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Microbial Fermentation by Traditional Process using Intrinsic Microflora Reduces the Cyanide Content of Bamboo Shoots

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Ethnic people living in the North-East India, Nepal and Bhutan prepare and consume a variety of domesticated and wild bamboo tender shoots. Fermented bamboo shoots are consumed as a component of regular delicacies. However, presence of potentially toxic compounds called cyanogenic glycosides has been a matter of concern in its consumption. In this study, several bamboo shoot samples (fermented and unfermented-succulent bamboo shoots) were collected from different sites of Manipur. The cyanide content of fermented bamboo shoot samples were analyzed and compared with succulent bamboo-shoots, boiled bamboo-shoots, soaked in saline water or without overnightsoaking. The pHs of all the fermented samples were found to be around 4.0 while that of unfermented bamboo shoot was recorded to be of pH 6.Bacterial counts of samples were determined and colony forming unit (CFU)ranges 3×10^7 - 724×10^7per gram. The nutritional parameters were also recorded. Protein content of succulent bamboo shoot and fermented bamboo shoot rangedbetween 500mg- 800mg/100g. The reducing sugar content ranged between 100 – 1200mg/100g. Unfermented - succulent bamboo shoot had high cyanide content (approx. 43.55 mg/g) as compared to the fermented samples (8.78-17.89mg/g). It was observed that boiling the succulent bamboo shoots as well as fermented bamboo shoots removed all the cyanide content from the samples.

Keywords: Cyanide, Fermented bamboo shoots, Nutritional.

Bamboo, belonging to the family *Poaceae* is natural resources in the world. Bamboo is intricately associated with humans from times immemorial. It is a plant which is widely distributed and grows wild in the fields and mountains from temperate zone of Japan to the tropical zone of India[1]. Bamboo shoots are the new culms that newly emerge from the ground and constitute various ranges of traditional delicacies[2]. Size and weight of fresh shoot depends upon climatic, pH and nutrition of soil, rainfall, drainage condition and harvesting period of bamboo shoot[3]. The freshly harvested bamboo shoot are creamy yellow in colour with pungent smell and bitter taste, due to the presence cyanogenic glycoside, named taxiphyllin which is toxic in nature[4]. The content of hydrogen cyanide (HCN) varies depending on the parts of the shoots and the bamboo species, ranging from less than 100 to nearly a 1000 mg HCN per kg of fresh shoot[5]. A glycosidase enzyme in the shoots hydrolyzes the glycoside and produces HCN, when the shoots are disrupted by cutting or peeling. Generally, traditional preparations including boiling can reduce the HCN content in bamboo shoots and make them edible[5,6].

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Bamboo shoots contain 0.3 to 0.8% HCN. Out of which, up to 0.16% of the total cyanide is contained in the tip, reducing to 0.01% in the base, with highest in leaves of young plants, but dropping rapidly after pollination. However, subsequent processing helps in fighting the cyanide concentration, though incomplete cooking result in glycoside hydrolysis and higher release of HCN, but the total amount of HCN in the shoots can be eliminated/ detoxified by boiling/cooking for two hours[7,8]. Though, succulent shoots of edible bamboo species have been consumed as food both in fresh and processed forms, particularly in the bamboo growing regions of the world. For centuries, bamboo shoot have lent unique flavors and a distinctive crunchy texture to traditional Asian dishes and is easily fermentable, people living in such places have been consuming fermented bamboo shoot as indigenous food[9]. The viability of integrating raw/processed bamboo shoot in modern diet and lifestyle for enhancing food nutritional security is explored[10]. Fermented bamboo shoot products are consumed as traditional food by Ethnic people of North-East states of India[11]. In Manipur, bamboo shoot is consumed as fresh or fermented. Fermented form, locally called *Soibum*, is a highly prized item and its consumption dates back time immemorial. There are classically two main types differing in their mode of fermentation; *Andro type* and *Noney/ Kwatha type*. *Andro type* of preparation of *Soibum*is practiced (only in Andro village) in the bulky roasted earthen pot by fed-batch fermentation and *Noney/Kwatha type*, by batch fermentation with more acidic taste is carried out in traditionally designed bamboo chamber. *Soibum* is produced from shoots of selected species of bamboo as *–Dendrocalamus hamiltonii* (Wanap, Unap, Pecha), *D. sekimensis* and *D.giganteus* (Maribop), *Melancona bambusoide* (Moubi/Muli), *Bambusa tulda* (Utang), *B.balcooa* (chingsaneibi) by natural fermentation[12]. In addition to preservation, fermented foods increases digestibility, improves nutritional and pharmacological values as well. Modern research has revealed that bamboo shoots have a number of health benefits includes improving appetite and digestion, weight loss, curing cardiovascular diseases, antioxidant activities and anti-inflammatory effects[13], and anti-cancer property[2]. Each fermented food is associated with a unique group of microflora which increases the level of proteins, vitamins, essential amino acids and fatty acids[7].

Lb. plantarum, Lb. brevis, Lb. corniformis, Lb delbrueckii, Leuc. fallax, Leuc. Lactis, Leuc. Mesenteroides, Enterococcus durans, Enterococcus faecium, Streptocaccus lactis, Bacillus subtilis, B. lichniformis, B. coagulans, Micrococcus luteus and yeast-*Candida, Saccharomyces* and *Torulopsis* have commonly been isolated from fermented bamboo shoot[14]. Lactic acid bacteria (LAB)has been reported as functional micro-organisms from some of the common fermented bamboo shoot of North-east states i.e., *Mesu* of Sikkim Himalyas, *Lung-siej* of Meghalayas, *Soibum* of Manipur, *Sinki* of Sikkim[15].

Therefore, the present study was undertaken to optimize the cyanogenic glycoside content of both the succulent and fermented bamboo shoot and to analyze their nutritional parameters.

MATERIALS AND METHODS

Study area

Samples were collected from different area of Manipur, located in North-Eastern region of India. Manipur lies in the Eastern Himalayan region of India at latitude of 23°83´N - 25°68´N longitude of 93°03´E - 94°78´E. The state is located at an average altitude of 790 meters above sea level. The state has a salubrious climate condition with approximate average annual rainfall varying from 933mm at Imphal to 2593mm at Tamenglong. The temperature ranges from sub-zero to 36°C.The study area selected for the present research covers an area of about 2238 sq km. The site includes the four valley districts i.e, Bisenpur, Imphal East, Imphal West, Thoubal and Chandel (Table No. 1).

Collections of samples

Fermented samples of *Bambusa balcooa* were collected from its production centre at four valley district places (Bishnupur, Kakching, Lamangdong, Singjamei, Kwatha, Andro) and one hill district (Lamkhang, Kongkhang, Shilet) of Manipur. The collected samples were packed in a sterile polythene bags and transported and stored in laboratory refrigerator for further analysis. Also

the succulent bamboo shoot of uniform size and maturity were procured from different places of Imphal valley of Manipur.

Microbiological analysis of the sample

The samples that are collected are then subjected to serial dilution for the isolation of bacteria. The samples were taken in test tube containing 9 ml of sterile water. Serial dilution technique was performed upto $10^{9.}$ From the tube with 10^6,$10^7$and 10^8 dilutions 0.5 ml of water containing *Soibum* samples was poured onto nutrient agar plate and de Man, Rogosa and Sharpe (MRS) agar plate spread over and colony forming unit (cfu) were counted.

Cfu count/gram = (No. of colony × dilution factor) / Weight of the sample

The plates were incubated at 37°C for 24 hour to observe bacterial colonies. Each of the morphologically different colonies was streak on sterile agar plate and incubated at 37°C for 24 hour.

Determination of pH

Samples (1.0 gram) of succulent bamboo shoot or the fermented bamboo shoot were blended in 10ml of sterile water and the pH was determined using a pH meter (Type 335, Systrinocs), as per the standard procedure defined by[16].

Toxicity assay

Determination of total cyanide in bamboo shoot was done with a modification of Picrate paper kit procedure. Cyanogenic glycocides estimation was done using the technique of the picrate impregnated paper[17]. The assay was performed in replicate. The new leaf matter of the bamboo shoot was removed exposing the edible bamboo shoot. The tip section contains more cyanide than the middle section which contains more than the base section. Each section was analyzed by cutting a thin section (about 0.5-1 cm thick across the bamboo shoot and then cutting a small sector from it. This sector was ground up in a pestle and mortar. (*The enzyme breaks down the taxiphyllin quite rapidly to produce HCN, hence these steps were carried as quickly as possible*). Immediately weighed out about 25 mg each of fermented bamboo shoot samples, boiled fermented bamboo shoot, unfermented boil and succulent bamboo shoot of the ground shoot in a flat bottomed plastic bottle. (Weight of sample = z mg). Immediately added 0.5 mL of 0.1 M phosphate buffer at pH 6. Further, added a yellow picrate paper attached to a plastic strip with precaution that the picrate paper must not touch the liquid in the bottle. Picrate papers were stored at -20°C. The bottle was closed with a screw capped lid. Another sample as above but with no bamboo shoot was processed to serve as a blank. The bottles were allowed to stand for 16-24 hour at room temperature (20-35^o C). The picrate paper was immersed in 5.0 ml of water (measured accurately with a pipette) for about 30 min with occasional gentle shaking. Took the blank picrate paper, removed its plastic sheet and immerse the yellow picrate paper in 5.0 ml of water for about 30 min with occasional gentle shaking. Measured the absorbance at 510 nm of the picrate solution against the blank. The total cyanogen content in ppm was calculated by the equation

Total cyanogen content (ppm) = 396 × absorbance × 100 / z.

Composition of nutrients

Component such as protein, amino acid, fat, carbohydrate and vitamins affects the nutrition issues and are considered to be important. Hence proximate analysis was carried out on the succulent bamboo shoot and fermented bamboo shoot samples.1gram of succulent bamboo shoots were homogenized with 10 ml of distilled water and the pH of the slurry was measured with a Systronics type 335 pH meter[17]. Moisture content was calculated by drying the sample at 130± 1°C to constant weight[17]. Ash content was measured by heating the sample at 600°C until the difference between two successive weighing was < 1 mg.

Estimation of soluble protein

The quantity of soluble protein was determined by colorimetric method[18]. 1.0 gram each of the succulent bamboo shoot, boiled succulent, fermented boiled or fermented bamboo shoot samples were crushed with 10 ml cold phosphate buffer (pH 7.5, 0.1M) using a chilled mortar and pestle. The homogenate was filtered through four layers of cheese cloth. The filtrate was centrifuged at 5000 rpm for 15 min in a centrifuge. The temperature of the centrifuge was maintained at 4°C. The supernatant was poured into a graduated tube and its volume was raised to a 10 ml with the same buffer. The pellet was discarded. The supernatant contains soluble proteins. 1 ml from this supernatant added with 1 ml of TCA solution. The precipitation (whitish in colour) was completed

in 5 min. The precipitate was centrifuged and the supernatant was discarded. 5 ml of ethyl alcohol (95%) was added to the soluble protein precipitate so as to remove the excess TCA from the surface of the soluble protein. The washed precipitate was mixed with a freshly prepared reagent mixture of 2 % sodium carbonatein 0.1N NaOH, 1% copper sulphate and 2% sodium potassium tartrate (10+0.1+0.1) at room temperature (25° C) and kept for 20 min with occasional shaking. Later 0.5 ml of folin-reagent was added and allowed to stand for 30 min at room temperature. The absorbance was read at 750 nm by using a spectrophotometer.The standard curve for soluble protein was prepared by using Bovine serum albumin.

Estimation of reducing sugars

The quantity of reducing was determined by standard method with minor modification. 1 gram each of the succulent bamboo shoot, boiled succulent, fermented boiled or fermented bamboo shoot samples were extracted with hot 80% ethanol twice (5 ml each time) and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and evaporated it by keeping it on water bath at 80°C. 10 ml of water was added to dissolve the sugars. 1 ml of extract was collected in the test tubes and amount of reducing sugar was determined by standard procedure[19].

RESULTS AND DISCUSSION

Traditional fermented foods of Manipur are prepared at household level through indigenous practices of food processing and preservation. *Soibum* is a fermented bamboo shoot product of Manipur traditionally consumed by the Meitei. It serves as a high consumer acceptance because of its unique flavor, texture and aroma. Indigenous food fermentation is one of the oldest food biotechnological process dependent on the biological activity of the micro organisms. The product with its exquisite taste and smell serve as a source of protein in the diets of the people[20]. Processing caused considerable changes in the composition of the micro flora in counts as well as in species found[21]. Fermentation is the traditional method for preserving the food. These methods of preservation are traditionally used with cultural identity and these household arts are handed down through generations. Bacteria play a very important role in fermentation. Functional effects of probiotic bacteria include adherence to the intestinal cell wall for colonization in the gastrointestinal tract with capacity to prevent pathogenic adherence or pathogen activation. Lactic acid bacteria are normal residents of the complex ecosystem of the gastrointestinal tract[22].

Several samples of *Soibum* were collected and analyzed for microbial load and nutritional parameter. The result showed presence of several microorganisms associated with the fermentation might play important role in fermentation process as fermentation depends on the biological activity of microorganisms. It was observed from analysis that the fermented bamboo shoot has a good source of nutritive value which has immense importance health benefits.

The proximate nutrient composition consists of moisture content, ash content, protein and carbohydrate (sugar). In the present study, several bamboo shoot samples, fermented as well as unfermented-succulent bamboo shoots were collected from different sites of Manipur. The cyanide content of fermented bamboo shoot samples were analyzed and compared with succulent bamboo-shoots, boiled bamboo-shoots, soaked in saline water or without overnight soaking.

Changes in pH were minimal. Unlike other fermentation, pH values decrease due to involvement of lactic acid bacteria. The pHs of all the fermented samples were found to be around 4.0 while that of unfermented bamboo shoot was recorded to be of pH 6. The cause of choosing this pH range was to determine whether the bacterial isolates can grow in acidic and alkaline condition so as to optimize the pH value for good growth[23]. pH values decrease due to involvement of lactic acid bacteria. Tamang[24] reported that the pHs of all the fermented samples were found to be around 4.0. The fermented sample were acidic in nature (pH 3.9- 5.3), which is due to the fermentation by lactic acid bacteria (LAB). Tamang[25] reported that during fermentation, some species of LAB lactic acid and acetic acid, which reduce pH of substrate making the product more acidic in nature and inhibit the growth of pathogenic micro-organism, thus food are safe for consumption.

Bacterial counts of samples were determined and colony forming unit (CFU) ranges

3 × 107 - 724 × 107per grams as shown in Table 1. The nutritional parameters were also recorded. The moisture content of different fermented bamboo shoot samples and succulent bamboo shoot varied from 84-94.8 mg/100g and the ash content varied from 2–2.2mg/100g in the samples investigated as given in Table 2.

Since time immemorial, bamboo shoots are consumed as fresh as well as fermented. These bamboo shoots are popular indigenous food product eaten by the Manipur. So therefore, it is equally important to check the level of cyanogenic content in these shoot as it is necessary for human health and human safety point of view[26, 27, 28] . The total cyanide levels are highest in the tip and lowest at the base of bamboo shoot. Haque[8] reported that, the cyanide content ranges from 100-1600ppm which represents a potential health hazards, but since the loss of HCN after cutting is quite rapid it may perhaps not present a problem for consumers. The occurrence of the compound taxiphyllin, which is a cyanogenic glycoside in raw shoots and its side effect on human health claim to introduce something new way of processing using scientific input to exclude the toxic compound without disturbing the nutrient reserve.The biosynthetic precursors of the cyanogenic glycosides are different L-amino acids, which are hydroxylated,

Table 1. Bacterial population in fermented bamboo shoots collected from different sites

Sl.No.	Sites	Samples	Colony Forming unit (Cfu)			
			1st month	2nd month	3rd month	4th month
1.	Bishnupur	FB1	572×10^{8}	$228x10^{6}$	$106x10^{6}$	$5x10^{6}$
2.	Lamangdong	FB2	11×10^{6}	$54x10^{6}$	$33x10^{6}$	$4x10^{6}$
3.	Singjamei	FB3	15×10^{6}	$184x10^{6}$	$76x10^{6}$	$5x10^{6}$
4.	Lamkhang	FB4	25×10^{6}	$91x10^{6}$	$13x10^{6}$	$3x10^{6}$
5.	Khongkhang	FB5	212×10^{6}	$70x10^{6}$	$15x10^{6}$	$6x10^{6}$
6.	Kwatha	FB6	312×10^{6}	$208x10^{6}$	$192x10^{6}$	$3x10^{6}$
7.	Shilet	FB7	22×10^{6}	$15x10^{6}$	$24x10^{6}$	$7x10^{6}$
8.	Andro	FB8	178×10^{6}	$207x10^{6}$	$114x10^{6}$	$18x10^{6}$

Table 2. Physiological parameters in succulent bamboo shoot as well as fermented bamboo shoot.SB -Succulent bamboo shoot, FB – Fermented bamboo shoot.

Sample	pH	Moisture content	Ash Content
SB	6	94.1	2
SB Boil	6	94.8	2.2
FB Boil	4.2	90	2.1
FB1	3.9	88.7	2.2
FB2	4	84	2.2
FB3	3.8	89.7	2
FB4	3.9	89.8	2
FB5	3.7	83.5	2.1
FB6	3.9	88.8	2
FB7	3.8	85.6	1.9
FB 8	4	84.5	2
FB 9	4.1	85.1	2.2
FB10	3.9	89.3	1.9
FB11	3.9	90.1	2

Values are in wet weight basis (mg/100g)

Table 3. Cyanogenic glycoside content in bamboo shoot.

Sample	Cyanogen content in mg/g of sample
SB	43.55
SB boil	0
FB boil	0
FB 1	12.14
FB 2	8.98
FB 3	12.58
FB 4	12.34
FB 5	8.98
FB 6	8.78
FB 7	14.18
FB 8	13.42
FB 9	13.90
FB 10	9.10
FB 11	17.89

then the N-hydroxylamino acids are converted to aldoximes and these are converted into nitriles and hydroxylated to á-hydroxy nitriles and then glycosylated to cyanogenic glycosides[29]. All known cyanogenic glycosides are â-linked, mostly with D-glucose. Advantages of picrate method over other method is that the developed yellow-orange-browncolour of the picrate paper was stable for 48 h and the colour can be quantitaively eluted off the paper and itsintensity determined spectrophotometrically[30]. In this study, the cyanogens content in succulent bamboo shoot has high cyanide content (approx. 43.55 mg/g) as compared to the fermented bamboo shoot *(Soibum)* (8.78-17.89 mg/g) while boiling the succulent and fermented bamboo shoot removes the cyanide content in all the samples (as seen in Table 3).

In all the fermented bamboo shoot, it showed a degradation of cyanogenic glycoside content as it is volatile, the loss of cyanogen content during fermentation processes like peeling, slicing, cutting, repeated washing. The most common traditional methods applied by the people of Manipur include chopping of tender shoots into small pieces, partial drying of fresh shoots, boiling in water or salt water and draining or keeping the tender shoot in hot water for 10- 15min[4]. Cynogenesis is a natural phenomenon occurs in plant to prevent themselves against their enemies.

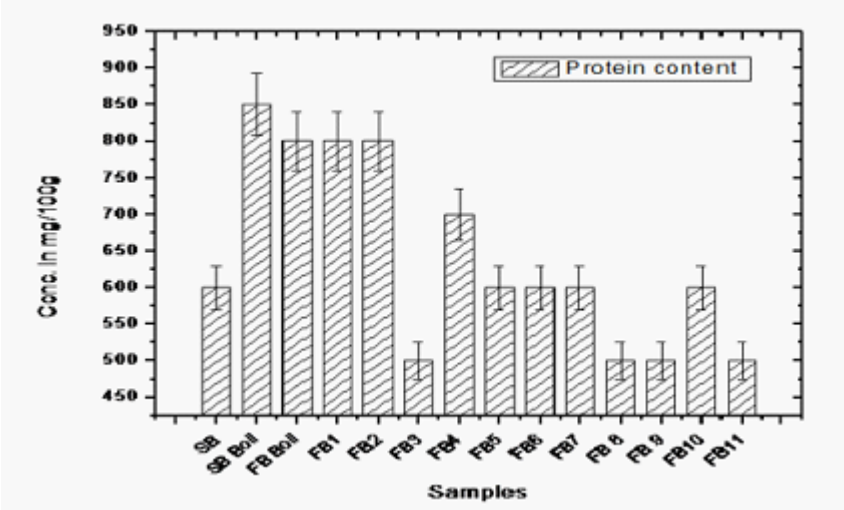

Fig. 1. Protein estimation of bamboo shoot samples in mg/100g

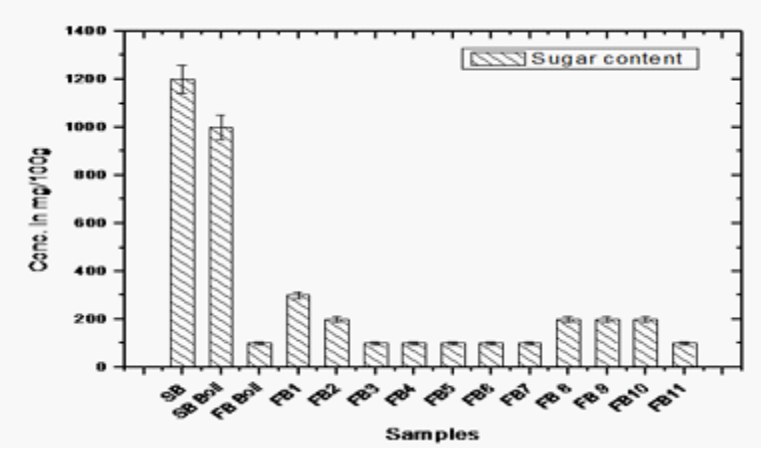

Fig. 2. Sugar estimation of bamboo shoot in mg/100g

The new shoots are brilliant for human consumption. To the best of our knowledge there is no report of poisoning and death due to cyanide intake from edible bamboo shoots. Many edible plants contain cyanogenic glycosides, whose concentrations vary widely as a result of location, season, genetic, soil type and environmental factors. A WHO report (1993) states that the concentration of cyanide in the immature shoot tip of bamboo is 8000 mg/kg of hydrogen cyanide, whereas Ferreira[31] report that bamboo shoots contain as much as 1000 mg/kg of hydrogen cyanide in the apical part. So, therefore fermentation resulted in the reduction of cyanogenic glycosides of fermented bamboo shoot *(Soibum*) samples making it more favorable for human consumption. The microbial community inhibits *Soibum* and resulted in the degradation of cyanogenic glycosides. Therefore, fermentation may be another means to improve the food value which eliminates the cyanogenic glycosides content in bamboo shoots besides increasing its flavor and texture[28].

The protein content of succulent bamboo shoot and fermented bamboo shoot ranged between 500mg - 800mg/100g (as seen in Fig. 1). A comparison of the protein content of succulent bamboo shoot with some of the fermented bamboo shoot reveal that protein content in succulent bamboo are lower than the fermented samples as shown above. The protein concentration in succulent bamboo shoot were found to be low (600mg/100g) comparing with the fermented samples (as seen in Fig 4). Among the fermented samples FB1 shows high protein content. Choudhury[32] reported that a comparison between fermented shoot and fresh shoot has also been reported. Protein content is 2.170 (g/100g) followed by fresh shoot 3.108 (g/100g). Crude protein content for some bamboo species ranged from 19.2 to 25.8g/100gm dry weight. The content of protein decreased upon boiling the shoot by 25% due to denaturation of protein[33]. Protein might have been degraded to amino acids by the activities of fermenting micro organism and used in the metabolic activities. This degradative process however brings out certain characteristics flavour that is essential for the quality of the final product. The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles[35]. LAB has a wide number of commercial applications in food industry with each fermented product being associated with unique groups of micro flora that increase protein content[27].

The reducing sugar content ranged between 100 – 1200mg/100g. As can be seen in Fig. 2, the succulent bamboo shoots have high content of sugar but are low in fermented bamboo shoot. The content of sugar was found to decrease after boiling and kept on decreasing after fermentation. Sugar like glucose is the most important substrate to undergo fermentation. Carbohydrates in the form of soluble sugar might be absorbed by the microflora present in the bamboo shoot for the supply of energy required for their general metabolism. Thus, fermentation showed to reduce the amount of reducing sugar to great extent converting them to acid resulting in the rise of acidity till reducing sugar bio-conversion get exhausted[28].

It was observed from the analysis that fermented bamboo shoot (*Soibum)*has a good source of nutritive values which have immense importance health benefits. Its increases digestibility, improves nutritional, pharmacological values as well. *Soibum*, therefore, is very good for weight-conscious and dieting people. It is also useful for the management of obesity and hypertension through its effect on energy density of food and its nutrients availability. High content of protein and low content of reducing sugars shows its health promoting nature. Fermentation also showed to reduce the amount of reducing sugar to a great extent converting them to acid resulting in the rise of acidity till reducing sugar bio-conversion get exhausted. Fermentation resulted in the reduction of cyanogenic glycosides of *Soibum* samples making it more favorable for human consumption.Cyanogenic glycosides are generally degraded by linamarase enzyme secreting lactic acid bacteria which are some major microbial community involve in fermentation. LAB has a large number of viable applications in food industry with each fermented product being associated with unique groups of micro flora that increases the nutritional content. There is a need of awareness and safety in production of *Soibum* as this has been using by consumers as a part of

the diet and a need of intensive multi-institutional collaborative research and improvement efforts to develop fermented food technology.

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