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Isolation and quantification of phytosterols in fresh and processed shoots of an edible bamboo *Bambusa bambos* (L.) Voss using GC-MS

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Bamboo shoot is considered a palatable delicacy in Southeast Asia and Northeastern India and is traditionally consumed in fresh, boiled, soaked and fermented forms. Despite having high nutritious value, bamboo shoot is a minimally explored food resource, particularly for bioactive compounds. The present study was conducted to explore the phytosterols in shoots of *Bambusa bambos* (L.) Voss and how they were affected by processing techniques such as boiling, soaking and fermentation. The total phytosterol content in fresh shoots was 198.69 mg/100g in terms of dry weight. Lipid extraction, followed by isolation of sterol fraction and examination through GC-MS, revealed the presence of three major free phytosterols with the highest content of β-sitosterol followed by campesterol and stigmasterol. All the processing techniques lead to the enhancement of phytosterol content for each of the free sterols and, in totality, where fermentation was most effective, bringing about a better constitution of phytosterols, which can provide many health functions. Thus, bamboo shoots could be used as a healthy food commodity in fresh as well as processed form, and its extracted sterols could be fortified in various supplements, drugs and health care products.

Keywords: Bamboo shoot, *Bambusa bambos* (L.) Voss, Bioactive compounds, Gas Chromatography, Phytosterol, Processing

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Introduction

Plant derivatives from various parts have been encompassed in phyto-medications since ancient times[1,2]. Knowing the chemical constituents of these plants can be informative for synthesising valuable complex chemical compositions. However, the continuously destructing biological diversity is pointing towards the urgency of expanding and exploring the resources of novel bioactive components, which could be utilised in health food and pharmaceutical industries[3,4].

One minimally explored resource is bamboo shoot, the juvenile tender part of bamboo culm emerging from an underground rhizomatous bud. Bamboo shoots have been recognised for their palatability in conventional delicacies of Southeast-Asian countries for centuries due to their exquisite flavour, fragrance and traditional knowledge of their health benefits. In India, although bamboo is highly exploited as a non-forest timber for multifaceted uses in households and construction, the knowledge of bamboo as food is restricted only to the Northeastern region and some Southern states. Customarily, shoots are preferred for consumption in fresh form. However, other processed forms like fermented, dried, pickled and boiled shoots are also used to prepare numerous dishes to extend the content and deliciousness of food[5,6]. Moreover, amid the presence of certain antinutrients, specifically cyanogenic glycosides, which release a toxin 'hydrogen cyanide' (HCN) upon hydrolysis, bamboo shoots need to be intoxicated before consumption. It is conventionally done by processing the shoots by boiling, soaking and fermentation methods.

Besides being a delicacy, bamboo shoot is considered a highly nutritious vegetable rich in proteins, carbohydrates, amino acids, vitamins and numerous mineral elements while having very low fat and calories. Bioactive compounds like dietary fibres, phenolic acids, and phytosterols are also prevalent in shoots[5-8]. Although much work has been done on nutrient contents, elaborative work on the composition of bioactive compounds, particularly phytosterols in bamboo shoots, is still deficient in processed shoots. Phytosterols can be used as an

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effective food additive and to concoct bioceuticals and drugs for health ailments.

Phytosterols are biologically active plants-derived steroid alcohols; members of the tri-terpene family; 27-30 carbon atom compounds with an attached side chain at position C-17; and are structurally identical to cholesterol, except for an additional sterol side chain at the C-24 position. The most prevalent free phytosterols in plant-derived food are sitosterol, campesterol and stigmasterol[9,10]. These molecules are capable of averting cholesterol absorption by displacing it during micelle formation in the gut region because of their higher hydrophobic nature[10-12]. It is also evident that functional foods with plant sterols might be preferred by hypercholesterolemic patients having intermediary and low global cardiovascular risk[13]. Phytosterols are also reported to have antidiabetic and anti-cancerous effects against breast, stomach, colon and prostate cancers. They can act as an anti-oxidant, a modest radical scavenger and a membrane stabilizer[10,14,15]. Numerous dairy products, such as margarine or yoghurt, are augmented with phytosterols and have been commercialised for several years[16]. Moreover, phytosterols are precursors of many pharmaceutically important steroid products[9].

In view of the enormous utility of phytosterols, the present study was conducted to i) determine total phytosterols in fresh juvenile bamboo shoots and to analyse the impact of traditional processing, *viz*. soaking, boiling and fermentation on the total content, ii) qualitatively analyse the free sterols in fresh and processed shoots, and iii) isolate and quantify the phytosterols using gas chromatography-mass spectrometry (GC-MS) technique.

Materials and Methods

Collection and identification of plant material

Shoots of *Bambusa bambos* (L.) Voss was collected from late August to early September, for three successive years (2014–2016), from the clumps maintained in Bambusetum of CSIR, IHBT (Institute of Himalayan and Bioresource Technology) Palampur, situated in Kangra district of Himachal Pradesh (32.11 °N, 76.53 °E), India. The species was identified by Dr Anil Sood, former Chief Scientist at CSIR, IHBT, Palampur. Shoots were procured at a stage of 10–14 days past their emergence from the ground.

Processing of bamboo shoots

After collection, bamboo shoots were washed, and the outer protective culm sheath was peeled. The inedible tip and hard basal portion were also removed to obtain the edible tender portion, which was used to analyse fresh samples. Furthermore, for processing, the shoots were chopped or sliced and subjected to boiling separately for three durations (10, 20, and 30 min), soaking for three durations (6, 12 and 24 h) and fermentation for three durations (1, 3, and 6 months). The fresh and processed shoots were placed at -80°C in a deep freezer (Skadi®, Green Line, Europe) for 24 hours. The frozen samples were then freeze-dried for 24 hours in a Lyophilizer (LYOQUEST 55, Skadi, Europe) with a condenser temperature of -55°C and vacuum pressure of 0.10 mbar. The dried samples were placed separately into glass containers with air-tight lids, labelled, and stored at room temperature (25±2°C) until further experiment use.

Reagents and Standards

The standards of cholesterol, β-Sitosterol, stigmasterol, and campesterol, having assay≥ 99%, were purchased from Sigma-Aldrich/Merck KGsA, Darmstadt, Germany. The chloroform used to dissolve sterols was HPLC-grade, and all other chemicals and reagents were of analytical grade and purchased from HiMedia Laboratories Pvt. Ltd., Maharashtra, India.

Estimation of total phytosterols

Total phytosterols were estimated by using the method of Srivastava[17], using ethanol: acetone (1: 1) solution for extraction, acid “Liebermann-Burchard reagent” for estimation and cholesterol as standard. Estimation of sterols was done by taking absorbance through PC-based Double Beam spectrophotometer 2202 (Systronics, India)

Analysis of free phytosterols

Extraction and saponification of bamboo shoot lipids

Extraction of lipids from bamboo shoots was done using the method of Feng *et al.*[18]. Dried and powdered bamboo shoot (1 g) was extracted in absolute ethanol for 4-5 h at a constant temperature of 50°C. The ethanol was filtered and vaporised at around 40°C and finally dried in a vacuum lyophiliser. The yield of lipids obtained was expressed as g lipids /g dry weight.

The lipids were subjected to saponification and solid phase extraction (SPE) as per the technique of

Lu *et al.*[19]. The dried lipid extract was saponified with 20 mL of ethanolic KOH (2 M) at 80°C for 2 h. The saponification mix was transferred from the flask to a separating funnel, rinsed with 20 mL of distilled water, and extracted twice with diethyl ether (20 mL each time), in which the residual saponification produced was insoluble. The diethyl ether phase was collected and washed twice with 20 mL of water. The ether extract was filtered through anhydrous sodium sulphate and evaporated at 30°C, dried in a vacuum lyophiliser and dissolved in 15 mL of hexane/ethyl acetate mixture (95:5, v/v). For SPE, a Sep-pak Vac silica cartridge (500 mg, 6 cc; Waters, New Delhi, India) was conditioned with hexane (around 15 mL) and the sample dissolved in hexane: ethyl acetate mixture (95:5, v/v) was loaded to it. The sterol fraction was eluted with 5 mL of hexane: ethyl acetate (60:40, v/v) solution. The eluted fraction was then subjected to deep freeze and dried in a vacuum lyophiliser, redissolved in 2 mL of chloroform and filtered through 0.45 µm membrane filters (Merck Millipore, India) before chromatography study.

GC-MS analysis of phytosterols

Gas chromatography combined with mass spectrometry (GC-MS) was used to analyse sterols by following the method of Thanh *et al.*[20] with slight modifications. Analysis of the isolated/fractioned sterols was done with a Thermo Trace 1300 GC coupled with Thermo TSQ 800 Triple Quadrupole MS having a Software (XCalibur 2.2SP1 with Foundation 2.0SP1), Column (TG 5MS; 30 m X 0.25 mm, 0.25 µm), Injector (S/SL; Split/ Splitless), Injection volume (1.0 µL), Injector temperature (250°C), MS transfer line temperature (280°C), Ion source temperature (230°C), Carrier Flow (1 mL/min), Detector (MS TSQ 8000), Flow rate (1 mL/min) and an oven program as follows:

Initial Temperature : 60°C; Hold time : 2 min
Final Temperature : 280°C; Hold time : 10 min
Temperature Rate : 10°C /min
The library used was NIST 2.0

Quantitative analysis of free sterols

The identified sterols were quantified in the extract using software with SIM (single ion monitoring) mode based on calibration curves. The calibration curves were drawn using different concentrations of standard solutions of β-Sitosterol, stigmasterol and campesterol.

Statistical analysis

Phytosterol estimation was done in triplicates; the results are shown in the form of an average of the replicates ± s.d. Data were subjected to one-way analysis of variance (ANOVA) using PASW statistics software version 18.0. Statistical difference was determined using Duncan's multiple range tests at a significance level of $P < 0.05$.

Results

A detailed analysis of sterols was conducted after estimating the total phytosterol content (Table 1) in fresh and processed bamboo shoots. For this, bamboo shoot lipids were extracted and saponified to obtain a non-saponified fraction containing free sterols.

Extraction of bamboo shoot lipids and non-saponified matter

Through the ethanol extraction method, different concentrations of lipids were extracted from dry bamboo shoot powder obtained from fresh, boiled (20 min), soaked (12 h) and fermented (6 months) samples. The % yield was calculated as indicated in Fig. 1. The lipid % for fresh shoots was 8.43±0.18% dry wt. The lipid yield increased in processed shoots: 11.57±0.22% for boiled shoots and 11.89±0.09% for soaked shoots, while maximum lipid yield was obtained from fermented shoots (14.87±0.16%).

After saponification of the extracted lipids, which converted glycerides to glycerol and fatty acid salts precipitating as soap, the complex lipids, including sterols, were left in non-saponified material, and the amount of non-saponified matter was reduced to a range of 0.54–0.98% for fresh and processed shoots

Table 1 — Effect of different durations of processing on total phytosterol content (mg/100 g dry wt.) in shoots of *Bambusa bambos* (L.) Voss

Treatments		Total phytosterol content (mg/100 g dry wt.)
Fresh		198.69±4.92[g]
Boiled (min)	10	213.65±3.81[f]
	20	244.94±2.22[e]
	30	250.24±2.78[de]
Soaked (h)	6	218.22±2.11[f]
	12	253.43±3.97[d]
	24	266.44±1.34[c]
Fermented (months)	1	251.21±4.28[d]
	3	275.29±2.93[b]
	6	316.41±2.77[a]

[a]Data are presented in mean values ± standard deviation (n=3).
[b]Letter with different alphabets in superscripts indicate significant difference ($P < 0.05$) in each column.

(Fig. 1). Maximum yield of non-saponified matter was obtained for fermented shoots (0.98±0.03%), followed by0.79±0.02% and 0.74±0.02% in soaked and boiled shoots respectively. While, a notably lower amount (0.54±0.01%) was obtained from fresh shoots of *B. bambos* compared to the processed ones.

GC-MS analysis of sterol fraction

The non-saponified stock of bamboo shoot lipids was analysed through GC-MS to detect whether the phytosterols exist in the fraction. The GC-MS chromatogram of non-saponified material displayed various peaks of multiple compounds (Table 2) (Fig. 2), which, on reckoning with the NIST library, affirmed the presence of three main phytosterols. The MS (mass spectrum) of peak 20 at RT 19.77 exhibited the molecular ion peak at m/z 400.37 matched with campesterol. The peak 21 at RT 20.27 was identified as stigmasterol, for which molecular ion peak was observed at m/z 412.40. The m/z 414.38 was observed at peak 22, recognised as β-sitosterol at RT 21.36. The MS of the identified sterols is also comparable to the MS of corresponding standards (Fig. 3).

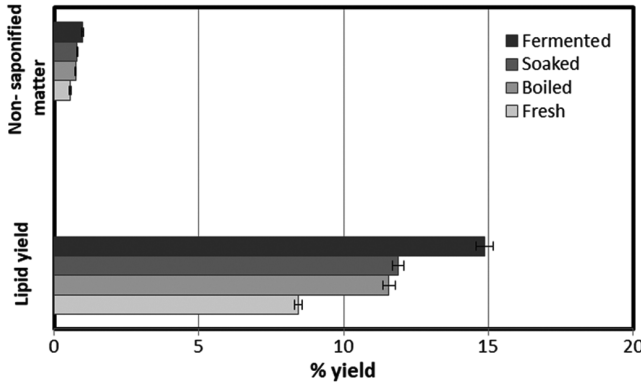


Fig. 1 — % lipid yield and % yield non-saponified matter in fresh and processed shoots of *B. bambos*.

Quantitative analysis of individual sterols

Further, to investigate the quantitative composition of the three identified phytosterols, a non-saponified stock of lipids from each bamboo shoot sample was purified to obtain sterol fraction, which was then subjected to chromatographic study. The calibration curves for β-Sitosterol, stigmasterol and campesterol standards were plotted for a concentration range of 20 – 500 ppm. A linear regression equation was obtained, and a correlation coefficient≥ 0.9998 was found for each standard. The identified sterols were quantified in the extract based on calibration curves. GC chromatogram for a mixture of sterol standards is given in Fig. 4.

Table 2 — Tentative identification of *B. bambos* metabolites in Non saponifiable fraction of lipid extract of bamboo shoots using GC-MS

Peak	RT	Peak area	Area %	Mol. formula	Exact mass	Compound
1	7.61	1110412731.26	1.38	$C_{14}H_{22}O$	206.16	Phenol, 2,4-bis(1,1-dimethylethyl)-
2	8.15	1089556895.95	1.35	$C_{16}H_{32}$	224.25	Cyclohexadecane
3	9.31	1493203974.70	1.85	$C_{19}H_{40}O$	270.29	1-Octadecanol
4	10.27	21638376772.28	26.85	$C_{14}H_8O_2$	208.05	Anthraquinone
5	10.74	1305479442.95	1.62	$C_{22}H_{44}O_2$	340.33	1-Heneicosyl formate
6	10.92	1991555192.62	2.47	$C_{15}H_{10}O_2$	222.06	9,10-Anthracenedione, 2-methyl-
7	11.29	1026356554.52	1.27	$C_{24}H_{50}O$	354.38	n-Tetracosanol-1
8	11.97	868908788.12	1.08	$C_{28}H_{42}O_2$	410.31	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-
9	12.74	1119105002.84	1.39	$C_{30}H_{62}$	422.40	Triacontane
10	13.30	2123138493.45	2.63	$C_{26}H_{54}$	366.42	Hexacosane
11	13.61	1156053824.22	1.43	$C_{28}H_{58}$	394.40	Octacosane
12	13.94	2239516584.57	2.78	$C_{34}H_{70}$	478.50	Tetratriacontane
13	14.33	1554145540.68	1.93	$C_{22}H_{43}NO$	337.33	13-Docosenamide, (Z)-
14	14.68	5111823849.34	6.34	$C_{28}H_{58}$	394.40	Octacosane
15	15.54	3946206350.62	4.90	$C_{29}H_{60}$	408.40	Nonacosane
16	15.83	1448123810.03	1.80	$C_{30}H_{62}$	422.40	Triacontane
17	16.53	4817357040.01	5.98	$C_{30}H_{62}$	422.40	Triacontane
18	17.12	1786318550.03	2.22	$C_{36}H_{74}$	506.57	Hexatriacontane
19	17.70	2462406025.00	3.05	$C_{36}H_{74}$	506.57	Hexatriacontane
20	19.77	5021969916.42	6.23	$C_{28}H_{48}O$	400.37	Campesterol
21	20.27	3939658877.95	4.89	$C_{29}H_{48}O$	412.40	Stigmasterol
22	21.36	13352917572.93	16.57	$C_{29}H_{50}O$	414.38	β- sitosterol

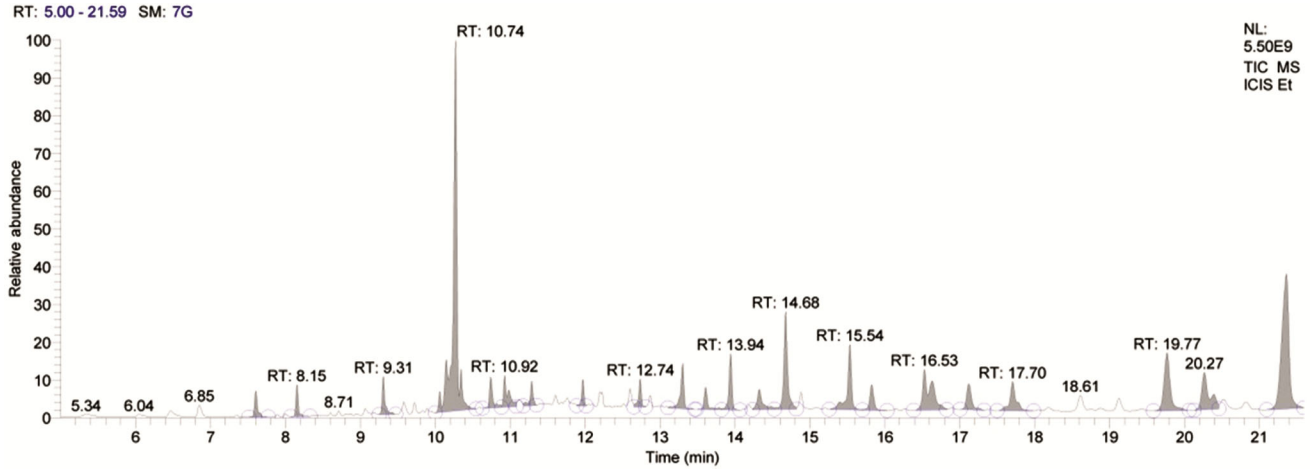


Fig. 2 — The GC chromatogram of the non-saponified fraction of bamboo shoot lipids showing different phytosterol peaks.

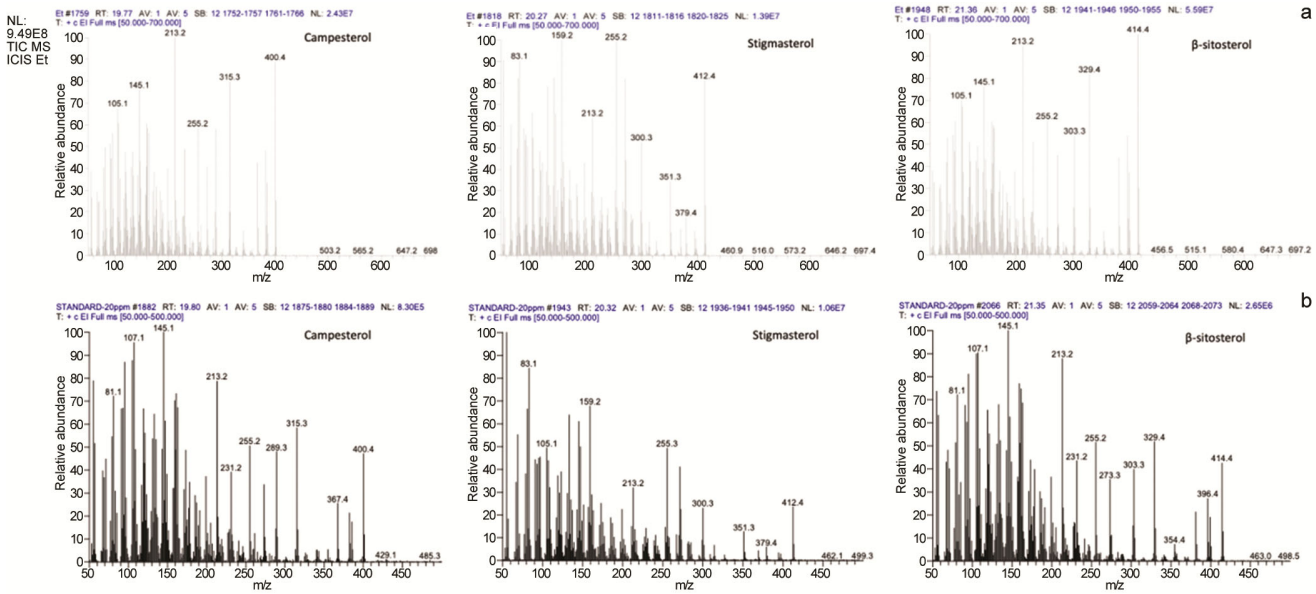


Fig. 3 — Comparison of MS of phytosterols in a) Bamboo shoots; and b) Standards.

Phytosterol content and composition in fresh shoots

Total phytosterol content in fresh shoots of *B. bambos* was estimated to be 198.69±4.92 mg/100g dry wt. The three free phytosterols quantified in fresh shoots were present in a descending sequence of β-sitosterol >campesterol>stigmasterol. The content of β-sitosterol was recorded to be high, *i.e.* 86.51±1.09 mg/100g dry wt. compared to campesterol and stigmasterol, which were found in the comparable range of 17.20±0.67 mg/100g dry wt. and 13.08±0.24 mg/100g dry wt., respectively (Table 1; Fig. 5).

Impact of processing on total and free phytosterols

Processing of shoots brings about certain changes in biochemical composition, of which phytosterols are also markedly affected. With currently applied processing methods, *viz*. boiling, soaking and fermentation, the quality composition of phytosterols remained infrangible as the shoots contained all three free sterols, *viz*. β-sitosterol, campesterol and stigmasterol in all the processed forms as in fresh shoots. However, the quantity of phytosterols in totality and individual sterol components was affected significantly (P>0.05) with notable enhancement in content after all the processing treatments, as displayed in Table 1 and Fig. 5 and 6.

Boiling

The total phytosterols showed elevation by around 8% in content after initial boiling of 10 min, which further enhanced significantly to 23% with an increase in the duration of boiling (20 min), while no

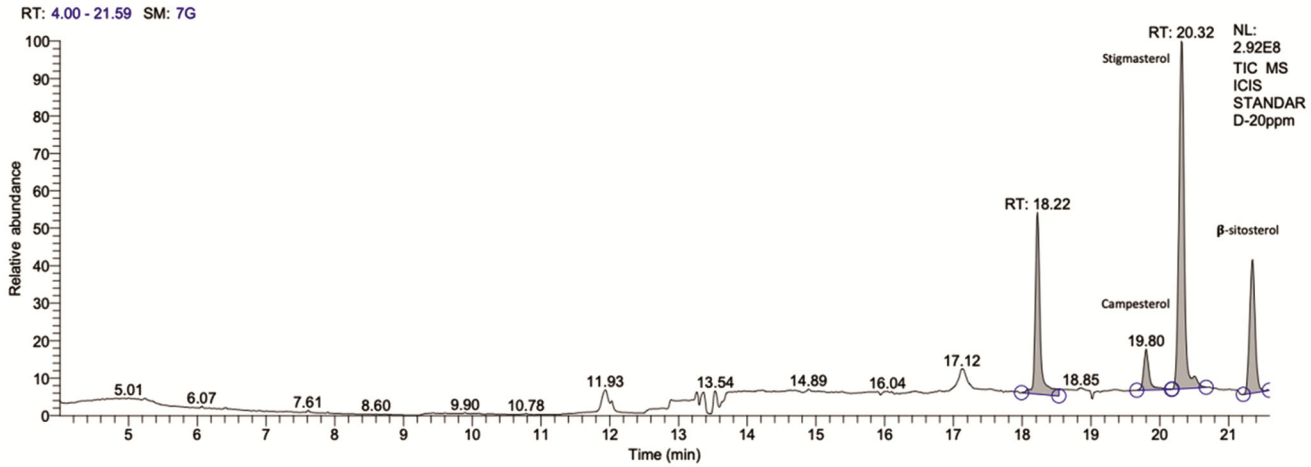


Fig. 4 — GC Chromatogram of mixture of phytosterol standards.

significant enhancement (P <0.05) was noticed with further increasing the duration of boiling. After 30 min of continuous boiling, the total content in shoots was 250.24±2.78 mg/100g dry wt., which, compared to fresh shoots, was notably high (P >0.05). A similar enhancement pattern was followed in the case of free sterols, but the rate of increase was different for each sterol. Among the three, β-sitosterol content exhibited maximum enhancement (111.90±2.13 mg/100g dry wt.) by around 29% in 20 min boiled shoots; campesterol also surged by a similar range of 26% from its initial content, which was recorded to be 21.62±0.55 mg/100g dry wt. On the other hand, stigmasterol showed a minimal increase of 12%, which equated to 14.63±0.68 mg/100g dry wt. content in boiled shoots.

Soaking

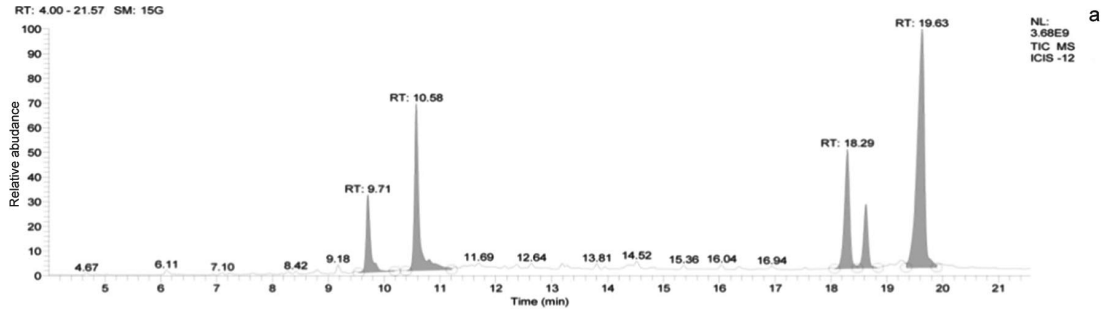
An apparent rise in total phytosterol content was observed in soaked shoots in all the durations of treatment. After water soaking the shoots for 6 h, the phytosterol content enhanced by 10%, similar to the content enhancement after 10 min boiling. With increased soaking duration, the amount of phytosterols also increased correspondingly up to 28 and 34% after 12 and 24 h of soaking, respectively. Amongst the free sterols, β-sitosterol content was evaluated as 115.57±1.14 mg/100g dry wt. after 12 h of soaking, corresponding to34% enhancement. Campesterol exhibited a greater increase of 48% with an estimated value of 25.44±0.83 mg/100g dry wt., while comparatively lesser elevation up to 16.44±0.38 mg/100g dry wt. was observed for stigmasterol, indicating a 26% increase in content. The overall impact of soaking depends upon the individual sterol of bamboo shoots compared to boiling treatment.

Fermentation

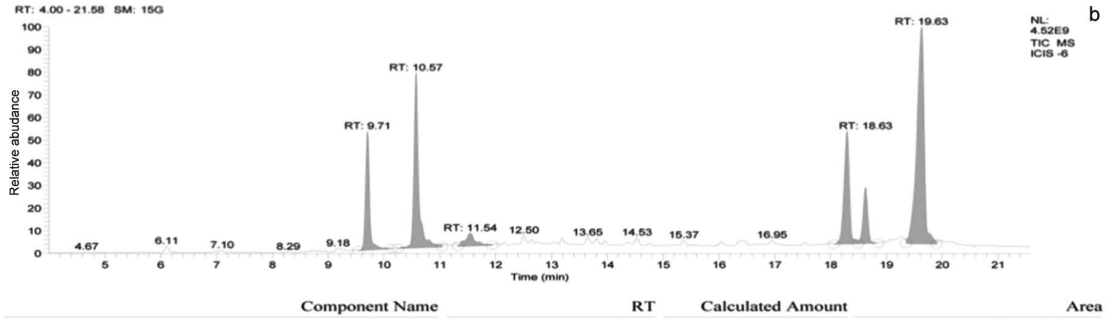
Of all the processing techniques investigated in the current study, fermentation has the highest impact on phytosterol content and composition. It led to a 26% enhancement in the total content of phytosterols after one month of processing, which appeared significantly similar to values obtained upon 30 min boiling and 12 h soaking of shoots. Like boiling and soaking, with an increase in the duration, the content also increased and showed a 39 and 59% surge in total phytosterols post 3 and 6 months of fermentation. In the present investigation, 316.41±2.77 mg/100g dry wt. was the highest recorded total phytosterols observed in 6 months of fermented bamboo shoots. The free sterols were analogously raised in fermented shoots, where β-sitosterol accounted highest with 141.25±2.44 mg/100g dry wt. content (63% enhancement); the enhancement rate was similar for campesterol, but the quantified amount was lesser (28.80±0.77 mg/100g dry wt.). The stigmasterol content was also maximum in fermented shoots (30.21±0.40 mg/100g dry wt.), more than double the values from its initial content in fresh shoots, indicating a 131% surge.

Discussion

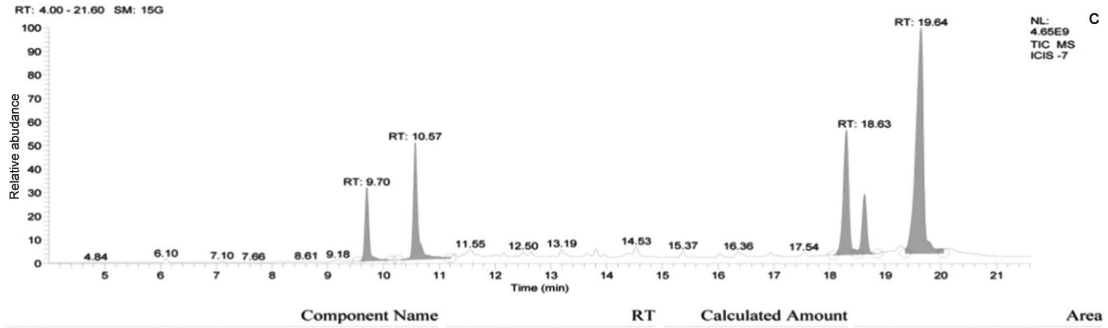
Juvenile bamboo shoots are a rich reservoir of dietary phytosterols, showing an appreciable amount of total phytosterol in presently investigated shoots of *B. bambos*. A comparable level was also affirmed with 64.25–321.80 mg/100g dry wt. phytosterol content in various bamboo species in previous observations[19,21,22]. In a recent study conducted on 12 bamboo species from Manipur, the phytosterol content in different parts of shoots was estimated in a range between 48.30-293.80 mg/100g, where the



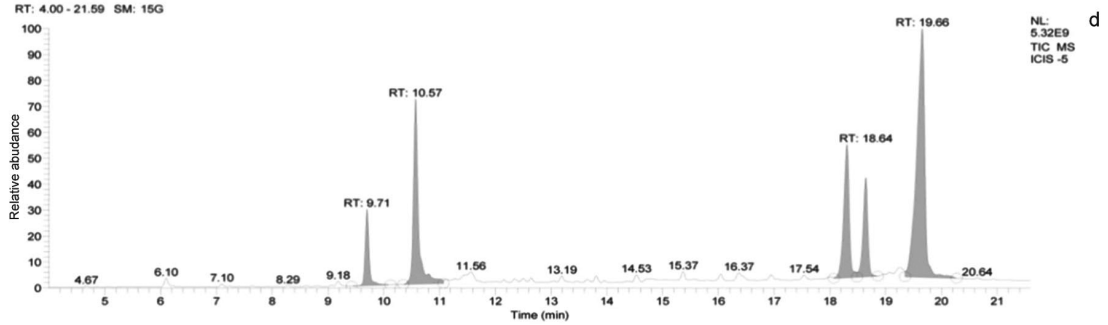
Component Name	RT	Calculated Amount	Area
CAMPESTEROL	18.29	171.967	352021926.66
STIGMASTEROL	18.62	130.800	127341438.46
SITOSTEROL	19.64	865.143	979446903.72



Component Name	RT	Calculated Amount	Area
CAMPESTEROL	18.29	216.154	442443694.93
STIGMASTEROL	18.63	146.328	142642125.09
SITOSTEROL	19.64	1118.985	1266091259.77



Component Name	RT	Calculated Amount	Area
CAMPESTEROL	18.31	254.476	520865796.97
STIGMASTEROL	18.63	164.383	160432880.48
SITOSTEROL	19.65	1155.755	1307612567.73



Component Name	RT	Calculated Amount	Area
CAMPESTEROL	18.31	288.134	589740213.23
STIGMASTEROL	18.64	302.113	296150282.94
SITOSTEROL	19.67	1412.523	1597561442.16

Fig. 5 — GC-MS chromatogram showing phytosterols in *B. bambos* shoots in a) fresh and after processing through; b) boiling; c) soaking; and d) fermentation.

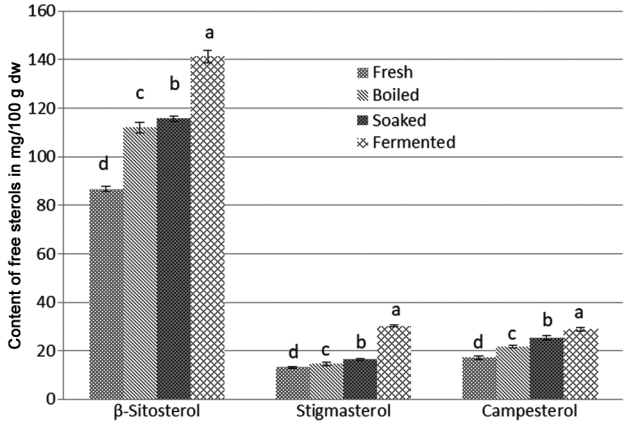


Fig. 6 — Impact of different processing treatments on the composition of free phytosterol. Different letters above the bars indicate significant difference (P <0.05) for each sterol separately.

highest content was found in apical portion and lowest in basal portion[23].

The identification of predominant sterol components in shoots was made, where the content found in this species was in the order of β-sitosterol>campesterol> stigmasterol. The documentation of sterol components was initially done by He and Lachance[24] and Lachance & He[25]. Further, Lu *et al.*[19] conducted a detailed investigation on sterol composition and their levels in shoots of four bamboo species, *viz*. *Dendrocalamus latiflorus, Phyllostachys pubescens, P. praecox* and *Pleioblastus amarus*, reported the presence of ergosterol, stigmastanol and cholesterol in addition to the previously reported three sterols *viz*. β-sitosterol, campesterol and stigmasterol ranging from 89.9-233.0, 13.6-28.3 and 6.4-13.5 mg/100g dry wt. which was consistent with present outcomes.

Unlike other biochemicals, total phytosterol and accumulated sterol components (β-sitosterol, stigmasterol and campesterol) of bamboo shoots elevated with an increase in the duration of various processing treatments. A simultaneous increase in phytosterol content after boiling and soaking of three *Dendrocalamus* species *viz D. latiflorus, D. giganteus* and *D. sikkimensis*, with a maximum upsurge by 78% in fermented shoots of *D. latiflorus* was also observed recently[21,26]. Phytosterol enrichment after fermentation was reported in *B. balcooa* and *D. hamiltonii* shoots[27,28] due to the biotransformation of organic matter by microorganisms that resulted in the elevation of total phytosterols. Zheng *et al.*[29] showed a twofold increase in total phytosterol content of bamboo shoots after fermentation using *Aspergillus niger* CTBU, where the accountability of β-sitosterol, stigmasterol and cholesterol was 80.1, 15.2, and 2.9% of total content in fresh shoots, and 79.9, 17.2, and 3.1% of total content in fermented shoots residue, indicating that the individual sterols also enhanced concurrently during fermentation.

Scanty data are available on the effect of plant sterols in different food processing procedures used at the traditional level. A significant increase in the level of total phytosterols was reported in cabbage, courgette, celery, yellow onion and red pepper after 30 min of boiling; the value of campesterol and sitosterol was also enhanced after processing, while stigmasterol showed decreased or increased values for these vegetables[30]. The sterols, especially stigmasterol, sitosterol and campesterol, are an integral part of plant membranes, and there might be some structural alterations due to stress on membranes during processing, which may further affect these components and promote the modification in the quantity of sterols during estimation. It was also indicated that some reactions influenced by oxidative stress during storage or through partial degradation, transformation and isomerisation processes lead to enhancing phytosterols and their conjugates[30,31].

The traditional processing beneficially augments the phytosterols in bamboo shoots, which can be advantageous, as they were clinically proven to constrain cancers in multiple organs and lower LDL-cholesterol, subsequently preventing coronary heart diseases[10,14]. According to WHO, the raised level of cholesterol is the foremost cause of disease burden in both developed and developing nations as a threat issue of ischemic heart disease and stroke. It is estimated to cause 2.6 million casualties and 29.7 million DALYs (disability adjusting life year). A reduction in serum cholesterol by 10% in men aged 40 and 70 leads to a reduction in heart ailments by 50 and 20%, respectively, in 5 years[32]. The consumption of phytosterols, according to the recommended intake of 1.5–3 g/day, allows a 30–40% reduction of cholesterol absorption from the gut region, which results in the decline of 7–12% of plasma LDL-cholesterol[33-35]. The cholesterol-reducing effect of phytosterols occurs through two main mechanisms, which involve, i) their competition with cholesterol molecules at the time of integration into micelles in the gastrointestinal tract and their co-crystallisation along with cholesterol, ensuing an increase in cholesterol excretion through defecation[33]; ii) the

competition among phytosterols and cholesterol for uptake by intestinal transporters (SR-BI and NPC1L1) and a biliary secretion due to an apical efflux of phytosterols from the enterocytes through ABCG5/G8[36].

Through multiple trials, it was also evident that supplementation of individual sterols, i.e. stigmasterol (23 mg/day), β-sitosterol (0.2%) or a mixture of phytosterols (β-sitosterol - 56%; campesterol - 28%; stigmasterol - 10%; dihydrobrassicasterol - 6%) in daily diet upto certain period can lower ovarian and colon cancers[37-38]. Moreover, β-sitosterol imparts multifarious activities such as cell apoptosis, angiogenic, non-genotoxic or non-cytotoxic, anthelminthic, anti-mutagenic, immunomodulatory, anti-oxidant, antidiabetic and neuroprotective properties[10,39]. Recently, the anti-osteoarthritic properties of stigmasterol and the significant effect of phytosterol supplementation on improving maternal and neonatal problems on gastrointestinal diabetes mellitus were also unveiled[40,41]. Therefore, incorporating plant sterols into nutraceutical formulations and diet has been markedly increased in the past decade[42,43]. The use of phytosterols as a cholesterol-lowering food was approved by Health Canada in 2010[44]. Canadian Cardiovascular Society dyslipidemia guidelines 2012 suggested considering phytosterols as a component of a broader cholesterol-lowering strategy[45]. Moreover, phytosterols provide steroidal intermediates and precursors for producing many hormone pharmaceuticals. They are useful emulsifiers for cosmetic manufacturers and inhibit oxidative deterioration of oils, which anti-polymerise the frying oils[9].

Conclusion

The present study revealed the profusion of phytosterols in fresh bamboo shoots with the predominance of β-sitosterol and moderate occurrence of campesterol and stigmasterol. With processing, the level of total phytosterols was continually enhanced with increasing boiling, soaking and fermentation durations. The free sterols also increased after all the processes, where fermentation showed a maximum increase in total and free phytosterol content. The high phytosterol profile in all shoots can provide better health by directly adding to the diet or extracting them to formulate various supplements. The extracted phytosterols can be incorporated as adjuncts in drugs and encapsulated assimilations, which may encourage a more pragmatic and economically worthwhile approach for nutraceuticals and pharmaceutical industries.

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