CXLIII. THE SELECTIVE FERMENTATION OF GLUCOSE AND FRUCTOSE BY BREWER'S YEAST.

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THE literature dealing with the subject of the selective fermentation of mixtures of glucose and fructose by living yeast contains many apparently contradictory and confusing statements. A summary of the earlier work is given by Lippmann [1904]. The description "fermentation élective" was used by Dubrunfaut and this title has frequently been used by subsequent workers. It is well established by the work of Slator [1908] that living yeast of various species and in various conditions of activity ferments glucose and fructose separately at approximately the same rate. The combined rates of fermentation of the two sugars when mixed is also this same rate, but the relative rates of disappearance of the respective sugars from a mixture of the two undergoing fermentation varies very widely with the species of yeast used and with other conditions. Thus, Sauterne yeasts are stated to ferment fructose faster than glucose, whereas with many yeasts, including *S. cerevisiae*, the reverse phenomenon has been observed. Most of the experimental evidence upon which these statements are founded has been the specific rotation of the mixed sugars remaining in the partly fermented solution. However, Schiller [1925] used the iodimetric method of Willstätter and Schudel as modified by Judd [1920] and by Baker and Hulton [1920] and obtained evidence of selectivity in various yeasts.

It was considered that it was of primary importance to confirm these phenomena of selective fermentation by methods other than those based upon specific rotation, and to consider any alternative explanations. It was shown by Neuberg and Kobel [1926] that when fructose is mixed with certain amino-acids an immediate change in rotation occurs, in the negative direction. Whilst such changes as were recorded are of an order quite inadequate to provide a simple explanation of the specific rotations of partly fermented solutions of invert sugar, they justify a careful reconsideration of the latter results. The possibility also arises that the nitrogen compounds excreted by the yeast during fermentation may possess an independent and appreciable specific rotation. Even when the yeast is vigorously reproducing, some excretion occurs, if only from old and dead cells which are inevitably present.

Unfortunately these substances absorb iodine and thus interfere with the iodimetric determinations of glucose, and their complete removal by precipitating reagents is not easy.

Solutions of (a) glucose, (b) fructose and (c) an equal mixture of the two sugars were fermented under exactly similar conditions with brewery (top) yeast (Exp. 1) and it was found that, throughout the fermentation:

(1) the specific rotation of the unfermented sugar in

(a) remained approximately $+52^\circ$;

(b) remained approximately -92° ;

(c) fell steadily from -19.6° to -50° ;

(2) in each of the three solutions the percentage of glucose obtained by iodimetric titration was in fair agreement with that obtained from rotation and reducing power, but exceeded it by a similar small amount in all cases. The fact that the glucose results obtained iodimetrically exceeded those obtained polarimetrically was due to the presence of excreted nitrogen compounds which could not be completely removed by such agents as normal lead acetate, phosphotungstic acid and animal charcoal. Since the three fermentations were performed under similar conditions, it may be presumed that the yeast excretions were equivalent in all three at the corresponding stages of the fermentations.

The above results were obtained at four stages of each fermentation. Multiplication of yeast occurred in these fermentations since malt culm extract was added as nutrient, but exactly similar results were obtained in three other fermentations, in one of which yeast water, in another synthetic nutrients, were employed and in the other no nutrients were added. In the last case more yeast autolysis and excretions might be expected, but the general tenor of the results was not affected. For evident reasons the iodimetric method could not be employed as a check where synthetic nutrients, which included ammonium salts, were used.

From these experiments it seems fairly evident that, under the respective conditions adopted, this brewery yeast removes glucose more rapidly than fructose from a solution containing equal quantities of the two. Similar results were also obtained using Burton brewery yeast (more attenuative) and Lager (bottom) yeast.

In some of the investigations the percentage of fructose present was confirmed by measuring the rotation at 20° and 82° respectively, and the results obtained were in fair agreement with the others.

In explanation of the phenomena so far described one needs to reconcile three apparently contradictory facts:

(1) that glucose and fructose in separate solutions are fermented at approximately equal rates by living yeast;

(2) that glucose and fructose in mixtures are fermented at unequal rates, glucose being fermented the faster by most yeasts;

(3) that fructose alone is specifically esterified at least as fast as glucose by yeast juice or zymine, and more rapidly than glucose in the presence of suitable concentrations of added phosphates [Harden and Young, 1909].

The work of Slator [1908] and Slator and Sand [1910] indicates that the rate of fermentation of sugars by living yeast depends upon factors operating within the cell, the rate of diffusion inwards being, except in very dilute solutions, such as to supply sugar faster than it can be dealt with inside. In view of this and of the fact that fermentation within living cells occurs at rates higher than those obtainable with corresponding quantities of non-living yeast preparations, even when phosphates are added, one would expect fructose to be fermented faster than glucose. This is not the case, and, as far as separate fermentations are concerned, the simplest explanation is that one reaction ultimately controls the rate of fermentation in all these cases. This reaction is, according to the theory of Harden and Young [1908], a splitting of hexosediphosphate by the enzyme hexosephosphatase, thereby releasing free phosphate to be used in esterifying further sugar. The constancy of the rate of fermentation over an appreciable period of time points to this being a typical enzyme action, the concentration of both the enzyme (hexosephosphatase) and of its substrate (hexosediphosphate) remaining approximately constant. It is the existence of this cycle of changes which maintains the uniform velocity of the reaction. The hexosediphosphate concentration is limited by the phosphorus content of the cell. In such circumstances a relatively rapid esterification of fructose as compared with that of glucose will be of no appreciable significance when the rates of fermentation of the separate hexoses are under consideration.

An explanation of the selective fermentation of glucose and fructose involves many considerations. The relative rates of diffusion of these two sugars through the cell membrane have been held by some writers to account for the phenomenon, but diverse opinions have been expressed. Slator [1908] summed up by saying, "It is improbable that the somewhat different rates of diffusion of dextrose and laevulose into the cell play any important part in the determining whether dextrose or laevulose disappears faster from the solution." In addition to the evidence available at that time we have the observation that the zymine prepared from brewery yeast exhibits the same phenomenon as the yeast itself in this respect [Fernbach, Schoen and Mori, 1927]. (See also Exp. 4.) Lastly, if the specific rates of diffusion were the only consideration we should have to postulate that glucose diffuses two and a half times as fast as fructose through the cell membrane of brewery yeast in certain cases, whilst fructose diffuses from two to three times as fast as glucose through the cell membrane of (*e.g.*) Sauterne yeasts. It is very much more unlikely that cell membranes should so differ, than that, for example, the relative activities of the various enzymes within the cells should differ.

It was suggested by Slator [1911] that when glucose alone is fermented by living yeast, fructose may be excreted from the cell and found in the partly

fermented solution. Attempts to obtain evidence of this under various conditions were unsuccessful (Exps. 1 to 3). To test for fructose, the specific rotation, the change of rotation of fructose with change of temperature, the colour reaction of fructose with orcinol and phosphoric acid [Löwe, 1916] were used. From this it appears that the excess of fructose found in partly fermented solutions of invert sugar does not owe its presence to excretions of fructose from within the cell.

Further experiments showed that the selective fermentation by top and bottom brewery yeasts is approximately independent of:

- (a) concentration of total sugars between the limits 3.6 % and 19 %;
- (b) temperature between the limits 12° and 26°;
- (c) reaction of medium between the limits of p_H 4.0 and 7.5, whether nutrient and buffering substances are present or not;
- (d) the fermentative power of the yeast as modified by the action of heat.

The relative rates of disappearance of the two sugars change as fermentation proceeds, since the proportion of the sugars in the medium is constantly changing. The ratio of the two specific rates is however more constant, and was calculated by means of the formula

$$K = \frac{\ln a_g - \ln(a_g - x_g)}{\ln a_f - \ln(a_f - x_f)},$$

where

a_g = initial concentration of glucose,

a_f = initial concentration of fructose,

$(a_g - x_g)$ = concentration of glucose at time t ,

$(a_f - x_f)$ = concentration of fructose at time t .

This formula was obtained by the following considerations. The formula for the velocity constant of a monomolecular reaction $k = \frac{1}{t} \ln \frac{a}{a-x}$ has often been misapplied to fermentations of single sugars by living yeast, but the formula is applicable relatively to the individual sugars in selective fermentations. Whilst the combined rate of fermentation of the two sugars is uniform for a considerable period if yeast concentration remains constant, for each individual sugar the rate of fermentation at any instant will be proportional to its concentration at that instant and the law will hold. If k_g , a_g and x_g represent the values of k , a and x respectively for glucose at time t , and k_f , a_f , x_f those of fructose, then

$$\frac{k_g}{k_f} = \frac{\frac{1}{t} \ln \frac{a_g}{a_g - x_g}}{\frac{1}{t} \ln \frac{a_f}{a_f - x_f}} = \frac{\ln a_g - \ln(a_g - x_g)}{\ln a_f - \ln(a_f - x_f)} = K,$$

which might be regarded as the "selectivity constant." This value, however, appears to increase somewhat during fermentation except in the very late stages when it approaches unity.

Using solutions containing approximately equal proportions of glucose and fructose, and a top brewery yeast, the following values of K were obtained:

18.0 % sugar (initially)	1.78	} at 25°;
9.5 % ,,	1.81	
3.6 % ,,	1.79	
3.6 % ,,	1.88	at 12°;
3.6 % ,,	1.76	at 25° and at p_H 7.0 throughout;
3.6 % ,,	1.68	at 25° using yeast, 60 % of the fermentative power of which had been destroyed by heat;
3.6 % ,,	1.80	at 25° using yeast which had been grown in a medium of fructose and nutrients.

The value of this constant does not appear to vary much beyond the limits of experimental error under the conditions. Perhaps selectivity is rather greater at low temperatures, a result obtained by Bourquelot [1885], and less in yeast of which the activity has been reduced by heat. Far greater differences exist between yeasts of different species, and yeasts under different conditions of nutrition. Whilst the Edinburgh brewery top yeast above gives the value $K = 1.8$ under normal conditions, a bottom yeast gave $K = 2.1$ at 20° under similar conditions, and the top yeast, when growing in synthetic nutrients rich in phosphates (see Exp. 3), gave $K = 2.8$ at 20°. From the results obtained by Schiller [1925] calculation shows K to be 0.5 or less for a Sauterne yeast, in which case the fructose disappears from the medium faster than the glucose.

The reaction of the medium was found by Schiller to have a definite influence, the fructose being fermented relatively faster in more alkaline media. This was not found to be the case with the two types of brewery yeast referred to above. Schiller's work seems to be open to criticism however. He fermented mixtures of glucose and fructose with various yeasts, using malt culm extract as nutrient and measuring glucose at intervals by the iodimetric method. The fructose was estimated by deducting the quantity of glucose so obtained from the total reducing sugar as measured by Bertrand's method. The nitrogenous compounds present in the malt culm extract were removed by treatment with animal charcoal. The results obtained indicated that, with most yeasts, whilst glucose is the more rapidly fermented in the more acid media, selectivity is less pronounced in more alkaline media, and, indeed, in the early stages of fermentation in alkaline media the fructose is the more rapidly removed of the two. On repeating Schiller's work with top and bottom brewery yeasts it was found that animal charcoal does not remove nitrogenous material excreted by the yeast, and that somewhat high results for glucose are thereby obtained by the iodimetric method. Further it was found that, on boiling solutions of p_H 7.0, if fructose is present this sugar is altered to some extent, the resulting products possessing a less negative rotation

and a higher iodine value than before boiling. During boiling of the partly fermented solutions, a procedure necessary to remove volatile matters before estimating the reducing sugars, carbonic acid is evolved and the liquid becomes more alkaline. If the reaction is beyond p_H 7.0 during boiling, the subsequent analysis will yield high results for glucose both polarimetrically and iodimetrically. These observations may be the explanation of Schiller's results, which could not be obtained with brewery yeast.

The possibility that yeasts which ferment fructose faster than glucose may have acquired such characteristics from long habitat in media rich in fructose suggested that a brewery yeast, normally living in wort rich in maltose, might on repeated cultivation in a medium containing fructose be "trained" to ferment fructose selectively faster than glucose. Such subculturing was continued for several weeks, but the results indicated no change in the properties of the yeast in this respect.

Experiment showed that, for the zymins prepared from the top and bottom brewery yeasts previously referred to, the ratio of *maximum* rates of esterification attained (as measured by CO_2 evolution) for fructose : glucose,

(1) increased with concentration of free phosphate when this was above a certain concentration for each zymine under the conditions adopted;

(2) did not vary between 15° and 30° when the phosphate concentration was that of the optimum for glucose;

(3) did not appreciably vary between p_H 5.9 and 6.8 when the phosphate concentration was that of the optimum for glucose.

Of these three the influence of phosphate concentration is the most important. With the zymine from the top yeast for example the ratio fructose : glucose was 1.1, for concentration of free phosphates within 0.15 *M*. Above this the ratio increased to 1.4 with 0.19 *M* phosphate. Comparatively slight variations in phosphate concentration over this zone have an appreciable effect on the ratio. With yeast juice, which shows greater susceptibility to phosphates, wider variations in the ratio are to be expected. This is illustrated by the results obtained by Harden and Young [1909]. One possible inference from these results, assuming the hexosephosphate theory of Harden and Young, is that variations in species and in conditions of nutrition of yeast, reflected by variations in the relative activity of hexosephosphatase and of the constituents of the esterifying complex, would be a factor in accounting for the different values of *K* in selective fermentations of glucose and fructose. Temperature and reaction of medium would have comparatively little influence.

The temperature coefficient of esterification by the zymine was found to be $\frac{V_{25^\circ}}{V_{15^\circ}} = 2.01$ for glucose with the optimum phosphate addition, and that for fructose 2.08 with the same phosphate addition. It is by no means certain that this coefficient would hold for different phosphate additions. These results are somewhat higher than those obtained by Euler and Ohlsén [1911] using sucrose and dried yeast extract. These authors measured the rate of dis-

appearance of free phosphate at 17.5° and 30° respectively. This however does not give the true temperature coefficient of esterification, since the continuous re-formation of free phosphate by the hexosephosphatase causes the rate of disappearance of free phosphate measured to be less than the true one. The temperature coefficient for hexosephosphatase $\frac{V_{24.5^\circ}}{V_{14.5^\circ}}$ was found to be 2.88 by Herzog (which was roughly confirmed in conjunction with the above experiments with zymin). It is in good agreement with that for yeast cells as found by Slator [1908] $\frac{V_{25^\circ}}{V_{15^\circ}} = 2.80$. Since this coefficient exceeds that of esterification, the velocities observed by Euler and Ohlsén would be below the actual at each temperature, and in greater proportion at the higher temperature than at the lower one, and consequently the temperature coefficient obtained by them must be lower than is actually the case.

The approximate equality of the temperature coefficient of esterification of glucose and fructose in the presence of small concentrations of free phosphate is not surprising since the same enzyme functions for both sugars. But the abnormal properties of fructose, and in particular its unique change in specific rotation with rise of temperature, suggest that an internal structural change is occurring in one of the isomeric (α) forms and that this change may be such as to produce a more or less labile form of the sugar, with possible effects upon the velocity of esterification.

The influence of the reaction of the medium between the limits of p_H 3.5 and 7.5 upon the p_H of the cell interior is very uncertain, but it is probably comparatively little. One would not then expect any decisive influence to be exerted upon selective fermentation in view of the esterification ratios found over the range of p_H 5.9 to 6.8.

EXPERIMENTAL.

Exp. 1. The sugars remaining in solution during the fermentations of glucose, fructose, and a mixture of the two by brewery (top) yeast.

Solutions of these sugars, boiled to destroy mutarotation, were treated with 5 % of fermented and boiled extract of malt culms to serve as yeast nutrient, and seeded with equal seed rates of pure culture yeast. Half of each was rendered faintly alkaline, p_H 7.5, and maintained at p_H 7.0 to 7.5 throughout the fermentation by occasional small additions of concentrated alkali. The fermentations were conducted at 20°. Samples were withdrawn at the intervals stated, filtered, if alkaline rendered faintly acid, boiled to destroy any possible mutarotation and to remove volatile reducing substances and made up to the original volume. Rotations were measured at 20° in a 200 mm. tube and reducing powers by the method of Lane and Eynon (using methylene blue). The iodimetric titrations were performed exactly as described by Baker and Hulton [1920]. The use of alumina cream, which is liable to contain ammonium salts, must be avoided in the solutions used for the

iodimetric titration. Allowance was made for the small absorption of iodine by fructose.

The solutions containing the residual sugars after 70 hours' fermentation were also read in the polarimeter at 82°, at which temperature an equal mixture of the two sugars should have no rotation, whilst the specific rotation of fructose should have fallen to about -52° and that of glucose should remain unchanged. The percentage fructose was calculated from the difference between the readings at 20° and 82° on the assumption that the specific rotation of fructose decreases by 0.6385° per degree rise in temperature. It was found necessary to maintain the polarimeter tube in its bath at 82° for over 1 hour to obtain constant readings. On cooling the rotations returned to normal.

Table I.

Sugar fermented	Glucose		Fructose		Glucose and fructose	
	5.0-4.0	7.5-7.0	5.0-4.0	7.5-7.0	5.0-4.0	7.5-7.0
p_H during fermentation						
Original solution. Conc. of sugar (g. per 100 cc.)	7.9	7.9	7.85	7.85	Glucose 4.20 Fructose 4.20	4.20 4.20
$[\alpha]_D^{20}$ after 16 hours	+51.7°	+51.6°	-92.5°	-92.5°	-23.8°	-23.9°
Conc. of sugar (g. per 100 cc.)	7.55	7.38	6.50	6.35	Glucose 3.35 Fructose 3.75	3.31 3.72
Iodine absorption expressed as glucose (g. per 100 cc.)	7.65	7.50	0.07	0.12	3.48	3.45
$[\alpha]_D^{20}$ after 22 hours	+51.5°	+51.5°	-92.1°	-91.7°	-27.0°	-25.8°
Conc. of sugar (g. per 100 cc.)	6.95	5.90	5.58	5.44	Glucose 2.74 Fructose 3.36	2.76 3.26
Iodine absorption expressed as glucose (g. per 100 cc.)	7.10	6.10	0.14	0.18	2.91	2.98
$[\alpha]_D^{20}$ after 46 hours	+50.6°	+49.9°	-94.0°	-92.6°	-34.5°	-35.7°
Conc. of sugar (g. per 100 cc.)	6.25	4.21	3.90	3.85	Glucose 1.71 Fructose 2.61	1.62 2.55
Iodine absorption expressed as glucose (g. per 100 cc.)	6.45	4.44	0.18	0.28	1.92	1.85
$[\alpha]_D^{20}$ after 70 hours	+50.1°	+49.7°	-93.0°	-94.5°	-49.4°	-49.4°
Conc. of sugar (g. per 100 cc.)	4.80	3.15	2.35	3.02	Glucose 0.70 Fructose 1.68	0.70 1.68
Iodine absorption expressed as glucose (g. per 100 cc.)	5.02	3.40	0.26	0.24	0.94	0.96
$[\alpha]_D^{82}$	+50.0°	+49.6°	-55.0°	-56.2°	-22.4°	-22.2°
Fructose calculated from $[\alpha]_D^{20}$ and $[\alpha]_D^{82}$ (g. per 100 cc.)	0.0	0.0	2.25	2.92	1.62	1.63

Exp. 2. A series of fermentations as in *Exp. 1* was performed without added nutrients with results similar to those in *Exp. 1*.

Exp. 3 (Table II). A mixture of glucose and fructose was fermented at 20° in the presence of the following synthetic nutrients:

NH_4NO_3	3.0 g. per litre	Na_2HPO_4	6.9 g. per litre
MgSO_4	0.75 "	ZnSO_4	Trace
KH_2PO_4	0.6 "	FeSO_4	"

The phosphate content of this medium was $M/20$. Included in the table are the values of K , the ratio of the specific rates of fermentation (glucose to fructose).

Table II. *Concentration of sugars present in g. per 100 cc.*

	Original solution	Hours				
		22	31	46	73	145
Glucose	7.50	6.41	5.62	4.45	2.90	0.20
Fructose	7.50	7.09	6.78	6.25	5.27	0.90
K	—	2.78	2.86	2.87	2.70	1.71

A similar fermentation of glucose alone with the same yeast and nutrients gave successive specific rotations: $+53.0^\circ$, $+51.1^\circ$, $+51.1^\circ$, $+49.6^\circ$, $+50.0^\circ$. The solutions were tested for fructose by means of the reaction with orcinol and phosphoric acid [Löwe, 1916], but no evidence of the presence of fructose was obtained, whereas control tests with 0.1% of fructose added gave positive results.

Exp. 4. Fermentation of mixtures of glucose and fructose by zymin.

75 cc. of solutions containing 5 g. of glucose and 5 g. of fructose were fermented with 10 g. of zymin prepared from

- (a) top fermentation brewery yeast,
- (b) bottom fermentation brewery yeast,

with toluene, without added phosphates, at 25° for 24 hours. The liquids were now partly cleared with alumina cream, diluted and boiled until all volatile substances were evolved, and cleared afresh with alumina cream. The rotations and reducing powers were determined, and calculating the latter as percentage of glucose the following specific rotations were obtained:

Using top yeast zymin $[\alpha]_D^{20} - 28.0^\circ$.

Using bottom yeast zymin $[\alpha]_D^{20} - 25.5^\circ$.

These rotations would have been more negative but for the presence of a substance of high dextrorotatory power and low reducing power such as was shown to be present by Harden and Young [1913]. The solutions were next fermented with living yeast as completely as possible and the above process repeated. The specific rotations were now $+112^\circ$ in each case. Some fermentable sugars and hexosephosphates would still be present, as it is difficult to ferment away the last traces of sugar, and hexosephosphates are apparently not fermentable by living yeast. It is fairly evident that the fermentable sugars remaining after the partial fermentation by zymin had a specific rotation more negative than the figures -25.5° and -28° , which were obtained in the presence of the highly dextrorotatory polysaccharide.

Velocities of esterification of glucose and fructose by zymin.

The velocity of esterification was measured by determining the loss of CO_2 . The zymin was added to the sugar solution in U-tubes (all of similar shape

and size) with well-fitting taps, and the side tubes plugged lightly with cotton wool. After incubation until the fermentation was steady and the system saturated with CO_2 , the phosphate (saturated with CO_2) was added and the air in the tube displaced by passing through a current of CO_2 . The taps were closed when weighing and when shaking. Weighings were taken as frequently and rapidly as possible, every few minutes, and losses of weight plotted graphically against time. After a short induction period, longer with glucose than with fructose, the velocity of gas evolution soon acquired a maximum which remained uniform for some time, depending upon the quantity of phosphate added. The curve here was remarkably straight, and the maximum rates were read off from this part of the curve. The losses of weight were all corrected for water vapour.

Exp. 5. Velocity of esterification of glucose and fructose by zymine in presence of varying concentrations of phosphates.

To 2 g. of zymine were added 8 cc. of a solution containing 2 g. of the sugar and 0.2 cc. toluene and the mixture was incubated at 25° until a constant rate of fermentation had been attained. The quantities of 0.6 M K_2HPO_4 (saturated with CO_2) were added and water (saturated) to total volume 15 cc. The rates of gas evolution are recorded in mg. per 5 mins. (1 mg. CO_2 = approx. 0.5 cc.).

Table III.

cc. 0.6 <i>M</i> K ₂ HPO ₄	Max. rate, mg. per 5 mins.		Ratio F./G.	Average rate over whole acceleration period		Ratio F./G.
	Glucose	Fructose		Glucose	Fructose	
0	Too transient for accurate measurement					
2.0	6.76	7.57	1.12	4.8	5.30	1.10
3.0	7.50	8.10	1.08	5.7	6.16	1.08
3.5	8.30	9.00	1.08	5.8	6.25	1.08
4.0	8.25	9.36	1.13	5.8	6.30	1.09
5.0	7.37	10.40	1.41	5.7	7.06	1.24
6.0	7.16	10.65	1.50	5.5	7.85	1.43

Exp. 6. Temperature coefficient of esterification of glucose and fructose.

2 g. zymine + 10 cc. of solution containing 2 g. sugar were incubated as before with toluene. 3.5 cc. 0.6 M phosphate and water to 15 cc. were added.

Table IV.

Temperature	Max. rates attained, mg. per 5 mins.		
	15°	25°	30°
Glucose	2.55	5.12	7.15
Fructose	4.83	10.05	13.90
	$\frac{V_{25^\circ}}{V_{15^\circ}}$	$\frac{V_{30^\circ}}{V_{25^\circ}}$	$\frac{V_{30^\circ}}{V_{15^\circ}}$
Glucose	2.01	1.40	2.80
Fructose	2.08	1.37	2.88

Exp. 7. Influence of hydrogen ion concentration on the relative velocities of esterification of glucose and fructose.

The hydrogen ion concentrations in this experiment were measured electrometrically, by the quinhydrone electrode, using the contents of control tubes

which were in all cases started at a known interval of time after the ordinary weighed tubes. A sample was withdrawn from the control tube just as the maximum rate of esterification was being attained, the time of this being the known interval after the contents of the weighed tube had arrived at the same stage. Another sample was withdrawn at the end of the period of esterification. The hydrogen ion concentration was determined immediately and rapidly in each case. The maximum velocities recorded do not represent the true maxima for the respective hydrogen ion concentrations, since a certain amount of CO_2 is released from the system as a result of the change in degree of ionisation of the phosphates during esterification. This amount is assumed to be identical for glucose and fructose in any one case (*i.e.* over the same range of p_{H}), since the same amount of free phosphate is esterified and the change in p_{H} should be the same. The *ratios* should not be affected. Further work is required to ascertain the true rates of esterification at the respective ranges of p_{H} . The concentration of phosphate added was the optimum for glucose under normal conditions.

To 2 g. zymin were added 10 cc. of solution containing 2 g. of sugar, and after incubation 3.5 cc. of 0.6 *M* phosphate, etc. (as stated in Table V), and the total volume was made up to 15 cc.

Table V.

cc. of 0.6 <i>M</i>				Max. rate, mg. CO_2 per 5 mins.		Ratio F./G.	p_{H}
K_2HPO_4	KH_2PO_4	KHCO_3	KCl	Glucose	Fructose		
1.2	2.3	0	1.5	5.00	5.40	1.08	6.04-5.91
1.5	2.0	0	1.5	5.40	6.06	1.12	6.21-6.06
2.0	1.5	0	1.5	6.80	7.80	1.15	6.30-6.20
2.8	0.7	0	1.5	7.95	9.15	1.15	6.36-6.30
3.5	0	0	1.5	8.90	10.70	1.20	6.60-6.50
3.5	0	1.0	0.5	10.50	13.30	1.26	6.88-6.80

SUMMARY.

1. Brewery yeasts (top and bottom types) ferment glucose faster than fructose in mixtures of the two, both when the yeast is multiplying freely in the presence of various kinds of nutrients, and when nutrients are absent.
2. The relative specific rates of fermentation of glucose and fructose in such cases are far more dependent upon the species of yeast used and the conditions of its nutrition, than upon such factors as concentration of sugars in the medium, temperature, and reaction of medium.
3. No fructose could be detected in a solution of glucose undergoing fermentation by living yeast.
4. Zymin, prepared from brewery yeast, ferments glucose faster than fructose in a mixture of the two.
5. A yeast "trained" by continual subculturing in a medium of fructose and nutrients showed no change in its properties as regards selectivity.

6. The temperature coefficients of esterification of glucose and fructose by zymine under conditions of phosphate concentration yielding the optimum esterification of glucose are approximately the same, viz. $\frac{V_{25^\circ}}{V_{15^\circ}} = 2.0$.

7. The velocities of esterification of both sugars vary with the reaction of the medium, but the ratio of the velocities varies much less.

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