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Priya Pokhariya
Department of Foods and Nutrition, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Priyanka Tangariya
Department of Foods and Nutrition, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Anushriya Sahoo
Department of Foods and Nutrition, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Pratima Awasthi
Department of Foods and Nutrition, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Anupama Pandey
Home-Science Extension Department, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Correspondence
Priya Pokhariya
Department of Foods and Nutrition, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Reducing hydrocyanic acid content, nutritional and sensory quality evaluation of edible bamboo shoot based food products

Priya Pokhariya, Priyanka Tangariya, Anushriya Sahoo, Pratima Awasthi and Anupama Pandey

Abstract
Bamboo shoots are emerging as a good food option owing to its high nutritional value. Therefore bamboo; an uncommon, underutilized and unexplored food was analyzed in the present study for its nutritive value, Hydrocyanic acid (HCN) content, sensory and nutritional quality of formulated food products. It was analyzed that fresh bamboo shoot contains 90.74% moisture, 2.39% protein, 0.33% fat, 1.11% total ash, 1.48% crude fiber, 3.95 % carbohydrate and 28 kcal/100g of energy. Though, HCN is present in considerable amount in fresh, raw bamboo shoots (944mg/kg in tip, 795mg/kg in mid portion and 551 mg/kg in basal portion); four different processing methods viz. boiling, steaming, soaking and dehydration at different temperatures and time; were studied to reduce the HCN content. Further, two products were formulated viz."Bamboo shoot *pakora*" and "bamboo shoot vegetable" for the sensory evaluation and it was found that bamboo shoot *pakora* was not only more acceptable than bamboo shoot vegetable but also had an edge over bamboo shoot vegetable in terms of all sensory characteristics. "Bamboo shoot *pakora"* was found to contain more protein, energy and carbohydrate as compared to "bamboo shoot vegetable" on the basis of nutritional analysis.

Keywords: Bamboo shoots, *Dendrocalamus strictus*, hydrocyanic acid, processing, sensory evaluation

1. Introduction

In the search of new and safe sources of food for humans and animals, many wild plants, which now have not been adequately utilized by man, are being investigated as food and bamboos are one of them. Bamboos are one of the most significant evergreen plants used under Agroforestry system with incredible nutritional characteristics. Bamboo shoot contains about 88.8% water, more than 3.9% protein and 17 amino acids including eight essential amino acids. Bamboo shoots have much higher amino acid content than other vegetables such as cabbage, carrot, onion and pumpkin. Eight essential amino acids which our human system is unable to synthesize are present in bamboo shoots. Bamboo shoot contains 17 different types of enzymes and over 10 mineral elements such as Cr, Zn, Mn, Fe, Mg, Ni, Co, Cu etc. In view of these nutritional characteristics (rich in certain vitamins, essential amino acids, mineral elements, dietary fiber and low in fat, etc.) bamboo shoot is considered as an ideal vegetable for healthy diet by RFRI, 2008. All this indicates a great potential of bamboo shoot as food resource which can help in reducing the problem of nutritional deficiency. Recent studies have also indicated its potential health benefits like; Antioxidant and anti-inflammatory effects of bamboo shoot extracts (Lu *et al*., 2005), Antimicrobial and antifungal activities of bamboo shoot pyrolysates (Fujimura *et al*., 2005), prevention against oxidative stress (Akao *et al*., 2004) [2], Antiapoptotic activities of bamboo shoot-derived pyrolysates (Hong *et al*., 2010), antiviral, antifatigue and cholesterol lowering activity (Fujimura *et al*., 2005; Zhang *et al*., 2006; Park and Jhon, 2009) [13, 29].

Apart from its various nutritional qualities bamboo shoot contains taxiphyllin, a cyanogenic glycoside, which under action of heat decomposes to release HCN (Seigler, 1991) [35] which is harmful for human consumption thus in turn decreases its consumer acceptability & nutritional value. In raw bamboo shoot HCN content varies from 0.05 –0.3% and sometimes the tips of immature shoots contain upto 0.8% of HCN. For human beings the acute lethal concentration of HCN has been analyzed as 0.5–3.5 mg/kg body weight (EFSA 2004) [9].

So, approximately 50–60 mg of free cyanide from bamboo shoot constitutes a lethal dose for an adult man (Satya *et al*. 2010) [32]. The consumption of foods with toxic cyanogens could result in acute or chronic toxicity which may result in high rate of mortality and morbidity. However, various processing methods can be applied for decreasing the HCN content to a significant level, thus reducing the potential health risk.

Since bamboo shoot is an uncommon food with a great potential, there is a need to promote this crop for human consumption and to check the acceptability of the food products. More than 1250 species, belonging to 75 genera, are being reported worldwide, to which India has contributed more than 125 species of edible types belonging to 23 genera. At present, over two million tons of edible bamboo shoots are consumed in the world in each year (Yang *et al*. 2008; Vaiphei 2005) [38, 37]. Bamboo shoots are consumed as vegetables, pickles, salads and in various other forms in several countries. Consumption of bamboo shoots is mainly concentrated in Southeast Asia, where they are a popular ingredient in the local cuisine. In India, shoots of *Bambusa bambos*, *B. multiplex*, *B. tulda*, *B. vulgaris*, *D. giganteus*, *D. hamiltonii*, *D. longispathus*, *D. strictus* and *Sinobambusa elegans* are used as vegetables and pickle products. The seeds are pickled and candied and used for making beer. Consumption of tender shoots is confined mainly to the Northeastern states of India where they are part of the traditional cuisine.

The present investigation, therefore, was undertaken with a view to probe further into the nutrient quality of bamboo shoot, reducing its HCN content using different processing methods and to explore its possibility of using in food product formulation and to judge the acceptability of formulated products by way of sensory evaluation.

2. Material and Methods

2.1 Experimental design and sample preparation

Fresh succulent tender shoots of *Dendrocalamus strictus* (15-30cm of height) were procured from the Agroforestry Research Centre (AFRC), Pantnagar. Shoots were dried in hot air oven at 60^0 C for 48hrs, ground to powder in a moisture free environment and stored in airtight plastic container. Raw bamboo shoot was analyzed for its nutrient content which included proximate composition using AOAC (2000) [1] method.

For determining the moisture content two gram of sample was dried in oven at 130±3°C for 1 hour, cooled in desiccator. After cooling, the loss in weight was taken as moisture content and expressed in terms of percentage.

The protein content was detrmined using the Kjeldahl method, based on the conversion of the organic nitrogen present in the sample to $(NH_4)_2SO_4$, using 6.25 as the conversion factor.

Crude fat of the sample was extracted with petroleum ether (B.P 60-80° C) in soxhlet assembly for about 1hour 10 min.

Ash was determined by ignition of 5g of sample at 550°C until all carbon has been burnt. The residue is the ash and is taken to represent the inorganic constituents.

Crude fibre was determined as the organic residue, which remains after the defatted material is boiled successively with dilute H_2SO_4 and dilute NaOH solution.

The carbohydrate content was determined by difference i.e., by subtracting the sum of the values (per 100 g) for moisture, total ash, crude fat, crude fibre and crude protein from hundred.

Physiological energy was calculated by the method as described by Mudambi *et al*. (1989) [23]. The calorific value (Kcal/100g) of sample was calculated by summing up the product of multiplication of per cent crude protein, crude fat and carbohydrate present in the sample by 4, 9, and 4, respectively.

2.2 Estimation of HCN content

The outer covering (sheath) was removed, inedible base of the shoot was also cut and then the fresh and succulent tender shoot was used for the estimation of HCN content. After this a portion of fresh tender shoot was processed using different methods. Boiling, steaming and soaking for different time intervals (5 min,15 min, 25 min for boiling and steaming and 5hrs, 10hrs, 15hrs and 20hrs for soaking) and dehydration of shoots at different temperatures (60˙ºC, 80˙ºC, and 100˙ºC) were the four different processing methods which were followed. HCN content was analyzed for both raw and processed shoots in order to measure the reduction in HCN content after each processing method. Three different parts of bamboo shoot i.e. tip, middle and the basal portion were chosen for estimating HCN content in fresh shoot. For comparison of HCN content of raw with the processed shoots, mean value of the HCN content of three portions (tip, mid, base) of raw bamboo shoot was taken. For the estimation of hydrocyanic acid, the test procedure as described by Hogg and Ahlgren (1942) [18] was followed. The test relies on the enzymatic release of HCN from cyanogenic plant tissues and it is estimated spectrophotometrically at the wavelength of 515 nm.

2.3 Product formulation, sensory and nutritional evaluation of formulated products

Fresh and processed bamboo shoots (boiled for 25 min) were used for preparation of bamboo shoot *pakora* and bamboo shoot vegetable.

For preparation of Bamboo shoot *pakora*; Onion (50g) and bamboo shoots (200g) were chopped into very fine pieces. Bengal gram flour (100g), semolina (50g), turmeric powder (2.5g), salt (to taste), were mixed with chopped onion and bamboo shoots and a thick batter was prepared using water. *Pakora* were fried in oil on simmer flame till it turned golden brown.

For preparation of Bamboo shoot vegetable; Oil (2tsp) was heated in a skillet and cumin seeds (1/4 tsp),chopped onion (100g) and chopped tomatoes (100g),were fried in heated oil for few minutes and then salt (to taste) and turmeric powder (1/2 tsp) were added to it and stirred for few minutes. Pre-boiled (25 min) and chopped bamboo shoots were added in the skillet and cooked till done. Formulated products were then evaluated using sensory score card method to test various attributes which contribute to acceptability of the products and Nine Point Hedonic Scale for judging the liking or disliking for the products (Amerine *et al*. 1965) [3].

The evaluation was done using the semi trained panel of fifteen members from the Department of Foods and Nutrition, in a tasting room, separated from the area where the products were prepared and maintained at room temperature (25 °C) during the evaluation.

For nutrient analysis of formulated products, the samples were dried in hot air oven at 60^0 for 48hrs, ground to powder in a moisture free environment and stored in airtight plastic containers. Standard analytical methods (AOAC 2000) [1] were used for analysis of products.

2.4 Data Analysis
All analyses were performed in triplicate and data were expressed as mean values ± standard deviations. For the estimation of HCN content three replicates were taken for all the samples and the data was statistically analyzed using Paired t-test for comparison of HCN content of raw and processed shoots. The mean sensory scores of bamboo shoot *pakora* and bamboo shoot vegetable were statistically analyzed using Paired t-test, for the sensory evaluation of formulated products.

3. Results and Discussion
3.1 Nutrient composition of raw bamboo shoot
Data on nutrient composition of raw bamboo shoot are given in Table 1. The moisture content of raw bamboo shoots was 90.74g/100g which shows that the major portion of bamboo shoots is water. Slightly lower values of moisture 88.88g/100g and 85.98g/100g was reported in bamboo shoots by Gopalan *et al*., 2004 [15] and NMBA, 2009 [25]. Crude protein content of raw bamboo shoots was 2.39g/100g. Similar value of 2.08g/100g of crude protein was reported in edible bamboo shoots i.e. by Pandey and Ojha, 2011. Crude fat content of bamboo shoots was 0.33g/100g which shows that bamboo shoots are very low in fat, thus it is suitable for the obese and heart patients. Similar value 0.5g/100g of crude fat in bamboo shoot was reported by Gopalan *et al*. (2004) [15]. Total ash was found to be 1.11g/100g in the present study. Slightly lower value of 0.90g/100g total ash was earlier reported by Kumbhare and Bhargava (2007) [20].

Table 1: Nutrient composition of raw bamboo shoots

Nutrient composition	Mean value*±S.D
Moisture (g/100g)	90.74±0.516
Crude protein (g/100g)	2.39±0.095
Crude fat (g/100g)	0.33±0.055
Total ash (g/100g)	1.11±0.042
Crude fiber (g/100g)	1.48±0.142
Carbohydrate (g/100g)	3.95±0.206
Energy (kcal/100g)	28±1.604

*= Expressed on fresh weight basis, all values are average of triplicates ± standard deviation (S.D.).

The crude fibre content in the raw shoots was found to be 1.48g/100g. According to earlier investigators the crude fibre content ranged from 0.7 to 1.9g/100g (Duke and Atchley 1986; Dransfield 1995) [7, 8]. The value of carbohydrate was calculated to be 3.95g/100g. This value of carbohydrate is in agreement with the values of previous workers who reported a wide range of carbohydrate content *from* 2.0 to 6.4g/100g (Duke and Atchley 1986; Dransfield *et al*. 1995; Bhargava *et al*. 1996; Tewari 1992; Pandey and Ojha, 2011) [7, 8, 4, 36, 28] in bamboo shoots of different species. The energy value of bamboo shoots was calculated to be 28kcal/100g which shows that bamboo shoot is a low caloric food thus can be adapted by person on a crash diet for losing weight.

3.2Analysis of variation in HCN content in fresh and processed bamboo shoots
Hydrocyanic acid content of the *Dendrocalamus strictus* was found to be the highest in the tip (944 mg/kg), followed by mid portion (795 mg/kg), and the base of the shoot had the lowest amount of HCN content (551 mg/kg) (Table 2.1). Similar results were reported regarding the HCN content in bamboo shoot, apical portion of shoot had higher HCN content (742 mg/kg) as compared to the basal portion (532 mg/kg) (Ferreira *et al*., 1992) [11]. In the present study the mean HCN content of the fresh bamboo shoot was calculated to be 763 mg/kg. This mean HCN content value is again in the agreement with the result of earlier report of 750 mg/kg of HCN in bamboo shoots (Ferreira *et al*., 1992) [11]. This mean HCN content was compared with HCN content of processed shoots. Earlier investigator also gave similar results reporting that tip of the bamboo shoot had the highest HCN content (920- 1460 mg/kg), followed by the mid portion (620-1140 mg/kg), and the least HCN content in the basal portion (114-380 mg/kg) (Haque and Bradburt, 2002) [16].

Table 2.1: HCN content in different parts of fresh bamboo shoots

Different parts	Mean HCN(mg/kg)±S.D
Tip	944±0.85
Mid	795±21.33
Base	551±29.31

All values, indicating the HCN content in tip, mid and basal portion, are average of triplicates ± standard deviation (S.D.)

3.2.1 Effect of boiling on HCN content
The results showed a significant reduction in HCN content of bamboo shoots when shoots were boiled at different time intervals. HCN content of bamboo shoot reduced to 278 mg/kg after boiling of shoots for 5 min i.e. a reduction of 63.56%. However it reduced to 127 mg/kg (Table 2.2) after boiling of shoots for 15 min i.e. a reduction of 83.35%. Maximum reduction in the HCN content of 94.72% was noticed after boiling it for 25 min and HCN content reduced to 40 mg/kg. Various workers showed similar results. It was reported that loss of cyanogens during boiling was more likely to be by leaching or solublization into boiling water (Sokari and Wachukwu 1993) [33]. A reduction of over 90% of HCN from cassava chips within 15 min of boiling has been reported (Cooke and Maduagwu 1978) [6]. They reported that probable processes of loss of cyanogenic glucosides on heating are for the loss of volatile cyanohydrins (the intermediate products of cyanogenic glucosides breakdown). Therefore, decrease in cyanogenic glucosides content in the cooked plant as observed in the present study might be due to hydrolysis of cyanogenic glucosides into HCN and the evaporation of the produced HCN and cyanohydrins. Other literatures also have somewhat similar results regarding the reduction of HCN. Losses of 95-100% and 50-80% have been reported. However, it was reported that boiling of bamboo shoots removed nearly 70% of HCN present in it (Ferreira *et al*. 1995) [12].

3.2.2 Effect of steaming on HCN content
Steaming of the bamboo shoots for different time intervals also gave good results in terms of reduction in HCN content. Reduction in HCN content through steaming of the bamboo shoots was comparatively less than that obtained through boiling. Reduced HCN content of bamboo shoots was 401, 297, and 213 mg/kg after 5, 15 and 25 min of steaming, respectively (Table 2.2).

A reduction of 72.08% in HCN was noticed after steaming of shoots for 25 min. It was observed that there was less reduction in HCN content after steaming the shoots for 5 and 15 min i.e. a reduction of 47.44% and 61.07%, respectively.

However, in each case (steaming of shoots for 5, 15and 25 min), there was a significant reduction in HCN content. These results are in agreement with the data of reporting a reduction of 40-70% of cyanogennic glucosides in cassava chips as a result of steaming (Sopade 2000) [2]. Taxiphyllin is a cyanogenic glucoside of bamboo shoot which under action of heat decomposes to release HCN. HCN being a volatile compound evaporates along with the vapors of water. This may be the probable reason for HCN reduction after steaming of shoots in the present study. Steaming is reported to be as effective as baking in detoxifying cassava but both are less effective than boiling (Nambisan and Sundaresan 1985) [24].

3.2.3 Effect of soaking on HCN content

Soaking of bamboo shoots for different time intervals gave variable results. Minimum reduction in HCN content was obtained after soaking of shoots for 5 hrs, i.e. a reduction of 30.93% only (527 mg/kg). However, significant reduction in HCN content was obtained by soaking of shoots for 5, 10, 15 and 20 hrs. Amount of HCN after soaking of shoots for 10, 15 and 25 hrs were reported to be 424, 270 and 235 mg/kg, respectively (Table 2.2). It has been reported that stirring of cassava chips in cold water for 4 hrs caused negligible decrease in cyanide content. However, stirring of the cassava chips overnight (18 hrs) in cold water caused a marked decrease in cyanide accompanied by sour smell indicating onset of fermentation. So it could be concluded that soaking as a processing method for the reduction of HCN is less effective until the onset of fermentation. Similarly in the present study onset of fermentation was noticed after 15-18 hrs which also marked the significant reduction in HCN. Percent reduction in HCN content obtained through soaking was quite less as compared to that of boiling and steaming. Maximum reduction in the HCN content was obtained by soaking of shoots for 20 hrs, i.e. a reduction of 69.20%, followed by 64.61% reduction obtained by soaking for 15 hrs and of 44.42% obtained by soaking for 10 hrs. Appreciable losses in HCN content was reported as a result of soaking of some Nigerian legumes for 24 hrs (Okoliet and Ugochukwu 1989) [27]. Further, it has also been reported by Ferreira *et al.* (1990) [10] that the HCN content of bamboo shoot was reduced by soaking either in the still or running water.

3.2.4 Effect of drying on HCN content

Drying of bamboo shoots at different temperatures gave different result in terms of the extent of HCN reduction. Maximum reduction in HCN content was observed when bamboo shoots were dried at a temperature of 60˙ ºC as compared to that after drying at temperatures of 80˙ºC and 100˙ºC. Insignificant losses of HCN content were observed by drying of shoots at 100˙ºC i.e. 691 mg/kg was the amount of HCN left after drying of shoots at 100˙ºC (9.43% reduction). However, drying of shoots at 80˙ ºC and 60˙ ºC showed a significant reduction in HCN content i.e. a reduction of 55.57% and 70.24% respectively. HCN left in the bamboo shoots after drying was observed to be 339 and 227 mg/kg at 80˙ºC and 60˙ºC respectively. A decrease in the reduction of HCN content of bamboo shoot was noticed with the increase in drying temperature. Similar trend was reported by earlier investigator, who showed that drying of rasped root at 60˙ºC removed up to 90% of the cyanide and that drying at higher temperatures was less effective (Charavanpavan 1944). This can be explained by the fact that the activity of enzymes generally increases with temperature up to an optimum value, above which the enzyme is inactivated (Heimann 1980). Earlier investigation showed that cassava β-glucosidase gave maximum linamarase activity at 55˙ºC (Yeoh 1989) [39]. There was a considerable loss of cyanide at 80˙ºC also but beyond the temperature of 100˙ ºC the percent loss of cyanide decreased rapidly and there was non-significant loss of cyanide at 100˙ºC. Another reason for greater reduction in HCN content in present study at lower temperature (60˙ºC) may be due to the longer time required for drying of shoots at lower temperature which causes longer exposure of shoots at optimum temperature thus shoots getting enough time for proper release of HCN into the environment.

Table 2.2: Variations in HCN content in fresh and processed bamboo shoots

Different processing methods	Mean HCN (fresh shoot mg/kg)	Mean HCN (processed shoot mg/kg)	t-value	Percent (%) reduction
Boiling (5min)	763	278±17.10	36.61*	63.56
Boiling (15min)	763	127±14.60	53.07*	83.35
Boiling (25min)	763	40±3.40	130.11*	94.72
Steaming (5 min)	763	401±44.80	12.32*	47.44
Steaming (15 min)	763	297±8.30	57.53*	61.07
Steaming (25 min)	763	213±4.50	89.45*	72.08
Soaking (5hrs)	763	527±24.10	22.81*	30.93
Soaking (10hrs)	763	424± 18.10	23.88*	44.42
Soaking (15hrs)	763	270±8.30	195.15*	64.61
Soaking (20hrs)	763	235±13.10	46.99*	69.20
Drying (60^0 c)	763	227±11.70	105.82*	70.24
Drying (80^0 c)	763	339±9.40	54.59*	55.57
Drying (100^0 c)	763	691±26.70	3.80ns	9.43

All values are average of triplicates ± standard deviation (S.D.) Tabulated t-value: 4.302

*indicates significant difference (p≤ 0.025) among the mean HCN content of fresh and processed bamboo shoots, Ns: not significant difference

3.3 Sensory evaluation of formulated products

Results of the sensory evaluation of products using score card method revealed that the overall acceptability for both the products was same however there was significant difference between the two. The mean sensory scores of bamboo shoot *pakora* and bamboo shoot vegetable are presented in Table 3. The bamboo shoot *pakora* scored high as compared to bamboo shoot vegetable in each of the sensory attributes i.e, colour, appearance, taste, flavor and texture/mouth feel. Data of Nine Point Hedonic scale showed that bamboo shoot *pakora* was "liked very much" (scored 8 out of 9) and bamboo shoot vegetable was "liked moderately"(scored 7 out of 9) by majority of panel members.

Table 3: Mean sensory scores of bamboo shoot *pakora* and bamboo shoot vegetable

Sensory characteristics	*Pakora*	vegetable	t-value
Colour	8.46	7.80	2.56*
Appearance	8.40	7.73	2.97*
Taste	8.66	7.93	3.31*
Flavour	8.60	8.00	2.55*
Texture /mouth feel	8.53	7.60	4.42*
Overall acceptability	8.73	8.73	3.07*

Tabulated t-value: 2.048

* indicates significant difference ($p \leq 0.025$) among the different sensory characteristics of bamboo shoot *pakora* and bamboo shoot vegetable

3.4 Nutrient analysis of bamboo shoot *pakora* and bamboo shoot vegetable

Nutrient composition of bamboo shoot *pakora* and bamboo shoot vegetable showed that crude protein content of bamboo shoot *pakora* (5.09%) was higher than vegetable (3.13%). Crude fat was also very high in *pakora* (26.11%) than in vegetable (10.51%), as *pakora* is a deep fried product. However, total ash content and crude fibre content were higher in case of bamboo shoot vegetable than *pakora*. Since, bamboo shoot *pakora* is a deep fried product so the energy value was high in case of *pakora* (334kcal/100g) than bamboo shoot vegetable (160kcal/100g) (Table 4).

Table 4: Nutrient composition of formulated products

Nutrient composition	Mean value* (*pakora*) ±S.D	Mean value* (vegetable) *±S.D
Crude protein (%)	5.09±0.18	3.135±0.13
Crude fat (%)	26.11±0.19	10.51±0.51
Total ash (%)	0.80±0.10	1.50±0.19
Crude fiber (%)	0.78±0.18	2.43± 0.20
Carbohydrate (%)	19.62±0.34	13.25±1.09
Energy (kcal/100g)	334±1.02	160±1.80

All values are average of triplicates ± standard deviation (S.D.)

4. Conclusions

Bamboo shoot was found to be good source of protein (2.39%) and low in calories (28kcal/100g) and fat (0.33%).That's why it can be considered as a good food option for those with protein deficiency and obesity. However, above results showed that one of the disadvantages associated with bamboo shoot is its high HCN content, which can be reduced by some simple processing methods of boiling, steaming, soaking and drying. Further it is evident that boiling of shoots (25 min) is the best method of reduction of HCN content as it gave the maximum reduction and it is simple and easy practice to follow. However, on the other hand, drying of shoots gave the least reduction in HCN.

From the above study it might be concluded that the formulated products; bamboo shoot *pakora* and bamboo shoot vegetable, were acceptable, though the *pakora* was a more acceptable and liked product as compared to the vegetable, which proves it's potential as a tasty, nutrient dense food item.

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