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Cyanogenic glycosides in Edible Succulent Bamboo Shoots of Manipur, India

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A B S T R A C T

Bamboo shoots have recently attracted significant research and commercial interest due to its use as a new health food and as a potential nutraceuticals. And it has been encouraged to supplement the rising food demands all over the world. In spite of the popular use of bamboo shoots, its associated toxicological properties are also well known. Uses of bamboo shoots are prone to hydrogen cyanide poisoning as bamboo shoots contain taxiphyllin, a cyanogenic glycoside. When fresh plant material is crushed ormacerated, enzymatic hydrolysis releases cyanohydric acid (HCN) and a ketone or aldehyde. The present paper exploresthe cyanohydric acid (HCN) content in the edible bamboo shoots so as to promote certain bamboo species having low cyanogenic glycoside content as safe for consumption. Present study was done on edible shoots of fourteen bamboo species found in Manipur. Amongst them *Bambusa balcooa* contain the highest level and lowest in *Chimonobam busacallosa* . The distribution of HCN varies in the portion of the bamboo shoots itself. Highest HCN content was found in the apex region and lowest at the basal portion of the fresh bamboo shoot. The cyanogenic glycosides content in bamboo shoots were found to decrease substantially in the fermented samples below the human toxic level.

Introduction

Cyanogenic glycosides are phytotoxins which occur as secondary plant metabolites in at least 2500 plant species of which a number of species are used as food(Conn, 1979; Nartey, 1980; Rosling, 1994). Among the cyanogenic containing plants, bamboo shoots has been cited as one of the highest plant containing cyanide (HCN) exceeding that of apricot, bitter almonds and that of cassava(WHO,2004). Most interestingly, bamboo shoots is consumed as one of the most favourite food items of the people of the Oriental countries and its consumption increase worldwide expanding from oriental to the western world. The emerging fresh young bamboo shoots are used in numerous Asian dishes and are available in markets in various sliced forms, fresh, fermented and canned version (Tai, 1985; Fu *et al*, 1987; Midmore, 1998). At present over two million

tons of edible bamboo shoots are consumed in the world in each year (Yang *et al*. 2008). In Manipur, a state located in the north eastern part of India, the fresh succulent bamboo shoots slices and the fermented shoot slices done in large scale are highly prized vegetable food items. More than 700,000 culms are extracted every year in Manipur (Statistical bulletin of Manipur forest, Govt. of Manipur, 1999-2000). Young delicate bamboo shoots are of favorite because of its high fiber content and its delicacy (Fuchigami,1990). They are rich in minerals, have adequate amount of glucose, low in fat and is brittle, tender, delicious and nutritive (Yamaguchi and Kusama, 1976; Yamaguchi, 1983; Park and John, 2009). Bamboo also contains many secondary metabolites which can be used as precursors of many pharmaceutical industries (Sarangthem and Singh, 2003). In spite of their high nutritive value, bamboo shoots are found to contain cyanogenic glycosides releasing hydrogen cyanide which is toxic to human being.When a plant tissue containing Cyanogenic glycosides The cyanogenic glycoside in bamboo is taxiphyllin. Taxiphyllin is hydrolyzed to glucose and hydroxyl benzaldehyde cyanohydrin. This benzaldehyde cyanohydrin then decomposes to hydroxyl benzaldehyde and HCN (Saunders and Conn ,1978;Nahrstedt, 1993; Nahrstedt, 1996;Schwarzmair, 1997; Vetter, 2000;Hunter and Yang,2002; Pandeyand Ojha, 2013). Taxiphyllin is a bitter compound (Ke-jun *et al*.,2005) making some bamboo shoots taste bitter to eat.

Hence, in the present study cyanogenic glycosides of edible succulent bamboo shoots of different species of bamboo which are consumed in their fresh and fermented forms in Manipur were studied to investigate the cyanogenic content and the likelihood of cyanide intoxication from consumption of the fresh and fermented bamboo shoots.

Materials and Methods

In the present investigation, the emerging young fresh succulent bamboo shoots of *Dendrocalamus hamiltonii* Nees & Arn.ex Munro, *D. strictus (Roxb.) Nees* ,*D. hookeri* Munro, *D. sikkimensis* Gamble ex *Oliv. Bambusa balcooa* Roxb., *B. khasian* Munro, *B. pallida* Munro, *Chimonobambusa callosa* (Munro) Nakai, *Thyrsostachysoliveri*, *Melocanna baccifera* (Roxb.) Kurz, *Schizostachyum dulloa* (Gamble) Majumdar, *Cephalostachyum latifolium* Munro, and *Pseudostachyum polymorphum* Munro were collected during peak sprouting season (May-August) from different district of Manipur, India For *Ochlandra wightii* (Munro) C.E.C. Fisch. (Nath) species (collected from Bishnupur dist., Manipur) the apical shoots (meristem) were harvested by shaking the bamboo plant and the young shoots toppled down to the ground, these were collected and the outer hard covering were removed and the inner delicate portions were used for the experiment.

Fermentation

Preservative methods of the fresh succulent bamboo shoots were done in large-scale in Manipur by traditional methods of fermentation process. The fermented bamboo shoot slices are locally called *soibum* and *soidon*.The soft portion of bamboo shoots of many Bamboo species were used for fermentation of *soibum. Ochlandra wightii* apex portion of the shoots were used in *soidon* fermentation

Traditional method of fermentation

The *soibum* is prepared traditionally by storing thin slices of fresh succulent and soft bamboo shoots in certain containers/ chambers for 2-3 months. The fermented chambers are either made of bamboo planks or roasted earthen pots. The inner surface of

bamboo chambers are lined with banana leaves and a thin polythene sheets. The upper surface is sealed with polythene sheet and weights are then put on top for proper pressing. At the initial stage of fermentation the exudates is leached/drained out of the tilted side of the bamboo plank chamber. After fermentation is completed, which is indicated by the smell, colour and texture, soibumcan be stored up to one year.

Laboratory fermentation

Fermentation of the fresh bamboo shoot slices were also carried out in the laboratory by a modified form (Sarangthem and Singh,2003) of the traditional method of fermentation which involves inoculating thin slices of succulent bamboo shoots (*Bambusa balcooa*)with the exudates obtained from already fermented slices of bamboo shoots (traditionally fermented) under aseptic condition using a Laminar flow. After inoculation, the samples were kept in an incubator at 30$\pm$2^{o}C for a period of 90 days.

Assessing the moisture content (%)

Moisture content were determined using the ISTA methods (1996) as follows-

$$\text{Moisture content (\%)} = \frac{\text{original weight- oven dry weight}}{\text{Original weight}} \times 100$$

Determination of pH

The pH of the samples was determined directly using a digital pH meter (Type 361, Systronics, India) calibrated with standard buffer solutions (Merck).

Estimation of cyanogenic glycosides:

Cyanogenic glycosides estimation was done using the technique of the picrate-impregnated paper (Bradbury *et al.*, 1999). The assay was performed in triplicate. Fresh plant material (bamboo shoots) was cut into small pieces and crushed in a pestle and motar and immediately placed into a small flat bottomed vial. 0.5 ml of phosphate buffer (0.1M, pH 7) and 6 drops of chloroform was added followed by brief crushing the materials with a glass rod. A picrate paper attached to a plastic backing strip was added and the vial immediately closed with a screw stopper and left for about 16h at 30^{0}C. The liberation of the HCN occurred rapidly after crushing the bamboo shoots. A colour change of picrate paper from yellow to brown-red or reddish colour, indicate the release of HCN by the plant samples. The change in the picrate paper is in proportion to the amount of hydrogen cyanic acid evolved. The picrate paper was then removed and eluted in 5.0 ml water for 30 min. The absorbance was measured at 510 nm and the total cyanide content was determined using potassium cyanide as the standard solution.

GC-MS analysis

The HCN present in the fresh bamboo shoot was analysed by a headspace GC technique using Varian Model 450GC-220MS with 1079 injector Auto sampler Combipal, column factor four (capillary column VF 5ms, 30m x 0.25mm ID.DF 0.25µm) . Injector temperature was 240^{0} c with 20min. hold time; column flow with 1ml/min. GC cycle time was 8 min. In order to analyse the HCN content in the fresh shoot, the shoot sample were ground separately and the sample was placed into a 15ml head space glass vial and the vial was immediately capped with a vial crimp seal fitted Teflon coated septum to avoid any loss of volatile. Then injection was made. Injection mode was GC headspace with 1ml syringe, with 120^{0}C, 5 min. incubation time. Extraction was carried out for 5 min. at 50^{0}Cto 250^{0}Cwith agitation at 250 rpm. Analysis

was carried out according to Wirthensohn *et al*., (2008).

Results and Discussion

From the present investigation, the moisture content for the fresh shoots ranges from 70.41% to 90.70% as indicated in Table 1. The highest was observed in the shoots of *Dendrocalamus hamiltonii* (90.70%) and lowest in *Pseudostachyum polymorphum* shoot (70.41%). The pH value for the fresh shoots was found to be the highest in *Ochlandra wightii* shoot slices with 6.52 and lowest with that of *Chimonobambusa callosa* with 5.38 (Table 1).

The delicate soft portion of the edible bamboo shoots of the fourteen different bamboo species were screened for hydrogen cyanide content Amongst them *Bambusa balcooa* contain the highest level (317.67mg/100g in apex, 262.67 mg/100g in middle portion and 88.33 mg/100g fresh wt. at the base of the fresh bamboo shoot) and lowest in *Chimonobambusa callosa* (4mg/100g in the apex, 3mg/100g in the middle portion and 2.67mg/100g fresh wt. at the base portion of the bamboo shoot) as shown in Table 2. The distribution of HCN varies in the portion of the bamboo shoots itself.

Table.1 Moisture and pH value in edible bamboo shoots of different bamboo species.

Name of the species	Moisture (%)	pH value
1.*Dendrocalamus hamiltonii*	90.70±1.20	6.05±0.12
2.*Dendrocalamus strictus*	89,30±1.06	6.05±0.12
3. *Dandrocalamus hookeri*	75.49±0.58	5.61±0.05
4. *Dandrocalamus sikkimensis*	75.62±1.20	5.41±0.15
5.*Bambusa balcooa*	86..47±1.10	5.98±0.36
6.*Bambusa khasiana*	87.57±1.10	5.98±0.36
7.*Bambusa pallida*	88,57±1.04	5.32±0.16
8. *Chimonobambusa callosa*	72.96±1.06	5.38±0.36
9. *Thyrsostachys oliveri*	76.94±1.29	6.36±0.41
10. *Ochlandra wightii*	71.82±1.56	6.52±0.30
11. *Melocanna baccifera*	72.74±0.52	5.59±0.33
12. *Schizostachyum dulloa*	76.44±3.64	5.72±0.22
13.*Cephalostachyum latifolium*	79.22±0.93	5.39±0.45
14.*Pseudostachyum polymorphum*	70.41±1.08	5.53±0.29

*Data presented as mean ± SD.

Table.2 Cyanogenic glycosides (HCN) content in fresh edible bamboo shoots of different bamboo species.

Name of species	Shoot portion	HCN(mg/100g fresh wt.)
	Apex	291.67±5.51
1.*Dendrocalamus hamiltonii*	Middle	224.67±6.51
	Base	155.33±4.04
2.*Dendrocalamus strictus*	Apex	214.67±3.51
	Middle	224.12±2.51
	Base	204.67±6.51
3, *Dandrocalamus sikkimensis*	Apex	255.33±4.04
	Middle	212.12±3.51
	Base	188.33±4.93
4. Dendrocalamus hookeri	Apex	191.67±3.06
	Middle	158.67±6.51
	Base	100.33±3.06
5. *Bambusa balcooa*	Apex	317.67±6.03
	Middle	262.67±7.64
	Base	88.33±4.93
6.*Bambusa khasiana*	Apex	287.67±4.03
	Middle	272.67±7.64
	Base	218.00±1.93
7.*Bambusa pallida*	Apex	213.21±2.09
	Middle	187.63±4.60
	Base	118.00±1.03
8. *Chimonobambusa callosa*	Apex	4.00±2.65
	Middle	3.00±1.15
	Base	2.67±1.15
9. *Thyrsostachys oliveri*	Apex	37.33±3.21
	Middle	25.33±3.21
	Base	18.00±5.57
10. *Ochlandra wightii*	Apex	28.33±2.52
	Middle	24.33±1.53
	Base	22.00±3.00
11. Melocanna baccifera	Apex	197.67±4.73
	Middle	192.33±6.11
	Base	125.00±4.00
12. Schizostachyum dullooa	Apex	44.33±1.53
	Middle	29.33±4.93
	Base	16.00±1.00
.13. Cephalostachyum latifolium	Apex	102.00±4.36
	Middle	29.67±4.16
	Base	14.00±2.00
14. *Pseudostachyum polymorphum*	Apex	28.67±6.66
	Middle	16.33±0.58
	Base	11.67±0.58

*Data presented as mean ± SD.

Fig.1 Chromatogram plot of *Dendrocalamus hamiltonii*.

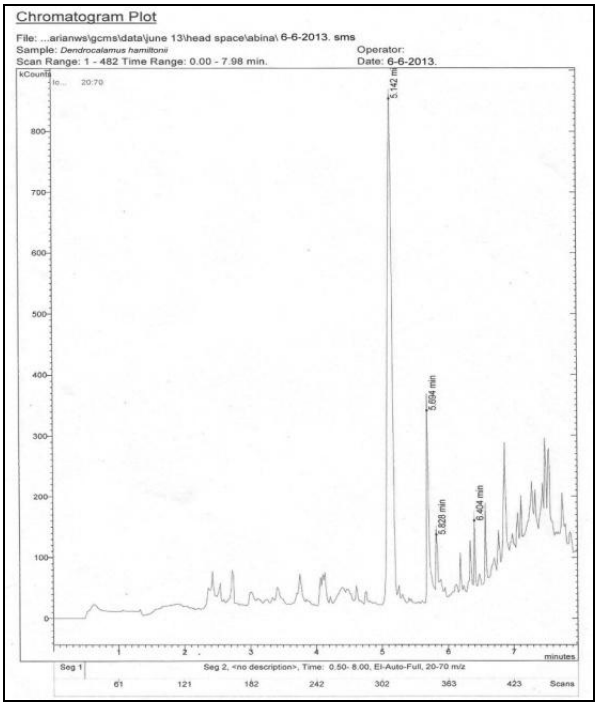
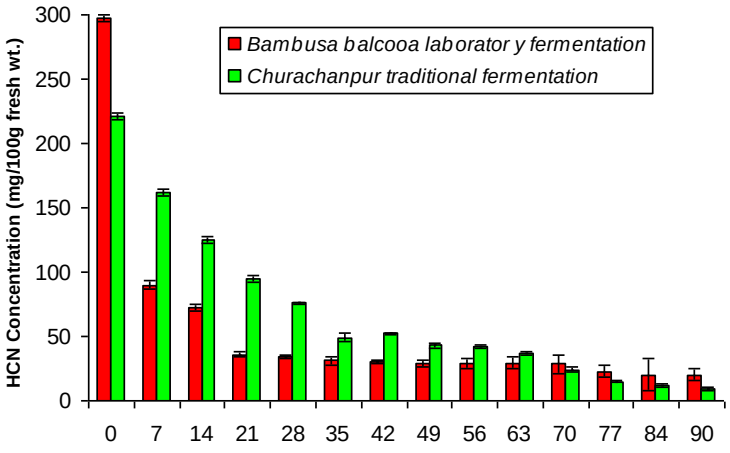


Fig.2 Changes in the cyanogenic glycosides (HCN) content during fermentation of the bamboo shoot slices of *Bambusa balcooa*. Laboratory fermentation and traditional fermentation



Highest HCN content was found in the apex region and lowest at the basal portion of the fresh bamboo shoots (Table 2). Many edible plants contain cyanogenic glycosides, whose concentrations vary widelyas a result of genetic and environmental factors, location, season and soil types (Ermans *et al*., 1980; JECFA 1993). Haque and Bradbury (2002) also reported minimum cyanogens content in the emerging shoots. An increase in the cyanogens content of bamboo shoots with

age was also reported by Fu *et al*., (2002) and NMBA (2004). This indicates that although there are reports elsewhere of bamboo species containing significant potentially verytoxic amounts of cyanogenic glycosides in their shoots (JECFA, WHO, 1993), however the available materials do not confirm that some bamboo species do indeed contain very high level of cyanogenic glycosides in their shoots. The acute lethal dose of HCN for human beings is0.5-3.5 mg/kg body weight, animals is 0.66 to 15mg/kg body weight (Reddy, 2006).

The analysis of HCN by GC-MS showed a number of calibration peaks/curves. The major peaks were then compared with MS workstation (version 6.9.1.) for identification. The chromatogram plot (Fig. 1) shows the peak at retention line 5.142 min is of the compound 1-bromo-3-(2-bromoethyl)-nonane; 5.694 min is of the compound benzaldehyde; 6.0404 min is of benzalhydrazine, N_2-(3,7-dimethyl-2-5-octadienylidene)-2-nitro were detected.

Changes in the cyanogenic content during fermentation of the bamboo shoot slices (both in traditional fermentation and scientifically modified laboratory fermentation) were conducted. The weekly analysis on the hydrogen cyanide content assessed in the laboratory fermentation (90 days) with the bamboo shoot slices of *Bambusa balcooa* shows a decreasing trend of hydrogen cyanide level. In the traditional fermentation done in Churchandpur for 12 weeks, it also shows a decreasing trend in the concentration of HCN. In all fermentation it shows a degradation of HCN content with the advance of fermentation as is shown in fig 2. The most probable reason for the decreasing trend of hydrogen cyanide level in fermentation may be that in the drained out exudates during fermentation, the content may contribute to the loss of cyanide content during fermentation. Moreover, since HCN are highly volatile (WHO, 2004), the loss of HCN during the fermentation processes like peeling, slicing, cutting, repeated washing and the involvement of microorganism may contribute to the loss of cyanide content during fermentation. Hence fermentation technology both in traditional and scientific methods should been couraged to reduce the consumption of toxic components present in fresh bamboo shoots. Further it is advisable to use the basal portion of the bamboo shoots so as to avoid cyanide intoxication

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