

Cyanide Content of Sorghum Sprouts

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ABSTRACT

The seeds of four cultivars of grain sorghum and four of sweet sorghum (*Sorghum bicolor* (L.) Moench) contained only traces (1 or 2 ppm) to 29 ppm of potential hydrocyanic acid (HCN) that could be generated as free HCN by digestion and steam distillation. Sprouts of the same cultivars grown for 3 days in the dark at 30°C, however, contained from 258–1030 ppm potential HCN relative to the weight of the ungerminated, dry seed. Drying at 50°C and grinding of sprouts to produce a meal did not reduce the potential HCN content. The consumption of sorghum sprouts or products made from them may be hazardous. The average amount (61.3 mg) of HCN obtained in our laboratory from sprouts grown from 100g of seed exceeds the average fatal dose for an adult.

INTRODUCTION

WANG AND FIELDS (1978) reported that a meal or "malt" prepared from sorghum sprouts dried at 50°C had increased relative nutritive values and increased levels of lysine, methionine, and tryptophan when compared to ungerminated seeds. Production of sorghum sprouts from seeds required only 3–5 days with rudimentary equipment. The authors recommended germination of sorghum seed in the home as a means of producing a more nutritious food ingredient and suggested that this practice would be valuable in developing countries and in the United States.

Dry sorghum seed is reported to contain low or undetectable amounts of dhurrin, a cyanogenic glycoside which yields hydrocyanic acid (HCN) upon hydrolysis (Akazawa et al., 1960; Zinmeister, et al., 1980). However, the sorghum plant contains appreciable amounts of dhurrin, and immature plants contain exceptionally high levels of the cyanogen (Gorz et al., 1977; Conn, 1979). Consumption of immature sorghum is a frequent cause of death of livestock, and it is common knowledge among farmers who grow sorghum that their cattle must not be allowed to graze on young plants until "the cane is belly-high on the cow" (Conn, 1973). The coleoptile and first leaf of sorghum seedlings are reported to contain as much as 25% (dry weight basis) of dhurrin (Conn, 1974).

Because the literature concerning the cyanogenic properties of sorghum suggested that sorghum sprouts or products derived from them might be potentially toxic, the objective of our study was to determine the HCN yield of sprouts of eight cultivars of sorghum germinated under the conditions described by Wang and Fields (1978). These conditions are essentially those used for producing edible sprouts from may types of seeds in the home or in commerce. A second objective was to compare the cyanide content of raw sprouts with that of a meal prepared by the procedure of Wang and Fields from dried sprouts.

MATERIALS & METHODS

EIGHT VARIETIES of sorghum seed (*Sorghum bicolor* (L.) Moench) were obtained from three sources. A grain sorghum (cv. Bird-a-boo)

was obtained from the Taylor-Evans Seed Company (Tulia, TX). Three other varieties of grain sorghum (ATX 623 × RTX 430, ATX 399 × RTX 430, and RS 610) were provided by the Dept. of Soil & Crop Sciences, Texas A&M Univ. Four varieties of sweet sorghum (Dale, Wray, Brandes, and M81E) were supplied by the U.S. Dept. of Agriculture's Sugar Crops Field Station (Meridian, MI). Sorghum seed was stored at 4°C until used in sprouting experiments.

To initiate sprouting, 15g of dry sorghum seed was soaked for 16–18 hr at room temperature in 4–5 volumes of water as recommended by Wang and Fields (1978). The seeds then were transferred to commercial sprouting trays (Biosnacky Miracle Sprouter) and washed twice daily during the sprouting period to prevent mold growth. Sprouts of all eight cultivars of sorghum were grown at 30°C in the dark and harvested at the end of 3 days. Sprouts of one cultivar of grain sorghum were grown at 25°, 30°, and 35°C for periods ranging from 2–6 days. Each time-temperature parameter was repeated in duplicate, except for the 5 and 6 day 35°C treatment, which produced sprouts that were judged overmature.

At the end of the growth period, sprouts were drained and blotted on absorbent paper. Sprout length was measured and percent germination was determined. Sprouts and any ungerminated seeds from the original 15g of dry seed were blended with 100 ml of water at high speed for 3 min in a Waring Blendor. The resulting homogenate was transferred to a 1-liter distillation flask with sufficient wash water to bring the volume to about 200 mL. Dry, unspouted sorghum seed was prepared for distillation by grinding 20g to No. 20 mesh in a Wiley mill and transferring the powder to the distillation flask with sufficient water to bring the volume to 200 ml.

The apparatus and procedures of Honig et al. (1983) were used, with minor modifications, for distilling HCN from the homogenate. A drop of Dow-Corning Antifoam B, a magnetic stirring bar, 10 mL 10% lead nitrate, and 10 mL 10% tartaric acid were added to the homogenate in the distillation flask. Steam was introduced into the distillation flask, and the contents were stirred constantly until 200 mL of distillate was collected in a 250 mL Erlenmeyer flask which contained 30 mL of 0.25N NaOH. During the entire distillation process, nitrogen was introduced into the steam generator and passed through the entire system at a rate of about 100 mL per minute. Care was taken to immerse the end of the distillation tube below the surface of the NaOH solution in the collecting flask before beginning the distillation. After 200 mL distillate had been collected, the collection flask was replaced with another 250 mL Erlenmeyer flask containing 30 mL 0.25N NaOH, and 20 mL 1N H₂SO₄ was added to the distillation flask through a separatory funnel fitted to the top of the flask. Steam flow was resumed and a second portion of 175 mL of distillate was collected.

The first 200-mL portion of distillate was filtered through a 0.45 μm Millipore filter and diluted to 250 mL with distilled water. Two 100-mL aliquots were titrated with 0.02N AgNO₃ by an AOAC Official Method (26.136, 1980) for cyanide in beans. The second 175-mL portion of the distillate was filtered, as described above, diluted to 200 mL, and titrated as above. The combined titration values were used to calculate the amount of HCN generated from sorghum sprouts or dry, ungerminated seeds.

Determinations were carried out on blanks containing only water and reagents and on controls containing known amounts of added cyanide to assure that there were no interfering substances and that the recovery of cyanide was complete. (CAUTION: Cyanide compounds are extremely toxic. Cyanide standards should be handled with care. Manipulations involving the production of HCN should be carried out in a hood.) The validity of the procedure for determining cyanide also was substantiated by analyzing a few samples, standards, and blanks by a spectrophotometric method (employing pyridine and barbituric acid) described by Honig et al. (1983).

The possibility that liberation of HCN from pulverized, dry sorghum seed might require a more rigorous procedure, as reported

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by Winkler (1958) for lima beans, was tested. An exhaustive distillation/digestion over a 5-hr period was applied to ground sorghum seed. In alternating periods of 10-15 min, steam was introduced and then the suspension was allowed to digest while being stirred. The results of this exhaustive procedure for determining the HCN content of sorghum seed agreed with the results obtained with the shorter procedure described above.

The potential HCN content of sprouts dried at 50°C in an airstream for 16 hr, as carried out by Wang and Fields (1978), was compared with the potential HCN content of raw sprouts.

RESULTS & DISCUSSION

AS REPORTED BY Akazawa et al. (1960), we found that relatively small amounts of HCN were derived by pulverizing, digesting, and steam distilling sorghum seed. At most, in the sorghum varieties investigated, the potential HCN content of seed was 29 ppm and was barely detectable in the four varieties of sweet sorghum seed (Table 1). In our hands, the lower limit of detection for the method was less than 1 ppm, an insignificant amount from the standpoint of food safety.

Data presented in Tables 1 and 2 represent averages of duplicate germinations (or duplicate samples or ungerminated seed) and analyses of the sorghum variety and treatment specified. Duplicate germination treatments generally yielded values for the cyanide content of sprouts that were within ±5% of the mean value. In all cases, sprouts yielded much higher amounts of HCN than the seeds from which they were grown (Table 1). Sprouts of one variety (Dale) grown at 30°C for 3 days following the initial soaking period of 16-18 hr had a potential HCN content equivalent to over 1,000 ppm in terms of the weight of the dry seeds from which they were produced. The least amount of HCN derived from sprouts grown at 30°C for 3 days was 258 ppm (variety ATX 623 x RTX 430), but even this represents almost a 13-fold increase when compared with the ungerminated seed, which contained only 20 ppm HCN.

Three days was chosen as the optimum time for harvesting sprouts incubated at 30°C, since more mature sprouts developed leaves by the 4th or 5th day and were less desirable in size and appearance as a food ingredient. The incubation temperature of 30°C was selected for screening all eight varieties of sorghum because it is the midtemperature of the three temperatures (25°, 30°, and 35°C) that Wang and Fields (1978) used for their study. It is also near the optimum temperature for producing edible sprouts from many varieties of seed.

One grain sorghum cultivar, Bird-a-boo, was sprouted at 25°, 30°, and 35°C and sprouts were harvested and analyzed at 2, 3, 4, 5, and 6 days. The potential HCN content of sprouts grown at 25°C increased in a nearly linear fashion, reaching a maximum of 614 ppm on the 6th day, while the potential HCN content of sprouts grown at 30° and 35°C

peaked on the 3rd and 2nd days, respectively, with maximum amounts of 671 and 385 ppm, respectively (Table 2).

Wang and Fields (1978) dried sorghum sprouts at 50°C in an airstream and then ground them to make a meal that could be used as a food ingredient. In comparisons of paired samples of fresh sprouts and a rehydrated meal that had been prepared by the method of Wang and Fields, we found no reduction of potential HCN on an absolute basis. Thorough rehydration of the meal was necessary prior to digestion and distillation in order to obtain a full yield of HCN. The rehydration procedure consisted of soaking meal prepared from 15g of seed overnight in 100 ml of water in a stoppered flask and then transferring the contents of the flask to the distillation apparatus. Since HCN has a boiling point of 25.7°C, the observation that drying at 50°C did not reduce the amount of cyanide suggests that cyanide in the sprouts exists largely in the form of the heat-stable, non-volatile cyanogenic glycoside. However, a simple qualitative test (AOAC, 26.134, 1980) readily detected free HCN in the atmosphere over dried meal that had been held for a few weeks at -8°C, and rehydrated meal produced increasing amounts of free HCN in the headspace with time. These observations indicate that the endogenous, autolytic enzymes, β-glucosidase and hydroxynitrile lyase, which are responsible for the breakdown of the cyanogenic glycoside, remained at least partially active in the dried meal. Since the autolytic enzymes persisted in the meal, the amount of free HCN at the time of consumption could represent a considerable portion of the potentially available HCN if the meal was not handled properly (e.g., if the meal was rehydrated and allowed to stand for some time before cooking or eating).

Even complete inactivation of the autolytic enzymes does not obviate the potential toxicity of cyanogenic foods. The enzymes themselves are readily inactivated by cooking or thermal processing in the neighborhood of 100°C, but the cyanogens are relatively heat-stable, and Montgomery (1965) reported that linamarin was not destroyed by several hours of boiling or autoclaving at 160°C. Thus, dietary cyanogens can remain intact, even in thermally processed foods, and there is ample evidence that they are broken

Table 1—Cyanide content of eight varieties of sorghum sprouts (*Sorghum bicolor* [L.] Moench) grown at 30°C for 3 days

Sorghum variety	HCN content (ppm) ^a			Sprout length (cm) ^b	
	Ungerminated seeds		Percent germination	Range	Avg
		Sprouts			
Bird-a-boo ^c	13	454	92	0.1-10.9	7.9
ATX 623 x RTX 430 ^c	20	258	74	0.1- 4.7	2.8
ATX 399 x RTX 430 ^c	11	458	91	0.1- 4.2	3.1
RS 610 ^c	29	508	97	0.1- 3.6	2.5
Brandes ^d	trace	737	83	0.1- 4.5	2.3
Dale ^d	trace	1030	99	0.1- 8.9	6.3
Wray ^d	trace	695	96	0.1- 4.2	3.0
M 81 E ^d	trace	769	95	0.1- 4.1	3.2

^a Reflects the amount of HCN obtained by digestion and distillation, presumably largely from hydrolysis of the cyanogenic glycoside, dhurrin. For sprouts, the ppm of HCN is in terms of the original dry weight of ungerminated seeds.

^b Excluding seeds that did not germinate.

^c Grain sorghum.

^d Sweet sorghum.

Table 2—Effect of time and temperature on the cyanide content of sorghum sprouts (*Sorghum bicolor* [L.] Moench cv Bird-a-boo)

Treatment	Temp (°C)	Time (days)	Percent germination	Sprout length (cm) ^a		HCN content (ppm) ^b
				Range	Avg	
Ungerminated (control)	—	0	0	0	0	13
Germinated (sprouts)	25	2	94	0.1- 2.1	1.3	152
		3	98	0.1- 3.5	2.4	275
		4	95	0.1- 5.5	3.7	376
		5	97	0.1- 6.9	5.8	508
		6	96	0.1- 9.6	7.3	614
Germinated (sprouts)	30	2	91	0.1- 7.7	5.2	580
		3	92	0.1-10.9	7.9	671
		4	98	0.1-13.2	8.4	666
		5	99	0.1-17.5	12.4	467
		6	93	0.1-16.0	11.6	390
Germinated (sprouts)	35	2	97	0.1- 5.2	3.2	385
		3	95	0.1- 9.5	4.8	342
		4	94	0.1-11.4	5.5	216
		5	93	0.1- 9.2	4.8	122
		6	90	0.1-10.0	5.5	84

^a Excluding seeds that did not germinate.

^b For sprouts, the ppm of HCN is in terms of the original dry weight of ungerminated seeds.

down to some extent in the digestive tract to yield HCN, probably by enzymes produced by intestinal flora, since man does not produce β -glucosidase (Conn, 1973). That the hydrolysis of cyanogens in the gut is less than complete is supported by the observation that some lima beans grown in Burma and Puerto Rico contain more potential HCN than the prescribed 200 ppm maximum, yet such beans are consumed with only occasional cases of acute poisoning (Conn, 1973).

Beans are cooked before consumption to make them palatable, but sprouts often are consumed raw. In raw bean sprouts or sorghum sprouts, the enzymes capable of producing HCN would be ingested with the cyanogenic substrate. If the sprouts were macerated or cut up and allowed to stand before being consumed, the indigenous enzymes and the cyanogen would be brought together, and HCN would be formed prior to consumption. In line with our observations, analogous situations exist for dried products that have received only mild heat during drying and contain active enzymes capable of hydrolyzing cyanogens.

Statistical conclusions cannot be drawn from such a small sampling of sorghum cultivars, but in this study sweet sorghum sprouts grown at 30°C contained, on the average, twice as much potential HCN as grain sorghum sprouts, while seeds of sweet sorghum (a sugar crop) contained less than grain sorghum. On a world basis, grain sorghum is grown more widely than sweet sorghum, but the use of sweet sorghum seed for the production of edible sprouts is likely, since sprouts of nontraditional food crops (e.g., alfalfa) now are being grown both commercially and in the home and consumed by increasing numbers of people in the United States.

The average potential HCN content of sprouts grown at 30°C for 3 days was 613 ppm in terms of the original dry weight of ungerminated seeds. By comparison, the maximum permissible level of potential HCN in lima beans in the United States and several other countries is 200 ppm (Conn, 1973). The average fatal dose of HCN is 50-60 mg (Windholz, 1976), and this amount was readily obtained under laboratory conditions from sprouts grown from 100g of sorghum seed — the average yield for sprouts of all eight cultivars was equivalent to 61.3 mg of HCN per 100g seed. The consumption in a single meal of sorghum sprouts or a dried product of sorghum sprouts derived from 100g of seed is entirely possible, especially in developing countries where the variety of foodstuffs may be limited.

Klim et al. (1981) estimated that upward of 1 billion people around the world are afflicted with chronic cyanide poisoning, which is manifested in ills such as ataxis neuropathies and goiter, and they attributed this widespread

toxicity largely to the consumption of two cyanogen-containing food staples, cassava and sorghum grain. If this is the case, the germination of sorghum seed to improve its nutritional quality could result in a widespread adverse, rather than favorable, effect on human health. Populations already under cyanide stress from the consumption of grain sorghum would increase their intake of cyanide by many-fold if they germinated the seed and consumed it in the form of fresh or dried sprouts. Taken on the average, our limited data for grain sorghum suggest that dietary cyanide might be increased in the neighborhood of 25 times. For sweet sorghum, the increase would be still greater, possibly as much as 500-1000-fold. Consequently, we believe that the consumption of germinated sorghum seed or dried products made from it is potentially hazardous, particularly in circumstances where sorghum is a staple of persons who are malnourished and suffering from chronic dietary exposure to cyanide.

REFERENCES

- Akazawa, T., Miljanich, P., and Conn, E.E. 1960. Studies on cyanogenic glycoside of *Sorghum vulgare*. *Plant Physiol.* 35: 535.
- AOAC. 1980. "Official Methods of Analysis," 13th ed., sections 26.134, and 26.136, Association of Official Analytical Chemists, Washington, DC.
- Conn, E.E. 1973. Cyanogenetic glycosides. In "Toxicants Occurring Naturally in Foods," 2nd ed., p. 302, (Ed.) F.M. Strong. National Academy of Sciences, Washington, DC.
- Conn, E.E. 1974. Biosynthesis of cyanogenic glycosides. *Biochem. Soc. Symp.* 38: 277.
- Conn, E.E. 1979. Cyanogenic glycosides. In "International Review of Biochemistry, Biochemistry of Nutrition," 1A, Vol. 27, p. 21, (Ed.) A. Neuberger and T.H. Jukes. Univ. Park Press, Baltimore, MD.
- Gorz, H.J., Haag, W.L., Specht, J.E., and Haskins, F.A. 1977. Assay of p-hydroxybenzaldehyde as a measure of hydrocyanic acid potential in sorghums. *Crop Sci.* 17: 578.
- Honig, D.H., Hockridge, M.E., Gould, R.M., and Rackis, J.J. 1983. Determination of cyanide in soybeans and soybean products. *J. Agric. Food Chem.* 31: 272.
- Klim, P., Kohanski, R.A., and Heinrichson, R.L. 1981. Rhodanese: Structure-function relationships relevant to the mechanisms of cyanide detoxication. In "Antinutrients and Natural Toxicants in Foods," p. 352, (Ed.) R.L. Ory. Food & Nutrition Press, Westport, CT.
- Montgomery, R.D. 1965. The medical significance of cyanogen in plant foodstuffs. *Am. J. Clin. Nutr.* 17: 103.
- Wang, Y-Y.D. and Fields, M.L. 1978. Germination of corn and sorghum in the home to improve nutritive value. *J. Food Sci.* 43: 1113.
- Windholz, M. (Ed.) 1976. "The Merck Index," 9th ed., p. 633. Merck & Co., Inc., Rahway, NJ.
- Winkler, W.O. 1958. Report on methods for glucosidal HCN in Lima beans. *J. Assoc. Off. Anal. Chem.* 41: 282.
- Zinmeister, H.D., Erb, N., and Lehman, G. 1980. Der Blausäuregehalt tropischer und subtropischer Getreidearten. *Z. Lebensm. Unters. Forsch.* 171: 170.

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