

after the rats were given a stock ration in lieu of the milk, copper, and iron diet.

A nitrogen partition in the urine of 3 groups of female rats fed stock ration, milk, and milk plus copper and iron, respectively, indicates that the addition of copper and iron to the milk diet affects the excretion of nitrogen bodies, particularly ammonia, creatine, and creatinine.

8423 P

Effect of Methods of Preparation upon Fermentative Activity of Yeast Zymin.

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Stavely, Christensen and Fulmer^{1, 2, 3, 4} presented data on the effect of several electrolytes and of ethanol upon the fermentative activity and phosphate content of yeast zymin. The zymin was prepared from brewers' yeast (furnished by Anheuser-Busch of St. Louis) by the procedure outlined by Harden.⁵ Five hundred grams of yeast and 3 liters of acetone were stirred together for 10 minutes and filtered on a Buchner funnel. The mass was then mixed with one liter of acetone for 2 minutes and again filtered. The residue was coarsely powdered, stirred with 250 cc. of ether for 3 minutes, filtered, and spread out on paper in a thin layer. After standing for one hour at room temperature it was dried for 24 hours at 40-45°.

The present communication deals with data on several factors as they affect the activity of the zymin preparation. The apparatus of Harden, Thompson and Young,⁶ with some modification, was employed in the determination of the evolution of carbon dioxide. The

¹ Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 877.

² Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 771.

³ Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 785.

⁴ Stavely, H. E., Christensen, L. M., and Fulmer, E. I., *J. Biol. Chem.*, 1935, **111**, 791.

⁵ Harden, A., *Alcoholic Fermentation*, p. 39. Longmans, Green and Co.

⁶ Harden, A., Thompson, J., and Young, W. J., *Biol. Chem. J.*, 1910, **5**, 230.

inorganic phosphate was determined colorimetrically by the revised procedure of Kuttner and Lichtenstein.⁷ Detailed procedure and technique are given in reference 2.

It was noted by Stavely, Christensen and Fulmer² that the fermentative activity of the zymín increased with the time of storage, in the refrigerator, of the yeast from which it was made. The increase was paralleled by increase in free inorganic phosphate and in the rate of esterification of the phosphate in the presence of dextrose. In Tables I and II are given more detailed data with reference to the points noted above. The yeast was stored at 5°. The reaction mixture consisted of 5 gm. zymín, 1 gm. dextrose, 1 cc. toluene, and 25 cc. of water. The fermentative activity increased up to 14 days' storage of the yeast, but at 20 days the preparation was inactive. This increase in fermentative ability is seen to be correlated with an increased rate of esterification of the inorganic phosphate

TABLE I.
Effect of Storage on CO₂ Evolution by Zymín (Mg. of CO₂ Evoluted).

No. Days Storage Time:	0	1	3	5	8	14	20
10	0.0	3.2	1.1	0.0	0.0	0.0	
20	3.3	9.1	15.1	7.4	3.2	5.7	
30	9.1	22.8	28.8	32.5	20.1	16.5	
40	12.2	34.7	42.2	45.2	35.6	30.5	Inactive
50	18.2	46.3	48.8	51.9	46.7	43.4	
60	23.4	52.3	54.1	57.2	55.7	55.6	
70	29.7	56.5	58.3	61.5	60.4	64.5	
90	43.5	66.0	66.4	70.5	70.8	74.3	

TABLE II.
Effect of Storage on Phosphate Esterifying Ability (Mg. Inorganic P per cc.).

No. Days Storage Time:	0	1	3	5	8	14	20
10	0.65	0.62	0.78	0.85	1.08	1.25	1.15
30	0.36	0.32	0.22	0.26	0.72	0.84	1.09
60	0.30	0.18	0.19	0.19	0.18	0.23	1.10
90	0.32	0.24	0.19	0.17	0.15	0.18	—
Change in P (10-90 min.)	0.33	0.38	0.59	0.68	0.93	1.07	—

In the procedure of Harden the material is dried at 40-45° for 24 hours. Two zymín preparations were made, one by the usual procedure, drying at 45° and the other by drying in a vacuum. Such preparations were made from yeast that had been stored 0 and 3 days at 5°. The fermentative activities of these preparations are given in Table III. It is evident that the vacuum drying gives a zymín which shows a greater initial evolution of carbon dioxide and

⁷ Kuttner, J., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

TABLE III.
Effect of Vacuum Drying on CO₂ Evolution by Zymin (Mg. CO₂ Evolved). Zymin 1 had been stored 0 days and Zymin 2 for 3 days.

Time (min.)	Zymin 1		Zymin 2	
	Control 45°	Vacuum Dried	Control 45°	Vacuum Dried
10	0.0	3.5	1.1	8.4
20	3.3	12.3	15.1	36.6
30	9.1	20.8	28.8	48.8
40	12.2	27.8	42.2	57.2
50	18.2	33.2	48.8	61.8
60	23.4	39.8	54.1	69.2
70	29.7	47.5	58.3	74.7
90	43.5	60.9	66.4	86.1

a more rapid decrease in rate after the maximum rate is reached. The maximum and steady rates are higher. The total evolution of carbon dioxide is 30-40% greater than in the control which had been dried at 45°. Parallel experiments also showed a greater rate of esterification of the inorganic phosphate in the presence of dextrose.

Summary. Storage of yeast at 5° varies the fermentative activity of zymin made therefrom. The activity increased up to 14 days' storage but the preparation from yeast which has been stored 20 days was inactive. Drying the preparation in vacuum, instead of the usual temperature of 45° markedly increases the activity of the zymin. The increase in activity with storage of the yeast or by employing vacuum drying is correlated with increased rate of esterification of the inorganic phosphate of the preparation in the presence of dextrose.

8424 C

Effect of Aeration and Lack of CO₂ on Growth of Bacteria-Free Cultures of Protozoa.*

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It has been known for some time that CO₂ is necessary for the growth of bacteria, yeast, and molds on solid media^{1, 2} and recent

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¹ Valley, G., and Bettger, L. F., *J. Bact.*, 1927, **14**, 101.

² Valley, G., *Biol. Rev.*, 1928, **3**, 209.