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Changes In Phenolic Content And Antioxidant Activity During Traditional Fermentationof Bamboo Shoot Of Noney District, Manipur, India.

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ABSTRACT

Introduction: Bamboo (*Bambusa balcooa*) is a plant that is widely distributed and grows in the valley and hilly areas of Manipur, a state located in the northeastern part of India. Young and tender bamboo shoot is consumed in fresh or fermented form, locally called soibum. In Manipur, there are two different modes of fermentation; Andro type and Noney /Kwatha type. **Aim:** The study aimed to investigate the changes in phenolic and flavonoid contents and antioxidant activity during traditional fermentation of bamboo shoots collected from Noney District of Manipur, a northeastern state of India. **Materials and methods:** Raw and fermented bamboo shoots extracts were prepared by using 80% (v/v) ethanol for evaluation of total phenolic content by Folin-Ciocalteau reagent method and total flavonoid content by aluminium chloride colorimetric method and methanol 80%(v/v) for antioxidant activity by using DPPH radical assay.**Results:** The fermented bamboo shoots showed a high level of phenolic contents, flavonoid contents, and antioxidant activity. The 60days fermented had the highest phenolic contents of 238.33 mg CE/100g and the highest flavonoid contents of 32.32 mg QE/100g, while the 25 -day fermented sample had the highest level of antioxidant activity of 32.77%. **Conclusion:** The study can conclude that consumption of fermentedshoots of *Bambusa balcooa* could be more beneficial to our health than raw one as it has more level of antioxidant activity and polyphenols.

Keywords: *Bambusa balcooa*, antioxidant activity, phenolics, flavonoids.

INTRODUCTION

Bamboo (*Bambusa balcooa*) is a plant that belongs to the family Poacea, and it is widely distributed and grows wild in the fields and mountains from the temperate zone of Japan to the tropical zone of India. [1] Bamboo shoot is one of the chief vegetables fermented prevalently in Asian countries. Apart from the consumption of bamboo shoots as a vegetable by the northeast people of India, it has been consumed by fermenting into different types.[2] All the practitioners of bamboo shoot fermentation in Northeast India, adopt traditional methods - fermented well by natural lactic acid fermentation. Soibum, an exclusive delicacy of fermented bamboo shoots of Manipur, a Northeast state of India, has been preserved by incubating the mash for the shortest duration of one month. Elongation of preservation beyond this can be reduced as aging, which enhances both value and quality.[3]The exudate that ran out or accumulated inside the fermentation chamber is usually discarded from consumption. It is well known that fermentation of plant materials has significance in increasing polyphenols, the potent antioxidants. Thus, for the health concern, the importance of fermentation of plant material is increasing worldwide. Giri (2014) reported a similar finding during bamboo shoot fermentation for the production of soibum, a chief fermented food of Manipur, a northeastern state of India. Many investigators have analyzed the correlation among polyphenols, flavonoids levels, and antioxidant activity of different fermenting plant mashes.[4] Therefore, the authors attempt to reveal the interrelationship of phenolic, flavonoid levels with antioxidant activity during the course of soibum fermentation done from a material used substantially for its commercial production.

Oxygen free radicals and other reactive oxygen species can cause oxidative injury to living organisms and thus play an important role in many lifestyle-related diseases such as arthritis, atherosclerosis, emphysema, and cancer.[5][6] Increased concern over the safety of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has led to an increased interest in the exploration of effective and economical natural antioxidants.[7] Natural polyphenols exert their beneficial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases. [8] Their high antioxidant capacities are thought to have links with the inhibition of oxidative damage diseases, such as coronary heart disease, stroke, and cancers. [9] As a result of these findings, the intake of food-derived antioxidants in our daily diet has been suggested as a strategy to reduce the incidence of disease due to oxidative damage and to exert a beneficial effect on human health.[10]

MATERIALS AND METHODS

Collection of plant samples: The tender, fresh and soft shoots of *Bambusa balcooa* (Ching saneibi in local name) were procured from Noney District, Manipur, where soibum has been processed on a commercial scale. The sheath of bamboo shoots was manually removed and cut longitudinally into thin slices with the help of a knife under neat and clean conditions. Soon after mixing up thoroughly, the slices were filled inside the earthen fermentation chambers up to the capacity and closed airtight with polythene sheets. For ensuing natural

bacterial fermentation, the fermentation chambers were left inside a room having an ambient temperature of 25 degrees Celsius. On intermittent days:5,10,15,20,25,30,45,60,75,and 90, a small portion of the fermenting mass was removed for the evaluation of total phenolic content, total flavonoid content and antioxidant activity.

Polyphenols extract preparation:For the preparation of extract, on intermittent days:0,5,10,15,20,25,30,45,60,75, and 90, a small portion of the fermenting mass was removed,and 5 grams of it was weighed and crushed in 20 mL aqueous ethanol (80% v/v) and methanol (80% v/v) using mortar and pestle for carrying out determination of total phenolic content, total flavonoid content and antioxidant activity respectively by using analytical grade chemicals. The crushed sample was centrifuged, and the supernatant of the centrifuged sample was collected as a plant extract, the final volume was made up to 20mL and was stored at 4°C for further analysis.

Chemicals: DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich Pvt.Ltd., Whereas, Folin Ciocalteu phenol reagent,catechol,quercetin,ferric chloride hexahydrate,sodium acetate, acetic acid,hydrochloric acid, sodium carbonate,methanol, aluminium chloride, etc., were purchased from Merck, Pvt., Ltd., India. All the chemicals used were of analytical grade and stored at 4°C in the refrigerator.

Determination of total phenolic content: Total phenolic content was determined by Folin-Ciocalteau reagent method. [11] The results were expressed as mg catechol equivalent per 100g sample.

Determination of total flavonoid content: The total flavonoid content was determined using aluminium chloride colorimetric method Kosalec *et al*. (2005) with slight modifications.[12] Quercetin was used to draw the calibration curve (standard solutions of 6.25, 12.5, 25.0, 50.0, 80.0 and 100.0 µg mL^{-1} in 80% ethanol (v/v). Sample extracts were evaporated to dryness and retreated with 80% ethanol.1mL of a sample was treated with 3 mL 95% ethanol (v/v), 0.2mL 10% aluminium chloride (m/v), 0.2 ml of 1 $molL^{-1}$ potassium acetate and 5.6 mL water. A volume of 10% (m/v) aluminium chloride was then substituted by the same volume of distilled water in preparing blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was read at 415nm. The results were expressed as mg Quercetin equivalent /100g sample.

DPPH radical assay: DPPH radical scavenging activity was performed by the method described by Barca, (2002) with some slight modification. [13] 4 mg of DPPH was dissolved in 20mL of 80% (v/v) methanol to get the stock solution. Then 0.5 mL of sample solution was added to 1 mL of DPPH solution. The solution mixture was kept at room temperature in dark for 30 min(incubation period). Its absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity according to the equation

% Scavenging DPPH free radical = 100 × (1-AE/AD)

AE = It is absorbance of the solution when extract has been added at a particular level.
AD = It is the absorbance of the DPPH solution with nothing added (blank without extract)

Statistical analysis: Experimental results were expressed as mean ± standard deviation (SD) of three parallel measurements. The statistical analysis was performed using the Statistical Package for Social Science (SPSS Version 20.0, SPSS Inc., Chicago, IL, USA). *P* values< 0.05 were regarded as significant.

RESULTS AND DISCUSSIONS

Total phenolic content: Total phenolic content of the raw and fermented bamboo shoot mashes was found to have fluctuated in the range of 42.66 to 238.33 mg expressed as catechol equivalent /100g. The total phenolic contents steadily increased during the early fermentation days and then decreased, as evident by the levels on days 15 and 20, followed by afterwards increase or decrease. The pattern of change of polyphenols reported by Giri 2014 for 30 days of soibum fermentation of *Bambusa nutans* and *Dendrocalamus gigantus* was agreeable with the present finding.**Table 1** predicts the following pattern of change of polyphenols during soibum processing closes up to 90 days. It seems that elongation of the processing period has significance in increasing polyphenols even at the highest as evident by the level of day 60 (5.58 times), and at the lowest (day 45), the level had been elevated by 1.45 times.From the narration of the data of the present contribution and those of Giri 2014, it can be predicted that a common pattern of change of polyphenols might be ensured irrespective of the bamboo species used for soibum processing. According to the view of certain authors,the increase of polyphenols might be due to the mobilization of phenolic components lignin to a certain extent as caused by the fermentation.[14]The attributes of the decrease of phenolic compounds during the soibum fermentation might be due to the consumption of polyphenols as a carbon source and leaching into the exudate of fermenting mash.

Table 1: Total phenolic content in ethanol extracts of raw and fermented bamboo shoots.

Fermentation period (day)	Total phenolic content (mg CE/100g)
0	42.66±11.54
5	128.6±21.12
10	188.33±14.43
15	79±1
20	70.33±1.52
25	191.33±12.66
30	93.66±16.25
45	62±29.86
60	238.33±16.072
75	211±10.14
90	97.66±4.04

Note: Experiments were performed in triplicates, and the values were expressed in mean± SD with a significance value of ($P<0.05$).CE-Catechol equivalent.

Total Flavonoid content: The total flavonoid content of the ethanol extracts of the raw and fermentedbamboo shoots were expressed as mg Quercetin equivalent /100g sample [**Table 2**]. Total flavonoid content was found to be in the range of 5.76 mg QE/100g to 32.32 mg QE/100g. The highest flavonoid contents 32.32 mg QE/100g, was recorded with the 60 -day fermented sample and the least high value was the unfermented sample, that is 0 day. The results showed the total flavonoid content increases with an increase in the fermentation periods. These results also correspond to the findings of other researchers on the correlation between fermentation and the rise in the level of flavonoid contents in legumes.[15][16][17]
Flavonoids are polyphenolic compounds known for their high antioxidant properties and free radical scavenging ability.[18] The abundance of flavonoids coupled with their low toxicity relative to other plant compounds means they can be ingested in large quantities by animals, including humans.[19]

Table 2: Total flavonoid content in ethanol extract of raw and fermented bamboo shoots.

Fermentation Period (Day)	Total flavonoid content (mg QE/100g)
0	5.76±0.57
5	8.89±2.71
10	30±10
15	27.16±4.64
20	22.11±5.60
25	32.77±11.0
30	14.43±1.25
45	13.43±1.50
60	32.32±1.23
75	30.45±1.12
90	26.11±2.192

Note: Experiments were performed in triplicates, and the values were expressed in mean± SD with a significance value of ($P<0.05$).QE-Quercetin equivalent.

Table 3: Percentage of flavonoid composition of polyphenols.

Fermentation period (Day)	Total Phenolic content (mg CE/100g)	Total Flavonoid content (mg QE/100g)	Percentage flavonoid Composition.
0	42.66	5.70	13.36
5	128.6	8.89	6.91
10	188.33	30.00	15.92
15	79.00	27.16	34.38
20	70.33	22.11	31.44
25	191.33	32.77	17.13
30	93.66	14.43	15.40
45	62.00	13.43	21.66
60	238.33	32.32	13.56
75	211.00	30.45	14.43
90	97.66	26.11	26.73

Table 3 evidences that unlike polyphenols flavonoids undergo a different pattern of change in which about 5 times increase took place during the early days (up to day 10)and remained hiked up by 2.33-5.61 times relative to the initial level during the following days with the highest attainment of level on day 25. The polyphenols of fermenting mashes had 6.91-

34.38% flavonoids composition the lowest and highest values being on days 5 and 15 respectively.

DPPH free radical scavenging ability: It was observed that antioxidant activity had been raised more or less double-fold during the investing action period with the attainment of peak value on day 25[**Table 4**]. The polyphenols level raised up steeply during the early 10days and it remained lowered most of the time after this. It seemed that antioxidant activity had a closer pattern of charge with flavonoids than did with that of polyphenols. The polyphenols of bamboo shoots include catechin,protocatechin acid,caffeic acids, chlorogenic acid,p- coumaric acid, ferulic acid,p- hydroxyl benzoic acid, and syringic acid. Since only the former two are flavonoids, the remaining polyphenols might not play a significant role in exhibiting antioxidant activity of soibum.

Table 4: DPPH Antioxidant activity of methanol extracts of raw and fermented *Bambusa balcooa*.

Fermentation period (Day)	DPPH radical scavenging effect (%) (mg/mL)
0	37.35±0.09
5	65.79±0.16
10	92.11±0.39
15	90.71±0.12
20	89.22±0.32
25	92.40±0.09
30	88.42±0.32
45	73.79±0.11
60	79.67±0.17
75	78.02±1.09
90	78.37±0.80

Note: Experiments were performed in triplicates and the values were expressed in mean ± SD with a significance value of ($P<0.05$).

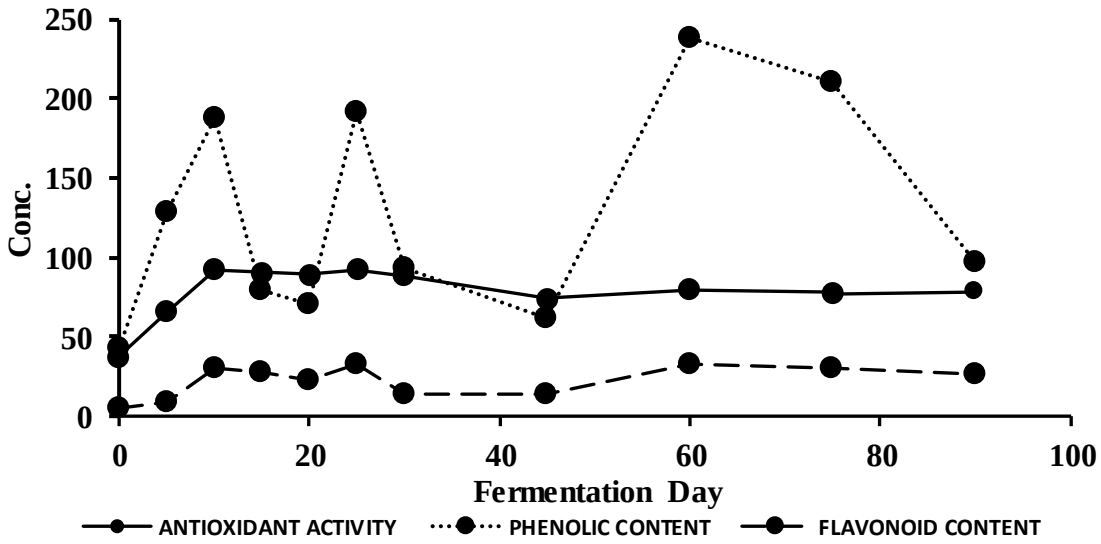

Fig.1: Correlation graph between antioxidant, phenolic, and flavonoid content of raw and fermented *Bambusa balcooa.*

CONCLUSION

The present study showed the changes in the contents of phenolic, flavonoids, and antioxidant activity during the fermentation of bamboo shoots*(Bambusa balcooa)*. The result showed that fermentation caused an increase in total phenolic content, total flavonoid content, and the antioxidant activity of the *Bambusa balcooa*. From the study, we can conclude that there is some correlation between the level of the phenolic, flavonoids and the increase in antioxidant activity. The higher content of flavonoids could be responsible for the increase in the antioxidant activity of the *Bambusa balcooa*. The present study concludes that the consumption of fermented bamboo shoots could be more beneficial to our health than raw bamboo shoots as it has a higher level of antioxidant activity and other phenolic compounds.

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CONFLICT OF INTEREST: There is no conflict of interest.

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