

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture
Organization of the
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World Health
Organization

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Agenda Item 3

NASWP17/CRD04

ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

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

17th Session

Nadi, Fiji

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(Prepared by Fiji in consultation with the Codex Secretariat)

Standard operating procedure (SOP) for the identification of kavalactones and flavokavains in fresh and dried kava products by high-performance thin layer chromatography (HPTLC) in the *Regional Standard for kava products for use as a beverage when mixed with water* (CXS 336R-2020)

1. The methods of analysis for provisions in the *Regional Standard for kava products for use as a beverage when mixed with water* (CXS 336R-2020) was presented at the 41st session of the Codex Committee on Methods of Analysis and Sampling (CCMAS41) in 2021, which requested CCNASWP to consider producing a single stepwise method or standard operating procedure (SOP) which would capture the necessary steps for each provision in one easy to follow document.
2. A draft SOP for the determination of kavalactones and flavokavains in fresh and dried kava products in CXS 336R-2020 was developed by Fiji, in consultation with Vanuatu. CCNASWP16 (2023) considered the draft SOP and agreed to forward it with revisions to CCMAS42 (2023) for endorsement.¹ The revised SOP focussed only on the high-performance thin layer chromatography (HPTLC) method as a method for identification of kavalactones and flavokavains, and clearly identified the different steps in the procedure.
3. CCMAS42 did not endorse the revised SOP due to the lack of description of how the final determination is to be made, and requested CCNASWP to provide further edits to address the lack of instruction on the final determination steps and assessment against a specification.
4. Fiji, in addressing the request of CCMAS42, proposes that the SOP be revised such that a qualitative assessment for kava nobility is conducted based on the UV absorbance reading of kava acetonetic extract, scanned at 440nm using a simple colorimeter (see Annex of this document).
5. The objective of the revised SOP is to provide more instruction on the final determination steps.
6. CCNASWP17 is invited to review the the revised SOP as contained in the Annex and consider forwarding it to CCMAS44 for endorsement.

¹ REP23/NASWP Appendix III

QUALITATIVE ASSESSMENT FOR KAVA NOBILITY BASED ON KAVA ACETONIC EXTRACT UV ABSORBANCE READING SCANNED AT 440NM USING A SIMPLE COLORIMETER

1 **REFERENCES:**

Main Reference:

Lebot V., and Legendre L. (2016) Comparison of kava (*Piper methysticum* Forst.) varieties by UV absorbance of acetonc extracts and high-performance thin-layer chromatography. Journal of Food Composition and Analysis, 48 (2016) : 25 -33

2 **SCOPE:**

The procedure is most accurate for dried kava samples but can also be used for fresh kava products provided moisture is removed prior to analysis.

3 **DEFINITIONS:**

Kava Nobility refers to the category of kava cultivars belonging to a specific genetic fingerprint having the desired kavalactones compounds and favorable kava chemotype lineup. Specifically, the UV absorbance value of Noble kava is found to be less than 0.90 Abs displaying a yellow color in their acetone extract. Non-noble kava is recorded to be equal to or more than 0.90 Abs reading, and color ranges from brick orange to dark red for their acetone extract.

4 **PRINCIPLE:**

1. A colorimeter is a light-sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. The device measures the intensity or concentration of the color that develops upon introducing a specific reagent into a solution.
2. The three main components of a colorimeter are a light source, a cuvette containing the sample solution and a photocell for detecting the light passed through the solution.
3. The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.
4. As pure kavalactones standards diluted in acetone give a translucent extract (for kavain) or light-yellow extract (for yangonin and demethoxyyangonin), darker colors are suspicious and can be due to non-kavalactones (pigments such as flavokavains for ex.) or other unsuitable compounds (alkaloids for ex.).
5. Dried kava is weighed and made into a solution with acetone, following a 1/3 dilution factor (10g for 30mL) followed by centrifugation. Following correct colorimetry procedure, the sample solution absorbance value is read and recorded. Reading below 0.90 Abs indicates the sample is of Noble character, any reading equal to and above 0.90 is classified Non-Noble.

5 **SAFETY NOTES:**

This procedure uses the acetone analytical reagent solvent. Analysts must follow the standard laboratory practice of using protective clothing, shoes, eye protection (safety glasses), and rubber hand gloves when carrying out the work. Appropriate material safety data sheets are also available in the MSDS cabinet and should be referred to prior to commencing analysis.

6 **SAMPLE STORAGE AND PREPARATION:**

Sample Preparation and Homogenization:

1. Obtain raw kava: Start with fresh or dried kava roots or rhizomes. Ensure that the kava is of good quality and free from any contaminants.
2. Cleaning and washing: Thoroughly clean the kava roots to remove any dirt or debris. You can use water to wash the roots and gently scrub them if needed. This step helps to ensure the purity of the final powder.
3. Drying: After cleaning, dry the kava roots. This can be done by spreading them out in a well-ventilated area or using an electric dryer cabinet with controlled temperatures set at 60°C drying overnight. The drying process helps to remove moisture from the roots and facilitates easier grinding.
4. Grinding: Once the kava roots are completely dry (ideally <12% Moisture), grind them into a fine powder. You can use a mortar and pestle, a blender, or a specialized grinder for this purpose. Ensure that the grinding equipment is clean and free from any contaminants.

5. Sieving: After grinding, pass the kava powder through a fine sieve or mesh to remove any larger particles or fibers. This step helps to achieve a more uniform and consistent powder with particle size <2mm.
6. Packaging and storage: Transfer the powdered kava into a clean, airtight container for storage. Label the container with relevant information such as the date of preparation and sample laboratory ID batch number

Sample storage

Prior to analysis, dried kava powder sample(s) should be properly sealed in zip log plastic bag(s) or equivalent and stored in a desiccator with lid having silica gels to remove any moisture, away from sunlight maintaining its quality

7 PRECISION AND ACCURACY

Precision

Use select kava in-house reference materials for checking the response of meter, in-house validated absorbance reading should be developed having an acceptable range of UV absorbance reading for the selected noble kava material (preferably year 3 kava roots of known origin) should be available for every batch of sample analysis.

Accuracy

Inter-laboratory exercise using the same model colorimeter instrument or equivalent to be employed for selected Pacific laboratories participating in analyzing the same blind kava sample of known origin. The exercise is to be coordinated annually and results documented and assessed by RC (Regional Chairperson CCNASWP). It should be ensured that results from participating labs falls within acceptable proficiency z-score made available by RC. The data will monitor the performance of the colorimeter over time and reliability of producing accurate data.

8 QUALITY CONTROL

All samples are to be done in duplicates. The meaning of the duplicate UV absorbance readings should be reported. The two absorbance values should not deviate $\pm 10\%$ from the mean value.

9 APPARATUS

- Colorimeter, biochrome Model CO700 or equivalence
- Food grade blender
- Food grade mortar and pistol
- Analytical balance or equivalence ($\pm 0.0001\text{g}$ weight)
- 50ml Centrifuge tubes with screw cap
- < 2mm Stainless steel sieve
- 10ml glass dispenser bottle (1L capacity)
- 10ml glass cuvettes or equivalent
- Centrifuge instrument with maximum rpm of 6000.
- Spatula
- 10ml Glass vial with screw caps
- Disposable Pasteur pipette
- Soft tissue
- Glass pipette
- Rubber tit for glass pipette
- Vortex

10 REAGENTS

- Acetone (analytical grade)

11 PROCEDURE

1. Place an empty 50ml centrifuge tube without lid on the analytical balance. Zero analytical balance.
2. Weigh 10 grams of kava powder with <2mm particle size into a 50ml centrifuge tube. Record weight of kava sample to four decimal places.
3. Remove centrifuge tube with sample and place on laboratory bench top for addition of 30ml analytical grade acetone.
4. Close the centrifuge cap and shake vigorously to extract the kava sample. A vortex can be used to aid in the process. Let rest overnight.

5. Collect with a glass pipette the supernatant of each sample acetonic extract (approximately 5ml) and transfer quantitatively to a transparent glass tube (10mm in diameter). Do not use plastic tubes.
6. Select the "440" wavelength of the colorimeter. If not, use the wavelength dial on the side of the Colorimeter to adjust to the required wavelength.
7. A Reference sample (acetone used as blank) should be measured between each single wavelength measurement. Press on "ON".
8. Wait for the message "0.00 Abs" to appear.
9. Place the reference sample into the cuvette holder and Press "T", the message "Ref Abs" appears. Press "R", the message "0.00 Abs" appears.
10. Remove the reference, place the kava sample and press "T".
11. Record the absorption reading (example 1.82 Abs), remove kava sample and replace with reference sample and press "R", should read as "0.00 Abs".
12. Remove reference sample and replace it with duplicate of kava sample, press "T", read and record the absorption (example 1.80 Abs) reading.
13. Continue through step 12.7 and 12.10 respectively until all kava samples with their duplicates are read.
14. Once analysis is complete, proceed to switch off colorimeter by pressing "OFF" on the right side of the colorimeter. Carefully discard sample extracts and any other waste in designated waste bottle for later disposable according to country waste disposable policy.
15. Turn off the main power of colorimeter and store back instrument into appropriate storage boxes. Always keep the instrument in a controlled laboratory environment with specific temperature and humidity conditions.
16. Identification of kava nobility using a biochrome CO7000 Colorimeter or equivalent.



- 1 - Absorbance reading screen
- 2 - Wavelength adjustable knob
- 3 - ON/OFF button
- 4 - Test button "T" is used for measuring the amount of light that passes through the sample extract.
- 5 - Reference button "R" used for calibrating the instrument.
- 6 - Cuvette holder

Figure 1.0: biochrom CO700 Colorimeter.

12 REPORTING OF RESULTS

Colorimeter

1. The colorimeter gives absorbance reading (Abs) which is displayed on the colorimeter LCD screen.
2. Report absorbance results to two decimal places on the developed worksheet for result reporting.