17265. Effect of Germination on Phytin Content and Phytase Activity of Some Common Indian Pulses.

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Phosphorus present in foodstuffs as phytin is largely unavailable for nutrition. Studies have been made by several workers of the availability of phytin phosphorus. Krieger, Bunkfeldt and Steenbock[1] observed that when rats were fed a cereal ration of normal calcium content the utilization of phosphorus from phytin was markedly enhanced by the addition of vitamin D. Boutwell, Geyer, Halverson and Hart[2] studied the availability of wheat bran phosphorus, which contains about 85% of phosphorus as phytin, in rats and observed that an adequate intake of vitamin D increased the utilization of phytin phosphorus as measured by bone ash. Pringle and Moran[3] and Widdowson[4] reported that phytin in wheat meal or white flour is partly destroyed during the process of preparation of bread. Pulses are rich in phytin. The vitamin content of pulses is found to increase during the process of germination.[5,6] It was, therefore, considered desirable to investigate whether the phytin content of pulses is diminished during germination.

Methods. One gram of clean and dry seeds of pulses were placed in a washed and sterilized petri dish. The seeds in each dish were soaked daily with 1 cc of redistilled water for a period of 5 days. Phytin phosphorus content of the seeds was estimated both before and during the course of germination up to 5 days. Total phosphorus content of the seeds was also estimated.

Total phosphorus. The seeds were finely ground and digested in a Kjeldahl flask with 10 cc of concentrated sulfuric acid and 1 cc of 60% perchloric acid for half an hour till the digestion was complete and the contents were diluted to a definite volume and the total phosphorus content was estimated by the method of Fiske and Subbarow.[7]

Phytin phosphorus. 1 g of the material was extracted with 50 cc of N/2 hydrochloric acid in a glass stoppered bottle for 2 hours and filtered. The phytin present in the filtrate was precipitated as the insoluble iron salt. The insoluble salt was converted to soluble sodium salt which was digested with sulfuric acid and perchloric acid mixture and the inorganic phosphate thus obtained was estimated by the method of Fiske and Subbarow.[7]

Phytase activity. 5 g of pulses, before or during the course of germination, were ground and extracted for 12 hours with 50 cc of water saturated with toluene. The extract was centrifuged. Inorganic phosphorus in a 10 cc portion of the centrifugate, representing 1 g of the pulse, was estimated and to another 10 cc of the extract was added acetate buffer of pH 5.2 and a substrate containing

TABLE I.
Mg of Phytin Phosphorus Present in 100 g of Pulses.

Pulses	Days of germination					
	0	1	2	3	4	5
Phaseolus mungo	156	156	101	96	95	95
Phaseolus radiatus	158	138	117	110	109	109
Cicer arietinum	138	125	117	97	80	80
Dolichos lablab	138	127	114	104	105	105

[1] Krieger, C. H., Bunkfeldt, R., and Steenbock, H., *J. Nutrition*, 1940, **20**, 7.

[2] Boutwell, R. K., Geyer, A. P., Halverson, A. W., and Hart, E. B., *J. Nutrition*, 1946, **31**, 193.

[3] Pringle, W. J. S., and Moran, T., *J. Soc. Chem. Indust.*, 1942, **61**, 108.

[4] Widdowson, E. M., *Nature*, 1941, **148**, 219.

[5] Ahmad, B., Qureshi, A. A., Babbar, I., and Sawhney, P. C., *Ann. Biochem. Exp. Med.*, 1946, **6**, 29.

[6] French, C. E., Berryman, G. H., Goorley, J. T., Harper, H. A., Harkness, D. M., and Thacker, E. J., *J. Nutrition*, 1944, **28**, 63.

[7] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

TABLE II
γ of Phosphorus Liberated from the Substrate Containing 10 mg of Phosphorus in the Form of Sodium Phytate by the Phytase Present in 1 g of the Pulse Either Before or After Germination.

Pulses	Days of germination					
	0	1	2	3	4	5
Phaseolus mungo	39	46	66	82	91	91
Phaseolus radiatus	167	179	200	206	208	208
Cicer arietinum	39	43	47	50	50	50
Dolichos lablab	172	186	191	192	191	192

10 mg of phosphorus as sodium salt of phytin. The mixture was incubated at 35°C for 6 hours. The phosphorus liberated from phytin was estimated by the method of Fiske and Subbarow.[7]

Results. The total phosphorus content in mg per 100 g of *phaseolus mungo, phaseolus radiatus, cicer arietinum* and *dolichos lablab* was respectively 375, 302, 219 and 247. Phytin phosphorus values and phytase activities of the pulses are given in Tables I and II.

Discussion. Phytin phosphorus values of all the 4 varieties of pulses studied were found to decrease gradually during the process of germination and the maximum lowering of the value occurred either on the third or on the fourth day of germination. The phytase activity of the pulses was found to increase during the course of germination and when the phytin content was minimum the phytase activity was maximum. This suggests that the enzyme phytase which is formed during the process of germination acts upon the phytin phosphorus and partly destroys it. It has been observed by French *et al.*[6] that 48 hours after germination of peas there is a considerable increase in the inositol value of the pulse. Phytin being the calcium or magnesium salt of inositol hexaphosphoric acid, the increased inositol value of the pulse after germination might be due to the breaking down of phytin by the enzyme phytase. The germinated pulse is therefore nutritionally superior to the ungerminated one.

Summary. Phytin content and phytase activity of 4 pulses have been estimated both before and for varying periods after germination. Phytin content gradually diminished along with the increase in the phytase activity of the germinating pulse which was maximum either on the third or on the fourth day of germination.

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17266. Creatinine, Potassium, and Virus Content of the Muscles Following Infection with the "Coxsackie Virus."*

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A number of specimens of feces from children exhibiting symptoms of poliomyelitis have yielded viruses that produce degeneration of the skeletal muscles of suckling mice and hamsters.[1,2] The anatomic response in man is not known but the clinical records of patients from whose feces virus was isolated suggest that muscle weakness or paralysis is common and sometimes persists for months. While considering means of testing patients for muscle degeneration, it occurred to us that creatine losses probably accompany the destruction of the striated muscle cells and,

* This agent is being called "Coxsackie Virus" since the first recognized human cases were residents of that New York village.

1 Dalldorf, Gilbert, and Sickles, G. M., *Science*, 1948, **108**, 61.

2 Dalldorf, Gilbert, Sickles, G. M., Plager, Hildegard, and Gifford, Rebecca, *J. Exp. Med.*, 1949, **89**, 567.