Food Chemistry **27** (1988) 167–175

Cyanide Profile of Component Parts of Sorghum (*Sorghum bicolor* L. Moench) Sprouts

Christopher O. Ikediobi, Olubukola Olugboji

Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

&

Patrick N. Okoh

Food Science and Technology Research Programme, Institute of Agricultural Research, Samaru-Zaria, Nigeria

(Received 27 April 1987; accepted 20 May 1987)

ABSTRACT

Sprouted sorghum from four Nigerian varieties were found to increase by 4000–7000% in cyanide after 2–6 days' sprouting. Separation of the seed, roots and shoots revealed that at least 99% of the cyanide is concentrated in the shoots and roots. Some local foods and beverages produced from sprouted sorghum grains contained negligible or undetectable levels of cyanide. Apparently, prior mechanical elimination of roots and shoots coupled with a heat or hot water treatment during processing is adequate for detoxifying sorghum-based food and beverage products.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the major cereals cultivated for food in Nigeria. It is, in fact, only surpassed by rice, wheat and maize in world-wide importance (Obilana, 1982). In a recent study, Obilana (1982) found that about 95% of all sorghum grown in Nigeria is used in the preparation of a variety of local foods like *tuwo*, *ogi*, *koko*, *agidi*, *kunu*, *fura* and the alcoholic beverages, *burukutu* and *pito* while only about 2% is used

Food Chemistry 0308-8146/88/$03·50

for animal feeds. The current decline in the Nigerian oil revenue, coupled with the devaluation of the country's currency, has forced industry to curtail importation and consider the use of locally grown sorghum in such industries as brewing, milling, baking, confectionery and beverages.

The use of sorghum as human food or for livestock feed is, however, seriously limited by the presence in its seeds, shoots and roots of dhurrin, a very poisonous cyanogenic glucoside. The level of this poisonous natural product depends, among other factors, on the variety of sorghum. Recently, Panasiuk & Bills (1984) reported a 3000% increase in cyanide content of sprouted sorghum seedlings. The figure of 613 ppm reported by these workers far exceeds the average fatal dose for an adult. Since a major potential industrial use for sorghum is in brewing and syrup production, in which the grain must first be germinated or malted, it is important to reexamine the cyanide question in selected Nigerian varieties and in various edible sorghum-derived foods and beverages.

MATERIALS AND METHODS

Sorghum samples

The sorghum varieties SSV_3, SSV_7, L187 and Mori were obtained from Dr C. C. Nwasike, Sorghum Breeder, Institute for Agricultural Research, Ahmadu Bello University, Zaria.

Chemicals

Chloramine T, bispyrazolone, 3-methyl-1-phenyl-5-pyrazolone and pyridine were products of British Drug Houses (BDH), Poole, Great Britain. Potassium cyanide and potassium hydroxide were purchased directly from May & Baker Ltd, Dagenham, Great Britain.

Sprouting of sorghum

About $\frac{1}{2}$ kg sample of each of the four sorghum varieties (SSV_3, SSV_7, L187 and Mori) was soaked overnight with 3 volumes of distilled water and with two changes of the water during the period. The water was removed and the wet grain further soaked in 1–2 volumes of 0·2% formaldehyde solution for $\frac{2}{3}$–1 h to retard mould growth during germination. The soaked grain was then washed several times with distilled water. The swollen grain was spread out thinly on Whatman filter paper saturated with distilled water in a shallow rectangular wooden container. Sprouted samples of grain were

removed after 2, 4, and 6 days and each seedling was carefully separated into the root, shoot and seed. Each component was then dried overnight in the oven at 80°C and subsequently pulverised by pounding in a laboratory mortar. Samples of whole seedlings were similarly dried without sectioning and subsequently powdered as described above.

Extraction of the cyanogenic glucoside

Approximately 0·1 g of each powdered sample of the root, shoot and whole seedling and 1 g of the powdered sample of the seed were separately extracted thrice with 5 ml aliquots of 0·1M sodium phosphate buffer, pH 6·8. The extract was then stored in a tightly stoppered vessel until analysed for total cyanide. Various edible sorghum-derived products prepared in the laboratory (Obilana, 1982) and purchased from the markets were similarly extracted and the extracts assayed for cyanide.

Preparation of a standard curve for cyanide

A stock solution was prepared by dissolving 4 mg of dry KCN in 100 ml of doubly-distilled water to give a solution of concentration 16 μg CN^-/ml. Eleven 15 ml tightly stoppered test tubes containing aliquots of the stock KCN solution to give a concentration range of 0·32–3·2 μg CN^- were carefully set up. Each tube was made up to 7 ml with 50 mM sodium phosphate buffer, pH 6, and then 1 ml of 0·1M NaOH solution was added and the tube and contents left for 30 min at room temperature (25–29°C).

To all the tubes prepared, each containing a total 8 ml of solution, 0·4 ml of 0·5% (w/v) chloramine-T was added and mixed thoroughly. The tubes were again allowed to stand for 5 min at 0°C, after which 1·6 ml of the bispyrazolone reagent (0·1% bispyrazolone and 0·5% 3-methyl-1-phenyl-5-pyrazolone in pyridine) was added, mixed and the tubes allowed to stand for 1 h at room temperature in a fume cupboard. The absorbance of the resulting soluble blue dyestuff was determined at 620 nm and subsequently used to plot a standard curve (Fig. 1).

Assay of total cyanide in sprouted sorghum and sorghum products

Exactly 0·1 ml of sorghum or sorghum product extract was pipetted in quadruplicate into 15 ml stoppered test tubes.

This was followed by addition of 1 ml of 0·1M NaOH and subsequent incubation for 30 min at room temperature to achieve total hydrolysis of the extracted cyanogenic glucoside, dhurrin. The HCN liberated (as CN^-) was determined as described above.

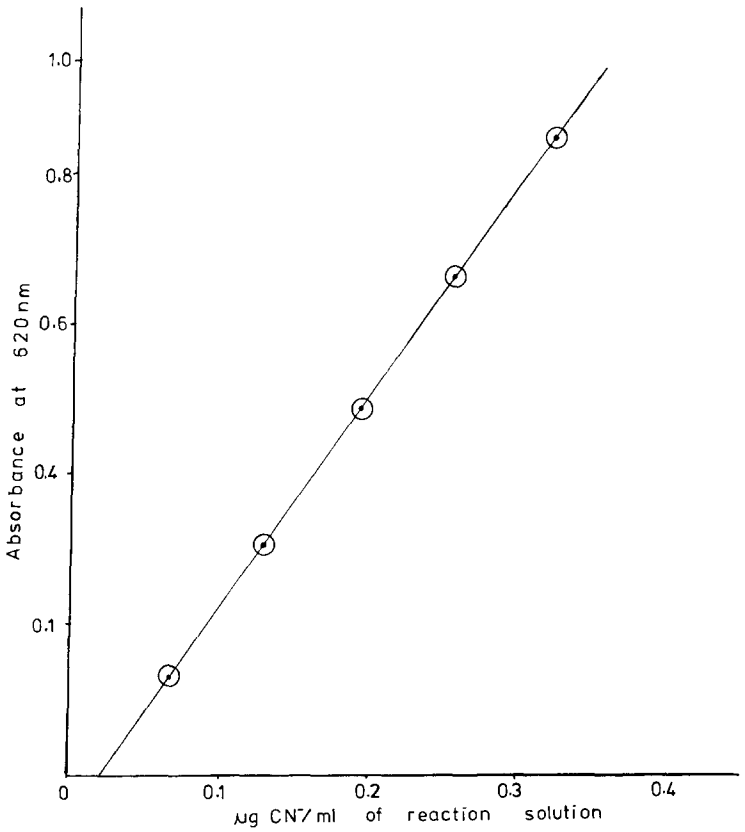

Fig. 1. Standard curve for cyanide estimation in sorghum and sorghum products.

RESULTS

Cyanide content of parts of sprouted sorghum grain

Table 1 shows the total cyanide contents of the component parts (shoot, root and seed) of sprouted sorghum, whole sorghum seedlings and ungerminated whole seeds of four popular varieties. It can be seen from the Table that there is a dramatic increase in the cyanide content of the shoot, root and whole seedling following sprouting while that of the seed remains fairly constant throughout the sprouting period. The shoot contains the highest amount of cyanide while the seed contains the least. The cyanide content of the shoot and root decreases as malting progresses from 2–6 days in all the varieties except the L187 which actually shows an increase in cyanide content. On the other hand, the cyanide content of the whole seedling increases steadily in all the varieties as malting progresses, attaining the highest value on the 6th day.

TABLE 1

Total Cyanide Content of the Shoot, Root and Seed of some Varieties of Sprouted Sorghum

Section of seedling	*Days of sprouting*	*Variety and cyanide content (ppm)*			
		SSV_3	*SSV_7*	*L187*	*Mori*
Shoot	2	3965 ± 89[a]	4694 ± 40[a]	3582 ± 45[a]	4853 ± 33[a]
	4	2342 ± 41	4635 ± 35	4244 ± 84	4406 ± 37
	6	2407 ± 47	4172 ± 43	4397 ± 121	4212 ± 44
Root	2	450 ± 0	1067 ± 9	554 ± 9	837 ± 0
	4	416 ± 14	1130 ± 9	756 ± 10	815 ± 0
	6	450 ± 0	963 ± 15	835 ± 17	675 ± 0
Seed	2	27 ± 1	23 ± 0·8	24 ± 1	23 ± 0
	4	18 ± 0·8	22 ± 0	23 ± 1	22 ± 0
	6	18 ± 0	22 ± 0	24 ± 0·6	23 ± 0
Whole seedling	2	420 ± 11	473 ± 7	588 ± 10	424 ± 7
	4	1005 ± 12	963 ± 2	1162 ± 0	795 ± 7
	6	1239 ± 0	1302 ± 8	1376 ± 13	963 ± 7
Ungerminated seed	—	17 ± 1	23 ± 0·8	22 ± 0·6	22 ± 0

[a] Mean of four determinations ± SD.

TABLE 2

Total Cyanide Content of some Commercially available Edible Sorghum-based Products

Food product	*Total cyanide (ppm)*
Burukutu	
Brand 1	9·6 ± 0·1[a]
Brand 2	11·1 ± 0·1
Brand 3	6 ± 0
Brand 4	3·5 ± 0·1
Tuwo	17 ± 0·5
Kunu (fermented)	ND
Kunu (unfermented)	ND
Composite flour bread	ND
Lager beer	
(a) Masters lager beer[b]	ND
(b) King lager beer[c]	ND

[a] Mean ± SD.

[b] Brewed from 50% sorghum malt and 50% barley malt by Premier Breweries, Ltd, Onitsha.

[c] Brewed from 50% unmalted sorghum and 50% barley malt by North Brewery Ltd, Kano.

ND = not detected.

TABLE 3
Changes in Total Cyanide at Different Stages during the Processing of Sorghum for *Burukutu*

Step/Product	*Total cyanide (ppm)*[a]
Unmalted whole grain	19 ± 0·5
After malting for 3 days	423 ± 0
Wort (unboiled)	5·1 ± 1
Wort (boiled)	2·5 ± 0
Fermented wort	2·5 ± 0
Burukutu	2·5 ± 0

[a] Mean ± SD.

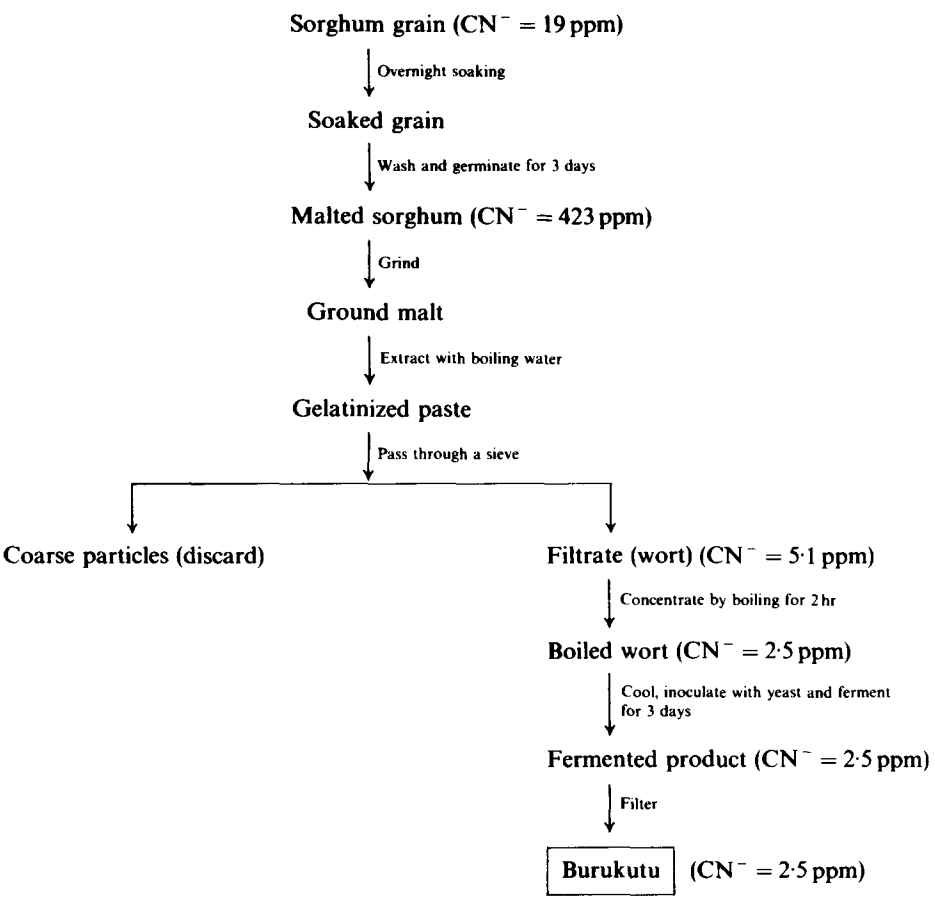

Fig. 2. Flow chart for the production of a popular local alcoholic beverage *burukutu* from sprouted sorghum grain.

There is little or no difference between the cyanide content of the germinated and that of the ungerminated seed.

Cyanide in sorghum-derived products

Table 2 shows the cyanide contents of four commercially available brands of the popular alcoholic beverage, *burukutu*, and other local food products prepared from sorghum grain. It is obvious from the data shown that all the products contain inocuous levels of cyanide. *Kunu*, bread and lager beer have no detectable amounts of cyanide. Table 3 depicts the changes in the total cyanide levels associated with the various intermediate products or steps during the production of *burukutu* from sorghum grain. Calculation shows that approximately 75% of the cyanide in the malted grain is lost during the extraction with boiling water. Figure 2 summarizes the steps involved in *burukutu* production.

DISCUSSION

The key to an accurate determination of total cyanide in plant extracts is the quantitative hydrolysis of the cyanogenic glycoside by chemical or enzymatic means (Zitnak, 1973; Cooke, 1978; Ikediobi *et al.*, 1980) to yield the assayable free cyanide (i.e. HCN). Normally enzymatic hydrolysis, where possible, is to be preferred in view of the high specificity of enzyme-catalyzed reactions. In this work, however, chemical, rather than enzymatic, hydrolysis was employed for three reasons: first, alkaline hydrolysis of dhurrin has been shown to be quantitative and fast (Wu & Wall, 1980; Olugboji, 1987). Second, a separate study has shown that β-glucosidase preparation from sorghum seeds achieved only 14% hydrolysis of dhurrin (Olugboji, 1987) presumably due to a deficiency, in sorghum seeds, of β-glucosidase II, known to be specific for dhurrin (Mao & Anderson, 1967). Third, plant β-glucosidases in general tend to sequester CN^- thus complicating the enzyme-based assay and requiring the preparation of a correction curve for each new batch of enzyme preparation (Tewe, 1975; Ikediobi *et al.*, 1986). Of the methods available for cyanide determination (Zitnak, 1973; Gorz *et al.*, 1977; Cooke, 1978; Ikediobi *et al.*, 1980; Ikediobi & Fashagba, 1985) the Cooke's modification of Epstein's method was adapted to dhurrin determination after alkaline hydrolysis on account of its sensitivity, specificity and reproducibility.

The tremendous increase in dhurrin synthesis during sprouting is explicable in terms of the usually increased metabolic activity associated with germination. In fact, during sprouting, starch, protein and lipids are

broken down by appropriate hydrolytic and oxidative enzymes to provide substrates and the energy for the syntheses of new cellular mass associated with the developing shoots and roots. In the presence of the appropriate enzyme system tyrosine from the seed proteins and glucose from seed starch are channelled into the biosynthetic pathway of dhurrin. The latter is subsequently translocated to the shoots and roots with very little remaining in the seeds as clearly demonstrated by the data in Table 1. Panasiuk & Bills (1984), in a recent study of some sorghum varieties, have made similar observations.

Because of the concentration of the cyanide in the shoots and roots, it is easy to detoxify malted sorghum grain by mechanical elimination of the roots and shoots from the dried malt. Considering the fatal doses in which cyanide exists in sprouted sorghum, the crop would be useless, or at best difficult to use as human food, were it not for this natural segregation or localized concentration of cyanide. As a result, foods usually prepared mainly from the seed (even when sprouted) are expected to contain subacute levels of cyanide as clearly shown in Table 2. The variation in the cyanide values shown for different samples of commercial *burukutu* arise from the non-uniformity of the largely unstandardised local processing methods. Nonetheless, the fact that *burukutu* is prepared (Fig. 2) with whole sorghum sprouts has continued to create concern in the minds of consumers about the long-term consequences of consuming this local drink. It would seem from Table 3 and Fig. 2 that considerable detoxification occurs during the boiling steps in the production process. Hogg & Ahlgren (1942) have reported on the facile hydrolysis of dhurrin in hot water. The consumption of sorghum in various forms, like that of cassava (another popular CN^--containing food crop) will no doubt continue to generate public concern and attract research interest because of the well documented chronic effects of cyanide ingestion in humans and livestock (Ekpechi *et al.*, 1966; Osuntokun, 1968, 1980; Ermans *et al.*, 1972; Delange, 1974; Tewe, 1975; Tewe & Maner, 1982; Wilson, 1983).

REFERENCES

Cooke, R. D. (1978). An enzymatic assay for the total cyanide content of cassava (*Manihot esculenta* Crantz). *J. Sci. Fd. Agric.*, **29**, 345.

Delange, F. (1974). Endemic goitre and thyroid function in Central Africa. *Monographs in paediatrics*, Vol. 2, S. Karger Publications, Switzerland.

Ekpechi, O. L., Dimitriadou, A. & Fraser, R. (1966). Goitrogenic activity of cassava (A staple Nigerian food). *Nature*, **210**, 1137.

Ermans, J. M., Delange, F., Van der Velden, M. & Kinthaert, J. (1972). *Human development and the thyroid gland.* Plenum Publishing Corporation, New York.

Gorz, H. J., Haag, W. L., Specht, J. E. & Haskins, F. A. (1977). Assay of *p*-hydroxybenzaldehyde as a measure of hydrocyanic acid potential in sorghums. *Crop Sci.*, **17**, 578.

Hogg, P. G. & Ahlgren, H. L. (1942). A rapid method for determining hydrocyanic acid content of single plants of Sudan grass. *J. Am. Soc. Agr.*, **34**, 199.

Ikediobi, C. O. & Fashagba, E. (1985). A method for cyanide assay in cassava and cassava products using the barbiturate-pyridine reagent. *Nig. J. Biochem.*, **2**, 124.

Ikediobi, C. O., Onyia, G. O. C. & Eluwah, E. C. (1980). A rapid and inexpensive enzymatic assay for total cyanide in cassava and cassava products. *Agric. Biol. Chem.*, **44**, 2803.

Ikediobi, C. O., Olugboji, O. O. & Okoh, P. N. (1986). Some problems associated with linamarase-based assay of cyanide. *Nig. J. Biochem.*, **3**, 56.

Mao, C. H. & Anderson, L. (1967). Cyanogenesis in sorghum vulgare: III. Partial purification and characterization of two β-glucosidases from sorghum tissues. *Phytochem.*, **6**, 473.

Obilana, A. T. (1982). Traditional sorghum foods in Nigeria: Their preparation and quality parameters. *Proceedings of the International Symposium on Sorghum Grain Quality*, 28–31 October 1981, Icrisat, Pantacheru AP, India.

Olugboji, O. O. (1987). *Biochemical studies on the cyanide content of malted sorghum (Sorghum bicolor) and sorghum products and the fate of sorghum dhurrin in the rat.* MSc Thesis, Ahmadu Bello University, Zaria, Nigeria.

Osuntokun, B. O. (1968). An ataxic neuropathy in Nigeria, a clinical, biochemical and electrophysiological study, *Brain*, **91**, 215.

Osuntokun, B. O. (1970). Cassava diet and cyanide metabolism in Wistar rats, *Brit. J. Nutr.*, **24**, 377.

Panasiuk, O. & Bills, D. D. (1984). Cyanide content of sorghum sprouts. *J. Fd. Sci.*, **49**, 791.

Tewe, O. O. (1975). *Implications of the cyanogenic glucoside fraction of cassava in the growth and reproductive performance of rats and pigs.* PhD Thesis, University of Ibadan, Ibadan, Nigeria.

Tewe, O. O. & Maner, J. H. (1982). Cyanide, protein and iodine interaction in the physiology and metabolism of rats. *Food Chem.*, **9**, 195.

Wilson, J. (1983). Cyanide in human disease: A review of clinical and laboratory evidence. *Fundament. Appl. Toxicol.*, **3**, 397.

Wu, Y. V. & Wall, J. S. (1980). *J. Agric. Food Chem.*, **28**, 455.

Zitnak, A. (1973). In: *Chronic cassava toxicity.* (Nestel, B. & Maclatyre, T. (Eds)). International Development Research Centre, Ottawa, Canada, 89–96.