[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

CHEMICAL FACTORS DETERMINING THE QUALITY OF TOBACCO[1]

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The tobacco plant is unique among the crop plants of the farm in many respects, but especially because differences in soil and climate may cause changes in the composition of the plant which make it more or less valuable or even useless for the purpose for which it was grown. This is true to some extent with wheat, but its low ash content compared with the tobacco plant makes the changes due to soil and climate less noticeable. Greater changes often take place in the composition of corn, oats, soy beans, etc., due to the same causes, but their composition because it does not determine their value usually goes unquestioned even though wide differences exist. The chemical changes which take place in a tobacco plant may not be more intricate than those in a corn or soy bean plant but the final product, tobacco, is used largely by human beings who have acquired a taste for a product having a certain aroma, color, pliability, toughness or burning property, and they demand these qualities.

Soil and Climate Control Composition

It has been impossible, as yet, to determine the soil factors and climatic forces that cause such great differences in composition as exist for example, between Turkish tobacco grown in Turkey, and the same variety grown in California or between Cuban tobacco grown in Cuba and the same variety grown in Florida, but the determination that such differences in composition usually do exist has been helped by chemical analysis. When a study is made in the principal tobacco growing regions of the relation of the types of soil to the kinds of tobacco grown, it is found that the wrapper-leaf soils, such as those in Connecticut and Florida, are of the sandy and sandy-loam types. Even the subsoils in these famous areas are sandy and hold but little water. The binder-leaf soils, such as those of the Wisconsin district, are light clay loams. The filler-leaf soils are mostly loams, are more fertile than the wrapper-leaf soils, and retain large amounts of water. The Hagerstown loams of the Lancaster district of Pennsylvania are considered typical of the filler-tobacco soil.

High Mineral Content a Feature

The outstanding feature of the tobacco plant as compared with other crop plants is its unusually high ash or mineral content, varying from 15 to 35%, but averaging about 20%. This is more than double the usual ash

[1] Presented before the Agricultural and Food Section of the American Chemical Society at New Haven, Conn., April 2–7, 1923.

percentage of the more common crops. It is partly because of this unusual mineral content that much study has been given to the fertilization of tobacco plants with the view of improving the quality of the tobacco.

Garner,[2] also Ames and Boltz,[3] and others, have made extensive investigation along this line and have shown that important changes in the ash composition are caused by the use of different fertilizers.

The data reported from the Ohio Station agree in general with those found by the writers, namely, that liming the soil lessens the amount of potassium and phosphorus in the tobacco, that potassium sulfate improves the burning qualities of the tobacco, whereas potassium chloride produces tobacco of poor burning qualities. The Ohio Station found also that the tobacco fertilized with manure contained large amounts of chlorine but this did not seem to impair the burning qualities. The aroma and flavor were somewhat improved in tobacco from soil treated with acid phosphate. The total nitrogen of plants grown in Indiana was usually about 3%, only a few having as high as 4%, or as low as 2%.

It was noted by the writers that, although the changes caused by fertilizers were considerable, they were of minor importance when compared with those effected by growing the plants on a different kind of soil or in a different climate. Therefore, it is evident that the final product seems to be the resultant of a series of factors and is not to be determined by the presence or absence of any one constituent.

Object of the Investigation

In view of these facts an effort was made to determine the chief features of tobacco grown on different soil types and in different climates, and also to detect variations in composition between the desirable and the less valuable forms of tobacco offered for sale. The part of the study relative to the amount of material extracted by the different solvents, and to the amount of nicotine contained in the different extracts, has proved helpful in interpreting the quality of tobacco used for different purposes.

Sources of Tobacco Investigated

In order to have available for study tobacco whose cultural history was known, Improved Burley plants were grown on Miami clay-loam soil in Switzerland County, and another set of the same variety on Sioux silt loam in Tippecanoe County, Indiana, during the summer of 1920 and again in 1921. Very little tobacco is grown in the latter county but it is an important crop in Switzerland County. Both lots of plants grew well, but soon developed differences in texture and color of the leaves; also, the ratio of leaf surface to stalk on the Switzerland County soils was about 10% and the ash 3% greater than that of the Tippecanoe County plants.

[2] Garner, U. S. Bur. Plant Ind., *Farmers' Bull.*, **571** (1920).

[3] Ames and Boltz, Ohio Exp. Sta., *Bull.*, **285** (1915).

Samples were secured from these different plants as well as from tobaccos grown in other States. Besides these, some of the more common brands of cigars and smoking tobaccos were secured from local stores for use in this investigation.

Treatment of Samples

The tobacco samples were finely ground (to about 40 mesh) and extracted in a Soxhlet extractor, first with petroleum ether (b. p., 45–50°), followed with ethyl ether (distilled over sodium), then with ethyl alcohol (98%), somewhat according to the method of Dragendorff,[4] except that a Soxhlet tube was used for making the extraction as in a fat determination. Six g. of the sample and 60 cc. of solvent were used for each extraction. The order in which the solvents were used was, first, petroleum ether, followed by ethyl ether, then alcohol, (acetone followed in some cases) and finally boiling water. The extraction was continued for 15 hours, after which the solvent was distilled from the extract, using a steam-bath; then the extract was dried in an oven at 80° and finally weighed. The water extraction was made by percolation without removing the sample from alundum shell used in the other extractions. A total of 500 cc. of boiling water was used. The acetone extracted less than 1% of solids and no nicotine. The water extract also contained no nicotine as determined by the (Kissling) official method. The combined extracts usually contained a little more nicotine than could be obtained by official method alone or by the Garner[5] method. Petroleum ether dissolves mostly ethereal and fatty oils, vegetable waxes and a little chlorophyll and nicotine, and does not seem to coagulate the albuminous material that may be present. Ethyl ether dissolves the resins, glucosides and much of the nicotine together with chlorophyll in unlimed soils. The alcohol extract contained the sugars, tannins, some of the resins and the remainder of the nicotine. The treatment with boiling water dissolved mucilage, acids, carbohydrates and albumins.

The data obtained from the extractions of tobacco grown in Switzerland County, Indiana, are given in Table I.

Discussion

It will be noted from the table that there is a great difference among tobaccos in the amounts of solids extractable by the different solvents, as well as distinct differences in the amounts of nicotine contained in the different extracts. In the limestone-treated plants there is about the usual amount of extractives in the petroleum ether extracts, but they contain very little nicotine. In the ethyl ether lots the amounts of extract and

[4] Dragendorff, "Plant Analysis," G. E. Stechert and Co., New York, 1921, p. 8, reprint.

[5] Garner, *U. S. Dept. Agr., Bur. Ind., Plant Bull.*, **102** (1907).

nicotine are very small. However, the plant contains considerable nicotine which shows up in the alcohol extract. The plants treated with the different fertilizer combinations without liming have more than double the solids soluble in ether and a large part of all nicotine present in the plant is to

TABLE I

THE PERCENTAGES OF EXTRACTIVES, NICOTINE, TOTAL NITROGEN AND ASH FOUND IN TOBACCO GROWN IN SWITZERLAND COUNTY, INDIANA, ON PLOTS TREATED WITH DIFFERENT AMOUNTS OF LIME COMPOUNDS AND FERTILIZERS

	Solids and nicotine extracted										
	Petroleum ether		Ethyl ether		Alcohol		Total from extracts				
Treatment of the different plots	Extract %	Nicotine %	Extract %	Nicotine %	Extract %	Nicotine %	Extract[a] %	Nicotine %	Nicotine Official method %	Total nitrogen %	Ash %
Limestone ½%[b]	5.07	0.00	1.71	0 39	12.53	2.46	40.71	2.85	4.95	3.57	20.71
Limestone 1%	4.22	.05	1.22	.08	16.32	2.18	41.06	2.81	3.56	3.43	21.27
Limestone 1½%	6.32	.13	2.43	.36	8.32	0.79	39.94	1.28	3.21	3.09	22.16
Limestone 2%	5.48	.13	2.36	.38	9.44	1.88	40.98	2.39	2.92	2.89	21.60
Limestone 2½%	5.57	.05	1.24	.13	20.16	3.53	47.49	3.71	3.99	4.48	19.55
Limestone 3%	3.90	.05	2.09	.33	19.32	3.54	47.38	3.92	2.56	3.77	21.08
Limestone 3½%	4.41	.08	2.02	.82	17.20	2.72	43.89	3.67	4.59	3.86	22.59
Limestone 4%	4.67	.05	1.45	.17	13.51	1.05	40.45	1.27	3.83	3.57	21.51
Ca. hyd. ½%	4.71	.10	3.04	.51	12.74	1.55	39.66	2.16	3.72	3.65	17.99
Ca. hyd. 1%	6.32	.13	2.64	1.01	16.40	1.50	46.11	2.64	2.65	4.62	17.96
Ca. hyd. 1½%	2.30	.09	2.13	1.14	16.78	1.72	48.27	2.95	2.80	4.15	18.50
Ca. hyd. 2%	6.26	.12	2.98	1.22	10.56	0.91	39.20	2.25	2.08	3.80	23.21
K_2SO_4 (90)[c]	6.73	.23	5.49	3.17	15.26	1.65	46.54	4.95	4.59	3.59	17.07
K_2SO_4 (360)	4.70	.18	4.78	2.48	16.21	2.28	45.44	4.94	4.32	3.21	20.87
KCl (90)	6.44	.16	3.66	1.29	11.87	1.90	48.16	3.35	2.73	2.52	21.14
KCl (360)	6.68	.15	5.37	2.33	19.67	0.43	50.92	2.91	3.05	3.14	22.40
$NaNO_3$ (90)	7.17	.15	3.23	2.71	11.98	1.52	44.37	4.38	2.94	3.68	16.60
$NaNO_3$ (360)	7.53	.13	5.55	2.53	12.28	0.76	53.85	3.42	2.54	3.88	18.31
Acid phos. (90)	4.82	.23	4.07	2.43	10.03	1.52	48.60	4.18	2.66	3.59	18.32
Acid phos. (360)	2.72	.08	6.01	2.21	9.52	1.49	36.02	3.78	2.66	3.39	18.81
4–6–10 (230)	3.85	.26	5.49	1.25	10.14	1.85	40.04	3.34	3.77	3.90	18.69
4–6–10 (360)	3.04	.15	4.89	1.60	13.07	2.05	45.22	3.80	1.57	4.09	22.42
4–6–10 (230)	5.03	.18	2.48	1.57	9.55	1.80	36.39	3.55	2.70	2.81	22.78
4–10–6 (360)	2.27	.15	4.78	1.13	9.14	0.90	36.44	2.18	2.86	2.72	19.54
Check	5.06	.56	3.15	1.95	16.43	0.57	44.49	3.08	3.05	4.48	16.77

[a] Total solids extracted by petroleum ether, ethyl ether, alcohol, acetone and water.
[b] Of weight of 28 cu. dm. (1 cu. ft.) of soil.
[c] Kg. per acre.

be found in this ether residue. The addition of acid phosphate to the soil seems to reduce the extractives soluble in petroleum ether and to enhance the aroma and flavor of the tobacco.

Aroma not Dependent upon Nicotine Content

It is well known that the aroma is not closely related to the amount of nicotine contained in the plant, as it has been shown by Garner[6] that the sharp, biting quality was removed after extraction with petroleum ether which does not usually remove much nicotine. Nicotine compounds are quite basic and seem to act like the corresponding compounds of ammonia, being volatile in some combinations and non-volatile in others, just as

[6] Garner, *U. S. Bur. Plant Ind., Bull.*, **141**, Part I (1908).

ammonia in ammonium carbonate is volatile as compared with ammonium sulfate, non-volatile; so nicotine in the free state, or as nicotine tannate, acetate or some loose combination is volatile, whereas nicotine malate, citrate and oxalate are non-volatile, and insoluble in petroleum ether. It is this free or loosely combined nicotine that is probably of greatest service as an insecticide when tobacco is used as a dust. This would include that part which is soluble in petroleum ether and probably some of that soluble in ethyl ether as shown in limed samples of Table I.

Calcium-Nicotine Combinations

It would seem, therefore, that when tobacco is grown on soil to which considerable limestone has been added it forms combinations which are not easily extractable with either petroleum ether or ethyl ether. When calcium chloride or calcium oxide are mixed with powdered tobacco of known nicotine content and extracted for nicotine by the official method, much less is obtained than when calcium compounds are absent. Also when nicotine sulfate ("black leaf 40") was absorbed in powdered calcium carbonate, as directed in the Official Methods, less nicotine was extracted than when the same nicotine compound had been absorbed in filter paper previous to extraction. It was found that when a 4% water solution of pure nicotine was added to a 10% solution of calcium chloride a slight precipitation resulted after a few minutes. When part of this solution is made basic with ammonium hydroxide no further precipitation is produced, but when sodium or potassium hydroxide is used a voluminous precipitate is formed containing most of the nicotine present. The precipitate thus obtained is insoluble in ether. When the sodium or potassium hydroxide is added to the nicotine solution without the addition of calcium chloride, nicotine separates in the form of an oily layer on top of the water solution, and then is easily soluble in ether. The calcium chloride solution used had a Sörensen value (P_H) of about 7.4 while the nicotine solution was more basic, but when the nicotine solution was added to the calcium chloride the solution became acid in reaction. The writers believe that the reason why the two basic solutions become acid when mixed is due to the combination of the calcium with the nicotine, producing a calcium salt of nicotine and hydrochloric acid.

Amide Nitrogen Linkage of Tobacco

When a powdered sample of tobacco is treated with a weak sodium hydroxide solution and heated gently the ammonia and other basic material volatilized are usually equivalent to 1 or 2% of the weight of the sample. An odor of ethyl acetate is quite evident after most of the ammonia has been released. In view of the release of so much ammonia from tobacco when treated with sodium hydroxide as in the official method for the

determination of nicotine, it is hardly to be expected that the amount of nicotine thus obtained would check closely with that obtained by the mild extraction method used in this investigation.

Tobaccos from Important Tobacco Districts

A study was made also of tobacco grown in several different States. The data obtained for some of these different varieties are given in Table II.

TABLE II

THE PERCENTAGES OF EXTRACTIVES, NICOTINE AND ASH OF TOBACCO FROM DIFFERENT LOCALITIES

	Extractives and nicotine extracted by									
	Petroleum ether		Ethyl ether		Alcohol		Total from extracts		Nicotine	
Tobacco used	Extract	Nicotine	Extract	Nicotine	Extract	Nicotine	Extract[a]	Nicotine	Official method	Ash
Wis. White Burley	3.56	0.14	1.48	0.43	13.48	0.41	42.02	0.98	1.48	34.37
Wis. Binder unfermented	3.20	.16	3.09	1.05	10.86	.57	41.48	1.78	3.02	25.22
Comstock Spanish	2.84	.08	2.09	0.68	9.28	.22	38.06	0.98	1.54	24.86
Wisconsin Pryor	2.93	.11	3.13	.89	9.91	.51	43.31	1.41	2.05	19.03
Resistant Wis. Binder	2.88	.08	3.03	.57	10.29	1.03	36.05	1.68	1.49	30.58
Wis. Binder fermented	2.93	.08	3.82	.73	11.34	0.97	39.94	1.78	1.65	24.30
Conn. unfermented	2.73	.22	1.88	.43	13.92	.65	36.38	1.30	1.16	22.74
Conn. Havana unfermented	3.54	.24	3.84	.43	15.30	.30	43.98	0.97	1.51	26.28
Ky. Dark, air cured	4.80	.89	3.64	1.19	19.43	1.75	47.13	3.83	3.65	28.52
Ky. Dark, fire cured	2.86	.19	4.16	1.92	16.25	2.56	40.89	4.67	3.00	20.37
Ky. White Burley	3.78	.19	2.64	0.46	17.86	0.76	40.71	1.41	1.30	21.90
Ind. Improved Burley	5.06	.56	3.15	.95	16.43	.57	44.49	3.08	3.05	16.77

[a] Total extract from petroleum ether, ethyl ether, alcohol and water.

Discussion

An inspection of Table II shows that in the case of Wisconsin tobaccos the percentages of extractives and to some extent the nicotine are all quite low, which seems to be characteristic of the binder and wrapper tobaccos as well as of the cigar tobaccos which were examined. Samples 9–12 from Kentucky and Indiana contain a high percentage of alcohol extractives, mostly glucoses, alkaloids and tannins, together with a large amount of nicotine. Most of the samples grown in Indiana which were tested give a relatively high petroleum ether extract and are characterized by having a waxy coating, some of which sticks to the hands. Garner has shown that the presence of considerable vegetable wax together with ethereal and fatty oils present in petroleum ether extract tends to give the tobacco a poor flavor and aroma and therefore it brings a lower price on the market.

Smoking Tobaccos and Cigars

In order to compare the pure leaf tobaccos studied with the smoking tobaccos and cigars on the market, samples were purchased locally and examined. The data from these samples are given in Tables III and IV.

TABLE III

THE PERCENTAGES OF EXTRACTIVES, NICOTINE AND ASH OF SOME COMMON SMOKING TOBACCOS

Tobacco	Extract and nicotine extracted by: Petroleum ether Extract	Petroleum ether Nicotine	Ethyl ether Extract	Ethyl ether Nicotine	Alcohol Extract	Alcohol Nicotine	Total from extracts Extract	Total from extracts Nicotine	Nicotine Official method	Ash
Tuxedo	7.40	0.41	2.32	0.44	15.38	0.08	42.70	1.93	1.23	16.50
Velvet	5.25	.21	4.47	.44	15.76	.49	47.69	1.14	0.92	16.41
Edgeworth	7.55	.28	6.93	.94	16.06	.13	50.91	1.35	1.39	15.24
City Club	7.12	.47	4.83	.59	14.22	.49	44.57	1.55	0.77	17.21
Prince Albert	3.99	.26	4.97	.68	22.64	.27	47.54	1.20	0.81	16.63
Blue Boar[a]	4.03	.23	4.37	.92	16.77	.22	43.62	1.37	1.15	15.26

[a] This retails at 52 g. (1¾ oz.) for 25 cents; all others given are 15 cents for the same weight.

TABLE IV

THE PERCENTAGES OF EXTRACTIVES, NICOTINE AND ASH OF DIFFERENT TOBACCOS (Arranged in Order of Increasing Selling Price)

Cigars used	Extract and nicotine extracted by: Petroleum ether Extract	Petroleum ether Nicotine	Ethyl ether Extract	Ethyl ether Nicotine	Alcohol Extract	Alcohol Nicotine	Total from extracts Extract	Total from extracts Nicotine	Nicotine Official method	Ash
American Citizen	8.74	0.37	2.54	0.30	6.05	0.40	37.29	1.07	1.29	23.89
Snap	2.22	.40	2.68	.40	8.19	.87	33.00	1.67	2.47	20.12
G. B.	3.86	.20	1.68	.20	7.36	.66	28.60	1.06	2.42	19.89
Kelly	5.08	.17	3.08	.51	8.60	1.77	35.10	2.45	1.30	18.84
Portima	3.00	.47	1.84	.14	7.74	0.82	27.30	1.43	1.35	25.78
Chancellor	4.12	.28	2.46	.28	5.61	.78	28.35	1.34	1.35	20.79

Discussion

The data from the smoking tobaccos (Table III) as well as cigar tobacco (Table IV) will be discussed together in order to contrast their differences. It will be noted that most of the smoking tobaccos give very high residues from the extract with petroleum ether, whereas those of most of the cigar tobaccos are quite low, signifying the presence of less wax and ethereal oils, and therefore affording a better aroma when smoked. The high solubilities in alcohol of the smoking tobaccos and the low percentages of extract from cigar tobaccos are outstanding. This may be due in some cases to the addition of licorice or some other sweetening material, but probably in most cases to the fact that a cheaper grade of filler tobacco was used in compounding. Most of the nicotine was extracted with the ethyl ether fraction in all but the tobacco from the limed soils, which would indicate that it may be connected in some way with the resin acids and can be extracted with the solvent most readily dissolving these compounds.

Summary

The extraction of tobacco samples with different solvents in the order of their relative solubilities has been found useful in classifying grades of tobacco. There is a relation between their grades and the order of their solubilities. Cigar tobacco of a good quality has a small percentage of

extractives and nicotine, while smoking tobacco has a high solubility in petroleum ether, ethyl ether and alcohol.

Plants having a high calcium content had most of their nicotine so combined in stable form as to be quite insoluble in either petroleum ether or ethyl ether.

Black Leaf 40, as well as powdered tobacco when mixed with different calcium compounds, had the nicotine so fixed as to make it difficult to extract it completely even by the official method.

The vegetable waxes, volatile oils and loosely combined nicotine contained in the petroleum ether extracts are responsible for much of the irritating effect of the tobaccos grown; tobaccos could be greatly improved by extraction with this solvent.

The ratio of leaf to stalk was much greater in Switzerland County (Miami clay loam) than in Tippecanoe County, Indiana (Sioux silt loam). The ash content averaged about 3% lower for tobacco grown in the latter county than for that from the clay loam soil.

Plants which had been treated with various amounts of acid phosphate fertilizer had a small petroleum ether extract and possessed the most agreeable aroma of any plants grown.

Although the composition of tobacco may be greatly modified and improved by different fertilizer treatments, the tobacco which commands the best price of any examined was grown on relatively poor sandy or clay soil, unsuited to corn, but producing a tobacco characterized by a low percentage of extractives, proteins and nicotine and suitable for making a cigar of such pleasing taste and aroma as to command a good market price.

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STUDIES ON CATALYSIS. IV. THE BEHAVIOR OF THE AMINO GROUP WHEN CYTOSINE AND NITRO-URACIL ARE REDUCED IN THE PRESENCE OF COLLOIDAL PLATINUM

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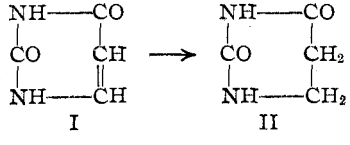
In the study of the application of catalytic reduction by means of colloidal platinum or palladium and molecular hydrogen to the pyrimidine series, the writers have previously reported the quantitative formation of hydro-uracil II by reduction of uracil I.[1] By the application of this method



[1] THIS JOURNAL, **45**, 2702 (1923).