Author: Polansky, Theodore Stephen

Title: The effects of temperature and moisture on carbon dioxide production of fermenting Pennsylvania cigarleaf tobacco

Place of Publication:

Copyright Date: 1936

Master Negative Storage Number: MNS# PSt SNPaAg031.4

<104596> * *OCLC* Form:manuscript item 2 Input:CHF Edit:FMD
008 ENT: 980111 TYP: s DT1: 1936 DT2: LAN: eng
035 (OCoLC)38092113
037 PSt SNPaAg031.4 $bPreservation Office, The Pennsylvania State University, Pattee Library, University Park, PA 16802-1805
090 20 Thesis 1936m $bPolan,TS $cst*7342806 $cax+(Archival)
090 20 Microfilm D344 reel 31.4 $cmc+(service copy, print master, archival master)
100 1 Polansky, Theodore Stephen $d1907-
245 14 The effects of temperature and moisture on carbon dioxide production of fermenting Pennsylvania cigarleaf tobacco $ca thesis by Theodore Stephen Polansky.
260 $c1936.
300 27 leaves $bill. $c29 cm.
502 Thesis (M.S.)--Pennsylvania State College.
504 Bibliography: leaves 26-27
533 Microfilm $bUniversity Park, Pa. : $cPennsylvania State University $d1997. $e1 microfilm reel ; 35 mm. $f(USAIN state and local literature preservation project. Pennsylvania) $f(Pennsylvania agricultural literature on microfilm).
590 Archival master stored at National Agricultural Library, Beltsville, MD : print master stored at remote facility.
590 This item is temporarily out of the library during the filming process. If you wish to be notified when it returns, please fill out a Personal Reserve slip. The slips are available in the Rare Books Room, in the Microforms Room, and at the Circulation desk.
650 0 Tobacco curing $zPennsylvania.
830 0 USAIN state and local literature preservation project. $pPennsylvania.
830 0 Pennsylvania agricultural literature on microfilm.

THE PENNSYLVANIA STATE COLLEGE

The Graduate School

Department of Agricultural and Biological Chemistry

THE EFFECT OF TEMPERATURE AND MOISTURE ON THE CARBON DIOXIDE PRODUCTION OF FERMENTING PENNSYLVANIA CIGAR-LEAF TOBACCO

A Thesis

By

Theodore Stephen Polansky

Submitted in partial fulfillment

for the degree of

Master of Science

February, 1936

Approved: 10-17-35

W. G. Haley

Professor of Soil and Phytochemistry

R. Adams Dutcher.

Head of Department of Agricultural and Biological Chemistry

Table of Contents

I. Introduction and Review of Literature

One of the chief interests of the Pennsylvania tobacco industry is to improve the quality of cigars made from Pennsylvania cigar-leaf. Although fertilizer experiments have accomplished much in the improvement of the leaf tobacco, it is of great importance that attention be given to its fermentation for the quality of the final product also depends upon this process.

It is clearly recognized that the flavor and aroma of the cigar-leaf tobacco are developed during its fermentation, although the changes are dependent upon the original quality of the cured leaf. Since the curing process also influences the quality of the final product, it is of interest to know that the following changes take place during this period (2):

1. - Disappearance of starch.
2. - Formation of sugar and its partial disappearance by respiration.
3. - Decomposition of protein with formation of amino compounds.
4. - Decrease of fatty matter.
5. - Decrease of tannin.
6. - Change of color and flavor.

After curing, cigar-leaf tobacco is then subjected to one or more definite fermentation periods. During this period there is an exchange of gases, a generation of heat, and a change in the flavor and aroma of the leaf.

The principle known changes which take place during the fermentation process may be summarized as follows (3):

1. - Loss of water.
2. - Almost complete loss of chlorophyll.
3. - Loss of carbohydrates.
4. - Splitting of proteins to amino acids.
5. - Increase in ammonia and amide nitrogen (showing a breakdown of amino acids).
6. - Decrease of total nitrogen, about 15%.
7. - Improvement of flavor and aroma.

At the present time the extent of fermentation is determined solely by the opinion of experienced men. There seem to be no scientific tests which are used to follow the progress of this process.

Today very little is known concerning the nature of the process of fermentation. It is known,

however, that the chemical changes occurring during this process depend upon two important factors, moisture and temperature. Yet today, there is no definite agreement as to the proper conditions to which tobacco should be subjected during fermentation. It is the object of this investigation to study the effect of these two factors upon the carbon dioxide production.

Whitney and Means (4), in 1899, claimed that it is difficult to properly ferment a small quantity of leaf, and that this should not be attempted with less than 1,000 pounds of leaf. They also claim that the temperature of the room should be maintained from 21°C. to 27°C. during active fermentation and with 23%-24% moisture content. A water content of less than 20% or more than 26% is undesirable, for less than 20% moisture inhibits enzyme action and more than 26% favors bacterial and fungus growths which may injure the leaf. There must be no actual standing water on the leaf and the relative humidity of room should be from 80%-90%, although it may occasionally run up to 100%. In contrast to this, Loew (2) reported that to bring about a normal sweat of tobacco

requires between 18%-25% moisture and 18°C. to 22°C. temperature.

In 1926, Fodor and Reifenberg (6) reported that the oxygen intake and the carbon dioxide output are dependent upon the moisture content of the tobacco, temperature, and amount of air introduced into the fermentation outfit.

Smirnov and Petrik, in 1930, (7) found that organic acids increase the carbon dioxide formation in some tobaccos but decrease it in others. Amino acids constantly increase the carbon dioxide output. They concluded that amino acids are fertile sources of carbon dioxide and that in some tobaccos organic acids are also good sources.

Loew, in 1899, (2) reported that 3 liters of air from the interior of a fermenting pile when drawn through 25 cc. of Nessler's reagent produced a light yellow color indicating about 0.05 milligram of ammonia. Very little carbon dioxide was found in this experiment.

In 1931, Vickery and Pucher (3) reported that a considerable part of the coagulable protein and insoluble carbohydrates passed into a soluble form and an equivalent quantity of substance then

underwent transformation ultimately to carbon dioxide, water, and ammonia or other volatile substances which escaped from the tissues.

Then, in 1934, Johnson (9) reported that carbon dioxide production is completely checked at low moisture contents and at the extremes of temperature. His estimation of the amount of carbon dioxide are based on the cloudiness of lime water. He stated that carbon dioxide formation is a result of oxidation and should therefore be closely correlated with the fermentation process.

II. Object of the Investigation

The object of the investigation was to make a study of the effect of temperature and moisture on the carbon dioxide production of fermenting Pennsylvania cigar-leaf tobacco.

As far as it is known today, there is no published study on the quantitative determination of carbon dioxide from the effect of varying temperature and moisture conditions of fermenting cigar-leaf tobacco.

III. Materials Used

The tobacco used was grown on the new experimental field of Lancaster, Pennsylvania. It is the 1932 crop of Pennsylvania cigar-leaf tobacco. The tobacco was harvested, air-cured, baled, and sent to The Pennsylvania State College where it was ground in a corn mill. All the ground tobacco was then mixed thoroughly to get a representative sample.

IV. Experimental Methods

The method used to determine the carbon dioxide production from fermenting tobacco under varying temperature and moisture conditions is as follows:

Fifty grams of ground dry tobacco were atomized with the number of cc. distilled water as designated in the data. After thorough mixing of tobacco with water, it was transferred into a wide mouthed Florence flask. Then the flask was tightly sealed with a rubber stopper containing two glass tubes. The air intake tube was connected with two wash bottles, the first one containing a 13% solution of KOH and the second one constant boiling point sulfuric acid. The outlet was connected to a soda-lime tube containing saturated boric acid solution with methyl red indicator which in turn was connected to another soda-lime tube having constant boiling point sulfuric acid. This last tube was then connected to three soda-lime tubes, the first two containing moist soda-lime and the third containing boiling point sulfuric acid with pumice stone. This end tube was connected to an

aspirator. After all connections were properly made, the flask containing the tobacco was inserted in a constant temperature bath where thermometers were kept to check the accuracy of the temperature. The stopcocks were then manipulated so that there was a slow constant flow of air passing through the system from the wash bottles through the tubes, at the rate of sixteen liters every twenty-four hours.

Each individual fermentation was allowed to proceed for a period of twenty-four hours on duplicate samples. After twenty-four hours, the system was closed and the determination ended. The tubes containing soda-lime and sulfuric acid with pumice stone were weighed and checked against the weight before the run was made. The tube containing saturated boric acid solution with methyl red was titrated against .01 N sulfuric acid. Blanks were run to check against any faulty connection and faulty reagents.

A series of moisture variations were run against a constant temperature and results were recorded on the data sheet. The change in temperature of the bath was manipulated by the adjustment of the thermostat. The sensitivity of

the thermostat is ± 1°C.

Bacterial counts were made in the usual way at three different temperatures and three different moisture contents.

Use of the Chemicals

1. - Wash bottles -- The one containing 13% KOH solution was used for taking out all carbon dioxide from the air entering into the system and the other containing constant boiling point sulfuric acid was used for absorbing the ammonia and moisture from the air.

2. - The saturated boric acid solution was used for absorbing the ammonia from the fermented tobacco.

3. - The sulfuric acid next to it was used for absorbing all the moisture coming from the tobacco and boric acid solution.

4. - The moist soda-lime was used for absorbing the carbon dioxide given off by the fermented tobacco.

5. - The sulfuric acid with the pumice stone was used for absorbing the moisture given off by the reaction between soda-lime and carbon dioxide.

V. Presentation of Data

All results regarding carbon dioxide are recorded as grams of carbon dioxide given off by the fermented tobacco in twenty-four hours. The amount of moisture added was calculated on the air dry basis of the tobacco. The moisture content of the tobacco was 5.97%.

After a twenty-four hour run, the ammonia was tested and recorded as negative or positive because of very small quantities given off. Positive signs indicate presence of ammonia and negative ones absence of ammonia.

The odor of the tobacco was recorded as indicated in the data after the twenty-four hour run.

Negative signs in the blank indicate that there were no faulty connections nor faulty reagents.

TABLE I

Fermentation of 50 grams Cured ground tobacco at 17° C

24 hours

Moisture	H_2O added	Sample No. 1	Sample No. 2	Sample No. 3	Ammonia	Odor	Blank
per cent	cc	gm CO_2	gm CO_2	gm CO_2			
15	8.7	.0053	.0040	.0037	—	Raw	—
20	12.5	.0087	.0097	.0101	—	Raw	—
25	16.6	.0125	.0115	.0129	—	Raw	—
30	21.4	.0175	.0196	.0181	—	Good	—
35	26.9	.0250	.0242	.0225	—	Good	—
40	33.3	.0150	.0165	.0175	—	Good	—
45	40.9	.0198	.0200	.0210	—	Bad	—
50	50.0	.0275	.0295	.0266	—	Bad	—
55	61.1	.0392	.0411	.0378	—	Bad	—

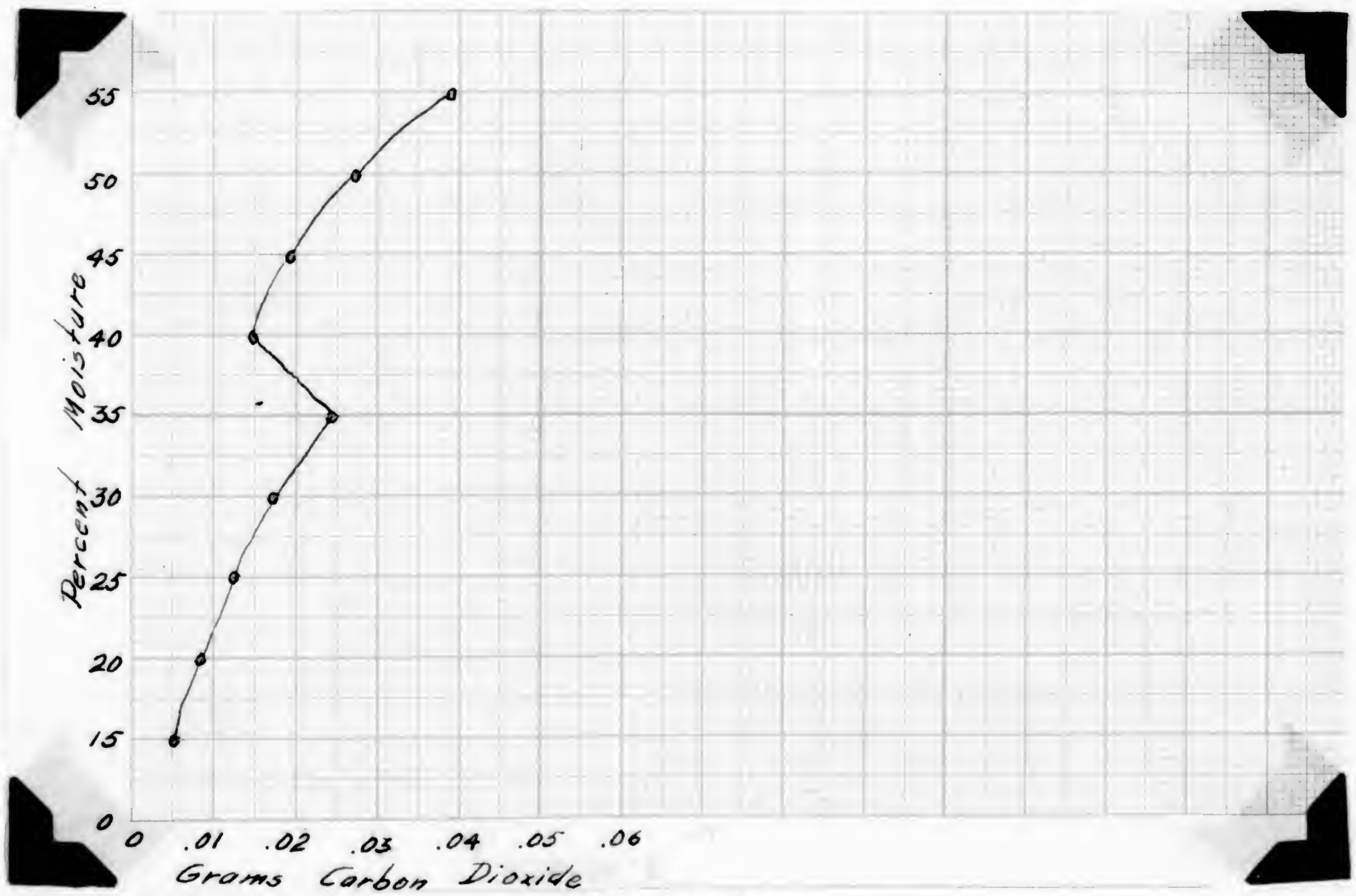


Figure I

TABLE II

Fermentation of 50 grams Cured-ground tobacco at 20° C.

24 Hours

Moisture	H_2O added	Sample No. 1	Sample No. 2	Sample No. 3	Ammonia	Odor	Blank
per cent	cc	gm CO_2	gm CO_2	gm CO_2			
15	0.7	.0125	.0142	.0150		Raw	
20	10.5	.0240	.0276	.0238		Raw	
25	16.3	.0190	.0330	.0356		Raw	
30	21.4	.0500	.0578	.0519		Good	
35	26.9	.0600	.0578	.0686		Good	
40	33.3	.0513	.0579	.0576		Good	
45	40.2	.0730	.0763	.0710		Bad	
50	50.0	.0833	.0861	.0806		Bad	
55	61.1	.0963	.0950	.0982		Bad	
60	75.0	.0950	.0982	.0995		Bad	
65	92.8	.1023	.1043	.1010		Bad	
70	116.0	.1301	.1463	.1235		Bad	
75	150.0	.1432	.1498	.1450		Bad	
80	200.0	.1428	.1561	.1560		Bad	

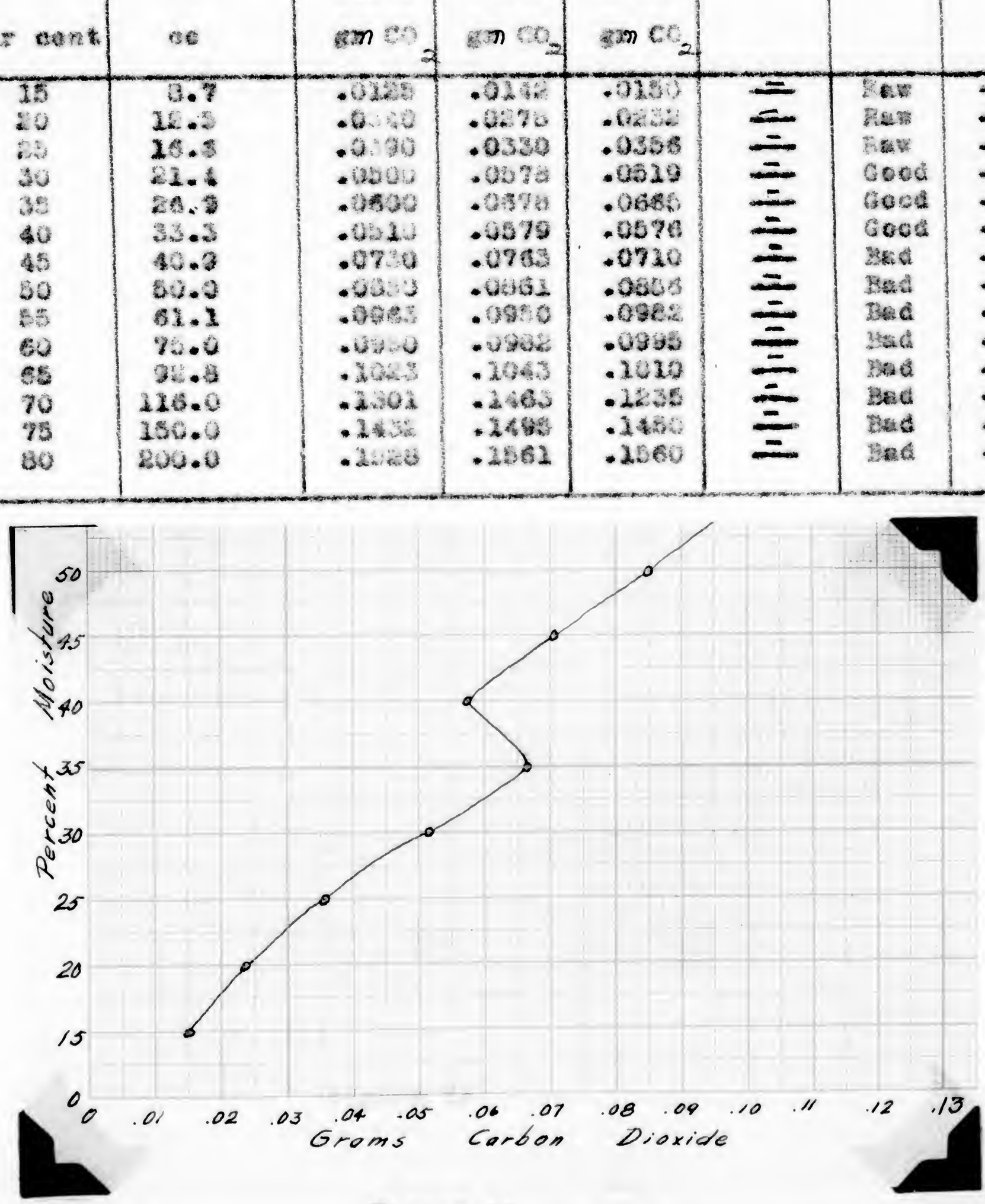


Figure II

TABLE III

Fermentation of 50 grams Cured ground tobacco at 25° C.

24 hours

Moisture	H_2O added	Sample No. 1	Sample No. 2	Sample No. 3	Ammonia	Odor	Blank
per cent	cc	gm CO_2	gm CO_2	gm CO_2			
15	8.7	.0251	.0189	.0376	±	Raw	—
20	12.5	.0395	.0376	.0410	±	Raw	—
25	16.6	.0469	.0357	.0505	±	Good	—
30	21.4	.0529	.0575	.0801	±	Good	—
35	26.9	.0871	.1284	.1128	±	Good	—
40	33.3	.0385	.0403	.0398	±	Bad	—
45	40.9	.0859	.1372	.1275	±	Bad	—
50	50.0	.1352	.1352	.1375	±	Bad	—
55	61.1	.1396	.1359	.1436	±	Bad	—
60	75.0	.1772	.1351	.1758	±	Bad	—

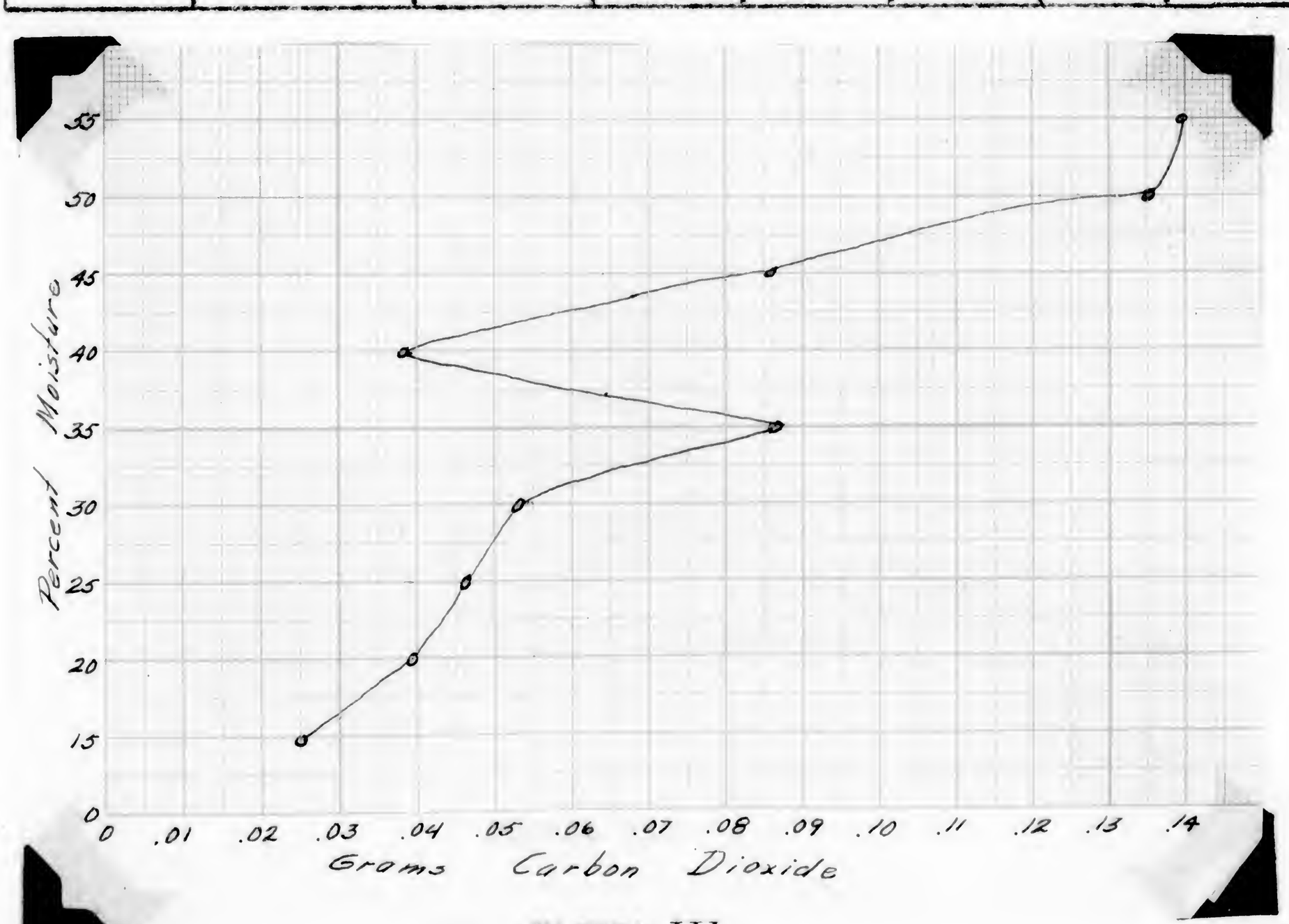


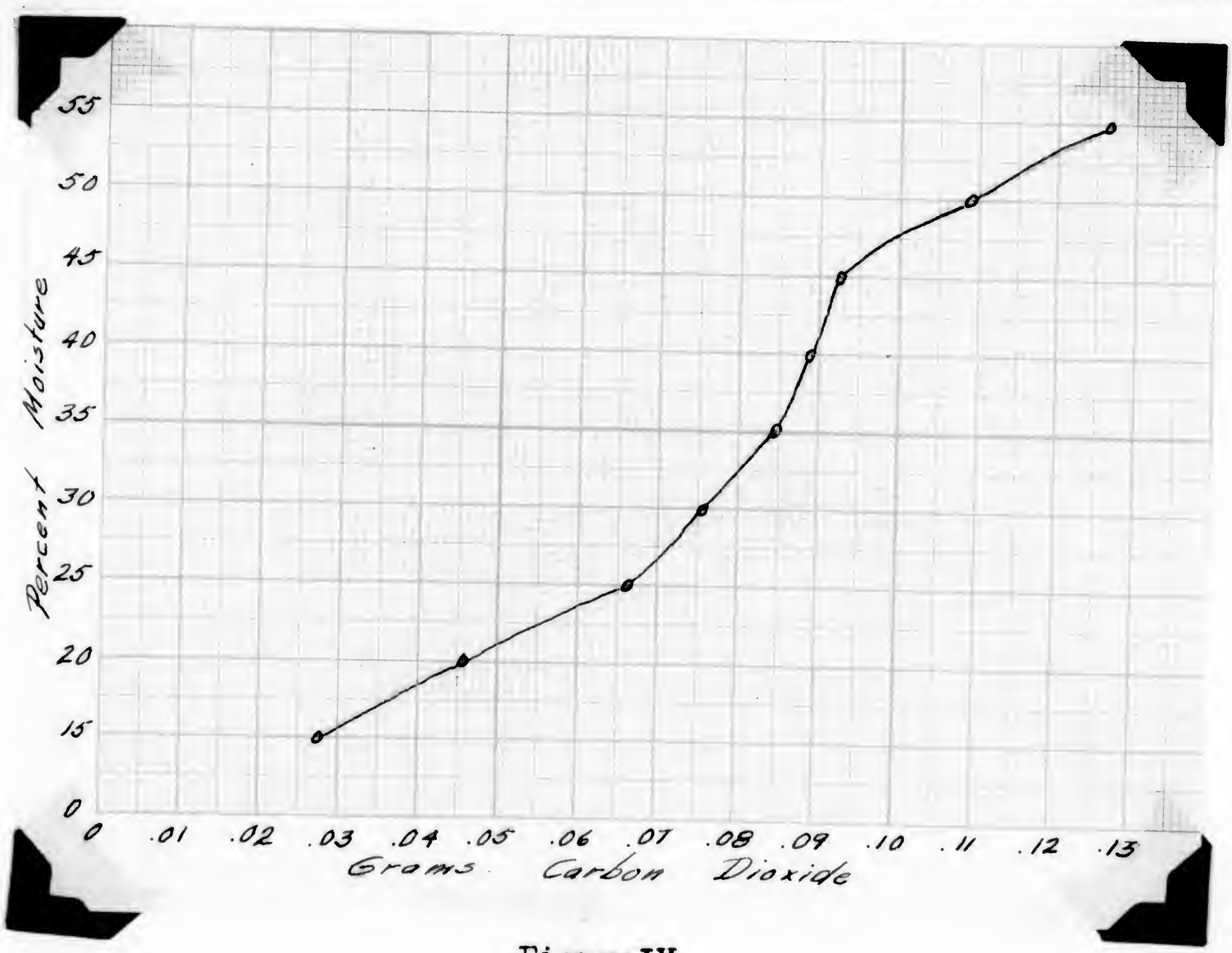
Figure III

TABLE IV

Fermentation of 50 grams Cured ground tobacco at 30° C.

24 hours

Moisture	H_2O added	Sample No. 1	Sample No. 2	Sample No. 3	Ammonia	Odor	Blank
per cent	cc	gm CO_2	gm CO_2	gm CO_2			
15	8.7	.0276	.0333	.0323	±	Raw	±
20	12.5	.0451	.0503	.0497	±	Raw	±
25	16.6	.0661	.0636	.0672	±	Good	±
30	21.4	.0762	.0762	.0775	±	Good	±
35	26.9	.0845	.0803	.0841	±	Good	±
40	33.3	.0886	.0775	.0732	±	Bad	±
45	40.9	.0929	.0959	.0952	±	Bad	±
50	50.0	.1092	.1261	.1012	±	Bad	±
55	61.1	.1267	.1342	.1241	±	Bad	±



FigureIV

TABLE V

Fermentation of 50 grams Cured ground tobacco at 35° C.

24 hours

Moisture per cent	H_2O added cc	Sample No. 1 gm CO_2	Sample No. 2 gm CO_2	Sample No. 3 gm CO_2	Ammonia	Odor	Blank
15	8.7	.0395	.0376	.0410	±	Raw	
20	12.5	.0595	.0480	.0510	±	Raw	
25	16.6	.0775	.0665	.0730	±	Good	
30	21.4	.0868	.0860	.0857	±	Good	
35	26.9	.0910	.0907	.0910	±	Good	
40	33.3	.0895	.0850	.0863	±	Good	
45	40.9	.0910	.0883	.0957	±	Bad	
50	50.0	.1130	.1075	.1072	±	Bad	
55	61.1	.1295	.1251	.1230	±	Bad	

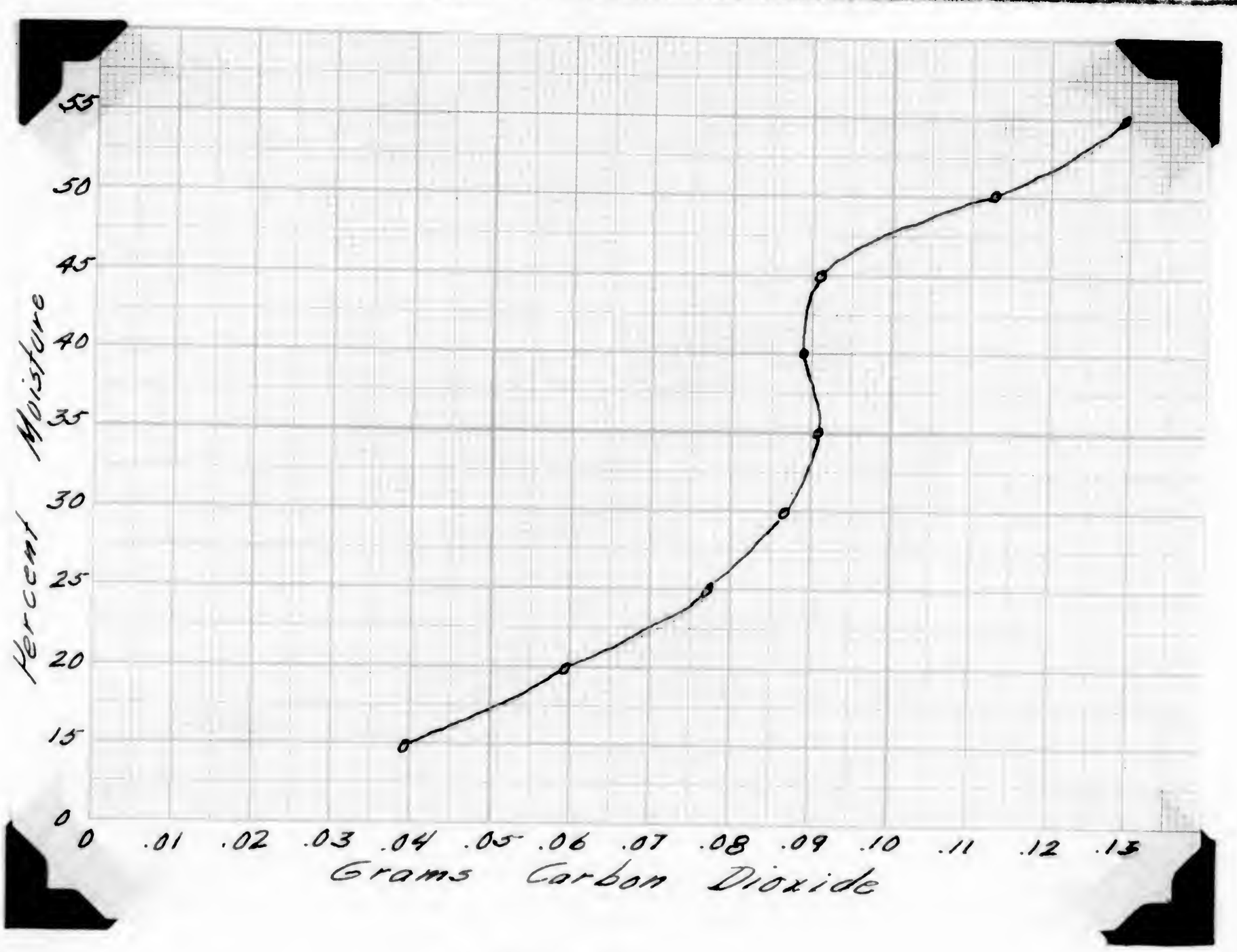


Figure V

TABLE VI

Fermentation of 50 grams Cured ground tobacco at 40° C.

24 hours

Moisture per cent	H_2O added cc	Sample No. 1 gm CO_2	Sample No. 2 gm CO_2	Sample No. 3 gm CO_2	Ammonia	Odor	Blank
15	8.7	.0530	.0433	.0521	±	Raw	—
20	12.5	.0803	.0703	.0775	+	Good	—
25	16.6	.0905	.0904	.0891	+	Good	—
30	21.4	.1047	.1032	.1050	+	Good	—
35	26.9	.1142	.1031	.1092	+	Good	—
40	33.3	.0815	.0840	.0921	+	Bad	—
45	40.9	.1148	.1030	.1063	+	Bad	—
50	50.0	.1606	.1766	.1654	+	Bad	—
55	61.1	.1865	.1841	.1801	+	Bad	—

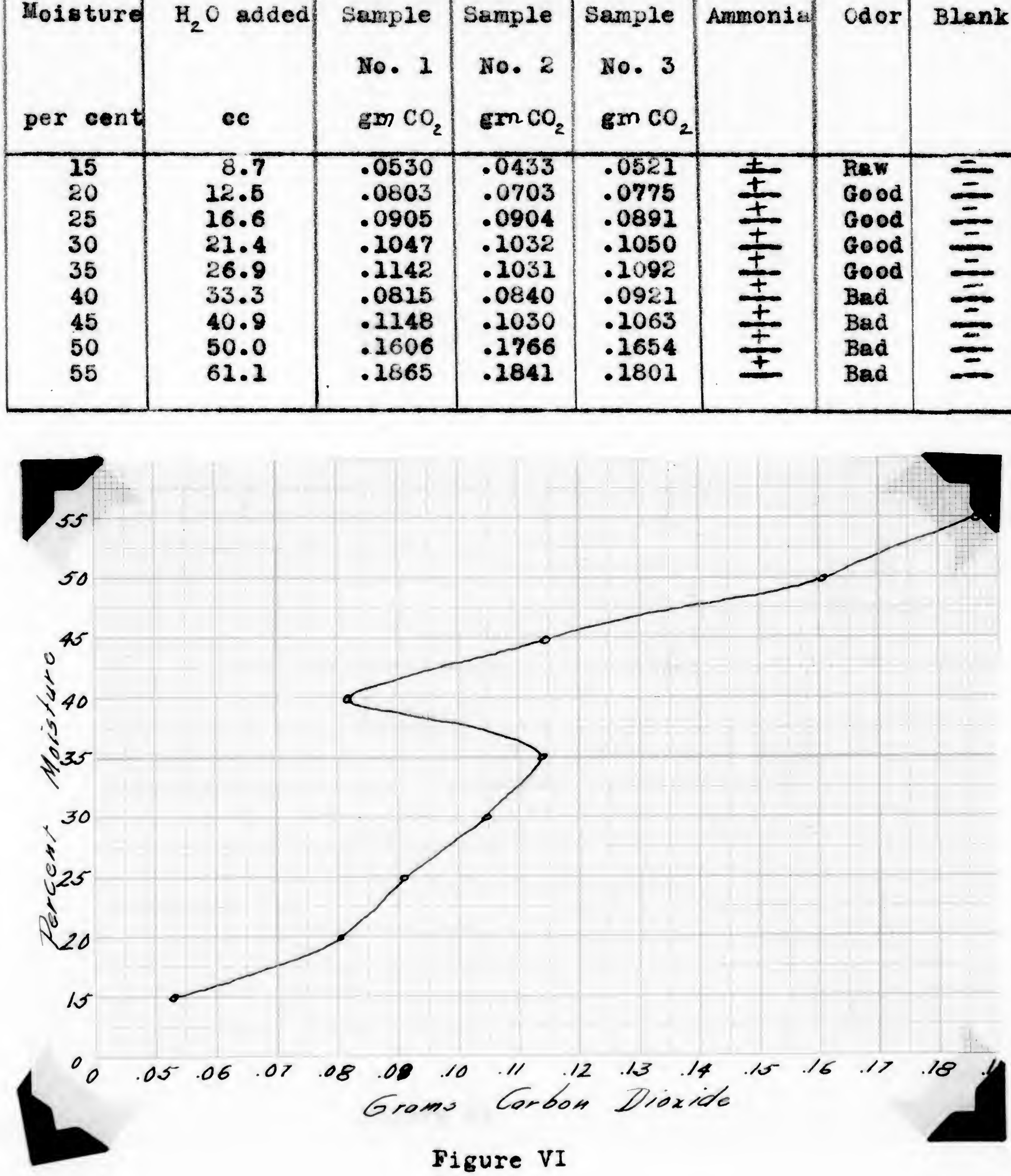


Figure VI

TABLE VII

Fermentation of 50 grams Cured ground tobacco at 50° C.

24 hours

Moisture	H_2O added	Sample No. 1	Sample No. 2	Sample No. 3	Ammonia	Odor	Blank
per cent	cc	gm CO_2	gm CO_2	gm CO_2			
15	8.7	.0675	.0673	.0592	±	Raw	=
20	12.5	.1232	.1140	.1072	±	Good	-
25	16.6	.1178	.1159	.1172	±	Good	-
30	21.4	.1556	.1348	.1451	±	Good	-
35	26.9	.1535	.1397	.1501	±	Bad	-
40	33.3	.1240	.1275	.1442	±	Bad	-
45	40.9	.1390	.1362	.1452	±	Bad	-
50	50.1	.1635	.1587	.1590	±	Bad	-
55	61.1	.1942	.1847	.1736			=

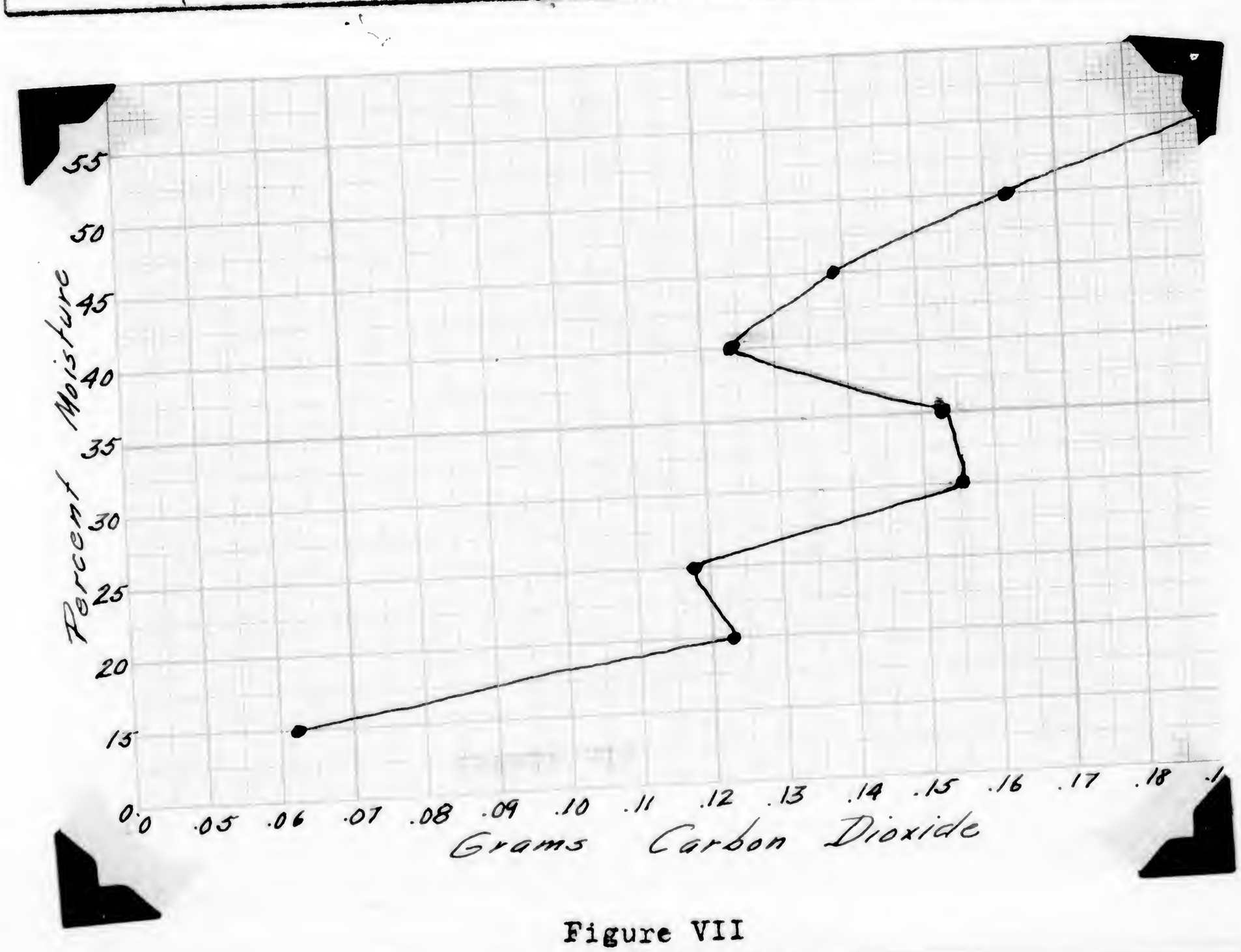
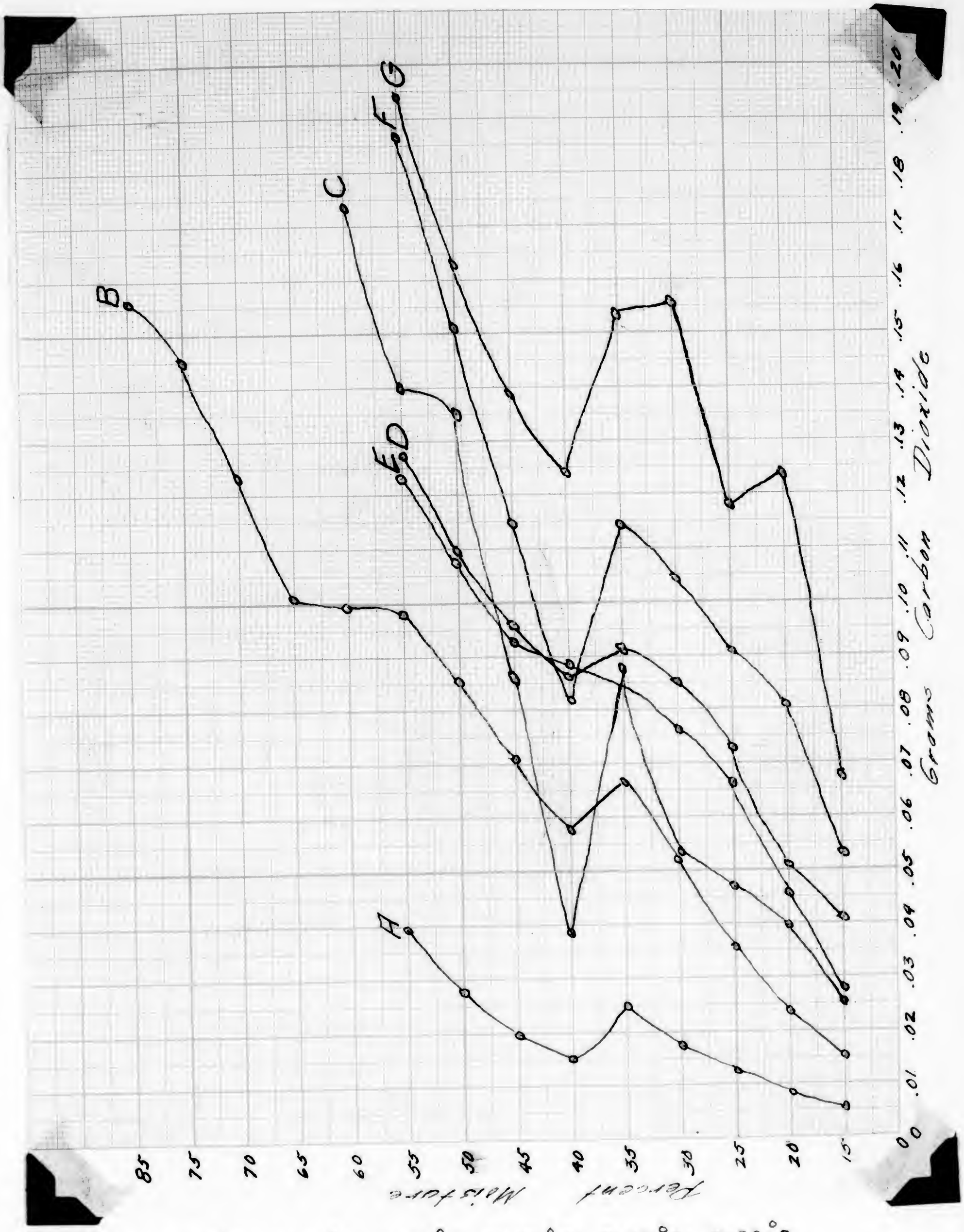


Figure VII



A=17°C; B=20°C; C=25°C; D=30°C; E=35°C; F=40°C; G=50°C

Figure VIII

Table VIII

Bacterial Counts on Tobacco after
24 hours Fermentation

Moisture %	Temperature Centigrade	No. of bacteria per gram of tobacco
35	30°	5,730,000
40	40°	6,100,000
45	50°	9,000,000

VI. Discussion of Results

Table I and Figure I indicate that very little carbon dioxide was given off at 15% moisture, but as the water content increased the amount of carbon dioxide increased gradually up to 35% and then dropped off at 40%. Above 40% water the carbon dioxide increases more rapidly. There was no ammonia detectable under any conditions. The odor of the tobacco was raw up to 25% moisture, but from 25% to 40% it was good. Above 40% the odor was stronger as the per cent of water was increased.

Table II and Figure II shows correspondingly similar results with higher amounts of carbon dioxide given off. The odor of the fermented tobacco varied with the increase of water as in the first case.

Table III and Figure III also show results comparable to Tables I and II except that a slight amount of ammonia was given off. The odor was pleasant between 25% and 35% moisture content and strong above 40%.

Tables IV and V and corresponding figures show similar results.

Table VI and Figure VI show higher results

in carbon dioxide production than any of the above tables. According to the color change of the methyl red more ammonia seemed to be given off. The odor was more pronounced and favorable between 20% and 35% moisture content. At 40% moisture the odor already was getting stronger.

Table VII and Figure VII showed the highest production of carbon dioxide. The odor was pleasant between 20% and 35% moisture content, but above that it became stronger.

Blanks indicated that there were no faulty connections in the setup nor faulty reagents.

Experimental work indicates that small amounts of tobacco can be fermented as advanced by Johnson (9).

The results indicate that from 15% to 35% moisture there is a gradual increase of carbon dioxide production and at 40°C. there is a marked decrease, but above that it increases rapidly.

Bacterial tests at three different temperatures showed that there were greater numbers of bacteria at 45% moisture content than at 35% or 40%. Perhaps the production of carbon dioxide is primarily due to the action of tobacco enzymes at the

lower water contents, the maximum activity being present at 30% to 35%. At this point the bacterial action may be relatively unimportant, but above the 40% level the carbon dioxide production may be almost entirely dependent on the bacteria present. This may possibly explain the unusual drop in carbon dioxide production at 40% moisture content.

VII. Summary

1. - Small quantities of tobacco as low as 50 grams can be fermented.
2. - Below 20°C. fermentation takes place very slowly.
3. - As the temperature increases the amount of carbon dioxide given off increases.
4. - Between 35°C. - 50°C. carbon dioxide production resulted in the largest yields.
5. - The results indicate that at 40% moisture there is less carbon dioxide given off than at 35% moisture. Above 40% the carbon dioxide production again increases rapidly. Bacterial tests show that there were many more bacteria present at 45% moisture than at either 35% or 40%.
6. - The amount of ammonia given off in any of the tests was very small.
7. - The odor of the tobacco was most pleasant generally at moisture contents between 20% and 35% and at temperatures between 25°C. and 35°C.

Acknowledgments

The author takes this opportunity to express his sincere appreciation to Dr. D. E. Haley for his valuable and many helpful suggestions during the progress of this work; to Dr. C. O. Jensen for his kind coöperation and advice; and to Dr. J. J. Thomas for his many timely suggestions.

Bibliography

1. Garner, W. W.
Tobacco curing. U.S.D.A. Farmers' Bul. 523:1-23. 1922.

2. Loew, Oscar
Curing and fermentation of cigar-leaf tobacco. U.S.D.A. Report No. 59, p. 13. 1899.

3. Vickery, H. B., and Pucher, G. W.
The chemical changes that occur during the curing of tobacco leaves. Science, Vol. 73, No. 1893, pp. 397-399. April, 1931.

4. Whitney, M., and Mean, T. H.
Temperature changes in fermenting piles of cigar-leaf tobacco. U.S.D.A. Report No. 60, pp. 16-17. 1899.

5. Tisdale, W. B.
Tobacco culture in Florida. University of Florida Experimental Agricultural Station. Bulletin 198, p. 399. June, 1928.

6. Fodor and Reifenberg, A.
Über die Atmungserscheinungen während der Trocknung von Tabaksblattern und über das

Wesen der sogenannten Tabakfermentation.
Zeitschrift für Physiologische Chemie.
Vol. 162, p. 1

7. Smirnov, A. I., and Petrik
Formation of carbon dioxide during the fermentation of tobacco. U.S.S.R. State Institute, Tobacco Investigations. Bull. 72, 18 pp. (1930).

8. Vickery, H. B., and Pucher, G. W.
Chemical investigation of the tobacco plant. Connecticut Experiment Station Bulletin 324. January, 1931.

9. Johnson, J.
Studies on the fermentation of tobacco. Journal of Agricultural Research, Vol. 49, No. 2. July, 1934.

End of Title