Changes of Starch, Crude Fiber, and Oligosaccharides in Germinating Dry Beans

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ABSTRACT

The effect of germination at 22°C on changes in carbohydrate components of red kideny, Gloria pink, and black eye dry beans was investigated. Starch, amylose, and amylopectin decreased gradually during the six-day sprouting period. The ratio of amylose to amylopectin also decreased during germination. Acid detergent fiber, cellulose, and lignin in the sprouts did not change appreciably during germination. The red kidney and Gloria pink varieties were higher in acid detergent fiber and cellulose contents than were the black eye beans. High pressure liquid chromatography was used to determine quantitative changes of oligosaccharides in the germinating beans. During the preliminary soaking period of 14 hr, an appreciable loss of sucrose, raffinose, and stachyose took place as a result of diffusion. The levels of raffinose and stachyose continued to decrease during germination. They disappeared after six days of germination in the Gloria pink and black eye beans. Sucrose increased in the four-day sprouts.

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The effect of germination on nutritive value of dry beans has been reported by several workers (Chen and Luh 1976, Chen and Thacker 1978, Chen et al 1975). During sprouting of dry beans, the storage materials are converted into other forms more usable to both plants and humans. The first stage in the germination process involves the breakdown of seed reserves and their utilization by the growing roots and shoots.

Fordham et al (1975) found that ascorbic acid content was higher in germinated beans and peas than in ungerminated dry seeds. Very little is known about the changes in starch and fiber in germinating bean seeds.

This work reports the effect of germination on changes in starch, crude fiber, and oligosaccharides in three dry bean varieties.

MATERIALS AND METHODS

Dry Beans

Red kidney (*Phaseolus vulgaris*), Gloria pink (*Phaseolus vulgaris* cv. Gloria), and black eye beans (*Vigna sinesis*) were supplied by Robert Ball of the Seed Laboratory of the University of California at Davis. The seeds were stored at 4°C in sealed polyethylene bags for two months before the germination study.

Germination Process

Triplicate samples of dry beans were rehydrated by soaking in deionized water for 14 hr at room temperature (22°C). After being washed, the soaked beans were placed on a cheesecloth seated in a screen basket. The basket was placed in a ceramic pot (10 in. in diameter and 12 in. high) covered with a piece of cheesecloth (Chen and Luh 1976). A wooden cover was used to minimize moisture loss. The beans were germinated at room temperature for a total of six days. During this period, they were rinsed with deionized water and sprayed with a 0.02% NaOCl solution at 4-hr intervals to inhibit microbial growth. Samples of bean sprouts were deep frozen at −10°F overnight on plastic trays and then freeze-dried in a Stokes freeze-drier. The freeze-dried products were milled to pass a 40–60 mesh screen and kept at 1°C in tightly covered glass jars.

Chemical Analysis

Moisture. The moisture content was determined by drying the ground samples at 100–102°C to constant weight according to the AOAC method (1975).

Starch. Total starch content was determined by the anthrone reagent method and the amylose content by the I_2 + KI method (McCready et al 1950). The amylopectin content was calculated by subtracting the amylose value from total starch content.

Acid Detergent Fiber. Acid detergent fiber (ADF) and its fractions, including cellulose and lignin, were determined by the method described by Goering and Van Soest (1970). Treatment of the ADF with 72% H_2SO_4 dissolved the cellulose fraction. The residue after washing and drying was the crude lignin fraction.

Sample Preparation of Sugars

A 10-g ground sample was extracted with 75 ml of 80% ethanol. A small quantity of $CaCO_3$ was added to neutralize any acidity. The mixture was refluxed for 1.5 hr. The alcoholic extract was cooled and filtered through Whatman No. 1 paper. The residue was washed three times with 100 ml of 80% ethanol. The combined washings were evaporated to dryness in vacuo in a rotary evaporator with a water aspirator at 50°C. The residue was brought to 100-ml volume with deionized water and filtered through a 0.45-μm celotate filter (Millipore Corp.). High pressure liquid chromatography (HPLC) was used to assay the aqueous solution for sugars (Palmer and Brandes 1974).

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TABLE I
Moisture and Solid Contents (%) of Dry Beans and Bean Sprouts

	Beans				Sprouts					
	Dry		Soaked		Two-Day		Four-Day		Six-Day	
Variety	Moisture	Solids	Moisture	Solids	Moisture	Solids	Moisture	Solids	Moisture	Solids
Red kidney	11.3	88.7	61.3	38.7	63.6	36.4	64.8	35.2	66.7	33.3
Gloria pink	10.4	89.6	59.7	40.3	61.7	38.3	73.4	26.6	73.7	26.3
Black eye	9.9	90.1	62.6	37.4	74.6	25.4	76.1	23.9	78.3	21.7

TABLE II
Changes in Starch, Amylose, and Amylopectin Contents (%) of Dry Beans and Bean Sprouts During Germination

Samples	Starch[a]	Amylose[a]	Amylopectin[a]	Ratio of Amylose to Amylopectin
Red kidney				
Beans				
Dry	46.95	17.46	29.50	0.59
Soaked	46.80	17.27	29.53	0.58
Sprouts				
Two-day	40.74	14.55	26.19	0.56
Four-day	37.62	13.11	24.51	0.54
Six-day	33.18	10.88	22.30	0.49
Gloria pink				
Beans				
Dry	42.31	14.93	27.38	0.55
Soaked	42.20	14.81	27.39	0.54
Sprouts				
Two-day	40.03	13.76	26.27	0.54
Four-day	38.10	12.90	25.20	0.51
Six-day	35.97	11.56	24.41	0.47
Black eye				
Beans				
Dry	41.18	15.75	25.43	0.62
Soaked	40.76	15.54	25.22	0.62
Sprouts				
Two-day	34.51	12.56	21.95	0.57
Four-day	31.24	10.95	20.29	0.54
Six-day	26.13	8.65	17.48	0.50
LSD ($P = 0.05$)	2.80	0.90	1.10	...

[a] On dry weight basis.

TABLE III
Acid Detergent Fiber (ADF), Cellulose, Lignin, and Ash in ADF of Dry Beans and Bean Sprouts

Sample	Total ADF	Cellulose[a] (%)	Lignin[a,b] (%)	Ash[a] in ADF (%)
Red kidney				
Beans				
Dry	6.10	5.86	0.12	0.13
Soaked	6.13	5.91	0.10	0.12
Sprouts				
Two-day	5.98	5.73	0.12	0.13
Four-day	5.83	5.60	0.11	0.12
Six-day	6.15	5.91	0.12	0.13
Gloria pink				
Beans				
Dry	6.28	6.02	0.16	0.10
Soaked	6.32	6.08	0.15	0.09
Sprouts				
Two-day	6.37	6.10	0.17	0.11
Four-day	6.26	5.99	0.16	0.12
Six-day	6.28	6.02	0.16	0.10
Black eye				
Beans				
Dry	5.06	4.89	0.09	0.08
Soaked	5.08	4.92	0.09	0.08
Sprouts				
Two-day	5.12	4.94	0.10	0.09
Four-day	5.03	4.87	0.09	0.07
Six-day	4.92	4.75	0.09	0.09

[a] On dry weight basis.
[b] Lignin is the fraction insoluble in 72% of H_2SO_4 after extraction for 3 hr at room temperature.

HPLC of the Oligosaccharides

A Waters Associates 6000A pump, a U6K Universal injector, and an R401 differential refractive index detector were used. The detector signal was recorded on an OmniScribe recorder. The column (4 mm id × 30 cm) was packed with μ Bondapak/carbohydrate packing material (Waters Associates). The eluant was acetonitrile (Burdick and Jackson Lab Inc., high purity grade) and distilled water (85:15, v/v). The acetonitrile was filtered through a 0.5-μl Millipore filter. Distilled water was filtered through a Millipore Milli-Q system. Samples of 40 μl each were injected with a syringe. The detector was operated at an attenuation setting of 8 X. Standard curves were constructed from peak areas generated by injecting standard solutions of fructose, sucrose, raffinose, and stachyose. The oligosaccharides were identified by comparing their retention times with those of the standard sugars run under the same conditions. The quantity of sugars present was determined from the peak areas as compared with those of the standard curves.

RESULTS AND DISCUSSION

Moisture and Solids Contents

The moisture and solids contents of the dry beans and sprouts are presented in Table I. Solids content gradually decreased as the germination procedures proceeded. The black eye beans were lower in solids content after germination than were the corresponding samples from red kidney and Gloria pink beans. This phenomenon may be explained by the faster sprouting process in the black eye beans.

Starch, Amylose, and Amylopectin

The total starch contents of the dry beans and their sprouts are presented in Table II.

During germination, starch content progressively decreased in all three varieties. Changes in starch during germination of soy beans have also been reported by Hsu et al (1973). The starch breakdown during germination may be attributed to the increase in amylase and phosphorylase activity in respiratory metabolism (Koller et al 1962). The difference in rates of starch changes among the varieties may be explained by the difference in amylase levels as well as in the rate of water penetration during germination.

Table II shows the changes in amylose and amylopectin contents of the beans during germination. After germination for six days, a decrease in the amylose-amylopectin ratio of all samples was found. In the early stages of hydrolysis of starch, α-amylase encounters the interior of the starch chain in an essentially random manner. Different amylases show different specificity toward the starch chain ends and the branch points within the interior of the starch chain. Because α-amylase cannot hydrolyze the α-1-6-glucosidic bonds of starch, hydrolysis-resistant chains containing branched glucoside chains are present in the hydrolyzed products. As hydrolysis proceeds, the ratio of linear starch chains to branched chains decreases as the chains become shorter. Alpha-amylase hydrolysates contain glucose, maltose, dextrins, and limit dextrins. During the early stages of starch hydrolysis, the action pattern of α-amylases of germinated seeds yields mainly dextrins.

ADF. The ADF, cellulose, lignin, and ash in ADF of the

TABLE IV
Effect of Germination on Oligosaccharides (mg/100 g) in Dry Beans and Bean Sprouts

	Fructose		Sucrose		Raffinose		Stachyose	
	Wet Basis	Dry Basis	Wet Basis	Dry Basis	Wet Basis	Dry Basis	Wet Basis	Dry Basis
Red kidney								
Beans								
Dry	0	0	1,455	1,641	337.1	380	353.0	398
Soaked	0	0	314	812	63.9	165	83.6	216
Sprouts								
Two-day	0	0	330	908	32.8	90	43.0	118
Four-day	57.7	164	350	997	12.7	36	16.2	46
Six-day	69.9	210	288	865	3.3	10	5.0	15
Gloria pink								
Beans								
Dry	0	0	1,319	1,473	389.8	435	349.4	390
Soaked	0	0	302	750	91.9	228	95.5	237
Sprouts								
Two-day	0	0	325	851	42.1	110	54.4	142
Four-day	31.9	120	246	927	14.1	53	17.8	67
Six-day	56.6	215	229	872	T[a]	T	T	T
Black eye								
Beans								
Dry	0	0	2,337	2,594	369.4	410	378.4	420
Soaked	0	0	411	1,101	69.2	185	81.5	218
Sprouts								
Two-day	0	0	303	1,193	20.8	82	28.4	112
Four-day	30.4	127	307	1,285	9.8	41	12.7	53
Six-day	54.3	250	249	1,150	T	T	T	T
LSD ($P = 0.05$)	...	21	...	81	...	15	...	18

[a] Trace.

dry beans and sprouts are shown in Table III. Dry beans contained 5.06–6.28% crude fiber, 4.89–6.02% cellulose, and 0.09–0.16% lignin. Rockland et al (1977) reported that the crude fiber contents of Gloria pink and black eye beans as determined by the AOAC method were 4.6% and 3.2%, respectively. The ADF procedure developed by Van Soest (1963) and described by Goering and Van Soest (1970) generally produces higher values than does the crude fiber method. Virtually all the fiber data on beans in the literature were determined by Thaer's method, in which crude fiber essentially represents the residue after sequential digestion with hot 1.25% H_2SO_4 and 1.25% NaOH solutions. This determination underestimates, in varying degrees, the amount of material left undigested by humans (Fisher 1973). Therefore, the crude fiber value cannot be considered an accurate estimate of dietary fiber. According to Van Soest (1963), the fiber isolated by the acid detergent method contains cellulose, lignin, and ash.

No significant change occurred (Table III) in the ADF, cellulose, and lignin on a dry basis. On a wet basis, ADF and its fractions decreased during germination because of the increase in the moisture content of the beans. The ash content of the ADF (dry basis) did not change during germination.

Identification and Quantitative Analysis of Oligosaccharides

The free sugar contents of the three varieties of beans are given in Table IV. Dry black eye beans contained more sucrose and stachyose than did red kidney and Gloria pink beans. The raffinose content of Gloria pink beans was higher than that of the other two varieties. Fructose was not detected in the dry beans but was measurable after four days of germination. A remarkable decrease in sucrose was observed after soaking; only 42.5% of the sucrose remained in the soaked red kidney, 50.9% in the Gloria pink, and 42.4% in the black eye beans. After soaking, the raffinose remaining was 43.4% of the original value in red kidney, 52.4% in Gloria pink, and 45.0% in black eye beans. Stachyose content decreased after soaking to 54.3% of the original value in red kidney, 60.8% in Gloria pink, and 51.9% in black eye beans. The inference can be made that appreciable amounts of oligosaccharides were removed by diffusion during the soaking process.

The levels of raffinose and stachyose in Gloria pink and black eye beans decreased during germination and disappeared completely in the six-day sprouts. In the red kidney beans, a small amount of raffinose and stachyose was detected in the six-day sprouts.

The sucrose content of sprouts increased after four days of germination and then decreased slightly thereafter (Table IV). The decrease in sucrose was accompanied by a slight increase in fructose.

Raffinose and stachyose appear to have been hydrolyzed by α-galactosidase into sucrose and galactose, and the latter metabolized through a galactose-utilization system. Such a system has been reported in navy beans, *Phaseolus vulgaris* L., with similar disappearance of raffinose and stachyose (Snauwaert and Markakis 1976).

No accumulation of glucose was found in the germinating beans. Any liberated glucose and galactose from hydrolysis of soluble oligosaccharides were probably metabolized to meet the requirement of the developing plant tissue. Such a system has been reported in germinating peas without accumulation of glucose (Swain and Dekker 1966). Snauwaert and Markakis (1976) reported the disappearance of raffinose and stachyose during germination of navy beans without galactose accumulation. However, they reported the presence of glucose in the sprouts. The difference in sugar accumulation between various varieties of germinating dry beans may be attributed to the varietal characteristics. More work is needed to explain the difference in the changes in various sugars in germinating beans.

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