CCXCVII. THE KINETICS OF ALCOHOLIC FERMENTATION OF SUGARS BY BREWER'S YEAST.

III. THE TEMPERATURE COEFFICIENTS OF THE RATES OF FERMENTATION OF GLUCOSE AND FRUCTOSE.

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THE temperature coefficient of the rate of fermentation by living yeast was investigated by Slator [1908]. His results refer to the reaction which limits that fairly uniform rate which is observed over a wide range of sugar concentration from 1 to 10 % in the case of glucose and from 2 to 10 % in the case of fructose. These uniform rates and their temperature coefficients were approximately the same for the two sugars. However, the marked difference between the rates of fermentation of the two sugars when the concentrations are low, below 1 %, and the phenomenon of selective fermentation of the mixed sugars give rise to the idea that other factors supervene at low concentrations. There are reasons for anticipating an abnormal temperature coefficient in the case of fructose, particularly. The change in optical rotation of solutions of this sugar with change in temperature is usually attributed to a change in the dynamic equilibrium of the various forms of the sugar which are supposed to be present in solution at equilibrium. If we suppose only one (or some) of these components to be specifically fermentable, three cases can arise when a sufficiently dilute solution is fermented, *i.e.* when the concentration of the fermentable component can influence the rate of fermentation. Firstly the fermentable component may increase with rise in temperature at the expense of the unfermentable one; in this case the observed temperature coefficient will be abnormally high. Secondly the fermentable component may decrease with rise in temperature and an abnormally low temperature coefficient be observed. Thirdly, despite changes in the dynamic equilibrium, the fermentable component itself may not change in concentration with rise in temperature, in which case the temperature coefficient should be normal and the same as at higher concentrations of fructose where the concentration has no appreciable effect on the rate of fermentation. These considerations do not apply in the case of glucose, so that a comparison between the behaviours of the two sugars, glucose being provisionally regarded as a standard, should enable us to decide between the three possible cases discussed above.

EXPERIMENTAL.

The technique employed was exactly as described by Hopkins and Roberts [1935]. For each concentration of sugar, the observations at all temperatures were made with the same stock of yeast on the same day. The figures given in

the table below are the ratio, velocity of fermentation at $t+5^\circ$: velocity of fermentation at t° . They are represented graphically in Fig. 1, as ordinates, plotted against t as abscissae.

Fig. 1. Temperature coefficients V_{t+5}/V_t (ordinates). Temperature t° (abscissae).

Exp. 1. Glucose or fructose 0.25–5.0 g. Pressed yeast 2 g. Temp. 15–40°. Total volume 100 ml.

Table I. Temperature coefficients V_{t+5}/V_t .

Concentration of sugar	0.25%	0.5%	1.0%	2.5%	5.0%
Temp. (t°)			Glucose		
15	2.03	2.03	1.97	1.97	2.01
20	1.72	1.75	1.70	1.73	1.75
25	1.55	1.66	1.59	1.61	1.59
30	1.42	1.47	1.48	1.44	1.44
35	1.20	1.22	1.28	1.23	1.25
			Fructose		
15	1.64	1.71	1.96	2.02	1.99
20	1.45	1.54	1.79	1.75	1.76
25	1.38	1.46	1.61	1.62	1.59
30	1.34	1.41	1.45	1.43	1.47
35	1.13	1.18	1.21	1.27	1.23

DISCUSSION.

The results show that the temperature coefficient of rate of fermentation of glucose over the range of temperature 15° – 40° is independent of the concentration of glucose between 0.25 and 5.0 %. The mean values, 2.0, 1.73, 1.60, 1.45 and 1.24 have been confirmed by other experiments. The corresponding results for fructose, however, exhibit an anomaly in the region of low concentration where the latter exerts the greatest effect on the rate of fermentation. At 1 % concentration and above, the curves for fructose coincide with those for glucose, but below 1 % concentration they fall below the latter, lower temperature coefficients being observed. The simplest explanation of this is that, with rise in temperature, the fermentable component in fructose solutions decreases in concentration. There are objections to this explanation, however, which may be dealt with in a later communication. However it can be shown here that there are good grounds for the view that the rate of fermentation of fructose at these low concentrations is actually limited by the rate of mutarotation, *i.e.* the rate at which the fermentable component is restored by change in the dynamic equilibrium.

If it be assumed that the sugar in solution at equilibrium contains one component which is specifically fermented and another or others which are not, then fermentation will result in the removal of the one component and its continual replacement from the other or others until exhaustion of the latter supervenes. If the concentration of total sugar is low enough, a limiting factor controlling the rate of fermentation will be the concentration of the fermentable component, and, unless its replacement is faster than its removal, the rate of mutarotation will become the controlling factor. The temperature coefficients of the latter factor are less than those of the rate of fermentation at normal concentrations of fructose but are reasonably close to those of fermentation of fructose at low concentrations. To ascertain the temperature coefficients of mutarotation of fructose, reference was made to the data furnished by Nelson and Beegle [1919], and the values calculated for p_H 6.0–6.2. This is the value of the p_H of the contents of brewer's yeast cells observed by Tait and Fletcher [1926] and by Mahdihassan [1930]. It was assumed that all components of the sugar diffuse into the cell, the removal of the fermentable one takes place within, and its replacement follows from the sugar in the immediate vicinity, *i.e.* within the cell and therefore at a rate controlled by the reaction of the highly buffered cell contents. The temperature coefficients so obtained were:

$$k_{25^{\circ}}/k_{15^{\circ}}=2.20; \quad k_{35^{\circ}}/k_{25^{\circ}}=1.86.$$

These refer to the velocity constants of the mutarotation unimolecularly of β (2, 6)-fructose ($[\alpha]_D - 130.8^{\circ}$ at all temperatures investigated) to equilibrium at the respective temperatures. But the equilibrium point varies with temperature so that the value of a in the equation $k = \frac{1}{t} \ln \frac{a}{a-x}$ is different for the different temperatures. In calculating their velocity constants Nelson and Beegle put

$$a = \rho_0 - \rho_{\infty} \quad \text{and} \quad a - x = \rho - \rho_{\infty},$$

where

ρ_0 = initial specific rotation,

ρ = specific rotation at time t ,

ρ_{∞} = specific rotation at equilibrium,

and

ρ_{∞} varies with temperature.

However the values of Nelson and Beegle's temperature coefficients can be shown to apply to this case as may be seen from the following considerations.

Consider a small quantity of fermentable component δx to have been removed from the solution at equilibrium, thus changing the specific rotation by an amount $\delta\rho$. The velocity at which this quantity will be restored will be $k\delta\rho$, since for a unimolecular reaction $\frac{dx}{dt} = k(a-x)$, and in this case $a-x$ is denoted by $\delta\rho$. At temperatures T_1 and T_2 let the corresponding unimolecular velocity constants be k_1 and k_2 . Then the velocities at which δx of fermentable component will be restored will be $k_1\delta\rho$ and $k_2\delta\rho$ respectively, and the temperature coefficient

$$\frac{V_{T_1}}{V_{T_2}} = \frac{k_1\delta\rho}{k_2\delta\rho} = \frac{k_1}{k_2},$$

or

$$\frac{V_{T_1}}{V_{T_2}} = \frac{\left(\frac{dx}{dt}\right)_1}{\left(\frac{dx}{dt}\right)_2} = \frac{k_1(a_1 - x_1)}{k_2(a_2 - x_2)}$$

Integrating,

$$\frac{k_1}{k_2} = \frac{\ln a_1 - \ln(a_1 - x_1)}{\ln a_2 - \ln(a_2 - x_2)} \cdot \frac{t_2}{t_1}.$$

Since $(a_1 - x_1)$ and $(a_2 - x_2) = \delta\rho$ are small, their value approaches zero, and the value of their logarithm approaches $-\infty$ so that this expression becomes $\frac{t_2}{t_1}$, or the inverse ratio of the times required at the respective temperatures to restore the same small quantity $\delta\rho$ of fermentable component.

Comparing the temperature coefficients of the velocity constants of mutarotation to equilibrium of β (2, 6)-fructose and those of α - or β -glucose, calculated similarly and derived from the data furnished by Nelson and Beegle, with those of the rate of fermentation reported above the following summary is obtained.

Mutarotation			Fermentation		
α - or β -Glucose	β (2, 6)-Fructose		$\alpha\beta$ -Glucose concentration	Equilibrium fructose concentration	
			0.25-5.0%	1-5%	0.25%
$V_{25^\circ}/V_{15^\circ}$	2.47	2.20	3.46	3.46	2.38
$V_{35^\circ}/V_{25^\circ}$	2.43	1.86	2.32	2.32	1.85

It will be seen that the values of fermentation temperature coefficients for fructose at 0.25 % concentration are much lower than the corresponding values for higher concentrations or the values for glucose. On the other hand they show a significantly close agreement with the corresponding temperature coefficients of rate of mutarotation of β (2, 6)-fructose. With concentrations not lower than 0.25 % the rate of fermentation of $\alpha\beta$ -glucose is not controlled by rate of mutarotation.

The general conclusion emerges that, of fructose in solution at equilibrium, one component only is specifically fermented, and this component is continually restored from the other component or components. If appropriate conditions are employed the rate of fermentation is actually limited by the rate of mutarotation. The conclusion is also implicit that the fermentable component is a relatively small proportion of the total sugar present.

SUMMARY.

1. The temperature coefficients of rate of fermentation of glucose and fructose solutions at equilibrium over the range 0.25 to 5.0 % concentration and 15 to 40° have been determined.

2. Except in the case of fructose at concentrations as low as 0.5 % or less, these temperature coefficients are in close agreement for either sugar and for any concentration.

3. In the case of fructose at 0.25 % concentration the temperature coefficients of rate of fermentation are in significantly close agreement with the corresponding temperature coefficients of velocity of mutarotation of β (2, 6)-fructose to equilibrium.

4. It is inferred that, of fructose in solution at equilibrium, one component only is specifically fermented, and that the rate at which it is restored from the other components is the factor limiting the rate of fermentation when the concentration of sugar is as low as 0.25 %.

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