CHARACTERIZATION OF OXIDATIVE BROWNING IN FLUE-CURED TOBACCO I. A METHOD FOR EVALUATING VARIETAL DISCOLORATION POTENTIALS[1]

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A method was developed and tested for characterizing oxidative browning in tobacco during curing, utilizing standardized procedures. Nearly identical cultural practices and curing conditions were imposed on three varieties of **Nicotiana tabacum** L. (Golden Wilt, Yellow Special A and Coker 139) to permit evaluation of color change phenomena as a variable of variety and leaf position. Oxidative browning was studied **in vivo** by thermally initiating the reaction at 80-85° C and allowing it to proceed at high leaf moisture content at 25° C. Tristimulus colorimetry was used to establish initial yellow color (Y_i), final brown color (Y_e), color change (ΔY) and browning rate constant (k). Both Y_i and Y_e varied among varieties and decreased for higher leaf positions. The browning rate constant (k) varied among varieties and increased by about 100% between bottom and top leaf positions. Polyphenol concentrations decreased by 85% during oxidative browning, implying a means for rapid and extensive reduction of this class of constituents, if desired. This method appears useful in quantifying effects due to varieties, cultural practices, environmental factors, and process conditions.

INTRODUCTION

One of the most important and observable changes during curing is that of color. The importance of this single factor is evidenced by the fact that leaf quality is to a great extent evaluated by color. The gradual change in color from the green-yellow at harvest, to yellow-orange for flue-cured, and on to uniform brown for air-cured, gives a direct visual indication of the stage of curing.

Unfortunately, in flue-cured tobacco oxidative browning is undesirable and lowers the apparent quality and market price. The discoloration is generally considered to be indicative of mismanagement during curing or of unfavorable growing conditions. For example, prolonged yellowing under high moisture conditions may enhance browning; furthermore, tobacco grown under high levels of nitrogen and abundant water supply may exhibit greater discoloration. Varieties may also differ in their innate genetic characteristics regarding browning, since it is more difficult to manage some varieties for the production of desirable cured leaf colors.

Varietal evaluations for discoloration during curing are generally inconclusive because of variability in results. Trends which at one time appear significant may later reverse to produce conflicting results. This variability may be expected, however, since curing introduces many variables with respect to temperature and drying rate of individual leaves as a result of spatial location within the curing chambers, proximity to heating units, etc. These factors may interact directly with the initiation and rate of browning and will no doubt contribute to overall variability. Furthermore evaluations are generally subjective and not based on fundamental response criteria.

The purpose of this investigation was twofold: (1) to develop criteria for characterization of oxidative browning in tobacco; and (2) to develop and test a method which involves a standardized procedure for evaluating varietal discoloration potentials.

LITERATURE REVIEW

Many investigators have studied the nature of oxidative browning in tobacco. The enzymatic oxidation of polyphenols (and particularly o-diphenols) produces the yellow-to-brown color changes characteristic of natural air curing (Frankenburg, 3; Asmaev and Yunoslev, 1; Shiroya *et al.*, 9; and Zucker and Stinson, 14). Penn and Weybrew (7) and Weaving (13) reported that principal polyphenols include chlorogenic acid, neochlorogenic acid, rutin, scopolin, and scopoletin.

The influence of temperature on the browning reaction was studied by Hassler (4), who measured leaf temperature with a thermocouple embedded within the leaf lamina. The reaction was slow and imperceptible below 44° C; however, at 57° C the reaction became sufficiently rapid to produce total discoloration within 6 minutes.

The dependence of the reaction at elevated temperatures on molecular oxygen was demonstrated by Watkins and Hassler (12) who found discoloration to be inhibited at reduced oxygen levels.

Enzymatic browning of plant tissues at elevated temperatures has been postulated to result from thermal injury which allows the oxidative enzymes, substrates, and oxygen to intermingle within the cell (Thomas *et al.*, 11). Alterations in the physical structure of the protoplasm are considered to remove obstacles that normally prevent free diffusion of suitable substrates, dissolved oxygen, or enzymes. Frankenburg (3) noted that as long as the cell membranes are intact, the phenols and oxidases remain separated, and no oxidation occurs.

Under specified conditions, the browning rate is critically influenced by the substrate concentration and activity of the oxidative enzyme. Penn and Weybrew (7) found that polyphenol concentration increased with

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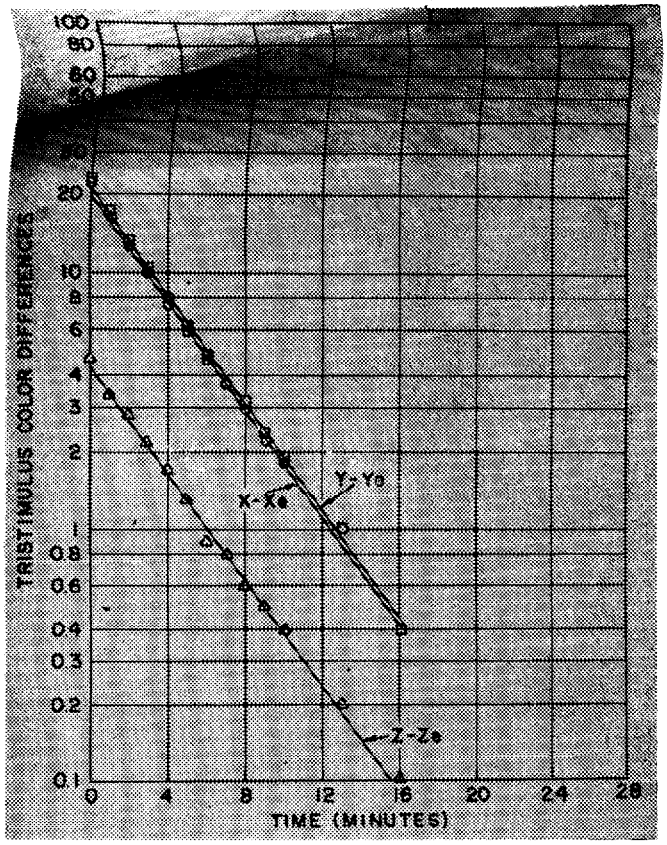

Figure 1—Exponential response of color parameters (Y-Y_e, X-X_e, Z-Z_e) with time.

Table 1. Influence of variety and leaf position on browning rate constant (k), initial color (Y_i), final color (Y_e) and color change ($\triangle Y$). Values within the table are averages over 80 replications.

	Variety[a]	Leaf Position 2	5	8	11	14	17	Means
Browning rate Constant (k)	1	.248	.319	.286	.348	.406	.457	.344
	2	.241	.238	.283	.313	.353	.454	.317
	3	.234	.241	.413	.363	.416	.475	.357
Means		.247	.266	.328	.341	.391	.462	
LSD (.01) = .03								
Initial Color (Y_i)	1	32.5	33.2	30.4	28.9	28.3	24.7	29.7
	2	30.8	32.1	29.9	28.0	27.0	23.1	28.5
	3	33.9	33.7	31.7	31.4	30.5	27.6	31.5
Means		32.4	33.0	30.6	29.5	28.6	25.2	
LSD (.01) = .42								
Final Color (Y_e)	1	12.9	12.0	10.7	9.1	8.3	7.8	10.1
	2	11.7	11.3	9.9	8.9	7.9	7.3	9.5
	3	14.9	14.4	12.0	11.1	9.6	8.6	11.7
Means		13.2	12.6	10.9	9.7	8.6	7.9	
LSD (.01) = .26								
Color Change ($\triangle Y$)	1	19.6	21.2	19.7	19.8	20.0	17.0	19.5
	2	19.1	20.7	20.0	19.1	19.1	15.8	19.0
	3	19.0	19.3	19.7	20.4	20.9	19.1	19.7
Means		19.3	20.4	19.8	19.8	20.0	17.3	
LSD (.01) = .46								

[a] *Variety 1: Golden Wilt*
2: Yellow Special A
3: Coker 139

maturity. Sisler and Johnson (10) reported o-diphenol oxidase to be significantly affected by leaf temperature and moisture content during curing. Activity was reduced at progressiveiy higher temperatures above 60° C and at higher levels of moisture content. Ishitoya and Matsuyama (5) also noted that the activity was influenced by the maturity of the leaf.

CRITERIA FOR OXIDATIVE BROWNING

The argument herein advanced is that oxidative browning should be characterized by parameters consistent with reaction rate theory. Normally, reaction kinetics are described by a specific rate constant, k, which expresses a proportionality between the reaction velocity and concentrations of the reactants (Prutton and Maron, 8). For simple uncatalized reactions, k is a constant which characterizes the particular reaction for a given temperature and is independent of concentration.

This situation becomes more complex for enzymatic reactions where an enzyme-substrate complex is formed which with further activation and reaction proceeds to form reaction products. The well-known Michaelis-Menton equation (Casey, 2) expresses the fact that reaction velocity depends upon both substrate and initial enzyme concentrations.

For tobacco leaves, it is proposed that the reaction velocity of oxidative browning can be studied on the basis of tristimulus colorimetry for intact leaf tissue undergoing browning. The method necessitates initiation of the reaction in yellow leaves followed by observation of color change with time. Johnson (6) found that oxidation of o-diphenols occurred as a first-order reaction, and that a linear relationship existed between the concentration of o-diphenols and colorimetric values. A mathematical relation was derived:

$$Y - Y_e = (Y_i - Y_e)e^{-kt} \qquad (1)$$

where Y is a color value during the reaction, Y_e is the equilibrium color value after completion of the reaction, Y_i is the color value of the yellow leaf at zero time, and t is time. **Figure 1** shows a plot of tristimulus color values ($X - X_e$, $Y - Y_e$, and $Z - Z_e$) with time, which indicated an exponential decrease in color as the reaction proceeded. The specific rate constant in the first-order oxidation reaction was the same constant k of equation (1). Hence, colorimetric determinations provide a method for evaluating the specific rate constant. However, it is emphasized that k is a constant only for a given sample under defined conditions. Variation of k among samples tested under defined conditions should therefore be indicative of differences in enzyme activity or of other factors influencing browning rate.

The colorimetric method also provides additional information important to characterizing the reaction. Y_i gives an initial color value which shows the lightness or brilliance of the yellow color and which may be related to maximum cured leaf color. Y_e, on the other hand, is indicative of maximum discoloration, since at this point the reaction is complete. Values of k, Y_i, and Y_e may be properly termed "discoloration potentials," since they may be correlated with factors which determine the rate and extent of the reaction.

EXPERIMENTAL METHODS

Throughout the experiment, care was given to producing test material having nearly identical growth and processing conditions in order that the measured color responses would be indicative of relative discoloration characteristics for the varieties tested.

Three varieties of tobacco (*Nicotiana tabacum* L.) which were considered to possess a range of browning characteristics were selected: (1) Golden Wilt, (2) Yellow Special A, and (3) Coker 139.

Plants were banded and selected for uniformity prior to transplanting and all plants were transplanted to the

field plots on the same day. One hundred and 50 plants per variety were used to provide adequate replication. A replication consisted of 3 plants, one of each variety. The planting order within each replication was chosen at random. At the time of harvest 80 replications of the most uniform plants were selected for the experiment.

Normal fertilization and cultural practices were followed during growth of the plants. Plants were suckered by hand to prevent any possible interaction with sucker control agents.

Preparation of plants prior to harvest was made to ensure identical leaf number per plant. Each plant was topped to 18 harvestable leaves when the first floret was open and in the pink stage. Bottom plant-bed leaves were removed to provide a uniform starting point for the first harvestable leaf at the bottom of the plant.

Tobacco was harvested at approximately weekly intervals to obtain normally maturing tobacco. Beginning at the bottom of the plants, the following leaves were tagged to indicate variety, plant number and leaf number: leaf numbers 2, 5, 8, 11, 14, and 17. For a given harvest the lowest tagged leaf was harvested from each plant, and the two adjacent leaves were discarded. Over a 6-week period this gave representative leaves from 6 stalk positions (with 3 leaves per position) from the bottom to the top of each plant. Leaves were strung individually on sticks to give around 60 leaves per stick.

Process Treatments Prior to Browning

Metabolic conversions of the coloring phase (yellowing) were accomplished under controlled conditions of 35° C dry bulb and 32° C wet bulb. The coloring time was approximately two days; however, adjustments were made for each stalk position to obtain essentially complete breakdown of the chlorophyll with maximum yellow color.

Near the end of coloring, the leaves were placed upright in aluminum trays with the petioles immersed in water to produce a state of turgor in all leaves. This served to minimize differences in leaf moisture among leaves and to establish a leaf condition whereby the browning reaction was enhanced upon initiation.

Following coloring and turgor conditioning, initial color measurements, Y_i, were made by using a Gardner tristimulus colorimeter[3] (model AC-2A-C.I.E.). A reflectance standard was utilized having tristimulus values approximating flue-cured tobacco, *i.e.* X = 26.1, Y = 27.6, and Z = 6.3 (Gardner, No. MY0090). Two circular leaf disks, 2 inches in diameter, were removed from opposite sides of the midrib of each leaf at diagonal positions from the lower two-thirds of the leaf. Color values, Y_i, for these disks established the initial yellowness or brightness prior to discoloration.

Initiating and Measuring the Browning Reaction

It is to be emphasized that the procedure at this point was designed to permit maximum browning under optimum conditions, and in such a way that each variety could completely "express" its discoloration tendency. This was accomplished by thermally triggering the reaction in yellow, turgid leaves and then permitting the reaction to occur under standard conditions.

The yellow leaves were suspended in mounting frames of a continuous infrared dryer and subjected to an infrared thermal treatment as described by Johnson (6). In a 20-second treatment, the leaf temperature of each leaf was elevated to approximately 80 to 85° C to initiate the reaction. Calibration tests were made to establish this time as an effective thermal treatment for maximum browning. Each leaf, passing through the apparatus, received the same thermal treatment. The beginning time for the treatment was recorded for each leaf and defined as the zero time for describing the browning reaction. After initiating browning, leaves were allowed to brown at room temperature of around 25° C.

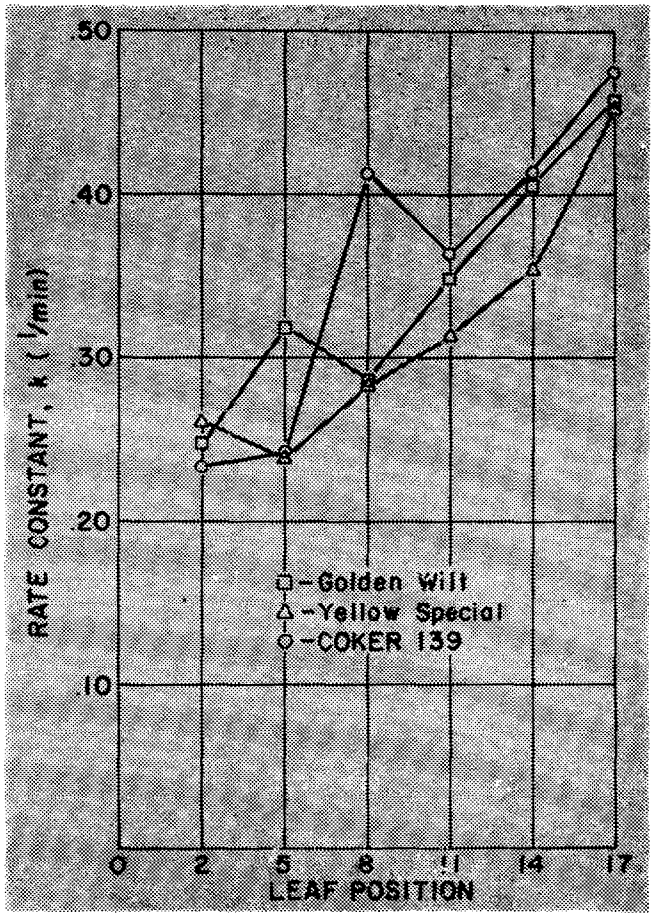

Figure 2—The effects of variety and leaf position on the browning rate constant, k.

At about 5 to 10 minutes after the thermal treatment, two disks of tobacco from positions comparable to those of the yellow samples were removed from each leaf. The intermediate color values, Y_t, were determined and the time recorded. At any time after 30 minutes, color values, Y_e, were measured for these same disks to establish the equilibrium or terminal color values. Previous work has established the reaction to be complete in most cases at 30 minutes or less. From data of Y_i, Y_t, and Y_e, the rate constant k was determined for each leaf.

Tobacco disks, from which Y_i (yellow) and Y_e (brown) color values were made, were freeze-dried and later evaluated for total polyphenol concentration by a cooperating tobacco company. This gave important data for correlations with the browning data.

RESULTS AND DISCUSSION

The procedure for evaluation consisted of examination of the effects of variety and leaf position on the browning rate constant k, initial color Y_i, final color Y_e, and color change ΔY. Furthermore, on the basis of polyphenol concentration data, inferences were obtained

[3] *Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.*

Table 2. Analyses of variance for the study on characterization of oxidative browning in tobacco.

Measured Variable	Source of Variation	Degrees of Freedom	Mean Square	F
Browning Rate Constant (k)	Variety (A)	2	0.201	12.25**
	Leaf Position (B)	5	1.525	93.02**
	Replication (C)	79	0.029	1.74**
	A x B	10	0.116	7.10**
	A x C	158	0.018	1.08ns
	B x C	395	0.020	1.24*
	Error	790	0.016	
Initial Color (Y_i)	Variety (A)	2	1081.744	331.41**
	Leaf Position (B)	5	1961.245	600.84**
	Replication (C)	79	7.090	2.17**
	A x B	10	31.125	9.51**
	A x C	158	4.431	1.36*
	B x C	395	4.120	1.26*
	Error	790	3.260	
Final Color (Y_e)	Variety (A)	2	624.231	524.35**
	Leaf Position (B)	5	1084.746	911.19**
	Replication (C)	79	2.078	1.75**
	A x B	10	10.494	8.81**
	A x C	158	2.003	1.68**
	B x C	395	1.239	1.04**
	Error	790	1.186	
Color Change (ΔY)	Variety (A)	2	71.889	18.68**
	Leaf Position (B)	5	295.014	76.64**
	Replication (C)	79	8.130	2.11**
	A x B	10	66.710	17.32**
	A x C	158	4.808	1.25**
	B x C	395	5.075	1.32**
	Error	790	3.845	

** *Significant at the .01 level.*
* *Significant at the .05 level.*
ns *Not Significant at the .05 level.*

Table 3. Polyphenol content[a] of tobacco before and after the browning reaction: comparison of varieties and leaf positions.

Variety:	1 Golden Wilt		2 Yellow Special A		3 Coker 139	
Leaf	mg polyphenol/g		mg polyphenol/g		mg polyphenol/g	
Position	Before	After	Before	After	Before	After
2	44.0	6.0	42.6	5.5	33.7	5.4
5	54.9	6.3	55.7	8.3	40.3	5.4
8	53.6	8.9	59.9	8.5	44.0	5.9
11	61.1	8.0	61.8	7.9	41.9	5.7
14	63.6	8.3	59.4	8.1	45.0	5.7
17	51.8	11.8	51.2	10.7	46.7	8.5
Average of 6 leaf positions	54.8	8.2	55.1	8.2	41.9	6.1
Change		-85.0%		-85.1%		-85.4%

[a] *Expressed as equivalent mg of chlorogenic acid*

between the level of substrate concentration and the browning parameters.

Table 1 summarizes the data of the major test showing the influence of variety and leaf position on the various browning parameters. Analyses of variance for the study are given in **Table 2.**

The Browning Rate Constant, k.

Both variety and leaf position produced large variations in the rate constant and tested highly significant at the .01 level of probability. Varietal means gave k values of .344 for Golden Wilt, .317 for Yellow Special A, and .357 for Coker 139. The larger the k value, the more rapid the reaction rate per unit of substrate concentration. This, however, does not imply the extent of color change associated with the reaction, which depends rather on substrate concentration levels available for reaction. Furthermore, the rate constant should be independent of substrate concentration, temperature, or factors which affect the degree of intermingling of enzyme with substrate. The fact that k is larger for a given variety therefore may imply a greater relative concentration of enzyme to substrate.

The effect of the leaf position was striking. Average values of k increased from .247 at leaf position 2 to .462 at leaf position 17. This magnitude of increase was unexpected but is also suggestive of larger enzyme concentrations for tobacco leaves higher on the plant. **Figure 2** shows the effects of variety and leaf position on k. A variety x leaf position interaction can be noted in the figure.

Initial Color, Y_i.

The initial yellow color prior to browning was found to be an important index to the characterization of the browning process. This will become apparent later in the discussion. Both variety and leaf position produced large variations in the initial color and tested "highly significant" at the .01 level of probability. Varietal means gave initial color values of 29.7 for Golden Wilt, 28.5 for Yellow Special A, and 31.5 for Coker 139 **(Table 1).** Throughout the study, it was apparent that the initial color of Coker 139 was brighter and clearer than for the other varieties.

The effect of leaf position was that of decreasing initial color with increasing level on the plant. The overall results gave Y_i of 32.4 at leaf position 2 and decreasing values to 25.2 at leaf position 17. **Figure 3** shows the effects of variety and leaf position on color values Y_i and Y_e. The trends for the three varieties were similar but at distinctly different levels. Variation in Y_i with leaf position was considered to result from variation in underlying pigment concentrations of carotene, xanthophyll, and other pigments.

Final Color, Y_e.

As with Y_i, the results showed that both variety and leaf position significantly influenced the values of Y_e, the color after completion of the browning reaction. Varietal means gave values of 10.1 for Goden Wilt, 9.5 for Yellow Special A, and 11.7 for Coker 139. In this case, the lower the value of Y_e, the darker and browner was the leaf color. It is of interest that Coker 139 did not brown as extensively, to a deep brown color, as Yellow Special A or Golden Wilt. This is consistent with general observations on curing behavior for this variety, which can be cured to bright yellow or a lemon color much easier and more frequently than most varieties. It appears that Y_e represents that point of maximum browning, and hence the lowest color value to which discoloration may be expected to proceed. Hence the lower the values of Y_e, the greater will be the discoloration "potential" for a variety.

Leaf position produced an effect on Y_e similar to that on Y_i. **Figure 3** shows the variation of Y_e with leaf position for all three varieties. With an increase in leaf position, average values of Y_e decreased from 13.2 at leaf position 2 to 7.9 at leaf position 17, showing that tobacco has a greater potential for discoloration at higher leaf positions.

Color Change, ΔY.

Data in **Table 1** show that ΔY, the color change associated with browning, was not affected as appre-

ciably as the other parameters. Variety and leaf position, however, tested significant at the .01 level of probability.

Average values of ΔY were 19.5 for Golden Wilt, 19.0 for Yellow Special A, and 19.7 for Coker 139. This is apparent in **Figure 3** by the similar character of the data for Y_i and Y_e for the three varieties. It is important to note that, although ΔY may be similar for different varieties, the underlying concentration changes of polyphenols may be quite different. This is because the ΔY's may be related differently to concentration changes, depending on the initial color and concentration at which the reaction is initiated.

Leaf position appeared to have a slight effect for positions 2-14; however, its greatest effect was noted for leaf position 17, for which ΔY decreased to an average value of 17.3. This is evident in **Figure 3** as the difference between values of Y_i and Y_e.

Polyphenol Content

As pointed out earlier, samples of tobacco prior to and following the browning process were freeze-dried and evaluated for polyphenol concentration. **Table 3** shows polyphenol content as influenced by variety, leaf position, and stage of reaction. Total polyphenols for this study were considered to contain only the o-dihydroxybenzene compounds consisting primarily of the chlorogenic acids and rutin in tobacco.

Average values of polyphenol content were similar for Golden Wilt and Yellow Special A with values of 54.8 mg/g and 55.1 mg/g, respectively, for the yellow (before) samples. Coker 139, however, had an initial content of 41.9 mg/g or about 25% less than the other varieties. This is interesting in view of the fact that Coker 139 gave higher values for Y_i and Y_e, as previously discussed.

Leaf position gave trends of increasing polyphenol content with higher leaf position. The data were not consistent, however, perhaps due to experimental error or natural biological variability.

A highly significant change in polyphenol content occurred between the samples taken before and after the browning reaction. The oxidative process produced a strikingly similar decrease in polyphenol content of around 85 percent. This implies that the reaction may have proceeded in each case to an equilibrium level in which the ratio of final products to initial reactants was a constant, characteristic of the reaction.

The magnitude of deacrease in polyphenol content, obtained by establishing optimum conditions for the reaction, is no doubt greater than decreases which normally occur even during air curing processes. This study implies a method for rapid and systematic reduction of polyphenols in flue-cured tobacco, if this is ever desired by the industry.

Correlations

In addition to the above aspects, polyphenol concentration before browning was considered to be functionally related to the browning reaction and possibly to the browning parameters. Linear regression and correlation analyses were therefore performed by using polyphenol concentration as the independent variable and k, Y_i, Y_e or ΔY as dependent variables. Results of this evaluation are given in **Table 4.**

Positive coefficients for k suggest that the higher the polyphenol concentration, the higher was the browning rate constant. Theoretically, k should be concentration independent; therefore, enzyme concentration must have increased concurrently with substrate concentration to produce a positive correlation.

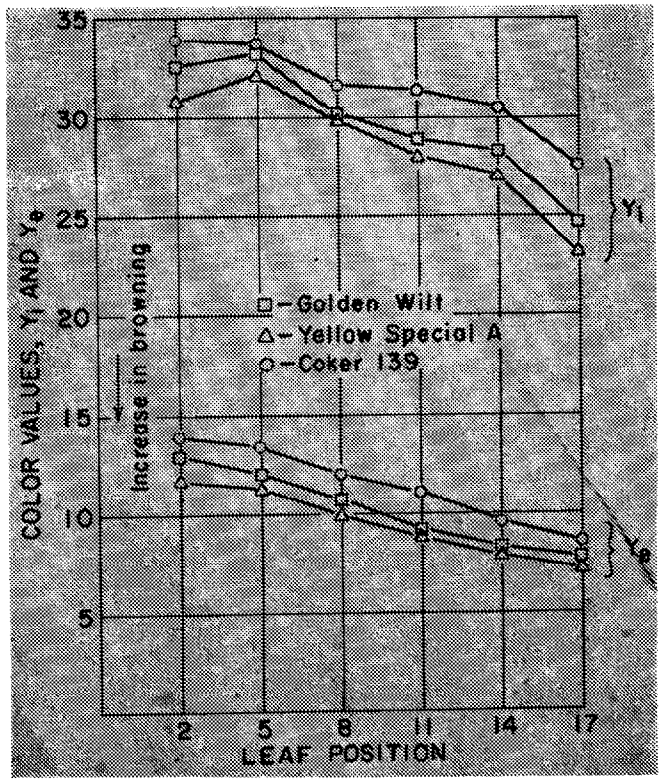

Figure 3. The effects of variety and leaf position on color values, Y_i and Y_e, prior to and following completion of oxidative browning.

Table 4. Regression and correlation coefficients for testing the relationship between either k_Q, Y_i, Y_e, or ΔY and polyphenol concentration. The equation $Y = a + bX$ is used where X is polyphenol concentration. Results are shown for three varieties.

Dependent Variable	Variety[a]	Regression Coefficient, b	Correlation Coefficient, r
k	1	.0054	.492
	2	.0003	.028
	3	.0192*	.898*
Y_i	1	−.1339	−.303
	2	−.0349	−.078
	3	−.4169*	−.833*
Y_e	1	−.1888	−.640
	2	−.1049	−.422
	3	−.4749*	−.868*
ΔY	1	.052	.259
	2	.072	.302
	3	.066	.374

a Variety 1: Golden Wilt; 2: Yellow Special A; 3: Coker 139.

** Rejection of the Ho: $\beta = 0$ or $\rho = 0$ at .95 level of significance.*

Color values Y_i and Y_e were both found to be negatively correlated with concentration, with Golden Wilt and Coker 139 showing greater flunctional dependence. Two factors support this strong inference: (1) Values of Y_i and Y_e decreased with increasing stalk position; however, concentration increased with stalk position, and (2) Coker 139 had higher color values Y_i and Y_e, but lower polyphenol content.

A positive correlation between ΔY and polyphenol content for each variety shows that concentration changes at different stalk positions were positively related with ΔY color changes. Note, however, that although Coker 139 has a lower polyphenol content, average ΔY for this variety was larger than for the other varieties. A given correlation may be restricted to *within* varieties

rather than *among* varieties.

SUMMARY AND CONCLUSIONS

This study was conducted to develop criteria for characterization of oxidative browning in tobacco during curing, and to develop and test a method for evaluating varietal discoloration potentials by a standardized procedure.

Three varieties of *Nicotiana tabacum* L. were evaluated: Golden Wilt, Yellow Special A, and Coker 139. Nearly identical cultural practices and curing conditions were imposed by using 80 field replications to permit evaluation of the color change phenomena as a variable of variety and leaf position.

The browning reaction was studied *in vivo* by thermally initiating the reaction and allowing it to proceed at high leaf moisture content at 25°C. Tristimulus colorimetry was used to determine the initial yellow color (Y_i), the final brown color (Y_e), and color change (ΔY). Calculations were made to determine the browning rate constant, k, for each leaf

On the basis of this study the following conclusions are made:

1. The criteria developed for characterizing oxidative browning enable quantitative comparisons between varieties, leaf positions, cultural factors, etc.
2. The browning rate constant, k in the equation

$$Y - Y_e = (Y_i - Y_e)e^{-kt} \qquad (2)$$

differs among varieties and increases by nearly 100% between bottom and top leaf positions.

3. The initial color, Y_i, at the maximum yellow state varies among varieties and decreases for higher leaf positions for a given variety. A negative correlation exists between Y_i and the polyphenol concentration prior to browning.
4. The final color, Y_e, after completion of the browning reaction, is significantly affected by variety and leaf position. For higher stalk tobacco, Y_e decreases, indicative of a deeper brown color. The brighter the initial color Y_i, the higher the value of Y_e or the lighter the brown leaf after the reaction. Y_e is also negatively correlated with initial polyphenol concentration.
5. By the method described for carrying out the browning reaction, polyphenol concentrations are reduced by up to 85 per cent for flue-cured tobacco.

This implies a method for rapid and systematic reduction of this class of constituents, if this is ever desired by the tobacco industry.

The major criteria for characterization of browning were found to be k, Y_i, and Y_e. These parameters can effectively quantify the rate and extent of the reaction, and hence can be used to study differences due to varieties, cultural practices and process conditions. This information will be of significance in providing predictability and control of this important class of compounds in tobacco.

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