XXIV. THE PRODUCTS OF THE "ACETONE: n-BUTYL ALCOHOL" FERMENTATION OF CARBOHYDRATE MATERIAL WITH SPECIAL REFERENCE TO SOME OF THE INTERMEDIATE SUBSTANCES PRODUCED.

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From the Royal Naval Cordite Factory, Holton Heath.

(*Received March 5th, 1920.*)

The production of butyl alcohol and other higher alcohols from carbohydrate material, directly by fermentation, has been well-established [compare Fitz, 1878, 1882, 1883, 1884; Gruber, 1887; Perdrix, 1891; Grimbert, 1893; Botkin, 1893; Schardinger, 1907; Bredemann, 1909; Buchner and Meisenheimer, 1908].

The presence of iodoform-producing substances among the products of fermentation has been recorded occasionally, but the substance yielding the iodoform has never been identified. The first observation of acetone as the result of fermentation of carbohydrates was made by Schardinger [1905], who obtained acetone together with acetic and formic acids. The isolation of an organism yielding acetone and n-butyl alcohol from amylaceous material was due to Fernbach and Strange [1912]. Since this initial discovery other processes have been described which produce acetone and n-butyl or ethyl alcohol by the breakdown of carbohydrates under bacterial action [Farbenfabr. vorm F. Bayer & Co.; Weizmann, 1915, 1919; Ricard, 1918; Northrop, 1919, 1 & 2]. While the technique of fermentations yielding acetone and n-butyl alcohol has improved so that it is possible to carry them out successfully on a large scale, no investigation on the mechanism of the process appears to have been recorded.

A quantitative examination was therefore undertaken of certain intermediate substances formed. The normal end-products of the fermentation, as estimated during the course of the present work, are shown in the following Table I. The total weight of end-products exceeds the weight of starch fermented, but the total carbon content is nearly equivalent to that of the starch. Hence it may be inferred that the starch undergoes hydrolysis.

Table I.

From Observations made at the Royal Naval Cordite Factory, Holton Heath.

1000 lb. maize containing 650 lb. starch. Volume of mash (6·5 % maize) = 1540 gallons.

		lb.	Carbon content
650 lb. starch yield		70 acetone	43·5
		163 n-butyl alcohcl	105·7
	3410 cu. ft. of CO_2 evolved—measured at 27° and 760 mm.	= 390 CO_2 evolved	106·3
1 vol. of mash dissolves 0·555 vol. CO_2 at 38°	135 cu. ft CO_2 in solution at 0° and 760 mm.	= 17 CO_2 in solution	4·6
	2090 cu. ft. H_2 at 27° and 760 mm.	= 11 H_2	—
Total gas evolved (Composition of gas assumed to be constant throughout the fermentation)	= 5·5 cu. ft. at 27° and 760 mm. of mixed gas per 1 lb. of maize fermented		
	Residual acidity*	= 12·0 containing	5·7
		663·0	265·8

Theoretically 650 lb. of starch is equivalent to 722 lb. of hexose. The carbon content of these amounts to 288·8 lb.

* The residual acidity is taken as acetic acid 56·5 % by weight, and butyric acid 43·5 % by weight. The non-volatile portion is recorded as butyric acid for the purpose of calculation. (See Table III, p. 232, and pp. 246–7.)

Calculated as Percentage of Starch Fermented.

100 g. starch gives 111·1 g. hexose and contains 44·4 g. carbon

	g.	Carbon
100 g. starch gives	10·77 acetone	6·68 g.
,, ,,	25·07 n-butyl alcohol	16·21
,, ,,	62·61 carbon dioxide	17·07
,, ,,	1·60 hydrogen	—
,, ,,	1·80 residual acidity	0·85
	101·85	40·81

In a normal fermentation several remarkable features may be observed. The acidity of the mash increases from a very small initial value until a maximum is reached in from 13–17 hours after inoculation. (The length of time taken to reach this point is influenced by the percentage of inoculant used, temperature of the mash, etc.) When the maximum acidity is reached in 6·5 % maize mash, 3·5–4·5 cc. of $N/10$ alkali are required to neutralise 10 cc. of mash. After this point is reached a very marked acceleration in the rate of production of acetone, n-butyl alcohol, carbon dioxide and hydrogen takes place. The acidity gradually falls to a constant value, 1·5–2·5 cc. of $N/10$ alkali being required to neutralise 10 cc. of mash. (See Fig. 1, Curve 2.)

The rate of gas evolution is shown in Curve 1, Fig. 1, and the composition of the gas in Table II, p. 232.

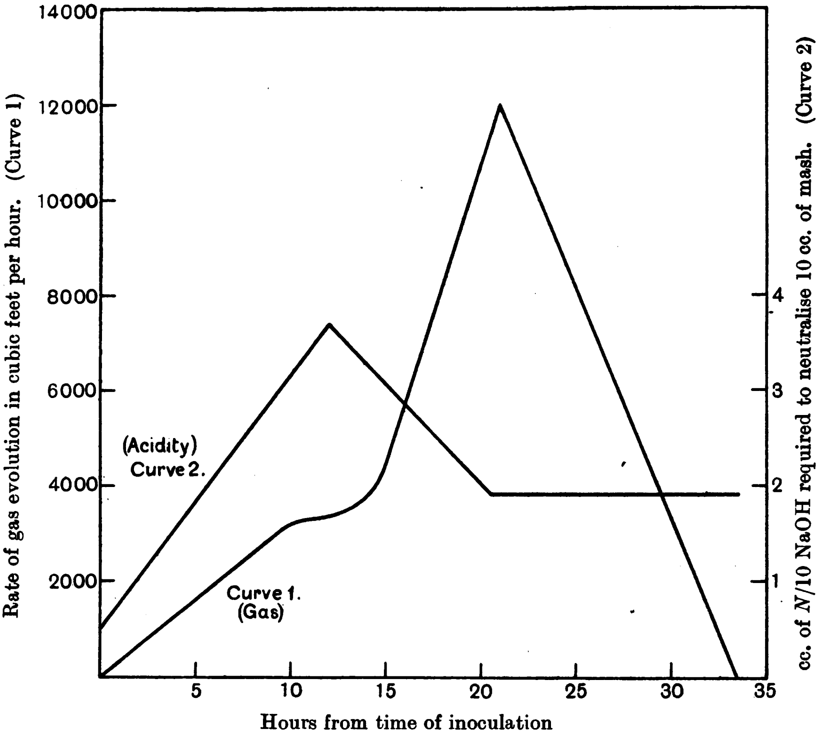


Fig. 1.

RATE OF GAS EVOLUTION, AND RISE AND FALL OF ACIDITY THROUGHOUT THE FERMENTATION.

The curves in Fig. 1 show the changes in

(1) the rate of gas evolution, and

(2) the acidity during a fermentation. The figures are the average of 12 fermentations of 6·5 % maize mash (40,000 gallons) with 4·7 % of inoculant.

The acidity is measured in cc. of *N*/10 alkali required to neutralise 10 cc. of mash after boiling the solution to remove carbon dioxide. Phenolphthalein is used as indicator.

It will be noted that the rate of gas evolution rises steadily with the increase of acidity for some time; it then becomes constant (in some cases it even slackens somewhat). As the acidity falls, the rate of gas evolution rises quickly to a maximum, and then falls rapidly until the end of the fermentation. Readings of the gas evolution were taken every hour. The acidity was estimated every three hours. The acidity at the end of the fermentation is generally higher than that of the mash when inoculated.

Composition of Gas.

Estimations were made of the composition of the gas evolved during fermentation. The following Table shows the results of one of these experiments.

The fermenting vessel was about two-thirds full of mash, the space above the latter being occupied by air before inoculation.

Table II.

Time 28.vi.'16	Gas evolved cubic feet per hour	CO_2 %	H_2 %	Air %	
4 p.m.	—	—	—	100	fermentation started
7 ,,	253	11·5	38·5	50	
8 ,,	834	27·1	57·9	15	
9 ,,	822	40·3	55·2	4·5	
10 ,,	660	46·0	59·0	3·0	
11 ,,	760	50·3	47·2	2·5	
29.vi.'16					
9.30 a.m.	1186	62	38	—	

The percentage of carbon dioxide did not vary from 9.30 a.m. 29. vi. '16 to the end of the fermentation, which lasted 36 hours. Total gas evolved 42,694 cubic feet. The high percentage of hydrogen in the gas evolved during the early hours is probably due to the greater solubility of the carbon dioxide.

The production of acetone and *n*-butyl alcohol at various stages in the fermentation is shown in Table III.

Table III.

Time	Acidity	Ratio, acetic to butyric acid	Amount of acetone and *n*-butyl alcohol mixture in 1 litre of mash
9.0 p.m.	1·0	1 : 0·5	None
11.0 ,,	1·5	1 : 0·62	None
12.15 a.m.	2·0	1 : 0·9	Trace
5.30 ,,	3·7	1 : 1·25	1 cc.
3.15 p.m.	2·0	1 : 0·28	4 cc.
4.30 ,,	1·6	1 : 0·25	5 cc.

Acidity is expressed in cc. of *N*/10 alkali required to neutralise 10 cc. of mash.

There appears to be an intimate connection between the production of acetone and *n*-butyl alcohol, and the fluctuations of acidity and rate of gas evolution.

Composition of Acids produced during Fermentation.

The acids present in the mash were examined in order to ascertain their nature and to determine if possible their function in the fermentation. The greater part of these acids was found to be volatile in steam. To study their nature the volatile acids were removed by distillation in a current of steam. The neutralised distillate was concentrated. The acids regenerated from the concentrated solution were found by Duclaux's method [1895] to be butyric and acetic acids. By fractional crystallisation of the barium salts of the mixed acids, a separation was effected into two acids of different molecular weights. The barium content of each of the two fractions agreed with that for barium acetate and barium butyrate respectively. Neither by the Duclaux method of distillation nor by fractional crystallisation of the barium salts was any indication obtained of the presence of a third volatile acid in more than traces.

Fermentation in presence of Calcium Carbonate.

The presence of acetic and butyric acids in the fermenting mash is significant. It lends support to the hypothesis that these acids are intermediate products in the formation of acetone and *n*-butyl alcohol from the carbohydrate of the mash. A fermentation was carried out in the presence of calcium carbonate to determine the effect of neutralising the acids as produced. Under these conditions the production of acetone and *n*-butyl alcohol was almost entirely suppressed, and the calcium salts of acetic and butyric acids were produced usually in the ratio of 1 molecular proportion of acetic acid to 1·8 molecular proportions of butyric acid. Evidence was also obtained of a third acid only very slightly volatile in steam, having a molecular weight equal to or greater than that of butyric acid. The relative proportions of acetic and butyric acids vary under certain conditions and in one experiment a ratio of ten molecules of acetic to nine molecules of butyric acid was obtained.

If it be assumed that two molecules of acetic acid yield one molecule of acetone, and that each molecule of butyric acid yields by reduction one molecule of *n*-butyl alcohol, then the ratio of acetic acid to butyric acid, when the fermentation is conducted in presence of calcium carbonate, should be 1 mol. : 1 mol., provided the balance of the reactions taking place is not upset in these circumstances. The result obtained in practice however is usually 1 molecular proportion of acetic acid to 1·8 molecular proportions of butyric acid.

RELATIVE RATE OF PRODUCTION OF ACETIC AND BUTYRIC ACIDS IN THE COURSE OF FERMENTATION.

Further information on the formation of the acids was obtained by determining the relative amounts of acids present at intervals throughout the fermentation. Shortly after inoculation acetic acid is found to be present in considerable amount compared with butyric acid, but as the acidity increases the relative proportion of butyric acid also increases until at the point of maximum acidity the ratio is about 1·1–1·5 molecular proportions of butyric acid to 1 molecular proportion of acetic acid.

From this point onwards the ratio of butyric acid to acetic acid falls progressively, until at the end of the fermentation the acids are in the ratio of 4–5 molecular proportions of acetic acid to 1 molecular proportion of butyric acid. (See Summary of Results, p. 246, and Fig. 2.)

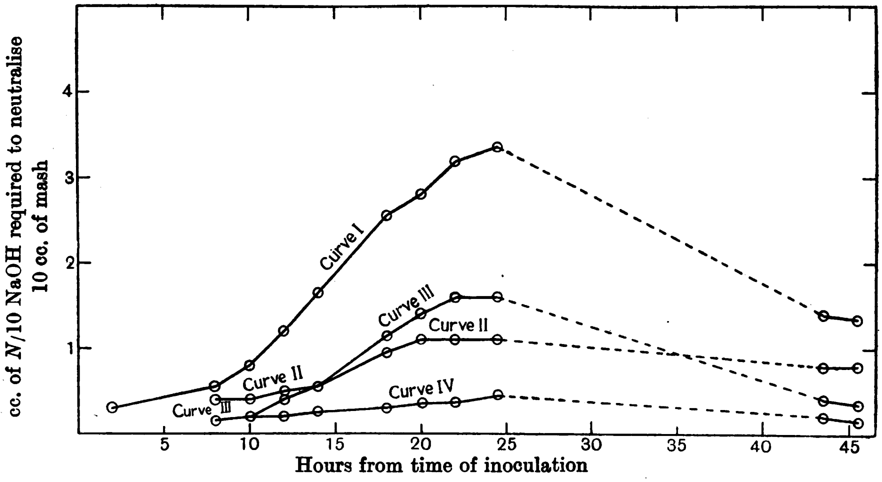


Fig. 2.

Explanatory:

Curve I. Total acidity of mash expressed in cc. $N/10$ NaOH per 10 cc. of mash.
Curve II. Acidity due to acetic acid.
Curve III. Acidity due to butyric acid.
Curve IV. Acidity due to slightly volatile acid.

A comparison of (*a*) the relative proportions of the two volatile acids in the mash at different periods and of (*b*) rise and fall of total acidity shows that during the production of acetone and *n*-butyl alcohol the proportion of butyric acid falls more rapidly than that of acetic acid, agreeing with the formation of a greater proportion of *n*-butyl alcohol than of acetone.

The addition of Acetic Acid and of other Acids to the Fermenting Mash.

When acetic acid is added to the fermenting mash an increase takes place in the amount of acetone obtained at the end of the fermentation.

A few preliminary experiments have been made in which other acids were added to the fermenting mash. Formic acid and trichloracetic acid tended to inhibit the fermentation. Propionic and butyric acids were converted into the corresponding alcohols. Valeric acid appeared to have no effect. The addition of aceto-acetic ester resulted in an increased yield of acetone.

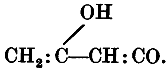
Production of Ethyl Alcohol.

A small proportion of ethyl alcohol is usually obtained. It is doubtful whether it is a normal product of the fermentation of the carbohydrate, or a by-product derived from non-carbohydrate material in the mash. The possibility of its production by foreign organisms is not excluded. When infection of the mash has been observed, this has generally been due to lactic acid-producing organisms.

The Chemical Changes involved in the Fermentation.

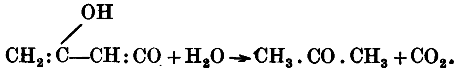
The acetic acid normally formed in the mash appears to yield acetone, for on neutralising the acid by means of calcium carbonate only traces of acetone are obtained. As already mentioned, the addition of acetic acid to the mash (fermenting in absence of $CaCO_3$) causes an increase in the yield of acetone. This action might be compared to the production of acetone by distilling calcium acetate, carbon dioxide and water being produced instead of calcium carbonate. The acetic acid may on the other hand be supposed to undergo a condensation to aceto-acetic acid, which subsequently produces acetone and carbon dioxide.

A tentative suggestion to explain the peculiar action on acetic acid is to consider acetic acid as a monoketide, $H.CH_2.CO.OH$. By condensation $CH_2:CO$ yields



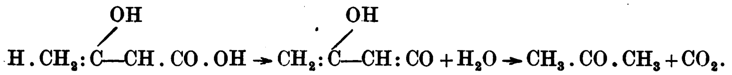
$$CH_2:\overset{OH}{C}\text{—}CH:CO.$$

The elimination of CO_2 and the addition of the elements of water would then yield acetone.



$$CH_2:\overset{OH}{C}\text{—}CH:CO+H_2O\rightarrow CH_3.CO.CH_3+CO_2.$$

Aceto-acetic acid is a diketide



$$H.CH_2:\overset{OH}{C}\text{—}CH.CO.OH\rightarrow CH_2:\overset{OH}{C}\text{—}CH:CO+H_2O\rightarrow CH_3.CO.CH_3+CO_2.$$

This hypothesis may be extended and is capable of explaining ordinary alcoholic fermentation.

In these reactions acetone and carbon dioxide are produced in the ratio of 58 to 44 by weight. It is obvious therefore from the relative amounts of acetone and carbon dioxide in the end-products of the fermentation that there must be some additional source of carbon dioxide. The 10·77 parts of acetone (see Table I) are only equivalent to 8·2 parts of carbon dioxide, but the total of the latter obtained is 62·61 parts. By comparing Curve 1, Fig. 1 and Table III, it will be seen that a large volume of gas has been evolved before any appreciable amount of acetone or *n*-butyl alcohol has been produced. On the large scale it has been observed that from 20 % to 30 % of the total volume of gas is evolved before the maximum acidity is reached, and the production of acetone and *n*-butyl alcohol in appreciable amounts begins.

A careful estimation of the total acids, carbon dioxide and hydrogen formed when the fermentation is carried out in presence of calcium carbonate, may throw light on the source of the carbon dioxide and hydrogen. All that can be said in this connection, without further experiments, is, that the weights of acetic and butyric acids calculated from the weight of mixed calcium salts obtained when a fermentation was carried out in presence of calcium carbonate were equivalent to only a part of the starch fermented. No measurements of the gas evolved nor of its composition have been made.

The proportions of the end-products obtained and their relation to the amount of carbohydrate fermented may be fairly accurately represented by the following two equations:

$$3\ C_6H_{10}O_5 + 3\ H_2O = 3\ C_6H_{12}O_6.$$
$$3\ C_6H_{12}O_6 = 2\ C_4H_{10}O + C_3H_6O + 7\ CO_2 + 4\ H_2 + H_2O.$$

The amounts of the end-products calculated as percentages on the starch fermented from the equations and actually obtained are as follows:

	From the equations	Found (Table I)
Acetone	11·9	10·77
n-Butyl alcohol	31·0	25·07
Carbon dioxide	63·0	62·61
Hydrogen	1·6	1·60
		(1·80) residual acidity

The nature of the reactions taking place to produce these results is obscure. Apparently acetic and butyric acids are intermediate products in the formation of acetone and *n*-butyl alcohol. No information has been obtained concerning the source of the acetic and butyric acids or of the hydrogen and carbon dioxide. A portion of the last-named substance may arise from the conversion of the acetic acid into acetone. The following theories may account for the origin of the carbon dioxide and hydrogen and of part of the *n*-butyl alcohol, but do not explain the production of acetic acid.

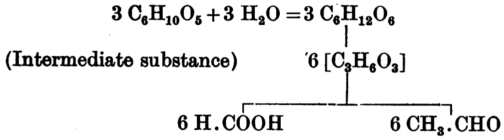
Buchner and Meisenheimer [1908], in dealing with a fermentation yielding butyric acid and butyl alcohol together with other products, suggested that acetaldehyde was first produced, which yielded aldol by condensation. The butyl alcohol was obtained by reduction from aldol, while rearrangement of the aldol yielded butyric acid thus:

A. $2\ CH_3.CHO = CH_3.CH(OH).CH_2.CHO.$
$CH_3.CH(OH).CH_2.CHO + 2\ H_2 = CH_3.CH_2.CH_2.CH_2.OH + H_2O.$

B. $2\ CH_3.CHO \rightarrow CH_3.CH(OH).CH_2.CHO \rightarrow CH_3.CH_2.CH_2.COOH.$

A similar theory has been put forward by Harden [1901], and Grey [1914], to explain the mechanism of the fermentation produced by *B. coli communis*. According to this theory the carbohydrate is first decomposed into an intermediate substance which gives rise to formic acid and acetaldehyde. The former is converted into carbon dioxide and hydrogen; the latter undergoes the Cannizzaro reaction and yields ethyl alcohol and acetic acid.

The following equations show the application of the above-mentioned theories to the acetone fermentation:



$$3\ C_6H_{10}O_5 + 3\ H_2O = 3\ C_6H_{12}O_6$$
(Intermediate substance) $6\ [C_3H_6O_3]$
$6\ H.COOH \qquad 6\ CH_3.CHO$

No direct evidence has yet been found of the formation of acetaldehyde or of formic acid in the acetone fermentation. It may, however, be pointed out that the following empirical equations, in which the end-products are underlined, agree closely with the quantities actually found.

1. $\mathrm{6\,H.COOH = 6\,\underline{CO_2} + \underline{4\,H_2} + 2\,H_2}$;
2. $\mathrm{2\,C_2H_4O + 2\,H_2 = \underline{CH_3.CH_2.CH_2.CH_2.OH} + \underline{H_2O}}$;
3. $\mathrm{4\,C_2H_4O = \underline{C_3H_6O} + \underline{CH_3.CH_2.CH_2.OH} + \underline{CO_2}}$.

Many bacteria are known to produce the change represented in equation (1), but the organism employed in this work has not been tested in this respect. Equation (2) corresponds with Buchner's theory of the formation of butyl alcohol. As to the mechanism of equation (3) nothing is known.

The fact that formic acid was not found when the fermentation was carried out in presence of calcium carbonate is not necessarily a proof that this theory is incorrect. It has been shown by Pakes and Jollyman [1901] and Grey [1914] that formic acid and formates are decomposed by *B. coli* and possibly the acetone-producing organism may act similarly. It is intended to make experiments to discover whether this is the case by the addition of formates to the fermenting mash. Careful measurements of the composition of the gas evolved when the fermentation takes place in presence of calcium carbonate are also necessary, and the effect of additions of acetaldehyde should be studied.

The possibility of the production of *n*-butyl alcohol from amino-acids is excluded, as the quantity of amino-acids present is totally insufficient to account for the quantity of alcohol produced.

EXPERIMENTAL.

Investigation of Acids produced during the Acetone : n-Butyl Alcohol Fermentation Process.

A portion of the mash was subjected to a steam distillation. 250 cc. of liquid, having an acidity equivalent to 43·8 cc. $N/10$ alkali, gave 450 cc. of distillate having an acidity equal to 27·4 cc. $N/10$ alkali. The presence therefore of a volatile acid or acids is evident.

In order to obtain larger quantities of the volatile acids free from sugars and other impurities present in the fermentation liquid, a larger quantity of the mash was steam distilled. The distillate was neutralised by means of a saturated solution of barium hydroxide and the solution evaporated to a small bulk. A stream of carbon dioxide was passed through the solution to precipitate any slight excess of barium hydroxide, and the solution was next raised to boiling point, filtered and afterwards evaporated to dryness. During evaporation of the neutralised distillate it became darker in colour, changing from light to a darker brown. The barium salt was usually obtained as an amorphous brown mass which only crystallised with difficulty. On warming with dilute sulphuric acid an odour resembling that of acetic acid was obtained. 0·1957 g. of salt dried in the oven gave 0·1599 g. of $BaSO_4$; Ba = 48·1 %.

With another sample, 0·1500 g. gave 0·1206 $BaSO_4$; Ba = 47·3 %.

The steam distillate of a sample of fermented acetone mash usually gave a barium salt which only crystallised slowly. In one experiment however a crystalline product was readily obtained and after one crystallisation it yielded a white salt which contained 45·85 % of Ba. By crystallising the mother-liquors a salt was obtained in the form of plates which contained 49·90 % of Ba, and on further crystallisation the barium content was reduced to 45·57 %. The barium salt on warming with dilute sulphuric acid gave an odour somewhat similar to acetic acid and the free acid was stable to oxidising agents and bromine water.

The quantity of material available was not sufficient for further crystallisations but from the general properties of the compound it would appear to be mainly barium butyrate (requires Ba = 44·1 %). The other barium salt, if it is a salt of a fatty acid, would appear to be an acetate rather than a propionate, for even by crystallising the mother-liquors containing a relatively high percentage of the butyrate, a salt is obtained containing 49·9 % of Ba (barium propionate requires Ba = 48·4 %; barium acetate, Ba = 53·7 %; barium formate, Ba = 60·35 %).

Another crystallisation brings the barium content of this salt nearer to that of barium butyrate owing to the somewhat smaller solubility of this salt in water than of the acetate. These figures indicate the probable absence of a large proportion of barium propionate. It should be noted that a phenylhydrazine compound was obtained as described below. On heating the crude crystalline barium salt under diminished pressure with syrupy phosphoric acid, an acid distillate was obtained which gave with phenylhydrazine a small amount of a white precipitate which was insoluble in water and ether, but soluble in absolute alcohol. It was purified by dissolving in absolute alcohol and precipitating with ether. It melted at 235–238°, yielding a brown oil. Evaporation of the alcoholic solution mentioned above gave the substance in the form of small needles. In order to determine if there were any β-hydroxybutyric acid present the crude barium salt was dissolved in a small amount of water, the barium removed by the addition of slight excess of sulphuric acid, and the solution extracted several times with ethyl acetate. On evaporating the solvent a thick brown oil remained which did not solidify on cooling. It had an acid reaction and was stable towards bromine water and Fehling's solution, but was decomposed by chromic acid and concentrated sulphuric acid. It yielded the iodoform reaction with sodium hydroxide and iodine and also dissolved silver oxide, yielding a solution which rapidly darkened on exposure to light. Extraction with amyl acetate instead of ethyl acetate yielded an oil similar in properties to that described above. An acetone extraction followed by a salting out with calcium chloride gave a solution which contained some mineral matter. On evaporation to dryness followed by a further extraction with acetone or ether a light coloured oil was obtained. By acidifying the barium salt with sulphuric acid, absorbing the mixture in anhydrous sodium sulphate, powdering and extracting with ether in a Soxhlet, a brown ethereal extract was obtained which had an odour similar to butyric acid. After removing most of the volatile fatty acids, the residue was tested for β-hydroxybutyric acid according to the method of Black [1908] using hydrogen peroxide and ferric chloride, and also by oxidising it with dichromate in presence of a mercuric salt. Neither method gave any positive result. These experiments would indicate the absence of β-hydroxybutyric acid.

In order to identify the volatile acids present, the barium salt dissolved in water was treated with the calculated amount of $N/1$ sulphuric acid to precipitate the barium, filtered from barium sulphate, and distilled according to Duclaux's method:

Vol. of distillate c.c.	$N/10$ KOH cc.	Percentage	Calculated for 1 mol. acetic acid: 1 mol. butyric acid
30	19·75	35·0	35·2
40	25·57	45·3	46·0
50	31·17	55·3	55·7
60	36·35	64·4	65·0
70	41·45	73·5	73·7
80	46·33	82·1	82·2
100	56·43	100·0	—

The results of the Duclaux estimation would appear to confirm our original observations that acetic and butyric acids were the only volatile acids present. The ratio of acetic acid to butyric was also determined by Dyer's method [1917]. About 1 gram of the salt dissolved in water was treated with a slight excess of $N/1$ sulphuric acid, the volume made up to 150 cc. and steam distilled.

The first 100 cc. of distillate required 29·52 cc. $N/10$ alkali for neutralisation and the second 100 cc. 14·9 cc. The distilling constant is 49·5.

Dyer's figures give 1 mol. acetic acid, 0·94 mol. butyric acid.

Stein's figures [1913] give 1 mol. acetic acid, 0·78 mol. butyric acid.

10 g. of the finely powdered barium salt were treated with a slight excess of phosphoric acid and distilled. The distillate was dried over phosphorus pentoxide and again distilled. It was collected in two fractions – 130° and 130–160°. Each fraction was then submitted to a Duclaux distillation.

Fraction – 130°			Fraction 130—160°		
Vol. of distillate cc.	$N/10$ KOH cc.	Percentage	Vol. of distillate cc.	$N/10$ KOH cc.	Percentage
30	4·75	34·4	30	19·95	38·1
40	6·18	44·8	40	25·45	48·5
50	7·53	54·6	50	31·15	59·4
60	8·90	64·5	60	35·85	68·3
70	10·05	72·8	70	40·55	77·3
80	11·25	81·5	80	44·75	85·3
100	13·80	100·0	100	52·47	100·0
1 mol. of acetic acid to 0·83 mol. of butyric acid.			1 mol. of acetic acid to 1·8 mols. of butyric acid.		

Thus by distilling the volatile acids and by collecting the distillate in two fractions the ratio in which the acids are present has been altered, and no appreciable amount of a third volatile acid was shown to be present.

Fermentation in presence of Calcium Carbonate.

For further examination of the acid produced, the fermentation was allowed to proceed in presence of calcium carbonate. A quantity of 2 litres of 5 % maize mash, sterilised at 30 lb. pressure for 2 hours, was inoculated with 2 % acetone inoculant. A slight excess of calcium carbonate was added, and the acidity was prevented from developing by the frequent addition of calcium carbonate, during the fermentation. Throughout the fermentation, the organism, examined microscopically, appeared normal. After 7 days' fermentation the suspended matter was removed, and the filtrate reserved for the examination of the calcium salts. 300 cc. of the filtrate were distilled, and the first 50 cc. of distillate shaken with an excess of anhydrous potassium carbonate. On "salting out," about 2 cc. of an oil were obtained. It had an odour similar to *n*-butyl alcohol and the oil also gave the iodoform test, when both sodium carbonate and ammonia were used.

For a further examination of its properties a fresh amount of calcium salt was made: 2 litres of 5 % maize mash were prepared as in the previous case. To this was added 40 cc. of inoculant and an excess of calcium carbonate. Probably the excess of calcium carbonate was too great, since the fermentation was not very satisfactory until a further amount of 40 cc. of acetone inoculant had been added. The fermentation was allowed to proceed for 7 days, and the liquid neutralised periodically by the addition of small amounts of calcium carbonate. The soluble calcium salts were filtered off, the insoluble portion washed with water and the washings added to the above solution. On evaporating to dryness (on the water-bath) the solution containing the calcium salts, 45 g. of solid were obtained (containing $H_2O = 7{\cdot}6\%$, $Ca = 17{\cdot}7\ \%$). The amount of sugar present was very small.

10 g. were distilled with an excess of glacial phosphoric acid—there was some charring and frothing. The distillate was strongly acid and was dried with calcium chloride and finally with phosphorus pentoxide. On redistillation it gave a fraction at 115–140° smelling strongly of acetic acid, whilst at 140–160° a second fraction was obtained, having an odour similar to butyric acid. From these two fractions the barium salts were prepared by neutralising with barium carbonate, filtering, and evaporating to dryness. The lower boiling fraction yielded a barium salt which contained $Ba = 47{\cdot}95\ \%$. On extracting the anhydrous salt several times with absolute alcohol to remove higher fatty acids if present, the barium content was increased to 51·57 %. By recrystallising the barium salt of the higher boiling fraction from water a salt was obtained containing 45·99 % Ba.

The results obtained by the fractional crystallisation of the barium salts indicate the presence of butyric and acetic acids. This was confirmed by a

Duclaux estimation. A weighed amount of the original calcium salt was dissolved in water, decomposed by the calculated amount of sulphuric acid and distilled:

Vol. of distillate cc.	N/10 KOH cc.	Percentage	Ratio, acetic acid : butyric acid
20	11·12	26·3	1 : 1·7
30	16·12	38·2	1 : 1·7
40	20·90	49·5	1 : 1·8
60	28·70	68·0	1 : 1·8
70	32·85	77·8	1 : 1·9
100	42·20	100·0	—
		Average ...	1 : 1·8

Ratio, acetic acid : butyric acid ... 1 : 1·8 mols.
1 : 2·6 by weight.

The ratio was also determined from the constants given by Dyer and by Stein [1913]. For this purpose two determinations were made. From the mean of these 58·3 % of the total acid distilled over in the first 100 cc. of distillate, giving from Dyer's constants, 1 mol. acetic acid : 2·4 mols. butyric acid; from Stein's constants, 1 mol. acetic acid : 2·0 mols. butyric acid.

In order to check these methods, 1 g. of calcium salt, after acidifying with $N H_2SO_4$, was steam distilled, the distillate being neutralised with a saturated solution of barium hydroxide. When all the volatile acid had been driven over, the neutralised distillate was evaporated to a small bulk. A stream of carbon dioxide was passed through the solution to remove any slight excess of barium hydroxide: the insoluble barium carbonate was removed by filtration after boiling, the filtrate evaporated to dryness and the residue heated in an oven until constant in weight. 0·1500 g. gave 0·1202 g. $BaSO_4$; Ba = 47·20 %.

This figure corresponds with the presence of acetic acid and butyric acid in the ratio 1 mol. acetic acid to 1·75 mols. of butyric acid.

Summary.

Method	Dyer	Stein	Duclaux	Ba salt
Ratio, acetic acid : butyric acid, mols.	1 : 2·4	1 : 2·0	1 : 1·8	1 : 1·8
Ratio, acetic acid : butyric acid, by weight ...	1 : 3·5	1 : 2·9	1 : 2·6	1 : 2·6

Disregarding the Dyer determinations it would seem as if the proportion in which acetic and butyric acids were present is approximately 1 : 1·8 (mol.) or 1 : 2·6 (weight). It should be pointed out that the above workers carried out their investigations on prepared mixtures of the pure acids. In our investigation, the mash liquor and a non-volatile acid have probably some effects on the distillation figures and the results may not be so uniform. In some other experiments higher proportions of acetic acid were obtained. Evidence was also obtained of the presence of a third acid only slightly volatile in steam.

1 g. of the calcium salt was dissolved in a small amount of water, and a solution of 4 g. of quinine sulphate dissolved in absolute alcohol added. After heating for a short time to 100° the mixture was allowed to stand for a few days. The precipitate was removed by filtration and the filtrate evaporated to dryness in vacuo and finally extracted with chloroform.

Although the quinine salts have not yet been examined in detail, a preliminary investigation points to the presence of another acid in addition to acetic and butyric acids. Some other evidence on this point is given later.

Nature of the Acids present during the course of a Normal Fermentation.

In order to investigate the nature of these acids samples were withdrawn periodically from a fermentation and a Duclaux estimation was carried out directly on the mash after determining the total acidity. This method was adopted as it was imperative to carry out all the determinations on the same fermentation, and it appeared to yield the most concordant results, while the time required to carry out a determination was short. The results are comparable among themselves, since the influence of substances in the solution is approximately constant. The suspension of carbohydrate material was allowed to settle, as far as possible, and the supernatant liquid decanted through purified glass-wool to remove any further amount of suspended matter. This investigation was only undertaken in the case of one or two fermentations and the results obtained may not be general. The following figures are therefore of the nature of a preliminary investigation.

In order to test the accuracy of the results obtained by direct distillation of the mash two experiments were carried out with the same sample of mash as follows:

A Duclaux estimation was carried out on the mash.

Acidity of mash, 5·1 cc. N/10[1].

Duclaux estimation				
Vol. of distillate cc.	N/10 KOH cc.	Percentage		
10	4·90	12·6	1·0	
20	9·48	24·1	0·9	Ratio acetic : butyric 1 : 1·0
30	13·93	35·7	1·1	
40	18·03	46·3	1·1	
50	21·86	56·1	1·1	
60	25·41	65·2	1·0	
80	31·90	81·9	0·9	
100	38·96	—	—	

Another portion was distilled in steam at about 60° under diminished pressure. The distillate was neutralised and concentrated; the calculated amount of sulphuric acid added, and the ratio of butyric and acetic acids determined by distillation at constant volume by a method which has been recently described by Reilly and Hickinbottom [1919].

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[1] The values recorded for the acidity of the mash refer to the number of cc. of N/10 NaOH required to neutralise 10 cc. of mash.

200 cc. of mash on steam distillation gave a distillate requiring 89·0 cc. $N/10$ $Ba(OH)_2$ for neutralisation. The volatile acid is 87 % of the total acidity.

Estimation of ratio of acetic and butyric acids by new method.

Vol. of distillate cc.	Percentage of total acid distilled	Ratio acetic acid	Ratio butyric acid
20	16·5	1	1·14
30	23·3	1	1·15
40	29·2	1	1·10
50	35·1	1	1·14
60	40·1	1	1·13
70	45·7	1	1·13
80	48·7	1	1·12
90	52·4	1	1·13
100	55·6	1	1·10
		Av. 1	1·12

The agreement between the two series is good. The difference is not more than that due to experimental error in determining the ratio by the Duclaux method.

The estimation on the mash was checked by distilling 1 litre to a small bulk, when it was diluted with distilled water, and again distilled. The distillate so obtained was neutralised with normal sodium hydroxide solution and evaporated to a small bulk. The volatile acids in the neutralised concentrated liquor were estimated by Dyer's method and the results calculated, using both Stein's and Dyer's figures.

During the removal of the volatile fatty acids from the mash, it is extremely difficult to distil all the acetic acid over without carbonising the residue and consequently the results from the Dyer estimations may be somewhat too low for acetic acid.

Sample I ... ... 7.30 p.m.
Acidity ... ... 0·33 cc. $N/10$
Sample II... ... 9.30 p.m.
Acidity ... ... 0·28 cc. $N/10$
Sample III ... 11.30 p.m.
Acidity ... ... 0·32 cc. $N/10$

The composition of the mixture of acids present in these three samples was not estimated

Sample IV ... 1.30 p.m.
Acidity ... ... 0·51 cc. $N/10$

Duclaux estimation

Vol. of distillate cc.	$N/10$ KOH cc.	Percentage	Calc. for 1 mol. acetic acid : 0·33 mol. butyric acid
30	1·51	29·7	29·4
40	1·98	39·0	39·0
50	2·47	48·6	48·8
60	2·95	58·1	57·7
80	3·85	75·8	77·0
100	5·08	100·0	—

Sample V ... 3.30 a.m.
Acidity... .... 0·77 cc. *N*/10

Duclaux estimation

Vol. of distillate cc.	N/10 KOH cc.	Percentage	Calc. for acetic acid 1 mol. : butyric acid 0·5 mol.
30	1·85	31·8	31·4
40	2·42	41·6	41·3
50	2·97	51·0	50·8
60	3·51	60·3	60·2
70	4·10	70·5	69·4
80	4·62	79·4	78·8
100	5·82	100·0	—

Sample VI ... 5.30 a.m.
Acidity... ... 1·20 cc. *N*/10

Duclaux estimation

			Ratio, acetic acid : butyric acid
30	3·15	33·7	1 : 0·71
40	4·15	44·4	1 : 0·71
50	5·09	54·5	1 : 0·84
60	5·94	63·6	1 : 0·77
70	6·89	73·8	1 : 1·0
80	7·67	82·1	1 : 1·0
100	9·34	100·0	—

The average proportion is 1 mol. of acetic acid : 0·83 mol. of butyric acid

Sample VII ... 7.30 a.m.
Acidity... ... 1·63 cc. *N*/10

Duclaux estimation

			Percentage calc. for 1 mol. acetic acid : 1 mol. butyric acid
30	4·58	34·6	35·2
40	6·01	45·5	46·0
50	7·31	55·3	55·7
60	8·60	65·1	65·0
70	9·80	74·1	73·7
80	11·01	83·3	82·2
100	13·22	100·0	—

Sample VIII ... 11.30 a.m.
Acidity... ... 2·55 cc. *N*/10

Duclaux estimation

			Ratio, acetic acid : butyric acid
30	7·53	35·7	1 : 1·1
50	11·93	56·5	1 : 1·2
60	13·89	65·8	1 : 1·2
70	15·78	74·8	1 : 1·2
80	17·55	83·1	1 : 1·3
100	21·10	100·0	—

1 mol. of acetic acid to 1·2 mols. of butyric acid

Sample IX ... 1.40 p.m.
Acidity... ... 2·80 cc. *N*/10

Duclaux estimation

Vol. of distillate cc.	*N*/10 KOH cc.	Percentage	Ratio, acetic acid : butyric acid	
30	8·78	36·0	1 : 1·2	1 mol. of acetic acid to 1·30 mols. of butyric acid
40	11·60	47·6	1 : 1·3	
50	14·00	57·4	1 : 1·3	
60	16·30	66·8	1 : 1·3	
70	18·45	75·6	1 : 1·4	
80	20·50	84·0	1 : 1·5	
100	24·40	100·0	—	

A Dyer estimation of the distillate from 1000 cc. required
27·8 cc. *N*/10 KOH to neutralise acid in 1st 100 cc.
12·45 „ „ „ 2nd „
These figures give a distilling constant of 55·2 which from
Dyer's figures give a ratio of 1 mol. of acetic acid : 1·7 mols. of butyric acid.
Stein's „ „ „ „ „ „ 1·4 „ „ „

Sample X ... 3.30 p.m.
Acidity... ... 3·25 cc. *N*/10

Duclaux estimation

			Ratio, acetic acid : butyric acid	
30	9·68	36·1	1 : 1·2	1 mol. of acetic acid to 1·4 mols. of butyric acid
40	12·78	47·6	1 : 1·3	
50	15·65	58·3	1 : 1·3	
60	18·10	67·4	1 : 1·5	
70	20·40	76·0	1 : 1·5	
80	22·61	84·2	1 : 1·6	
100	26·85	100·0	—	

A Dyer estimation on the distillate from 1000 cc. required
52·6 cc. *N*/10 KOH to neutralise the acid in 1st 100 cc.
23·8 „ „ „ „ 2nd „
Distilling constant = 54·8.

Dyer's figures give a ratio of 1 mol. of acetic acid : 1·6 mols. of butyric acid.
Stein's „ „ „ „ „ „ 1·4 „ „ „

Sample XI ... 6.0 p.m.
Acidity... ... 3·35 cc. *N*/10

Duclaux estimation

			Ratio, acetic acid : butyric acid	
30	10·10	35·9	1 : 1·2	1 mol. of acetic acid to 1·3 mols. of butyric acid
40	13·20	47·0	1 : 1·2	
50	16·00	56·9	1 : 1·3	
60	18·72	66·6	1 : 1·3	
70	21·30	75·9	1 : 1·5	
80	23·60	84·0	1 : 1·5	
100	28·10	100·0	—	

A Dyer estimation on the distillate from 1000 cc. required
33·0 cc. *N*/10 alkali to neutralise acid in 1st 100 cc.
14·0 „ „ „ „ 2nd „
Distilling constant = 57·6.

Dyer's figures give a ratio of 1 mol. of acetic acid : 2·1 mols. of butyric acid.
Stein's „ „ „ 1 „ „ 1·9 „ „

Sample XII ... 1.0 p.m.
Acidity... ... 1·4 cc. $N/10$

Duclaux estimation

Vol. of distillate cc.	$N/10$ KOH cc.	Percentage	
30	2·43	27·3	1 mol. of acetic acid to 0·22 mol. of butyric acid
40	3·35	37·6	
50	4·15	46·6	
60	4·98	56·0	
70	5·80	65·1	
80	6·75	75·8	
100	8·90	100·0	

A Dyer estimation on the distillate from 1000 cc. required
11·1 cc. $N/10$ alkali to neutralise acid in 1st 100 cc.
6·4 „ „ „ „ 2nd „
Distilling constant = 42·4.

Dyer's figures give the ratio 1 mol. of acetic acid : 0·40 mol. of butyric acid
Stein's „ „ „ 1 „ „ 0·33 „ „ „

Sample XIII ... 3.0 p.m.
Acidity ... ... 1·35 cc. $N/10$

Duclaux estimation

30	2·52	28·0	1 mol. of acetic acid to 0·2 mol. of butyric acid
40	3·45	38·3	
50	4·09	45·4	
60	4·85	53·9	
70	5·85	65·0	
80	6·71	74·5	
100	9·00	100·0	

Summary of Results.

		Ratio acetic acid : butyric acid as determined by method of			
Age of fermenting mash in hours	Acidity*	Duclaux	Dyer	Duclaux	Stein
		Determined in the mash	Determined in the distillate from the mash		
2	0·33	—	—	—	—
4	0·28	—	—	—	—
6	0·32	—	—	—	—
8	0·51	1 : 0·33	—	—	—
10	0·77	1 : 0·5	—	—	—
12	1·20	1 : 0·83	—	—	—
14	1·63	1 : 1	—	—	—
18	2·55	1 : 1·2	—	—	—
20	2·80	1 : 1·3	1 : 1·7	—	1 : 1·4
22	3·25	1 : 1·4	1 : 1·6	—	1 : 1·4
24½	3·35	1 : 1·3	1 : 2·1	—	1 : 1·9
43½	1·4	1 : 0·22	1 : 0·43	(1 : 0·4)	1 : 0·33
45½	1·35	1 : 0·20	—	—	—

* Expressed in cc. of $N/10$ alkali required to neutralise 10 cc. of mash.

To test the view that the production of "oil" and the diminution of butyric acid were connected, the amount of "oil" produced was determined on samples taken during the progress of a fermentation. The ratios in which the volatile acids were present in the same sample were also determined.

Time	Acidity	Ratio determined by Duclaux estimation, acetic acid : butyric acid	Volume of oil from one litre of mash
9.0 p.m.	1·0	1 : 0·5	None
11.0 ,,	1·5	1 : 0·62	,,
12.15 a.m.	2·0	1 : 0·9	Trace
5.30 ,,	3·7	1 : 1·25	1 cc.
3.15 p.m.	2·0	1 : 0·28	4 ,,
4.30 ,,	1·6	1 : 0·25	5 ,,

These results indicate that traces of acetone and *n*-butyl alcohol are produced before the maximum acidity has been attained. The production of "oil" in the fermentation using calcium carbonate appears to confirm this result.

As far as can be judged from the limited number of experiments carried out, these ratios indicate that while the acidity of the mash is increasing previous to the production of *n*-butyl alcohol and acetone, the production of butyric acid proceeds at a faster rate than that of acetic acid. In the particular fermentation examined the maximum amount of butyric acid appears to be present at the time of maximum acidity, and when the production of acetone and *n*-butyl alcohol takes place, the amount of butyric acid diminishes. It would seem that the production of butyric and acetic acids, of *n*-butyl alcohol, and of acetone is intimately connected.

In the presence of calcium carbonate fermentation apparently proceeds as far as the point corresponding to the maximum acidity in a normal fermentation.

Evidence of the presence of a Third Acid only slightly volatile in Steam.

Indications have been obtained of the presence of another acid in the mash; it has not been isolated. In determining the ratio of butyric acid and acetic acid by Duclaux's method, it was observed that the amount of acid present in the original mash, calculated from Duclaux's table, did not agree with the total acid present, as estimated by titration.

The following tables and Fig. 2 show the percentage of the total acidity which is accounted for by butyric and acetic acids:

Acidity of the mash	Acetic and butyric acids, percentage of total acidity
1·0	105 (carbon dioxide present in addition to fatty acids)
1·5	—
2·0	90
3·7	86
2·0	70
1·6	74

Another mash gave the following results:

Age of fermenting mash in hours	Acidity of the mash	Acetic and butyric acids, percentage of total acidity
8	0·51	107 (carbon dioxide present)
10	0·77	80
12	1·20	80
14	1·63	83
18	2·55	84
20	2·80	89
22	3·25	83
24½	3·35	88
43½	1·4	70
45½	1·35	73

This agreement between two series points to the existence of an acid not readily volatile in steam which increases in proportion to the volatile acids after the maximum acidity has been reached. The calcium salt obtained by fermenting in presence of chalk, on distillation by the Dyer method, yields only about 53–55 % of its weight as volatile acid. The moisture and calcium oxide only account for about 30–35 % of the salt. The amount of sugar present in the crude salt was extremely small. There was, therefore, about 10–15 % not accounted for.

The quinine salt prepared from the calcium salt, on hydrolysis with baryta, gives a mixture of barium salts which points to the existence of another acid with an equivalent equal to or greater than that of butyric acid.

Addition of Acetic Acid to the Fermenting Mash. [See Desborough etc., 1918.]

105 lb. of maize meal were used to prepare 168 gallons of mash: the mash was inoculated with half a gallon of fermenting mash. Total volume of mash 168·5 gallons.

The acetic acid was added to the mash in the concentrated form, but dilute acid may equally well be used. During the addition of the acid the mash was vigorously stirred and the stirring was continued for 15 minutes after the acid had been added. The first lot of 500 cc. of acid was added 24 hours after inoculation, as soon as the normal acidity had passed its maximum and was beginning to fall.

Subsequent additions of 500 cc. of acid were made as soon as the acidity of the mash had fallen to the same point at which the first lot of acid was added. In all, seven lots of 500 cc. of acid were added, making a total of 3500 cc. of acid added.

Specific gravity of 97 % acetic acid used, 1·0625.

Total weight added, 3718·7 g.; so that 3607·1 g. of acetic acid were added.

The total acetone produced at the end of the fermentation was 27·27 g. per gallon. The volume of fermented mash at the end of the fermentation amounted to 163 gallons, as 4 gallons were taken out for a control immediately after inoculation and 1·5 gallons were drawn off at intervals for estimating the acidity.

The total acetone produced was therefore 163 × 27·27 = 4445·01 g.

The acetone produced in the control to which acetic acid was not added amounted to 19·54 g. per gallon. The total acetone produced in 163 gallons of mash to which acetic acid was not added would be 163 × 19·54 g. = 3185·02 g.

Hence the acetone produced from the acetic acid = 4445·01 − 3185·02 = 1260 g.

Assuming that two molecules of acetic acid produce one molecule of acetone, the 3607 g. of acetic acid added should give 1743·4 g. of acetone. The amount actually found was 1260 g. or 72·2 % of the theoretical amount.

The yield would probably have been better had less acetic acid been added, as apparently more acetic acid was added to the mash than could be converted.

The acidity of the mash at the end of the fermentation remained abnormally high, 10 cc. of mash requiring 3·0 cc. $N/10$ alkali for neutralisation, instead of the normal amount of 1·9 cc.

In another experiment made about the same time, a similar result was obtained. In this case also, too much acetic acid was added, the yield of acetone was 79·9 % of the theoretical amount that should have been obtained from the total acetic acid added.

There were no indications of infection by lactic acid producing organisms in either of these fermentations. The volume of gas evolved was slightly above the normal.

A large scale experiment was carried out in which over a ton of acetic acid was added to 86,000 gallons of fermenting mash.

Unfortunately the mash became infected by lactic acid producing organisms before the fermentation was completed. In spite of this the weight of acetone produced was much above the normal amount, the excess of acetone obtained, over the normal yield from the grain, showing a yield of 61·27 % of the theoretical from the acetic acid added.

Examination of an Infected Mash.

A sample was taken from a fermentation in which the acidity had become high. The sample contained "oil" and had an acidity = 6·3 cc. $N/10$ NaOH. Part of the acidity was due to the presence of volatile acids, which were estimated by the Duclaux method.

Vol. of distillate cc.	$N/10$ KOH cc.	Percentage
30	9·20	28·9
40	12·20	38·4
50	15·30	48·2
60	18·20	57·3
70	21·35	67·3
80	24·50	77·5
100	31·75	100·0

The distillation figures agree with a mixture of butyric and acetic acids in the ratio of approximately 3 mols. of acetic acid to 1 mol. of butyric acid

From this estimation approximately 55 % of the total acidity is accounted for as acetic and butyric acids. For the examination of the non-volatile acid, about 3 litres of the mash were evaporated down to a very small bulk. The residue, which was very dark in colour and sticky when cold, was mixed with fine silica while still hot, and finally made into a hard mass by stirring in finely-powdered anhydrous sodium sulphate. The mass was then broken up and extracted with ether in a Soxhlet extraction apparatus. A brown syrupy extract was obtained, which had an acid reaction. Oxidation by means of dilute potassium permanganate solution in presence of sulphuric acid yielded acetaldehyde. The brown liquid gave a *zinc* salt containing Zn = 26·6 %. (Zinc lactate requires Zn = 26·9 %.)

Another portion gave a *barium* salt which did not crystallise. The *calcium* salt crystallised in small nodules. A sample of the calcium salt after 3 crystallisations showed a slight laevo-rotation $[\alpha]_D = -0·73°$; after 8 crystallisations $[\alpha]_D = -1·34°$. The *copper* salt crystallised in long blue prisms.

By distilling the acid under moderately diminished pressure a solid distillate was obtained having a constant m.p. of 125° after 3 crystallisations from chloroform (lactide from lactic acid, m.p. 125°). A mixture with a specimen of pure lactide made from lactic acid melted at the same temperature. The acid cooled in ice was oxidised by the action of dilute hydrogen peroxide solution, in presence of a trace of ferrous acetate, in the manner described by Fenton and Jones [1900]. On the addition of a few drops of phenylhydrazine acetate solution, a red precipitate was produced which after washing well with water and small amounts of ether melted at 177–178° with decomposition. The melting point was not depressed by the addition of some pure pyruvic phenylhydrazone. The acid is therefore lactic acid.

A high acidity mash contains lactic, acetic and butyric acids.

SUMMARY.

In the fermented mash, acetic and butyric acids are present in varying proportions. During the progress of the fermentation the ratio of butyric acid to acetic acid increases with the increase in the acidity of the mash, reaching a maximum when the acidity is at its highest. With the production of "oil" the ratio of butyric acid to acetic acid diminishes, until the mash contains an excess of acetic acid. If the acidity is neutralised by the addition of calcium carbonate, the calcium salts of the acids present in the mash replace the acetone and *n*-butyl alcohol, and these latter are produced in almost negligible amount. In the presence of calcium carbonate, however, the fermentation proceeds as far as the point corresponding to the maximum acidity in a normal fermentation. It is extremely probable that acetic and butyric acids are not the only acids present. The type of infection most frequently observed in the fermenting mash produces lactic acid. When the mash is

infected in this way the amount of volatile acid accounted for by the Duclaux estimation is lower than in the case of a normal fermentation.

When acetic acid is added to the fermenting mash an increased yield of acetone is obtained; the yield of *n*-butyl alcohol being unchanged. Propionic and butyric acids when added appear to be converted into the corresponding alcohols. An increased yield of acetone has been obtained by the addition of aceto-acetic ester.

In conclusion the authors take this opportunity of expressing their thanks to Capt. Desborough, R.A., Superintendent, and Mr W. T. Thomson, Manager of the Royal Naval Cordite Factory, Dorset, both for the interest they have taken in this investigation, and for granting permission to publish the results obtained. In addition we are indebted to Dr A. Harden for suggestions which may help to explain the mechanism of the reactions taking place.

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