and 75 g. aspartic acid were obtained after 36 hours of electrolysis. The acid was then recrystallized and analyzed.

STUDIES ON ACIDS FORMED BY RHIZOPUS SPECIES. PART II.

FORMATION OF ETHYL ALCOHOL FROM TARTARIC OR FUMARIC ACID.

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Further five species of *Rhizopus* of various origin were studied. One of them, *Rh. japonicus* Saito, formed mainly fumaric acid with trace of lactic acid, while *Rh. shangheiensis* Yamazaki gave both these acids in a reversed proportion. The other three species, *Rh. nodosus* Yam., *Rh. Batatas* Nakazawa, *Rh. Tritici* Saito formed both lactic and fumaric acids.

Lactic acid formed by these strains was *l*-lactic acid as we have mentioned in the previous report.

In the volatile products there were found beside ethyl alcohol both formic and acetic acids. The quantitative determination of acetic acid was made of this distillate after the destruction of formic acid by K-bichromate and sulphuric acid. The quantity of formic acid was calculated from the difference of the total acidity and that due to the acetic acid.

In a previous report the presence of malic acid was mentioned from the result of qualitative test. This acid has now been isolated and accurately identified by Dakin's⁽¹⁾ method. Cinchonin-*l*-malate thus obtained melted at 197–198°C (uncorr.), as given by Dakin.⁽²⁾

The results of the analyses are shown in the following table :—

Species of Rhizopus.	R. japonicus	R. nodosus	R. Batatas	R. Tritici	R. shang- heiensis
Weight of fungus in 1 L. medium.	1.109 gr.	1.320	1.268	1.455	1.002
Sugar consumed.	38.4 gr.	47.5	46.8	37.8	54.0
Acidity of medium expressed in c.c. of $\frac{1}{10}N$ NaOH to neutralize 10 c.c. of it.	14.4 c.c.	22.2	24.6	18.6	21.6
Quantity of acid pptd as Pb-salt in the residue of ether extract expressed in c.c. of $\frac{1}{10}N$ NaOH.	32.8 c.c.	6.8	3.3	24.2	4.9
Quantity of volatile acid from 1 L. medium expressed in { Formic acid. c.c. of $\frac{1}{10}N$ NaOH. Acetic acid.	1.0 c.c.	7.8 12.2	2.0 8.0	13.4 11.6	2.3 1.7
Alcohol formed (wt. %)	0.67%	0.16	0.29	0.54	0.23
Acid in ether extract from 1 L. medium. { Fumaric acid. Lactic acid.	6.07 gr. trace	0.105 17.1 gr.	0.059 18.0	0.041 13.9	trace 16.5
Zn-lactate { Water of crystallisation. [α] _D ²⁰		13.05% + 6.75°	13.14 + 7.1	12.91 + 6.81	13.21 + 6.85
Remarks	Ba-salt, soluble in 80% alcohol, obtained from the ether extract was 0.03 g. Malic acid obtained as cinchonin-salt was 0.02 g.				
	Ba-salt insoluble in 80% alcohol obtained from the ether extract weighed 0.36g.				

The formation of lactic acid and alcohol from fumarate was affirmed in the culture of Rh. G. 36 in the medium consisting of: water 100 c.c., peptone 0.3 g. and K-fumarate 2.5 g. besides mineral matters. Lactic acid amounting to 0.063 g. was determined by Ripper's method.⁽³⁾ The quantity of ethyl alcohol was too small to be determined quantitatively although it

(1) and (2). Dakin: Jourual of Biol. chem. Vol LIX, No 1, p. 7. 1924.

(3) Ripper: Bioch. Zeit. 42, S. 91–104, 1912.

gave iodoform when treated by iodine and sodium hydroxide. The change of fumaric acid into lactic acid may be shewn in accordance with equation :-

As to the production of ethyl alcohol the most probable explanation is the intermediate formation of pyruvic acid from lactic acid, as already proved by Kayser⁽⁴⁾ in the case of yeasts.

The change of tartarate into fumarate and alcohol was confirmed by the culture of Rh. Oryza in the medium consisting of: water 1000 c.c., K₂HPO₄ 0.15 g., KH₂PO₄ 0.15 g., MgSO₄ 0.1 g., CaCl₂ 0.1 g., Fe₂Cl₆ and NaCl trace, Na or K-tartarate 20 g., peptone either 1 or 3 gr., or some times 2 g. and CaCO₃ 20 g.

Fumaric acid isolated from this culture melted at 279°C (uncor.) in a sealed tube and gave a characteristic colour reaction found by the authors.⁽⁵⁾ Its silver salt dried at 110°C was analysed and gave the following data :-

Subst. taken.	AgCl.	Ag	as	C ₄ H ₂ O ₄ Ag ₂
0.2485 g.	0.2242 g.	Found		65.57 %
		Calcul.		65.44 %

Its dimethyl ester melted at 100-101°C. The production of the fumaric acid from tartaric acid may be expressed by the following mode :-

The formation of ethyl alcohol by this fungus seems to take place most reasonably in accordance with the equations I and II. If we accept ~~ethers~~ the old theory that lactic acid is an intermediate product of alcoholic fermentation is proved in this case too, as in the case of yeast done by Kayser.⁽⁴⁾

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(4) Kayser : Comp. R. Tome 176. No. 22. p. 1663. 1923.

(5) Authors method will be published later.