adsorption approaches this state. Assuming a Maxwellian distribution of energy among the adsorbed molecules of oxygen, then only those molecules possessing the necessary energy of activation, E, will react with the carbon surface; thus,

$$Ae^{-E/RT} = K\,(\Omega' - \Omega)$$
$$\text{and } \log \frac{A}{\Omega' - \Omega} = \frac{E}{R}\left(\frac{1}{T}\right) + \log K$$

Since the energy of activation, E, of the reaction is probably constant after the induction period during which the active points on the surface are destroyed, then $\log A/(\Omega' - \Omega)$ should be practically a linear function of $1/T$. The values of $A/(\Omega' - \Omega)$ are plotted semi-logarithmically against the reciprocal temperature in Figure 3 for various types of carbon.

Literature Cited

(1) Adkins, H., and Connor, R., *J. Am. Chem. Soc.*, 53, 1092 (1931).
(2) Cain, J. R., and Maxwell, L. C., J. Ind. Eng. Chem., 11, 852 (1919).
(3) Day, J. E., and co-workers, *Ibid.*, 28, 234 (1936).
(4) Detling, K. D., and Day, J. E., paper presented before Division of Physical and Inorganic Chemistry at 89th Meeting of American Chemical Society, New York, N. Y., April 22 to 26 (1935); Detling, doctor's dissertation, Ohio State Univ., 1934.
(5) Hitchcock, L. B., Ind. Eng. Chem., 26, 1158 (1934).
(6) Leopold, H. G., and Luck, J. M., *J. Am. Chem. Soc.*, 55, 2655 (1933).
(7) Mayers, M. A., *Ibid.*, 56, 1879 (1934).
(8) Taylor, H. S., and Kistiakowsky, G. B., *Ibid.*, 49, 2470 (1927).

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PRODUCTION OF KOJIC ACID FROM XYLOSE BY ASPERGILLUS FLAVUS

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A NUMBER of investigators (*4, 7, 9, 10, 13, 14, 22, 23, 26, 28–31*) have shown that kojic acid, 2-hydroxymethyl-5-hydroxy-γ-pyrone, is produced during the growth of different species of *Aspergilli* upon solutions of a variety of organic substances. Of the carbon sources tested, xylose was used with varying success as to the yields obtained (*1, 2, 3, 5, 8, 11, 27*).

The purpose of this investigation was to determine the effects of environmental changes upon the quantity of kojic acid produced by a strain of *Aspergillus flavus* growing upon xylose solutions and to learn if any change occurs in the stability of that strain of *A. flavus* over a period of 4 years with regard to kojic acid production. One series of experiments was carried out in 1931, a second in 1935.

The mold employed was probably introduced into the laboratory on pods of *Gymnocladus sp.* and was identified as an *A. flavus* by Charles Thom of the Bureau of Chemistry, United States Department of Agriculture.

Xylose, procured from the Swann Corporation, Birmingham, Ala., was a technical product containing a slight acid residue and a small quantity of a second impurity which reduced Fehling solution and was optically active. Quantities obtained at different times varied to some extent as to the quantity of acid required to bring fermentation media to a given initial pH.

The experimental results of a study of the effects of environmental changes upon the ability of a strain of Aspergillus flavus to produce kojic acid from xylose are presented. Yields corresponding to over 20 per cent weight per weight conversion of xylose were obtained consistently. The fermentation process is similar to that in which citric acid is made commercially and, likewise, could probably be adapted to the production of kojic acid in commercial quantities.

The structure of kojic acid is such that it lends itself to the preparation of numerous derivatives; probably this acid will prove a useful substance either through its known derivatives or those to be prepared in the future.

Methods

Stock cultures were maintained continuously on a dextrose–corn meal agar medium of the following composition in grams per liter: ammonium nitrate, 1.0; potassium dihydrogen phosphate, 0.6248; $MgSO_4{\cdot}7H_2O$, 0.5; dextrose, 200; gelatin powder, 10; and Bacto corn meal agar, 19. Transfers were made at regular intervals. Previous to the fermentation experiments, the stock mold was transferred to and grown for several generations upon a xylose–corn meal agar medium, the composition of which was the same as that just given, except that the dextrose was replaced by 150 grams of xylose per liter. Spores developed on this medium were used to inoculate the xylose solutions to be fermented.

Fifty cubic centimeters of prepared xylose solutions were fermented in 125-cc. Erlenmeyer flasks in which the ratio of area to volume was approximately 0.6, and the flasks and contents were autoclaved for 15 minutes at 15 pounds per square inch (1.05 kg. per sq. cm.) pressure before inoculation. All fermentations were run in duplicate and all runs were repeated.

Evaporation and absorption by the mycelial mats somewhat reduced the volume of the solutions from the original 50 cc. When fermentation was complete, the mats were removed and washed with water sufficient to restore the original volume. Two separate 5-cc. aliquots were taken from the fermented liquor of each flask, and each one was diluted with water to 50 cc. The kojic acid in one aliquot was measured by titrating with 0.1 N sodium hydroxide, using phenolphthalein as an indicator, and titrating to full red color over a source of diffused light. In the second aliquot the kojic acid was neutralized with 0.1 N sodium hydroxide and precipitated with 0.1 N copper acetate; the precipitate was dried at 100° C. and weighed as its copper salt, $Cu(C_6H_5O_4)_2{\cdot}1/2\ H_2O$ (*13*). With solutions of carefully purified kojic acid, these methods checked well with each other and with

TABLE I. EFFECT OF PHOSPHATE VARIATION (1931)

(Composition of media, grams per liter: xylose, 150; NH_4NO_3, 2.25; $MgSO_4 \cdot 7H_2O$, 0.5)

	KH_2PO_4, Grams per Liter:							
Time, Days	0.469	0.625	0.781	0.937	1.093	1.250	1.406	1.562
	Kojic Acid, Grams per Culture							
6	0.19	0.47	0.62	0.61	0.62	0.67	0.47	0.50
7	0.46	0.69	0.71	0.75	0.82	0.89	0.69	0.74
9	0.79	0.97	0.99	0.98	0.98	1.09	1.04	0.93
11	1.01	1.11	1.12	1.07	1.11	1.10	1.05	0.99
12	1.19	1.30	1.26	1.10	1.14	1.14	1.05	1.02
13	1.41	1.44	1.29	1.13	1.06	1.09	1.11	1.04
14	1.37	1.31	1.23	1.10	1.04	0.94	1.08	..

TABLE II. EFFECT OF PHOSPHATE VARIATION (1935)

(Composition of media, grams per liter: initial pH extremes, 3.5 to 3.7; xylose, 150; NH_4NO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5)

	KH_2PO_4, Grams per Liter:									
Time, Days	0.0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
	Kojic Acid, Grams per Culture									
4	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
6	[a]	0.24	0.55	0.55	0.43	0.43	0.30	0.42	0.38	0.31
8	[a]	0.60	0.95	1.05	0.89	0.80	1.12	0.89	1.21	0.80
10	[a]	1.17	1.37	1.33	1.31	1.20	1.21	1.48	1.06	1.09
13	[a]	1.48	1.27	1.31	1.26	1.26	1.30	1.38	1.27	1.32
16	[a]	1.04	0.72	0.82	0.75	0.73	1.09	0.80	0.83	0.82

[a] No more than traces of kojic acid.

TABLE III. EFFECT OF AMMONIUM NITRATE VARIATION (1931)

(Composition of media, grams per liter: xylose, 150; KH_2PO_4, 0.625 and 1.250; $MgSO_4 \cdot 7H_2O$, 0.5)

	NH_4NO_3, Grams per Liter:							
Time, Days	0.50	1.00	1.50	1.75	2.25	2.75	3.25	3.75
	Kojic Acid, Grams per Culture							
	0.625 Gram KH_2PO_4 per Liter							
7	..	..	..	..	0.21	0.07	0.04	
9	..	..	..	..	0.60	0.64	0.56	
10	0.12	1.70	0.98	1.31	..	..	..	
11	..	..	..	..	1.29	0.99	0.85	
13	0.23	2.07	1.65	1.66	1.45	1.02	0.67	
16	..	..	..	..	1.31	0.77	0.43	
17	0.29	1.93	1.41	1.17	..	..	..	
	1.250 Grams KH_2PO_4 per Liter							
7	..	..	..	..	0.67	0.73	0.57	0.33
8	..	..	..	..	0.83	0.79	0.58	0.28
10	0.09	1.45	1.54	1.46	..	..	..	..
11	..	..	..	..	1.01	0.68	0.34	0.04
13	0.12	1.88	1.37	1.22	..	..	..	..
17	0.20	1.60	0.82	0.74	..	..	..	..

TABLE IV. EFFECT OF AMMONIUM NITRATE VARIATION (1935)

(Composition of media, grams per liter: initial pH extremes, 3.45 to 3.50; xylose, 150; KH_2PO_4, 0.625; $MgSO_4 \cdot 7H_2O$, 0.5)

	NH_4NO_3, Grams per Liter:									
Time, Days	0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25
	Kojic Acid, Grams per Culture									
4	..	..	..	..	..	..	..	..	..	..
6	..	..	..	0.24	0.85	0.80	0.55	0.40	0.50	0.43
8	..	..	..	0.38	1.35	1.11	1.11	1.04	0.71	0.40
10	..	..	..	0.84	1.49	1.29	0.92	0.92	0.84	0.86
13	..	..	..	1.25	1.24	0.87	0.43	..	..	..
16	..	..	0.46	1.27	0.82	0.40	..	..	..	..

TABLE V. EFFECT OF pH VARIATION (1935)

(Composition of media, grams per liter: xylose, 150; NH_4NO_3, 1.0; KH_2PO_4, 0.625; $MgSO_4 \cdot 7H_2O$, 0.5)

	Initial pH:									
Time, Days	2.25	2.50	2.75	3.20	3.45	4.60	5.10	5.40	5.75	5.90
	Kojic Acid, Grams per Culture									
4	..	..	..	..	..	..	..	..	..	..
6	0.81	0.57	0.52	0.42	0.37	0.28	..	..	..	..
10	1.59	1.76	1.01	1.41	1.57	1.30	1.45	1.16	1.02	0.77
13	1.82	1.79	1.11	1.14	1.50	1.64	1.65	1.70	1.66	1.42

calculated values. With experimental solutions, however, the titration method gave results which were high approximately to the extent of the buffer effect, which varied with the conditions of the experiment from 0.4 to 1.5 cc. After the sixth day of fermentation the difference between analyses of a sample by the two methods was no greater on the average than 0.7 cc. The kojic acid analytical data listed in the tables were obtained by the copper precipitation method and represent averages.

Residual xylose, when measured, was determined by means of the polariscope. The pH adjustments of solutions to be fermented were made by additions of 0.1 *N* sulfuric acid or 0.1 *N* potassium hydroxide. The pH measurements of fermented solutions were made with the quinhydrone electrode. The temperature of fermentation was 35° C.

The following factors affecting mold growth were varied individually: potassium dihydrogen phosphate, ammonium nitrate, pH, xylose, $MgSO_4 \cdot 7H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, and time of fermentation. The results obtained in 1931 and 1935 are presented. Because of the necessity of checking back on the variables in the earlier part of the work, the 1931 results cannot always be compared with each other or with those of 1935. The 1935 results are comparable.

Influence of Phosphate Concentration

The ammonium nitrate concentration used in the 1931 phosphate experiments was chosen because it had been found to give the best kojic acid yield over an arbitrarily chosen 10-day period. In subsequent experiments of the same year, the smaller concentration (1.0 gram per liter) was found to be optimal and was used, therefore, in the 1935 experiments.

The general effect of phosphate increase from a certain minimum was to increase the rate of fermentation and to decrease the yield. However, within the range of phosphate concentrations used, there developed consistently in the 1931 experiments (Table I) two concentrations favorable to kojic acid formation at roughly 0.6 and 1.2 grams potassium dihydrogen phosphate per liter. It was thought desirable to use both of these concentrations as a basis for the variation of the other constituents even though there was considerable difference between the kojic acid yields corresponding to the two apparent maxima. A repetition of the phosphate experiments in 1935 (Table II) showed a widening of the interval between the maxima.

Influence of Ammonium Nitrate Concentration

In preliminary experiments, different inorganic salts containing the ammonium and nitrate radicals were tested for their suitability in this fermentation. Salts containing the ammonium group were found to be much the better (*6*), and ammonium nitrate seemed to be particularly well adapted (*3*, *13*, *26*).

The data given in Tables III and IV afford a good comparison between the 1931 and 1935 fermentations. The most favorable ammonium nitrate concentration was 1.0 gram per liter, above which the yield gradually decreased. This behavior held for both low and high phosphate concentrations, although the low phosphate gave a slightly higher maximum yield. Increased kojic acid production resulting from the use of lower concentrations of available nitrogen than are usually employed in culture media appears to bear out the observations of Kinoshita (*10*), Kluyver (*11*), and Currie (*6*).

Influence of pH Variation

Results of the 1931 experiments led to the conclusion that the optimum pH for kojic acid production was the lowest that the strain of mold would tolerate. In almost all trials the most favorable initial pH was approximately 3.5; however, in a few isolated cases yields were obtained as high as that corresponding to 36 per cent weight per weight conversion of xylose in solutions where the initial pH was well down toward 2.0. Tamiya (*26*) concluded 5.5 to be the optimum pH.

The 1935 data (Table V) confirm this conclusion generally but give the additional information that good yields resulted not only at the initial pH 3.5 but also consistently within the lower 2.0 to 3.0 pH range (*8*, *9*, *11*).

Influence of Xylose Concentration

In the conversion of xylose into kojic acid, there was little difference between the 150 and 200 grams per liter xylose concentrations on the low phosphate concentration (Tables VI and VII); however, on the high phosphate concentration the 200-gram concentration was the more efficient (Table VI). Where only 50 grams of xylose per liter were used, all of the sugar was utilized by the organism for vegetative growth and as a source of energy. Xylose disappeared entirely from those solutions containing 150 grams or less per liter when fermentation was allowed to proceed 22 days.

Influence of Magnesium Concentration

After a minimum concentration of $MgSO_4 \cdot 7H_2O$ (0.1 gram per liter) was reached, further increase had relatively little effect although a continuous increase or decrease in yield did not follow a continuous increase of $MgSO_4 \cdot 7H_2O$ (Table VIII).

The fermentation of xylose solutions with this strain of *A. flavus* was almost always accompanied by the formation of a small quantity of a yellow compound with indicator properties. The quantity of this substance was appreciably increased in those solutions, the $MgSO_4 \cdot 7H_2O$ concentration of which ranged between 0.00 and 0.05 gram per liter.

Influence of Iron and Calcium Concentrations

Additions of iron as $Fe(NO_3)_3 \cdot 9H_2O$ and calcium as $Ca(NO_3)_2 \cdot 4H_2O$ were inhibitory in an irregular manner except at one calcium and two iron concentrations where there were no appreciable effects. This behavior does not eliminate possible stimulating influences of traces of iron and calcium which might have been present in the materials of which the solutions were made.

These results are in harmony with Currie's conclusion (*6*) regarding the influence of iron in citric acid production but not with that of Di Capua (*7*) regarding the influence of iron in kojic acid production.

The data in Table IX show also that continuous increases in the concentrations of iron and calcium did not occasion a continuous increase or decrease in yields.

Influence of Zinc Concentration

An accumulation of experimental evidence indicates that a small concentration of zinc stimulates the growth of *A. niger* (*12, 16, 18, 20*) and *A. flavus* (*15*), and is even required for normal growth and development (*19, 25*). Porges (*17*) showed that zinc favors acid production by *A. niger* although Currie (*6*) had concluded earlier that zinc is unnecessary for that purpose.

Because of the seeming importance of zinc in mold metabolism, comparative experiments were carried out to find what influence zinc might have upon kojic acid production from xylose by *A. flavus*. For this purpose a concentration of 2 to 3 parts per million of zinc ion furnished by $ZnSO_4 \cdot 7H_2O$ was chosen. Previously this concentration of zinc with the same strain of *A. flavus* had markedly increased kojic acid yields with another carbon source (*24*).

The results of the experiments with xylose media containing zinc were identical with those without zinc, showing that zinc at this concentration range has no effect upon the production of kojic acid from xylose. Spore formation was retarded to some extent; the mats were somewhat thinner and possessed a whitish appearance, especially in the earlier stages of fermentation.

TABLE VI. EFFECT OF XYLOSE VARIATION (1931)

(Composition of media, grams per liter: NH_4NO_3, 2.25; KH_2PO_4, 0.625 and 1.250)

	Xylose, Grams per Liter:							
	150	200	250	300	150	200	250	300
Time, Days	Kojic Acid, Grams per Culture				Per Cent Yield Kojic Acid, per Weight			Weight
	0.625 Gram KH_2PO_4 per Liter							
6	0.29	0.14	0.03	..	3.90	1.40	0.25	...
8	0.63	0.56	0.33	0.21	8.40	5.60	2.60	1.40
10	1.13	1.16	0.96	0.87	15.10	11.60	7.70	5.80
12	1.42	1.60	1.79	1.27	18.90	16.00	14.30	8.50
16	1.63	2.17	2.54	2.50	21.70	21.70	20.30	16.70
18	1.34	2.05	2.39	2.54	17.90	20.50	19.10	16.90
	1.250 Grams KH_2PO_4 per Liter							
6	0.31	0.20	0.22	..	4.10	2.00	1.75	...
8	0.92	0.69	0.84	0.62	12.30	6.90	6.70	4.10
10	0.95	1.03	1.41	1.17	12.70	10.30	11.30	7.80
12	1.04	1.68	2.11	1.99	13.90	16.80	16.90	13.30
16	0.93	1.85	2.29	2.71	12.40	18.50	18.30	18.10
18	0.91	1.65	1.74	2.45	12.10	16.50	13.90	16.30

TABLE VII. EFFECT OF XYLOSE VARIATION (1935)

(Composition of media, grams per liter: initial pH extremes, 3.5 to 3.9; NH_4NO_3, 1.0; KH_2PO_4, 0.625; $MgSO_4 \cdot 7H_2O$, 0.5)

	Xylose, Grams per Liter:									
	50	100	150	200	250	50	100	150	200	250
Time, Days	Kojic Acid, Grams per Culture					Per Cent Yield Kojic Acid, per Weight				Weight
4	..	..	..	..	..	..	...	...	...	...
6	..	0.70	0.85	0.67	..	..	14.00	11.30	6.70	...
10	..	0.62	1.56	1.80	1.52	..	12.40	20.80	18.00	12.20
13	..	..	1.31	1.93	2.19	..	...	17.40	19.30	17.50

TABLE VIII. EFFECT OF $MgSO_4 \cdot 7H_2O$ VARIATION (1935)

(Composition of media, grams per liter: initial pH, 3.55; xylose, 150; NH_4NO_3, 1.0; KH_2PO_4, 0.625)

	$MgSO_4 \cdot 7H_2O$, Grams per Liter:									
Time,	0.00	0.005	0.01	0.05	0.10	0.30	0.50	0.70	0.90	1.10
Days	Kojic Acid, Grams per Culture									
4	..	..	..	..	..	..	..	..	..	..
6	..	..	..	0.47	0.60	0.54	0.60	0.63	0.64	0.65
10	..	..	0.80	1.26	1.58	1.51	1.65	1.53	1.65	1.60
13	..	..	1.66	1.14	1.36	1.36	1.56	1.65	1.55	1.56

TABLE IX. EFFECT OF $Fe(NO_3)_3 \cdot 9H_2O$ and $Ca(NO_3)_2 \cdot 4H_2O$ VARIATION (1935)

(Composition of media, grams per liter: xylose, 150; NH_4NO_3, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KH_2PO_4, 0.625; pH extremes, 2.05 to 3.60 when iron was varied, 3.50 to 3.55 when calcium was varied)

		$Fe(NO_3)_3 \cdot 9H_2O$ or $Ca(NO_3)_2 \cdot 4H_2O$, Grams per Liter:								
	Time,	0.005	0.01	0.05	0.25	0.50	1.00	1.50	2.00	2.50
Metal	Days	Kojic Acid, Grams per Culture								
Fe	6	0.43	0.57	0.41	0.21	0.26	0.65	..	..	..
	13	1.41	1.24	1.26	1.07	0.94	1.32	1.47	1.36	..
Ca	6	0.57	0.60	0.37	..	0.35	0.18	..	..	..
	13	1.36	1.43	1.20	0.40	1.14	0.92	0.80	0.72	0.58

TABLE X. EFFECT OF TIME VARIATION (1931)

(Composition of media, grams per liter: initial pH, 3.4; xylose, 150; NH_4NO_3, 2.25; KH_2PO_4, 0.625; $MgSO_4 \cdot 7H_2O$, 0.5)

Time	Kojic Acid per Culture	Xylose Consumed	Weights of Mats	pH
Days	*Grams*	*Per cent*	*Gram*	
3	..	10.7	0.0896	3.06
4	..	21.2	0.2960	2.11
5	0.14	32.0	0.3840	2.13
6	0.48	43.8	0.3845	2.29
7	0.56	48.3	0.3820	2.50
8	0.93	68.0	0.4600	3.60
10	1.43	92.3	0.6679	4.17
11	1.54	93.1	0.7902	4.19
12	1.63	93.4	0.7973	4.29
13	1.58	94.5	0.8365	4.04
14	1.44	94.5	0.8787	4.04
15	1.33	96.1	0.8401	3.90
16	1.32	97.5	0.8451	3.85

TABLE XI. EFFECT OF TIME VARIATION (1935)

(Composition of media in grams per liter: initial pH, 3.5; xylose, 150; NH_4NO_3, 1.0; KH_2PO_4, 0.625; $MgSO_4 \cdot 7H_2O$, 0.5)

Time	Kojic Acid per Culture	pH	Time	Kojic Acid per Culture	pH
Days	*Grams*		*Days*	*Grams*	
4	..	2.45	10	1.65	4.00
6	0.60	3.50	13	1.36	4.10
8	1.15	3.90	16	0.77	4.25

Influence of Time Variation

The rate of kojic acid production was not constant during the progress of fermentation. In 1931 experiments (Table X) an initial 5–7 day unproductive period was usually found. This period was accompanied by a slow rate of sugar consumption and a slow rate of increase in mycelial weight. During this period there was considerable pH adjustment. In the 1935 experiments (Table XI) the lag was formed somewhat earlier. There was also a marked drop in pH consistently from the original over the first 3 or 4 days, and, until there had been a certain amount of pH recovery, fermentation proceeded slowly.

In 1931, maximum yields were obtained on the twelfth or thirteenth day; in 1935 they were obtained on about the tenth day. The fact that the 1931 and 1935 maximum yields are approximately equal is not interpreted as indicating that the concentrations of 1.00 and 2.25 grams of ammonium nitrate per liter are equally favorable, since these and other data (Tables III and IV) appear to show that 4 years of culturing decreased moderately the maximum yield and increased the rate of fermentation.

Discussion of Results

The fermented solutions were analyzed for kojic acid by two different methods—titrating with standard alkali and weighing as the copper salt. The fact that the analytical data for individual samples checked within the limits of the buffer effect leads us to the conclusion that acidic organic compounds other than kojic acid, if present, were in negligible quantities.

Examination of the 1931 and 1935 results shows that 4 years of culturing modified moderately the strain of *A. flavus* in its ability to convert xylose into kojic acid. The interval between the phosphate maxima increased from about 0.6–1.2 in 1931 to 0.2–1.4 in 1935, the organism became tolerant to a greater hydrogen-ion concentration, and the maximum yield was lowered appreciably.

In a number of instances during the course of these experiments, a continuous increase or decrease in yield of kojic acid did not accompany a continuous increase of one of the variants. This behavior was particularly apparent in the variation of the initial pH, iron, calcium, and, to some extent, phosphate and magnesium. It may be objected that in some cases the differences noted are too small to be significant. But in view of the consistency with which repeated experiments indicated this behavior, such variations in yields can scarcely be fortuitous although they may be relatively unimportant numerically. Similar observations were made by Lohmann (*12*) who found optimum zinc concentrations for *A. niger* at 0.8 and at 132 mg. per hundred, and by Rossi and Scandellari (*21*) who found that continuously increasing ammonium nitrate and potassium dihydrogen phosphate concentrations gave first an increase, then a decrease, and finally an increase of invertase production by *A. niger*. A possible explanation may be suggested for this behavior. It is recognized that the availability of phosphate to plant life in general is dependent upon the form in which the phosphate exists which, in turn, is partially dependent upon the heavy metal and hydrogen-ion concentrations. It is conceivable that the combined effects of the heavy metals and hydrogen ion may modify the availability or utilization of phosphate in fermentation processes which may govern the production of invertase or other necessary enzymes, as suggested by the work of Rossi and Scandellari (*20*, *21*).

Summary

1. The fermentation of xylose solutions with a strain of *A. flavus* was carried out over a period of 4 years. The acidic organic product was almost exclusively kojic acid. The medium most favorable to kojic acid production at 35° C. had the following composition in grams per liter: xylose, 150; ammonium nitrate, 1.0; potassium dihydrogen phosphate, 0.625; and $MgSO_4 \cdot 7H_2O$, 0.5.

2. There were two optimal pH values, approximately 2.5 and 3.5.

3. Maximum yields were obtained over intervals of 13 (1931) and 10 (1935) days.

4. A minimum of 150 grams of xylose per liter was required for good kojic acid yields. When fermented for 22 days, the sugar disappeared entirely.

5. The ammonium radical was much better than the nitrate radical in furnishing nitrogen. Ammonium nitrate served excellently for this purpose.

6. Iron and calcium were inhibitory except at one calcium and two iron concentrations where there was no appreciable effect. Zinc exerted no influence.

7. In the phosphate, iron, calcium, magnesium, and pH experiments the continuous increase of a variant was not followed by a continuous increase or decrease in yield of kojic acid, possibly because of variations in the availability of the phosphate.

8. Low magnesium concentrations favor the formation of a yellow compound with indicator properties.

9. Over a period of 4 years there was a moderate change in the stability of the mold with respect to kojic acid production. The interval between phosphate maxima widened, the tolerance of the mold to acid increased, and the maximum yield decreased.

Literature Cited

(1) Birkinshaw, J. H., Charles, J. H. V., Lilly, C. H., and Raistrick, H., *Trans. Roy. Soc.* (London), **B220**, 127–38 (1931).
(2) Challenger, F., *Ind. Chemist*, **6**, 97–100 (1930).
(3) Challenger, F., Klein, L., and Walker, T. K., *J. Chem. Soc.*, **1929**, 1498–1505.
(4) *Ibid.*, **1931**, 16–23.
(5) Corbellini, A., and Gregorini, B., *Gazz. chim. ital.*, **60**, 244–56 (1930).
(6) Currie, J. N., *J. Biol. Chem.*, **31**, 15–37 (1917).
(7) Di Capua, A., *Gazz. chim. ital.*, **63**, 296–302 (1933).
(8) Katagiri, H., and Kitahara, K., *Bull. Agr. Chem. Soc. Japan*, **5**, 38–47 (1929).
(9) Katagiri, H., and Kitahara, K., *Mem. Coll. Agr. Kyoto Imp. Univ.*, No. **26**, 1–29 (1933).
(10) Kinoshita, K., *Acta Phytochim.* (Japan), **3**, 31–50 (1927).
(11) Kluyver, A. J., and Perquin, L. H. C., *Biochem. Z.*, **266**, 82–5 (1933).
(12) Lohmann, G., *Arch. Mikrobiol.*, **5**, 31–56 (1934).
(13) May, O. E., Moyer, A. J., Wells, P. A., and Herrick, H. T., *J. Am. Chem. Soc.*, **53**, 774–82 (1931).
(14) May, O. E., Ward, G. E., and Herrick, H. T., *Zentr. Bakt. Parasitenk.*, II, **86**, 129–34 (1932).
(15) McHargue, J. S., and Calfee, R. K., *Botan. Gaz.*, **91**, 183–93 (1931).
(16) Pirschle, K., *Planta* (*Abt. E, Z. wiss. Biol*), **23**, 177–224 (1934).
(17) Porges, N., *Am. J. Botany*, **19**, 559–67 (1932).
(18) Porges, N., *Botan. Gaz.*, **94**, 197–205 (1932).
(19) Roberg, M., *Zentr. Bakt. Parasitenk.*, II, **84**, 196–230 (1931).
(20) Rossi, G., and Scandellari, G., *Biochem. terap. sper.*, **19**, 157–61 (1932).
(21) *Ibid.*, **22**, 87–91 (1935).
(22) Saito, K., *Botan. Mag.* (Tokyo), **21**, No. 249 (1907).
(23) Sakaguchi, K., *J. Agr. Chem. Soc. Japan*, **7**, 748–62 (1931).
(24) Smits, B. L., unpublished data.
(25) Steinberg, R. A., *Bull. Torrey Botan. Club*, **62**, 81–90 (1935).
(26) Tamiya, H., *Acta Phytochim.* (Japan), **3**, 51–173 (1927).
(27) *Ibid.*, **6**, 1–129 (1932).
(28) Traetta-Mosca, F., *Ann. chim. applicata*, **1**, 477–92 (1914).
(29) Traetta-Mosca, F., *Gazz. chim. ital.*, **51**, II, 269–77 (1921).
(30) Wijkman, N., *Z. physiol. Chem.*, **132**, 104–8 (1924).
(31) Yabuta, T., *Orig. Com. 8th Intern. Congr. Appl. Chem.* (*Appendix*), **25**, 455–62 (1912).

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