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Essential Amino Acids and Nutrients Found in Tender Bamboo Shoots and Products Available in Arunachal Pradesh, India

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Abstract

Bamboo is a versatile grass with multiple applications and yielding edible tender shoots. Juvenile shoots are a delicacy for many ethnic communities all over the globe and are rich in essential nutrients for human health. A study was conducted to identify the concentrations of essential amino acids *viz*. Lysine, Methionine, and Tryptophan with Crude protein, Crude fiber, and Carbohydrates in both fresh and processed tender shoots of seven commercial bamboo species available in Arunachal Pradesh, India. Concentrations of all nutritional parameters considered for this study are comparable with conventional foods and some values were higher in processed bamboo shoot products than those of fresh tender shoots. Findings of this study revealed that bamboo shoots and their processed products are highly nutritious which can provide opportunities in both nutritional as well as economic aspects to the people of the country in general and the state in particular. It may provide opportunities to combat poverty and hunger by opting for new re*adi*ly available food sources and may also provide scope to overcome malnutrition, especially in the areas where it is widely prevalent.

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Processed Products.

Introduction

Bamboo - a giant grass belongs to the sub-family *bambusoideae* of *poaceae* family. In addition to more than 1500 traditional and scientific applications, its young succulent shoots are an important delicacy for many ethnic and continental cuisines. In Northeast India, tender bamboo shoots in fresh, fermented, and roasted forms are consumed in large quantities as food in several preparations such as pickles, curry, salad, fermented and roasted products, etc.[1,2] Stewed bamboo shoots and *Mesu*, a form of bamboo shoot pickle are also regarded

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as a delicacy.[3,4] In the recent past, bamboo shoots and their products are gaining the attention of the mass due to their high nutritive values.[5-7] There are reports on the presence of essential amino acids *viz*. tyrosine, arginine histidine, and leucine in bamboo shoots, whereas, qualitative and quantitative data on lysine, tryptophan, and methionine are very meagre.[8] Lysine is important for lowering cholesterol levels, absorption of calcium and formation of collagen, etc. whereas, methionine can dissolve fats and prevent liver damage due to acetaminophen (Tylenol) poisoning, and tryptophan plays a very important role in the improvement of normal growth in infants and adults also finds its usage in treating many diseases and disorders including arguably its utilities in one of the most challenging fields in modern times ADHD (Attention deficit hyperactive disorder).[9-13] Although there have been reports on the nutritional mak*eup* of several bamboo species from throughout the world, their values differ among the species, products, and locality due to many significant reasons.[2,5,8] Notable works on crude protein, fiber, fat, carbohydrates, vitamins, and minerals for the fresh tender shoots of many bamboo species were also carried out.[3,5,6] Not only bamboo shoot has 17 types of amino acids, 10 types of important minerals, but compared to most vegetables, it also contains higher amount of selenium, sometimes known as the "Mineral of life.".[14] Recent studies also showed that fermentation of the shoots helped in increasing the level of phenols, flavonoids and antioxidative capacity significantly indicating the importance of value addition to bamboo shoots.[15] Through this study, an attempt was made to determine a few of these important nutritional parameters such as Lysine, Methionine, and Tryptophan with Crude protein, Crude fiber, and Carbohydrates in fresh tender shoots as well as processed products namely *Hidung*, *Eup* and *Ekung* of seven bamboo species of Arunachal Pradesh bearing high commercial values throughout the state.

Materials and Methods

For the study, the selection of the bamboo species and their processed products was done based on availability and their edible nature. As per the survey, 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 *Hidung*, *Eup* and *Ekung* were selected for detailed analysis of essential amino acids namely, lysine, methionine, tryptophan and crude protein, crude fiber, and carbohydrates.

Indigenous Methods Adopted for the Preparation of *Hidung, Eup* And *Ekung* by the Local Inhabitants of Arunachal Pradesh

The methods employed to prepare *Eup* (moist fermented), *Ekung* (dried fermented), and *Hidung* (partial fermented and roasted) are purely indigenous and are popularly adopted by major tribes namely *Nyshi, Adi*, and *Galo* on regular basis and occasionally by *Apatanis* and *Mishings* of the state. All the tender shoots were utilized for the preparation of processed products by the people of the region with an exception of P. b*ambusoides*. The samples were collected from the local major markets of Papumpare, East Kameng and East Siyang Districts of the state. The sales and consumption of the collected fresh shoots and its processed products are done throughout the state.

A. Preparation of *Hidung* (Roasted Bamboo Shoots)

Hidung is prepared from the species like *Dendrocalamus hamiltonii*, *Bambusa tulda*, *Bambusa pallida*, *Dendrocalamus giganteus*, *Bambusa balcooa*, and *Gigantochloa macrostachya*. Firstly, the outer sheath is partially peeled off then the whole shoots are placed inside a bamboo basket (fermenter) which offers spaces to leach out the liquid liberated from the shoots. A pit measuring 2-3m long and 1.5 m wide is dug out and bamboo shoots containing baskets of varying sizes are laid down. On an average, only two or three baskets are kept together at a time traditionally but may actually increase in some cases. The basket is covered with banana leaves followed by a polythene layer then tied with ropes, and covered the baskets with the soil. After a gape of two to three weeks, the whole basket is removed and a partially fermented shoot is roasted giving the final product *Hidung*. Afterward, the item is dispatched for selling in primary vendors as well as secondary vendors. Sometimes the unsold *Hidung* is washed and removed from the stains of burnt marks by a knife, pressed in the bamboo basket, and kept

for further fermentation for around 1-2 months. Then it is packed in polythene and made available in the market for resale.

B. Preparation of *Eup* (Moist fermented bamboo shoots)

Most of the species considered for the present study are used for preparing *Eup*, however, *Phyllostachys bambusoides* is not used for making *Eup.* Collected bamboo shoots are entirely freed from outer sheaths and then washed. Following this, the shoots are cut into smaller pieces and put into the polythene-lined basket. Then banana leaves are used to cover the basket and fastened with little cord around the neck to secure it. Later the basket containing chopped shoots is laid down inside a pit and covered by soil. And to give weight, heavy stones are piled up just above the area where the basket containing shoots was buried. For a period of one to three months fermentation is undertaken. Then the fermented shoots are collected along with the juice and bottled for selling directly or indirectly.

C. Preparation of *Ekung* (Dried bamboo shoots)

Only *Phyllostachys bambusoides* is not considered for preparing *Ekung* while the other species namely *Dendrocalamus hamiltonii*, *Bambusa tulda*, *Bambusa pallida*, *Dendrocalamus giganteus*, *Bambusa balcooa*, and *Gigantochloa macrostachya* are utilized for the preparation of Ekung. Outer sheaths of the collected bamboo shoots from the wild are completely taken off and then washed. The shoots are then cut into smaller pieces and placed inside a basket without polythene layering. The preparation of *Ekung* also follows a similar method as in preparation of the *Eup*. But a finer cut is made for the preparation of *Ekung* as it is going to be sun dried for five to ten days. Then the dried *Ekung* is ready for sale in primary or secondary vendors.

Estimation of Amino Acids

Essential amino acids such as lysine, methionine, and tryptophan were determined through colorimetry following the methodologies as outlined by Sadasivam and Manikam.[16]

A. Lysine

100 mg of the defatted sample was mixed with 5 ml of the papain solution, which was then incubated at 65 °C overnight then centrifuged and decanted the clear digest. One millilitre of digest was mixed with 0.5 millilitres of copper phosphate buffer and carbonate buffer before centrifuging. 0.1 ml of the pyridine reagent was added to 1 ml of supernatant and thoroughly mixed by shaking for two hours. After that, 5 ml of 1.2 N HCl was added, extraction was done with 5 ml of ethyl acetate thrice and discarded the top layer. At 390 nm, the aqueous layer's absorbance was measured. The identical process was used to prepare a blank using just 5ml of papain. In order to create a standard curve, 62 mg of lysine monohydrochloride was dissolved in 50 ml of carbonate buffer (1 mg of lysine/ml), and five concentration grades (0.2, 0.4, 0.6, 0.8, and 1 ml) were made from the same solution.

Calculation

$$\text{Lysine content} = \frac{Lysine\ value\ from\ graph\ in\ \mu g\ \times 0.16}{Percent\ of\ N\ in\ the\ sample}$$

= g per 16g N

B. Methionine

On a 0.5 g defatted sample, 6 ml of 2 N HCl was added, and the mixture was autoclaved at 15 lb pressure for one hour. Activated charcoal was added to the hydrolysate, brought to boil, filtered, and then the charcoal was washed with hot water. It was neutralised with 10 N NaOH to pH 6.5 and was brought up to 50 ml volume. Then, 25 ml of the solution was then mixed with 3 ml of 10% NaOH and 0.15 ml of sodium nitroprusside. One millilitre of glycine solution was added after waiting 10 minutes, followed by two millilitres of orthophosphoric acid, all of which were violently shaken. After 10 minutes, the red color's intensity was measured at 520 nm. By pipetting out 0, 1, 2, 3, 4, and 5 ml of standard methionine solution and making up to 25 ml with water, several concentration classes were created. Standard curve was prepared following the above mentioned procedure.

Calculation

$$\text{Methionine content} = \frac{Methionine\ content\ from\ the\ graph\ \times 6.4}{Percent\ of\ N\ in\ the\ sample}$$

= g per 16g N

C. Tryptophan

To 100 mg of the defatted sample, 5 ml of the papain solution was added and incubated at 65 °C. 4 ml of reagent C was then added to the clear supernatant after it had been centrifuged. Then add the vortex mixture and continue to incubate for 15 more

minutes at 65 °C. After allowing it to thaw at room temperature, the orange-red color's absorbance was measured at 545 nm. By applying the same method, a standard curve was created from standard tryptophan solutions of various concentrations.

Calculation

$$\text{Tryptophan content} = \frac{Tryptophan\ value\ from\ graph\ in\ \mu g}{Percent\ of\ N\ in\ the\ sample} \times 0.096$$

= g per 16g N

Using the Anthrone methode, quantitative analysis of total carbohydrate was carried out. Total nitrogen content was determined by Kjeldahl method using the KEL Plus Nitrogen Analyzer (Pelican, India), later the value was multiplied by 6.25 to calculate crude protein concentration.[17] Sulphuric acid and sodium hydroxide were used as an acid-based digestion technique to measure dietary fibre under standard conditions.[16]

Statistical Analysis

All the data were statistically analysed for their significant levels (one-way ANOVA) using SYSTAT software Ver 13.0 and Graphical presentations were generated using ORIGIN software version 7.0.

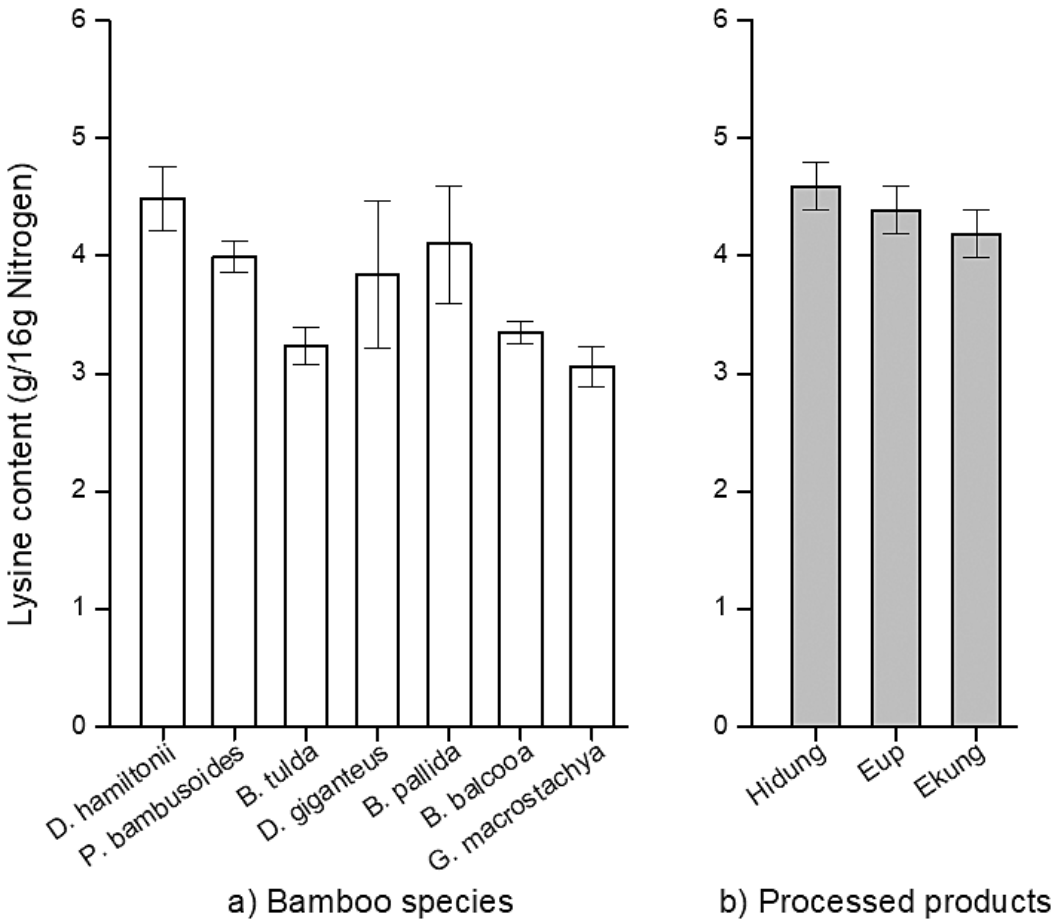

Fig. 1: Variation in Lysine content in (a) fresh tender shoots of seven bamboo species (Mean ± SD) and in (b) processed products of tender shoots (Mean ± SD, p< 0.01).

Results & Discussion

Lysine content in the fresh tender shoot was highest in *Dendrocalamus hamiltonii* than in the rest six bamboo species considered for the study whereas, *Hidung* – the roasted and partially fermented bamboo shoot showed the greatest

value of lysine among the processed products (F= 21.313, p<0.01) (Fig. 1). Processed bamboo shoot products were considerably rich in lysine than fresh tender shoots (F= 9.175, p<0.001). The observed values of lysine contents may be compared with what was recorded in *Phyllostachys manii*[18] and *Phyllostachys pubescens*[19] as well. The results were also comparable with other conventional foods and vegetables as per the Nutritive value of Indian foods.[18] Relatively high concentration of lysine recorded in processed bamboo shoot products to that of the fresh tender shoot may be attributed to the liberation of lactic acid when fermentation took place. A similar finding was reported by many workers, where an increase in lysine in foodstuffs was observed during lactic acid fermentation.[20-26]

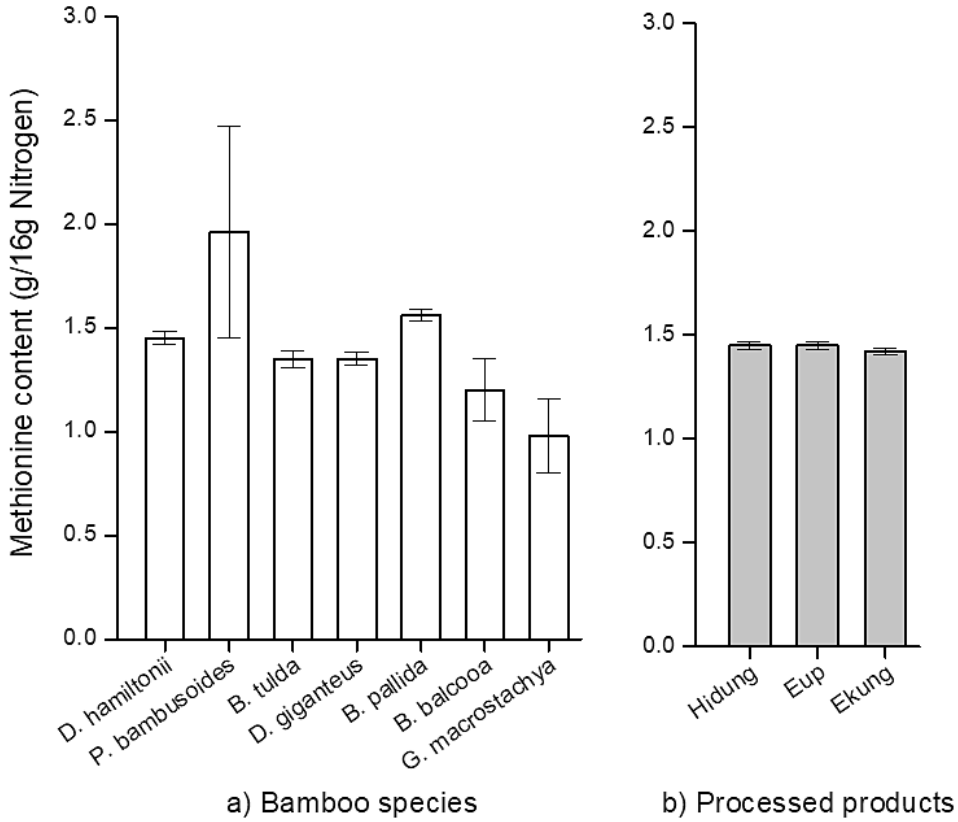

Fig. 2: Variation in Methionine content in (a) fresh tender shoots of seven bamboo species (Mean ± SD, p<0.005) and in (b) processed products of tender shoots (Mean ± SD).

Significantly high methionine content in the fresh tender shoot was observed for *Phyllostachys bambusoides* than the rest six bamboo species (F= 6.26, p <0.005). Though, there was no significant variation in methionine content among the three processed products (Fig. 2). Variation in methionine content between fresh and processed bamboo shoot products was significant (F = 5.936, p< 0.001). The value was greater in processed products than in fresh shoots. This may be due to the liberation of lactic acid when fermentation takes place as in lysine content.[24] It has been observed that methionine can be liberated from the foodstuff by the microbial action.[27,28] More or less similar results were also obtained from tender shoots of different bamboo species.[18,19]

Similar to methionine, tryptophan content in the fresh tender shoot was also significantly high for *Phyllostachys bambusoides* (F = 27.789, p < 0.001)

whereas, in the case of processed products, the value of tryptophan was significantly high in *Hidung* - a roasted and partially fermented bamboo shoot product (F= 31.941, p < 0.001) (Fig. 3).

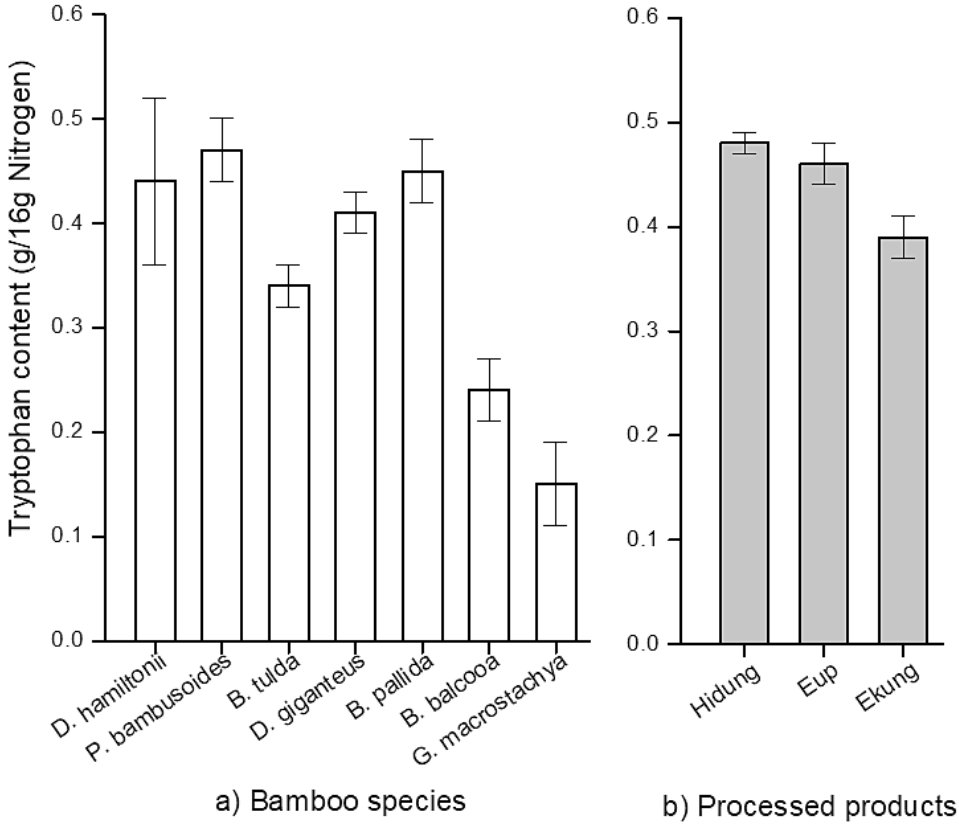

Fig. 3: Variation in Tryptophan content in (a) fresh tender shoots of seven bamboo species (Mean ± SD, p< 0.001) and in (b) processed products of tender shoots (Mean ± SD, p< 0.001).

Table 1: Variation in Crude protein, Carbohydrates and Crude fibre content in fresh tender shoots and processed products (Mean ± SD) (one-way ANOVA)

Bamboo Species	Crude Protein (%)	Carbohydrates (%)	Crude Fibre (%)
Dendrocalamus hamiltonii	$20.8^{a,ns}$ ± 0.1	$4.8^{a,a}$ ± 0.4	$4.18^{a,ns}$ ± 0.1
Phyllostachys bambusoides	$22.7^{a,ns}$ ± 0.2	$6.3^{a,a}$ ± 0.17	$3.68^{a,ns}$ ± 0.2
Bambusa tulda	$18.7^{a,ns}$ ± 3.1	$4.6^{a,a}$ ± 0.2	$5.29^{a,ns}$ ± 0.1
Dendrocalamus giganteus	$17.3^{a,ns}$ ± 0.1	$5.5^{a,a}$ ± 0.2	$4.70^{a,ns}$ ± 0.2
Bambusa pallida	$30.3^{a,ns}$ ± 0.1	$4.9^{a,a}$ ± 0.2	$4.24^{a,ns}$ ± 0.1
Bambuda balcooa	$14.5^{a,ns}$ ± 0.6	$5.1^{a,a}$ ± 0.2	$4.88^{a,ns}$ ± 0.2
Gigantochloa macrostachya	$7.1^{a,ns}$ ± 0.1	$4.4^{a,a}$ ± 0.2	$3.52^{a,ns}$ ± 0.2
Processed products			
Hidung	$20.6^{b,ns}$ ± 0.1	$4.3^{a,a}$± 0.15	$4.25^{c,ns}$ ± 0.2
Eup	$20.9^{b,ns}$ ± 0.1	$3.8^{a,a}$ ± 0.2	$3.70^{c,ns}$ ± 0.5
Ekung	$19.5^{b,ns}$ ± 0.3	$3.6^{a,a}$ ± 0.2	$4.20^{c,ns}$ ± 0.1

$^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$, ^{ns}Non significant

Tryptophan content in processed products was considerably high than that of fresh tender shoot irrespective of bamboo species and different processed products ($F= 30.861$, $p < 0.001$). The significant increase of tryptophan content in fermented products may be explained by the formation of probiotic bacteria in the fermentation process and experimental studies have shown that the administration of probiotic bacteria increased peripheral tryptophan levels.[22,23] Tryptophan content in bamboo shoots and processed product mostly show a greater value than those in vegetables which are known as a rich source of amino acids.[5,20,21,29,30]

Variations in crude protein, total carbohydrate, and dietary fibre content in selected fresh tender bamboo shoots and their processed products are presented in Table 1. Fresh tender shoot of *Bambusa tulda* show considerably high crude protein content than the rest six bamboo species ($F= 110.387$, $p < 0.001$) whereas, among the processed products, crude protein was highest in *Eup* - a moist fermented bamboo shoot product ($F= 67.282$, $p < 0.01$). There was a little increase in average crude protein content when fresh tender shoots were processed into different products. It may be justified as there are reports on the increment of crude protein content with an increase in the fermentation period in other crops.[29] A seemingly higher value in crude protein content in processed products may be explained due to the accumulation of protein compounds by microbial activities during the fermentation process.[31] Values of crude protein observed in tender bamboo shoots during this study were observed within the range as reported of other bamboo species.[31-33]

Total carbohydrate content in the fresh tender shoot was significantly high for *Phyllostachys bambusoides* than the rest six bamboo species ($F= 23.725$, $p < 0.001$) whereas, *Hidung* - a roasted and partially fermented product was considerably rich in total carbohydrate ($F= 9.948$, $p < 0.001$). In contrast to the crude protein content, there was a considerable decline in total carbohydrate content while fresh tender shoots were converted to different processed products ($F= 34.857$, $p < 0.001$). This phenomenon may be attributed due to microbial conversion of carbohydrates to other organic compounds including secondary metabolites.[34] Total carbohydrate content in both fresh and processed bamboo shoot products observed was within the range of findings as reported and comparable with other conventional food crops.[20,26,35]

Percent dietary fibre content in the fresh tender shoot was considerably high in *Bambusa tulda* than the rest six bamboo species ($F=41.793$, $p< 0.01$). Variation in dietary fibre among the three processed products was also significantly higher in *Hidung* - a roasted and partially fermented bamboo shoot product ($F=3.151$, $p< 0.05$). However the difference of dietary fibre content between fresh tender shoots and products was non-significant. Overall it was found that both fresh tender shoots and their processed forms were rich in dietary fibre and comparable with other bamboo species and conventional foods.[20,24,26,35-37]

Conclusion

Bamboo is a plant of many utilities, it helps in carbon sequestration at a much faster rate as it is the fastest growing land plant and its young tender nutritious shoot serves as food in many countries. In fact when the climate crisis is scaling up in the world in an unprecedented rate, perhaps bamboo may provide a solution in feeding more people when the crops are dying and in absorbing the excess carbon dioxide from the air. It is noteworthy that bamboo shoots are also consumed in many marginalized areas. One such area is the North eastern part of India. The largest state in the region, Arunachal Pradesh also consumes bamboo shoots which are collected purely from the wild. The study was conducted to understand the nutritional aspect of these edible bamboo shoots and their process products available in the state and revealed the presence of a sizable quantity of three essential amino acids *viz*. lysine, methionine, and tryptophan, and other nutritional components such as crude protein, crude fiber, and carbohydrates in seven fresh tender shoots and their three processed products. It is evident from the findings that the concentration of lysine and tryptophan was higher in processed bamboo shoots, whereas methionine was highest in fresh tender shoots. This study also revealed the presence of higher crude protein and carbohydrate content in processed bamboo shoots than the fresh tender shoots, and a considerable fiber percent in both fresh and processed bamboo shoots. Therefore, this finding may help to select suitable bamboo species

as well as processed bamboo shoot products for tapping essential nutrients and their utilization as food & nutrient supplements to fulfill the daily requirements for a healthy life. Overall it can be concluded that bamboo shoot can be promoted as a future food for all and not restricted to Asian ethnicity only as it can be produced in a mass scale providing options of food security and its rich nutritional profile will certainly help in accommodating global nutritional security too.

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Conflict of Interest
The authors declare that there is no actual or potential conflict of interest

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