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Biochemical Composition of Fermented Bamboo Shoot of *Cephalostachyum Capitatum (L.)* Munro *of Bishnupur District*, Manipur

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Abstract: ***Biochemical composition of raw bamboo shoot extract, fermented product extract and fermented media of a locally very popular bamboo species, Cephalostachyum capitatum (L.) Munro found in Bishnupur district, Manipur was studied. Parameters studied include moisture, protein, glycogen, amino acids, riboflavin, vitamins B and C, minerals, phenol, alkaline phosphatase, aspartate aminotransferase (ASAT/GOT), alanine aminotransferase (ALAT/GPT), and Bacterial flora. The protein content was found to be highest in the fermented media compared to raw bamboo shoot extract and fermented product extract. The highest glycogen content was found in the raw bamboo shoot extract. Amino acids and Riboflavin were found to be highest in fermented media. Minerals like Ca, Fe, Na and K were found in maximum in fermented media. Organic acids - ascorbic acid and total acid (% lactic acid) were found to be higher in fermented products and media. Bioactive compounds like phenol, alkaline phosphatase and aspartate aminotransferase were also found to be highest in fermented media. ALAT/GPT content in fermented media in 12 days is more than 5 times the amount present in raw bamboo shoot. In the fermented media, the counts of Staphylococcus aureus and Bacillus cereus were 10^5 and 10^6, respectively. The analysis of biochemical composition indicates that the fermented media and the fermented product of bamboo shoot of Cephalostachyum capitatum (L.) Munro is more nutritious compared to the raw bamboo shoot and thus they could form a rich supplementary food for mankind.***

Keywords: *Cephalostachyum capitatum*, glycogen, minerals, fermented media, fermented product

1. Introduction

Bamboo shoot fermentation is an age-old technological procedure used for the preservation and value addition of food. Bamboo shoots are edible, highly nutritious and have medicinal value. Chauhan *et al.,* 2016 reported that fermented bamboo shoots contain minerals, proteins, vitamins, antioxydants and phytochemicals. Fermented bamboo shoot also contain phytochemicals such as phenols, phytosterols, dietary fibres, flavonoids, lectins, glycosides, etc. which show anti-cancerous properties (Nirmala *et al., 2011*). Different forms of fermented bamboo shoot products locally known as *Soidon, Soibum, Soijin* and *Bamboo pickle* undergo natural fermentation by *Lactobacillus sp.* (Tamang *et al*. 2008). There are various reports on the health benefits of fermented bamboo shoots as they are anti-oxidant, anti-free radical, anti-cancer, and antimicrobial. It was also reported that they prevent cardiovascular diseases and decrease blood pressure. The bamboo shoots and their fermented products form an important food source in Manipur, thus holding both industrial and economic value.

Recent investigations revealed that fermented bamboo shoots can be a source of phytosterols (Srivastava and Sarangthem, 1994). They are synthesized as secondary plant metabolites and can be used as an alternative source of starting material for the synthesis of steroidal drugs (Goswami *et al*., 1983, Srivastava, 1990). Plant products can be used for steroidal drug after its microbial bioconversion (Mathur, 1991; Mathur *et al*., 1995). Steroidal hormones and drugs are important in curing human disorders as well as in the control of fertility. Human beings are directly or indirectly dependent on steroidal drugs in treating various diseases such as cancer, skin diseases, and other metabolic disorders. Phytosterols are precursors of many pharmaceutically important steroidal drugs. There are reports on the biotransformation of sterols into various steroids by different microbes in fermented bamboo shoots (Srivastava and Sarangthem, 1994, Srivastava, 1990). Naito *et al*., 1966; and Goswami, 1982 reported the microbial conversion of sterols to active steroid hormones and drugs by a variety of microorganisms. The bamboo shoot products are used as food component in various countries in the form of raw, dried, boiled, canned, fermented products or as medicine. Investigations in recent few years revealed that Bamboo shoots have potential of healthy foods (Singh *et al.,* 2007). The fermented media (i.e. acidic liquid obtained during fermentation, locally known as *soijim*) can be used as condiment or flavouring agent mostly by tribal people of Manipur in the preparation of various local dishes. In Nagaland, the fermented bamboo shoot liquid extract represents an important part of daily cuisine (Nongdam, 2015). During the preparation of daily cuisine, a small quantity of it, is usually added to impart a special flavor. Some Tribes of Arunachal Pradesh have the practice of consuming the acidic liquid drained from bamboo shoot fermentation which they called it *eku* (Nongdam and Tikendra, 2014). *Eku* is a favourite condiment for these tribes and they used it particularly in cooking fish, meat and vegetables.

Different species or varieties of bamboo are used in fermentation and in the preparation of other bamboo shoot products. Accordingly, the properties of the fermented bamboo shoot products also vary. *Bambusa balcooa, B. nutans, Dendrocalamus hamiltonii, D. latiflorus, Chimonobambusa callosa* (Munro) are some of the bamboo species commonly used for preparation of different bamboo shoot products. *Soidon* and *Soijin* are two different bamboo shoot products which are specially prepared from the bamboo species, *Cephalostachyum capitatum* (L) Munro locally known as *naat* in Manipuri. The *soidon* prepared from young tender stems and shoot tips of *naat* is considered tastier, popular and accepted as best fermented food than *soidon* prepared from other bamboo species. The villages of Bishnupur district of Manipur are famous for the preparation of best quality *Soidon*.

2. Materials and Method

Pieces of young shoots of the bamboo species *Cephalostachyum capitatum* (L) Munro measuring approximately 28±1 cm in length and about 2 cm in diameter were collected from Bishnupur market, 30 kms. away from Imphal, the capital city of Manipur.

Figure 1: Raw bamboo shoot of *Cephalostachyum capitatum* (L.) Munro

Preparation of Raw bamboo shoot extract:
Young and fresh bamboo shoots were cut into thin slices. They were washed two or three times with distilled water. One gram of the raw thin slices was ground by using mortar and pestle in 10 ml of distilled water (1:10 W/V) and filtered. The filtrate was at pH 6.5±1.0 and used for different biochemical analysis.

Preparation of fermented media:
For the preparation of fermented media, the young bamboo shoots were cut into thin slices and soaked in water at the ratio (1:10 W/V) in a glass jar with a lid for 10 days at 37^0C. After 10 days the liquid portion become milky white. It is filtered. The filtrate is acidic having a pH of 3.8 ± 0.6, and it's used as fermented media for further biochemical analysis.

Preparation of fermented product extract:
The solid residue containing fermented bamboo shoots in the above process so obtained after filtration was used for the preparation of fermented product extract. 1 gram of the fermented product was homogenized with 10 ml of distilled water by using mortar and pestle and filtrate was used for biochemical analysis. The filtrate is having pH 4.4±0.8.

Biochemical analysis:
Moisture percentage was measured by hot air oven method (Hart and Fisher, 1971). Acidity was estimated following AOAC (1970); Reducing sugars by using the method of Somogyi (1952). Estimation of Ascorbic acid and calcium were done following Ranganna (1977). Total acid was estimated following the method of Moore and Stein (1948). Riboflavin content was estimated by the method of Bray and Thorpe (1954). Sodium, Potassium and Iron were determined by the method of Corning (flame photometer, USA). pH was measured using the Systronics digital pH meter following the method described by Valsan (1975). Total amino acid was estimated by Ninhydrin method following the procedure described by Moore and Stein (1948). The total protein content was determined by Biuret method. Trinder's reagent was used for the estimation of Glucose-by-Glucose oxidase enzymatic method. Alkaline phosphatase was determined by following the modified Kind and King's method (1954). The estimation of ASAT was done by following the modified method of Reitman (1957). Estimation of Alanine aminotransferase was done following Reitman (1957). Total counts of bacteria were determined by the dilution plate method of APHA (1976) using tryptone glucose agar medium (pH-7.0). *Escherichia coli* (*E. coli)* was determined by using brilliant green lactose broth Eosine methylene blue method. Coliform culture tube was isolated from EMB medium using the methods of APHA (1976) and Kis (1984).

Coliform (MPN)
By incubating diluted sample in brilliant green lactose, broth coliform count was determined using Durham's tubes positive tubes. *E. coli, Staphylococcus aureus, Salmonella* were determined following the method of APHA (1976) and Kis (1984).

Bacillus cereus
It was estimated by using polymyxin Pyruvate Egg yolk Mannitol Bromothymol Blue agar (PEMBA) medium. Prior to pouring into the plates, sterile egg Yolk and polymyxin B sulphate are added and incubated for 36 to 48 hrs at 37^0C. Peacock coloured colonies were observed and isolated on slant for further test. For identification of *Bacillus cereus*, IMVIC test, gelatine liquefaction, gram stain, cell shape, starch hydrolysis, endospore formation, motility and growth on 10% NaCl were conducted.

3. Results

Biochemical composition of the Raw bamboo shoot extract, Fermented product extract and Fermented media were represented in Table 1. All parameters were recorded in quintuplicate. Only the Mean values and Standard deviation are shown in the table. Fermented media had a higher percentage of nutritive substances, like amino acids, proteins, riboflavin, calcium, iron and potassium. Aspartate amino transferase (ASAT), Alanine amino transferase (ALAT), Alkaline phosphatase, protein and glycogen were increased during fermentation.

Mean values of Alkaline phosphatase recorded in raw bamboo shoot extract, fermented product extract and fermented media were 25.89±0.56 IU/L, 16.6±4.04 IU/L and 34.6±21.77 IU/L respectively. The enzyme ASAT in raw bamboo shoot extract,

Fermented product extract and fermented media were 79.938±3.45 IU/L, 14.09±5.03 IU/L and 50.71±43.71 IU/L respectively. Similarly, Enzyme ALAT in raw bamboo shoot extract, fermented product extract and fermented media were recorded as 21.01±2.75 IU/L, 27.03±26.27 IU/L and 44.62±42.69 IU/L respectively. Reducing sugar (Glycogen) level drastically decreases from raw bamboo shoot (53.53±0.09 mg/100 ml) to fermented product (6.39±0.37 mg/100 ml) while the fermented media contains 8.12±0.66 mg/100 ml. Protein content was 0.83±0.03 mg/100 ml in raw bamboo shoot extract, 4.01±0.03 mg/100 ml in fermented product extract and 5.11±0.04 mg/100 ml in fermented media.

Table 1: Biochemical composition of the Raw bamboo shoot extract, Fermented product extract and Fermented media

Parameter	Raw bamboo shoot extract	Fermented product extract	Fermented media
Moisture content (mg/100ml)	30.18±0.04	35.85±0.09	72.16±0.6
pH	6.50±1.00	4.40±0.80	3.80±0.60
Protein (mg/100ml)	0.83±0.03	4.01±0.03	5.11±0.04
Total Amino acids (mg/100ml)	2.40±0.04	3.21±0.04	4.30±0.17
Reducing sugar (Glycogen) (mg/100ml)	53.53±0.09	6.39±0.37	8.12±0.66
Ascorbic acid (mg/100ml)	0.45±0.04	0.95±0.04	1.98±0.03
Riboflavin (mg/100ml)	1.16±0.04	1.32±0.12	8.12±0.66
Calcium (mg/100ml)	0.51±0.05	0.80±0.03	1.20±0.04
Iron (mg/100ml)	0.36±0.02	0.56±05	0.97±0.03
Potassium (mg/100ml)	0.08±0.04	1.02±0.03	1.36±0.04
Sodium (mg/100ml)	0.41±0.04	0.72±0.5	0.86±0.04
Phenol (mg/100ml)	1.05±0.10	1.80±0.03	2.21±0.04
Alkaline Phosphatase IU/L	25.89±0.56	16.6±4.04	34.6±21.77
Aspartate Amino Transferase (ASAT) IU/L	79.94±3.45	14.09±5.03	50.71±43.71
Alanine Amino Transferase (ALAT) IU/L	21.01±2.75	27.03±26.27	44.62±42.69
Total acid (% Lactic acid)	0.14±041	0.24±038	0.28±036

In the Fermented media, *Staphylococcus aureus* and *Bacilus cereus* count were 10^5 and 10^6 respectively. In both the samples, *Bacillus cereus* was observed and the highest count was obtained from fermented media.

Moisture content of Raw Bamboo shoot, Fermented product extract and Fermented media were 30.18 mg., 35.85 mg. and 72.16 mg./100ml respectively.

4. Discussion

The Raw bamboo shoot extract was less acidic (pH -6.5±1.0) compared to the Fermented product extract (pH – 4.4±0.8) and Fermented media (pH – 3.8±0.6). The increased in acidity may be due to the excretion of lactic acid bacteria like Bacillus sp. (Soyiri, 2003) involved in the fermentation.

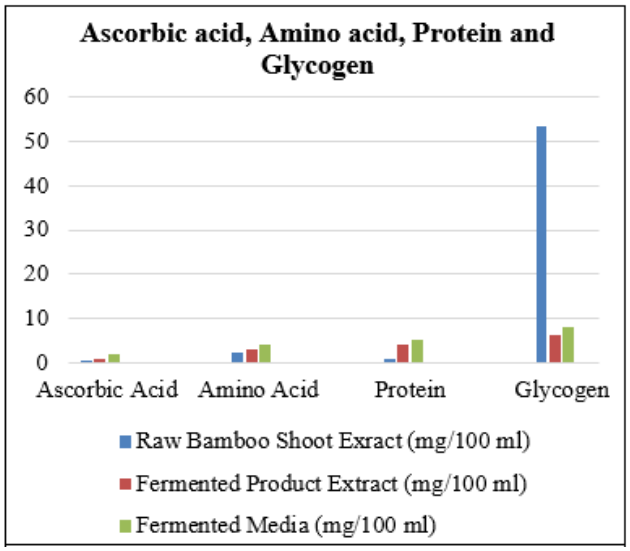

Figure 2: Graph showing comparative amount of Ascorbic acid, Amino acid, Protein and Glycogen

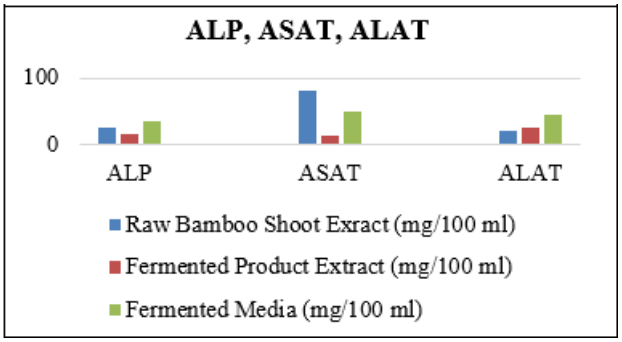

Figure 3: Graph showing comparative amount of ALP, ASAT, ALAT and Phenol

The moisture content of Raw Bamboo shoot extract was lower than the fermented media. It may be due to the micro bacterial conversion of sour acidic liquid produced during fermentation of Bamboo shoots. They have rich sources of minerals that are required by human body in trace amount as suggested by various research studies (Devi and Singh, 1986).

The concentration of mineral elements vary with fermentation and higher concentration was found in the fermented products of Bamboo shoots. Bamboo shoots are good sources of Sodium, potassium and calcium which reveal its nutritional status and importance (Chauhan *et al*., 2008, Devi and Singh, 1986). It may be a good food specially for children and lactating mothers who have deficiency of these minerals. Due to the high content of Potassium in Bamboo shoot, it is recommended for keeping blood pressure under control. It possesses antiaging properties due to the presence of Germaclanium (Chauhan *et al*., 2008). The daily requirement of Calcium and Potassium are met with adequate consumption of Bamboo shoots for children and adults (Devi and Singh, 1985; Nirmala *et al.*,2007). The highest Sodium (Na) content was found in fermented media (0.858±0.04 mg/100ml) while the fermented bamboo shoot extract contains 0.722±0.05 mg/100ml and the raw bamboo shoot contains 0.414±0.04 mg/100ml. Sodium and Potassium is associated in maintaining acid-base balance and nerve transmission in human body. Sodium is an essential element in human body to maintain the balance of the physical fluids system, and nerve and muscle functions. Calcium concentration also increases from 0.51±0.04 mg/100ml in raw

bamboo shoot to 0.80±0.03 mg/100ml in fermented bamboo shoot and to 1.202±0.04 mg/100ml in fermented media in this particular bamboo species. Iron content was highest in fermented media extract (0.974 mg/100ml), higher (0.564 mg/100ml) in Fermented product extract and lowest in raw bamboo shoot (0.356 mg/100ml). The increase in Fe content in fermentation of Bamboo shoot could be due to the microbial bioconversion of soluble solids with chemically stable products as reported by Devi and Singh, 1985; Nirmala *et al*. 2007. Bamboo shoots fermentation are good sources of Iron which promotes the nutritional values and health aspects (Thakur *et al*., 2016, Chauhan *et al*.,2008). It has immense potential of playing healthy food and have a significant role in traditional Asian medicine (Thakur *et al*., 2016).

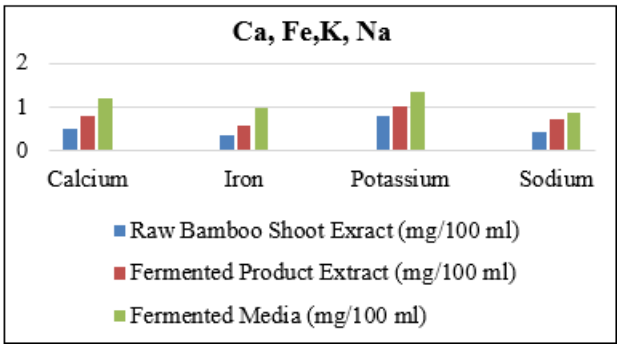

Figure 3: Graph showing comparative amounts of Calcium, Iron, Potassium and Sodium

The content of total amino acid was highest in Fermented media (4.292±0.17 mg/100ml, higher in fermented bamboo shoot extract (3.208±0.04 mg/100ml) while it is only 2.398±0.04 mg/100ml in raw bamboo shoot extract. In fermentation of Bamboo shoots, the production of organic acid and other metabolites have produced characteristic taste, flavour, texture etc. due to the occurrence of Lactic acid bacteria (Sonar and Halami MP. 2014). There are various functional properties of Lactic acid bacteria isolated from fermented Bamboo shoots such as acidifying activities, degradation of phytic acid, enzymatic activities, biogenic amine production, etc. (Choudhury *et al*, 2011). The higher content of Amino acids was found in fermented products of Bamboo shoots and distinct changes were observed with advancement of fermentation. During fermentation of Bamboo shoots, the relative increase in high acidity could be due to the lactic acid production (Badwaik *et al*.2014; Park and John 2009; Nirmala *et al*.,2011).

The phenol content varies from Raw Bamboo shoots (1.05±0.10 mg/100ml) to fermented media (2.208±0.04 mg/100ml) while the fermented product contains 1.802±0.03 mg/100ml. The increase in phenol content might be due to the activities of micro-organisms in metabolic bio conversion. Phenolic compounds play an important role in the treatment of cancer. In vegetable and dietary plants commonly contain phenolic acids, flavonoids, tannins, anthocyanins, lignin, stilbenes, curcuminoids etc. (Giada 2013; Huang *et al*.,2009). Phenolic compounds can prevent the reaction of free radicals with other molecules in the body preventing damage of various bio molecules by scavenging various reactive radicals (Mira *et al*., 2002, Gonzalez *et al*., 2008). Bamboo shoots consumption imparts various health benefits and helps in the prevention of several chronic diseases such as cardio vascular diseases and certain types of cancers. Scientific basis of preventive action of bamboo shoot has been attributed to the presence of bioactive compounds such as phenols, phytosterol and dietary fibres (Nirmala *et al*. 2011). Phenolic compounds are the natural antioxidant because it has strong hydrogen donating properties of their hydroxyl groups which prevents damage of various micro molecules. Several studies confirmed the positive effects of phenols on the reduction of many cancer types by the epidemiological studies (Sailu *et al* 2012; Landete, 2012).

The fermented products of Bamboo shoots contain phytosterol which is used to decrease blood pressure, cholesterol and increase appetite. It is labelled as heart protecting vegetables and Bamboo shoots have anti-inflammatory properties which inhibit various Chronic diseases. Phytosterols have a protective effect on the lipid profile, bowel function and total serum cholesterol level keep in reduce state (Nirmala *et al*. 2011; Chauhan *et al* 2008). Besides their cholesterol lowering activities, many investigators have shown that phytosterol possesses potent anti-cancerous properties against certain cancer types such as lung cancer, liver cancer, stomach cancer, ovary cancer and breast cancer (Mendilaharsu *et al*, 1998; De Stefani *et al* 2000). The highest concentration of Ascorbic acid (Vitamin C) was found in Fermented media (1.982 ±0.03 mg/100ml) while fermented product extract contains 0.954±0.04 mg/100ml and the raw bamboo shoot extract contains 0.452±0.04 mg/100ml. Vitamin C can encapsulate spreading cancer and it also serves as a barrier of dense fibrous tissue. Singh *et al*., (2012) and Chongtham *et al*., (2011) reported that Bamboo shoots are considered to have therapeutic values due to presence of law fat, high edible fibres content, vitamins such as vitamin C and vitamin E. Antioxidant capacity of Bamboo shoot fermentation is closely related to the higher content of Ascorbic acid reported by Chauhan *et al*., (2008); Zhang *et al*., (2011). A significant quantity of potent antioxidant compounds such as Vitamin C and Vitamin E found in the Bamboo shoot fermentation are involved in the prevention of various cancer types such as gastric, oesophageal pancreatic, lung, oral and rectal cancer (Block, 1992).

Protein content varies from Raw bamboo shoot to Fermented media. The highest protein content was recorded (6.00 mg/100ml) in 9 days old Fermented media, decreased after 12 days of fermentation and the colour of the product was also changed into dark brown. The increased in protein content might be due to the degradation of protein into amino acids by the activities of fermenting microorganisms used in the metabolic bioconversion. The decrease in protein content was also related with the decrease in the number of micro-organisms found in the Fermented media. It is well known that fermentation plays an important role in the production of more proteins and enzymes because of the favourable energies of the fermentation (Sundermann, 1971). It is also found similar in case of Ngari (a fermented dried fish) fermentation as reported by Sarojnalini and Vishwanath, (1988).

The glycogen content was drastically reduced from raw bamboo shoot (53.532±0.03 mg/100ml) to (6.39±0.04 mg/100ml) in fermented bamboo product. The decreased glycogen content in Fermented product extract might be due to the utilization of sugar by the microorganisms during

fermentation. The reduction in the glycogen content was also found similar during *Ngari* fermentation as reported by Sarojnalini and Vishwanth (1988). Glycogen provides major sources of energy responsible for breaking down of fatty acids and preventing raised levels of Ketone in the body tissues (Landete 2012). Fermentation of Bamboo shoots display various changes in physiochemical composition of macro and micro molecules of food constituents (Badwaik *et al*., 2014). Glycogen is the most important substrate for microbes to undergo fermentation. Understanding the activities of glycogen will enable to understand the function of fermentation mechanism. The high content of glycogen in Raw Bamboo shoot decreases with the advancement of fermentation and this report is similar with the observation of mulberries fermentation (Perez -Gregorio *et al*.,2011).

The highest Alkaline phosphatase (34.16±21.77 IU/L) was recorded in fermented media while the lowest was recorded in fermented product (16.6±4.03 IU/L) while the raw bamboo shoot contains 25.886±0.56 IU/L. The increased Alkaline phosphatase in Fermented media might be due to micro-organisms which secrete strong proteolytic enzymes, volatile bases, from the maturing Fermented media. These findings are similar to the report of Beddows (1976), that the halotorant Bacteria secrete strong proteolytic enzymes, acids, volatile bases to the production of higher enzyme content from the maturing Fermented products. Activity of Aspartate amino transferase (ASAT) decreases from raw bamboo shoot (79.938±3.45 IU/L) to fermented product extract (14.092±5.03 IU/L). During fermentation the Alanine amino transferase (ALAT) in both the samples (Fermented product extract and media) increased gradually. The highest ALAT concentration was found in fermented media (44.622 ±42.69 IU/L) and the lowest was found in raw bamboo shoot extract (21.01±2.75 IU/L) while the fermented product extract contains 27.026±26.263 IU/L. The increased in enzyme ALAT might be due to the activities of the fermenting microorganisms by producing aminotransferases, transaminases, volatile bases etc. These changes lead to the improvement of digestibility and nutritional value of food. The microbial interactions in the fermentation bring about an appreciable increase in free amino acids, amylolytic and proteolytic, enzymatic activities and these changes also lead to an improvement of digestibility and nutritional value of foods (Soni and Sandhu, 1990). Total acid (% Lactic acid) slightly changes from 0.14±041 in raw bamboo shoot to 0.24±038 in fermented product extract and 0.28±036 in fermented media.

The total plate count of bacteria in Raw Bamboo shoot extract ranged from 10^2 to 10^4 cfu/g. In Fermented media, a value of 10^6 cfu/g was obtained. A value of 10^5-10^6 was obtained from laboratory samples at different temperature. In fermented food, the growth of bacteria is necessary for the development of characteristic odour, flavours and textures. The absence of large numbers of micro-organism associated with the absence of characteristic organoleptic attributes (Sillikar, 1963). Hence, the increased micro-organism is responsible for the production of flavour and taste. Unhygienic processing, handling and transportation might also impart bacteria but it does not permit speculation about the probability that such food may constitute a health hazard (Sillikar,1963).

5. Conclusion

In the present study, fermented media were found to have higher nutritive value than the Raw bamboo shoot extract and fermented product extract in respect of various parameters studied. The fermented bamboo shoot product contains higher nutritive values compared to raw bamboo shoot. Since time immemorial, the Bamboo Shoots have served as a sustainable food source. It is one of the most valued vegetables and it plays an important role in providing higher nutritional and medicinal values to mankind.

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