

content of 0.5 to 1% which may be removed by recrystallization. For this purpose a 50% aqueous solution of the crystals is prepared, filtered, and to it is added the previously mentioned acidified alcohol, nearly to cloudiness, and the solution stirred. Crystallization of raffinose hydrate is rapid at room temperature and the crystals, which may be washed first with 75% and later with 95% alcohol, contain less than 0.05% ash, give a specific rotation of 123.2° (anhydrous sugar) in 10% aqueous solution at 20° , and the yield is about 2.5% of the weight of the original meal. A further crystallization of 0.5% of equally pure raffinose may be obtained by cooling the mother liquor to zero.

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THE OCCURRENCE OF METHYL ALCOHOL IN CORN SILAGE.

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In 1912, Hart and Willaman published a paper on the volatile fatty acids and alcohols of corn silage.¹ From the data secured in the separation of the alcohols by the Duclaux method, it was concluded that corn silage contains, besides ethyl and propyl alcohols, a small amount of methyl alcohol. The quantity found may be approximately expressed as 0.05% of the silage mass, or about 20 g. in 100 pounds of silage.

In the same year there appeared a paper by Dox and Neidig,² in which the main conclusions of Hart and Willaman were substantiated, but which did not agree on the presence of methyl alcohol. The question of the occurrence of methyl alcohol in corn silage is therefore taken up in this paper.

Experimental Part.

There are numerous methods in the literature, of varying degrees of reliability, for the detection of methyl in the presence of ethyl alcohol. Many of these were tested with various known mixtures of alcohols, and only those which appeared always to be trustworthy were used. Of these, the Trillat test,³ and the resorcin test, as modified by Scudder,⁴ seemed to be most reliable. The gallic acid test,⁵ the morphine test,⁶ the phenol test,⁷ the Haigh phloroglucin test, which is discussed by Scudder,⁸ and

¹ THIS JOURNAL, 34, 1619 (1912).

² *Research Bull.* 7, Iowa Expt. Sta.

³ *Bull.* 107, Bureau of Chem., p. 99.

⁴ THIS JOURNAL, 27, 892.

⁵ *Ann. de Chim. analyt. appl.*, 4, 156 (1899).

⁶ Hinkel, *Analyst*, 33, 417.

⁷ Leach, "Food Inspection and Analysis," 2d ed., p. 820.

⁸ *Loc. cit.*

the Sanglé-Ferrière-Cumiassé test, as modified by Scudder and Riggs,¹ were used as confirmatory tests. A number of the most trustworthy tests for methyl alcohol have been thoroughly tried out by Scudder,² and his conclusions as to the reliability of the tests and proper conditions for their use have been substantiated in this work.

The following method was used in demonstrating the presence of methyl alcohol in normal corn silage: About 300-400 g. of silage were comminuted in a food grinder, suspended in water, and distilled with steam. The first sample was distilled at atmospheric pressure, but the later ones were distilled under reduced pressure at from 30-40 mm., to eliminate the possibility of chemical changes taking place at the higher temperature. Only about one liter was usually distilled over, thus getting less than half of the volatile acids and alcohols in the distillate, as was determined by titrating the distillate and the total acidity, and comparing with the average acidity of normal silage, as determined by Hart and Willaman. The distillate, having been neutralized with NaOH, was redistilled, also under reduced pressure, to liberate the alcohols, and the distillate containing the alcohols concentrated by repeated distillation, with the aid of a fractionating column. In each case, the samples were distilled, and the distillates concentrated uniformly, so that the results of the tests could be compared. In one case a water extract was made, by grinding up a sample of about 400 g., triturating in a mortar, and filtering rapidly on a Büchner funnel. The filtrate, about 1.5 liters, was distilled in steam under reduced pressure, neutralized, and redistilled as above. Table I shows the results of the tests made on each sample.

TABLE I.—RECORD OF TESTS FOR METHYL ALCOHOL IN NORMAL CORN SILAGE.

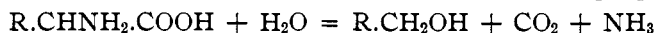
Sample.	Temp. of dist.	Resorcin test.	Trillat test.	Phenol test.	Gallic test.	Haigh test.	Morphine test.	Sanglé-Ferrière-Cumiassé.
1 Stave silo.	100°	+	+	+				
2 Stave silo.	35°		+					
			(strong)					
3 Stave silo.	30°	+	+	+		+		
		(strong)						
4 Stave silo water extract....	28°	+	+	+				
			(very strong)	(strong)				
5 Concrete silo.....	40°	+	+	+	+		+	+
		(very strong)						

The presence of methyl alcohol in normal silage having been established,

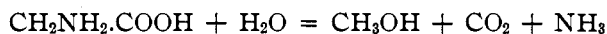
¹ THIS JOURNAL, 28, 1202 (1906).

² *Ibid.*, 27, 892 (1905).

an attempt was made to determine the source of it. F. Ehrlich has shown¹ that certain yeasts have the power of fermenting amino acids with the formation of the corresponding alcohols, as in the following equation:



He has further shown that one of the specific sources of methyl alcohol in a protein-carrying fermentation mixture is glycine, as shown by the following equation:



To determine whether or not the methyl alcohol in normal silage has its source in the glycine of the corn proteins, a number of water cultures with synthetic media were set up and inoculated with a small piece of normal silage, obtained under antiseptic conditions from the large concrete silo on the University farm, the sample being taken about two feet below the surface of the silage. A typical medium was composed as follows:

CaCO ₃	1 g.
K ₂ HPO ₄	1 g.
MgSO ₄	0.1 g.
FeCl ₃	Trace
NaCl.....	Trace
Glucose.....	2 g.
Glycine.....	2 g.
Distilled water.....	1 liter

The object of this method of inoculation was to carry into the cultures all of the typical silage flora. These cultures were arranged, according to the composition of the media, into pairs, each pair being similar in composition, except that one contained glycine as the sole source of nitrogen, and the other some other nitrogenous substance. All contained either glucose or a tartrate as the source of carbon, except cultures I and II, which con-

TABLE II.—TESTS FOR METHYL ALCOHOL IN WATER CULTURES.

Cultures.	Resorcin test.	Trillat test.	Phenol test.	Gallic acid test.	Sanglé-Ferrière-Cumiasse.
I. Glycine.....	+	+	+	+	+
II. Glycine.....	+				
	(strong)				
III. Glycine.....	+	—			
IV. No glycine.....	—	—			
VII. Glycine.....	—				
VIII. No glycine.....	No growth				
IX. Glycine.....	+			+	+
X. Glycine.....	+				
	(strong)				
XI. No glycine.....	+				
	(weak)				
XII. Glycine.....	+		+		+

¹ *Wochschr. Brau.*, 30, 561; *Z. angew. Chem.*, 27, 48 (1914).

tained only glycine and inorganic salts. Considerable growth and formation of methyl alcohol was obtained even in these two cultures. Moreover, no other organic substance which might be fermented to methyl alcohol was present. After being allowed to stand at room temperature for three or four weeks, the cultures were distilled in steam, at ordinary pressure, and the distillates concentrated and tested for methyl alcohol. The results, which are shown in Table II, seem to indicate the possibility of glycine being at least one of the sources of the methyl alcohol.

The objection, however, may be justly raised, that under different conditions of environment, the flora of the silo might not be typically represented in these cultures. To secure more definite evidence, silage was made in the laboratory, in sealed jars, using corn which had been grown to maturity in the greenhouse. Jar No. 1 contained normal silage, jar No. 2 contained normal silage to which a solution containing 2 g. of glycine had been added, and jar No. 3 normal silage to which 2 g. of glycine and 50 cc. of ether had been added. The silage in these jars was excellent, and was perfectly normal in appearance, odor, taste, and acidity, except that in jar No. 3 the ether had inhibited the action of organisms, and there was much less acidity developed. Otherwise the silage in this jar had practically the same appearance and odor as that in the others, after the ether had been allowed to evaporate. During the ripening of the silage, the pressure of gas was relieved at intervals by opening a pinchcock. Considerably less gas was developed in jar No. 3 than in the others.

At the end of about six weeks samples of 350 g. were ground and distilled as in the case of the normal silage. The titer of volatile and fixed acid was practically the same, in jars No. 1 and No. 2, the total acidity being equivalent to about 225 cc. of 0.1 *N* alkali per 100 g. of silage, but the total acidity in jar No. 3 was only about one-third as great as in the other two. The alcohols were distilled off, concentrated somewhat, and the tests for methyl alcohol applied, with the results as shown in Table III.

TABLE III.—TESTS FOR METHYL ALCOHOL IN EXPERIMENTAL SILAGE.

Sample.	Temp. of dist. C.	Resorcin test.	Trillat test.	Phenol test.	Gallic acid.	Haigh test.	Morphine test.
1. Normal silage...	35°	+	+	+	+	+	+
		(weak)	(very weak)				(very weak)
2. Glycine silage...	40°	+	+	+	+	+	+
		(very strong)	(strong)	(strong)			(strong)
3. Glycine-ether silage.....	40°	—	—	—	—	—	—

From the data shown, it is evident that more methyl alcohol was formed in the silage to which glycine had been added, which fact supports the hypothesis that its source is the glycine. In these two jars, the experimental conditions were exactly the same throughout, except the addition

of the glycine, and the consequent increase in the amount of methyl alcohol formed. The difference in the strength of the tests, as indicated in the table, was very pronounced. The fact that in jar No. 3, the silage which ripened in the presence of an antiseptic contained no trace of methyl alcohol, even though a similar amount of glycine had been added, seems to show that the formation of this alcohol is brought about by some organism. This is in harmony with the results secured from the water cultures.

Of course, at this stage of the work, we can point, with certainty, only to the presence of an appreciable amount of methyl alcohol in normal silage, and merely indicate the probability of its formation by the hydrolysis of glycine. It is also possible that other substances than glycine may furnish some methyl alcohol in the presence of certain organisms, as for example, the fermentation of glycerine by *B. Boöcopricus*.¹ Indeed, the presence of methyl alcohol in fermentation mixtures is not new. Wolff² found it present in the distillate from fermented fruits, such as apples, cherries, grapes, and plums. Sanglé-Ferrière and Cumiasse³ found it in absinthe. Takahashi⁴ discovered methyl alcohol among the products produced by several varieties of mycoderma yeasts in fermenting rice mixtures. Evidently a number of different species of microorganisms possess the power of producing methyl alcohol in fermenting mixtures.

Summary.

Normal silage is shown to contain small amounts of methyl alcohol. A number of tests were made on the distillates from several samples of silage, the tests having first been standardized by using various known mixtures of alcohols. The number of different tests used precludes the possibility of positive reactions being given by some other substance than methyl alcohol.

The hypothesis is advanced that at least part of the methyl alcohol is formed by the action of microorganisms on glycine. All work done thus far with water cultures and experimental silage shows results which support that hypothesis.

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THE VOLATILE SUBSTANCES OF URINE.

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Freshly voided, normal urines possess low vapor pressures⁵ and little odor, thus indicating the presence of only traces of volatile substances.

¹ Emmerling, *Ber.*, 29, 2726.

² *Compt. rend.*, 131, 1323.

³ *Ann. chim. analyst.*, 8, 82 (1903).

⁴ *Bull. Coll. Agr. (Tokyo)*, 6, 387 (1905).

⁵ *Vide infra*.