of silica on repeated treatment; this he regards as indicating the presence of free alumina which is dissolved at once by the alkali. That soil was gravelly, however, and much of the alumina was extracted from the coarser materials separated by mechanical analysis; the land was fertile. Owing to lack of material, it has been impossible to apply this treatment to the Cuban soils; many other interesting observations have been omitted for the same reason. It is hoped that the peculiarities of these widely extended West Indian soils here reported, may lead others to more thoroughly study them.

A few words respecting the fertility of these Cuban soils: Their present content of calcium and magnesium carbonates is sufficient to keep them permeable, to provide for nitrification and to furnish all of these elements that may be needed to keep the soil sweet and the plants well fed. Magnesia is less abundant than lime, a condition very commonly reversed in the soils of the Gulf States. Potash is low but, owing to the presence of the lime, may not exhibit deficiency for some time. Phosphoric acid is unusually abundant, but of low availability because of its combination with iron or aluminum. These lands will, for this reason, as well as to insure continuance of permeability, require occasional calcareous manuring. Nitrogen is present in liberal supply.

[CONTRIBUTION FROM THE LABORATORY OF THE PENNSYLVANIA STATE COLLEGE AGRICULTURAL EXPERIMENT STATION, NO. 9.]

THE EFFECTS OF FERMENTATION UPON THE COMPOSITION OF CIDER AND VINEGAR.

BY C. A. BROWNE, JR.

Received October 24, 1902.

THE present work comprises the results obtained in connection with an experiment begun at this Experiment Station in the Fall of 1898, which had for its object a chemical study of the cask methods of producing cider and vinegar, as ordinarily practiced in the rural sections of this country. So far as known, no experiments have been published thus far towards this end.[1]

PLAN OF THE EXPERIMENT.

The juice for the experiment was prepared wholly from one

[1] Reference should be made to Bulletin No. 127, of the Va. Agr. Expt. Station, by W. B. Alwood and R. J. Davidson, entitled "Observations on the Production of Vinegar in Cellars," which has since come to the writer's attention.

kind of apples of an unknown variety. The apples were picked November 15, 1898, and were taken at once to a cider mill near-by where they were ground and pressed. A 25-gallon cask was filled with juice and then taken immediately to a cool, dry cellar, where it was kept throughout the experiment. The bung of the cask was loosely drawn to allow the escape of carbon dioxide during fermentation. The first sample for analysis was drawn the day after pressing. Samples were then drawn about every two weeks during the early stages of the alcoholic fermentation; afterwards during the latter part of the alcoholic fermentation and throughout the acetic fermentation, when chemical changes advanced less rapidly, analyses were made only monthly or bimonthly.

In sampling, a quart jar was usually filled, the cider being drawn off through a rubber siphon from about the middle of the cask. Care was taken not to disturb the sediment which settled out during the fermentation. The temperature of the cellar and cider were taken at the time of sampling, and the cask containing the cider was weighed occasionally in order to ascertain the losses from evaporation and seepage. The samples after taking to the laboratory were also weighed before beginning the analysis, to determine the amounts removed from the cask. The samples were drawn always in the morning, and the analytical work was begun immediately after taking to the laboratory to avoid vitiation of results due to the progress of the fermentation. If turbid or containing matter in suspension, the samples were filtered before analyzing. The results of the analytical work, covering a period of over three years, are presented in Table I.

THE ALCOHOLIC FERMENTATION.

The determinations of solids in Table I were unfortunately all made by the old method of drying at 100° C. in a water-bath. A constancy in weight could never be secured, and the percentages given represent simply the solid matter after ten to twelve hours' drying. As compared with a method[1] used later upon fruit products of drying *in vacuo* at 70° C., this procedure gives too low results, owing to the decomposition of levulose.

Looking at the percentages of the sugars, sucrose, dextrose, and levulose, in Table I, we notice during the first two weeks a

[1] This Journal, **23**, 873.

TABLE I.[1]—EFFECTS OF FERMENTATION UPON THE COMPOSITION OF CIDER AND VINEGAR.

	Date of analysis.	Temperature of cellar. °F.	Temperature of cider. °F.	Weight of sample. Grams.	Weight of cider in cask after sampling. Grams.	Specific gravity 17.5° C.	Solids at 100° C. Per cent.	Ash. Per cent.	Rotation, Ventzke, 400 mm. tube.	Levulose. Per cent.	Dextrose. Per cent.	Sucrose. Per cent.	Total sugar after inversion. Per cent.	Alcohol. Per cent.	Volatile acid as acetic. Per cent.	Fixed acid as malic. Per cent.	Pectin. Per cent.	Ester number.
Alcoholic fermentation.	1898, Nov. 16	44	49	2004	95070	1.0577	13.75	0.26	-44.18	7.38	2.90	2.93	13.36	0.43	0.04	0.42	0.12	..
	1898, Nov. 30	50	46	1017	.....	1.0462	12.25	0.24	-53.78	7.29	2.86	1.20	11.43	1.67	0.05	0.43	...	..
	1898, Dec. 16	51	50	1000	.....	1.0331	9.48	0.24	-50.45	6.28	2.06	0.50	8.87	3.05	0.06	0.43	...	..
	1899, Jan. 5	60	58	1033	.....	1.0133	5.92	0.24	-29.60	2.88	1.21	0.09	4.19	5.32	0.08	0.40	...	..
	1899, Jan. 19	52	47	1050	.....	1.0067	4.53	0.24	-18.80	2.02	0.44	0.01	2.47	6.03	0.09	0.38	0.09	..
	1899, Feb. 2	34	35	1062	.....	1.0026	3.03	0.25	-13.00	1.41	0.29	0.00	1.70	6.49	0.10	0.31	...	..
	1899, Feb. 16	55	47	830	82440	1.0013	2.89	0.25	-11.06	1.17	0.21	...	1.38	6.70	0.11	0.30	...	5.8
	1899, March 9	50	49	1045	80850	0.9982	2.24	0.25	- 5.30	0.54	0.07	...	0.61	6.92	0.11	0.23	...	5.9
	1899, April 3	47	45	1038	79040	0.9968	2.10	0.25	3.40	0.30	0.00	...	0.30	6.93	0.17	0.21	...	9.0
	1899, May 4	72	64	1941	76770	0.9969	1.94	...	- 2.30	0.24	...	...	0.24	7.00	0.27	0.21	0.03	10.1
Acetic fermentation.	1899, July 27	67	62	1917	74960	0.9959	1.80	0.26	- 1.50	0.11	...	...	...	6.86	0.52	0.20	...	8.2
	1899, Aug. 14	68	66	1060	.....	0.9965	1.53	0.26	- 1.20	0.11	...	...	...	6.66	1.11	0.17	...	5.4
	1899, Oct. 2	55	50	1000	.....	0.9988	1.48	0.27	.....	...	...	...	...	6.01	1.77	...	...	..
	1899, Nov. 27	47	43	1079	.....	1.0017	1.42	0.26	.....	...	...	...	...	4.80	2.59	...	...	..
	1900, Feb. 14	54	46	1080	.....	1.0037	1.45	0.26	.....	...	...	...	...	4.54	3.67	...	...	..
	1900, April 20	65	58	1095	64980	1.0060	1.37	0.26	- 1.40	0.18	...	...	...	3.77	4.31	0.14	...	..
	1900, July 31	70	66	1090	63620	1.0113	1.70	0.25	- 1.20	0.22	...	...	...	2.61	5.98	...	...	..
Destructive fermentation.	1902, Feb. 10	..	..	....	44570	1.0162	2.40	0.48	- 5.00	1.20	...	...	...	0.00	3.33	0.06	...	..
	1902, March 9	..	..	....	.....	.....	...	...	.....	...	...	...	...	...	3.12	...	...	..
	1902, April 7	..	..	....	.....	.....	...	...	.....	...	...	...	...	...	2.76	...	...	..
	1902, May 19	..	..	....	.....	....	...	...	.....	...	...	...	...	...	2.25	...	...	..
	1902, June 16	..	..	....	.....	.....	...	...	.....	...	...	...	...	...	1.98	...	...	..
	1902, July 22	..	..	....	.....	.....	...	...	.....	...	...	...	...	...	1.28	...	...	..
	1902, Aug. 15	..	..	....	.....	.....	. .	...	.....	...	...	...	...	...	0.78	...	...	..
	1902, Oct. 13	..	..	....	.....	.....	...	...	.....	...	...	...	...	...	0.15	...	...	..

[1] All determinations, except that of solids, were made according to methods described in this Journal. **23**, 869 (1901).

rapid fall in the per cent. of sucrose, but that the percentages of dextrose and levulose remain nearly constant. This would indicate that in the beginning, by the action of the invertase upon the sucrose, the amounts of dextrose and levulose are restored nearly as fast as fermented. The following diagram will show the rates of decrease in the sugars, and their relations to alcohol and rotation more graphically.

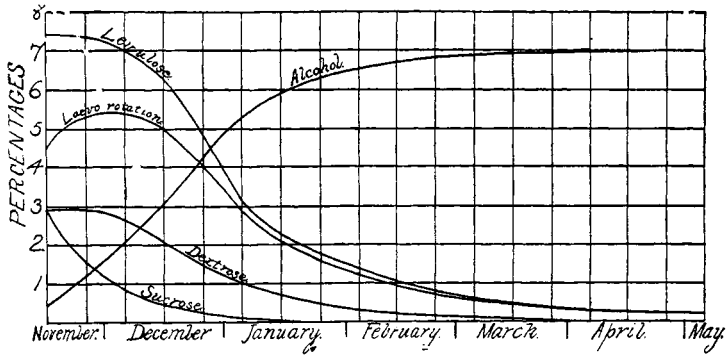


Diagram No. 1. Each division represents ten days.

The effect of the removal of the sucrose, which is strongly dextro-rotatory, is shown most strikingly by the marked rise at first in the levo-rotation; the maximum is soon reached, however, and the rotation then begins to fall, gradually at first,—the removal of sucrose and dextrose at this time very nearly counterbalancing the decrease in rotation that would be produced by the fermentation of levulose—, and then more suddenly owing to the increased rapidity in the fermentation of the levulose. The period of greatest chemical activity is from the fourth to the seventh week; we notice here the largest drop in the percentage of levulose, attended by the greatest rise in the percentage of alcohol. Another important fact is that while the sucrose and dextrose are completely removed by fermentation, a small amount of levulose remains unaffected. This is due to the formation of acetic acid, which arrests the alcoholic fermentation, as will be explained later. The levulose remains largely in excess of the dextrose during the whole fermentation, as is indicated by the marked levo-rotation at all times.

In plotting the curve for rotation one-tenth of the actual read-

ings given in Table I were used, in order to bring the curve within the range of the others. We note here that the rotation curve, as thus plotted, follows from its point of maximum the course of the levulose curve quite closely, gradually approaching the latter as the sucrose and dextrose diminish, until, with the elimination of these restraining factors the two curves merge, continuing afterwards together. The fact that the percentages of levulose, as calculated from the copper-reducing power, in the latter part of the alcoholic fermentation agreed so closely with one-tenth the observed polariscopic readings (Ventzke), in the 400 mm. tube, led the writer to calculate the theoretical Ventzke readings (20° C.) for a number of levulose solutions of different concentrations.

The formula[1] used was $v = \frac{4\,WP}{34.68}$, in which v = the Ventzke reading sought, W = grams of substance in 100 cc. solution, and P = the specific rotatory power at 20° C. for the given concentration. In calculating the value of P for levulose, the formula of Jungfleisch and Grimbert[2] was employed, $[\alpha_D] = -[101.38 - 0.56t + 0.108(c - 10)]$, in which t is the temperature of the solution and c the grams of levulose in 100 cc.

TABLE II.

Concentration. Levulose in 100 cc. Grams.	Calculated specific rotatory power. 20° C.	Calculated polariscope reading, Ventzke, 400 mm. 20° C.
10	— 90.18	— 104.00
5	— 89.64	— 51.69
2	— 89.33	— 20.60
1	— 89.21	— 10.26
0.5	— 89.15	— 5.14
0.2	— 89.12	— 2.06
0.1	— 89.11	— 1.02

Thus, for quantities of levulose up to 2 grams per 100 cc., one-tenth of the polariscopic reading in the 400 mm. tube at 20° C., gives a result agreeing within a few hundredths of the amount of levulose actually taken. This fact seems to offer a ready and easy means of determining levulose in the absence of other sugars. For low concentrations of levulose the differences between grams in 100 cc. and actual percentages are negligible. The close agree-

[1] This Journal, **23**, 881.

[2] Landolt: "Das optische Drehungsvermögen," 2 Auflage, p. 524.

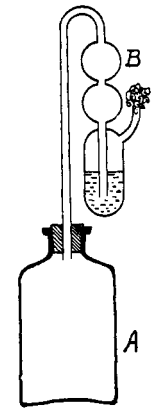
ment between the above calculated results and those actually observed in Table I affords not only a check upon the work, but also an indication that levulose is the only important optically active body in completely fermented ciders.

As regards other chemical changes produced during the period of alcoholic fermentation, we observe a slow, but gradual increase in the percentage of acetic acid, while as regards the percentage of fixed or malic acid, the reverse is true. Just how the malic acid is destroyed is not well understood. A similar diminution takes place with tartaric acid in the fermentation of grape juice. The amount of pectin or gummy matter precipitated by alcohol was determined in a few cases during the alcoholic fermentation, and also seems to undergo a decrease, due no doubt to sedimentation.

Determinations of the ester number, or cubic centimeters of N/10 sodium hydroxide necessary to saponify the esters distilled from 100 cc. of cider were made in the latter part of the period of alcoholic fermentation. The results seem to indicate an increase in the volatile esters up to the point of greatest alcohol content; with the development of acidity, however, as shown by later analyses, these esters seem to be partly broken up. The maximum ester number of 10.1 would be equivalent to 0.09 per cent. of ethyl acetate.

THE SUGAR-ALCOHOL RATIO.

Kulisch[1] in an experiment upon the fermentation of apple juice calculates that 100 parts of sugar after inversion yield 49.5 parts of alcohol, but does not state whether the experiment was conducted in a cask or bottle.[2] The results of Kulisch are based



[1] Kulisch: *Landw. Jahrb.* **19**, 111, (1890).

[2] Larger yields of the alcohol are, of course, obtained where fermentation bottles are used since losses from evaporation are prevented. In some fermentation experiments carried out by the writer under the direction of Professor Alfred Koch, at the University of Göttingen, there were obtained in one experiment from 100 parts of sugar, 50.49 parts alcohol and 48 22 parts carbon dioxide. Sterilized grape juice and a pure yeast culture (Reinhefe No. 13) were employed, so that all injurious fermentative changes, acetic, etc., were excluded. The fermentations were carried out in flasks of the form shown in the accompanying figure. After sterilizing the apparatus the bottle *A*, holding about 500 cc., is filled about half full with a known quantity of the sterilized medium (must, cider, wine, etc.) and then, after inoculating, tightly closed with the ar-

upon the supposition that the volume of liquid remains constant throughout the fermentation; while this assumption is practically correct as is shown by the results of Stein[1] and others, it would not hold where there were losses from evaporation and seepage, as is always the case where the fermentation takes place in casks.

To determine the yield of alcohol from sugar in the experiment, the calculation was made as follows, using the data given in Table I.

Date.	Weight of cider. Grams.	Sugar in cider. Per cent.	Sugar in cider. Grams.	Alcohol in cider. Per cent.	Alcohol in cider. Grams.
Nov. 16	97070	13.36	12968.6	0.43	417.4
May 4	76770	0.24	184.2	7.00	5373.9

12968.6 grams = total weight of sugar at beginning of fermentation.
deduct 184.2 " = " " " " " end " "

12784.4
deduct 585.6 grams = total weight of sugar removed by samples.

12198.8 grams = weight of sugar actually fermented.
5373.9 grams = total weight of alcohol at end of fermentation.
deduct 417.4 " = " " " " " beginning of fermentation.

4956.5
add 579.0 grams = total weight of alcohol removed by samples.

5535.5 grams = weight of alcohol formed during period of fermentation.

$$\frac{5535.5}{12198.8} = 0.454, \text{ sugar-alcohol ratio.}$$

In other words 100 parts of sugar in the experiment gave an actual yield of 45.4 parts alcohol. This is 88.8 per cent. of the theoretical yield and about 90 per cent. of the best results of laboratory experiments.

The principal cause of this loss in alcohol is that from volatilization. A small laboratory experiment upon the effects of evaporation, at room temperature, on a dilute alcohol solution showed the following:

	Weight of solution. Grams.	Specific gravity. 15.5° C.	Alcohol. Per cent.	Alcohol in solution. Grams.
At beginning	188.5	0.98647	8.11	15.29
After forty-eight hours ..	142.2	0.99802	1.04	1.48
Loss	46.3	.....	....	13.81

rangement *B*. The bottom bulb is filled about half full with sulphuric acid (1 : 1), which seals the flask from without, but does not interfere with the escape of carbon dioxide. The mouth of the bulb is closed with a small wad of cotton. By weighing the apparatus from time to time and noting the loss in weight, one is enabled to follow the rate of fermentation very closely.

[1] *J. Soc. Chem. Ind.*, **19**, 127-8 (1900).

Thus 30 per cent. of the loss on evaporation was due to alcohol, while of the total amount of alcohol originally present 90 per cent. disappeared by volatilization at room temperature. This illustration is, of course, an extreme case; the experiment was conducted in an open beaker and a relatively large surface (10 square inches) was exposed to evaporation in proportion to the amount of solution.

The loss from evaporation in the cider experiment during the 169 days of the alcoholic fermentation was calculated as follows:

97070 grams = weight of cider at the beginning.
deduct 76770 grams = weight of cider at the end of the alcoholic fermentation.
20300
deduct 12020 grams = weight of cider removed by samples.
8280 grams = loss from evaporation and carbon dioxide.
deduct 5964 grams = theoretical weight of carbon dioxide by fermentation [of 12198 8 grams sugar.
2316 grams = the calculated loss from evaporation and seepage.

The outer surface of the cider cask showed no signs of leakage, so that the greater part of this loss, which amounts to about 2.4 per cent. of the total amount of liquor, was probably due to evaporation,—this being increased no doubt by the abundant evolution of carbon dioxide early in the fermentation. In this loss from evaporation a considerable quantity of alcohol must have escaped.

Very serious losses of alcohol may result in cider-making, from the development of the acetic ferment. During the period of alcoholic fermentation in the experiment, there was formed 0.23 per cent. acetic acid, equivalent to about 0.2 per cent. alcohol; and sufficient to cause a decrease of 0.7 in the percentage of alcohol formed from sugar.

The average percentage of acetic acid in a number of ciders, obtained from farmers and analyzed at this station, was found to be 0.69, the results ranging from 0.24 to 1.96 per cent. Kulisch[1] found in the analysis of many German ciders an average of only 0.04 per cent. acetic acid, the range being from 0.01 to 0.14 per cent. This low degree of acidity is especially noteworthy and is an indication of the extreme care employed in the fermentation.

[1] Kulisch: *Landw. Jahrb.*, **19**, 88 (1890).

By racking ciders off after the main fermentation into other casks and bunging tightly, a considerable part of the losses of alcohol from evaporation and acetification may be prevented, and a much better quality of cider obtained.

THE ACETIC FERMENTATION.

The cider obtained by the alcohol experiment was used for the experiment on acetification. The cider was not transferred; but to facilitate the process of oxidation the bung of the cask, which had been only loosely inserted during the alcoholic fermentation, was now removed entirely. The results of the analytical work are tabulated in the second part of Table I. The writer, owing to a temporary absence of eighteen months, was unable to carry the experiment through to the completion of acetification. The last analysis shows the presence of 2.61 per cent. alcohol and this would correspond to about 3 per cent. more acetic acid, so that the vinegar at the end of acetification showed probably about 9 per cent. acetic acid. The work was, however, carried far enough to illustrate the principal chemical changes of acetification and to establish an alcohol-acetic acid ratio.

Inspecting Table I we notice that with the progress of acetification the specific gravity, which had been steadily decreasing since the beginning of the alcohol fermentation, now begins to increase. The percentage of solids determined by drying at 100° C. undergoes a slight diminution: the last analysis, however, seems to show an increase, due, perhaps, to a concentration of the liquid from evaporation. Ash remains constant. The percentage of malic or fixed acid continues to decrease, as during the alcoholic fermentation. The percentage of levulose continues to decrease at first slightly,—the result of the last remaining efforts of the alcoholic ferment,—but upon the percentage of acetic acid reaching 0.5 undergoes no further diminution. The alcoholic fermentation proceeds best in the presence of a minimum amount of acetic acid. A percentage of 0.5 acetic acid will retard the alcoholic fermentation considerably, and experiments by Lafar[1] show that out of fifteen different varieties of yeasts only three were able to produce any fermentation at all when the

[1] Lafar: *Landw. Jahrb.*, **24**, 445 (1895).

amount of acetic acid exceeded 1 per cent. Many farmers and vinegar manufacturers make a foolish practice of adding fresh apple-juice to old vinegar stock, in the hope of thus securing a more rapid conversion of the product into vinegar and then complain that their vinegar "won't make." The sugar of the juice must first undergo the alcoholic fermentation before the acetic fermentation can begin, and by adding apple-juice to old vinegar, the alcoholic fermentation may not only be checked, but even absolutely prevented. The writer knows of instances where such mixtures have remained for years without fermenting. This fact no doubt explains the high percentage of reducing sugars found occasionally in samples of cider vinegar.

The relation between alcohol and acetic acid in the fermentation experiment is illustrated graphically in the following diagram.

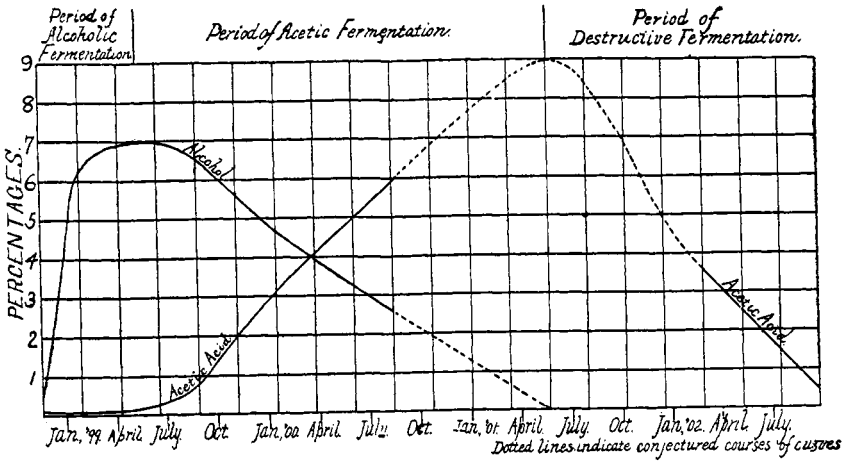


Diagram No. 2. Each division represents ten weeks.

The process of acetification advances very slowly until about July when it receives a sudden start, probably through the influence of the warm summer temperature. The conjectured courses of the alcohol and acetic acid curves, for the time when no analyses were made, are indicated by dotted lines.

THE ALCOHOL-ACETIC ACID RATIO.

Calculations made from the specific-gravity tables of alcohol and acetic acid, indicate that there is a slight increase in volume

during the acetic fermentation, provided no loss occurs from evaporation. Assuming that the volume remains unchanged during the fermentation, a calculation of the ratio of alcohol to acetic acid, from the data in Table I, gives a value of 1.33, which instead of being lower is higher than the theoretical figure 1.30. This is to be explained by a concentration of the acetic acid, during the experiment, from evaporation. A laboratory experiment[1] upon the effects of evaporation on a dilute solution of acetic acid at room temperature showed the same effect.

	Weight of solution. Grams.	Specific gravity. 15.5° C.	Acetic acid by titration. Per cent.	Acetic acid in solution. Grams.
At beginning.....	188.5	1.01184	8.53	16.08
After forty-eight hours ..	155.5	1.01326	9.04	14.06
Loss	33.0	.....	....	2.02

This shows a concentration of the solution from evaporation, there being an increase of 0.5 per cent. acetic acid in forty-eight hours. A calculation, based upon the actual amounts of acetic acid in the solution, shows, however, a loss of 2.02 grams acetic acid from evaporation or 12.5 per cent of the original amount. Vinegars may, therefore, become stronger through evaporation, notwithstanding that there is an actual loss of acetic acid.

To determine the true ratio of alcohol to acetic acid in the experiment, the following calculation was made, using data from Table I.

Date.	Weight of vinegar.	Alcohol in vinegar. Per cent.	Alcohol in vinegar. Grams.	Acetic acid in vinegar. Per cent.	Acetic acid in vinegar. Grams.
1899, May 4	76770	7.00	5373.9	0.27	207.3
1900, July 31	63620	2.61	1660.5	5.98	3804.5

5373.9 grams = total weight of alcohol at beginning of experiment.
deduct 1660.5 " = " " " " " " end of experiment.

3713.4
deduct 432.8 grams = weight of alcohol removed by samples.

3280.6 " = " " " actually fermented.
3804.5 grams = total weight of acetic acid at end of experiment.
deduct 207.3 " = " " " " " " beginning of experiment.

3597.2
add 219.4 grams = weight of acetic acid removed by samples.

3816.6 " = " " " " formed during period of experiment.

$$\frac{3816.6}{3280.6} = 1.163, \text{ ratio of alcohol to acetic acid.}$$

[1] This experiment was conducted at the same time and under similar conditions as the experiment upon evaporation of alcohol in dilute solution, page 22.

In other words 100 parts of alcohol in the experiment gave an actual yield of 116.3 parts acetic acid, instead of 133.2 parts as calculated according to constant volumes. This yield is 89.2 per cent. of the theoretical. The greater part of this loss occurred no doubt from volatilization of both alcohol and acetic acid. In the experiment during the period of acetification the bung was withdrawn from the cask entirely, and considerable loss from evaporation might easily take place.

The loss from evaporation during the fifteen months of the acetification experiment was computed as follows.

	76770 grams = weight of vinegar at beginning of experiment.	
deduct	63620 grams = weight of vinegar at end of experiment.	
	13150 grams.	
deduct	8321 grams = weight of vinegar removed by sample.	
	4829 grams.	
add	2296 grams = calculated weight of oxygen absorbed to oxidize	[3280.6 grams alcohol.
	7125 grams = calculated loss from evaporation and seepage.	

This loss or shrinkage is over 9 per cent. of the weight of cider at the end of the alcoholic fermentation, and if calculated to the completion of acetification would equal nearly 15 per cent.

Another source of loss of acetic acid, in addition to that from volatilization, occurs as a result of certain destructive fermentative changes. This will be spoken of more fully under the subject of deterioration of vinegar. The ratio of sugar to acetic acid in the experiment would be equal to the product of the sugar-alcohol and alcohol-acetic acid ratios, $0.454 \times 1.163 = 0.528$. That is, 100 parts of sugar produced 52.8 parts acetic acid, which is about 80 per cent. of the theoretical yield 66.6.

THE DETERIORATION OF VINEGAR.

It is a fact well known among vinegar manufacturers that vinegars after long standing deteriorate and lose their strength. Such vinegars they say become "overaged."

A good example of a deteriorated vinegar is afforded in the fermentation experiment. After an interval of eighteen months from the analysis on July 31, 1900, an examination of the vinegar was made with the results shown at the bottom of Table I. The vinegar, when drawn, contained a large amount of sediment,

mixed with mother and had to be filtered. There were also present many dead larvae of vinegar flies (*Drosophila*); whether these may have played any important part in the process of deterioration is uncertain.

Comparing the analytical results of the deteriorated vinegar with those of eighteen months before, we notice some very decided changes. The solids and ash have both undergone a marked increase, due to a concentration of the vinegar from evaporation. The bung-hole of the vinegar cask had remained open since the time of the former analysis and the weight of vinegar had decreased from 63 to 44 kilos, a loss of over 30 per cent. We note also in Table I that the alcohol has entirely disappeared in the deteriorated vinegar, as was to be expected, and that the percentage of malic acid, which had been decreasing from the very first, has fallen off to 0.06 per cent.

The acetic acid, it is noticed, has decreased over 2.5 per cent. since the last determination. The loss in acetic acid from deterioration is, however, much greater than this, as the process of acetification at the time of the former analysis was incomplete. Determinations of acidity in the deteriorated vinegar were made from month to month, and the decrease continued quite regularly, though somewhat faster in the summer months, at the rate of about 0.1 per cent. per week. This is also shown graphically in diagram No 2.

This marked decrease in the percentage of acetic acid is due mostly to a destructive fermentation. There are a number of acetic acid-consuming organisms, but of first importance among these are the acetifying bacteria themselves. It was first established by Pasteur that the acetic acid bacteria, after converting the alcohol into acetic acid, then consume the latter, forming carbon dioxide and water, $CH_3COOH + 4O = 2H_2O + CO_2$. The saying sometimes heard that vinegar on long standing "eats itself up" is therefore really true. This secondary action of the acetic ferment sets in no doubt before the complete acetification of the alcohol, and this would diminish somewhat the yield of acetic acid from alcohol.

THE NATURE OF THE ORGANISMS PRODUCING DETERIORATION.

A sample of the deteriorated vinegar poured off into a large

bottle continued to develop mother in large quantities, and the question arose as to the exact nature of the ferment producing the deterioration. A chemical examination of the mother, which was taken before the period of deterioration and of that found in the degenerated sample, showed that the organisms, which had produced acetification, and those causing the deterioration were of an entirely different nature, although both belonged to the class of acetic bacteria. Pieces of the mother from the deteriorated vinegar were boiled with successive portions of dilute sulphuric acid and soda, to remove incrusting substances, and then, after washing, treated with chloride of zinc and iodine; an intense blue coloration developed, characteristic of cellulose. The same reaction was also produced with sulphuric acid and iodine. This proves the ferment to be not the ordinary *mycoderma aceti* but *bacterium xylinum*[1] or an allied form,—the slimy envelopes of *mycoderma aceti* or *bacterium Pasteurianum* not containing cellulose. The presence of a cellulose-forming organism in the deteriorated vinegar is especially noteworthy, inasmuch as the mother taken from the vinegar before deterioration gave no cellulose reaction, showing that the ferment, which produced acetification, was of the ordinary *mycoderma* type. Bertrand[2] states that the small red vinegar flies (*Drosophila cellaris*), which frequent places where fruit juices, etc., are fermenting, inoculate the latter with the sorbose bacterium[3] (*bacterium xylinum*). As was before stated many larvae of vinegar flies were found in the deteriorated vinegar and we may have here a source of the organisms, which seem to have supplanted the original acetifying bacteria.

THE FORMATION OF REDUCING SUBSTANCES IN "OVERAGED" VINEGAR.

A singular fact in connection with the results upon deterioration is the high increase in the percentage of reducing sugar, which is also attended by a considerable increase in the levorotation. This cannot be explained altogether by the concentration of the vinegar from evaporation, since with a reduction in volume of only 30 per

[1] Lafar: *Technische Mykologie*, Bd. I, p. 346. The *Bacterium xylinum*, first studied by A. J. Brown, forms a very tough, thick mother, popularly designated in England as "vinegar plant."

[2] Bertrand: *Compt. Rend.*, **122**, 900; Maquenne: "Les Sucres," Paris, (1900), p. 581.

[3] The Sorbose bacterium and *Bacterium xylinum*, at first considered different, have been proved by Emerling to be identical. *Ber. d. chem. Ges.*, **32**, 541.

cent., we observe that the percentage of sugar, calculated as levulose, has increased to over five times its original amount. The percentage of sugar as levulose no longer agrees with one-tenth the rotation Ventzke in the 400 mm. tube, as was the case early in the process of acetification, but is over twice the same, so that other reducing substances besides levulose must be present,—the result no doubt of certain fermentative changes. This latter assumption does not seem improbable, when we consider the fact that the solids of vinegar are made up to a great extent of glycerol, pectin, and other substances, probably of a carbohydrate nature, and hence easily subject to chemical transformation.

To ascertain more fully the nature of the reducing bodies in the deteriorated vinegar, a quantity of the sample, after filtering from the mother, was treated with a solution of phenylhydrazine dissolved in acetic acid. After heating for twenty minutes on the steam-bath, a flocculent precipitate began to form and, on cooling, a thick yellowish colored magma was obtained. This was filtered off, and by fractional crystallization from alcohol was found to contain two well-defined osazones.

Osazone No. 1, present in considerable amounts, was easily soluble in alcohol, but less soluble in water. After recrystallizing several times from dilute alcohol, a yellowish colored product was obtained melting at 142°-143°. The compound turned slightly brown on long exposure to the air and light.

Osazone No. 2, present in only small amounts, was almost insoluble in alcohol and very difficultly soluble in ether. Its quantity was so small that no attempt was made to recrystallize it. It was purified by washing with small amounts of alcohol and ether. The compound consisted of a pale yellow crystalline powder, melting at 240°-242°.

An elementary analysis of osazone No. 1 showed it to have the composition of a phenyl hexosazone.

	Found. Osazone No. 1. Per cent.	Calculated. Phenylhexosazone, $C_{18}H_{22}N_4O_4$. Per cent.
Carbon	60.45	60.27
Hydrogen	6.22	6.20

Of the some twelve known isomeric phenylhexosazones only one has a melting-point agreeing with that of osazone No. 1 and

that is the phenylformosazone, described by Fischer,[1] melting at 144°. Formose,[2] a body of great interest in the synthesis of sugars, has not been found hitherto in any natural product. It has been prepared synthetically by the condensation of formaldehyde in alkaline solution. In this condensation a mixture of substances is formed, but the name formose is applied by Fischer only to the compound giving the above osazone. The arrangement in composition and melting-point of osazone No. 1 with phenylformosazone, while not an absolute proof of the identity of the two compounds, yet furnishes an indication of the presence in the deteriorated vinegar either of formose or of some unknown hexose sugar.

An elementary analysis of osazone No. 2 showed that it was not an osazone of any of the sugars. Its composition agrees, however, with that of phenyldiacetylosazone.

	Found. Osazone No. 2. Per cent.	Calculated. Phenyldiacetylosazone, $C_{16}H_{18}N_4$. Per cent.
Carbon	71.90	72.10
Hydrogen.....	7.08	6.82

Diacetylosazone, first prepared by Pechmann,[3] is described as a yellowish colored, finely crystalline compound, melting at 245°, almost insoluble in alcohol or water, and difficultly soluble in ether. These properties agree exactly with those of osazone No. 2. This osazone can be formed either from diacetyl, $CH_3COCOCH_3$, or from dimethyl ketol, CH_3—CHOH—CO—CH_3. The latter compound benig a ketone, alcohol is closely allied to the sugars and, as described by Pechmann,[4] reduces Fehling's solution strongly, even in the cold.

The separation of the two osazones just described from the deteriorated vinegar goes to confirm, what was previously surmised from the changes in reducing power and rotation, *viz.*, that other reducing substances besides levulose must be present as a consequence of fermentative changes. As a matter of fact, the levulose seems to have disappeared completely, since no traces of other osazones besides the two mentioned could be found.

As a remedy against deterioration, vinegars intended for storage should be racked off into clean casks, the latter filled full

[1] Fischer: *Ber. d. chem. Ges.*, **21**, 989–991.

[2] See Tollens: "Die Kohlenhydrate," Bd. II, p. 39 and p. 135; also Maquenne: "Le Sucres," p. 398.

[3] Pechmann: *Ber. d. chem. Ges.*, **21**, 2754.

[4] Pechmann: *Ibid.*, **22**, 2214.

and tightly bunged. Since none of the acetic organisms can thrive without air, their further development is thus prevented, and the loss from destructive fermentation reduced to a minimum.

MISCELLANEOUS RESULTS.

In the following tables a few miscellaneous results are given, which may have a certain interest, though not bearing directly upon the main part of the fermentation experiment.

The Composition of Vinegar Settlings. During the alcoholic and acetic fermentation a large quantity of matter settles to the bottom of the cask. A quantity of this material, as obtained from the cask at the close of the fermentation experiment, was filtered off, washed, and submitted to an examination.

The material, which was of a brownish color, was seen under the microscope to consist largely of dead yeast cells; fragments of mother, particles of pomace, and amorphous flocks of a pectinous character were also observed. In addition to these was noted a number of spherical bodies, more or less marked with a linear depression, which were stained blue with iodine. Measurements showed these to range in size from 0.004 mm. to 0.016 mm., which thus identifies them with the starch granules of the apple.[1] This starch, which was removed from the apple at the time of pressing the cider, settles out and remains unaffected by the fermentation.

An analysis of the dried settlings showed them to have the following composition:

TABLE III.

	Per cent.
Moisture	4.91
Fat	1.69
Protein	20.13
Ash	2.65
Crude fiber	5.69
Nitrogen-free extract	64.93

A determination of pentosans showed only 1.48 per cent., so that the high percentage of nitrogen-free extract remains unaccounted for. The amounts of starch and pectin present were not determined, and these no doubt would make up a considerable part of the deficiency.

Composition of Ash.—Analyses were made of the ash from the apples and cider used for the fermentation experiment and of the ash from the settlings, taken from the cask after closing the experiment.

[1] Bulletin No. 58, Pennsylvania Department of Agriculture, p. 12.

TABLE IV.

	Ash of apples. Per cent.	Ash of cider. Per cent.	Ash of settlings. Per cent.
K_2O	58.31	60.60	19.94
Fe_2O_3 and Al_2O_3	12.26	6.00	19.97
CaO	undetermined.	1.15	4.17
MgO	undetermined.	undetermined.	2.22
P_2O_5	12.51	7.12	29.64
SO_3	6.87	4.15	0.14
SiO_2	1.65	1.90	24.23
Undetermined CO_2, etc	8.40	19.08	....

The high percentage of silica and phosphoric acid in the ash of the settlings is noteworthy; no carbonates were present.

In concluding, the writer expresses acknowledgment to Messrs. C. P. Beistle and A. N. Diehl, former assistants at this experiment station, for help in much of the analytical work of the foregoing experiments, and also to Dr. William Frear for his encouragement and many kindly suggestions, and under whose initiative the work was first begun. The writer owes an especial debt of gratitude to his former teacher, Professor Alfred Koch, of Göttingen University, for much valuable advice and information upon this subject of fermentation.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF TEXAS, AUSTIN, TEXAS.]

A CONTRIBUTION TO THE CHEMISTRY OF FATIGUE.

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INTRODUCTION.

SOME years since, while engaged in the study of the phenomena of fatigue and breathlessness, the senior author of this contribution was struck with an apparent resemblance to the phenomena encountered when caffeïne is administered in toxic doses. It seemed clear that the phenomena of fatigue were largely due to the action of a chemical substance present in toxic quantity in the blood-stream, and that this substance had its origin in the muscles, *i. e.*, that the metabolism within the muscles undergoing excessive exercise was accompanied with a production of incompletely