LVI. DIGESTIBILITY OF GERMINATED BEANS.

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It is a well known fact that beans contain a comparatively high percentage of protein which is not readily utilised by animals. Recent work by Chick and Hume [1919] has shown that germinated pulses are much richer in vitamins than are the ungerminated seeds. This result suggested the possibility that on germination of a seed changes may occur which affect the digestibility of the protein; experiments were therefore made in order to discover the effect of germination on the digestibility of bean protein.

Haricot, Brown Dutch and Soya Beans were used and they all gave the same results. In the experiments beans were boiled and digested by means of Benger's "Liquor Pancreatin." After regular intervals portions of the digesting mixture were tested by a method adapted from Sørensen's formaldehyde method.

Method of Digestion.

The liquor in which the beans were boiled was poured off and the beans were washed with cold water (1000 cc.) and powdered.

Sodium chloride (0.75 g.) and toluene (10 cc.) were added. Alkali was added until the reaction of the mixture was alkaline to cresol red and acid to phenolphthalein. Benger's pancreatic fluid (25 cc.) was added and the whole made up with water at 37° to a known volume (500 cc. for 50 g. of beans). The flask was shaken well and 50 cc. of the mixture removed for immediate titration. The neck of the flask was plugged with cotton wool and it was placed in a thermostat at 37°. From this digesting mixture portions were removed when required for titration.

Method of Titration.

The portion of the digesting mixture to be tested was filtered. Five drops phenolphthalein were added to 10 cc. of the filtrate, which was then titrated against dilute NaOH till only very faint pink.

To this 5 cc. of a 40 % solution of formaldehyde (containing one drop phenolphthalein and dilute NaOH till faint pink) were added. The resulting mixture was acid and was titrated against $N/5$ NaOH. This titration was

noted; since the excess of formaldehyde caused any free amino-groups to be neutralised and thus rendered the solution acid, the number of cc. in the last titration gave a measure of the amount of free amino-acids formed by hydrolysis of the protein by the enzyme.

In calculating the amount of nitrogen digested it is assumed that 1 cc. of $N/5$ NaOH used in the titration in presence of formaldehyde corresponds with $0.014/5$ g. of N.

Calculation of Percentage Increase in Digestibility.

Average nitrogen digested at end of 24 hrs.

(a) Ungerminated beans (29 expts) 34.6 mg.

(b) Germinated beans (27 expts) 61.7 mg.

(c) Germinated and dried beans (20 expts) 49.1 mg.

Average increase obtained by germinating = 27.1 mg. N. This is from 100 cc. bean solution. Increase from 1000 cc. bean solution, which includes 100 g. beans = 271 mg. N.

By Kjeldahl experiments it was shown that 100 g. beans contain 3.18 g. N, so that the percentage increase in digestibility due to germination is 8.5.

Similarly the average increase by germination followed by drying is 4.6 %.

Preliminary experiments were made in order to ascertain:

- (1) The correct degree of alkalinity for digestion by pancreatic extract.
- (2) The most suitable length of time for boiling the beans.
- (3) The effect of boiling NaOH and fat with beans on the digestibility of bean protein. (In both cases the increase obtained was negligible.)

Results.

As a result of 29 experiments the average increase in nitrogen digestibility, as indicated by resistance to tryptic activity, of germinated over ungerminated beans was 8.5 %.

The increase varied from 15.3%, when the beans were germinated rapidly, to 4.3 %, when only slow growth took place.

The increase in nitrogen digestibility of dried germinated beans over ungerminated beans varied from 7.8 % to 1.2 % according to the rapidity with which drying occurred. The best method of drying appeared to be to expose the beans to heat such as strong sunlight for one day and then to keep them at a temperature of 30°.

In all cases when the germinated beans were even partially dried, their protein became more resistant to tryptic digestion.

From these experiments it is clear that when beans are germinated their protein is rendered appreciably more digestible—on drying, the protein becomes again less digestible.

Tabulated Results.

No. of Expt.	Beans (50 g.) Series 1	Milligrams of Nitrogen digested after			
		(1) 0 hrs.	(2) 24 hrs.	(3) 48 hrs.	(4) 72 hrs.
(1)	Germinated in saturated air for 24 hrs. at 34°	5.9	80.1	96.6	101.9
(2)	Germinated similarly to (1)	7.0	75.0	95.3	101.2
(3)	Ungerminated	6.4	47.9	66.1	71.9
(4)	Ungerminated	6.4	45.1	64.9	71.1
<i>Series 2</i>					
(1)	Germinated in saturated air for 48 hrs. at 34°	6.7	76.9	110.6	130.0
(2)	Germinated similarly to (1)	7.6	90.0	114.3	127.6
(3)	Ungerminated	6.1	56.0	79.3	98.0
(4)	Ungerminated	5.6	59.8	84.0	98.0
<i>Series 3</i>					
(1)	Germinated: dried in hot sun for 1 day then at 30° for 4 weeks	7.0	70.0	82.0	88.9
(2)	Germinated: dried similarly to (1)	8.1	69.2	84.3	92.0
(3)	Ungerminated	5.8	52.1	56.2	70.3
(4)	Ungerminated	5.8	50.1	62.1	67.4
<i>Series 4</i>					
(1)	Germinated in open air in warm weather for 3 days (radicles 1" long)	3.9	57.1	80.0	95.9
(2)	Ungerminated	2.0	31.9	52.1	61.8
(3)	Germinated similarly to (1)	3.9	63.8	84.3	89.5
(4)	Ungerminated	3.1	36.1	54.1	63.8
<i>Series 5</i>					
(1)	Germinated in greenhouse (30° in day- time) for 3 days	5.0	58.5	74.2	82.9
(2)	Germinated similarly to (1)	5.0	48.2	70.0	80.1
(3)	Ungerminated	3.4	36.1	47.9	52.1
(4)	Ungerminated	3.1	32.2	45.4	47.9
<i>Series 6</i>					
(1)	Germinated in open air for 3 days in warm weather	7.0	45.6	65.0	75.9
(2)	Germinated similarly to (1)	5.9	40.0	58.5	73.9
(3)	Ungerminated	2.0	28.6	47.9	61.9
(4)	Ungerminated	2.0	24.1	31.4	44.0
<i>Series 7</i>					
(1)	Germinated for 2 days: dried in hot sun for 1 day, then at 30° for 6 weeks	3.1	54.0	66.1	72.5
(2)	Germinated: dried similarly to (1)	3.1	47.9	61.3	68.0
(3)	Ungerminated	2.0	28.3	42.0	49.8
(4)	Ungerminated	2.0	30.8	42.6	47.9
<i>Series 8</i>					
		(1) 0 hrs.	(2) 12 hrs.	(3) 24 hrs.	(4) 48 hrs.
(1)	Germinated 2 days: dried for 6 weeks at 30°	3.1	23.0	30.8	33.9
(2)	Germinated 2 days: dried for 4 weeks at 30°	3.4	21.0	29.1	36.1
(3)	Ungerminated	2.0	10.9	17.6	24.9
(4)	Ungerminated	2.0	9.0	16.5	23.0

Tabulated Results (continued).

No. of Expt.	<i>Series 9</i>	Milligrams of Nitrogen digested after			
		(1) 0 hrs.	(2) 12 hrs.	(3) 24 hrs.	(4) 48 hrs.
(1)	Germinated in open air in very warm weather for 2 days	5.9	31.9	45.9	61.9
(2)	Germinated similarly to (1)	5.0	30.8	42.0	60.4
(3)	Ungerminated	3.1	12.9	22.1	31.1
(4)	Ungerminated	2.0	17.1	23.0	29.1
<i>Series 10</i>					
(1)	Germinated in greenhouse for 2 days at 24° in daytime	5.0	31.1	37.0	42.0
(2)	Germinated similarly to (1)	5.9	30.8	36.1	43.1
(3)	Germinated similarly to (1): dried in room (15° in daytime) for 4 weeks	3.0	17.9	26.0	33.0
(4)	Ungerminated	2.0	15.1	22.1	26.9
<i>Series 11</i>					
(1)	Germinated in greenhouse for 2 days at 26° in daytime	5.0	40.0	47.0	52.6
(2)	Germinated similarly to (1)	3.9	35.0	46.5	49.0
(3)	Ungerminated	2.5	21.0	26.0	28.3
(4)	Ungerminated	2.0	18.8	23.0	26.6
<i>Series 12</i>					
(1)	Germinated in saturated air at 22° for 2 days	5.9	52.1	63.9	70.0
(2)	Germinated in saturated air at 20° for 2 days	5.0	34.2	56.0	73.9
(3)	Germinated similarly to (1): dried at 30° for 4 weeks	3.9	28.0	42.0	62.2
(4)	Ungerminated	2.0	22.1	30.0	36.1
<i>Series 13</i>					
		(1) 0 hrs.	(2) 24 hrs.	(3) 48 hrs.	(4) 72 hrs.
(1)	Germinated: dipped into boiling water for 2 seconds, dried at 30° for 4 weeks	6.4	41.2	50.7	56.8
(2)	Germinated: dipped, dried in laboratory (15° in daytime) for 8 weeks ...	7.0	33.0	38.1	40.9
(3)	Germinated: dried in laboratory (15° in daytime) for 8 weeks	7.3	29.1	33.0	35.7
(4)	Ungerminated	4.2	26.9	31.9	35.8
<i>Series 14</i>					
		(1) 0 hrs.	(2) 12 hrs.	(3) 24 hrs.	(4) 48 hrs.
(1)	Germinated: dried in hot sun for 1 day then at 30° for 4 weeks	8.4	50.1	61.3	72.0
(2)	Germinated: dipped into boiling water, dried in laboratory for 4 weeks ...	5.9	38.1	48.4	57.7
(3)	Germinated: dried at 30° for 4 weeks ...	6.7	47.6	58.5	65.0
(4)	Ungerminated	3.9	30.5	38.9	44.0
<i>Series 15</i>					
(1)	Germinated in laboratory at 15° in daytime for 3 days	6.4	31.9	50.1	68.3
(2)	Germinated in saturated air for 24 hrs. at 30°	9.0	57.7	71.1	80.6
(3)	Germinated in saturated air for 24 hrs. at 34°	8.4	54.0	75.3	91.6
(4)	Ungerminated	3.9	24.1	33.6	43.4

Tabulated Results (continued)

No. of Expt.		Milligrams of Nitrogen digested after			
		(1) 0 hrs.	(2) 12 hrs.	(3) 24 hrs.	(4) 48 hrs.
<i>Series 16</i>					
(1)	Germinated: dried in hot sun for 1 day then at 30° for 4 weeks	5.9	54.0	66.4	78.1
(2)	Germinated: dipped into boiling water, dried at 30° for 4 weeks	3.9	38.1	47.6	58.0
(3)	Germinated: dried at 30° for 4 weeks	5.3	47.9	59.4	66.1
(4)	Ungerminated	3.9	32.2	41.7	47.6
<i>Series 17</i>					
(1)	Germinated in greenhouse for 2 days (30° in daytime)	8.4	61.9	82.6	96.0
(2)	Germinated in open air in warm weather in shade for 3 days	7.3	47.0	63.8	79.2
(3)	Germinated similarly to (2)	8.1	52.4	69.2	77.6
(4)	Ungerminated	4.5	35.0	44.5	50.1
<i>Series 18</i>					
(1)	Germinated by placing in funnels plugged with moist cotton wool. A little damp cotton wool was placed over the beans—kept at 27° for 24 hrs.	5.9	52.4	63.8	75.0
(2)	Germinated similarly to (1) but for 48 hrs.	7.6	68.0	88.5	101.9
(3)	Ungerminated	3.9	25.5	39.8	54.0
(4)	Germinated similarly to (2)	7.8	69.7	82.3	90.4
<i>Series 19</i>					
(1)	Germinated: dried in laboratory (at 15° in daytime) for 6 weeks	5.9	39.8	52.1	62.4
(2)	Germinated: dried in laboratory (at 15° in daytime) for 6 months	5.6	39.5	54.0	58.5
(3)	Germinated: dried in laboratory (at 15° in daytime) for 3 months	7.0	51.8	62.2	64.4
(4)	Ungerminated	3.9	28.3	38.1	44.5

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REFERENCE.

Chick and Hume (1919). *Biochem. J.* 13, 199.