of this class, being indeed more nearly like that in the case of some mucin substances, while the sulfur found more nearly coincides with the amount found in most globulins, rather than in keratins.

A. Argiris[1] obtained on analysis of his neurokeratin:

C, 56.60%; H, 7.15 and 7.40%; N, 14.16%; and S. 2.24 to 2.31%, calculated to the ash-free material.

His analysis of the hydrolytic products also gave different results, but was performed after the older methods. On the other hand, one of the analyses quoted by Hammersten gives nitrogen as low as 11.5%.

Whether or not the long standing of the tissues in 85% alcohol and the subsequent extraction with boiling alcohol, might have permanently rendered other of the proteins indigestible and insoluble, or what portion, if any, of similar indigestible proteins might possibly have been left from the cerebral vessels, we are unable to say definitely; but these last then must probably also have been present in preparations of neurokeratin obtained by Kühne and Chittenden's method.

## Conclusions.

Neurokeratin, as obtained by us, is a protein of unusually low nitrogen and sulfur content, as compared with more commonly known keratins, and appears to differ from these latter also in the structural make up of its molecule.

BINGHAMTON, N. Y.

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[CONTRIBUTION FROM THE CHEMISTRY SECTION OF THE IOWA AGRICULTURAL EXPERIMENT STATION.]

# AN ACCURATE AERATION METHOD FOR THE DETERMINATION OF ALCOHOL IN FERMENTATION MIXTURES.

BY ARTHUR W. DOX AND A. R. LAMB.

Received August 28, 1916.

## Introduction.

A great deal of work has been done upon methods for the determination of ethyl alcohol by physical means, principally the specific gravity, the index of refraction, or the boiling point of alcoholic solutions. Physical methods, however, are all open to the same objection, *viz.*, that the presence of any other substance than ethyl alcohol in the solution tested will always cause incorrect—usually high—results. The production of alcohol in fermentation processes, in whose products it is generally desired to estimate the alcohol, is invariably accompanied by the formation of by-products in greater or less amount, even in pure yeast cultures. Many of these other products are volatile, and cannot be separated from the alcohol by distillation, especially in dilute solutions. The estimation

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[1] *Loc. cit.*

of the alcohol in such a mixture by physical methods must therefore be very difficult to free from error.

Chemical methods have not received nearly so much attention, perhaps because the physical methods generally consume less time. However, the list of interfering substances should not be nearly so long in the case of the determination of ethyl alcohol by oxidation to acetic acid, since it would include only volatile substances which yield a volatile acid on oxidation. The latter method should therefore be more reliable, and may perhaps be susceptible of development to as great a degree of accuracy.

It is not the purpose of this paper, however, to enter into a discussion of the relative merits of physical and chemical methods, but merely to present the results of a considerable experience with chemical methods for the determination of alcohol, which may be of value to others engaged with fermentation problems.

## The Oxidation of Alcohol.

In the oxidation method there are two possible sources of error, which may be considered separately. The first, which is common to all methods, lies in the difficulty in quantitatively removing the alcohol from the original fermented solution, and in getting it into a convenient volume for oxidation. Direct distillation of a fermented solution is likely to be troublesome, especially when nearly all of the solution must be distilled over in order to get all of the alcohol into the distillate. Distillation with steam in the usual manner will volatilize all of the alcohol only after a very long time, and it is difficult to concentrate the large distillate without losing a considerable amount of the alcohol. The second possible source of error is in the oxidation itself, where the oxidation may be incomplete, yielding aldehyde, or may go too far, with the production of carbon dioxide and water. Other substances than alcohol, which might yield a volatile acid as an oxidation product, might also be present.

The most commonly used oxidizing agent is a sulfuric acid solution of potassium dichromate. Potassium permanganate solution has not often been suggested. A recent paper by Evans and Day[1] has shown that varying amounts of oxalic acid and carbon dioxide are formed in the oxidation, in addition to the acetic acid. The sulfuric acid-dichromate method was used by Hehner,[2] and by Benedict and Norris,[3] who determined the amount of dichromate reduced, and by Dupre,[4] who distilled and titrated the acetic acid formed. Various modifications of Dupre's method have been used by Suzuki, Hastings, and Hart,[5] and by Hart,

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[1] This Journal, **38**, 375–381 (1916).

[2] *Analyst*, **12**, 25 (1887).

[3] This Journal, **20**, 293 (1898).

[4] *J. Chem. Soc.*, **20**, 495 (1867).

[5] *J. Biol. Chem.*, **7**, 431–458 (1910).

Hastings, Flint, and Evans,[1] in work on cheese, and by Hart and Willaman,[2] and by Dox and Neidig,[3] in work on corn silage.

## Experimental Part.

**Oxidation Trials.**—Dupre's method, as originally published,[4] is unsupported by data checking it against known alcohol solutions, and has apparently not been standardized by other workers who have used the method. Since a reliable and accurate method was necessary for use in the silage investigations in progress in this laboratory, a careful study of Dupre's method was begun. It seemed that the distillation and titration of the acetic acid formed would be much more trustworthy than the determination of the reduced dichromate, as done by Benedict and Norris,[4] since various organic substances might reduce the dichromate, while the formation of a volatile acid in the oxidation would be much less likely to occur. Therefore the oxidation of standard alcohol solutions by Dupre's method was first studied.

The standard alcoholic solutions were made by weighing out about 10 g. of redistilled, nearly absolute, alcohol, and making up to exactly 1000 cc. with $CO_2$-free distilled water. The densities of the original alcohol and of the dilute solutions were determined with a Sprengel pycnometer, and the strength of the dilute alcohol solutions calculated. Samples were taken with a carefully calibrated pipet.

Various mixtures of sulfuric acid and dichromate have been used by other workers, but the exact proportions seem to be of minor importance. The oxidizing solution decided upon and used in most of this work was made up in the following proportions: 10 g. $K_2Cr_2O_7$, 20 g. $H_2SO_4$, 70 g. $CO_2$-free distilled water. Oxidation in a pressure flask in a boiling water bath was first tried. It was soon found that 30 minutes' oxidation would give as good results as 3 hours. Later it was found that if the solution was merely allowed to stand in the distilling flask a few minutes at room temperature, before distillation, the results obtained were fully as accurate. The reaction seems to be complete in a very few minutes on heating, during the time necessary to bring the solution in the distilling flask to the boiling point. In the case of oxidation at room temperature, the first few cubic centimeters of the distillate sometimes have a slight odor of acetaldehyde, but this small loss is apparently no greater than the loss attendant upon transferring the liquid from the pressure flask to the distilling flask in the case of oxidation with heat.

Dupre and later workers with his method have reduced the excess dichromate in the oxidizing solution with zinc, before distillation. This

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[1] *J. Agr. Res.*, **2**, 193 (1914).

[2] THIS JOURNAL, **34**, 1619 (1912).

[3] Iowa Agr. Exp. Sta., *Research Bull.* 7 (1912) and 16 (1914).

[4] *Loc. cit.*

extra step is not only entirely unnecessary, but it is often a source of trouble in the distillation. Therefore it has not been done in this work.

The distillation of the volatile acid from the oxidizing solution is carried on carefully over a free flame, using an asbestos board with a small circular opening for the bottom of the distilling flask. The combination of a glass bead with two small pieces of pumice stone in the distilling flask has been found the most satisfactory preventive of both bumping and foaming during the distillation. If care is taken not to permit the volume of liquid in the distilling flask to become low enough to allow sulfuric acid to be carried over, the distillation gives no trouble whatever. If barium hydroxide solution is used for the titration, the presence of any sulfuric acid in the distillate will at once be detected. The oxidizing solution is repeatedly distilled with additions of $CO_2$-free distilled water, until the last titration is 0.5 cc. of a decinormal solution, or less. This is generally after four or five distillations. If care is taken, the error due to carbon dioxide is negligible. Some representative data secured on this part of the process are shown in Table I.

TABLE I.—OXIDATION OF KNOWN ALCOHOL SOLUTIONS.

| Conditions. | Calc. g. alcohol. | Found g. alcohol. | Recovery. %. |
|---|---|---|---|
| Oxidation at 100°, 30–40 min. | 0.238 | 0.236 | 99.2 |
| | 0.238 | 0.235 | 98.7 |
| | 0.257 | 0.257 | 100 |
| | 0.257 | 0.256 | 99.6 |
| Oxidation at room temperature | 0.238 | 0.237 | 99.6 |
| | 0.257 | 0.256 | 99.6 |
| | 0.257 | 0.257 | 100 |

Only when the method of oxidation had been perfected, and reliable results in that part of the process assured, were the more uncertain preliminary steps studied.

**Distillation of Alcohol Solutions.**—Previous investigators have concentrated the alcohol solution obtained from steam distillation by successive redistillations, distilling off each time about one-half to three-fourths of the volume in the flask. This method is very tedious and far from quantitative. Bacon,[1] of the Bureau of Chemistry, has shown that it is possible, by nearly saturating the alcohol solution with common salt, to distill off all the alcohol in the first one-fifth of the volume distilled, Bacon's suggestion was carried further in this work, by adding the salt to the original solution to be distilled with steam, thus liberating both the alcohols and the volatile acids much more readily, and getting them over practically completely in the first 500 cc. of distillate. It is noteworthy that although lactic acid was present in the solution, no hydrochloric acid was liberated. Solid phenolphthalein was added to the distillate, the volatile acids titrated and fixed, and the alcoholic solution easily

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[1] U. S. Dept. Agr., Bur. Chem., *Circ.* 74 (1911).

concentrated in one or two distillations with salt to a convenient volume for oxidation. A few typical results obtained with known solutions by this method are shown in Table II. This table also shows the result obtained by the concentration of a known solution from 500 cc. to 50 cc. with the use of salt, to compare with steam distillation with salt followed by concentration. In one distillation shown the salt used was ammonium sulfate.

TABLE II.—DISTILLATION AND OXIDATION OF KNOWN ALCOHOL SOLUTIONS.

| Conditions. | Calc. g. alcohol. | Found g. alcohol. | Recovery. %. |
|---|---|---|---|
| Distilled and concentrated with NaCl...... | 0.238 | 0.210 | 88.2 |
| | 0.238 | 0.225 | 94.6 |
| | 0.238 | 0.216 | 90.8 |
| Concentrated only, with NaCl............ | 0.238 | 0.228 | 96.6 |
| Distilled and concentrated with $(NH_4)_2SO_4$ | 0.238 | 0.218 | 91.6 |

Steam distillation of alcohol solutions generally gives just such variable results as are shown in this table. It is evident that the greatest loss of alcohol in this method occurs during the distillation with steam.

## Aeration Method.

Since the saturation of an alcoholic solution with a soluble salt brings about the liberation of the alcohol by distillation so readily, it seemed probable that alcohol could be volatilized by aeration at room temperature from a similarly saturated solution. Ammonium sulfate was chosen as a suitable salt for the purpose, and was found more efficient than sodium chloride. A known amount of alcohol in 100 cc. of water was placed in a Drexel gas-washing bottle with 80 g. of ammonium sulfate. This was connected to a series of two bottles, each containing 50 cc. of oxidizing solution, which were followed by a flask containing a definite amount of 0.1 *N* barium hydroxide solution. A soda-lime tube and a guard bottle containing oxidizing solution were placed at the beginning of the train, and a stream of air was drawn through at a moderate rate with a water pump. This method gave at first more or less variable results. It was necessary to determine the amount of volatile acid, exclusive of carbon dioxide, which had been caught by the barium hydroxide solution, and to distill off the rest of the acetic acid from the oxidizing solution. However, the volatilization of the alcohol was found to be complete in about 8 hours' aeration, and the amount of alcohol recovered averaged from 90% to 96%.

Benedict and Norris[1] make the statement that concentrated sulfuric acid takes up alcohol as readily as it does water. Therefore a bottle containing 20 cc. of the acid was substituted for the oxidizing solution, and the barium hydroxide and soda-lime were eliminated. After aeration the sulfuric acid was carefully added to 100 cc. of saturated solution of

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[1] *Loc. cit.*

potassium dichromate, oxidized, and distilled as before. More accurate and more uniform results were obtained than before, and other advantages were secured. As the alcohol is not oxidized until after the aeration is completed, there is no danger of loss of volatile acid. The sulfuric acid takes up the alcohol more completely than does the oxidizing solution. The alcohol is brought into a small volume, and may be left in that condition indefinitely, if not convenient to make the oxidation at once. A smaller number of bottles is necessary in the train, and less attention is required.

Instead of a regular gas-washing bottle as a container for the sulfuric acid, it is preferable to use an ordinary 100 cc. graduated cylinder, fitted with a rubber stopper carrying an inlet and an outlet tube. The inlet tube may be slightly constricted at the lower end, or a bulb with small perforations, similar to a Folin bulb, may be blown at the end, to secure still more efficient absorption. However, it is necessary in any case to use two cylinders of sulfuric acid, in order to secure the most complete recovery of the alcohol. It seems that this is not because the stream of air is too large or too fast, but because the reaction between the alcohol and the sulfuric acid is reversible, and therefore a small amount of the alcohol passes over into the second cylinder. The recovery of alcohol was increased by 1%–2% when the second cylinder was first used.

A few representative results showing the average accuracy of the method are given in Table III. In nearly all cases, the oxidation was at room temperature.

TABLE III.—THE DETERMINATION OF ALCOHOL IN KNOWN SOLUTIONS BY THE AERATION METHOD.

| Conditions. | Calc. g. alcohol. | Found g. alcohol. | Recovery. %. |
|---|---|---|---|
| Oxidizing solution used to fix alcohol... | 0.238 | 0.227 | 95.4 |
| | 0.238 | 0.226 | 95.0 |
| | 0.238 | 0.229 | 96.2 |
| Concentrated $H_2SO_4$ used to fix alcohol. | 0.257 | 0.248 | 96.5 |
| | 0.257 | 0.250 | 97.3 |
| | 0.257 | 0.252 | 98.1 |
| | 0.257 | 0.254 | 98.8 |
| | 0.103 | 0.101 | 98.0 |
| | 1.620 | 1.594 | 98.4 |
| | 0.0202 | 0.0201 | 99.5 |

The aeration train, as now made up, consists of a guard bottle of oxidizing solution, followed by the saturated ammonium sulfate solution containing the alcohol, and by two cylinders of concentrated sulfuric acid, the first containing about 18 cc. and the second 8–10 cc. of acid. The rate at which the air is drawn through is about 25 liters per hour. The aeration is generally complete in 8–10 hours, but slightly higher results are obtained by aeration at a slower rate for 24 hours, minimizing the spattering of the sulfuric acid. After the aeration is complete, the sulfuric acid is mixed with 10–15 g. of dichromate in water in the distilling flask, and the cylinders well rinsed with $CO_2$-free water. The mixture is allowed to stand about 15 minutes, and then distilled as described above.

The ammonium sulfate used should be as nearly neutral as possible. The commercial C. P. salt sometimes contains a considerable amount of bisulfate.

It is interesting to KNOW that volatile fatty acids cannot be determined by aeration, either with ammonium sulfate or sodium chloride, although they distill over from a saturated salt solution at 100° faster than the alcohol.

Quite large amounts of alcohol, up to two grams, can be determined accurately by this method. Larger amounts can be determined by using a smaller sample. In the presence of half a gram of alcohol or more, the sulfuric acid and dichromate solutions must be mixed carefully and cooled.

The entire determination requires from 12 to 30 hours, but requires personal attention amounting in time to less than one hour. The results obtained are as accurate and more trustworthy than those obtained by any other method applicable to fermentation mixtures which is known to the authors. This method has been used for the determination of alcohol in various kinds of silage with very satisfactory results.

**Interfering Substances.**—Acetone is oxidized but slightly under the conditions of this method. It is carried over by aeration, but most of it distils unchanged from the oxidizing solution. About 0.6 g. of acetone yielded only 0.003 g. of acetic acid. If oxidized for 2 or 3 hours in a pressure flask at 100°, however, it is nearly all oxidized to acetic acid.

Certain esters, especially ethyl acetate, interfere with the determination of alcohol by this method, but these may be determined separately.

Acetaldehyde is readily volatilized and oxidized to acetic acid, but aldehydes generally occur in fermentation mixtures only in small quantities as intermediate products.

Methyl alcohol is almost completely oxidized under these conditions to carbon dioxide and water. Some recent investigators have apparently overlooked this fact in dealing with mixtures of alcohols, which were oxidized and determined as the corresponding acids by the Duclaux method. Only 0.012 g. of methyl alcohol was recovered as formic acid from 0.185 g. by aeration and oxidation.

According to Thorpe and Holmes,[1] pyridine is not changed by the sulfuric acid-dichromate mixture, allyl alcohol is completely oxidized to carbon dioxide and water, and methyl acetate yields both carbon dioxide and acetic acid. They also state that ethyl alcohol yields constantly

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[1] *J. Chem. Soc.*, **85**, 1 (1904).

0.5% of its weight as carbon dioxide. The correction may be applied, if desired. The presence of toluol does not effect the accuracy of the method, as the toluol is not oxidized.

## Summary.

The determination of ethyl alcohol by oxidation with sulfuric acid-dichromate solution has been carefully studied. It is considered that the best results are obtained when the alcohol is finally determined by distillation and titration of the acetic acid formed.

The preparation of the solution by distillation, even from a saturated salt solution, is tedious and troublesome and far from quantitative.

The alcohol solution may be saturated with ammonium sulfate and the alcohol carried over into concentrated sulfuric acid by a current of air at room temperature. The alcohol-sulfuric acid solution may then be mixed with a solution of potassium dichromate and the acetic acid distilled off at once. Results accurate within 1.5% of the amount used have been obtained by this method.

Necessary precautions and interfering substances are discussed.

AMES, IOWA.

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[FROM THE N. Y. STATE DEPARTMENT OF HEALTH LABORATORIES, ALBANY, N. Y.]

# THE EFFICIENCY OF THE AERATION METHOD FOR DISTILLING AMMONIA; IN ANSWER TO CERTAIN CRITICISMS.

BY PHILIP ADOLPH KOBER.

Received September 3, 1916.

From his "Comparative Study of Aeration and Heat Distillation in the Kjeldahl Method for the Determination of Nitrogen" Falk[1] concludes that "the aeration procedure in the ordinary Kjeldahl method for nitrogen very often gives inaccurate and therefore unreliable results, and should not be used."

This conclusion is based on his failure to remove all of the ammonia by aeration. Dillingham[2] has also recorded failure to obtain satisfactory results for the same reasons, while Bock and Benedict[3] and Greenwald[4] report great variations in the results of their determinations without accounting for them.

Accordingly, I have studied carefully the records of these observers in their published articles in the hope of discovering the sources of error, whether in the method or in the manner in which it was used.

Falk attempts to employ the results of Davis, as confirmatory data, in saying that:

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[1] THIS JOURNAL, 38, 916 (1916).

[2] *Ibid.*, 36, 1310 (1914).

[3] *J. Biol. Chem.*, 20, 47 (1915).

[4] *Ibid.*, 21, 61 (1915).