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RESEARCH ARTICLE

Estimation of lesser known anti-nutrients; Phytic acid and Trypsin inhibitor from the edible bamboo shoots and their processed products of Arunachal Pradesh, India

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Abstract: Bamboo shoot is well known for the high nutritive values it possesses. People in Northeastern India, the majority of which are tribal and lives in rural area consume bamboo shoot extensively without knowing its benefits and demerits. The present study was undertaken in Arunachal Pradesh, India considering the possible threats it may pose to the people of the region in particular by estimating the anti-nutritional elements such as Phytic acid and Trypsin inhibitor from bamboo shoots of seven species and its three processed forms that are widely consume. Phytic acid content was observed within a range of 0.81 to 0.31 *P*mg/100g and 0.75 to 0.38 *P*mg/100g in bamboo shoots and processed products respectively whereas trypsin inhibitor in tender shoots of all the seven species (0.53-0.24 TIU mg/protein) and processed products (0.37-0.19 TIU mg/protein) was also recorded. Similarly, there were also significant variations in phytic acid ($F=41.509$, $P<0.001$) and trypsin inhibitor ($F=16.631$, $P< 0.001$) concentration between the fresh shoots and processed forms. The amount of antinutrients estimated from these samples are considerably less and far from any harm.

Keywords: bamboo shoots, phytic acid, trypsin inhibitor, processed products

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Introduction

For ages, young, succulent bamboo shoots are considered to be one of the most appetising foods due to their delicate taste. More than two million tonnes of edible bamboo shoots are eaten each year throughout the world (Yang *et al*., 2008). Farooquee *et al.,* (2007) earlier assessed the size of India's domestic bamboo economy somewhere at 450 million Indian rupees during the year 2007 and predicted to grow to *ca*, 26,000 million Indian rupees by 2015. By 2021, Indian Union Minister of Micro, Small and Medium Enterprise (MSME) had officially estimated the value to 30,000, crore or 300,000 million Indian rupees (The Economic Times, 2021). Among all the other important utilities the bamboo possesses, its fresh tender shoot is one important aspect which can be given special attention as it is proved to be a very nutritive product that can benefit to health. Tender bamboo shoots are sold in different forms in market laces all over North Eastern India also, and inhabitants of the urban areas get the opportunity to enjoy the taste of these products through the marketplaces.

This activity plays a key role in generating livelihoods for the rural poor who are the majority in the region. Though bamboo shoot is regarded as a very good and easily available food, some of the chemicals present in them may often render their qualification as quality food. Most of the toxicological studies on bamboo shoots have been directed at the presence of cyanogens (Schwarzmaier 1976, 1977; Tjon 1978; Wu *et al.,* 1982, 1983). Reports on other notable works on the oxalate and benzoic acid contents (Nakahara, 1974; Thilasut *et al.,* 1980 and Ogawa *et al.,* 1980), acrid components (Maruyama *et al.,* 1979, 1982), and trace elements (Wang *et al.,* 1987) in the edible bamboo shoots of different species are available. There are also reports on the estimation of phytic acid (Tamang *et al*., 2009; Kananbala & Nabakumar, 2013; Sonar & Halami 2014; Paul *et al.,* 2015) and a very few on trypsin inhibitor (Anonymous, 2017) from bamboo shoots also.

Phytic acid is a unique substance as its occurance is limited only in plants (Arnason, 2018). And because of its impact on mineral absorption, it has drawn a lot of attention. It hinders the body's ability to absorb vital minerals like calcium, iron, and zinc, which could lead to mineral shortages (Schlemmer *et al*., 2009; Wang & Guo, 2021). More importantly, the inclusion of high phytate in diets may develop detrimental effects on vegetarians, vegans, and who have ailments of iron deficiency as well (Gibson *et al.,* 2014). The reason may be given as non-heme iron from plants derived diet is poorly absorbed whereas heme iron can be absorbed efficiently (Péneau *et al*., 2008). This is furthermore highly affected by the presence of phytic acid. As it proves to be an obstacle to the benefits our health could gain from these essential elements, the term anti-nutrient is frequently associated with it.

Trypsin inhibitor is one kind of serine protease inhibitor that lowers the biological activity of the crucial enzyme trypsin. Trypsin aids in the digestion process in both humans and other animals by breaking down a wide variety of proteins. Upon consumption, trypsin inhibitor functions as a competitive and irreversible substrate. (Silverman *et al.,* 2001). Therefore, our body experiences an anti-nutritional effect from protease inhibitors that interfere with its function.. Studies suggested that chronic ingestion of trypsin inhibitor can developed adenomas as well as carcinomas of the exocrine pancreas (Hathcock, 1991).

Materials and methods

For determination of both antinutrients; Phytic acid and Trypsin inhibitor, tender shoots seven bamboo species namely *Dendrocalamus hamiltonii* Nees & Arn. ex Munro*, Phyllostachys bambusoides* Siebold and Zucc.*, Bambusa tulda* Roxb.*, Dendrocalamus giganteus* Munro*, Bambusa pallida* Munro*, Bambusa balcooa* Roxb.*,* and *Gigantochloa macrostachya* Kurz and processed products of tender bamboo shoots, such as *Eup, Ekung* and *Hidung*, were selected which are widely consumed by the tribal people of Arunachal Pradesh. *Eup* is the wet fermented shoot and *Ekung* is the dried form of the bamboo shoot and *Hidung* is the roasted as well as partially fermented bamboo shoot. Majority of tribes in the state have been known to use these entirely indigenous methods of product preparation for processing fresh tender shoots. (Sadananda, *et al.,* 2023a). It may be mentioned that only *Phyllostachys bambusoides* is not use for the preparation of these processed products. Freshly harvested shoots (not more than 24 hrs) of aforementioned seven selected bamboo shoots were brought from local market as well as from their habitats from Papumpare, East Siang, and Lower Subanshiri districts of Arunachal Pradesh. Any associated dirt was washed off thoroughly and inedible portions of culm sheets were peel off. For the analysis of Phytic acid, shoots were chopped in smaller and thin slices and later dried it in room temperature and grounded to powder in Willy's mill. But for determination of Trypsin inhibitor, fresh shoots were grounded in a pre-chilled mortar and pestle and required amount of samples were taken for the analysis. The samples were analysed using three replicates.

Quantitative analyses of Phytic acid and Trypsin inhibitor were carried out through standard colorimetric methods as described by Sadasivam and Manikam (2008).

Phytic acid

50ml of 3% TCA and 5 mg of each finely ground (40 mesh) sample were extracted for 30 min with 45 min of mechanical shaking into 125ml Erlenmeyer flasks. After the suspension was centrifuged, an aliquot of 10 ml of the supernatant was transferred to a 40 millilitre conical flask. It was heated through water bath for 45 min after adding 4ml of $FeCl_3$ solution. After 30 min of heating, added two drops of 3% TCA till the supernatant remains clear and

decanted the clear supernatant after centrifuge (10 -15min). After thoroughly dispersing the precipitate in 20–25 ml of 3% TCA, the residue was heated for five to ten minutes before being centrifuged once more. 3ml 1.5 N NaOH was added with few ml of water to disperse the precipitate. Afterwards, the volume was made to 30 ml with water, and the water bath was done for an additional 30 minutes. The precipitate was then rinsed with 60–70 ml hot water after being filtered through Whatmann No. 2, and the filtrate was disposed of. In a 100 ml volumetric flask, the precipitate from the paper was dissolved using 40 ml of hot 3.2 N HNO_3. An additional 100 ml volumetric flask was filled with 5 ml of the aliquot, which was then diluted to about 70 ml. After adding 20 ml of 1.5 M KSCN diluted to volume, the colour was read at 480 nm right away (within a minute). For each sample set blank were also run.

Standard solution

In a volumetric flask, 433 mg $Fe(NO_3)_3$ was dissolved in 100 ml of distilled water. To make 250 ml in a volumetric flask, 2.5 ml of this stock standard was diluted. 2.5, 5, 10, 15, and 20 millilitres of this working standard were pipetted into a series of 100 millilitre volumetric flasks, and 5 millilitre solutions were taken out of each flask, and the rest of procedure was followed as before in the samples .

Calculation

Colorimetric analysis is used to assess the precipitate's iron content, and the value of this analysis is used to compute the phytate-phosphorus content, presuming that the molecular ratio of 4 Fe:6 P in the precipitate remains constant.

$$\text{Phytate P mg/100g sample} = \frac{\mu g\ Fe \times 15}{\text{Weight of sample}}$$

Colorimetric analysis is used to assess the precipitate's iron content, and the value of this analysis is used to compute the phytate-phosphorus content, presuming that the molecular ratio of 4 Fe:6 P in the precipitate remains constant.

Trypsin inhibitor

Source of trypsin inhibitor (TI): To fully extract the trypsin inhibitor (TI), 0.5 g of the sample was ground in 25 ml of water in a mortar that had been previously refrigerated. The ground sample was then placed in the refrigerator for two hours, stirring it occasionally. The homogenate was centrifuged for 20 minutes at 4°C at 12,000 rpm. After that, 1 ml of the supernatant was diluted with 10 ml of distilled water as the TI source.

Pipetted extracts ranging from 0 to 1 ml were placed into duplicate test tube sets labelled as test (T) and endogenous (E). The endogenous set's volume was increased to 2 ml using the buffer solution and 1 ml in the test set. Each test set tube then received 1 ml of the trypsin solution (20µg). One millilitre of trypsin solution and one millilitre of buffer were pipetted into a separate test tube for the standard solution (S). After a few minutes in a water bath set at 37°C, 2.5 ml of substrate (1 mg BAPNA) was added to each test tube, and the water bath was kept at that temperature for an additional half hour. 0.5 millilitres of glacial acetic acid were added to halt the process. In the spectrophotometer, the absorbance was measured at 410 nm. The extract's protein content was established by utilising the procedure outlined by Lowry *et al*., (1951).

Calculation

T-S absorbance was examined. A plot was made between the absorbance and the extract volume. It was established that one unit of trypsin inhibitor was equal to the aliquot size of the extract needed to inhibit 50% of the trypsin activity (S/2).

In the context of experimentation, one unit of activity is equivalent to 0.5 µg of trypsin inhibitor, which results in a 50% suppression of enzyme activity. Trypsin inhibitor units (TIU) per g sample or per mg of protein are used to express the trypsin inhibitor activity. For the experiment, the trypsin inhibitor source dilutions were prepared so that 0.5 ml resulted in 50% inhibition.

Statistical analyses of the collected data were carried out by using one-way ANOVA to understand their significant level of variation and changes in anti-nutrients among bamboo samples and their processed forms.

Results

Fig 1. A & B presents the phytic acid concentration products respectively. Variations in phytic acid in tender shoots of seven species and available processed concentration among the shoot samples was significantly different ($F=48.049$, $p<0.001$),

where the highest concentration was recorded in *D. hamiltonii* (0.81 *P*mg/100g), and the lowest concentration was observed in *B.tulda* (0.31 *P*mg/100g). Phytic acid concentration in the rest of the bamboo species was in the order of *P. bambusoides>D. giganteus >B. balcooa>B. pallida>G. macrostachya.* Significant variation in phytic acid content among the processed forms was also observed (F=40.554, p <0.001). *Hidung* exhibited the highest phytic acid content (0.75 *P*mg/100g), followed by *Eup* (0.52 *P*mg/100g) and lowest by *Ekung* (0.38 *P*mg/100g). Similarly, there were also significant variations in phytic acid concentration between the fresh shoots and processed forms (F=41.509, p <0.001).

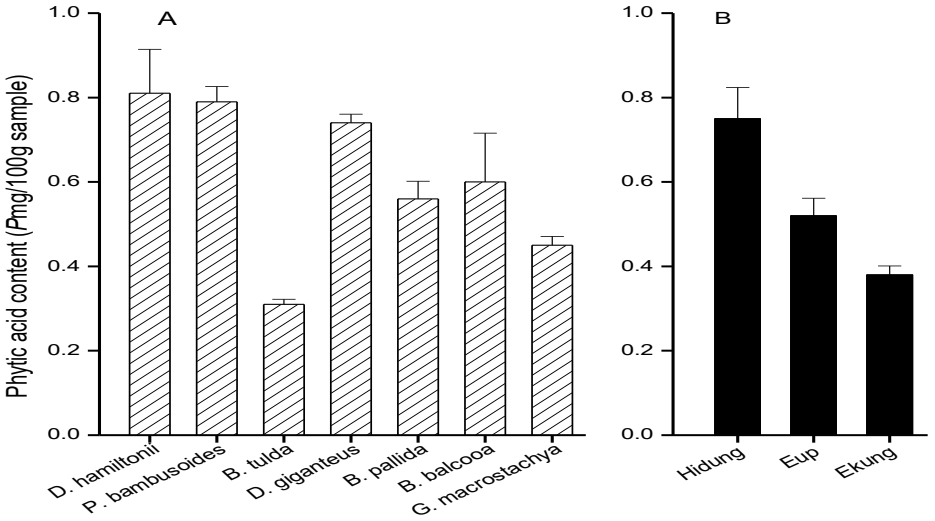

Fig 1. Variation in Phytic acid content in (A) seven bamboo shoots (mean ± SD, $p < 0.001$), (B) and in processed forms (mean ± SD, $p < 0.001$)

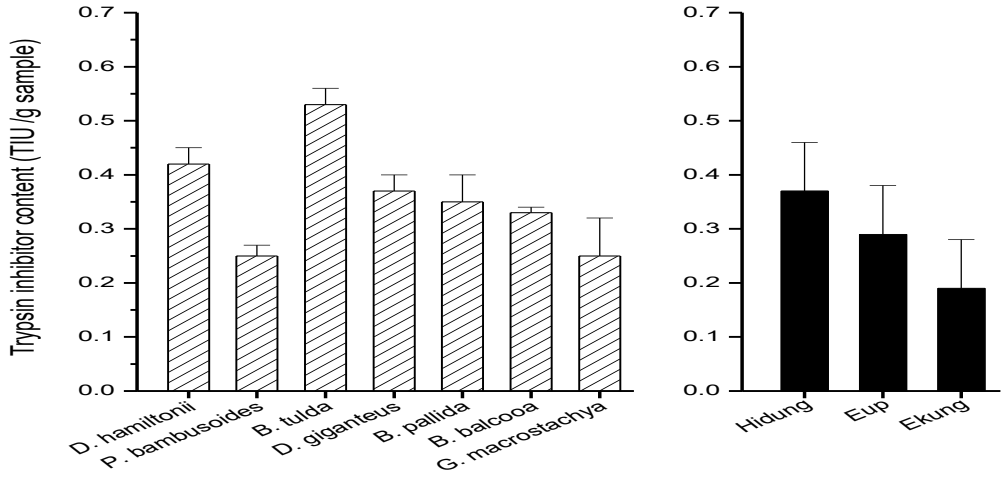

Fig 2. Variation in Trypsin inhibitor content in (A) seven bamboo shoot (mean ± SD, $p < 0.001$), (B) and in processed forms (mean ± SD, $p < 0.001$).

Data on trypsin inhibitor content in bamboo shoots as well as their processed forms is given in Fig. 2. A & B. The result show a significant variation in trypsin inhibitor content among the tender shoots of seven bamboo species where *B. tulda* showed the highest concentration (0.53 TIU mg/ sample) and the lowest value was recorded in *P. bambusoides* (0.24 TIU mg/ sample) ($F = 13.367$, $p < 0.001$). Trypsin inhibitor content in rest of bamboo species was in trend as *D. hamiltonii>D. giganteus>B. pallida>B. bulcooa> G. macrostachya.* Variation in mean trypsin inhibitor content among the processed forms was also significantly different ($F= 39.224$, $p< 0.001$) where it was highest in *Hidung* (0.37 TIU mg/ sample) followed by *Eup* (0.29 TIU mg/ sample) and *Ekung* (0.19 TIU mg/ sample). Trypsin inhibitor content between tender shoots and the processed product was also significantly different where a much higher value was recorded in fresh shoots than in processed form ($F=16.631$, $P< 0.001$).

Discussion

The presence of antinutritional elements in food-stuffs always hinders the good prospect of any food. However, it is also important to note that up to what extent the element can pose threat to health. There are health concerns associated with consuming more than 800 mg of phytic acid per day, even if the Recommended Daily Intake (RDI) varies from one country to another country (Abdoulaye, 2015). The present study revealed that phytic acid content which showed a range of 0.81 to 0.31 *P*mg/100g and 0.75 to 0.38 *P*mg/100g in both bamboo shoots and processed products respectively is well below the potential of posing any threat to health. This result is comparatively very less than the findings in tender shoots of other bamboo species (Kananbala & Nabakumar, 2013). However, the present observation of a much lower phytic acid content in processed products can draw a similar pattern (Kananbala & Nabakumar, 2013).

According to Tamang *et al.,* (2009), there is additional evidence that fermentation causes foods' phytic acid content to decrease. It was also documented that during the fermentation of bamboo shoots, *Lacto bacillus bravia* degraded the phytic acid content by a considerable amount (Sonar & Halami, 2014).

Data obtained from the present study on trypsin inhibitor in tender shoots of all the seven species (0.53-0.24 TIU mg/protein) and processed products (0.37-0.19 TIU mg/protein) is comparatively very less than those observations made from other food vegetables (Doell, *et al.,* 1981; Venderjagt, *et al.,* 2000; Elemo, *et al.,* 2011). The investigation additionally demonstrated that the processed goods had a notable decrease in trypsin inhibitor. Similarly, several workers (Vidal, *et al.,* 1993; Egounlety & Aworh, 2003; Hong, *et al.,* 2004) also observed a significant reduction of trypsin inhibitor during and after fermentation.

Conclusion

Despite having a very rich bamboo resource, India is lacking by far in terms of exploiting its benefits in general and utilization of bamboo shoots as quality food in particular. Without a doubt, bamboo shoots have a comparatively high nutrient content and a huge potential for health benefits. (Sadananda, *et al.* 2021; 2023a,b). With a few notable exceptions across the nation, eating bamboo shoots as food is primarily reserved for indigenous states and communities.. Without having much knowledge of the pros and cons, people had been consuming bamboo shoots from time immemorial. But it becomes imperative to have a well-informed awareness, especially when the presence of certain elements in foodstuffs may pose a health hazard. Presence of cyanogenic glycoside; taxiphyllin is well documented in bamboo shoots. Apart from this, the presence of other antinutrients like phytic acid and trypsin inhibitor needs to pay attention too. Also it can be mentioned that all the examined species and products contain very less amount of targeted toxins that it poses no threat to human health and can be claimed that all are suited for edible purpose. The result may look insignificant from the point of an attempted discovery but it is very significant by determining the shoots and products are free from harm. Furthermore, there are ways to mitigate the addressed problem as it is well-established fact that the fermentation procedure can significantly lower these anti-nutritional components, enhancing the shelf life and quality of the bamboo shoots (Chongtham *et al*., 2021). It further opens the possibility to market these fresh shoots and products to international community as it will be fed at higher prices. For comparison, retail price range of bamboo shoot in Yuan Renminbi is CNY 20.97 to CNY 63.04 per kg (*ca* 239- 720 India rupees) whereas in North-eastern India its prices ranged from 40 to 50

Indian rupees per kg even though China produces fairly larger amount of bamboo shoots than India. Thus it can be concluded that, North eastern part of India has enormous potential in merchandising its bamboo shoot that can attract masses and building a better economic opportunity.

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