Spectrophotometric Method for the Differentiation Between 'Noble' and 'Non-noble' kava (Piper Methysticum).

Garry Stoner - T.K. Group Labs, Ankeny IA – December 22, 2016

Abstract: Current and developing laws which forbid exportation of two day kava from South Pacific nations have made accurate determination of kava types essential to the industry. HPLC and HPTLC methods are available, but such methods are costly and time consuming. The photometric method described here is intended to provide a fast, accurate, and inexpensive means of distinguishing between noble kava, two day kava, and noble kava adulterated with two day kava.

Sample preparation: Dried ground kava root and rhizome was combined with acetone at a ratio of 1g/3ml and sonicated for 15 minutes. Samples were centrifuged at 3250rpm for 15 minutes, and supernatant transferred to Bausch & Lomb Colorimetric round tubes with a light path of 10mm for analysis. Separate samples were prepared for adulteration analysis.

Analysis: Samples were tested for transmittance 400-700nm at ~3nm intervals and spectra converted to dominant wavelength using CIE standard algorithms (*Appendix A*). This dominant wavelength (expressed in nm) serves as the parameter for indication of kava type.

Results: Verified samples of noble kava, two day kava, and noble kava intentionally adulterated with two day kava returned values as shown in Tables 1 & 2.

Table 1

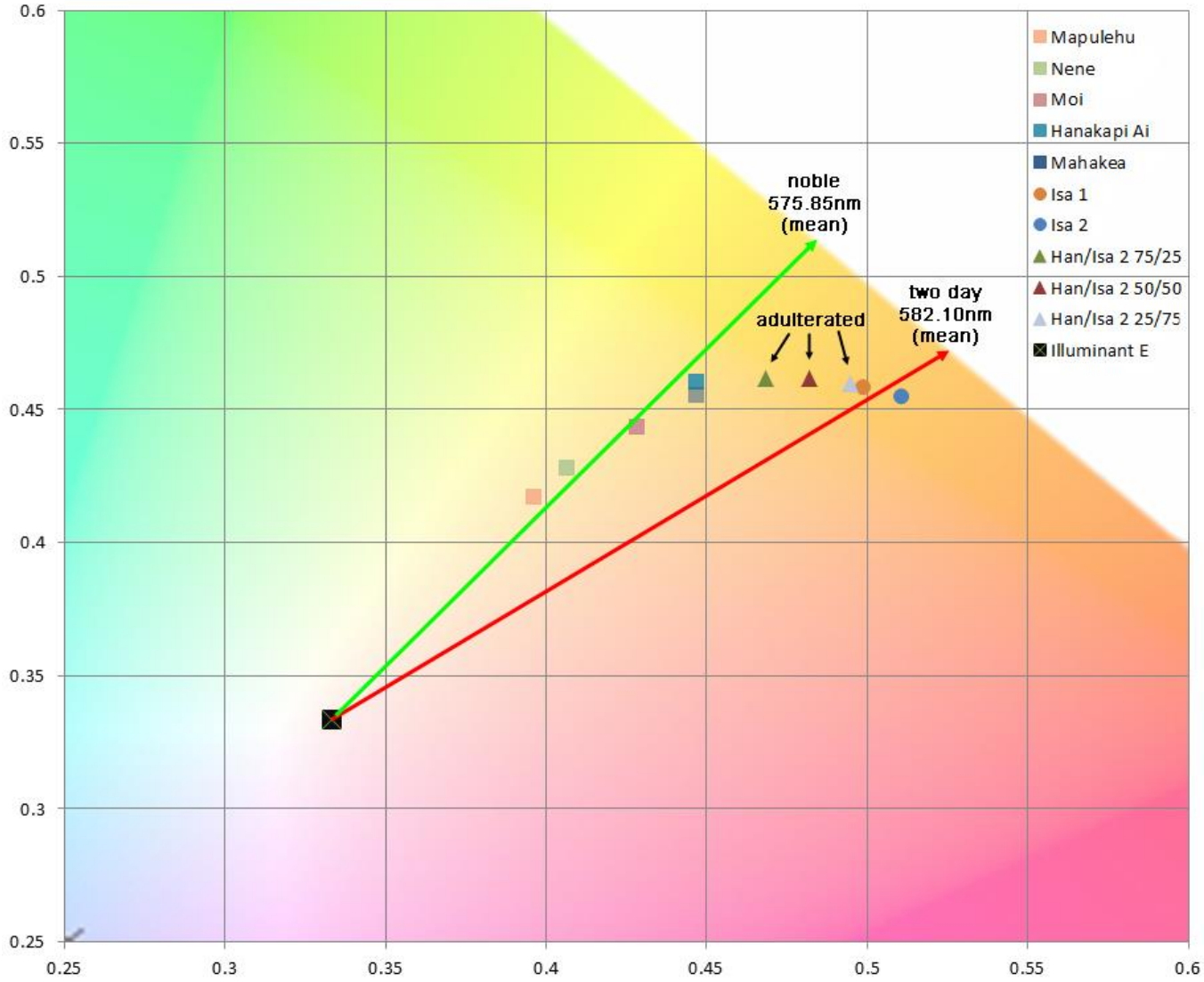
sample #	pure cultivars	Origin	Island	Supplier	% chips	% roots	domWL
1	Mapulehu	Hawaii	Hilo	Chris Allen	65	35	574.4592
2	Nene	Hawaii	Hilo	Chris Allen	65	35	574.8404
3	Moi	Hawaii	Hilo	Chris Allen	65	35	576.1878
4	Hanakapi Ai	Hawaii	Hilo	Chris Allen	0	100	576.6236
5	Mahakea	Hawaii	Hilo	Chris Allen	65	35	577.1588
6	Isa 1	Hawaii	Hilo	Chris Allen	60	40	581.4816
7	Isa 2	Hawaii	Hilo	Uka Kava	60	40	582.7218

Table 2

sample #	content	Noble	75 / 25	50 / 50	25 / 75	two day
2 & 7	Nene/Isa 2	574.7154	577.1101	579.0450	580.7442	582.3508
1 & 6	Mapulehu/Isa 1	574.7163	577.2739	579.2106	581.0762	582.5098
4 & 7	Hanakapi Ai / Isa 2	577.3354	578.7188	579.8944	581.0778	582.3015

Discussion: The basis for this method is the current understanding that coloration of acetonic kava extracts is due to specific pigments present in two day kava but absent from noble kava. Due to the presence or absence of these pigments, the coloration of noble or two day kava extracts may vary in *saturation*, but each category displays a fairly narrow range of *hue* irrespective of saturation. Further, these ranges are quite well separated between noble and two day kava cultivars, as illustrated by the CIE 1931 chart in Figure 1. Dominant wavelength was chosen as the indicating parameter since it represents hue only, is most indicative of perceived color, and is fairly insensitive to minor errors in sample ratio/concentration. In these trials, a dominant wavelength ≤577.5nm indicated noble kava, a dominant wavelength ≥581.0nm indicated non-noble kava, and ranges between these parameters indicated a potential for noble kava adulterated with non-noble kava. Further trials using a much wider variety of samples are in progress, and these parameters will be updated in future releases of this Method.

Fig.1



Software: To assist in implementation of this method, dedicated software has been developed which is capable of performing the necessary calculations using spectrophotometer output files containing transmittance or absorbance data in multiple formats. Since the calculations performed include resampling and prediction, a wide range of non-specific input intervals may be used. Though results presented in this article utilize 97 data points at approximately 3nm intervals from 400-700nm, equivalent results have been obtained using as few as ten data points in the same range. As a result, this method can easily be adapted for use with low cost instrumentation. This software is provided as open source under MIT license, and is fully supported and updated by its authors. Sample screenshots, input files, and output files are provided in *Appendix B*.

Conclusion: The author acknowledges that the small number and variety of samples used in this study is insufficient to establish validated standards, and that the exact molecule(s) and/or mechanism responsible for the coloration described in this and other studies has not been determined.

Even so, sufficient evidence is available to support the colorimetric approach, and by applying this method to a larger cross section of samples representative of available kava cultivars standards could easily be developed. These standards, along with the method and accompanying software, could provide a much needed tool for the developing kava industry.

Appendix A

Method of Calculation

If a spectrum is in percent transmittance, it is converted to fractional transmittance, T, by:

$$T = \frac{\%T}{100}$$

If a spectrum is in absorbance format, it is converted to transmittance by:

$$T = 10^{-A}$$

where T is fractional transmittance, and A is absorbance.

The input spectrum is resampled by linear interpolation to yield a spectrum between 400 nm and 700 nm, with 5 nm integral intervals.

After resampling the spectrum, the transmittance can be expressed as a function of wavelength $T(\lambda_i)$ where λ is the wavelength in nanometers, and i is the index of the point relative to 400 nm (in 5 nm steps).

The transmittance at each wavelength measured is represented by the vector:

$$\vec{T} = \begin{bmatrix} T(\lambda_1) \\ \vdots \\ T(\lambda_i) \\ \vdots \end{bmatrix}$$

The illuminant function is $I(\lambda)$ for each value of $\lambda_i$for which it is tabulated. The illuminant function is represented by the vector:

$$\vec{I} = \begin{bmatrix} I(\lambda_1) \\ \vdots \\ I(\lambda_i) \\ \vdots \end{bmatrix}$$

Note in this program CIE standard illuminant "E" [1] is used which corresponds to an arbitrary constant value for each element of the illuminant vector. Therefore the illuminant vector becomes:

$$\vec{I} = \mathrm{c}\vec{\mathbf{1}}$$

where $\vec{\mathbf{1}}$ is the unit vector.

The observer, or color matching, functions are represented by the set of vectors:

$$\vec{x} = [\overline{x}(\lambda_1) \quad \cdots \quad \overline{x}(\lambda_i) \quad \cdots]$$

$$\vec{y} = [\overline{y}(\lambda_1) \quad \cdots \quad \overline{y}(\lambda_i) \quad \cdots]$$

$$\vec{z} = [\overline{z}(\lambda_1) \quad \cdots \quad \overline{z}(\lambda_i) \quad \cdots]$$

The values of the color matching function are given by the CIE 1931 2-degree data set, which are documented in *ISO 11664-1:2007 (CIE S 014-1/E:2006) Colorimetry -- Part 1: CIE standard colorimetric observers.* [2]

and obtained from CVRL - Colour Matching Functions [3]

Define the value N as:

$$N = \vec{y} \cdot \vec{I}$$

Then, for Standard Illuminant E,

$$N = \vec{y} \cdot \vec{I} = c\left(\vec{y} \cdot \vec{\mathbf{1}}\right)$$

and the tristumulus X value is found from:

$$X = \frac{(\vec{x}\vec{T}) \cdot \vec{I}}{N} = \frac{(\vec{x}\vec{T}) \cdot (c\vec{\mathbf{1}})}{c(\vec{y} \cdot \vec{\mathbf{1}})} = \frac{c(\vec{x} \cdot \vec{T})}{c(\vec{y} \cdot \vec{\mathbf{1}})}$$

so that

$$X = \frac{\vec{x} \cdot \vec{T}}{\vec{y} \cdot \vec{\mathbf{1}}}$$

and similarly for Y and Z:

$$Y = \frac{\vec{y} \cdot \vec{T}}{\vec{y} \cdot \vec{\mathbf{1}}}$$

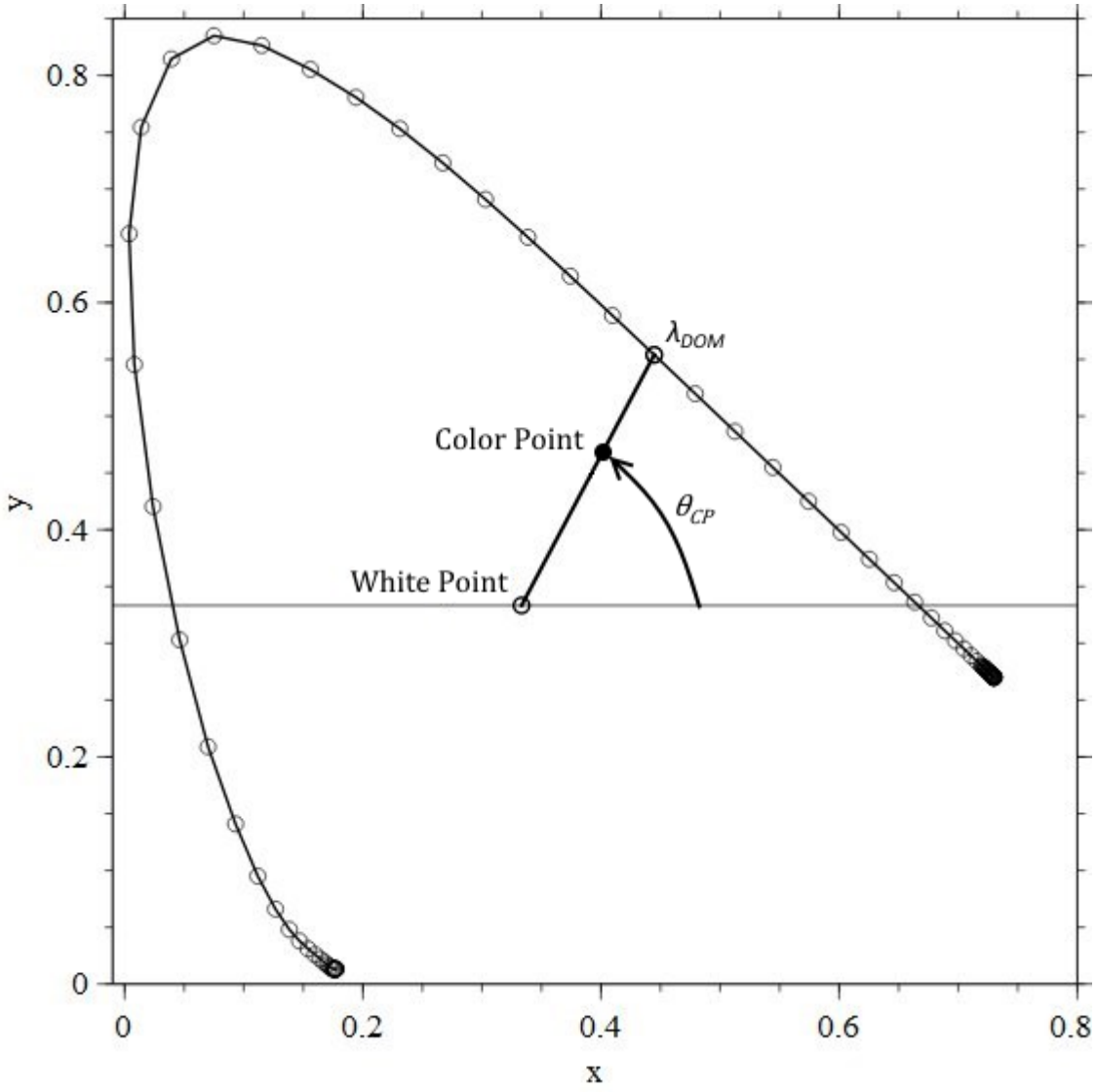
$$Z = \frac{\vec{z} \cdot \vec{T}}{\vec{y} \cdot \vec{\mathbf{1}}}$$

The XYZ values are converted to xy(Y) fomat using the following formulas:

$$x = \frac{X}{X + Y + Z}$$

$$y = \frac{Y}{X + Y + Z}$$

The "Horseshoe" shaped chromaticity curve in xy coordinates is derived from the color matching functions by using the above equations for x and y for each X, Y and Z triplet provided in the standard data set. The angle of each point, $\begin{bmatrix} x \\ y \end{bmatrix}$, on the chromaticity curve relative to the the white point of the illuminant is calculated:



A cubic spline interpolation relating angle θ to wavelength λ of the CIE "horseshoe" curve is constructed from this data yielding an approximate function:

$$\lambda = f(\theta)$$

To find the dominant wavelength, the angle of the color point relative to the white point, θ_{CP} is calculated. Then the dominant wavelength is given by

$$\lambda_{DOM} = f(\theta_{CP})$$

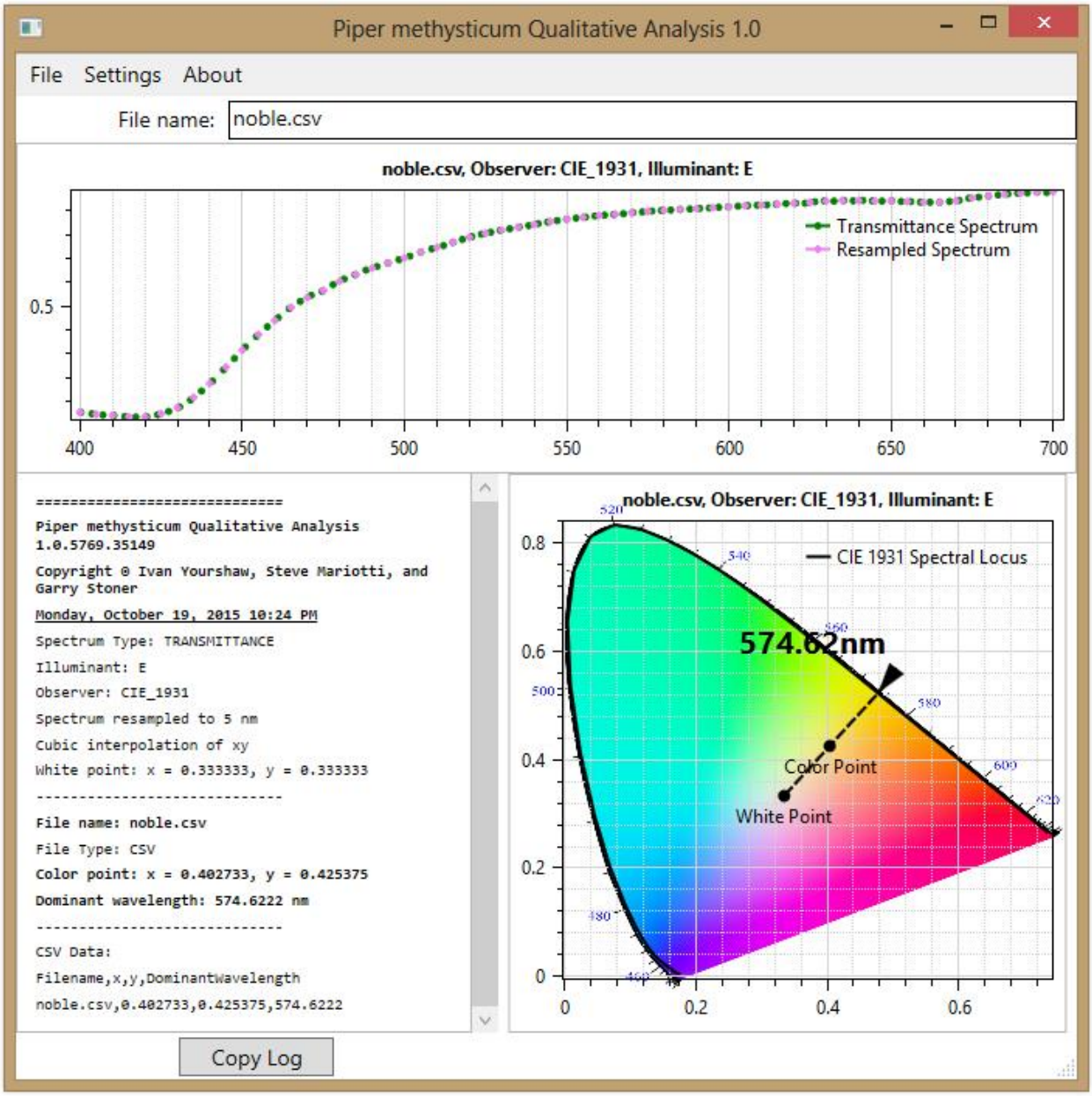
[1] http://www.image-engineering.de/library/technotes/753-cie-standard-illuminants

[2] http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=52495

[3] http://cvrl.ioo.ucl.ac.uk/cmfs.htm

Appendix B

Piper methysticum Qualitative Analysis 1.0, written by Ivan Yourshaw and Steve Mariotti, 2015



Sample input file "noble.csv"

400.24 5.39014373717
403.62 4.80333152685
407.00 4.34535666003
410.38 4.12899274016
413.76 3.66673309299
417.14 3.46472925568**.**
.
.
683.00 96.5245100467
686.00 96.9466589233
689.00 97.2992302336
692.00 97.4313551816
695.00 97.7702405628
698.00 97.5609756098

Sample output file "SA Output.txt"

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Piper methysticum Qualitative Analysis 1.0.5769.35149
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Monday, October 19, 2015 10:24 PM
Spectrum Type: TRANSMITTANCE
Illuminant: E
Observer: CIE_1931
Spectrum resampled to 5 nm
Cubic interpolation of xy
White point: x = 0.333333, y = 0.333333

File name: noble.csv
File Type: CSV
Color point: x = 0.402733, y = 0.425375
Dominant wavelength: 574.6222 nm

CSV Data:
Filename,x,y,DominantWavelength
noble.csv,0.402733,0.425375,574.6222