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Quantification of Kavalactones and Determination of Kava (*Piper methysticum*) Chemotypes Using Near-Infrared Reflectance Spectroscopy for Quality Control in Vanuatu

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Kava (*Piper methysticum* Forst f., Piperaceae) has anxiolytic properties and the ability to promote a state of relaxation without the loss of mental alertness. The rapid growth of the nutraceutical market between 1998 and 2000 has been stopped by a ban in Europe and Australia because of some suspicion of liver toxicity. It is now important to develop a fast, cheap, and reliable quality test to control kava exports. The aim of this study is to develop a calibration of the near-infrared reflectance spectroscopy (NIRS) using partial least-squares (PLS) regression. Two hundred thirty-six samples of kava roots, stumps, and basal stems were collected from the Vanuatu Agricultural Research and Technical Centre germplasm collection and from four villages. These samples, representing 45 different varieties, were analyzed using NIRS to record their absorption spectra between 400 and 2500 nm. A set of 101 selected samples was analyzed for their kavalactone content using HPLC. The results were used for PLS calibration of the NIRS. The NIRS prediction of the kavalactone content and the dry matter were in agreement with the HPLC results. There were good correlations between these two series of results, and coefficients (R^2) were all close to 1. The measurements were reproducible and had repeatability on par with the HPLC method. The NIRS system has been calibrated for the six major kavalactone content measurements, and it is suggested that this method could be used for quality control in Vanuatu.

KEYWORDS: Chemotypes; kavalactones; kava; *Piper methysticum*; near-infrared reflectance spectroscopy; quality control

INTRODUCTION

Kava (*Piper methysticum* Forst. f., Piperaceae) is a species having a geographic distribution that is exclusively limited to the Pacific, and it is the only cultivated species of economic importance to be so distributed. In Vanuatu, the major producer and exporter, the beverage is prepared by cold water extraction of the freshly harvested roots, but in Fiji, Samoa, Tonga, and Wallis-Futuna it is prepared from the sun-dried roots. Historic use shows that kava is safe and effective under the strict control of the rituals of Pacific cultures. The traditional beverage is presently consumed on a daily basis without apparent side effects. Overall, about 15000 ha are now in production in the Pacific.

The 1990s saw an explosion of interest in kavalactones, the active ingredients, by European pharmaceutical and herbal industries. Kavalactones, which are lipid-like compounds extracted using organic solvents, have long been recognized as a natural, nonaddictive, alternative to benzodiazepines (*1–3*). Pacific Island countries have benefited from the recent Western interest in phytomedicines, with kava exports commodity reaching 70 million euros in the late 1990s (*4, 5*). However, in 1998−2000, the consumption of kava-based products in Europe was implicated in several liver failure cases (*6–9*). In 2001, the German health authority (BfArM) declared that there were grounds to believe that kava preparations sold in Europe could cause hepatotoxic reactions (*10*). Although BfArM recognized that it is not known whether kavalactones or other components, which are also contained in the extracts, are responsible for the adverse effects, it decided in 2002 to withdraw authorization for all pharmaceutical preparations containing *P. methysticum* extracts. France and other European countries have also banned the marketing and sale of kava-based products. This has caused severe damage to the economies of the small Pacific nations. However, a comprehensive review prepared on behalf of one of the German companies producing kava pills was unable to link kava to the cases (*11*). More recently, a comprehensive

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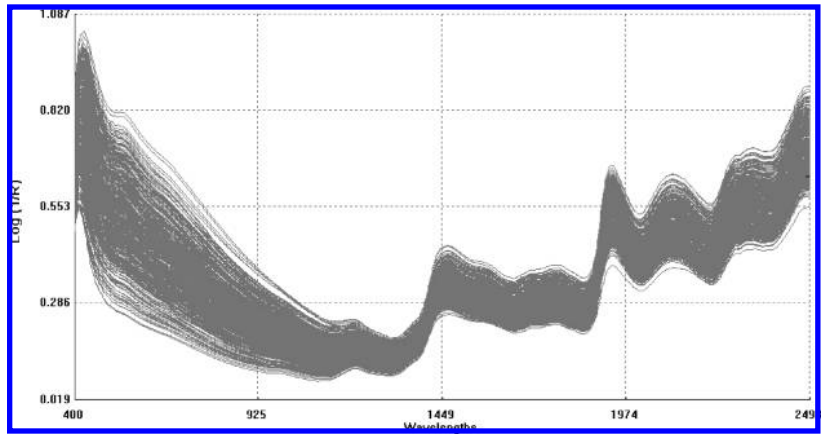

Figure 1. NIRS spectra (472) corresponding to 236 samples.

WHO report has shown that kavalactones could not be implicated in such cases (*12*).

In the Pacific, the dry material intended for exportation can include very different products (cultivars and/or organs). In Vanuatu, a law (The Kava Act) was enacted by Parliament in December 2002 to regulate the cultivation and use of noble varieties and to declare illegal the sale of others. It is, however, impossible to recognize the varieties and therefore their chemical signature, their chemotype, which is an important quality factor, when the kava is chopped into pieces and dried.

The selection of the cultivar, its organ, and the geographical area of its cultivation are factors contributing to quality control (*13*). For a given cultivar, chemotype is stable across locations, but there are differences between organs. The total kavalactone concentration is highest in the roots and stumps and progressively decreases toward the aerial portions of the plant. Differences in chemotype and kavalactone content between roots, stumps, and basal stems are maintained while the plant matures (*14, 15*).

However, quality standards based on chemotype are impossible to enforce without an efficient analytical method for the routine determination of chemotype of numerous samples. Numerous adulterations or frauds are possible, and there are currently no means of controlling the chemotype of exported products at the point of origin. At the moment, analysis of the chemical composition of the exported lots can be made, at great expense, only in Fiji, the United States, Australia, or Europe, where laboratories are equipped with HPLC. It is therefore urgent to set up an efficient system of quality control before kava is again accused of properties due to malpractices.

Two studies (*16, 17*) showed that it is possible to determine the composition in kavalactones of the powder of dried kava using near-infrared reflectance spectroscopy (NIRS). These results constitute a hope for kava quality control and particularly for the identification of the cultivar used. However, both were conducted with samples presenting relatively low variation because the first one dealt with extracts and the second with Hawaiian material, which presents limited chemical variation (*18*). Vanuatu is the area of origin of *P. methysticum*, and tremendous chemical variation exists due to the diversity of cultivars. The objective of the present study is to calibrate NIRS for the routine analysis of dry kava intended for export from Vanuatu and to test its reliability to determine chemotypes.

MATERIALS AND METHODS

Sample Preparation. Overall, 236 samples of kava were collected and prepared. Two hundred and fifteen samples of different organs (roots = 72, stumps = 71, basal stems = 72) were taken from 45 different kava cultivars grown in the germplasm collection of the Vanuatu Agricultural and Research and Technical Center (VARTC) on Santo Island. These cultivars were planted together in a common plot and were all mature plants over 3 years of age. Another 21 samples (roots = 7, stumps = 7, basal stems = 7) were taken from plants collected from kava producers in four villages of Santo (Butmas, Fanafo, Narango, and Sara). Roots, stumps, and basal stems were separated, washed, and cut into small cubic pieces of approximately 2 × 2 cm. These samples (n = 236) were then dried at 60−80 °C until constant weight. Dry matter was ground in Vanuatu using a Forplex F00 1218 (Boulogne, France) hammer mill into a kava powder with <2 mm particle size. Powder samples were then packaged and sent to CIRAD, Montpellier, France, via airplane. In Montpellier the samples were stored at room temperature before use. Samples were ground again using a Retsch ZM200 mill (Haan, Germany) with a 0.5 mm screen and then split into two subsamples; one subsample was used for kavalactone extraction. The other subsample was used for NIRS measurements. Approximately 5 g of kava powder was weighed and dried for 24 h in an oven at 105 °C. Percent moisture value was calculated on a dry weight basis. The water content of the samples varied from 7 to 11%.

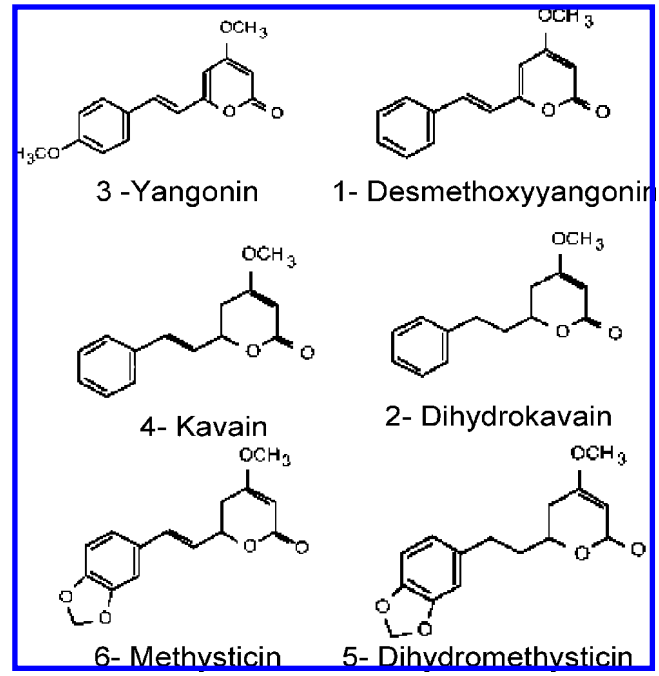

Figure 2. Structures of six major kavalactones.

Near-Infrared Spectrometry Data Pretreatment and Measurements. A NIRS instrument (Foss-Perstorp 6500) using a NIRS 2 version 4.11 software (InfraSoft International, Port Matilda, PA) was used to scan 22 mm ring cups (50 mm) in a linear transport attachment. These cups were filled with 3−5 g of kava powder and measured in diffuse

Table 1. HPLC Analysis Results of 52 Root Samples[a]

accession no.	cultivar	DMY (1)	DHK (2)	Y (3)	K (4)	DHM (5)	M (6)	KL%	chemotype
9	Puariki	0.71	1.49	0.99	2.70	0.63	0.43	6.96	423156
15	Kelai	0.91	2.21	1.57	4.05	0.71	0.64	10.08	423156
8	Gimonlagakris	0.87	2.97	2.42	4.72	1.05	0.88	12.9	423561
12	Pade	0.77	1.98	2.05	3.38	1.08	0.8	10.06	423561
17	Nikawa Pia	0.80	2.46	1.78	3.75	1.04	0.93	10.76	423561
213	Tafanda	0.99	3.06	2.67	4.71	1.49	1.04	13.96	423561
218	Malog Velablalas	1.16	3.44	2.65	5.41	1.61	1.24	15.52	423561
B1N11	Borogu	0.74	2.33	2.06	3.26	0.95	0.71	10.05	423516
1	Borogu	0.84	2.16	1.94	3.53	0.96	0.86	10.30	423561
5L8	Borogu	0.70	2.29	1.88	3.21	0.96	0.79	9.82	423561
206	Borogu	0.51	2.05	1.74	2.74	0.95	0.69	8.68	423561
503	Borogu	0.67	1.94	1.69	2.88	0.82	0.71	8.72	423561
B6N7	Borogu	0.61	1.44	1.34	2.42	0.74	0.69	7.24	423561
Narango	Borogu	0.78	2.17	1.91	3.73	1.19	1.13	10.9	423561
Sara	Borogu	0.38	1.12	0.74	1.92	0.66	0.63	5.44	423561
1yr	Borogu	0.47	1.48	0.88	2.35	0.72	0.67	6.56	423561
2yrs	Borogu	0.46	1.17	0.84	2.38	0.48	0.67	6.00	423651
3yrs	Borogu	0.43	1.24	0.80	2.01	0.69	0.66	5.85	423561
Fanafo	Borogu	0.45	1.47	0.70	2.23	0.57	0.6	6.02	423651
212	Silese	0.64	1.50	1.43	2.69	0.75	0.75	7.76	423651
25	na	0.78	2.84	1.76	2.93	1.83	1.82	11.96	425361
19	Ahouia	0.78	1.83	1.84	3.27	0.86	0.73	9.32	432516
13	Bongania	0.94	5.31	1.28	3.86	0.72	0.7	12.83	243156
5	Marino	0.63	2.46	1.49	1.93	1.71	0.96	9.17	245361
11	Rongrongwul	0.84	4.04	1.62	2.65	2.44	1.32	12.92	245361
104	Fabularalara	0.89	3.41	2.32	3.06	2.48	1.97	14.13	245361
106	Malogu bwagongo	0.73	3.66	1.96	2.68	2.24	1.49	12.75	245361
117	Tarivoravora	0.71	2.70	1.66	2.46	1.78	1.42	10.73	245361
139	Long han	0.56	1.73	1.02	1.70	1.07	0.96	7.03	245361
158	Abogae	0.61	2.63	1.35	1.89	1.77	1.15	9.40	245361
170	Gelav	0.80	3.03	1.98	2.7	2.07	1.46	12.04	245361
207	Vambu	0.77	2.97	1.94	2.64	2.00	1.41	11.74	245361
130	Malavoke	0.65	2.57	1.49	2.12	2.13	1.22	10.17	245361
103	Fabukhai	0.87	3.11	1.93	2.70	2.53	1.8	12.94	245361
P, D	Palisi	0.59	2.23	1.41	1.83	1.77	1.39	9.22	245361
P, A	Palisi	0.57	1.95	1.43	1.74	1.71	1.39	8.80	245361
P, C	Palisi	0.41	2.24	1.29	1.69	1.52	1.30	8.48	245631
Narango	Palisi	0.48	2.12	1.31	1.62	1.74	1.45	8.71	254631
Sara	Palisi	0.57	2.06	1.10	1.91	1.93	1.46	9.04	254631
10	Sentender	0.42	2.73	1.45	2.01	1.65	1.33	9.60	245361
124	Tarivarus	0.91	5.21	1.86	2.60	3.09	1.31	14.98	254361
156	Isa	0.54	3.76	1.79	1.96	3.41	1.58	13.04	254361
214	Ranapapa	0.72	3.97	1.52	2.17	2.63	1.28	12.29	254361
215	Malogrock	0.49	3.51	1.84	2.54	2.88	1.61	12.87	254361
3	Malogro	0.36	2.17	1.24	1.60	1.66	1.38	8.42	254631
2	Laklakh	0.59	0.44	1.47	0.87	1.72	1.21	6.29	536412
175	Maewo	0.70	0.53	0.44	0.28	1.31	0.24	3.51	512346
123	Meleliap	2.71	2.57	1.15	0.47	4.25	0.59	11.75	512364
200	Malohubora	1.11	0.48	0.62	0.15	2.10	0.22	4.67	513264
6	Sinibo	2.36	2.78	0.84	0.34	3.12	0.51	9.95	521364
131	Malovuro	2.04	2.12	0.73	0.3	2.87	0.33	8.39	521364
153	Ring	0.81	0.10	0.45	0.13	1.98	0.37	3.84	521364
SEL		0.01	0.04	0.03	0.05	0.04	0.03	0.16	
min		0.36	0.10	0.44	0.13	0.48	0.22	3.51	
max		2.71	5.31	2.67	5.41	4.25	1.97	15.52	
mean		0.79	2.37	1.49	2.40	1.64	1.02	9.70	
SD		0.44	1.07	0.53	1.16	0.84	0.43	2.85	
CV%		56.1	45.0	35.7	48.2	51.6	42.5	29.4	

[a] DMY, desmethoxyyangonin; DHK, dihydrokavain; Y, yangonin; K, kavain; DHM, dihydromethysticin; M, methysticin; KL, total kavalactone (kavalactones are numbered in their HPLC elution order); SEL, standard error of laboratory.

reflectance. The spectra wavelength range was 400−2500 nm. Each spectrum was obtained by averaging 32 scans of each sample with approximately 72° rotation between each scan (automatic conveyor), and all of the spectra were recorded as log (1/*R*) with respect to a highly reflective ceramic standard. All samples were scanned with NIRS and their spectra recorded. Two spectra were obtained for each of the 236 samples prepared, and overall 472 spectra were recorded (**Figure 1**).

The spectra were mathematically transformed according to the WINISI software (Infrasoft International): second derivative of standard normal variate and detrend corrected spectrum (SNVD) calculated on five datapoints and smoothed (Savitzky and Golay smoothing) on five data points. Calibration of the kavalactone content was performed using partial leastsquares regression (PLSR). Calibration statistics used to evaluate models performances include the standard error of calibration (SEC), the coefficient of determination (R^2), and the standard error of cross-validation (SECV). The cross-validation method (*19*) resulted in the calculation of the SECV. In addition to R^2, the RPD ratio (RPD = SD/SECV (*20*) was also used to evaluate performances of each equation.

A principal component analysis (PCA) was used to extract the relevant information from the spectral matrix ($n = 472$). The generalized Mahalanobis distance (H) was calculated on the extracted PCs for each sample. This statistical distance is useful for defining boundaries of

Table 2. Correlation Coefficients between Major Kavalactones and Total Kavalactones Content[a]

	DMY	DHK	Y	K	DHM	M
DHK	+*0.1689* ns					
Y	−*0.0422* ns	+0.5746 **				
K	−*0.2165* ns	+0.4499 **	+0.7857 **			
DHM	+*0.5590* **	+0.4416 **	+0.0758 ns	−0.4097 **		
M	−*0.2848* *	+0.5473 **	+0.5514 **	+0.2350 ns	+0.3958 **	
KL%	+*0.2440* ns	+*0.9010* **	+0.8193 **	+0.5994 **	+0.4546 **	+0.6272 **

[a] **, significant at 1% level, tabular value = 0.3541; *, significant at 5% level, tabular value = 0.2732; ns, not significant; DMY, desmethoxyyangonin; DHK, dihydrokavain; Y, yangonin; K, kavain; DHM, dihydromethysticin; M, methysticin; KL%, total kavalactones.

Table 3. Distribution of Total Kavalactones between Organs of Different Cultivars[a]

	roots		stumps		basal stems	
cultivar	Total KL%	Chemotype	Total KL%	Chemotype	Total KL%	Chemotype
Borogu	10.30	423561	5.14	423561	6.03	245416
Malog velablalas	15.52	423561	9.79	425361	6.02	243561
Nikawa Pia	10.76	423561	5.61	423516	5.27	243561
Long han	7.03	245361	6.21	245631	5.95	254631
Melomelo	9.17	245361	7.41	254361	5.52	254361
Sentender	9.60	245361	7.33	254361	5.16	254361
Bogania	12.83	243156	6.48	421356	5.80	243516
Tarivarus	14.98	254361	6.40	254361	7.49	254361
Isa	13.04	254361	8.16	254361	10.64	254361
Palisi	6.29	536412	5.49	254361	5.34	254631
Maewo	3.51	512346	1.32	521364	1.34	521634
Meleliap	11.75	512364	7.40	521364	8.40	521346
Malovuro	8.39	521364	6.75	521364	5.88	521346
Malohubora	4.67	513264	2.95	521364	2.77	521346
min	3.51		1.32		1.34	
max	15.52		9.79		10.64	
mean	9.85		6.17		5.83	
SD	3.64		2.11		2.21	
CV%	37.0		34.1		37.8	

[a] KL%, total kavalactones.

the population and a similarity index between spectra (*21*). The 27 principal components (PC) extracted from the PCA explained 100% of the total inertia of the initial matrix. The first three PCs explained 57.18, 16.64, and 9.40% of initial inertia, respectively. The mean spectrum was calculated for each sample of kava powder, leading to a total of 236 spectra. A new PCA was done on these data to investigate the homogeneity of the population. The Mahalanobis distance (H) of each spectrum to the mean spectrum of the group was calculated. The removal of spectral outliers was based on Mahalanobis distance $H > 3$ from the average spectrum of the file, and four samples of roots and two samples of stump were removed because of their heterogeneity.

Finally, to reduce time and cost of reference analysis (HPLC), a core sample of only 101 accessions (roots = 39, stumps = 23, basal stems = 39) representing the 230 remaining samples were selected from their spectral fingerprint. The spectral selection of sample was done by clustering using distance between samples. By hypothesis, a single sample is sufficient to represent all of the samples of its neighborhood (*22*). According to the fixed number of samples (n = 101), the sample having most neighbors is selected, and these neighbors are eliminated. Among the remaining samples, another sample having most neighbors is selected and its neighbors are eliminated. The calculation continued until all 101 samples were selected. This selection was completed with 19 samples constituted of the 6 H distance outliers and 13 random selected samples. This set of 19 samples was used as a validation file. The total of selected samples was 120 (roots = 52, stumps = 27, basal stems = 41).

Extraction and HPLC Analysis. HPLC is the reference method used for kavalactone quantification (*17, 18*). The selected kava powder samples (n = 120 and 0.5 mm particle sizes) were used for kavalactone extraction and HPLC analysis. Approximately 0.5 g of each sample was suspended in 17 mL of methanol and agitated for 1 h. After filtration through a paper filter, extracts were quantitatively transferred in a measuring flask and made up to a 20 mL final volume with methanol. An aliquot was then filtered through a 450 nm filter and analyzed via HPLC.

Table 4. Descriptive Statistics of the Calibration Set Composed of 101 Samples[a]

	DW	DMY	DHK	Y	K	DHM	M	KL%
min	89.11	0.13	0.10	0.07	0.00	0.44	0.01	1.32
max	93.34	1.29	5.31	2.67	5.41	3.41	1.97	15.52
mean	91.34	0.51	2.03	1.09	1.74	1.32	0.69	7.37
SD	0.82	0.28	0.95	0.58	1.14	0.70	0.44	3.13
CV%	0.89	53.83	47.05	53.42	65.59	53.31	64.18	42.42

[a] DMY, desmethoxyyangonin; DHK, dihydrokavain; Y, yangonin; K, kavain; DHM, dihydromethysticin; M, methysticin; KL%, total kavalactones; SEL, standard error of laboratory.

A normal phase HPLC system, using a 250 × 3 mm Nucleosil 100-5 column (Macherey-Nagel, Düren, Germany) at a constant 30 °C, with a Spectra-System P1000XR pump (ThermoSeparation Products, Riviera Beach, FL), a multidetection of waves UV detector set in scanning mode between 400 and 800 nm, was used to separate the kavalactones. Chromatograms were recorded and quantified on a Spectra Focus IM000239 detector (Spectra-Physics, Mountain View, CA). The mobile phase was n-hexane 94.5%, dioxane 1%, and methanol 4.5% on a volume basis with a flow rate of 0.6 mL/min. Overall, 120 samples were analyzed by HPLC, representing cultivars from the germplasm collection and clones collected in four different villages for comparison.

Chemicals and Reagents. n-Hexane, dioxane, and methanol of chromatographic grade were purchased from Sigma-Aldrich (Munich, Germany). Known concentration analytical standards were interspersed between samples to verify that no time-related changes affected the HPLC results. The final percentage dry weight content of each kavalactone was calculated from standard curves obtained from the

Table 5. Statistical Parameters of the NIRS Calibration[a]

constituent	N	mean	SEC	R^2	ltc	slope	SECV	1-VR	SEL	SEP	RPD	PLS terms
dried material	97	91.34	0.26	0.90	0.00	1.00	0.29	0.87	0.07	0.51	2.7	4
desmethoxyyangonin	93	0.49	0.07	0.92	0.00	1.00	0.10	0.84	0.01	0.24	2.5	7
dihydrokavain	96	1.98	0.29	0.87	0.02	1.01	0.33	0.84	0.04	0.40	2.5	4
yangonin	100	1.07	0.16	0.92	0.00	1.00	0.19	0.89	0.03	0.16	3.0	5
kavain	101	1.74	0.30	0.93	−0.02	1.01	0.35	0.90	0.05	0.37	3.2	5
dihydromethysticin	99	1.33	0.16	0.95	0.00	1.00	0.22	0.91	0.04	0.32	3.3	8
methysticin	94	0.70	0.08	0.96	0.01	0.96	0.10	0.95	0.03	0.11	4.3	8
total kavalactones	94	7.32	0.81	0.93	0.01	1.01	0.85	0.93	0.16	1.06	3.7	2

[a] *n*, number of samples used; R^2, coefficient of determination; SEC, standard error of calibration; ltc, intercept and slope of the regression of wet chemistry values versus NIRS values; SECV, standard of cross-validation; SEP, standard error of prediction; SEL, standard error of the reference method; RPD, ratio of performance to deviation = ET/SEP.

individual analytical standards. The standards of kavalactones (**Figure 2**) were purchased from Phytolab (Vestenbergsgreuth, Germany): desmethoxyyangonin (DMY), 100% purity; dihydrokavain (DHK), 99% purity; yangonin (Y), 98% purity; kavain (K), 100% purity; dihydromethysticin (DHM), 97% purity; and methysticin (M), 99% purity.

RESULTS AND DISCUSSION

Variability of the Kavalactone Content and Composition. The order of elution of the six major kavalactones was the same as previously described (*14, 15*). DMY was first to elute, followed by DHK, Y, K, DHM, and M, respectively, after 11.28, 12.28, 15.25, 17.57, 19.57, and 25.75 min.

Previous work has shown that the different organs of the plant have different kavalactone contents. To assess the variation existing between cultivars in Vanuatu, it is therefore necessary to compare the kavalactone contents and compositions of the same organ. **Table 1** presents the kavalactone compositions of 52 root samples analyzed by HPLC. There is a remarkable variation of the concentration for every individual kavalactone, with CV% ranging from 35.7 (Y) to 56.1% (DMY), and for the total kavalactones, with a CV% of 29.4%. Total kavalactone concentrations in the roots varied from 3.51 to 15.52% with an average of 9.7%. Most of these samples (47 accessions) are cultivars from the germplasm collection, and 5 samples came from the villages of Fanafo, Narango, and Sara (3 different clones of Borogu and 2 different clones of Palisi).

These concentrations were lower compared to previous studies, which showed that kavalactone concentration varies between 3 and 20% (*14*). This could be explained by the diminution of kavalactone concentration during the preparation of the kava powder samples in Vanuatu. Kavalactone concentrations could also diminish during the time of storage of the samples and their transportation from Vanuatu to Montpellier, France. In decreasing order of importance in the dry matter, the most important kavalactones were kavain (mean = 2.40), dihydrokavain (2.37), dihydromethysticin (1.64), yangonin (1.49), methysticin (1.02), and desmethoxyyangonin (0.79) (**Table 1**).

Chemotypes were identified following the simple system described by Lebot and Lévèsque (*14, 15*). Six major kavalactones account for approximately 96% of the lipid extract. They differ quantitatively and qualitatively in their physiological action (*1*). These six major kavalactones are used to define the chemotype (1 = demethoxy-yangonin, DMY; 2 = dihydrokavain, DHK; 3 = yangonin, Y; 4 = kavain, K; 5 = dihydromethysticin, DHM; and 6 = methysticin, M). Chemical compositions are coded by listing in decreasing order of proportion these kavalactones to identify different chemotypes (**Table 1**).

Because different kavalactones have different physiological properties, the physiological effect of kava has been demonstrated to result from the kavalactone content and the chemotype (*14*). Good cultivars used for daily drinking and those known for their rapid effect, such as Borogu, have chemotypes rich in K (423561, 423651). This can be explained by the fast absorption of kavain, which causes a sudden high, compared to the much slower absorption of DHK and DHM, which frequently produce nausea. Chemotypes 521634 and 526341 represent cultivars that are rarely consumed and that belong to var. *wichmannii*. When ingested, the beverage induces an unpleasant nausea due to the very high proportions of DHM (5). The same is true for a group of cultivars of var. *methysticum* famous for their long-lasting effect and called "two-days", which present chemotype 254631, such as Palisi. Correlation coefficients obtained between the six major kavalactones and the total kavalactone content are presented in **Table 2**. Lebot and Lévèsque (*14*) showed that kavain and dihydromethysticin were significantly negatively correlated (−0.9222 **), and this negative correlation is maintained in the present study, although to a lesser extent (−0.4097 **).

Chemotype is genetically controlled. The chemotype of a given cultivar is fairly stable as indicated by the different plants of cultivars Borogu (a noble variety) and Palisi (an illegal variety) collected from the germplasm collection and from different villages. The chemotype is also fairly stable for a given cultivar independent of its age, as indicated by the values obtained from different plants of different ages (1, 2, and 3 years) of the same cultivar (**Table 1**).

Total kavalactone concentration also varied according to the cultivar and the organ of the kava plant (**Table 3**). The samples prepared from roots displayed higher total kavalactone concentration compared to samples of stump. On average, the samples of stumps displayed higher kavalactone concentration compared to samples of basal stem organs.

NIRS Calibration. The core sample of 101 accessions submitted to the calibration set (roots = 39, stumps = 23, basal stems = 39) covered the expected values of the dry weight, the six major kavalactones, and the total kavalactone content (**Table 4**). PLS regression was applied to develop a predictive model using HPLC values of 101 samples and second derivative of the near-infrared part of the spectra (1100−2500 nm). The statistical parameters for eight equations obtained for the six major kavalactones, the total kavalactones, and dry matter content displayed R^2 values between 87 and 96% (**Table 5**). This indicates that the equations explained the majority of the variation for kavalactones and the dried material.

The SECVs observed for each kavalactone were close to the SECs, which means fair and robust fitting. These values were good estimations of the equation accuracies as they were close to the standard error of prediction (SEP) obtained on the 19 validation samples (**Table 5**). In fact, the SEPs observed were slightly higher than the SECVs, but in our case, SEPs were

overestimated (extrapolation) because of six outlier samples in the validation set.

The equation for dihydrokavain explained the least amount of variation (89%) within the reference values (HPLC results) with a SECV equal to 0.33%, whereas the equation of methysticin presented an R^2 equal to 0.96 with a SECV error equal to 0.1%. The R^2 for the equation of total kavalactones was equal to 0.93 with a SECV of 0.85%. In terms of predictive performance these two last equations were high, with RPD parameters respectively equal to 4.3 and 3.7.

The statistical parameters obtained for kavain and dihydromethysticin are of special interest for quality control in Vanuatu. These kavalactones are negatively correlated and are the most important ones in determining chemotypes. Their respective R^2 values (0.93 and 0.95) are high and allow good estimates of their content in kava powders. When the kavain content is higher than that of the five other kavalactones, this is an indication that the dry powder sample originates from a noble variety. On the other hand, if the dihydromethysticin content is higher than that of the other five kavalactones, the sample originates from an undesirable variety.

Overall, the equations obtained displayed a good correlation between the HPLC value and the NIRS prediction. The coefficients of correlation were close to 1, and the equations are very satisfactory. RPD is >2 in all cases, and the number of terms is ≤ 8.

The confrontation of the NIRS spectra and the HPLC value allowed the establishment of equations of calibration for the prediction of the level of each of the six major kavalactones and total kavalactones and dry materials. The coefficients of determination were $>90\%$ except those of the dried material and dihydrokavain (respectively, 89 and 87%), but they were all sufficient to appreciate the quality of the calibration. These results are very satisfactory and show that NIRS could be efficient for the routine evaluation of kava quality.

The results presented in this paper confirm those obtained previously (*16, 17*) and indicate that NIRS is a reliable technique for measuring kavalactone composition and content on dry kava powder. The overall number of samples studied and the R^2 values of the six major kavalactones and their total (**Table 5**) are, however, higher than those presented in the two previous studies. Furthermore, it is shown that the equations developed can be applied over many varieties and, in fact, can determine the chemotype reliably. Also, the equations are valid for all plant parts. These results also indicate that NIRS could be used in Vanuatu for controlling the export of noble varieties by determining their chemical signature (chemotype) with dry powder samples. This work represents a significant contribution to the development of a routine quality control system.

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