Processing Techniques for Reduction of Cyanogenic Glycosides from Bamboo Shoots

Kanchan Rawat[1*], C. Nirmala[1] and M.S. Bisht[2]

[1]Department of Botany, Panjab University, Chandigarh -160014, India

[2]Centre for Science Education, North Eastern Hill University, Shillong -793002, India

[*]Email: kanchanr580@gmail.com

Abstract

Bamboo shoots have a long history of its utilization as food and medicine and are rich in nutrient components such as proteins, carbohydrates, minerals, vitamins and bioactive compounds. Due to presence of bioactive compounds phenols, phytosterols and dietary fibres, they play a vital role in health promotion as well as prevention of cardiovascular, cancer and chronic diseases. But, due to presence of cyanogenic glycoside i.e. taxiphyllin, the shoots have to be eaten with caution. Cyanogenic glycosides are the bioactive plant products present in many food crops, derived from amino acids. On hydrolysis, they release HCN which cause deleterious effects on human health. Studies revealed that juvenile shoots of bamboo show a variation in cyanogen level depending on species, harvesting age, altitude, rainfall, temperature. Even, different parts (tip, middle, base) of shoots show a large variation in the cyanogen concentrations. As the shoots of some bamboo species contain cyanogen level above the range of permissible limit, they need to be processed before consumption. This paper describes the various processing methods such as peeling, slicing, boiling, soaking, drying and fermentation applied directly or indirectly for the reduction of cyanogen content in the bamboo shoots used for food.

Keywords: Bamboo shoots, Cyanogenic glycosides, Processing, Boiling, Fermentation

Introduction:

Bamboo shoot has a long history of its usage as an ingredient in traditional food and medicine particularly in East and South-East Asian cuisines. The edible young shoots of bamboo are delicious and nutritionally rich. Bamboo shoots can be consumed in various forms as raw, dried, canned, boiled, and fermented. Now it has been determined that bamboo shoots are a good source of nutrition and getting the status of health food due to presence of various bioactive compounds and good profile of minerals (like Se, Si and K) and vitamins (Shi and Yang 1992; Nirmala et al. 2007). In fact, in the 21st century, bamboo in general and shoots in particular are posed to go beyond nutritious and tasty vegetable to functional food and nutraceuticals due to presence of bioactive compounds such as dietary fibres, phenol and phytosterol (Nirmala et al. 2011, 2014 a, b).

However, the young shoots also contain an antinutrient, cyanogenic glycosides, which is responsible for the acridity and sometimes peculiar smell in the shoots. Cyanogenic glycoside is defined as glycoside of α-hydroxynitrile. The amount of cyanogenic glycoside in plants is usually referred to the level of releasable hydrogen cyanide (HCN). They belong to a group of amino acid-derived secondary metabolites which are widely distributed in plants (Conn 1991; Seigler 1991; Vetter 2000).Till now

approximately 25 cyanogenic glycosides are identified and their content has been reported in various parts of food plants (Table 1), major ones are Linamarin in roots of cassava (*Manihot esculenta*), amygdalin in seeds of apple (*Malus spp*.), kernels of peach (*Prunus persica)* and apricot (*Prunus armeniace*), Dhurrin in leaves of sorghum (*Sorghum vulgare*) and triglochinin in leaves of giant taro (*Alocasia macrorrhizos*). The cyanogenic glycoside present in young shoots of bamboo known as taxiphyllin (Jones 1998). The structures of taxiphyllin and few other cyanogenic glycosides are given in Figure 1. Plant synthesizes cyanogen glycosides as a defence mechanism against attack of herbivores, insects and pathogens. Additional roles of cyanogenic glycosides include improvement of plant plasticity, i.e., establishment, robustness and viability with response to environmental challenges (Gleadow and Moller 2014). The level of cyanogen glycosides in plant depends on developmental (endogenous) and ecological (exogenous) factors (Vetter 2000). The variation in cyanogenic concentration also results from genetic, environment factors, location, season and soil types (JEFCA 1993). In plants, cyanogenic glycosides accumulate almost in all parts. Cyanogen glycosides types and their content may vary in above and underground parts. This pattern of variation also found in reproductive and vegetative tissues of plant. HCN is typically higher in plants growing at nonoptimal temperatures, e.g., below 15◦C or above 25◦C (Gleadow and Moller 2014).

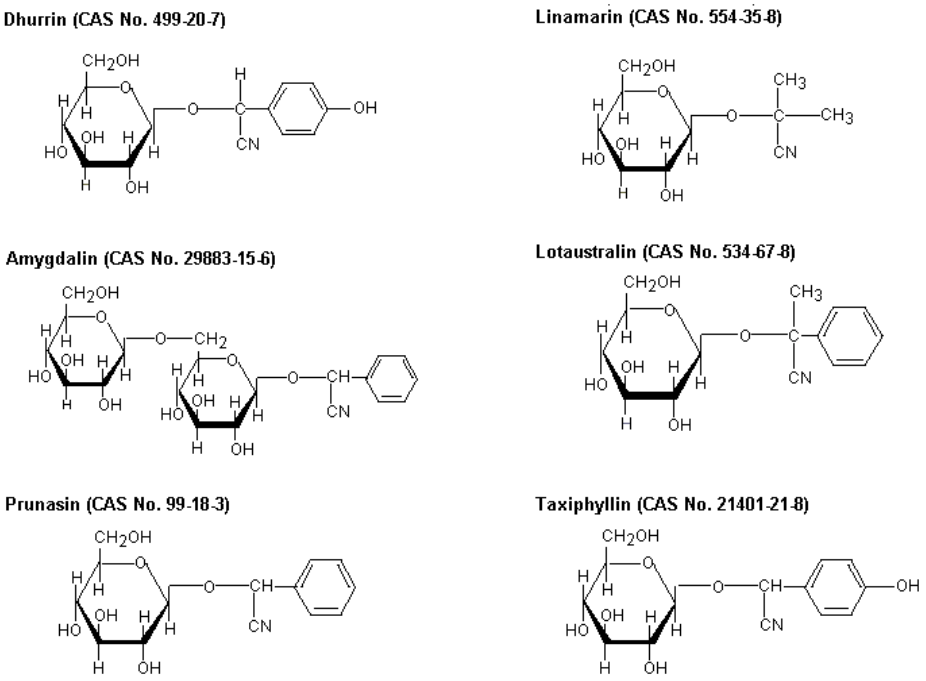

Figure 1 Structures of some commonly found glycosides in food plant

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Biosynthesis and enzymatic degradation of cyanogenic glycosides

Cyanogenic glycosides are biosynthesized from closely related amino acid precursors such as tyrosine converted into dhurrin and taxiphyllin, phenylalanine into prunasin, valine into linamarin and isoleucine into lotaustralin (Conn 1980; Seigler 1991) Figure 2. In intact cells, cyanogen glycosides are stored in vacuoles and protected from degrading enzyme. But, when the plant is disturbed, as caused by chewing herbivores or when cell integrity is destroyed by physical processes, such as by freezing or maceration, the two components come into contact and in this process; cyanogen glycoside is hydrolysed in two steps (Figure 3). First β-glucosidase enzyme converts the cyanogen into cyanohydrin which is further converted into aldehyde or ketone and hydrogen cyanide by hydroxynitrilelyase enzyme (Gleadow and Moller 2014).

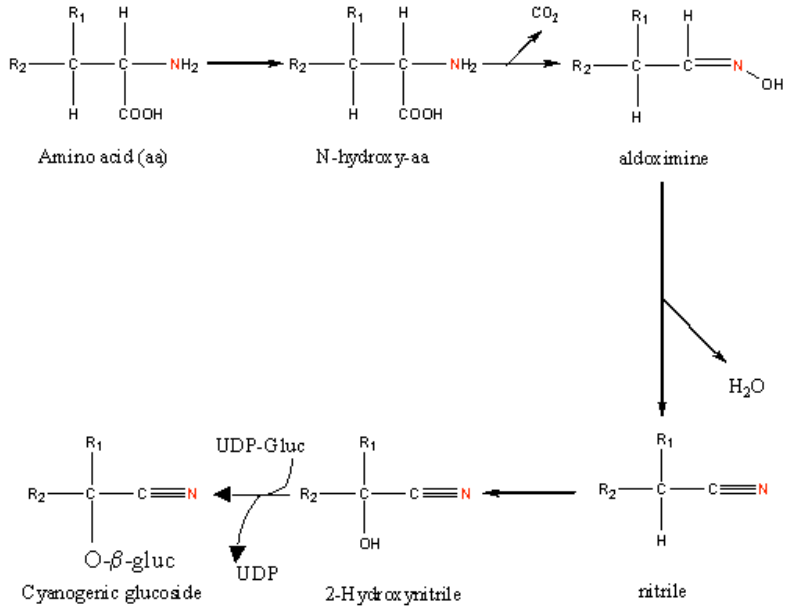

Figure 2 Biosynthetic pathways of cyanogenic glycoside

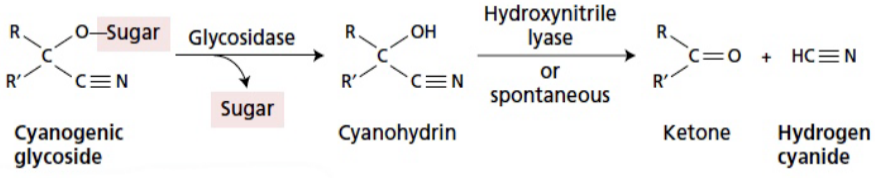

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Figure 3 Enzymatic breakdown pathways of cyanogenic glycoside

Table 1 Different types of cyanogenic glycosides and their content in some commonly consumed plant food

Food Plant	Plant Part	Cyanogenic glycosides	Cyanogenic content (mg/kg fresh weight)
Alocasia macrorrhizos (Giant taro)	Leaves	Triglochinin	29-32
Linum usitatissimum (Flax)	Seed meal	Linamarin, linustatin, neolinustatin	360-390
Malus spp. (Apple)	Seed	Amygdalin	690-790
Manihot esculenta (Cassava)	Leaves	Linamarin	200-1300
Phaseolus lunatus (Lima beans)	Seed	Linamarin	2000-3000
Prunus armeniace (Apricot)	Seed	Amygdalin	400-4000
Prunus dulcis (Bitter Almond)	-	Amygdalin	2900
Prunus persica var. mucipersica (Nectarine)	Kernel	Amygdalin	196-209
Sorghum vulgare (Sorghum)	Leaves	Dhurrin	750-790

(Adapted from Haque and Bradbury 2002; Simeonova and Fishbein 2004; Zhang et al. 2014)

Cyanogenic glycosides in bamboo shoots

Bamboo is a plant, having a high content of cyanogenic glycoside, a well-known food plant antinutrient, compared to other plants like cassava. However, the cyanogen content varies in different species of bamboo as well as in different parts of plants. In bamboo it is the young juvenile shoots where maximum amount of cyanogenic content is found. In bamboo the cyanogenic glycoside is taxiphyllin, which is structurally p-hydroxylated mandelonitrile tiglochinin (Moller and Seigler 1999; FSANZ 2004). On hydrolysis, taxiphyllin yields glucose and hydroxybenzaldehyde cyanohydrins which further decomposes to hydroxybenzaldehyde and hydrogen cyanide (Moller and Seigler 1999; FSANZ 2004). The symptoms of cyanide intoxication in humans include rapid respiration, low blood pressure, dizziness, headache, stomach pains, vomiting, diarrhoea, convulsions and mental confusion. Cyanide inhibits mitochondrial enzyme cytochrome oxidase, through combination with their copper and iron ions respectively which result in inhibition of respiration (Onwuka 2005). In humans, cyanide is detoxified into thiocynate (SCN^-) by mitochondrial enzyme rhodanese, which is further excreted in the urine. The detoxification of cyanide needs amino acids rich in sulphur like cysteine and methionine in the diet. Taxiphyllin is an unusually least stable among other similar antinutrient compounds and also thermolabile, hence can be decomposed readily by the action of heat (Davies 1991).

Cyanogen content in fresh shoots of bamboo

There are around 125 species of bamboo in India and the cyanogen content in bamboo shoots might vary from species to species. The acrid taste or smell in bamboo shoots due to presence of cyanogenic glycosides is one of the deterrence for many people to have bamboo shoots as food. In the current study cyanogenic glycosidic content in the fresh shoots and after various processing methods were analysed by the method of Haque and Bradbury (2002) using linamarin as standard. It was found that fresh shoots of some species like *Chimonobambusa callosa*, *Phyllostachys mannii*, *Melocanna baccifera* have very less content of cyanogenic glycoside ranged from 31.68- 285.12 mg/kg fresh weight which is below permissible limit of cyanogen i.e 500 mg/kg (Table 2).The shoots of these species can be eaten raw without any processing treatments. In some other species like *Bambusa jaintia*, *B. mizorameana*, *B. bambos*, *Dendrocalamus membranaceus*, *D. calostachys*, *D. hamiltonii* and *D. sikkimensis* the cyanogenic glycoside content ranged from 285.12 to 778.27 mg/kg of fresh shoots (Table 2). The cyanogen content was observed to be more than 1000 mg/kg in number of the species like *D. longispathus, Thyrsostachys oliveri*, and *D. flagellifer.* The content of cyanogens also varies in different parts of the fresh shoots. Waikhom et al. (2013) worked out in the fresh shoots of 15 bamboo species and reported that total cyanogen content varied from 300-2604 ppm (tip portion), 210-2243 ppm (middle portion) and 199-920 ppm (basal portion). Generally the tip portion contains comparatively higher amount of cyanogenic content than the middle and base portion of the young edible shoot. Various groups also observed similar trends of cyanogenic contents in the different parts of fresh shoots of bamboo species (Haque and Bradbury 2002; Haorongbam et al. 2009). Cyanogenic content in the fresh shoot of bamboo also varies according to genotype, geographic location and age of shoot. Young shoots of bamboo growing at lower altitude were high in cyanogen content as compared to species growing on higher altitude (Waikhom et al. 2013). The age of harvesting of shoots was found to be related with cyanogen toxicity. In the newly emerging shoots cyanogen content has been reported to be minimum, while an increasing trend was seen with age or days of the harvested shoot (Haque and Bradbury 2002; Haorongbam et al. 2009; Pandey and Ojha 2013). Hence, to avoid cyanogen toxicity and acridity the shoots should be harvested at right maturity stage.

Table 2 Total cyanogenic glycoside content (Result =mean ± SD of three replicates) in fresh shoots of bamboo

S. No	Species	Cyanogen content (mg/kg fresh weight)
1.	*Bambusa balcooa*	1108.32 ± 18.54
2.	*B. bambos*	678.39 ± 12.45
3.	*B. jaintia*	434.02 ± 8.14
4.	*B. mizorameana*	670.03 ± 6.42
5.	*B. nutans*	1709.66 ±18.35
6.	*B. tulda*	1412.40 ±16.30
7.	*Chimonobambusa callosa*	31.68 ± 2.12
8.	*Dendrocalamus asper*	766.66 ± 8.12
9.	*D. calostachys*	636.77 ± 6.10
10.	*D. flagellifer*	1893.67 ± 22.16
11.	*D. giganteus*	988.17 ± 18.21
12.	*D. hamiltonii*	733.92 ± 9.41
13.	*D. hookerii*	1315.78 ± 8.40
14.	*D. longispathus*	1951.49 ± 28.20
15.	*D. manipureanus*	1347.98 ± 21.10
16.	*D. membranaceus*	514.80 ± 3.15
17.	*D. sikkimensis*	778.27 ± 6.17

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18.	*D. strictus*	1717.85 ± 11.28
19.	*Melocanna baccifera*	285.12 ± 4.84
20.	*Phyllostachys mannii*	36.32 ± 2.18
21.	*Thyrsostachys oliveri*	1097.71 ± 11.14

Processing methods for reduction of cyanogen toxicity

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Fresh juvenile shoots of bamboo contain a high amount of cyanogen content which is considered as toxic for human consumption. European Food Society authority (EFSA 2004) stated that cyanogen level up to 10mg/kg HCN is safe and not associated with any acute toxicity. In general, plants having cyanogen content above 20mg/100g of fresh plant material are considered harmful for human health (kingsbury 1964). The permissible limit of cyanogen content in food is 500mg/kg (Anon 2005; FAO 2005). But, shoots of some bamboo species have cyanogen level much higher than the permissible limit. Therefore, appropriate processing methods prior to consumption are needed to reduce or remove cyanogen toxicity. Reduction in cyanogen level can be achieved by several processing methods such as slicing, peeling, soaking, cooking (boiling, roasting), fermentation, drying and canning.

Boiling

Cooking and boiling greatly reduces the antinutrients from the vegetables and fruits. During boiling or cooking, cell walls rupture which permit leakage of cell content including antinutrients and toxic substances (Ogbadoyi et al. 2006). Duration of boiling and amount of water used for boiling greatly affect the reduction of cyanogenic glycoside (Montagnac et al. 2009). Present analysis showed that cyanogenic glycoside in the shoots of *D. hamiltonii* and *D. giganteus* were reduced by 67.84 and 76.92 per cent respectively after 10 minutes boiling (Table 3). Further, cyanogen content decreased up to 87% when shoots were boiled for 20 minutes (Table 3). Similarly, Oke (1994) reported that boiling method for cyanogen reduction will be more efficient when small-sized cassava pieces boiled in large volume of water. Boiling of bamboo shoots in an open vessel at 98 - 102^{0}C for 148 - 180 min can reduce the toxicity by 97% (Ferreira et al. 1995). Steaming can also reduce the HCN from shoots up to the permissible limit (Tripathi 1998). Subsequently, reduction of cyanide content up to 91% was observed after slicing and cooking bamboo shoot in boiling water for 15 minutes. The cyanide content lowered from 40 mg/kg to 3.7 mg/kg when boiled for 15 minutes and 1.9 mg/kg when boiled for half an hour. In canned and packaged bamboo shoots samples cyanide value ranged from non-detected to 5.3 mg/kg (RAS 2007). Boiling of bamboo shoots in different concentration of brine also been reported to reduce the cyanogenic content efficiently (Pandey and Ojha 2014; Rana et al. 2012).

Soaking

Soaking is a simple traditional practice which is followed in the processing of shoots for food in almost all the species of bamboo. Soaking of shoots is quite effective in eliminating cyanogens particularly in those species which have low content (Vidal-Valverde et al. 1998). The soaking of shoots can be for few hours as in case of *Chimonobambusa callosa* and *Phyllostachys mannii* which have very low cyanogen content in fresh shoots to long term treatment in closed containers or in running water in rivers and streams in those species which have very high content of cyanogen in the fresh shoots. The Khasi-Jaintia tribes of Meghalaya have a unique method of removing the antinutrients from the shoots of *Dendrocalamus hamiltonii* which has around 733 ppm cyanogenic glycoside in fresh shoots (Table 2). The shoots are chopped in small chips and soaked in plain water for more than six months, after which the shoots lose all anti-nutrient elements and become palatable. The decrease in cyanogen also depends on some factors like temperature, time and soaking medium in

which the material is soaked. Overnight soaking of bamboo shoot slices results in enzymatic hydrolysis of taxiphyllin by ß-glucosidase to glucose and 4- hydroxyl mandelonitrile, which is further hydrolyzed to HCN and benzaldehyde by the activity of hydroxynitrilelyase enzyme (Bhardwaj et al. 2007). Experimental studies showed that shoots of *Dendrocalamus hamiltonii* and *D. giganteus* showed around 49.52 and 63.61% reduction in cyanogenic glycoside when soaked for 12 hours in plain water and more than 80% reduction when soaked for 24 hours (Table 3). Similarly, Bhargava et al. (1996) reported that removal of antinutrients during cooking of shoots by changing water several times or by pre-soaking for a long time by subsequent changing 2% salt solution. Thus, Soaking is one of the best methods for reducing the cyanogenic content below permissible limit.

Table 3 Effect of different time durations of boiling and soaking on the total cyanogenic glycoside content (mg/kg HCN fresh weight) in the shoots of *Dendrocalamus giganteus* and *Dendrocalamus hamiltonii* (Result = mean ± SD of three replicates)

Shoot Treatments	*Dendrocalamus giganteus*		*Dendrocalamus hamiltonii*	
	Content	**Reduction (%)**	**Content**	**Reduction (%)**
Unprocessed (Raw)	988.17± 18.21	-	733.91 ± 9.41	-
Boiled (10 min)	228.09 ± 12.11	76.92	236.02 ±2.98	67.84
Boiled (20 min)	137.80 ± 5.23	86.05	95.04 ±2.39	87.05
Soaked (12 hrs)	359.58 ± 8.46	63.61	370.45 ±3.22	49.52
Soaked (24 hrs)	178.14 ± 7.31	81.97	110.46 ±2.32	84.95

Drying

Drying is also an appropriate processing method for removal of cyanogenic glycosides in food plants up to the permissible limits. Drying is mass transfer process which removes water from the product by evaporation and keeps the product free from microorganisms and quality decay. Drying methods such as sun, oven, freeze and superheated steam can be employed for the reduction of cyanogen. In bamboo shoots around 80% of cyanogen glycoside was reduced after vacuum freeze drying for 24hours at -50ºC temperature. Superheated steam drying at 120-160 ^{0}C decomposes the taxiphyllin which causes bitterness in shoots (Wongasakpaiorod 2000). Oven drying after grating at 60ºC even for brief (8 hrs) drying periods leads to very high reduction of cyanogen content up to 95% of the initial cyanide content (Lambri and Fumi 2014). Oven drying at 50 ºC removes around 81 per cent of cyanogen glycoside within 24 hours (Iwuoha et al. 1997). In cassava, 80 to 99 per cent of cyanogen glycoside is removed by sun drying (Nambisan and Sundaresan 1985).

Fermentation

Fermentation is one of the ancient methods of food preservation and became widely accepted in many cultures due to its nutritional value and variety of sensory attributes (Chaves-Lopez et al. 2014). Fermentation enhances the nutritive value of food through biosynthesis of vitamins, essential amino

acids and degradation of anti-nutrients (Nwosu 2010). Since time immemorial the method is being followed in the North eastern regions for bamboo shoot preservation, removal of anti-nutrients and making shoots palatable and nutritious (Sarangtham and Hoikhokim 2010). To remove bitterness in the shoots Adi women of Arunachal Pradesh used to do semi-fermentation of shoots by covering the shoots with banana leaves and pressing under stones near water stream for 3 - 4 months (Bhardwaj et al. 2005). Cyanogen content was analysed in raw and fermented shoot of *D. hamiltonii* and content was found to be 410.27mg/kg fresh weight in fermented shoots which was less than the unprocessed shoots. Similar findings has been reported from Sarangthem and Hoikhokim (2010) who observed that fermentation of bamboo shoots decrease the cyanide content from 2.42mg/g(fresh shoots) to a non-significant toxic level ranged from 0.21-0.29mg/g of HCN. Sarangthem and Singh (2013) investigated cyanogenic glycoside (HCN) content in fresh and fermented bamboo shoots and reported that fresh samples have more cyanogen content than the fermented shoot slices. This drop in cyanide content is due to loss of HCN during peeling, slicing, washing and draining of exudates in the fermentation process. The reduction in HCN present in shoots may be due to volatile nature of taxiphyllin. Darmayanti et al. (2014) reported that fermented pickle of *Gigantochloa nigrociliata* (tabah) bamboo shoot reduces cyanogen content upto 20.52 ppm on 13th day from initial 37.8 ppm on 0 day of fermentation process. They also found that lactic acid bacteria play an important role in cyanogen reduction and make shoots safe for consumption. During natural fermentation of shoots of *D. giganteus* and *B. tulda* as the pH drops, the lactic acid bacteria indirectly convert taxiphyllin into HCN and other components by accumulating acid (Singh and Singh, 1994). Prolonged fermentation also decrease the taxiphyllin content by lowering the pH through microbial activity (Bhardwaj et al., 2007). Similar reduction in cyanogenic glycosides content has also been reported during cassava root fermentation. Cyanogenic glycosides are hydrolysed by the action of both endogenous linamarase and linamarase produced by lactic acid bacteria such as *Leuconostoc mesenteroides* and *Lactococcus lactis* (Kobawila et al. 2005). During fermentation, hydrogen cyanide which is easily soluble in water can be reduced by 99.96% (Ugwu and Oranye 2006).

Conclusion

Bamboo shoot is a well-known vegetable for its nutritional and functional properties but presence of cyanogen in the shoots is deterrent for its popularity as food. Study reveals that shoot of some of the bamboo species have cyanogen content below the permissible limits which can be eaten raw without any processing. However, most of the species of bamboo have very high content of cyanogenic content in the shoots which need processing for safer consumption. The recent study reveals that simple processing methods such as boiling, soaking, drying and fermentation are effective for cyanogen reduction up to permissible limit. The study will be helpful in establishing guidelines and strategies to eliminate antinutritional factors. Subsequently, the study will provide consumer a safer food which would be free from toxicity and thus prevent diseases which were caused due consumption of raw or unprocessed shoots.

Acknowledgements

The authors acknowledge the University Grant Commission (RFSMS, F.7-151/2007) and Department of Biotechnology, Govt. of India, New Delhi, for providing financial assistance to conduct this research work.

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