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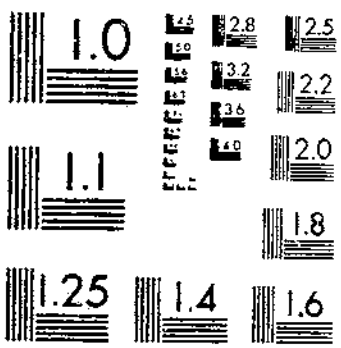
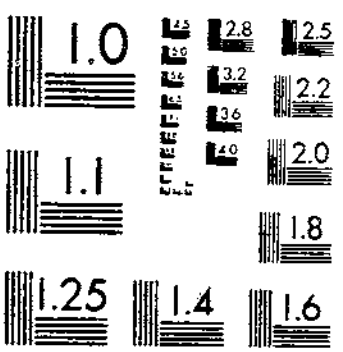


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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CHEMICAL COMPOSITION OF REPRESENTATIVE GRADES OF THE 1951 and 1952 CROPS OF BURLEY TOBACCO

√ including chemical methods

by Max Phillips

and Aubrey M. Bacot

Technical Bulletin No. 1186

Agricultural Marketing Service

UNITED STATES DEPARTMENT OF AGRICULTURE

contents

Issued August 1958

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 30 cents

SUMMARY

The investigation described in this publication was undertaken to determine the relationship between the United States standard grade classifications of tobacco and the chemical composition of the respective grades. The data apply to the 1951 and 1952 crops of Burley tobacco. The method of sampling and grading is described, and a brief description of the United States grades of this type is included for the information of those unfamiliar with the grade specifications.

At the outset there was no definite guide as to which constituents would be most useful for the purpose of determining the chemical composition of the various grades, and for this reason all components for which there was a reasonably accurate analytical method were determined. Other laboratories in the tobacco industry were invited to join in the project on a collaborative basis.

The data on all of the components determined are tabulated and the analytical method used for the determination of each component, as submitted by the laboratory which made the determination, is described. Instances in which there appears to be a significant relationship between the grades and chemical composition are pointed out by comment, as are those in which apparently no such relationship exists.

The grade data are tabulated according to group, quality, and color. The average content of each component for the two crop years, and for each of the groups, is calculated for the purpose of comparison.

All of the various nitrogen determinations were relatively significant, with the exception of protein nitrogen and nitrate nitrogen. Alpha amino nitrogen is one of the most significant analyses in the series of constituents determined. There is a difference in the alpha amino nitrogen content in most of the grade classifications on the basis of group, quality, and color comparisons. Total volatile bases as ammonia also are closely associated with grade.

The nicotine, nornicotine, and total alkaloids (as nicotine) all varied in content according to grade, which follows the pattern of growth and development of the plant.

The difference between grades in the content of total reducing substances (as glucose) and of total reducing sugars (as glucose) is too small in this type of tobacco to draw fine distinctions. However, there is a gradual increase, groupwise, in the content of total reducing sugars (as glucose) in proceeding from the Flyings group to the Tip group.

There is a small but consistent decrease, groupwise, in the polyphenols content in proceeding from the Flyings group to the Tip group.

The amounts of plastid pigments both total chlorophyll and total carotenoid, show a relationship to grade, with the content in both cases generally in inverse proportion to maturity.

Protopectin, pectic acid and pectates (as calcium pectates) are also constituents which are associated with plant maturity. As the plant matures, the protopectin content decreases and the amount of pectic acid and pectates increases. However, this relationship is evident only in a groupwise comparison.

Uronic acids (as anhydrides) and pentosans are shown to have a possible relationship to body and to color, respectively.

The structural components crude fiber, cellulose, and lignin are closely associated with each other in their distribution in the plant, according to grade. The analytical results of each of these constituents are proportional to the relative maturity of the different grades. The determination of methoxyl in lignin showed a consistent difference in content between the second and fourth qualities throughout the range of grades.

The content of both tannin and polyphenols was determined by empirical methods. The most obvious fact concerning these two determinations is that the maximum amount of each class of compounds as determined by these methods is at the opposite extreme of the grade classifications; that is, the maximum tannin content is in the Tip group of grades and the maximum polyphenols content is in the Flyings group.

Total volatile acids (as acetic acid) and formic acid content followed a general trend of decreasing slightly in proceeding from the Flyings group of grades to the Tip group. The water-soluble acids content followed the opposite general trend of increasing through the same groups, and the amount of water-soluble acids was proportionately greater.

There was no apparent significant difference in the content of petroleum ether extractives or of waxes from the usual standpoints of comparison.

The solvent properties of 95 percent ethanol and hot water in extracting soluble material from the different grades showed a parallel trend, with the hot water dissolving the greater amount. The use of either solvent was almost equally effective in showing the difference in soluble material of the respective grades.

The pH values decreased in the order of group, as follows: Flyings, Cutter, Leaf, and Tips. The data does not appear to show significant distinctions in grade on a quality or color basis.

The content of sulphur and chlorine, like sodium, phosphorus, and manganese, was so irregularly distributed among the grades that no significant grade relationship could be stated.

the CHEMICAL COMPOSITION of REPRESENTATIVE GRADES of the 1951 and 1952 CROPS of BURLEY TOBACCO including chemical methods

Max Phillips [1] and Aubrey M. Bacot, Standards Branch, Tobacco Division, Agricultural Marketing Service.[2]

INTRODUCTION

This publication is one of a series from this Division on the chemical composition of the standard grades of various American tobacco types (*30, 31, 32, 33, 34, 48*).[3] The object of these studies is to determine the chemical composition of the various grades of tobacco and to observe the relationship of the composition to grade.

The chemical composition of Burley tobacco, Type 31, has been the subject of investigation over a long period of time by many research workers in various State experiment stations in the United States Department of Agriculture, and in research laboratories of the tobacco companies.

In their selection of tobacco for chemical analysis, chemists in the past have been influenced by the subdivisions made by growers in the preparation of tobacco for market, or by manufacturers' classifications of tobacco into smoking and nonsmoking grades. As a result, it is extremely difficult, or perhaps impossible, for present-day investigators to interpret accurately these chemical findings in terms of present-day grades of tobacco.

Official United States grades for Burley tobacco were established in 1936, and for a number of years these grades have been applied to all Burley tobacco moving to market. These grades, as well as the official standard grades for other kinds of tobacco, have been found practicable and workable. Slight modifications and additions have been made from time to time to keep pace with changes in production and curing methods. United States standard grades have been accepted by producers and manufacturers as a practical basis for classifying tobacco according to quality.

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[1] Deceased.

[2] Frank B. Wilkinson, former Chief of the Standards Branch, presently retired, initiated and planned this project, and the collaborators, listed on pages 3 and 4, contributed not only their efforts but also their wholehearted cooperation.

[3] Italic numbers in parentheses refer to Literature Cited, pages 90, 91, 92, and 93.

In order that our chemical findings might be expressed in terms of a common denominator, all chemical determinations in this investigation were made on tobacco graded according to the official standard grades. The purpose of this investigation was to determine the chemical composition of Burley tobacco and relate these findings to the physical factors and the various elements of quality upon which the standard grades are based.

The grades selected to be analyzed represent the range of tobacco quality and were taken from grades sold on tobacco auction markets. After the analysis of the 1951 crop, it seemed advisable to add the additional grades X2F, C2F, and B2FR, in order to represent more completely the quality pattern and system of grading.

The processing of the grades for analysis is described in the section "Sample Selection and Preparation." These grades, after preparation in the manner described in this section, represent the combined appraisal of several judges and do not include any tolerance for injury or off-grade tobacco that is permissible to a stated limited extent in United States standard grades. The object of adhering to the grade specifications with the elimination of all tolerances was to find the composition as nearly characteristic of the grade as was practicable to obtain. With the composition of the various grades determined, a mixture of permissible tolerances or of blends of grades, might better be calculated. Also with the composition of the grade determined after grading according to this procedure, the variation in composition due to other factors, such as the change in weather from year to year, could be more nearly established.

Total ash and the ash constituents are generally considered to be some of the most fundamental for plant analysis and are included in our project as probably some of the most basic and useful for this purpose. The ash content of the different grades, the related determinations of water-soluble and water-insoluble ash, and the alkalinity of the water-soluble and of the water-insoluble ash all proved to be significant in varying degrees in relation to grade. Of the five ash constituents determined, potassium and calcium proved to be more closely related to grade than sodium, phosphorus, and manganese.

In the absence of criteria as to which of the tobacco constituents were or were not significant for this purpose, we concluded that every tobacco constituent for which a reasonably satisfactory analytical method was available must be considered important, or at least potentially important.

As a means of expediting the work involved in such an undertaking, a voluntary cooperative research project was organized and designated as the "Tobacco Research Pool." Taking part in the project, in addition to the Standards Branch laboratory of the Tobacco Division, Agricultural Marketing Service, USDA, were other Federal research laboratories, State experiment station laboratories, and research laboratories of tobacco companies, 16 in all. Each laboratory selected the particular determination

which it was in a position to make. As a result of this voluntary program, we have, in a relatively short period of time, made determinations and are sharing findings which otherwise would have required quite a considerable time. The Tobacco Division is deeply grateful to each participating laboratory. A list of the collaborating laboratories and the determinations which they made follows:

COOPERATING LABORATORY	CONSTITUENT OR PROPERTY DETERMINED
American Sumatra Tobacco Corp.	Phosphorus Manganese
The American Tobacco Co.	Moisture Moisture Equilibrium pH Total Volatile Bases Petroleum Ether Extract Waxes
Brown & Williamson Tobacco Corp.	Formic Acid Total Volatile Acids
The Imperial Tobacco Co., Ltd. of Great Britain and Ireland	Moisture Crude Fiber
Larus & Brother Co., Inc.	Total Nitrogen
Liggett and Myers Tobacco Co.	Moisture Alpha Amino Nitrogen pH Nitrate Nitrogen Water-Soluble Acids Protein Nitrogen
P. Lorillard Co.	Polyphenols Total Reducing Substances Total Reducing Sugars 95 percent Ethanol Extractives
North Carolina State College	Burn Rate Whiteness of Ash Plastid Pigments
The Pennsylvania State University	Calcium Moisture
Philip Morris, Inc.	Total Ash Water-Soluble and Insoluble Ash Alkalinity of Water-Soluble and Insoluble Ash Moisture Equilibrium Sulfur Chlorine

R. J. Reynolds Tobacco Co.	Nicotine Nornicotine Total Alkaloids as Nicotine
United States Department of Agriculture ARS, Eastern Utilization Research and Development Division	Tannins Sodium Potassium Soluble Extractives
United States Department of Agriculture ARS, Crops Research Division Tobacco and Sugar Crops Research Branch Tobacco Section	Total Alkaloids
United States Tobacco Co. (Nashville Branch)	Protein Nitrogen
University of Kentucky Agricultural Experiment Station	Oxalic, Malic, and Citric Acids
University of Tennessee Tobacco Laboratory	Resins and Waxes

REVIEW OF LITERATURE

Moore (*22*) in 1883 reported on the chemical composition of Burley tobacco from Mason County, Ky. He did not sort the tobacco into grades, or into different quality groups. The percentages of the several constituents determined were as follows: Nicotine, 3.12; resinous and fatty substances, 5.34; starch, 4.45; glucose, 0; albuminoids (N x 6.25) 15.98; pectic acid (anhydride) 7.49; citric acid (anhydride), 4.05; malic acid (anhydride), 9.26; oxalic acid (anhydride), 2.18; acetic acid (anhydride), 0.64; nitric acid (anhydride), 0; ammonia, 0.48; crude fiber, 12.18; sand, 0.66; ash (exclusive of sand and carbonic acid), 16.06; undetermined, 18.11.

Shedd (*40*) determined total nitrogen, nicotine, nitrate nitrogen, and some ash constituents in good, medium, and common tobacco samples. His data showed a higher potash content for the better grades, while the proportion of the anions, including nitrate, chloride, and sulfate did not show any trend among the grades. This was considered as indicating that the quantity of potassium in organic combination was greatest in the better grades. This finding was in accord with the observations of Schlösing (*38*) and Garner (*14*) who noted the beneficial effects of potassium in organic combination on the burning quality of tobacco.

Heggestad and Bowman (*17*) made comparisons of the chemical composition of Burley tobaccos at seven stages of maturity. Harvests were made at 6-day intervals beginning 1 week after topping. The data showed that, irrespective of harvest date, the lower leaves had the physical properties and chemical composition desired for use in cigarette manufacture. In gen-

eral, the duration of burn was better in tobaccos from the early harvests than from the later ones. The potassium concentration in the leaves decreased from the first harvest to a minimum at the fourth harvest, and then increased thereafter. The values for the alkalinity of ash followed approximately the same pattern as the potassium concentration, both factors being closely associated with leaf burn. Total volatile bases were considerably lower in trash than in lugs and bright leaf at all harvest dates.

Graham and Carr (*15*) subjected Burley tobacco, ground to about 40 mesh (A.S.T.M.), to successive extraction with petroleum ether, ether, 98 percent ethanol, acetone, and boiling water. Results indicated a relationship between grade and order of solubility in the solvents listed above. Thus, about 40 percent of the tobacco could be dissolved, with the ethanol extract amounting to about 18 percent of the tobacco.

Moseley, Harlan, and Hanmer (*23*) studied the chemical composition of the 1938 and 1939 crops of Burley tobacco from several districts of this tobacco belt. In all cases, the midribs were removed and the leaf tissue was analyzed separately. The results were reported on a moisture-free basis. In addition to total nitrogen, various nitrogenous constituents and fractions were determined. Their data showed a fairly regular increase in total nitrogen over the entire stalk range, with the lowest amount in Flyings, and considerably higher percentages in the red Leaf and Tips. The ammonia content followed a similar trend. The percentage of nicotine was also found to be higher in the leaves from the upper portions of the stalk, attaining a maximum in the leafy grades and declining in the immature Tips.

Good quality Burley tobacco was found to be related to a low content of total volatile bases.

SAMPLE SELECTION AND PREPARATION

COLLECTION OF SAMPLES

Samples of the 1951 and 1952 crops of Burley tobacco, Type 31, were collected on the auction warehouse floors in eight states. An effort was made to collect, from different producing areas, a substantially equal quantity of tobacco of each grade from 100 or more different growers' lots. The samples of the 1951 crop were collected from 39 markets and those of the 1952 crop from 43 markets. In case of the smaller one-buyer markets, 20 two-hand samples were collected, while on the larger markets, 20 four-hand samples of each grade were collected.

Special care was taken that each hand selected was reasonably uniform and typical of the grade. If one-third or more of the leaves of any hand was not typical of the grade in group, quality, and color, it was not chosen. Tobacco having unusual characteristics not commonly found on the market was not selected.

These samples were brought together in the Tobacco Division's sample room in Lexington, Ky., and were divided into lots according to the grades assigned by the head inspectors on the several markets. Supervising inspectors then checked each sample to see if it was true to grade. Four 4-hand samples of each grade were then reviewed by representatives of several tobacco companies.

The samples were then shipped to the laboratory of the Tobacco Division in Washington, D. C., where the tie leaf of each hand was removed and discarded. Each leaf in the hand was opened and carefully examined to determine if it was properly graded. All leaves of each hand which were not truly representative of the grade were eliminated.

Representatives of the tabacco companies cooperating in this investigation reviewed the samples from the standpoint of uniformity of the lots of each grade. Consequently the grade-samples selected for this investigation represented the composite judgment of USDA tobacco inspectors and leaf men of the tobacco industry. These samples are believed to have been as carefully selected and to have been as truly representative of each respective grade as this composite judging could make possible.

The leaves of each grade were stemmed by hand, and the web portion to be used for analysis was granulated. Sand and other earthly material was removed by passing the granulated material over a rotary sieve having square mesh openings of 0.5 mm. The cleaned, coarsely granulated material of each grade was thoroughly blended, using a Jones sampler for this purpose. The blended, granulated tobacco was air-dried at 95° F. until its weight was nearly constant, and it was then ground in a Wiley mill provided with a 1 mm. round mesh sieve. The ground material was thoroughly blended in a specially designed blender, transferred to glass containers provided with tight closures, and labeled for grade and crop identification.

The stems from three grades of leaf were similarly ground, blended, placed in glass jars, and distributed for general analysis.

ANALYTICAL METHODS

MOISTURE [4]

The American Tobacco Company

Weigh accurately duplicate samples of approximately 5 grams each and dry at 99° to 100° C. for 3 hours in a forced-draft oven. Cool to room temperature in a desiccator containing anhydrous calcium chloride and reweigh. Calculate the loss in weight as the percent of moisture.

[4] The moisture content data of all samples used in this investigation were determined by the method of The American Tobacco Co. For comparative purposes, the percentage of moisture was determined also by the other methods described here.

Standards Branch, Tobacco Division, AMS

Weigh accurately duplicate samples of approximately 2.5 grams each and dry at 99° to 100° C. for 4 hours in a convection type oven. Cool to room temperature in a desiccator containing anhydrous calcium chloride and reweigh. Calculate the loss in weight as the percent of moisture.

Liggett and Myers Tobacco Company

Weigh accurately duplicate samples of approximately 2 grams each and place in a desiccator containing fresh concentrated (sp. gr. 1.84) sulfuric acid, and allow to dry for 9 days at 30° C., and reweigh. (Place no more than six samples in one desiccator.) Calculate the loss in weight as the percent of moisture.

The Pennsylvania State University

Weigh accurately in duplicate approximately 2 grams of sample and dry at 80° C. for 3 hours in a vacuum oven maintained at a pressure of less than 50 mm. of mercury. Cool in a desiccator over anhydrous $CaCl_2$ and reweigh. Calculate the loss in weight as the percent of moisture.

The Imperial Tobacco Company, Ltd.

Weigh accurately duplicate samples of approximately 6.5 grams each and dry for 16 hours at 100° C. under standard ventilation conditions. Determine the dry weight and calculate the loss in weight as the percent of moisture.

TOTAL ASH

Standards Branch, Tobacco Division, AMS

Weigh accurately duplicate samples of approximately 2.5 grams each in a tared porcelain dish of 75 ml. capacity, and heat in the hood over a low flame of a bunsen burner until smoking ceases, taking care that the sample does not flame. Then heat in an electric muffle furnace provided with a temperature control for a period of 2 hours after the temperature reaches 550° C. Cool the sample to room temperature in a desiccator containing anhydrous calcium chloride and weigh. Calculate the increase in weight of the dish as percent total ash.

SAND

Standards Branch, Tobacco Division, AMS

Cover the dish containing the total ash from the preceding determination with a cover glass to prevent loss by spattering, and add 10 ml. of cencentrated hydrochloric acid slowly to minimize spattering. Boil the mixture over a small flame for about 1 minute, then evaporate on the steam bath to dryness, and finally bake on the steam bath for an additional three hours after reaching dryness. Add 5 ml. of concentrated hydrochloric

acid, cover the dish with a cover glass, and boil the mixture over a small flame for another minute. Add 30 ml. of distilled water, heat the mixture on the steam bath for 5 minutes, and decant through an ignited and tared Gooch crucible. Wash the insoluble material in the dish with hot water by decantation two or three times, decanting the washings into the crucible. Add 15 ml. of a hot saturated sodium carbonate solution (previously filtered through an asbestos mat in a Gooch crucible) to the dish, cover with a watch glass, and heat the mixture to boiling. Remove from burner and add five drops of 10 percent sodium hydroxide solution. Allow the mixture to settle, and decant through the Gooch crucible. Add another 15 ml. portion of the sodium carbonate solution to the dish, cover, and heat the mixture to boiling, treat as in the previous step, and allow to settle. Decant the solution into the crucible, and wash the insoluble material in the dish by decantation with several portions of hot water, pouring the washings into the crucible. Transfer the remaining insoluble material in the dish to the crucible using a hot water wash bottle, and wash with two small portions of dilute HCl (1 volume of concentrated HCl to 4 of water), and finally with hot water until free of HCl. Dry crucible and contents in an oven at 100° C. for one hour and then heat in the muffle furnace at 600° C. for one-half hour or longer. Allow to cool in a desiccator over anhydrous calcium chloride to room temperature and weigh. Calculate the increase in weight of the crucible as sand.

$$\frac{\text{Weight of Sand} \times 100}{\text{Weight of moisture-free tobacco}} = \% \text{ Sand (moisture-free basis)}$$

WATER-SOLUBLE AND WATER-INSOLUBLE ASH

Philip Morris, Inc.

Transfer the ash obtained in the determination of total ash as described above to a 250 ml. beaker, add 25 ml. of water, and heat the mixture to boiling with frequent stirring. Filter on an ashless filter paper, wash with 25 ml. of hot water, and retain the combined filtrate and washings for the determination of the alkalinity of the water-soluble ash. Return the filter paper containing the insoluble ash to the original porcelain dish and dry in a 100° C. oven before placing in muffle. Heat at 550° C. for 2 hours, cool to room temperature in a desiccator over anhydrous $CaCl_2$, weigh, and calculate the increase in weight as the percentage of insoluble ash. Obtain the percentage of water-soluble ash by subtracting the percentage of water-insoluble ash from the percentage of total ash.

ALKALINITY OF WATER-SOLUBLE ASH

Philip Morris, Inc.

Titrate the combined filtrate and washings containing the soluble portion of the total ash, as above-described, with N/10 HCl to a pH of 4.3, using

a Fisher titrimeter. Calculate the alkalinity of the soluble ash as the number of ml. of N/10 HCl per gram of moisture-free and sand-free tobacco.

ALKALINITY OF WATER-INSOLUBLE ASH

Philip Morris, Inc.

To the water-insoluble ash, obtained as above-described, add a measured quantity of N/10 HCl in excess of that required to make the mixture definitely acid in reaction. Heat the mixture to boiling, and titrate to a pH of 4.3 with N/10 NaOH solution, using a Fisher titrimeter. Calculate the alkalinity of the water-insoluble ash as the number of ml. of N/10 HCl required to neutralize the water-insoluble ash from one gram of moisture-free and sand-free tobacco.

SODIUM

Eastern Utilization and Development Division, ARS

Place a 1-gram sample of tobacco in a platinum dish, moisten with 10 ml. of 0.5 percent sulfuric acid solution, and char under an infrared lamp until fumes of SO_3 cease to be given off. Heat at 600° C. for 8 hours, and finally at 750° C. for 2 hours. After cooling to room temperature, add 10 ml. of 3N (approx.) HCl, and evaporate the mixture to dryness on the steam bath. To the residue, add 20 ml. of the 3N HCl, and warm the mixture on the steam bath while stirring to dissolve the ash. Decant the solution through a washed filter paper into a funnel inserted in the neck of a 100 ml. volumetric flask. Repeat this operation of dissolving the ash, using a second 20 ml. portion of 3N HCl. Transfer the insoluble material to the filter, and wash the dish and filter with water until the volume of filtrate approaches 100 ml. Cool the solution to room temperature and make to volume with water. Aspirate this solution of the ash into the flame, and read the intensity (I) of emission at 589.0 mμ on the spectrophotometer. Compare this reading with that of a standard curve prepared with pure NaCl solution (ppm. Na vs. I 589.0 mμ). Determine the ppm. of Na in the solution containing the sample by interpolation from the standard curve. Calculate the percent Na in the sample as follows:

$$\frac{(\text{ppm. Na}) \times (\text{volume of solution}) \times 100}{\text{Weight of moisture-free sample} \times 10^6} = \%\ \text{Na (moisture-free basis)}$$

POTASSIUM

Eastern Utilization and Development Division, ARS

Ash a 1-gram sample of tobacco, according to the same procedure described above for sodium, and dissolve the ash following also the procedure described for the determination of sodium.

Aspirate the solution of the ash into the flame, and read the intensity of emission (I) at 766.5 mμ on the spectrophotometer. Prepare a standard curve (ppm. K vs. 766.5 mμ) using matrix solutions containing known

amounts which bracket the amount in the sample, and which contain amounts of Ca, Mg, SO_4, and PO_4 estimated to be present in the tobacco ash, plus 40 ml. of 3N HCl per 100 ml. of solution. Determine K (ppm.) in the sample solution by interpolation from the standard curve, and calculate the percent of potassium as follows:

$$\frac{\text{(ppm. K) (volume of solution)} \times 100}{\text{Weight of moisture-free sample} \times 10^6} = \% \text{ Potassium (moisture-free basis)}$$

In making a series of analyses, measure the flame intensity of a single standard potassium solution with approximately the same amount of K as in the sample, at regular intervals. Compare also the standard solution with the matrix solutions so that variations due to changes in atomization rate and flame intensity can be corrected, and in order that the effect of the extraneous salts on the intensity of the flame can be evaluated.

CALCIUM

The Pennsylvania State University

REAGENTS AND SOLUTIONS

(1) Hydrochloric acid (one volume of concentrated hydrochloric acid plus four volumes of water); (2) Glacial acetic acid; (3) Aqueous ammonium oxalate solution (saturated); (4) Sulfuric acid solution (one volume of concentrated sulfuric acid plus four volumes of water).

DETERMINATION

Place a 4.4-gram sample in a silica dish, and heat overnight in a muffle furnace maintained at 500° C. Allow to cool to room temperature, and dissolve the ash in about 25 ml. of the dilute hydrochloric acid. Transfer the solution to a 100 ml. beaker, heat to boiling, filter into a 110 ml. volumetric flask, and dilute the filtrate to the mark with water. Transfer a 50 ml. aliquot to a 250 ml. beaker, and add ammonium hydroxide solution until the iron and aluminum hydroxides begin to precipitate. Add 10 ml. of glacial acetic acid, heat the solution to boiling, and add 10 ml. of the ammonium oxalate solution. Continue the boiling of the solution until the precipitate is coarsely granular. Allow the solution to stand overnight. Filter the solution through a S. and S. No. 589 Blue Ribbon filter paper, and wash the precipitate with water until the filtrate is free of oxalates. Break the point of the filter paper with a pl[illegible]um wire, and wash the precipitate into the beaker in which the calc[illegible] was precipitated, using hot 1:4 sulfuric acid solution. Wash the filter paper with hot water, and add 10 ml. of the dilute sulfuric acid solution to the filtrate. Heat the solution to about 90° C., and titrate with N/10 $KMnO_4$ solution. Finally add the filter paper to the solution and complete the titration. Calculate the percentage of calcium as follows:

$$\frac{\text{ml. N/10 } KMnO_4 \text{ required} \times 0.2004}{\text{Weight of sample (moisture-free basis)}} = \% \text{ Calcium (moisture-free basis)}$$

PHOSPHORUS

American Sumatra Tobacco Corporation

APPARATUS

Fisher Electrophotometer No. 7–106 with extra micro cells No. 7–102–65.

REAGENTS AND SOLUTIONS

Buffer solution.—Dissolve 100 grams of sodium acetate ($CH_3COONa \cdot 3H_2O$) in 500 ml. of water, add 30 ml. of 99.5 percent acetic acid, and dilute with water to 1 liter.

Standard phosphorus stock solution. (100 ppm. of P.) Dissolve 0.0439 gram of KH_2PO_4 in 100 ml. of water.

Sulfonic acid reagent.—Dissolve approximately 0.5 gram of pure, dry 1-amino-2-naphthol-4 sulfonic acid (Eastman Kodak Co.) in 195 ml. of 15 percent sodium bisulfite solution in a 250 ml. volumetric flask. Add 5 ml. of 20 percent sodium sulfite solution, stopper the flask, shake, and dilute to volume with water. After again shaking, allow the solution to stand overnight, filter, and store in a brown bottle.

Molybdate reagent.—Place 15 grams of ammonium molybdate in a 1 liter volumetric flask and dissolve in about 300 ml. of water. Add 800 ml. of concentrated hydrochloric acid (sp. gr. 1.18) slowly, cool to room temperature, dilute with water to 1 liter, and store in a brown glass-stoppered bottle. Prepare a fresh supply of this reagent every 3 months.

DETERMINATION

Ashing of sample.—Place a 2-gram sample in a 150 ml. Pyrex beaker, add 20 ml. of nitric acid (69–71%), cover beaker with a watch glass, and allow the mixture to stand for one-half hour. Rotate the beaker a few times until the sample is completely wetted or nearly so. Place the beaker on a hot plate and heat gently until no visible signs of solid material, except silica, remain. Remove the beaker from the hot plate, add 5 ml. of 70 percent perchloric acid, and cover the beaker with a watch glass. Boil the solution gently until it is clear and fumes copiously.

Solution A.—Add about 50 ml. of water to the ash prepared as above-described. Heat to boiling and filter through Whatman No. 40 filter paper into a 200 ml. volumetric flask. Wash the residue on the filter paper with hot water and collect the washings in the same volumetric flask. Cool the combined filtrate and washings to room temperature and dilute to 200 ml. with water. Designate this as "Solution A."

Solution B.—Pipette 1 ml. of "Solution A" into a test tube and add 9 ml. of the buffer solution. Designate this as "Solution B."

Standard curve.—Plot a standard curve in the range between 1 and 4 ppm. of P for interpreting color transmittancy of the sample from this standard curve.

Phosphorus standards.—Prepare a series of standards of 1, 2, 3, and 4

ppm. of P, respectively, by diluting 1, 2, 3, and 4 ml. of standard phosphorus stock solution to 100 ml. with buffer solution. Plot a standard curve in the range between 1 and 4 ppm. of P.

Colorimeter procedure.—Pipette 4 ml. of "Solution B" into a test tube, add 0.3 ml. of the sulfonic acid reagent, and mix. Add 1 ml. of the molybdate reagent rapidly against the side of the test tube and mix. Allow the solution to stand for 10 minutes. Transfer this solution to an extra micro cell and read the color transmittancy in the electrophotometer at wave length 650 mμ. Determine the ppm. of P in "Solution A" by reference to a standard curve prepared simultaneously.

Calculate the percentage of phosphorus as follows:

$$\frac{(\text{ppm. P})\,(\text{ml. Solution A})\,(\text{dilution of Solution A}) \times 100}{\text{Weight of sample} \times 10^6} = \%\ \text{Phosphorus}$$

MANGANESE

American Sumatra Tobacco Corporation

REAGENTS AND SOLUTIONS

Standard manganese stock solution.—(100 ppm. of Mn.) prepared as follows: Place 0.0288 gram of $KMnO_4$ in a 125 ml. Erlenmeyer flask, add 10 ml. of water, 6 drops of concentrated sulfuric acid, and a few glass beads. Heat to boiling and add sodium bisulphite in sufficient quantity to discharge the color. Concentrate the solution until fumes of sulfuric acid begin to appear. Dissolve the residue in water and dilute to a volume of 100 ml.

DETERMINATION

(a) *Ashing of sample.*—Place a 2-gram sample (as-is) into a 150 ml. Pyrex beaker, add 20 ml. of nitric acid (69–71%), cover the beaker with a watch glass, and allow to stand for about one-half hour. During this period rotate the beaker a few times until the sample is completely wetted or nearly so. Place the beaker on a hot plate and heat gently until no visible signs of solid material, except silica, remain. Remove the beaker from the hot plate, add 5 ml. of 70 percent perchloric acid, cover the beaker again with a watch glass, and boil gently until the solution is clear and fumes copiously. Take care not to evaporate the solution to complete dryness.

Solution A.—Add about 50 ml. of water to the ash prepared as described in paragraph (a), bring to a boil, and filter through a Whatman No. 40 filter paper into a 200 ml. volumetric flask. Wash the silica on the filter paper with hot water and collect the washings in the same flask. Cool the filtrate and washings to room temperature and dilute with water to 200 ml. Designate this as "Solution A."

Standard curve.—Prepare a series of standards of 1, 2, 3, and 4 ppm., respectively, of Mn by diluting 1, 2, 3, and 4 ml. of the standard Manganese stock solution to 100 ml. with water. Plot a standard curve in the range

between 1 and 4 ppm. of standard manganese stock solution for interpreting color transmittancy of the sample from the standard.

Colorimeter procedure.—Transfer 40 ml. of "Solution A" to Nessler tubes, add 4 ml. of ortho phosphoric acid (85%), stir, and then add about 200 mg. of potassium periodate, and stir again. Place the Nessler tubes in a wire basket, immerse in a galvanized pot half-filled with water, and boil for 1 hour. Stir the contents of the Nessler tubes a few times during this period. Allow the Nessler tubes to cool and dilute to 50 ml. with water. Transfer this solution to a rectangular absorption cell and read the color transmittancy in the electrophotometer at wave length 325 mμ. Determine the ppm. of Mn in "Solution A" by reference to a standard curve, prepared in the same manner and at the same time.

Calculate the percentage of Mn as follows:

$$\frac{\text{ppm. of Mn. (ml. of Solution A)} \times 100}{\text{Weight of sample (as-is)} \times 10^6} = \% \text{ Manganese (as-is basis)}$$

TOTAL NITROGEN (INCLUDING NITRATE NITROGEN)

Liggett and Myers Tobacco Company

The method is essentially the one given in the A.O.A.C. Book of Methods (*6*).

DETERMINATION

Place 1.4000 grams (as-is) of tobacco in a 650 ml. Kjeldahl digestion flask. Add 35 ml. of an acid solution, consisting of 28.6 grams of salicylic acid in 1 liter of H_2SO_4 (sp. gr. 1.84). Shake until thoroughly mixed and allow to stand for at least one hour. Add 5 grams of $Na_2S_2O_3 \cdot 5H_2O$, place on digestion rack, turn on electric heaters to low heat, and digest until all danger of frothing has passed (about 30 minutes). Increase heat until acid boils briskly and continue boiling until white fumes of SO_3 no longer escape from the flask. Add 0.7 gram of metallic mercury, 0.1 gram of $CuSO_4 \cdot 5H_2O$, and 5 grams of K_2SO_4, and continue boiling for 2 hours, at which time the liquid is colorless. If the contents of the flask are likely to become solid before this point is reached, add 5 ml. of H_2SO_4 (sp. gr. 1.84) and continue heating. Allow the flasks to cool for 20 to 30 minutes, add 250 ml. of distilled water and a few pieces of granulated zinc to prevent bumping. Pour 70–80 ml. of a sodium hydroxide-sodium thiosulfate solution (consisting of 200 ml. of sodium thiosulfate, made by dissolving 1400 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 1250 ml. of distilled water, mixed with 5 liters of 40% NaOH solution) down the side of the flask so that it does not mix at once with the acid solution. Connect flask to condenser by means of a Kjeldahl connection bulb, taking care that the tip of the condenser extends below the surface of an accurately measured volume of N/10 H_2SO_4 solution contained in the receiver. Mix contents by shaking, and distill until all the NH_3 has passed over into the measured quantity of the standard acid. The first 150 ml. of distillate normally contains all the NH_3. Titrate the

excess acid with N/10 NaOH solution, alizarin red S indicator being used, and calculate the results as follows:

$$\frac{\text{ml. of N/10 } H_2SO_4\text{—ml. of N/10 NaOH}}{\text{Weight of sample (as-is)}} \times 0.140 = \%\ \text{Total Nitrogen}$$

NITRATE NITROGEN

Liggett and Myers Tobacco Company

The method is essentially that described in the A.O.A.C. Book of Methods (7).

DETERMINATION

Place 1.4000 grams (as-is) of tobacco in a 650 ml. Kjeldahl digestion flask. Add 5 grams of $FeSO_4 \cdot 7H_2O$, 10 ml. of distilled H_2O, and shake flask until all particles of tobacco are thoroughly wet. Wash down the sides of the flask with 25 ml. of distilled water. Let this mixture stand for one hour, mixing it frequently by rotating flask. Add 25 ml. of H_2SO_4 (sp. gr. 1.84), and mix thoroughly by rotation. Wash down neck and sides of flask with 15 ml. of water. After sample has been in contact with the sulfuric acid solution for one hour, place the flask on the digestion rack and heat until dense fumes of SO_3 no longer appear. Add 0.7 gram of metallic mercury, 0.1 gram of $CuSO_4 \cdot 5H_2O$, and 5 grams of K_2SO_4, and continue boiling for 2 hours. If the contents of the flask are likely to become solid before this point is reached, add 5 ml. of H_2SO_4 (sp. gr. 1.84), and continue heating. Allow the flask to cool for 20 to 30 minutes, add 250 ml. of water and a few pieces of granulated zinc to prevent bumping. Pour 70–80 ml. of a sodium hydroxide-sodium thiosulfate solution (consisting of 200 ml. of sodium thiosulfate solution, made by dissolving 1400 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 1250 ml. of distilled water, mixed with 5 liters of 40% NaOH solution) down the side of the flask so that it does not mix at once with the acid solution. Connect flask to condenser by means of a Kjeldahl connection bulb, taking care that the tip of the condenser extends below the surface of an accurately measured volume of N/10 H_2SO_4 solution contained in the receiver. Mix contents by shaking, and distill until all the NH_3 has passed over into the measured quantity of the standard acid. The first 150 ml. of distillate normally contains all the NH_3. Titrate the excess acid with N/10 NaOH solution, alizarin red S indicator being used. Calculate the nonnitrate nitrogen and nitrate nitrogen as follows:

$$\frac{\text{ml. of N/10 } H_2SO_4\text{—ml. of N/10 NaOH}}{\text{Weight of sample (as-is)}} \times 0.140 = \%\ \text{Nonnitrate Nitrogen}$$

$$\%\ \text{Total Nitrogen} - \%\ \text{Nonnitrate Nitrogen} = \%\ \text{Nitrate Nitrogen}$$

AMMONIA NITROGEN

Agricultural Experiment Station, University of Kentucky

APPARATUS

A Van Slyke and Cullen (*44*) aeration apparatus is used.

REAGENTS

Phosphate-borate buffer solution.—Prepare this solution according to the directions of Pucher, Vickery, and Leavenworth (*35*), as follows: Mix thoroughly 750 ml. of 0.1 M potassium dihydrogen phosphate (13.6 grams per liter) with 250 ml. of 0.05 M borax (19.1 grams per liter).

Tashiro's indicator.—Prepare by adding 0.375 gram of methyl red and 0.245 gram of methylene blue to 95 percent ethanol, and diluting to 300 ml. with 95 percent ethanol.

DETERMINATION

Transfer a finely ground sample, equivalent to 0.5 gram of moisture-free tobacco, to a 38 × 200 mm. test tube. Add 10 ml. of the phosphate-borate reagent and 10 drops of capryl alcohol. Just before the stoppers are tightened, add 5 ml. of N/2 NaOH, containing 5 grams of borax per 100 ml. Aerate the reaction mixture for 2.5 hours at a rapid rate during which time the ammonia is absorbed in 15 ml. of N/50 HCl. At the end of the aeration period wash the aeration tubes, inside and out, with distilled water into the receiving acid. Add one drop of the indicator, and titrate the solution to a gray or slightly green color with N/50 NaOH solution. Make a separate blank determination with each set of samples. Calculate the percentage of ammonia nitrogen as follows:

$$\frac{\text{Normality of NaOH} \times (\text{Sample} - \text{Blank}) \text{ ml. NaOH} \times 1.4}{\text{Weight of sample (grams)}} = \% \text{ Ammonia Nitrogen}$$

PROTEIN NITROGEN

Liggett and Myers Tobacco Company

DETERMINATION

Place 2.000 grams (as-is) of ground tobacco in a 250 ml. Erlenmeyer flask, add 100 ml. of 0.5 percent acetic acid solution, heat the mixture to boiling, and reflux for 15 minutes. Filter with suction while hot, using a Buchner funnel, size 2A, and Whatman No. 1 filter paper, diam. 15 cm. Press filter paper into the funnel so that about 2 cm. extends up on the inside wall of the funnel. Wash the residue with hot 0.5 percent acetic acid solution until the filtrate becomes colorless, usually about 450 ml. Place the filter paper and residual tobacco in a 650 ml. Kjeldahl digestion flask, add 0.7 gram of metallic mercury, 0.1 gram of $CuSO_4 \cdot 5H_2O$, 5 grams of K_2SO_4 and 25 ml. H_2SO_4 (sp. gr. 1.84). Mix thoroughly by rotating flask, place on digestion rack, heat slowly until frothing ceases, and then increase heat until solution boils briskly. Continue digestion for 2 hours, at which time the mixture is colorless. If the contents of the flask are likely to become solid before this point is reached, add 5 ml. of H_2SO_4 (sp. gr. 1.84), and continue heating. Allow the flask to cool for 20 to 30 minutes, add 250 ml. of distilled water and a few pieces of granulated zinc to prevent bumping. Pour 70–80 ml. of a sodium hydroxide-sodium thiosulfate solution (consisting of 200 ml.

of sodium thiosulfate, made by dissolving 1400 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 1250 ml. of water, mixed with 5 liters of 40% NaOH solution) down the side of the flask so that it does not mix at once with the acid solution. Connect flask to condenser by means of a Kjeldahl connection bulb, taking care that the tip of the condenser extends below the surface of an accurately measured volume of N/10 H_2SO_4 solution contained in the receiver. Mix contents by shaking, and distill until all the NH_3 has passed over into the measured quantity of the standard acid. The first 150 ml. of distillate normally contains all the NH_3. Titrate the excess acid with N/10 NaOH solution, alizarin red S indicator being used, and calculate the results as follows:

$$\frac{(\text{ml. of N/10 } H_2SO_4 - \text{ml. of N/10 NaOH})}{\text{Weight of sample (as-is)}} \times 0.140 = \% \text{ Protein Nitrogen}$$

Calculate the results on a moisture-free and sand-free basis. Multiply percent protein nitrogen by 6.25 to obtain percent protein.

ALPHA AMINO NITROGEN

Liggett and Myers Tobacco Company

DETERMINATION

Preparation of Extract A.—Place 6.67 grams (as-is) of ground tobacco in a 250 ml. Erlenmeyer flask, add 40 ml. of distilled water, mix until all particles of the tobacco are wet, and then add 60 ml. of distilled water, washing down the sides of the flask. Close flask with rubber stopper and let it stand for 16 hours at room temperature. Filter mixture through a fluted filter paper and designate filtrate as "Extract A."

Alpha amino nitrogen.—Determine alpha amino nitrogen using 10 ml. aliquots of the "Extract A" by the Van Slyke Method (Methods of Analysis, A.O.A.C. 6th ed., p. 429 (1945) or 7th ed., p. 364 (1950)). Calculate the results as follows:

$$\frac{(\text{ml. } N_2 - \text{blank}) \times F \times 100}{\text{Weight of sample (as-is)}} = \% \text{ Alpha Amino N (as-is)}$$

Where:

$$F = \tfrac{1}{2}\left[\frac{(P - W)}{760}\right] \times \frac{273}{T} \times .00125$$

P = barometric pressure in mm.

W = vapor pressure of H_2O at t°C.

T = absolute temperature (273 + t°C.)

t° C. = temperature at which the volume of nitrogen obtained is measured.

Average duplicate determinations and convert the percentage from an as-is to a moisture-free basis or to a moisture and sand-free basis, using the appropriate factors.

TOTAL VOLATILE BASES (AS NH_3)

The American Tobacco Company

APPARATUS

The apparatus required for this determination is described and illustrated in the articles by Bradford *et al* (*8*) and Moseley *et al* (*23*).

DETERMINATION

Transfer a 5-gram sample of tobacco to an 800 ml. Kjeldahl flask. Add 75 ml. of standard trisodium phosphate solution (consisting of 58.67 grams of anhydrous trisodium phosphate and 3.33 grams of sodium hydroxide per liter) and connect the flask to an apparatus (see References) arranged for the distillation in a current of steam. Collect the distillate in a 1000 ml. Erlenmeyer flask containing an excess of N/10 hydrocholoric acid. Turn on the burner beneath the reaction flask and adjust to a medium height until the liquid within the flask begins to boil. Admit steam from a low pressure line (5 to 10 pounds) and adjust to the rate which will yield 800 ml. of distillate in 45 minutes. Adjust the flame beneath the reaction flask so that the volume within the flask remains constant throughout the distillation. Titrate the excess of hydrochloric acid with N/10 sodium hydroxide using 7 to 8 drops of methyl red-methylene blue, an achromatic indicator. Calculate the results as follows:

$$\frac{\text{ml. N/10 HCl required} \times 0.17032}{\text{Weight of moisture-free sample}} = \% \text{ T.V.B., as ammonia (moisture-free basis)}$$

NICOTINE, NORNICOTINE AND TOTAL ALKALOIDS (AS NICOTINE)

R. J. Reynolds Tobacco Company

The method submitted is a description of a published article (*12*) by Cundiff and Markunas.

APPARATUS

Precision-Shell titrimeter with calomel and glass electrodes (used for the potentiometric titrations). Wrist-action shaker, Model BB, Burrell Corporation, Pittsburgh, Pa., or equal.

REAGENTS AND SOLUTIONS

(1) Barium hydroxide, $Ba(OH)_2 \cdot 8H_20$, A.C.S. grade; (2) Barium hydroxide solution, a saturated aqueous solution; (3) Benzene-chloroform solution, consisting of 900 ml. of benzene and 100 ml. of chloroform; (4) Celite, Johns-Manville Corporation's analytical filter aid; (5) Acetic anhydride, A.C.S. grade; (6) Crystal violet indicator, one gram of crystal violet dissolved in 100 ml. of glacial acetic acid (A.C.S. grade); (7) 0.025 N Perchloric acid solution, 2.1 ml. of 72 percent perchloric acid (A.C.S. grade) diluted to one liter with glacial acetic acid (A.C.S. grade). Standardize the perchloric acid solution against potassium acid phthalate (primary

standard grade) according to the procedure of Seaman and Allen (Anal. Chem., *23*, 592 (1951).

DETERMINATION

Accurately weigh a sample of 2.5 to 3.5 grams of the finely ground tobacco and transfer to a 250 ml. glass-stoppered Erlenmeyer flask. Add approximately one gram of granular barium hydroxide and 15 ml. of the barium hydroxide solution. Swirl the flask until the tobacco is thoroughly wetted, adding more barium hydroxide solution if necessary. Pipette 100 ml. of benzene-chloroform solution into the flask, stopper tightly, and agitate vigorously for 10 minutes, using the Wrist-action shaker, or for 15 minutes if shaken by hand. Add approximately 2 grams of celite, swirl flask until the filter aid is well dispersed, allow the two liquid phases to separate, and filter the benzene-chloroform layer through Whatman No. 2 filter paper into a second flask. Pipette 25 ml. aliquots of the filtrate into each of two 125 ml. Erlenmeyer flasks. Pass a stream of air over the surface of the solution in the first flask for 5 minutes to remove any free ammonia that might be present in the filtrate. Add 0.5 ml. of acetic anhydride to the second flask. To each flask, add one drop of crystal violet indicator and titrate to a green end point with the 0.025 N perchloric acid. If the nornicotine content is found to be as high as 25 percent of the nicotine content, acetylate another portion of the filtrate and titrate potentiometrically to obtain the equivalence point. Calculate the results as follows:

$$\% \text{ Total Alkaloids} = \frac{V_1 \times N \times 32.45}{\text{Weight of moisture-free sample}}$$

$$\% \text{ Nicotine} = \frac{2V_2 - V_1 \times N \times 32.45}{\text{Weight of moisture-free sample}}$$

$$\% \text{ Nornicotine} = \frac{2(V_1 - V_2) \times N \times 29.64}{\text{Weight of moisture-free sample}}$$

Where:

V_1 = ml. of perchloric acid required to neutralize nonacetylated aliquot.

V_2 = ml. of perchloric acid required to neutralize acetylated aliquot.

N = normality of perchloric acid solution.

The factors 32.45 and 29.64 in the above equations are based on the fact that nicotine and nornicotine are dibasic in the nonaqueous solvents used. Accordingly, 1 ml. of N/1 perchloric acid = 0.081114 grams of nicotine or 0.0741 grams of nornicotine. Inasmuch as 25 ml. aliquots (corresponding to ¼ of the total extract) are taken for titration, above figures are multiplied by 4. To get percentages, they are multiplied further by 100.

TOTAL ALKALOIDS

Tobacco Section, Tobacco & Sugar Crops Research Branch, Crops Research Division, ARS

APPARATUS

(a) A slight modification of the Griffith and Jeffrey (*16*) improved steam distillation apparatus was used; (b) Beckman spectrophotometer, Model DU.

DETERMINATION

Distillation.—Accurately weigh a sample of about 0.3 gram (as-is) and place it in the receiver of the distillation apparatus, together with about one gram of sodium chloride and 1 ml. of 30 percent sodium hydroxide. Place 2 ml. portion of 1 + 4 hydrochloric acid in the receiver and distill nearly 250 ml. in about 15 minutes. Make to a volume of 250 ml.

Spectrophotometric analysis.—Determine nicotine in the distillate by the spectrophotometric method of Willets *et al* (*49*), except that the specific extinction value of 33.4 at 259 mμ obtained experimentally on the instrument in this laboratory is used in the calculation instead of the value 34.3 given in the original method.

TOTAL REDUCING SUBSTANCES, TOTAL REDUCING SUGARS, AND POLYPHENOLS

P. Lorillard Company

REAGENTS AND SOLUTIONS

(R1)—Fehling's solution A.—82.4 grams of copper sulfate pentahydrate dissolved in water to make 1 liter of solution.

(R2)—Fehling's solution B.—346 grams of Rochelle Salt (potassium sodium tartrate) dissolved in water in a 1-liter volumetric flask. Sufficient concentrated sodium hydroxide solution is added to give 130 grams of sodium hydroxide. The strength of this concentrated sodium hydroxide is determined by titration with standard acid.

(R3)—Iodide-Iodate solution.— 120 grams of potassium iodide, 10.8 grams of potassium iodate, and 5 ml. of a saturated sodium hydroxide solution, made up to 2,000 ml. with water in a volumetric flask.

(R4)—5N Sulfuric acid.—135 ml. of concentrated sulfuric acid (sp. gr. 1.835–1.840) cautiously mixed with 800 ml. of water, and made up to 1 liter in a volumetric flask at room temperature.

(R5)—Potassium oxalate solution.—330 grams of potassium oxalate dissolved in water and made to 1 liter in a volumetric flask at room temperature.

(R6)—Sodium thiosulfate solution.—95 grams of sodium thiosulfate pentahydrate dissolved in 3,000 ml. of water. One ml. of chloroform is added as a preservative. Standardize against 0.1250 N $K_2Cr_2O_7$ solution (6.1291 grams $K_2Cr_2O_7$ per liter).

(R7)—Neutral lead acetate solution.—100 grams of neutral lead acetate trihydrate dissolved in 160 ml. of water.

PART 1—TOTAL REDUCING SUBSTANCES

(a) Preparation of filtrate A.—Place a 3-gram sample (as-is) and 0.3-gram $CaCO_3$ in a 500 ml. volumetric flask, add 200 ml. of water, and reflux gently for 1 hour with occasional manual shaking. Allow flask and contents to cool to room temperature, make up to volume with water, mix, filter by gravity through a fluted circle of Whatman No. 44 filter paper, and designate as filtrate "A."

(b) Determination.—Pipette a 20 ml. aliquot of filtrate A (equivalent to a 0.12-gram sample), and transfer to a 500 ml. Erlenmeyer flask containing 30 ml. of water.

(bb).—Pipette 25 ml. of each (R1) and (R2) into the Erlenmeyer flask, and mix by gentle manual shaking. Invert a 100 ml. beaker over the mouth of the flask, and place in a thermostatically controlled water bath at 80° C. for 30 minutes. Remove and pipette 25 ml. of (R3) to the hot solution. Swirl and add 25 ml. of (R4). Swirl for 5 seconds, then add 20 ml. of (R5). Titrate with (R6), using 5 percent starch indicator (1 ml. added near end point). Record ml. of sodium thiosulfate used. In the same manner, run a blank on 50 ml. of water, using the above procedure.

(c) Calculation.—Subtract ml. of thiosulfate used in blank titration from ml. of thiosulfate used in sample titration.
Then,

$$\text{Ml.} \times \text{Normality} \times 63.57 = \text{Mg. Cu reduced.}$$

From Quisumbing and Thomas Table* determine corresponding amount of d-glucose in aliquot and convert to grams.

$$\frac{\text{Grams d-glucose}}{\text{Grams of sample in aliquot}} \times 100 = \% \text{ Total Reducing Substances.}$$

PART 2—TOTAL REDUCING SUGARS

(a) Preparation of filtrate B.—Extract a 5-gram sample (as-is) for 16 hours with 80 percent ethanol in a Soxhlet extraction apparatus. Use an alundum extraction thimble (34 × 100 mm., medium porosity) for the extraction. Insert a tared plug of glass wool in the thimble after weighing out the 5-gram sample. (Save the extracted tobacco for the determination of starch.) Transfer the alcoholic extract to a 250 ml. volumetric flask and make up to volume with 80 percent ethanol. Transfer 100 ml. of the alcoholic extract (equivalent to a 2-gram sample) to a 250 ml. beaker, and evaporate on the steam bath until the odor of alcohol can no longer be detected. Transfer the residual aqueous solution to a 250 ml. volumetric flask, wash beaker several times with small portions of hot (about 80° C.) water and add washings to the aqueous solution in the volumetric flask. Cool solution to room temperature and clarify with 1.5 ml. of (R7). Delead

* From the Assoc. Off. Agr. Chem., "Methods of Analysis," (1955).

with 0.3420 gram of solid sodium oxalate, make up to volume with water, mix, filter by gravity through Whatman No. 44 filter paper, and designate as filtrate "B." Test a 10 ml. portion of this filtrate with a few crystals of sodium oxalate to make sure that all the lead has been removed.

(b) Determination.—Pipette a 25 ml. aliquot of filtrate B (equivalent to a 0.2-gram sample), transfer to a 500 ml. Erlenmeyer flask containing 25 ml. of water, and continue the determination as described in paragraph (bb) of Part 1.

(c) Calculation.—Subtract ml. of thiosulfate used in blank titration from ml. of thiosulfate used in sample titration.

Then,

$$\text{Ml.} \times \text{Normality} \times 63.57 = \text{Mg. Cu reduced.}$$

From Quisumbing and Thomas Table determine corresponding amount of d-glucose in aliquot and convert to grams.

$$\frac{\text{Grams d-glucose}}{.200} \times 100 = \% \text{ Total Reducing Sugars}$$

PART 3—POLYPHENOLS

Polyphenols.—Percent total reducing substances (as d-glucose) minus % total reducing sugars (as d-glucose) = % polyphenols (as d-glucose).

References.—(1) C. A. Browne and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," 3d. ed., pp. 828 and 1235, John Wiley and Sons, New York (1941). (2) P. A. Shaffer and A. F. Hartman, J. Biol. Chem., *45*, 349–390 (1921). (3) M. A. Joslyn, "Methods in Food Analysis," p. 149, Academic Press, New York (1950). (4) Methods of Analysis, A.O.A.C., 8th. ed., p. 549 (1955).

PECTIC SUBSTANCES

Standards Branch, Tobacco Division, AMS

APPARATUS

The apparatus used is essentially a Weihe-Phillips (*46*) extractor arranged as illustrated in figure 1.

It consists of a modified extraction crucible (A), designated as a "Pectin Extractor,"[6] the bottom of which is rimmed to fit a one-hole, No. 9, rubber stopper (E), carrying the 6 mm. O. D. glass tube (F). The pectin extractor is held in position by means of a clamp attached to a ring stand. The fritted-glass disk (D) is of "C" porosity. The 2-liter beaker (C) serves as a water bath. The glass rod (B), having fire-polished ends, is kept in the pectin extractor throughout the successive operations, and is used for stirring the sample with the solvent. The suction flask (H), of 1-liter capacity,

[6] The pectin extractor may be purchased from the Kontes Glass Co., Vineland, N. J., under the designation and number "Pectin Extractor No. 2721–E."

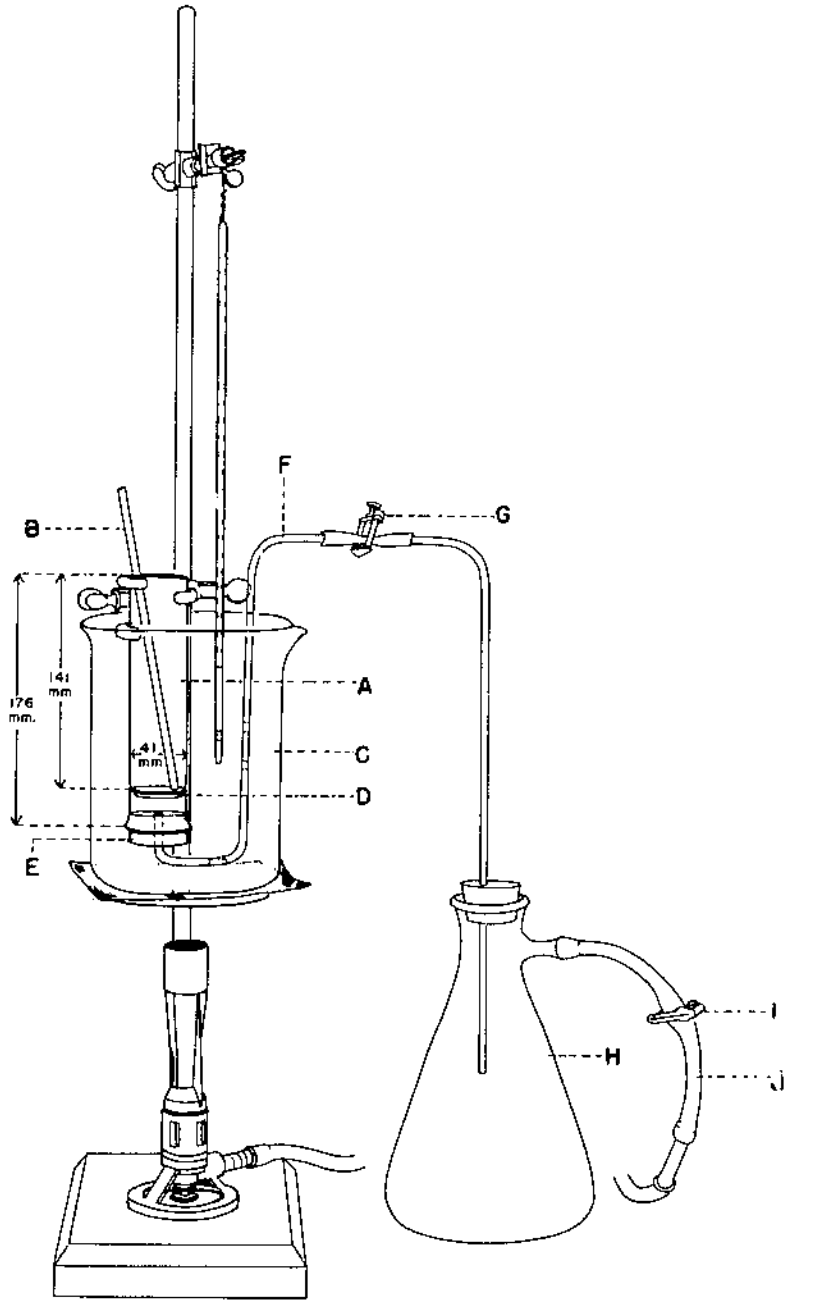

FIGURE 1.—Apparatus for extraction of pectic substances.

is connected to the vacuum line through the rubber tube (J). By manipulation of the screw clamps (G) and (I), the solvent is withdrawn from the pectin extractor through tube F, into the suction flask. It is advantageous to open the screw clamps only partially and to apply a gentle suction. Otherwise, the ground tobacco packs on the fritted-glass disk and thus slows up filtration.

REAGENTS

(1) 10 percent Hydrochloric acid solution; (2) Ethanol solution, 1 volume of distilled water to 2 volumes of 95 percent ethanol; (3) N/5 (approx.) Sodium hydroxide solution; (4) N/1 (approx.) Acetic acid solution; (5) $\frac{\text{Molar}}{10}$ (approx.) aqueous calcium chloride solution; (6) 2 Molar (approx.) aqueous calcium chloride solution; (7) N/20 (approx.) Hydrochloric acid solution; (8) 0.5 percent Aqueous ammonium citrate solution; and (9) 2 percent Aqueous ammonium citrate solution.

DETERMINATION

Part 1—Preliminary extraction with alcohol.—Dry the pectin extractor and glass rod for 1 hour or longer at 100° C., and allow to cool to room temperature in a desiccator, and determine their combined tare weight. Assemble the apparatus, close the screw clamps, and heat the water in the beaker to 50° C. Weigh a sample, equivalent to 2 g. of moisture-free tobacco, place in the pectin extractor, and add 50 ml. of 95 percent ethanol, previously heated to 50° C. Stir mixture with the glass rod from time to time during a period of approximately 30 minutes. Apply gentle suction through screw clamp to draw solvent into suction flask. Close screw clamps and repeat the 50° C. alcohol extraction, using 25 ml. of solvent for a period of approximately 15 minutes. Discard the combined alcoholic extracts.

Part 2—Pectinic acids or "pectin".—(a) EXTRACTION WITH WATER: Siphon off the water from the beaker. Close the screw clamp and add 90 ml. of distilled water to the residual tobacco in the pectin extractor, stirring the mixture from time to time at room temperature for 30 minutes. Draw off the extract to the suction flask by gentle suction and repeat the 30-minute extraction with water twice more. Transfer the combined aqueous extract in the suction flask to a 500 ml. volumetric flask, dilute to the mark with water and mix.

(b) PRECIPITATION OF PECTIC MATERIAL: Pipette a 100 ml. aliquot of the 500 ml. solution (corresponding to 0.4 g. of moisture-free tobacco) into a 400 ml. beaker, and add 5 ml. of the 10 percent hydrochloric acid, while stirring. Add, while stirring, 200 ml. of 95 percent ethanol, and allow to stand overnight. Filter the solution on a filter paper (11 cm. S & S No. 597)[6].

[6] No water-soluble pectinic acids were found in any of the Burley samples from the 1951 and 1952 crop years. Accordingly, the remainder of Part 2 procedure was not carried out, and the solution of the water extract of Part 2 (a) was discarded. The determinations as described in Parts 3, 4, and 5 were completed.

Wash the pectic material on the paper three times with 1:2 aqueous alcoholic solution, and once with 95 percent ethanol. During this operation, do not allow the gelatinous precipitate to dry on the filter paper. Dissolve the pectic precipitate on the paper completely by pouring through successive portions of a hot aqueous ammoniacal solution (approximately 1.0–1.5%). Collect the filtrate in an 800 ml. beaker on which a 200 ml. mark has been made. Wash the filter paper three more times with hot water, and collect the washings in the beaker containing the main filtrate. Dilute the combined filtrate with water to 200 ml., add 150 ml. of the N/5 sodium hydroxide solution, while stirring, and allow the solution to stand overnight. Add 60 ml. of N/1 acetic acid solution with stirring and allow to stand for a few minutes. Add dropwise 25 ml. of $\frac{M}{10}$ $CaCl_2$ solution while stirring (preferably with a mechanical stirrer), and follow with the addition of 25 ml. of 2M $CaCl_2$, also added dropwise, and stirred in the same manner. Heat to boiling, with occasional stirring, and boil the mixture for two minutes over a reduced flame. Filter the hot solution through a filter paper (11 cm. S & S No. 597), and wash the calcium pectate precipitate thoroughly with hot water. Wash the precipitate into the 800 ml. beaker with at least 100 ml., but not over 200 ml. of water, and boil the mixture for 2 minutes. Filter through a dried and weighed 30 ml. fritted glass crucible of porosity "M." During the filtration and washing in the crucible do not allow the crucible to drain completely until the very last, otherwise, the precipitate packs and the filtration is slowed considerably. Wash the precipitate in the crucible several times with hot water, then three times with 95 percent ethanol. During washing with the ethanol, the precipitate may be stirred with a small glass rod having fire-polished ends, and any precipitate adhering to the rod washed into the crucible with ethanol. Finally, wash the precipitate twice with ether. Warm the crucible on the steam bath until the odor of ether is no longer noticeable, and dry overnight at 100° C. Cool to room temperature in a desiccator over calcium chloride and weigh.

(c) DETERMINATION OF IMPURITIES IN CALCIUM PECTATE: Place the fritted-glass crucible with its contents on its side in a 400 ml. beaker, and add 2 percent aqueous ammonium citrate solution in sufficient quantity to cover the crucible. Cover the beaker with a cover glass, and heat on the steam bath (stirring occasionally with a glass rod) until the calcium pectate is dissolved. Filter the solution, while still hot, through a tared Gooch crucible containing an asbestos mat, and transfer the undissolved material into the crucible with a stream of hot water from a wash bottle. Wash the undissolved material several times with hot water, dry at 100° C. for 3 hours, cool in a desiccator containing calcium chloride, and weigh.

(d) CALCULATION OF RESULTS: Calculate the percentage of water-soluble

pectinic acids (pectin), as calcium pectate, as follows:

$$\frac{(\text{Weight of Calcium Pectate} - \text{Weight of Impurities}) \times 100}{0.4}$$

= % Pectinic Acids (pectin), as calcium pectate, in the moisture-free sample

Part 3—Protopectin.—(a) PECTIC FRACTION SOLUBLE IN HOT N/20 HCl SOLUTION: Close screw clamps, fill the beaker with water, and heat to 80–85° C. Add 90 ml. of the N/20 HCl solution, previously heated to 80–85° C. to the residual tobacco in the pectin extractor, and stir the mixture occasionally with stirring rod for 30 minutes. Draw off the acid extract into the suction flask under slight suction. Repeat the 30-minute extraction with N/20 HCl solution four more times. Transfer the combined acid extract to a 500 ml. volumetric flask, cool to room temperature, dilute to the mark with water, and mix.

(b) PRECIPITATION OF PECTIC MATERIAL AND DETERMINATION OF IMPURITIES: Determine the protopectin and impurities as described in paragraphs (b) and (c) of Part 2.

(c) CALCULATION OF RESULTS: Calculate the percentage of protopectin (the pectic substance soluble in hot N/20 HCl solution), as calcium pectate as follows:

$$\frac{(\text{Weight of Calcium Pectate} - \text{Weight of Impurities}) \times 100}{0.4}$$

= % Protopectin (as calcium pectate) in moisture-free sample

Part 4—Pectic acid and pectates.—(a) EXTRACTION WITH 0.5% AMMONIUM CITRATE SOLUTION: Close the screw clamps and add 90 ml. of the 0.5 percent aqueous ammonium citrate solution, previously heated to 80–85° C., to the residual tobacco in the pectin extractor. Stir the mixture from time to time with the glass rod during the extraction for 30 minutes. Draw off the extract into suction flask as in extractions above, and repeat the 30-minute digestion with hot 0.5 percent aqueous ammonium citrate solution four more times. Transfer the combined extract to a 500 ml. volumetric flask, cool to room temperature, dilute to the mark with water, and mix.

(b) PRECIPITATION OF PECTIC MATERIAL AND DETERMINATION OF IMPURITIES: Precipitate the pectic material and determine the impurities following the exact procedure described in paragraphs (b) and (c) of Part 2.

(c) CALCULATION OF RESULTS: Calculate the percentage of pectic acid and pectates, as calcium pectate, as follows:

$$\frac{(\text{Weight of Calcium Pectate} - \text{Weight of Impurities}) \times 100}{0.4}$$

= % Pectic Acid and Pectates, as calcium pectate, in moisture-free sample

Part 5—Tobacco residue.—Wash the tobacco residue and stirring rod in

the pectin extractor with three successive 5 to 10 ml. portions of water, using suction. Disconnect the pectin extractor from the rest of the apparatus, and dry the outside with a towel. Dry the extractor containing the tobacco residue and the glass rod overnight at 100° C., cool in a desiccator containing anhydrous calcium chloride to room temperature, and weigh. Calculate the percentage of residue remaining from the original 2-gram moisture-free sample. Retain the tobacco residue for the determination of pentosans.

TOTAL PECTIC SUBSTANCES (AS CALCIUM PECTATE)

Add the percentages of pectinic acids (if present), protopectin, and pectic acid and pectates (all as calcium pectate) to obtain the total pectic substances.

URONIC ACIDS (AS ANHYDRIDES)

Standards Branch, Tobacco Division, AMS

APPARATUS

The apparatus described by Browning (*9*) was used. However, anhydrous calcium chloride was used in place of anhydrone in the long drying tube, absorption tube, and guard tube. Treat the anhydrous calcium chloride before using as follows: Place the material in a tube of suitable size and pass a slow stream of dry carbon dioxide through for 30 minutes. Then pass a stream of dry air, free of carbon dioxide, through for one hour. Preserve the anhydrous $CaCl_2$, thus threated, in a well-stoppered bottle.

Fill the trap to a depth of about 7mm., above the inlet tube, with the silver phosphate solution. Renew the solution in the trap after each determination.

REAGENTS

(1) *12 percent Hydrochloric acid (by weight).*—Add 1,000 ml. of concentrated hydrochloric acid (d. 1.19) to 2380 ml. of distilled water with stirring.

(2) *Silver phosphate solution.*—Add 10 grams of silver carbonate to 300 ml. of 85 percent ortho phosphoric acid. Heat the mixture on the steam bath for one hour, and at the same time pass through it a stream of air, free of carbon dioxide. Filter the solution through a fritted-glass Buchner funnel of porosity "M."

DETERMINATION

Place a sample equivalent to 2 grams of moisture-free tobacco in the reaction flask, and add 60 ml. of the 12 percent hydrochloric acid solution and two boiling chips. Connect the reaction flask to the water-cooled condenser, and heat the flask in a glycerol bath at such a rate that the temperature is raised to 70° C. in 20 minutes. During this time, as well as throughout the determination, pass dry air, free of carbon dioxide, through the apparatus at the rate of two to three bubbles per second. The heating at

70° C. is for the purpose of removing CO_2 from carbonates that may be present (*28*). Disconnect the absorption tube, place in a stoppered test tube, and allow to remain near the balance for five minutes. Weigh the absorption tube and again connect it to the apparatus. Raise the temperature of the glycerol bath slowly to 137–140° C. over a period of 30 to 45 minutes, and maintain this temperature for 5 hours. Disconnect the absorption tube from the apparatus without interrupting the flow of air (carbon dioxide-free), place in a stoppered test tube, allow to remain near the balance for 5 minutes, and weigh.

Conduct a blank determination, following exactly the procedure above-described, except that no tobacco sample is added to the reaction flask. Deduct the weight of carbon dioxide obtained in the blank determination from the weight of carbon dioxide obtained in the actual determination. The difference represents the weight of carbon dioxide given off by the uronic acids.

Calculate the percentage of uronic acids (as anhydrides) as follows:

$$\frac{\text{Weight of } CO_2 \text{ given off by the uronic acids} \times 400}{\text{Weight of moisture-free sample}} = \%\ \text{Uronic Acids (as anhydrides) (moisture-free basis)}$$

PENTOSANS (5)

Standards Branch, Tobacco Division, AMS

APPARATUS

The apparatus consists of a 500 ml. distilling flask, a West type condenser having a cooling jacket 400 mm. long, and a dropping funnel of about 40 ml. capacity. The distilling flask and condenser are connected by a standard taper 19/38 ground-glass joint, and held together by two bronze springs fastened to glass hooks fused onto the condenser and onto the distilling flask. The dropping funnel is connected to the top of the distilling flask through a standard taper 24/40 ground-glass joint, and similarly held fast with two bronze springs. When thus connected, the stem (6 mm. O.D.) of the dropping funnel extends about 30 mm. below the outlet tube of the distilling flask. The end of the stem is constricted to 2 to 3 mm. inside diameter, and bent so that the stream of the 12 percent hydrochloric acid could be directed against the wall of the distilling flask to wash down the plant material adhering to the wall.

REAGENTS

(1) *Hydrochloric acid (12 percent by weight).*—Add 1,000 ml. of concentrated hydrochloric acid (d. 1.19) to 2,380 ml. of water with stirring.

(2) *Phloroglucinol solution.*—Heat 300 ml. of the 12 percent hydrochloric acid solution in a beaker, and add 11 grams of phloroglucinol in small quantities at a time, stirring constantly until it is nearly dissolved. Pour the hot solution into a sufficient quantity of 12 percent hydrochloric

acid (cold) to make a total volume of 1,500 ml. Allow the solution to stand at least overnight, but preferably for several days, to permit the diresorcin to crystallize. Filter the solution before use.

DETERMINATION

Weigh, in a weighing bottle, a sample of the tobacco residue from the determination of the pectic substances (preferably corresponding to 1.5 to 1.7 g. of the original 2-gram moisture-free sample), and transfer to the distillation flask. Add 100 ml. of the 12 percent hydrochloric acid solution and two boiling chips (Boileezers). Connect the apparatus, the distillation flask being supported on a wire gauze at a convenient height above a bunsen burner. Connect the dropping funnel to the distillation flask, and connect the latter to the condenser. After inserting the dropping funnel, heat the distillation flask and contents, rather slowly at first and then so regulate the distillation rate that 30 ml. of distillate comes over in 10 minutes. Collect the distillate in a 50 ml. graduated cylinder provided with a small funnel and a folded filter paper (12½ cm. S & S No. 588). When 30 ml. distills over, add 30 ml. of the 12 percent hydrochloric acid solution rapidly, by means of the dropping funnel, while rotating the funnel in such a manner as to wash down particles adhering to the sides of the distilling flask, and continue the distillation. At this point replace the 50 ml. graduated cylinder containing the distillate with another 50 ml. graduated cylinder provided also with a small funnel and a folded filter paper.

Continue the distillation and collection of the distillate in 30 ml. quantities in the manner described above until the total distillate amounts to 360 ml. Add, with stirring, approximately twice the amount of phloroglucinol reagent as is considered necessary to precipitate the amount of furfural expected, and dilute the volume of the solution to 400 ml. with 12 percent HCl solution. The solution turns progressively yellow, green, and finally almost black. After again stirring, allow the solution to stand overnight.

Filter the amorphous black precipitate into a tared Gooch crucible having an asbestos mat, and wash with 150 ml. of distilled water in such a manner that the water is not entirely removed from the crucible until the very last. Dry the crucible and contents for four hours at 100° C., cool in a desiccator over anhydrous calcium chloride, and weigh in a weighing bottle. The increase in weight of the Gooch crucible is considered to be furfural phloroglucide.

Calculate the weight of pentosans from the weight of phloroglucide, using the following formulas given by Kröber (*19*):

(1) For a weight of phloroglucide, designated by "a" in the following formulas, under 0.03 gram.

$$\text{Pentosans} = (a + 0.0052) \times 0.8949$$

(2) For a weight of phloroglucide "a" between 0.03 and 0.3 gram then:

$$\text{Pentosans} = (a + 0.0052) \times 0.8866$$

(3) For a weight of phloroglucide "a" over 0.3 gram then:

$$\text{Pentosans} = (a + 0.0052 \times 0.8824$$

Calculate the percentage of pentosans on the basis of the original unextracted moisture-free tobacco sample.

CRUDE FIBER (*1*)

The Imperial Tobacco Company, Ltd.

DETERMINATION

Place a 2-gram (as-is) sample into a one-liter Erlenmeyer flask, add 50 ml. of petroleum ether (boiling range 40° to 60° C.), cover the flask with a watch glass, and allow to stand overnight. Pour the petroleum ether extract through a filter paper, and retain the filter paper for subsequent use. Add another 50 ml. portion of petroleum ether to the sample, mix, and allow to stand for a few minutes. Filter through the original filter paper and allow to dry. Brush all adhering particles from the filter paper into the flask containing the tobacco. Warm the flask on the water bath until all the petroleum ether vapor has evaporated.

Bring to a boil in a beaker 200 ml. of sulfuric acid solution (12.5 g. H_2SO_4 per liter), and add this acid solution to the tobacco in the Erlenmeyer flask. Attach the flask to a reflux water-cooled condenser, bring the mixture to a boil quickly, and allow to boil gently for 30 minutes. Rotate the Erlenmeyer flask every few minutes to mix contents and to remove particles from the sides. Filter the mixture through a Buchner funnel having a Whatman No. 541 filter paper with a disc of butter muslin under it. Carry out the filtration fairly rapidly, so that the filtration of the bulk of the solution is completed within 10 minutes. Wash the residual material on the filter paper with hot water until the washings are acid-free.

Heat to boiling 200 ml. of aqueous sodium hydroxide solution (12.5 g. NaOH per liter) in a wash bottle. Wash the entire contents in the Buchner funnel with a stream of the hot sodium hydroxide solution from the wash bottle into the original Erlenmeyer flask, and pour the remainder of the sodium hydroxide solution into this flask. Connect the Erlenmeyer flask to the reflux condenser, bring the reaction mixture to a boil quickly, and boil gently for 30 minutes. Filter the solution through the same Buchner funnel using Whatman No. 541 filter paper (diam. 7 cm.), and transfer the entire contents of the Erlenmeyer flask into the Buchner funnel with hot water. No muslin is used this time for the filtration. Wash the material in the funnel once with 1 percent hydrochloric acid solution and follow with hot water, washing until acid-free. Wash three times with ethanol, once with ether, and finally draw air through until the paper is dry. Detach the fiber quantitatively from the paper and place in a tared silica dish. Dry

in an electric oven at 100° C. for one hour, allow to cool in a desiccator to room temperature, and weigh. Return the dish and contents to the oven for one-half hour and reweigh. Repeat this operation until a constant weight is obtained.

Char the dried crude fiber over a low flame, then bring to a dull red heat and maintain until the ignition is complete. Cool the dish and contents in a desiccator to room temperature, and determine the weight of the ash.

Calculate the percentage of crude fiber as follows:

$$\frac{(\text{Weight of Crude Fiber} - \text{Weight of Ash}) \times 100}{\text{Weight of sample (moisture-free basis)}} = \% \text{ Crude Fiber in moisture-free sample}$$

CELLULOSE (*20, 38*)

Standards Branch, Tobacco Division, AMS

DETERMINATION

Weigh a sample, equivalent to 2 grams of moisture-free tobacco, and transfer through a powder funnel to a 200 ml. Erlenmeyer flask provided with a standard taper ground-glass joint. Add 80 ml. of 95 percent ethanol and 20 ml. of nitric acid (sp. gr. 1.42), while rotating the flask. Connect the flask to a water-cooled reflux condenser, and heat the flask in a water bath maintained at near boiling temperature for one hour, stirring occasionally by lifting the flask and rotating. Transfer the contents of the flask to a 250 ml. beaker, using a stream of 95 percent ethanol from a wash bottle. Decant, under reduced suction, through a tared 50 ml. fritted-glass crucible of porosity "C," which has been fitted to a 1,000 ml. suction flask. (Before using the same crucible for another sample, determine its tare weight again, since the normal loss in weight of the crucible in this procedure was in the range of 2 to 6 mg.) Complete the transfer of the residual material in the beaker into the crucible with a stream of 95 percent ethanol from the wash bottle. Wash the material in the crucible by filling with 95 percent ethanol and draining three times under reduced suction. Transfer the material from the crucible to the beaker and then to the original Erlenmeyer flask, using 80 ml. of 95 percent ethanol from a wash bottle for the purpose. Add 20 ml. of nitric acid as before, and repeat the above-described refluxing and washing twice, or a total of three times. Transfer the cellulosic material into the crucible and wash three times with 95 percent ethanol. Finally, wash the material with water five times in the same manner. During the washing with water, stir the material with a fire-polished glass rod several times. Allow the crucible to stand in the 250 ml. beaker during the soaking and stirring period to catch the drainage.

If the successive 95 percent ethanol and water washes cannot be completed without overnight interruption, allow the material to remain in the Erlenmeyer flask until the next day, because if left in the crucible it dries to a consistency that is difficult to disintegrate and wash effectively with water.

Dry the crucible and contents overnight at 100° C. in an oven, and allow to cool in a desiccator over anhydrous calcium chloride for one-half to one hour. Weigh in a tared weighing bottle, and determine the weight of the "crude cellulose." Place the crucible and contents in an electrically heated and thermostatically controlled muffle furnace, and heat for one hour after the temperature of the furnace reaches 550° C. Place the crucible and contents on a wire gauze for 3 to 5 minutes for preliminary cooling, and then in a desiccator over anhydrous calcium chloride for one hour. Determine the weight of the ash and subtract this weight from the weight of the "crude cellulose" to obtain the weight of "ash-free cellulose." Calculate the percentage of "ash-free cellulose" as follows:

$$\frac{\text{Ash-free Cellulose} \times 100}{\text{Weight of moisture-free sample}} = \% \text{ Cellulose in moisture-free sample}$$

LIGNIN (27)

Standards Branch, Tobacco Division, AMS

REAGENTS

(1) *1% Hydrochloric acid.*—Add 111 g. of concentrated hydrochloric acid (d. 1.19) to 3,890 ml. of water.

(2) *72% Sulfuric acid.*—Add 660 ml. of concentrated sulfuric acid (d. 1.84) to 411 ml. of water.

DETERMINATION

Transfer a 7.5-gram sample (moisture-free equivalent weight) into an 80 × 33 mm. paper extraction thimble, insert a wad of absorbent cotton in the mouth of the thimble, extract with 95 percent ethanol for 8 hours in a Soxhlet extraction apparatus. Then extract for 4 hours in the same apparatus with a 95 percent ethanol-benzene solution (1:2 by weight). Dry the thimble and contents on the steam bath until the odor of ethanol and benzene can no longer be detected. Transfer the extracted tobacco quantitatively to a one-liter Erlenmeyer flask, add 750 ml. of the 1 percent hydrochloric acid solution and a few drops of capryl alcohol and boil the mixture under a reflux condenser for 3 hours. (Caution: When the mixture reaches the boiling point, it has a tendency to foam and shoot up through the condenser. This may be avoided by regulating the flame under the flask, rotating the mixture, and adding a few more drops of capryl alcohol.) Allow the mixture to cool to room temperature and filter through a dried (at 100° C.) and weighed 50 ml. fritted-glass crucible of porosity "C." Wash with water until the filtrate is free of acid and, finally, with 95 percent ethanol. Dry the crucible and contents overnight in an oven at 100° C., allow to cool in a desiccator over anhydrous calcium chloride to room temperature, and weigh in a weighing bottle. Calculate the percentage of loss due to the total of the three successive extractions.

Combine the extracted tobacco of duplicate samples and grind the

material, first in a small hand-operated mill [7] to reduce particle size, and then pulverize in a mortar, and dry for 2 hours at 100°C.

Weigh accurately triplicate samples, ranging from 0.5 to 0.7 gram, from a weighing bottle, and designate as (a), (b), and (c). Calculate from the weight of each of these three samples the corresponding weight of the original, unextracted, moisture-free tobacco. Transfer each sample to a 50 ml. Erlenmeyer flask provided with a one-hole rubber stopper through which passes a glass rod 12 cm. long, the end of which has been flattened. For every 0.1 gram of the extracted sample add portionwise 5 ml. of 72 percent sulfuric acid, which has been previously cooled to 5° C. Stir the mixture with the glass rod, which has been lubricated with a drop of glycerol to facilitate moving it through the hole in the rubber stopper. Close the Erlenmeyer flask with the rubber stopper carrying the glass rod, and allow the mixture to stand for 24 hours in a refrigerator at a temperature of approximately 5° C., stirring occasionally with the glass rod.

Transfer the mixture quantitatively to a one-liter Erlenmeyer flask, and add sufficient distilled water to make an approximately 5 percent sulfuric acid solution (add 109.5 ml. of water for every 5 ml. of 72 percent sulfuric acid used). Insert a boiling tube [8] about 18 cm. long into the Erlenmeyer flask, add a few drops of capryl alcohol to prevent foaming, and boil the mixture under a reflux condenser for 2 hours. Allow to cool to room temperature.

Filter sample (a) into a tared Gooch crucible, which has been ignited for one hour at 600° C. in an electric muffle furnace. Filter sample (b) into a tared Gooch crucible, and sample (c) into a 30 ml. fritted-glass crucible of porosity "M," both crucibles having been previously dried at 100° C. Wash the crude lignin of each sample with water until free of acid, dry overnight in an oven at 100° C., allow to cool to room temperature in a desiccator containing anhydrous calcium chloride, and weigh in a weighing bottle.

Ignite the crucible containing sample (a) for 2 hours at 600° C. in an electric muffle furnace, determine the weight of ash, and calculate the percentage of ash in the crude lignin. Using the percentage of ash as determined for sample (a), calculate the weights of ash in samples (b) and (c). Transfer the crude lignin of sample (b) quantitatively to a Kjeldahl digestion flask, and determine the percentage of nitrogen in the crude lignin by the Kjeldahl-Gunning-Arnold (3) method, using HgO as the catalyst. Calculate the percentage of crude protein (N × 6.25) in the crude lignin. Using the percentage of crude protein determined in sample (b), calculate the weights of crude protein in sample (a) and (c). Use the

[7] A small spice mill was used for this purpose.

[8] This is made of Pyrex glass tubing, approximately 2 mm. internal diameter and 18 cm. long, by sealing at one end and sealing also about 2 cm. from the other end. The open end is fire-polished. The boiling tube is introduced open end down.

crude lignin of sample (c) for the determination of the percentage of methoxyl in the lignin.

Calculate the percentage of lignin in each sample as follows:

$$\frac{\text{Weight of crude lignin minus weights of ash and protein} \times 100}{\text{Weight of original moisture-free, unextracted tobacco sample}} = \% \text{ Lignin in moisture-free sample}$$

METHOXYL IN LIGNIN (27)

Standards Branch, Tobacco Division, AMS

APPARATUS

The apparatus used is illustrated in figure 2. It consists of reaction flask D, which is immersed in glycerine bath C. D is connected through a standard taper ground-glass joint to the condenser E, which is connected by a standard taper ground-glass joint to scrubbing flask G. This flask is about one-third filled with a thin suspension of red phosphorus in 5 percent aqueous cadmium sulfate solution. G is immersed in water bath F, the temperature of which is maintained at 50–55° C., and is connected through a standard taper ground-glass joint to tube A, the delivery tube to absorption tubes H and I, and flask J. The methyl iodide absorption tubes H and I contain the reagent consisting of a solution of bromine and potassium acetate in glacial acetic acid. J is half-filled with water to which a few drops of the formic acid reagent has been added to absorb the vapors of bromine. H, I, and J are supported by wooden holder K attached to ring stand R. All ground-glass joints are held fast by means of bronze springs O. Water from Erlenmeyer flask B, heated to 50–55° C. circulates through condenser E. The water overflowing from E passes through tube N into flask M. A small wad of cotton P is inserted lightly into the mouth of flask M. A stream of carbon dioxide from a tank provided with a reducing valve is passed through a gas wash-bottle containing concentrated sulfuric acid, and into the apparatus. The apparatus is supported on ring stand Q.

REAGENTS

(1) Hydriodic acid (sp. gr. 1.70); (2) Red phosphorus, special for micro-analysis; (3) Phenol, U.S.P. grade; (4) Potassium acetate-acetic acid reagent, (made by dissolving 20 grams of potassium acetate in sufficient glacial acetic acid to make 200 ml. of solution); (5) Bromine, free of iodine; (6) 85–90 percent Formic acid solution; (7) Sodium acetate solution, (made by dissolving 50 grams of sodium acetate in sufficient water to make 200 ml. of solution); (8) Potassium iodide, free of iodate; (9) 10 percent Sulfuric acid solution; (10) 0.05N Sodium thiosulfate solution; and (11) 0.5 percent Starch solution.

DETERMINATION

Heat containers B and F with micro burners and maintain the temperatures at 50–55° C. Add 15 drops of bromine to 10 ml. of the potassium

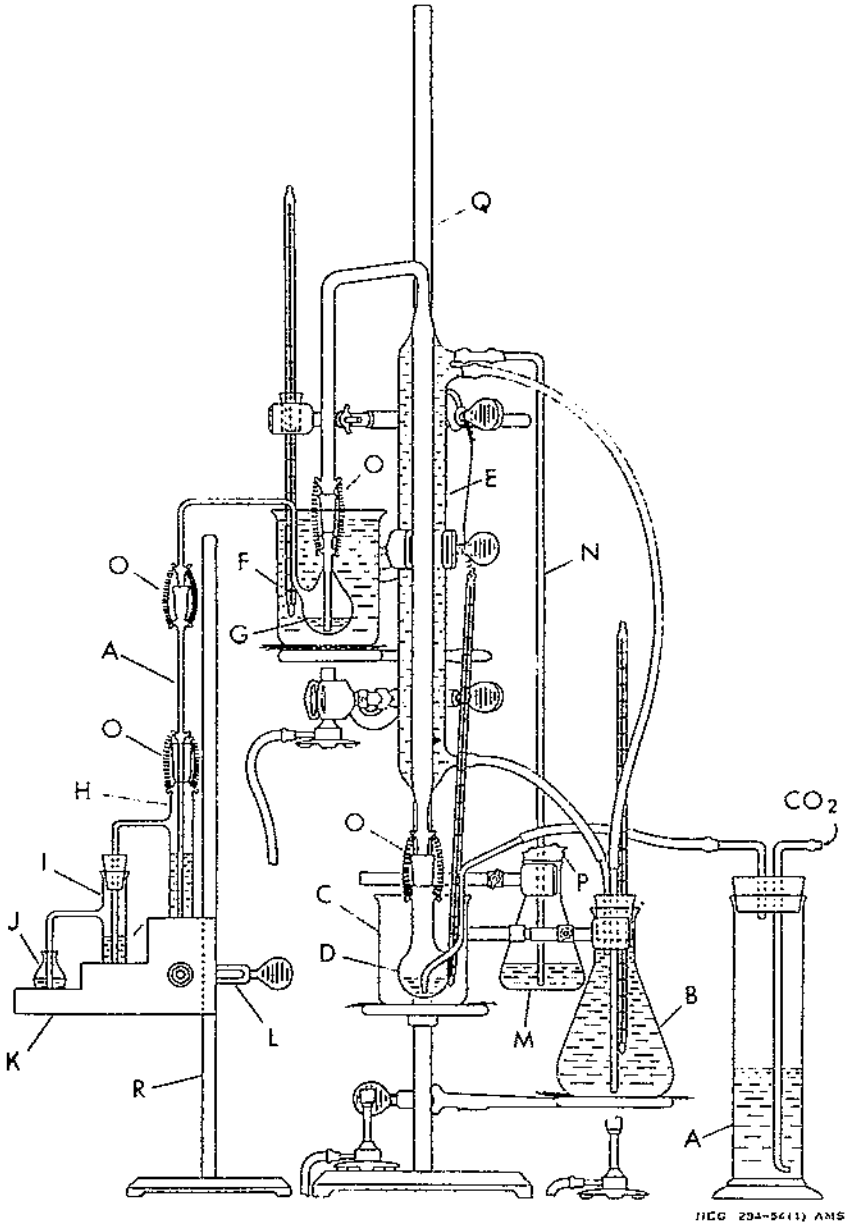

FIGURE 2.—Apparatus for determination of percentage methoxyl.

acetate-acetic acid reagent, and mix. Add about 3 ml. of this solution to absorption tube I, and 7 ml. to tube H. Connect absorption assembly A, H, I, and J to the apparatus as shown in the drawing.

Weigh in a weighing bottle 50 to 100 mg. sample of the dry crude lignin from the triplicate sample of crude lignin, which is subdivision (c), as described previously in the procedure for the determination of lignin. Calculate the weight of the sample of lignin on a crude-protein-free and ash-free basis. Transfer the sample to flask D, and add 2 to 3 ml. of phenol, 5 ml. of hydriodic acid, and two boiling chips (Boileezers). Connect flask D to condenser and immerse in glycerine bath C heated to 135–140° C. Maintain the temperature at this level for one hour, while passing a stream of carbon dioxide through the apparatus at the approximate rate of one bubble per second. During the last 10 minutes of the heating period, increase the rate of passage of carbon dioxide through the apparatus so as to sweep all of the methyl iodide into the absorption tubes.

Disconnect tubes H and I from the apparatus and wash the contents into a 300 ml. Erlenmeyer flask containing 15 ml. of the sodium acetate solution. Add the formic acid reagent, dropwise, to the solution in the Erlenmeyer flask, with stirring, until the bromine color is discharged. Blow a gentle stream of air into the Erlenmeyer flask to remove residual vapors of bromine. Add one gram of potassium iodide and 20 ml. of the 10 percent sulfuric acid, and titrate the liberated iodine with N/20 sodium thiosulfate solution using starch solution as the indicator. Conduct a blank determination following the above-described procedure, and deduct the number of ml. of N/20 sodium thiosulfate solution required for the blank from that used for the actual determination.

Calculate the percentage of methoxyl in the lignin as follows:

$$\frac{\text{ml. N/20 } Na_2S_2O_3 \text{ required (corrected for blank)} \times 0.0002586 \times 100}{\text{Weight of lignin sample}} = \%\ OCH_3 \text{ in lignin}$$

TANNIN (2)

Eastern Utilization and Development Division, ARS

REAGENTS

(1) *Kaolin.*—A 1 percent suspension in water after digestion for one hour at 23° C. should not yield more than 1 mg. of soluble solids per 100 ml. of filtrate.

(2) *Hide powder.*—The Official American Leather Chemists Association hide powder approved by the A.L.C.A. Hide Powder Committee.

DETERMINATION

Preparation of Extract A.—Extract a 40-gram sample, as received, at a uniform rate with boiling water for seven hours in a continuous extraction apparatus. The Pyrex extractor should be steam-jacketed so that the

material being extracted is at the temperature of boiling water throughout the extraction. Collect approximately 2 liters of extract and allow it to remain overnight in a room at 23–24° C. Dilute with water to exactly 2 liters, and designate solution as "Extract A."

Soluble extractives.—The operations described below should be conducted in an air conditioned room maintained at a temperature of 23–24° C.

Add 2 grams of kaolin to 225 ml. of Extract A, stir the suspension and filter through S & S No. 610 filter paper 21.5 cm. in diameter, pleated to contain 32 evenly divided creases. When approximately 40 ml. have passed through the filter, return the filtrate to the funnel. Continue this operation for one hour, and then siphon the solution from the paper, taking care not to disturb the kaolin film on the paper. Refill the prepared filter paper with 225 ml. of Extract A and continue the filtration. After 40 ml. of the filtrate have passed through, collect the next 125 ml. of filtrate into a clean, dry, glass container. Pipette 100 ml. of the clear filtrate into a weighed flat-bottomed dish, 70 mm. in diameter, then evaporate and dry for 17 hours at 100° C. (± 0.5°) in a circulating-air type electric oven. Transfer the dish and residue to a desiccator containing Drierite, cool, and weigh.

Nontannins.—Calculate the quantity of air-dried hide powder which will be required for the number of determinations to be made, on the basis of 12.5 grams of moisture-free powder for each determination. Increase this calculated amount by 10 grams to provide a sufficient quantity for the determination of moisture in the wet, chromed, hide powder and also for a working leeway. Digest the total quantity of air-dried hide powder with 10 times its weight of water until thoroughly soaked. For each gram of air-dried hide powder so digested, add 1 ml. of 3 percent chrome-alum solution, $K_2SO_4 \cdot Cr_2(SO_4)_3 \cdot 24H_2O$. Agitate frequently for two hours and let stand overnight. Transfer the hide powder to a cotton cloth (Indianhead) and squeeze thoroughly. Using the cloth as a bag, digest the hide powder for 15 minutes in a quantity of water equal to 15 times the weight of the air-dried hide powder used. Then squeeze the hide powder in the bag to about 75 percent moisture. Repeat the digestion and squeezing three times and at the last pressing adjust the moisture as nearly as possible to 72.5 percent (not less than 71 percent and not more than 74 percent).

To 10 grams of the wet chromed hide powder, add 200 ml. of Extract A, shake the mixture in a shaker for exactly 10 minutes, then pour it onto a perforated porcelain plate held in a 125 ml. funnel. Add 2 grams of kaolin to the filtrate and refilter through paper. Pipette 100 ml. of the new filtrate into a weighed flat-bottomed dish, 70 mm. in diameter, then evaporate and dry for 17 hours at 100° C. in a circulating-air type electric oven. Transfer the dish and residue to a desiccator containing Drierite, cool, and weigh. Correct the nontannin residue weight for dilution caused by water remaining in the wet hide powder and calculate the percentage of nontannins.

Tannin.—The percentage of tannin is the difference between the percentage of soluble extractives and the percentage of nontannins.[9]

TOTAL VOLATILE ACIDS (AS ACETIC ACID) (*41*)

Brown and Williamson Tobacco Corporation

APPARATUS

The apparatus consists of a 500 ml. Kjeldahl flask provided with a two-hole rubber stopper. Through one hole passes a 6 mm. O. D. glass tube "A" bent at a right angle, which extends nearly to the bottom of the Kjeldahl flask. Through the other hole, pass one end of a spherical connecting bulb such as is used in the determination of nitrogen by the Kjeldahl method (Fisher Scientific Co. catalogue No. 13—177A or equal). The upper end of the connecting bulb is connected to a glass condenser by means of a rubber stopper. The Kjeldahl flask is immersed in a glycerine bath. A conventional type of steam generator is used.

DETERMINATION

Place a sample of 5 grams (moisture-free equivalent weight) in the Kjeldahl flask of the apparatus and add 100 ml. of distilled water and 2 grams of tartaric acid. Heat glycerine bath, in which the Kjeldahl flask is immersed, to 100° C., connect tube "A" to the steam generator, pass in a current of steam, and collect distillate in a flask containing 25 ml. of N/10 sodium hydroxide solution. Continue the distillation until all of the volatile acids (approximately 700 ml.) are distilled over. Throughout the distillation, keep volume of mixture in the Kjeldahl flask approximately constant by heating the glycerine bath with a small flame. Determine the excess of sodium hydroxide by titration with N/10 sulfuric acid solution, phenolphthalein being used as the indicator. Calculate total volatile acids (as acetic acid) as follows:

$$\frac{\text{ml. of N/10 sodium hydroxide required} \times 0.0060 \times 100}{\text{Weight of moisture-free sample}} = \% \text{ Total Volatile Acids (moisture-free basis)}$$

FORMIC ACID

Brown and Williamson Tobacco Corporation

APPARATUS

The apparatus is described and illustrated in the A.O.A.C. Book of Methods, 6th ed. (1945) p. 536.

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[9] Since the tannin content of the tobacco samples was quite low resulting in a low tannin concentration in the extract, 10 grams of wet, washed hide powder were used instead of 46 grams $\frac{(12.5 \times 100)}{100 - 72.5}$ as are normally used for commercial tanning materials.

REAGENTS

(1) *Sodium acetate solution.*—Dissolve 50 grams of dry sodium acetate in sufficient water to make 100 ml. of solution and filter.

(2) *Mercuric chloride solution.*—Dissolve 100 grams of mercuric chloride and 150 grams of sodium chloride in sufficient water to make one liter of solution and filter.

DETERMINATION

Place a sample of 10 grams (moisture-free equivalent weight) in reaction flask A and add 100 ml. of water and 2 grams of tartaric acid. Add to flask B, 2 grams of barium carbonate and 100 ml. of water. Connect apparatus and heat contents of flasks A and B to boiling, and distill with steam from generator S, vapor passing first through sample in flask A, then through the boiling suspension of barium carbonate in B, after which it is condensed and collected in 1,000 ml. volumetric flask C. Continue the distillation until one liter of distillate is collected, maintaining the volume of liquids in flasks A and B as nearly constant as possible by heating with small Bensen flames and avoiding charring of sample in flask A. Disconnect apparatus and filter contents of flask B while hot, and wash the barium carbonate with a little hot water. Filtrate and washings should measure about 150 ml., if they do not, they should be boiled down to that volume. Add to this 10 ml. of the sodium acetate solution, 2 ml. of 10 percent hydrochloric acid, and 25 ml. of the mercuric chloride solution. Mix thoroughly and immerse container in boiling water or place on steam bath for 2 hours. Filter through a dried (100° C.) and weighed Gooch crucible, and wash precipitate thoroughly with cold water and finally with a little 95 percent ethanol. Dry in oven at 100° C. for 30 minutes, cool, and weigh. If weight of mercurous chloride precipitate obtained exceeds 1.5 grams, repeat the determination, using more mercuric chloride solution or a smaller quantity of sample. Conduct a blank determination on the reagents, using 150 ml. of water, one ml. of 10 percent barium chloride solution, 2 ml. of the 10 percent hydrochloric acid solution, 10 ml. of the sodium acetate solution, and 25 ml. of the mercuric chloride solution, and heating mixture in boiling water or steam bath for 2 hours. Deduct weight of mercurous chloride precipitate obtained in this blank test from that obtained in regular determination. Calculate the percentage of formic acid as follows:

$$\frac{\text{Weight of mercurous chloride precipitate} \times 0.0975 \times 100}{\text{Weight of moisture-free sample}} = \% \text{ Formic Acid (moisture-free basis)}$$

WATER-SOLUBLE ACIDS

Liggett and Myers Tobacco Company

DETERMINATION

Preparation of Extract A.—Place 6.67 grams (as-is) of ground tobacco

in a 250 ml. Erlenmeyer flask, add 40 ml. of distilled water, mix until all particles of the tobacco are wet, and then add 60 ml. of distilled water, washing down the sides of the flask. Close flask with rubber stopper and let it stand for 16 hours at room temperature. Filter mixture through a fluted filter paper and designate filtrate as "Extract A."

Dilute 5 ml. aliquot of extract A to 100 ml. with distilled water and titrate to a pH of 8.1 with N/30 NaOH, using a glass electrode and a Beckman pH meter, Model H2 or equal. Express results as ml. of N/10 NaOH required to neutralize the acidity in 1 gram of tobacco.

PLASTID PIGMENTS (*47*)

Tobacco Laboratories, North Carolina State College

The plastid pigments were determined by a modification of the spectrophotometric procedure described for flue-cured tobacco by Stinson (*42*) and by Pack (*26*).

DETERMINATION

Weigh 2.5 grams of sample, in duplicate, and extract alternately with 95 percent ethanol and acetone in a Waring Blender. Filter the extract, dilute with water, and transfer to ether. Dry the ether extract, after scrubbing with water, by trickling through a bed of anhydrous Na_2SO_4 and dilute to 100 ml. with ether.

Determine the pigment concentrations on a Warren Spectracord, or equivalent instrument, by computation from the interpolated optical densities (D) at wave lengths of 665, 649, 642.5, 485, 474, and 470 mμ.

The simplified estimating equations, when the final volume of extract is 200 ml. and read in a cell having a path-length of 0.998 cm., follow:

$$\text{Total Chlorophyll} = 5566.5\ D_{649}$$

$$\text{Chlorophyll (a)} = 1994.5\ D_{665} - 173.4\ D_{642.5} = (a)$$

$$\text{Chlorophyll (b)} = 3528.0\ D_{642.5} - 607.0\ D_{665} = (b)$$

$$\text{Total Carotinoid} = 982.1\ D_{474} - 0.255(a) - 0.2250(b)$$

$$\text{"Carotene"} = 2518.2\ D_{485} - 1198.5\ D_{470} - 0.0298(a) + 0.3356(b)$$

$$\text{"Xanthophyll"} = 2026.1\ D_{470} - 2288.6\ D_{485} + 0.0036(a) - 0.6518(b)$$

In this method of estimation, "carotene" is defined as a 68:32 mixture of beta-carotene and neo-beta-carotene, and "xanthophyll" as a 60:22:18 mixture of lutein, neoxanthin, and violoxanthin, respectively.

Although the pigment constituents were determined separately, only the total chlorophyll and total carotenoids are reported in the table since the totals appear to show more significance to grade distinction than the individual pigments on the same basis of comparison.

RESINS AND WAXES

Tobacco Laboratory, University of Tennessee

The method used for the determination of resins and waxes is essentially that of Pyriki (*36*).

DETERMINATION

Weigh 3 grams of tobacco and mix with 30 grams of sand, which has been previously treated several times with hot HCl solution and ignited at 700° C. for approximately one hour. Transfer the mixture to a paper extraction thimble, and extract with 95 percent ethanol for 24 hours in a Soxhlet extraction apparatus. Filter the hot extract through filter paper into a 250 ml. beaker, and wash the extraction flask with hot 95 percent ethanol, pouring the washings through the filter paper into the same beaker. Evaporate the filtrate to dryness on the steam bath, and dry for one hour at 75° C. in the oven. Add 30 to 40 ml. of water which has been previously heated to 45° C. to the resinous material, mix with a stirring rod, and filter through filter paper into a 250 ml. beaker. Repeat the washing with hot water and filtration until the filtrate gives a negative test for nicotine with silicotungstic acid reagent. Add 5 ml. of concentrated HCl to the filtrate, designate the solution as "A," and retain for subsequent extraction with ether.

Place the filter paper used in the preceding filtration in the 250 ml. beaker containing the resinous material, and add 75 ml. of 95 percent ethanol. Cover the beaker with a cover glass and heat on the steam bath until substantially all of the resinous material dissolves. Filter through filter paper into a 250 ml. beaker. Repeat the solvent treatment of the resinous material twice more, using 50 ml. and 25 ml. portions of hot 95 percent ethanol, and filter the solution into the same beaker. Evaporate the filtrate in a weighed 50 ml. Erlenmeyer flask on the steam bath, adding the filtrate to the flask portionwise. The time of evaporation may be reduced by evaporating the major portion of the filtrate while in the 250 ml. beaker to a small volume, transferring the solution to the 50 ml. Erlenmeyer flask, rinsing the beaker with small portions of hot 95 percent ethanol and adding the rinsings to the Erlenmeyer flask.

Extract filtrate "A" with three successive portions of ether in a separatory funnel (using a volume of ether approximately one-fourth the volume of the filtrate). Combine the ether extracts, and wash once with 25 ml. of water. Filter the washed ether solution and evaporate the filtrate on the steam bath, adding the filtrate portionwise to the 50 ml. Erlenmeyer flask used above. After evaporating to dryness on the steam bath, dry the flask and contents at 75° C. for 45 minutes in the oven. Allow to cool to room temperature in a desiccator containing calcium chloride, and weigh. Calculate the combined weight of the dry extracts as resins and waxes, as follows:

$$\frac{\text{Weight of resins and waxes} \times 100}{\text{Weight of moisture-free sample}} = \% \text{ Resins and Waxes in moisture-free sample}$$

PETROLEUM ETHER EXTRACTIVES

The American Tobacco Company

DETERMINATION

Weigh a 5-gram (as-is) sample and transfer to a 25 × 80 mm. Whatman extraction thimble, and insert a small plug of fat-free cotton into the top of the thimble. Extract with approximately 125 ml. of petroleum ether (boiling range 35–37° C.) in a Soxhlet extraction apparatus equipped with a tared 250 ml. flask with ground-glass joint containing three glass beads. Adjust the heat so that siphoning occurs five or six times per hour, and continue the extraction for 20 hours. Evaporate the solvent on the steam bath while directing a small stream of air onto the surface of the extract. Rotate the flask frequently during evaporation in order to distribute the extracted material on the walls of the flask, and to facilitate drying. After the odor of petroleum ether has completely disappeared, dry the flask and contents for one hour in a convection type oven at 99–100° C. Allow to cool to room temperature in a desiccator containing anhydrous calcium chloride and weigh. Calculate the percent of petroleum ether extractives, as follows:

$$\frac{\text{Weight of petroleum ether extractives} \times 100}{\text{Weight of moisture-free sample}} = \% \text{ Petroleum Ether Extractives in moisture-free sample}$$

WAXES

The American Tobacco Company

DETERMINATION

Dissolve the petroleum ether extract in the extraction flask, obtained as described above, in 50 ml. of warm absolute ethanol. Warm the solution on the steam bath while rotating the flask to facilitate solution. Chill the flask and a wash bottle containing absolute ethanol in an ice bath. Filter the contents of the flask through a No. 1 Whatman filter paper, and wash with cold absolute ethanol from the wash bottle until free of pigment.

Dissolve the waxes on the filter paper in diethyl ether, receiving the solution in a tared dish or in the original tared Soxhlet flask with the beads. Evaporate the ether on the steam bath, directing a small stream of air onto the surface to aid evaporation. Rotate the flask to facilitate drying. After the odor of ether has disappeared entirely, dry for one hour at 99–100° C. in a convection type oven. Allow to cool in a desiccator containing anhydrous calcium chloride to room temperature and weigh. Calculate the percent of waxes as follows:

$$\frac{\text{Weight of Waxes} \times 100}{\text{Weight of moisture-free sample}} = \% \text{ Waxes in moisture-free sample}$$

95% ETHANOL EXTRACTIVES

P. Lorillard Company

DETERMINATION

Weigh a 2-gram sample (as-is) and transfer to a Soxhlet extraction thimble. Insert a wad of cotton into the mouth of the thimble, and extract with 95 percent ethanol for 24 hours in a Soxhlet extraction apparatus. Adjust the boiling rate of the solvent so that siphoning takes place about every 5 minutes. After completion of the extraction, distill the alcohol off until 15–20 ml. of solution remain in the Soxhlet flask. Evaporate this concentrated alcoholic solution on the steam bath until it reaches a semi-solid state. Dry the Soxhlet flask and contents in a vacuum oven for three hours at 50–53° C., under a pressure of 5–10 mm. of mercury, while air (dried by passing through a concentrated H_2SO_4 trap) is passed through the system. Allow the Soxhlet flask and contents to cool for one hour in a desiccator over anhydrous calcium chloride, and weigh. Calculate the percent of 95 percent ethanol extractives as follows:

$$\frac{\text{Weight of 95\% ethanol extractives} \times 100}{\text{Weight of sample}} = \text{\% 95\% Ethanol Extractives}$$

Convert the result to moisture-free basis, using the appropriate factors for the sample.

pH

The American Tobacco Company

DETERMINATION

From a sample, as ground and prepared for chemical analysis, prepare an aqueous solution by infusing a 2.000 gram sample for one hour in 100 ml. of water and filter. Determine the pH of the solution using a glass electrode. Convert the duplicate pH values to moles of H ions per liter, average the values in moles of H ions per liter, and convert this average value to a pH unit.

Liggett and Myers Tobacco Company

DETERMINATION

Determine the pH of extract "A," prepared for the determination of alpha-amino nitrogen, described elsewhere in this publication, using a Beckman pH meter, Model H2, and a glass electrode.

MOISTURE EQUILIBRIUM

The American Tobacco Company

DETERMINATION

Place a sample of 10 grams in a tared aluminum moisture dish, 9 cm. in diameter by 3.5 cm. deep. Place dish and contents in a cabinet in which the atmospheric condition is maintained at 60 percent R. H. and 80° F. Allow sample to remain in cabinet until the moisture content of the tobacco

has reached equilibrium, then remove sample from cabinet and determine the weight of the moist tobacco. Dry the sample for 3 hours in a forced-draft oven at 99° to 100° C., reweigh, and calculate the percentage of moisture which the sample acquired under the specified atmospheric conditions.

Philip Morris, Inc.

DETERMINATION

Place the samples of 3 grams each into tared aluminum moisture dishes, 85 mm. in diameter and 50 mm. deep. Evenly distribute the dishes containing the samples on the shelves of a forced-air-type humidity chamber in which the atmospheric conditions are controlled at 25° C. (77° F.) and 40 percent relative humidity. Allow the samples to remain in the cabinet until the moisture content of the tobacco has reached equilibrium. Then remove the samples from the cabinet and determine the moist weight of each sample. Replace the samples in the cabinet and change the atmospheric conditions to 25° C. and 60 percent R. H. When the moisture content of the tobacco has reached equilibrium at this condition, again remove the samples from the cabinet and weigh them. Follow the same procedure with the cabinet adjusted at 25° C. and 70 percent and 80 percent R. H. After the moist weights of the samples have been determined following exposure under the four different atmospheric conditions, dry the samples for 3 hours in a forced-draft oven at 99° to 100° C. and determine their dry weights. Calculate the percent of moisture in each sample after being exposed to each atmospheric condition, based upon the several moist weights and dry weight of the sample.

Note.—Ground samples as prepared for chemical analysis were used. The data listed are the results of duplicate determinations.

DISCUSSION OF RESULTS

The X-group of grades, which is composed of leaves from the lower part of the tobacco stalk, contains a greater percentage of sand in varying quantities because of its proximity to the soil, than the grades above this group in stalk position. While the leaves above the lowest position normally contain proportionately less sand according to height, the sand content is also variable and uncertain in the upper leaves. The moisture content of all grades also varies to some extent according to circumstances. Both the sand and moisture content depend somewhat upon cultivation and harvesting practices, and subsequent handling. The amount of sand and moisture, therefore, are important factors to consider in the analysis of tobacco, and for these reasons the analytical results reported in the tables of data are on a moisture and sand-free basis.

TOTAL ASH

In most cases, the lighter colored grades of the same group and quality contained a higher percentage of ash. The grades of Flyings of the two crops averaged 22.31 percent; Cutters, 20.47 percent; Leaf, 18.12 percent; and Tips, 16.13 percent.

TABLE 1.—TOTAL ASH

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	23.68	22.51	23.10
	X4L	23.92	24.90	24.41
	X2F	——	21.70	——
	X4F	22.06	21.78	21.92
	X4R	20.36	18.95	19.66
	N1L	23.11	23.32	23.22
	N1F	21.37	21.74	21.56
				Av. 22.31
Cutter	C2L	21.28	22.47	21.88
	C4L	21.30	22.63	21.96
	C2F	——	21.15	——
	C4F	20.20	17.89	19.04
	C4R	18.94	19.06	19.00
				Av. 20.47
Leaf	B2F	18.38	19.20	18.79
	B2FR	——	17.87	——
	B4F	18.39	18.48	18.44
	B4FR	18.13	17.84	17.98
	B4R	18.04	17.47	17.76
	B4D	17.41	17.87	17.64
				Av. 18.12
Tip	T4F	16.29	18.09	17.19
	T4FR	15.98	15.62	15.80
	T4R	15.78	16.16	15.97
	T4D	15.83	14.76	15.30
	N1D	16.25	16.53	16.39
				Av. 16.13

WATER-SOLUBLE ASH

The average percentages of water-soluble ash of the grades of the two crops ranged from 5.40 percent in T4R to 9.16 percent in X2L, the average of all grades of the two crops being 7.08 percent. Flyings averaged 7.93 percent; Cutters, 7.90 percent; Leaf, 6.62 percent; and Tips, 5.89 percent.

In a comparison of the Flyings group of grades of like color and of different degrees of quality, the grades of lower quality generally contain a lower percentage of water-soluble ash.

TABLE 2.—WATER-SOLUBLE ASH

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	10.20	8.11	9.16
	X4L	9.08	7.17	8.12
	X2F	——	7.07	——
	X4F	8.26	6.68	7.47
	X4R	8.24	6.47	7.36
	N1L	7.59	7.62	7.60
	N1F	8.56	7.20	7.88
				Av. 7.93
Cutter	C2L	8.25	8.35	8.30
	C4L	7.81	8.68	8.24
	C2F	——	7.67	——
	C4F	7.46	9.03	8.24
	C4R	6.94	6.66	6.80
				Av. 7.90
Leaf	B2F	6.83	6.65	6.74
	B2FR	——	6.74	——
	B4F	5.95	6.17	6.06
	B4FR	6.31	6.49	6.40
	B4R	6.53	7.11	6.82
	B4D	6.70	7.44	7.07
				Av. 6.62
Tip	T4F	5.78	5.85	5.82
	T4FR	6.13	5.67	5.90
	T4R	5.69	5.12	5.40
	T4D	5.92	5.79	5.86
	N1D	6.66	6.28	6.47
				Av. 5.89

WATER-INSOLUBLE ASH

Flyings averaged 14.38 percent; Cutters, 12.58 percent; Leaf, 11.51 percent; and Tips, 10.24 percent. X4L contained a much larger percentage of insoluble ash than any of the other grades, while the darker Tip grade T4D contained the smallest percentage.

A comparison of the grades of the same color and group but of different quality shows that the grade of lower quality generally contained a greater percentage of water-insoluble ash than the one of higher quality. A comparison of the grades in which the group and quality are the same but the color different, the lighter colored grades contain the higher water-insoluble ash content.

TABLE 3. WATER-INSOLUBLE ASH

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	13.48	14.40	13.94
	X4L	14.84	17.73	16.28
	X2F	—	14.63	—
	X4F	13.80	15.10	14.45
	X4R	12.12	12.48	12.30
	N1L	15.52	15.69	15.60
	N1F	12.81	14.54	13.68
				Av. 14.38
Cutter	C2L	13.03	14.12	13.58
	C4L	13.49	13.95	13.72
	C2F	—	13.48	—
	C4F	12.74	8.86	10.80
	C4R	12.00	12.39	12.20
				Av. 12.58
Leaf	B2F	11.55	12.56	12.06
	B2FR	—	11.13	—
	B4F	12.44	12.31	12.38
	B4FR	11.82	11.35	11.58
	B4R	11.51	10.36	10.94
	B4D	10.71	10.43	10.57
				Av. 11.51
Tip	T4F	10.51	12.25	11.38
	T4FR	9.85	9.95	9.90
	T4R	10.09	11.04	10.56
	T4D	9.91	8.98	9.44
	N1D	9.59	10.25	9.92
				Av. 10.24

ALKALINITY OF WATER-SOLUBLE ASH

In the Flyings group of grades of like color, the alkalinity of the water-soluble ash increased with the increase in quality of the grade.

TABLE 4.—ALKALINITY OF WATER-SOLUBLE ASH

Alkalinity in ml. of N/10 HCl per gram of M. and S. F. Tobacco.

	UNITED STATES GRADE	1951 CROP (ML.)	1952 CROP (ML.)	AVERAGE OF 1951 AND 1952 (ML.)
Flyings	X2L	5.10	4.77	4.94
	X4L	5.32	3.83	4.58
	X2F	——	4.54	——
	X4F	4.60	3.63	4.12
	X4R	3.47	3.53	3.50
	N1L	4.77	3.77	4.27
	N1F	4.80	3.82	4.31
				Av. 4.29
Cutter	C2L	4.94	5.49	5.22
	C4L	4.63	5.30	4.96
	C2F	——	4.64	——
	C4F	5.02	9.23	7.12
	C4R	4.20	4.12	4.16
				Av. 5.36
Leaf	B2F	4.33	3.94	4.14
	B2FR	——	4.70	——
	B4F	5.26	3.53	4.40
	B4FR	5.47	3.46	4.46
	B4R	5.55	4.64	5.10
	B4D	5.32	3.78	4.55
				Av. 4.53
Tip	T4F	6.01	2.37	4.19
	T4FR	5.99	3.50	4.74
	T4R	5.94	2.12	4.03
	T4D	5.96	3.06	4.51
	N1D	6.05	3.42	4.74
				Av. 4.44

ALKALINITY OF WATER-INSOLUBLE ASH

There was no consistent relationship between the alkalinity of water-insoluble ash and the qualities of the grades within each group.

TABLE 5.—ALKALINITY OF WATER-INSOLUBLE ASH

Alkalinity in ml. of N/10 HCl per gram of M. and S. F. Tobacco.

	UNITED STATES GRADE	1951 CROP (ML.)	1952 CROP (ML.)	AVERAGE OF 1951 AND 1952 (ML.)
Flyings	X2L	27.15	30.36	28.76
	X4L	30.53	34.93	32.73
	X2F	—	34.43	—
	X4F	27.84	32.34	30.09
	X4R	28.51	27.52	28.02
	N1L	30.76	33.82	32.29
	N1F	29.69	31.09	30.39
				Av. 30.38
Cutter	C2L	29.70	29.16	29.43
	C4L	29.79	29.15	29.47
	C2F	—	28.48	—
	C4F	29.40	27.66	28.53
	C4R	29.10	26.47	27.78
				Av. 28.80
Leaf	B2F	30.37	32.80	31.58
	B2FR	—	35.14	—
	B4F	29.83	37.51	33.67
	B4FR	29.44	35.17	32.30
	B4R	29.40	33.35	31.38
	B4D	28.79	33.63	31.21
				Av. 32.03
Tip	T4F	30.71	24.54	27.62
	T4FR	30.60	20.84	25.72
	T4R	30.66	21.33	26.00
	T4D	30.55	19.75	25.15
	N1D	30.56	22.04	26.30
				Av. 26.16

SODIUM

The percentage of sodium in all grades was extremely small, ranging from 0.015 percent for B4D to 0.046 percent in X4L. There was no consistent relationship between the content of sodium and the various groups of grades or between the qualities of the grades within each group.

TABLE 6.—SODIUM

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.016	0.062	0.039
	X4L	0.017	0.075	0.046
	X2F	——	0.115	——
	X4F	0.016	0.032	0.024
	X4R	0.016	0.024	0.020
	N1L	0.025	0.034	0.030
	N1F	0.014	0.034	0.024
				Av. 0.030
Cutter	C2L	0.011	0.031	0.021
	C4L	0.019	0.042	0.030
	C2F	——	0.028	——
	C4F	0.016	0.038	0.027
	C4R	0.015	0.026	0.020
				Av. 0.024
Leaf	B2F	0.012	0.032	0.022
	B2FR	——	0.026	——
	B4F	0.010	0.027	0.018
	B4FR	0.014	0.025	0.020
	B4R	0.010	0.024	0.017
	B4D	0.008	0.022	0.015
				Av. 0.018
Tip	T4F	0.011	0.028	0.020
	T4FR	0.007	0.038	0.022
	T4R	0.006	0.025	0.016
	T4D	0.011	0.022	0.016
	N1D	0.010	0.026	0.018
				Av. 0.018

POTASSIUM

The average percentage of potassium in the grades of the two crops was 3.62 and ranged from 2.79 percent in T4R to 4.55 percent in X2L. Flyings averaged 4.00 percent; Cutters, 3.95 percent; Leaf, 3.50 percent and Tips, 3.01 percent. Generally, the better qualities and lighter colors of each group contained a larger percentage of potassium. This is in agreement with the findings of Shedd (*40*). Corresponding grades of Tips contained less potassium than the Leaf grades, while corresponding grades of Flyings and Cutters contained about the same percentage of potassium.

TABLE 7.--POTASSIUM (as Potassium)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	4.76	4.34	4.55
	X4L	4.62	4.15	4.39
	X2F	——	3.79	——
	X4F	3.96	3.21	3.59
	X4R	3.28	3.38	3.33
	N1L	4.38	3.69	4.04
	N1F	4.65	3.55	4.10
				Av. 4.00
Cutter	C2L	4.45	4.30	4.38
	C4L	4.42	4.00	4.21
	C2F	——	4.00	——
	C4F	4.04	3.49	3.77
	C4R	3.71	3.20	3.46
				Av. 3.95
Leaf	B2F	3.63	3.23	3.43
	B2FR	——	3.40	——
	B4F	3.33	4.03	3.68
	B4FR	3.55	3.04	3.30
	B1R	3.64	3.45	3.55
	B4D	3.62	3.50	3.56
				Av. 3.50
Tip	T4F	3.18	2.76	2.97
	T4FR	3.35	2.81	3.08
	T4R	3.13	2.45	2.79
	T4D	3.37	2.60	2.99
	N1D	3.75	2.71	3.23
				Av. 3.01

CALCIUM

The average calcium content was found to be greatest (5.30%) in the Flyings (X's plus N1L and N1F), and the average for each of the other groups decreased in the following order: Cutters (C's) 4.83 percent; Leaf (B's) 4.17 percent; and Tips (T's + N1D) 3.64 percent. There was no significant relationship between the calcium content and the quality of the grades within each group.

TABLE 8.—CALCIUM (as Calcium)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	5.22	5.54	5.38
	X4L	5.27	6.09	5.68
	X2F	—	5.48	—
	X4F	5.22	5.67	5.45
	X4R	4.99	4.57	4.78
	N1L	5.41	5.86	5.64
	N1F	4.49	5.31	4.90
				Av. 5.30
Cutter	C2L	4.70	5.22	4.96
	C4L	4.78	5.09	4.94
	C2F	—	4.97	—
	C4F	4.64	4.80	4.72
	C4R	4.34	5.01	4.68
				Av. 4.83
Leaf	B2F	4.34	4.64	4.49
	B2FR	—	4.11	—
	B4F	4.47	4.54	4.51
	B4FR	4.18	4.15	4.17
	B4R	4.12	3.75	3.94
	B4D	3.79	3.68	3.74
				Av. 4.17
Tip	T4F	3.78	4.27	4.03
	T4FR	3.52	3.83	3.68
	T4R	3.60	3.61	3.61
	T4D	3.40	3.30	3.35
	N1D	3.32	3.73	3.53
				Av. 3.64

PHOSPHORUS

The amount of phosphorus in all of the grades was very small, all of which were under 0.25 percent. There was no apparent relationship between the phosphorus content of the different groups, considered as units, or between the several grades within each group.

TABLE 9.—PHOSPHORUS

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.223	0.212	0.218
	X4L	0.214	0.213	0.214
	X2F	——	0.211	——
	X4F	0.205	0.210	0.208
	X4R	0.204	0.194	0.199
	N1L	0.223	0.206	0.214
	N1F	0.234	0.208	0.221
				Av. 0.212
Cutter	C2L	0.220	0.220	0.220
	C4L	0.219	0.227	0.223
	C2F	——	0.211	——
	C4F	0.226	0.216	0.221
	C4R	0.186	0.220	0.203
				Av. 0.217
Leaf	B2F	0.209	0.213	0.211
	B2FR	——	0.201	——
	B4F	0.206	0.207	0.206
	B4FR	0.202	0.211	0.206
	B4R	0.207	0.220	0.214
	B4D	0.207	0.236	0.222
				Av. 0.212
Tip	T4F	0.242	0.243	0.242
	T4FR	0.226	0.226	0.226
	T4R	0.238	0.217	0.228
	T4D	0.235	0.236	0.236
	N1D	0.244	0.228	0.236
				Av. 0.234

MANGANESE

The manganese content was very small in all of the grades, averaging only 0.041 percent (± 0.02%). There appears to be no significant relationship between the manganese content and grade, with the exception that the darker colored grades generally contained more than the lighter colored grades.

TABLE 10.—MANGANESE

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.049	0.022	0.036
	X4L	0.031	0.022	0.026
	X2F	—	0.026	—
	X4F	0.039	0.035	0.037
	X4R	0.047	0.080	0.064
	N1L	0.045	0.052	0.048
	N1F	0.047	0.062	0.054
				Av. 0.044
Cutter	C2L	0.021	0.017	0.019
	C4L	0.023	0.017	0.020
	C2F	—	0.023	—
	C4F	0.021	0.027	0.024
	C4R	0.039	0.047	0.043
				Av. 0.026
Leaf	B2F	0.024	0.024	0.024
	B2FR	—	0.025	—
	B4F	0.023	0.021	0.022
	B4FR	0.028	0.026	0.027
	B4R	0.034	0.065	0.050
	B4D	0.124	0.114	0.119
				Av. 0.048
Tip	T4F	0.019	0.021	0.020
	T4FR	0.027	0.020	0.024
	T4R	0.034	0.032	0.033
	T4D	0.079	0.096	0.088
	N1D	0.069	0.101	0.085
				Av. 0.050

TOTAL NITROGEN

In total nitrogen the Flyings group of grades averaged 3.46 percent; Cutters, 3.69 percent; Leaf, 4.91 percent; and Tips, 5.01 percent. Moseley, Harlan, and Hanmer (23) in their study of the chemical composition of Burley tobacco made a similar observation. They pointed out that the percentage of total nitrogen increased regularly over the entire stalk range.

In all groups, the grades of darker color generally contained a greater percentage of total nitrogen than those of lighter color. From the standpoint of quality within the different groups, a comparison of the grades shows that the higher the numerical designation of the grade, the less the total nitrogen content.

TABLE 11.—TOTAL NITROGEN (as Nitrogen)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	3.28	3.45	3.37
	X4L	3.21	3.31	3.26
	X2F	—	3.89	—
	X4F	3.63	3.66	3.65
	X4R	3.97	4.19	4.08
	N1L	3.13	3.23	3.18
	N1F	3.25	3.21	3.23
				Av. 3.46
Cutter	C2L	3.36	3.63	3.50
	C4L	3.28	3.32	3.30
	C2F	—	3.85	—
	C4F	3.53	3.86	3.70
	C4R	4.16	4.36	4.26
				Av. 3.69
Leaf	B2F	4.61	4.65	4.63
	B2FR	—	4.88	—
	B4F	4.26	4.34	4.30
	B4FR	4.42	5.04	4.73
	B4R	5.33	5.48	5.41
	B4D	5.47	5.46	5.47
				Av. 4.91
Tip	T4F	4.48	4.14	4.31
	T4FR	4.48	4.92	4.70
	T4R	5.36	5.42	5.39
	T4D	5.71	5.75	5.73
	N1D	4.89	4.94	4.92
				Av. 5.01

NITRATE NITROGEN

The percentages of nitrate nitrogen were relatively small, averaging only 0.36 percent in the grades of the two crops. The average percentage was greater in the Flyings group of grades (0.41%) than in the Cutters (0.34%), Leaf (0.39%), or Tips (0.27%). Moseley, Harlan and Hanmer (23) found a greater percentage of nitrate nitrogen in the Flyings and Trash leaves, which correspond to the X-group of grades in the Federal classification system of tobacco.

TABLE 12.—NITRATE NITROGEN

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.47	0.49	0.48
	X4L	0.53	0.42	0.48
	X2F	—	0.55	—
	X4F	0.45	0.31	0.38
	X4R	0.49	0.29	0.39
	N1L	0.39	0.39	0.39
	N1F	0.32	0.36	0.34
				Av. 0.41
Cutter	C2L	0.37	0.34	0.36
	C4L	0.38	0.31	0.34
	C2F	—	0.36	—
	C4F	0.33	0.28	0.30
	C4R	0.40	0.29	0.34
				Av. 0.34
Leaf	B2F	0.52	0.32	0.42
	B2FR	—	0.37	—
	B4F	0.37	0.32	0.34
	B4FR	0.39	0.30	0.34
	B4R	0.57	0.39	0.48
	B4D	0.48	0.23	0.36
				Av. 0.39
Tip	T4F	0.27	0.18	0.22
	T4FR	0.14	0.21	0.18
	T4R	0.29	0.28	0.28
	T4D	0.46	0.19	0.32
	N1D	0.33	0.27	0.30
				Av. 0.26

AMMONIA NITROGEN

The average percentage of ammonia nitrogen was almost twice as great in the Cutter as in the Flyings grades. The Leaf and Tip grades contained

four times as much ammonia nitrogen as the Flyings group of grades.

The grades of the Flyings group for the two crops averaged 0.075 percent; Cutters, 0.143 percent; Leaf, 0.318 percent; and Tips, 0.310 percent.

In all cases represented, the darker colored grades of the same group and quality contained a greater percentage of ammonia nitrogen.

There are six instances in the table in which a comparison between grades can be made on a quality basis with the group and color constant factors. In five of these six cases the second quality contained a greater amount of ammonia nitrogen than the fourth quality.

Moseley, Harlan, and Hanmer (*23*) in their study of the chemical composition of Burley tobacco found a regular increase in ammonia content over the entire stalk range, with the lowest content in the Flyings and a considerably higher content in the red Leaf and Tips.

TABLE 13.—AMMONIA NITROGEN (as Nitrogen)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.102	0.081	0.091
	X4L	0.075	0.065	0.070
	X2F	——	0.097	——
	X4F	0.085	0.084	0.085
	X4R	0.122	0.113	0.117
	N1L	0.041	0.033	0.037
	N1F	0.065	0.034	0.050
				Av. 0.075
Cutter	C2L	0.149	0.121	0.135
	C4L	0.121	0.102	0.112
	C2F	——	0.142	——
	C4F	0.142	0.135	0.138
	C4R	0.181	0.189	0.185
				Av. 0.143
Leaf	B2F	0.258	0.281	0.269
	B2FR	——	0.288	——
	B4F	0.247	0.231	0.239
	B4FR	0.265	0.327	0.296
	B4R	0.349	0.389	0.369
	B4D	0.414	0.419	0.416
				Av. 0.318
Tip	T4F	0.235	0.233	0.234
	T4FR	0.264	0.291	0.277
	T4R	0.356	0.390	0.373
	T4D	0.368	0.421	0.394
	N1D	0.261	0.285	0.273
				Av. 0.310

PROTEIN NITROGEN

The protein nitrogen content of the two crops ranged from 1.24 percent in C2L to 2.20 percent in T4D. The darker grades contain comparatively greater amounts of protein nitrogen than the companion grades of the same group and quality.

TABLE 14.—PROTEIN NITROGEN (as Nitrogen)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	1.46	1.48	1.47
	X4L	1.51	1.36	1.44
	X2F	—	1.63	—
	X4F	1.71	1.55	1.63
	X4R	1.78	1.72	1.75
	N1L	1.85	1.71	1.78
	N1F	1.81	1.85	1.83
				Av. 1.65
Cutter	C2L	1.21	1.27	1.24
	C4L	1.31	1.26	1.28
	C2F	—	1.22	—
	C4F	1.38	1.44	1.41
	C4R	1.65	1.67	1.66
				Av. 1.40
Leaf	B2F	1.38	1.38	1.38
	B2FR	—	1.38	—
	B4F	1.47	1.44	1.46
	B4FR	1.58	1.47	1.52
	B4R	1.72	1.74	1.73
	B4D	1.94	1.93	1.94
				Av. 1.61
Tip	T4F	1.60	1.49	1.54
	T4FR	1.73	1.73	1.73
	T4R	1.88	1.91	1.90
	T4D	2.21	2.18	2.20
	N1D	2.14	2.13	2.14
				Av. 1.90

ALPHA AMINO NITROGEN

The content of alpha amino nitrogen bears a close relationship to grade from the standpoint of group, quality, and color. Flyings averaged 0.195 percent; Cutters, 0.299 percent; Leaf, 0.679 percent; and Tips, 0.696 percent. In the grades of the same group and color the second quality was

consistently higher than the fourth quality in alpha amino nitrogen content.

In the grades of the same group and quality the darker colored grades had the greater percentage of this constituent, without exception.

TABLE 15.—ALPHA AMINO NITROGEN (as Nitrogen)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.204	0.230	0.217
	X4L	0.171	0.204	0.188
	X2F	——	0.238	——
	X4F	0.198	0.228	0.213
	X4R	0.227	0.278	0.253
	N1L	0.120	0.148	0.134
	N1F	0.172	0.158	0.165
				Av. 0.195
Cutter	C2L	0.283	0.309	0.296
	C4L	0.246	0.277	0.212
	C2F	——	0.348	——
	C4F	0.292	0.323	0.308
	C4R	0.348	0.407	0.378
				Av. 0.299
Leaf	B2F	0.570	0.550	0.560
	B2FR	——	0.675	——
	B4F	0.500	0.521	0.511
	B4FR	0.570	0.743	0.657
	B4R	0.724	0.902	0.813
	B4D	0.787	0.921	0.854
				Av. 0.679
Tip	T4F	0.573	0.524	0.549
	T4FR	0.639	0.744	0.692
	T4R	0.768	0.889	0.828
	T4D	0.792	0.923	0.858
	N1D	0.578	0.526	0.552
				Av. 0.696

TOTAL VOLATILE BASES (AS AMMONIA)

The method of analysis for the determination of total volatile bases is an empirical one developed in the Research Laboratory of The American Tobacco Company (*8*). By means of this method various basic substances are determined which are volatile under the experimental conditions prescribed in the method. This determination has been found useful in the estimation of Burley tobaccos from the standpoint of strength, smoking

quality, and adaptability to blending with other types. It has also been found to be closely related to what tobacco judges designate as "body" (*23*).

The content of total volatile bases ranged from 0.330 percent in N1L to 1.314 percent in B4D, a ratio of nearly four to one. Flyings group averaged 0.502 percent; Cutters, 0.763 percent; Leaf, 1.199 percent; and Tips, 1.105 percent. On the basis of quality comparison in the same group and color the second quality is higher than the fourth quality in total volatile bases, except in the comparison of B2FR with B4FR.

On the basis of color comparison in the same group and quality the darker colored grade is consistently higher in total volatile bases than the corresponding lighter colored grade, except in the comparison between T4R and T4D.

TABLE 16.—TOTAL VOLATILE BASES (as Ammonia)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.517	0.553	0.535
	X4L	0.440	0.486	0.463
	X2F	—	0.633	—
	X4F	0.555	0.609	0.582
	X4R	0.697	0.748	0.723
	N1L	0.320	0.339	0.330
	N1F	0.416	0.346	0.381
				Av. 0.502
Cutter	C2L	0.733	0.741	0.737
	C4L	0.633	0.630	0.632
	C2F	—	0.879	—
	C4F	0.727	0.793	0.760
	C4R	0.884	0.963	0.924
				Av. 0.763
Leaf	B2F	1.163	1.167	1.165
	B2FR	—	1.235	—
	B4F	1.037	1.058	1.048
	B4FR	1.039	1.292	1.166
	B4R	1.227	1.373	1.300
	B4D	1.299	1.329	1.314
				Av. 1.199
Tip	T4F	0.998	0.983	0.991
	T4FR	1.003	1.184	1.094
	T4R	1.245	1.294	1.270
	T4D	1.192	1.315	1.254
	N1D	0.853	0.976	0.915
				Av. 1.105

NICOTINE, NORNICOTINE, AND TOTAL ALKALOIDS (*12*) (AS NICOTINE)

Alkaloids as determined by the extraction and titration procedure described in (*12*) varied from a low of 1.19 percent for N1L to a high of 6.00 percent for B2F, a range of 4.81 percent. With the group and quality constant factors the darker colored grades almost invariably contained the greater alkaloid content. With the group and color constant factors the second quality contained more alkaloid than the fourth quality, with one exception (the comparison of X2F with X4F).

The average content of alkaloids, groupwise, of 2.49 percent for Flyings, 3.90 percent for Cutter, 5.18 percent for Leaf, and 4.12 percent for Tips follows the variation in maturity pattern of some other constituents, such as Total Volatile Bases, Water-Soluble Acids, and 95 percent Ethanol Extract.

TABLE 17.—TOTAL ALKALOIDS (as Nicotine)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	2.27	2.91	2.59
	X4L	1.94	2.64	2.29
	X2F	——	3.41	——
	X4F	2.59	3.54	3.06
	X4R	3.66	4.39	4.02
	N1L	1.19	1.52	1.36
	N1F	1.68	1.52	1.60
				Av. 2.49
Cutter	C2L	3.55	4.02	3.78
	C4L	3.07	3.36	3.22
	C2F	——	4.90	——
	C4F	3.48	4.44	3.96
	C4R	4.31	5.01	4.66
				Av. 3.90
Leaf	B2F	5.32	6.00	5.66
	B2FR	——	6.32	——
	B4F	4.72	5.58	5.15
	B4FR	4.40	5.84	5.12
	B4R	4.77	5.58	5.18
	B4D	4.54	5.00	4.77
				Av. 5.18
Tip	T4F	3.86	4.69	4.28
	T4FR	3.57	5.09	4.33
	T4R	4.21	5.08	4.64
	T4D	3.97	4.70	4.34
	N1D	2.57	3.47	3.02
				Av. 4.12

ALKALOIDS (*16*)

Alkaloids as determined by steam distillation and spectrophotometric procedure according to the method described in (*16*), varied from a low of 1.18 percent for N1L to a high of 6.00 percent for B2F, a range of 4.82 percent. This range in alkaloid content is practically a duplication of the range as determined by the above extraction and titration procedure (*12*). However, the alkaloid content as determined by steam distillation followed by spectrophotometric measurement (*16*) is consistently higher than the content as determined by the extraction and titration procedure.

The comparison between the darker colored and lighter colored grades of the same group and quality, and between the second and fourth quality of grades in the same group and color shows the same relationship by either of these two methods used for the determination of alkaloids.

The group averages for alkaloid content determined by this method are 2.54 percent for Flyings, 4.00 percent for Cutters, 5.27 percent for Leaf, and 4.19 percent for Tips which is a difference of 0.05 percent to 0.10 percent higher than the group averages that were determined by the extraction and titration procedure.

TABLE 18.—TOTAL ALKALOIDS (as Nicotine)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	2.23	2.98	2.60
	X4L	1.93	2.68	2.30
	X2F	——	3.44	——
	X4F	2.67	3.74	3.20
	X4R	3.71	4.57	4.14
	N1L	1.18	1.58	1.38
	N1F	1.69	1.54	1.62
				Av. 2.54
Cutter	C2L	3.60	4.16	3.88
	C4L	3.17	3.37	3.27
	C2F	——	4.84	——
	C4F	3.62	4.61	4.12
	C4R	4.43	5.02	4.72
				Av. 4.00
Leaf	B2F	5.50	6.00	5.75
	B2FR	——	6.53	——
	B4F	4.79	5.69	5.24
	B4FR	4.52	5.90	5.21
	B4R	4.99	5.61	5.30
	B4D	4.68	5.03	4.86
				Av. 5.27
Tip	T4F	3.93	4.79	4.36
	T4FR	3.73	4.66	4.20
	T4R	4.37	5.37	4.87
	T4D	4.08	4.73	4.40
	N1D	2.68	3.55	3.12
				Av. 4.19

TOTAL REDUCING SUBSTANCES (AS GLUCOSE)

The average content of total reducing substances (as glucose) ranged from 1.22 percent for N1L to 2.09 percent for T4F. In the entire series of samples there was, therefore, an extreme difference of only 0.87 percent, and this difference was so distributed among the grades that there was no apparent pattern of total reducing substances content by which the grades could be distinguished.

TABLE 19.—TOTAL REDUCING SUBSTANCES

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	1.76	1.69	1.72
	X4L	1.27	1.58	1.42
	X2F	—	1.77	—
	X4F	1.53	1.68	1.60
	X4R	1.26	1.94	1.60
	N1L	1.17	1.29	1.23
	N1F	1.54	1.49	1.52
				Av. 1.52
Cutter	C2L	1.32	1.60	1.46
	C4L	1.36	1.71	1.54
	C2F	—	1.57	—
	C4F	1.22	1.90	1.56
	C4R	1.52	2.00	1.76
				Av. 1.58
Leaf	B2F	1.42	1.80	1.61
	B2FR	—	1.67	—
	B4F	1.14	1.66	1.40
	B4FR	1.32	1.62	1.47
	B4R	1.37	1.46	1.42
	B4D	1.62	1.74	1.68
				Av. 1.52
Tip	T4F	2.39	1.81	2.10
	T4FR	1.79	1.89	1.84
	T4R	1.40	1.76	1.58
	T4D	1.42	1.56	1.49
	N1D	1.32	1.56	1.44
				Av. 1.69

TOTAL REDUCING SUGARS (AS GLUCOSE)

Total reducing sugars ranged from 0.78 percent in N1L to 1.94 percent in T4F. This range of 1.15 percent between the two extremes was somewhat greater than in the case of total reducing substances. The Flyings group averaged 1.08 percent; Cutters, 1.20 percent; Leaf, 1.23 percent; and Tips, 1.47 percent. Thus, the percentages of total reducing sugars increased gradually from Flyings to Tips. Aside from this gradual difference groupwise, the content of this component does not draw further distinctions among the grades.

TABLE 20.—TOTAL REDUCING SUGARS

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	1.60	1.19	1.40
	X4L	1.10	1.00	1.05
	X2F	——	1.05	——
	X4F	1.46	0.96	1.21
	X4R	0.95	1.26	1.10
	N1L	0.80	0.77	0.78
	N1F	1.03	0.98	1.00
				Av. 1.09
Cutter	C2L	1.07	1.31	1.19
	C4L	1.14	1.29	1.22
	C2F	——	1.32	——
	C4F	1.16	1.34	1.25
	C4R	0.89	1.34	1.12
				Av. 1.20
Leaf	B2F	1.14	1.32	1.23
	B2FR	——	1.33	——
	B4F	1.07	1.23	1.12
	B4FR	1.20	1.34	1.27
	B4R	1.15	1.29	1.22
	B4D	1.22	1.38	1.30
				Av. 1.23
Tip	T4F	2.24	1.64	1.94
	T4FR	1.55	1.42	1.48
	T4R	1.26	1.64	1.45
	T4D	1.25	1.16	1.20
	N1D	1.31	1.21	1.26
				Av. 1.47

POLYPHENOLS (AS GLUCOSE)

The amount of polyphenols was rather small in all cases. The average of all grades of the two crops was 0.33 percent, and it ranged from 0.13 percent in T4R to 0.65 percent in C4R. Flyings averaged 0.43 percent; Cutters, 0.39 percent; Leaf, 0.28 percent; and Tips, 0.22 percent. Thus, on a comparative basis by groups, the Flyings group of grades contained a larger percentage, while the Tip grades contained the smallest percentage. There was no consistent relationship between the content of polyphenols of t e various grades on a quality or a color basis.

TABLE 21.—POLYPHENOLS

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.16	0.50	0.33
	X4L	0.16	0.58	0.37
	X2F	----	0.72	----
	X4F	0.07	0.72	0.40
	X4R	0.32	0.68	0.50
	N1L	0.37	0.53	0.45
	N1F	0.51	0.51	0.51
				Av. 0.43
Cutter	C2L	0.25	0.29	0.27
	C4L	0.22	0.41	0.32
	C2F	----	0.25	----
	C4F	0.06	0.56	0.31
	C4R	0.63	0.66	0.64
				Av. 0.38
Leaf	B2F	0.28	0.48	0.38
	B2FR	----	0.33	----
	B4F	0.07	0.43	0.25
	B4FR	0.12	0.28	0.20
	B4R	0.22	0.17	0.20
	B4D	0.40	0.36	0.38
				Av. 0.28
Tip	T4F	0.15	0.17	0.16
	T4FR	0.24	0.45	0.34
	T4R	0.14	0.12	0.13
	T4D	0.17	0.40	0.28
	N1D	0.01	0.34	0.18
				Av. 0.22

PROTOPECTIN, (AS CALCIUM PECTATE)

Protopectin, as its name indicates, is the first pectic substance produced by the plant. In the course of growth and development of the plant, the protopectin is converted, through the action of enzymes produced by the plant, into water-soluble pectinic acids or pectin. As the plant matures, the pectinic acids are converted into pectic acid. Therefore, the greatest percentage of protopectin should be in the grades consisting of relatively immature tobacco, while the greatest percentage of pectic acid would be expected in the mature, overripe tobacco. The results, in the main, substantiate this assumption.

The percentage of protopectin in all grades of the two crops ranged from 3.62 percent in N1L to 7.79 percent in T4R. N1L and N1F contained much less protopectin than any of the other grades, while T4R and T4FR contained much more. Flyings averaged 4.53 percent; Cutters, 5.92 percent; Leaf, 5.68 percent; and Tips, 6.99 percent. There was no consistent relationship between the content of protopectin on either a quality or color basis. The only relationship appeared to be by group, where Flyings contained the least, and Tips contained the most protopectin.

TABLE 22.—PROTOPECTIN (as Ca Pectate)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	4.89	4.37	4.63
	X4L	4.61	6.20	5.41
	X2F	——	4.26	——
	X4F	4.65	4.42	4.54
	X4R	5.79	4.45	5.12
	N1L	3.58	3.66	3.62
	N1F	4.04	3.63	3.84
			Av.	4.53
Cutter	C2L	6.60	4.62	5.61
	C4L	6.16	6.40	6.28
	C2F	——	6.43	——
	C4F	6.09	5.98	6.04
	C4R	5.38	6.07	5.73
			Av.	5.92
Leaf	B2F	5.42	4.76	5.09
	B2FR	——	4.72	——
	B4F	5.74	5.01	5.38
	B4FR	4.85	7.59	6.22
	B4R	4.77	6.78	5.78
	B4D	4.61	7.26	5.94
			Av.	5.68
Tip	T4F	5.00	7.11	6.06
	T4FR	7.76	7.53	7.65
	T4R	8.79	6.79	7.79
	T4D	7.64	6.12	6.88
	N1D	7.64	5.49	6.57
			Av.	6.99

Note: All grades were free of pectin, or water-soluble pectinic acids.

PECTIC ACID AND PECTATES (AS CALCIUM PECTATE)

The percentage of pectic acid and pectates of all grades of the two crops ranged from 3.71 percent in T4D to 7.40 percent in N1F, which is a two-fold difference. The Flyings group of grades contained the largest amount of pectic acid and pectates, averaging 5.83 percent; Cutters averaged 5.60 percent; Leaf, 4.48 percent; while the Tips contained the smallest percentage, averaging 4.17 percent. The results on pectic acid and pectates were closely associated with group, but there was no consistent relationship with grade quality within the group. Generally, the grades of lighter colors contained larger percentages than those of darker colors.

TABLE 23.—PECTIC ACID & PECTATES (as Ca Pectate)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	5.16	6.54	5.85
	X4L	5.37	4.84	5.11
	X2F	——	5.97	——
	X4F	4.74	5.38	5.06
	X4R	4.20	5.18	4.69
	N1L	6.80	6.89	6.85
	N1F	7.46	7.34	7.40
				Av. 5.83
Cutter	C2L	5.49	6.21	5.85
	C4L	6.43	5.84	6.14
	C2F	——	5.02	——
	C4F	6.59	4.89	5.74
	C4R	4.41	4.88	4.65
				Av. 5.60
Leaf	B2F	5.00	5.93	5.47
	B2FR	——	6.10	——
	B4F	4.71	5.61	5.16
	B4FR	4.64	3.36	4.00
	B4R	4.16	3.72	3.94
	B4D	3.96	3.71	3.84
				Av. 4.48
Tip	T4F	4.82	4.57	4.70
	T4FR	4.25	3.40	3.83
	T4R	3.73	3.90	3.82
	T4D	3.70	3.72	3.71
	N1D	4.68	4.85	4.77
				Av. 4.17

TOTAL CHLOROPHYLL

There is a gradual increase in the total chlorophyll content, groupwise, in proceeding from the Flyings group through the Cutter and Leaf groups to the Tip group. On a quality comparison basis among grades of the same color, the chlorophyll content does not show a consistent difference between grades. On a color comparison basis among the grades of the same quality, the darker colored grades are higher in most cases in total chlorophyll content than the lighter colored grades.

TABLE 24.—TOTAL CHLOROPHYLL

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (*MCG./GM.)	1952 CROP (MCG./GM.)	AVERAGE OF 1951 AND 1952 (MCG./GM.)
Flyings	X2L	26	34	30
	X4L	25	26	26
	X2F	—	29	—
	X4F	37	29	33
	X4R	56	37	46
	N1L	34	16	25
	N1F	59	36	48
				Av. 35
Cutter	C2L	41	40	40
	C4L	26	41	38
	C2F	—	36	—
	C4F	54	30	42
	C4R	69	42	56
				Av. 44
Leaf	B2F	60	33	46
	B2FR	—	42	—
	B4F	51	42	46
	B4FR	67	69	68
	B4R	76	52	64
	B4D	104	39	72
				Av. 59
Tip	T4F	61	44	52
	T4FR	84	43	64
	T4R	118	51	84
	T4D	116	54	85
	N1D	100	55	78
				Av. 73

* Micrograms per gram.

TOTAL CAROTENOIDS

The total carotenoids content follows the same trend from one group to the next as in the case of the total chlorophyll content, except between the Leaf and Tip groups in which case the amounts were equal. The second quality is consistently higher than the fourth quality in total carotenoids content on a comparison of grades in the same group and color. On comparisons of grades in the same group and quality, the darker colored grades are almost invariably higher in total carotenoids content than the lighter colored grades.

TABLE 25.—TOTAL CAROTENOIDS

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (*MCG./GM.)	1952 CROP (MCG./GM.)	AVERAGE OF 1951 AND 1952 (MCG./GM.)
Flyings	X2L	39	25	32
	X4L	40	17	28
	X2F	——	27	——
	X4F	49	25	37
	X4R	61	36	48
	N1L	36	12	24
	N1F	62	20	41
				Av. 35
Cutter	C2L	88	44	66
	C4L	74	36	55
	C2F	——	53	——
	C4F	91	46	68
	C4R	101	58	80
				Av. 67
Leaf	B2F	115	68	92
	B2FR	——	71	——
	B4F	111	57	84
	B4FR	122	61	92
	B4R	128	57	92
	B4D	111	49	80
				Av. 88
Tip	T4F	101	52	76
	T4FR	114	63	88
	T4R	127	57	92
	T4D	136	53	94
	N1D	130	48	89
				Av. 88

* Micrograms per gram.

URONIC ACIDS (AS ANHYDRIDES)

The average content of uronic acids (as anhydrides) of the two crops ranged from 15.21 percent for T4D to 17.59 percent in N1F. Flyings averaged 17.02 percent; Cutters, 16.70 percent; Leaf, 16.30 percent; and Tips, 15.65 percent. The results showed a gradual decrease in uronic acid content in proceeding groupwise from the Flyings group to the Tip group. Phillips and Bacot (*30*) in an investigation of the content of uronic acids (as anhydrides) in certain grades of Type 12 tobacco (which is a flue-cured type), found that the grades that were heavier in body and had the greatest tensile strength had the lowest content of uronic acids (as anhydrides), while those which were thinner in body and had the least tensile strength had the highest content of uronic acids (as anhydrides). It appears from the data that this is equally true for Burley tobacco, which is an air-cured instead of a flue-cured type.

Generally, the lighter colored grades in each group contained a larger percentage of uronic acids (as anhydrides), but there was no consistent relationship between the amount of uronic acids on a quality basis within the several groups.

TABLE 26.—URONIC ACIDS (as Anhydrides)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	17.02	17.39	17.21
	X4L	17.70	17.07	17.39
	X2F	—	16.95	—
	X4F	16.74	16.03	16.39
	X4R	16.38	15.80	16.09
	N1L	17.78	17.15	17.47
	N1F	17.97	17.20	17.59
				Av. 17.02
Cutter	C2L	17.17	17.16	17.17
	C4L	17.33	16.74	17.04
	C2F	—	16.71	—
	C4F	17.14	16.27	16.71
	C4R	16.36	15.42	15.89
				Av. 16.70
Leaf	B2F	16.62	16.56	16.64
	B2FR	—	16.02	—
	B4F	16.98	16.04	16.51
	B4FR	16.65	15.59	16.12
	B4R	16.80	15.43	16.12
	B4D	16.19	16.02	16.11
				Av. 16.30
Tip	T4F	16.00	15.40	15.70
	T4FR	16.08	15.37	15.73
	T4R	15.26	15.58	15.42
	T4D	15.60	14.81	15.21
	N1D	16.63	15.57	16.20
				Av. 15.65

PENTOSANS

The content of pentosans in the two crops ranged from 2.02 percent for B4D to 2.96 percent for C4L. The Flyings group averaged 2.46 percent; Cutters, 2.55 percent; Leaf, 2.15 percent; and Tips, 2.26 percent. Thus the Cutters contained somewhat larger percentages of pentosans than any other group, while the Leaf grades contained the smallest. Generally, the lighter colors contained higher percentages of pentosans than the darker colors in the same group and quality.

TABLE 27.—PENTOSANS

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	2.34	2.41	2.38
	X4L	2.14	2.41	2.28
	X2F	—	2.30	—
	X4F	2.32	2.36	2.34
	X4R	2.05	2.33	2.19
	N1L	2.88	2.74	2.81
	N1F	2.84	2.67	2.76
				Av. 2.46
Cutter	C2L	2.40	2.47	2.44
	C4L	3.15	2.76	2.96
	C2F	—	2.39	—
	C4F	2.58	2.37	2.48
	C4R	2.44	2.21	2.33
				Av. 2.55
Leaf	B2F	2.17	2.26	2.22
	B2FR	—	2.20	—
	B4F	2.31	2.38	2.35
	B4FR	2.16	2.09	2.13
	B4R	1.97	2.13	2.05
	B4D	2.14	1.90	2.02
				Av. 2.15
Tip	T4F	2.44	2.20	2.32
	T4FR	2.51	1.93	2.22
	T4R	2.27	2.03	2.15
	T4D	2.36	1.97	2.17
	N1D	2.61	2.29	2.45
				Av. 2.26

CRUDE FIBER

The crude fiber determination is one of the recognized standards in the analysis of various agricultural products. Various investigators of the chemical composition of tobacco have included this determination in their analytical data. The analytical determination is an empirical method, and the product known as crude fiber is essentially a mixture of cellulose and lignin (27).

The grades of the Leaf group contained the smallest amount of crude fiber, averaging 9.08 percent, while the grades of the Flyings group contained the largest amount, averaging 11.06 percent. Cutters averaged 10.36 percent, and the Tip grades averaged 9.87 percent. Crude fiber ranged from 8.57 percent in B4R to 12.98 percent in N1L. N1L and N1F, which contained higher percentages of injury and waste, contained much greater percentages of crude fiber than any of the other grades. The grades of lighter colors contained more than those of darker colors, with the exception of B4D and T4D, which contained somewhat more crude fiber than the corresponding grades of B4R and T4R. There was a rather consistent relationship between the crude fiber content of the grades according to group and color.

TABLE 28.—CRUDE FIBER

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	10.18	10.81	10.50
	X4L	11.27	11.11	11.19
	X2F	——	10.37	——
	X4F	10.48	10.18	10.33
	X4R	8.94	9.39	9.17
	N1L	12.40	13.38	12.98
	N1F	11.68	12.67	12.18
				Av. 11.06
Cutter	C2L	10.50	11.01	10.76
	C4L	10.80	11.01	10.91
	C2F	——	10.59	——
	C4F	10.17	10.38	10.28
	C4R	9.18	9.82	9.50
				Av. 10.36
Leaf	B2F	9.09	9.51	9.30
	B2FR	——	9.20	——
	B4F	9.15	9.58	9.37
	B4FR	9.18	8.98	9.08
	B4R	8.18	8.96	8.57
	B4D	8.75	9.43	9.09
				Av. 9.08
Tip	T4F	10.10	10.32	10.21
	T4FR	9.79	9.89	9.84
	T4R	9.08	9.23	9.16
	T4D	9.09	9.89	9.49
	N1D	10.45	10.86	10.66
				Av. 9.87

CELLULOSE

The results on the amount of cellulose parallel very closely the results on crude fiber on the same basis of comparison, although the cellulose results are consistently higher than the crude fiber results.

TABLE 29.—CELLULOSE

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	12.47	11.66	12.07
	X4L	12.50	11.12	11.81
	X2F	——	11.02	——
	X4F	12.19	10.27	11.23
	X4R	10.73	10.55	10.64
	N1L	13.98	12.65	13.32
	N1F	13.44	12.67	13.06
				Av. 12.02
Cutter	C2L	12.27	10.79	11.53
	C4L	12.45	11.62	12.04
	C2F	——	10.82	——
	C4F	12.19	10.32	11.26
	C4R	11.16	9.58	10.37
				Av. 11.30
Leaf	B2F	10.60	9.55	10.08
	B2FR	——	9.32	——
	B4F	10.80	9.82	10.31
	B4FR	10.94	9.19	10.07
	B4R	10.07	9.18	9.63
	B4D	10.60	9.27	9.94
				Av. 10.01
Tip	T4F	12.08	10.10	11.09
	T4FR	12.23	9.97	11.10
	T4R	10.66	9.87	10.27
	T4D	11.06	9.72	10.39
	N1D	12.97	10.73	11.85
				Av. 10.94

LIGNIN

The lignin content in the grades of the two crops ranged from 2.24 percent in B2F to 3.88 percent in N1F. Flyings averaged 3.25 percent; Cutters, 2.61 percent; Leaf, 2.41 percent; and Tips, 2.89 percent. Generally, the content of lignin was higher in the fourth quality grades of each group, but there was no consistent relationship between the lignin content and the color of the tobacco. The three Nondescript grades, N1L, N1F, and N1D, contained much higher percentages of lignin than the remaining grades.

TABLE 30.—LIGNIN

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	2.69	3.43	3.06
	X4L	2.89	3.13	3.01
	X2F	——	2.94	——
	X4F	2.88	3.19	3.04
	X4R	2.57	2.76	2.67
	N1L	3.67	3.98	3.83
	N1F	3.64	4.12	3.88
				Av. 3.25
Cutter	C2L	2.32	2.38	2.35
	C4L	2.48	2.63	2.56
	C2F	——	2.37	——
	C4F	2.55	2.92	2.74
	C4R	2.61	2.92	2.77
				Av. 2.61
Leaf	B2F	2.13	2.34	2.24
	B2FR	——	2.21	——
	B4F	2.38	2.53	2.46
	B4FR	2.44	2.30	2.37
	B4R	2.34	2.23	2.29
	B4D	2.88	2.49	2.69
				Av. 2.41
Tip	T4F	2.68	2.72	2.70
	T4FR	2.79	2.68	2.74
	T4R	2.83	2.47	2.65
	T4D	3.05	2.69	2.87
	N1D	3.89	3.12	3.51
				Av. 2.89

METHOXYL IN LIGNIN

The content of methoxyl in lignin in all grades of the two crops ranged from 3.13 percent in N1D to 4.57 percent in X4R. Flyings group averaged 4.06; Cutter, 4.16 percent; Leaf, 3.76 percent; and Tips, 3.33 percent. Without exception, the percentages of methoxyl in lignin were higher in the second qualities of each group and color than in the fourth qualities, and, in most cases, the percentages were higher in the lighter colors than in the darker colors of each comparison of the same group and quality. Corresponding grades of Flyings and Cutters contained about the same percentage of methoxyl in lignin. Corresponding qualities of the Leaf group contained a larger percentage than the grades of the T-group. The most consistent relationship between the content of methoxyl in lignin and grade was on a quality basis.

TABLE 31.--METHOXYL IN LIGNIN

Calculated on ash-free and crude-protein-free Lignin.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	4.58	3.71	4.15
	X4L	4.44	3.68	4.06
	X2F	——	4.00	——
	X4F	4.49	3.50	4.00
	X4R	5.06	4.08	4.57
	N1L	4.33	3.30	3.82
	N1F	3.80	3.75	3.78
			Av.	4.06
Cutter	C2L	4.54	4.27	4.41
	C4L	4.55	3.76	4.16
	C2F	——	4.03	——
	C4F	4.42	3.61	4.02
	C4R	4.59	3.47	4.03
			Av.	4.16
Leaf	B2F	4.45	3.72	4.09
	B2FR	——	3.80	——
	B4F	3.97	3.71	3.84
	B4FR	3.96	3.66	3.81
	B4R	3.91	3.69	3.80
	B4D	3.26	3.24	3.25
			Av.	3.76
Tip	T4F	3.65	3.33	3.49
	T4FR	3.57	3.41	3.49
	T4R	3.37	3.35	3.36
	T4D	3.14	3.20	3.17
	N1D	3.15	3.11	3.13
			Av.	3.33

TANNIN

The average tannin content of the two crops was 2.90 percent; Flyings averaged 2.82 percent; Cutters, 2.79 percent; Leaf, 2.86 percent; and Tips, 3.13 percent. Thus on a group basis, the Tip grades contained the larger average percentage. In the case of polyphenols, it was the Flyings grades which averaged the greater percentage, as compared with the other grades.

There was no significant relationship between the amount of tannin and the several grades in quality or color.

TABLE 32.—TANNIN

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	2.75	2.67	2.71
	X4L	2.93	2.42	2.68
	X2F	——	2.97	——
	X4F	2.28	3.43	2.86
	X4R	3.07	2.63	2.85
	N1L	2.72	2.55	2.64
	N1F	3.44	2.87	3.16
				Av. 2.82
Cutter	C2L	3.32	2.49	2.90
	C4L	2.69	2.73	2.71
	C2F	——	3.26	——
	C4F	2.67	2.42	2.54
	C4R	2.86	3.08	2.97
				Av. 2.78
Leaf	B2F	3.93	2.76	3.34
	B2FR	——	2.14	——
	B4F	2.93	2.24	2.58
	B4FR	2.58	1.82	2.20
	B4R	2.97	2.47	2.72
	B4D	3.07	3.76	3.42
				Av. 2.85
Tip	T4F	3.66	3.42	3.54
	T4FR	3.43	2.62	3.02
	T4R	2.36	2.65	2.50
	T4D	3.04	2.73	2.88
	N1D	4.41	2.97	3.69
				Av. 3.13

TOTAL VOLATILE ACIDS (AS ACETIC ACID)

The Flyings group of grades averaged 1.48 percent; Cutters, 1.38 percent; Leaf, 1.31 percent; and Tips, 1.24 percent. The total volatile acids content decreased progressively groupwise from the Flyings group of grades to the Tip group, but there was no significant difference between the grades on a quality basis, or on a color basis.

TABLE 33.—TOTAL VOLATILE ACIDS

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	1.93	1.28	1.60
	X4L	1.24	1.33	1.28
	X2F	——	1.25	——
	X4F	1.19	1.38	1.28
	X4R	1.70	1.36	1.53
	N1L	1.67	1.50	1.58
	N1F	1.23	1.92	1.58
				Av. 1.48
Cutter	C2L	1.02	1.51	1.26
	C4L	1.43	1.52	1.48
	C2F	——	1.52	——
	C4F	1.42	1.56	1.49
	C4R	1.34	1.21	1.28
				Av. 1.38
Leaf	B2F	1.39	1.10	1.24
	B2FR	——	1.08	——
	B4F	1.31	1.13	1.22
	B4FR	1.28	1.51	1.40
	B4R	1.24	1.48	1.36
	B4D	1.38	1.27	1.32
				Av. 1.31
Tip	T4F	1.58	1.14	1.36
	T4FR	1.37	1.14	1.26
	T4R	1.00	1.26	1.13
	T4D	1.05	1.23	1.14
	N1D	1.33	1.28	1.30
				Av. 1.24

FORMIC ACID

The Flyings group averaged 0.066 percent; Cutters, 0.018 percent; Leaf. 0.017 percent; and Tips, 0.020 percent. While Flyings contained a slightly greater percentage of formic acid than the other groups, there was otherwise no significant difference among the grades according to group or according to quality or color within each group.

TABLE 34.—FORMIC ACID

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.037	0.006	0.022
	X4L	0.045	0.004	0.024
	X2F	——	0.004	——
	X4F	0.035	0.027	0.031
	X4R	0.036	0.036	0.036
	N1L	0.047	0.045	0.046
	N1F	0.042	0.024	0.033
				Av. 0.032
Cutter	C2L	0.023	0.009	0.016
	C4L	0.015	0.013	0.014
	C2F	——	0.017	——
	C4F	0.024	0.011	0.018
	C4R	0.026	0.024	0.025
				Av. 0.018
Leaf	B2F	0.024	0.024	0.024
	B2FR	——	0.004	——
	B4F	0.015	0.012	0.014
	B4FR	0.010	0.005	0.008
	B4R	0.025	0.001	0.013
	B4D	0.034	0.018	0.026
				Av. 0.017
Tip	T4F	0.027	0.005	0.016
	T4FR	0.025	0.025	0.025
	T4R	0.026	0.004	0.015
	T4D	0.033	0.009	0.016
	N1D	0.019	0.022	0.020
				Av. 0.018

WATER-SOLUBLE ACIDS

The average percentage of all grades of the two crops ranged from 1.02 percent in N1L to 5.15 percent in B4R, a ratio of approximately five to one. The Flyings group averaged 1.90 percent; Cutters, 3.52 percent; Leaf, 4.87 percent; and Tips, 4.43 percent. In all cases, the second quality in the grades of the same group and color contained more water-soluble acids than the corresponding fourth quality. With the exception of B4D and T4D, the grades of darker color of the same group and quality contained more water-soluble acids than the corresponding grades of lighter color. Thus, there is evidently a significant relationship between the water-soluble acid content and grade according to group, quality, and color. N1D, which is a very low quality grade of Tips, was much lower in water-soluble acids than the other Tip grades. N1L and N1F, which are very low quality grades of Flyings, also contained much less water-soluble acids than the other Flyings group of grades.

TABLE 35.—WATER-SOLUBLE ACIDS (Ml. N/10 NaOH per gram of tobacco)

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (ML.)	1952 CROP (ML.)	AVERAGE OF 1951 AND 1952 (ML.)
Flyings	X2L	1.55	2.56	2.06
	X4L	1.51	2.05	1.78
	X2F		2.87	—
	X4F	2.05	2.80	2.43
	X4R	2.15	3.85	3.00
	N1L	0.93	1.10	1.02
	N1F	1.19	0.99	1.09
				Av. 1.90
Cutter	C2L	2.88	3.71	3.30
	C4L	2.67	3.19	2.93
	C2F		4.24	—
	C4F	3.37	4.14	3.76
	C4R	3.61	4.55	4.10
				Av. 3.52
Leaf	B2F	4.36	5.38	4.87
	B2FR		5.80	—
	B4F	4.25	5.07	4.66
	B4FR	4.24	5.70	4.97
	B4R	4.62	5.68	5.15
	B4D	4.73	4.65	4.66
				Av. 4.87
Tip	T4F	4.04	4.76	4.40
	T4FR	4.26	4.96	4.61
	T4R	4.36	5.38	4.87
	T4D	4.20	5.27	4.74
	N1D	3.21	3.83	3.52
				Av. 4.43

PETROLEUM ETHER EXTRACT

The content of petroleum ether extract in all grades of the two crops ranged from a low of 5.06 percent in N1D to 7.98 percent in T4F, which is a difference of 2.91 percent. The Flyings group averaged 6.50 percent; Cutter, 7.63 percent; Leaf, 6.84 percent; and Tips, 6.36 percent. The Cutter grades are an intermediate group between Flyings and Leaf, and the content of petroleum ether extractives was higher in this than in any of the other groups. There was no difference in the content of petroleum ether extract between the second and fourth quality when the group and color were the same. However, on a color comparison basis the lighter colored grades in the Flyings and Cutter group in the same quality contained higher quantities of petroleum ether extract, and the darker colored grades in the same quality contained the higher quantities in the Leaf and Tip grades.

TABLE 36.—PETROLEUM ETHER EXTRACT

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	6.25	6.57	6.41
	X4L	6.14	6.50	6.32
	X2F	—	6.99	—
	X4F	6.57	7.20	6.88
	X4R	6.79	7.69	7.24
	N1L	5.55	6.13	5.84
	N1F	6.33	6.27	6.30
				Av. 6.50
Cutter	C2L	7.70	7.47	7.58
	C4L	7.54	7.59	7.56
	C2F	—	8.43	—
	C4F	8.00	7.90	7.95
	C1R	7.35	7.53	7.44
				Av. 7.63
Leaf	B2F	8.22	7.66	7.94
	B2FR	—	6.88	—
	B4F	7.54	7.76	7.65
	B4FR	6.97	7.14	7.10
	B4R	6.00	6.28	6.14
	B4D	4.80	6.03	5.42
				Av. 6.85
Tip	T4F	7.44	8.52	7.98
	T4FR	6.47	7.58	7.02
	T4R	6.01	6.78	6.40
	T4D	4.95	5.71	5.33
	N1D	4.88	5.25	5.06
				Av. 6.36

RESINS AND WAXES

In grades of the same group and color, the fourth quality is generally higher in resins and waxes content than the second quality. In resins and waxes content, there is no apparent difference between the light and dark shades of color of the same group and quality.

TABLE 37.—RESINS AND WAXES

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	9.24	7.89	8.56
	X4L	9.15	8.37	8.76
	X2F	——	8.29	——
	X4F	9.28	8.30	8.79
	X4R	8.79	8.98	8.88
	N1L	9.99	8.99	9.49
	N1F	9.53	8.38	8.96
				Av. 8.91
Cutter	C2L	9.15	7.83	8.49
	C4L	9.75	8.72	9.24
	C2F	——	7.89	——
	C4F	9.54	8.98	9.26
	C4R	8.66	8.93	8.80
				Av. 8.95
Leaf	B2F	8.22	8.25	8.24
	B2FR	——	7.65	——
	B4F	8.74	8.59	8.66
	B4FR	7.89	7.86	7.88
	B4R	7.06	7.05	7.06
	B4D	6.01	6.55	6.28
				Av. 7.62
Tip	T4F	8.74	9.35	9.04
	T4FR	7.97	8.17	8.07
	T4R	7.21	7.46	7.34
	T4D	6.53	6.66	6.60
	N1D	6.57	6.62	6.60
				Av. 7.53

WAXES

The average waxes content for the Flyings, Cutter, Leaf, and Tip groups of grades was 0.46 percent, 0.45 percent, 0.36 percent, and 0.41 percent, respectively. There was no consistent relationship between the amount of waxes and the qualities or colors within each group, nor was there a significant difference between groups. In several cases, widely different grades had approximately the same amount of waxes.

TABLE 38.—WAXES

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.45	0.43	0.44
	X4L	0.52	0.35	0.44
	X2F	——	0.46	——
	X4F	0.56	0.47	0.52
	X4R	0.50	0.45	0.48
	N1L	0.51	0.38	0.44
	N1F	0.47	0.39	0.43
				Av. 0.46
Cutter	C2L	0.62	0.39	0.50
	C4L	0.44	0.40	0.42
	C2F	——	0.24	——
	C4F	0.51	0.43	0.47
	C4R	0.42	0.39	0.40
				Av. 0.45
Leaf	B2F	0.42	0.37	0.40
	B2FR	——	0.27	——
	B4F	0.38	0.42	0.40
	B4FR	0.32	0.35	0.34
	B4R	0.36	0.31	0.34
	B4D	0.31	0.28	0.30
				Av. 0.36
Tip	T4F	0.49	0.47	0.48
	T4FR	0.55	0.41	0.48
	T4R	0.39	0.36	0.38
	T4D	0.36	0.31	0.34
	N1D	0.39	0.35	0.37
				Av. 0.41

95% ETHANOL EXTRACT

The amount of 95 percent ethanol extract varied from a low of 27.23 percent in N1L to a high of 35.92 percent in B2F, a range of 8.69 percent. The average content of extract for the Flyings group was 29.52 percent; Cutter, 32.61 percent; Leaf, 34.95 percent; and Tips, 33.61 percent. In most cases of comparisons between the second and fourth qualities in grades of the same group and color, the second quality contained more extractive material than the fourth quality. Differences in 95 percent ethanol extract content were almost evenly divided between the light and dark colors when group and quality were the same.

TABLE 39.- 95% ETHANOL EXTRACT

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	30.51	29.99	30.25
	X4L	29.82	28.39	29.11
	X2F	——	30.86	——
	X4F	31.25	30.90	31.08
	X4R	31.15	32.28	31.72
	N1L	28.19	26.26	27.23
	N1F	29.50	25.91	27.71
				Av. 29.52
Cutter	C2L	31.58	32.58	32.08
	C4L	32.05	30.99	31.52
	C2F	——	33.23	——
	C4F	32.88	33.20	33.04
	C4R	33.96	33.66	33.81
				Av. 32.61
Leaf	B2F	36.75	35.08	35.92
	B2FR	——	35.69	——
	B4F	34.99	34.65	34.82
	B4FR	32.54	35.07	33.81
	B4R	34.80	36.61	35.71
	B4D	35.08	33.87	34.48
				Av. 34.95
Tip	T4F	34.24	35.08	34.66
	T4FR	34.30	34.87	34.59
	T4R	34.55	36.00	35.28
	T4D	33.59	33.38	33.49
	N1D	29.85	30.18	30.02
				Av. 33.61

HOT-WATER SOLUBLE EXTRACT

The quantity of hot-water soluble substances ranged from a low of 38.59 percent in N1L to a high of 50.72 percent in B2F, a difference of 12.13 percent. Flyings averaged 42.78 percent; Cutter, 46.87 percent; Leaf, 49.06 percent; and Tips, 44.63 percent. Thus, the greatest percentage of tobacco material was removed from the heavier Leaf grades by this solvent, while the least was removed from the thinner Flyings grades. In all cases of comparison between the second and fourth qualities in the same group and color, the second quality invariably contained the greater amount of extract. In the comparisons of different colors in the same group and quality, the variations in the amount of extract did not follow a significant trend.

TABLE 40.—HOT-WATER SOLUBLE EXTRACT

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	45.15	45.08	45.12
	X4L	43.84	44.46	44.15
	X2F	——	45.05	——
	X4F	44.02	44.74	44.38
	X4R	44.34	45.77	45.06
	N1L	39.17	38.01	38.59
	N1F	40.70	38.07	39.39
				Av. 42.78
Cutter	C2L	48.56	46.35	47.46
	C4L	46.14	46.89	46.52
	C2F	——	48.29	——
	C4F	45.65	46.86	46.26
	C4R	46.02	48.48	47.25
				Av. 46.87
Leaf	B2F	50.19	51.25	50.72
	B2FR	——	52.76	——
	B4F	46.40	48.70	47.55
	B4FR	45.75	50.42	48.09
	B4R	48.71	50.70	49.71
	B4D	48.49	49.95	49.22
				Av. 49.06
Tip	T4F	44.14	45.67	44.91
	T4FR	43.07	46.67	44.87
	T4R	44.33	46.72	45.53
	T4D	44.29	46.14	45.22
	N1D	41.00	44.25	42.63
				Av. 44.63

pH

Since pH values are stated as logarithmic numbers, they were converted to grams of hydrogen ions per liter before averaging and then recalculated to the corresponding pH in obtaining the average pH value.

Flyings had the greatest pH values, and these then decreased by groups in the following order: Cutter, Leaf, and Tips. There were no significant differences in the pH values within each group on a quality basis.

TABLE 41.—pH

	UNITED STATES GRADE	1951 CROP (pH)	1952 CROP (pH)	AVERAGE OF 1951 AND 1952 (pH)
Flyings	X2L	5.90	5.85	5.87
	X4L	6.20	6.00	6.09
	X2F	——	5.80	——
	X4F	6.03	5.80	5.90
	X4R	5.75	5.60	5.67
	N1L	7.05	6.77	6.90
	N1F	6.80	7.00	6.90
				Av. 6.02
Cutter	C2L	5.85	5.65	5.74
	C4L	5.85	5.75	5.80
	C2F	——	5.60	——
	C4F	5.73	5.62	5.67
	C4R	5.68	5.52	5.59
				Av. 5.70
Leaf	B2F	5.48	5.45	5.46
	B2FR	——	5.40	——
	B4F	5.45	5.44	5.44
	B4FR	5.55	5.40	5.46
	B4R	5.43	5.40	5.41
	B4D	5.40	5.37	5.38
				Av. 5.44
Tip	T4F	5.45	5.47	5.46
	T4FR	5.40	5.35	5.37
	T4R	5.35	5.32	5.33
	T4D	5.35	5.30	5.32
	N1D	5.65	5.55	5.60
				Av. 5.41

SULFUR

The sulfur content by groups for the two crop years averaged for Flyings, 0.69 percent; Cutter, 0.79 percent; Leaf, 0.78 percent; and Tips, 0.71 percent. The range between groups was 0.10 percent. The lowest content for grade was 0.58 percent for X4L and the highest was 0.86 percent for T4R, or a range of 0.28 percent. There appeared to be no grade differentiation on the basis of sulfur content from either a quality or color standpoint.

TABLE 42.—SULFUR

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.76	0.77	0.76
	X4L	0.64	0.53	0.58
	X2F	——	0.66	——
	X4F	0.68	0.67	0.68
	X4R	0.68	0.56	0.62
	N1L	0.66	1.02	0.84
	N1F	0.76	0.56	0.66
				Av. 0.69
Cutter	C2L	0.76	0.86	0.81
	C4L	0.77	0.79	0.78
	C2F	——	0.85	——
	C4F	0.79	0.86	0.82
	C4R	0.74	0.78	0.76
				Av. 0.79
Leaf	B2F	0.72	0.77	0.74
	B2FR	——	0.73	——
	B4F	0.87	0.82	0.84
	B4FR	0.84	0.80	0.82
	B4R	0.78	0.66	0.72
	B4D	0.71	0.87	0.79
				Av. 0.78
Tip	T4F	0.82	0.81	0.82
	T4FR	0.88	0.82	0.85
	T4R	0.87	0.85	0.86
	T4D	0.78	0.77	0.78
	N1D	0.79	0.73	0.76
				Av. 0.81

CHLORINE

The average content of chlorine according to group was as follows: Flyings, 0.74 percent; Cutter, 0.50 percent; Leaf, 0.61 percent; and Tips, 0.51 percent. The chlorine content ranged from a low of 0.29 percent for T4FR to a high of 1.25 percent for B4D. Although the ratio of the high to the low in chlorine content was over four to one, the amounts in the various grades were so scattered that no relationship to grade was evident from the standpoint of quality or of color.

TABLE 43.—CHLORINE

All results calculated on a moisture-free and sand-free basis.

	UNITED STATES GRADE	1951 CROP (PERCENT)	1952 CROP (PERCENT)	AVERAGE OF 1951 AND 1952 (PERCENT)
Flyings	X2L	0.99	0.64	0.82
	X4L	0.81	0.65	0.73
	X2F	——	0.47	——
	X4F	0.54	0.49	0.52
	X4R	0.48	0.57	0.52
	N1L	0.98	0.88	0.93
	N1F	1.02	0.78	0.90
				Av. 0.74
Cutter	C2L	0.68	0.40	0.54
	C4L	0.46	0.65	0.56
	C2F	——	0.43	——
	C4F	0.35	0.49	0.42
	C4R	0.47	0.47	0.47
				Av. 0.50
Leaf	B2F	0.75	0.41	0.58
	B2FR	——	0.44	——
	B4F	0.48	0.36	0.42
	B4FR	0.57	0.41	0.49
	B4R	0.63	0.57	0.60
	B4D	1.25	0.70	0.98
				Av. 0.61
Tip	T4F	0.57	0.31	0.88
	T4FR	0.54	0.29	0.42
	T4R	0.57	0.38	0.48
	T4D	0.73	0.43	0.58
	N1D	0.72	0.54	0.63
				Av. 0.60

MOISTURE EQUILIBRIUM

There are noteworthy changes in the moisture equilibrium of different grades, and groups of grades, corresponding to changes in relative humidity at 25° C. as shown in Table 44. The increase in moisture content with the increase in relative humidity indicates the relative moisture absorption properties of the different grades, which is a significant factor in the technology of tobacco.

COMPOSITION OF STEMS

The chemical composition of the stems from three grades of tobacco is given in Table 45.

In explanation of the grade designations used here for stems, it is necessary to point out that there are no Federal grades for stems. The grade designations N1L, C2L, and B4R mean that the stems were taken from leaves graded N1L, C2L, and B4R, respectively.

In comparing the chemical composition of the three samples of stems among themselves, it will be noted that N1L had the smallest percentages of total nitrogen, nitrate nitrogen, and nicotine, while B4R had the greatest percentages of these components. The percentage of protopectin (as calcium pectate) was the smallest in N1L and greatest in B4R. However, in case of pectic acid and pectates (as calcium pectate) this relationship was reversed. N1L contained the greatest percentages of pentosans, crude fiber, cellulose, and lignin as compared with C2L and B4R. The methoxyl in lignin was the greatest in C2L and the smallest in N1L. The percentages of 95% ethanol extractives and the acidity values increased in the following order: N1L, C2L, and B4R.

In comparing the chemical composition of the stems with the web of the same grade, it is evident that the stems contained much greater percentages of uronic acids, crude fiber, cellulose, and methoxyl in lignin. Lignin from a more mature plant tissue generally contains a greater percentage of methoxyl.

The pH values are also greater in the case of the stems.

Because of the woody nature of stems, it was expected that they would contain a much greater percentage of lignin. However, the percentages of lignin in the stems were not much different from those of the corresponding grades of web.

TABLE 44.—MOISTURE EQUILIBRIUM

	UNITED STATES GRADE	40 PERCENT RELATIVE HUMIDITY		60 PERCENT RELATIVE HUMIDITY		70 PERCENT RELATIVE HUMIDITY		80 PERCENT RELATIVE HUMIDITY	
		1951 CROP (PERCENT)	1952 CROP (PERCENT)	1951 CROP (PERCENT)	1952 CROP (PERCENT)	1951 CROP (PERCENT)	1952 CROP (PERCENT)	1951 CROP (PERCENT)	1952 CROP (PERCENT)
Flyings	X2L	8.10	7.35	10.15	9.30	11.85	11.65	16.60	15.60
	X4L	8.20	7.60	10.20	9.35	11.65	11.75	15.60	15.15
	X2F	——	7.50	——	9.60	——	11.90	——	15.80
	X4F	7.80	7.75	9.60	9.40	11.20	11.85	15.50	15.40
	X4R	7.40	7.35	9.60	9.50	11.10	12.35	15.50	16.35
	N1L	8.40	7.85	10.15	9.50	11.50	11.50	15.40	14.60
	N1F	8.40	8.45	10.30	9.75	11.80	12.10	16.00	15.45
Average		8.05	7.69	10.00	9.48	11.51	11.87	15.77	15.48
Cutter	C2L	7.60	7.15	10.25	9.20	11.80	12.00	16.20	16.30
	C4L	7.75	7.25	10.05	9.50	11.80	12.40	16.30	17.00
	C2F	——	7.25	——	9.55	——	12.50	——	16.95
	C4F	7.50	7.15	9.70	9.65	11.45	12.20	16.00	16.50
	C4R	7.75	7.40	10.00	9.70	11.70	12.60	15.80	17.00
Average		7.65	7.24	10.00	9.52	11.68	12.34	16.08	16.75
Leaf	B2F	7.52	7.22	10.38	9.75	12.18	12.65	16.70	17.38
	B2FR	——	7.35	——	10.05	——	12.95	——	17.75
	B4F	7.50	7.05	9.95	9.45	11.80	12.30	16.70	16.70
	B4FR	7.60	7.00	10.05	9.70	11.80	12.50	15.90	17.20
	B4R	7.65	7.10	10.45	9.90	12.35	13.40	17.50	18.50
	B4D	7.50	7.20	10.75	9.95	13.40	12.80	19.50	17.90
Average		7.55	7.15	10.31	9.80	12.30	12.77	17.26	17.57
Tip	T4F	7.15	6.95	9.75	9.35	11.50	12.00	16.30	16.25
	T4FR	7.30	6.90	9.85	9.05	11.60	12.30	15.50	16.00
	T4R	7.35	7.20	10.15	10.00	12.10	12.75	16.80	16.70
	T4D	7.40	7.25	10.25	9.90	12.10	12.80	17.00	16.85
	N1D	7.70	7.60	10.15	10.00	11.90	12.75	16.70	16.75
Average		7.38	7.18	10.03	9.66	11.84	12.52	16.45	16.51

Note: Data in terms of percent moisture in equilibrium at 25° C.

TABLE 45.—COMPOSITION OF STEMS

All results calculated on a moisture-free and sand-free basis.

CONSTITUENT OR PROPERTY	GRADES		
	N1L	C2L	B4R
	Percent	*Percent*	*Percent*
Sand-free Ash	27.90	29.08	24.54
Water-Soluble Ash	11.76	19.70	15.19
Alkalinity of Water-Sol. Ash[1]	9.63	4.96	12.46
Total N	1.98	2.24	3.33
Nitrate N	0.92	1.24	1.46
Protein N	0.77	0.73	0.82
Nicotine	0.27	0.55	1.14
Protopectin (as Ca pectate)	2.53	2.86	4.16
Pectic Acid and Pectates (as Ca pectates)	13.98	12.48	10.26
Uronic Acids (as anhyd.)	28.08	27.45	29.80
Pentosans	4.92	4.59	4.82
Crude Fiber	26.09	22.86	20.08
Cellulose	27.29	23.57	22.15
Lignin	3.45	2.07	2.17
Methoxyl in Lignin	10.40	13.08	11.60
Tannins	2.24	2.18	0.53
Total Volatile Acids (as acetic acid)	2.17	1.49	1.22
95% Ethanol Extractives	26.89	28.11	30.81
pH	8.65	7.60	6.20

[1] ml. N/10 HCl per gram moisture and sand-free tobacco.

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BRIEF OF BURLEY GRADES

Type 31: Burley; produced principally in Kentucky, Tennessee, Ohio, Indiana, West Virginia, Virginia, North Carolina, and Missouri.

Key to Standard Grade Marks for Burley Tobacco

Group	Quality	Color	Special factor
B–Leaf	1–Choice	L–Buff	V–Greenish
T–Tips	2–Fine	F–Tan	K–Variegated
C–Lugs or Cutters	3–Good	R–Red	FR–Reddish Tan
X–Flyings	4–Fair	D–Dull Red	GF–Light Green
M–Mixed Group	5–Low	M–Mixed Color	GR–Dark Green
N–Nondescript		G–Green	
			W–Unsafe Order [1]
			U–Unsound [2]

Summary of Standard Grades and Subgrades

17 Grades of Leaf

B1F	B1FR	B1R	
B2F	B2FR	B2R	
B3F	B3FR	B3R	
B4F	B4FR	B4R	B4D
B5F	B5FR	B5R	B5D

11 Subgrades of Tips

T3F	T3FR	T3R	
T4F	T4FR	T4R	T4D
T5F	T5FR	T5R	T5D

15 Grades of Cutters or Lugs

C1L	C1F		
C2L	C2F		
C3L	C3F	C3R	
C4L	C4F	C4R	C4G
C5L	C5F	C5R	C5G

15 Grades of Flyings

X1L	X1F		
X2L	X2F		
X3L	X3F	X3R	
X4L	X4F	X4R	X4G
X5L	X5F	X5R	X5G

10 Subgrades of Green

B3GF	B3GR		
B4GF	B4GR	T4GF	T4GR
B5GF	B5GR	T5GF	T5GR

7 Mixed Color Subgrades

B3M		
B4M	C4M	X4M
B5M	C5M	X5M

6 Mixed Group

M3F	M3R
M4F	M4R
M5F	M5R

12 Greenish Subgrades (V's)

B3FV	T3FV	C3FV
B3RV	T3RV	C3RV
B4FV	T4FV	C4FV
B4RV	T4RV	C4RV

4 Variegated Subgrades (K's)

B3FK	C3FK
B4FK	C4FK

11 Grades of Nondescript

N1L		Botched
N1F	N2L	Nested
N1D	N2D	Offtype
N1C	N2C	N–Dec.

[1] Unsafe order—Sound but containing excessive moisture which is likely to damage unless unusual precaution is taken.

[2] Unsound—Damaged under 20 percent.

Description and specifications for Official Standard Grades are available upon request, Tobacco Division, Agricultural Marketing Service, U. S. Department of Agriculture, Washington 25, D. C.

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