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Evaluation of underutilized fern *Cyathea gigantea* from Northeast India for its potentiality in food processing

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Cyathea gigantea is a species of tree fern found in the North-Eastern part of India in Arunachal Pradesh. Pith of *Cyathea gigantea* fern is a useful but underutilized part, popularly known as tashe, has traditionally been consumed by the natives of Arunachal Pradesh. From time immemorial, tashe rich in carbohydrates has been consumed as a supplementary food by several tribes like *Solungs, Nyishis, Tagins* and *Adis* when faced under natural calamities like famine and earthquake. Tashe being an important source of healthy diet was studied to determine its physico-chemical characteristics to validate the traditional knowledge about its goodness in consumption. The physical and functional properties selected for evaluation were bulk density, colour and pasting properties and nutritional properties selected for evaluation were the content of moisture, ash, protein, fat, crude fibre, carbohydrate, energy value and antioxidant levels as per the standard procedure. From the study it has been found that moisture content in tashe flour is less offering a longer shelf life. The lower fat per cent of tashe makes it an ideal health food option, particularly for those who are overweight. Tashe flour has a low bulk density of less than 1 g/cm^3, making it ideal for preparation of complementary foods. It has been concluded from the study that tashe flour can be used to replace traditional flours in a number of flour-based products due to its promising properties.

Keywords: Carbohydrate rich, Complementary food, Nutritional properties, Pasting properties, Starch, Underutilized fern

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Cyathea gigantea (Wall. Ex Hook.) belonging to the genus *Alsophila* and family Cyatheaceae, is a species of giant tree fern native to India, Indonesia, Malaysia, Vietnam, Laos, Thailand, Myanmar, Nepal, Sri Lanka and Southeast Asia[1]. It thrives primarily in damp, open places at elevations of 600 to 1000 meters. The family includes the tallest tree ferns in the world, which can grow up to 20 meters tall[2]. This huge fern has five to ten fronds with a crown at the top. Fronds are bipinnate and usually 2 to 3 meters long. After the scales fall, the rachis turns long, darkish and rough in texture. These scales are lustrous, dark brown, and have frail edges and small whiter margin. Including the remains of earlier leaf bases, the tall stem can grow up to 5 meters or more. The scales on the stipe are lustrous and dark brown. The sporangia are abundant, and the sori are spherical, developing a zigzag row sub marginally.

Cyathea gigantea have been found to contain several active constituents such as hentriacontane, β-sitostenone, β-sitostanone, diploterol, sitosterol, hopan-29-ol, and oleanolic acid[3]. Further studies confirmed the existence of alkaloids and glycosides[4]. The starchy trunk pith is consumed, brewed, and also used to make home remedies. The dried pith material is mixed with pulverized cereals or millets for preparation of bread[5]. The starch-rich pith of *Cyathea gigantea*, popularly known as tashe, has traditionally been consumed by the natives of Arunachal Pradesh from a long time[6]. Tashe rich in carbohydrates is used as a major foodstuff for several tribes like Sulungs and a supplementary food for the Nishis, Tagins and Adis. It is largely consumed during April to August when traditional crops are scarce[7]. Its collection is

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important and almost necessary when there is no paddy especially during natural calamity such as famine and earthquake. Tashe has a short shelf-life but can be stored underground up to a month[7].

The northeastern part of India is rich in indigenous plant produce. However, due to local challenges, linguistic barriers, poor infrastructure, and limited connectivity with other parts of India, many traditionally consumed plants with potential as alternatives for market-oriented food products remain unexplored. Although tashe is a promising potential food source, there is no existing literature reporting its antioxidant and nutritional properties. Therefore, this study was conducted to determine its physical characteristics, such as density, specific gravity, and colour, as well as its nutritional properties, including moisture, ash content, protein, fat, crude fibre, carbohydrate, and antioxidant activity.

Materials and Methods

Collection of *Cyathea gigantea* and extraction of starch rich tashe flour

The fern was obtained from forest of Nyapin, Kurung Kumey district, Arunachal Pradesh, India as shown in (Fig. 1a & Fig. 1b). The fern was then cut into blocks of 5-6 ft length followed by removing the hard bark from the fern blocks as shown in (Fig. 1c & Fig. 1d). The blocks of the fern obtained were further pounded to remove the starch granules encapsulated within the fibre as depicted in (Fig. 1e & Fig. 1f). The crushed fibre containing the starch granules is then washed in a big container so as to let the starch gets along with the water easily as shown in (Fig. 1g). The filtered water is then drawn out to obtain the starch settled down at the bottom of the container as depicted in (Fig. 1h). The settled starch obtained, locally known as tashe, is then weighed followed by drying in a mechanical dryer at a temperature of 55°C for 6 h. The dried tashe flour obtained was then sealed in airtight plastic bags (clear low-density polyethylene) and stored in freezer for further evaluation of its functional and physico-chemical properties.

Analyses of physical and functional properties of tashe flour

Physical and functional properties, *i.e.,* bulk density, colour and pasting properties were determined as per the standard procedure. All analyses were carried out in triplicates on moisture free basis.

Bulk density

Tashe samples were loaded in a standardized measuring cylinder of 25 mL and filled up to 5 mL by continuous pressing unless any volume changes occur. The measuring cylinder with and without contents, were weighed, and the bulk density of the sample was estimated using the difference in weight. The bulk density is measured in g of flour/cubic cm[8].

Colour

Colour was determined using colorimeter (Hunter Lab). The colorimeter was calibrated with a standard white ceramic plate before use. The colour of tashe flour is measured using the Hunter L*, a*, b* scale where the degree of brightness to darkness is represented by the L* value, the degree of redness (+) to greenness (–) is

Fig. 1 — (a) *Cyathea gigantea* (tashe) fern, (b) cutting and cleaning of the fern, (c) cutting into small blocks, (d) peeling of hard bark, (e) & (f) pounding of fern blocks, (g) washing and allowing to settle, (h) draining of the water to obtain the tashe

represented by a* value, and the degree of yellowness (+) to blueness (–) is represented by b* value. Additionally, from the values of L, a, and b, the chroma (C), hue angle (α), and total colour change (ΔE) was determined (3) based on equation (1) to (3)[9].

$$C = \sqrt{a^2 + b^2} \quad \dots (1)$$

$$\alpha = tan^{-1}\left(\frac{b}{a}\right) \quad \dots (2)$$

$$\Delta E = \sqrt{(L_{bd} - L_{ad})^2 + (a_{bd} - a_{ad})^2 + (b_{bd} - b_{ad})^2} \quad \dots (3)$$

where, L_{bd}, a_{bd}, b_{bd} = values of L*, a*, b* before drying and

L_{ad}, a_{ad}, b_{ad} = values of L*, a*, b* after drying.

Pasting properties of tashe flour

The pasting properties of tashe flour were tested using RVA Starch Master 2. A measuring cylinder of 50 mL was weighed followed by weighing of 3.50±0.01 g of tashe flour and 25.00±0.05 g of distilled water (DW). The measured DW was transferred to the cannister; subsequently the flour was added to the water and was allowed to disperse using the plastic paddle preventing formation of lumps. The cannister was then loaded into the RVA Starch Master 2 and left to complete the process. The data obtained were then analysed for its pasting temperatures, peak viscosities, and viscosity at 95°C, stability, holding time and setback viscosities[10].

Analyses of nutritional properties of tashe flour

Tashe flour was evaluated for nutritional composition (moisture, ash, protein, fat, crude fibre, carbohydrate, and antioxidant) using the usual approach recommended by AOAC (Association of Official Analytical Chemists)[11]. All of the tests were performed in triplicate on a moisture-free basis.

Moisture content

The Sartorius MA 160 Moisture Analyzer was used to measure the amount of moisture in the tashe flour. To calibrate the moisture analyzer, the disposable aluminium pan is first loaded blank. Following calibration, a 5 g sample is placed in the aluminium pan, and the hood is securely closed to allow the moisture analyzer to operate at 105°C. The moisture analyzer automatically stops after 5 to 10 min of testing and provides the moisture content of tashe flour in percentage[12]. Subsequently, the moisture content was also evaluated using oven drying method recommended by AOAC[11].

Ash content

The percentage of ash in a substance is an indicator of the amount of inorganic residue that is left over after organic matter or mineral content has been destroyed. A crucible containing 5 g of tashe flour was stored in a muffle furnace at 450°C for 4 h. After 4 h the sample is taken out and place in a desiccator to cool. After cooling the weight of the crucible containing ash was determined. The ash percentage of tashe was determined using equation (4).

$$Ash\ Content\ (\%) = \frac{Wt.of\ ash\ (g)}{Wt.of\ sample\ (g)} \times 100 \quad \dots (4)$$

Protein content

The protein content was estimated using Kjeldhal method. In this method 0.5 g tashe flour was heated with 20 mL of sulphuric acid (H_2SO_4 of 98% concentration) for 115 min in speed digester (BUCHI K-439), which decomposes the organic substance of the tashe flour. To increase the boiling temperature of the solution from 337-373°C, 0.02 g $CuSO_4$ and 4.98 g K_2SO_4 were added during this stage. The chemical decomposition of the sample is completed when the dark coloured medium turns clear and colourless.

The solution was then distilled in kjelflex (BUCHI K-360) with 320 g NaOH in 1000 mL DW for 4 min, leading to conversion of ammonium salt to ammonia. Back titration is used to determine the concentration of ammonia, and consequently the nitrogen content (N) in the sample. The condenser's end is immersed in a boric acid solution (32 g H_3BO_3 in 800 mL DW) with a pH of 4.65. The ammonia reacts with the acid, and the remaining acid is titrated in titrino plus (BUCHI) with a 0.1 M HCl solution. The percent nitrogen (N) was calculated with titre value using equation (5).

$$N = \frac{[Sample\ titre\ (mL) - Blank\ titre\ (mL)] \times Normality\ of\ HCl \times 14 \times 100}{Weight\ of\ sample\ (g) \times 1000} \quad \dots (5)$$

The protein percentage was calculated using a conversion ratio of 6.25, as given in equation (6).

$$Protein\ (\%) = N\ (\%) \times 6.25 \quad \dots (6)$$

Fat content

Fat content is estimated in dried and powdered tashe flour by solvent extraction using a soxhlet apparatus. Moisture free tashe flour of 10 g was loaded inside a moisture free thimble and closed tightly with cotton, which is then loaded into the main chamber of the soxhlet extractor. The soxhlet extractor is then inserted into a flask containing n-hexane as the extraction solvent covering nearly half of the round-bottom flask. The heating mantle temperature is set in such a way that at least 100-150 drops/min. of solvents flow through the thimble. The unit was then operated for 6 to 8 h, for complete extraction of fat from the sample. The solvent was evaporated after extraction to obtain fat using a rotary evaporator, which involves placing the flask containing the solvent in a water bath at 70°C. The obtained fat was then weighed along with the moisture free flask, and the weight of fat recovered was obtained. Percent fat is determined by using equation (7).

$$Fat\ Content\ (\%) = \frac{Wt.of\ fat\ recovered\ (g)}{Wt.of\ sample\ (g)} \times 100 \qquad \ldots(7)$$

Crude fibre

Crude fibre was determined using the standard approach[13]. It is important to note that crude fibre is not synonymous with total dietary fibre, which is the more appropriate measure for human nutrition analysis. Fat free tashe flour was hydrolyzed with H_2SO_4 solution (0.255 N) and NaOH solution (0.313 N) to estimate crude fibre. 200 mL H_2SO_4 (0.255 N) was added in a 500 mL conical flask containing 2.5 g of defatted moisture free sample and refluxed for 1 h. The sample was then filtered through muslin fabric followed by washing with boiling water so that the samples were no longer acidic. Subsequently, 200 mL NaOH (0.313 N) was added in a 500 mL conical flask containing the filtered sample and again refluxed for 1 h. The mixture obtained is filtered through muslin cloth again followed by washing with 25 mL of boiling H_2SO_4 (1.25%), water (50 mL) and alcohol (25 mL) successively. The residue obtained was then dried at 130°C for 2 h and weighed with ashing dish (W_1). The residue was then ignited at 600°C for 30 min and then allowed to cool in a dessicator thereafter taking the weight of the ash with ashing dish (W_2). The percentage of crude fibre is calculated by using the equation (8).

$$Crude\ fibre\ (\%) = \frac{W_1 - W_2}{Wt.of\ sample\ (g)} \times 100 \qquad \ldots(8)$$

Where, W_1 = Weight of residue after drying

W_2 = Weight of residue after ignition

Total carbohydrates

The carbohydrate content was determined using Anthrone's method[13]. HCl (5 mL) of 2.5 N was added to a boiling tube containing 100 mg of tashe flour and hydrolyzed for 3 h in a boiling water bath. After the process completes the boiling tube containing the sample is allowed to cool at room temperature followed by neutralizing it with solid Na_2CO_3 till the effervescence ceases. It was then made up to 100 mL volume by adding DW and centrifuged. The supernatant was then collected from which 0.5 mL and 1 mL aliquots were taken for tests. The standards were prepared by taking 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard. Volume of all the standards in the tubes including the sample tubes were made up to 1 mL by adding DW followed by addition of 4 mL anthrone reagent and heating all the tubes in a boiling water bath for 8 min followed by cooling. After cooling, the tubes were measured for green to dark green absorbance at 630 nm. The concentration of the standard on the X-axis was plotted against absorbance on the Y-axis to create a standard graph. Based on the final graph, the amount of carbohydrates in the sample tube was determined.

$$Carbohydrate\ content\ in\ 100\ mg\ sample = \frac{mg\ of\ glucose}{Volume\ of\ test\ sample} \times 100 \qquad \ldots(9)$$

Energy value

The energy value (Kcal/100 g) of the tashe flour was estimated using the Atwater factor method[14] and was calculated using the equation (10).

$$Energy\ Value = (4 \times Protein\ \%) + (9 \times Fat\ \%) + (4 \times Carbohydrate\ \%) \qquad \ldots(10)$$

Antioxidant test (Determination through DPPH activity)

Tashe flour's antioxidant activity was determined using the DPPH assay. After dissolving 4.3 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) in 6.6 mL of methanol, the test tube was covered with aluminium foil to prevent light. Absorbance was immediately measured at 517 nm after 150 µL of DPPH solution was added to 3 mL of methanol for the control reading. 150 µL of DPPH solution was added after 3 mL of methanol was used to dilute test samples at various concentrations (50, 100, 150, 200, and 250 g/mL). Using methanol as a blank, the absorbance of each sample was measured in a UV-visible spectrophotometer at 517 nm after 15 min. The DPPH free radical antioxidant activity was determined as shown in equation (11).

$$Antioxidant\ activity\ (\%) = \frac{A_o - A_s}{A_o} \times 100 \quad \dots (11)$$

Where, A_o = absorbance of control blank (nm), and A_s = absorbance of sample extract (nm).

Results and Discussion

Physical properties

The physical properties of tashe flour including bulk density and colour (L*, a*, b*, hue angle and chroma) are shown in Table 1. The bulk density of food products influences its porosity. Tashe flour has a bulk density of 0.40±0.02. Flours with a bulk density of less than 1 g/cm^3 can be used for preparation of complementary and healthy foods. Furthermore, the low volume of packing material required for storing the flour, makes storage, marketing and transportation easier[15].

In comparison, studies on composite flours from wheat-breadfruit-cassava starch have reported bulk densities ranging from 0.82 to 0.85 g/cubic cm[16], indicating that tashe flour has a relatively lower bulk density. This lower bulk density suggests that tashe flour could be advantageous in applications where reduced dietary bulk is desired, such as in weaning foods.

Pasting properties

The pasting profile of tashe flour is shown in (Table 2 and Fig. 2). Tashe flour achieves pasting at a temperature of 70.57±0.16°C, indicating the minimum temperature required for cooking. This temperature is lower compared to other flours, such as cocoyam flour (87.33-92.53°C)[17], making tashe flour more energy efficient and time saving during food preparation. Additionally, (Fig. 2) displays the amylograph, emphasizing key characteristics such as peak viscosity (7038 cP) and final viscosity (4488 cP), which suggest the flour's potential for producing consistent and stable food textures. Pasting temperature is one of the pasting qualities that determine the minimum amount of temperature required for cooking, as well as the cost involved for energy consumption and stability of food nutrients[18]. The findings confirmed that due to its lower pasting temperature, tashe flour cooks more quickly, consuming less energy and thereby saving both time and money.

Nutrtional properties

The nutritional properties (moisture content, ash content, protein content, fat content, crude fibre, carbohydrate and energy value) and antioxidant levels of tashe flour are shown in Table 3. At room temperature, flour holding less than 14% moisture can resist microbial growth ensuring storage stability[19]. Tashe flour has a moisture level of 9.35±0.81%, which falls within limit accepted for further processing without the risk of microorganism contamination. When the initial moisture content of tashe (45.66%) and dried tashe flour (9.35%) are

Table 1 — Physical characteristics of tashe flour

Parameter		Value
Bulk Density (g/cm^3)		0.40±0.02
	L*	79.86±0.31
	a*	3.77±0.23
Colour	b*	23.45±0.13
	Chroma (C)	80.87±0.27
	Hue angle (α)	23.75±0.01

Values are mean ± standard deviation from triplicate analyses

Table 2 — Pasting properties of tashe flour

Pasting characteristics	Tashe flour
Pasting temperature	70.57±0.16
Peak viscosity (cP)	7038±0.53
Hold viscosity (cP)	3400±0.28
Final viscosity (cP)	4488±0.08
Breakdown (cP)	3638±0.21
Setback 1 (cP)	1088±0.35

Values are mean ± standard deviation from triplicate analyses

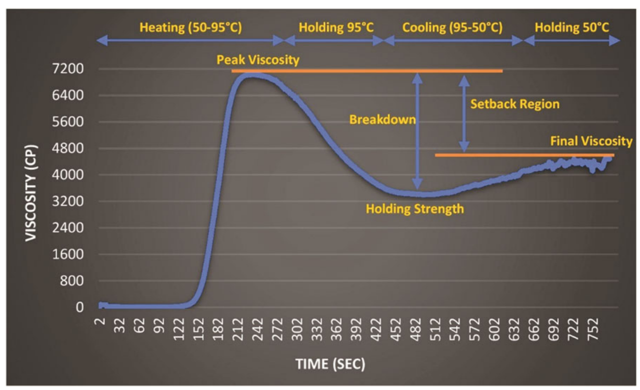

Fig. 2 — Amylograph pasting characteristics of tashe flour

Table 3 — Nutritional composition of tashe flour

Parameter	Value
Moisture content (% db)	9.35±0.81
Ash content (%)	1.79±0.12
Protein content (%)	2.32±0.25
Fat content (%)	0.83±0.01
Crude fibre (%)	0.40±0.29
Carbohydrate (%)	85.20±0.16
Energy, Kcal/100 g	357.55
Antioxidant levels (% Scavenging activity)	32.51±0.12

Values are mean ± standard deviation from triplicate analyses compared, it is clear that oven drying of the samples proved to be effective in reducing the moisture content of the flour ensuring longer shelf life.

The inorganic mineral content of flour samples is represented by the ash content. Tashe flour has an ash content of 1.79±0.12%. Protein is an essential macronutrient that also serves as a functional element in food recipes. Tashe flour has reported to contain 2.32±0.25% protein content, which is relatively low compared to other flours. For instance, sweet potato flours have been reported to have protein contents ranging from 3.40 to 8.60 g/100 g dry weight[20]. The lower protein content in tashe flour suggests it may be more suitable for applications where lower protein levels are desired or can be complemented with other protein sources in food formulations.

Fat content of tashe flour is found to be 0.83±0.01%. The low-fat content observed in this study helps to conclude tashe flour as desirable as it reduces the possibility of oxidation and thus prevents the formation of unpleasant odors caused by rancidity. In comparison, Maya nut flour has been reported to have higher fat content, which could lead to quicker rancidity[21]. Therefore, the lower fat content in tashe flour could be considered a beneficial attribute for longer shelf life and suitability in low-fat dietary applications. Furthermore, the flour's low-fat level makes it a good choice for health-conscious people as well as people who are overweight and wish to cut down on their calorie and fat intake.

The crude fibre content of tashe flour was found to be 0.40±0.29%. As crude fibre was estimated rather than total dietary fibre, it is important to note that the carbohydrate content of 85.20±0.16%, includes fibre fractions, potentially leading to a slight overestimation of the energy value (357.55 Kcal/100 g). Future studies should incorporate total dietary fibre estimation to provide a more accurate nutritional profile.

Antioxidant properties

Antioxidants level of tashe flour is found to be 32.51±0.12%. Antioxidants play a vital role in neutralizing free radicals, thereby contributing to health benefits such as reduced risk of chronic diseases. The antioxidant activity in tashe flour suggests its potential as a functional food ingredient with health-promoting properties.

Studies on other flours, such as those from sweet potatoes, have reported varying antioxidant activities depending on the variety and processing methods[20]. The antioxidant activity in tashe flour indicates its potential contribution to health benefits, aligning with findings in other studies that highlight the importance of phenolic compounds in antioxidant properties.

Implications and study limitations

The unique physical and nutritional properties of tashe flour, including its low bulk density, suitable pasting temperature, low moisture and fat contents, high carbohydrate content, and significant antioxidant levels, suggest its potential as a valuable ingredient in health foods. These attributes make it particularly suitable for developing weaning foods and products aimed at health-conscious consumers.

However, this study is limited by the exclusive use of oven drying as the dehydration method. Different drying techniques can significantly influence the structural and functional properties of flours. For instance, freeze-drying has been shown to affect the microstructure and functional properties of banana flours[22]. Similarly, the choice of drying method can impact the physicochemical properties and antioxidant activity of flours[23]. Future research should explore the effects of various drying methods on tashe flour to provide a more comprehensive understanding of its properties and potential applications.

Conclusion

Tashe is traditionally consumed by native tribes in Arunachal Pradesh and is also marketed in its raw form; however, there is only a limited literature reporting its traditional consumption and nearly no study evaluating its nutritional composition. In this present work, from the preliminary analysis of starch-rich pith of *Cyathea gigantea* locally called tashe in Arunachal Pradesh, it was found that tashe is a good source of carbohydrates and has a great potential to be used in food processing industry for preparation of value-added food products and can be used as a substitute of starches obtained from other sources to meet domestic demands. Tashe flour has a low moisture content, which ensures a long shelf life. Its low-fat level also decreases the risk of oxidation and the formation of off-flavors caused by rancidity. This flour's low-fat level makes it an excellent health food option, especially for people who are overweight. Bulk density of less than 1 g/cm^3 makes tashe flour quite suitable for preparation of complementary foods. Because of these favourable characteristics, tashe flour can be used to replace typical flours in the creation of a variety of flour-based products. Further research work can be carried out to document the effect of different drying techniques for long term storage and utilization in order to contribute to nation's targeted growth rate since tashe is rich in starch and starch has been identified as one of the potential products of food processing industry.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

DS: Conceptualization, Validation, Investigation, Methodology, Writing - review & editing; PKN: Supervision, Formal analysis; AT: Supervision, Conceptualization, Validation, Investigation; RK: Supervision, Formal analysis; FAP: Writing - review & editing, Formal analysis; GB: Data curation, Validation, Formal analysis.

Informed Consent

All individuals whose images are included in this manuscript have provided their consent for publication. The authors affirm that written consent was obtained prior to the inclusion of these images.

Data Availability

Data will be made available by the corresponding author upon reasonable request.

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