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Effect of Cultivation and Drying Methods on the Drying Efficiency and Nutritive Composition of Azolla Species

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ABSTRACT

Background: Azolla, a sustainable and nutrient-dense biomass, has significant potential for human nutrition and as a functional ingredient in seafood products. This study aimed to compare biomass yield, nutrient composition and drying efficiency between two species, *Azolla pinnata* and *Azolla caroliniana.*

Methods: Both Azolla species were cultivated under controlled aquatic conditions. The harvested biomass was processed using three drying methods: sun drying, cabinet tray drying and hot-air oven drying (40-45°C for 4-6 hours). Drying efficiency was evaluated based on nutrient retention, rehydration capacity and product stability. Culture medium parameters such as dissolved ammonia and total hardness were monitored for correlation with biomass yield and protein content. Proximate composition analysis was conducted on dried samples.

Result: Cabinet tray drying showed the highest nutrient retention and rehydration capacity among the drying methods tested. Improved biomass yield and protein content corresponded with reduced dissolved ammonia and total hardness in the culture medium. On a dry-weight basis, proximate composition ranged as moisture 4.70-5.50%, ash 16.53-17.85%, protein 22.05-22.99%, fat 4.19-4.72%, carbohydrates 49.54-51.72% and fiber 13.53-18.07%. Azolla pinnata exhibited higher nutrient density compared to *A. caroliniana*. Overall, cabinet tray-dried *A. pinnata* was identified as a promising functional ingredient for enhancing the nutritional profile of seafood formulations.

Key words: *Azolla caroliniana,* Azolla cultivation, *Azolla pinnata*, Drying, Nutritional composition, Rehydration.

INTRODUCTION

Azolla, one of the fastest-growing aquatic plants globally, has garnered significant attention as a sustainable, nutrient-dense biomass with expanding applications in functional foods, animal feed and sustainable agricultural systems (Chandrababu and Parvathy, 2025). Azolla is a genus of free-floating freshwater ferns in the family Salviniaceae, commonly called water fern, mosquito fern, duckweed fern, or fairy moss and is found in temperate and subtropical regions across the world. This fern can multiply rapidly, increasing its biomass several thousand-fold within a month, which makes it ideal for fast biomass production (Zhang *et al*., 2025). Azolla's nutritional richness is its symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena azollae*, which resides within foliar cavities. This cyanobiont fixes atmospheric nitrogen and carries out photosynthesis, supplying essential nitrogen and carbon resources to the host plant, thereby substantially enhancing Azolla's protein content and growth rate. Conversely, Azolla benefits from a protected habitat and a stable carbon supply. This mutualism has earned *Azolla* the epithet of a "green gold mine" because of its dual role: as a biofertilizer improving soil fertility and suppressing weeds in rice paddies (Wagner, 1997).

Azolla offers a broad nutritional profile inclusive of high-quality protein, dietary fiber, essential macro- and micronutrients (such as calcium, iron, potassium), vitamins and bioactive carotenoids. Nonetheless, advancements in processing technologies have enabled the conversion of *Azolla* biomass into powders, extracts and fortified ingredients suitable for diverse food products such as soups, stews, baked goods and protein-rich snacks. Its rich amino acid profile, combined with potent antioxidant activity supports its role as a promising health-promoting ingredient in functional diets aimed at combating malnutrition and chronic diseases (Thakur and Mehta, 2025). Ecologically resilient, Azolla flourishes in varied freshwater habitats, including paddy fields, irrigation canals, lakes and stagnant, slow-moving waters. It reproduces predominantly via vegetative propagation, allowing rapid population growth under favorable conditions (Singh and Patel, 2025). Environmental stresses, such as high salinity or temperature fluctuations, can induce sexual

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reproduction, leading to morphological changes like elongated fronds, particularly observed in species such as *A. nilotica*. However, saline stress notably impairs the photosynthetic activity and nitrogen-fixing efficiency of its cyanobiont, which may detrimentally affect biomass productivity and nutritional quality (Fernandez *et al*., 2025). Understanding these stress-induced physiological and biochemical dynamics is critical for optimizing *Azolla* cultivation under diverse agro-ecological settings.

This study specifically investigates *Azolla pinnata* and *Azolla caroliniana*, which are among the most widely cultivated species in South Asia due to their adaptability and high biomass yield (Kumar *et al*., 2025). Cultivated under controlled aquatic conditions with potable water, this research evaluates their growth performance, nutrient composition and responses to various drying methods. The drying efficiency directly impacts the retention of primary nutrients and bioactive compounds, crucial for ensuring the stability and functionality of Azolla powders intended as ingredients in seafood-based and other food formulations. The findings should add to the body of knowledge on how *Azolla* species can be used as nutrient-enriched, sustainable functional components in the expanding field of plant-based nutrition and alternative protein sources to support worldwide sustainability and food security objectives (Rajendran *et al*., 2025).

MATERIALS AND METHODS

Time and place

The study was conducted from January to March, 2025 in Department of Food Science and Technology at Kerala University of Fisheries and Ocean Studies, Kochi.

Experimental site and tank specifications

The study was conducted from January to March 2025 at the Department of Food Science and Technology, Kerala University of Fisheries and Ocean Studies, Kochi. The Azolla cultivation tank was constructed using high-density polyethylene (HDPE) sheets, reinforced with supporting stones to maintain the tank's shape and ensure waterproofing. The tank measured 2 metres in length, 1 metre in width and 0.5 metres in height. For optimal Azolla growth, water was filled to a depth of 25 cm. The tank was installed in Panangad, Kochi (Latitude: 9.895994°N; Longitude: 76.326094°E), at an elevation of 9 metres above mean sea level. The cultivation site experiences ambient temperatures ranging from 24°C to 33°C, with relative humidity of 80-86% and annual rainfall between 2,882 and 3,015 mm. Two species of Azolla, *Azolla pinnata* and *Azolla caroliniana*, were used for the study.

Collection of samples

Fresh samples of Azolla pinnata, characterized by oval to spherical-shaped leaflets measuring 0.7-1.22 mm and Azolla caroliniana, identified by clustered or rhomboidal-shaped leaflets measuring 0.4-0.7 mm, were obtained from Kerala Agricultural University, Mannuthy, Kerala, India. The samples were thoroughly washed to remove attached debris and stored in polyethylene pouches under refrigerated conditions at 4°C until use.

Water quality testing parameters

Water quality was analyzed before Azolla cultivation to ensure suitability and minimize the risk of harmful heavy metal accumulation. Potable water was used for culture preparation. The water quality parameters measured included pH, hardness, salinity, alkalinity, nitrate and ammonia. Ammonia concentration was determined by the phenol-hypochlorite method as described by Standard Methods (APHA, 2017), which involves reacting 25 mL of water with 1 mL phenol alcohol, 1 mL sodium nitroprusside and 2.5 mL oxidizing agent, followed by incubation for 1 hour and absorbance measurement at 620 nm using a UV spectrophotometer. Nitrite content was measured colorimetrically following the method of Miranda *et al*. (2001), by reacting 25 mL water sample with 0.5 mL sulfanilamide (4 min reaction), then 0.5 mL N-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) (10 min reaction) and measuring absorbance at 540 nm according to standard methods 4500-H+B (EPA, 1994). Salinity was determined by Mohr's titration method, titrating 25 mL of sample against silver nitrate solution using potassium chromate as an indicator; the endpoint corresponds to a color change from wine red to violet (Edmunds, 1992). Total hardness was assessed by titration of 25 mL sample buffered at pH 8.5 with ammonium chloride buffer against 0.01 N EDTA using Eriochrome Black T as the indicator, with the endpoint marked by a color change from wine red to blue. Alkalinity was measured by titrating 25 mL of sample against 0.02 N sulfuric acid in the presence of phenolphthalein indicator; the endpoint is noted by a change from pink to colorless (Tiwari and Mishra, 2024). Water pH was recorded using a calibrated pH meter according to standard methods 4500-H+B (EPA, 1994).

Cultivation and propagation of azolla species

Azolla is predominantly propagated through vegetative means (Adzman *et al*., 2022). *Azolla pinnata* and *Azolla caroliniana* were cultured separately in tanks. An initial inoculum of 150 g of each species was introduced into the respective tanks and cow dung slurry was applied to the water surface during the first two days of cultivation. Light intensity under field conditions reached a maximum of 50,000 lux. Partial shading using palm leaves was provided to protect Azolla from direct sunlight and prevent heat stress. Azolla was harvested after 8–10 days of cultivation, which is the typical interval for optimal biomass yield (Csambalik *et al*., 2023). The yield of Azolla during the culture period was calculated as:

$$\text{Yield of azolla (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

W_1 = Initial weight of Azolla used for culture.
W_2 = Final weight of Azolla after harvesting.

Drying methods

Sample preparation

Fresh cultures of *Azolla caroliniana* and *Azolla pinnata* had been harvested and completely cleaned to get rid of dust with lukewarm distilled water, debris and visible organisms. The cleaned samples were air-dried for 15-30 minutes to eliminate excess surface moisture. For each replicate, 200 g of fresh biomass was accurately weighed using a digital balance. Samples were labelled with the species name, drying method and replicate number before further processing.

Sun drying

For sun-drying, 200 g of *Azolla* biomass was placed on a tray lined with filter paper and positioned in an open area receiving direct sunlight throughout the day. Ambient temerature and relative humidity were recorded at the start of drying and at hourly intervals. Samples were weighed hourly to monitor moisture loss and drying was continued until a constant weight was obtained, indicating complete dehydration.

Cabinet tray drying

The cabinet tray dryer was preheated to the desired temperature (40-50°C). A 200 g portion of *Azolla* biomass was spread evenly on a tray and placed inside the dryer. The operating temperature and airflow were recorded at the start of the process. Samples were weighed at 30-minute intervals to monitor moisture loss and drying was continued until a constant weight was achieved.

Hot air oven drying

The hot-air oven had been preheated to 60°C. A 200 g portion of *Azolla* biomass was spread evenly on a tray and placed inside the oven. The operating temperature was recorded at the start of the process. Samples were weighed at 30-minute intervals to monitor moisture loss and drying was continued until a constant weight was obtained.

Optimization

Drying experiments for the cabinet tray dryer and hot-air oven were repeated at varying temperatures and drying durations to identify optimal conditions. For the cabinet tray dryer, temperatures were tested in 5°C increments, with variations in airflow. For the hot-air oven, temperatures were tested in 5-10°C increments. For each treatment, drying time and final dry weight were recorded. Optimal conditions were determined based on the combination of temperature and time that yielded the highest quality biomass, as evaluated by color, texture and subsequent nutrient analysis.

Proximate analysis

The Association of Official Analytical Chemists' official methods were used to determine the dried Azolla samples' proximate analysis (AOAC, 2023).

Determination of moisture content

A pre-dried Petri dish was filled with around 1.5 g of air-dried sample, which was then heated to 105±1°C for 4 hrs. After cooling in a desiccator, the dish was weighed. At 30-minute intervals, drying, cooling and weighing were repeated until the difference between two subsequent weights was less than 1 mg; the lowest recorded weight was considered the final.

Calculations

$$\text{Moisture, \% by mass} = \frac{(W_1 - W_2)}{(W_1 - W)} \times 100$$

Where,
W_1 = Weight, in g, of the dish with material before drying.
W_2 = Weight, in g, of the dish with material after drying.
W = Weight, in g, of empty dish.

Determination of total ash content

A sample weighing around 2 g was placed in a silica crucible that had been pre-ignited and burned for 4 hours at 500°C in a muffle furnace until white ash was produced. A desiccator was used to cool the crucible before it was weighed.
Calculations:

$$\text{Total ash, \% by mass} = \frac{(W_2 - W_1)}{W} \times 100$$

Where,
W_1 = Weight, in g, of the empty crucible.
W_2 = Weight, in g, of the empty crucible with ash.
W = Weight, in g, of the test sample.

Determination of crude fat

Soxhlet extraction was used to calculate the crude fat. Two grams of ground, dried sample were placed in a thimble lined with cotton and extracted with 150 mL petroleum ether for 16 h using a Soxhlet apparatus. After the solvent was removed using a water bath, the residue-containing flask was dried for 5 hours at 105°C, cooled in a desiccator and weighed.
Calculations:

$$\text{Crude fat, \% by mass} = \frac{(M_1 - M_2)}{M} \times 100$$

Where
M_1 = Mass, in g, of the Soxhlet flask with extracted fat.
M_2 = Mass, in g, of the empty Soxhlet flask.
M = Mass, in g, of the material taken for the test.

Determination of crude protein

The Kjeldahl method was used to quantify the total nitrogen concentration and determine the crude protein content. The crude protein % was determined by multiplying the acquired nitrogen value by a conversion factor of 6.25.

Determination of carbohydrate

The formula outlined in AOAC (2023) was used to determine the carbohydrate content by difference.

Carbohydrate content = 100 - % (Total moisture + Crude protein + Crude fat + Ash)

Determination of crude fiber

The organic residue that remains after a food sample has been processed under conventional conditions using boiled acid and alkali solutions is referred to as crude fiber. The determination was carried out using a Fibro-tron apparatus, an advanced device designed for crude fiber analysis employing these standard treatments. The procedure followed the official AOAC (2023) methodology.

$$\% \text{ Crude fiber} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

Determination of rehydration ratio

Rehydration ratio is an important quality parameter for dehydrated products, indicating their ability to absorb water and regain their original texture. For the dried Azolla leaves, the rehydration ratio ranged between 2 and 5. The analysis was conducted following the standard method described by Mir (2022).

$$\text{Rehydration ratio} = \frac{W}{W_1} \times 100$$

Where,
W = Weight of rehydrated sample.
W1 = Weight of dehydrated sample.

Statistical analysis

The results were presented as mean ± standard deviation (SD) and each analysis was performed in triplicate. The means of the three groups were compared using a completely randomized design (CRD) and $p<0.05$ was considered statistically significant. The General R-based Analysis Platform Empowered by Statistics (GRAPES 1.0.0), created by Kerala Agricultural University in Thrissur, India, was used to analyze the data.

RESULTS AND DISCUSSION

Evaluation of culture water quality

The potable water used for culturing exhibited low concentrations of ammonia (2.254 ppm), nitrite (0.137 ppm), salinity (120 mg/L), hardness (90 mg/L) and alkalinity (24.4 mg/L), with a pH of 7.2. These water quality parameters indicate its suitability for culturing the nitrogen-fixing cyanobacterium Azolla and facilitating its propagation. The findings are consistent with the observations of Mohamed *et al*. (2018), who reported that lower levels of total hardness, alkalinity and salinity provide favorable conditions for the optimal growth and biomass production of Azolla.

Yield of azolla

Azolla is recognized for its rapid reproductive capacity under favorable conditions, which makes it a highly prolific species. This rapid growth and high biomass yield render Azolla a promising candidate for controlled cultivation, particularly for integration into human diets. Starting from an initial biomass of 150 g, *Azolla pinnata* propagated to 450 g, while *Azolla caroliniana* increased to 300 g in 8-10 days, corresponding to yield increases of 200% and 100%, respectively (Fig 1). These findings align with those of Regar *et al*. (2017), who reported a doubling time of approximately 2.16 days for *Azolla pinnata* under conditions of high light intensity (20,000 lux), high humidity, sufficient nitrogen availability and balanced pH levels. Similarly, *Azolla caroliniana* has demonstrated high productivity, with yields reaching up to 173 grams of fresh weight per square meter per day.

Drying of Azolla species

Three drying techniques *viz*., sun drying, hot air oven drying and cabinet tray drying were used to dry two species of Azolla: *Azolla pinnata* and *Azolla caroliniana*. Prolonged exposure to sunlight during sun drying can lead to significant degradation of heat and light sensitive compounds. However, the high temperature in the hot air oven can cause thermal degradation of volatile and polyphenolic compounds, resulting in a loss of functionality and a darker color in the images (Fig 5) of the hot air oven-dried samples compared to the others. Cabinet tray drying, often close to the oven line or as a balanced intermediate, showed the most balanced approach (Fig 2). Visual and

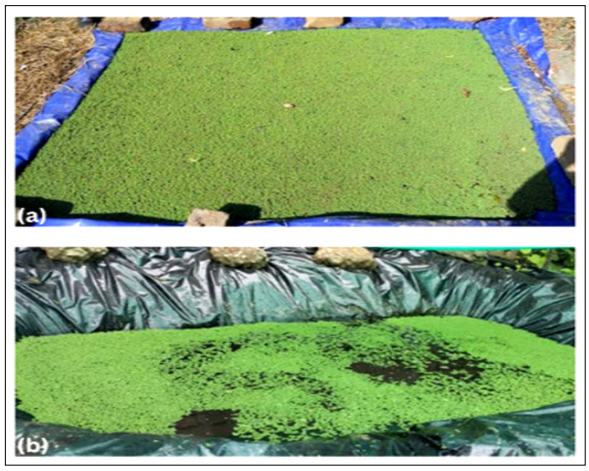


Fig 1: Culture of *Azolla pinnata* (a) and *Azolla caroliniana* (b).

analytical data confirmed that cabinet tray drying resulted in the best combination of efficiency and quality preservation.

Drying time

Sun drying produced the longest dehydration times for both *Azolla pinnata* and *Azolla caroliniana*, typically spanning 10 to 14 hours, depending on ambient weather conditions. In contrast, hot-air oven drying was the quickest method, usually completed within 2 to 4 hours at elevated temperatures of 55-60°C. Cabinet tray drying offered a balanced approach, with intermediate drying durations of 4 to 6 hours maintained at a more moderate temperature of 40-45°C. Drying temperatures between 40°C and 60°C are commonly reported, wherein temperatures higher than 40°C can improve speed but may compromise quality, while lower temperatures preserve quality at the cost of longer drying times (Paryanto *et al*., 2023).

Across all methods, *A. caroliniana* tended to dry slightly slower than *A. pinnata*, likely due to its relatively higher initial moisture content reported or structural differences in its rhomboidal leaflet clusters.

Dry biomass quality

Sun drying caused significant degradation of Azolla biomass, evident through bleaching, nutrient loss and an increased risk of microbial contamination (Fig 3). Prolonged exposure to direct sunlight and fluctuating environmental conditions result in quality deterioration, including greater losses of chlorophyll, vitamins and essential nutrients, alongside increased browning and contamination hazards, as confirmed by Atere *et al*. (2023). Cabinet tray drying consistently produced the highest quality dry biomass among the three methods evaluated. This method preserved color retention, nutrient content and structural integrity most effectively. The dried Azolla maintained a dark green hue, indicating minimal chlorophyll degradation (Fig 4). Studies on leafy vegetables similarly report that cabinet tray drying retains more nutrients, better preserves color and conserves functional properties compared to sun or oven drying (Satwase *et al*., 2013).

Although hot-air oven drying was effective in shortening drying time, it resulted in excessive browning and brittle texture (Fig 5). Elevated temperatures likely caused degradation of heat-sensitive compounds and pigments, leading to nutrient loss and undesirable textural changes. These findings align with previous reports detailing increased browning and reduced carotenoid stability at high drying temperatures (Bi *et al*., 2022). Overall, cabinet tray drying emerged as the most efficient drying method,

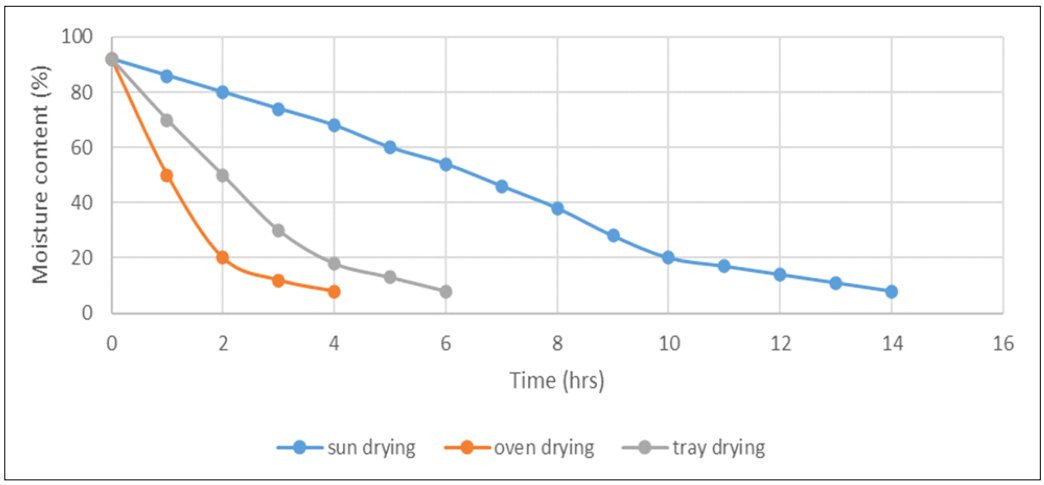


Fig 2: Drying curve of sun, oven and tray dried azolla.



Fig 3: Sun dried *Azolla pinnata* (a) and *Azolla caroliniana* (b).

balancing optimal drying time with superior nutritional and physical quality preservation of Azolla biomass.

Proximate analysis

The proximate composition of *Azolla caroliniana* and *Azolla pinnata* dried using hot-air oven drying, sun drying and cabinet tray drying had been assessed and presented in Table 1 and 2. Across all dehydrated samples, moisture content ranged from 4.7-5.5%, with *A. caroliniana* showing the lowest levels, aligning with observations by Bhaskaran and Kannapan (2015). Among the drying methods, sun-dried samples retained the most moisture (5.3-5.5%), while cabinet tray drying produced the driest samples with moisture ranging from 4.7-4.9%.

Ash content was similar in cabinet tray-dried and oven-dried specimens, ranging from 17.12-17.85%, but notably lower in sun-dried ones (16.53% -16.63%). Significantly more ash was retained in cabinet-tray-dried samples than in oven- or sun-dried ones ($p<0.05$). Protein levels ranged from 22.05 to 22.99%, with the highest levels observed in cabinet tray-dried *A. pinnata*; differences among the six samples were statistically significant ($p<0.05$). Fat content (4.19-4.72%) followed a similar trend, being highest in cabinet tray-dried (4.5-4.72%) and lowest in sun-dried samples (4.4-4.5%).

Carbohydrates were most abundant in sun-dried samples, especially in *A. caroliniana*. Overall, *A. pinnata* demonstrated higher nutritional value than *A. caroliniana*. Crude fiber content varied greatly between species, with cabinet tray-dried *A. pinnata* exhibiting the highest fiber content. High crude fiber levels suggest beneficial bulk-forming effects that support digestive motility and may help prevent colon cancer (Nielsen *et al*., 2010). Recent literature by Nimbalkar and Patil (2024) found that Azolla can contain up to 25.9% crude protein and rich mineral content (Ca 1220 mg/100g, P 704 mg/100 g, K 562 mg/100 g), confirming its advantages for protein supplementation and micronutrient delivery.



Fig 4: Cabinet tray dried *Azolla pinnata* (a) and *Azolla caroliniana* (b).



Fig 5: Hot air oven dried *Azolla pinnata* (a) and *Azolla caroliniana* (b).

Table 1: Nutritional Composition of dried *Azolla caroliniana*.

Nutrients (%)	Sun dried	Hot air oven dried	Cabinet tray dried
Moisture	5.3±0.01[a]	4.9±0.201[b]	4.7±0.04[b]
Ash	16.53±0.02[b]	17.12±0.009[a]	17.34±0.02[a]
protein	22.05±0.02[b]	22.34±0.03[a]	22.48±0.007[a]
Fat	4.4±0.04[a]	4.19±0.8[b]	4.5±0.01[a]
Carbohydrate	51.45±0.02[a]	51.72±0.05[a]	50.12±0.008[b]
Fiber	13.81±0.04[b]	13.53±.0.06[b]	14.11±.01[a]

Values expressed as mean ± standard deviation (n=3).

Values in the same row with the same superscript letter are not statistically significant from each other ($p<0.05$).

Table 2: Nutritional Composition of dried *Azolla pinnata*.

Nutrients (%)	Sun dried	Hot air oven dried	Cabinet tray dried
Moisture	5.5±0.05^{a}	5.1±0.01^{a}	4.9±0.008^{b}
Ash	16.63±0.04^{b}	17.33±0.04^{a}	17.85±0.01^{a}
protein	22.38±0.01^{b}	22.69±0.05^{ab}	22.99±0.02^{a}
Fat	4.5±0.02^{b}	4.68±0.03^{a}	4.72±0.007^{a}
Carbohydrate	50.9±0.05^{a}	50.2±0.02^{a}	49.54±0.04^{b}
Fiber	16.56±0.01^{c}	17.92±0.05^{b}	18.07±0.039^{a}

Values expressed as mean ± standard deviation (n=3).

Values in the same row with the same superscript letter are not statistically significant from each other ($p<0.05$).

Comparatively, cabinet tray drying emerged as the superior method for maintaining nutrient integrity and overall quality. Sun drying, though simple, risks nutrient degradation and discoloration due to prolonged exposure to variable environmental conditions and microbial contamination. Oven drying, while fast, may cause overheating, leading to damaged pigments, browning and brittle textures. In contrast, cabinet tray drying provided controlled temperature and airflow, preserving color and minimizing thermal and oxidative damage.

The slightly longer drying time for *A. caroliniana* may be due to its larger leaflets and higher water-holding capacity affecting moisture release (Sonowal *et al*., 2024). These findings have practical implications: cabinet tray drying is a reliable, efficient method for preserving Azolla's nutritional and functional qualities, supporting its use in animal feed, biofertilizer production and bioremediation.

CONCLUSION

The study clearly identifies cabinet tray drying as the most effective method for dehydrating *Azolla pinnata* and *Azolla caroliniana*, than hot-air oven and sun drying in preserving nutrients. It achieved the highest retention of essential macronutrients, *viz*., carbohydrates, proteins and fats. The resulting Azolla powders showed great promise for use in various processed foods, particularly because *A. pinnata* offers a higher nutritional profile, enhancing its appeal as a functional food ingredient. Beyond extending shelf life, dehydration boosts the extraction efficiency of bioactive compounds by increasing surface area, elevating the value of Azolla as a sustainable, nutrient-rich food supplement to conventional leafy vegetables. Further, should undertake on detailed profiling of vitamins, minerals and antioxidants and assess sensory acceptability and nutrient bioavailability across diverse food systems.

Author contributions

Anokhi Chandrababu K. - Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing - Original draft.

Parvathy U. - Conceptualization; Investigation; Methodology; Project administration; Supervision; Validation; Writing - review and editing.

Meenu B. - Data curation; Formal analysis

Binsi P.K. - Conceptualization; Project administration; Supervision; Validation.

Conflict of interest

The authors declare that they have no conflict of interest.

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