$$BrCH_2CH_2N(CH_3)_3Br + AgOH \longrightarrow BrCH_2CH_2N(CH_3)_3OH + AgBr,$$

and

$$BrCH_2CH_2N(CH_3)_3^+ + OH^- \longrightarrow CH_2{=}CHN(CH_3)_3^+ + Br^- + H_2O.$$

Neurine iodide was obtained in a similar manner from iodocholine iodide.

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VOLATIL FATTY ACIDS AND ALCOHOLS IN CORN SILAGE.

BY E. B. HART AND J. J. WILLAMAN.

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When green herbage of any kind is put into a silo, certain physical and chemical changes are noticed. There is a considerable rise in temperature, evolution of gases, a decrease in true protein and in carbohydrates, and a marked increase in acidity and aroma. The causes of these changes in corn have been studied in some detail by several investigators, principally by Burrill and Manns,[1] Babcock and Russell,[2] and E. J. Russell,[3] of England. As this paper deals only with the volatil fatty acids and alcohols, the work already done on them will be briefly reviewed.

The first to state the cause of the increase of acidity was Burrill, in 1888. He explained it all by bacterial action. He found many species of bacteria in the silage, and from what was known at that time of the action of bacteria, he concluded that all the acids in the silage were formed by the microbic decomposition of the carbohydrates in the fodder. He called the non-volatil acid lactic, and the volatil, acetic. He said there was no ethyl alcohol present, but probably butyl, although he made no definite analyses. He found great numbers of the yeast *Saccharomyces mycoderma* Rees, but concluded it formed no alcohol, as it does so only under certain conditions not found in the silo. In 1900 Babcock and Russell made extensive laboratory experiments on ensiling fodder. They showed quite conclusively that the major changes in corn silage formation were due to direct respiration of the living cells, and to intra-molecular respiration. They made silage under antiseptic conditions by the use of chloroform, ether or benzene, and found it perfectly normal, even to the characteristic silage aroma. They noticed that the acids and aroma were formed within a couple of days after ensiling the material, and before bacteria would have time to work to any extent.

The conclusions of Babcock and Russell were corroborated in 1908 by E. J. Russell at the Rothamsted station. He found that the volatil

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[1] Ill. Expt. Sta., *Bull.* 7 (1888).

[2] Wis. Agric. Expt. Sta., *Seventeenth Ann. Rpt.*, 1900.

[3] *J. Agric. Sci.*, 2 (1907–8).

acids were formed by the anaërobic, or intra-molecular respiration of the cells, and that the action of bacteria, molds, and fungi was of secondary importance. He made detailed chemical analyses of the silage, and found that the acidity was due mainly to lactic, acetic and butyric acids, with small amounts of formic, propionic and valeric. He found ethyl alcohol, but no higher ones, and no aldehydes.

Experimental.

The object of this work was to determin the kinds of volatil fatty acids found in corn silage, and the amounts of each; also the kinds and amounts of alcohol. It was believed, at the beginning of the experiment, that the aroma was due to esters, and the system of analysis was conducted accordingly. But it was soon found that there were no esters present, but simply the free alcohols. Both Burrill and Manns, and Russell identified their acids by fractionally crystallizing the silver or barium salts, and then determining the amount of metal in each fraction. Hence their results were not quantitative. In this investigation Duclaux's method was used for determining quantitatively both the acids and the alcohols.

The silage was taken from the silo on the University farm. This silo is of concrete, about forty feet high, fifteen feet in diameter, and has a capacity of about 150 tons. It was filled about the first of September (1911) with the whole corn fodder cut into pieces an inch or two in length. The first sample was not taken until November 15, 1911, by which time the silage was completely ripened, and was being fed to the stock. The silage retained a noticeably high temperature until January (1912), due probably to slow radiation of heat formed during the initial stages of change.

Considerable difficulty was experienced in getting for analysis a sample that would be representative of the whole mass of silage. In filling the silo, there is a tendency for the heavier cob and stalk to be dropped nearest to the side at which the fodder enters, and for the lighter leaves to be carried to the opposit side. In securing a sample, several portions of two or three pounds each were taken from different places in the silo, a foot or more beneath the surface. These portions were, thoroughly mixed, and then about three pounds were ground in a food chopper. The first sample was chopped in a chopping bowl; but, as will be seen from the data, this did not comminute the tissues sufficiently to allow the extraction of all the acids. The mass of ground material was again thoroughly mixed, and 100 grams of it were taken as a sample for analysis.

The acids and alcohols were distilled out with steam. This was done by suspending the sample in two liters of water in a five liter flask, adding 10 cc. of concentrated sulfuric acid to insure the liberation of all acids, and then passing in steam until three liters of distillate had been collected. Even then there were traces of acid still coming over, probably formic. The total distillate was mixed, and one liter taken for analysis. This distillate contained the volatil acids, the alcohols, and any esters. By neutralizing with barium hydroxide and distilling to one-third its volume, the alcohols and esters were entirely removed to the distillate, and the barium salts of the acids remained behind. The solution of alcohols and esters was set aside in a refrigerator for future analysis.

The solution of barium salts was evaporated to 150 cc., and fractionally distilled. This was done by adding 20 cc. of 0.1*N* sulfuric acid and distilling over 100 cc. This distillate was designated Fraction I. To the residue was added 100 cc. of water, and another 20 cc. of 0.1*N* sulfuric acid, and 100 cc. distilled over for Fraction II. This was repeated until sufficient sulfuric acid had been added to free all the fatty acids from their barium salts. In the last fraction, this was indicated by the action of free sulfuric acid on Congo red paper. By thus fractionating, the number of different acids in each fraction was lessened, and this simplified the succeeding computation. Duclaux's method for the volatil fatty acids was then applied to each fraction.

The method depends upon the fact that each acid has its own rate of vaporization in dilute solutions (*i. e.*, less than 0.5%). Duclaux took 110 cc. of a solution containing a single acid, and distilled it from a 200 to a 300 cc. distilling flask. By titrating each 10 cc. of distillate up to 100 cc., and then determining the percentage of the total distillate found in each 10 cc., he obtained a curve that was characteristic of that acid. In a mixture of several acids, each retains its own rate of vaporization; and by manipulating the known curves until one is found that corresponds to the curve for the unknown mixture, the kinds of acids and their respective amounts can be determined. The tables of constants, from which the calculations in these experiments were made, were obtained from Duclaux's "Traite de Microbiologie," Vol. III, 1900.

In analyzing the alcohols and esters, the distillate from the barium salts of the acids was concentrated by distilling two or three successive times to one-half its volume; that is, 1000 cc. were distilled to 600 cc., this to 350 cc., and this to 200 cc. As this solution was thought to contains esters and not free alcohols, it was saponified with potassium hydroxide. Fifty cc. portions of the solution were put into pressure flasks, 10 cc. of 30% potassium hydroxide added to each, and then heated on a steam bath for two to three hours. The alcohols were distilled off and the residue acidified with sulfuric acid and distilled to drive off any volatil acids that had been united to the alcohols as esters. But no acids were found, showing that the alcohols had existed free and not in acid combination.

The solution of the alcohols was concentrated as before, and then oxidized to the acids. This was done by treating 75 cc. portions of the solution with 30 cc. of oxidizing solution in tightly stoppered pressure flasks, and heating on the steam bath about three hours. The oxidizing solution was made by dissolving 10 grams potassium dichromate and 11 cc. concentrated sulfuric acid in water and making up to 100 cc. The flasks were cooled, emptied into a 500 cc. distilling flask, about 5 grams of zinc dust added to remove the excess of chromic acid, and the volatil acids removed by distilling to 500 cc. with repeated additions of water. The acids were neutralized with barium hydroxide, concentrated by evaporation, fractionated, and "duclauxed" as in the other acid determinations. The solutions of alcohols and esters after separating from the barium salts of the free acids had to be kept two or three months before they were analyzed. In spite of the fact that they were kept in a refrigerator, the molds attacked them to such an extent that they were spoiled for any very exact analysis. Sample VI, Table I, is the only one in which an exact analysis of the alcohols could be made. In this case, the sample was prepared, the acids determined, and the alcohols analyzed, all within three or four days, so that the results obtained are truly representative of the silage.

The data obtained are given in Table I. As has been said, the first sample was chopped and does not show the true acidity. It is not included in the averages. The time of taking each sample is included simply for purposes of completeness, but the age of the silage does not seem to have any bearing on the quantity of acid present. The last column is computed with the total volatil acidity represented as acetic acid. The determination of the total acidity, both volatil and nonvolatil, was neglected in the first samples. For the sake of comparison a new sample was taken on May 14, and the total acidity determined. As the concrete silo, from which the other samples were taken, was empty, silage was taken from the University stave silo. The sample was prepared as before, and 50 grams of the ground material digested with water by boiling under a reflux condenser for an hour. It was then cooled, made up to one liter, and 50 cc. aliquots titrated with barium hydroxide. This gave the total acidity. The volatil acids were determined as before by distilling 100 grams with steam to three liters and titrating 60 cc. aliquots.

The steam distillate was tested for aldehydes by means of Fehling's solution and by alkalin silver nitrate. Fehling's solution gave no reaction. The silver gave no mirror, but the solution turned a delicate purple color, showing that a slight amount of aldehyde was present.

The sample taken from the stave silo on May 14 appeared to be like the other samples in color, character of fodder, and odor, and on analysis was found to have a total acidity equivalent to 160 cc. 0.1 *N* alkali, and a

TABLE I.—VOLATIL ACIDS AND ALCOHOLS IN 100 GRAMS OF CORN SILAGE.

		Formic.		Acetic.		Propionic.		Butyric.			
Sample.	Date.	Cc. 0.1 N alk.	Per cent of tot. vol. acid.	Cc. 0.1 N alk.	Per cent of tot. vol. acid.	Cc. 0.1 N alk.	Per cent of tot. vol. acid.	Cc. 0.1 N alk.	Per cent of tot. vol. acid.	Total vol. acid. Cc. 0.1 N alk.	Per cent. of vol. acid as acetic in silage.
I. Chopped	Nov. 15	17.56	32.5	30.81	56.2	5.76	10.4	0.65	1.2	54.78	0.33
II. Ground	Jan. 11	27.74	16.6	124.32	74.3	13.30	7.9	1.92	1.2	167.18	1.00
III. Ground	Jan. 15	17.97	12.6	111.18	77.8	13.56	9.5	..	.	142.71	0.85
IV. Ground	Jan. 17	9.81	6.3	129.48	84.2	14.49	9.4	..	.	153.78	0.92
V. Ground	Jan. 18	11.82	7.8	127.77	84.2	9.99	6.6	2.04	1.3	151.62	0.91
VI. Ground	Apr. 9	28.80	22.9	87.90	70.1	8.70	7.0	..	..	125.40	0.74
	Average	22.81	16.8	105.90	75.2	10.76	7.6	0.99	0.6	139.61	0.85
VII. Alcohols		14.61	21.4	49.26	72.3	4.26	6.2	..	.	68.13	..

VIII. From stave silo, May 14, 1912; total acidity, 160 cc. 0.1N; vol. acidity, 145 cc. 0.1N.

volatil acidity of 145 cc. 0.1 N alkali. As the latter corresponds very well with the volatil acidity of the other six samples, it may be assumed that the total acidity was also very nearly the same. Then taking the 15 cc. of non-volatil acid as lactic and multiplying by the factor 0.009, we have 0.13% of the silage in the form of non-volatil acids, as compared to 0.84% in the form of volatil. This gives a total acidity of 0.97%. The non-volatil acids were much lower in amounts than was reported by Burrill and Manns. They found as high as 1-2% of non-volatil acids in the silage examined.

It will be seen that acetic acid forms about 75% of the total volatil acidity. Burrill and Manns, in their determinations by fractional crystallization, estimated that the acetic acid constituted 79% and butyric, about 18% of the whole. E. J. Russell also found that acetic and butyric were the principal acids present. In these experiments, butyric was found in only three out of the six samples, and then in very small amounts. It is undoubtedly present, but in the other three cases escaped notice in the manipulation of the curves. Burrill and Manns, and Russell both found valeric, but none was found in these experiments.

In the table, the alcohols are expressed as their corresponding acids. It will be noticed that their titrated acids amount to about one-half of the volatil acids, and that the different alcohols are in nearly the same proportions as the acids. No butyl alcohol was found. If the total volatil acids from the alcohols are calculated to ethyl alcohol, the percentage of this in the silage becomes 0.31%.

The difference in results noticed between these analyses and those of other experimenters are accounted for by the differences in silage. Experiments have shown that the acidity in silage depends very largely upon the maturity, the variety of corn, the coarseness of the cut material, and the percolation of air into the silo. Since all these factors are somewhat variable, the resulting products may be different. No two silages are ever exactly alike in composition; and this, coupled with the difficulty of obtaining a representative sample for analysis, makes concordant results very difficult.

In these experiments no attempt was made to find the causes of the formation of the acids and alcohols. But the fact that comminuting the tissues in a food grinder liberated more acid than simply chopping with a knife, points to the conclusion that the acids are formed within the tissues, that is, by anaërobic respiration of the living cells, rather than without the tissues by means of foreign organisms. The experiments of Russell, and of Babcock and Russell, prove this quite conclusively.

Summary.

1. It was found that the total acidity of the corn silage examined was equivalent to about 160 cc. 0.1N alkali, or 0.97% by weight.

2. The volatil acidity was equivalent to about 140 cc. 0.1 N alkali, or 0.84% by weight.

3. Of the total volatil acids, 17% was formic, 75% was acetic, 8% was propionic, and 0.6% butyric.

4. No esters were found in this silage, but simply the free alcohols. A trace of aldehyde was also found.

5. The volatil alcohols, expressed as their corresponding acids, amounted to 68 cc. 0.1N alkali, or 0.31% by weight of ethyl alcohol.

6. Of the total alcohols, 21% was methyl,[1] 72% was ethyl, and 7% propyl.

THE OCCURRENCE OF LACTIC ACID IN SISAL.

By Wm. McGeorge.

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It has been known ever since the sisal plant (*Agave Sisalana*) has been in use as a source of fiber that the juice from these leaves which is crushed out during the decortication is very destructive to the parts of the decorticating machinery with which it comes in contact, as well as to the hands of the laborers who are required to handle the wet fiber as it emerges from the mill. In spite of this fact, few investigators have attempted an identification of this acid. During a recent study made by this laboratory upon the chemical nature of the sisal plant,[2] the identification of this acid was given considerable attention.

So far as I have been able to ascertain from literature at hand, only one claim has been made as to the nature of this acid and that by M. D'Herelle,[3] who is calling attention to the strong acidity of this plant juice, says it is due in part to oxalic acid. However, he cited no proof as to this statement, and probably made the statement only as a theory. I was unable to obtain a test for oxalic acid from a solution of the plant juice, but upon strongly boiling the leaves with hydrochloric acid a very faint test for oxalate was obtained, indicating the possibility of the presence of this acid in minute quantities.

On extracting the leaves with ether, then alcohol, then water, all three extracts gave an acid reaction to litmus, but from none of these extracts was I able to effect a crystallization of any organic acid. Fermentation of the juice goes on quite rapidly and the acidity increases in so doing.

On failure to obtain any crystallization a decision was reached that the acid was one that normally had no crystallin form. Accordingly 500

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[1] While the method of analysis used showed the presence of methyl alcohol, it is barely possible that the formic acid may have arisen from the oxidation of substances other than this alcohol.

[2] Hawaii Exp. Sta., *Press. Bull.* **35**.

[3] *J. Agriculture Tropicale*, June, 1910.