CC. SELECTIVE FERMENTATION.

II. FERMENTATION OF SUGAR MIXTURES BY SAUTERNE YEAST.

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Two strains of "Sauterne" yeast, reported in a preceding communication [Sobotka and Reiner, 1930] preferred glucose to fructose in the fermentation of a glucose-fructose mixture. The value of $K_{G/F}$ [Willstätter and Sobotka, 1922], the measure of this preference, was similar to the $K_{G/F}$ of all other strains of *Saccharomyces* investigated. This was at variance with the findings of several French authors [for references, cf. Sobotka and Reiner, 1930], who ascribed to Sauterne yeast a preference for fructose.

We were able to secure a pure culture of authentic Sauterne yeast. This yeast, tested after two passages on a fructose medium, showed a marked preference for fructose ($K_{F/G} = 2-3$), which after five additional passages was greatly increased. The high figures obtained for $K_{F/G}$ ranged from 6 to 12; they show greater variations than the lower values in other experiments for analytical reasons. Besides, $K_{F/G}$ drifted towards lower values during the rapid disappearance of fructose from the fermenting solution.

When this yeast, after twelve passages on fructose, was subjected to two passages on maltose, its $K_{F/G}$ value dropped to 1.15, rising to 3.6 during 5 hours' contact with invert sugar. Thus, the Sauterne yeast may be said to prefer fructose to glucose at an average rate of 4-6, but this ratio is not stable and might readily be changed by adaptation to values varying from little more than unity to very high ones.

When using a mixture of equal amounts of glucose and mannose, $K_{G/M}$ started at 1.5 and dropped to unity. In a fructose-mannose experiment, $K_{F/M}$ varied between 4.25 and 3.15. The figures in both experiments suggest an adaptation to mannose. From $K_{F/G} = K_{F/M}/K_{G/M}$, a $K_{F/G}$ of about 3 might be derived in agreement with the average values obtained in the direct experiment.

Separate fermentations.

The rates of fermentation of glucose, fructose and invert sugar did not differ in most instances more than 10 % from each other with the yeasts previously investigated [Sobotka and Reiner, 1930, Table II]. The rates for the present strain of Sauterne yeast are given in Table II. The differences in

favour of fructose are very pronounced, fructose being fermented more than 20 % faster than invert sugar and from 50 to 100 % faster than glucose.

It is possible that the capacity of this yeast to initiate the alcoholic fermentation of glucose is so much reduced that the cells in a pure glucose solution cannot utilise to their full extent the enzymes involved in the liberation of carbon dioxide and ethyl alcohol. In other yeasts, both glucose and fructose are converted at such a rate into a three-carbon intermediate compound that the latter is supplied in excess to the enzymic apparatus governing the last stages of alcoholic glycolysis.

The bearing of the anomalies in alcoholic fermentation by Sauterne yeast upon other peculiarities in the metabolism of this micro-organism is being investigated.

EXPERIMENTAL.

The methods used were essentially the same as those described in the previous paper [1930]. The medium contained 5–7 % of the sugar, besides 1.0 % KH_2PO_4 , 1.0 % peptone, 0.1 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.04 % CaSO_4 . The yeast was transferred every 3 days to fresh media. The method of Bertrand was used for the titrimetric determinations.

Table I gives representative experiments for the fermentation of invert sugar by various specimens of Sauterne yeast. For convenience, the $K_{F/G}$ values were used instead of their reciprocals $K_{G/F}$. The column headed $K'_{F/G}$ gives this value in those experiments in which the value of $K_{F/G}$ changed, signifying an acute change of specificity. While

$$K_{F/G} = \frac{\log f_0 - \log f_n}{\log g_0 - \log g_n}, \quad K'_{F/G} = \frac{\log f_n - \log f_{(n+1)}}{\log g_n - \log g_{(n+1)}}.$$

Exps. 1 and 2 illustrate the increasing preference for fructose.

As the concentration of alcohol is 3 %, after fermentation of the 6 % sugar solution used in these experiments, we tried to ascertain whether or not the drop of $K_{F/G}$ from 12 to about 6 in Exp. 2 was due to the increasing amount of alcohol. Exp. 3 parallels Exp. 2 except that 3 % ethyl alcohol was added at the beginning. Since the selectivity for fructose and its drop during fermentation coincided within the limits of error, we conclude that alcohol has no influence.

Exp. 4 demonstrates the loss of fructose specificity when the yeast was kept in the ice-chest for 3 days after removal of the medium by centrifuging and washing.

Exp. 5 shows adaptation to glucose in a maltose medium, but subsequent re-adaptation to fructose during the invert sugar experiment.

The fermentation of glucose-mannose and fructose-mannose mixtures by crop "Fructose IV" is demonstrated in Exps. 7 and 8. When compared to Lager yeast, glucose and fructose seemed to have changed their rôle. With Lager yeast, $K_{G/M}$ varied between 4.5 and 3.0, while $K_{F/M}$ was little more than unity; with Sauterne yeast, $K_{F/M}$ ranges between 4.25 and 3.15, while glucose is fermented only slightly faster than mannose.

Table I. *Fermentation of sugar mixtures with Sauterne yeast.*

Yeast and temp.	Time min.	Sugar (actual %)	Optical rotation $l=2$ dm.	Specific rotation	% of total original sugar unfermented			K_F/G	K'_F/G	CO_2 %
					Total sugar	Glucose	Fructose			
1. Sauterne, fructose II and III, 30°	0	6.00	-1.90	-15.8	100.0	51.1	48.9	—	—	0
	90	5.50	-1.50	-13.6	91.7	48.3	43.4	2.10	—	10.7
	215	3.79	-0.23	-3.0	63.2	38.1	25.1	2.20	—	25.3
	510	1.73	+1.23	+35.6	28.8	25.3	3.5	(3.70)	—	50.9
2. Sauterne, fructose VII, 29°	0	6.29	-2.04	-16.2	100.0	51.2	48.8	—	—	0
	140	4.70	+0.29	+3.1	74.8	48.5	26.3	11.45	—	8.0
	290	4.23	+0.96	+11.4	67.3	47.6	19.7	12.60	15.2	18.5
	470	3.12	+1.51	+24.2	49.6	39.7	9.9	6.20	3.7	26.5
	1440	1.02	+1.06	+52.0	16.2	16.2	0.05	(6.00)	—	81.5
3. Sauterne, fructose VII +3% alcohol, 29°	0	6.40	-2.00	-15.6	100.0	51.6	48.4	—	—	0
	140	5.22	-0.11	-1.6	81.5	50.2	31.3	15.80	—	9.3
	290	4.49	+0.61	+6.8	70.2	47.4	22.8	8.90	5.6	20.4
	470	3.35	+1.69	+25.2	52.4	42.2	10.2	7.75	6.9	29.6
	1440	1.72	+1.67	+48.7	26.9	26.2	0.7	(6.25)	—	78.4
4. Sauterne, fructose VII, 3 days in ice-chest, 29°	0	6.00	-2.21	-18.4	100.0	50.3	49.7	—	—	0
	165	4.18	-1.04	-12.4	69.7	38.0	31.7	1.60	—	21.5
	255	3.53	-0.30	-5.2	58.9	35.0	23.9	2.00	—	35.3
	485	1.48	+0.40	+13.6	22.7	16.5	6.2	1.85	—	57.0
5. Sauterne, maltose II, 27°	0	5.90	-2.26	-19.2	100.0	49.7	50.3	—	—	0
	105	4.64	-1.66	-17.9	78.7	39.8	38.9	1.15	—	14.1
	205	3.91	-0.91	-11.6	66.3	36.4	29.9	1.70	2.9	29.5
	265	3.14	-0.31	-4.9	53.1	31.6	21.5	1.90	2.4	36.2
	340	2.65	+0.13	+2.4	44.9	29.1	15.8	2.20	3.6	44.2
K_G/F										
6. Berlin strain, fructose, 27°	0	5.94	-2.44	-20.5	100.0	48.5	51.5	—	—	0
	90	4.71	-2.57	-27.3	79.3	34.7	44.6	2.30	—	15.4
	160	3.76	-2.43	-32.3	63.3	25.4	37.9	2.10	—	28.4
	205	3.08	-2.55	-41.4	51.8	17.5	34.3	2.50	—	37.6
	245	2.60	-2.30	-44.3	43.8	13.8	30.0	2.30	—	45.7
Glucose Mannose K_G/M										
7. Sauterne, fructose IV, 27°	0	6.55	+4.18	+31.9	100.0	45.7	54.3	—	—	0
	155	5.59	+3.50	+31.3	85.3	37.6	47.7	1.50	—	11.1
	570	3.35	+2.15	+32.1	51.1	23.6	27.5	1.05	—	38.9
	1185	1.63	+1.05	+32.2	24.9	11.5	13.4	1.00	—	59.2
Fructose Mannose K_F/M										
8. Sauterne, fructose IV, 27°	0	6.23	-4.48	-36.0	100.0	48.7	51.3	—	—	0
	155	5.29	-3.24	-30.6	84.9	36.9	48.0	4.20	—	19.8
	400	3.72	-1.34	-18.0	59.7	18.7	41.0	4.25	—	48.8
	570	2.67	-0.51	-9.6	42.7	9.9	32.8	3.55	—	61.2
	1185	1.19	+0.14	+5.9	19.1	1.6	17.5	3.15	—	72.2

Table II. *Ratio of separate fermentation of hexoses with various specimens of Sauterne yeast.*

Yeast	No.	K_F/G (for mixture)	Ratio for separate fermentation			
			Fructose Glucose	Invert Glucose	Fructose Mannose	Glucose Mannose
"Fructose II, III"	1	2.15	1.36	1.12	—	—
"Fructose IV"	7, 8	3*	1.75	—	1.30	0.74
"Fructose XVI"	—	>10	2.18	1.61	1.62	0.75
"Fructose XVII"	—	>10	2.18	—	1.27	0.58
"Fructose VII"	4	1.8	1.46	1.30	—	—
3 days in ice-chest						
"Maltose II"	5	1.15-2.2	1.53	1.37	—	—
Berlin strain	6	0.44	0.98	1.00	—	—

* Extrapolated from $K_F/M/K_G/M$.

After the conclusion of this series, several passages were made from the pure culture of Berlin Sauterne used in our previous communication. It had retained its preference for glucose as shown in Exp. 6 of the present Table I.

The rates of separate fermentation from which the figures in Table II are derived were determined in the Kluyver apparatus. The concentrations of sugar (6 %) and yeast (2 % dry weight) were the same as in the experiments of Table I.

SUMMARY.

Genuine Sauterne yeast ferments fructose preferably to glucose in a mixture. $K_{F/G}$ varies from 1.2 to values above 10 depending on adaptation phenomena. It ferments fructose alone 50 to 100 % faster than glucose alone. The mechanism of this anomalous behaviour is discussed.

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REFERENCES.

- Sobotka and Reiner (1930). *Biochem. J.* **24**, 926.
Willstätter and Sobotka (1922). *Z. physiol. Chem.* **123**, 170.