larva. In both respects it resembles the effect of docosahexaenoic acid from cod-liver oil.

2. Ascorbic acid, in combination with the saponifiable fraction of wheat-germ oil, has a positive growth effect which is attributed to an antioxidant action.

3. The linoleic acid content of various insect oils has been estimated by testing their effect on the wings of *Ephestia*. Oils from *Tenebrio* and *Ephestia* grown on a diet containing linoleic acid were

assessed to contain 20 and 15% linoleic acid, and from a diet without linoleic acid, 10 and less than 1% respectively. It is concluded that linoleic acid is synthesized by *Tenebrio* but not by *Ephestia*.

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Further Observations on the Non-fermentability of *d*-Fructopyranose by Yeast

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In recent years we have investigated the fermentation rates of the various *d*-fructose, *d*-glucose and *d*-mannose modifications at low concentration (0.005M) under conditions (0°, pH 4.5) which reduce the rate of mutarotation to a minimum. On solution in water β -*d*-fructopyranose is reversibly converted to β -*d*-fructofuranose, the keto form contributing only a minute proportion to the equilibrium mixture

(Purves & Hudson, 1934; Isbell & Pigman, 1938; Bednarczyk & Marchlewski, 1938). The proportion of fructofuranose increases with temperature. The mutarotation of *d*-glucose, on the other hand, consists almost exclusively in an α -pyranose $\rightleftharpoons$ β -pyranose change.

The results obtained by this technique (for review, see Gottschalk, 1947b) seemed to substantiate the hypothesis, first proposed by Hopkins (1931), that only the furanose form of *d*-fructose is fermentable by yeast, whereas the pyranose modification is unfermentable. This interpretation implies that the concentration of *d*-fructofuranose, and not the total fructose concentration, has to be considered when the fermentation rate is referred to substrate concentration.

As an alternative interpretation of the slower fermentation rate, as compared with *d*-glucose, of *d*-fructose in low concentration at 30° and at 0° (cf. nos. 1 and 3 of the summary at the end of section 'Discussion') the following explanation may be offered: A lower concentration of *d*-fructose inside the cell resulting in a slower fermentation rate may be due to a higher energy barrier encountered by *d*-fructopyranose at the interface suspending fluid-cell membrane. Thus it was recently shown (Gottschalk, 1947a) that at 10° *d*-mannopyranose in low concentration (0.026M) is fermented at only half the rate of *d*-glucose by living yeast, whereas equal rates of CO₂ production are observed with yeast extract as fermenting agent under otherwise similar conditions. In view of this observation it seemed desirable to test the correctness of the alternative interpretation of the abnormal behaviour of yeast towards *d*-fructose in low concentration. If, by resisting the penetration of *d*-fructose more than that of *d*-glucose, the membrane of the yeast cell causes the slower fermentation of low concentrations of fructose compared with glucose, then the fermentation rates both of *d*-fructose and *d*-glucose may be expected to vary in the same way with yeast concentration. The present paper is concerned with the effect of yeast concentration on the fermentation rate of *d*-fructose and of *d*-glucose at low concentrations at 25°.

MATERIALS AND METHODS

The experiments were performed with baker's yeast (Effront), stored for 24 hr. at 1–2° after having been compressed. The yeast (2.0 g.) was suspended in 0.1M-KH₂PO₄

(total vol. 40 ml.; initial pH 4.5). From this stock solution a 1:1 dilution was prepared with 0.1M-KH₂PO₄, and 20 ml. of the required suspension were taken for each experiment.

The substrates added were pure, anhydrous substances:

$[\alpha]_D^{20}$ *D*-glucose: +52.4° (water; *c*=4, *l*=2);

$[\alpha]_D^{20}$ *D*-fructose: -91.7° (water; *c*=4, *l*=2).

The rate of fermentation at 25.0° ± 0.1 was determined with the apparatus described previously (Gottschalk & Rawlinson, 1942). After thermal and gaseous equilibration of the suspension at 25° with N₂ (freed from O₂) the sugar, dissolved in 0.5 ml. of 0.1M-KH₂PO₄ and allowed to mutarotate overnight at 25°, was tipped into the yeast suspension. Readings were taken at 2 min. intervals over a period of 20 min.; the values observed were reduced to N.T.P.

RESULTS

It may be seen (Table 1) that on doubling the amount of yeast the maximum rate of CO₂ production from *D*-fructose (0.005M) increases only by one-third, whereas with glucose the fermentation rate is proportional to the yeast concentration. It may be mentioned that with these concentrations of yeast and substrate 77 % of the hexose consumed is fermented to CO₂ and ethanol.

mentation rates of *D*-fructose and of *D*-glucose is, however, to be expected if the fermentation of *D*-fructose, in contrast to that of *D*-glucose, is preceded by a reversible chemical reaction which is not controlled by the enzyme system of the yeast:

It is evident from (1) that fermentation interferes with the rate of mutarotation in that it cuts down the back reaction by transforming fructofuranose into CO₂ and ethanol. If yeast is added to an equilibrated solution of *D*-fructose, the preformed fructofuranose will be fermented at a rate proportional to the yeast concentration (cf. Table 1, time interval 0-2 min.). Thereafter no proportionality between fermentation rate and yeast concentration will obtain because the effect on the fermentation rate of any increase in yeast concentration is partly counterbalanced by a lower concentration of *D*-fructofuranose. With higher yeast concentration the concentration of *D*-fructofuranose is lower since initially more of the preformed amount is removed and this removal at a higher rate is continued throughout the process.

Table 1. Fermentation rates of *D*-fructose and *D*-glucose (0.005M) with various yeast concentrations

Temp. 25°.										
Exp. no.	Yeast (g.)	Hexose added (mg.)	CO ₂ produced (ml. in successive 2 min. periods)							Maximum fermentation rate (ml. CO ₂ /2 min.)
			0-2	2-4	4-6	6-8	8-10	10-12	12-14	
			A. With <i>d</i> -fructose as substrate							
1	0.5	19.3	0.24	0.37	0.36	0.36	0.30	0.24	0.24	0.37
	1.0	19.4	0.49	0.48	0.49	0.36	0.30	0.24	0.18	0.49
2	0.5	19.3	0.24	0.36	0.36	0.36	0.36	0.24	0.18	0.36
	1.0	19.3	0.49	0.48	0.48	0.36	0.30	0.17	0.18	0.49
3	0.5	19.5	0.24	0.37	0.36	0.37	0.36	0.24	0.24	0.37
	1.0	19.6	0.48	0.49	0.48	0.42	0.29	0.24	0.24	0.49
4	0.5	18.9	0.24	0.37	0.36	0.36	0.36	0.30	—	0.37
	1.0	19.2	0.42	0.47	0.48	0.47	0.29	0.30	—	0.48
B. With <i>d</i> -glucose as substrate										
5	0.5	19.1	0.37	0.48	0.49	0.54	0.54	0.42	0.18	0.54
	1.0	19.3	0.72	0.96	0.96	0.58	0.18	—	—	0.96
6	0.5	19.7	0.37	0.48	0.49	0.48	0.48	0.48	0.24	0.49
	1.0	19.6	0.73	0.84	0.96	0.60	0.24	—	—	0.96
7	0.5	20.1	0.31	0.41	0.52	0.52	0.48	0.47	0.24	0.52
	1.0	20.0	0.61	0.86	0.97	0.72	0.24	—	—	0.97

DISCUSSION

The finding that at low substrate concentration the fermentation rate of *D*-glucose varies directly with yeast concentration, while that of *D*-fructose does not, disproves the interpretation that differences in the permeability of the cell membrane for the two sugars may be responsible for the peculiar reaction of yeast towards *D*-fructose at low concentrations. The observed effect of yeast concentration on the fer-

In conclusion the differences so far established between *D*-glucose and *D*-fructose as fermentation substrates for yeast may be summarized:

(1) At 30° and at substrate concentrations less than 0.03M, *D*-fructose (equilibrated) is fermented markedly slower than *D*-glucose, whereas at higher concentrations (above 0.1M) both sugars are fermented at about equal rates (Hopkins & Roberts, 1935*a*).

(2) Between 0.056 and 0.278M-substrate concentration the temp. coefficients (from 15 to 40°) of fermentation of

d-glucose and *d*-fructose are the same. With 0.014M-fructose concentration, however, the temp. coefficients of fermentation are lower than those for fructose at higher concentration or for glucose at equal concentration; they approach closely the corresponding temp. coefficients of the rate of mutarotation of *d*-fructose (Hopkins & Roberts, 1935b).

(3) At 0° and at 0.005M-substrate concentration the fermentation rate of β -*d*-fructopyranose is only a small fraction of that of α -*d*-glucopyranose; it coincides practically with the rate of mutarotation of *d*-fructopyranose at 0° (Gottschalk, 1943a). Under the same conditions fructofuranose, set free enzymatically from sucrose or raffinose, is readily fermented (Gottschalk, 1945).

(4) Living yeasts of the industrial type (baker's, brewer's, spirit yeasts), and dried yeasts prepared from them, respond uniformly to invert sugar as substrate by fermenting preferentially *d*-glucose at room temperature (for literature see Gottschalk, 1947c). However, by raising the proportion of fructofuranose without altering the total concentration of *d*-fructose and *d*-glucose the relative rates at which *d*-glucose and *d*-fructose are fermented from invert sugar can be equalized (Gottschalk, 1945).

(5) The capacity of Sauternes yeast selectively to ferment fructose from invert sugar is confined to the intact cell. Dried Sauternes yeast preferentially ferments glucose from invert sugar (Gottschalk, 1946).

(6) At 25° and at 0.005M-substrate concentration increase in yeast concentration does not result in a proportional increase in the rate of fructose fermentation, whereas this is the case with glucose as substrate (present paper). The

same holds for experiments performed at 0° (Gottschalk, 1943a).

The concept that, of the various *d*-fructose modifications present in a fructose solution at equilibrium, only *d*-fructofuranose (22% of the total at 25°; Gottschalk, 1943b) is a suitable substrate for ethanolic fermentation seems to be the only interpretation consistent with the relevant facts. Obviously *d*-fructopyranose is unfermentable, because it has no esterifiable —OH group at C atom 6. The α - and β -pyranose forms of *d*-glucose and *d*-mannose and the furanose form of *d*-fructose have as common features the same side-chain and the same spatial distribution of the addenda to C atoms 3, 4 and 5; therefore they can combine with yeast hexokinase by the same mechanism (Gottschalk, 1947b).

SUMMARY

1. At 25° and low substrate concentration (0.005M) the fermentation rate of *d*-glucose is proportional to the concentration of yeast, whereas that of *d*-fructose is not, increasing only by one-third for a twofold increase in yeast concentration.

2. This phenomenon conforms to the concept that of the various *d*-fructose modifications present in solution only *d*-fructofuranose is a suitable substrate for alcoholic fermentation.

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