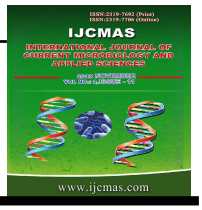
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 11 (2013) pp. 361-369**
http://www.ijcmas.com



Original Research Article

Fermentation decreases the antinutritional content in bamboo shoots

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A B S T R A C T

The emerging fresh young delicate bamboo shoots are used as delicious and nutritive food. However it contain anti-nutritive component likephytate, saponin, tannin alkaloid and cyanogenic glycoside (HCN)which are toxic to consumers . Fermentation of the bamboo shoot slices significantly reduces the anti-nutrient content.Microbial study was conducted on the exudates of the traditionally fermented bamboo shoot slices to investigate the role of specific microorganisms in the fermentation. Eleven types of bacterial strains were isolated. From these eleven isolates, the role of *Micrococcus luteus, Bacillus coagulans, Bacillus licheniformis, and Bacillus subtilis* in reducing the anti-nutritional component of hydrogen cyanide were assessed during fermentationso as to promote the bamboo shoots as ideal food for consumption .Fermentation is one of the most economic and effective measures for reducing the content of anti- nutritional factors.

Keywords

Anti-nutritional;bamboo shoots; Micro-organism; fermentation.

Introduction

The emerging fresh young bamboo shoots are harvested and used as vegetables. They are used in numerous Asian dishes and are available in markets in various sliced forms, fresh, fermented and canned version (Tai, 1985; Fu *et al.*, 1987; Midmore, 1998). The fermented form of bamboo shoots was used as a highly prized vegetable by the Tibeto-Mongoloid of Asian countries (Yamaguchi. 1983). In Manipur, the fresh succulent bamboo shoots slices and the fermented shoot slices done in large scale in the state is a highly prized vegetable item. The content of edible fiber in bamboo shoot is high. They are also rich in mineral, have adequate amount of glucose, low in fat and is brittle, tender, delicious and nutritive (Yamaguchi and Kusama, 1976; Yamaguchi, 1983). Besides nutrients, bamboo shoots also contain anti-nutrients that are toxic and need to be removed before human consumption. In this present paper comparative studies on the content of anti-nutrient in fresh bamboo shoot and processed bamboo shoot slices were conducted and the role of micro-organism in reducing the anti-nutritional components were assessed so as to promote the bamboo shoots as ideal food for consumption .Fermentation is one of the most economic and effective

measures for reducing the content of anti- nutritional factors.

Materials and Methods

Materials

Fresh succulent bamboo shoots were collected during their growing season (May to September). The outermost scales of the bamboo shoots were removed manually and the delicate shoot apex was sliced. For biochemical analysis the sliced shoots were oven dried at $60^{o}C \pm 2^{o}C$ for 12h and the dried samples of the delicate shoot apex were then crushed to powder form and kept for the analysis.

Fermentation

Preservative methods of the fresh succulent bamboo shoots were done in large scale in Manipur by traditional methods of fermentation process. The fermented bamboo shoot slices are locally called *soibum*. Bamboo shoots of many species like *Bambus atulda , B. balcooa, Dendrocalam ushamiltonii, D. hookeri, Thyrsostachys oliveri, Chimonobambusa callosa,Schizostachyum dullooa, Cephalostachyum latifolium, etc.* were used for fermentation (*soibum*).

Traditional method of fermentation

The *soibum* is prepared traditionally by storing thin slices of fresh succulent and soft bamboo shoots in certain containers/chambers for 2-3 months. The fermented chambers are either made of bamboo planks or roasted earthen pots. The inner surface of bamboo chambers are lined with banana leaves and a thin polythene sheets. The upper surface is sealed with polythene sheet and weights are then put on top for proper pressing. At the initial stage of fermentation the exudates is leached/drained out of the tilted side of the bamboo plank chamber. After fermentation is completed, which is indicated by the smell, colour and texure, soibumcan be stored up to one year. For the present studies traditionally fermented bamboo shoot slices of *Dendrocalamus hamiltonii* and *Bambus abalcooa* fermentation samples of 60days old were taken as the research samples.

Laboratory fermentation

Fermentation was also carried out in the laboratory by a modified form of the traditional method of fermentation which involves inoculating thin slices of succulent bamboo shoots of *Bambusabalcooa*with the exudates obtained from fermented slices of bamboo shoots sold in the local market in the name of 'soibum' After inoculation the samples were kept in an incubator at $30^{o}C \pm 2^{o}C$ for a period of 60 days, during which the period of fermentation took place. The whole process of fermentation (from slicing the shoot till sealing the polythene bag) was undertaken under sterile condition in a laminar flow chamber.

Microbial studies

Isolation of Bacterial strains from fermented samples:

Using the exudates (soibum extract) from the traditionally fermented bamboo shoots as the source of inoculants (containing mixed microbial population), microbial study was conducted (Skerman 1976, Cappuccino and Sherman 1983). The mixed population of micro-organism from the exudates of traditionally fermented bamboo shoots were isolated on agar plates containing nutrient agar media(g/l):

beef extract,1; yeast extract,2; peptone,5;NaCl,5 and Agar,15 ; pH was adjusted to 7.2. The cultures were grown in an incubator at $30^{o}C\pm 2^{o}C$ for 72h. When growth appeared, representative colonies were picked up and further purified through single colony isolation. The isolates obtained were then characterized partially by following Bergey's Manual of Determinative Bacteriology 9th Ed. (John *et al.*, 1994). Characterization included observations on the morphological and biochemical characteristics of the micro-organisms. Gram staining test (Skerman 1967) was also conducted for each bacteria isolated from the exudates of fermenting bamboo shoot slices.

Characterization of the Bacterial strains

To ascertain similarity or dissimilarity among the different organisms, a clinical sensitive test was conducted with antibiotic resistance discs called intrinsic antibiotic resistance (IAR) test by Bauer *et al*. 1966. Clinical sensitivity test was done with the antibiotic resistance disc (Span diagnostics) to study the IAR of the bacterial isolates. The nutrient agar plates were inoculated with turbid cultures of isolated organism. After inoculation, antibiotic discs, each having a fixed concentration of the desired antibiotic (chloramphenicol, doxycycline, erythromycin, gentamycin, neomycin, nitrofurantoin, streptomycin and tetracycline) was aseptically placed on the agar plates (11cm diameter).

The plates were maintained at $37^{o}C$ in an incubator for 18h. The diameter of the clear zone around the disc measured in mm by a ruler and compared with the standard interpretation chart in value for the respective antibiotic discs. The antibiotic sensitivity was classed as sensitive, intermediate or resistant according to the response of a strain for any particular antibiotic. Biochemical analysis, pH and temperature optimization of these isolated strains were also conducted. The isolated strains were then sent to the Institute of Microbial Technology, Chandigarh (India), for confirmation of the identification.

Assessment of the bacterial strains during fermentation:

From the different bacterial isolates, isolated in pure culture, the four bacterial strains identified up to species level were then used as inoculum in inoculating fresh shoot slices of *Bambus abalcooa*Roxb(shoots approximately of equal size and maturity). After inoculating with the different pure micro-organism under aseptic conditions, fermentation was done by keeping the inoculated slices in pure sterilized thin polythene bags in an incubator at $30^{o}C\pm 2^{o}C$ for 60days. The fermentation processes (from slicing till sealing the bags) was done under sterile condition in a laminar flow chamber.

Analysis of tannin contents

Tannin contents were determined by Folin-Denis method (Sadasivam and Manickam, 1992) which is based on the non-stoichiometric oxidation of the molecules containing a phenol hydroxyl group. Tannin like compounds reduced phosphotungstomolybdic acid in alkaline sodium carbonate solutions to produce highly blue coloured solutions. The intensity of which is proportional to the amount of tannin. The absorbance was measured at 700nm using tannic acid as the standard compound.

Analysis of saponin contents

Saponin content was estimated following Hiai*et al.*, method (1976). 500mg of the fresh sample was weight and stirred overnight in 10ml of 80% ethanol in a magnetic stirrer and centrifuged at 1000rpm for 15min. The supernatant was collected and the residue was washed three times with 5ml of 80% ethanol. The total volume was collected in a conical flask followed by centrifugation. To 1ml of the sample extract 1ml of vanillin reagent and 0.5ml of sulphuric acid (72%) was added. The whole volume was made up to 5ml with distilled water. Then the absorbance was measured at 570 nm using diosgenin as the standard compound.

Analysis of phytate content

Estimation of phytate content was done following Sadasivam and Manickam,1992. The phytate is extracted with trichloroacetic acid as ferric salt. The iron content of the precipitate is determined colorimetrically by taking absorbance at 480 nm and the phytate phosphorus content calculated from this value assuming a constant 4Fe:6P molecular ratio in the precipitate. $Fe(NO_3)$ was used as the standard solution to find out the µgFe present and phytate P was calculated as per the equation

$$\text{PhytateP (mg/100g)} = \frac{\mu g\,Fe \times 15}{\text{Weightof thesample(g)}}$$

Analysis of alkaloids content

Alkaloids content were determined by titration method (Rastogi and Mehrotra, 1993) using 0.1N NaOH as the titrating reagent. The samples were prepared in 90% ethanol thoroughly mixed with 0.1N HCl. Methyl red was used as the indicating reagent for the colour changed from red to yellow as the end point.

Estimation of cyanogenic glycosides content

Cyanogenic glycosides (hydrogen cyanide) estimation was done using the technique of the picrate-impregnated paper according to Bradbury *et al.*, 1999. The assay was performed in triplicate. Fresh plant material was cut into small pieces and crushed in a pestle and motar and immediately placed into a small flat bottomed vial. 0.5ml of phosphate buffer (0.1M, pH 7) and 6 drops of chloroform was added followed by brief crushing the materials with a glass rod.

A picrate paper attached to a plastic backing strip was added and the vial immediately closed with a screw stopper and left for about 16h at 30^{o} C. The liberation of the hydrogen cyanide (HCN) occurred rapidly after crushing the bamboo shoots. A colour change of picrate paper from yellow to brown-red or reddish colour, indicate the release of HCN by the plant samples. The change in the picrate paper is in proportion to the amount of hydrogen cyanic acid evolved. The picrate paper was then removed and the colour is eluted by immersing the paper in a clean test tube containing 5.0ml water for 30 min. The absorbance was measured at 510 nm and the total cyanide content was determined using potassium cyanide as the standard solution

Results and Discussion

The anti-nutritional component like phytate, saponin, tannin alkaloid and cyanogenic glycoside(HCN) were studied in fresh and fermented bamboo shoots and were found to have a higher content of the

anti-nutrient component in the fresh bamboo shoot and a decreasing trend was found in fermented bamboo shoot slices with the exception of the tannin content as is shown in table 1. Phytate has been reported to reduce the bioavailability of trace element and minerals (Apata and Ologhobo, 1989; Kubmarawa *et al.,* 2008).

From the present observations, it was found that the phytate content of 35.95mg/100g and30.67mg/100g fresh wt. in fresh bamboo shoots of *Dendrocalamus hamiltonii* and *Bambusa balcooa* respectively were found to reduce to 22.46mg/100g and 24.12 mg/100g fresh wt. in the traditional fermented and laboratory fermented samples respectively. The reduction of phytate after fermentation could be due to hydrolysis of phytate by microbial phytase(Lopez *et al.*, 1983)

Likewise the saponin content with 103.32mg/100g and 93.32mg/100g fresh wt. in the fresh shoots respectively decreases to 76.47mg/100g and 68.21 mg/100g fresh wt. in the traditional and laboratory fermented samples. Alkaloid content in fresh bamboo shoots (0.98mg/100g and 0.87mg/100g dry wt.) was decreased in both the fermented samples (0.63mg/100g and 0.34 mg/100g dry wt.). When alkaloids occur in high level in food, they cause gastro-intestinal upset and neurological disorders.Harvey *et al*. (1985) reported that alkaloids of more than 1to 3mg per kg body weight is considered a toxic dose for human being. But the present finding of alkaloid content in both fresh and fermented bamboo shoots is below the toxic level.

However, tannin content of 31.49mg/100g and 45.49mg/100g fresh wt. in fresh bamboo shoots of *Dendrocalamus hamiltonii* and *Bambus abalcooa* respectively increases to 68.21mg/100g and 52.00mg/100g fresh wt. in the traditional and laboratory fermented samples. Similar to the present finding, that tannin content increases after fermentation was also in agreement with the information reported by other workers (Olatunde and Mofoluso, 2007;Nuha*et al.*,2009) where tannin content increases after fermentation.

The hydrogen cyanide content also decreased significantly in fermented sample. The HCN content (224mg/100g and 317mg/g100g fresh wt.) in fresh bamboo shootsof *Dendrocalamushamiltonii* and *Bambusabalcooa*also decreases in thetraditional and laboratory fermented samples (33mg/100g and 30.11mg/100g fresh wt.).

In all fermentation it shows a degradation of HCN content with the advance of fermentation. Since HCN are highly volatile, the loss of HCN during the fermentation processes like peeling, slicing, cutting,repeated washing (3-4 times) is quite rapid. This may explain the reason that though bamboo shoots may contain significantly higher levels of HCN, however, the HCN content is reduced substantially during fermentation (both in traditional and modified laboratory fermentation) processing prior to consumption.The decreases in HCN concentration may be due to the volatile nature of the cyanogenic glycosides (Taxiphyllin) present in bamboo. Bamboo shoots become edible because of this instability (Schwarzmaier, 1977).

Microbial study was conducted on the exudates of the traditionally fermented bamboo shoot slices to investigate the role of specific microorganisms in the fermentation.

Table.1 Anti-nutritional composition in bamboo shoots of different bamboo species and in traditionally fermented bamboo shoot samples.

Name of the species	Phytate mg/100g fresh wt.	Saponin mg/100g fresh wt.	Tannin mg/100g fresh wt.	Alkaloid mg/100g dry wt.	HCN mg/100g fresh wt.
Dendrocalamus hamiltonii	35.95±1.17	95.32±0.55	45.49±0.67	0.87±0.07	224± 6.51
Bambusa balcooa	30.67±0.69	103.32±1.58	31.49±1.50	0.98±0.06	317± 6.03
Traditionally fermented bamboo shoot samples	22.46±1.19	76.47±0.31	68.21±0.55	0.34±0.01	33±2.65
Lab fermented bamboo shoots samples	24.12±0.12	84.21±1.53	52.00±1.58	0.63±0.03	30.23±0.05

*Data presented as mean ± SD.

Table.2 Microbial sensitivity test (intrinsic antibiotic resistance test) with different isolates of bacteria obtained from fermented bamboo shoots "Soibum".

Sl. No.	Bacterial isolate (tentative)	Gram's staining	Response to the micro-organism to the antibiotics for chemotherapeutic agents impregnated in paper discs							
			C	F	E	J	N	S	T	VB
	Micrococcus luteus	+ve	S	R	I	S	I	R	R	R
	Bacillus coagulans	+ve	I	R	I	R	R	R	R	R
	Bacillus licheniformis	+ve	R	R	R	S	S	R	R	R
	Bacillus subtilis	+ve	S	S	I	S	S	R	R	S
	Bacillus species$_1$	+ve	R	S	R	R	R	R	R	S
	Micrococcus species	+ve	S	R	S	R	R	R	R	R
	Bacillus species$_2$	+ve	S	R	R	R	R	S	R	S
	Bacillus species$_3$	+ve	S	S	R	S	S	R	S	S
	Bacillus species$_4$	+ve	S	S	S	S	S	R	I	S
	Bacillus species$_5$	+ve	S	S	I	S	S	R	R	S
	Bacillus species$_6$	+ve	S	S	I	S	S	R	R	S

Responses : I = intermediate; S = sensitive ; R = resistant
Antibiotics : C = chloramphenicol; N = neomycin; VB = doxycycline;
F = nitrofurantoin; T = tetracycline; E = erythromycin;
S = streptomycin; J = gentamycin.

Table.3 Cyanogenic glycosides (HCN) content in the fermenting samples of *Bambus abalcooa* Roxb. inoculated with different bacterial isolates isolated from the 'soibum' exudates.

Bacterial isolates (tentative)	Cyanogenic glycosides (HCN) content (mg/100g fresh wt.) Fermentation period (days)				
	0	7	14	21	30
Fresh delicate shoot slices (uninoculated)	295±2.08 *	290±3.00	278±2.65	266±2.00	254±1.44
Bacillus subtilis	290±1.44	268±2.00	183±2.43	163±2.08	132±1.58
Bacillus licheniformis	290±1.01	264±2.01	264±1.58	250±2.01	247±2.01
Baiclluscoagulans	293±2.65	261±2.65	231±3.01	230±2.08	211±1.28
Micrococcus luteus	294±2.03	231±2.01	203±3.01	188±2.41	167±2.02
Shoots inoculated with the above four bacterial isolates	294±2.03	190±3.00	175±2.43	98±2.03	74±1.03

*Data presented as mean ± SD.

Eleven types of bacterial strains were isolated based mainly on the morphological and biochemical characteristics. Morphological characteristics of the microorganisms include the colour, size, margin, surface elevation, shape of the cells and the Gram stain response .A critical study of the intrinsic antibiotic resistance test result revealed that of the eleven isolates, ten were different and isolates No.4 and 11 shows similar response (sensitive to C, F, J, N and VB, resistant to S and T and intermediate response to E antibiotics). Hence, these two were considered as one isolate (table 2).

For further confirmation/identification, these isolates were sent to the Institute of Microbial Technology, Chandigarh (India) and so far four isolates have been identified up to species level and the remaining six up to genus level. They are: *Micrococcus luteus, Bacillus coagulans, Bacillus licheniformis, Bacillus subtilis, Micrococcus* species and six *Bacillus* species. These result shows that fermentation is indeed caused by the activity of microorganism (Prescott and Dunes 1959, Frazier and Westhoft 1978). The role of the four bacterial strains (*Micrococcus luteus, Bacillus coagulans, Bacillus licheniformis, Bacillus subtilis)* in reducing the anti-nutritional hydrogen cyanide content which is the most toxic compound among the anti-nutritional compounds were conducted by fermenting shoot slices of*Bambusabalcooa*which showed a decreasing trendin the HCN concentration from the initial (0-day) fermentation period to 30 days fermentation period (table 3). *Bambus abalcooa* fresh bamboo shoot slices to 247mg/100g to 132mg/100g fresh wt in fermented shoot slices .Fermentation with the shoot slices inoculated with the mixture of the pure four bacterial isolated further decreases the HCN content as shown in table 3. This shows that the

The fermentation of the bamboo shoot slices inoculated with the pure isolate of *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans* and *Micrococcus luteus* was effective in decreasing the hydrogencyanide (HCN) from 295mg/100g to 290mg/100g fresh wt. in

involvement of all the micro-organism in fermenting the bamboo shoot are more efficient in reducing the anti-nutritional compound of HCN.

Fermentation probably eliminated the toxic or other undesirable characteristics making the food palatable by enhancing its organoleptic properties, aroma, texture and flavor (Wang and Hesseltine, 1981;Steinkraus, 1998;Chelule*et al*., 2010). Hence to improved nutritional quality and effective utilization of bamboo shoots to their full potential as food, removal of anti-nutritional factors fermentation is one of the most economic and effective measures for reducing the content of anti- nutritional factors.

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