸ REP20/NASWP15, paragraphes 80, 83(ii) et annexe II

⁹ REP21/MAS41, paragraphes 13-14.

¹⁰ REP23/NASWP16, paragraphe 73(ii) et annexe VII, Partie B

¹¹ <https://www.fao.org/fao-who-codexalimentarius/meetings/detail/fr/?meeting=CCMAS&session=42>

régionale pour le jus de noni fermenté à l'étape 8, en indiquant que la méthode HPLC-DAD sera disponible à l'avenir.

Révision de la procédure opérationnelle standard pour l'identification des kavalactones et des flavokavaïnes dans les produits du kava frais et séchés, par chromatographie sur couche mince à haute performance dans la Norme régionale pour les produits du kava à usage de boisson lorsqu'ils sont mélangés à de l'eau (CXS 336R-2020)

13. À sa 15^e session (2019), le CCNASWP est convenu¹² d'envoyer les dispositions pour les méthodes d'analyse et d'échantillonnage au CCMAS pour confirmation.
14. À sa 41^e session (2021), le CCMAS a noté que l'examen des références n'avait pas permis de dégager de procédure claire pour déterminer les teneurs en kavalactones ou flavokavaïnes, qu'il semblait y avoir différentes sections à suivre dans chaque référence et que la référence de 2016 n'était pas forcément nécessaire pour les flavokavines.
15. À sa 41^e session, le CCMAS est convenu de demander au CCNASWP d'envisager d'élaborer une seule méthode par étape ou une procédure opérationnelle standard qui englobe les étapes essentielles pour chaque disposition dans un document unique facile à suivre.¹³
16. À sa 16^e session (2023), le CCNASWP a examiné la demande du CCMAS, à sa 41^e session, et a révisé la procédure opérationnelle standard en conséquence. Le Comité est convenu de transmettre au CCMAS, pour confirmation, la révision de la procédure opérationnelle standard pour l'identification des kavalactones et des flavokavaïnes dans les produits du kava frais et séchés au moyen de la méthode HPTLC¹⁴.
17. Le CCMAS **est invité à confirmer** la procédure opérationnelle standard révisée à l'annexe V.

¹² REP20/NASWP15, paragraphe 96(iii)

¹³ REP21/MAS41, paragraphes 15-17

¹⁴ REP23/NASWP16, paragraphe 28(iii), annexe III

ANNEXES
ORIGINAL LANGUAGE ONLY

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	<u>Vitamin B12</u>	<u>AOAC 2014.02</u>	<u>LC-UV</u>	<u>III</u>
	<u>Total amino acids (excluding taurine and tryptophan)</u> <u>For use according to Section 3.1.3 (a) footnotes 3 and 4 of CXS 72-1981</u>	<u>AOAC 2018.06 / ISO 4214 IDF 254 /AACC 07-50.01</u>	<u>UHPLC- UV</u>	<u>II</u>
	<u>Tryptophan</u> <u>For use according to Section 3.1.3 (a) footnotes 3 and 4 of CXS 72-1981</u>	<u>AOAC 2017.03</u>	<u>HPLC</u>	<u>II</u>

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) <u>MPM: V-8. Spices</u>	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) <u>MPM: V-8. Spices (For whole)</u>	Visual Examination followed by Gravimetry	IV
Mould visible	Method V-8 Spices, Condiments, Flavours and Crude Drugs (Macroanalytical Procedure Manual) <u>MPM: V-8. Spices</u>	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 <u>Method V-8 Spices, Condiments, Flavors and Crude Drugs</u>	Visual Examination Visual Examination	IV IV
Insects/Excreta/Insect fragments	ISO 927	Visual Examination	IV
Crude fibre	ISO 5498	Gravimetry	I
Mould visible	<u>Method V-8 Spices, Condiments, Flavours and Crude Drugs</u>	Visual Examination	IV
Live insect	ISO 927	Visual Examination	IV
Mammalian or/and Other excreta	<u>Method V-8 Spices, Condiments, Flavours and Crude Drugs</u>	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	<u>Method V-8 Spices, Condiments, Flavours and Crude Drugs</u> (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	<u>Method V-8 Spices, Condiments, Flavours and Crude Drugs</u> (Macroanalytical Procedure Manual, FDA Technical Bulletin Number 5)	Visual examination (for whole)	IV
Mammalian Excreta, And Other Excreta	<u>Method V-8 Spices, Condiments, Flavours and Crude Drugs</u> (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/LaboratoryMethods/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) USDA 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)	Microscopic examination	I
	AOAC 977.25 B (Ground paprika)	Microscopic examination	I

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

²According 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.”

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 Analysis¹⁵**

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 ($\geq 1.0\%$)	ISO 1841-1	Volhard method	III
Chloride as Sodium Chloride ($\geq 0.25\%$)	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.”

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 SAMPLING¹⁶**

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.

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 SAMPLING¹⁷**

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

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.

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 R_f 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 R_t 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.

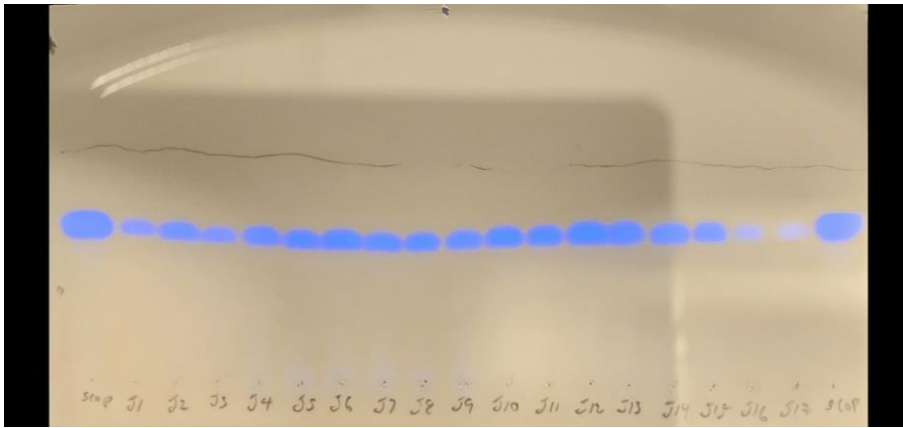


Figure 2. Scopoletin standard plus SPE extraction & TLC with UV@365nm visualization of fermented Noni juice samples, with left to right, standard; fermented Noni juice samples J1-17; standard.

Standard and Pacific Island samples J18-J19.

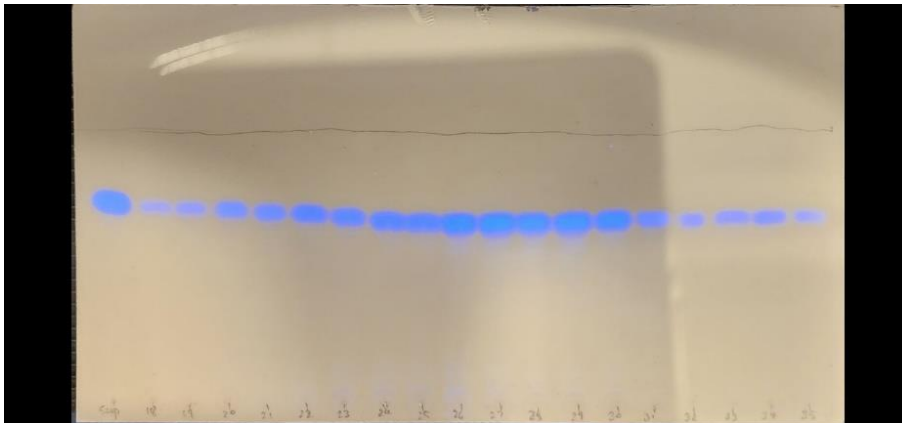


Figure 3. Scopoletin standard plus SPE extraction & TLC with UV@365nm visualization of fermented Noni juice samples, with left to right, standard; fermented Noni juice samples 18-35.

- 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.

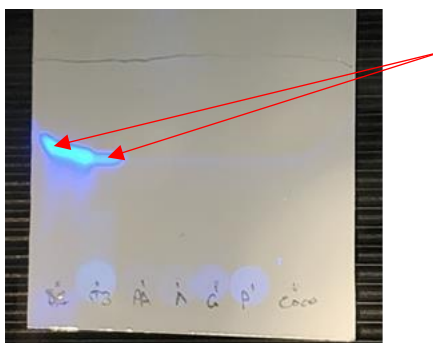


Figure 4. Scopoletin ID TLC for a Noni juice plus various other juices; from left to right, Scopoletin (0.1mg/mL), Noni Juice#3(J3), Pineapple juice (PA), Apple and Blackcurrant juice(A), Grape juice(G), Pear juice(P), and Coconut juice (Coco). Scopoletin band for standard and Noni Juice#3 indicated 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.

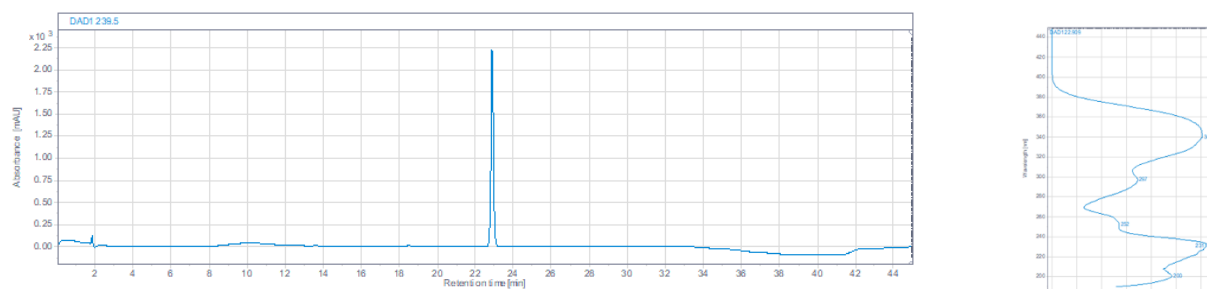


Figure 5. Scopoletin standard, HPLC-DAD chromatogram, 10 μ L injection, @ 239.5nm and peak UV spectra.

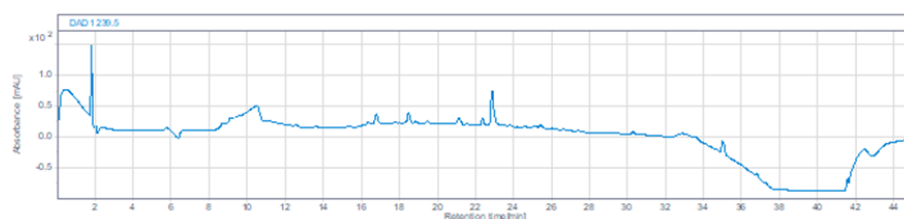


Figure 6. Juice#3, filtered, HLB-SPE 100% MeOH elution solution and injected 10 μ L on HPLC-DAD @ 239.5nm

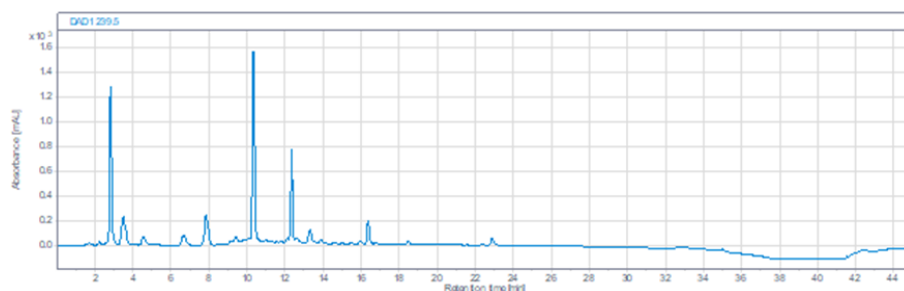


Figure 7. Juice#3, filtered 0.45 μ m, no SPE, and direct injected 10 μ L on HPLC-DAD @ 239.5nm.

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.

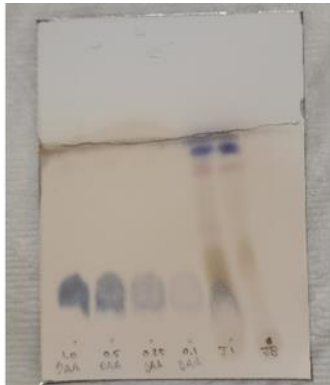


Figure 8. TLC standard solutions spots 1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL; Juice 1; Juice 8.

- 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.

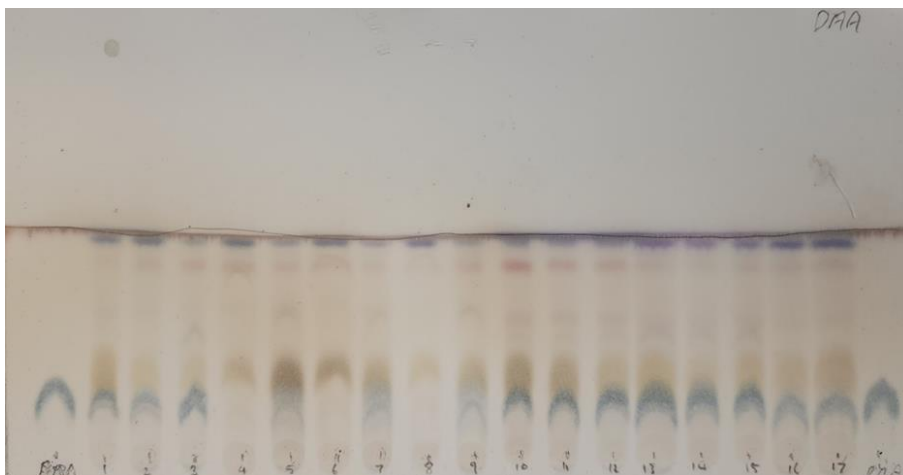


Figure 9. From left to right, DAA standard; fermented Noni juice samples 1-17; DAA standard; with TLC visualised with 2% anisaldehyde / 4% sulfuric acid-ethanol (EtOH) solution then heating.

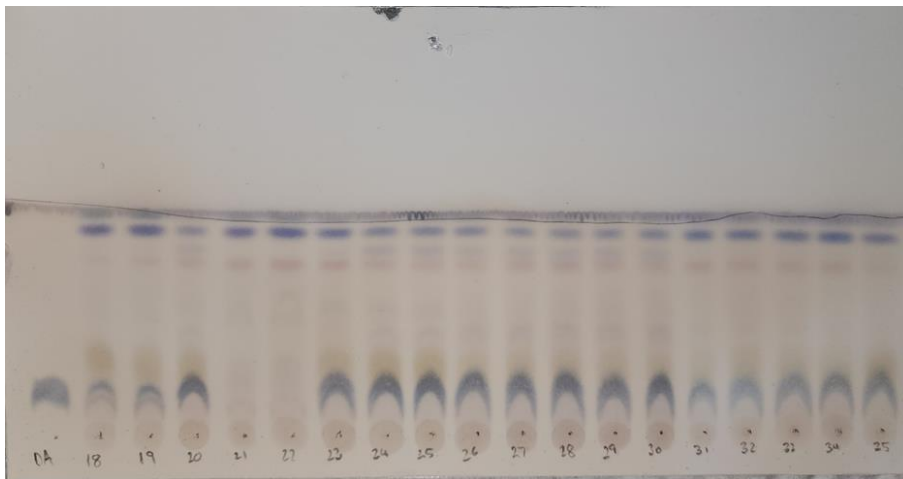


Figure 10. From left to right, DAA standard, fermented Noni juice samples 18-35; with TLC visualisation with 2% anisaldehyde / 4% sulfuric acid-ethanol (EtOH) solution then heating.

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.

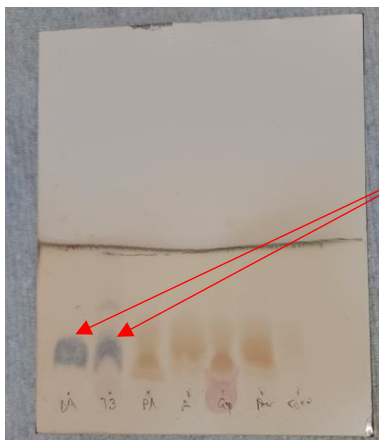


Figure 11. Deacetylasperulosidic acid ID TLC for a Noni juice plus various other fruit juices; from left to right, Deacetylasperulosidic acid (0.5mg/mL DA), Noni Juice#3(J3), Pineapple juice (PA), Apple and Blackcurrant juice(A), Grape juice (Gp), Pear juice (Pear), and coconut juice (Co). Deacetylasperulosidic 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.

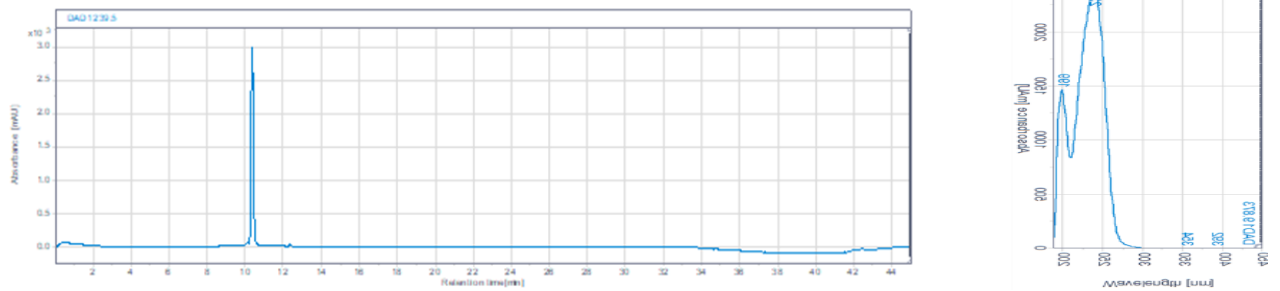


Figure 12. Deacetylasperulosidic acid 2 mg/mL; HPLC-DAD chromatogram, 10µL injection, @ 239.5nm; and peak UV spectra.

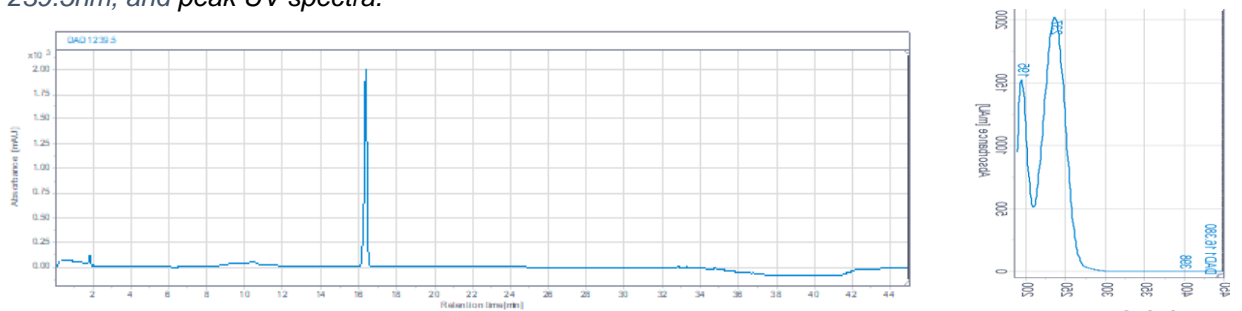


Figure 13. Asperulosidic acid; HPLC-DAD chromatogram, 10µL injection, @ 239.5nm; and peak UV spectra.

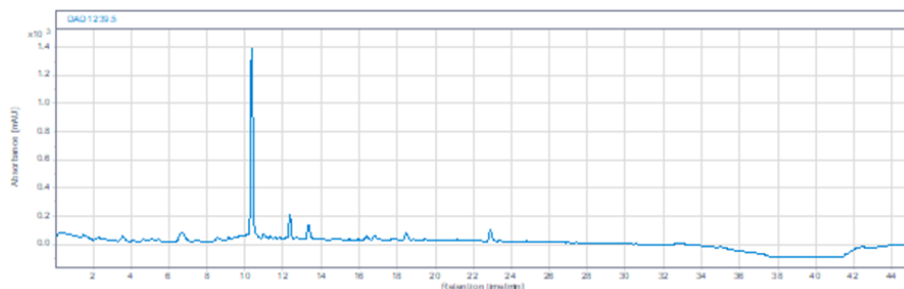


Figure 14. Juice 1, 0.45µm filtered & no SPE, direct injected 10µL on HPLC-DAD @ 239.5nm
See section f) for HPLC-DAD conditions.

- 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.

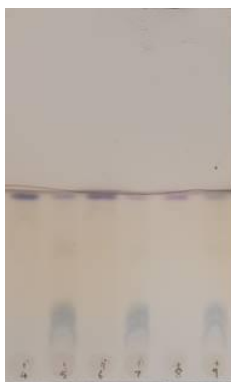


Figure 15 Cropped section of Fig 10, highlighting Deacetylasperulosidic acid ID by TLC for a selection of various Pacific Island Noni Juice samples where ‘negative’ and ‘positive’ DAA IDs were observed.

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 $R_t=22.8$ mins, thus positive ID for Scopoletin. Note the 10x reduction in absorbance scale for the negative results for DAA.

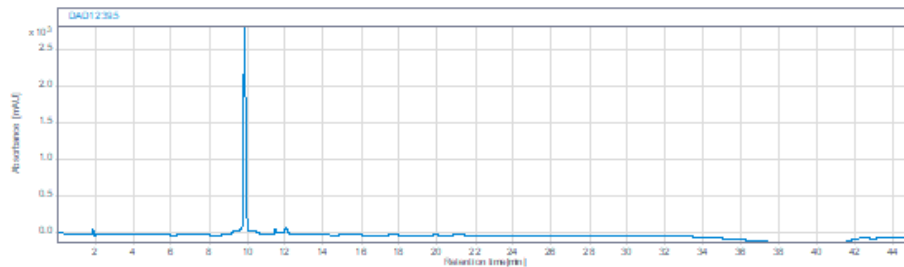


Figure 16. HPLC of DAA standard 2mg/mL with peak at 9.9mins.

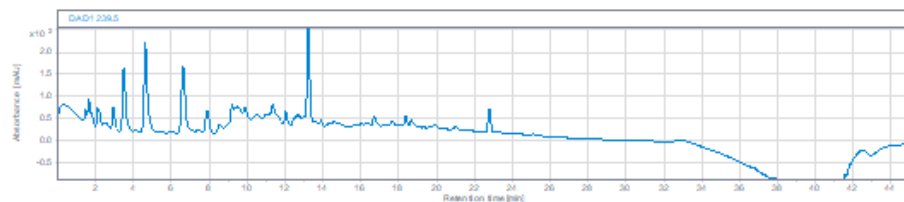


Figure 17. HPLC injection of Pacific Island juice#4, confirming 'negative' result for DAA.

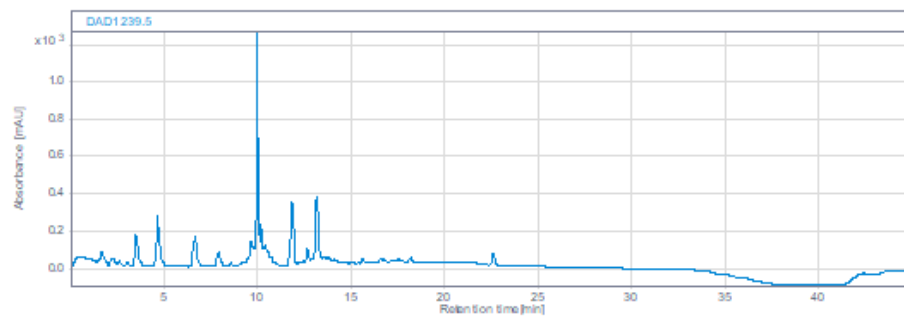


Figure 18. HPLC injection of Pacific Island juice#5, confirming 'positive' result for DAA.

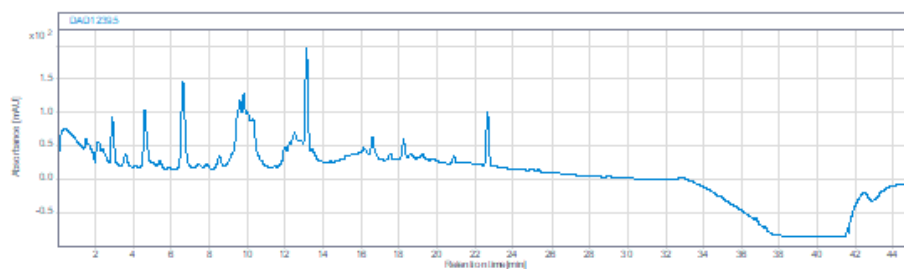


Figure 19. HPLC injection of Pacific Island juice#6, confirming 'negative' result for DAA.

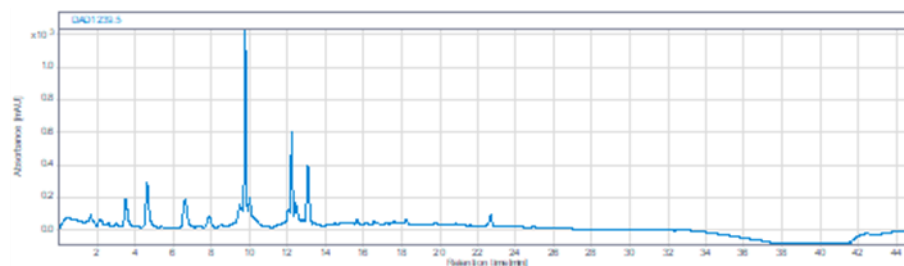


Figure 20. HPLC injection of Pacific Island juice#7, confirming 'positive' result for DAA.

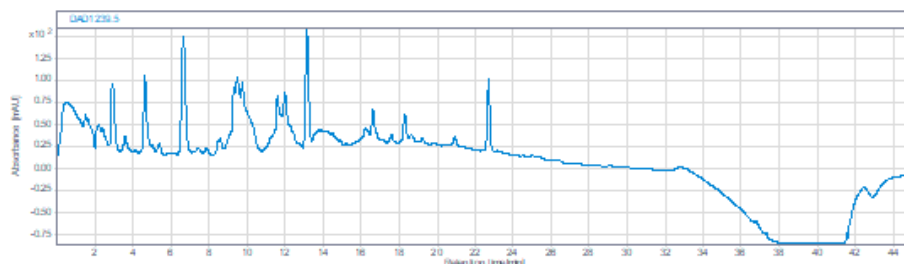


Figure 21. HPLC injection of Pacific Island juice#8, confirming 'negative' result for DAA.

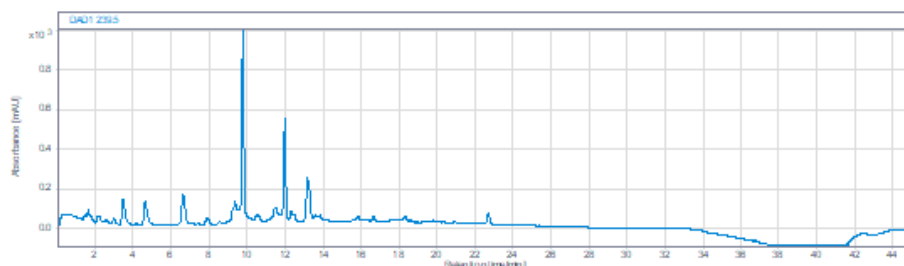


Figure 22. HPLC injection of Pacific Island juice#9, confirming 'positive' result for DAA.

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 °C

Column: Agilent, Zorbax Eclipse Plus C18. 5µm, 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 Kavalactones 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:

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

Three flavokavain:

- o flavokavain A (FKA),
 - o flavokavain B (FKB) and
 - o 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 at 4500 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 phase to 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 x 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). Kavalactone and Flavokavins Profile Contribute to Quality Assessment of Kava (*Piper methysticum* G. Forst), the Traditional Beverage of the Pacific. *Beverages*. 2019, 1-14.