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A COMPREHENSIVE STUDY OF THE CHEMICAL AND PHYSICAL PROPERTIES OF B3FR BURLEY TOBACCO AND THEIR RELATIONSHIP TO SMOKE STRENGTH

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Summary or Abstract:

A set of five B3FR burley tobaccos were studied in an attempt to elucidate the mechanisms of smoke strength. The study encompassed both smoke and leaf chemical analyses. The significant data variables were incorporated into a multi-variable strength prediction equation.

The ratio of leaf nicotine to the sum of oxalic and citric acid concentrations was observed to be the significant parameter affecting strength perception. As the data base is increased for burley tobacco, it may be possible to develop a universal strength prediction equation for both domestic and off-shore burley tobaccos. The results of this study may also provide insight into smoke character and off taste.

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INTRODUCTION

Those in Product Development who evaluate burley grade samples have long been interested in a method of predicting and/or altering the strength characteristics of certain burleys. Such a procedure could upgrade an otherwise unacceptable tobacco and perhaps provide a favorable economic incentive. Normally, one would expect smoke strength to increase with higher levels of leaf nicotine. Generally, this the expected behavior of tobacco with respect to stalk position.

Yet in some instances, tobaccos with similar leaf nicotine concentrations and stalk positions may exhibit significant variations in smoke strength. Also certain tobaccos with high leaf nicotine levels have exhibited a lower strength rating than did a similar tobacco grade of lower leaf nicotine. The elucidation of this anomalous behavior was the initial focus of this project.

We felt that such an investigation could result in a technique that would aid us in the systematic prediction of smoke strength and perhaps a method capable of adjusting the strength ratios of burley tobacco. Ultimately, our research goal encompassed the ideal of a cost savings at the burley market. Normally, the inexpensive burley grades available from foreign markets or tobacco stabilization do not possess the smoking qualities required for our blends. Any physical or chemical technique capable of positively modifying the smoke parameters of such tobaccos might be appealing. A future aspect of the burley project would be the development of technology capable of upgrading previously unacceptable burley tobaccos by modifying strength and/or character features.

We realize that such an undertaking could be extremely complex and time consuming involving multiple samples for analysis. We initially met with Mr. T. L. Jessup and Mr. H. E. Smith of Product Development in order to develop an understanding of their needs for burley improvement in the future. We were aware of an earlier work that attempted to correlate throat and chest impact with leaf and smoke chemistry data based upon a large set of domestic burley tobaccos.[1] In this report, the author developed a multiple regression equation capable of predicting smoke impact (strength). A careful review of the data revealed unrealistically low strength ratings assigned to each burley tobacco. Based upon this information, we decided to conduct a similar study limited to a smaller set of burley tobacco samples in which abnormal strength behavior was apparent. Such a sample set would perhaps contain high nicotine/low impact and low nicotine/high impact tobacco samples. We would proceed to study the basic differences of a small sample set and correlate the significant parameters with smoke strength. Then, the sample set would be enlarged in order to encompass our domestic and investigational off-shore burley tobaccos.

We envision the development of a universal prediction equation and strength scale capable of ranking burley, domestic and off-shore tobaccos into strength catagories with a high degree of confidence.

DISCUSSION

Product Development sought a set of five burley samples for our study possessing the aforementioned properties. After screening a number of grade samples, five composite samples of B3FR burley tobacco from Stabilization were analyzed and later purchased. A composite sample was removed from each hogshead prior to purchase. Cigarettes were prepared and average strength and character ratings were assigned after multiple smokings. These data are given in Table 1. The tobacco from each respective hogshead was re-blended in order to generate a homogeneous sample. A portion of the tobacco was cased with 2% glycerine, cut, and 85 mm non-filter cigarettes were machine prepared in the Research Center's pilot plant. The cigarettes were repeatedly smoked by the expert panel and an average rating was assigned. These data are given in Table 2. A major discrepancy in strength was noted for burley sample 41159. Hand-made cigarettes prepared from the pre-purchased hogshead No. 41159 exhibited significantly greater strength than did the cased machine made cigarettes. A reevaluation of the strength characteristics ten months later revealed that the strength of 41159 compared favorably with the smoke evaluation generated from the cigarettes prepared from the pre-purchased hogsheads. For the final correlations, we used the latest strength data generated on January 20, 1981. These data are presented and compared to the values assigned to the pre-purchased cigarettes in Table 2.

In the following section, the experimental data will be presented and discussed. Each measured independent variable was computer correlated with the observed aforementioned strength rating. A correlation was deemed significant if the correlation coefficient (R^2 max = 1.00) was greater than or equal to 0.5. A listing of experimental variables for all of the data and their respective correlation coefficients was compiled in Table 3 for the convenience of the reader.

EXPERIMENTAL INVESTIGATIONS

Leaf Analysis Data

Leaf analysis data were correlated with the strength ratings assigned to the B3FR burley cigarettes. Leaf nicotine did not appear to correlate with strength ($R^2 = 0.17$, linear). The maximum correlation coefficient was observed for strength versus percent leaf chloride ($R^2 = 0.63$, linear). By observation of the data, it is apparent that leaf data alone cannot explain the observed

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deviations of smoke strength. Since our data set is somewhat limited, our confidence in the significance of chloride content will have to be extrapolated to a greater data base. Because the leaf nicotine did not relate as well to strength as we would have believed, we decided to explore the mechanisms of strength that may be related to free and bound leaf and smoke alkaloids. Earlier studies have indicated that strength modifications occur upon altering smoke pH.[2] Smoke pH has been related to the ratio of free (unprotonated) and bound (protonated) nicotine in tobacco smoke by a pH dependent equilibrium equation.[3]

Free/Bound Leaf Alkaloids, Smoke pH, Smoke Nicotine

A simple method was developed utilizing a methylene chloride wash that would remove the free alkaloids on the tobacco leaf. The tobacco was submitted for alkaloid analyses before and after solvent extraction. The percentage of free nicotine removed from the leaf was calculated and correlated with the strength ratings. The correlation coefficient was calculated to be 0.41 ($y = ax + b$). It should be noted that free leaf alkaloids correlate considerably better than did total leaf alkaloids. The quantity of free smoke nicotine should be related to the smoke pH and perhaps the perception of strength is related as well. Multiple smoke pH determinations of our cigarettes were made by Dr. A. M. Ihrig and revealed no significant differences between samples. The range of smoke pH for the burley samples was 7.88 - 8.06. Utilizing a computer program, we related smoke pH and free smoke nicotine in order to calculate the percentage and quantity of free nicotine in the smoke of each cigarette. Although it appears that smoke pH correlates reasonably well, it should be noted that this occurs because of small pH changes (0.1 unit) which are within experimental error of the method. A redetermination of smoke pH of the B3FR cigarettes confirmed the inherent variability of the method. Smoke nicotine correlated better than did leaf nicotine exhibiting a correlation coefficient of 0.52.

Total Alkaloid Profiles

Another aspect of our research regarding the burley tobacco alkaloids involved gas chromatographic profiling and quantitation of the major tobacco alkaloids on our burley samples. We were interested in determining if total alkaloid composition varied among our samples. A previously published method was used to extract the alkaloids from the tobacco leaf.[6] Subsequent gas chromatographic analysis was used to profile each tobacco alkaloid. The alkaloid profile of each sample was observed to be fairly constant. Nicotine constitutes the overwhelming contribution to the total alkaloid fraction. Although the three minor alkaloids (nornicotine, myosmine, anabasine) are somewhat more variable, it does not appear that their presence exerts any appreciable influence upon the strength characteristics of the burley samples.

Indole-Skatole and Smoke Myosmine Studies

In a previously published report, Bell reported a relationship between smoke indoles and strength for two burley samples.[5] During our investigation, we felt it necessary to explore this avenue of study. We were also interested in determining whether smoke myosmine played a role in the mechanism of strength.

Indole and skatole were isolated from smoke condensate by a procedure published by Hoffman and Rubin.[6] Sixteen cigarettes were smoked per analysis and their condensate was collected in cooled traps. C^{14} labelled indole was used to calculate the efficiency of indole extraction. There does not appear to be a significant correlation between strength and indole/skatole content but smoke character exhibited a maximum correlation coefficient of 0.61 for the equation $y = a + \frac{b}{x}$. Additional indole studies will be carried out in the Burley Character project in order to determine the contribution to character.

Smoke myosmine was determined by using a method by Saint-Jalm and Testa.[7] We were aware that myosmine is pyro-synthesized by the destruction of nornicotine during smoking. This was confirmed by the absence of nornicotine in the smoke condensate. Gas chromatographic analysis of the resultant extract confirmed the pyro-synthesis of myosmine. In some burley samples, the smoke condensate contained significantly greater quantities of myosmine than would be expected based upon previously calculated quantities of leaf myosmine. Correlations of these quantities with observed strength did not reveal a significant relationship.

Atomic Absorption Spectroscopy - Calcium

In our early discussions regarding potential factors that could influence smoke strength, Mr. H. E. Smith felt that the strength of some burleys was controlled by the amount of calcium in the leaf. Such examples are the ones grown in Kentucky limestone regions. These burleys usually exhibit a mild strength compared to those that are grown in low calcium containing soils. We analyzed the B3FR burley tobaccos for calcium using a previously published procedure.[8] The values ranged from 4.1 to 5.5% calcium. Based upon a simple regression analysis, no significant relationship between calcium content and strength was discovered.

Cigarette Burn Rate

The static burn rate of each burley cigarette grade sample was measured by a previously published procedure.[9] The basis for this experiment was to determine whether any chemical or physical burn accelerators could be responsible for actual changes in strength.

The procedure involved the measure of cigarette weight loss over a predetermined interval. Multiple determinations were made for each sample. The data revealed significant differences between the burn rates of the grade samples. Subsequent correlation with strength data indicated no significant relationship. Yet, the importance of burn rate and its effect upon certain aspects of smoke chemistry should not be overlooked. Such parameters will be evaluated during the burley character studies.

Smoke Nicotine Transfer Rate

The basis for impact or strength undoubtedly includes smoke nicotine. Yet the quantity or amount that is required to give sufficient strength is debatable. If follows that if sufficient nicotine transfers to the mainstream smoke then the cigarette should exhibit strength whose degree of perception is governed by a number of unknown variables. A comprehensive smoke nicotine study was initiated in order to determine if a relationship between strength and smoke nicotine exists. Surprisingly, no significant relationship exists between smoke nicotine transfer rate and perceived smoke strength.

Ionic Conductivity

During our investigation, we studied the role of ionizable species on the leaf and attempted to relate the data to strength. An aqueous extract of each burley leaf was obtained and the resistance of the solution was measured with a Wheatstone Bridge. The reciprocal of this measurement is regarded to be conductance. Within experimental error, all of the burley samples exhibited the identical ionic conductivity. No significant relationship was therefore observed.

Gas and Organic Vapor Phase Analysis

In the first phase of our study, we studied parameters such as leaf data and smoke nicotine. Additional analyses were centered around these variables. The second phase of this work involved the study of the gas and organic vapor phase components. The analyses were conducted by Dr. A. M. Ihrig upon conditioned machine made cigarettes. A number of significant correlations are reported. Strength correlated linearly with acrolein ($R^2 = 0.74$) and acetone ($R^2 = 0.71$) contents. 2-Methylfuran also linearly correlated exhibiting a correlation coefficient of 0.55. The components comprising the permanent gas phase such as oxygen, nitrogen and carbon dioxide did not correlate with strength. The significance of these correlations for other burley samples will be revealed upon expansion of our data base. For the present, we will be able to incorporate the aforementioned significant organic phase data into a final multiple regression equation.

Organic Acid Analyses

Burley tobaccos unlike flue-cured, do not exhibit significant quantities of total reducing sugars. This particular feature along with elevated nicotine and nitrogen contents tends to make burley tobacco a harsher, stronger smoke than flue-cured. Burley tobaccos do contain significant quantities of oxalic, malic and citric acids. It has been demonstrated that addition of malic acid to an off-shore burley was successful in significantly reducing smoke strength.[10] We opted to analyze the quantity of organic acids on our burley samples to determine whether the acids play a significant role in the regulation of strength.

The sample set we were studying exhibited a leaf nicotine range from 3.5 - 4.1%. The low strength sample (S = 6.5) exhibited a nicotine value comparable to the samples with a strength rating of 8.5. The determination of organic acids seemed to be a logical path of investigation. The samples containing significantly higher quantities of organic acids with similar nicotines might exhibit a lower strength rating.

A procedure developed by Shoffner was used to extract, esterify and analyze oxalic, malic and citric acids from tobacco.[11] The tobaccos were pulverized prior to drying at 100°C for twenty four hours. Gas chromatographic analyses of the resultant esterfied extract was used to quantitate the acids. The data revealed a significant relationship between total organic acids and smoke strength. The burley sample containing the higher quantities of organic acids possessed the lower strength rating. The major organic acid on burley was citric followed by oxalic acid and malic acid. To further extend the correlation coefficient, a ratio was developed that incorporated leaf nicotine which took the form (% leaf nicotine/% oxalic + citric acid). The malic acid contribution was not included since its simple correlation coefficient versus strength was only 0.36. The previously mentioned ratio exhibited a correlation coefficient of 0.90 for the linear equation of $y = ax + b$. Such a strong relationship appears reasonable since such a relationship implies an acid-base balance effect. The natural buffering effect of tobacco could allow a range of organic acids to exist on tobacco without altering the strength characteristics. Once the buffering capacity has been exceeded, the strength could be affected. This point is well illustrated by the B3FR sample No. 1465. The tobacco contains approximately 18% total organic acids which is 5 to 7% more than the other B3FR samples with similar leaf nicotine values. It is possible that the buffering capacity of 1465 was exceeded and the smoke strength was lowered.

An experiment was performed to confirm the observation. A high strength B3FR tobacco (1464) was sprayed with approximately 6% total organic acids. The acid level was elevated to approximately 17%.

Cigarettes for smoking evaluation were prepared on the hand maker and were submitted to Mr. Terry Jessup for evaluation. The smoking results confirmed our expectations. A drastic lowering of the smoke strength was observed. The adjusted strength value was reported to be approximately 6. This value is in line with the strength rating assigned to sample 1465 which exhibited 18% total organic acids. Mr. Jessup also noted character changes which is reasonable. Character and strength values for B3FR burley were discovered to linearly correlate with a coefficient of 0.65 ($y = ax + b$). Alterations of smoke strength should therefore alter the taste character as well. The interrelationship of character, strength, nicotine and organic acids should provide a sound basis for additional research involving our domestic burley tobaccos that we presently use.

Multiple Regression Analysis - Correlation of Data

In such a study where large amounts of data are generated for a small set of data, the statistical validity of the data is of importance. Usually, a statistically valid experiment involves using a sufficiently large set of samples. In our case, we wished to pick a limited set of samples that represented the properties we wanted to study. In this case, this would be tobacco samples exhibiting similar nicotine values but with fluctuating strength. Thus, we did not desire leaf nicotine values to correlate with smoke strength. By imposing this limitation, our sample domain was limited somewhat. Product Development wanted to supply us with samples that were anomalous with respect to strength. By elucidating those factors other than leaf nicotine that contributed to the control of smoke strength on B3FR burley tobacco, we felt the mechanism of strength for the burley samples such as those used in our blend or off-shore burleys would be elucidated.

In our data analysis, each measured variable was correlated with observed smoke strength using a computer program on the Tektronix 4051. A correlation coefficient (R^2) is generated which measures the data degree of "fit" with several equations. We chose a regression coefficient of 0.5 to represent a significant correlation in this instance. We measured the degree of variance of our data sets by carrying out duplicate or triplicate measurements. In some instances a significant regression ($R^2 \geq 0.5$) had to be disallowed due to flux in the measurement of the variable. Usually this variability is due to the non-homogeneity of the tobacco sample. The significant variables with an acceptable variance range were used to develop a set of multivariable equations capable of predicting strength for the B3FR burley samples based upon leaf and smoke data. These computer derived equations are reported in Figure 1. The multiple correlation coefficient is reported for each equation.

The primary feature in these equations is the interaction between nicotine and the organic acids. Such an acid-base relationship is no doubt significant in this instance. Certain variables

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deemed significant were not included in the multivariable expression due to their dependence on the selected variables. We can also utilize the organic phase analysis data of acrolein and 2-Methylfuran which correlated well. In most instances, one does not expect to discover a single parameter which relates smoke behavior. Usually such a relationship is a multifaceted one that incorporates additional independent variables.

The statistical validity of the equations described in Figure 1 is of importance. Such an equation adequately explains the behavior of the B3FR burley tobaccos under investigation. In order for such an equation to universally explain the mechanism of strength for a majority of samples, the existing data base will have to be expanded. This particular study enabled us to successfully determine the aberrations of strength behavior in certain samples. As the sample data base is increased, we expect to discover additional characteristics that will explain the prediction and perception of strength in domestic and eventually off-shore burley tobaccos.

As the domestic burleys are added to the existing data base, we might expect to see a better correlation of leaf nicotine and strength. This trend follows from a low stalk (LS) to a high stalk (#HH). The utility of a prediction equation would be to differentiate between subsets of a similar stalk region exhibiting similar nicotine levels. Samples such as these could exhibit significant differences of other leaf or vapor phase components. Any significant deviation from that relationship could be compensated by the quantity of organic acids or other components on the tobacco which would be incorporated into the prediction equation. The present equation used to predict strength for the B3FR burley tobaccos would thus be refined and enlarged so as to adequately profile smoke strength.

As mentioned previously, we hope to develop a universal equation system capable of predicting relative smoke strength. Such a system might include all domestic burleys and appropriate variations of the equation could be capable of integrating the resultant character changes. The interrelationship of character and strength will be further explored in the Burley Character project B-418. If successful, our work will also include off-shore burleys whose modifications and subsequent usage would be a substantial cost savings.

CONCLUSIONS/SUMMARY

The mechanism of smoke strength for a set of B3FR burley tobaccos was successfully elucidated. Although leaf nicotine was not a significant factor for prediction of strength, the quantities of oxalic and citric acids were discovered to be primary contributing factors. Acrolein and 2-methylfuran from the organic vapor phase were discovered to significantly correlate with smoke strength as well. A set of multiple regression equations were developed that accurately predicted the strength of the B3FR cigarette samples based upon leaf and smoke chemistry. The basis of this equation will be utilized to study the interrelationship of character and strength for a set of domestic and off-shore burley samples. As the data base is expanded, the strength prediction equation will become more universal and should allow one to predict relative strength within a selected band of burley tobaccos. Present studies include domestic and off-shore burley tobaccos whose differences in smoke strength may be attributed to the content of organic acids.

REFERENCES

1 C. W. Kelley to A. B. Hudson, "Organoleptic Correlation of Burley Tobacco" (September 28, 1974).

2 Paul D. Schickedantz, Lorillard Report Accession No. 994, "Gas Phase Ammoniation of Tobacco" (April 14, 1977).

3 G. P. Morie, Tobacco Science, 16, 167 (1972).

4 J. J. Piade, J. D. Adams and D. Hoffman, "Alkaloids and Non-Volatile N-Nitrosaminesof Tobacco and Smoke of Dark Cigarettes," 33rd Tobacco Chemists' Research Conference, Lexington, Ky. (October 29-31, 1979).

5 J. H. Bell to C. I. Lewis, "Comparison of the Indole-Skatole-Carbazole Fraction Among Flue-Cured, Cased Burley and Uncased Burley Condensate" (February 28, 1972).

6 D. Hoffman and P. Rubin, Beitrage zur Tabakforschung, Band 3, Heft 6 (September, 1966).

7 Y. Saint-Jalm and P. Moree-Testa, Journal of Chromatography, 198, 188-192 (1980).

8 R. W. Dale, "The Determination of Ca, Mg and Other Trace Metals in Tobacco Using Atomic Absorption Chromatography," R & D Division, Gallagher Limited, Belfast, Ireland (1970).

9 R. L. Rice, R. S. Perrier, V. C. Runickles, "A Weight Loss Technique for Determining Rate of Static Burn," Tobacco Science, 14, 173 (1970).

10 Lorillard Research Center Notebook Entry CSC-37-18.

11 Rose A. Shoffner and Jimmy H. Bell, Lorillard Report Accession No. 1214, "An Improved Gas Chromatographic Method for the Determination of Oxalic, Malic and Citric Acid in Tobacco as Their Methyl/Esters" (June 15, 1978).

Figure 1.

Development of a Multivariable Smoke Strength Equation for B3FR Burley Cigarettes

(1) Strength Equation Using Leaf Data

(2) Strength Equation Using Smoke Chemistry Data

(3) Combined Equation (Leaf & Smoke Data)

(1) <u>Strength</u> = 11.18(x4) - 8.12(x5) - 0.28(x9) + 11.21 $R^2 = 0.943$

(2) <u>Strength</u> = - 0.025(x6) + 0.046(x7) + 0.008(x8) - 0.659 $R^2 = 0.987$

(3) <u>Strength</u> = 10.98(x4) + 0.085(x7) + 0.35(x9) - 9.62 $R^2 = 0.996$

x4 = % Leaf Nicotine ÷ % (Oxalic + Citric) Acid

x5 = % N ÷ % (Oxalic + Citric) Acid

x6 = conc. 2-MeFuran (μg/cig.)

x7 = conc. Acrolein (μg/cig.)

x8 = conc. Acetone (μg/cig.)

x9 = % Total Organic Acids

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Table 1.

Smoke Strength and Character Ratings of Composite Pre-purchased Handmade B3FR Burley Cigarettes

Sample No.	Character	Strength
26389	4.5	7
	5.5 (5.2 avg.)	6 (7.8 avg.)
	5.5	10.5
26405	4.5	6.0
	5.5 (4.8 avg.)	7.5 (6.5 avg.)
	4.5	6.0
40351	5.0	11
	5.5 (5.3 avg.)	6 (7.8 avg.)
	5.5	6.5
41159	6.0	10
	6.0 (6.0 avg.)	8.0 (9.2 avg.)
	6.0	9.5
44082	5.0	6.0
	6.5 (5.8 avg.)	10.0 (8.0 avg.)
	6.0	8.0

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Table 2.

Average Strength Ratings of Glycerine Cased Machine Made Non-Filter B3FR Cigarettes

Hogshead No.	PD No.	Strength (avg.)	Strength (avg.) reevaluated January 1981
26389	1464	8.5	8.5
26405	1465	6.5	6.5
40351	1466	7.0	8.5
41159*	1467	5.5	9.0
44082	1468	8.5	8.5

* Discrepancy with strength value assigned to pre-purchased sample (see Table 1)

Note: When January 1981 strength evaluations are correlated with the values obtained from the pre-purchased hogshead samples (Table 1), a good correlation ($R^2 = 0.82$) resulted.

Table 3.

Leaf Data Versus Smoke Strength
Simple Regression Analyses

Independent Variable	Max. Correlation Coefficient	Equation
Leaf Ash	0.47	(1)
Leaf Alkaloids	0.16	(1)
Leaf Nitrate	0.34	(2)
Leaf Nitrogen	0.001	(1)
Protein Nitrogen	0.08	(1)
Leaf pH	0.001	(1)
Total Volatile Bases	0.03	(1)
Water Soluble Nitrogen	0.28	(3)
Chloride*	0.65	(2)
Free Leaf Nicotine	0.45	(3)
Smoke pH	0.75[a]	(3)
Smoke Nicotine*	0.53	(3)
Leaf Nicotine	0.17	(2)
Leaf Nornicotine	0.05	(3)
Leaf Myosmine	<0.10	(1)
Leaf Anabasine	0.69[a]	
Indole & Skatole	<0.5	(1)
Smoke Myosmine	0.03	(3)
Calcium (%)	0.06	(1)
Burn Rate (sec.)	0.001	(1)
Oxygen	0.03	(3)
Nitrogen	0.05	(1)
Carbon Monoxide	0.005	(1)
Carbon Dioxide	0.09	(3)

Table 3.

Leaf Data Versus Smoke Strength
Simple Regression Analyses
(continued)

Independent Variable	Max. Correlation Coefficient	Equation
Nitric Oxide	0.01	(3)
Hydrogen Cyanide	0.02	(1)
Isoprene	<0.5	(1)
Acetaldehyde	<0.5	(1)
Acetone*	0.75	(4)
Acrolein*	0.77	(1)
2-MeFuran*	0.55	(1)
Methyl Ethyl Ketone	<0.5	(1)
Benzene	<0.5	(1)
Acetonitrile	<0.5	(1)
Toluene	<0.5	(1)
Oxalic Acid*	0.70	(3)
Malic Acid	0.36	(3)
Citric Acid*	0.82	
Total Organic Acids*	0.78	(5)
% Leaf Nic./Oxalic + Citric**	0.90	(1)
% Leaf Nic./Total Organic Acids*	0.84	(3)

[a] Due to data variability; non-significant
* Denotes significant variable ($R^2 \geq 0.5$)
** Denotes maximum R^2 value

Equation Legend

(1) $y = Ax + b$
(2) $y = Ae^{bx}$
(3) $y = A + \frac{b}{x}$
(4) $y = \frac{1}{A + Bx}$
(5) $y = \frac{x}{A + Bx}$

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