CHANGES IN MOISTURE AND CHEMICAL COMPOSITION OF FLUE-CURED TOBACCO DURING CURING[1]

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The present study represents a portion of an effort to develop mathematical models for predicting changes in moisture and chemical composition of flue-cured tobacco during curing. In two experiments, concentrations of chlorophyll, starch, reducing sugars, and total alkaloids (as well as moisture contents) were measured. In the first experiment, tobacco was yellowed at temperatures of 30°C, 35°C, and 40°C with 3.33°C wet-bulb depression. In the second experiment, tobacco was yellowed at 35°C with wet-bulb depressions of 1.67°C, 3.33°C, and 5°C (or approximately 89, 81, and 70% relative humidity, respectively). In general, concentrations of chlorophyll and starch in the leaves decreased during curing, whereas concentrations of reducing sugars increased, and total alkaloids remained relatively unchanged. Yellowing temperature had no significant effect on moisture content at the end of the yellowing and leaf drying stages, nor on chlorophyll at the end of the yellowing stage and starch at the end of the cure. Starch tended to degrade faster at higher yellowing temperatures. Reducing sugar concentration at the end of the cure was significantly affected by yellowing temperature with the highest temperature resulting in the lowest reducing sugar concentration. Total alkaloid concentration was not affected by yellowing temperature. Yellowing wet-bulb depression had a significant effect on moisture content at the end of the yellowing stage, where the highest wet-bulb depression gave the lowest moisture content. On the other hand, concentrations of chlorophyll, starch, reducing sugars, and total alkaloids were not affected by wet-bulb depression.

ADDITIONAL KEY WORDS: chlorophyll, starch, sugars, total alkaloids, yellowing relative humidity, and yellowing temperature.

INTRODUCTION

Curing is one of the most important steps in the flue-cured tobacco (*Nicotiana tabacum*) production system. Its ultimate goal is to bring the leaf to a desired state without sacrificing its potential quality present at harvest. To achieve this goal, curing should allow continuation of biological activities in the leaf (12). At the beginning of the process, moisture should be removed slowly to allow biological reactions to proceed. Then the rate of moisture removal should be increased to arrest the reactions and complete curing in a relatively short time (5 to 7 days).

In general, curing can be divided into three distinct stages: yellowing, leaf drying, and stem drying. The first stage can be described as a period of major chemical conversions and color development. Air temperature in the barn is maintained between 30 and 40°C, with relative humidity of 80 to 95%, (5,12) for about 48 h or until the leaves turn yellow. In the second stage, air temperature in the barn is increased gradually to 50 or 60°C, while relative humidity is lowered to allow more rapid moisture removal. This stage lasts for 36 to 72 h (12). The last stage (stem drying) generally requires 36 to 48 h. Air temperature is increased to 74°C with further decrease of relative humidity to permit rapid drying of the midrib.

Chemical changes mediated by enzymatic activity (6) during the yellowing stage lead to the formation of desired compounds in the cured tobacco. Starch is converted into reducing sugars during yellowing and early leaf drying. As starch degrades, reducing sugar concentration increases and reaches its peak by the end of the yellowing stage. It then declines due to respiration, which oxidizes reducing sugar into carbon dioxide and water. Reducing sugars contribute up to approximately 22% of flue-cured leaf dry weight and are major components of cured leaf quality (22).

Chlorophyll degradation, noted by the disappearance of green and the emergence of yellow colors, is widely used to judge the curing progress. Full development of yellow color is often used to mark the end of the yellowing process, which is usually associated with completion of certain chemical reactions, especially starch to sugar conversions. This is possible because the degradation of starch and chlorophyll occur at about the same rate (5), although the reactions are independent. However, in some cases tobacco leaves appear yellow before desirable chemical changes have been completed. This might lead to low quality tobacco and a poor smoke taste (5).

Color has a strong influence on flue-cured market value. A clear yellow to orange color has been accepted, to some extent, as an indicator of quality (1). A uniform yellow color with absence of chlorophyll assures the buyer that curing was not grossly mismanaged (24). Nevertheless, color may have little correlation with tobacco chemical quality because various leaf compounds may contribute more to the final smoke quality than color (1,22).

During the yellowing stage, starch should be hydrolyzed as much as possible while achieving a high level of reducing sugar concentration. However, temperature and moisture content, which can accelerate chemical conversions, also influence respiratory loss of reducing

[1]Paper No. BAE2000-11 of the Journal Series of the Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh, NC 27695-7625. The use of trade names in this publication does not imply endorsement by the North Carolina State University of the products named, nor criticism of similar ones not mentioned.

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sugars. Respiration rate is high at high moisture content and increases as temperature rises (16). Unfortunately, how these parameters influence the final chemical composition of the leaf are not completely understood. Comprehensive understanding of the nature of chemical conversions in the leaf, especially in relation to temperature and humidity levels, will lead to the design of improved curing schedules that produce tobacco with more desirable color and leaf chemistry.

Rates of chemical conversion in the leaf can be manipulated by controlling leaf temperature and moisture content because these variables, along with pH, influence enzymatic reactions. Johnson (12) stated that the major process variables encountered during curing are leaf temperature, relative humidity, air velocity, and time variation of these variables. Adjusting curing air temperature can regulate leaf temperature, while adjusting curing air relative humidity can control leaf moisture content.

Considerable research on chemical composition and changes in tobacco leaves during curing has been conducted (3,7,14,19,25), but only a few studies (11,23) have focused on chemical changes during the yellowing stage with respect to temperature and relative humidity. Information on manipulation of these factors to create optimum conditions for desirable chemical conversions is very limited. Further knowledge of the nature of chemical changes during yellowing will contribute to efforts aimed at improving cured leaf quality.

The objective of this study was to investigate and obtain data on the effect of yellowing temperature and relative humidity (wet-bulb depression) on the moisture content and major chemical compounds during curing. This information will be used to develop mathematical models for moisture removal and chemical changes during curing, and to evaluate the effect of curing factors on chemical compositions through simulation, and thus improve and optimize curing schedules as a function of curing factors and initial moisture and chemical concentrations.

Figure 1. **A typical curing schedule having 35°C yellowing temperature, 3.33°C wet-bulb depression, and 48 h yellowing time.**

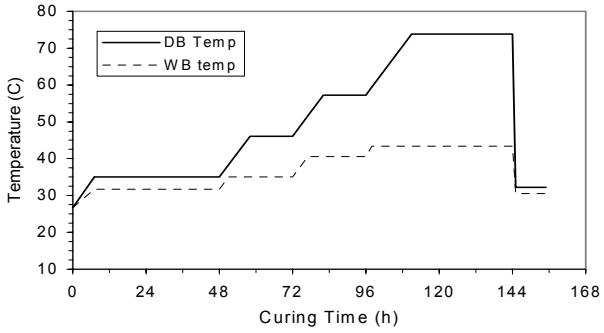
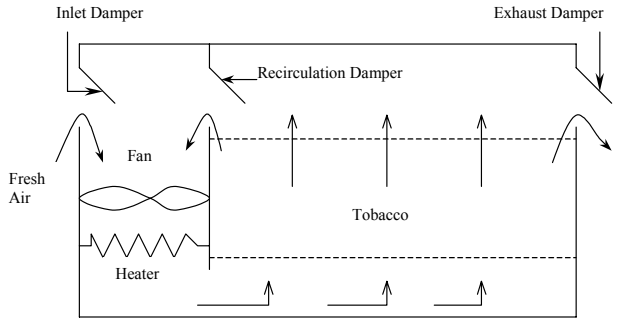


Figure 2. **Schematic view of the curing barn.**



MATERIALS AND METHODS

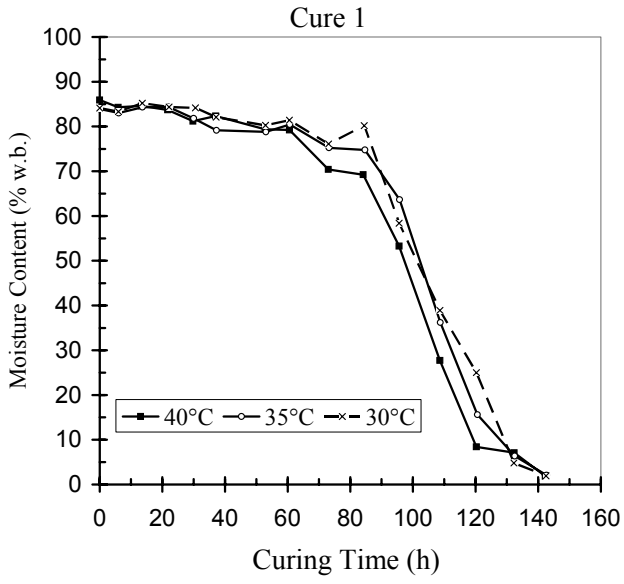
A preliminary experiment was conducted in two small two-tier rack barns at Weaver Laboratory, North Carolina State University, in August and September 1996, using tobacco obtained from the Oxford Tobacco Research Station, Oxford, North Carolina. The tobacco was yellowed at 35°C and 3.33°C wet-bulb depression (relative humidity in the barn) for 48 h, with a total curing time of 160 h (Figure 1). This experiment provided preliminary information on the effect of yellowing temperature and wet-bulb depression on chemical changes during curing.

A primary experiment was carried out at the Oxford Tobacco Research Station, Oxford, North Carolina, in September and October 1997. Tobacco, cv. 'K 149', was grown using recommended cultural practices for flue-cured tobacco production in NC (18).

Curing equipment utilized consisted of three box barns with walls constructed of two layers of plywood spaced about 10 centimeters apart. The barns were of identical design with inside dimensions of 1.42 m depth, 1.42 m width, and 2 m height. Each barn held two metal boxes, each with dimensions of 0.71 m depth, 1.35 m width, and 1.41 m height. The boxes were placed on a frame, 0.31 m above the floor, to let drying air flow freely into the tobacco.

Each barn was equipped with a backward curve centrifugal fan located in the rear chamber and a three-stage electrical heater mounted below the fan. The barn had three

Figure 3. **Moisture content of tobacco leaves from lower stalk position (cure 1) during curing at 40, 35, and 30°C yellowing temperatures, and 3.33°C wet-bulb depression.**

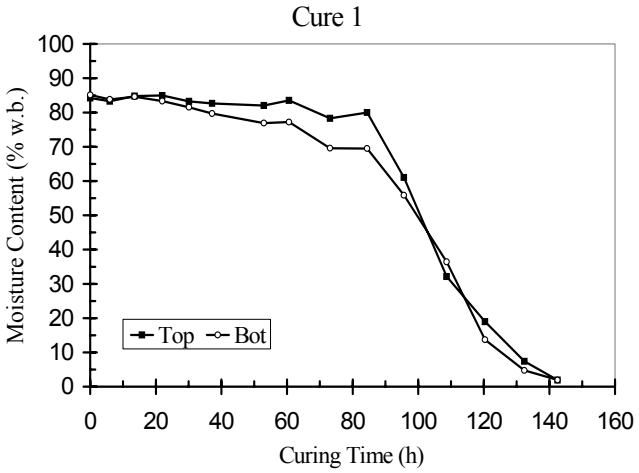


dampers: front, inside and rear. Rear and front dampers provided louvered openings for air intake and exhaust that were regulated by the inside damper. The inside damper was connected to a motor-driven actuator that was controlled digitally to control air recirculation. The fan forced the drying air through the heater, to the bottom of the barn, and up through the tobacco in the boxes (Figure 2).

Temperature in the curing system was recorded using a PC-based data acquisition system (Cyber Research, DAS 800 and EXP 16). Four Type-T thermocouples were placed in each barn to measure dry-bulb and wet-bulb temperatures of air entering the tobacco at the bottom and of air exiting the tobacco at the top of the boxes. Temperatures were collected every 5 sec for control purposes, and recorded every 15 min for documentation throughout the cure. Heaters and dampers in the barns were controlled automatically using a microcomputer through the utilization of a Cyber Research DIO-96 digital input and output board. Wet-bulb temperature was measured using the wick method. One end of the wick was immersed in a PVC tube container that refilled with water every two hours automatically, while the other end was pointed up and attached to a thermocouple.

Barn temperatures and humidities were controlled using a software control system from Laboratory Technology Control, Inc. The control was based on dry-bulb and wet-bulb temperature at the bottom of the barn. If dry-bulb temperature was below a predetermined setpoint, the computer sent a digital signal to turn the heater on; if the reverse was true, the computer turned the heater off. If the wet-bulb temperature was above a predetermined setpoint, the computer sent a digital signal to the actuator to close the inside damper, which then forced the fan to bring in fresh air. If wet-bulb temperature was below a predetermined setpoint, the computer opened the inside damper; thus, the fan recirculated the air within the barn to increase wet-bulb temperature (relative humidity). In the case of low initial tobacco moisture content, water mist was sprayed into the barn to help maintain the humidity within the system.

Figure 4. **Moisture content of tobacco leaves from top and bottom of the curing boxes at 35°C yellowing temperatures and 3.33°C wet-bulb depression (cure 1).**



Tobacco was hand-harvested and loaded into the boxes on the same day. Each box was rotated 90 degrees and loaded uniformly with about 259 kg (192.2 kg m^{-3}) of green tobacco leaves that were arranged horizontally. A door was then attached and rods were inserted to provide vertical support for leaves during curing. The filled container was then rotated 90 degrees to the upright position so that leaf arrangement was parallel relative to airflow in the barn and moved by a gantry with a chain hoist into the curing barn.

Yellowing temperature and wet-bulb depression were studied. In the temperature tests, three yellowing temperatures (30, 35 and 40°C) were maintained for 64 h at a constant wet-bulb depression of 3.33°C. In the wet-bulb

Figure 5. **Total chlorophyll concentrations in tobacco leaves from lower stalk position (cure 2) during the yellowing stage at 40, 35, and 30°C yellowing temperatures.**

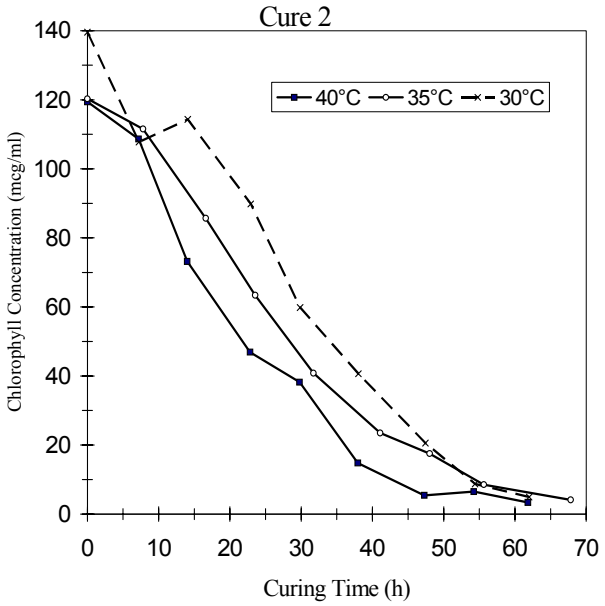
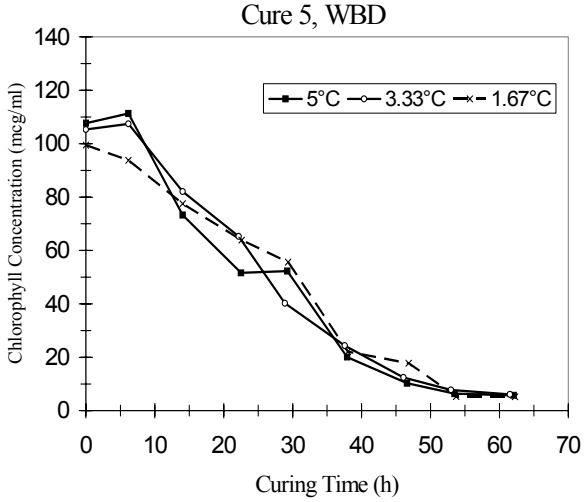


Figure 6. **Total chlorophyll concentrations in tobacco leaves from upper stalk position (cure 5) during the yellowing stage at 5, 3.33, and 1.67°C yellowing wet-bulb depressions.**



depression tests, three levels of wet-bulb depression (1.67, 3.33, and 5°C, which represent 89, 81, and 70% relative humidity, respectively), were maintained for 64 h at a constant temperature of 35°C. After yellowing (the first 64 h), temperature and wet-bulb depression in the barn were returned to the typical curing schedule values (Figure 1).

Six curing tests were performed. Four were dedicated to temperature treatments (cures 1, 2, 4, and 6) and two to wet-bulb depression treatments (cures 3 and 5). Treatments were assigned randomly to barns prior to each test. Due to immaturity of cv K 149 leaves prior to cures 4 and 6, it was decided to use cv K 326 instead to prevent delay in the experiment. Cures or tests 1 & 2, 3 & 4, and 5 & 6 were harvested from lower, middle, and upper stalk positions, respectively.

Samples were taken from the front box of each experimental barn by opening the barn doors. The front side of the box was divided into four sampling sites, two on the top and two on the bottom, with about 0.15-m margins along the horizontal lines. One sample of 70 - 100 g was taken from each site. A 3.75-cm diameter bore corer sampler was used to obtain representative samples. Samples were weighed immediately, placed in plastic bags, labeled, and frozen until analyses were performed. Samples were taken every eight hours during the yellowing (the first 64 h), and every 12 h during leaf and early stem drying.

Leaf disk samples for chlorophyll analysis were taken from the main samples by employing a 1.1-cm diameter bore corer. Ten leaf disks were collected and immediately transferred to a 20-ml scintillation vial which was capped, labeled, weighed, and stored in a freezer (in the field) until transferred to the laboratory for analyses. Chlorophyll samples were taken at 8-h intervals for the first 64 h.

Moisture content was determined by freeze drying samples for four days. After freeze-drying, the lamina were separated from the midribs and ground for chemical analyses.

Table 1. Yellowing temperature and wet-bulb depression (WBD) effects on moisture contents at the beginning, end of yellowing (EOY), and end of leaf drying (ELD).

Yellowing Treatment		Moisture Content[a] (% wb)			Sampling time (h)	
Temp. (°C)	WBD (°C)	Initial	EOY	ELD	EOY	ELD
40	3.3	83.8	73.7	31.4	61.8	109.2
35	3.3	83.6	74.7	29.8	63.3	107.8
30	3.3	83.3	75.5	37.8	61.7	109.1
Average		83.6	74.6	33.0	62.2	108.7
35	5.0	82.0	72.3	35.0	61.7	108.5
35	3.3	83.0	77.2	39.0	61.6	108.0
35	1.7	82.6	78.2	43.0	62.3	108.7
Average		82.5	75.9	39.0	61.9	108.4

[a] Values for temperature and WBD were average of four and two cures, respectively.

Table 2. Yellowing temperature effect on moisture (MC) and chlorophyll at the end of yellowing (EOY), moisture at the end of leaf drying (ELD), and starch, reducing sugars, and total alkaloids at the end of curing.

Chemicals[a]		Temperature		
		40°C	35°C	30°C
MC-EOY	(% wb)	73.7	74.7	75.5
MC-ELD	(% wb)	31.4	29.8	37.8
Chlorophyll	(mcg/ml)	5.2	6.3	8.7
Starch	(% db)	2.9	2.2	2.5
Red. Sugars	(% db)	9.8	10.5	13.0*[b]
T. Alkaloid	(% db)	2.1	2.4	2.2

[a] Values are average of four cures (1, 2, 4, & 6).

[b] * = mean significantly different at P<0.05.

Chemical Analyses

Lamina samples were analyzed to determine chlorophyll, calcium, starch, reducing sugars and total alkaloid concentrations over time. The chlorophyll and calcium analyses were performed using the procedures outlined by Moran (17) and Bacon et al. (4), respectively, while the analyses of starch and reducing sugars and total alkaloids were carried out using Technicon Auto Analyzer by the procedure of Rosa (20) and Harvey et al. (9), respectively. The chlorophyll analyses were carried out at the Environmental Analysis Laboratory, Biological and Agricultural Engineering Department, while the others were performed at the Tobacco Chemistry Laboratory, Crop Science Department, North Carolina State University. A SAS General Linear Model Procedure was used to analyze the data for statistical significance (SAS, Cary, NC).

RESULTS AND DISCUSSION

Moisture Content

In the yellowing temperature experiment, moisture in the leaf decreased only slightly during yellowing and the first half of leaf drying (hour 88) before it dropped sharply during the second half of leaf drying and first half of stem drying or until about hour 124 (Figure 3). Average moisture content was reduced from approximately 84% to about 75% by the end of the yellowing (hour 62), and to about 33% by the end of leaf drying (Table 1). Similarly, in the yellowing wet-bulb depression experiment, average moisture content was reduced from approximately 83% to about 76% by the end of yellowing (hour 62), and to about 39% by the end of leaf drying.

Table 3. Yellowing wet-bulb depression effect on moisture (MC) and chlorophyll at the end of yellowing (EOY), moisture at the end of leaf drying (ELD), and starch, reducing sugars, and total alkaloids at the end of curing.

Chemicals[a]		Wet-Bulb Depression		
		5°C	3.33°C	1.67°C
MC-EOY	(% wb)	72.3*[b]	77.2	78.2
MC-ELD	(% wb)	35.0	39.0	43.0
Chlorophyll	(mcg/ml)	6.30	5.26	4.7
Starch	(% db)	3.3	3.44	3.8
Red. Sugars	(% db)	15.6	14.4	14.9
T. Alkaloid	(% db)	2.3	2.3	2.2

[a] Values are average of two cures (3 & 5).

[b] * = mean significantly different at P<0.05.

Average leaf moisture contents at the end of the yellowing for each yellowing temperature treatment were comparable (Table 2) because relative humidity in the barns was maintained at approximately 81% by setting the wet-bulb depression constant at 3.33°C. There were no significant effects of yellowing temperature on moisture removal rate at the end of yellowing and leaf drying. In contrast, the wet-bulb depression or relative humidity treatment significantly affected moisture content at the end of yellowing, with the lowest relative humidity resulting in the highest average moisture removal (Table 3). This was expected because relative humidity of curing air is a driving force for moisture removal from a wet biological material (10). At constant airflow rate, the lower the curing air relative humidity, the higher the moisture removal rate. While a similar trend occurred at the end of leaf drying, the differences were not significant (Table 3).

Moisture content of leaves at the bottom of the curing boxes became progressively lower than that at the top of the boxes during yellowing and leaf drying (Figure 4). This difference was due to the fact that as curing air was forced through the tobacco from the bottom to the top of the boxes, the air became increasingly saturated with water vapor and possessed reduced drying potential. During stem drying, most moisture had been removed and moisture differences between leaves located at the top and bottom of the curing boxes were small. Tobacco positions within a box had significant effects on moisture content at the end of yellowing, but the differences were not significant by the end of leaf drying (Table 4).

Total Chlorophyll

Chlorophyll degradation and the progressive appearance of yellow pigments were the most visible of the biological changes that took place during yellowing. Chlorophyll, abundant prior to curing, was degraded gradually (Figures 5, 6). Degradation was slow for a short period at the beginning of the yellowing, and then increased sharply until hour 48. Although the trend was not consistent (2 out of 6 tests), this initial lag in chlorophyll degradation has been reported previously (3,8,15). By the end of yellowing the decomposition slowed considerably and chlorophyll concentration leveled off at 10 μg/ml or less.

Chlorophyll degradation rate varied with temperature. At the highest temperature, it degraded faster and leveled off earlier. In all but one test at the highest yellowing temperature (40°C), chlorophyll concentrations leveled off earlier (at about hour 48) than at lower temperatures. If one assumes that low chlorophyll concentration corresponds closely to the appearance of yellow color, then the tobacco yellowed faster at the higher yellowing temperatures. Moreover, as temperature decreased, the chlorophyll concentration at the end of the yellowing stage increased, although the effects of temperature on chlorophyll concentrations were not statistically significant (Table 2).

Although relative humidity during yellowing did not consistently affect chlorophyll degradation (Figure 6), visual observation indicated that a greenish color was often associated with low relative humidity treatment. Moreover, yellowing relative humidity did not have any significant effects on chlorophyll concentration at the end of yellowing.

Our results also indicate that after about 50 h, hydrolysis of chlorophyll was practically completed and

Table 4. **Effect of leaf position in the curing box on moisture (MC) and chlorophyll at the end of yellowing (EOY), moisture at the end of leaf drying (ELD), and starch, reducing sugars, and total alkaloids at the end of curing.**

Chemicals [a]		Position in the box	
		Bottom	Top
MC-EOY	(% wb)	71.3* [b]	77.9
MC-ELD	(% wb)	29.7	36.3
Chlorophyll	(mcg/ml)	5.3	8.2
Starch	(% db)	2.5	2.5
Red. Sugars	(% db)	11.2	11.0

[a] Values were average of all cures.

Figure 7. **Starch concentrations in tobacco leaves from upper stalk position (cure 6) during curing at 40, 35 and 30°C yellowing temperatures.**

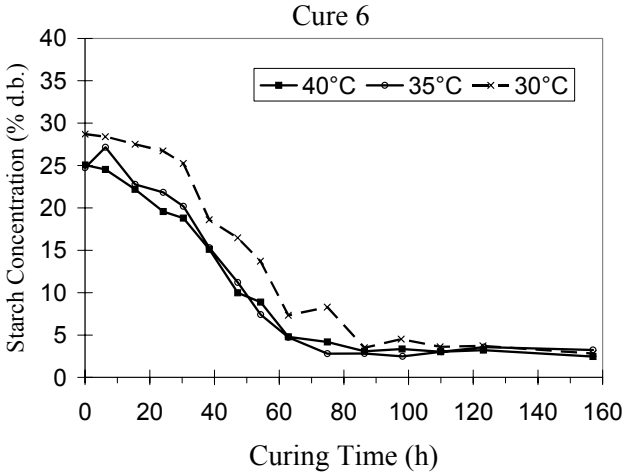
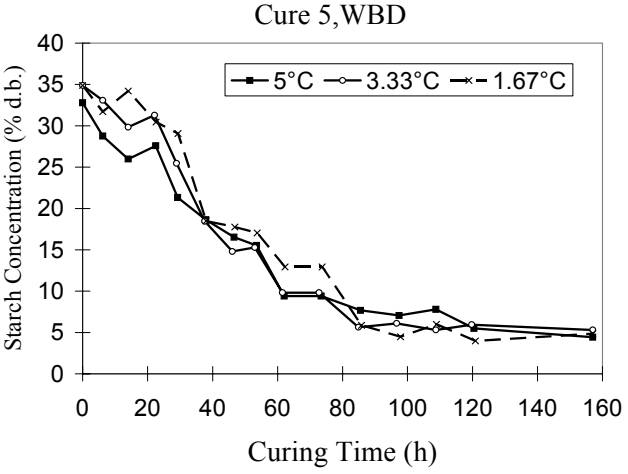


Figure 8. **Starch concentrations in tobacco leaves from upper stalk position (cure 5) during curing at 5, 3.33, and 1.67°C yellowing wet-bulb depressions.**



further degradation was very limited. The latter was probably caused by limited substrate and an adverse effect of prolonged exposure to yellowing temperature and lower moisture content on the enzymatic activities associated with the degradation (3).

Tobacco position in the curing box also tended to influence the rate of chlorophyll degradation. Chlorophyll in leaves near the bottom of the box tended to degrade faster than in leaves near the top, perhaps due to leaf partial drying and slight differences in temperatures. However, chlorophyll concentrations at the end of yellowing were not significantly different.

Starch

Starch was hydrolyzed rapidly during the first 60 - 80 h of yellowing; the degradation process then declined slowly until the end of curing (Figures 7, 8). This rapid degradation was due to accelerated enzyme activity during yellowing. The primary enzyme that actively converts starch to reducing sugars is α-amylase, which is influenced by leaf temperature and moisture content. Starch hydrolysis usually ceases during the leaf drying phase due to substrate limitation and enzyme inactivation (3).

Temperature influenced the starch degradation rate with the fastest degradation occurring at the highest temperature (Figure 7). This result agreed with a previous study (21) which reported that α-amylase activity increased as temperature increased and peaked at 50°C, slowed down above 50°C, and stopped (inactivated) above 60°C. In one test, however, starch degradation at high (40°C) temperature was slow. This might have been due to a mechanical problem encountered during this particular test, in which barn temperature dropped at around hour 40 to about 29°C (instead of to 40°C) for 6 h.

At the highest (5°C) wet-bulb depression (relative humidity about 70%), starch degradation tended to slow down earlier and remained at a higher concentration than it did at the lower wet-bulb depressions or higher relative humidities (Figure 8). This might be due to a relatively faster rate of moisture removal at the lowest yellowing relative humidity, which has an adverse effect on α-amylase activity (3). This was in agreement with the effect of position in the curing box on starch hydrolysis. Starch in leaves at the bottom of the box, where the tobacco usually dried earlier, leveled off earlier than those of the top of the box, although statistical analysis did not show any significant effects of box positions (Table 4). The accelerated rate of starch degradation in tobacco near the bottom of the boxes might be due to interactive effects of leaf temperature and moisture gradients. However, there were no significant effects of yellowing temperature or yellowing relative humidity on the starch concentration in the tobacco at the end of curing (Tables 2 and 3).

Reducing Sugars

In general, reducing sugars increased during yellowing, then leveled off, and then decreased toward the middle of leaf drying before leveling off again (Figures 9, 10). The increase in reducing sugar concentration resulted from the conversion of starch into reducing sugars during yellowing (6). As starch hydrolysis ceased during the drying stage, reducing sugar concentration started to plateau before decreasing due to respiration (12).

Reducing sugar concentrations at the highest yellowing temperature tended to have the lowest peak and plateaued earlier than at lower temperatures. In contrast, reducing sugar concentration at the lowest yellowing temperature tended to have the highest peak at a later hour (around hour 76) and leveled off last at a higher concentration, particularly in leaves from the middle and upper stalk positions (2). This indicated that respiration rate was relatively high at the 40°C yellowing temperature compared to the other temperature treatments. It was apparent that toward the end of yellowing, respiration consumed reducing sugars at a rate higher than the rate at which they were converted from starch. Long and Weybrew (14) mentioned that elevated temperature and relative humidity during curing are conducive to high respiration rate. Respiration increases approximately 1.88 fold as temperature rises 10°C within the range of 27 to 44°C (3). Statistical analysis of final reducing sugar concentration supported those trends.

Figure 9. **Reducing sugar concentrations in tobacco leaves from middle stalk position (cure 4) during curing at 40, 35, and 30°C yellowing temperatures.**

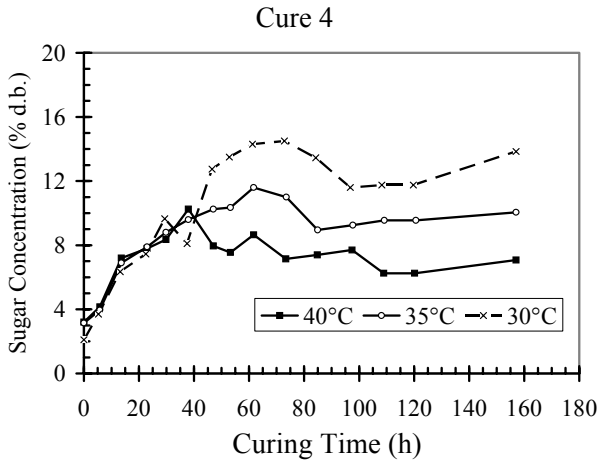
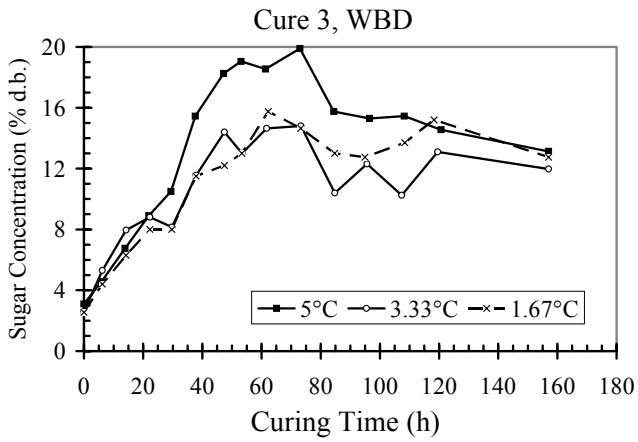


Figure 10. **Reducing sugar concentrations in tobacco leaves from middle stalk position (cure 3) during curing at 5, 3.33, and 1.67°C yellowing wet-bulb depressions.**



Yellowing temperature had a significant effect on the final reducing sugar level, with the highest temperature giving the lowest concentration (Table 2).

Reducing sugar concentrations in leaves that were yellowed at 5°C wet-bulb depression were higher than in those yellowed at other humidity levels throughout curing until the end of leaf drying (Figure 10). This might be caused by both a relatively rapid degradation of starch at 5°C wet-bulb depression (Figure 8) and a possible decrease of respiration rate due to moisture removal.

Moreover, reducing sugar concentrations at the bottom of the curing boxes were consistently higher, especially during the yellowing, than those at the top of the boxes (Figure 11). Although there were no significant effects of box position on the final reducing sugar concentration (Table 4), the data indicated that rapid starch degradation or comparatively lower rate of respiration had occurred during yellowing at the bottom of the curing boxes. Walker (23) reported that there were insignificant chemical and visual differences in cured tobacco leaves within the boundaries of 75 to 90% yellowing relative humidity.

Total Alkaloids

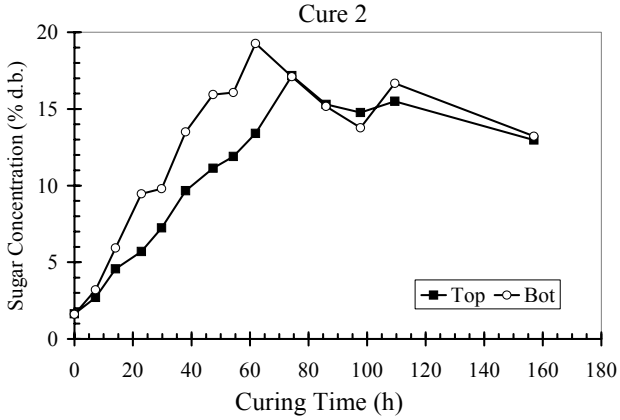
Total alkaloid concentrations tended to increase slightly during yellowing and leaf drying (up to hour 120), and then tended to decline thereafter, perhaps due to evaporation at the high stem drying temperature (Figures 12). Statistical analysis showed there were no significant effects of yellowing temperature and wet-bulb depression on the final concentration of total alkaloids (Tables 2 and 3).

Goins (7) and Peele (19) reported that there was little change in total alkaloids with the same level of ripeness; however, total alkaloid concentrations of cured leaves were significantly increased as ripeness increased. Similar results have been reported by Collins and Hawks (5), and Weybrew et al. (25).

CONCLUSIONS

Varying yellowing temperatures from 30 to 40°C at a constant wet-bulb depression did not significantly affect moisture contents at the end of yellowing or leaf drying. In contrast, varying yellowing relative humidity from 70 to 89% at a constant temperature significantly affected moisture content at the end of yellowing. The lowest relative humidity resulted in the lowest moisture content. Although yellowing temperature did not significantly affect chlorophyll concentration at the end of yellowing, it influenced the degradation rate. As yellowing temperature increased, chlorophyll degradation rate increased. Yellowing relative humidity did not significantly affect chlorophyll concentration. Neither yellowing temperature nor relative humidity significantly affected starch concentration, although starch tended to degrade faster at the highest yellowing temperature. Yellowing temperature had a significant effect on the final reducing sugar concentration. As yellowing temperature increased, reducing sugar concentration decreased. However, varying yellowing relative humidity did not affect reducing sugar concentration. Yellowing temperature and yellowing relative humidity did not have significant effects on total alkaloid concentration.

Figure 11. **Reducing sugar concentrations in tobacco leaves from top and bottom of the curing boxes during Cure 2 (lower stalk position) at 35°C yellowing temperatures, and 3.33°C wet-bulb depression**



LITERATURE CITED

1. Abdallah, F.M. 1970. Can Tobacco Quality Be Measured? Lockwood Publishing Company, Inc., New York City, NY.

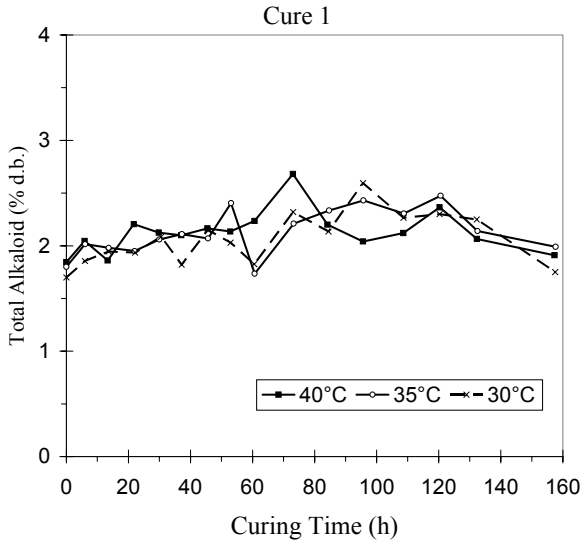
2. Abubakar, Y. 2000. Predicting moisture and chemical changes during bulk curing of flue-cured tobacco. Ph.D. Dissertation, N.C. State University, Raleigh, NC.

3. Amin, A.N. 1979. Dynamic transformation of chemical constituents during flue curing of *Nicotiana tabacum*. Ph.D. Dissertation, N.C. State University, Raleigh, NC.

4. Bacon, C.W., R. Wenger, and J.F. Bullock. 1952. Chemical changes in tobacco during flue curing. Ind. Eng. Chem. 44:292-296.

5. Collins, W.K., and S.N. Hawks, Jr. 1993. Principles

Figure 12. **Total alkaloid concentrations in tobacco leaves from lower stalk position (cure 1) during curing at 40, 35, and 30°C yellowing temperatures.**



of Flue-Cured Tobacco Production. 2nd ed. W.K. Collins and S.N. Hawks, Jr., Raleigh, NC.

6. Frankenburg, W.G. 1946. Chemical changes in the harvested tobacco leaf. I. Chemical and enzymatic conversions during the curing process. Adv. Enz. 6:309-387.

7. Goins, G.D. 1993. Influence of ethylene gas on the chemistry and quality of underripe and ripe flue-cured tobacco. Ph.D. Dissertation, N.C. State University, Raleigh, NC.

8. Gopalam, A., and N.C. Gopalachari. 1979. Biochemical changes in leaf pigments and chemical constituents during flue-curing of tobacco. 1. Changes in leaf pigments. Tob. Res. 5:117-124.

9. Harvey, W.R., H.M. Stahr, and W.C. Smith. 1969. Automated determination of reducing sugar and nicotine alkaloids on the same extract of tobacco leaf. Tob. Sci. 13:13-15.

10. Henderson, S.M., R.L. Perry, and J.H. Young. 1997. Principles of Process Engineering. 4th ed. American Society of Agricultural Engineers, St. Joseph, MI.

11. Johnson, W.H. 1965. Influence of harvesting and process variables on bulk curing of bright leaf tobacco. Trans. ASAE 8:354-357.

12. Johnson, W.H. 1974. Curing. Rec. Adv. Tob. Sci. Inaugural Volume (unnumbered): 63-78.

13. Johnson, W.H., and F.J. Hassler. 1963. Carbon dioxide liberation and carbohydrate accumulation during the yellowing phase of tobacco curing. Tob. Sci. 7:85-92.

14. Long, R.C., and J.A. Weybrew. 1981. Major chemical changes during senescence and curing. Rec. Adv. Tob. Sci. 7:40-74.

15. McClure, F.W., and G.R. Gwynn. 1973. Technique for evaluating the yellowing rates of flue-cured tobacco. Tob. Sci. 17:131-133.

16. Mohapatra, S.C., and W.H. Johnson. 1980. Postharvest physiology of bright leaf tobacco. I. Comparative biochemical changes during yellowing and drying phases of curing. Tob. Sci. 24:37-39.

17. Moran, R. 1982. Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol. 69:1376-1381.

18. Peedin, G.F., W.D. Smith, T.A. Melton, P.S. Southern, A.B. Brown, W.D. Eikhoff, M.D. Boyette, A.D. Worsham, F.H. Yelverton, and J.M. Moore. 1997. Flue-cured Tobacco Information. N.C. Coop. Ext. Serv. Bull. AG-187 (revised).

19. Peele, D.M. 1994. Effect of twice-over harvesting and ethephon and/or ethylene gas on flue-cured tobacco. Ph.D. Dissertation, N.C. State University, Raleigh, NC.

20. Rosa, N. 1971. An automated method for analyzing starch in green and mature leaf lamina. Tob. Sci. 15:58-61.

21. Spencer, R., and T.J. Weston. 1966. Alpha-amylase activity in tobacco leaf during and after flue-curing. Tob. Sci. 10:94a-99.

22. Tso, T.C. 1990. Production, Physiology, and Biochemistry of Tobacco Plant. Ideals, Inc., Beltsville, MD.

23. Walker, E.K. 1973. Effect of relative humidity in yellowing flue-cured tobacco under forced-air conditions. Tob. Sci. 17:131-133.

24. Watkins, R.W., and F.J. Hassler. 1962. Effect of oxygen stress on tobacco discoloration. Tob. Sci. 6:92-97.

25. Weybrew, J.A., W.G. Woltz, and R.J. Monroe. 1984. Harvesting and curing of flue-cured tobacco. The effects of ripeness at harvest and duration of yellowing on yield, physical characteristics, chemical composition and smoker preference. N.C. Agric. Res. Serv. Tech. Bull. 275.