

Spectral Characteristics of Tobacco In the Near-Infrared Region From 0.6 to 2.6 Microns¹

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Development of optical instrumentation for studying specific biophysical and biochemical properties presupposes knowledge of the spectral characteristics of the biological specimen involved. For example, a study of the spectral characteristics of peanuts revealed a specific property, namely, the differences between light absorption at wavelengths of approximately .480 μ and .510 μ was proportional to maturity. From these studies an instrument was developed to rapidly measure this property (4)³. By observing the visible spectra of whole tomatoes BIRTH *et al.* (2) discovered that the transmittance peak shifted to longer wavelengths with increasing maturity. The same observations were made for peaches (3), and as a result an instrument was constructed to monitor the wavelength of peak transmittance throughout the maturation phase.

Each biological material has its own unique transmittance spectrum. The absorption bands vary according to the physical and chemical characteristics of the material. The time dependent behavior of the spectrum will vary for different biological entities, and in order to obtain useful spectral information the characteristic spectra must be carefully analyzed for features related to the

parameters under study. It is the primary purpose of this paper to present characteristic spectra of tobacco and to discuss them from a classical spectrophotometric viewpoint.

Experimental

Plant Material

Flue-cured tobacco (*Nicotiana tabacum* L.) of several varieties was used in these experiments. All samples were 1.5 by 4.5 cm sections of the leaf lamina cut in such a way as to avoid the lateral veins of the leaf. Since the top of the leaf reflects less light than the bottom, all spectra were taken with the top of the leaf toward the source side of the optical path. The physical state of the tobacco will be indicated in the legend of the figure as the spectra are presented and discussed in the following paragraphs.

Equipment

The near infrared (NIR) spectra of the samples were determined using a Perkin-Elmer Model 450 spectrophotometer (PE 450). The instrument was designed to overcome many of the problems associated with measuring the transmittance spectra of dense light scattering material, such as intact tobacco. The use of large end-on cathode photomultipliers placed near the sample (approx. 4.0 cm) in a direct optical path collects essentially all of the light after it passes through the sample, minimizing losses from scattering. Spectro-

photometric scale expansion (up to 50X) permits the instrument to emphasize small peaks in the transmittance curves which otherwise might be missed. The baseline of the instrument may also be varied without altering the relative response of the recorder. This permits the operator to alter the base line in order to compensate for scattering changes which may occur even during a scan. The instrument also has a repetitive scan accessory which permits periodic monitoring of spectra over long periods of time.

The precise location of any absorption band is dependent on the bandpass of the monochromator⁴ in the spectrophotometer. The bandpass of the monochromator in the PE 450 was .0115, .0249, .0378, and .0887 μ at wavelengths of 0.6, 1.43, 1.93 and 2.5 μ respectively. Hence, the bandpass was sufficiently narrow to define any of the absorption peaks in any of the spectra discussed in this paper.

Cured Tobacco

Typical spectra of three samples of cured tobacco are shown in Figure 1. Physical parameters of the three samples are given in Table 1. The thickness of the samples were 77.7, 92.7 and 111.0 microns (μ) for samples 1 through 3 respectively. The moisture content of the samples also varied as follows: sample 1, 12.36%; sample 2, 28.45%; and sample 3, 61.98%.

The effects of both moisture and thickness are readily observed in Figure 1. The spectra show two

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³ Numbers in parentheses refer to the appended references.

prominent water absorption bands at 1.43 and 1.93 μ . Other water bands are known to occur in the NIR spectrum at 0.77, 0.97, 1.17 and 2.7 μ for most materials. The band at 1.17 μ for tobacco (sample No. 3 in Figure 1) shows only a slight trace of absorption. The other bands are not noticeable. The band at 2.7 μ is not well defined due to the low sensitivity of the lead sulfide detectors in this region.

Absorption bands due to other constituents appear at 1.7, 2.08 and 2.21 μ with a shoulder at 2.47 μ . The spectrum of the high moisture sample (No. 3) exhibits only a shoulder at 2.08 and 2.21 μ due to the overlapping effects caused by water absorption. When the moisture content is very high, such as in green and yellow uncured leaves, the band at 2.08 μ does not appear (see Figure 2).

The relatively constant spacing between the three curves in Figure 1 is indicative of an apparent absorption which is proportional to sample thickness, which was measured with a wide face micrometer at the point where the optical readings were taken. Samples 1 and 2 (as well as samples 2 and 3) differ by 20 μ in thickness. A closer investigation of the curves in the regions between 1.0-1.3 μ , 1.6-1.8 μ , and at 2.17 μ reveals a correlation between the vertical distance between the curves and the difference in sample thickness.

The absolute transmittance values at the 1.43 or 1.93 μ peaks show no relationship to moisture content of the sample due to light scattering. However, since scattering is directly proportional to thickness, choosing a reference measurement at a point in one of the regions between 1.0-1.3 μ , 1.6-1.8 μ or at 2.17 μ will permit the absolute readings to be corrected for scattering. For example, a difference reading between transmittance at 1.80 μ ($T_{1.80}$) and transmittance at 1.93 μ ($T_{1.93}$) serves as a good index of moisture content (see Table 1).

A noticeable deviation from the equidistant spacing occurs in the 0.6 to 1.0 μ region due to color differences. While samples 2 and 3 were

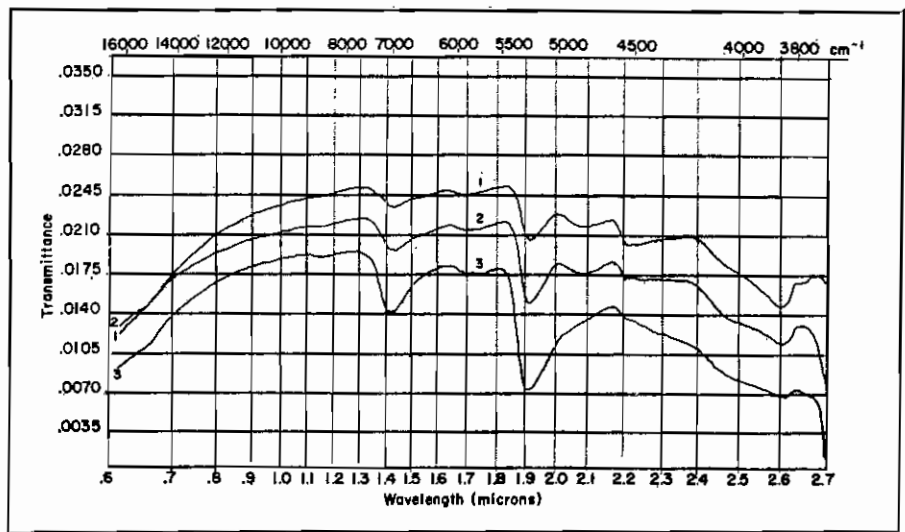


Figure 1. Spectra of cured tobacco in the intact form. Physical parameters for the three samples are given in Table 1.

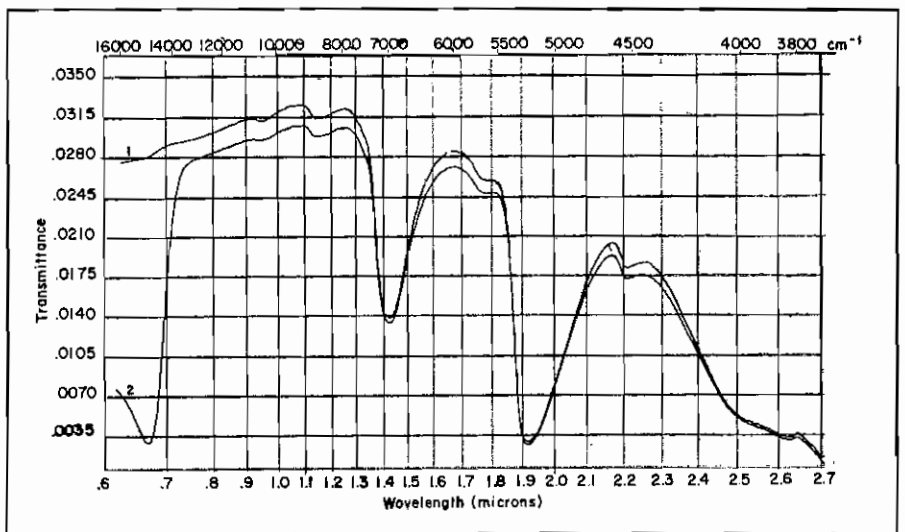


Figure 2. Near-infrared spectra of uncured green (curve 2) and yellow (curve 1) tobacco leaves after imbibition with water.

very similar in color (indicated by the equidistant spacing between the spectra in 0.6 to 1.0 μ region) sample 1 was noticeably darker than either 2 or 3 (resulting in a steeper slope in the 0.6 to 1.0 μ band).

Green and Yellow Tobacco

Figure 2 shows the near-infrared spectra of green and yellow uncured tobacco. The yellow leaves were pile-

yellowed until all traces of chlorophyll had completely disappeared. Both green and yellow leaves were imbibed with water prior to cutting the samples and making the measurements.

The high moisture level of the samples made the water absorption bands at 1.43 and 1.93 μ much more prominent. The bands at 0.97 and 1.17 μ are much more noticeable in Figure 2 than in Figure 1. However,

Table 1. Physical parameters of three cured tobacco samples. Spectra of these samples are given in Figure 1.

Sample Number	Initial Weight	Dry Weight	Percent Moisture	$(T_{1.30} - T_{1.43})$	$(T_{1.8} - T_{1.93})$	Thickness (μ)
1	.0354	.0315	12.38	.049	.140	77.7
2	.0447	.0348	28.45	.080	.200	92.7
3	.0588	.0363	61.98	.151	.304	111.0

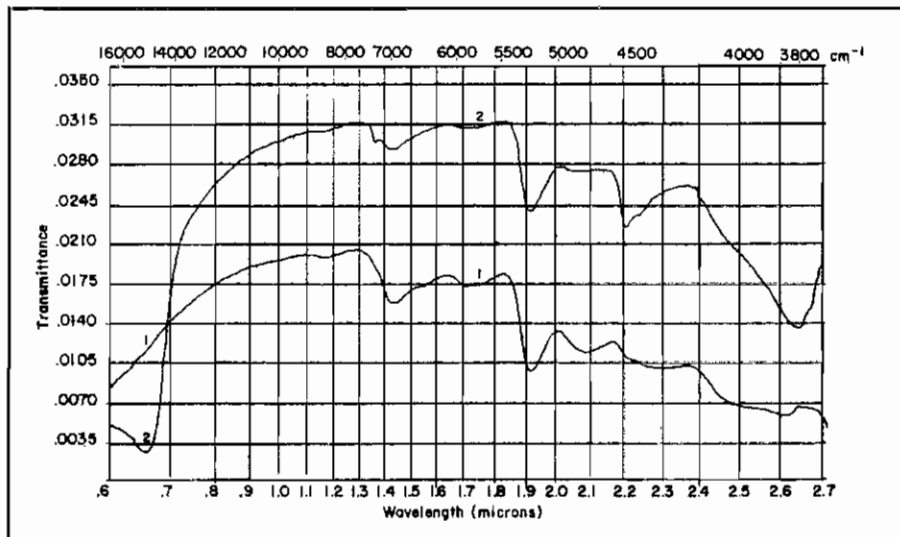


Figure 3. Spectra of green (curve 2) and yellow (curve 1) leaves quick dried and ordered to approximately 30% moisture.

the 0.77 μ -band still does not appear due to the very small absorption coefficient at this wavelength.

Sample No. 2 has a strong chlorophyll absorption peak at 0.67 μ . This band disappears during yellowing and is barely perceptible in the spectrum of yellow leaves (Curve 1). Comparing Figure 2 with Figure 1, the absorption peak at 2.21 μ was slightly better defined in the spectra of green and yellow uncured leaves than the spectra of cured leaves.

The effects of rapid drying on the spectra of the samples in Figure 2 are illustrated in Figure 3. After quick drying in a forced air oven at 250°F for five minutes the samples were ordered to approximately 30% moisture content (moisture contents in this paper are calculated on a dry basis). It appears that rapid drying of green (unyellowed) tobacco accentuated by two absorption bands, one at 1.37 μ and the other at 2.21 μ (see Curve 1, Figure 3). The constituents causing these bands are not known. However, they apparently disappear during the yellowing phase for these peaks are barely noticeable in the yellow-dried sample (Curve 1, Figure 3).

Brown vs. Yellow Tobacco

The effects of brown pigmentation, associated with extended yellowing and with thermally induced color changes, on the spectra of tobacco can be seen in Figure 4. The major difference between yellow and brown leaves occurs in the region between 0.6 and 1.3 μ . In this region the slope of the spectrum of brown tobacco is greater than that of yellow tobacco. Measurements of the slopes of curves in this region were observed to be

good indices of the concentration of brown pigmentation. Work is presently being conducted to design an instrument which will rapidly determine the slope. The instrument will be used to investigate the extent of browning due to thermal treatments.

NIR Spectra of Tobacco During Yellowing

The spectral response of tobacco at various times during the yellowing phase of curing is shown in Figure 5. The spectra represent repetitive scanning of the same sample under yellowing conditions of 90°F and 90% r.h. Curves 1 through 5 represent 0, 15, 23, 40 and 59 hours of yellowing time respectively. During this time the moisture content decreased from approximately 700% to 400%. The chlorophyll concentration of this particular sample decreased from approximately 300

micrograms per gram of fresh weight ($\mu\text{g/gfw}$) to 10 $\mu\text{g/gfw}$.

It is of interest to note that the apparent absorption decreases as yellowing progresses. This shift to lower absorption is illustrated in Figure 5 by the shift of the curves toward higher overall transmittance as the sample progresses through yellowing from 0 to 59 hours. Concerning the mechanisms which may influence this pattern, there is no doubt that light scattering takes place due to the interaction of the light with components which have structural dimensions of the same order of magnitude as the wavelength of transmitted energy. It is unlikely that cells cause much scattering since their dimensions are generally too large. However, the chloroplasts (5-10 μ) and grana (0.2-1.0 μ) are dimensionally suitable for scattering (5). Notwithstanding the fact that there is little pigment selectivity in the NIR region (except for the region from 0.6 to 1.0 μ) the decrease in apparent absorption is most probably associated with the change in the refractive index of the grana as chlorophyll disappears.

The results of this section imply a possible extension of the principle for monitoring changes which occur in tobacco during growth as well as curing. Changes in the chlorophyll concentration and moisture content of the growing leaves were the most notable changes found to occur. However, in order to take data on the PE 450, samples had to be cut and brought to the instrument to be analyzed. Since determinations were not repeated on the same leaf it is possible that other spectral changes did occur which could have been masked by the between sample bi-

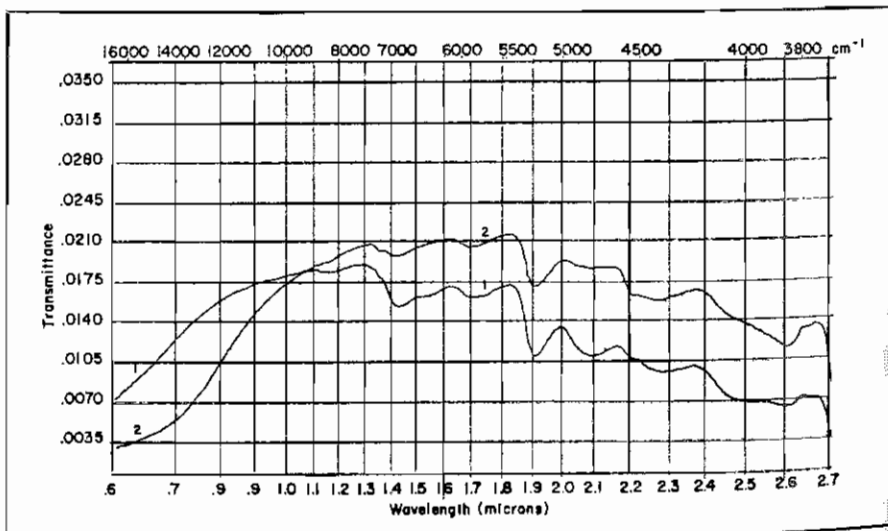


Figure 4. Influence of brown pigmentation on the near-infrared spectra of tobacco (curve 1, yellow cured tobacco; curve 2, brown cured tobacco).

ological variability. However, this variability could be largely eliminated if an instrument were designed to make measurements in the field.

NIR Spectra of Cured Tobacco Undergoing Desorption

Figure 6 shows NIR transmittance obtained from a single section of cured tobacco undergoing desorption. The sample was ordered to a moisture content of approximately 75.3% before determining the spectra. Ambient conditions for desorption were 75°F and 40% rh, the conditions of the laboratory. Curve 1 in Figure 6 was the initial plot at the 75.3% moisture level. Curves 2 through 6 were plotted at 45 minute intervals using the PE 450 repetitive scan accessory. Curves 7 and 8 were plotted after oven drying the sample for 15 and 30 minutes respectively (in 250°F forced air oven).

Moisture removal was noted by decreasing absorption (increasing transmittance) at 1.93 and 1.43 μ . The 1.93 μ bands appear to be the best index of moisture for samples with moisture contents between 0-100%. The band at 1.43 μ appeared to be more suited to moisture levels between 100-200%. Five isobestic points (1) occur at 1.38, 1.58, 1.83, 2.14, and 2.35 μ . These reference points occurred in all samples that underwent desorption tests.

The curves in Figure 6 show the sensitivity of the NIR technique for measuring the moisture content of tobacco leaves. The more intense absorption band at 1.93 μ is presently being investigated for measuring the moisture content in cured leaves. The results of these investigations will be discussed in another paper.

Summary

The near-infrared (NIR) transmittance spectra of flue cured tobacco (*Nicotiana tabacum* L.) in various physical states have been obtained. The spectra have been discussed with reference to moisture content, leaf thickness, brown pigmentation, and certain dynamic spectral characteristics. Apparent absorption caused by light scattering was found to be a function of leaf thickness. Brown pigmentation influenced the NIR spectra only in the short wavelength region from 0.6 to 1.3 μ . The primary difference between cured and yellowed tobacco was the intensity of the water absorption bands, with the yellowed leaf exhibiting the strongest band due to its higher moisture level. In addition to strong water absorption bands,

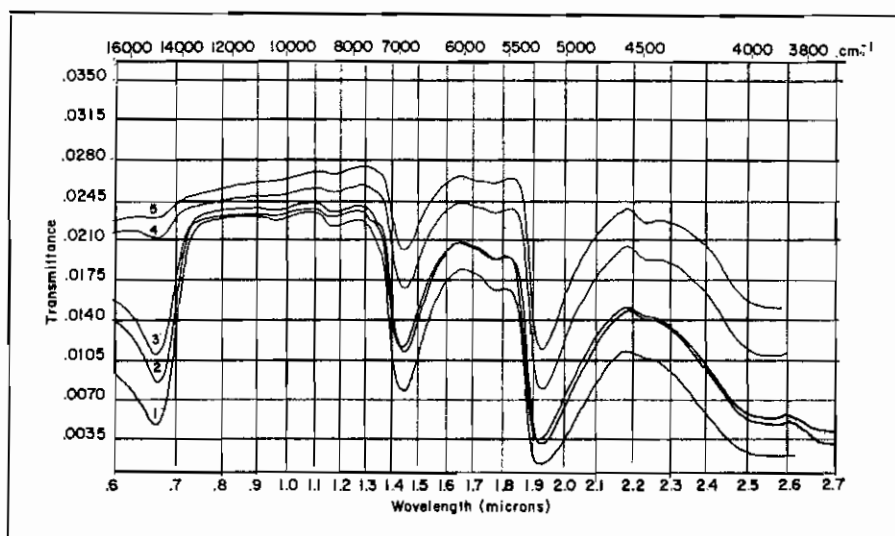


Figure 5. Spectral response of tobacco during the yellowing phase of curing (yellowing time per curve was: curve 1-0 hrs, 2-15 hrs, 3-23 hrs, 4-40 hrs, and 5-59 hrs).

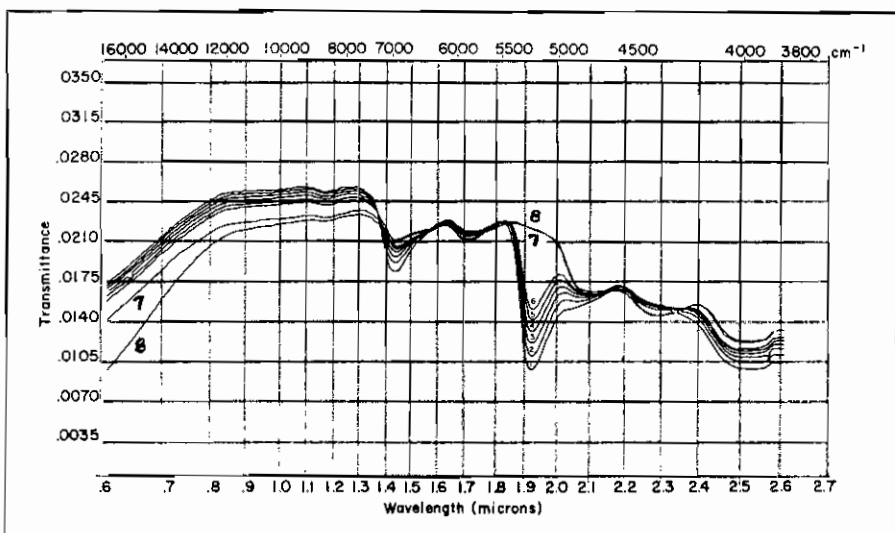


Figure 6. Spectral characteristics of tobacco undergoing moisture desorption (curves 1-6 plotted at 45-minute desorption intervals; curves 7 and 8 plotted after quick drying at 250° F for 15 and 30 minutes respectively).

the spectra of green leaves have a chlorophyll absorption band at .675 μ . The time dependent behavior of the spectra during curing was discussed with reference to developing analytical instruments for monitoring certain optical attributes of the leaf.

Acknowledgement

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