CLIII.—*The By-Products of Alcoholic Fermentation.*

By Olive Eveline Ashdown and John Theodore Hewitt.

The formation of small quantities of carbon compounds other than carbon dioxide and alcohol during the fermentation of various sugars by yeast has attracted much attention, and during the past few years considerable light has been thrown on the formation of some of these by-products. Whilst many endeavours have been made to solve the problem as to why and how succinic acid and the higher alcohols seem to be almost invariable constituents of crude alcohol, comparatively little attention has been devoted to the acetaldehyde which accompanies the alcohol in varying quantity, and which sometimes is formed in considerable amount.

If, however, one desires to throw light on the mechanism of the reaction by which alcohol is produced from dextrose and similar sugars, it is fairly evident that this substance is worthy of much greater attention, since whilst yeast normally produces alcohol with extremely small amounts of acetaldehyde, the fact that the latter substance is occasionally formed in much greater amount during abnormal fermentations is likely to lead to information of value if only the nature of the abnormality is understood.

The occurrence of notable amounts of aldehyde might be referred to one of several possible causes:

1. Subsequent oxidation of alcohol by atmospheric oxygen. This hypothesis necessitates the presence of a catalytic agent, which might either be derived from an inanimate source, for example, some metallic salt the presence of which had been overlooked, or might be of an enzymatic nature and be derived from the yeast or some other organism.

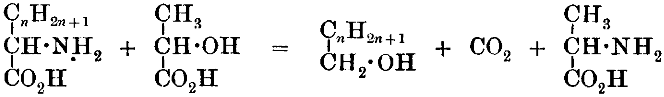
2. The acetaldehyde may be produced from the sugar by a fermentation due to some other organism.

3. Sugars when fermented by yeast normally give ethyl alcohol and carbon dioxide, but owing to malnutrition of the yeast or some similar cause, acetaldehyde may make its appearance in place of ethyl alcohol to a greater or less extent.

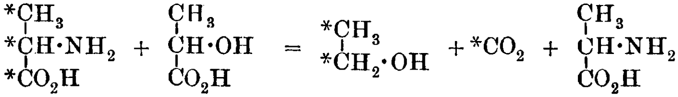
The first or second of these hypotheses may at first sight appear more probable, and one of the present authors at one time inclined to the view that the occurrence of larger amounts of aldehyde than usual was due to some agency outside the yeast. But, as will be seen later, the same yeast will give very varying amounts of acetaldehyde, according to the materials on which it is nourished being altered, other conditions remaining the same, so that the idea of the aldehyde being due to some other agency is out of the question. Moreover, the results obtained by the present authors with yeasts from different breweries show the same result, the production of aldehyde varying at different times with yeast from the same brewery, whilst any possibility of the aldehyde having been produced by subsequent catalytic oxidation owing to the presence of small amounts of salts of heavy metals was carefully excluded by performing all fermenting and distilling operations in glass vessels.

Evidently, then, the aldehyde must be a product derived from the sugar under the action of the yeast, the cause of the variation in amount being due to the different food the yeast is supplied with during fermentation. In order to obtain an idea as to which of the other constituents of the mash could affect the greater or lesser production of aldehyde, systematic experiments were carried out, solutions being made up of known composition. Generally, crystallised sucrose or dextrose was employed, together with necessary mineral constituents, nitrogen being also supplied either in the form of ammonium salts or as amino-acids. In view of the work of F. Ehrlich (*Zeitsch. Ver. deut. Zuckerind.*, 1905, **55**, 539; *Biochem. Zeitsch.*, 1906, **1**, 8; **2**, 52; *Ber.*, 1907, **40**, 1027, etc.) on the production of higher alcohols from the amino-acids with extra carbon atoms (for example, of ordinary *iso*amyl alcohol from leucine), complications were avoided by using alanine as amino-acid, since this can give no other alcohol but ethyl alcohol. In fact, the present authors started the work quite expecting to find that deficiency in available nitrogen would mean increase in aldehyde content, and it was thought not at all unlikely that the function of amino-acids and their conversion into alcohols during fermentation might be connected with the formation of ethyl alcohol and carbon dioxide from some intermediate product, possibly lactic acid.

Assuming that lactic acid is formed as an intermediate product during the alcoholic fermentation of sugars, one might imagine the chemical change involved to be capable of representation in the following (or similar) manner:

I. 

$$\text{I.}\quad \begin{array}{l} C_nH_{2n+1} \\ | \\ CH\cdot NH_2 \\ | \\ CO_2H \end{array} + \begin{array}{l} CH_3 \\ | \\ CH\cdot OH \\ | \\ CO_2H \end{array} = \begin{array}{l} C_nH_{2n+1} \\ | \\ CH_2\cdot OH \end{array} + CO_2 + \begin{array}{l} CH_3 \\ | \\ CH\cdot NH_2 \\ | \\ CO_2H \end{array}$$

II. 

$$\text{II.}\quad \begin{array}{l} {}^*CH_3 \\ | \\ {}^*CH\cdot NH_2 \\ | \\ {}^*CO_2H \end{array} + \begin{array}{l} CH_3 \\ | \\ CH\cdot OH \\ | \\ CO_2H \end{array} = \begin{array}{l} {}^*CH_3 \\ | \\ {}^*CH_2\cdot OH \end{array} + {}^*CO_2 + \begin{array}{l} CH_3 \\ | \\ CH\cdot NH_2 \\ | \\ CO_2H \end{array}$$

That a plentiful supply of nitrogen in the form of ammonium salts or as alanine would reduce the amount of aldehyde to any appreciable extent was immediately negatived by the results obtained with a series of fermentations carried out on sucrose solutions to each of which equal amounts of potassium phosphate, magnesium sulphate, and calcium phosphate had been added, but which differed respectively in containing (*a*) no added nitrogenous material, (*b*) ammonium sulphate, (*c*) ammonium tartrate, and (*d*) alanine. A reference to table II, which records the results obtained with a Yorkshire yeast at the ordinary temperature (Jan., 1909) over a period of nineteen days, shows that whilst the largest yield of alcohol was obtained when alanine was added, and the next best yield with ammonium sulphate, least aldehyde was formed when the yeast had to depend on its own nitrogen, and the greatest amount (absolutely—the amount relatively to the alcohol formed was slightly greater in the case of ammonium tartrate) was produced when alanine was added. Evidently lack of nitrogenous food is not the cause of aldehyde formation, and a systematic search for ferments, whether inorganic or enzymic, which can effect a catalytic decomposition of alanine with production of acetaldehyde would seem desirable in view of the observation made by E. Drechsel (*Ber.*, 1892, **25**, 3502), that alanine heated alone, or, still better, with an excess of concentrated phosphoric acid, to 220—230°, is decomposed into acetaldehyde, carbon monoxide, and ammonia, according to the equation:

$$CH_3\cdot CH(NH_2)\cdot CO_2H = CH_3\cdot CHO + CO + NH_3.$$

Drechsel draws particular attention to the wandering of an oxygen atom of the carboxyl group (presumably the hydroxylic oxygen) to the α-carbon atom, and it seems not unreasonable to suppose that, given a suitable enzyme, a similar transformation, resulting in the formation of acetaldehyde and formamide or ammonium formate, might be effected at the ordinary temperature.

During the progress of this work we fortunately turned our attention to a paper by H. Schade (*Zeitsch. physikal. Chem.*, 1906, **57**, 1), who had discovered a method of fermenting dextrose to ethyl alcohol and carbon dioxide without the aid of any organism or organic enzyme. Briefly, this author found that dextrose is decomposed by alkalis with the formation of acetaldehyde and a formate, whilst acetaldehyde and formic acid yield ethyl alcohol and carbon dioxide under the influence of rhodium sponge. Schade thinks it not improbable that yeast effects the conversion of dextrose into ethyl alcohol and carbon dioxide in a somewhat similar manner, dextrose decomposing into an intermediate product, which in turn yields formic acid and acetaldehyde, an enzyme functioning in a similar manner to rhodium sponge then coming into play.

The nature of the intermediate compound which furnishes the acetaldehyde and formic acid (if the hypothesis of Schade is correct) is not quite clear; presumably the dextrose must first decompose to yield two molecular proportions of a compound, $C_3H_6O_3$, and perhaps the simplest assumption would be to regard this compound as lactic acid (compare Buchner and Meisenheimer, *Ber.*, 1904, **37**, 417; 1905, **38**, 620), which may furnish either ethyl alcohol and carbon dioxide directly, or else give acetaldehyde and formic acid, which may in turn react to give the end-products mentioned. It should, however, be noticed that a comparatively recent paper by P. Boysen Jensen (*Ber. Deut. bot. Ges.*, 1908, **26**, *A*, 666) assumes that dihydroxyacetone is an intermediate product in the fermentation of dextrose to ethyl alcohol and carbon dioxide, for the oxime of this ketone is stated to have been isolated when hydroxylamine was added to a fermenting dextrose solution. Jensen consequently divides the fermentation of dextrose into stages:

$$C_6H_{12}O_6 \longrightarrow 2OH{\cdot}CH_2{\cdot}CO{\cdot}CH_2{\cdot}OH \longrightarrow 3CH_3{\cdot}CH_2{\cdot}OH + 2CO_2.$$

and supposes that zymase consists of two enzymes, the first capable of effecting the conversion of dextrose into dihydroxyacetone, the second decomposing the latter into ethyl alcohol and carbon dioxide. He further states that an addition of glycerol inhibits the second reaction, so that in these circumstances dihydroxyacetone is isolated as the final product. This ketone may be decomposed by an oxydase, but in this case carbon dioxide and water are produced, no alcohol being formed.

Quite recently E. Buchner and J. Meisenheimer (*Ber.*, 1910, **43**, 1773) have published the results of exhaustive experiments on the action of yeast, both living and as yeast juice, on a number of compounds containing a chain of these carbon atoms; their results are interpreted as disposing of the possible fermentation of lactic acid, methylglyoxal, or glyceraldehyde to ethyl alcohol and carbon dioxide, although with dihydroxyacetone and yeast juice, boiled yeast juice having been added (see A. Harden and W. Young, Proc., 1905, **21**, 189; E. Buchner and H. Haehn, *Biochem. Zeitsch.*, 1909, **19**, 192), no less than 80 to 90 per cent. was fermented to alcohol and carbon dioxide. This result is even more striking than that of G. Bertrand (*Ann. Chim. Phys.*, 1904, [viii], **3**, 257), who succeeded in fermenting 25 per cent. of this triose.

There seems to be a consensus of opinion that some intermediate product, probably of the formula $C_3H_6O_3$, is produced, and that this gives rise to alcohol and carbon dioxide either directly or possibly with the intermediate formation of acetaldehyde and formic acid, although Buchner and Meisenheimer consider Schade's view quite untenable (*Ber.*, 1906, **39**, 4218; 1910, **43**, 1782).

This objection is based on the negative results obtained in attempting to ferment a mixture of acetaldehyde and formic acid; of course, the harmful influence of these materials might be the cause of the enzyme being put out of action, but later experiments, in which yeast juice has been allowed to act on ethylidene oxide diacetate, $[CH_3\cdot CH(O\cdot CHO)]_2O$, in which the aldehyde and formic acid are only produced slowly by a gradual hydrolysis, have proved no more successful.

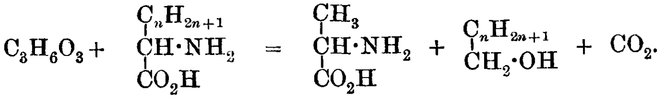
From these experiments of Buchner and Meisenheimer, it would appear that any possibility of formic acid playing a part in fermentation was out of the question, but, as our experiments show, the amount of acetaldehyde formed is largely reduced when a formate is present, whilst the use of small quantities of formic acid as an aid to good fermentations has been strongly advocated by H. Lange (*Zeitsch. f. Spiritusind*, 1905, **28**, 341).

It seems to us not improbable that some substance, possibly alanine, which should ferment normally to give ethyl alcohol and carbon dioxide, may be formed either as a product of the decomposition of the dextrose or perhaps as a transformation product of dihydroxyacetone in presence of nitrogenous material. Such a substance can give rise to acetaldehyde (Drechsel, *loc. cit.*); it may be that in presence of formic acid this latter reaction takes place to a more limited extent; certainly the fact remains that the presence of formates strongly diminishes the amount of acetaldehyde produced in alcoholic fermentation.

Without in any way committing ourselves to an explanation of the mechanism of the process, we think the following scheme may perhaps represent the course followed during the fermentation of dextrose to ethyl alcohol and carbon dioxide:

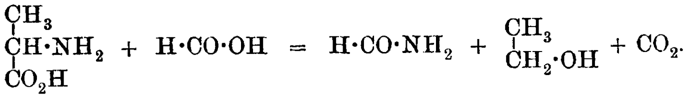
I. $C_6H_{12}O_6 \longrightarrow 2C_3H_6O_3$ (? $HO\cdot CH_2\cdot CO\cdot CH_2\cdot OH$).

II.



$$C_3H_6O_3 + \begin{array}{l} C_nH_{2n+1} \\ | \\ CH\cdot NH_2 \\ | \\ CO_2H \end{array} = \begin{array}{l} CH_3 \\ | \\ CH\cdot NH_2 \\ | \\ CO_2H \end{array} + \begin{array}{l} C_nH_{2n+1} \\ | \\ CH_2\cdot OH \end{array} + CO_2.$$

III.



$$\begin{array}{l} CH_3 \\ | \\ CH\cdot NH_2 \\ | \\ CO_2H \end{array} + H\cdot CO\cdot OH = H\cdot CO\cdot NH_2 + \begin{array}{l} CH_3 \\ | \\ CH_2\cdot OH \end{array} + CO_2.$$

IV. $C_3H_6O_3 + H\cdot CO\cdot NH_2 = H\cdot CO_2H + CH_3\cdot CH(NH_2)\cdot CO_2H$.

This represents small quantities of formic acid or formamide acting together with an enzyme of the yeast as catalytic agent; the occurrence of small amounts of one or other of these substances being quite possible in the light of Drechsel's work. In fact, the migration of oxygen from the carboxyl group to the α-carbon atom in Drechsel's reaction can best be explained by the assumption of the following intermediate actions:

I. $CH_3\cdot CH(NH_2)\cdot CO_2H = CH_3\cdot CH{:}NH + H\cdot CO_2H$ (or $CO + H_2O$).
II. $CH_3\cdot CH{:}NH + H\cdot CO_2H = CH_3\cdot CH{:}O + H\cdot CO\cdot NH_2$.
III. $H\cdot CO\cdot NH_2 = NH_3 + CO$.

The first of these reactions will be observed to be fully analogous to the well-known decomposition of an α-hydroxycarboxylic acid into an aldehyde and formic acid or its decomposition products. With reference to the possibility of formic acid playing a part in alcoholic fermentation, attention may be drawn to F. Ehrlich's views on the decomposition of leucine and the occurrence of valeraldehyde as a by-product (*Ber.*, 1907, **40**, 1046).

The view now advanced, that dextrose decomposes into half-molecules (? dihydroxyacetone), and that these in turn furnish alanine under the action of small amounts of formamide, the alanine then reacting with formic acid under the influence of an enzyme to give ethyl alcohol and carbon dioxide with the regeneration of formamide, would gain much in probability if direct synthesis of α-amino-acids from aldehydes and formamide were known; despite a careful search of chemical literature, we have found no synthesis of such a kind, and we are unfortunately prevented at the present time from carrying out a research in this direction. We may, however, sum up the arguments in favour of the suggested hypothesis.

1. The production of ethyl alcohol from alanine during fermentation by yeast brings the formation of the former substance

into line with that of its higher homologues from the higher amino-acids.

2. Acetaldehyde is always formed during the fermentation of dextrose by yeast; our experiments show that the quantity is in no way diminished by an increased supply of nitrogen, whilst Drechsel has shown that alanine yields acetaldehyde and the decomposition products of formamide (carbon monoxide and ammonia) on heating.

3. If alanine is an intermediate product of the conversion of dextrose into ethyl alcohol and carbon dioxide, the formation of lactic acid, which Slator * regards as a by-product, is easily accounted for.

4. The hypothesis that formic acid and formamide play a part in the production of ethyl alcohol receives support from Schade's observation of the reaction between aldehyde and formic acid with rhodium as catalytic agent, and our experiments clearly show that with small quantities of formates initially present, the amount of acetaldehyde formed during fermentation is very markedly decreased.

We may incidentally draw attention to the greater amount of higher alcohols produced when no ammonium salts or alanine were added to the sugar solutions; seeing that in this case the yeast must draw on its own store of nitrogen with concomitant degradation of different amino-acids (leucine, etc., not merely alanine), one has a striking, if somewhat unnecessary, confirmation of Ehrlich's views as to the formation of the higher alcohols.

EXPERIMENTAL.

The yeasts employed in these experiments were not pure cultures, but of known origin, and the authors desire to express their warm thanks to Messrs. J. L. Baker, A. R. Ling, and J. H. Millar for their kindness in supplying them with the necessary material. The usual procedure was to mix 5 grams of the pressed yeast with 10 c.c. of water, and add the resulting cream to a solution of 100 grams of sugar in 1 litre of water.

The use of solutions made up artificially with either sucrose or dextrose, potassium phosphate, magnesium sulphate, and calcium phosphate, and when nitrogenous material was also added, either alanine or ammonium sulphate or tartrate, was preferred to the employment of beer wort, as a complete check on the material

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* Lactic acid seems to be out of the question as the intermediate product between dextrose and ethyl alcohol and carbon dioxide. See A. Slator, Trans., 1906, **89**, 141; 1908, **93**, 231; *Ber.*, 1907, **40**, 123; and E. Buchner and J. Meisenheimer, *Ber.*, 1910, **43**, 1776, 1785.

supplied to the yeast was thus obtained. The solutions were made with boiling water, and subsequently aerated, but any access of air during the fermentation was excluded, owing to the necessity of connecting the Winchester-quart bottles in which the operation was conducted with washing tubes, so that all the escaping carbon dioxide passed through about 50 c.c. water to retain any aldehyde or other vapour carried off by the stream of gas. It is probable that the lack of aeration explains the somewhat small yield of alcohol obtained in many cases. In some experiments fermentation was allowed to proceed at the prevailing temperature, in others the temperature was maintained at 30°. In the latter case all the Winchester-quart bottles of one set of experiments were immersed in the same water-bath, so that temperature conditions should be strictly comparable.

At the end of a fermentation experiment, the contents of the bottle and the attached washing-tube were transferred to a flask and distilled with the aid of a rod-and-disk stillhead furnished with a thermometer; the distillation was always allowed to proceed several minutes after the temperature-reading had shown that water alone was passing over, thus ensuring that all volatile by-products were carried over in the current of steam. A second distillation was usually resorted to in order to concentrate the alcohol sufficiently for the necessary estimations. It is scarcely necessary to remark that considerable pains were exercised in cooling the vapours; the end of the condenser passed into a flask through a cork, which also carried a long reflux tube, so as to ensure volatile constituents being retained.

Besides estimating alcohol in the distillates, the amounts of volatile acid, esters, aldehyde, and higher alcohols were also determined; furfuraldehyde proved to be absent or present only in indeterminable traces. The estimations of the secondary constituents were carried out essentially according to the methods recommended by Girard and Cuniasse (*Manuel pratique de l'Analyse des Alcools et des Spiritueux*, Paris, 1899), and since comparative results were chiefly desired, the colorimetric method for estimating the higher alcohols, using an *iso*butyl alcohol standard, was adopted. Both in this case and in estimating the acetaldehyde colorimetrically, several standard solutions were always employed, and the colour developed was matched against the nearest standard. In this way much of the uncertainty experienced in using two liquids of very different intensity of colour was avoided.

The lack of any very close connexion between the supply of nitrogen and the amount of aldehyde produced is shown by the set of experiments recorded in table I.

TABLE I.

Five grams of Yorkshire yeast, stirred up with 10 c.c. of water, were added to each of five solutions. Each of these five solutions contained 200 grams of sucrose, 10 grams of potassium phosphate, 10 grams of magnesium sulphate, and 1·0 gram of calcium phosphate (for added nitrogenous material see the table itself). The yeast was added on Jan. 6th, 1909, the temperature maintained at 30° for sixty-six hours, and, after a further sixty-five hours at 10—15°, the fermented liquids were distilled on Jan. 11th.

None of the results are good as regards alcohol production; the temperature employed was unsuitable for the yeast in question; the highest yield of alcohol was, however, obtained when nitrogenous material was added in the form of alanine. The much greater production of higher alcohols where the yeast had merely its own nitrogen to depend on (experiment 2) is very noticeable.

Experiment	1.	2.	3.	4.	5.
Added nitrogenous material.	16 grams ammonium sulphate.	None.	20 grams alanine.	30 grams ammonium tartrate.	20 grams ammonium tartrate.
Yield of alcohol (in grams)	66·2	35·4	67·5	33·5	42·8
By-products; expressed as grams per 100 litres of absolute alcohol.					
Acids	—	27·1	30·3	53·8	41·4
Esters	—	45·7	19·4	55·2	73·5
Aldehydes	71·3	52·5	53·2	81·3	66·1
Higher alcohols..............	120·0	255·2	141·0	44·4	80·0

TABLE II.

In another set of experiments, using exactly the same materials and some of the same sample of yeast, but where the fermentation was allowed to proceed at the ordinary temperature for nineteen days (Jan. 6th to 25th, 1909), much better yields of alcohol were obtained.

Experiment	1.	2.	3.	4.	5.
Added nitrogenous material.	16 grams ammonium sulphate.	None.	20 grams alanine.	30 grams ammonium tartrate.	20 grams ammonium tartrate.
Yield of alcohol (in grams)	80·7	41·2	84·75	52·4	56·0
By-products; expressed as grams per 100 litres of absolute alcohol.					
Acids	40·6	29·1	23·3	45·4	30·5
Esters	67·3	34·1	63·3	76·0	67·0
Aldehydes	28·2	6·9	84·3	86·0	91·3
Higher alcohols........	34·2	38·2	—	14·7	46·0

The amounts of alcohol produced under these more favourable conditions as to temperature are considerably larger; the small amount obtained in experiment 2 is only to be expected, the yeast being starved with regard to nitrogen. The particular experiment is instructive, so small an amount of aldehyde having been produced, a result with which we were much surprised at the time, as we thought the increased production of aldehyde probably depended on the yeast obtaining insufficient nitrogenous food. It is also interesting that the absolutely largest quantity of aldehyde was produced when alanine was supplied.

TABLE III.

In order to control the foregoing results, experiments were made using dextrose solutions and fermenting with a London beer yeast at 30°. Each solution contained 100 grams of dextrose (crystallised, colourless), 5 grams of potassium phosphate, 5 grams of crystallised magnesium sulphate, and 0·5 gram of calcium phosphate in 1 litre of water. To each solution 5 grams of yeast, stirred up with 10 c.c. of water, were added on Jan. 28th, 1909, and the solutions were distilled on Feb. 2nd.

Experiment	1.	2.	3.
Added nitrogenous material	8·0 grams ammonium sulphate.	None.	10·0 grams ammonium tartrate.
Alcohol produced (in grams)..............	37·8	31·8	40·9
Grams of aldehyde per 100 litres alcohol	50·0	13·1	51·8

Again the small yield of alcohol where no nitrogenous material was added is noticeable, and, as before, least aldehyde was also formed. The yeast from experiment 2 was therefore used to ferment a fresh dextrose (100 grams) solution (in 1 litre of water), salts being added as before, and also 10 grams of ammonium tartrate. 39·4 Grams of absolute alcohol were then obtained, and the aldehyde figure had risen to 41·0. In this experiment fermentation proceeded for ninety hours at 30°.

Thus there can be no doubt that a plentiful supply of nitrogen, instead of diminishing the aldehyde, rather tends to increase the amount produced. We, consequently, in the light of Schade's work, turned our attention to the influence of formates on the production of aldehyde, and found that the quantity was considerably reduced; instead of 30 to 90 parts of aldehyde per 100,000 of absolute alcohol, numbers from 5 to 20 (sometimes more) were usually obtained. The amounts of sodium formate employed were much larger than necessary in our earlier experiments (1 to 9 in table IV), so that occasionally slightly more aldehyde was produced when 10 instead of 5 grams of sodium formate were employed to 100 grams of sucrose, the excess material being probably without much influence.

TABLE IV.

In the following four sets of experiments, in each separate experiment a solution was made up with 100 grams of sucrose, 5 grams of potassium phosphate, 5 grams of magnesium sulphate, and 0·5 gram of calcium phosphate to 1 litre of boiling water. The amount and form in which nitrogen was added or not is shown in the table. After cooling, the solutions were aerated, and 5 grams of yeast stirred up with 10 c.c. water added in each case.

In experiments 1 to 5 a beer yeast was added on March 13th, 1909, and a temperature of 30° was maintained for about ninety hours (see table). Experiments 6 to 9 were started on the same day with some of the same yeast, but fermentation was allowed to proceed at the prevailing laboratory temperature until March 25th.

In experiments 10 to 17 a porter yeast was added on May 5th, 1909, the temperature was kept at 30° for three days, and fermentation stopped after a further two days at the laboratory temperature.

A beer yeast was again employed for experiments 18 to 25, the fermentation was started on June 3rd, 1909, the temperature maintained for two days at 30°, and the fermentation stopped after a further two days at the laboratory temperature.

The results recorded in the following table demonstrate the far larger amounts of aldehyde usually produced when formates are absent, and evidently comparing the first nine experiments with the succeeding ones, quantities of sodium formate ranging from 5 to 10 per cent. of the weight of the sucrose are quite unnecessary. Turning to the strictly comparable set of experiments 10 to 13, the increasing amount of aldehyde with the decreasing amount of sodium formate is very noticeable; in another strictly comparable set, namely, 22 to 25, exactly the same phenomenon is noticed. In set 14 to 17, slight irregularities are observed, but here in each case the aldehyde-content was fairly low, and the same remark applies to experiments 18 to 21, in which, compared with the other three experiments, No. 18 has given a somewhat anomalous result.

The record of the determinations of volatile acids (calculated as acetic acid) and esters (as ethyl acetate) has been given, but although care was taken to obtain all the alcohol and by-products produced, no attempt was made to get all the distillates of the same alcoholic strength, or time given to allow the equilibrium:

$$\text{alcohol} \times \text{acid} / \text{ester} \times \text{water} = \text{a constant}$$

to be attained, so that varying ratios of acids to esters are not to be wondered at.

			Other materials added to solution (grams).				By-products; grams per 100 litres absolute alcohol.				
Expt.	Duration of fermentation.	Temperature.	Sodium formate.	Ammonium sulphate.	Ammonium tartrate.	Alcohol produced (grams)	Acids.	Esters.	Aldehyde.	Higher alcohols.
1	88 hours	30°	5	8	—	41·4	37·4	67·5	16·7	75·8
2	90½ ,,	30	10	8	—	45·6	22·4	77·9	22·0	139·0
3	91½ ,,	30	5	—	—	45·3	20·8	61·0	50·0	99·9
4	92¼ ,,	30	10	—	—	45·1	12·4	67·5	3·8	33·65
5	95½ ,,	30	10	—	10	47·5	12·7	99·5	20·5	64·8
6	12 days	15	5	8	—	45·6	22·8	58·1	40·6	71·4
7	12 ,,	15	10	8	—	47·6	14·5	91·0	33·0	70·9
8	12 ,,	15	5	—	—	36·2	17·1	100·2	21·9	182·0
9	12 ,,	15	10	—	—	37·3	14·5	113·2	29·0	100·0
10	3 ,, 2 ,,	30 15	2	8	—	43·0	23·3	150·0	11·1	58·8
11	3 ,, 2 ,,	30 15	0·5	8	—	46·0	38·2	88·0	15·6	105·0
12	3 ,, 2 ,,	30 15	0·1	8	—	42·7	67·4	98·3	41·7	160·0
13	3 ,, 2 ,,	30 15	0	8	—	41·2	58·9	88·4	53·2	140·0
14	3 ,, 2 ,,	30 15	2	—	—	43·9	18·9	85·0	24·1	180·0
15	3 ,, 2 ,,	30 15	0·5	—	—	41·9	26·9	73·2	30·0	130·0
16	3 ,, 2 ,,	30 15	0·1	—	—	43·5	11·4	48·4	11·4	129·0
17	3 ,, 2 ,,	30 15	0	—	—	41·0	18·6	46·1	14·75	165·0
18	2 ,, 2 ,,	30 15	2	8	—	37·2	33·5	129·0	24·0	70·8
19	2 ,, 2 ,,	30 15	0·5	8	—	43·3	32·4	190·3	8·8	30·2
20	2 ,, 2 ,,	30 15	0·1	8	—	39·4	60·6	228·6	20·0	95·2
21	2 ,, 2 ,,	30 15	0	8	—	37·0	56·5	162·6	23·7	74·1
22	2 ,, 2 ,,	30 15	2	—	—	33·6	28·7	46·1	24·6	79·4
23	2 ,, 2 ,,	30 15	0·5	—	—	32·5	18·1	122·3	27·4	76·9
24	2 ,, 2 ,,	30 15	0·1	—	—	30·8	14·7	109·1	32·0	99·0
25	2 ,, 2 ,,	30 15	0	—	—	32·5	14·5	—	51·3	191·0

The yields of alcohol, as we have previously mentioned, are far from ideal, but it has to be remembered that after the first aeration following the making up of the solutions, the fermentations were carried on without any access of air, and in some cases there is no doubt that an insufficient supply of nitrogen contributed to lack of vigour on the part of the yeast. This is well seen by comparing experiments 8 and 9 (mean amount of alcohol 36·7 grams) with 6 and 7 (47·1 grams), or experiments 22 to 25 with 18 to 21.

We think, however, our experiments conclusively show that the amount of aldehyde is markedly diminished by the presence of formates during fermentation, and although Schade's original theory as to the rôle played by formic acid in fermentation may need modification, it should not too lightly be set on one side.

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