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RESEARCH ARTICLE

# Comparative assessment of oxalic acid levels in fresh, dried and fermented forms of water spinach (*Ipomoea aquatica* Forsk.)

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## Abstract

The current study aimed to comparatively evaluate oxalic acid levels in fresh, dried and fermented forms of water spinach (*Ipomoea aquatica* Forsk.) (var. Kashi Manu). The field trial was conducted at the Experimental Farm of ICAR-IIVR, Varanasi, India, July, 2024 and estimation of oxalic acid was carried out at the Biochemical Laboratory of same research institute. The oxalic acid content was assessed by titrimetric analysis using standardized potassium permanganate ($KMnO_4$) solution. The results showed significant differences among treatments ($p \leq 0.01$). Fresh samples showed the highest oxalic acid concentration (250.24 ± 3.08 g/100 g) whereas, the lowest value was observed in the fermented sample prepared with salt water (FSW; 22.17 ± 0.68 mg/100 g). Drying reduced oxalic acid levels to 107.66 ± 4.86 mg/100 g whereas, fermentation distinctly reduced the concentration in all treatments with reductions of 83-91 % compared to fresh samples. The decline in oxalic acid concentration during fermentation is attributed to enzymatic degradation and microbial decarboxylation processes. This result revealed that traditional fermentation particularly salt water fermentation, serves as an effective approach for reducing anti-nutritional oxalic acid in water spinach, thereby enhancing its nutritional safety and value for human consumption.

**Keywords:** anti-nutritional factor; fermentation; oxalic acid; water spinach

## Introduction

Water spinach (*Ipomoea aquatica* Forsk.), is a herbaceous perennial semi-aquatic important leafy vegetable normally cultivated in subtropical-tropical regions belongs to family Convolvulaceae having chromosome number 2n = 30 (1). This vegetable is a rich source of protein, fibre, vitamins (A, C, K and B9) and minerals (2). This vegetable is a primary ingredient in the cuisine of various countries like Vietnam, Cambodia, Thailand, Laos, the Philippines, Burma, Malaysia and China, mainly in rural areas (3). Despite its high nutritional value, due to the content of oxalic acid may limit its safe consumption, mainly when consumed raw or inadequately processed. Plant foods are crucial elements of a balanced diet, contributing several health benefits and adding essential nutrients and bioactive compounds which help human well-being (4). Still, they also contain naturally occurring anti-nutritional factors (like oxalic acid) that can restrict with nutrient metabolism and absorption (5). These anti-nutrients contain protease inhibitors, phytates, lectins, cyanogenic glycosides and oxalates, many of which are frequently ignored in dietary studies (6). Though oxalate accumulation in most plant foods is normally low but certain botanical families exhibit particularly high levels. These contain Convolvulaceae (water spinach), Araceae (taro, aroid), Amaranthaceae (spinach, amaranth), Oxalidaceae (wood sorrel), Polygonaceae (buckwheat, rhubarb), Portulacaceae (purslane), Aizoaceae (ice-plant), Theaceae (tea) and Malvaceae (cocoa) (7). Additionally, numerous factors affect the oxalate content within the same plant species. These contain agricultural as well as physiological parameters like plant genotype (green tea leaves vs. black tea and diverse taro leaf cultivars), crop harvesting season (tea leaves picked in autumn season compared to those harvested in spring season) and stage of maturity of crop (mature leaves of *Beta vulgaris* containing about 58 % total oxalates whereas, young immature leaves may hold up to 89 %) (8). Oxalic acid and its salts/ oxalates are widespread in the plant kingdom, have been identified in over 215 plant families with many generally consumed crops (9). The contain of oxalic acid can adversely affect the sensory and nutritional values of various edible plants (10). Calcium oxalate (the needle-shaped raphide crystals) is responsible for irritation and acridity in the throat and mouth (11).

Oxalates excreted through urine significantly lead to the development of urinary stones, mostly calcium oxalate calculi (75 to 90 %) (12). Hyperoxaluria is a condition marked by high urinary oxalate which is closely linked with high consumption of high oxalate containing foods (13). It has been studied that dietary oxalate contributes up to 50 % of urinary oxalate excretion and in individuals having high oxalate absorption rates, this proportion may reach 67 % (oxalate binds strongly with calcium in the kidneys to form kidney stone) (14). The worldwide occurrence of kidney stones has

been increasing steadily among all genders and all age groups, associating strongly with consumption of oxalate-rich diets (15). From a nutritional viewpoint, oxalic acid is of concern because extensive consumption mainly in soluble form may lead to adverse health outcomes (16). Soluble oxalates might form complexes with vital minerals, inhibiting the absorption of calcium, iron and magnesium which can result in mineral deficiencies during digestion (17). The anti-nutritional effect of oxalic acid is mainly linked with its ability to chelate essential minerals like calcium, iron, potassium, sodium and magnesium, thereby reducing their bioavailability and absorption in the human body (18).

Subsequently endogenous oxalate synthesis mostly resulting from vitamin C and glyoxylate metabolism cannot be altered by outdoor interference. Reducing dietary oxalate consumption remains the utmost effective means of minimizing urinary oxalate levels (19). Processing techniques like drying, boiling, soaking and fermentation techniques have been shown to significantly reduce oxalate contents in several plant-based foods (20). Knowing the influence of common processing techniques such as fermentation and drying on oxalate reduction is therefore crucial to enhance its nutritional safety and utilization. The present study was conducted to assess the oxalic acid levels in fresh, dried and fermented water spinach and to determine the effect of these processing methods on oxalate reduction. This research will contribute to the growing body of knowledge on safe dietary practices and promote the effective utilization of water spinach as a sustainable and healthful food crop.

## Materials and Methods

### Experimental site and design

The present research was carried out at the Biochemical Laboratory of the ICAR-IIVR, Varanasi, Uttar Pradesh, India, during 2024 in the month of July (the metrological data during investigation are illustrated in Table 1 and Fig. 1). The field trial experiment was conducted in a randomized block design with three replications under upland field condition. The seeds of water spinach variety Kashi Manu were sown directly on the main field without establishment of nursery.

### Sample preparation

#### Fresh sample material

Fresh samples were harvested from the fields (the samples were harvested 30 days after seed sowing) along with stems (approximately 40 cm length). The harvested sample were washed thoroughly with normal tap water at first to remove soil and debris from the sample. After washing with normal tap water, it was again washed with distilled water at last. After removal surface moisture (remained during cleaning), fresh material was taken for immediate analysis.

#### Drying sample material

For the dry treatment samples analysis of oxalic acid accumulation, sample of 1.0 kg fresh material per replication (three replication) was dried in a hot-air oven at 65 °C for 48 hr to get complete moisture removal from the sample.

**Table 1.** Meteorological data table during fermentation period (6th July- 15th July, 2024)

| Dates | Max. Temperature (°C) | Min. Temperature (°C) | Morning Relative Humidity (6:00) % | Evening Relative Humidity (18:00) % | Rainfall (mm) |
|---|---|---|---|---|---|
| 06 Jul 2024 | 25.5 | 35.5 | 90 | 80 | 0 |
| 07 Jul 2024 | 25.2 | 34.4 | 88 | 78 | 0 |
| 08 Jul 2024 | 25.8 | 34.5 | 85 | 75 | 0 |
| 09 Jul 2024 | 28.0 | 38.6 | 92 | 82 | 0 |
| 10 Jul 2024 | 27.6 | 37.8 | 94 | 85 | 0 |
| 11 Jul 2024 | 25.5 | 37.0 | 90 | 80 | 0 |
| 12 Jul 2024 | 25.2 | 32.2 | 88 | 76 | 0 |
| 13 Jul 2024 | 26.4 | 35.0 | 89 | 77 | 0 |
| 14 Jul 2024 | 23.2 | 34.3 | 93 | 83 | 0 |
| 15 Jul 2024 | 26.5 | 36.0 | 90 | 79 | 0 |

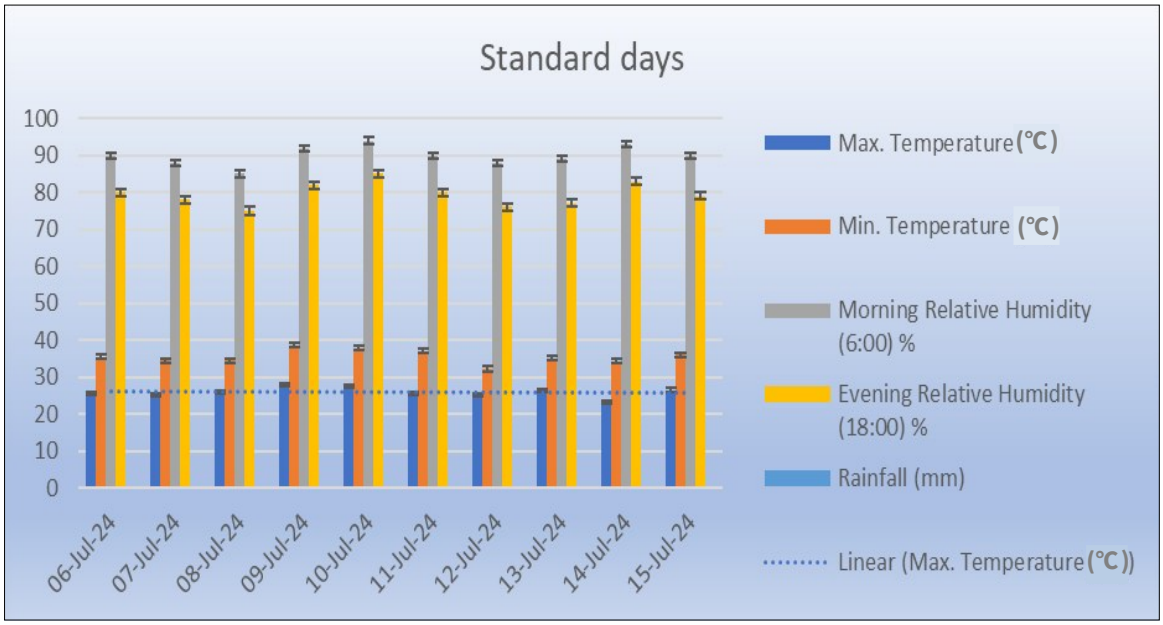


**Fig. 1.** Standard days weather conditions during fermentation period (6th July- 15th July, 2024).

**Fermentation treatments**

Four fermentation treatments were evaluated following a traditional Nepalese method with minor modifications (21). In all treatments, fresh material (1.0 kg per replication) was first wilted in a hot-air oven for 3 hr to reduce surface/moisture content (see treatment-specific temperatures below), mildly crushed, packed tightly into a 1000 mL dry glass bottle and filled with the specified liquid. Bottles were pressed to expel trapped air and left to ferment at ambient laboratory temperature (meteorological data during fermentation period ($6^{th}$ July- $15^{th}$ July, 2024 are shown in Table 1 and Fig. 1) for 10 days. After 10 days completion of fermentation, the fermented samples were taken for oven dry (as same drying procedure followed in dry sample).

**Treatment details**

**T1= Fermented with mustard powder (FMP):** At first the sample was wilted at 65 °C for 3 hr then mildly crushed using mortar and pestle. Just after mild crush mixed with 20 g mustard powder (grinded with mortar and pestle), packed into 1000 mL glass bottle and at last filled to top with distilled water, kept for fermented for 10 days, after 10 days fermentation, it was dried at 105 °C for 24 hr to get complete dry.

**T2= Fermented with boiled water (FBW):** In this treatment technique, wilt at 65 °C for 3 hr (same as FMP) mildly crushed the wilted sample packed into 1000 mL glass bottle, then finally added freshly boiled water (then cooled) and let it for fermentation for 10 days. After 10 days fermentation process the samples were dried same as FMP.

**T3= Fermented with normal water (FNW):** In this treatment all the procedure were followed same as that followed in FMP except added normal tap water for fermentation.

**T4= Fermented with salt water (FSW):** In this technique also followed same procedure that followed in FMP except mixed 20 g common salt in crushed sample then finally added distilled water (it gets full in the sample containing bottle).

After oven drying, the dried fermented samples were crushed with a ceramic mortar and pestle to a fine powder the finally sieved through a fine mesh sieve (to get fine powder). Powders were packed in air tight polyethylene bags and stored at -20 °C until chemical analyses.

## Reagents and standards, sample extraction and estimation of oxalic acid (titrimetric analysis)

For the estimation of oxalic acid content in *Ipomoea aquatica* samples, 0.05 N oxalic acid and 0.05 N $KMnO_4$ solution were prepared as standard reagents for the estimation process (Shown in Fig. 2). The oxalic acid standard was prepared by taking 1.20 g of oxalic acid and made up to 200 mL standard flask using distilled water. The burette was filled up with potassium permanganate solution. Then pipette out 20 mL of standard oxalic acid solution into a clean conical flask and added an equal amount of diluted sulfuric acid and heated the mixture at 60 °C temperature for 10 min. Then titrated against KMnO4 solution for the standardization of $KMnO_4$. The end point of the titration is the appearance of permanent pale pink color (repeated the titration to get concordant value and calculated the normality of $KMnO_4$ solution). For the standardization of free oxalate ion present sample, taken 0.5 g of fresh crushed sample with mortar and pestle (for fresh sample), taken 0.5 g of finely powdered sample (for dry and fermented dry) then transferred to clean beaker (separately for each sample) then added 50 mL of dil. $H_2SO_4$ in it. The contents were boiled for 10 min, cooled and filtered into 100 mL volumetric flask. The final volume was made up to 100 mL by adding distilled water. 10 mL of this solution was pipette out into the other 100 mL standard measuring flask. This aliquot was transferred into a titration flask and added 20 mL of dil. $H_2SO_4$ acid to it and heated the mixture to about 60 °C temperature then titrated it against 0.05 $KMnO_4$ solution taken in a burette (the end point in appearance of permanent pale pink color during titration time). The total oxalic acid content in water spinach sample was calculated by using following formula (22).

$$\text{Normality of oxalic Acid (N1)} = \frac{\text{Weight/Litre}}{\text{Equivalent weight of oxalic acid}}$$

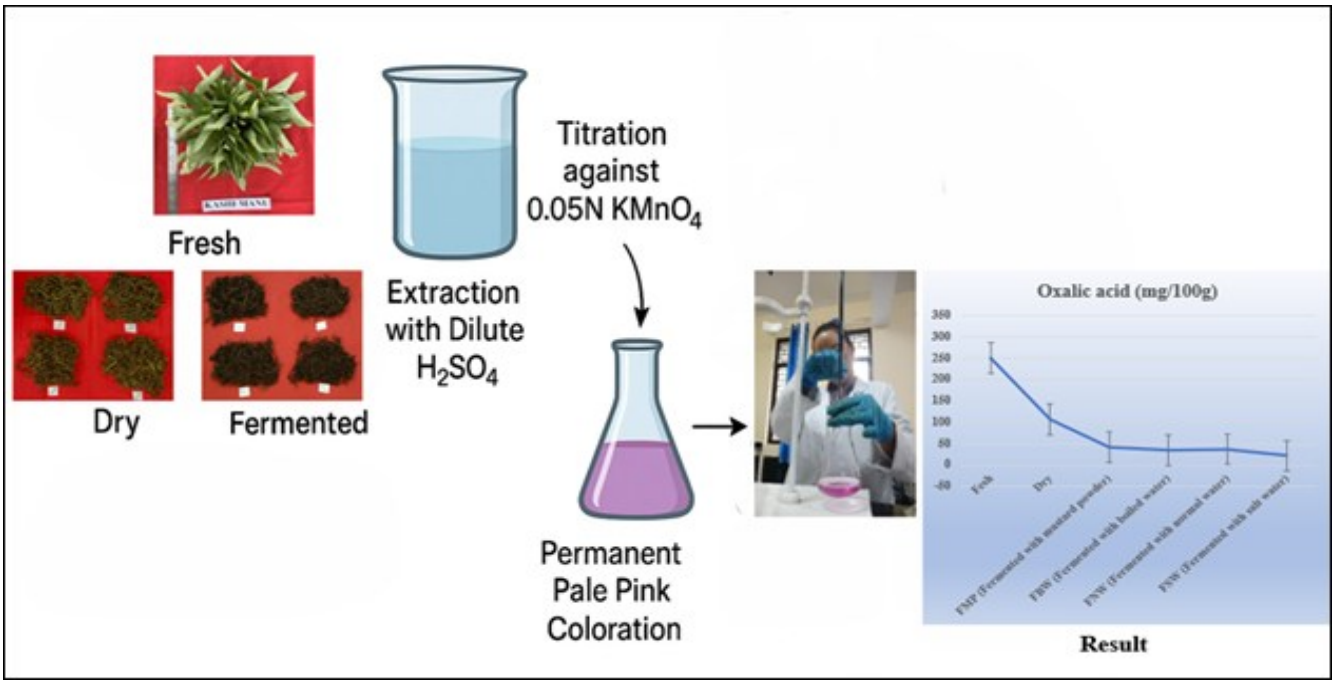


**Fig. 2.** Overall view of oxalic acid analysis.

Volume of KMnO4 Solution (V1)

Normality of KMnO4 solution (N1)

Volume of water spinach extract V2

Normality of the oxalate ions in water spinach extract N2 =?

N2= V1 × N1 / V2

Equivalent weight of oxalate ion = 44

Amount of oxalate ions in 1 kg water spinach sample =

$$N2 \times 44 \times \frac{100}{1000} \times \frac{100}{50} \quad g/L$$

The results that in g/L were converted into g /100 g.

### Statistical analysis

The results are presented as the average value ± standard deviation (SD) of the data (four replications). The results were analyzed by Duncan's multiple range test (DMRT) analysis by using the statistical software OPSTAT (23) .

## Results

Oxalic acid is present in leafy vegetables and is considered an anti-nutrient due to it reducing mineral bioavailability in foods and in extreme cases can create many diseases in human body such as renal failure because of kidney stones formation, cardiovascular disease and osteoporosis (24). The oxalic acid content of water spinach varied significantly among the applied treatments shown in Table 2 and Fig. 3. The highest oxalic acid accumulation was recorded in the fresh sample (250.24 ± 3.08 mg/100 g) which was followed by the dried sample (107.66 ± 4.86 mg/100 g). Various fermentation treatments resulted in a notable reduction in oxalic acid concentration. The values ranged from 41.90 ± 0.80 mg/100 g to 22.17 ± 0.68 mg/100 g. The treatment FMP showed the highest 41.90 ± 0.80 mg/100 g oxalic acid content followed by treatments, FNW 37.74 ± 0.53 mg/100 g, FBW 35.15 ± 0.27 mg/100 g and significantly lowest reported in treatment FSW 22.17 ± 0.68 mg/100 g, shown in Fig. 1. The treatment, fermentation with salt water (FSW) was recorded as the lowest oxalate level, showing a 91.1 % reduction compared to the fresh sample, dry and other fermented forms. The

**Table 2**. DMRT analysis of oxalic acid levels in fresh, dried and fermented forms of water spinach

| Treatments | Treat Mean | Least Sign. Diff |
|---|---|---|
| Fesh | 250.24[a] | 0 |
| Dry | 107.66[b] | 23.343 |
| FMP (Fermented with mustard powder) | 41.9[c] | 24.455 |
| FBW (Fermented with boiled water) | 35.15[c] | 25.418 |
| FNW (Fermented with normal water) | 37.74[c] | 24.973 |
| FSW (Fermented with salt water) | 22.17[c] | 25.64 |

The reduction order was as follows:

Fresh > Dry > FMP > FNW > FBW > FSW

These results illustrate that both drying and fermentation techniques significantly reduce the oxalic acid accumulation in studied water spinach sample. Salt-water fermentation was the most effective method in reducing oxalic acid content.

## Discussion

The oxalic acid's anti-nutrient effect is mostly attributed to its capacity to chelate minerals such as potassium, calcium, sodium, iron and magnesium, dropping their bioavailability (25). Oxalate is necessary however, higher oxalate concentration has consequently been related to various diseases such as acute renal breakdown because of kidney stone development which is mainly composed of calcium oxalate and calcium phosphate. Previous researchers have found that hyperoxaluria and systemic oxalosis have been cited as potential causes of kidney stone. Owing to such negative impact on health, oxalic acid is considered as an anti-nutrient. The observed reduction in oxalic acid content across treatments can be attributed to the impact of thermal and microbial degradation mechanisms. Drying at 65 °C for 48 hr reduced oxalate concentration by approximately 57 %, likely due to the decomposition of soluble oxalates and partial oxidation during prolonged heat exposure (26).

Fermentation further intensified the decline in oxalate content, consistent with earlier reports in leafy vegetables (27). The significant reduction observed in salt-water fermented samples (FSW) may result from the synergistic effect of osmotic stress and microbial metabolism. Lactic acid bacteria involved in natural

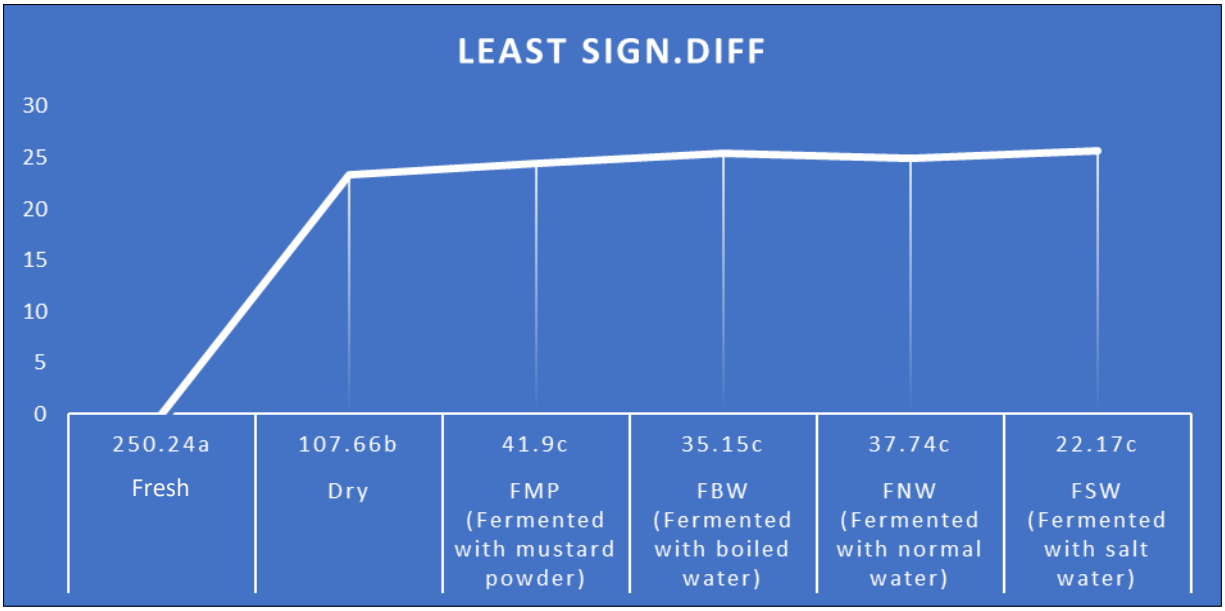


**Fig. 3.** Comparative oxalic acid content in fresh, dried and fermented forms of water spinach.

fermentation are known to break down oxalate through enzymatic decarboxylation and degradation pathways. In addition, the acidic environment during fermentation promotes the solubilization and leaching of calcium oxalate crystals, reducing total oxalate concentration (28).

The comparatively lower oxalate content in FSW (22.17 mg/100 g) suggests that sodium chloride may enhance oxalate solubilization or stimulate oxalate-degrading microbial activity, as reported in traditional fermented vegetables like sinkhi and gundruk (29). Fermentation with mustard powder (FMP) also found a considerable decrease oxalic acid concentration (41.90 mg/100 g), possibly because of the presence of glucosinolate-hydrolyzing enzymes and accompanying microflora from mustard seeds powder (30).

Overall, fermentation techniques resulted in an average reduction of 85-91 % in oxalic acid level relative to the fresh form indicating its efficiency in lowering the anti-nutritional effects associated with oxalic acid rich leafy vegetables like water spinach. Since oxalic acid forms insoluble complexes with minerals such as magnesium, calcium and iron (dropping their bioavailability), the noticeable decrease following the different fermentation technique recommends improved nutritional quality of water spinach for safe dietary use.

## Conclusion

This investigation demonstrated that both fermentation techniques and drying significantly decrease the oxalic acid concentration in water spinach (var. Kashi Manu). The fermentation method proved particularly effective, with salt water fermentation (FSW) achieving the highest reduction in oxalic acid content (up to 91 %). The reduction of oxalic acid during fermentation might be attributed to acidification and microbial enzymatic activity which enhances oxalic acid solubility and breakdown. Thus, traditional fermentation methods not only extend shelf life but also help improve the nutritional safety and quality of water spinach by minimizing oxalic acid. This study supports the application of salt water fermentation as a practical, cost effective (low cost) bioprocessing technique for improving the dietary value of water spinach in both rural and urban food systems.

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## Authors' contributions

KMW conducted the experiments, analyzed the data, and prepared the first draft of the manuscript. RKD assisted in data collection and contributed to manuscript writing and revision, conceptualized the study, designed the methodology, supervised the research work, and provided critical inputs for analysis. AS contributed to the preparation of tables and figures. $RK^1$ provided critical inputs for data interpretation. KB and RKD assisted in manuscript preparation and finalization. AKS and NR guided the overall research and validated the results. $RK^2$ further contributed to manuscript refinement. [$RK^1$ stands for Rajeev Kumar and $RK^2$ stands for Rajesh Kumar].

## Compliance with ethical standards

**Conflict of interest:** The authors declare that they have no conflicts of interest regarding the publication of this research article.

**Ethical issues:** None

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