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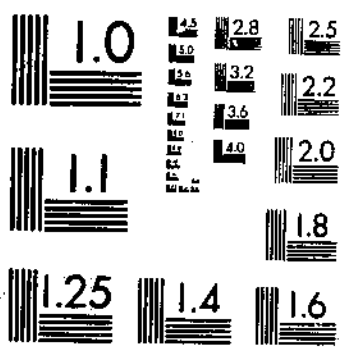

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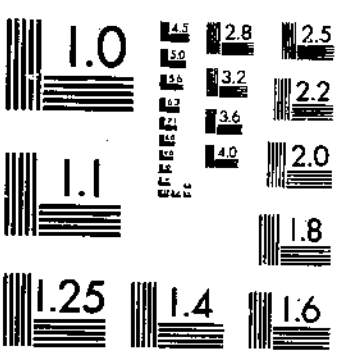

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CHEMICAL COMPOSITION OF REPRESENTATIVE GRADES OF THE 1952 and 1954 CROPS OF FLUE-CURED TOBACCO

√ including chemical methods

Technical Bulletin No. 1225

UNITED STATES DEPARTMENT OF AGRICULTURE

the CHEMICAL COMPOSITION OF REPRESENTATIVE GRADES OF THE 1952 and 1954 CROPS OF FLUE-CURED TOBACCO

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by Aubrey M. Bacot

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The author further acknowledges the wholehearted cooperation given by the collaborators.

Issued November 1960

contents

SUMMARY

This publication consists of the chemical analysis of representative U.S. grades of Flue-cured tobacco, the chemical methods used for the analysis of the tobacco, and comments in some instances on the relationship of the chemical analysis to grade characteristics.

The samples used for analysis were taken from the 1952 and 1954 crops. Total monthly rainfall data for the 1952 and 1954 growing seasons for the area in which the tobacco was grown are tabulated and graphically shown. The difference in rainfall distribution for the two crops, as well as the difference in amount of rainfall, is evident as an influence in the composition of the tobacco in some of the constituents determined.

Chemical analysis of the two crops was accomplished through the cooperation of our collaborators, who are listed and are shown with the method each used for the determination. Data tables on each of the chemical constituents give comparisons in content for the respective crop years. The tables give also the averages for the different groups of grades, the yearly average, high, low, ratio of high to low, standard deviation, and coefficient of variation for each crop.

A correlation study shows some of the most significant components as related to physical characteristics to be alpha-amino nitrogen, proteins, total reducing sugars, total volatile bases, and nicotine. In the same study some of the components found to be relatively less significant to physical characteristics are pectic acid and pectates, waxes, lignin, petroleum ether extract, and alkalinity of ash. Some other constituents found to be of doubtful variation in content according to grade are starch, sulfur, methoxyl in lignin, total volatile acids, and formic acid.

The moisture content at equilibrium under different conditions of relative humidity for this series of grades are tabulated and are also shown graphically.

the CHEMICAL COMPOSITION of REPRESENTATIVE GRADES of the 1952 and 1954 CROPS of FLUE-CURED TOBACCO including chemical methods

Aubrey M. Bacot, Standards Branch, Tobacco Division, Agricultural Marketing Service.

INTRODUCTION

The purpose in undertaking chemical analysis of representative grades of Flue-cured tobacco was to determine more closely the differences among the grades from the standpoint of grading practices, to determine the relationship of chemical composition to physical characteristics, and to indicate possible modifications of the U.S. grade specifications.

Twenty-four representative grades of Flue-cured tobacco, grown in the Old and Middle Belts of Virginia and North Carolina, were selected for analysis from each of the 1952 and 1954 crops. The samples of each crop were obtained from the sales floors of 26 tobacco markets representating a cross section of the Flue-cured tobacco area.

One of the most important factors in this study is the care and judgment used in the selection of samples. The classification of the samples according to the grade specifications of the U.S. Department of Agriculture represents the combined opinion of several experienced judges of tobacco.

Due mostly to weather conditions, all of the grades selected for analysis were not equally available in both crop years. For this reason, U.S. grades C2F and B2F are not represented in the 1952 crop but are represented in the 1954 crop, and grades B2R and B6S are not represented in the 1954 crop but are represented in the 1952 crop. Since these grades are not included in both crops, the results of the chemical analysis of these grades are not used in calculating group averages or yearly averages for either crop.

Each data table gives the amount of the chemical component in each grade analyzed for each of the two crops, and also shows the difference between the two crops. Averages for each of the groups are also given. Accompanying each table is a summary giving the high, low, range, average, ratio of high to low, standard deviation, and coefficient of variation for each crop. This provides an outline of the limits and variations in content of the component. (The number of the analytical method by which the data were determined appears at the head of each data table.)

Moisture and sand are variable ingredients of tobacco as it is grown and marketed. Tobacco is variable in moisture content because its hygroscopic nature responds to changes in relative humidity. It is variable in sand due to cultivation practices, handling, and rainfall. Moisture and sand are not considered constituent parts of the tobacco plant for the purpose of judging the chemical composition, and for this reason, the chemical components are reported on a moisture-free and sand-free basis. (The term "moisture-free and sand-free" is abbreviated as "M-&S-F".)

A brief on the U.S. system of grading Flue-cured tobacco is appended for the information of those unfamiliar with the grades. The X group consists of tobacco from the lower part of the plant. Above the X group, the C group is next in order, then the H or the B group, depending upon the ripeness of the tobacco. The B group becomes H-group tobacco if allowed to attain a sufficient degree of ripeness on the stalk before harvesting.

The two Nondescript grades, N1L and N1D, are composed of leaves which do not meet the minimum specifications of any other group. The N1L grade is placed in the tables before the X group because it is composed of tobacco from the lower part of the stalk. The N1D group is placed after the B group because it is composed of tobacco from the upper part of the stalk. Since each Nondescript grade is treated as a group in this case, neither is used in calculating any group average, but both are included in calculating the respective crop averages. In chemical composition, the Nondescript grades indicate in general the trend of some constituents in the tobacco plant and show the effect of the comparatively high proportion of waste which is characteristic of the Nondescript grades.

The chemical analysis of these two crops of Flue-cured tobacco is a continuation of the collaborative work initiated with the analysis of the 1951 and 1952 crops of Burley tobacco. Results of the previous collaborative study were reported by Phillips and Bacot (*22*).[1]

The analyses and determinations for both studies were made by a group of collaborators from the tobacco industry, colleges, agricultural experiment stations, and the U.S. Department of Agriculture. The determinations were in some cases exploratory since it was not known in the beginning which might prove to be the most useful to those interested in the cultivation and technology of tobacco.

A list of the collaborating laboratories follows:

American Sumatra Tobacco Corp.
The American Tobacco Co.
Brown & Williamson Tobacco Corp.
General Cigar Co., Inc.[2]

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[1] Italic numbers in parentheses refer to Literature Cited, p. 124.

[2] The name of the General Cigar Co., Inc. was inadvertently omitted from the list of the collaborators in a similar bulletin published on Burley tobacco (*22*).

The Imperial Tobacco Co., Ltd.
Liggett & Myers Tobacco Co.
P. Lorillard Co., Inc.
Philip Morris Inc.
North Carolina State College
The Pennsylvania State University
R. J. Reynolds Tobacco Co.
U.S. Department of Agriculture, ARS, Eastern Utilization Research and Development Division
U.S. Department of Agriculture, ARS, Crops Research Division, Tobacco and Sugar Crops Research Branch
United States Tobacco Co.

SAMPLE PREPARATION

Samples were collected from 26 auction markets and sent to Washington, D.C., to be appraised by several judges of tobacco for conformity to grade specifications. These samples were sorted and any leaves not conforming to grade specifications were discarded. At this point in sample preparation, collaborating members of the tobacco trade were invited to review the samples and were asked for their evaluation, suggestions, and comments

After the leaves were allowed to dry sufficiently at room temperature, the web portion was stripped from the midrib by hand and granulated by rubbing through a ¼-inch-square mesh wire screen. Only the web portion was used for analysis. The granulated web was sieved in a 1-mm. square mesh wire rotary sieve to remove excess sand. Then it was placed in paper bags and allowed to dry further at room temperature until the moisture content reached approximately 5 percent in order to minimize the likelihood of chemical change during the time necessary to complete the series of analyses.

The samples were passed several times through a Jones riffle sampler until they were thoroughly mixed, after which they were ground in a Wiley mill fitted with a standard 1-mm. sieve. Then they were blended in a tumbler-type diamond-shaped mixer and transferred to screw-top glass bottles to preserve them for chemical analysis.

RAINFALL DATA

Areas from which the 1952 and 1954 Flue-cured samples were collected had considerable differences in total rainfall between the time the seedlings were transplanted in the field and the time the tobacco was harvested. Rainfall data are limited to the reports of the 13 weather bureau stations nearest the auction markets from which the samples were obtained, and are given only for the months during which the plants were set, cultivated, and harvested. The data for the two growing seasons were taken from the Climatological Data of the U.S. Department of Commerce Weather Bureau (*25*, *26*, *27*, *28*) and are as follows:

Month	Average rainfall		Departure from normal	
	1952	1954	1952	1954
	Inches	*Inches*	*Inches*	*Inches*
May	4. 87	5. 64	—0. 03	0. 93
June	3. 29	3. 85	—1. 75	—. 92
July	5. 52	4. 43	—. 34	—1. 27
August	8. 10	3. 74	3. 34	—2. 05
September	7. 85	1. 24	. 35	—2. 16
Total	29. 63	18. 90	1. 57	—5. 47

A study was made at the Oxford, N.C., Experiment Station in 1936 by Darkis et al. (*14*). This study showed the variations in several chemical components under known conditions of fertilization and rainfall and demonstrated the influence of rainfall difference on the quantity and character of Flue-cured tobacco.

Our collaborative results parallel the findings of the Darkis investigation for the 11 chemical components common to both analyses. The present study, however, covers some 50 additional determinations on commercially

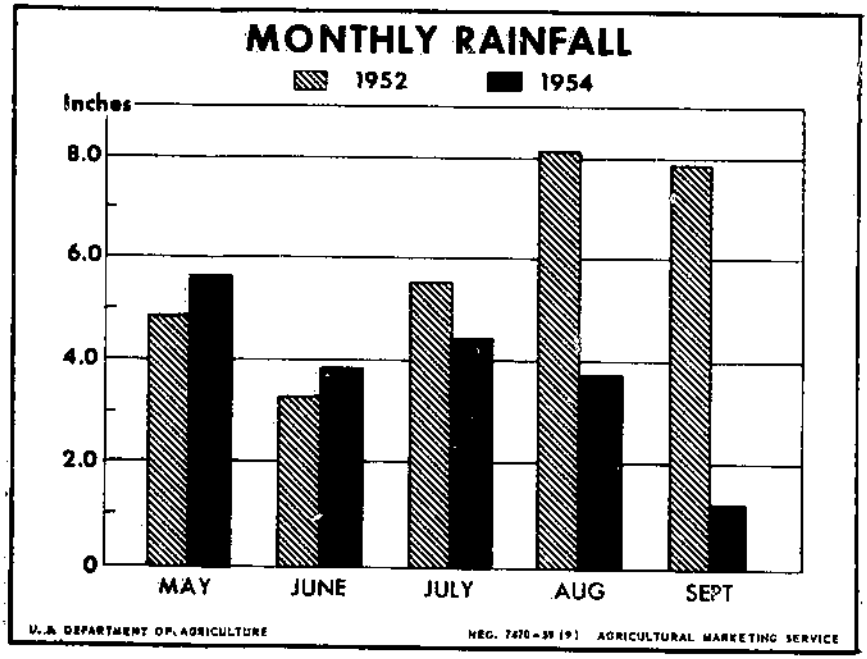

FIGURE 1.—Monthly rainfall, 1952 and 1954.

grown Flue-cured tobacco, and it covers a wider and more diversified area of cultivation. Most of the determinations made in this study revealed that the content of the various components differed between the two crops. In some instances, quite a marked difference is shown.

In the Darkis study the whole leaf was used for analysis, while in the analyses reported here only the portion of the leaf exclusive of the midrib was used. A further difference between the two studies is in the criteria used in grading the tobacco analyzed. In the Darkis publication the range of stalk position was graded into eight divisions according to the harvesting or priming of the tobacco. For this series of analyses, the tobacco was graded into the 24 applicable U.S. standard grades.

DISCUSSION OF RESULTS

Total Ash

The most noticeable variation in total ash content (table 51, p. 67) was found in group comparison. The comparison showed a decrease in the amount of ash from the lowest part of the plant to the top. Calcium (tables 7, 8, and 9, pp. 22, 23, and 24) constituted slightly more than 20 percent of the total ash and followed a trend similar to that of total ash. Potassium (tables 39 and 40, pp. 55 and 56) compared generally with calcium in distribution.

Copper (table 15, p. 30) was limited in quantity and was localized mainly in the top leaves. In grades of the same group and quality, it was consistently higher in the darker colored leaves.

The amount of manganese (tables 22 and 23, pp. 37 and 38) increased with the darkness of color in the same group and quality, but magnesium (table 21, p. 36), except for being slightly higher in content in the X group, appeared to be evenly distributed through the rest of the grades.

The excessive amount of aluminum (table 5, p. 20) in the X group probably was due to the nearness of this group to the soil where the leaves were most exposed to soil particles disturbed by cultivation, rain, and wind. These leaves received less direct rainfall since they were protected from it by the higher leaves. The X group of leaves consistently contained considerably more sand than the leaves of higher stalk positions, also because of the proximity of this group to the soil.

Water-soluble and water-insoluble ash and the respective alkalinities of water-soluble and water-insoluble ash (tables 62, 61, 3, and 2, pp. 78, 77, 18, and 17) did not appear to vary significantly between grades.

Cellulose, Crude Fiber, Lignin, and Methoxyl in Lignin

The determination of cellulose, crude fiber, and lignin were empirical. Determination of the methoxyl group in lignin was made on the assumption

that it might provide an indication of grade distinction. The methoxy-group content of lignin (table 24, p. 39) was apparently different in the various tobacco groups but was not significantly different for grades that are subdivisions of the groups. Lignin (table 20, p. 35) did not appear to vary with differences in grade.

Cellulose and crude fiber are closely related determinations showing the distribution of the structural material of the leaves of the plant. In the 1952 crop, which was produced during a comparatively wet season, the cellulose content (table 11, p. 26) was consistently higher than the crude fiber (table 16, p. 31); but in the 1954 crop, which was produced during a dry season, the cellulose content was consistently lower than the crude fiber.

Pectic Substances and Pentosans

The range of protopectin content (table 42, p. 58) was greater in the 1954 crop than in the 1952 crop, and the variation in the amount between grades also was greater in the 1954 crop. A progressive increase in the amount of protopectin from the X group to the H group was evident in a group comparison. The B group was lower in protopectin content than the H group.

As the plant matures, the protopectin decreases and the pectic acid and pectates increase. This transition can be observed by noting the variation in the amount of pectic acid and pectates (table 32, p. 48) in a comparison of the X group with the B group. In this comparison, the most mature group (the X group) contained the greatest amount of pectic acid and pectates, and the most immature group (the B group) contained the least amount. This trend was more evident in the 1954 crop than in the 1952 crop.

There does not appear to be sufficient difference in the data on pentosans (table 33, p. 49) to make a significant grade distinction.

Total Reducing Sugars, Sucrose, Starch, and Polyphenols

The determination of total reducing sugars is one of the measures of grade appraisal most helpful to the tobacco technologist. The table of data on this determination (table 56, p. 72) shows consistent variations in content according to group and quality. In the same group and quality, quantity of sugar also varies consistently with color, the lighter colored grades almost invariably containing a somewhat higher amount of sugar.

The 1954 crop had a consistently higher sucrose content (table 47, p. 63) than the 1952 crop when corresponding groups were compared. The second quality of both crops was generally higher in sucrose than the corresponding fourth quality in the same group. The L grades were usually higher than the F grades in the same group and quality. The starch content (table 46, p. 62) from a grade standpoint did not appear to be significant.

The data on polyphenols (table 38, p. 54) did not show a significant relationship to grades. The reference to polyphenols is mentioned in this carbohydrate discussion because polyphenol data are calculated from data on the determinations of total reducing substances and total reducing sugars.

Total Nitrogen, Protein Nitrogen, Water-Soluble Nitrogen, Nitrogenous Fractions, and Alpha Amino Nitrogen

Differences in total nitrogen content in the 1952 and 1954 tobaccos of corresponding grades from the upper stalk position again demonstrated the second growth effect due to the later rainfall in the crop year (*14*). The upper stalk position grades of the 1952 crop were consistently lower in total nitrogen content than the corresponding grades of the 1954 crop. As shown in the tabulation of rainfall data (p. 4), the greatest amount of rain during the two growing seasons fell in the months of August and September of 1952.

The total nitrogen content (table 54, p. 70) was greatest in the darker colored grades of the same group and quality in both tobacco crops.

Protein nitrogen and water-soluble nitrogen content (tables 41 and 63, pp. 57 and 79) was not significantly different among the grades.

The nitrogenous fractions (tables 28 and 29, pp. 44 and 45) were determined on two grades, N1L and B4R, of the two crops. These two grades represent the approximate extremes of chemical composition for most of the other constituents determined. The definitions and methods of determination of the various nitrogenous fractions are described by Frankenburg and others (*15*), but they are too lengthy to be included in this bulletin.

The amount of alpha amino nitrogen (table 4, p. 19) was higher in the lower quality grades when grades of the same group and color in each of the two crops were compared. The 1954 crop, which received the lesser amount of rainfall, was consistently higher in alpha amino nitrogen than the 1952 crop in comparisons of the same grades.

Nicotine, Total Alkaloids (as Nicotine), and Total Volatile Bases (as Ammonia)

Content of nicotine, total alkaloids, and total volatile bases (tables 26, 27, 50, and 58, pp. 42, 43, 66, and 74) paralleled one another very closely in the variations with grade and, therefore, are discussed collectively. The 1954 crop consistently contained more of these components than the 1952 crop in grade comparisons.

The darker colored grades contained more of these constituents than the lighter colored grades of the same group and quality. Comparing the second and fourth qualities, with color acting as a constant factor, in almost all the groups the fourth quality contained a larger amount of these constituents than the second quality.

Plastid Pigments

Total Chlorophyll, Chlorophyll (a), and Chlorophyll (b)

From the top stalk position to the bottom stalk position, there is a general decrease in the amount of total chlorophyll in leaves of the plant. A similar conclusion was published by Weybrew (*30*). The total chlorophyll content (table 53, p. 69) of the Flue-cured grades from these comparative stalk positions differs further according to variations in color and quality.

The total chlorophyll content was generally higher in the darker colored grades of the same group and quality above the C Group in stalk position. In grades of the same group and color, the lower quality grades were consistently higher in total chlorophyll content than the higher quality grades.

The 1954 crop, which was produced during a comparatively dry season, had a consistently higher total chlorophyll content than the 1952 crop.

The ratio of chlorophyll (*a*) (table 13, p. 28) to total chlorophyll varied from approximately 0.7 to almost 1.0 in the series of samples analyzed. The distribution of chlorophyll (*a*) paralleled the distribution of total chlorophyll in the plant.

The chlorophyll (*b*) determination (table 14, p. 29) is less useful for grade differentiation than either the total chlorophyll or chlorophyll (*a*) values. This chlorophyll (*b*) determination is probably sufficient to account for the difference between the total chlorophyll and chlorophyll (*a*) within experimental error.

Total Carotenoid, Carotene, and Xanthophyll

Comparisons of the difference in amount of total carotenoid (table 52, p. 68) between grades showed that the amount was greater in the darker colored grades of the same group and quality.

In the same group and color, the total carotenoid content was generally greater in the fourth quality than in the second quality. The total content was also greater in the sixth quality than in the fourth quality, except for the B group, in which it was greater in the fourth quality than in the sixth quality in the F, R, and S colors.

The greatest variation in total carotenoid content was that displayed in comparisons of the 1954 and 1952 crops. The comparative amount was indicated by the ratio of the content of total carotenoid for the respective group averages of these two crops. The ratios of the 1954 amount to the 1952 amount for the groups were: X group, 2.3; C group, 2.7; H group, 2.5; and B group, 2.6. Therefore, the amount of total carotenoid in Flue-cured tobacco was in inverse proportion to the amount of rainfall during the crop year, the 1954 crop year being relatively dry as compared with the 1952 crop year.

The difference in the amount of carotene and xanthophyll (tables 10 and 65, pp. 25 and 81) between grades was less significant than the total

carotenoid content. However, the proportion of carotene to xanthophyll for each of the two crops was consistently different on a group comparison basis. The ratios of carotene to xanthophyll in the 1952 crop in the X, C, H, and B groups were respectively: 1.86, 1.73, 1.30, and 1.70. The ratios in the 1954 crop for the same groups were respectively: 0.96, 0.88, 0.90, and 0.98. Thus, the amount of rainfall apparently influenced not only the total carotenoid content but also the proportion of carotene to xanthophyll in Flue-cured tobacco.

Alcohol, Hot-water, and Petroleum Ether Extracts, Waxes, and Resins and Waxes

The purpose of using the 95 percent alcohol extraction was to determine whether this procedure would be a more direct method of ascertaining grade differences than some of the more involved procedures, or to what extent it could serve this purpose.

In both crops, grades of the same group and color showed a greater content of alcohol extract (table 1, p. 16) in the second quality than in the fourth quality, and a greater content in the fourth quality than in the sixth quality. In grades of the same group and quality, almost without exception, the L-colored grades had a higher content of alcohol extract than the F-colored grades, and the F-colored grades had more than the comparable R-colored grades.

The hot-water extraction was a preliminary step or procedure in the determination of tannin. The object of tabulating the data separately was to determine whether or not this procedure had any advantages over the 95 percent alcohol extraction for the determination of grade differences. Comparison of the two extraction procedures showed that, although the amount of hot-water extract (table 18, p. 33) was consistently higher in a grade comparison, each of the two determinations showed the same grade differences and was of practically equal value for this purpose.

The comparatively dry 1954 crop year produced tobacco which consistently had more petroleum ether extract than the 1952 crop. However, the difference in the content of this constituent between grades did not appear to show a significant relationship to grade in either crop.

The amount of both petroleum ether extract and waxes (tables 34 and 64, pp. 50 and 80) tends to decrease from the grades in the lower stalk position to the grades in the upper stalk position. Neither the content of waxes nor the amount of petroleum ether extract varied significantly with differences in grade characteristics.

The analytical procedure used for the determination of resins and waxes gives the total amount of both these constituents, whereas the petroleum ether extract and the waxes are reported separately. As in the case of petroleum ether extract and waxes, the amount of resins and waxes (table 43,

p. 59) was higher in the 1954 crop than in the 1952 crop. Also, no significant relationship to grade was apparent.

Tannin and Nontannin

The tannin content (table 49, p. 65) of the 1954 crop was only slightly higher than that of the 1952 crop. However, the content of the 1954 crop was somewhat more evenly distributed through the range of grades. The majority of comparisons in the same group and quality revealed that the darker colored grades contained more tannin than the lighter colored grades.

Grades of the same group and color had a nontannin content (table 30, p. 46) which was higher in the second quality than in the fourth quality, and higher in the fourth quality than in the sixth quality. In grades of the same group and quality, the lemon-colored grades were higher in nontannin content than the orange-colored grades, the orange-colored grades were higher than the red-colored grades, and the red-colored grades were higher than the mahogany grades.

pH

The hydrogen ion concentration (table 35, p. 51) in the overall range of grades increased gradually from the X group through the B group, with considerable variation between some grades within the same group. The variation of pH with quality in the same group and color was, for the majority of comparisons, probably not sufficiently consistent to establish a definite grade relationship. However, in the same group and quality, for both crops, the hydrogen ion concentration usually increased with an increase in the darkness of the color. The only exceptions were the B4L, B4F, B4R, and B4S grades of the 1952 crop.

Water-soluble Acids

The amount of water-soluble acids (table 60, p. 76) increased gradually from grades in the lower stalk position to grades in the higher stalk position. The relative amount of these components switched from a predominance in the lower stalk grades of the 1952 crop to a predominance in the upper stalk grades of the 1954 crop.

The water-soluble acids content followed the same relationship to grade as the hydrogen ion concentration with respect to color. As a rule, the darker the color, the greater the quantity of these acids.

Uronic Acids (as Anhydrides)

Uronic acids content (table 59, p. 75) was, in most instances, in inverse proportion to quality. Eighty percent or more of the comparisons of qualities in the same group and color showed that the uronic acids content was

greater in the fourth quality than in the second quality, and greater in the sixth quality than in the fourth quality.

The relationship of uronic acids content to color showed the most consistency in the B group of grades in which uronic acids were proportional to the darkness of color in the grades of the same quality.

Moisture Equilibrium

The variations in moisture content under different conditions of relative humidity were studied (table 25, p. 40 and figures 2, 3, 4, and 5). The greatest differences in moisture content among the grades were found among the different conditions of relative humidity.

Some differences in moisture content were consistent with differences in grade. When differences in moisture content were compared with differences in quality, in grades of the same group and color, the second quality was found to contain more moisture than the fourth quality in 28 of the 40 comparable instances, or 70 percent. The fourth quality was found to contain more moisture than the sixth quality in 27 of the 32 comparable instances, or 84 percent. These comparisons of differences in quality include both crop years under all four conditions of relative humidity.

In the same group and quality of both crops, the L-colored grades were more hygroscopic than the corresponding F-colored grades in seven of the eight comparisons. The F-colored grades were likewise more hygroscopic than the corresponding R-colored grades in the same group and quality in six of the eight comparisons.

Differences between the two crops in the same group and in grades of the same quality and color, showed the 1954 crop to be consistently more hygroscopic than the 1952 crop, except in the B group. The B6F and B6R grades in the 1952 crop showed considerably higher moisture content than the same grades in the 1954 crop. This difference in hygroscopic property coincided with the late rainfall difference (August and September of the two crop years as shown under Rainfall Data, p. 3), and was evidently an effect of the rainfall difference.

Insignificant Components

From a grade differentiation standpoint, some of the components which were determined in this series of analyses proved to be more significant than others. Those which appear to show a comparatively insignificant relationship to grade are: Boron (table 6, p. 24), chlorine (table 12, p. 27), formic acid (table 17, p. 32), iron (table 19, p. 34), nornicotine (table 31, p. 47), phosphorus (tables 36 and 37, pp. 52 and 53), sodium (tables 44 and 45, pp. 60 and 61), sulfur (table 48, p. 64), and total volatile acids (table 57, p. 73). The data on these components, as on the rest of the components, are shown alphabetically.

553471—60——2

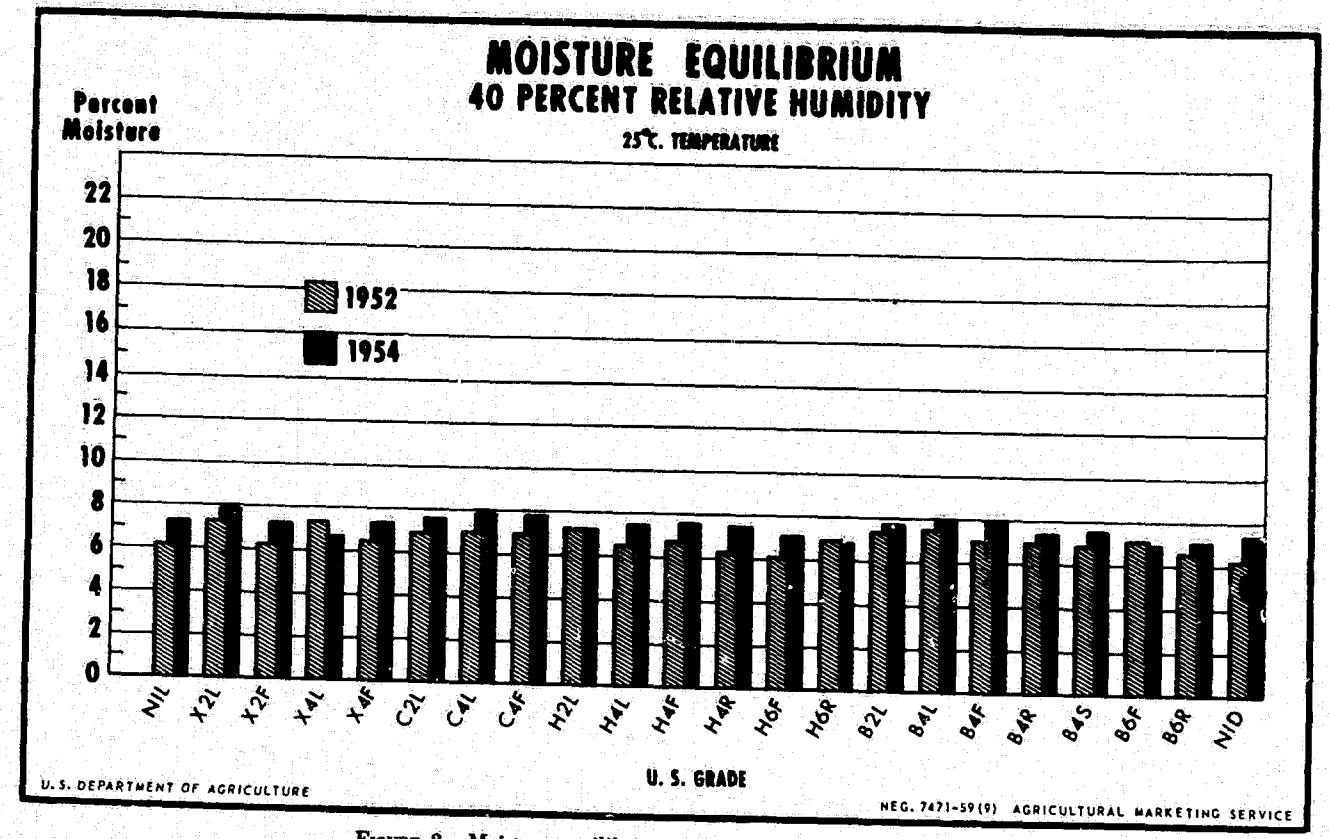

FIGURE 2.—Moisture equilibrium at 40 percent relative humidity.

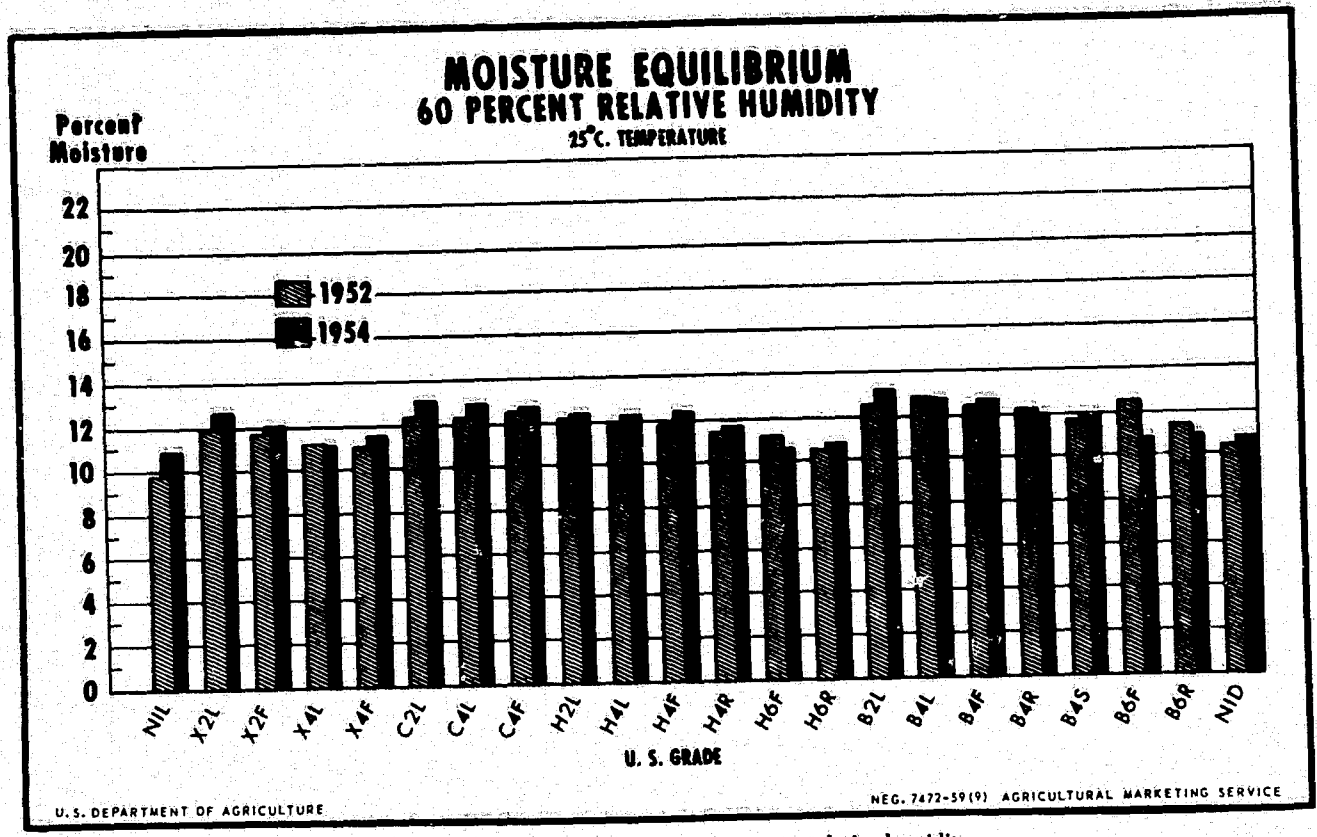

FIGURE 3.—Moisture equilibrium at 60 percent relative humidity.

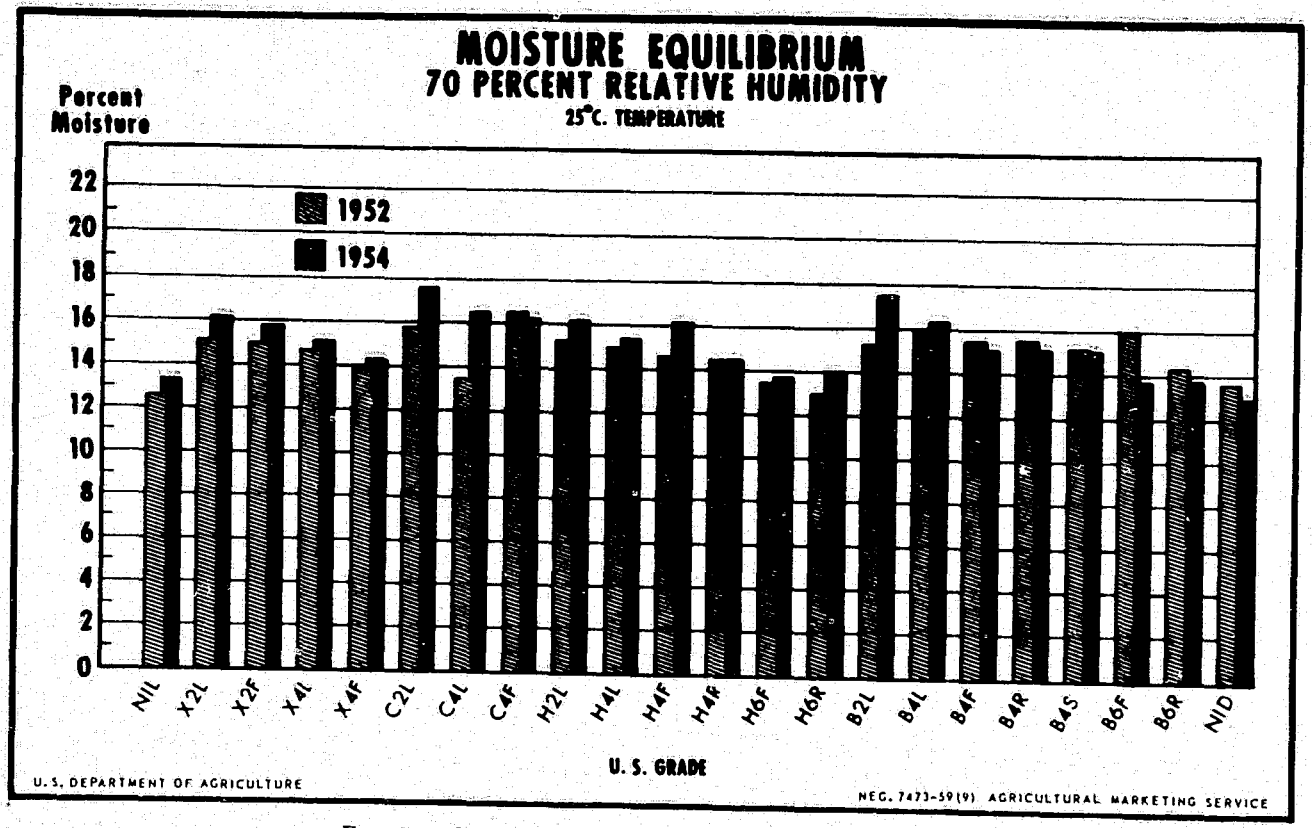

FIGURE 4.—Moisture equilibrium at 70 percent relative humidity.

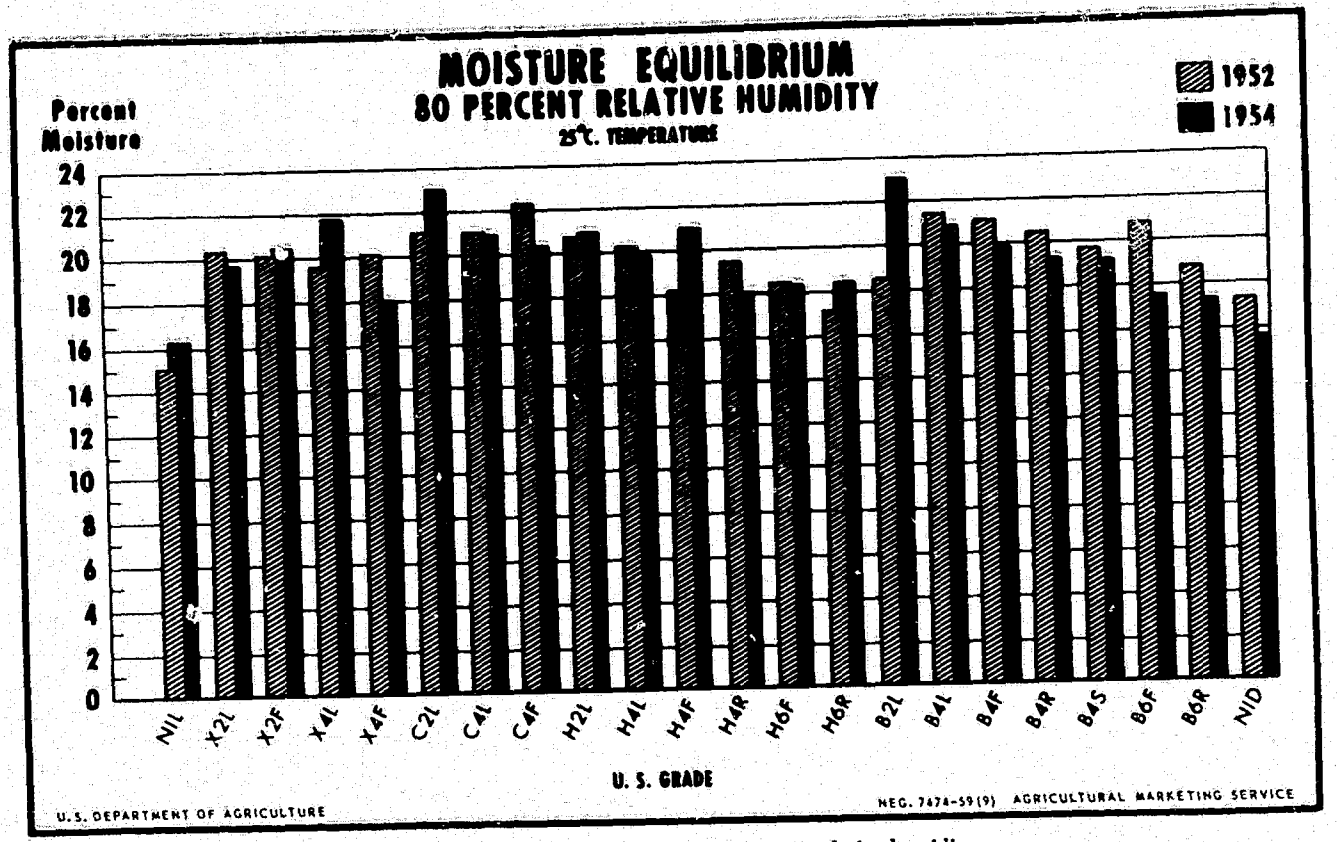

FIGURE 5.—Moisture equilibrium at 80 percent relative humidity.

DETERMINATIONS AND DATA

Table 1.—Alcohol Extract (Method 1)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	38. 91	37. 29	1. 62
Lugs	X2L	55. 53	58. 03	2. 50
	X2F	54. 84	55. 56	. 72
	X4L	48. 77	48. 89	. 12
	X4F	48. 43	47. 96	. 47
Average		51. 89	52. 61	. 72
Cutters	C2L	61. 62	59. 13	2. 49
	C2F		60. 08	
	C4L	59. 47	59. 08	. 39
	C4F	59. 28	59. 59	. 31
Average		60. 12	59. 27	. 85
Smoking Leaf	H2L	58. 60	57. 99	. 61
	H4L	56. 88	57. 70	. 82
	H4F	55. 95	53. 90	2. 05
	H4R	52. 18	52. 45	. 27
	H6F	49. 68	50. 14	. 46
	H6R	45. 25	46. 51	1. 26
Average		53. 09	53. 12	. 03
Leaf	B2L	60. 51	62. 27	1. 76
	B2F		62. 39	
	B2R	57. 49		
	B4L	60. 49	60. 25	. 24
	B4F	58. 70	58. 69	. 01
	B4R	55. 02	53. 98	1. 04
	B4S	51. 96	53. 38	1. 42
	B6F	55. 66	53. 86	1. 80
	B6R	49. 93	50. 31	. 38
	B6S	46. 20		
Average		56. 04	56. 10	. 06
Nondescript	N1D	39. 52	42. 06	2. 54

Analysis of data	1952 crop	1954 crop
High	61. 62	62. 39
Low	38. 91	37. 29
Range	22. 71	25. 10
Average	53. 51	53. 59
High/Low ratio	1. 58	1. 67
Coefficient of variation (percent)	8. 6	8. 2
Standard deviation	4. 61	4. 40

Table 2.—Alkalinity of Water-Insoluble Ash (Method 32)

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

Group	U.S. grade	Milliliters		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	21.4	28.4	7.0
Lugs	X2L	19.4	17.1	2.3
	X2F	18.3	17.0	1.3
	X4L	38.6	21.9	16.7
	X4F	36.8	20.5	16.3
Average		28.3	19.1	9.2
Cutters	C2L	43.1	13.2	29.9
	C2F		13.1	
	C4L	22.2	15.0	7.2
	C4F	24.6	14.6	10.0
Average		30.0	14.3	15.7
Smoking Leaf	H2L	27.1	14.4	12.7
	H2L	33.0	14.3	18.7
	H4F	31.8	14.6	17.2
	H4R	27.6	14.3	13.3
	H6F	32.0	15.4	16.6
	H6R	30.8	16.6	14.2
Average		30.4	14.9	15.5
Leaf	C2L	22.2	12.4	9.8
	B2F		12.5	
	B2R	27.2		
	B4L	22.2	12.1	10.1
	B4F	24.7	13.5	11.2
	B4R	13.8	14.0	.2
	B4S	11.2	12.3	1.1
	B6F	14.4	12.4	2.0
	B6R	19.4	13.7	5.7
	B6S	26.2		
Average		18.3	12.9	5.4
Nondescript	N1D	33.9	16.2	17.7

Analysis of data	1952 crop	1954 crop
High	43.1	28.4
Low	11.2	12.1
Range	31.9	16.3
Average	25.8	15.6
High/low ratio	3.8	2.3
Coefficient of variation (percent)	32.6	16.7
Standard deviation	8.4	2.6

Table 3.—Alkalinity of Water-Soluble Ash (Method 32)

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

Group	U.S. grade	Milliliters		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.5	1.6	0.9
Lugs	X2L	.7	1.4	.7
	X2F	1.2	1.5	.3
	X4L	2.3	1.3	1.0
	X4F	2.2	1.6	.6
Average		1.6	1.4	.2
Cutters	C2L	.6	1.8	1.2
	C2F		1.5	
	C4L	1.7	1.3	.4
	C4F	1.2	1.4	.2
Average		1.2	1.5	.3
Smoking Leaf	H2L	3.0	1.8	1.2
	H4L	2.5	1.7	.8
	H4F	.7	1.7	1.0
	H4R	3.1	1.3	1.8
	H6F	4.3	1.4	2.9
	H6R	4.4	1.3	3.1
Average		3.0	1.5	1.5
Leaf	B2L	2.4	1.3	1.1
	B2F		1.3	
	B2R	2.7		
	B4L	2.2	1.3	.9
	B4F	1.9	1.2	.7
	B4R	1.2	1.0	.2
	B4S	1.0	1.0	
	B6F	1.4	.8	.6
	B6R	1.4	.8	.6
	B6S	1.0		
Average		1.6	1.1	.5
Nondescript	N1D	1.1	.7	.4

Analysis of data	1952 crop	1954 crop
High	4.4	1.8
Low	.6	.7
Range	3.8	1.1
Average	2.0	1.3
High/low ratio	7.3	2.6
Coefficient of variation (percent)	55.0	23.1
Standard deviation	1.1	.3

Table 4.—Alpha Amino Nitrogen (Method 2)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0.328	0.340	0.012
Lugs	X2L	.168	.174	.006
	X2F	.166	.173	.007
	X4L	.216	.253	.037
	X4F	.211	.253	.042
Average		.190	.213	.023
Cutters	C2L	.096	.142	.046
	C2F		.136	
	C4L	.121	.165	.044
	C4F	.108	.151	.043
Average		.108	.153	.045
Smoking Leaf	H2L	.121	.155	.034
	H4L	.155	.205	.050
	H4F	.165	.199	.034
	H4R	.207	.283	.076
	H6F	.246	.261	.015
	H6R	.292	.364	.072
Average		.198	.244	.046
Leaf	B2L	.107	.127	.020
	B2F		.133	
	B2R	.198		
	B4L	.130	.160	.030
	B4F	.159	.188	.029
	B4R	.210	.253	.043
	B4S	.241	.276	.035
	B6F	.193	.244	.051
	B6R	.284	.313	.029
	B6S	.331		
Average		.189	.223	.034
Nondescript	N1D	.328	.367	.039

Analysis of data	1952 crop	1954 crop
High	0.331	0.367
Low	.096	.127
Range	.235	.240
Average	.193	.229
High/low ratio	3.45	2.89
Coefficient of variation (percent)	29.5	27.1
Standard deviation	.057	.062

Table 5.—Aluminum (Method 28)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Parts per million		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	>1,071	>1,090	19
Lugs	X2L	>996	>984	12
	X2F	>1,015	>1,067	52
	X4L	>1,050	>1,081	31
	X4F	>1,068	>1,078	10
Average		>1,032	>1,052	20
Cutters	C2L	334	673	339
	C2F		705	
	C4L	516	>998	482
	C4F	737	>981	244
Average		529	884	355
Smoking Leaf	H2L	415	533	118
	H4L	687	706	19
	H4F	551	802	251
	H4R	471	507	36
	H6F	439	763	324
	H6R	363	669	306
Average		488	663	175
Leaf	B2L	252	381	129
	B2F		395	
	B2R	197		
	B4L	317	343	26
	B4F	464	458	6
	B4R	235	401	166
	B4S	237	436	199
	B6F	292	542	250
	B6R	239	589	350
	B6S	306		
Average		291	450	159
Nondescript	N1D	404	720	316

Analysis of data	1952 crop	1954 crop
High	>1,071	>1,090
Low	197	343
Range	874	747
Average	552	718
High/low ratio	5.44	3.18
Coefficient of variation (percent)	53.6	35.0
Standard deviation	296	251

> Amount present is greater than amount shown; analysis exceeded the calibration range.

Table 6.—Boron (Method 28)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Parts per million		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	42	48	6
Lugs	X2L	34	34	0
	X2F	34	34	0
	X4L	31	38	7
	X4F	39	42	3
Average		34	37	3
Cutters	C2L	25	25	0
	C2F		34	
	C4L	26	37	11
	C4F	32	34	2
Average		28	32	4
Smoking Leaf	H2L	33	35	2
	H4L	52	29	23
	H4F	29	29	0
	H4R	33	46	13
	H6F	30	39	9
	H6R	35	54	19
Average		35	39	4
Leaf	B2L	28	25	3
	B2F		34	
	B2R	29		
	B4L	26	29	3
	B4F	29	40	11
	B4R	33	60	27
	B4S	36	49	13
	B6F	29	46	17
	B6R	38	59	21
	B6S	35		
Average		31	44	13
Nondescript	N1D	43	49	6

Analysis of data	1952 crop	1954 crop
High	52	60
Low	25	25
Range	27	35
Average	34	40
High/low ratio	2.1	2.4
Coefficient of variation (percent)	17.6	25.0
Standard deviation	6	10

Table 7—Calcium (Method 3)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	3.60	4.16	0.56
Lugs	X2L	2.69	2.51	.18
	X2F	2.51	2.65	.14
	X4L	3.06	3.50	.44
	X4F	3.05	3.16	.11
Average		2.83	2.96	.13
Cutters	C2L	1.87	1.95	.08
	C2F		2.14	
	C4L	2.21	2.30	.09
	C4F	2.18	2.25	.07
Average		2.09	2.17	.08
Smoking Leaf	H2L	2.34	2.12	.22
	H4L	2.46	2.25	.21
	H4F	2.48	2.41	.07
	H4R	2.26	2.34	.08
	H6F	2.28	2.53	.25
	H6R	2.19	2.73	.54
Average		2.34	2.40	.06
Leaf	B2L	1.68	1.69	.01
	B2F		1.76	
	B2R	2.19		
	B4L	1.67	1.82	.15
	B4F	1.93	2.17	.24
	B4R	2.03	2.17	.14
	B4S	2.00	1.88	.12
	B6F	1.76	2.03	.27
	B6R	2.18	2.33	.15
	B6S	2.10		
Average		1.89	2.01	.12
Nondescript	N1D	2.79	2.63	.16

Analysis of data	1952 crop	1954 crop
High	3.60	4.16
Low	1.67	1.69
Range	1.93	2.47
Average	2.33	2.44
High/low ratio	2.16	2.46
Coefficient of variation (percent)	20.3	23.0
Standard deviation	.47	.56

Table 8.—Calcium (Method 4)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	3. 87	4. 35	0. 48
Lugs	X2L	2. 72	2. 66	. 06
	X2F	2. 76	2. 76	0
	X4L	3. 38	3. 52	. 14
	X4F	3. 23	3. 39	. 16
Average		3. 02	3. 08	. 06
Cutters	C2L	1. 90	2. 12	. 22
	C2F		2. 22	
	C4L	2. 11	2. 43	. 32
	C4F	2. 25	2. 37	. 12
Average		2. 09	2. 31	. 22
Smoking Leaf	H2L	2. 27	2. 26	. 01
	H4L	2. 39	2. 34	. 05
	H4F	2. 39	2. 49	. 10
	H4R	2. 28	2. 38	. 10
	H6F	2. 35	2. 55	. 20
	H6R	2. 29	2. 73	. 44
Average		2. 33	2. 46	. 13
Leaf	B2L	1. 75	1. 80	. 05
	B2F		1. 99	
	B2R	2. 19		
	B4L	1. 68	1. 92	. 24
	B4F	1. 95	2. 24	. 29
	B4R	2. 07	2. 31	. 24
	B4S	2. 10	2. 01	. 09
	B6F	1. 76	2. 17	. 41
	B6R	2. 11	2. 36	. 25
	B6S	2. 15		
Average		1. 92	2. 12	. 20
Nondescript	N1D	2. 82	2. 75	. 07

Analysis of data	1952 crop	1954 crop
High	3. 87	4. 35
Low	1. 68	1. 80
Range	2. 19	2. 55
Average	2. 38	2. 54
High/low ratio	2. 30	2. 42
Coefficient of variation (percent)	16. 4	17. 3
Standard deviation	. 39	. 44

Table 9.—Calcium (Method 5)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	4.40	2.58	1.82
Lugs	X2L	3.18	2.54	.64
	X2F	3.36	2.66	.70
	X4L	3.72	2.84	.88
	X4F	3.72	2.84	.88
Average		3.50	2.72	.78
Cutters	C2L	2.38	2.28	.10
	C2F		2.44	
	C4L	2.54	2.38	.16
	C4F	2.81	2.70	.11
Average		2.58	2.45	.13
Smoking Leaf	H2L	2.30	2.52	.22
	H4L	2.80	2.65	.15
	H4F	3.02	2.67	.35
	H4R	2.65	2.53	.12
	H6F	3.30	2.77	.53
	H6R	2.78	2.44	.34
Average		2.81	2.60	.21
Leaf	B2L	2.27	2.48	.21
	B2F		2.31	
	B2R	2.73		
	B4L	2.23	2.04	.19
	B4F	2.40	2.51	.11
	B4R	2.58	2.42	.16
	B4S	2.40	2.06	.34
	B6F	2.19	2.41	.22
	B6R	2.73	2.58	.15
	B6S	2.53		
Average		2.40	2.36	.04
Nondescript	N1D	3.52	2.77	.75

Analysis of data	1952 crop	1954 crop
High	4.40	2.84
Low	2.19	2.04
Range	2.21	.80
Average	2.88	2.53
High/low ratio	2.01	1.39
Coefficient of variation (percent)	20.0	8.3
Standard deviation	.58	.21

Table 10.—Carotene (Method 22)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Micrograms per gram		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	23	69	46
Lugs	X2L	26	47	21
	X2F	26	40	14
	X4L	31	48	17
	X4F	30	55	25
Average		28	48	20
Cutters	C2L	22	50	28
	C2F		48	
	C4L	29	49	20
	C4F	26	48	22
Average		26	49	23
Smoking Leaf	H2L	19	47	28
	H4L	29	52	23
	H4F	33	66	33
	H4R	35	70	35
	H6F	32	59	27
	H6R	34	72	38
Average		30	61	31
Leaf	B2L	27	47	20
	B2F		51	
	B2R	43		
	B4L	26	51	25
	B4F	27	52	25
	B4R	33	65	32
	B4S	33	63	30
	B6F	26	51	25
	B6R	31	62	31
	B6S	28		
Average		29	56	27
Nondescript	N1D	32	65	33

Analysis of data	1952 crop	1954 crop
High	43	72
Low	19	40
Range	24	32
Average	29	56
High/low ratio	2. 26	1. 80
Coefficient of variation (percent)	14. 0	16. 0
Standard deviation	4. 1	8. 8

Table 11.—Cellulose (Method 6)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	10.74	9.58	1.16
Lugs	X2L	8.65	7.20	1.45
	X2F	8.55	6.90	1.65
	X4L	9.31	7.70	1.61
	X4F	9.55	7.43	2.12
Average		9.02	7.31	1.71
Cutters	C2L	7.82	7.29	.53
	C2F		6.49	
	C4L	8.32	7.11	1.21
	C4F	8.22	7.13	1.09
Average		8.12	7.18	.94
Smoking Leaf	H2L	8.39	7.94	.45
	H4L	8.70	7.55	1.15
	H4F	8.38	7.70	.68
	H4R	8.75	7.40	1.35
	H6F	9.11	8.40	.71
	H6R	8.96	7.74	1.22
Average		8.72	7.79	.93
Leaf	B2L	7.21	6.24	.97
	B2F		4.79	
	B2R	7.35		
	B4L	7.53	5.74	1.79
	B4F	7.27	5.77	1.50
	B4R	7.81	5.79	2.02
	B4S	7.35	6.28	1.07
	B6F	7.41	7.03	.38
	B6R	8.47	6.78	1.69
	B6S	8.11		
Average		7.58	6.23	1.35
Nondescript	N1D	9.52	7.81	1.71

Analysis of data	1952 crop	1954 crop
High	10.74	9.58
Low	7.21	4.79
Range	3.53	4.79
Average	8.46	7.20
High/low ratio	1.50	2.00
Coefficient of variation (percent)	8.0	10.1
Standard deviation	.68	.73

Table 12.—Chlorine (Method 7)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	1.73	1.45	0.28
Lugs	X2L	1.19	.85	.34
	X2F	1.26	.98	.28
	X4L	1.38	1.13	.25
	X4F	1.60	1.27	.33
Average		1.36	1.06	.30
Cutters	C2L	1.06	.79	.27
	C2F		.86	
	C4L	1.09	.88	.21
	C4F	1.29	.88	.41
Average		1.15	.85	.30
Smoking Leaf	H2L	.99	.82	.17
	H4L	1.06	.75	.31
	H4F	1.04	.84	.20
	H4R	.98	.86	.12
	H6F	1.08	.87	.21
	H6R	1.06	.96	.10
Average		1.04	.85	.19
Leaf	B2L	.82	.64	.18
	B2F		.68	
	B2R	.95		
	B4L	.81	.64	.17
	B4F	.88	.73	.15
	B4R	.99	.84	.15
	B4S	.90	1.14	.24
	B6F	.85	.96	.11
	B6R	.98	1.12	.14
	B6S	.89		
Average		.89	.87	.02
Nondescript	N1D	1.04	.98	.06

Analysis of data	1952 crop	1954 crop
High	1.73	1.45
Low	.81	.64
Range	.92	.81
Average	1.09	.93
High/low ratio	2.14	2.26
Coefficient of variation (percent)	17.4	17.2
Standard deviation	.19	.16

Table 13.—Chlorophyll (a) (Method 22)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Micrograms per gram		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	56	76	20
Lugs	X2L	38	45	7
	X2F	40	37	3
	X4L	45	56	11
	X4F	47	59	12
Average		42	49	7
Cutters	C2L	31	38	7
	C2F		40	
	C4L	38	36	2
	C4F	38	42	4
Average		36	39	3
Smoking Leaf	H2L	24	33	9
	H4L	36	39	3
	H4F	40	47	7
	H4R	39	48	9
	H6F	38	44	6
	H6R	42	52	10
Average		36	44	8
Leaf	B2L	43	39	4
	B2F		49	
	B2R	71		
	B4L	37	45	8
	B4F	43	49	6
	B4R	58	65	7
	B4S	65	77	12
	B6F	42	46	4
	B6R	48	56	8
	B6S	59		
Average		48	54	6
Nondescript	N1D	59	60	1

Analysis of data	1952 crop	1954 crop
High	65	77
Low	24	33
Range	41	44
Average	43	50
High/low ratio	2.7	2.3
Coefficient of variation (percent)	20.0	21.2
Standard deviation	8.6	10.6

Table 14.—Chlorophyll (b) (Method 22)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Micrograms per gram		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	11	24	13
Lugs	X2L	11	14	3
	X2F	12	17	5
	X4L	16	16	0
	X4F	13	17	4
Average		13	16	3
Cutters	C2L	11	13	2
	C2F		11	
	C4L	15	16	1
	C4F	11	12	1
Average		12	14	2
Smoking Leaf	H2L	8	9	1
	H4L	14	15	1
	H4F	13	22	9
	H4R	18	21	3
	H6F	13	15	2
	H6R	17	24	7
Average		14	18	4
Leaf	B2L	14	16	2
	B2F		14	
	B2R	18		
	B4L	12	10	2
	B4F	10	16	6
	B4R	10	18	8
	B4S	10	15	5
	B6F	11	15	4
	B6R	16	20	4
	B6S	11		
Average		12	16	4
Nondescript	N1D	18	24	6

Analysis of data	1952 crop	1954 crop
High	18	24
Low	8	9
Range	10	15
Average	13	17
High/low ratio	2.2	2.7
Coefficient of variation (percent)	20.0	20.0
Standard deviation	2.6	3.4

Table 15.—Copper (Method 28)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Parts per million		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	6.4	6.9	0.5
Lugs	X2L	4.7	4.2	.5
	X2F	6.5	5.0	1.5
	X4L	6.9	4.7	2.2
	X4F	7.2	6.2	1.0
Average		6.4	5.0	1.4
Cutters	C2L	3.6	3.0	.6
	C2F		8.8	
	C4L	3.5	3.4	.1
	C4F	4.8	5.2	.4
Average		4.0	3.9	.1
Smoking Leaf	H2L	4.2	6.3	2.1
	H4L	4.9	6.7	1.8
	H4F	5.3	7.4	2.1
	H4R	10.2	10.5	.3
	H6F	8.7	11.6	2.9
	H6R	22.6	14.4	8.2
Average		9.3	9.5	.2
Leaf	B2L	4.3	3.9	.4
	B2F		5.0	
	B2R	11.0		
	B4L	6.2	7.6	1.4
	B4F	8.6	7.9	.7
	B4R	15.6	17.3	1.7
	B4S	31.2	23.8	7.4
	B6F	20.5	27.1	6.6
	B6R	45.3	25.3	20.0
	B6S	44.7		
Average		18.8	16.1	2.7
Nondescript	N1D	34.0	35.6	1.6

Analysis of data	1952 crop	1954 crop
High	45.3	35.6
Low	3.5	3.0
Range	41.8	32.6
Average	12	11
High/low ratio	15.1	11.9
Coefficient of variation (percent)	94.2	80.9
Standard deviation	11.3	8.9

Table 16.—Crude Fiber (Method 8)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	9.24	10.50	1.26
Lugs	X2L	7.59	7.43	.16
	X2F	7.12	7.50	.38
	X4L	7.95	8.54	.59
	X4F	8.35	8.23	.12
Average		7.75	7.92	.17
Cutters	C2L	7.11	7.31	.20
	C2F		6.85	
	C4L	7.34	7.46	.12
	C4F	7.09	7.32	.23
Average		7.18	7.36	.18
Smoking Leaf	H2L	7.54	8.33	.79
	H4L	7.72	8.33	.61
	H4F	7.48	8.29	.81
	H4R	7.51	8.10	.59
	H6F	8.42	8.69	.27
	H6R	8.44	9.11	.67
Average		7.85	8.48	.63
Leaf	B2L	6.09	6.40	.31
	B2F		6.33	
	B2R	6.17		
	B4L	6.18	6.36	.18
	B4F	6.55	7.55	1.00
	B4R	6.62	7.12	.50
	B4S	6.55	6.74	.19
	B6F	6.59	7.51	.92
	B6R	7.25	8.12	.87
	B6S	7.13		
Average		6.55	7.11	.56
Nondescript	N1D	8.32	9.52	1.20

Analysis of data	1952 crop	1954 crop
High	9.24	10.50
Low	6.09	6.33
Range	3.15	4.17
Average	7.41	7.93
High/low ratio	1.52	1.66
Coefficient of variation (percent)	9.2	9.2
Standard deviation	.68	.73

Table 17.—Formic Acid (Method 9)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0. 047	0. 049	0. 002
Lugs	X2L	. 017	. 026	. 009
	X2F	. 031	. 024	. 007
	X4L	. 037	. 012	. 025
	X4F	. 099	. 012	. 087
Average		. 046	. 018	. 028
Cutters	C2L	. 038	. 030	. 008
	C2F		. 023	
	C4L	. 070	. 014	. 056
	C4F	. 056	. 020	. 036
Average		. 055	. 021	. 034
Smoking Leaf	H2L	. 048	. 014	. 034
	H4L	. 044	. 022	. 022
	H4F	. 051	. 032	. 019
	H4R	. 060	. 024	. 036
	H6F	. 037	. 036	. 001
	H6R	. 032	. 036	. 004
Average		. 045	. 027	. 018
Leaf	B2L	. 044	. 035	. 009
	B2F		. 027	
	B2R	. 076		
	B4L	. 030	. 043	. 013
	B4F	. 038	. 027	. 011
	B4R	. 012	. 051	. 039
	B4S	. 036	. 039	. 003
	B6F	. 023	. 041	. 018
	B6R	. 036	. 016	. 020
	B6S	. 064		
Average		. 031	. 036	. 005
Nondescript	N1D	. 073	. 025	. 048

Analysis of data	1952 crop	1954 crop
High	0. 10	0. 05
Low	. 01	. 01
Range	. 09	. 04
Average	. 044	. 029
High/low ratio	8. 2	4. 2
Coefficient of variation (percent)	45. 0	34. 0
Standard deviation	. 02	. 01

Table 18.—Hot-Water Extract (Method 10)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	47.01	46.65	0.36
Lugs	X2L	56.79	60.62	3.83
	X2F	57.45	58.61	1.16
	X4L	54.31	55.34	1.03
	X4F	52.37	53.55	1.18
Average		55.23	57.03	1.80
Cutters	C2L	62.43	61.83	.60
	C2F	----------	62.28	----------
	C4L	60.09	61.13	1.04
	C4F	59.08	59.88	.80
Average		60.53	60.95	.42
Smoking Leaf	H2L	59.01	58.51	.50
	H4L	56.78	57.99	1.21
	H4F	55.83	56.72	.89
	H4R	54.92	51.82	3.10
	H6F	52.24	50.40	1.84
	H6R	49.69	46.92	2.77
Average		54.74	53.73	.01
Leaf	B2L	60.97	64.98	4.01
	B2F	----------	63.18	----------
	B2R	58.51	----------	----------
	B4L	62.99	60.61	2.38
	B4F	60.22	60.78	.56
	B4R	59.38	53.01	6.37
	B4S	56.70	53.44	3.26
	B6F	60.64	52.58	8.06
	B6R	53.72	49.11	4.61
	B6S	52.56	----------	----------
Average		59.23	56.36	2.87
Nondescript	N1D	48.20	43.96	4.24

Analysis of data	1952 crop	1954 crop
High	62.99	64.98
Low	47.01	43.96
Range	15.98	21.02
Average	56.40	55.38
High/low ratio	1.34	1.48
Coefficient of variation (percent)	6.3	8.6
Standard deviation	3.55	4.74

Table 19.—Iron (Method 23)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Parts per million		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	595	>1,013	418
Lugs	X2L	453	491	38
	X2F	464	630	166
	X4L	509	635	126
	X4F	569	815	246
Average		499	643	144
Cutters	C2L	141	245	104
	C2F		248	
	C4L	195	402	207
	C4F	287	418	131
Average		208	355	147
Smoking Leaf	H2L	170	231	61
	H4L	276	251	25
	H4F	261	285	24
	H4R	216	248	32
	H6F	208	357	149
	H6R	247	344	97
Average		230	286	56
Leaf	B2L	132	153	21
	B2F		189	
	B2R	135		
	B4L	146	166	20
	B4F	190	197	7
	B4R	153	239	86
	B4S	136	264	128
	B6F	191	258	67
	B6R	160	255	95
	B6S	191		
Average		158	219	61
Nondescript	N1D	260	335	75

Analysis of data	1952 crop	1954 crop
High	595	>1,013
Low	132	153
Range	463	860
Average	271	374
High/low ratio	4.5	6.6
Coefficient of variation (percent)	53.1	32.4
Standard deviation	144	121

>Amount present is greater than amount shown; analysis exceeded the calibration range.

Table 20.—Lignin (Method 11)

[All results calculated on an ash-free and crude-protein-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	3.05	3.10	0.05
Lugs	X2L	1.63	1.45	.18
	X2F	1.67	1.80	.13
	X4L	2.03	1.83	.20
	X4F	2.12	1.94	.18
Average		1.86	1.76	.10
Cutters	C2L	1.36	1.61	.25
	C2F		1.93	
	C4L	1.55	1.61	.06
	C4F	1.60	1.41	.19
Average		1.50	1.54	.04
Smoking Leaf	H2L	1.62	2.63	1.01
	H4L	1.86	2.46	.60
	H4F	1.88	2.05	.17
	H4R	2.26	3.08	.82
	H6F	2.62	3.24	.62
	H6R	3.11	3.12	.01
Average		2.22	2.76	.54
Leaf	B2L	1.54	1.40	.14
	B2F		1.44	
	B2R	1.64		
	B4L	1.65	1.55	.10
	B4F	1.66	1.49	.17
	B4R	1.95	1.77	.18
	B4S	1.94	1.70	.24
	B6F	1.95	2.10	.15
	B6R	2.35	2.56	.21
	B6S	2.54		
Average		1.86	1.80	.06
Nondescript	N1D	3.50	3.19	.31

Analysis of data	1952 crop	1954 crop
High	3.50	3.19
Low	1.36	1.40
Range	2.14	1.79
Average	2.04	2.14
High/low ratio	2.57	2.28
Coefficient of variation (percent)	20.0	27.6
Standard deviation	.41	.59

Table 21.—Magnesium (Method 5)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0.745	0.558	0.187
Lugs	X2L	.519	.358	.161
	X2F	.559	.393	.166
	X4L	.607	.467	.140
	X4F	.506	.471	.035
Average		.548	.422	.126
Cutters	C2L	.368	.317	.051
	C2F		.329	
	C4L	.343	.336	.007
	C4F	.436	.331	.105
Average		.382	.328	.054
Smoking Leaf	H2L	.419	.333	.086
	H4L	.426	.346	.080
	H4F	.468	.327	.141
	H4R	.463	.330	.133
	H6F	.442	.353	.089
	H6R	.436	.420	.016
Average		.442	.352	.090
Leaf	B2L	.345	.288	.057
	B2F		.295	
	B2R	.426		
	B4L	.277	.305	.028
	B4F	.354	.286	.068
	B4R	.382	.346	.036
	B4S	.405	.336	.069
	B6F	.336	.329	.007
	B6R	.419	.374	.045
	B6S	.435		
Average		.360	.323	.037
Nondescript	N1D	.532	.411	.121

Analysis of data	1952 crop	1954 crop
High	0.745	0.558
Low	.277	.286
Range	.468	.272
Average	.445	.364
High/low ratio	2.69	1.95
Coefficient of variation (percent)	23.4	18.1
Standard deviation	.104	.066

Table 22.—Manganese (Method 12)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0. 058	0. 070	0. 012
Lugs	X2L	. 025	. 023	. 002
	X2F	. 045	. 029	. 016
	X4L	. 039	. 031	. 008
	X4F	. 058	. 030	. 028
Average		. 042	. 028	. 014
Cutters	C2L	. 025	. 019	. 006
	C2F		. 022	
	C4L	. 029	. 022	. 007
	C4F	. 040	. 026	. 014
Average		. 031	. 022	. 009
Smoking Leaf	H2L	. 025	. 020	. 005
	H4L	. 028	. 025	. 003
	H4F	. 034	. 033	. 001
	H4R	. 042	. 032	. 010
	H6F	. 038	. 028	. 010
	H6R	. 049	. 037	. 012
Average		. 036	. 029	. 007
Leaf	B2L	. 020	. 014	. 006
	B2F		. 020	
	B2R	. 030		
	B4L	. 020	. 019	. 001
	B4F	. 029	. 023	. 006
	B4R	. 042	. 022	. 020
	B4S	. 018	. 041	. 023
	B6F	. 023	. 028	. 005
	B6R	. 039	. 025	. 014
	B6S	. 048		
Average		. 027	. 025	. 002
Nondescript	N1D	. 049	. 068	. 019

Analysis of data	1952 crop	1954 crop
High	0. 058	0. 070
Low	. 018	. 014
Range	. 040	. 056
Average	. 035	. 030
High/low ratio	3. 22	5. 00
Coefficient of variation (percent)	28. 6	20. 0
Standard deviation	. 010	. 006

Table 23.—Manganese (Method 28)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Parts per million		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	466	559	93
Lugs	X2L	344	260	84
	X2F	457	288	169
	X4L	408	289	119
	X4F	469	362	107
Average		420	300	120
Cutters	C2L	257	196	61
	C2F	----	283	----
	C4L	249	255	6
	C4F	429	273	156
Average		312	241	71
Smoking Leaf	H2L	252	237	15
	H4L	399	319	80
	H4F	328	351	23
	H4R	207	304	97
	H6F	386	337	49
	H6R	389	372	17
Average		327	320	7
Leaf	B2L	182	145	37
	B2F	----	211	----
	B2R	340	----	----
	B4L	219	188	31
	B4F	286	261	25
	B4R	334	329	5
	B4S	311	369	58
	B6F	281	289	8
	B6R	396	345	51
	B6S	394	----	----
Average		287	275	12
Nondescript	N1D	383	538	155

Analysis of data	1952 crop	1954 crop
High	469	559
Low	182	145
Range	287	414
Average	338	312
High/low ratio	2. 6	3. 9
Coefficient of variation (percent)	25. 1	30. 4
Standard deviation	85	95

Table 24.—Methoxyl in Lignin (Method 13)

[All results calculated on an ash-free and crude-protein-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	5.61	4.30	1.31
Lugs	X2L	5.36	5.00	.36
	X2F	5.89	4.62	1.27
	X4L	5.71	5.21	.50
	X4F	5.83	5.24	.59
Average		5.70	5.02	.68
Cutters	C2L	6.06	4.14	1.92
	C2F		3.61	
	C4L	5.23	4.33	.90
	C4F	5.45	5.05	.40
Average		5.58	4.51	1.07
Smoking Leaf	H2L	5.39	3.27	2.12
	H4L	5.02	3.66	1.36
	H4F	5.04	4.35	.69
	H4R	5.24	3.73	1.51
	H6F	4.80	3.64	1.16
	H6R	5.07	4.09	.98
Average		5.09	3.79	1.30
Leaf	B2L	4.31	4.24	.07
	B2F		4.10	
	B2R	4.73		
	B4L	3.58	3.85	.27
	B4F	4.92	4.93	.01
	B4R	4.19	[1]	
	B4S	4.91	[1]	
	B6F	4.30	4.13	.17
	B6R	4.26	4.10	.16
	B6S	4.83		
Average		4.27	4.25	.02
Nondescript	N1D	4.65	4.39	.26

Analysis of data	1952 crop	1954 crop
High	6.06	5.24
Low	3.58	3.27
Range	2.48	1.97
Average	5.03	4.31
High/low ratio	1.69	1.60
Coefficient of variation (percent)	12.5	13.7
Standard deviation	.63	.59

[1] Insufficient sample.

Table 25.—Moisture Equilibrium (Method 15)

Group	U.S. grade	40 percent relative humidity		60 percent relative humidity		70 percent relative humidity		80 percent relative humidity	
		1952 crop	1954 crop	1952 crop	1954 crop	1952 crop	1954 crop	1952 crop	1954 crop
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Nondescript	N1L	6.2	7.3	9.8	10.9	12.6	13.4	15.1	16.4
Lugs	X2L	7.4	7.9	12.0	12.7	15.2	16.1	20.4	19.7
	X2F	6.3	7.5	11.7	12.1	15.0	15.8	20.2	20.5
	X4L	7.4	6.7	11.2	11.1	14.7	15.2	19.6	21.8
	X4F	6.5	7.4	11.0	11.5	13.9	14.3	20.2	17.9
Average		6.9	7.4	11.5	11.8	14.7	15.4	20.1	20.0
Cutters	C2L	7.0	7.7	12.4	13.2	15.7	17.6	21.2	23.2
	C2F		7.7		12.9		16.7		21.4
	C4L	7.0	7.9	12.3	12.9	13.5	16.5	21.0	20.9
	C4F	7.0	7.8	12.5	12.7	16.5	16.2	22.3	20.4
Average		7.0	7.8	12.4	12.9	15.2	16.8	21.5	21.5
Smoking Leaf	H2L	7.3	7.3	12.2	12.3	15.3	16.1	20.8	20.9
	H4L	6.6	7.6	11.8	12.2	14.9	15.4	20.3	19.8
	H4F	6.7	7.7	11.7	12.4	14.6	16.1	18.3	21.0
	H4R	6.3	7.5	11.3	11.6	14.5	14.5	19.5	18.1
	H6F	6.2	7.2	11.1	10.6	13.5	13.7	18.5	18.4
	H6R	7.0	6.8	10.5	10.7	13.0	13.9	17.2	18.4
Average		6.7	7.4	11.4	11.6	14.3	15.0	19.1	19.4

Leaf	B2L	7.4	7.7	12.5	13.2	15.3	17.5	18.5	23.1
	B2F		8.2		13.5		17.4		22.2
	B2R	7.2		12.6		15.8		20.8	
	B4L	7.5	7.9	12.8	12.7	16.0	16.3	21.5	20.8
	B4F	7.0	8.0	12.4	12.6	16.1	15.9	21.2	19.8
	B4R	6.9	7.4	12.2	11.8	15.5	15.1	20.5	19.3
	B4S	6.8	7.5	11.6	11.8	15.2	15.0	19.7	19.2
	B6F	7.1	6.9	12.5	10.7	16.0	13.7	20.8	17.5
	B6R	6.6	7.0	11.3	10.8	14.4	13.7	18.8	17.3
	B6S	6.4		11.0		14.0		18.4	
Average		7.0	7.5	12.2	11.9	15.5	15.3	20.1	19.6
Nondescript	N1D	6.2	7.4	10.4	10.7	13.6	12.9	17.3	15.6

Analysis of data	1952 crop	1954 crop	1952 crop	1954 crop	1952 crop	1954 crop	1952 crop	1954 crop
High	7.5	8.2	12.8	13.5	16.5	17.6	22.3	23.2
Low	6.2	6.7	9.8	10.6	13.0	12.9	15.1	15.6
Range	1.3	1.5	3.0	2.9	3.5	4.7	7.2	7.6
Average	6.8	7.5	11.8	12.0	14.9	15.4	20.0	19.9
High/low ratio	1.21	1.22	1.31	1.27	1.27	1.36	1.48	1.49
Coefficient of variation (%)	5.3	5.1	5.2	7.0	6.2	7.5	6.2	8.3
Standard deviation	.36	.38	.61	.84	.92	1.16	1.24	1.65

Table 26.—Nicotine (Method 16)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2. 51	3. 33	0. 82
Lugs	X2L	2. 04	2. 79	. 75
	X2F	2. 64	3. 44	. 80
	X4L	2. 22	2. 90	. 68
	X4F	2. 59	3. 62	1. 03
Average		2. 37	3. 19	. 82
Cutters	C2L	1. 85	2. 76	. 91
	C2F		3. 27	
	C4L	2. 01	2. 89	. 88
	C4F	2. 25	3. 05	. 80
Average		2. 04	2. 90	. 86
Smoking Leaf	H2L	2. 81	3. 01	. 20
	H4L	2. 28	3. 24	. 96
	H4F	2. 66	3. 89	1. 23
	H4R	2. 79	5. 18	2. 39
	H6F	2. 47	3. 97	1. 50
	H6R	2. 23	4. 71	2. 48
Average		2. 54	4. 00	1. 46
Leaf	B2L	2. 13	2. 75	. 62
	B2F		3. 22	
	B2R	3. 89		
	B4L	2. 05	3. 18	1. 13
	B4F	2. 55	4. 19	1. 64
	B4R	3. 29	5. 99	2. 70
	B4S	3. 62	6. 35	2. 73
	B6F	2. 02	4. 37	2. 35
	B6R	2. 71	5. 35	2. 64
	B6S	2. 54		
Average		2. 62	4. 60	1. 98
Nondescript	N1D	2. 51	5. 16	2. 65

Analysis of data	1952 crop	1954 crop
High	3. 89	6. 35
Low	1. 85	2. 75
Range	2. 04	3. 60
Average	2. 46	3. 91
High/low ratio	2. 10	2. 31
Coefficient of variation (percent)	20. 3	44. 8
Standard deviation	. 50	1. 75

Table 27.—Nicotine (Method 31)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.64	3.06	0.42
Lugs	X2L	2.35	2.49	.14
	X2F	2.76	3.13	.37
	X4L	2.45	2.79	.34
	X4F	2.84	3.51	.67
Average		2.60	2.98	.38
Cutters	C2L	1.98	2.26	.28
	C2F		2.93	
	C4L	2.13	2.44	.31
	C4F	2.38	2.66	.28
Average		2.16	2.45	.29
Smoking Leaf	H2L	2.38	2.49	.11
	H4L	2.46	2.79	.33
	H4F	2.86	3.45	.59
	H4R	3.05	4.90	1.85
	H6F	2.63	3.67	1.04
	H6R	2.44	4.50	2.06
Average		2.64	3.63	.99
Leaf	B2L	2.29	2.42	.13
	B2F		2.91	
	B2R	4.24		
	B4L	2.17	2.76	.59
	B4F	2.77	3.83	1.06
	B4R	3.75	5.51	1.76
	B4S	4.01	6.16	2.15
	B6F	2.30	3.90	1.60
	B6R	2.98	5.07	2.09
	B6S	2.78		
Average		2.90	4.24	1.34
Nondescript	N1D	2.83	4.78	1.95

Analysis of data	1952 crop	1954 crop
High	4.24	6.16
Low	1.98	2.26
Range	2.26	3.90
Average	2.66	3.57
High/low ratio	2.14	2.73
Coefficient of variation (percent)	18.8	31.4
Standard deviation	.50	1.12

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Table 28.—Nitrogen and Nitrogenous Fractions [1]

U.S. GRADE NIL [2]

Nitrogenous constituents	Percent		
	1952 crop	1954 crop	Difference between 1952 and 1954
Total nitrogen	2.475	2.331	0.144
Insoluble nitrogen	.982	.912	.070
Soluble nitrogen	1.465	1.448	.017
Identified nitrogen subfractions:			
Ammonia N (reference notation A)	.054	.058	.004
Glutamine amide N (reference notation B)	.052	.056	.004
Asparagine amide N (reference notations B and M)	.127	.137	.010
Nicotine N (reference notation G)	.416	.453	.037
Alpha amino N (reference notation P)	.172	.159	.013
Secondary alkaloid N (reference notation Kb)	.020	.034	.014
Nitrate N (reference notation O)	.040	.044	.004
Total identified N	.881	.941	.060
Unidentified nitrogen subfractions:			
Acid precipitate N (reference notation E)	.007	.048	.041
Decomposition NH_3 N (reference notation M)	.037	.021	.016
Volatile base N (reference notation G)	(3)	.018	.018
Magnesium oxide N (reference notation I)	.135	.117	.018
Silicotungstic acid N (reference notation K)	.139	.140	.001
Devarda mud N (reference notation Q)	.053	.039	.014
Rest N (reference notation P)	.137	.119	.018
Total unidentified N	.508	.502	.006
Total of all subfractions	1.389	1.443	.054
Other determinations:			
Van Slyke N obtained after 6N hydrolysis	.354	.302	.052
Soluble solids	50.7	50.3	.4
pH of aqueous extract	5.40	5.37	.03

[1] The methods used in determining the above data are described by Frankenburg, Gottscho, Kissinger, and others (*15*).

[2] The data shown on this page were based upon the following drying method: The powdered sample was spread in a layer 2 to 4 mm. thick and dried for ½ hour at 68° C. in a forced-draft oven.

[3] Negative.

Table 29.—Nitrogen and Nitrogenous Fractions [1]

U.S. GRADE B4R [2]

Nitrogenous constituents	Percent		
	1952 crop	1954 crop	Difference between 1952 and 1954
Total nitrogen	2. 557	2. 977	0. 420
Insoluble nitrogen	. 737	. 814	. 077
Soluble nitrogen	1. 781	2. 144	. 363
Identified nitrogen subfractions:			
Ammonia N (reference notation A)	. 025	. 040	. 015
Glutamine amide N (reference notation B)	. 061	. 047	. 014
Asparagine amide N (reference notations B and M)	. 085	. 106	. 021
Nicotine N (reference notation G)	. 591	. 855	. 264
Alpha amino N (reference notation P)	. 119	. 127	. 008
Secondary alkaloid N (reference notation Kb)	. 032	. 056	. 024
Nitrate N (reference notation O)	. 002	. 003	. 001
Total identified N	. 915	1. 234	. 319
Unidentified nitrogen subfractions:			
Acid precipitate N (reference notation E)	. 077	. 085	. 008
Decomposition NH_3 N (reference notation M)	. 059	. 007	. 052
Volatile base N (reference notation G)	(3)	(3)	none
Magnesium oxide N (reference notation I)	. 191	. 202	. 011
Silicotungstic acid N (reference notation K)	. 225	. 257	. 032
Devarda mud N (reference notation Q)	. 038	. 024	. 014
Rest N (reference notation P)	. 317	. 304	. 013
Total unidentified N	. 907	. 879	. 028
Total of all subfractions	1. 822	2. 113	. 291
Other determinations:			
Van Slyke N obtained after 6N hydrolysis	. 380	. 386	. 006
Soluble solids	66. 8	62. 6	4. 2
pH of aqueous extract	5. 02	5. 08	. 06

[1] The methods used in determining the above data are described by Frankenburg, Gottscho, Kissinger, and others (*15*).

[2] The data shown on this page were based upon the following drying method: The powdered sample was spread in a layer 2 to 4 mm. thick and dried for ½ hour at 68° C. in a forced-draft oven.

[3] Negative.

Table 30.—Nontannin (Method 30)

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

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	43.88	43.88	0
Lugs	X2L	55.41	57.98	2.57
	X2F	54.46	55.54	1.08
	X4L	50.75	52.64	1.89
	X4F	48.71	50.61	1.90
Average		52.33	54.19	1.86
Cutters	C2L	59.05	59.04	.01
	C2F		59.44	
	C4L	57.76	58.46	.70
	C4F	56.87	57.19	.32
Average		57.89	58.23	.34
Smoking Leaf	H2L	57.02	55.47	1.55
	H4L	54.99	55.15	.16
	H4F	53.56	53.46	.10
	H4R	51.15	47.59	3.56
	H6F	49.07	45.67	3.40
	H6R	45.66	43.08	2.58
Average		51.91	50.07	1.84
Leaf	B2L	59.58	62.30	2.72
	B2F		59.95	
	B2R	54.98		
	B4L	59.13	56.98	2.15
	B4F	56.08	57.79	1.71
	B4R	56.06	49.09	6.97
	B4S	51.48	49.52	1.96
	B6F	55.87	47.82	8.05
	B6R	48.89	44.90	3.99
	B6S	50.23		
Average		55.30	52.63	2.67
Nondescript	N1D	43.90	39.08	4.82

Analysis of data	1952 crop	1954 crop
High	59.58	62.30
Low	43.88	39.08
Range	15.70	23.22
Average	53.15	51.96
High/low ratio	1.36	1.59
Coefficient of variation (percent)	7.3	10.2
Standard deviation	3.89	5.30

Table 31.—Nornicotine (Method 31)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	NIL	0. 20	0. 11	0. 09
Lugs	X2L	. 07	. 11	. 04
	X2F	. 12	. 11	. 01
	X4L	. 13	. 10	. 03
	X4F	. 21	. 13	. 08
Average		. 13	. 11	. 02
Cutters	C2L	. 07	. 08	. 01
	C2F		. 11	
	C4L	. 10	. 10	0
	C4F	. 08	. 14	. 06
Average		. 08	. 11	. 03
Smoking Leaf	H2L	. 10	. 10	0
	H4L	. 09	. 12	. 03
	H4F	. 16	. 16	0
	H4R	. 14	. 21	. 07
	H6F	. 14	. 18	. 04
	H6R	. 10	. 20	. 10
Average		. 12	. 16	. 04
Leaf	B2L	. 07	. 08	. 01
	B2F		. 15	
	B2R	. 21		
	B4L	. 12	. 15	. 03
	B4F	. 17	. 21	. 04
	B4R	. 14	. 23	. 09
	B4S	. 23	. 31	. 08
	B6F	. 12	. 19	. 07
	B6R	. 18	. 27	. 09
	B6S	. 15		
Average		. 15	. 21	. 06
Nondescript	N1D	. 18	. 29	. 11

Analysis of data	1952 crop	1954 crop
High	0. 23	0. 29
Low	. 07	. 08
Range	. 16	. 21
Average	. 13	. 16
High/low ratio	3. 3	3. 6
Coefficient of variation (percent)	38. 5	37. 5
Standard deviation	. 05	. 06

Table 32.—Pectic Acid and Pectates (Method 17)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	4.57	4.09	.48
Lugs	X2L	3.03	2.50	0.53
	X2F	2.87	2.37	.50
	X4L	3.29	2.91	.38
	X4F	3.86	2.93	.93
Average		3.26	2.68	.58
Cutters	C2L	2.57	2.27	.30
	C2F		2.14	
	C4L	2.75	2.39	.36
	C4F	2.83	2.37	.46
Average		2.72	2.34	.38
Smoking Leaf	H2L	3.02	2.54	.48
	H4L	3.16	2.55	.61
	H4F	2.87	2.72	.15
	H4R	2.94	1.86	1.08
	H6F	3.67	2.22	1.45
	H6R	3.64	2.23	1.41
Average		3.22	2.35	.87
Leaf	B2L	2.66	1.95	.71
	B2F		2.02	
	B2R	2.30		
	B4L	2.09	2.13	.04
	B4F	2.01	2.62	.61
	B4R	1.73	2.11	.38
	B4S	1.90	2.01	.11
	B6F	3.46	2.09	1.37
	B6R	3.41	1.92	1.49
	B6S	3.54		
Average		2.47	2.12	.35
Nondescript	N1D	5.30	2.58	2.72

Analysis of data	1952 crop	1954 crop
High	4.57	4.09
Low	1.73	1.86
Range	2.84	2.23
Average	3.07	2.43
High/low ratio	2.64	2.20
Coefficient of variation (percent)	19.2	12.8
Standard deviation	.59	.31

Table 33.—Pentosans (Method 18)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.52	2.45	0.37
Lugs	X2L	2.21	1.76	.45
	X2F	2.20	1.74	.46
	X4L	2.41	2.10	.31
	X4F	2.51	2.13	.38
Average		2.33	1.93	.40
Cutters	C2L	1.83	1.79	.04
	C2F		1.65	
	C4L	1.93	1.81	.12
	C4F	1.88	1.93	.05
Average		1.88	1.84	.04
Smoking Leaf	H2L	2.05	2.02	.03
	H4L	2.17	2.02	.15
	H4F	2.09	2.09	0
	H4R	2.11	2.06	.05
	H6F	2.50	2.18	.32
	H6R	2.60	2.12	.48
Average		2.25	2.08	.17
Leaf	B2L	1.68	1.58	.10
	B2F		1.73	
	B2R	1.66		
	B4L	1.74	1.79	.05
	B4F	1.73	1.99	.26
	B4R	1.79	2.08	.29
	B4S	1.82	1.96	.14
	B6F	1.50	2.06	.56
	B6R	1.61	2.01	.40
	B6S	1.61		
Average		1.70	1.92	.22
Nondescript	N1D	1.94	2.26	.32

Analysis of data	1952 crop	1954 crop
High	2.60	2.45
Low	1.50	1.73
Range	1.10	.72
Average	2.04	2.00
High/low ratio	1.73	1.42
Coefficient of variation (percent)	15.2	8.0
Standard deviation	.31	.16

Table 34.—Petroleum Ether Extract (Method 19)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	9.72	10.97	1.25
Lugs	X2L	9.22	9.30	.08
	X2F	9.32	9.99	.67
	X4L	10.04	10.67	.63
	X4F	10.28	11.23	.95
Average		9.72	10.30	.58
Cutters	C2L	8.12	8.73	.61
	C2F		8.48	
	C4L	8.12	8.28	.16
	C4F	8.36	8.23	.13
Average		8.20	8.41	.21
Smoking Leaf	H2L	8.38	7.91	.47
	H4L	8.44	8.62	.18
	H4F	8.58	8.92	.34
	H4R	8.07	10.41	2.34
	H6F	7.90	9.91	2.01
	H6R	7.52	10.65	3.13
Average		8.15	9.40	1.25
Leaf	B2L	7.73	7.77	.04
	B2F		8.00	
	B2R	7.47		
	B4L	7.56	7.72	.16
	B4F	7.88	8.90	1.02
	B4R	7.55	9.58	2.03
	B4S	7.04	9.23	2.19
	B6F	7.32	9.20	1.88
	B6R	7.72	10.00	2.28
	B6S	6.61		
Average		7.54	8.91	1.37
Nondescript	N1D	7.85	10.78	2.93

Analysis of data	1952 crop	1954 crop
High	10.28	11.23
Low	6.61	7.72
Range	3.67	3.51
Average	8.31	9.41
High/low ratio	1.56	1.45
Coefficient of variation (percent)	9.6	10.6
Standard deviation	.80	1.00

Table 35.—pH (Method 20)

Group	U.S. grade	pH		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	5. 21	5. 30	0. 09
Lugs	X2L	5. 03	5. 13	. 10
	X2F	5. 02	5. 05	. 03
	X4L	5. 08	5 14	. 06
	X4F	5. 03	5. 08	. 05
Average		5. 03	5. 09	. 06
Cutters	C2L	5. 01	5. 13	. 12
	C2F		5. 07	
	C4L	4. 90	5. 13	. 23
	C4F	4. 83	5. 06	. 23
Average		4. 90	5. 10	. 20
Smoking Leaf	H2L	5. 05	5. 11	. 06
	H4L	5. 01	5. 01	0
	H4F	4. 98	5. 00	. 02
	H4R	4. 89	4. 86	. 03
	H6F	4. 98	4. 96	. 02
	H6R	4. 90	4. 92	. 02
Average		4. 96	4. 96	0
Leaf	B2L	5. 03	5. 11	. 08
	B2F		5. 05	
	B2R	4. 73		
	B4L	4. 89	5. 05	. 16
	B4F	4. 86	4. 96	. 10
	B4R	4. 89	4. 84	. 05
	B4S	4. 90	4. 79	. 11
	B6F	5. 02	4. 74	. 28
	B6R	4. 94	4. 71	. 23
	B6S	4. 87		
Average		4. 89	4. 88	. 01
Nondescript	N1D	5. 07	4. 90	. 17

Analysis of data	1952 crop	1954 crop
High	5. 21	5. 30
Low	4. 73	4. 71
Range	. 48	. 59
Average [1]	4. 93	4. 94
High/low ratio	3. 00	3. 90

[1] The pH values were calculated to grams of Hydrogen ions per liter before averaging.

Table 36.—Phosphorus (Method 21)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0. 184	0. 149	0. 035
Lugs	X2L	. 190	. 191	. 001
	X2F	. 190	. 187	. 003
	X4L	. 193	. 175	. 018
	X4F	. 184	. 148	. 036
Average		. 189	. 175	. 014
Cutters	C2L	. 202	. 186	. 016
	C2F		. 176	
	C4L	. 213	. 171	. 042
	C4F	. 190	. 161	. 029
Average		. 202	. 173	. 029
Smoking Leaf	H2L	. 213	. 180	. 033
	H4L	. 207	. 181	. 026
	H4F	. 195	. 181	. 014
	H4R	. 182	. 171	. 011
	H6F	. 223	. 183	. 040
	H6R	. 234	. 175	. 059
Average		. 209	. 178	. 031
Leaf	B2L	. 226	. 194	. 032
	B2F		. 184	
	B2R	. 205		
	B4L	. 217	. 189	. 028
	B4F	. 202	. 185	. 017
	B4R	. 201	. 185	. 016
	B4S	. 190	. 182	. 008
	B6F	. 233	. 207	. 026
	B6R	. 208	. 188	. 020
	B6S	. 208		
Average		. 211	. 190	. 021
Nondescript	N1D	. 226	. 189	. 037

Analysis of data	1952 crop	1954 crop
High	0. 234	0. 207
Low	. 182	. 148
Range	. 052	. 059
Average	. 205	. 180
High/low ratio	1. 29	1. 40
Coefficient of variation (percent)	7. 3	7. 2
Standard deviation	. 015	. 013

Table 37.—Phosphorus (Method 5)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0.183	0.167	0.016
Lugs	X2L	.195	.201	.006
	X2F	.181	.191	.010
	X4L	.191	.196	.005
	X4F	.183	.162	.021
Average		.188	.188	0
Cutters	C2L	.202	.207	.005
	C2F		.187	
	C4L	.222	.182	.040
	C4F	.180	.177	.003
Average		.201	.189	.012
Smoking Leaf	H2L	.205	.214	.009
	H4L	.205	.207	.002
	H4F	.187	.208	.021
	H4R	.202	.204	.002
	H6F	.214	.206	.008
	H6R	.218	.189	.029
Average		.205	.205	0
Leaf	B2L	.217	.203	.014
	B2F		.205	
	B2R	.197		
	B4L	.217	.215	.002
	B4F	.196	.212	.016
	B4R	.207	.218	.011
	B4S	.189	.212	.023
	B6F	.233	.214	.019
	B6R	.224	.199	.025
	B6S	.216		
Average		.212	.210	.002
Nondescript	N1D	.223	.197	.026

Analysis of data	1952 crop	1954 crop
High	0.223	0.218
Low	.180	.162
Range	.053	.056
Average	.203	.199
High/low ratio	1.29	1.35
Coefficient of variation (percent)	7.9	7.5
Standard deviation	.106	.015

Table 38.—Polyphenols (Method 34)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	1.16	0.02	1.14
Lugs	X2L	2.43	1.12	1.31
	X2F	2.24	1.42	.82
	X4L	2.48	1.60	.88
	X4F	2.45	.68	1.77
Average		2.40	1.20	1.20
Cutters	C2L	2.54	2.16	.38
	C2F		2.68	
	C4L	1.07	1.82	.75
	C4F	2.69	1.10	1.59
Average		2.10	1.69	.41
Smoking Leaf	H2L	3.01	1.59	1.42
	H4L	1.77	1.88	.11
	H4F	1.06	2.58	1.52
	H4R	1.22	2.71	1.49
	H6F	2.95	2.12	.83
	H6R	1.94	2.65	.71
Average		1.99	2.26	.27
Leaf	B2L	1.84	1.89	.05
	B2F		1.90	
	B2R	1.82		
	B4L	2.97	1.84	1.13
	B4F	2.93	2.70	.23
	B4R	1.42	1.26	.16
	B4S	2.24	1.06	1.18
	B6F	1.57	1.62	.05
	B6R	2.10	.42	1.68
	B6S	2.13		
Average		2.15	1.54	.61
Nondescript	N1D	2.76	.91	1.85

Analysis of data	1952 crop	1954 crop
High	3.01	2.71
Low	1.06	.02
Range	1.95	2.69
Average	2.13	1.60
High/low ratio	2.84	135.5
Coefficient of variation (percent)	29.1	40.6
Standard deviation	.62	.65

Table 39.—Potassium (Method 5)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.81	2.42	0.39
Lugs	X2L	2.27	2.00	.27
	X2F	2.33	2.01	.32
	X4L	2.20	2.04	.16
	X4F	2.54	1.96	.58
Average		2.34	2.00	.34
Cutters	C2L	2.27	2.18	.09
	C2F		2.04	
	C4L	2.19	2.14	.05
	C4F	2.42	2.08	.34
Average		2.29	2.13	.16
Smoking Leaf	H2L	2.41	2.18	.23
	H4L	2.47	2.03	.44
	H4F	2.50	2.08	.42
	H4R	2.62	1.97	.65
	H6F	2.43	1.85	.58
	H6R	2.32	1.84	.48
Average		2.46	1.99	.47
Leaf	B2L	2.07	1.74	.33
	B2F		1.96	
	B2R	2.24		
	B4L	1.92	1.73	.19
	B4F	2.14	1.76	.38
	B4R	2.05	1.85	.20
	B4S	2.12	2.09	.03
	B6F	1.97	1.52	.45
	B6R	1.83	1.40	.43
	B6S	2.12		
Average		2.01	1.74	.27
Nondescript	N1D	2.15	1.54	.61

Analysis of data	1952 crop	1954 crop
High	2.81	2.42
Low	1.83	1.49
Range	.98	.93
Average	2.27	1.93
High/low ratio	1.54	1.62
Coefficient of variation (percent)	10.1	11.9
Standard deviation	.23	.23

Table 40.—Potassium (Method 23)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	3.33	2.14	1.19
Lugs	X2L	2.45	1.69	.76
	X2F	2.47	1.68	.79
	X4L	2.46	1.78	.68
	X4F	2.82	1.73	1.09
Average		2.55	1.72	.83
Cutters	C2L	2.63	1.88	.75
	C2F		1.75	
	C4L	2.45	1.92	.53
	C4F	2.53	1.82	.71
Average		2.54	1.87	.67
Smoking Leaf	H2L	2.67	1.94	.73
	H4L	2.61	1.75	.86
	H4F	2.59	1.78	.81
	H4R	2.77	1.74	1.03
	H6F	2.51	1.61	.90
	H6R	2.56	1.52	1.04
Average		2.62	1.72	.90
Leaf	B2L	2.23	1.50	.73
	B2F		1.54	
	B2R	2.15		
	B4L	1.99	1.44	.55
	B4F	2.13	1.44	.69
	B4R	2.03	1.52	.51
	B4S	2.03	1.73	.30
	B6F	1.93	1.29	.64
	B6R	2.00	1.20	.80
	B6S	2.08		
Average		2.05	1.45	.60
Nondescript	N1D	2.26	1.29	.97

Analysis of data	1952 crop	1954 crop
High	3.33	2.14
Low	1.93	1.20
Range	1.40	.94
Average	2.43	1.65
High/low ratio	1.73	1.78
Coefficient of variation (percent)	7.0	12.1
Standard deviation	.17	.20

Table 41.—Protein Nitrogen (Method 24)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	1.05	0.80	0.25
Lugs	X2L	.82	.76	.06
	X2F	.79	.76	.03
	X4L	.83	.83	0
	X4F	.87	.84	.03
Average		.83	.80	.03
Cutters	C2L	.67	.69	.02
	C2F		.69	
	C4L	.77	.72	.05
	C4F	.79	.76	.03
Average		.74	.72	.02
Smoking Leaf	H2L	.77	.72	.05
	H4L	.82	.80	.02
	H4F	.80	1.00	.20
	H4R	.89	.93	.04
	H6F	1.00	.94	.06
	H6R	1.15	1.08	.07
Average		.90	.91	.01
Leaf	B2L	.75	.67	.08
	B2F		.72	
	B2R	.95		
	B4L	.74	.77	.03
	B4F	.85	.79	.06
	B4R	.94	.98	.04
	B4S	1.06	1.13	.07
	B6F	.90	1.00	.10
	B6R	.97	1.10	.13
	B6S	1.23		
Average		.89	.92	.03
Nondescript	N1D	1.15	1.31	.16

Analysis of data	1952 crop	1954 crop
High	1.23	1.31
Low	.67	.69
Range	.56	.62
Average	.88	.88
High/low ratio	1.84	1.90
Coefficient of variation (percent)	12.5	15.9
Standard deviation	.11	.14

Table 42.—Protopectin (Method 17)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	6.33	6.93	0.60
Lugs	X2L	6.30	6.03	.27
	X2F	6.44	6.78	.34
	X4L	6.72	6.52	.20
	X4F	6.38	6.63	.25
Average		6.46	6.49	.03
Cutters	C2L	6.29	6.86	.57
	C2F		6.95	
	C4L	6.03	7.01	.98
	C4F	6.16	6.30	.14
Average		6.16	6.72	.56
Smoking Leaf	H2L	7.39	7.38	.01
	H4L	7.46	7.42	.04
	H4F	7.45	7.38	.07
	H4R	8.16	8.88	.72
	H6F	6.71	8.44	1.73
	H6R	7.12	8.94	1.82
Average		7.38	8.07	.69
Leaf	B2L	5.47	6.81	1.34
	B2F		6.29	
	B2R	6.84		
	B4L	6.00	6.43	.43
	B4F	6.58	6.78	.20
	B4R	7.55	8.21	.66
	B4S	7.19	8.04	.85
	B6F	6.35	9.00	2.65
	B6R	7.77	9.18	1.41
	B6S	7.55		
Average		6.70	7.78	1.08
Nondescript	N1D	6.86	9.40	2.54

Analysis of data	1952 crop	1954 crop
High	7.77	9.40
Low	6.00	6.03
Range	1.77	3.37
Average	6.76	7.52
High/low ratio	1.30	1.56
Coefficient of variation (percent)	10.1	13.0
Standard deviation	.68	.98

Table 43.—Resins and Waxes (Method 25)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	10.33	10.81	0.48
Lugs	X2L	9.20	9.43	.23
	X2F	8.92	10.01	1.09
	X4L	8.40	11.04	2.64
	X4F	10.31	10.10	.21
Average		9.21	10.14	.93
Cutters	C2L	8.68	8.78	.10
	C2F		9.54	
	C4L	8.49	9.96	1.47
	C4F	8.38	9.86	1.48
Average		8.52	8.53	.01
Smoking Leaf	H2L	8.69	9.55	.86
	H4L	9.62	10.37	.75
	H4F	9.28	10.68	1.40
	H4R	8.25	11.77	3.52
	H6F	8.63	12.21	3.58
	H6R	8.27	12.88	4.61
Average		8.79	11.24	2.45
Leaf	B2L	8.19	8.97	.78
	B2F		8.93	
	B2R	8.05		
	B4L	8.28	9.42	1.14
	B4F	8.64	9.91	1.27
	B4R	7.76	11.20	3.44
	B4S	7.37	11.16	3.79
	B6F	7.80	11.51	3.71
	B6R	8.59	11.93	3.34
	B6S	8.97		
Average		8.09	10.59	2.50
Nondescript	N1D	9.14	12.43	3.29

Analysis of data	1952 crop	1954 crop
High	10.33	12.88
Low	7.37	8.78
Range	2.96	4.10
Average	8.69	10.64
High/low ratio	1.40	1.47
Coefficient of variation (percent)	8.2	10.8
Standard deviation	.71	1.15

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Table 44.—Sodium (Method 28)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Parts per million		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	394	393	1
Lugs	X2L	319	203	116
	X2F	288	228	60
	X4L	369	429	60
	X4F	333	359	26
Average		327	305	22
Cutters	C2L	248	185	63
	C2F		202	
	C4L	240	203	37
	C4F	342	199	143
Average		277	196	81
Smoking Leaf	H2L	190	188	2
	H4L	243	184	59
	H4F	195	153	42
	H4R	165	127	38
	H6F	178	191	13
	H6R	187	166	21
Average		193	168	25
Leaf	B2L	140	124	16
	B2F		140	
	B2R	107		
	B4L	131	109	22
	B4F	137	130	7
	B4R	142	107	35
	B4S	122	129	7
	B6F	157	132	25
	B6R	130	123	7
	B6S	125		
Average		137	122	15
Nondescript	N1D	135	115	20

Analysis of data	1952 crop	1954 crop
High	394	393
Low	107	107
Range	287	286
Average	218	190
High/low ratio	3.7	3.7
Coefficient of variation (percent)	26.6	25.8
Standard deviation	58	49

Table 45.—Sodium (Method 27)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0. 053	0. 041	0. 012
Lugs	X2L	. 041	. 023	. 018
	X2F	. 039	. 027	. 012
	X4L	. 046	. 042	. 004
	X4F	. 046	. 034	. 012
Average		. 043	. 032	. 011
Cutters	C2L	. 038	. 025	. 013
	C2F		. 027	
	C4L	. 038	. 027	. 011
	C4F	. 043	. 026	. 017
Average		. 040	. 026	. 014
Smoking Leaf	H2L	. 029	. 025	. 004
	H4L	. 034	. 024	. 010
	H4F	. 031	. 021	. 010
	H4R	. 028	. 019	. 009
	H6F	. 043	. 028	. 015
	H6R	. 037	. 022	. 015
Average		. 034	. 023	. 011
Leaf	B2L	. 031	. 018	. 013
	B2F		. 022	
	B2R	. 024		
	B4L	. 020	. 017	. 003
	B4F	. 021	. 019	. 002
	B4R	. 022	. 018	. 004
	B4S	. 020	. 020	
	B6F	. 022	. 019	. 003
	B6R	. 025	. 016	. 009
	B6S	. 025		
Average		. 023	. 018	. 005
Nondescript	N1D	. 023	. 020	. 003

Analysis of data	1952 crop	1954 crop
High	0. 053	0. 042
Low	. 020	. 016
Range	. 033	. 026
Average	. 033	. 024
High/low ratio	2. 65	2. 62
Coefficient of variation (percent)	27. 3	25. 0
Standard deviation	. 009	. 006

Table 46.—Starch (Method 34)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.58	2.12	0.80
Lugs	X2L	4.09	5.82	1.73
	X2F	3.93	5.37	1.44
	X4L	3.50	4.26	.76
	X4F	3.43	3.77	.34
Average		3.74	4.80	1.06
Cutters	C2L	6.37	3.45	2.92
	C2F		3.41	
	C4L	5.36	3.20	2.16
	C4F	4.91	3.36	1.55
Average		5.55	3.34	2.21
Smoking Leaf	H2L	3.93	2.73	1.20
	H4L	3.61	2.58	1.03
	H4F	3.17	2.32	.85
	H4R	3.69	2.44	1.25
	H6F	3.63	2.66	.97
	H6R	4.15	2.43	1.72
Average		3.70	2.53	1.17
Leaf	B2L	7.83	4.73	3.10
	B2F		4.17	
	B2R	4.37		
	B4L	7.81	4.10	3.71
	B4F	3.77	3.07	.70
	B4R	4.78	2.19	2.59
	B4S	4.47	2.18	2.29
	B6F	6.17	2.93	3.24
	B6R	4.84	2.28	2.56
	B6S	4.39		
Average		5.67	3.07	2.60
Nondescript	N1D	3.56	2.10	1.46

Analysis of data	1952 crop	1954 crop
High	7.83	5.82
Low	2.58	2.10
Range	5.25	3.72
Average	4.53	3.19
High/low ratio	3.03	2.77
Coefficient of variation (percent)	28.9	32.9
Standard deviation	1.35	1.05

Table 47.—Sucrose (Method 34)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0	0.36	0.36
Lugs	X2L	2.45	4.13	1.68
	X2F	.92	2.93	2.01
	X4L	1.19	2.80	1.61
	X4F	.48	1.47	.99
Average		1.26	2.83	1.57
Cutters	C2L	3.05	4.38	1.33
	C2F	----------	4.03	----------
	C4L	.80	4.36	3.56
	C4F	1.48	2.98	1.50
Average		1.78	3.91	2.13
Smoking Leaf	H2L	1.97	3.27	1.30
	H4L	.36	2.56	2.20
	H4F	.23	1.35	1.12
	H4R	.04	0	.04
	H6F	.67	.38	.29
	H6R	.80	0	.80
Average		.68	1.26	.58
Leaf	B2L	.67	3.65	2.98
	B2F	----------	2.65	----------
	B2R	.34	----------	----------
	B4L	3.35	2.71	.64
	B4F	1.22	.46	.76
	B4R	.60	0	.60
	B4S	0	0	0
	B6F	.73	.66	.07
	B6R	0	.04	.04
	B6S	.41	----------	----------
Average		.94	1.07	.13
Nondescript	N1D	0	.38	.38

Analysis of data	1952 crop	1954 crop
High	3.35	4.38
Low	0	0
Range	3.35	4.38
Average	.96	1.77
High/low ratio	3.35	4.38
Coefficient of variation (percent)	98.0	90.0
Standard deviation	.94	1.59

Table 48.—Sulfur (Method 29)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0.61	0.60	0.01
Lugs	X2L	.52	.59	.07
	X2F	.49	.54	.05
	X4L	.53	.66	.13
	X4F	.58	.56	.02
Average		.53	.59	.06
Cutters	C2L	.51	.52	.01
	C2F		.54	
	C4L	.53	.62	.09
	C4F	.54	.57	.03
Average		.53	.57	.04
Smoking Leaf	H2L	.51	.51	0
	H4L	.47	.51	.04
	H4F	.48	.52	.04
	H4R	.50	.57	.07
	H6F	.49	.54	.05
	H6R	.55	.57	.02
Average		.50	.54	.04
Leaf	B2L	.44	.47	.03
	B2F		.51	
	B2R	.47		
	B4L	.44	.46	.02
	B4F	.46	.53	.07
	B4R	.31	.61	.30
	B4S	.53	.60	.07
	B6F	.48	.63	.15
	B6R	.58	.68	.10
	B6S	.55		
Average		.46	.57	.11
Nondescript	N1D	.63	.66	.03

Analysis of data	1952 crop	1954 crop
High	0.63	0.68
Low	.31	.46
Range	.32	.22
Average	.51	.57
High/low ratio	2.03	1.48
Coefficient of variation (percent)	11.8	10.5
Standard deviation	.06	.06

Table 49.—Tannin (Method 30)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	3. 13	2. 78	0. 35
Lugs	X2L	1. 38	2. 64	1. 26
	X2F	2. 99	3. 07	. 08
	X4L	3. 56	2. 70	. 86
	X4F	3. 66	2. 94	. 72
Average		2. 90	2. 84	. 06
Cutters	C2L	3. 38	2. 79	. 59
	C2F		2. 84	
	C4L	2. 33	2. 67	. 34
	C4F	2. 21	2. 69	. 48
Average		2. 64	2. 72	. 08
Smoking Leaf	H2L	1. 99	3. 04	1. 05
	H4L	1. 79	2. 84	1. 05
	H4F	2. 27	3. 26	. 99
	H4R	3. 77	4. 23	. 46
	H6F	3. 17	4. 73	1. 56
	H6R	4. 03	3. 85	. 18
Average		2. 84	3. 66	. 82
Leaf	B2L	1. 39	2. 68	1. 29
	B2F		3. 23	
	B2R	3. 53		
	B4L	3. 86	3. 62	. 24
	B4F	4. 14	3. 00	1. 14
	B4R	3. 32	3. 93	. 61
	B4S	5. 22	3. 93	1. 29
	B6F	4. 77	4. 76	. 01
	B6R	4. 83	4. 21	. 62
	B6S	2. 33		
Average		3. 93	3. 73	. 20
Nondescript	N1D	4. 30	4. 89	. 59

Analysis of data	1952 crop	1954 crop
High	5. 22	4. 89
Low	1. 38	2. 64
Range	3. 84	2. 25
Average	3. 25	3. 42
High/low ratio	3. 78	1. 85
Coefficient of variation (percent)	32. 6	22. 2
Standard deviation	1. 06	. 76

Table 50.—Total Alkaloids (as Nicotine) (Method 31)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.86	3.18	0.32
Lugs	X2L	2.43	2.61	.18
	X2F	2.89	3.25	.36
	X4L	2.60	2.90	.30
	X4F	3.07	3.65	.58
Average		2.75	3.10	.35
Cutters	C2L	2.05	2.35	.30
	C2F		3.05	
	C4L	2.24	2.55	.31
	C4F	2.47	2.81	.34
Average		2.25	2.57	.32
Smoking Leaf	H2L	2.50	2.60	.10
	H4L	2.56	2.92	.36
	H4F	3.03	3.63	.60
	H4R	3.20	5.13	1.93
	H6F	2.78	3.87	1.09
	H6R	2.55	4.72	2.17
Average		2.77	3.81	1.04
Leaf	B2L	2.37	2.51	.14
	B2F		3.07	
	B2R	4.47		
	B4L	2.30	2.92	.62
	B4F	2.95	4.06	1.11
	B4R	3.90	5.76	1.86
	B4S	4.26	6.50	2.24
	B6F	2.43	4.11	1.68
	B6R	3.18	5.37	2.19
	B6S	2.95		
Average		3.06	4.46	1.40
Nondescript	N1D	3.02	5.10	2.08

Analysis of data	1952 crop	1954 crop
High	4.47	6.50
Low	2.05	2.35
Range	2.42	4.15
Average	2.80	3.75
High/low ratio	2.18	2.77
Coefficient of variation (percent)	18.2	16.3
Standard deviation	.51	.61

Table 51.—Total Ash (Method 32)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	19.2	18.4	0.8
Lugs	X2L	14.1	12.0	2.1
	X2F	14.3	12.4	1.9
	X4L	16.4	15.2	1.2
	X4F	16.2	14.2	2.0
Average		15.2	13.4	1.8
Cutters	C2L	10.6	10.6	0
	C2F		10.7	
	C4L	11.1	11.8	.7
	C4F	11.9	11.2	.7
Average		11.2	11.2	0
Smoking Leaf	H2L	12.0	11.2	0.8
	H4L	12.5	10.8	1.7
	H4F	12.6	11.4	1.2
	H4R	12.5	11.0	1.5
	H6F	12.2	11.6	.6
	H6R	12.0	11.7	.3
Average		12.3	11.3	1.0
Leaf	B2L	9.6	8.9	.7
	B2F		9.5	
	B2R	10.8		
	B4L	9.4	9.0	.4
	B4F	10.4	10.0	.4
	B4R	10.5	10.5	0
	B4S	10.6	10.2	.4
	B6F	9.5	9.7	.2
	B6R	10.4	10.3	.1
	B6S	10.8		
Average		10.0	9.8	.2
Nondescript	N1D	13.2	11.6	1.6

Analysis of data	1952 crop	1954 crop
High	19.2	18.4
Low	9.4	8.9
Range	9.8	9.5
Average	12.3	11.5
High/Low ratio	2.0	2.1
Coefficient of variation (percent)	16.3	13.0
Standard deviation	2.0	1.5

Table 52.—Total Carotenoid (Method 22)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Micrograms per gram		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	41	151	110
Lugs	X2L	39	91	52
	X2F	41	82	41
	X4L	41	103	62
	X4F	46	112	66
Average		42	97	55
Cutters	C2L	33	106	73
	C2F		108	
	C4L	40	96	56
	C4F	42	111	69
Average		38	104	66
Smoking Leaf	H2L	38	108	70
	H4L	48	117	69
	H4F	54	131	77
	H4R	54	139	85
	H6F	58	136	78
	H6R	56	148	92
Average		51	130	79
Leaf	B2L	38	94	56
	B2F		101	
	B2R	59		
	B4L	38	110	72
	B4F	44	111	67
	B4R	55	133	78
	B4S	52	122	70
	B6F	39	103	64
	B6R	38	119	81
	B6S	48		
Average		43	113	70
Nondescript	N1D	45	120	75

Analysis of data	1952 crop	1954 crop
High	59	148
Low	33	82
Range	26	66
Average	45	116
High/low ratio	1.8	1.8
Coefficient of variation (percent)	16.5	14.8
Standard deviation	7.4	17.2

Table 53.—Total Chlorophyll (Method 22)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Micrograms per gram		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	58	92	34
Lugs	X2L	46	56	10
	X2F	49	54	5
	X4L	59	68	9
	X4F	56	73	17
Average		52	63	11
Cutters	C2L	41	49	8
	C2F		49	
	C4L	51	52	1
	C4F	48	51	3
Average		47	51	4
Smoking Leaf	H2L	31	39	8
	H4L	49	53	4
	H4F	51	71	20
	H4R	58	68	10
	H6F	48	57	9
	H6R	59	76	17
Average		49	61	12
Leaf	B2L	54	54	0
	B2F		57	
	B2R	80		
	B4L	47	50	3
	B4F	48	61	13
	B4R	59	77	18
	B4S	64	81	17
	B6F	49	60	11
	B6R	63	76	13
	B6S	61		
Average		55	66	11
Nondescript	N1D	73	81	8

Analysis of data	1952 crop	1954 crop
High	80	92
Low	31	39
Range	49	53
Average	53	64
High/low ratio	2.6	2.4
Coefficient of variation (percent)	14.5	17.7
Standard deviation	7.7	11.3

Table 54.—Total Nitrogen (Method 33)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.63	2.58	0.05
Lugs	X2L	2.00	1.88	.12
	X2F	2.15	2.09	.06
	X4L	2.11	2.13	.02
	X4F	2.32	2.35	.03
Average		2.15	2.11	.04
Cutters	C2L	1.67	1.62	.05
	C2F		1.87	
	C4L	1.81	1.81	0
	C4F	1.96	1.90	.06
Average		1.81	1.78	.03
Smoking Leaf	H2L	1.93	1.84	.09
	H4L	1.99	2.06	.07
	H4F	2.26	2.28	.02
	H4R	2.51	3.02	.51
	H6F	2.50	2.66	.16
	H6R	2.72	3.17	.45
Average		2.32	2.50	.18
Leaf	B2L	1.75	1.64	.11
	B2F		1.89	
	B2R	2.84		
	B4L	1.76	1.93	.17
	B4F	2.10	2.32	.22
	B4R	2.69	3.17	.48
	B4S	3.05	3.77	.72
	B6F	2.14	2.68	.54
	B6R	2.67	3.39	.72
	B6S	3.02		
Average		2.31	2.70	.39
Nondescript	N1D	2.87	3.57	.70

Analysis of data	1952 crop	1954 crop
High	3.05	3.77
Low	1.67	1.62
Range	1.38	2.15
Average	2.25	2.45
High/low ratio	1.83	2.33
Coefficient of variation (percent)	16.4	24.9
Standard deviation	.37	.61

Table 55.—Total Pectic Substances (Method 17)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	10.90	11.02	0.12
Lugs	X2L	9.33	8.53	.80
	X2F	9.31	9.15	.16
	X4L	10.00	9.43	.57
	X4F	10.24	9.56	.68
Average		9.72	9.17	.55
Cutters	C2L	8.86	9.13	.27
	C2F		9.09	
	C4L	8.79	9.40	.61
	C4F	8.98	8.67	.31
Average		8.88	9.07	.19
Smoking Leaf	H2L	10.41	9.92	.49
	H4L	10.62	9.97	.35
	H4F	10.32	10.10	.22
	H4R	11.10	10.74	.36
	H6F	10.38	10.66	.28
	H6R	10.77	11.17	.40
Average		10.60	10.43	.17
Leaf	B2L	8.13	8.76	.63
	B2F		8.31	
	B2R	9.15		
	B4L	8.09	8.56	.47
	B4F	8.59	9.40	.81
	B4R	9.28	10.32	1.04
	B4S	9.10	10.05	.95
	B6F	9.82	11.09	1.27
	B6R	11.18	11.10	.08
	B6S	11.09		
Average		9.17	9.90	.73
Nondescript	N1D	12.16	11.98	.18

Analysis of data	1952 crop	1954 crop
High	12.16	11.98
Low	8.13	8.31
Range	4.03	3.67
Average	9.83	9.94
High/low ratio	1.50	1.44
Coefficient of variation (percent)	9.5	8.5
Standard deviation	.93	.84

Table 56.—Total Reducing Sugars (Method 34)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	4. 99	2. 71	2. 28
Lugs	X2L	18. 54	19. 64	1. 10
	X2F	18. 11	18. 60	. 49
	X4L	13. 11	12. 19	. 92
	X4F	12. 49	12. 41	. 08
Average		15. 56	15. 71	. 15
Cutters	C2L	25. 60	22. 82	2. 78
	C2F		22. 58	
	C4L	24. 40	20. 90	3. 50
	C4F	21. 40	21. 64	. 24
Average		23. 80	21. 79	2. 01
Smoking Leaf	H2L	20. 59	20. 63	. 04
	H4L	20. 07	17. 08	2. 99
	H4F	19. 66	15. 83	3. 83
	H4R	15. 91	10. 66	5. 25
	H6F	13. 52	10. 60	2. 92
	H6R	10. 68	6. 28	4. 40
Average		16. 74	14. 96	1. 78
Leaf	B2L	25. 40	23. 14	2. 26
	B2F		23. 10	
	B2R	17. 51		
	B4L	24. 28	21. 14	3. 14
	B4F	21. 82	17. 79	4. 03
	B4R	18. 02	12. 61	5. 41
	B4S	15. 42	10. 87	4. 55
	B6F	22. 87	13. 18	9. 69
	B6R	15. 19	8. 68	6. 51
	B6S	13. 21		
Average		20. 43	15. 34	5. 09
Nondescript	N1D	7. 75	3. 64	4. 11

Analysis of data	1952 crop	1954 crop
High	25. 60	22. 82
Low	4. 99	2. 71
Range	20. 61	20. 11
Average	17. 72	14. 68
High/low ratio	5. 13	8. 42
Coefficient of variation (percent)	24. 9	34. 2
Standard deviation	4. 41	5. 02

Table 57.—Total Volatile Acids (as Acetic Acid) (Method 35)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	2.99	1.98	1.01
Lugs	X2L	3.07	1.67	1.40
	X2F	3.08	1.58	1.50
	X4L	2.97	1.54	1.43
	X4F	3.01	1.63	1.38
Average		3.03	1.60	1.43
Cutters	C2L	3.01	2.03	.98
	C2F		1.11	
	C4L	2.64	1.81	.83
	C4F	2.34	1.66	.68
Average		2.66	1.83	.83
Smoking Leaf	H2L	1.90	1.64	.26
	H4L	2.54	1.53	1.01
	H4F	1.97	1.76	.21
	H4R	2.79	1.63	1.16
	H6F	2.57	1.77	.80
	H6R	2.40	1.82	.58
Average		2.36	1.69	.67
Leaf	B2L	2.12	1.36	.76
	B2F		1.45	
	B2R	2.30		
	B4L	2.41	1.37	1.04
	B4F	2.54	1.32	1.22
	B4R	2.53	1.57	.96
	B4S	2.19	1.48	.71
	B6F	2.24	1.50	.74
	B6R	2.41	1.95	.46
	B6S	2.26		
Average		2.35	1.51	.84
Nondescript	N1D	2.25	1.96	.29

Analysis of data	1952 crop	1954 crop
High	3.08	2.03
Low	1.90	1.11
Range	1.18	.92
Average	2.54	1.66
High/low ratio	1.62	1.83
Coefficient of variation (percent)	13.8	11.4
Standard deviation	.35	.19

Table 58.—Total Volatile Bases (as Ammonia) (Method 36)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0. 554	0. 502	0. 038
Lugs	X2L	. 401	. 399	. 002
	X2F	. 436	. 475	. 039
	X4L	. 439	. 482	. 043
	X4F	. 491	. 563	. 072
Average		. 442	. 480	. 038
Cutters	C2L	. 301	. 349	. 048
	C2F		. 421	
	C4L	. 341	. 383	. 042
	C4F	. 366	. 413	. 047
Average		. 336	. 382	. 046
Smoking Leaf	H2L	. 373	. 395	. 022
	H4L	. 406	. 451	. 045
	H4F	. 467	. 527	. 060
	H4R	. 521	. 761	. 240
	H6F	. 495	. 613	. 118
	H6R	. 537	. 788	. 251
Average		. 466	. 589	. 123
Leaf	B2L	. 336	. 351	. 015
	B2F		. 422	
	B2R	. 631		
	B4L	. 341	. 418	. 077
	B4F	. 424	. 541	. 117
	B4R	. 593	. 805	. 212
	B4S	. 671	. 942	. 271
	B6F	. 405	. 613	. 208
	B6R	. 558	. 827	. 269
	B6S	. 627		
Average		. 475	. 642	. 167
Nondescript	N1D	. 589	. 865	. 276

Analysis of data	1952 crop	1954 crop
High	0. 671	0. 942
Low	. 301	. 349
Range	. 370	. 593
Average	. 457	. 571
High/low ratio	2. 23	2. 70
Coefficient of variation (percent)	19. 7	29. 8
Standard deviation	. 09	. 17

Table 59.—Uronic Acids (as Anhydrides) (Method 37)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	13. 48	12. 85	0. 63
Lugs	X2L	9. 35	9. 40	. 05
	X2F	10. 27	9. 58	. 69
	X4L	10. 75	10. 30	. 45
	X4F	10. 66	10. 68	. 02
Average		10. 26	9. 99	. 27
Cutters	C2L	9. 42	9. 21	. 21
	C2F		9. 28	
	C4L	9. 60	9. 41	. 19
	C4F	9. 21	9. 14	. 07
Average		9. 41	9. 25	. 16
Smoking Leaf	H2L	10. 06	10. 18	. 12
	H4L	10. 40	10. 11	. 29
	H4F	10. 39	10. 52	. 13
	H4R	10. 88	11. 38	. 50
	H6F	11. 94	11. 33	. 61
	H6R	11. 68	11. 68	0
Average		10. 89	10. 87	. 02
Leaf	B2L	9. 00	8. 05	. 95
	B2F		8. 49	
	B2R	10. 06		
	B4L	8. 72	9. 33	. 61
	B4F	9. 46	9. 94	. 48
	B4R	10. 26	10. 69	. 43
	B4S	10. 36	10. 80	. 44
	B6F	9. 39	11. 02	1. 63
	B6R	10. 93	11. 26	. 33
	B6S	11. 41		
Average		9. 73	10. 16	. 43
Nondescript	N1D	12. 76	12. 11	. 65

Analysis of data	1952 crop	1954 crop
High	13. 48	12. 85
Low	8. 72	8. 05
Range	4. 76	4. 80
Average	10. 41	10. 41
High/low ratio	1. 55	1. 60
Coefficient of variation (percent)	8. 1	8. 8
Standard deviation	. 84	. 92

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Table 60.—Water-Soluble Acids (Method 38)

[Ml. of 0.1N NaOH per gram of moisture-free and sand-free tobacco]

Group	U.S. grade	Milliliters		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	3. 86	3. 33	0 53
Lugs	X2L	4. 26	3. 61	. 65
	X2F	4. 46	4. 09	. 37
	X4L	4. 26	3. 90	36
	X4F	4. 48	4. 19	. 29
Average		4. 36	3. 95	. 41
Cutters	C2L	4. 21	3. 34	. 87
	C2F		. 73	
	C4L	4. 52	3. 48	1. 04
	C4F	4. 32	3. 74	. 58
Average		4. 35	3. 52	. 83
Smoking Leaf	H2L	4. 20	3. 69	. 51
	H4L	4. 09	3. 98	. 11
	H4F	4. 51	4. 43	. 08
	H4R	4. 92	5. 50	. 58
	H6F	4. 50	4. 48	. 02
	H6R	4. 28	4. 99	. 71
Average		4. 42	4. 51	. 09
Leaf	B2L	3. 85	3. 25	. 60
	B2F		3. 77	
	B2R	5. 85		
	B4L	3. 75	3. 72	. 03
	B4F	4. 27	4. 53	. 26
	B4R	5 42	5. 86	. 44
	B4S	6. 36	6 44	. 08
	B6F	4. 16	4. 96	. 80
	B6R	4. 90	5. 67	. 77
	B6S	5. 01		
Average		4. 67	4. 92	. 25
Nondescript	N1D	4. 20	4. 84	. 64

Analysis of data	1952 crop	1954 crop
High	6. 36	6. 44
Low	3. 75	3. 25
Range	2 61	3. 19
Average	4. 44	4. 36
High/low ratio	1. 70	1. 98
Coefficient of variation (percent)	12. 8	20. 0
Standard deviation	. 57	. 87

Table 61.—Water-Insoluble Ash (Method 32)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	13.4	12.6	0.8
Lugs	X2L	10.0	7.4	2.6
	X2F	10.0	8.0	2.0
	X4L	11.1	10.0	1.1
	X4F	11.0	9.5	1.5
Average		10.5	8.7	1.8
Cutters	C2L	5.9	6.1	.2
	C2F		6.3	
	C4L	6.5	6.9	.4
	C4F	7.1	6.6	.5
Average		6.5	6.5	0
Smoking Leaf	H2L	7.0	6.5	.5
	H4L	7.6	6.6	1.0
	H4F	7.9	7.1	.8
	H4R	7.4	6.8	.6
	H6F	7.5	7.4	.1
	H6R	7.2	7.8	.6
Average		7.4	7.0	.4
Leaf	B2L	5.6	5.1	.5
	B2F		5.9	
	B2R	6.5		
	B4L	5.4	5.7	.3
	B4F	6.3	6.2	.1
	B4R	6.0	6.5	.5
	B4S	6.3	5.7	.6
	B6F	5.5	6.0	.5
	B6R	6.4	6.4	0
	B6S	6.5		
Average		5.9	5.9	0
Nondescript	N1D	8.7	7.4	1.3

Analysis of data	1952 crop	1954 crop
High	13.4	12.6
Low	5.4	5.1
Range	8.0	7.5
Average	7.7	7.2
High/low ratio	2.5	2.5
Coefficient of variation (percent)	23.4	16.7
Standard deviation	1.8	1.2

Table 62.—Water-Soluble Ash (Method 32)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	5.7	5.8	0.1
Lugs	X2L	4.1	4.6	.5
	X2F	4.3	4.4	.1
	X4L	5.2	5.2	0
	X4F	5.2	4.7	.5
Average		4.7	4.7	0
Cutters	C2L	4.7	4.5	.2
	C2F		4.4	
	C4L	4.6	4.9	.3
	C4F	4.8	4.6	.2
Average		4.7	4.7	0
Smoking Leaf	H2L	5.0	4.7	.3
	H4L	4.9	4.2	.7
	H4F	4.7	4.3	.4
	H4R	5.1	4.2	.9
	H6F	4.7	4.2	.5
	H6R	4.8	3.9	.9
Average		4.8	4.2	.6
Leaf	B2L	4.0	3.8	.2
	B2F		3.6	
	B2R	4.2		
	B4L	3.9	3.3	.6
	B4F	4.1	3.8	.3
	B4R	4.5	4.0	.5
	B4S	4.3	4.5	.2
	B6F	3.9	3.7	.2
	B6R	4.0	3.9	.1
	B6S	4.2		
Average		4.1	3.8	.3
Nondescript	N1D	4.5	4.2	.3

Analysis of data	1952 crop	1954 crop
High	5.7	5.8
Low	3.9	3.3
Range	1.8	2.5
Average	4.6	4.3
High/low ratio	1.5	1.8
Coefficient of variation (percent)	10.9	11.6
Standard deviation	.5	.5

Table 63.—Water-Soluble Nitrogen (other than Nitrate Nitrogen) (Method 39)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	1.85	1.67	0.13
Lugs	X2L	1.44	1.32	.12
	X2F	1.62	1.50	.12
	X4L	1.62	1.54	.08
	X4F	1.77	1.60	.17
Average		1.61	1.49	.12
Cutters	C2L	1.35	1.22	.13
	C2F		1.34	
	C4L	1.41	1.26	.15
	C4F	1.48	1.37	.11
Average		1.41	1.28	.13
Smoking leaf	H2L	1.47	1.36	.11
	H4L	1.55	1.41	.14
	H4F	1.71	1.58	.13
	H4R	1.85	2.15	.30
	H6F	1.96	1.79	.17
	H6R	1.97	2.12	.15
Average		1.75	1.74	.01
Leaf	B2L	1.50	1.18	.32
	B2F		1.38	
	B2R	2.20		
	B4L	1.46	1.35	.11
	B4F	1.74	1.73	.01
	B4R	1.98	2.13	.15
	B4S	2.24	2.58	.34
	B6F	1.75	1.72	.03
	B6R	2.02	2.07	.05
	B6S	2.26		
Average		1.81	1.82	.01
Nondescript	N1D	2.10	2.26	.16

Analysis of data	1952 crop	1954 crop
High	2.26	2.58
Low	1.35	1.18
Range	.91	1.40
Average	1.72	1.68
High/low ratio	1.67	2.19
Coefficient of variation (percent)	14.0	22.0
Standard deviation	.24	.37

Table 64.—Waxes (Method 19)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Percent		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	0.38	0.40	0.02
Lugs	X2L	.36	.33	.03
	X2F	.31	.38	.07
	X4L	.38	.42	.04
	X4F	.43	.45	.02
Average		.37	.40	.03
Cutters	C2L	.45	.27	.18
	C2F		.29	
	C4L	.27	.38	.11
	C4F	.29	.36	.07
Average		.34	.34	0
Smoking Leaf	H2L	.10	.36	.26
	H4L	.18	.37	.19
	H4F	.23	.29	.06
	H4R	.23	.29	.06
	H6F	.25	.32	.07
	H6R	.35	.34	.01
Average		.22	.33	.11
Leaf	B2L	.34	.26	.08
	B2F		.22	
	B2R	.29		
	B4L	.16	.23	.07
	B4F	.29	.25	.04
	B4R	.27	.22	.05
	B4S	.23	.22	.01
	B6F	.12	.32	.20
	B6R	.30	.34	.04
	B6S	.32		
Average		.24	.26	.02
Nondescript	N1D	.27	.36	.09

Analysis of data	1952 crop	1954 crop
High	0.45	0.45
Low	.10	.22
Range	.35	.23
Average	.28	.33
High/low ratio	4.5	2.0
Coefficient of variation (percent)	32.1	18.2
Standard deviation	.09	.06

Table 65.—Xanthophyll (Method 22)

[All results calculated on a moisture-free and sand-free basis]

Group	U.S. grade	Micrograms per gram		
		1952 crop	1954 crop	Difference between 1952 and 1954
Nondescript	N1L	20	82	62
Lugs	X2L	15	44	29
	X2F	16	42	26
	X4L	12	56	44
	X4F	18	59	41
Average		15	50	35
Cutters	C2L	13	56	43
	C2F		61	
	C4L	14	48	34
	C4F	18	64	46
Average		15	56	41
Smoking Leaf	H2L	21	61	40
	H4L	21	64	43
	H4F	24	63	39
	H4R	21	68	47
	H6F	28	77	49
	H6R	25	76	51
Average		23	68	45
Leaf	B2L	14	46	32
	B2F		50	
	B2R	19		
	B4L	14	58	44
	B4F	19	59	40
	B4R	25	68	43
	B4S	23	60	37
	B6F	16	50	34
	B6R	10	56	46
	B6S	23		
Average		17	57	40
Nondescript	N1D	17	55	38

Analysis of data	1952 crop	1954 crop
High	28	82
Low	10	42
Range	18	40
Average	18	60
High/low ratio	2.8	2.0
Coefficient of variation (percent)	26.7	16.0
Standard deviation	4.8	9.6

ANALYTICAL METHODS

Method 1.—Alcohol Extract

P. Lorillard Co., Inc.

DETERMINATION

Heat an alundum extraction thimble (34-mm. diameter, 100-mm. high, medium porosity) at 450° C. in a muffle furnace for 1 hour, cool in a desiccator containing anhydrous $CaCl_2$, and weigh. Transfer a 2-gram sample (M-&S-F basis) to the extraction thimble and extract for 24 hours in a Soxhlet extraction apparatus with 95 percent ethanol. Place extraction thimble and contents in a 150-ml. beaker and heat on the steam bath until substantially all of the alcohol has evaporated. Dry the extraction thimble and contents overnight at 100° C. in an air-circulating electric oven, cool in desiccator over anhydrous $CaCl_2$, and weigh. Calculate the percentage loss due to the extraction with 95 percent ethanol.

Method 2.—Alpha Amino Nitrogen

Liggett & Myers, Tobacco Co.

DETERMINATION

Preparation of extract A.—Place 6.67 grams of ground tobacco (M-&S-F basis) 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 allow to 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 [3] by the Van Slyke Method (*3*). Calculate the results as follows:

$$\frac{(\text{ml. } N_2 - \text{blank}) \times F \times 100}{\text{weight of M-\&S-F sample}} = \% \text{ alpha amino nitrogen (M-\&S-F)}$$

Where:

$$F = \tfrac{1}{2}\left[\frac{(P-W)}{760}\right] \times \frac{273}{T} \times 0.00125$$

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.

[3] Save remainder of extract A for the determination of water-soluble acids (method 30) and pH (method 20).

Method 3.—Calcium

Eastern Utilization Research and Development Division, ARS

APPARATUS

(1) Atomizer and burner of our design.

(2) No. 3486 yellow shade yellow plus No. 5120 pidymium plus 550 mμ interference filters.

(3) Photovolt multiplier photometer equipped with 1P21 multiplier phototube.

DETERMINATION

Ashing of sample.—Weigh accurately about 1 gram of M-&S-F sample into a platinum dish, moisten with 10 ml. of 5 percent sulfuric acid, char under an infrared lamp until SO_3 fumes cease, and ash overnight at 600° C.

Solution of ash.—Add approximately 30 ml. of 1+9 HCl, evaporate to dryness on steam bath, add 20 ml. of 1+9 HCl, warm on steam bath while stirring to dissolve ash, decant into a funnel containing washed filter paper, and collect filtrate in a 100-ml. volumetric flask. Repeat solution step with a second 20-ml. portion of 1+9 HCl. Transfer residue to filter, wash dish and filter with 1+9 HCl until volume of filtrate approaches 100 ml., cool solution to room temperature, and make to volume with 1+9 HCl.

Flame spectrophotometric procedure.—To eliminate the effect of anions on the emission of the calcium, treat the solutions with an anion exchange resin (Amberlite IR–4B). Add approximately 4 grams of the resin to a 100-ml. beaker; then 10 ml. of the sample and 20 ml. of water. Stir the mixture and let stand for 5 minutes. Filter through a dry filter paper and use the filtrate for calcium determination. Aspirate the treated solution into flame and read the intensity of emission of the light that passes through the three filters.

Prepare a standard curve (p.p.m. Ca vs. Intensity), using solutions of Ca containing similar amounts of HCl and treated in the same way as the samples. If the standard curve approximates a straight line, within estimated experimental error, use the equation for a straight line ($y=mx+b$) to calculate the amount of Ca in the sample. If the curve does not approximate a straight line, the p.p.m. of Ca in the sample solution may be determined by interpolation. In either case, the observed intensity must be corrected for variations in operating conditions (atomization rate, flame temperature, etc.) by determining the flame intensity of a single standard calcium solution (Z) at regular intervals during a series of determinations.

The solution Z is also compared with the solutions used to obtain the standard curve. The observed intensity of the sample solution is adjusted by multiplying by the factor I_1/I_2, where I_1 is the flame intensity for solution Z determined at the same time and under the same conditions as the standard solutions and I_2 is the flame intensity of solution Z determined at the

same time the sample solutions were analyzed. Calculate the calcium content as follows:

$$\frac{\text{(p.p.m. Ca) (vol. of sample solution)} \times 100}{\text{weight of M-\&S-F sample} \times 10^6} = \%\ \text{calcium (M-\&S-F)}$$

Method 4.—Calcium

The Pennsylvania State University

REAGENTS AND SOLUTIONS

(1) *Hydrochloric acid,* 1 volume of concentrated acid and 4 volumes of water.

(2) *Acetic acid,* concentrated.

(3) *Ammonium oxalate solution,* saturated aqueous.

(4) *Sulfuric acid solution,* 1 volume of sulfuric acid and 4 volumes of water.

DETERMINATION

Ignite a 4.4-gram sample (M-&S-F basis) in silica dish in muffle furnace maintained at 500° C. overnight. Dissolve ash in about 25 ml. of the dilute hydrochloric acid and transfer to 100-ml. beaker, heat to boiling, filter into a 110-ml. volumetric flask, and dilute to mark with water. Transfer a 50-ml. aliquot to a 250-ml. beaker, add ammonium hydroxide until the iron and aluminum hydroxides start to precipitate, and add immediately 10 ml. of acetic acid. Heat to boiling, add 10 ml. of ammonium oxalate solution, and boil until the precipitate is coarsely granular. Cool and allow to stand overnight.

Filter through S. & S. No. 589 Blue Ribbon filter paper and wash with water at room temperature until the filtrate is free from oxalates. Break the point of the filter with a platinum wire and wash the precipitate into the beaker in which the calcium was precipitated with hot sulfuric acid solution. Then wash with hot water. Add about 10 ml. of the sulfuric acid solution, heat to about 90° C., and titrate with $N/10$ potassium permanganate solution. Finally, add the filter paper to the solution and complete the titration. Calculate the results as follows:

$$\frac{\text{ml. of } N/10\ KMnO_4 \text{ required} \times 0.2004}{\text{weight of M-\&S-F sample}} = \%\ \text{calcium (M-\&S-F)}$$

Method 5.—Calcium, Magnesium, Potassium, and Phosphorus (*32, 33*)

North Carolina State College

APPARATUS

Beckman model B Spectrophotometer or equal.

Reagents and Solutions

(1) *Nitric acid,* C.P., concentrated (sp. gr. 1.42).

(2) *Perchloric acid,* C.P., 75 percent.

(3) *Sodium acetate,* C.P. solution, 20 percent, preserved by adding a crystal of thymol to the solution.

(4) *Brom cresol green indicator solution.*—Grind 0.0160-gram brom cresol green in a glass mortar with 2.3 ml. of *N*/100 NaOH solution. When dissolved, dilute to 100 ml. with distilled water.

(5) *Ammonium oxalate,* C.P. solution, 4 percent. Store in the refrigerator.

(6) *Sulfuric acid,* approximately *N*/1.

(7) *Wash solution* (for calcium determination). Mix together 500 ml. of ether, 500 ml. of ethanol (95 percent), 500 ml. of distilled water, and 30 ml. of concentrated ammonium hydroxide, C.P. (sp. gr. 0.90).

(8) *Cerous sulfate,* 0.2 percent in approximately *N*/1 sulfuric acid. Dissolve 4 grams of $Ce_2(SO_4)_3 \cdot 8H_2O$ (G. Frederick Smith Chemical Co., Columbus, Ohio) in approximately 300 ml. of distilled water and 55.8 ml. of concentrated sulfuric acid with heating. Cool and dilute to 2 liters.

(9) *Ceric sulfate stock solution.*—Weigh 3.2072 grams of oven-dried $Ce(HSO_4)_4$ (G. Frederick Smith Chemical Co., Columbus, Ohio). Dissolve and dilute to 1 liter with the 0.2 percent cerous sulfate-sulfuric acid solution. This solution is approximately 0.0081 *N* as standardized against sodium oxalate and is stable for at least 10 months.

(10) *Ceric sulfate working solution.*—Dilute 3 volumes of the ceric sulfate stock solution to 20 volumes with the 0.2 percent cerous sulfate solution. Ceric sulfate solutions prepared in this manner give a working range between 0.10 to 0.20 mg. Ca.

(11) *Primary standard sodium oxalate.*—Weigh exactly 334.3 mg. of oven-dried Bureau of Standards sodium oxalate and dissolve in 1 liter of approximately normal sulfuric acid. Two ml. of this solution is equivalent to 0.20 mg. Ca. For the preparation of calibration curves, 10-, 12-, 14-, 16-, and 18-ml. portions of the primary standard are diluted to 20 ml. with *N*/1 H_2SO_4. Two ml. portions of these dilutions are equivalent to 0.10, 0.12, 0.14, 0.16, and 0.18, mg. Ca, respectively. Two ml. of the undiluted standard furnishes the sixth datum for the calibration.

(12) *Ammonium molybdate,* 5 percent aqueous solution.

(13) *Ammonium vanadate,* 0.25 percent solution. Dissolve 2.5 grams of ammonium vanadate in about 500 ml. of boiling water. Cool and add 250 ml. of concentrated nitric acid. Cool and make to 1 liter.

(14) *Primary standard phosphate stock solution.*—Dissolve in distilled water exactly 0.3509 gram of reagent grade KH_2PO_4, which has been previously dried to constant weight over anhydrous calcium chloride in a desiccator. To this, add 10 ml. of 10 *N* sulfuric acid, cool, and dilute to 1 liter. One ml. of this solution contains 80 mcg. phosphorus.

(15) *Phosphate working standard,* 16 mcg. per ml. Transfer 200 ml. of the phosphate stock solution to a 1-liter flask. Add 38 ml. of 10 *N* H_2SO_4, cool, and dilute to 1 liter.

(16) *Phosphate working standard,* 12 mcg. per ml. Transfer 150 ml. of the phosphate stock solution to a 1-liter flask. Add 38.5 ml. of 10 *N* H_2SO_4, cool, and dilute to 1 liter.

(17) *Phosphate working standard,* 8 mcg. per ml. Transfer 100 ml. of the phosphate stock solution to a 1-liter flask. And 39 ml. of 10 *N* sulfuric acid, cool, and dilute with water to 1 liter.

(18) *Phosphate working standard,* 4 mcg. per ml. Transfer 50 ml. of the phosphate stock solution to a 1-liter flask. Add 39.5 ml. of 10 *N* sulfuric acid, cool, and dilute to 1 liter.

(19) *Potassium dihydrogen phosphate,* KH_2PO_4, 2 percent aqueous solution.

(20) *Wash solution* (for magnesium determination). To 200 ml. of 95 percent ethanol, add 50 ml. of ammonium hydroxide and make to a volume of 1 liter with distilled water.

DETERMINATION

Wet Ashing.—(Conduct ashing in a well-ventilated hood.) Transfer a 300-mg. sample of the ground tobacco to a clean Pyrex test tube (25 by 250 mm.) and add 5 ml. of concentrated nitric acid. Insert the test tube to a depth of approximately 5 inches, at about a 45° inclination, into a sand bath. Heat the sand bath on an electric hot plate to medium heat, and digest until the sample is well gelatinized (usually about 20 to 30 minutes). Remove the tube from the sand bath, allow to cool, and add 3 ml. of 75 percent perchloric acid.

Insert again in the sand bath (protect face with a plastic shield), turn the hot plate to high heat, and continue the digestion until the solution is colorless and the volume has been reduced to 1 to 2 ml. Allow to cool, add about 10 ml. of distilled water, heat, and filter through ashless paper, collecting the filtrate in a 100-ml. volumetric flask. Wash any residual material from the tube and rinse three times with distilled water. Wash the filter paper 10 times with hot distilled water, collecting the washings in the same 100-ml. volumetric flask. Allow to cool and dilute with distilled water to the mark. This is solution A.

Calcium.—Pipet 2 to 5 ml. aliquots (depending upon the amount of calcium expected) of solution A into a conical-tipped centrifuge tube. If less than 5 ml. is used, add distilled water to make the total volume 5 ml. Add in turn 1 ml. of the 20 percent sodium acetate solution, 0.25 ml. of the brom cresol green indicator, and 1 ml. of 4 percent ammonium oxalate solution. Mix by blowing a gentle stream of air through a fine capillary immersed to the bottom of the tube. Adjust to the sky-blue color of the indicator at pH 5.0 to 5.5 by adding drops of dilute HCl or NH_4OH as required, mixing after each addition.

For ash solutions prepared as above, a few drops of dilute acid will usually suffice. This pH adjustment for precipitation is not extremely criti-

cal. However, if the solution is too acid, complete precipitation of the calcium will not be effected; if too alkaline, coprecipitation of magnesium may result. Cover the tubes and allow to stand overnight.

Centrifuge at 2,000 r.p.m. for 8 minutes. Carefully decant the supernatant liquid into another conical tube and retain for magnesium determination. This is filtrate B. Retain the tube in an inclined position, at a sufficient angle to prevent dislodgment of the precipitate, and allow to drain for 5 minutes. Rinse down the walls of the tube with approximately 3 ml. of the ether-alcohol wash solution from a wash bottle. Stir the precipitate by twirling a hook-shaped fine stirring rod, rinsing the hook in the upper layers of the wash solution. Centrifuge at 2,000 r.p.m. for 8 minutes. Decant by inclining the tube as before and drain for 5 minutes in the inclined position. Repeat the washing. After the tubes have drained for 10–15 minutes, dry in an oven at 100° C. for 1 hour.

Dissolve the washed and dried calcium oxalate in exactly 2 ml. of N/1 sulfuric acid. Solution of the precipitate may be facilitated by immersing the tubes in a boiling water bath for 5 minutes. Add exactly 10 ml. of the ceric sulfate working solution and mix the contents thoroughly by stoppering the tube and inverting repeatedly. The presence of the cerous ion catalyzes the reaction so that oxidation is rapid and complete at room temperature. After 5 minutes determine the residual ceric concentration spectrophotometrically. These solutions are stable for at least 3 to 4 hours.

Prepare at the same time 2-ml. portions of the sodium oxalate standards as indicated under reagent No. 11, oxidize with 10-ml. volumes of the ceric solution, and determine the residual optical densities. Plot a calibration curve from the optical density values. The calcium content may be read directly from the curve or, preferably, may be computed from the linear regression equation representing the calibration data.

The maximum absorption of the ceric ion is at 315 mμ. However, it has been demonstrated that the precision of the determination is approximately as good at 370 mμ. The calcium data in this case were determined at 370 mμ, using the Beckman model B spectrophotometer.

Phosphorus.—Pipet 5 ml. of solution A into a test tube. Measure into other test tubes 5 ml. of each of the phosphate standard solutions. In another tube, include a blank of 5 ml. of distilled water. Add 1 ml. of ammonium vanadate to each tube, including the blank. Next add 1 ml. of the ammonium molybdate to each tube but stopper and shake vigorously for 30 seconds immediately following the addition of the molybdate before proceeding to the next tube.

The yellow color that develops is stable for several hours. Allow the tubes to stand for 5 minutes, and measure their optical densities at 420 mμ. on the model B spectrophotometer, using the reagent blank as the reference solution.

The phosphorus content of the analytical solution can be read directly from the calibration curve which was plotted from the data for the standard solutions. The preferred procedure, however, is to determine the regression equation from the calibration data and calculate the phosphorus contents of the unknowns from this equation.

Magnesium.—Add 1 ml. of 2 percent KH_2PO_4 solution to the decanted filtrate (filtrate B) from the calcium precipitation, followed by 1 ml. of concentrated ammonium hydroxide. Shake vigorously for 30 seconds and allow to stand overnight. Centrifuge at 2,000 r.p.m. for 15 minutes. Decant the tube at a sufficient angle to retain the precipitate and allow to drain for 5 minutes. Wash down the sides with about 5 ml. of the wash solution. Centrifuge, decant, and drain as before. Repeat the washing. After the final draining, dry the tubes in an oven at 100° C. for 20–30 minutes.

Dissolve the washed and dried magnesium ammonium phosphate in 1 ml. of the acidified ammonium vanadate solution. Add 5 ml. of distilled water. Proceed as in the phosphorus determination above, adding 1 ml. of the ammonium molybdate reagent. Prepare a calibration curve at the same time, in the terms of phosphorus, using 5 ml. of each of the phosphate standard solutions. Convert the phosphorus found in the analytical samples to magnesium by multiplying by the factor 0.7850.

Potassium.—Determine the potassium in solution A by flamephotometry method 23.

Method 6.—Cellulose (*18, 24*)

Standard Branch, Tobacco Division, AMS

DETERMINATION

Transfer 2 grams (M-&S-F equivalent weight) of tobacco to a 200-ml. Erlenmeyer flask which has a standard ground-glass taper, and add 80 ml. of 95 percent ethanol. Add, while swirling the flask, 20 ml. of concentrated nitric acid (sp. gr. 1.42). Connect flask to reflux condenser and reflux in a boiling water bath for 1 hour, stirring occasionally by lifting the flask and rotating. Transfer the material to a 250-ml. beaker, using a stream of alcohol from a wash bottle. Decant under suction through a tared 50-ml. fritted-glass crucible of porosity *M*, which has been fitted to a 1000-ml. suction flask. (Before using the same crucible for another sample, recheck its tare weight since the normal loss in this procedure is approximately 2 to 6 mg.)

Finally wash the residual material from the beaker into the crucible with a stream of alcohol from the wash bottle. Measure 80 ml. of alcohol into the wash bottle and use this amount to transfer the material from the crucible back into the original Erlenmeyer flask. Add 20 ml. of nitric acid as before and repeat the above refluxing and washing twice more, or a total of three times. After the third refluxing, transfer the cellulose material to the crucible, and wash the material in the crucible three times with alcohol.

During each alcohol wash, fill the crucible about two-thirds full and allow to stand at atmospheric pressure from 3 to 5 minutes, stirring occasionally with a fire-polished glass rod before drawing off the alcohol. In the washing procedure do not draw off the liquid so completely that the material packs. Then wash in the same manner 5 times with distilled water. Allow the crucible to stand in the 250-ml. beaker during the soaking and stirring period to catch the leakage. If the successive alcohol and water washes cannot be completed without overnight interruption, the material should be allowed to stand in the Erlenmeyer flask, since the material tends to dry to a consistency that is difficult to disintegrate and wash effectively with water.

Dry crucible with contents overnight in an oven at 100° C. and allow to stand in a desiccator over calcium chloride for 1 hour. Weigh in a tared weighing bottle, as the material is hygroscopic, and determine the net weight of the crude cellulose. Ash by placing crucible and contents in a thermostatically controlled muffle furnace and heating for 1 hour after the temperature reaches 550° C. Place crucible on a wire gauze for 3 to 5 minutes for preliminary cooling and then put it in a desiccator over calcium chloride for 1 hour. Determine weight of ash, and subtract the ash from the weight of the crude cellulose to obtain the weight of ash-free cellulose. Calculate the percentage of ash-free cellulose as follows:

$$\frac{\text{ash-free cellulose} \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{cellulose (M-\&S-F)}$$

Method 7.—Chlorine (as chlorides)

Philip Morris, Inc.

APPARATUS

Fisher Titrimeter equipped with silver and glass electrodes.

REAGENTS AND SOLUTIONS

(1) *Nitric acid,* dilute. Add 1 volume of concentrated nitric acid to 9 volumes of distilled water.

(2) *Silver nitrate solution,* 0.05 *N*.

DETERMINATION

Weigh accurately 2 grams of ground tobacco (M-&S-F basis) into a 300-ml. beaker and add 100 ml. of distilled water. Allow the mixture to stand for at least 5 minutes, stirring two or three times during that period. Pipet 5 ml. of the dilute nitric acid into the mixture and titrate potentiometrically with the standard silver nitrate solution to a potential of −105 mv. Calculate the results as follows:

$$\frac{\text{normality of } AgNO_3 \times \text{ml. } AgNO_3 \times 3.55}{\text{weight of M-\&S-F sample (grams)}} = \%\ \text{chlorine (M-\&S-F)}$$

Method 8.—Crude Fiber (*1*)

The Imperial Tobacco Co., Ltd.

DETERMINATION

Place a 2-gram sample (M-&S-F basis) into a 1-liter Erlenmeyer flask. Add 50 ml. of petroleum ether (boiling range 40°–60° C.), cover with watch glass, and allow to stand overnight.

Filter off and keep paper. Add another 50 ml. of petroleum ether to sample, mix gently, and allow to stand for a few minutes. Filter through original paper and allow to dry. Brush all adhering particles from paper into flask containing the tobacco. Warm flask on water bath until all petroleum ether vapor has entirely evaporated.

Heat 200 ml. of sulfuric acid solution (12.5 grams per liter) in a beaker to boiling and add to the tobacco. Connect to the condenser and bring to boil again within 1 minute and boil exactly for 30 minutes. Rotate the flask frequently to mix contents and to remove particles from the sides.

Filter into a Buchner funnel using Whatman No. 541 paper with a disc of butter muslin under it. The filtration of the bulk of solution must be completed within 10 minutes. Wash with hot water until acid free.

Previously heat 200 ml. of caustic soda (12.5 grams per liter), transfer to a wash bottle and wash all contents of funnel back into the original flask with the caustic soda solution, pouring the remainder of the solution into the flask. Bring to boil quickly as before and boil for exactly 30 minutes.

Filter through Whatman No. 541 filter paper (7-cm. diameter), washing contents of flask into the funnel with hot water. No muslin is used this time. Wash successively: once with 1 percent HCl, with hot water until acid free, three times with ethanol, and once with ether. Aspirate until dry. Transfer the fiber quantitatively from the paper to a small silica dish.

Dry the crude fiber in an electric oven at 100° C. for 1 hour, cool in desiccator, and weigh. Return it to the oven for one-half hour and reweigh. If necessary to obtain constant weight, dry for a further one-half hour and reweigh.

Char the dried crude fiber over a low flame and complete the combustion at a dull red heat. Cool in desiccator and determine the weight of the ash.

Calculation.—Calculate the results as follows:

$$\frac{\text{weight of crude fiber} - \text{weight of ash} \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{crude fiber (M-\&S-F)}$$

Method 9.—Formic acid

Brown & Williamson Tobacco Corp.

APPARATUS

The apparatus is described and illustrated in the A.O.A.C. Book of Methods (*4*).

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 1 liter of solution and filter.

DETERMINATION

Place a sample of 10 grams (M-&S-F 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, allowing vapor to pass first through sample in flask A and 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 1 liter of distillate is collected, maintaining the volume of liquids in flasks A and B as nearly constant as possible by heating with small Bunsen flames and avoiding charring of sample in flask A.

Disconnect apparatus and filter contents of flask B while hot, then wash the barium carborate with a little hot water. Filtrate and washings should measure about 150 ml., and 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. 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 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, 1 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. Heat 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:

$$\frac{\text{wt. of mercurous chloride precipitate} \times 0.0975 \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{formic acid (M-\&S-F)}$$

Method 10.—Hot-water Extract

Eastern Utilization Research and Development Division, ARS

DETERMINATION

Hot-water extract.—Thoroughly mix extract A, as prepared for the determination of tannin (method 30), and pipet at once a 100-ml. aliquot into a

weighed, flat-bottomed, glass dish 70 mm. in diameter. Evaporate and dry in a circulating-air-type electric oven at 100° C. (±0.5°) for 17 hours. Transfer dish and contents to a desiccator (inside diameter 4 inches) containing Drierite; cool and weigh. Calculate the percentage of hot-water extract.

Method 11.—Lignin (*21, 34*)

Standards Branch, Tobacco Division, AMS

REAGENTS

(1) *1 percent hydrochloric acid,* 111 grams of concentrated hydrochloric acid (d. 1.19) in 3,890 ml. of distilled water.

(2) *72 percent sulfuric acid,* 660 ml. of concentrated sulfuric acid (d. 1.84) in 411 ml. of distilled water.

DETERMINATION

Place a sample of 7.50 grams (M-&S-F equivalent weight) in an 80 by 33 mm. paper extraction thimble, insert a piece of absorbent cotton in the mouth of the thimble, and extract with 95 percent ethanol in a Soxhlet extraction apparatus for 8 hours. Then extract sample for 4 hours in the same apparatus with an ethanol-benzene solution (1: 2 by weight). Dry thimble and contents on steam bath until the odor of ethanol and benzene can no longer be detected.

Transfer the extracted tobacco, quantitatively, to a 1-liter Erlenmeyer flask and add 750 ml. of the 1 percent hydrochloric acid and a few drops of capryl alcohol. Boil under a reflux condenser for 3 hours. (The solution has a tendency to foam excessively upon reaching the boiling point with the consequent loss of sample passing through the top of the condenser. This may be prevented by rotating the Erlenmeyer flask frequently during the preheating period, with additional drops of capryl alcohol as required, until an even ebullition is established.)

Allow the solution to cool to room temperature, filter through a dried (at 100° C.) and weighed 50-ml. fritted-glass crucible of porosity C, and wash with distilled water until the filtrate is free of acid. Dry crucible and contents overnight at 100° C., cool in a desiccator, and weigh in an aluminum weighing bottle. Calculate the percentage of loss due to the three successive extractions.

Combine the extracted tobacco of duplicate samples and grind the horn-like material first in a small hand-operated mill, then pulverize with a mortar and pestle to a fine powder. Dry for 2 hours at 100° C. Weigh accurately from a weighing bottle triplicate subdivisions of from 0.5 to 0.7 gram each, and designate the subdivisions as (*a*), (*b*), and (*c*). Calculate for each subdivision, from the percentage it represents of the total extracted tobacco, the equivalent weight of the original, unextracted, M-&S-F tobacco.

Transfer each subdivision to a 50-ml. Erlenmeyer flask provided with a one-hole rubber stopper, through which passes a glass rod 12 cm. long and

the end of which has been flattened. For each 0.1 gram of the subdivision add, portionwise, 5 ml. of 72 percent sulfuric acid, which has been cooled to 5° C. Mix 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 reaction mixture to stand for 24 hours in a refrigerator at a temperature of approximately 5° C., with occasional stirring.

Transfer the reaction mixture to a 1-liter Erlenmeyer flask, and add sufficient distilled water to make approximately 5 percent sulfuric acid solution (which requires 109.5 ml. of water for each 5 ml. of 72 percent sulfuric acid used). Insert a boiling tube about 18 cm. long, add a few drops of capryl alcohol to prevent foaming, and boil under a reflux condenser for 2 hours. Allow to cool to room temperature.

Filter subdivision (*a*) through a tared Gooch crucible, which has been ignited for 1 hour at 600° C. in a muffle furnace. Filter subdivision (*b*) in a tared Gooch crucible and subdivision (*c*) in a 30-ml. fritted-glass crucible of porosity M, both crucibles having previously been dried at 100° C. Wash the crude lignin of each subdivision with distilled water, dry overnight at 100° C., and weigh in a weighing bottle.

Ignite the crucible containing subdivision (*a*) at 600° C. in the muffle furnace for 2 hours, determine the weight of ash, and calculate the percentage of ash in the crude lignin. Use the ash percentage as determined for subdivision (*a*) in determining the weights of ash in subdivisions (*b*) and (*c*). Transfer, quantitatively, the crude lignin of subdivision (*b*) to a Kjeldahl flask and determine the percentage of *N* in the crude lignin by the Kjeldahl-Gunning-Arnold method, using HgO as the catalyst. Calculate the percentage of protein (N × 6.25) in the crude lignin. Use the percentage of protein determined in subdivision (*b*) to calculate the weights of protein in subdivisions (*a*) and (*c*). The crude lignin of subdivision (*c*) is used for the determination of the percentage of methoxyl in the lignin.

Calculate the percentage of lignin in each subdivision as follows and average the results.

$$\frac{\text{weight of crude lignin} - \text{weights of ash and protein} \times 100}{\text{weight of M-\&S-F sample (original, unextracted tobacco)}} = \% \text{ lignin (M-\&S-F)}$$

Method 12.—Manganese

American Sumatra Tobacco Corp.

APPARATUS

Electrophotometer with rectangular absorption cell, 60 ml., Fisher catalog No. 7–102–40. Nessler tubes, 50 ml., Fisher catalog No. 7–052. Test tube wire basket, catalog No. 14–965. Galvanized pot, round, 8-liter capacity.

REAGENTS AND SOLUTIONS

Standard manganese stock solution (100 p.p.m. of Mn).—Prepare as follows: Introduce 0.0288 gram of $KMnO_4$ into a 125–ml. Erlenmeyer flask and

add 10 ml. of water, 6 drops of concentrated sulfuric acid, and a few glass beads. Heat to boiling and add sufficient sodium bisulfite (avoid large excess) to discharge the color. Evaporate until fumes of sulfuric acid appear. Cool, dissolve the residue with water, and dilute to 100 ml.

DETERMINATION

(*a*) *Ashing of sample.*—Place a 2-gram sample (M-&S-F basis) into a 150-ml. Pyrex beaker, add 20 ml. of nitric acid (69–71 percent), cover with a watch glass, and let stand for about one-half hour. Rotate beaker a few times during this period until 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. (Usually the volume at this point is 1 to 3 ml.) Remove the beaker from the hot plate, add 5 ml. of 70 percent perchloric acid, cover again with watch glass, and boil gently until the solution is clear and is fuming copiously. Do not evaporate the solution to complete dryness.

(*b*) *Solution A.*—Add about 50 ml. of water to the beaker as prepared in paragraph (*a*), bring to a boil and filter through Whatman No. 40 filter paper into a 200-ml. volumetric flask, wash silica on the filter paper with hot water, and collect the filtrate in the same flask. Cool the filtrate to room temperature and dilute to volume with water. Designate as solution A.

(*c*) *Standard curve.*—Prepare a series of standards of 1, 2, 3, and 4 p.p.m. of manganese by diluting 1, 2, 3, and 4 ml. of standard manganese stock solution to 100 ml. with water. Plot a standard curve in the range between 1 and 4 p.p.m. for interpreting color transmittancy of the sample from the standard.

(*d*) *Colorimeter procedure.*—Transfer 40 ml. of solution A into Nessler tubes, add 4 ml. of phosphoric acid (ortho 85 percent), and mix. Add about 200 mg. of potassium periodate and mix again. Place the Nessler tubes in a wire basket, immerse in the galvanized pot about half-filled with water, and boil for 1 hour. Stir the contents of the Nessler tubes a few times during this period. Remove from hot water, 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 525 mμ. Determine p.p.m of manganese in solution A by reference to a standard curve prepared in the same manner and at the same time.

(*e*) *Calculation.*—Calculate the percentage of manganese as follows:

$$\frac{(\text{p.p.m. of Mn})\ (\text{ml. of solution A}) \times 100}{\text{weight of M-\&S-F sample} \times 10^6} = \%\ \text{manganese (M-\&S-F)}$$

Method 13.—Methoxyl in Lignin (*20, 23*)

Standard Branch, Tobacco Division, AMS

APPARATUS

The apparatus used is illustrated in figure 6.

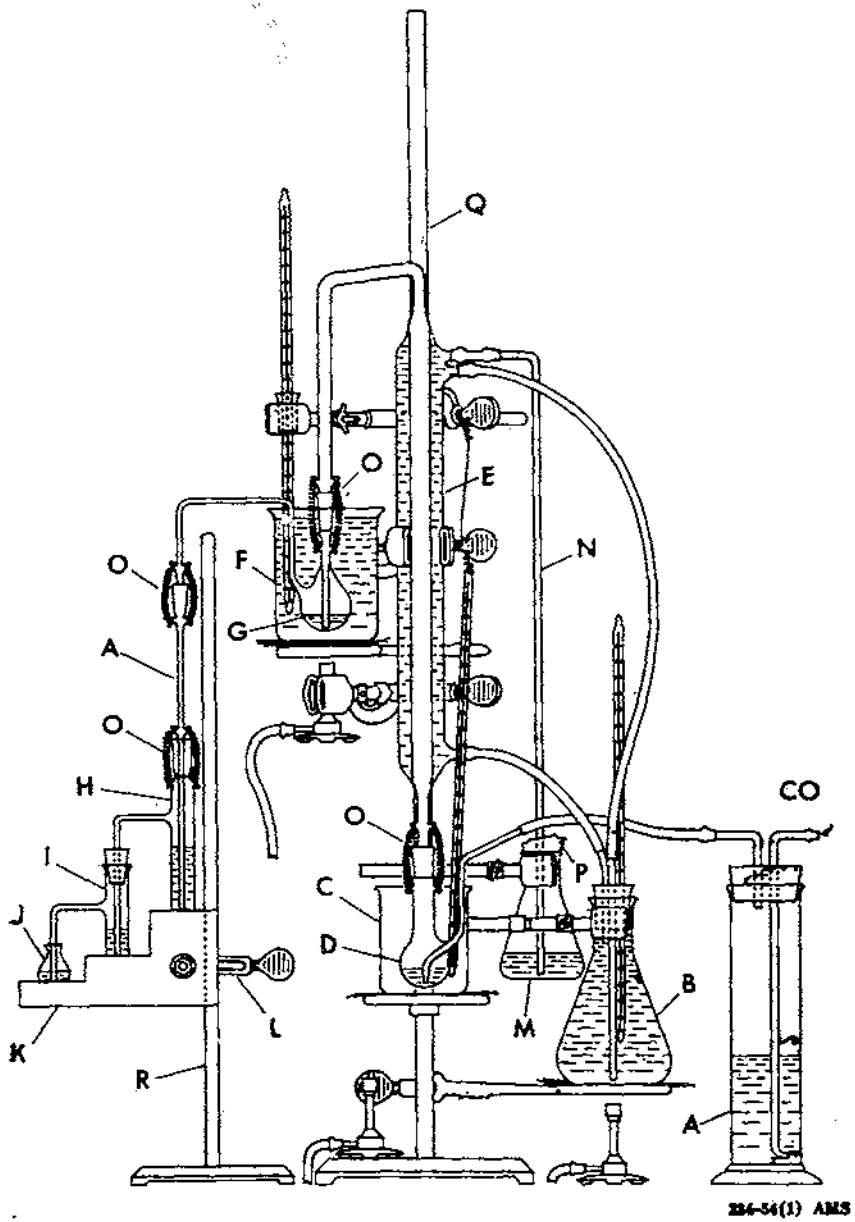

FIGURE 6.—Apparatus for determination of percentage of methoxyl.

REAGENTS

(1) *Hydriodic acid* (sp. gr. 1.70).
(2) *Red phosphorus*, special for microanalysis.
(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.*

(11) *0.5 percent starch solution.*

DETERMINATION

Heat containers B and F with microburners and maintain the temperatures at 50°–55° C. Add 15 drops of bromine to 10 ml. of the potassium 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 glycerin bath C heated to 135°–140° C. Maintain the temperature at this level for 1 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 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 1 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:

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

Method 14.—Moisture [4]

The American Tobacco Company

DETERMINATION

Weigh accurately duplicate samples of 5 grams each in aluminum dishes which are approximately 90 mm. in diameter and 20 mm. in depth and which are provided with covers. Distribute the sample evenly over the bottom of the dish and dry uncovered at 99°–100° C. in a forced-draft oven for 3 hours. Cover dishes, place in a desiccator over anhydrous calcium chloride, and weight again soon after the samples reach room temperature. Calculate the loss in weight as percent of moisture.

Method 15.—Moisture Equilibrium

Philip Morris, Inc.

DETERMINATION

Place ground samples, consisting of approximately 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. 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°–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.

Method 16.—Nicotine

North Carolina State College

DETERMINATION

The nicotine determination is a modification of the Garner (*16*) procedure. Transfer 6 grams of tobacco (calculated to a M-&S-F basis) to a 1-pint Ball fruit jar, paste with 10 ml. of 7.5 percent aqueous NaOH solution,

[4] Of several methods for the determination of moisture, this method was selected for use in this series of determinations since it was considered to follow the typical procedure currently used in commercial practice.

and cover with 100 ml. of petroleum ether (boiling range 60°–90° C.). Seal, rotate until mixed, and allow to stand overnight.

Transfer a 25-ml. aliquot of the clear extract to an Erlenmeyer flask and allow to stand for 1 hour, with occasional rotation, to allow ammonia to escape. Add 75 ml. of 0.025*N* H_2SO_4 to the Erlenmeyer flask and shake in a mechanical shaker for 10 minutes. Allow to separate clearly and withdraw a 25-ml. aliquot of the acid phase for back titration. (6 g. sample × 25/100×25/75=0.5 g.) Add 10 ml. of 0.04*N* NaOH to the 25-ml. aliquot and complete the back titration with 0.007*N* NaOH to endpoint of pH 5.6, using a Beckman Automatic Titrator or equal. Titrate a 25-ml. portion of the standard 0.025*N* H_2SO_4 solution to endpoint of pH 7.0, using the same instrument.

Calculate the back titration in mls. of 0.025*N* solution required and subtract from the 25-ml. standard blank titration. (1 ml. 0.025*N* H_2SO_4 =0.0041 g. nicotine).

$$\frac{(25 \text{ ml. titn.} - \text{back titn.}) \times 0.0041 \times 100}{0.5} = \% \text{ nicotine (M-\&S-F)}$$

Method 17.—Pectic Substances (*12, 17*)

Standards Branch, Tobacco Division, AMS

APPARATUS

The apparatus is essentially a Weihe-Phillips (*29*) extractor arranged as illustrated in figure 7.

The apparatus consists of a modified extraction crucible (A), designated as a "Pectin Extractor," [5] 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 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.

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

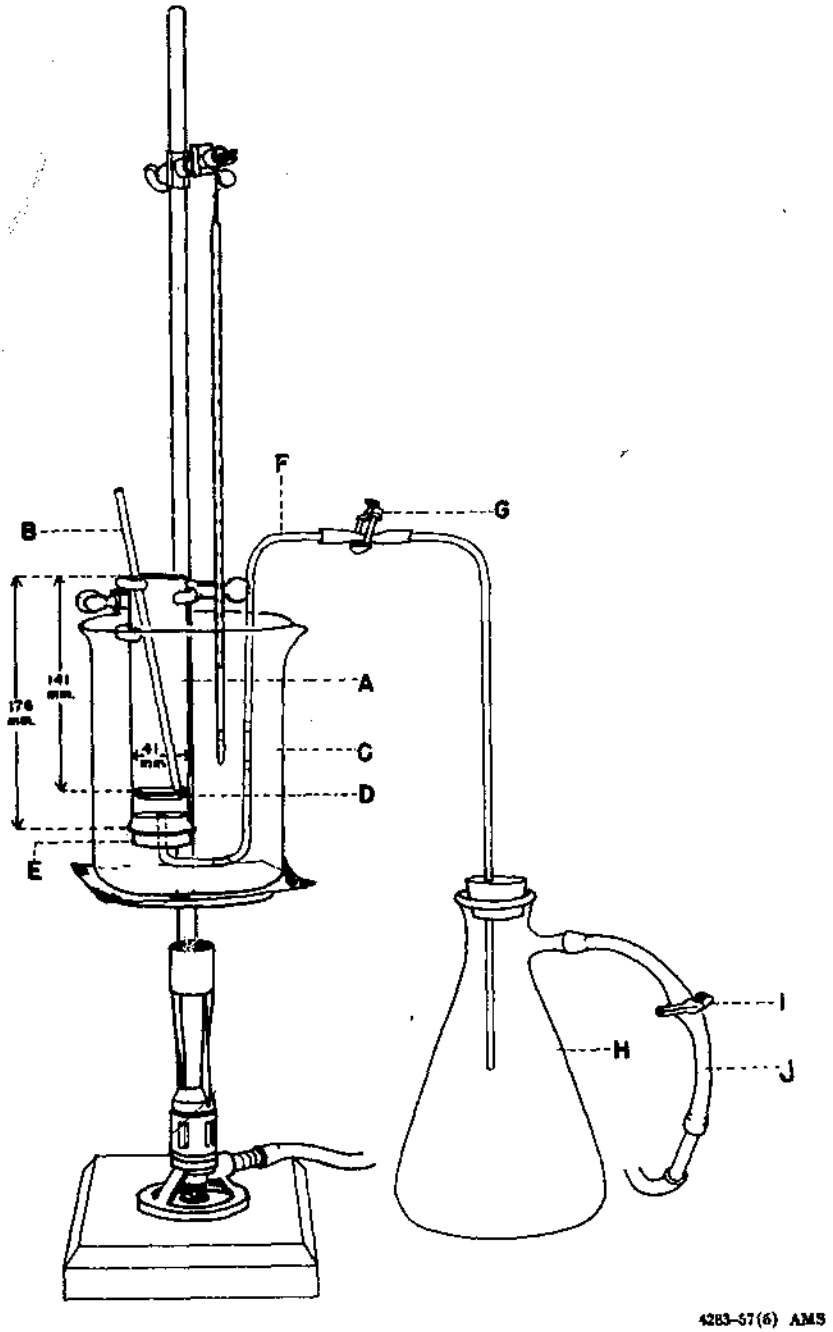

FIGURE 7.—Apparatus for extraction of pectic substances.

(3) *N/5 (approx.) sodium hydroxide solution.*
(4) *N/1 (approx.) acetic acid solution.*
(5) $\frac{Molar}{10}$ *(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.*
(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., 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: Pipet 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] 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 pectin precipitate on the paper completely by pouring through successive portions of a hot aqueous ammoniacal solution (approximately 1.0–1.5 percent).

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

[6] No water-soluble pectinic acids were found in any of the Flue-cured samples from the 1952 and 1954 crops. 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.

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 25 ml. of *M*/10 $CaCl_2$ solution by drops while stirring (preferably with a mechanical stirrer) and follow with the addition of 25 ml. of 2*M* $CaCl_2$, also added by drops, and stirred in the same manner.

Heat to boiling, with occasional stirring, and boil the mixture for 2 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 is 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:

$$\frac{(\text{weight of calcium pectate} - \text{weight of impurities}) \times 100}{0.4} = \%\ \text{pectinic acids (pectin), as calcium pectate, in the moisture-free sample}$$

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:

$$\frac{(\text{weight of calcium pectate} - \text{weight of impurities}) \times 100}{0.4} = \%\ \text{protopectin (as calcium pectate) in moisture-free sample}$$

Part 4—Pectic acid and pectates.—(*a*) Extraction with 0.5 percent 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:

$$\frac{(\text{weight of calcium pectate} - \text{weight of impurities}) \times 100}{0.4} = \%\ \text{pectic acid and pectates, as calcium pectate, in moisture-free sample}$$

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.

Method 18.—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 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 using.

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 or three 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, slowly at first, and then regulate the distillation rate so 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. Stir thoroughly and 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 4 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:

(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 M-&S-F tobacco sample.

Method 19.—Petroleum Ether Extract and Waxes

The American Tobacco Co.

DETERMINATION

Petroleum ether extract.—Place a 5-gram sample (M-&S-F basis) of the tobacco into a 25- by 80-mm. Whatman extraction thimble and cover with a small plug of fat-free cotton. Extract with approximately 125 ml. of petroleum ether (B.P. 35°–37° C.) in a Soxhlet extraction apparatus equipped with a tared 250-ml. ground-glass joint flask 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 a steam bath which may be hastened by a gentle stream of air on the surface. Rotate the flask frequently during evaporation to distribute the extracted material on the walls of the flask and to facilitate drying. Dry for 1 hour in a convection-type oven at 99°–100° C. Cool, weigh, and calculate the percent of petroleum ether extract as follows:

$$\frac{\text{weight of petroleum ether extract} \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{petroleum ether extract (M-\&S-F)}$$

Waxes.—Dissolve the petroleum ether extract in the extraction flask with 50 ml. of warm absolute ethyl alcohol. Warm on steam bath while rotating the flask to facilitate complete solution. Cool flask and wash bottle containing alcohol in an ice bath. Filter through No. 1 Whatman filter paper. Wash with cold absolute ethyl alcohol until free of pigment. Dissolve the waxes with ethyl ether and transfer to a tared dish. (The original tared Soxhlet flask with beads may be used.) Evaporate the ether on a steam bath using a gentle stream of air. Rotate the flask to facilitate drying. Dry for 1 hour in a convection-type oven at 99°–100° C. Cool, weigh, and calculate the percent of waxes as follows:

$$\frac{\text{weight of waxes} \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{waxes (M-\&S-F)}$$

Method 20.—pH

Liggett & Myers Tobacco Co.

DETERMINATION

Determine pH of extract A (as obtained in the determination of alpha amino nitrogen, method 2) using a Beckman pH meter, model H2, and a glass electrode, or equal.

Method 21.—Phosphorus

American Sumatra Tobacco Corp.

APPARATUS

Fisher Electrophotometer No. 7–106 with extra microcells No. 7–102–65.

REAGENTS AND SOLUTIONS

(1) *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.

(2) *Standard phosphorus stock solution (100 p.p.m. of P).*—Dissolve 0.0439 gram of KH_2PO_4 in 100 ml. of water.

(3) *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. Mix, allow the solution to stand overnight, filter, and store in a brown bottle.

(4) *Molybdate reagent.*—Place 15 grams of ammonium molybdate in a 1-liter volumetric flask and dissolve in about 300 ml. of water. Slowly add 500 ml. of concentrated hydrochloric acid (sp. gr. 1.18), 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 M-&S-F sample in a 150-ml. Pyrex beaker, add 20 ml. of nitric acid (69–71 percent), cover beaker with a watch glass, rotate the beaker until the sample is completely wetted, and allow the mixture to stand for one-half hour. Place the beaker on a hot plate and heat gently until no solid material except silica remains. Remove the beaker from the hot plate, allow to cool, add 5 ml. of 70 percent perchloric acid, and cover the beaker with a watch glass. Boil the solution until it is clear and fumes copiously.

Solution A.—Add about 50 ml. of water to the solution of ash material 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 collecting the washings in the same volumetric flask. Cool the combined filtrate and washings to room temperature and dilute to 200 ml. with water. Mix and designate as solution A.

Solution B.—Pipet 1 ml. of solution A into a test tube and add 9 ml. of the buffer solution. Mix and designate this as solution B.

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

Colorimeter procedure.—Pipet 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 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 p.p.m. of P in solution A by reference to the standard curve prepared at the same time.

Calculate the percentage of phosphorus as follows:

$$\frac{(\text{p.p.m. } P)\ (\text{ml. solution A})\ (\text{dilution of solution A}) \times 100}{\text{weight of M-\&S-F sample} \times 10^6} = \%\ \text{phosphorus (M-\&S-F)}$$

Method 22.—Plastid Pigments (*31*)

North Carolina State College

The plastid pigments were determined by a modification of the spectrophotometric procedure described for flue-cured tobacco by Stinson [7] and Pack.[8]

[7] Stinson, F. A. the distribution of plastid pigments in flue-cured tobacco during maturation and curing. Thesis, Ph. D. degree. North Carolina State College, Raleigh. 1949.

[8] Pack, A. B. the curing and quality of flue-cured tobacco; the effect of certain cultural and curing practices on the plastid pigment and carbohydrate content. Thesis, Ph. D. degree. North Carolina State College, Raleigh. 1950.

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 wavelengths 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.

Method 23.—Potassium

Eastern Utilization Research and Development Division, ARS

APPARATUS

Flame spectrophotometer equipment.—(1) Atomizer and burner of own design. (2) Filters No. 5850 blue purple plus No. 2404 dark red. (3) Photovolt multiplier photometer equipped with a 1P21 multiplier phototube.

DETERMINATION

Ashing of sample.—Weigh accurately about 1 gram of sample in a platinum dish, moisten with 10 ml. of 5 percent sulfuric acid, char under infrared lamp until SO_3 fumes cease, ash at 600° C. overnight (approximately 14 hours).

Solution of ash.—Add approximately 30 ml. of 1+9 HCl and evaporate to dryness on steam bath. Add 20 ml. of 1+9 HCl, dissolve and decant into a funnel through washed filter paper, collecting the filtrate in a 100-ml. volumetric flask. Repeat solution step with a second 20-ml. portion of 1+9 HCl. Transfer residue to filter, wash dish and filter with 1+9 HCl until volume of filtrate approaches 100 ml., cool solution to room temperature, and make to volume with 1+9 HCl.

Flame spectrophotometric procedure.—Aspirate the solution of sample ash into flame and read intensity of emission of the light that passes through the two filters. Prepare a standard curve (p.p.m. K vs. Intensity) using

matrix solutions containing known amounts of potassium which bracket those in the sample and also containing known amounts of Ca and HCl which are estimated to be present in the tobacco ash solution. If this curve approximates a straight line within estimated experimental error, an equation for a straight line is calculated using the points that bracket the intensities of the sample. This linear equation is used to calculate the p.p.m. of potassium in the solution of the sample ash. If the curve does not approximate a straight line, the p.p.m. of K in the sample solution is determined by interpolation.

In either case, the observed intensity must be corrected for variations in operating conditions (atomization rate, flame temperature, etc.). This is done by determining the flame intensity of a single standard potassium solution (Z) at regular intervals during a series of determinations. The standard solution Z is also compared with the matrix solutions used to obtain the standard curve. The observed intensity of the sample solution is adjusted by multiplying it by the factor I_1/I_2, where I_1 is the flame intensity for solution Z determined at the same time and under the same conditions as the matrix solutions and I_2 is the flame intensity of solution Z determined at the same time the sample solutions were analyzed.

The percent of potassium is calculated as follows:

$$\frac{(\text{p.p.m. K})\ (\text{vol. of sample soln.}) \times 100}{\text{weight of M-\&S-F sample} \times 10^6} = \%\ \text{potassium (M-\&S-F)}$$

Method 24.—Protein Nitrogen and Proteins

United States Tobacco Co.

DETERMINATION

Place 2 grams (M-&S-F equivalent weight) of tobacco in a 250-ml. Erlenmeyer flask. Add 100 ml. of 0.5 percent acetic acid solution, heat mixture to boiling, and reflux over low flame for 10 to 15 minutes. Filter with suction while hot using a Fisher filtrator funnel (with Whatman No. 30, 9 cm. diameter, filter paper), or use Buchner funnel, size 2A, and Whatman No. 1, 15 cm. diameter, filter paper. In using the Buchner funnel, press filter paper into the funnel so that about 2 cm. extend 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 filter paper with residual tobacco in an 800-ml. Kjeldahl flask and add 10 grams K_2SO_4, 0.7 gram HgO, and 25 ml. H_2SO_4. Heat gently until frothing ceases then boil briskly until solution clears and then for at least 30 minutes longer. If the contents of the flask appear likely to become solid before this point is reached, add 5 ml. H_2SO_4 (sp. gr. 1.84) and continue heating.

Allow to cool to room temperature and then add 200 ml. of distilled water and a few pieces of granulated zinc to prevent bumping. Add 50 ml. of 4 percent K_2S solution (or 50 ml. of 8 percent $Na_2S_2O_3\ 5H_2O$ solution) and

mix. Add 90 ml. of 40 percent NaOH, pouring it down the side of the flask slowly so that it does not mix. Connect flask to condenser with an Iowa-State-type Kjeldahl bulb, extending the tip of the condenser below the surface of an accurately measured volume of $N/10$ HCl in the receiver. Mix contents of the Kjeldahl flask by rotating flask and distill until all of 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 excess acid with $N/10$ NaOH solution, using methyl red as an indicator, and calculate the results as follows:

$$\frac{\text{ml. of } N/10 \text{ HCl required to neutralize } NH_3 \times 0.14008}{\text{weight of M-\&S-F sample}} = \%\ \text{protein nitrogen (M-\&S-F)}$$

$$\%\ \text{protein nitrogen (M-\&S-F)} \times 6.25 = \%\ \text{proteins (M-\&S-F)}$$

Method 25.—Resins and Waxes

Tobacco and Sugar Crops Research Branch, Crops Research Division, ARS

DETERMINATION

Weigh accurately a sample equivalent to 3 grams of M-&S-F tobacco and mix with 30 grams of sand, which has been acid-extracted and ignited at 700° C. for about 1 hour.

Transfer the mixture to a paper extraction thimble and extract with 95 percent ethanol for 24 hours in a Soxhlet extraction apparatus. Filter alcoholic extract into a 250-ml. beaker. Wash extraction flask with boiling alcohol, pouring alcoholic washings through filter, and collect filtrate from washings in the same 250-ml. beaker. Evaporate alcoholic filtrate to dryness on a steam bath and dry residual resinous material for 1 hour at 75° C. To the resinous extract, add 30 to 40 ml. of water heated to 45° C., mix with glass rod, and filter through a filter paper. Repeat this operation of washing and filtration until filtrate A gives a negative test for nicotine with silicotungstic acid reagent. Add 5 ml. of concentrated HCl to the filtrate 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. Rinse funnel used in above filtration with 95 percent ethanol and add rinsings to the beaker. Cover beaker with a watch glass and heat on the steam bath until the resinous material is dissolved. (A few particles may remain undissolved which are subsequently filtered off.) Remove the filter paper from beaker, rinse the paper with a few ml. of 95 percent ethanol, and add rinsings to the main alcoholic solution. Filter the main alcoholic solution into another 250-ml. beaker, wash filter paper and funnel with a few ml. of 95 percent ethanol, and add washings to the main alcoholic filtrate. Add the alcoholic filtrate portionwise to a weighed 50-ml. Erlenmeyer flask and evaporate alcohol on the steam bath.

Extract filtrate A with three successive portions of ether, and wash the combined ether extract once with 25 ml. of distilled water. Filter the washed ether solution, add it by portions to the weighed 50-ml. Erlenmeyer flask, and evaporate to dryness on a steam bath. Dry Erlenmeyer flask containing residue for 45 minutes at 75° C., cool in desiccator over anhydrous calcium chloride, and weigh. Calculate the result as follows:

$$\frac{\text{weight of resins and waxes} \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{resins and waxes (M-\&S-F)}$$

Method 26.—Sand (6)

Standards Branch, Tobacco Division, AMS

DETERMINATION

Add slowly 10 ml. of concentrated HCl to the dish containing the total ash determination. Cover the dish partially with a cover glass to prevent loss by spattering. Boil over a low flame about 2 minutes, evaporate on steam bath to dryness, and bake on steam bath 3 hours after evaporation is complete. Moisten residue with 5 ml. of concentrated HCl, cover, and boil over low flame for about a minute or two. Add approximately 30 ml. of distilled water, and heat on steam bath a few minutes. Decant through an ignited and tared Gooch crucible and wash insoluble material in dish with hot water by decantation two or three times, pouring through the Gooch crucible.

Add 15 ml. of hot saturated sodium carbonate solution to dish. If the sodium carbonate solution is not clear, filter it through asbestos before using. Cover, heat to boiling, and remove burner. Add about five drops of 10 percent sodium hydroxide solution, allow mixture to settle, and decant through the crucible. Repeat treatment with another 15-ml. portion of sodium carbonate solution. Wash the insoluble material in the dish with hot water by decantation, pouring through the crucible.

Transfer remaining insoluble material in dish to crucible with the aid of a rubber-tipped glass rod. Wash the material in the crucible with 2 or 3 small portions of dilute HCl (1 volume of HCl to 4 of H_2O), and finally with hot water several times until free of HCl. Dry crucible and contents in oven at 100° C. for 30 minutes or more. Cool to room temperature in desiccator over calcium chloride, weigh, confirm residue as sand, and calculate as percentage of sand on a M-&S-F basis, as follows:

$$\frac{\text{weight of sand} \times 100}{\text{weight of M-\&S-F tobacco}} = \%\ \text{sand (M-\&S-F)}$$

Method 27.—Sodium

Eastern Utilization Research and Development Division, ARS

APPARATUS

(1) Atomizer and burner of own design. (2) 600 mμ. interference filter set at the appropriate angle plus a No. 3480 red shade yellow filter. (3) Photovolt multiplier photometer equipped with a 1P21 multiplier phototube.

DETERMINATION

Ashing of sample.—Weigh accurately a sample of about 1 gram (as-is) in a platinum dish and calculate its M-&S-F weight. Moisten sample with 10 ml. of 5 percent sulfuric acid, char under infrared lamp until SO_3 fumes cease, and continue ashing at 600° C. overnight (approximately 14 hours).

Solution of ash.—Add approximately 30 ml. of 1+9 HCl, evaporate to dryness on steam bath, add 20 ml. of 1+9 HCl and warm on steam bath while stirring to dissolve ash. Decant into a funnel containing washed filter paper and collect filtrate in a 100-ml. volumetric flask. Repeat solution step with a second 20-ml. portion of 1+9 HCl. Transfer residue to filter, wash dish, and filter with 1+9 HCl until volume of filtrate approaches 100 ml. Cool solution to room temperature, make to volume with 1+9 HCl, and mix.

Flame spectrophotometric procedure.—Aspirate solution of sample ash into flame and read intensity of emission of the light that passes through the two filters. Prepare a standard curve (p.p.m. Na vs. Intensity) using NaCl solutions made up with 1+9 HCl. Since the relationship between the concentration of sodium and the intensity is linear, a simple proportion is used to calculate the p.p.m. of sodium in the samples. Calculate the percent of sodium as follows:

$$\frac{(\text{p.p.m. Na})\ (\text{vol. of soln.}) \times 100}{\text{weight of M-\&S-F sample} \times 10^6} = \%\ \text{sodium (M-\&S-F)}$$

The sample solutions were compared with standard solutions containing NaCl and HCl, with the HCl concentration being equal to that in the sample solutions. It was determined experimentally that extraneous ions in concentrations which were encountered in the sample solution had little or no effect on the sodium emission.

The values obtained may be high because of sodium concentration from handling the leaves, from the acid and water used in dissolving the ash, from the glass of the flasks, and from the dust in the laboratory air.

Method 28.—Spectrographic Analysis

North Carolina State College

DETERMINATION

Weigh accurately a subsample of approximately 20 mg. and place in a recessed cup in the end of a carbon electrode for arcing. Compare the intensity of the line of a particular element to the standard spectrum of a graded series of the particular element being measured.

The data in the tables are the averages of quadruplicate determinations of the respective elements, and the replicate determinations were made on successive days.

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Method 29.—Sulfur (7)

Philip Morris Inc.

REAGENTS

(1) Magnesium nitrate solution.—Dissolve 150 g. of MgO in HNO_3 (1:1) avoiding excess of the acid; add a small amount of MgO in excess, boil, filter from the excess of MgO, and dilute to 1 liter.

DETERMINATION

Place 1 gram of tobacco (M-&S-F basis) in a large porcelain crucible. Add 7.5 ml. of the magnesium nitrate solution, taking care that all tobacco is in contact with solution. Heat crucible and contents on an electric hot plate (180° C.) until no further action takes place. Transfer hot crucible to electric muffle furnace and allow to remain at low heat (muffle must not show any red) until charge is thoroughly oxidized. (No black particles should remain; it may be necessary to break up charge and return to muffle.)

Remove crucible from muffle and allow to cool. Add H_2O, then HCl in excess (approx. 0.5 ml. HCl per 200-ml. solution). Bring solution to boil, filter, and wash thoroughly. Heat filtrate to boiling and add 10 ml. of a 10 percent $BaCl_2$ solution by drops while constantly stirring. Continue boiling ca. 5 minutes and allow to stand 5 hours or longer in a warm place. Decant through an ignited and weighed Gooch crucible. Treat precipitate with 15–20 ml. of boiling water and transfer to crucible. Wash with boiling water until filtrate is free of Cl. Dry Crucible and precipitate at 100° C., ignite, cool to room temperature in desiccator over calcium chloride, and weigh as $BaSO_4$. Weight of $BaSO_4 \times 0.1374$ = weight of S. Report as percent S.

Method 30.—Tannin (2)

Eastern Utilization Research and Development Division, ARS

REAGENTS

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

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

DETERMINATION

Preparation of extract A.—Extract a 40-gram sample (M-&S-F basis) at a uniform rate with boiling water for 7 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 to remain overnight in a room at 23°–24° C. Dilute with water to exactly 2 liters and designate solution as extract A.

Soluble extract.—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 evently divided creases. When approximately 40 ml. have passed through the filter, return the filtrate to the funnel. Continue this operation for 1 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. Pipet 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.

Nontannin.—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 2 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. Pipet 100 ml. of the new filtrate into a weighed flat-bottomed dish, 700 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.

Since the tannin content of the tobacco samples was quite low, resulting in a low tannin concentration in the extracts, 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.

Method 31.—Total Alkaloids (as Nicotine) (*13*)

R. J. Reynolds Tobacco Co.

APPARATUS

Precision-Shell Titrimeter with calomel and glass electrodes (used for the potentiometric titrations). Wrist-action shaker, model BB, Burrell Corp., Pittsburgh, Pa.

REAGENTS AND SOLUTIONS

(1) *Barium hydroxide,* $Ba(OH)_2 \cdot 8H_2O$, 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 Corp.'s analytical filter aid.

(5) *Acetic anhydride,* A.C.S. grade.

(6) *Crystal violet indicator,* 1 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 1 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.

DETERMINATION

Weigh accurately 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 1 gram of granular barium hydroxide and 15 ml. of the saturated barium hydroxide solution. Swirl the flask until the tobacco is thoroughly wetted, adding more barium hydroxide solution if necessary. Pipet 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. Pipet 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 1 drop of crystal violet indicator and titrate to a green endpoint 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:

$$\% \text{ total alkaloids} = \frac{V_1 \times N \times 32.45}{\text{weight of M-\&S-F sample}}$$

$$\% \text{ nicotine} = \frac{2V_2 - V_1 \times N \times 32.45}{\text{weight of M-\&S-F sample}}$$

$$\% \text{ nornicotine} = \frac{2(V_1 - V_2) \times N \times 29.64}{\text{weight of M-\&S-F sample}}$$

Where:

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

V_2 = ml. of perchloric acid required to neutralize the 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 equals 0.0811 gram of nicotine or 0.0741 gram of nornicotine. Inasmuch as 25 ml. aliquots (corresponding to one-fourth of the total extract) are taken for titration, above figures are multiplied by 4. To get percentages, they are multiplied further by 100.

Method 32.—Total Ash and Its Solubility and Alkalinity (*8*)

Philip Morris Inc.

DETERMINATION

Total ash.—Weigh accurately a sample equivalent to 2 grams of M-&S-F tobacco in a tared porcelain dish of 75-ml. capacity. Heat at a temperature of about 300° C. for 2 hours. Ignite at 500° C. for 2 hours in an electric muffle furnace provided with a temperature control. Cool to room temperature in a desiccator over anhydrous calcium chloride and determine the weight of the ash. Calculate the percentage of total ash as follows:

$$\frac{\text{weight of ash} \times 100}{\text{weight of M-\&S-F sample}} = \% \text{ total ash (M-\&S-F)}$$

Water-insoluble ash.—Transfer the ash obtained from the total ash determination to a 250-ml. beaker, add 25 ml. of water, and heat to boiling. Filter on an ashless filter paper and wash with 25 ml. of hot water. (Save the combined filtrate and washings for the determination of alkalinity of water-soluble ash.) Dry the insoluble ash and filter paper and ignite at 550° C. for 2 hours. Cool in desiccator to room temperature and determine the weight of the insoluble ash. (Save the insoluble ash for the determination of alkalinity.) Calculate the percentage of insoluble ash as follows:

$$\frac{\text{weight of insoluble ash} \times 100}{\text{weight of M-\&S-F sample}} = \% \text{ water-insoluble ash (M-\&S-F)}$$

Water-soluble ash.—Subtract the percentage of water-insoluble ash from the percentage of total ash to determine the percentage of water-soluble ash.

Alkalinity of water-soluble ash.—Titrate combined filtrate and washings obtained in part 2 with $N/10$ hydrochloric acid to a pH of 4.3, using a Fisher Titrimeter. Calculate the alkalinity of soluble ash as ml. of $N/10$ HCl per gram of M-&S-F tobacco.

Alkalinity of water-insoluble ash.—To the water-insoluble ash obtained in part 2 add a measured quantity of *N*/10 hydrochloric acid in excess of that required to make the mixture definitely acid in reaction. Bring the mixture to boiling. Determine the excess acid by titration with *N*/10 sodium hydroxide to a pH of 4.3, using a Fisher Titrimeter. Calculate the alkalinity of water-insoluble ash as ml. of *N*/10 hydrochloric acid required to neutralize the water-insoluble ash of 1 gram of M-&S-F tobacco.

Method 33.—Total Nitrogen and Nitrate Nitrogen (*9*)

Liggett & Myers Tobacco Co.

DETERMINATION

Part 1—Total nitrogen including nitrate nitrogen.—Place 1.4 grams (M-&S-F) of tobacco in a 650-ml. Kjeldahl digestion flask. Add 35 ml. of an acid solution which consists of 28.6 grams of salicylic acid in 1 liter of H_2SO_4 (sp. gr. 1.84). Rotate flask until thoroughly mixed and allow to stand for at least 1 hour.

Add 5 grams of $Na_2S_2O_3 \cdot 5H_2O$, place on digestion rack, turn heaters to low heat, and digest until all danger of frothing has passed (about 30 minutes). Increase heat until acid boils briskly and continue heating until white fumes of SO_3 no longer escape from the flask. Add 0.7 gram of HgO, 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 and add 250 ml. of distilled water and a few pieces of granulated zinc to prevent bumping. Pour 70–80 ml. of sodium hydroxide-sodium thiosulfate solution (consisting of 200 ml. of sodium thiosulfate solution, made by dissolving 1,400 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 1250 ml. of distilled water, mixed with 5 liters of 40 percent 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 rotating flask 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 being used as the indicator.

Calculate the results as follows:

$$\frac{\text{ml. of } N/10\ H_2SO_4 - \text{ml. of } N/10\ \text{NaOH}}{\text{weight of M-\&S-F sample}} \times 0.140 = \%\ \text{total nitrogen (M-\&S-F)}$$

Part 2—Nitrate nitrogen.—Place 1.4 grams (as-is) of tobacco in a 650-ml. Kjeldahl digestion flash. Add 5 grams of $FeSO_4 \cdot 7H_2O$ and 10 ml. of distilled

H_2O. 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 1 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 1 hour, place the flask on the digestion rack and heat until dense fumes of SO_3 no longer appear. Add 0.7 gram of HgO, 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 and then 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 1,400 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 1,250 ml. of distilled water, mixed with 5 liters of 40 percent 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 of Kjeldahl flask 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 being used as the indicator.

Calculate the nonnitrate nitrogen and nitrate nitrogen as follows:

$$\frac{\text{ml. of } N/10\ H_2SO_4 - \text{ml. of } N/10\ \text{NaOH}}{\text{weight of M-\&S-F sample}} \times 0.140 = \%\ \text{nonnitrate nitrogen (M-\&S-F)}$$

$$\%\ \text{total nitrogen} - \%\ \text{nonnitrate nitrogen} = \%\ \text{nitrate nitrogen}$$

Method 34.—Total Reducing Substances, Total Reducing Sugars, Polyphenols, Sucrose, and Starch

P. Lorillard Co., Inc.

REAGENTS AND SOLUTIONS

(*R1*)—*Fehling's solution A.*—207.8 grams of copper sulfate pentahydrate dissolved in water to make 3 liters of solution.

(*R2*)—*Fehling's solution B.*—1,038 grams of Rochelle Salt (potassium sodium tartrate) and 309.6 grams of sodium hydroxide dissolved in water to make 3 liters of solution at room temperature.

(*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.*—74.6 grams of sodium thiosulfate pentahydrate dissolved in 3,000 ml. of water and allowed to stand for three weeks. Standardize against $N/10$ $K_2Cr_2O_7$ solution, 4.9033 grams of $K_2Cr_2O_7$ per liter.

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

DETERMINATION

Part 1—Total reducing substances.—(*a*) Preparation of filtrate A: Place a 3-gram sample (M-&S-F basis) and 0.3 gram of $CaCO_3$ in a 500-ml. flask, add 200 ml. of water, and reflux for 1 hour with occasional shaking. Allow flask and contents to cool to room temperature and transfer to a 500-ml. volumetric flask. Make up to volume with water, mix, and filter by gravity through a fluted circle of Whatman No. 44 filter paper. Designate as filtrate A.

(*b*) Procedure: Pipet 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*) Procedure: Pipet 25 ml. of each (R1) and (R2) into the Erlenmeyer flask and mix. Invert a 100-ml. beaker over the mouth of the flask and place over a Bunsen flame previously adjusted to bring the contents of the flask to a rolling boil in 4 minutes, ±5 seconds. Continue boiling for 2 minutes, remove the flask, and quench for 1½ minutes under running tap water. After quenching, pipet 25 ml. of (R3) into the flask. Mix for about 5 seconds and pour into the flask from a graduated cylinder 20 ml. of (R4). Mix again and pour into the flask from a graduated cylinder 20 ml. of (R5). After mixing the contents, titrate the sample with (R6), using 1 ml. of a freshly prepared 5 percent starch solution as an indicator.

In the same manner, run a control on 50 ml. of water, using the above procedure. The number of ml. of $N/10$ $Na_2S_2O_3$ used in the control, minus the number of ml. of $N/10$ $Na_2S_2O_3$ consumed by the unknown, gives the number of ml. of $N/10$ $Na_2S_2O_3$ equivalent to the weight of cuprous oxide deposited. A difference of 1.0 ml. of $N/10$ $Na_2S_2O_3$ between control and sample corresponds to 7.15 mg. of cuprous oxide. The *d*-glucose equivalent to the cuprous oxide (Cu_2O) is obtained from the Munson-Walker table (A.O.A.C., Ed. 8, sect. 42.11, p. 890).

Calculate the percentage of total reducing substances (as *d*-glucose) as follows:

$$\frac{B}{W} \times 100 = \%\ \text{total reducing substances (as-is)}$$

When B=mg. of *d*-glucose equivalent to the volume of $N/10$ $Na_2S_2O_3$ used in the titration, and W=120 mg. or 1/25 of the weight in mg. of the original sample.

Part 2—Total reducing sugars.—(*a*) Preparation of filtrate B: Extract a 5-gram sample (M-&S-F basis) for 16 hours with 80 percent ethanol in a Soxhlet extraction apparatus. Use an alundum extraction thimble (34x100 mm., medium porosity) for the extraction. Insert a tared plug of glass wool into the thimble after weighing 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 with 0.342 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 of the lead has been removed.

(*b*) Procedure: Pipet 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.

Calculate the percentage of total reducing sugars (as *d*-glucose) as follows:

$$\frac{B}{W}\times 100 = \%\ \text{total reducing sugars (M-\&S-F)}$$

When B=mg. of *d*-glucose equivalent to volume of $N/10$ $Na_2S_2O_3$ used in titration, and W=200 mg.

Part 3—Polyphenols.—The percentage of total reducing substances (as *d*-glucose) minus the percentage of total reducing sugars (as *d*-glucose) equals percentage of polyphenols (as *d*-glucose).

Part 4—Sucrose.—(*a*) Preparation of filtrate C: Pipet a 50-ml. aliquot of filtrate B, prepared as described in paragraph (*a*) of Part 2, to a 100-ml. volumetric flask and invert with 10 ml. of hydrochloric acid solution (546 ml. of hydrochloric acid of *d*. 1.19 diluted with water to 1 liter) for 24 hours at room temperature. Transfer this solution to a 250-ml. beaker, neutralize with sodium carbonate, return to the 100-ml. volumetric flask, dilute to the mark with water, mix, and designate as filtrate C.

(*b*) Procedure: Pipet a 25-ml aliquot of filtrate C (equivalent to a 0.1-gram sample), transfer to a 500-ml. Erlenmeyer flask containing 25 ml. of water, and determine the reducing sugars (as invert sugar) following the procedure described in paragraph (bb) of part 1.

(*c*) Calculation: The difference between the percentage of invert sugar before inversion and the percentage of invert sugar after inversion when multiplied by 0.95 equals the percentage of sucrose in the sample.

Part 5—Starch.—(*a*) Preparation of filtrate D: Transfer the tobacco quantitatively remaining in the extraction thimble after the extraction with the 80 percent ethanol, as described in paragraph (*a*) of part 2, to a 1,000-ml. Erlenmeyer flask. Wash down the glass-wool plug used in the extraction with distilled water and transfer the washings and suspended tobacco particles to the flask. Add 500 ml. of water and digest the mixture at room temperature for 24 hours with occasional stirring. Filter mixture by gravity through Whatman No. 44 filter paper. Wash the residual tobacco with cold water, and discard the filtrate and washings. Dry the residual tobacco at 100° C. for 3 hours, cool in desiccator, and weigh. Designate the remaining tobacco substance as residue A and determine the weight equivalent of the original sample. Pulverize residue A in a mortar to a fine powder and redry at 100° C. for 1 hour.

To ca. 1.5 grams of the dried residue A, add 100 ml. of a 1 percent aqueous potassium oxalate solution, and digest for 18 hours at room temperature, shaking frequently. Filter mixture, wash residual material with water, and discard the filtrate and washings. Transfer the residual tobacco quantitatively to a small mortar, add 10 ml. of water and 0.5 gram of powdered pumice, and triturate tissue to a pulp. Transfer the mixture quantitatively to a 250-ml. volumetric flask with several rinsings of water from a wash bottle, the total volume of water not exceeding 200 ml.

Heat flask and contents for 30 minutes in a boiling water bath in order to gelatinize the starch. Cool mixture to room temperature, add to it about five drops of toluene and 10 ml. of a 1 percent solution of dialyzed taka-diastase, and incubate for 48 hours at 22°–24° C. Dilute the mixture to the mark with water, mix, filter through a Whatman No. 44 filter paper, and designate this final filtrate as filtrate D.

(*b*) Procedure: Pipet a 50-ml. aliquot of filtrate D, transfer to a 500-ml. Erlenmeyer flask, and continue the determination as described in paragraph (*bb*) of part 1.

Calculate the percentage of starch (as *d*-glucose) as follows:

$$\frac{B\times 0.9}{W}\times 100 = \%\ \text{starch (M-\&S-F)}$$

When B = mg. of *d*-glucose equivalent to the volume of $N/10$ $Na_2S_2O_3$ used in the titration, and W = the weight in mg. on a M-&S-F basis.

Method 35.—Total Volatile Acids (as Acetic Acid)

Brown & Williamson Tobacco Corp.

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, and extending nearly to the bottom of the Kjeldahl flask. Through the other hole, passes one end of a spherical connecting bulb, such as is used in the determination of nitrogen by the Kjeldahl method (Fisher Scientific Co. catalog No. 13—177A or equal). The upper end of the connecting bulb is connected to a glass condenser by a rubber stopper. The Kjeldahl flask is immersed in a glycerin bath. A conventional type of steam generator is used.

DETERMINATION

Place a sample of 5 grams (M-&S-F equivalent weight) in the Kjeldahl flask of the apparatus and add 100 ml. of distilled water and 2 grams of tartaric acid. Heat glycerin 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 0.1*N* sodium hydroxide solution. Continue the distillation until almost 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 glycerin bath with a small flame. Determine the excess of sodium hydroxide by titration with *N*/10 sulfuric acid solution, using phenolphthalein as the indicator. Calculate total volatile acids (as acetic acid) as follows:

$$\frac{\text{ml. of } 0.1N \text{ sodium hydroxide required} \times 0.0060 \times 100}{\text{weight of M-\&S-F sample}} = \%\ \text{total volatile acids (M-\&S-F)}$$

Method 36.—Total Volatile Bases (as Ammonia) (*19*)

The American Tobacco Co.

DETERMINATION

Transfer a 5-gram sample of tobacco (M-&S-F basis) 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 (*10*) arranged for the distillation in a current of steam. Collect the distillate in a 1,000-ml. Erlenmeyer flask which contains an excess of *N*/10 hydrochloric 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 distillation. Titrate excess 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:

$$\frac{\text{ml.}\ N/10\ \text{HCl required} \times 0.17032}{\text{weight of M-\&S-F sample}} = \%\ \text{total volatile bases, as ammonia (M-\&S-F)}$$

Method 37.—Uronic Acids (as Anhydrides) (*23*)

Standards Branch, Tobacco Division, AMS

APPARATUS

The apparatus is described by Browning (*11*). However, anhydrous calcium chloride is used in place of anhydrone in the long drying tube, absorption tube, and guard tube. Pretreat the anhydrous calcium chloride as follows: Place the anhydrous calcium chloride in a tube of suitable size and pass a slow stream of dry carbon dioxide through the tube for one-half hour. Then pass a stream of dry air, free of carbon dioxide, through for 1 hour. Keep the anhydrous calcium chloride in a well-stoppered bottle. Fill the trap to a depth of about 7 mm. above the inlet tube with the silver phosphate solution. Renew 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 2,380 ml. of distilled water and mix.

(2) *Silver phosphate solution.*—Add 10 grams of silver carbonate to 300 ml. of 85 percent orthophosphoric acid. Heat mixture on the steam bath for 1 hour and at the same time pass through it a stream of air which is free of carbon dioxide. Filter through a sintered-glass Buchner funnel of porosity *M*.

DETERMINATION

Weigh accurately in a weighing bottle 1 to 1.3 grams of the dry tobacco which has been extracted with 95 percent ethanol in a Soxhlet extraction apparatus for 16 hours. Calculate the equivalent weight of the unextracted sample on an M-&S-F basis. Place the sample in the reaction flash and add 60 ml. of 12 percent hydrochloric acid and two boiling chips. Connect reaction flask to a 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 which is free of carbon dioxide, through the apparatus at the rate of two to three bubbles per second.

Disconnect the absorption tube, place in a sufficiently large stoppered test tube, and allow to remain near the balance for 5 minutes. Weigh absorption tube and connect again to the apparatus. Slowly raise the temperature of the glycerol bath to 137°–140° C., over a period of 30 to 45 minutes, and maintain this temperature for 5 hours. Without interrupting the flow of carbon dioxide-free air, disconnect the absorption tube from the apparatus, place in a stoppered test tube as before, allow to remain near the balance for 5 minutes, and weigh.

Conduct a blank determination following exactly the procedure described above, except do not add sample to 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 rep-

resents the weight of carbon dioxide from uronic acids. Calculate the percentage of uronic acids (as anhydrides) as follows:

$$\frac{\text{weight of } CO_2 \text{ given off by uronic acids} \times 400}{\text{weight of unextracted M-\&S-F sample}} = \% \text{ uronic acids (M-\&S-F)}$$

Method 38.—Water-Soluble Acids

Liggett & Myers Tobacco Co.

DETERMINATION

Water-soluble acids.—Dilute 5 ml. aliquot of extract A (as obtained in the determination of alpha amino nitrogen, method 2) 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 0.1*N* NaOH required to neutralize the acidity in 1 gram of tobacco.

Calculate the results as follows: Milliliters read from buret equal ml. 0.1*N* NaOH per gram of tobacco (M-&S-F). Average duplicate determinations.

Method 39.—Water-Soluble Nitrogen (other than Nitrate Nitrogen)

P. Lorillard Co., Inc.

DETERMINATION

Reflux gently for 1 hour a 3-gram sample (M-&S-F basis) in 200 ml. of distilled water, using an air condenser. Filter mixture through a mat of filtercel on a Whatman No. 30 filter paper in a Buchner funnel. Wash mat and sample with ten 20-ml. portions of distilled water and combine washings and filtrate. Allow extract to cool to room temperature, transfer to a 500-ml. volumetric flask, dilute to the mark with distilled water, and mix. Measure 100 ml. of the solution with a pipet and transfer to a 500-ml. Kjeldahl flask containing 10 grams of Na_2SO_4 and 0.2 gram of $CuSO_4$. Add 25 ml. of concentrated H_2SO_4 to Kjeldahl flask and digest for 1 hour after the color of the reaction mixture becomes clear green.

Allow to cool and add 250 ml. of distilled water and 10–15 pieces of granular 10-mesh zinc. Add 50 ml. of a saturated sodium hydroxide solution, pouring it down the side of the flask so that it does not mix at once with the acid solution. Pipet 50 ml. of *N*/10 HCl into the receiver at end of condenser. Connect the flask to the condenser by means of Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of the acid in the receiver. Mix contents in Kjeldahl flask by rotating flask and distill until a minimum of 150 ml. of distillate are obtained. Back-titrate with *N*/10 NaOH, using three drops of methyl red indicator.

Calculate the results as follows:

$$\frac{\text{ml. of } N/10 \text{ HCl required} \times 0.14008}{\text{one-fifth weight of original sample}} = \% \text{ water-soluble nitrogen (M-\&S-F basis)}$$

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BRIEF OF FLUE-CURED GRADES

Type 11: Flue-cured, produced principally in the Piedmont sections of Virginia and North Carolina.

Key to Standard Grdemarks for Flue-cured Tobacco

Groups	Qualities	Colors	Special Factors
A—Wrappers	1—Choice	L—Lemon	V—Greenish
B—Leaf	2—Fine	F—Orange	KR—Dappled
H—Smoking Leaf	3—Good	R—Red	GL—Light green
C—Cutters	4—Fair	S—Mahogany	GF—Medium green
X—Lugs	5—Low	D—Walnut	GR—Dark green
P—Primings	6—Poor	K—Variegated	W—Unsafe keeping
N—Nondescript		M—Mixed	U—Unsound
		G—Green	

Summary of Standard Grades and Subgrades

6 Grades of Wrappers

A1L A1F A1R
A2L A2F A2R

25 Grades of Leaf

B1L B1F B1R
B2L B2F B2R
B3L B3F B3R B3S
B4L B4F B4R B4S B4D
B5L B5F B5R B5S B5D
B6L B6F B6R B6S B6D

16 Smoking-leaf Grades

H1L H1F
H2L H2F
H3L H3F H3R
H4L H4F H4R
H5L H5F H5R
H6L H6F H6R

10 Cutter Grades

C1L C1F
C2L C2F
C3L C3F
C4L C4F
C5L C5F

10 Lug Grades

X1L X1F
X2L X2F
X3L X3F
X4L X4F
X5L X5F

6 Priming Grades

P3L P3F
P4L P4F
P5L P5F

7 Variegated Grades

B4K C4K X4K
B5K C5K X5K
B6K

3 Subgrades of Dappled

B4KR
B5KR
C5KR

12 Greenish Subgrades

B3LV B3FV X3LV X3FV
B4LV B4FV C4LV X4LV X4FV
B5LV B5FV C5LV

8 Mixed Grades

X3M
B4M C4M X4M
B5M C5M X5M
B6M

14 Grades and Subgrades of Green

X3G
B4GL B4GF B4GR X4G P4G
B5GL B5GF B5GR X5G P5G
B6GL B6GF B6GR

12 Subgrades of Nondescript

N1L N2L Botched
N1D N2D Nested
N1GL N2GL Offtype
N1GR N2GR N-Dec.

For example: B3L designates Leaf, good quality, in lemon color.

END