CII. SELECTIVE FERMENTATION. I. ALCOHOLIC FERMENTATION OF GLUCOSE, FRUCTOSE AND MANNOSE MIXTURES.

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THE knowledge of the chemical reactions involved in alcoholic fermentation and related glycolytic processes has been augmented in recent years by numerous researches on the later stages leading to the final products, carbon dioxide and alcohol. Although the individual enzymes forming the complex catalytic system "zymase" have not been separated, nor their scope, specificity, mode of action, and other properties definitely established, future findings may be expected to conform with our general conception of the mechanism of fermentation. Comparatively little is known of the primary reactions leading from carbohydrate to structures of three carbon atoms. The observation of Harden and Young coupling the evolution of carbon dioxide with the phosphorylation of a quantitatively correlated amount of sugar is the bridge between the two stages of alcoholic fermentation. Analogies occur in the carbohydrate metabolism of animal cells.

The most striking feature of glycolysis, its high stereochemical specificity, remains to be explained. A number of investigators, Dubrunfaut [1847], and especially Bourquelot [1886] and Slator [1908], demonstrated that the "zymohexoses," glucose, fructose and mannose are fermented at about equal rates when offered singly to yeast, but from a mixture like invert sugar, glucose is preferred to fructose. Gayon and Dubourg [1890] were the first to observe that some Sauterne wine yeasts behaved differently and preferred the laevo-rotatory moiety of invert sugar. The selectivity of dried yeast (zymin) and yeast juice (Buchner's zymase) usually parallels that of the fresh yeast from which they are prepared, but in some cases Fernbach, Schoen and Mori [1927] observed a reversion of selectivity. Fernbach and Schiller [1924] noted that culturing in alkaline media will influence the selective power of a "glucose"-yeast in the direction of increased fructose fermentation. The laws of these phenomena and of similar ones observed in other sugar mixtures will offer a clue to the mechanism of fermentation.

The speed of fermentation is independent of the sugar concentration within wide limits, and depends only on the concentration of the yeast. As the final

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step of alcoholic fermentation, the evolution of carbon dioxide and alcohol, is common for all fermentable sugars, it is not surprising that the formation of the end-products from a mixture should be controlled by the amount of carboxylase present. Carbon dioxide is released at a slower rate from the mixture than the sum of the rates in separate fermentation; the rate of fermentation is barely greater for the mixture than that of the faster fermenting component (cf. Table II, last column). The fermentation of glucose and fructose was treated quantitatively by Willstätter and Sobotka [1922]. The selectivity of a yeast is expressed by a factor $K_{G/F} = \frac{\ln y_0 - \ln y}{\ln z_0 - \ln z}$, where y_0 and z_0 are the initial concentrations of glucose and fructose and y and z their concentrations at any given time. It was found that the average factor was $K_{G/F} = 2.94$ for Munich Loewenbräu yeast. This factor changed to 1.89 when the yeast was cultured on a fructose medium. Hopkins [1928; cf. Ivecovich, 1930], using the same formula, found $K_{G/F}$ about 1.8 for an English top brewery yeast; this figure was unaffected by culturing on fructose.

In view of the great variations of enzymic content with type and strain, we determined the selectivity of various yeasts in the present study. We found 2.10–2.21 for a bottom brewery yeast (New York Lager), 2.46 for American baker's yeast, 2.98 for a top brewery yeast (New York Ale), and 4.63 for *Saccharomyces Marxianus*. We tried to verify the preference for fructose in two strains of Sauterne yeast which we obtained from the Versuchs-Anstalt für Brauwesen in Vienna and from the Institut für Gärungsgewerbe in Berlin. However, they showed a preference for glucose. Culturing the Vienna strain on fructose reduced $K_{G/F}$ from 4.0 to 2.6 and subsequent cultures on grape juice reached $K_{G/F} = 2.33$, which still signifies a preference for glucose. This limited adaptation to fructose resembles the behaviour of Munich brewery yeast. The $K_{G/F}$ of the New York Lager yeast was not reduced by culturing on fructose; it is analogous in this respect to Hopkins's brewery yeast which was unchanged by fructose treatment. $K_{G/F} = 2$ seems to be the lower limit of fructose adaptation. This limit was not reached by the Berlin strain which showed $K_{G/F} = 2.8$ to 2.9 when cultured on fructose. The yeasts which were transferred from fructose to maltose media reached $K_{G/F} = 4.0$ and 3.6 and the Berlin yeast also reached $K_{G/F} = 3.85$ when removed from an active fermentation of saccharose before the glucose component had disappeared.

Relation of selective fermentation to saccharase and maltase.

According to recent investigations of Weidenhagen [1930] the multiplicity of saccharase, maltase, raffinase and related enzymes can be reduced to one α -glucosidase and one β -fructosidase. As these enzymes are instrumental in the formation of fructose and glucose from higher saccharides, we surmised a correlation between these specific hydrolysing enzymes and the analogous specific zymases. However, treatment of yeast as devised by Willstätter, Lowry and Schneider [1925] to increase or decrease the saccharase content

failed to change the selectivity for glucose-fructose fermentation. This selectivity was also unaffected by the addition of large quantities of purified saccharase.

S. Marxianus contains fructosidase but no glucosidase, as it does not hydrolyse or ferment maltose; nevertheless, it has a decided preference for glucose over fructose.

Fermentation of mixtures containing mannose. The average $K_{G/M}$ was 3.8 with wide variations for the Lager yeast, although the rate of fermentation for the two aldoses was practically equal under the experimental conditions. Even fructose was preferred to mannose at a rate of $K_{F/M} = 1.39$. The value of 2.73 for $K_{G/F}$ calculated from the two mannose factors tallies with those obtained experimentally in glucose-fructose mixtures.

EXPERIMENTAL.

The specific rotation of the sugars used was verified and $[\alpha]_D = -93.6 + 0.64(t - 20)$ [Laborde, 1913] was used for the temperature correction of fructose. The original brewery yeasts contained 75 % water on the average. All the other yeasts were cultured in synthetic media containing 4 % of the respective sugar besides 0.25 % KH_2PO_4 , 0.25 % peptone, 0.025 % MgSO_4 , 7 aq and 0.01 % CaSO_4 [Willstätter and Steibelt, 1921] and isolated after a number of 2-3 day passages by centrifugation (average moisture = 75 %).

The fermentations were carried out at room temperature. 50 cc. of a 20 % yeast suspension were mixed with 75 cc. of a 10 % sugar solution and samples withdrawn at the times given. The progress of fermentation was followed in the van Iterson-Kluyver apparatus [van der Haar, 1920] with 1 cc. of the fermentation mixture containing 60 mg. sugar. The theoretical amount of carbon dioxide from this amount of sugar with the necessary corrections for absorption, temperature and pressure is 15.6 cc. at 20° and 760 mm. The figures given in Table I under CO_2 represent volumes measured in % of the theoretical total. Fermentation in the samples was stopped by addition of a few crystals of sulphosalicylic acid and rapid centrifugation. The clarified supernatant liquid was analysed polariscopically and by the Hagedorn-Jensen method in Hanes's modification or by the Bertrand method. Corrections were applied for the yeast blank when necessary. The reduction of the sugar mixtures was interpolated from the reduction values of their constituents.

Table I gives examples for each yeast used. $K_{G/F}$ was computed by using the decimal logarithms of the sugar concentrations as given in the preceding columns in % of total sugar originally present.

A synopsis of our experiments is given in Table II. The time necessary for evolution of half the theoretical amount of carbon dioxide was noted in the Kluyver apparatus. The relative speeds of fermentation for glucose, invert sugar and fructose were referred to that of fructose = 1. These figures are recorded in the last two columns of Table II. The deviation from unity is, with very few exceptions, less than 10 %, in accordance with previous investigations.

Table I. *Determination of $K_{G/F}$ (ratio glucose fermentation/fructose fermentation) in various types of yeast.*

Yeast and temp.	Time (min.)	Sugar (actual %)	Optical rotation $l = 2 \text{ dm.}$	Specific rotation	Not fermented in % of total original sugar			K_G/F	CO_2 %
					Total sugar	Glucose	Fructose		
1. Lager 30°	0	6.30	- 2.26°	- 17.92°	100.0	49.6	50.4	—	—
	60	5.04	- 2.37	- 23.51	80.0	36.5	43.5	2.08	—
	90	4.40	- 2.38	- 27.07	69.8	30.0	39.8	2.13	—
	120	3.74	- 2.32	- 31.01	59.4	23.9	35.5	2.08	—
								2.10	
2. Ale 21°	0	6.48	- 2.02	- 15.6	100.0	53.2	46.8	—	—
	120	5.57	- 2.31	- 20.75	86.0	42.7	43.3	2.85	—
	210	4.66	- 2.62	- 28.1	71.9	32.1	39.8	3.12	—
								2.98	
3. Baker's 20°	0	6.55	- 2.99	- 22.8	100.0	48.65	51.35	—	—
	130	3.37	- 3.04	- 45.1	51.5	17.25	34.25	2.56	30.3
	215	1.40	- 1.89	- 67.7	21.3	3.85	17.45	2.35	54.5
								2.46	
4. <i>S. Marx.</i> 21.5°	0	6.14	- 2.57	- 20.9	100.0	49.55	50.45	—	—
	150	4.58	- 3.14	- 35.25	74.6	29.5	45.1	4.65	18.6
	255	3.64	- 3.43	- 47.1	59.35	18.75	40.6	4.49	30.1
	335	2.96	- 3.49	- 58.8	48.35	11.35	37.0	4.76	39.8
								4.63	
5. Vienna Saut. on gr. juice 17.5°	0	6.31	- 2.59	- 20.5	100.0	50.5	49.5	—	—
	70	5.73	- 2.64	- 23.0	90.8	44.4	46.4	2.17	—
	115	5.20	- 2.75	- 26.45	82.4	38.3	44.1	2.40	—
	160	4.53	- 2.81	- 31.0	71.8	31.1	40.7	2.48	—
	245	4.00	- 2.70	- 33.75	63.4	26.35	37.05	2.25	—
								2.33	
6. Berlin Saut. on fructose 20°	0	5.60	- 2.62	- 23.4	100.0	48.0	52.0	—	—
	95	4.54	- 2.81	- 30.9	81.0	34.75	46.35	2.82	16.0
	145	3.60	- 2.93	- 40.7	64.3	23.3	41.0	3.04	34.0
	197	3.08	- 2.77	- 44.7	55.0	18.4	36.6	2.73	42.3
								2.90	
7. Berlin Saut. on sucrose 20°	0	5.74	- 2.60	- 22.7	100.0	48.35	51.65	—	—
	50	4.66	- 2.99	- 32.1	81.4	34.3	47.1	3.74	14.7
	85	3.50	- 2.99	- 42.75	61.1	19.9	41.2	3.93	26.3
	130	3.01	- 3.01	- 50.0	52.6	14.1	38.5	4.19	40.4
	190	1.99	- 2.46	- 62.0	34.7	6.05	28.65	3.53	53.8
								3.85	
8 a. Lager + sac- charase 21°	0	6.46	- 2.44	- 18.9	100.0	50.9	49.1	—	—
	90	3.78	- 2.56	- 33.85	58.5	23.8	34.7	2.19	32.7
	135	2.28	- 2.32	- 50.9	35.3	10.2	25.1	2.41	50.0
								2.30	
8 b. Lager (control) 21°	0	6.40	- 2.49	- 19.45	100.0	50.55	49.45	—	—
	90	3.79	- 2.62	- 34.55	59.2	23.8	35.4	2.25	33.3
	135	2.26	- 2.22	- 49.15	35.3	10.6	24.7	2.25	51.2
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Table II. *Ratio of glucose fermentation/fructose fermentation in separate and mixed solutions.*

Yeast	Exp.	Remarks	$K_{G/F}$ (for mixture)	Ratio for rate of separate ferm. gluc./fruct.	Ratio invert sugar/ fructose
Lager	No. 1.	Orig. (12. vii. 1929)	2.10	1.06	1.03
		Orig. (22. xi. 1929)	2.18	—	—
		Orig. (4. xii. 1929)	2.21	0.91	1.07
		Grown on fructose	2.50	0.98	1.11
	No. 8 a.	Saccharase added	2.30	0.98	1.32
	No. 8 b.	Control	2.25	1.02	1.20
		Enriched in saccharase	2.24	0.77	1.00
		Poor in saccharase (acid treatment)	2.13	1.00	—
Vienna Sauterne		Grown on maltose	3.98	0.98	0.98
		Grown on fructose I	3.12	1.06	1.00
		Grown on fructose II	2.60	1.04	1.01
		Grown on glucose	2.89	0.95	0.95
	No. 5.	Grown on grape juice	2.33	1.15	—
		Recultured on maltose	3.97	1.00	1.03
Berlin Sauterne		Grown on fructose	2.80	0.87	1.16
		Grown on maltose	3.62	1.00	0.97
		Grown on saccharose	2.80	0.89	0.98
	No. 7.	Grown on saccharose (interrupted)	3.85	0.98	1.19
	No. 6.	Grown on fructose	2.90	0.99	1.01
Ale	No. 2.	Orig. (5. xii. 1929)	2.98	1.21	0.85
Baker's	No. 3.	Orig. (10. xii. 1929)	2.46	1.00	0.99
<i>S. Marxianus</i>	No. 4.	Grown on saccharose	4.63	0.98	1.40

The saccharase determination of the Lager yeast yielded a saccharase value [Schneider, 1929] of 0.0044. By the stimulating treatment of Willstätter, Lowry and Schneider [1925] this value was more than doubled; by acid treatment it was reduced to 0.0017. The $K_{G/F}$ of these yeasts did not differ from the values for the original yeasts.

In Exp. 8, 166 mg. purified "Invertase scales" of a saccharase value 0.325, comprising the saccharase content of more than 10 g. yeast, were added to 100 cc. of a Lager yeast fermentation. No change of $K_{G/F}$ was noted.

SUMMARY.

1. The factor $K_{G/F}$ for several types of yeast was determined. All types preferred glucose to fructose at a rate of 2 : 1 or higher.
2. This factor is subject to changes depending on the culture medium used.
3. No correlation was found between selective fermentation and saccharase or maltase content.
4. The selective fermentation of mixtures of mannose plus glucose and mannose plus fructose was studied.

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