Biomass 8 (1985) 1–11

Near-Infrared Analysis of Hydrocarbon Producing Plant Species*

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(Received: 20 October, 1984)

ABSTRACT

*Fifty samples of native plants and 66 samples of milkweeds (*Asclepias*) were used to develop three equations using near-infrared reflectance (NIR) spectroscopy for the analysis of percent hexane, methanol and total yield extractables. The equations were then used to analyze samples not used for equation development. The native and milkweed-native (milkweed plus native plants) equations analyzed percent hexane, methanol and total yield extractables with a higher degree of accuracy than did the milkweed equation. The greatest errors occurred when analyzing percent methanol extractables. This may be due to a greater laboratory error. Analysis of percent total yield extractables had errors and r^2 values somewhat intermediate to those for percent hexane and methanol extractables due to the fact that percent methanol extractables made up part of the percent total yield values. NIR equations, using milkweed samples, for the analysis of percent hexane, methanol and total yield extractables are recommended only for milkweed samples, whereas equations developed using samples of a more diverse population can be used on a wider range of plants.*

Key words: Extractables, methanol, hexane, milkweed, native plants, near-infrared.

Research conducted in cooperation with Utah Agricultural Experiment Station, journal paper number 30377.

INTRODUCTION

The several shortages of liquid fuels have spurred a search for alternative sources of hydrocarbons from plants.[1–13] Screening plant species for extractable yields has now been accomplished for approximately 1500 species (Balandrin *et al.*[5] (543); Adams and McChesney[3] (80); Adams and Seiler[4] (39); Buchannan *et al.*[6] (100); Buchanan *et al.*[7] (100); McLaughlin and Hoffman[10] (195); Roth *et al.*[12] (508). Each of these surveys used liquid extraction (Soxhlets) by traditional wet laboratory techniques. Adams and McChesney[3] reviewed the different extraction methods and subsequent screening results. The need to screen thousands of individuals in a breeding program demands a faster method than is currently available. In view of these facts, we began to examine alternatives to approximate various yield parameters in plants.

USDA has recently developed computer algorithms to use near-infrared reflectance (NIR) spectroscopy to estimate the whole-plant composition of protein and seed oil yields.[14–16] This methodology has been extended to forage analyses of *in vitro* dry matter disappearance, acid-detergent fiber, lignin, cellulose, mineral analysis (Ca, P, K, B),[16, 17] *in vivo* digestibility,[18] carotene,[19] and the chemical composition of selected food products.[20]

NIR analyses within the ARS, USDA Forage Testing Network use a Pacific Scientific* model 6100 scanning monochromator coupled to a DEC PDP 11/ series computer. Spectra (from 1100 to 2500 nm at every 2 nm) of the ground plant material are stored for each sample. Approximately 50 samples of known composition are needed for calibration purposes. During equation development, multiple stepwise regression is used to correlate spectral and chemical data on the 50 samples and derive the analysis equations.

The NIR method for forage analyses is currently being made more portable and affordable with the addition of personal computers. Also, newly developed programs have made it possible for equations developed on one instrument to be transferred to other instruments, thus eliminating the need for recalibration on different instruments for the same chemical analysis.[21]

* Mention of a tradename does not imply an endorsement or recommendation by the US Department of Agriculture or Science Research Center, Hardin-Simmons University over similar companies or products not mentioned.

The purpose of this study was to examine the performance of NIR to estimate non-polar extractable yields (hexane extractables) and polar extractables (methanol extractables) for: (1) plants of a single species from various geographic sources; (2) plants chosen from many different families and geographic areas; and (3) a combination of the preceding two. Plant materials used for this study were available from a large screening by USDA.[5]

EXPERIMENTAL

The 66 samples of *Asclepsias speciosa* Torr. (milkweed) were collected in September, 1981, from (location, number of plants): Grand Junction, Colorado (17); Rogue River, Oregon (3); LaJunta, Colorado (20); Santa Fe, New Mexico (15); and Salt Lake City, Utah (11). The 50 samples of various species (referred to as 'native' in this paper) came from the following families: Agavaceae (3); Asteraceae (28); Boraginaceae (1); Chenopodiaceae (3); Euphorbiaceae (2); Fabaceae (1); Fagaceae (1); Fourquieriaceae (1); Liliaceae (1); Loaganiaceae (1); Onagraceae (1); Poaceae (1); Polemoniaceae (1); Polygonaceae (1); Ranunculaceae (1); Scrophulariaceae (2); and Verbenaceae (1).

Whole plant material was dried for 48 h at 70°C. The plant material was then ground in a Wiley mill to pass a 2 mm screen.

A plug of glass wool was placed in a Whatman paper thimble (33 × 94 mm) and both were dried for 48 h at 100°C. The thimble and glass wool plug were then placed in a desiccator for 4 h to prevent rehydration before pre-weighing.

Disposable aluminum pans were used for evaporation of the solvents from each extraction but these were found to contain a volatile coating that would contribute a source of error. Therefore, the aluminum pans were baked at 100°C for 24 h, placed in a desiccator for 4 h and then pre-weighed.

The dry, ground plant material was first extracted for 20 h with hexane in a Soxhlet, dried for 2 h at 100°C to remove traces of the hexane from the extracted residue, and then extracted for 20 h with methanol in a Soxhlet apparatus. The extracts were placed in pre-weighed aluminum pans and the solvents were evaporated in an externally vented oven. Hexane extracts were evaporated at 100°C for 48 h before weighing. Methanol extracts were evaporated at 100°C for 48 h and placed in a desiccator for 4 h before weighing. The extraction

thimble, glass wool and extracted residues were then dried for 48 h at 100°C and placed in a desiccator for 4 h before final weighing. Although some volatiles were lost in the extraction drying procedure, these would also probably be lost in a commercial harvesting and field drying process.

The hexane extracts contained pigments (mainly chlorophylls), some low molecular weight rubber, small amounts of fatty acids, alcohols, hydrocarbons (alkanes and squalene), monoglycerides and phytosterols (major amounts in *Asclepias*).[2, 5] The hexane extracts of the Asteraceae species often contained chiefly small carbohydrates such as inositol, glucose and sucrose as well as simple organic acids (malic acid, citric acid, pyroglutamic acid, etc.), some free amino acids and numerous other carbohydrates present in trace quantities in addition to chlorophyll that was not extracted by the hexane solvent.[2, 5]

The milkweed and native samples were scanned with a Pacific Scientific model 6100 monochromator linked to a DEC PDP 11/03 computer and files containing the chemical and spectral data were saved. The files were copied, split in a random fashion and recombined in a file containing milkweed and native samples (milkweed–native). This file was then split such that approximately 71 samples were used for development of a milkweed–native equation.

Three equations were developed using the milkweed, native, and milkweed–native samples. The milkweed and milkweed–native files were split such that the equations were developed on two-thirds of the samples and tested on the remaining one-third. Final equation selection and development for the milkweed and milkweed–native samples were based on the math treatment and number of wavelengths producing the lowest standard error of calibration (SEC), highest R^2, lowest standard error of analysis (SEA), highest r^2 and lowest bias. All of the native samples were used for equation development. Final equation selection and development on these samples was performed by using the math treatment and number of wavelengths producing the lowest SEC and still maintaining an F-test greater than 5·0.

The final milkweed equation was then used to analyse the native samples. A statistical comparison (standard error of difference (SED)) and squared simple correlation (r^2) between actual laboratory analysis and near-infrared reflectance (NIR) was performed. The same operation was performed on the milkweed samples analyzed by the native equation. The milkweed–native equation was used to analyze those

milkweed–native samples (approximately 33) not used for equation development, all the milkweed samples and all the native samples. Statistical comparisons were made as described above.

RESULTS AND DISCUSSION

Hexane extractables

Standard errors of calibration (SEC) were comparable for all three equations (Table 1) with milkweed having the lowest followed by native and milkweed–native (0·56, 0·79, 0·80, respectively). The SEC is a good indicator of laboratory error associated with the analysis. The R^2, which reflects the amount of variation in the samples, for equation development was highest for native followed by milkweed–native and milkweed. The native samples represent a more diverse population resulting in a higher R^2. Derivatization of the spectral data was required for all the equations. Milkweed and milkweed–native equations required second derivatization in order to produce the best analysis. Although the wavelengths are all different for the equations, there are some areas that are common. Common wavelength regions for all three equations were 1700, 2000 and 2300 nm. In addition, milkweed–native and native equations shared the 1300 nm region. Nine wavelengths appeared to be optimum for the native and milkweed–native equations; however, the milkweed equation only required six wavelengths for development.

Standard errors of analysis (SEA) were lowest for the milkweed–native equation analyzing milkweed samples (Table 2). The milkweed–native equation analyzing native samples had the highest SEA and r^2 of the three analyses by the milkweed–native equations. The reason for these differences is not understood since the milkweed–native equation was developed using some of the native samples and should have had an SEA similar to the milkweed–native equation analyzing milkweed samples. The SEA is still acceptable since it is not more than three times the SEC (0·80) of equation development. The r^2 values were similar for all milkweed–native equations analyzing other samples (0·82, 0·81 and 0·86).

The highest SEA occurred when the milkweed equation analyzed the native samples (3·43). The SEA is more than three times the SEC and the milkweed equation should not be used to analyze anything other than milkweed samples. The high SEA is expected since the

TABLE 1

Calibration Data, Math Treatments and Wavelengths Selected for the Different Equations

Measured component	*Calibration data*			*Math treatment*[d]	*Wavelength*[e] *(nm)*								
	Mean[a]	*SEC*[b]	R^{2}[c]										
Milkweed													
Hexane	6·34	0·56	0·85	2	2054	1694	1574	2034	2354	1714			
Methanol	15·76	2·16	0·71	1	1458	2078	2318						
Total yield	22·07	2·21	0·75	1	2444	1944	2084	2364	1784	1184	1464		
Native													
Hexane	5·48	0·79	0·95	1	1278	2258	1738	2078	2278	1678	2238	1158	2198
Methanol	16·68	2·01	0·81	2	2228	1448	2348	1648	1408	2268	2388	1688	2468
Total yield	22·16	2·13	0·86	1	1464	2224	1924	1564	2064	2364	2424	1724	1784
Milkweed–native													
Hexane	5·77	0·80	0·90	2	1808	1768	1388	2028	1688	1348	2128	2268	1848
Methanol	15·79	2·39	0·70	2	1568	1848	1268	1768	2028	2288	2268	1788	1408
Total yield	22·12	3·67	0·56	1	1214	1754	2094	1534	2274				

[a] Mean of known percent extractables.

[b] Standard error of calibration from the least squares regression of known percent extractables on NIR percent extractables.

[c] Squared coefficient of multiple determination from the least squares regression of known percent extractables on NIR percent extractables.

[d] Best data treatment for the variable; 1 = first derivative of log $1/R$; 2 = second derivative of log $1/R$.

[e] Wavelengths needed for the best analysis equation.

TABLE 2
Analysis on Samples not used for Equation Development

Measured component	N^a	*Actual mean*	SEA^b	r^{2c}	SD^d	
					Actual	*Analyzed*
Milkweed equation analyzing native samples						
Hexane	50	5·45	3·43	0·79	3·43	6·08
Methanol	50	16·67	5·48	0·13	4·64	5·00
Total yield	50	22·16	6·42	0·30	5·78	7·41
Native equation analyzing milkweed samples						
Hexane	61	6·41	1·23	0·33	1·46	1·14
Methanol	61	15·58	3·43	0·22	3·84	2·33
Total yield	61	21·98	7·08	0·35	4·25	4·74
Milkweed-native equation analyzing milkweed-native samples						
Hexane	33	5·95	0·96	0·82	2·24	1·99
Methanol	33	16·59	3·17	0·45	3·60	4·13
Total yield	33	22·54	3·79	0·31	4·36	3·56
Milkweed-native equation analyzing milkweed samples						
Hexane	61	6·41	0·63	0·81	1·46	1·35
Methanol	61	15·58	2·54	0·57	3·84	3·33
Total yield	61	21·98	3·42	0·35	4·25	2·52
Milkweed-native equation analyzing native samples						
Hexane	50	5·45	1·48	0·86	3·43	3·86
Methanol	50	16·67	2·70	0·69	4·64	4·68
Total yield	50	22·16	3·50	0·65	5·78	5·36

[a] Number of samples analyzed.
[b] Standard error of analysis of actual values by NIR.
[c] Squared simple correlation of NIR analyzed values versus known quality values from conventional laboratory analysis.
[d] Standard deviation of percent extractables.

native samples cover a broader range of percent hexane extractables than the milkweed samples that made up the equation. The SEA was reduced when the native equation analyzed milkweed samples and this SEA is within the acceptable range of accuracy. However, the r^2 was lower than when the milkweed equation analyzed native samples.

Methanol extractables

All three equations had similar SEC values for percent methanol extractables, but they were inflated compared to those for percent hexane extractables (Table 1). The high SEC values suggest a high standard error for the analysis of percent methanol extractables. The R^2 values were similar with the native equation again being the highest. Both native and milkweed-native equations required second derivatization of the spectra for the equation development. First derivatization was used for the milkweed equation. The wavelength regions of 1400 and 2300 nm were optimum for all three equations. The milkweed-native and milkweed equations also shared the 2000 nm region. Three wavelengths were used for the milkweed equation while the native and milkweed-native equations required nine wavelengths.

The SEA values were higher than those for percent hexane extractables, but are acceptable since they are within three times the SEC for percent methanol extractables (Table 2). The inflated SEA values are a reflection of the SEC if the instrument is capable of analyzing that component. Again the milkweed equation analyzing native samples had the highest SEA. The lowest SEA values were when the milkweed-native equations were analyzing samples, reflecting the diversity of the samples used for equation development which leads to a more robust equation. The r^2 values were considerably lower than those for percent hexane extractables, with the milkweed equation analyzing native being the lowest ($r^2 = 0{\cdot}13$).

Total yield

Percent total yield extractables is the sum of percent hexane and methanol extractables. The SEC values were higher than percent methanol extractables SEC (Table 1). The native equation had the lowest SEC followed by milkweed and milkweed-native equations (2·13, 2·21 and 3·67, respectively). The R^2 values for equation development followed a reverse trend compared to the SEC values, with the native equation being the highest followed by the milkweed and milkweed-native equations.

First-order derivatization of the spectra was optimum for all three equations. Three wavelengths were common for all three equations

(1500, 1800 and 2100 nm), with the milkweed and native equations also sharing the 1900 and 2400 nm regions. The milkweed and milkweed–native equations shared the 1200 region. The milkweed–native equation required five wavelengths while the milkweed and native equations required seven and nine wavelengths, respectively.

The standard errors of analysis were higher than for percent hexane or methanol extractables since the errors present in analyzing for these two components would be carried across to percent total yield extractables (Table 2). The native equation analyzing milkweed samples had a 10% larger SEA than the milkweed equation analyzing native samples. The error increased to 87% compared to the milkweed–native equations analyzing samples. Again, by including both milkweed and native samples into the calibrations, the SEA values were lower than with having two separate equations and analyzing samples not representative of the population used for equation development. The squared simple correlations followed the same pattern as the r^2 for percent methanol extractables with the milkweed–native equation analyzing native plants ($r^2 = 0{\cdot}65$). The r^2 values were higher than those for percent methanol extractables, but lower than those for percent hexane extractables. This is due to the presence of the added errors from percent methanol extractables in the percent total yield analysis.

The standard errors of calibration and analysis were high for percent methanol and total yield extractables which may be due to laboratory error, or to the fact that the samples were analyzed in the laboratory and with NIR using a 2 mm grind. The ranges of values for percent hexane, methanol and total yield extractables in the milkweed samples were 3·11–8·85, 5·69–31·38 and 10·96–39·56, respectively. The ranges for native plants were 0·95–16·36, 5·87–34·41 and 6·73–37·25, respectively. The diversity in the native plants may indicate why the NIR equations using native plants seemed to do a better job of analyses than using just milkweed samples.

In conclusion, NIR appears able to analyze polar and non-polar extractables in plants of a single species and from different families accurately enough for screening purposes. The time involved is reduced compared to regular laboratory analysis. NIR more accurately analyzes samples from a wide range of species and locations if the equation is developed from a broad spectrum of plant material with a large amount of variation.

ACKNOWLEDGEMENTS

We wish to thank USDA, Peoria (Dr M. O. Bagby) for furnishing the samples in conjunction with the hydrocarbon screening project (59-2495-1-6-047) at Native Plants, Inc. of Salt Lake City, Utah, and DOE (Contract DE-AC03-81CS4009, to Plant Resources Institute).

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