Comparative Study of Chemical Properties of Soibum- A Traditional Fermented Bamboo Shoot Product and Its Biological Investigation

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Abstract—**This research was carried out to quantify and compare the proximate composition, vitamins (ascorbic acid and riboflavin), fatty acid (saturated, mono-saturated, poly-saturated and trans-fatty acids) and mineral concentration between two types of *Soibum,* fermented bamboo shoot viz. Kwatha and Andro types. Total cyanogenic glycosides content was estimated in 11 nos. of *Soibum* samples on monthly interval. *Soibum* showed a high content of Cyanogenic glycosides in the initial stage which degraded with variable rate from samples to samples during the course of fermentation upto 80-85 % after 15 months of fermentation. Antioxidant activity test in 11 nos. of samples were carried out using DPPH Assay Method. The result show the presence of anti-oxidant activities in the range of 1.23% to 3.23% in all samples tested so far. In conclusion, *Soibum* was found to be a good nutritive source.**

Index Terms—**Anti-oxidant, Bamboo Shoot, Cyanogenic glycoside, DPPH, Soibum.**

I. Introduction

Bamboo belongs to the family *Poacea* and is a natural resource in the world. It is a plant which is widely distributed and grows wild in the fields and mountains from the temperate zone of Japan to the tropical zone of India. Broadly, the temperate climate bamboos are runners, which shoot in the spring, while the tropical and subtropical varieties are clumpers, which shoot in the late summer and fall. Bamboo shoots are young, new canes that are generally 8-12 inches long, taper to one end and grow extraordinarily. However, their size and weight depend considerably upon the location, depth and nutrition of the soil, watering and drainage conditions, rainfall, temperature, pH and soil fertility. The young and tender bamboo plant is utilized as one of the food items in many countries. It is harvested for food before they are two weeks old or one-foot tall. It is consumed in dried, canned, boiled, fermented or medicinal forms. Bamboo shoots are crisp and tender, comparable to asparagus, with a flavor similar to corn. For centuries, fermented bamboo shoots have lent unique flavors and a distinctive crunchy texture to traditional Asian dishes. They are often combined with other ingredients such as ginger, garlic, bell paper, white sesame and red chilli and then stir fried with leak, scallions, poultry, stock and anise to make soup [1]. Commercially, canned bamboo shoots are common, but fresh, locally grown bamboo has far better flavor and texture. Some of the common edible bamboos are from which shoots can be extracted are Bambusa *bambos, Bambusa tulda, B. polymorpha, B. balcooa, Dendrocalamus hemiltonii, D. gigentius* and *Melocanna baccifera.*

In Manipur, a state located in the north eastern part of India, 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. They have their unique taste and texture [2].

Organic acid, sugar, amino acid and vitamin composition of bamboo shoots were reported. The major organic acids in bamboo shoot ranged from 462 (top) to 157 mg (base) per 100 gm fresh weight. Citric acid was rich in the upper half, while malic acid was rich in the lower $3/4^{th}$. Fructose, glucose and sucrose were contained with approximately equal amounts in the top quarter section, the former two sugars were abundant in the lower half [3]. Total lipids ranged from from 800 (top) to 380 mg (base) per 100 gm fresh weight. The main fatty acids of the three lipid classes were palmitic, linoleic and linolenic acids [4]. The apical portion was richest in vitamin C and dehydroascorbic acid while in the internodal joints and the basal portion was richest in vitamin C [5].

Bamboo shoots are low in cholesterol and saturated fats contents (total fats 0.5%), are high in carbohydrates (5.70%), protein (3.9%), minerals (1.1%) and moisture (88.8%) [6]. It is also a good source of Vitamin E (α-Tocopherol), Vitamin C, B6, thiamin, riboflavin, niacin, and dietary fibers like hemicelluloses, cellulose, pectin, lignin [7]. It has been reported that bamboo shoots can significantly decrease serum total and serum LDL cholesterol in rats and total liver lipids including liver cholesterol by 16.1 mg/dl. With 17 different types of amino acids, it contains over 10 kinds of mineral elements i.e., Cr, Zn, Mn, Mg, Ni, Co, Cu; Lysine,

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Germaclinium, many nutritious and active materials.

Cyanogenic glycosides are nitrogeneous phytoanticipins [8] and are used by various plants as effective defensive mechanism against predators [9]. A mechanisms responsible for the formation of HCN has been formulated by Miller and Conn [10], and it has been found that in most of the species it is the degradation of the cyanogenic glycosides [11] that produces HCN; and the enzyme responsible for this are found out to be â-cyanoalanine synthase (EC 4.4.1.9) which is found in a number of plant species [12], apart from Rhodanese (thiosulphate-cyanide sulphur transferase EC 2.8.1.1) and Formamide hydrolyase (EC 4.2.1.66). The steps that catalyze the reaction through â-cyanoalanine synthase are [10]:

$HSCH_2CHNH_2CO_2H + HCN$ '! $NCCH_2CHNH_2CO_2H + H_2S$
Cystein cyanoalanine

$S_2O_3^{2-} + CN^{--}$ '! $S_2O_3^{2-} + SCN$

$HCN + H_20$ '! $HCONH_2$

Bamboo shoots contain 0.3 to 0.8% HCN [13, 14]. Out of which, up to 0.16% of the total cyanide is contained in the tip, reducing to 0.01% in the base [15], 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 [14].Studies were conducted on changes in nutrient composition and sensory properties of *Bambusa tulda* shoots during traditional fermentation process [16]. Even more, studies on fermented bamboo shoot are rather scanty.

Our research aims to investigate the biological properties, nutritional content and to analyze the chemical changes occurring during fermentation. Taking into consideration that bamboo is the fastest growing plant and rampant malnutrition in the developing countries, our study is imperative.

II. Material and Methods

A. Collection of Samples

Two primary production sites, based on pedigree analysis, of *Soibum* were selected. In each site again, three spots/ vessels were randomly selected. From each vessel production spots, sample was collected every week starting time = 0 i.e freshly sliced sample before keeping for fermentation. The collected samples were packed in 500 ml coded PET bottles and transported and stored in the laboratory refrigerator for further analysis.

The first production site is situated about 60 km from the laboratory, Kwatha (N 24˚ 19', E 94˚16', 450 msl) and second production site is located about 25 km from the laboratory, Andro (N 24˚ 19', E 94˚16', 450 msl). The raw materials used in both model (Kw, Kwatha and **As**, Andro) was *Bambusa balcooa* (*Local Name: Ching Saneibi*). Andro model uses earthern pot with holding capacity of around 50 L. It is a fed batch fermentation system where the raw materials are added per week for three to four times. Kwatha model uses a large bamboo basket lined with plastic on its wall and at the bottom. Exudate can leach out from the leaked areas. Leaves were said to be used before the advent of plastic.

B. Chemicals and Reagents

2, 2- Diphenyl-1-picryl hydrazyl (DPPH) was purchase from Sigma-Aldrich, India. Cyanogenic Glycoside Determining kit (Picrate paper kit) was obtained from Dr. J. Howard Bradbury, Division of Botany and Zoology, Australian National University, Australia. All other chemicals/reagents and solvents used in the study were from Merck, India unless stated otherwise. All reagents/solvents used in the study were of analytical/reagent grade.

C. Preparation of Methanolic Extract

Methanolic Extract of *Soibum* was prepared using Soxhlet apparatus. 10 g of dried samples were taken in 60 ml of methanol for extraction. After 2 hrs extraction at 60 ºC the extract was collected and stored at 4 ºC in airtight bottles.

D. Determination of Proximate Nutrient Composition

Proximate chemical composition was estimated using the following methods: protein by Lowry Method [17], fat by Folch Method [18], moisture by Oven drying method, minerals by ashing in muffle furnace and carbohydrate by difference method. Crude fibre was estimated by method of Bidwell [19] and reducing sugar by method of Nelson and Semogyi [20]. Fatty acid profile like saturated fat, monounsaturated fat, polyunsaturated fat and trans fatty acid were determined adopting the procedure of AOAC [21].

E. Determination of Elemental Composition

Elements (trace and major) were estimated by EDXRF (Energy Dispersive X-ray fluorescence) spectrometer; model ERWIN-3600 with a silicon drift chamber and semi-conductor. The sample collected were first cleaned and dried. The dried sample was crushed using agate mortar. About 200 mg crushed samples were made into pallets, 13 mm diameter and 3 mm thick, using a hydraulic press and subsequently used as targets. Five replicates of each target were prepared. A pallet of the NIST Apple Leaf Standard (SRM) was also prepared in the same way.

F. Toxicity Assay

Cyanogenic Glycoside content was determined following Picrate paper kit procedure.

G. Anti-oxidant Activity Assay

Antioxidant activity test of the methanolic extracts of *Soibum* samples were carried out using DPPH Assay Method. Briefly, 2.98 ml of 100 µM DPPH solution was transferred into two test tubes and added 20µl of methanolic extract of *Soibum* samples in one tube and 20µl of methanol for blank in the second tube. The tubes were shaken properly and immediately measured the O.D at 517 nm (T=0). After 30 min incubation the O.D was again measured. Percentage of DPPH Scavenging activity was calculated as follows:

% of DPPH Scavenging

$$= \frac{\text{Absorbance at T=0 – Absorbance at T=30}}{\text{Absorbance at T-=0}} \times 100$$

III. Result And Discussion

A. Proximate Nutrient Composition

Proximate composition, as determined, consists of moisture, dry matter, crude protein, ash, crude fibre, crude fat and carbohydrate (sugar). The proximate composition and gross energy of the two types of samples are given in table IA. The average moisture content of the two samples **Kw** and **As** were 91.5 and 90.73 % respectively with respective standard deviation of 1.83 and 0.71%. This indicates high moisture content of the samples. **As**, however, contains slightly higher moisture on the average of about 1.17 %. Dry matter therefore, is in the range of 7 -10%. Crude fibre and protein are found to be in the range of 2 – 3%. Crude fibre is defined as insoluble carbohydrate composed of cellulosic, hemicelllulosic and ligneous matter. **As** contains relatively higher quantity i.e. 3.54 and 3.09 % respectively for protein and fibre. However, consistent result was found in **Kw** sample showing respective standard deviation of 0.41 and 0.58 %. Crude fat is higher in case of **Kw** (0.6%) compared to **As** (0.35%). Sugar is slightly higher in **Kw** (2.01%) while **As** has about 1.84%.

Comprehensive study on dietary fiber and other components of fruits and vegetable was conducted. Neutral detergent fibre of most samples was found to be between 0.9 – 1.2 %. Sample analysed include pineapple, carambola, sapodilla, papaya, mango, grapefruit, sweet potato and yam. [22] Compared to this data range, *Soibum* seems to contain higher amount of fibre. The average fibre content of the two samples analysed viz. Kw and As were 2.61 and 3.09%. Thus, *Soibum* is a good source of dietary fibre. In another study dried bamboo shoot was found to contain around 29.3% neutral detergent fibre (NDF) [23]. In-vitro test revealed that most NDF can bind more dihydroxy acids than trihydroxy acids. Bamboo shoot exhibited good binding capacity. However, its fermented form, *Soibum*, still needs o be investigated. Many studies have demonstrated that dietary fibre is a very important beneficial substance for decreasing serum and/ or hepatic lipids, especially cholesterol [24].

Energy content was calculated on the basis that 1 gm each of sugar, fat and protein are equivalent to 4, 9 and 4 Kcal respectively. The mean energy content of both the samples was remained approximately same (22.44 and 24.66 % for **Kw** and **As** respectively).

Estimated saturated fat, mono-saturated, poly-unsaturated and trans fat are shown in the table I. b. The two samples showed no significant difference in mono saturated and poly unsaturated fat but **Kw** showed slightly higher amount of saturated fatty acid (0.31%) as compared to **As** (0.18%). Absence of trans fatty acid indicates its safety for human consumption. Estimated ascorbic acid and riboflavin were shown in table I. c. Content of Vit. C is higher than many fruits and vegetables. Concentration of ascorbic acid and riboflavin in both the samples are similar and showed little differences. Though the concentration of riboflavin is small, it may provide significant role in human health.

TABLE I A. Proximate Composition (g/ 100g) and Gross Energy of *Soibum*

[a] Energy in kilocalorie per 100 gm [b] SD Standard deviation.

Spots (Kw)	*Moisture*	*Dry matter*	*Crude Protein*	*Crude fibre*	*Ash*	*Crude fat*	*Sugar*	*Energy*
1	93.10	6.9	2.29	2.21	0.4	0.53	1.47	19.81
2	89.50	7.5	2.94	2.35	0.56	0.71	3.94	33.91
3	91.90	8.1	3.04	3.28	0.61	0.55	0.62	19.59
Mean	91.50	7.50	2.76	2.61	0.52	0.60	2.01	24.44
SD[b]	1.83	0.60	0.41	0.58	0.11	0.10	1.72	8.20
Spots (As)								
1	91.5	5.5	2.11	2.33	0.48	0.42	3.16	24.86
2	90.1	9.9	4.8	3.37	0.32	0.25	1.16	26.09
3	90.6	6.4	3.7	3.57	0.55	0.38	1.2	
Mean	90.73	7.27	3.54	3.09	0.45	0.35	1.84	24.66
SD[b]	0.71	2.32	1.35	0.67	0.12	0.09	1.14	1.55

TABLE I B. Fatty Acids Profile [A]

[A] Values are in % wet wt. basis

Fatty acids	*Kw*	*As*
Saturated fat	0.31	0.18
Mono-saturated fat	0.05	0.03
Poly-unsaturated fat	0.17	0.1
Trans fat	0	0

TABLE I C. Contents Of Vitamins (Microgram/gm) [A]

[A] Values are in wet wt. basis

Vitamins	*Kw*	*As*
Ascorbic Acid	174.7	170.17
Riboflavin	11.23	13.55

A. Changes in Reducing Sugar and Activity

Reducing sugar like glucose is the most important substrate for microbes to undergo fermentation. Understanding the dynamics of reducing sugar will also enable to understand the fermentation mechanism. An interesting observation was found in the Kwatha (**Kw**) and Andro (**As**) model. In Kw, a sharp and consistent decrease in the concentration is found (Fig. 1). On the contrary, **As** showed zig-zag pattern (Fig. 1), concentration suddenly decrease in the first 10 days and again shoot up. This happened for number of times as long as new substrate was added. However, an average trend indicates decrease over a period of time. It fits average trend of decrease. On the other hand, decrease in concentration of reducing sugar in Kwatha model seems to obey a certain uniform trend.

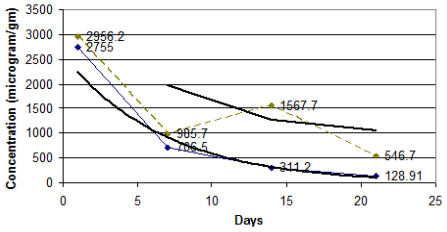

Figure 1. Reducing sugar changes during the course of fermentation in Kwatha (----) and Andro (—) type. (—) indicates trendline. Values of reducing sugar are in microgram/gm.

Acidity was measured on the basis of lactic acid. Increases in the acidity concentration were shown on both the fermentation model Fig. 2 and 3. However, distinct differences were observed in the behavior and pattern in their changes over time. On the day to day basis, **As** rise its acidity more steeply than **Kw**.

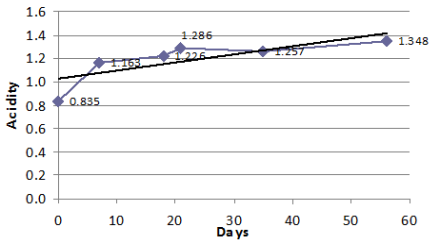

Figure 2. Acidity (percentage total acid) changes during the course of fermentation in Kwatha type. (◆—) indicates actual curve. (—) indicates linear trendline.

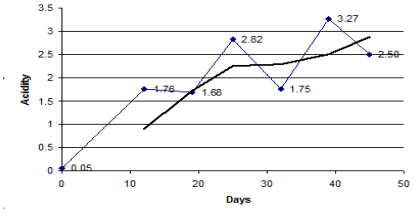

Figure 3. Acidity (percentage total acid) changes during the course of fermentation in Andro type. (◆—) indicates actual curve. (—) indicates linear trendline.

B. Elemental Analysis

EDXRF analysis of the product sample revealed presence of number of elements (table ii). Potassium is exceptionally higher in both the samples about 34127.5 ± 3.55 in **Kw** and 29551 ± 10.5 ppm in **As**. P, S, Cl and Ca are also present in high amount. Eighteen major and trace elements concentration were given table ii. To our knowledge, this is the first time, elemental analysis was done by EDXRF in *Soibum*. It can be corroborated from the data that *Soibum* is a good source of minerals.

TABLE II. MINERAL CONCENTRATION (IN PPM)[A]

[A]Mean value of three spots. Values in dry basis.

Sl. No.	*Element*	*Kw*	*As*
1	**P**	3540.5±2.12	3005.1±5.1
2	**S**	2782±4.24	2859.5±3.9
3	**Cl**	6944.5±3.54	7455.3±10.5
4	**K**	34127.5±3.55	29551.2±6.7
5	**Ca**	1283.5±2.13	1463.8±2.3
6	**Rb**	104.25±0.07	65.1±3.7
7	**Mn**	85.11±0.54	91.2±0.67
8	**Zn**	47.08±0.61	37.4±0.55
9	**Cu**	12.03±0.4	15.8±0.9
10	**Br**	16.72±0.56	19.33±1.2
Sl. No.	*Element*	*Kw*	*As*
11	**Sr**	6.63±0.42	2.6±0.56
12	**Fe**	219.3±4.9	189.4±5.7
14	**Cr**	2.29±	1.6±0.05
15	**V**	0.49±	0.25±0.07
16	**Co**	0.12±	0.05±.01
17	**Se**	0.48±	1.2±.08
18	**Pb**	0.13±	0.47±0.1

C. Cyanogenic Glycosides Content

Bamboo shoots contain cyanogenic glycoside taxiphyllin, which is a p-hydroxylated mandelonitrile tiglochinin. Taxiphyllin is hydrolysed to glucose and hydroxybenzaldehyde cyanohydrin. Benzaldehyde cyanohydrin then decomposes to hydroxybenzaldehyde and hydrogen cyanide.

Cyanogenic glycosides degrade during the course of fermentation as has revealed from the data observed (Table iii). During the 15th month fermentation period cyanogenic glycoside degrade upto 80-85% from its initial value. However, variation in the degradation was observed from samples to samples. Here, fermentation may be another means to improve the food value which eliminates the cyanogenic glycoside besides increasing its taste and texture.

TABLE III. MONTHLY CYANOGENIC GLYCOSIDES CONTENT IN BAMBOO SHOOTS.

SAMPLES	*VALUES (in ppm)* MONTHS														
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th
BLANK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CONTROL	200	200	200	180	200	200	180	160	180	200	200	200	180	160	160
SAMPLE 1	180	160	120	100	80	40	32	20	100	80	80	40	32	20	20
SAMPLE 2	200	180	160	140	120	100	80	40	140	120	80	80	80	40	32
SAMPLE 3	220	200	160	160	120	80	60	40	160	120	100	80	60	40	32
SAMPLE 4	640	620	600	360	240	160	105	120	360	240	160	160	140	120	80
SAMPLE 5	200	180	160	160	140	100	20	20	160	140	140	100	80	20	20
SAMPLE 6	160	140	120	100	80	60	40	28	100	80	60	60	40	28	28
SAMPLE 7	300	260	240	80	180	100	80	40	200	200	120	100	80	40	32
SAMPLE 8	360	380	300	200	160	200	180	40	280	240	220	200	180	160	120
SAMPLE 9	280	260	240	180	160	120	80	60	180	160	120	100	80	60	40
SAMPLE 10	200	180	160	160	120	160	100	100	160	160	160	120	100	100	80
SAMPLE 11	360	320	300	200	240	100	80	60	240	220	180	100	80	60	40

Microbial community inhabiting *Soibum* fermentation may be responsible for the reduction in cyanogenic glycosides content. Cyanogenic glycosides are generally degraded by linamarase enzyme secreting lactic acid bacteria which are some major microbial community involve in fermentation [25]. It can be confirmed by subsequent analysis if these bacteria also inhabit *Soibum*

D. Anti-oxidant Activity

Eight phenolic compounds namely protocatechuic acid, p-Hydroxybenzoic acid, catechin, caffeic acid, chlorogenic acid, syringic acid, p-Coumaric acid and ferulic acid were identified by high-performance liquid chromatography in two species of *P. pubescens* and *P. nigra.* It was determined that the antioxidant capacity was highly correlated with the total phenolic content [26].

2, 2- Diphenyl-1-picryl hydrazyl (DPPH) was used as a substrate to evaluate the antioxidant potential of *Soibum* extract. Methanolic extract of *Soibum* samples showed significant antioxidant activity ranging from 1.23% to 3.23% (Table iv). Phenolic compounds in the *Soibum* extract may be responsible for these anti-oxidant potential.

TABLE IV. ANTI-OXIDANT ACTIVITY OF *SOIBUM*

SAMPLES	*RESULT*
SAMPLE 1	3.72%
SAMPLE 2	1.54%
SAMPLE 3	1.29%
SAMPLE 4	1.55%
SAMPLE 5	2.5%
SAMPLE 6	2.35%
SAMPLE 7	2.55%
SAMPLE 8	1.72%
SAMPLE 9	3.23%
SAMPLE 10	2.75%
SAMPLE 11	3.06%

IV. CONCLUSIONS

It was observed from the analysis that *Soibum* is a potential source of dietary fibers which have immense importance health benefits. It is useful in the management of hypertension and obesity through its effect on energy density of food and the extent of interference with the nutrients of bioavailability. Low content of lipid and absence of trans fatty acid also indicated its health promoting nature. *Soibum*, therefore, is very good for weight-conscious and dieting people. Elements like K, Na, Cl, Mn, Cu, etc. were present in significant quantity. These elements take definite and specific role in the metabolism of human body. So, it will be useful if we can find the molecular structure of the compounds containing the trace elements in bamboo shoots. 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. The microbial community inhabiting *Soibum* and resulting in the degradation of cyanogenic glycosides is under investigation. *Soibum* possess anti-oxidant potential which may be further explored. The phenolic compounds responsible for these activities may be analysed.

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