Changes in Oligosaccharides During Germination and Cooking of Black Gram and Fermentation of Black Gram/Rice Blend[1]

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ABSTRACT

The oligosaccharides of the raffinose family of sugars account for 61% of total sugars in black gram seeds and cotyledons. The black gram seeds studied had 3.4% verbascose, 0.9% stachyose, a trace of raffinose, and 1.5% sucrose, whereas the black gram cotyledons contained 4.0% verbascose, 0.7% stachyose, a trace of raffinose, and 1.5% sucrose on a dry weight basis. Germination of black gram seeds for 48 hr resulted in complete disappearance of verbascose, stachyose, and raffinose; the sucrose content was unchanged after 24 hr of germination. Increased α-galactosidase activity was observed during the first 48 hr of germination and throughout the 45-hr fermentations of black gram/rice and of black gram blends. Cooking for 40 min at 10 psi (116°C) caused a decrease in the oligosaccharide content of black gram cotyledons. Polished Texas long-grain rice did not contain the verbascose, stachyose, and raffinose but had a trace of sucrose. Fermentation of black gram/rice and of black gram blends for 45 hr decreased the original oligosaccharides to 28 and 71%, respectively. Steaming of batter made from black gram/rice blend fermented for 20 hr produced an acceptable product, Idli, that contained about 1.0% verbascose and 0.2% stachyose, representing 44% of the total sugars.

Oligosaccharides of the raffinose family of sugars are found in appreciable amounts in mature legume seeds such as beans—California small white, Great Northern, navy, pinto, kidney, soy, faba, lima, field, and mung; peas; cowpeas; chick peas; pigeon peas; horse gram; lentils; and lupines (Akpapunam and Markakis 1979, Cerning-Beroard and Filiatre 1976, Naivikul and D'Appolonia 1978, Olson et al 1975, Rackis 1975). The sugars are chiefly verbascose, stachyose, and raffinose, which contain α-galactosido-glucose and α-galactosido-galactose bonds (Fig. 1) and are nonreducing.

When the oligosaccharides of the raffinose family of sugars are ingested by humans, two enzymes (invertase and α-galactosidase) are required for complete hydrolysis. Because the human gastrointestinal tract does not possess an α-galactosidase enzyme (Gitzelmann and Auricchio 1965) and because mammalian invertase is an α-glucosidase, the metabolic fate of raffinose family sugars is uncertain. Many studies have shown that these oligosaccharides are involved in flatulence production in man and animals, which is characterized by the production of high amounts of carbon dioxide, hydrogen, and small amounts of methane gases (Rackis 1975). Consequently, the presence of substantial amounts of these oligosaccharides impedes full nutritional utilization of the bean. Yet very little information is available about the effects on oligosaccharide content of processing procedures such as germination, cooking, and fermentation. A few studies are reported from India; they deal mainly with the effects of cooking and germination (Rao and Belavady 1978) and soaking (Iyengar and Kulkarni 1977) on the oligosaccharide content of black gram. Our earlier study (Reddy et al 1980) examined the extent of flatulence production by cooked beans and bean cotyledons, germinated beans, and a fermented product of black gram and rice. The present investigation reports the effects of germination, cooking, and fermentation on the oligosaccharide content of black gram (*Phaseolus mungo* L.) seeds.

MATERIALS AND METHODS

Whole black gram seeds and black gram cotyledons were purchased from Indo-European Inc., Hollywood, CA. Polished Texas long-grain rice was procured from a local market.

Germination

Black gram seeds (40 g) were germinated in petri dishes at room temperature (22°C) with distilled water. Samples were collected every day until the fifth day of germination and were freeze-dehydrated. The dried samples were powdered by a Udy Cyclone Mill (Tecator, Inc., Boulder, CO) to obtain a 60-mesh flour.

Cooking

One hundred grams of black gram cotyledons were cooked with 400 ml of distilled water at 10 psi (temperature 116°C) for varying lengths of time (5, 10, 15, 20, 25, 30, 35, and 40 min). The bean/water ratio was 1:4 (w/v). After cooking, the samples and water were freeze-dehydrated and milled to obtain a 60-mesh flour.

Fermentation

The procedure of Padhye and Salunkhe (1978) was followed. Equal parts (500 g each) of black gram cotyledons and polished Texas long-grain rice were used in the fermentation studies. Each ingredient was washed with distilled water to remove surface microorganisms and then soaked for 2 hr in distilled water. Each was then ground to a fine paste in a Waring Blendor (high speed, 2 min) and mixed with the addition of 1% salt. The mixed black gram and rice batter was placed into plastic cups, covered, and allowed to ferment in a 30°C incubator. Samples in duplicate were taken at 4-hr intervals. One set was used for the determination of batter volume and pH. The other set of samples was freeze-dehydrated immediately and used for determination of total sugars, individual oligosaccharides, and α-galactosidase activity. Fermentation experiments with black gram and rice alone were also conducted under the same conditions and used for determinations of physicochemical properties.

Total Sugars and Oligosaccharides

The total sugars and oligosaccharides (verbascose, stachyose, raffinose, and sucrose) from raw bean samples, cooked bean

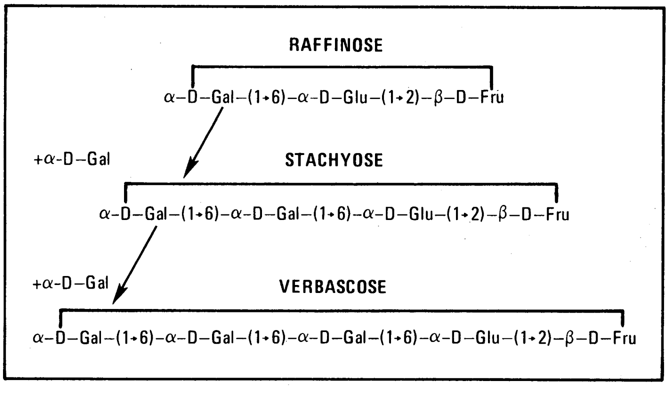

Fig. 1. Structural relationships of the raffinose family oligosaccharides.

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cotyledons, fermented black gram/rice samples, fermented black gram samples, fermented rice samples, germinated samples, and a steamed fermented product (Idli) were extracted according to the method of Hymowitz et al (1972). Total sugars in the aqueous extract were determined by the phenol-sulfuric acid method (Dubois et al 1956) and expressed in terms of dextrose. The oligosaccharides were then separated for 80 hr on Whatman chromatography paper 3^{M}_{M} by the solvent system and method of Shallenberger and Moores (1957) and were quantitatively measured after elution from the paper strips by the method of Dubois et al (1956).

Sources for the oligosaccharides standards were: verbascose (a gift from E. Cristofaro), Nestlé Products Technical Assistance Ltd., La Tour-De-Peilz, Switzerland; stachyose tetrahydrate (a gift from R. S. Shallenberger), New York Agricultural Experiment Station, Geneva, NY; raffinose pentahydrate, Mann Research Laboratories, Inc., New York, NY; sucrose, ICN Nutritional Biochemicals, Cleveland, OH.

Determination of α-Galactosidase Activity

The lyophilized samples (5 g) were blended with 100 ml of cold citrate-phosphate buffer (0.05 *M*, pH 5.20) for 1 min in the cold (4°C). The resulting slurry was left overnight in the refrigerator and then centrifuged at $10{,}000 \times g$ for 30 min; the enzymatically active supernatant solution was retained. The enzyme activities were measured at 37°C by the method of Barham et al (1971). *p*-Nitrophenyl-α-D-galactopyranoside (Calbiochem, Lajolla, CA) was used as a substrate for measuring the α-galactosidase activity. Appropriate blanks included boiled supernatant.

One unit of enzyme activity is defined as the amount of supernatant that would liberate 1 μmole of *p*-nitrophenol per minute per milligram of protein at 37°C.

RESULTS AND DISCUSSION

In black gram seeds and cotyledons, the oligosaccharides of the raffinose family of sugars represent about 61% of the total sugars. Verbascose was the major oligosaccharide, followed by sucrose and stachyose, which accounted for about 51.3% and 48.6% of total sugars in black gram cotyledons and seeds, respectively (Table I). Black gram cotyledons had higher amounts of verbascose than did the whole seed. Raw black gram cotyledons on a dry weight basis contained about 4.0% verbascose, 0.7% stachyose, a trace of raffinose, and 1.5% sucrose. The raw whole seeds had about 3.4%

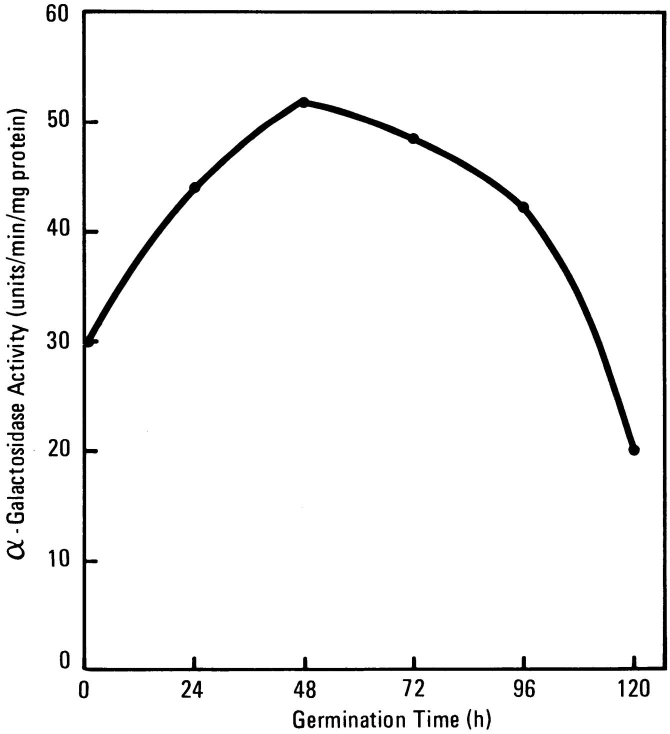

Fig. 2. Changes in α-galactosidase activity during germination of black gram seeds.

TABLE I
Oligosaccharide and Total Sugars Contents[a] of Whole Black Gram Seeds and Cotyledons, Rice, and Black Gram/Rice Fermented Product

Component	Total Sugars (mg/g)[b]	Raffinose Family Oligosaccharides, mg/g[b]			Sucrose (mg/g)[b]	Oligosaccharides[c] as Percent of Total Sugars
		Verbascose	Stachyose	Raffinose		
Whole black gram seed	70.8 ± 4.5	34.4 ± 1.1	8.9 ± 0.3	Trace	14.6 ± 0.5	61.2
Black gram cotyledon	78.5 ± 1.1	40.3 ± 2.0	7.2 ± 0.1	Trace	15.1 ± 0.5	60.5
Long-grain rice	4.9 ± 0.3	ND[d]	ND	ND	Trace	···
Black gram/rice fermented product	28.8 ± 1.9	10.3 ± 0.3	2.4 ± 0.1	ND	5.1 ± 0.2	44.1

[a]Each value is the mean of six determinations.
[b]Mean plus or minus standard deviation.
[c]Includes oligosaccharides of the raffinose family of sugars.
[d]Not detectable.

TABLE II
Effects of Germination on Total Sugars and Oligosaccharide Contents[a] in Black Gram

Germination (hr)	Total Sugars (mg/g)[b]	Raffinose Family Oligosaccharides, mg/g[b]			Sucrose (mg/g)[b]	Percent Oligosaccharides[c] Hydrolyzed
		Verbascose	Stachyose	Raffinose		
0	70.8 ± 4.5	34.4 ± 1.1	8.9 ± 0.3	Trace	14.6 ± 0.5	0.0
24	68.5 ± 2.1	2.6 ± 0.2	3.1 ± 0.1	ND[d]	33.6 ± 1.1	86.8
48	46.0 ± 1.4	ND	ND	ND	17.9 ± 0.8	100.0
72	56.9 ± 2.9	ND	ND	ND	13.3 ± 0.6	···
96	90.7 ± 4.1	ND	ND	ND	15.3 ± 0.4	···
120	103.0 ± 3.7	ND	ND	ND	15.8 ± 0.4	···

[a]Each value is the average of four determinations.
[b]Mean plus or minus standard deviation.
[c]Includes oligosaccharides of the raffinose family of sugars.
[d]Not detectable.

verbascose, 0.9% stachyose, a trace of raffinose, and 1.5% sucrose (dry weight basis). These results are in agreement with previously reported values (Nigam and Giri 1961). Germination, cooking, cooking after germination, roasting after germination, and fermentation processes are commonly used for processing beans before consumption. These processes reduce and/or eliminate the oligosaccharides (Cristofaro et al 1974, Iyengar and Kulkarni 1977, Shallenberger et al 1967).

Germination

During germination, the seeds undergo marked metabolic changes, and the reserve carbohydrates, including the oligosaccharides of the raffinose family, are hydrolyzed (Adjei-Twum et al 1976). The changes in the oligosaccharides, ie, verbascose, stachyose, raffinose, and sucrose, and in the total sugars content of black gram during a 5-day germination period are presented in Table II. The total sugars content gradually decreased from 70.8 to 46.0 mg/g during the first 48 hr of germination and increased thereafter up to 103.0 mg/g after 120 hr of germination. The increase in the total sugars content between 72 and 120 hr of germination probably resulted from the hydrolysis of starch and higher oligosaccharides by glycosidases. The sucrose content increased during the first 24 hr of germination and remained fairly constant thereafter. Raffinose was detected in trace amounts in raw beans but not in germinated seeds, probably because of hydrolysis by an α-galactosidase. Both verbascose and stachyose decreased in concentration as germination progressed and disappeared completely in the later stages (48 hr). The disappearance of verbascose and stachyose during the first 48 hr of germination was caused by enzymatic degradation, as confirmed by the increased α-galactosidase activity in germinated seeds (Fig. 2). The α-galactosidase activity was high in resting seeds and increased during the first two days of germination. This is in contrast to the results of Rao and Belavady (1978). Those authors did not observe complete disappearance of oligosaccharides (verbascose, stachyose, and raffinose) until after 72 hr of germination. Silva and Luh (1979) observed complete disappearance of raffinose and stachyose sugars in black eye and pink beans after germination for four days. East et al (1972) and Hsu et al (1973) reported that raffinose and stachyose in soybeans disappeared as a result of germination. About 70% of the raffinose plus stachyose was removed from soybeans by a combination of various treatments, involving pH adjustments, soaking, and germination (Kim et al 1973). In our study, no such attempts were made to remove the oligosaccharides of the raffinose family of sugars. The germinated black gram seeds (24 hr) produced low flatulence, ie, about 4.6 ± 0.6 ml of H_2 gas in rats, compared to cooked and fermented black gram products, which produced 22.8 and 8.4 ml of H_2, respectively, when fed at the 50% level in the diet (Reddy et al 1980). The low flatus production in rats by germinated seeds (24 hr) may be attributed to an 87% reduction in oligosaccharides (verbascose and stachyose).

Cooking

Cooking for 40 min at 10 psi (116° C) caused a slight disruption of the cotyledons, and the cotyledons absorbed most of the water. Changes in the oligosaccharide and total sugars contents of the cooked cotyledons of the black gram bean are presented in Table III. Total sugars were reduced from 78.5 to 52.6% during 40 min of cooking, with corresponding decreases in verbascose, stachyose, and sucrose contents to 73, 81, and 80% of the original, respectively. Cooking of bean cotyledons with water in a 1:4 ratio for 40 min did not eliminate all of the oligosaccharides but reduced them to 74.5% of the original amount. In contrast, Rao and

TABLE III
Effects of Cooking on Total Sugars and Oligosaccharide Contents[a] in Black Gram Cotyledons

Cooking Time (min)	Total Sugars (mg/g)[b]	Raffinose Family Oligosaccharides, mg/g[b] Verbascose	Stachyose	Raffinose	Sucrose (mg/g)[b]	Percent Oligosaccharides[c] Reduced
0	78.5 ± 1.1	40.3 ± 2.0	7.2 ± 0.1	Trace	15.1 ± 0.5	0.0
5	59.5 ± 1.1	37.2 ± 1.0	7.4 ± 0.4	ND[d]	16.2 ± 0.8	6.1
10	59.3 ± 3.0	35.7 ± 1.0	7.6 ± 0.4	ND	16.0 ± 0.6	8.8
15	58.6 ± 1.1	35.0 ± 1.0	7.5 ± 0.6	ND	15.1 ± 0.4	10.5
20	56.6 ± 2.4	34.8 ± 1.1	7.5 ± 0.4	ND	14.2 ± 0.6	10.9
25	55.1 ± 2.4	34.1 ± 0.9	6.7 ± 0.3	ND	13.8 ± 0.5	14.1
30	54.9 ± 2.1	33.9 ± 0.4	6.8 ± 0.3	ND	13.2 ± 0.9	14.3
35	53.8 ± 1.3	31.7 ± 1.0	6.2 ± 0.3	ND	12.9 ± 0.7	20.2
40	52.6 ± 0.4	29.6 ± 0.7	5.8 ± 0.3	ND	12.0 ± 0.4	25.5

[a] Each value is the average of six determinations.
[b] Mean plus or minus standard deviation.
[c] Includes oligosaccharides of the raffinose family of sugars.
[d] Not detectable.

TABLE IV
Physicochemical Changes Occurring in the Fermentation of Black Gram, Rice, and Black Gram/Rice Blends

Fermentation Time (hr)	Fermentation Media: Black Gram Batter Volume (ml)	Black Gram pH	Rice Batter Volume (ml)	Rice pH	Black Gram/Rice Batter Volume (ml)	Black Gram/Rice pH
0	100	6.00	100	6.10	100	5.90
4	100	5.90	100	6.10	100	5.90
8	101	5.88	103	6.05	102	5.88
12	102	5.85	105	5.65	108	5.86
16	104	5.85	122	5.52	140	5.70
20	105	5.81	120	5.52	173	5.60
24	108	5.72	115	5.48	155	5.40
45	178	5.20	108	4.40	126	5.00

TABLE V
Effects of Fermentation on Total Sugars and Sucrose Contents[a] in Rice Blend

Fermentation Time (hr)	Total Sugars (mg/g)[b]	Sucrose (mg/g)[b]
0[c]	4.7 ± 0.1	Trace
4	4.5 ± 0.8	ND[d]
8	4.5 ± 0.8	ND
12	4.0 ± 0.8	ND
16	3.0 ± 0.5	ND
20	2.1 ± 0.5	ND
24	3.0 ± 0.5	ND
45	15.5 ± 1.9	ND

[a] Each value is the average of four determinations.
[b] Mean plus or minus standard deviation.
[c] Samples soaked for 2 hr before blending.
[d] Not detectable.

Belavady (1978) obtained a significant increase in oligosaccharides (verbascose, stachyose, raffinose, and sucrose) content of 134, 114, 92, and 57% in red gram, black gram, bengal gram, and green gram, respectively, after cooking at 15 psi for 15 min. Ku et al (1976) reported that boiling of soybeans in a 1:10 bean/water ratio removed 33–59% of oligosaccharides depending on the time in the soaking water. A loss of 82.5% sucrose, 75.6% raffinose, 60.0% verbascose, and 52.2% stachyose in black gram seeds over a period of soaking in water for 12 hr was obtained by Iyengar and Kulkarni (1977). No such attempts were made in this study to remove the oligosaccharides by soaking.

Fermentation

A number of physiochemical changes including leavening (batter volume increase) and acid production (pH depression) are known to occur during fermentation (Mukherjee et al 1965). The changes in batter volume and pH depend on the type of microflora present and the substrate available. The changes in the batter volume and pH during fermentations of black gram/rice, black gram, and rice blends are summarized in Table IV. The batter volume reached its maximum in black gram/rice, black gram, and rice fermentations at 20, 45, and 16 hr, respectively. At 20 hr, the increase in batter volume of the black gram/rice fermentation was about 73%, compared with increases in black gram and rice fermentations of 5 and 20%, respectively. The pH values consistently decreased in each case as the fermentation progressed. The drop in pH values for black gram/rice, black gram, and rice fermentations at 20 hr were 0.30, 0.19, and 0.58, respectively. Similar results were also obtained by Padhye and Salunkhe (1978). On the basis of volume increase, appearance, and appealing sour flavor, the 20-hr black gram/rice fermented batter was judged to be optimum for preparation of Idli. In this investigation, no attempt was made to identify the microorganisms responsible for gas and acid production. Mukherjee et al (1965) reported that changes in leavening and acid production (pH) in black gram/rice fermentation are caused by the action of the heterofermentative lactic bacterium, *Leuconostoc mesenteroides*. They also showed that this organism is a natural microflora in black gram.

The changes in oligosaccharides (verbascose, stachyose, and sucrose) and total sugars contents and α-galactosidase activity during fermentations of black gram/rice, black gram, and rice are presented in Tables V–VII and Fig. 3. Verbascose, stachyose, and

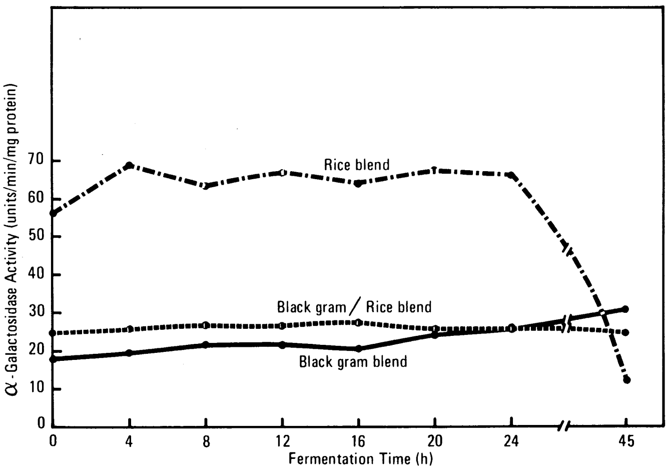

Fig. 3. Changes in α-galactosidase activity during fermentation of black gram, rice, and black gram/rice blends.

TABLE VI
Effects of Fermentation on Total Sugars and Oligosaccharide Contents[a] in Black Gram Blend

Fermentation Time (hr)	Total Sugars (mg/g)[b]	Raffinose Family Oligosaccharides, mg/g[b] Verbascose	Stachyose	Raffinose	Sucrose (mg/g)[b]	Percent Oligosaccharides[c] Hydrolyzed
0[d]	63.2 ± 3.1	36.7 ± 1.1	5.5 ± 0.3	Trace	12.7 ± 1.0	0.0
4	60.3 ± 2.1	35.9 ± 0.9	5.4 ± 0.3	ND[e]	12.2 ± 0.9	2.1
8	60.9 ± 1.2	35.7 ± 0.9	5.0 ± 0.5	ND	10.1 ± 0.6	3.6
12	60.8 ± 1.2	33.2 ± 1.0	4.8 ± 0.2	ND	9.5 ± 0.6	10.0
16	61.4 ± 1.2	32.7 ± 0.5	4.8 ± 0.3	ND	9.2 ± 0.3	11.1
20	59.4 ± 1.2	32.1 ± 1.0	4.1 ± 0.0	ND	11.2 ± 0.6	14.2
24	61.3 ± 1.2	30.3 ± 1.4	4.5 ± 0.2	ND	10.7 ± 0.3	17.5
45	38.8 ± 1.0	24.8 ± 0.8	5.4 ± 0.3	ND	ND	28.4

[a] Each value is the average of six determinations.
[b] Mean plus or minus standard deviation.
[c] Includes oligosaccharides of the raffinose family of sugars.
[d] Samples soaked for 2 hr before blending.
[e] Not detectable.

TABLE VII
Effects of Fermentation on Total Sugars and Oligosaccharide Contents[a] in Black Gram/Rice Blend

Fermentation Time (hr)	Total Sugars (mg/g)[b]	Raffinose Family Oligosaccharides, mg/g[b] Verbascose	Stachyose	Raffinose	Sucrose (mg/g)[b]	Percent Oligosaccharides[c] Hydrolyzed
0[d]	43.2 ± 1.6	15.5 ± 0.6	3.1 ± 0.2	ND[e]	8.3 ± 0.5	0.0
4	40.4 ± 4.1	15.3 ± 0.5	3.1 ± 0.2	ND	7.2 ± 0.7	1.1
8	41.1 ± 2.3	15.0 ± 0.8	2.9 ± 0.1	ND	6.0 ± 0.5	3.8
12	33.0 ± 0.8	14.3 ± 0.7	2.8 ± 0.2	ND	6.4 ± 0.2	8.1
16	33.6 ± 1.0	13.6 ± 0.7	3.1 ± 0.1	ND	6.8 ± 0.3	10.2
20	33.8 ± 0.7	12.7 ± 0.6	2.9 ± 0.2	ND	5.6 ± 0.2	16.1
24	25.4 ± 1.6	11.2 ± 0.3	2.2 ± 0.1	ND	2.9 ± 0.1	28.0
45	11.6 ± 0.5	4.7 ± 0.2	0.6 ± 0.0	ND	ND	71.5

[a] Each value is the average of six determinations.
[b] Mean plus or minus standard deviation.
[c] Includes oligosaccharides of the raffinose family of sugars.
[d] Samples soaked for 2 hr before blending.
[e] Not detectable.

raffinose were absent in polished Texas long-grain rice, but sucrose was present in traces (Table I). The total sugars content during rice fermentation decreased between 8 and 24 hr and increased thereafter (Table V). As the black gram fermentation progressed, the total sugars and verbascose decreased, but the stachyose concentration was fairly constant (Table VI). The constant concentration of stachyose was due to release of the stachyose component of verbascose through hydrolysis by an α-galactosidase. The sucrose content was completely depleted after 45 hr of black gram fermentation. Black gram/rice fermentation batter contained equal amounts (w/w) of rice and black gram cotyledons. The total sugars, verbascose, stachyose, and sucrose were progressively reduced in concentration during 45 hr of black gram/rice fermentation (Table VII). Sucrose had completely disappeared after 45 hr, whereas the total sugars, verbascose, and stachyose had reached 26.8, 20, and 19%, respectively, of their original values after 45 hr. Shallenberger et al (1967) observed that stachyose content decreased markedly and sucrose decreased slightly in tempeh fermentation during a 72-hr period. They also found that raffinose concentration remained unchanged throughout the fermentation because stachyose hydrolysis replenished raffinose as rapidly as it was hydrolyzed. The verbascose and stachyose concentrations declined to 82 and 95% of the original values, respectively, during black gram/rice fermentation at 20 hr. Steaming of black gram/rice batter fermented 20 hr further decreased the contents of verbascose and stachyose, ie, to 66.5 and 79%, respectively, of their original values. The steamed product, Idli, contained about 1.0% verbascose and 0.2% stachyose and produced 8.4 ml of H_2 in rats as compared to cooked black gram products, which produced from 16.6 to 22.9 ml of H_2 when fed at the 50% level in the diet (Reddy et al 1980).

The α-galactosidase activity successively increased during the black gram/rice, black gram, and rice fermentations (Fig. 3). In rice fermentation, the α-galactosidase activity decreased to 21% at 45 hr. The decrease in the concentrations of the oligosaccharides verbascose and stachyose noted during 45-hr fermentations of black gram/rice and black gram is a result of their hydrolysis by α-galactosidase. This was further corroborated by the increased α-galactosidase activity in black gram/rice and black gram fermented samples.

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