



RESEARCH ARTICLE

Influence of agronomic micronutrient biofortification on growth, post-harvest shelf life and quality of chickpea microgreens

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Abstract

Microgreens are tender plant-based foods typically consumed fresh as seasonings, garnishes or as whole foods. Their elevated respiration and transpiration rates after harvest induce senescence and deterioration of fresh biomass. Their high surface-area-to-volume ratio and active post-harvest metabolism render them highly perishable and accelerate nutritional losses. The aim of the present study was to standardise the most suitable post-harvest protectant amongst aloe vera gel, glycerol, chlorine water, ascorbic and citric acid for chickpea genotype HK-4 microgreens. Further, the study aimed to understand the influence of iron-ethylenediaminetetraacetic acid (Fe-EDTA) amendment in vermiculite during growth, combined with the application of the standardised protectant after harvest, on the biochemical and organoleptic parameters during storage at 5 °C in zip-lock pouches. Among the various post-harvest applications, 0.5 % ascorbic acid exhibited maximum fresh weight retention (79 %) and maintained visual appeal for up to 15 days of storage. Therefore, 0.5 % ascorbic acid was utilised as a protectant for chickpea microgreens grown in control (distilled water) and Fe-EDTA-amended vermiculite. Fresh weight declined during storage under all treatments; although losses were significantly lower following post-harvest application of 0.5 % ascorbic acid regardless of the growing condition. While the moisture content declined during storage, the ash content increased until the 10th day. The ascorbic acid content, antioxidant activity and total soluble solids (TSS) decreased substantially during storage, however growth under Fe-EDTA amended conditions and post-harvest application of ascorbic acid retained the highest content i.e. 70.6 mg/100 g, 8.4 % and 15.6 % respectively. On the other hand, the tannin concentration increased by 5.7 times, whereas the phytic acid content showed a 0.7-fold decline during storage, with the greatest changes observed in control-grown microgreens treated with distilled water. Organoleptic attributes diminished during storage; however, post-harvest application of ascorbic acid helped maintain them better. The results revealed that the shelf life of chickpea microgreens is influenced by nutrient manipulations during growth and post-harvest application of ascorbic acid, which retained the maximum nutritional and organoleptic attributes.

Keywords: antioxidant capacity; ascorbic acid; chickpea microgreens; fertilisation effect; fresh weight retention; iron-ethylenediaminetetraacetic acid amendment; storage stability

Introduction

Microgreens are potential plant-based vegetarian foods that are full of nutrients, fibre and antioxidants (1). These are juvenile greens grown for 7–28 days and cultivated from the seeds of herbs, cereals, pseudocereals and vegetables (2). Their levels of vitamins (A, C, D, E and K), phenolics (flavonoids), antioxidant potential and minerals (potassium (K), calcium (Ca), zinc (Zn), iron (Fe) and magnesium (Mg)) are higher than those of fully grown mature greens (3). Thus, nutritionally enriched microgreens possess anti-inflammatory, anti-carcinogenic, anti-diabetic and immunomodulatory attributes, as evident from studies on Caco-2 cell lines (4, 5). They also offer protective benefits against cardiovascular issues and support bone health (1). However, the genotype/cultivar, growing conditions, including light, temperature, humidity, substrate and the stage of harvest, have a pronounced effect on the concentration of nutrients (2, 6).

They are preferably consumed fresh, either whole or as garnishes and seasonings. Their cultivation at home can be an excellent and practical approach in terms of affordability and sustainability (7). Their short shelf-life (3–4 days), perishable nature and post-harvest management are the major challenges that limit their commercial application. Since they are harvested at an early growth stage, their accelerated transpiration and respiration rates, enhanced senescence and high surface area-to-volume ratio restrict storage and drive nutritional and biochemical alterations in the metabolically active fresh harvest (8). Maintaining food safety standards without compromising the nutritional and sensory qualities is of utmost importance, with minimal or no microbial contamination. Therefore, optimised storage conditions, including temperature and humidity, along with suitably modified packaging strategies and pre- and post-harvest interventions, may prolong shelf life while minimising the depletion of nutritive compounds (9). The application of 10 mM calcium chloride to broccoli microgreens

prior to harvest was effective in delaying senescence, enhancing visual appearance, extending shelf life and diminishing the growth of microorganisms during storage (10, 11).

Chickpea is a vital food legume crop and a significant source of proteins, antioxidants, Fe, Mg, Ca and Zn. It is widely consumed to overcome micronutrient (Fe and Zn) deficiency, popularly known as “hidden hunger” (12). Our earlier studies have also reported high levels of micronutrients in chickpea microgreens (2). Despite the fact that microgreens are rich in micronutrients, strategies for enhancing their concentrations, particularly Fe and Zn, through biofortification are of great interest to researchers and are being extensively explored (13). In a similar attempt, the authors of the present study analysed the effect of nutrient amendment in the growth substrate on the nutritional status of chickpea microgreens of the genotype HK-4 (data unpublished). Furthermore, reports on the post-harvest quality and storage stability of chickpea microgreens are still limited.

To maintain the post-harvest quality of microgreens, several chemical treatments Generally Recognised as Safe (GRAS) are also utilised. The United States Food and Drug Administration has defined GRAS chemicals as alternatives with the least known toxic or harmful effects on mammals as well as the environment and are therefore deemed safe for consumption (14). These include edible coatings, organic acids such as citric and ascorbic acid, ethanol vapour and acetic acid. The potential of aloe vera gel as an edible coating has also been explored to enhance shelf-life of radish (*Raphanus sativus* L.), roselle (*Hibiscus sabdariffa* L.), mustard (*Brassica juncea* L.) and lettuce (*Lactuca sativa* L.) microgreens (8, 15). Post-harvest treatment with ethanol spray or vapour, as well as ascorbic and citric acid sprays, reportedly improved the shelf life of sunflower (*Helianthus annuus* L.) microgreens (16). Washing with citric acid, calcium chloride and sodium hypochlorite, in addition to metallised polyester packaging and storage at 5 °C, improved the shelf-life of radish sango microgreens by minimising microbial spoilage and retaining highest antioxidant activity (17).

The optimum quality and highest shelf life of buckwheat microgreens can be maintained by storing them at 5 °C through moderately high O₂ and low CO₂ conditions, combined with chlorinated washing, which reduce microbial counts (10). In addition to above-mentioned studies, technologies such as modified atmosphere packaging, UV-C, ozone, cold plasma, chitosan-based nanoparticles and nano-coatings have also been evaluated for extending the shelf-life of microgreens (18). While several pre- and post-harvest chemical treatments, storage temperature, packaging conditions and nanotechnology-derived coatings have been extensively explored in several microgreen species, studies on the post-harvest quality of chickpea microgreens remain comparatively scarce. Furthermore, the influence of substrate amendment on the post-harvest physiology of microgreens, both alone and in combination with a suitable protectant, remains underexplored and requires further investigation. Based on the results of previous experiments, in which Fe-ethylenediaminetetraacetic acid (EDTA) nutrient supplementation enhanced content of several phytochemicals (data unpublished) in chickpea genotype HK-4 microgreens compared with those grown under distilled water (control) amendment, similar growth conditions were selected for the present study. It was also of interest to determine whether nutrient supplementation in the growth substrate influenced the shelf-life and changes in the nutrient profile of chickpea microgreens during storage.

In view of this, the present study was undertaken with the objective to elucidate shelf life of chickpea (*Cicer arietinum* L., genotype: HK-4) microgreens grown under control (distilled water) and Fe-EDTA-amended nutrient conditions, along with the post-harvest application of protectants, in terms of fresh weight retention (%), nutritional, antinutritional and organoleptic attributes during a storage period of 15 days.

Materials and Methods

The present study was conducted under two phases:

- i) Preliminary analysis to identify the most suitable post-harvest protectant for chickpea microgreens.
- ii) Experimental analysis involving the application of the post-harvest protectant identified in phase (i) to chickpea microgreens cultivated under nutrient supplementation with distilled water or Fe-EDTA at 10 mg/L.

The details of the protectant applications, the parameters studied and the methodology followed are described below.

Plant material

Seeds of chickpea (*C. arietinum*) genotype HK-4 were procured from Pulse Division of Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, Haryana in October 2025. Seeds of this variety are bold (32 g/100 seeds) while the plants are semi-erect having broad leaves, dark foliage and profuse branching. Before sowing, these were washed with Cedepol (2–3 drops) and sodium hypochlorite (5 %) for 5 min, followed by thorough rinsing and overnight soaking in autoclaved distilled water. Based on the results of previous studies, microgreens were grown on a soilless substrate i.e. vermiculite (100 %) using (a) distilled water (control) and (b) an Fe-EDTA-amended nutrient solution (at 10 mg/L) in trays (dimensions: 30 × 22.5 × 3.3 cm) arranged in a randomised block design. Each treatment was replicated three times in individual trays, which served as biological replicates. The first dose of the amendment was applied to the substrate by moistening it on day 0. The second dose was applied as a spray on the 6th day after sowing. The trays were periodically irrigated with RO water.

In a single tray, 300 seeds were evenly spread on a 1.5 cm-thick vermiculite bed at a sowing density of approximately 44 seeds per 100 cm². The trays were kept under naturally ventilated greenhouse conditions at Sharda University, Greater Noida, Uttar Pradesh (28.4731° N, 77.4829° E) during the first week of December 2025 for the growth of microgreens. The position of the trays was rotated during the experiment to avoid any positional bias. The environmental conditions were 23 °C/10 ± 2 °C (day/night) for temperature and 40 % relative humidity. The durations of the day and night were 10 hr 30 min and 13 hr 30 min, respectively. The biomass from individual trays was harvested on the 7th day and processed independently for shelf-life and nutrient profiling studies.

Vermiculite is a natural mineral composite with remarkable water-retention and aeration capacity. It was a 3 mm fine horticultural-grade granular substrate with a pH 6.4 and an electrical conductivity (EC) as 0.03 dS m⁻¹. The composition of constituent minerals included silicon dioxide (SiO₂, 38–46 %), aluminium oxide (Al₂O₃, 10–16 %), magnesium oxide (MgO, 16–35 %), ferric oxide (Fe₂O₃, 6–13 %), potassium oxide (K₂O, 1–6 %), titanium dioxide (TiO₂, 1–3 %) and calcium oxide (CaO, 1–5 %).

2.2. Shelf-life studies

Preliminary analysis

Based on the literature surveyed, the following protectants and concentrations were used as post-harvest treatments:

- 1) Distilled water (control)
- 2) Aloe vera gel (100 %) was extracted using a knife, followed by homogenisation in a mixer-grinder for 5–7 min.
- 3) Glycerol solution (v/v) 3 % and 6 % concentrations were prepared with distilled water using 3 mL and 6 mL of concentrated glycerol, respectively.
- 4) Sodium hypochlorite (NaOCl; 4 % available chlorine) was used to prepare two concentrations of chlorine water (50 mg/L and 100 mg/L; pH 9) were prepared. Aliquots of 125 and 250 μ L sodium hypochlorite were diluted to a final volume of 100 mL with distilled water to obtain the respective concentrations.
- 5) Citric acid (w/v) 0.5 % and 1.0 % solutions were prepared by dissolving 0.5 g and 1.0 g of citric acid, respectively, in 100 mL of distilled water.
- 6) Ascorbic acid (w/v) 0.5 % and 1.0 % solutions were also prepared following the same procedure as that used for the citric acid solutions.

A known amount i.e. 10 g of biomass, was subjected to these treatments along with the control individually to decipher the most effective treatment for prolonged shelf life in terms of fresh biomass appearance. Microgreen shoots were dipped in 100 mL of distilled water or freshly prepared individual treatment solutions (biomass-to-solution ratio: 1 : 10) for 5 min, followed by blot drying at room temperature (-18 ± 2 °C) under ambient air conditions for 15 min. These were then packed in zip-lock pouches (dimensions: 15 × 13 cm; thickness: 100 gauge) and stored at 5 °C in a refrigerator. A constant temperature was maintained throughout the 15-day storage period. Observations were recorded for fresh weight retention and visual appeal at 5 days intervals for 2 weeks.

Experimental details

Based on the maximum fresh weight retention (%) observed in the preliminary analysis, 0.5 % ascorbic acid was selected as the post-harvest treatment for chickpea microgreens. Harvested biomass (10 g each) was dipped in distilled water or ascorbic acid solution (100 mL) while maintaining a similar biomass-to-solution ratio (1 : 10) for 5 min, followed by blot drying at room temperature (-18 ± 2 °C) under ambient air conditions for 15 min. The treated samples were packed in zip-lock pouches (dimensions: 15 × 13 cm; thickness: 100 gauge) and stored at 5 °C in a refrigerator. Destructive sampling was followed and separate sampling bags were used at each day of analysis i.e. day 0, followed by days 5, 10 and 15, to study shelf-life parameters.

Fresh weight retention: The fresh weight (g) of microgreens was recorded on each sampling day using an analytical balance and the percentage of fresh weight retention was calculated relative to day 0 using the following formula:

$$\text{Fresh weight retention (\%)} = \frac{\text{Weight on day 0} - \text{Weight on sampling day}}{\text{Weight on day 0}} \times 100$$

Proximate analysis: Moisture and ash concentrations were determined using standard AOAC methods (19).

Biochemical attributes:

(i) Ascorbic acid: Ascorbic acid content of microgreen samples was assessed following the methodology of Di Bella et al. (20) and expressed as milligrams/100 g fresh weight. One gram of fresh sample was macerated in 10 mL of 3 % metaphosphoric acid and filtered with Whatman filter paper No. 1. The filtrate (4 mL) was titrated against 2,6-dichlorophenolindophenol (DCPIP) dye and the volume of dye used was recorded until the endpoint (pink colour) was obtained. The ascorbic acid content was calculated using the following formula:

Ascorbic acid content =

$$\frac{\text{Titre value} \times \text{dye factor} \times \text{Volume of sample extract} \times 100}{\text{Volume of aliquot} \times \text{Weight of fresh sample}}$$

(ii) Antioxidant activity: Antioxidant activity was estimated as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging potential and expressed as DPPH radical scavenging activity (%) on a fresh weight basis (21). Fresh leaves (250 mg) were macerated in 10 mL methanol and centrifuged at 4000 rpm for 5 min. The supernatant was collected and diluted 10 times for analysis. A 500 μ L sample extract was incubated with 5 mL of DPPH dye (25 mg/10 mL, diluted 100 times) for 10 min and the change in colour due to reduction was observed by recording absorbance at 517 nm using a UV-Vis spectrophotometer (Model-LMSP-UV1900S, Labman Scientific Instruments Pvt. Ltd., Chennai).

DPPH radical scavenging activity =

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

(iii) Total soluble solids: Total soluble solids (TSS) were determined using an Abbe refractometer (Relitech RT 780, India), which was calibrated with distilled water prior to use. A drop of microgreen extract, prepared using a known weight of sample in distilled water, was placed on the prism of the refractometer as per the protocol (22). The values were expressed as percentage TSS on a fresh weight basis.

(iv) Tannins: A standardised methodology was followed to determine the tannin concentration in the biomass of microgreens (23). Tannins were extracted in methanol using the same protocol as that used for the extraction of antioxidants. About 1 mL of extract was incubated with 5 mL vanillin-HCl reagent, prepared by dissolving equal volume of 4 % vanillin and 8 % HCl solution. After 20 min, the absorbance was recorded at 500 nm, and the concentration was estimated using a standard curve of catechin (0–2 mg/mL). The results were expressed as mg/g fresh weight.

(v) Phytic acid: Phytic acid concentration was determined using a standard curve (0–0.8 mg/mL) of sodium phytate with Wade's reagent (24) and expressed as mg/g dry weight. Briefly, 1 g of dried microgreen leaf powder was incubated with HCl (2.4 %) for 16 hr and then centrifuged at 6000 rpm for 10 min at 10 °C. The supernatant was collected and the volume was made up to 10 mL

using 2.4 % HCl. The extract was reacted with 1 g of sodium chloride at 4 °C for 1 hr, followed by centrifugation under similar conditions for 20 min. About 1 mL of supernatant was added to 4 mL of Wade's reagent for the development of pink colour (15 min) and the absorbance was recorded at 500 nm.

Organoleptic parameters: Organoleptic parameters were assessed by 20 semi-trained individuals to determine consumer acceptability on different days of storage. A nine-point hedonic scale was used for ranking of samples ranging from 9 (like extremely) to 1 (dislike extremely), for colour and appearance, taste, aroma, mouthfeel and overall acceptability. The participants were undergraduate and postgraduate students, with a random proportion of male and female participants aged between 18 and 23 years. They were briefly informed about the product to evade risks associated with food-related allergies (if any), the sensory evaluation procedure and the ranking methodology to be followed (25). Informed consent was obtained from all participating individuals prior to the evaluation. Samples were presented as coded packets to all participants, who were trained to carefully assess the samples for colour and appearance, place them in their mouth, chew adequately to evaluate taste and mouthfeel and expectorate them into the provided container. To continue assessing the next sample participants were asked to rinse their mouths with purified water to maintain a neutral palate. They were then instructed to record their responses as scores on the provided performa against each sample code for each organoleptic parameter. The evaluation procedure was conducted under laboratory conditions. Ethical approval for conducting the sensory analysis was obtained from the Institutional Ethics Committee (IEC) vide Reference No. SU/SMS&R/76-A/2025/313.

Statistical analysis: Three replications were used and the results were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). One-way ANOVA was performed for the data obtained from the preliminary analysis, while two-way ANOVA was used for the analysis of variance of data from the experimental analysis. Significant differences were determined based on Tukey's test (post-hoc) at 95 % probability, using Origin Pro version 2026 software (OriginLab Corporation, Northampton, MA, USA).

The correlation study was performed using Pearson's correlation coefficient at $p < 0.05$ and bubble heat maps of correlation matrix were generated. The analysis was carried out for each day of storage to determine the direction and strength of relationships (positive or negative) between pairs of calibrated parameters. Principal components (PCs) were extracted and analysed to reduce the dimensionality of the dataset and identify the major components explaining the observed variance. Scree plots were examined to determine the adequate number of PCs required to summarise the dataset and understand the contribution of each PC to the observed variation. Biplots depicting interactions between treatments i.e. pre- and post-harvest treatments (scores) and parameters (loadings), were also constructed using Origin Pro version 2026 software.

Results

Preliminary analysis

Fresh-weight retention, calculated relative to day 0, was evaluated in chickpea microgreens subjected to different post-harvest treatments (aloe vera gel, glycerol, chlorine water, citric acid and ascorbic acid) compared with distilled water as the control at 5-day intervals over 15 days of refrigerated storage. The fresh weight declined gradually and the calculated cumulative mean showed that 0.5 % ascorbic acid treatment retained the maximum fresh weight of 90.44 % during storage, followed by 86.15 % with 1 % ascorbic acid and citric acid treatments (Table 1). The effect of treatment, days of storage and their interaction were significant on the fresh-weight retention of chickpea microgreens. Therefore, 0.5 % ascorbic acid was selected as the post-harvest treatment for further shelf-life studies.

Experimental details

The biomass from chickpea genotype grown under two conditions was harvested on the 7th day of growth and a known fresh weight (10 g) was subjected to post-harvest application of ascorbic acid (0.5 %) and analysed in comparison with treatment with distilled water.

Table 1. Fresh weight retention (%) after post-harvest applications at different days of storage in chickpea microgreens

Treatment (T)	Days of storage (D)			Cumulative mean
	5	10	15	
Distilled water	60.57 $\pm$ 0.47 ^f	56.31 $\pm$ 0.84 ^{fg}	31.98 $\pm$ 3.02 ⁱ	49.62 $\pm$ 1.44
Aloe vera gel (100 %)	81.32 $\pm$ 3.28 ^f	52.94 $\pm$ 2.59 ^g	47.10 $\pm$ 3.39 ^h	60.52 $\pm$ 3.09
3 % Glycerol	92.95 $\pm$ 1.61 ^f	89.47 $\pm$ 0.54 ^{ab}	69.02 $\pm$ 2.46 ^{de}	83.81 $\pm$ 1.54
6 % Glycerol	84.11 $\pm$ 0.70 ^f	80.38 $\pm$ 3.33 ^c	69.50 $\pm$ 1.49 ^{de}	74.66 $\pm$ 1.84
50 mg/L Chlorine water	90.69 $\pm$ 3.10 ^f	87.91 $\pm$ 2.91 ^b	74.70 $\pm$ 1.66 ^{cd}	84.43 $\pm$ 2.56
100 mg/L Chlorine water	94.21 $\pm$ 0.47 ^f	89.51 $\pm$ 3.09 ^{ab}	66.05 $\pm$ 2.33 ^e	83.26 $\pm$ 1.96
0.5 % Citric acid	94.39 $\pm$ 0.88 ^f	91.40 $\pm$ 0.78 ^{ab}	67.06 $\pm$ 1.30 ^e	84.29 $\pm$ 0.99
1 % Citric acid	95.63 $\pm$ 0.54 ^f	91.96 $\pm$ 0.14 ^{ab}	71.70 $\pm$ 2.11 ^{de}	86.43 $\pm$ 0.93
0.5 % Ascorbic acid	97.11 $\pm$ 1.92 ^f	95.08 $\pm$ 1.16 ^a	79.12 $\pm$ 0.77 ^c	90.44 $\pm$ 1.28
1 % Ascorbic acid	95.62 $\pm$ 1.61 ^f	91.78 $\pm$ 1.38 ^{ab}	71.06 $\pm$ 1.24 ^{de}	86.15 $\pm$ 1.41
	<i>p</i> -value			
T	<1 $\times$ 10 ⁻⁴			
D	<1 $\times$ 10 ⁻⁴			
T $\times$ D	<1 $\times$ 10 ⁻⁴			

All values are depicted as mean $\pm$ standard deviation ($n = 3$).

Within each column, means with different superscript alphabets differ significantly whereas means sharing the same superscript alphabet are not significantly different, according to Tukey's post hoc test ($p < 0.05$).

Fresh weight retention

Retention in fresh weight (%) in chickpea microgreens, calculated relative to day 0 at 5-day intervals up to the 15th day of storage, showed a declining trend under both distilled water and ascorbic acid post-harvest applications. The absolute cumulative mean indicated that microgreens grown under Fe-EDTA amendment in the substrate and subjected to ascorbic acid post-harvest application retained the maximum fresh weight (94.30 %). The effect of substrate was significant on the 10th and 15th day of storage, while the effect of post-harvest application was significant on all days. The interaction effect between these two variables was significant only on the 10th day (Table 2).

Proximate analysis

Moisture content (%) declined linearly under both control and ascorbic acid post-harvest application conditions. The calculated cumulative mean indicated more or less similar values in plants grown with distilled water and those grown under Fe-EDTA-amended conditions. The cumulative mean was highest (88.07 %) with ascorbic acid treatment under Fe-EDTA amended growth conditions. The effect of substrate (amendment) was significant on 0 and 5th day, while post-harvest application significantly influenced moisture content on 5th day. The interaction between the variables was non-significant on all days of storage (Fig. 1a).

Ash concentration (%) showed a gradual and variable increase under distilled water application with increasing days of storage. When ascorbic acid was applied, the ash concentration increased up to the 10th day, followed by a slight decrease. The effect of growing condition (substrate) was significant on 0 and 10th day, while effect of post-harvest application was significant only on day 0. The interaction between substrate and post-harvest application was significant only on 5th days. The cumulative mean exhibited more or less similar ash concentrations under all the treatments used (Fig. 1b).

Biochemical attributes

Ascorbic acid: Ascorbic acid content (mg/100 g), calculated during storage up to the 15th day, showed a decrease under post-harvest application with distilled water and ascorbic acid on a fresh weight basis. The effects of nutrient conditions (substrate) and post-harvest application were significant on different days of storage. Furthermore, the interaction effect was significant only on the 5th and 10th days. The cumulative mean showed that post-harvest application of 0.5 % ascorbic acid resulted in the highest ascorbic acid content (93.94 mg/100 g) under Fe-EDTA-grown conditions, whereas the lowest content (56.45 mg/100g) was observed under control storage conditions in distilled water-grown plants (Fig. 2a).

Table 2. Fresh weight retention (%) at different days of storage in chickpea microgreens

Treatment (T)		Days of storage (D)			Cumulative mean
Substrate (S)	Post-harvest (P)	5	10	15	
Control	DW	96.44 ± 0.62 ^a	89.49 ± 0.70 ^c	85.90 ± 1.06 ^b	90.61 ± 0.79
	0.5 % AA	97.67 ± 0.35 ^a	91.74 ± 0.47 ^b	87.78 ± 0.49 ^{ab}	92.40 ± 0.44
Fe-EDTA	DW	95.94 ± 1.57 ^a	91.31 ± 0.11 ^b	87.04 ± 0.86 ^b	91.43 ± 0.85
	0.5 % AA	97.71 ± 0.21 ^a	95.02 ± 0.17 ^a	90.17 ± 0.41 ^a	94.30 ± 0.26
		<i>p</i> -value			
S		NS	<1 × 10 ⁻⁴	1.05 × 10 ⁻⁴	
P		4.00 × 10 ⁻²	<1 × 10 ⁻⁴	1.50 × 10 ⁻⁴	
S × P		NS	4.58 × 10 ⁻⁴	NS	

All values are depicted as mean ± standard deviation (*n* = 3).

Within each column, means with different superscript alphabets differ significantly, whereas means sharing the same superscript letter do not differ significantly, according to Tukey's post hoc test (*p* < 0.05).

Fe-EDTA: iron-ethylenediaminetetraacetic acid; DW: distilled water; AA: ascorbic acid; NS: non-significant.

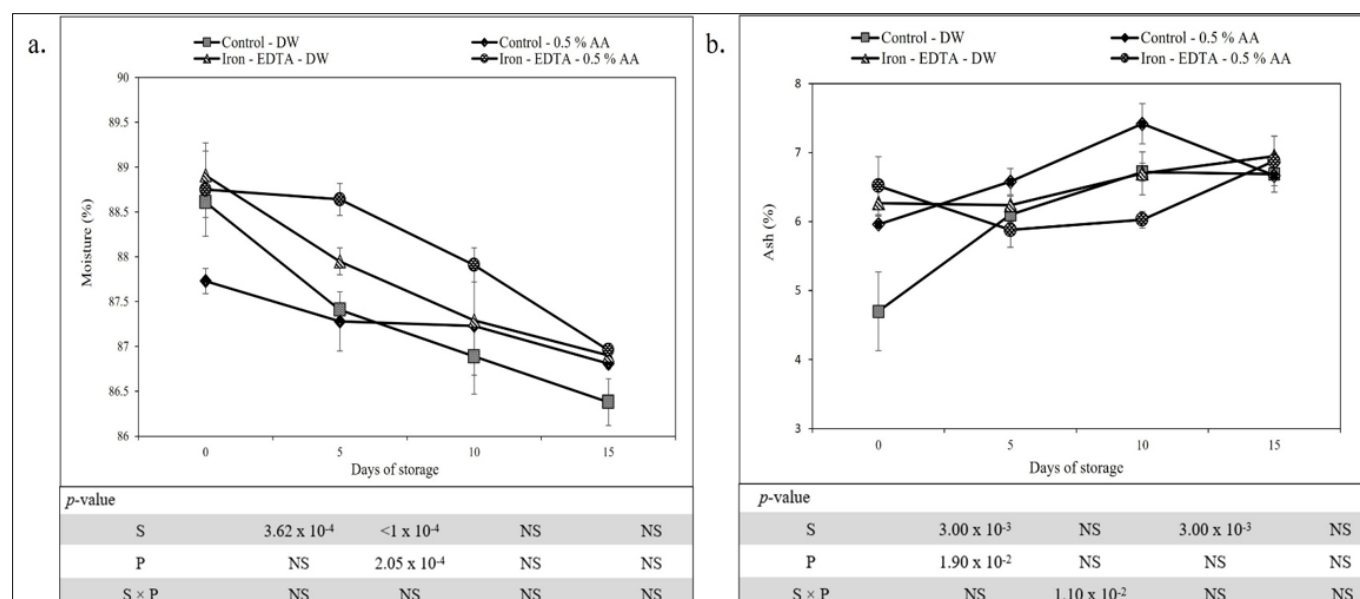


Fig. 1. (a) Moisture content (%) and (b) ash content (%) of chickpea microgreens during storage. The table presents the *p*-values from the two-way ANOVA (**p* < 0.05). All values are expressed as mean ± standard deviation (*n* = 3).

S: substrate; P: post-harvest; NS: non-significant.

Antioxidant activity: Antioxidant activity (% DPPH scavenging potential) was significantly affected by the substrates (growing conditions) used for the growth of microgreens at different days of storage. It also showed a pronounced reduction with increasing storage duration, irrespective of the post-harvest treatment. The post-harvest application of ascorbic acid exhibited a significant effect on the 5th, 10th and 15th day. The cumulative mean over the storage period showed the highest antioxidant activity (14.3 %) with 0.5 % ascorbic acid application in Fe-EDTA-grown plants, whereas distilled water post-harvest application exhibited the lowest antioxidant activity (7.8 %) in control-grown plants (Fig. 2b).

Total soluble solids: The TSS (%) reduced gradually during storage under both distilled water and ascorbic acid post-harvest applications. Total soluble solids was significantly influenced by the substrate (S), post-harvest application (P) and their interaction on different days of storage. The cumulative mean showed the best effect of 0.5 % ascorbic acid application (17.28 %) in Fe-EDTA grown microgreens, whereas the lowest effect was observed under distilled water application (13.65 %) in control-grown microgreens (Fig. 2c).

Tannins: Tannins (mg/g) showed an increase during storage under both distilled water and ascorbic acid post-harvest applications; however, the increase was more pronounced under distilled water treatment. Tannin concentrations were significantly affected by the growing conditions (substrate) and post-harvest application on different days of storage. Their interactive effect was significant only on the 15th day of storage for this attribute. The cumulative mean showed the highest tannin content (14.95 mg/g) under post-harvest distilled water application in control-grown microgreens, whereas the lowest content (9.60 mg/g) was observed with 0.5 % ascorbic acid application in microgreens grown with Fe-EDTA nutrient supplementation (Fig. 3a).

Phytic acid: The concentration of phytic acid (mg/g) decreased steadily during storage under both control and ascorbic acid post-harvest application. The effect of substrate under both growing conditions was significant on phytic acid concentration. Furthermore, post-harvest application also showed a significant effect, while the interaction between two factors was non-significant except on 5th day of storage. The cumulative mean of phytic acid was highest (5.45 mg/g) with ascorbic acid application in Fe-EDTA grown plants, whereas the effect of other treatments was more or less similar (Fig. 3b).

Organoleptic parameters

Colour and appearance: When organoleptic parameters were assessed in terms of colour and appearance, the scores showed a gradual decline, indicating steady deterioration in the freshness, green appearance and quality of microgreens during storage. The effect of growing conditions (substrate) on the scores was non-significant, whereas post-harvest application showed a significant influence on the 10th and 15th days of storage. The cumulative mean scores were more or less similar irrespective of the amendment used for growth and the post-harvest application of protectants (Fig. 4 and 5a).

Taste: The score for taste exhibited a steady decline during storage up to the 15th day under both control and ascorbic acid post-harvest applications. The effect of growing conditions (substrate) on taste score was significant only on day 0, whereas post-harvest application did not influence taste on any day of storage. The interactive effect was significant on 0 and 5th day of storage. The

cumulative mean score showed that taste was highest (7.09) with 0.5 % ascorbic acid application in control-grown plants and lowest (5.26) with the same post-harvest application when microgreens were grown in a Fe-EDTA supplemented substrate (Fig. 5b).

Mouthfeel: Mouthfeel scores exhibited a reduction under both post-harvest applications (distilled water vs ascorbic acid). The substrates used for growth significantly influenced the mouthfeel scores on days 0 and 10 of storage, whereas post-harvest application and the interactive between substrate and post-harvest application showed non-significant effects on all days. The cumulative mean scores remained more or less similar with post-harvest applications throughout the storage period (Fig. 5c).

Overall acceptability: Overall acceptability scores also decreased steadily during storage, irrespective of the post-harvest applications. The scores were not significantly affected by the post-harvest application or the substrate used for the growth of microgreens; however, the substrate exhibited a significant effect on day 0. The cumulative mean scores remained more or less similar in terms of overall acceptability (Fig. 5d).

Correlation studies

Pearson's correlation coefficient (r) was calculated to study the correlation between the observed nutritional, anti-nutritional and organoleptic parameters at different days of storage in chickpea microgreens. The details of correlation coefficients among various parameters tested on days 0, 5, 10 and 15 are presented in Fig. 6. At day 0 of storage, moisture content exhibited a strong and positive correlation with the organoleptic parameters, including taste ($r = 0.94$), mouthfeel ($r = 0.87$) and overall acceptability ($r = 0.87$). It also showed a strong positive correlation with ascorbic acid content and antioxidant activity on day 5 ($r = 0.95$ and 0.86) and day 10 ($r = 0.99$ and 0.99), as well as with phytic acid on days 5, 10 and 15 ($r = 0.86$, 0.93 and 0.84) and fresh-weight retention only on day 10 ($r = 0.99$).

A strong negative correlation existed between moisture content and overall acceptability on day 5 ($r = -0.85$) and between moisture content and tannins on day 10 ($r = -0.85$) and day 15 ($r = -0.97$) of storage. Fresh weight retention (FWR) exhibited a strong and linear correlation with ascorbic acid, antioxidant activity, TSS, phytic acid and colour and appearance on day 10 ($r = 0.99, 0.99, 0.91, 0.90$ and 0.86) and day 15 ($r = 0.94, 0.95, 0.96, 0.99$ and 0.91) of storage. On day 0 of storage, ash content showed a strong and positive correlation with TSS ($r = 0.98$) and phytic acid ($r = 0.96$). A strong negative correlation was observed between ash content and tannins ($r = -0.90$) on day 0 and taste ($r = -0.87$) on day 15 of storage. Furthermore, ascorbic acid showed a strong and positive correlation with antioxidant activity ($r = 0.99, 0.97, 0.95$ and 0.99) and phytic acid ($r = 0.94, 0.95, 0.81$ and 0.95) on all days of storage. It exhibited a strong and negative correlation with tannins ($r = -0.99, -0.90, -0.83$ and -0.85) on the corresponding days. The correlation between ascorbic acid and taste, mouthfeel and overall acceptability was strong and positive on day 0 ($r = 0.86, 0.94$ and 0.93) whereas its correlation with colour and appearance was strong and positive on day 10 ($r = 0.89$) and day 15 ($r = 0.99$). The correlation between antioxidant activity and phytic acid was strong and positive on day 0 ($r = 0.95$), day 5 ($r = 0.99$), day 10 ($r = 0.95$) and day 15 ($r = 0.96$) of storage. In contrast, antioxidant activity exhibited a strong negative correlation with tannins on the corresponding days ($r = -0.99, -0.98, -0.88$ and -0.87). On day 0, antioxidant activity also showed a strong positive correlation with organoleptic attributes such as taste ($r = 0.84$), mouthfeel ($r = 0.93$) and overall acceptability ($r = 0.92$).

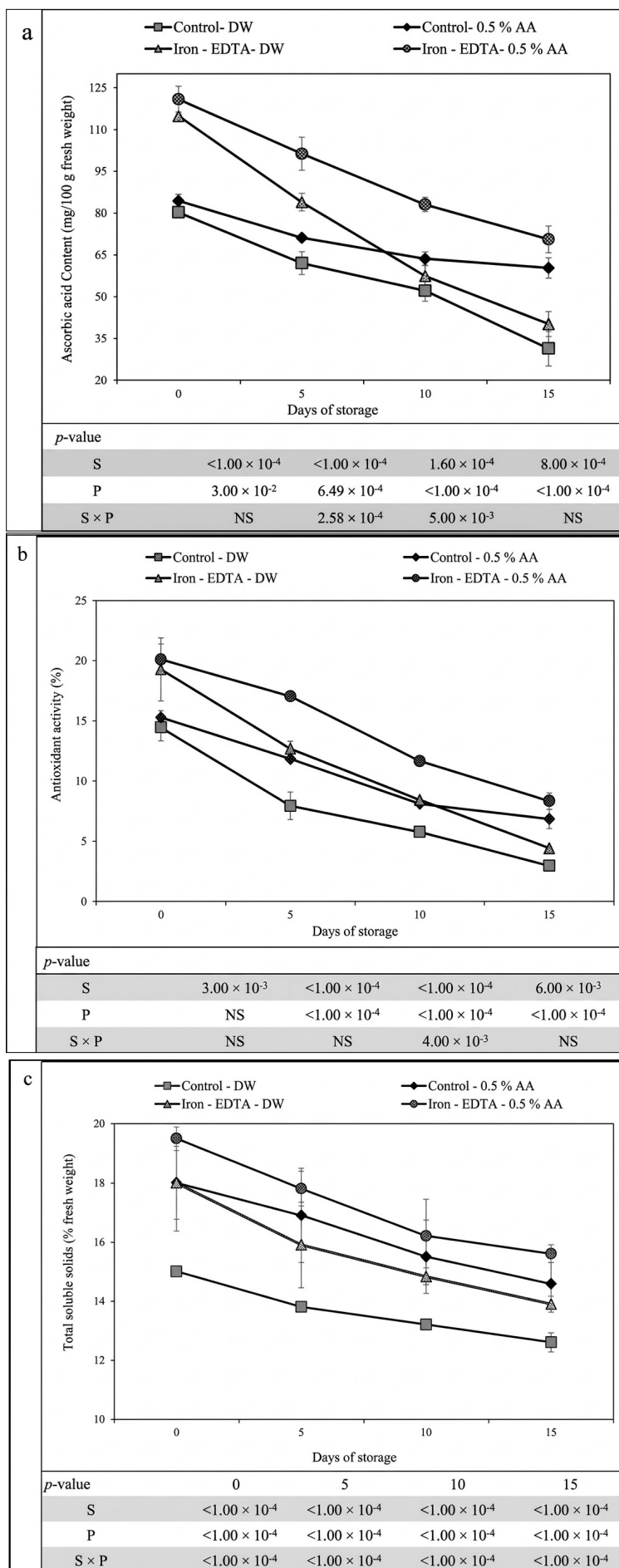


Fig. 2. (a) Ascorbic acid content, (b) antioxidant activity and (c) total soluble solids (TSS) of chickpea microgreens during storage. The table presents the p -values from the two-way ANOVA ($*p < 0.05$). All values are expressed as mean $\pm$ standard deviation ($n = 3$).

S: substrate; P: post-harvest; NS: non-significant.

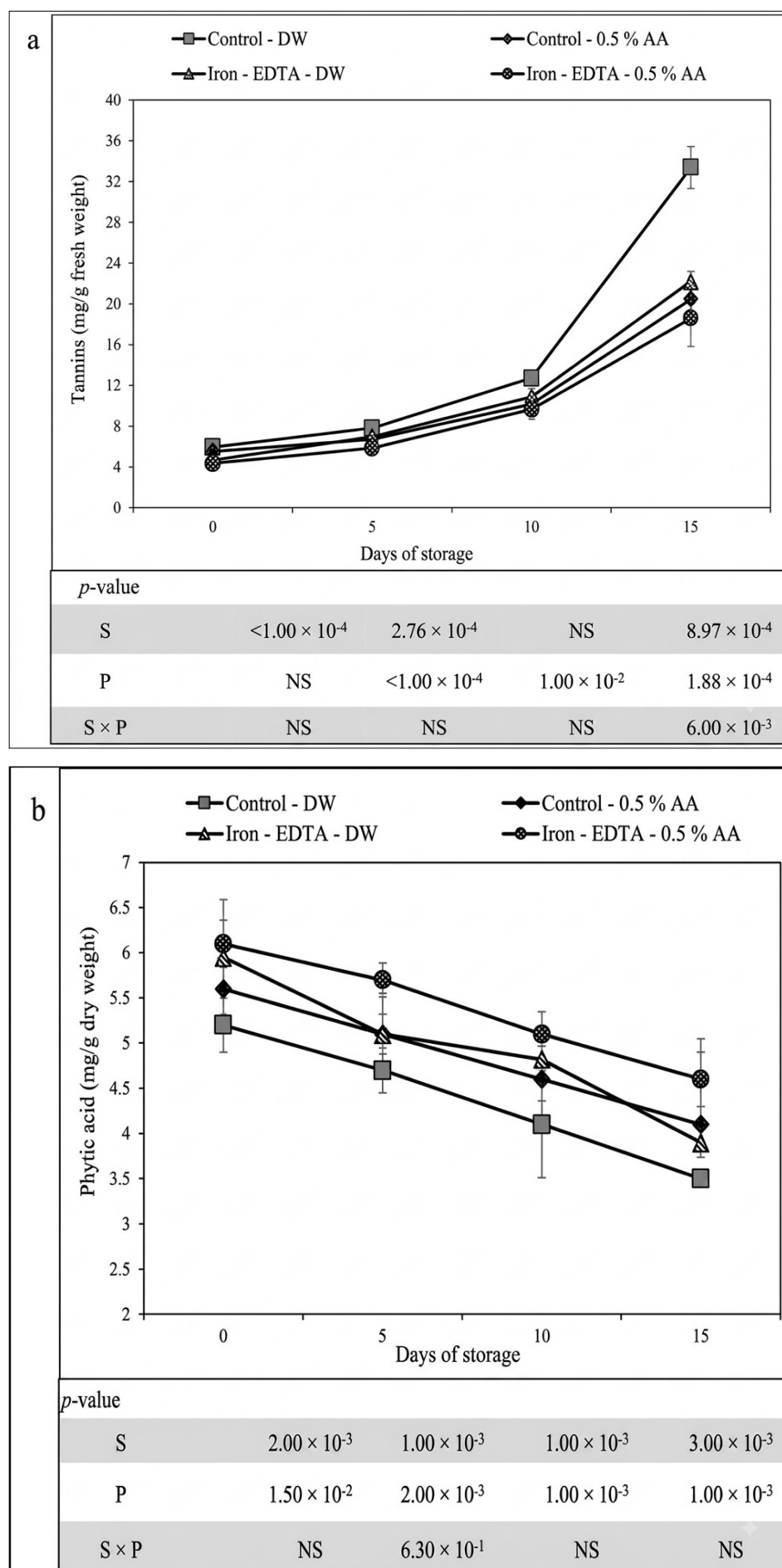


Fig. 3. (a) Tannins and (b) phytic acid content of chickpea microgreens during storage. The table presents the *p*-values from the two-way ANOVA ($*p < 0.05$). All values are expressed as mean $\pm$ standard deviation ($n = 3$).

S: substrate; P: post-harvest; NS: non-significant.

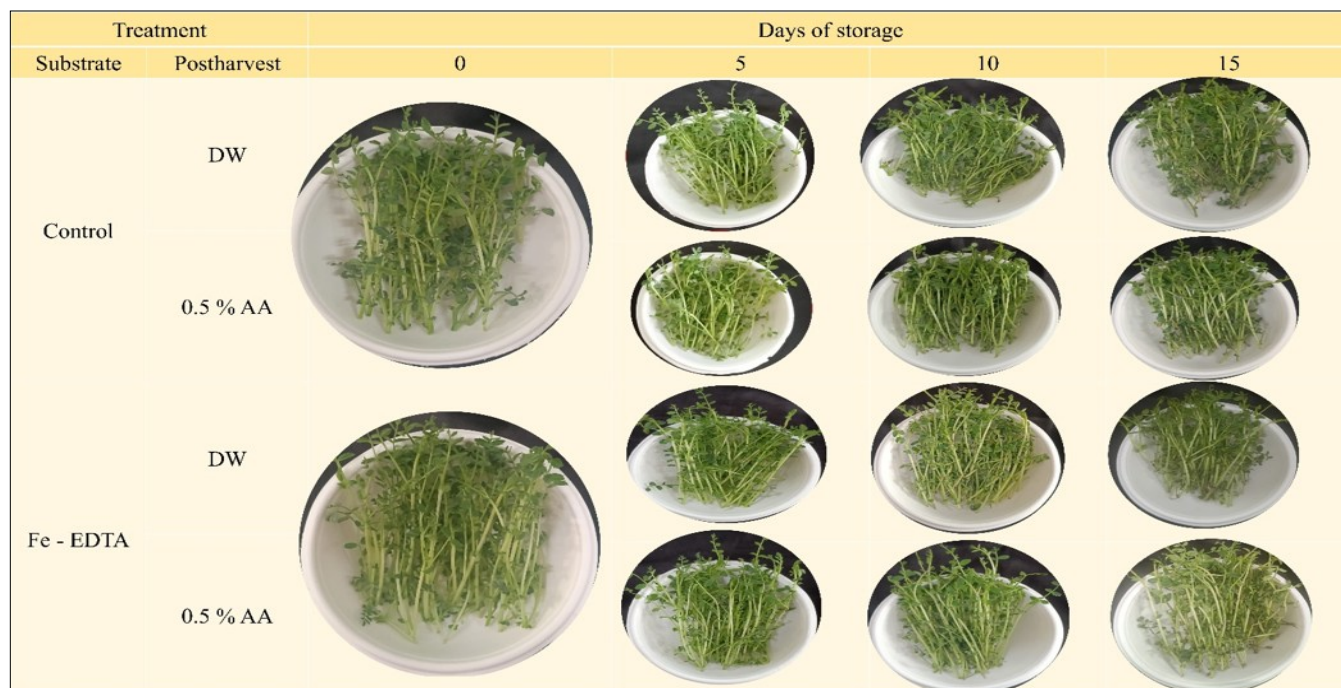
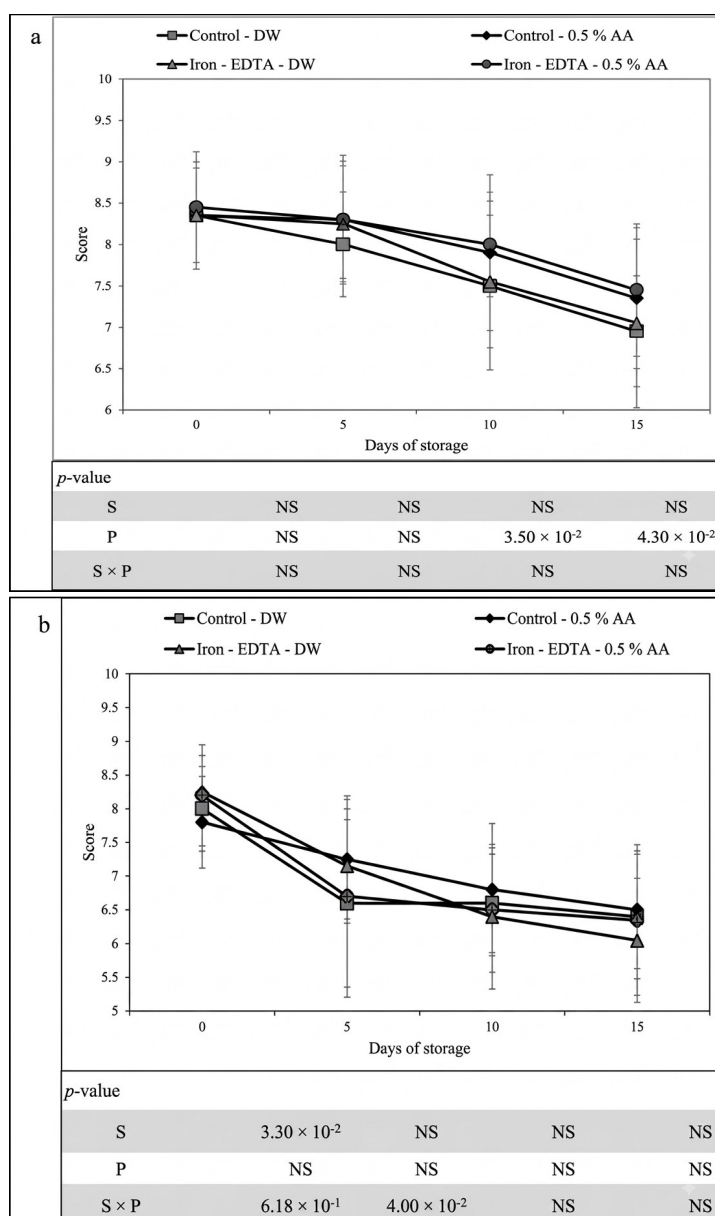


Fig. 4. Visual appearance with post-harvest applications in chickpea microgreens during storage.

DW: distilled water; AA: ascorbic acid; Fe-EDTA: iron-ethylenediaminetetraacetic acid.



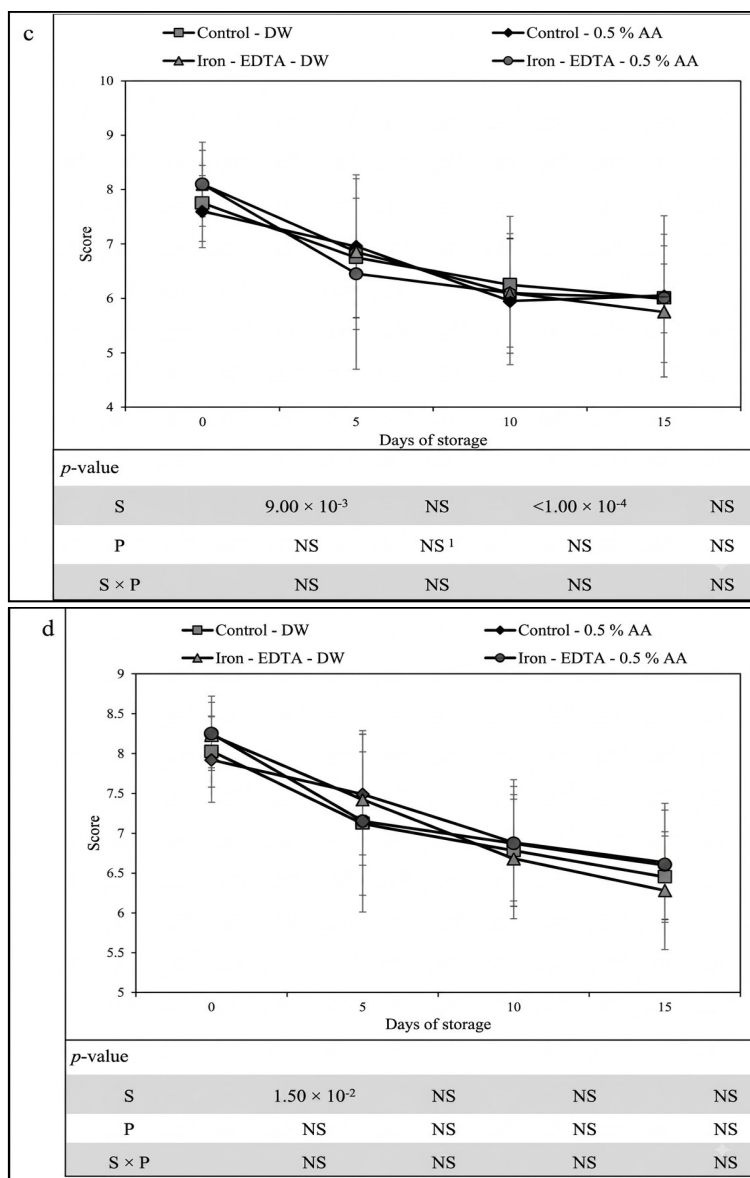


Fig. 5. (a) Colour and appearance, (b) taste, (c) mouthfeel and (d) overall acceptability scores of chickpea microgreens during storage. The table presents the *p*-values from the two-way ANOVA (**p* < 0.05). All values are expressed as mean ± standard deviation (*n* = 3).

S: substrate; P: post-harvest; NS: non-significant.

A similar positive correlation was observed with colour and appearance on day 5 ($r = 0.82$) and day 15 (0.99), as well as TSS on day 5 ($r = 0.92$) and day 10 ($r = 0.91$) of storage (Fig. 6).

The TSS exhibited a strong positive linear correlation with phytic acid on day 0 ($r = 0.93$), day 5 ($r = 0.91$), day 10 ($r = 0.91$) and day 15 ($r = 0.99$) and a strong negative correlation with tannins on the corresponding storage days ($r = -0.87, -0.97, -0.99$ and 0.93). The correlation between TSS and colour and appearance were also strong and positive on day 5 ($r = 0.95$), day 10 ($r = 0.89$) and day 15 ($r = 0.95$) of storage. Tannins exhibited a strong negative correlation with phytic acid on all days of storage ($r = 0.98, 0.98, 0.90$ and 0.89), with mouthfeel and overall acceptability on day 0 ($r = -0.87$ and -0.86) and with colour and appearance on day 5 ($r = -0.87$), day 10 ($r = -0.87$) and day 15 ($r = -0.83$). Colour and appearance showed a strong positive correlation with overall acceptability ($r = 0.84$) on day 10 and with phytic acid ($r = 0.93$) on day 15. Taste also exhibited a strong positive correlation with mouthfeel and overall acceptability on day 0 ($r = 0.98$ and 0.98) and day 15 ($r = 0.98$ and 0.88) and solely with overall acceptability on day 5 ($r = 0.99$). Mouthfeel also showed a strong and positive correlation with overall acceptability on day 0 ($r = 0.99$) and day 15 ($r = 0.91$) day of storage.

Principal component analysis

The biochemical (nutritional and anti-nutritional) and organoleptic parameters studied in chickpea microgreens during storage i.e. from day 0 to day 15, were subjected to principal component analysis (PCA) to extract the major principal components (PCs) explaining most of the variance in the dataset on each day of storage. The analysis revealed that the eigenvalues of the four extracted PCs ranged 8.49–0.54 on day 0, 7.03–4.01 on day 5, 8.03–2.86 on day 10 and 7.90–3.80 on day 15 of storage. The extracted PCs accounted for a cumulative variance of 100 %, with most of the variation in the dataset explained by PC1 and PC2 on all days of storage.

The scree plot shows the distribution of the calibrated eigen values with respect to each principal component as well as the differential contribution of each to the overall variation in the dataset (Fig. 7). The scree plot showed the highest eigen value for PC1, suggesting that it captured the maximum variability in the dataset. While a pronounced decline was observed from PC1 to PC2 in the scree plot from day 0 to day 10, on day 15 the decline was more prominent from PC1 to PC3, as indicated by the elbow around PC2 and PC3.

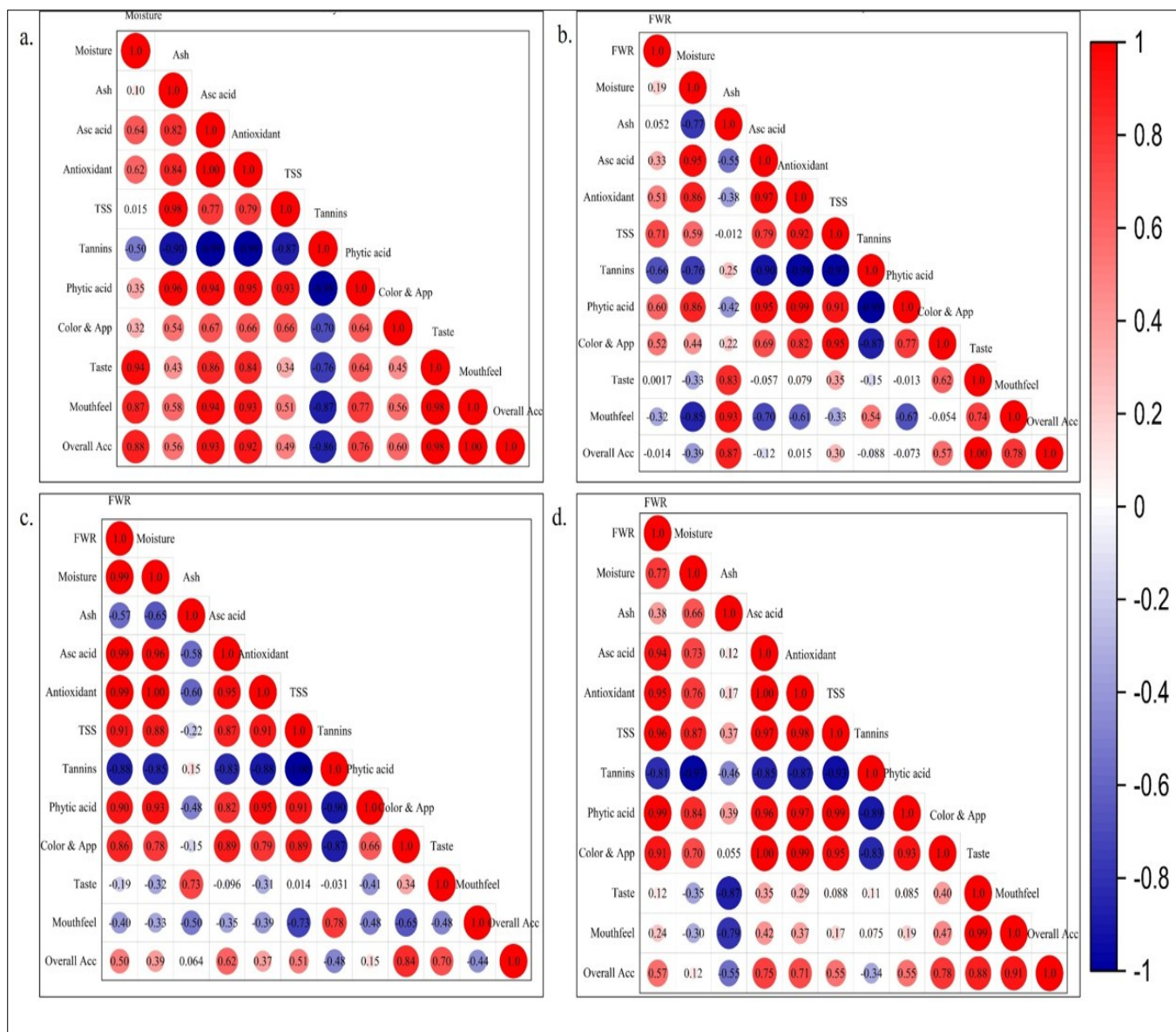
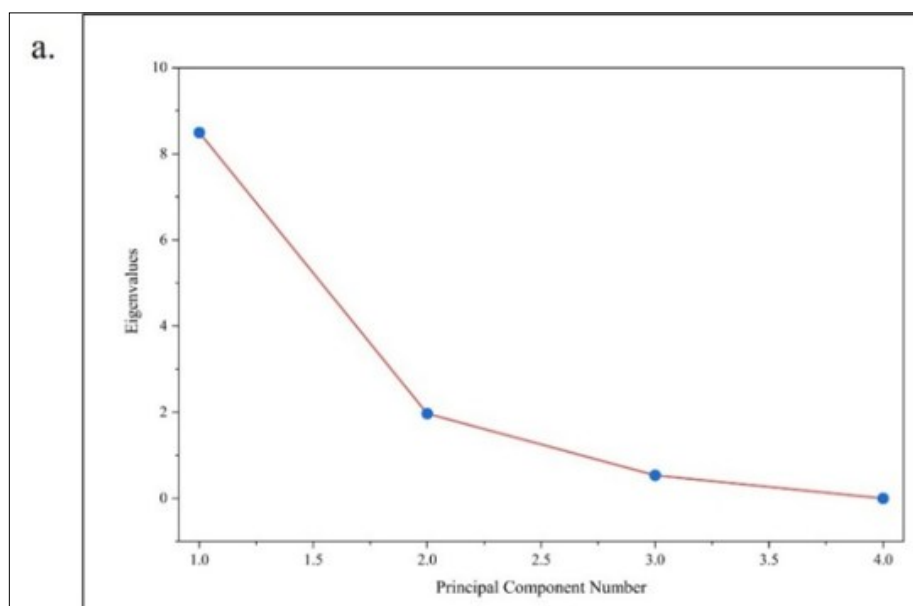


Fig. 6. Bubble heat maps of the correlation matrix based on Pearson's correlation coefficient (r), showing the degree of correlation among nutritional, anti-nutritional and organoleptic parameters in chickpea microgreens during storage ($*p \leq 0.05$). The colour gradient on the right represents the correlation coefficient, while the size of the circles indicates the strength of the correlation: (a) 0 day, (b) 5th day, (c) 10th day and (d) 15th day of storage.

FWR: fresh weight retention; Asc acid: ascorbic acid; TSS: total soluble solids; Colour & App: colour and appearance; Overall Acc: overall acceptability.



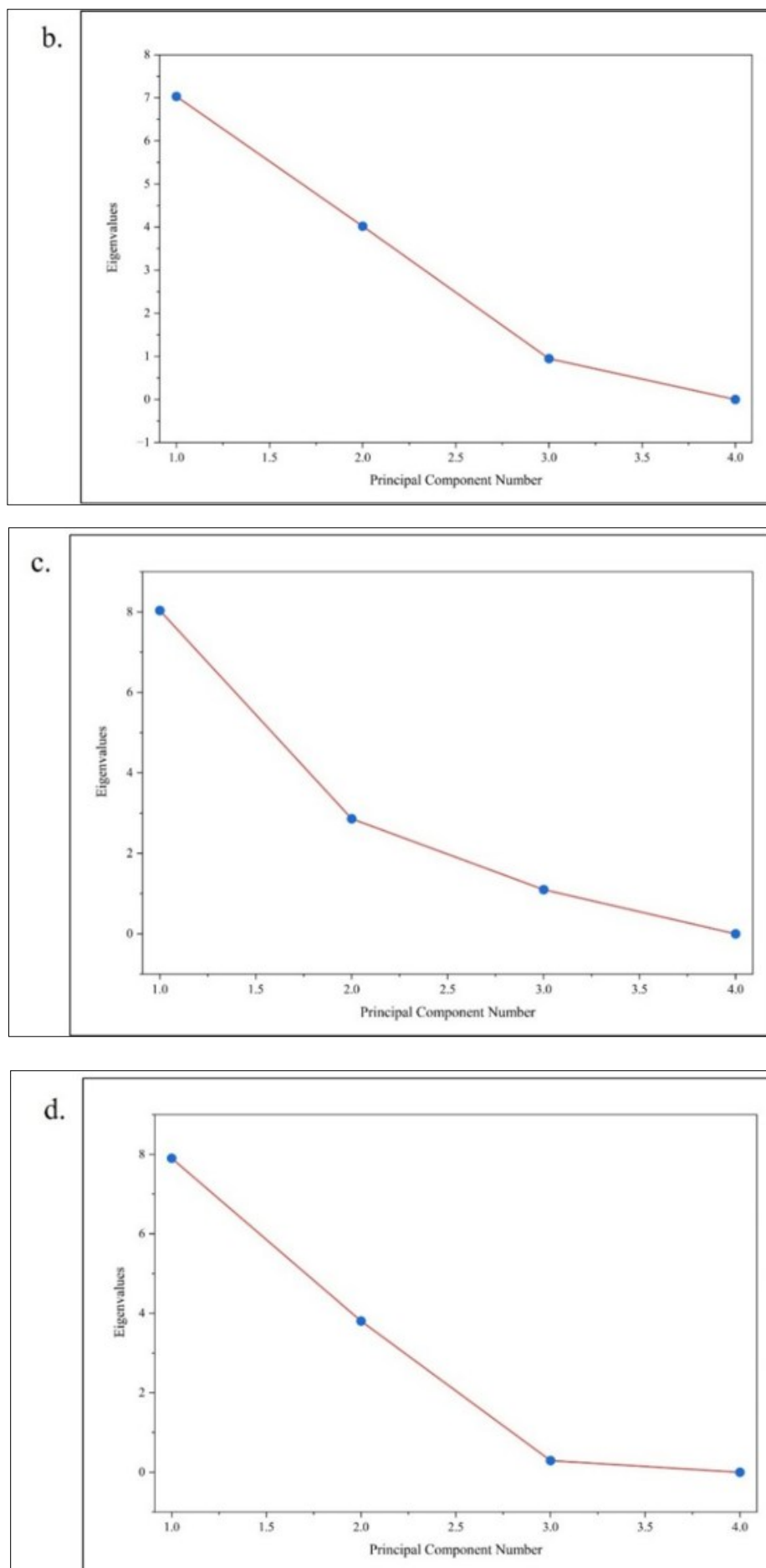


Fig. 7. Scree plots showing the eigenvalues of the first four principal components (PC1-PC4) obtained from principal component analysis (PCA) of biochemical (nutritional and anti-nutritional) and organoleptic parameters in chickpea microgreens during storage: (a) 0 day, (b) 5th day, (c) 10th day and (d) 15th day of storage.

On day 0, a cumulative variation of 95.12 % was explained by PC1 (77.21 %) and PC2 (17.91 %). The parameters formed two clusters: one in quadrant 1 (Q1), representing positive PC1 and PC2, and the other in quadrant 4 (Q4), representing positive PC1 and negative PC2 (Fig. 8). The organoleptic parameters (taste, mouthfeel and overall acceptability) and moisture content formed the first cluster (Q1) and showed an association with Fe-EDTA amendment in the growing medium and post-harvest application with distilled water (Fe-DW). The second cluster, comprising antioxidant activity, phytic acid, ash, TSS and colour and appearance in Q4, exhibited an association with the same substrate amendment and ascorbic acid post-harvest application (Fe-AA).

On the 5th day of storage, PC1 explained 58.62 % of the variation, while PC2 explained 33.50 %, accounting for a cumulative variation of 92.12 %. Phytic acid, antioxidant activity, FWR, TSS, colour and appearance clustered together in the positive regions of both PCs (Q1). In contrast, ash content, taste, mouthfeel and overall acceptability clustered in the negative PC1 and positive PC2 region (Q2), showing an association with control-grown microgreens subjected to ascorbic acid post-harvest application (control-AA), as well as microgreens grown under Fe-EDTA-amended conditions with distilled water post-harvest application (Fe-DW).

On 10th day of storage, PC1 and PC2 accounted for 90.83 % of the variation in the dataset, with each component depicting 67 % and 23.83 % of the variation respectively. The clustering pattern of the attributes on the 10th and 15th days of storage was similar to that on day 0. TSS, colour and appearance and overall acceptability clustered together in Q1 (positive PC1 and PC2), whereas ascorbic acid, FWR, antioxidant activity and moisture clustered in Q4 (positive PC1 and negative PC2). The attributes in quadrant 1 depicted a close association with control-grown microgreens subjected to ascorbic acid post-harvest application (control-AA). Cultivation under Fe-EDTA-amended growing conditions with post-harvest application of ascorbic acid (Fe-AA) was associated with the attributes in the positive PC1 and negative PC2 quadrant.

The cumulative variation on the 15th day was 97.56 %, with PC1 and PC2 accounting for 65.85 % and 31.71 % of the variation respectively. The organoleptic attributes of colour and appearance, taste, mouthfeel and overall acceptability, along with ascorbic acid and antioxidant activity, formed distinct cluster in the positive PC1 and PC2 quadrant. These attributes showed a strong association with microgreens cultivated under control conditions and subjected to ascorbic acid post-harvest application (control-AA). Furthermore, ash, moisture, fresh weight retention and phytic acid formed a cluster in the positive PC1 and negative PC2 and exhibited an association with Fe-EDTA amendment and ascorbic acid post-harvest application (Fe-AA). Tannins were associated with control cultivation and post-harvest application of distilled water (control-DW) on all days of storage.

Discussion

Post-harvest quality of microgreens is a crucial factor governing their consumer acceptance and marketability (26). Since they are young greens and their development relies on the breakdown of seeds-stored food reserves, physiological processes such as respiration and transpiration are accelerated, leading to senescence and consequently affecting their storage life (27). The traditional practice of sprinkling water to maintain freshness and texture is widely

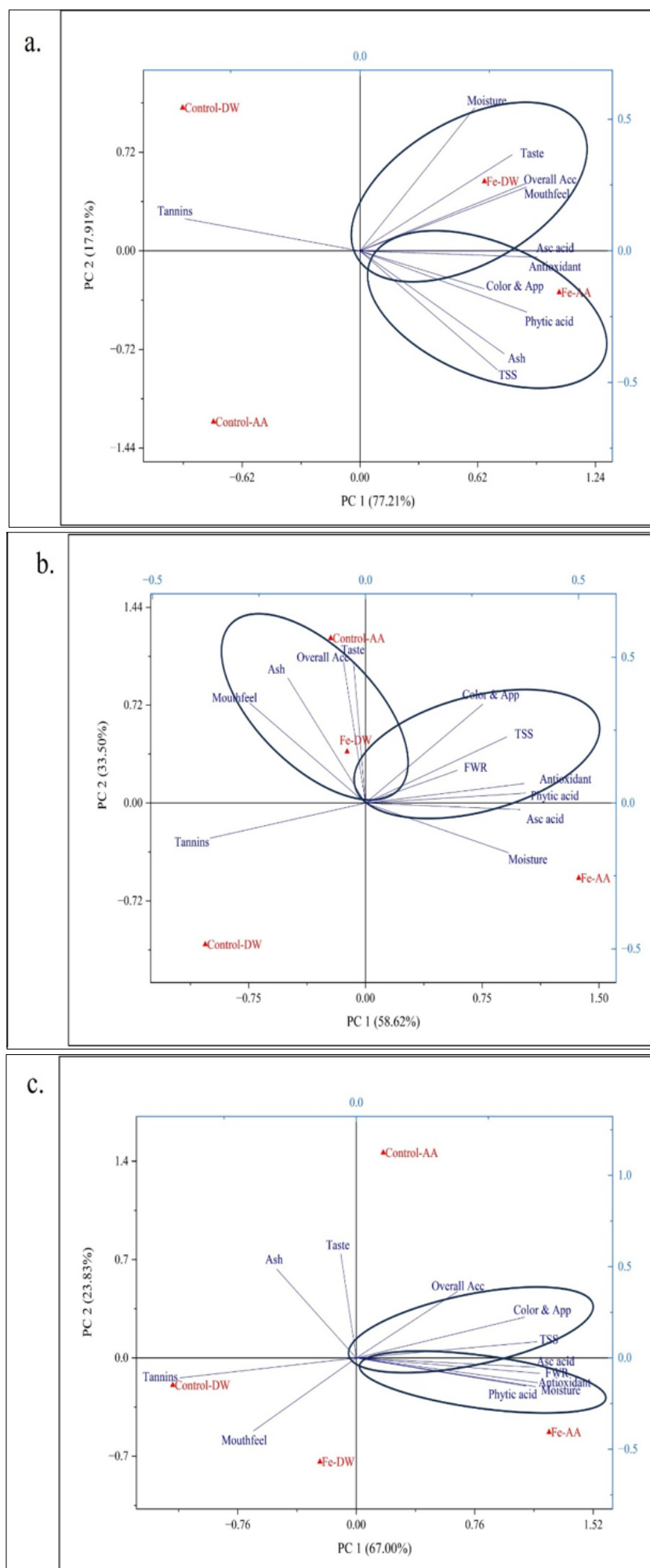
adopted locally, however, it may facilitate microbial proliferation, resulting in quality deterioration (28). This necessitates standardised storage temperature, post-harvest packaging under modified atmosphere for regulated oxygen (O₂) and carbon dioxide (CO₂) transmission and post-harvest treatment with chemicals belonging to GRAS category. The present study was conducted to elucidate shelf life of chickpea microgreens (genotype HK-4) and to understand nutrient profiling during storage under different cultural conditions. Seedlings were raised and the biomass was harvested on the 7th day of growth for shelf-life studies.

Harvested biomass was stored at 5 °C, as low temperature improves longevity by decelerating respiration and thereby reducing the cellular metabolism rate. This, in turn, slows down the degradation of pigments, phytoactive components and microbial growth, thereby maintaining visual quality and food safety, resulting in enhanced shelf-life (29, 30). Storage at low temperature (5 °C) also maintained quality and sensory attributes in mustard microgreens (31), reduced microbial proliferation and maintained maximum antioxidant activity in radish sango microgreens (17) and exhibited maximum retention of bioactive compounds in broccoli microgreens (14).

Utilisation of edible coatings of aloe vera gel and glycerol have been studied to enhance shelf life of fruits and vegetables by reducing microbial spoilage, weight loss and ion leakage, while maintaining high levels of ascorbic acid, anthocyanins, acidity and enzymes such as catalase, ascorbate peroxidase and superoxide dismutase (32). These coatings are biodegradable and harmless in nature and create an effective semi-permeable barrier by regulating stomata closure, moisture loss, regulating respiration and gas composition internal headspace, thereby curtailing the degradation of fresh produce (33). Application of aloe vera gel coating for shelf-life enhancement have been studied in radish, roselle, mustard and lettuce microgreens (8, 15), whereas the potential of glycerol for shelf-life improvement remains underexplored. Chlorine wash or dip treatment represents the most common and economical disinfectant approach to reduce microbial load and provide moisture to dehydrating greens (6). Chlorine wash enhanced the visual quality and reduced electrolyte leakage compared with unwashed ruby radish and daikon radish microgreens (6, 34). Citric and ascorbic acid are GRAS as they help retain nutritive properties and minimise microbial load without substantially affecting organoleptic attributes.

In view of this, chemical treatments including edible coatings of aloe vera gel and glycerol, along with chemical treatments such as chlorine wash and organic acids (citric acid and ascorbic acid), were tested for their efficacy as protectants for shelf-life enhancement. The preliminary analysis revealed that 0.5 % ascorbic acid application retained maximum fresh weight and maintained overall visual quality and acceptability under low temperature storage; hence, it was selected for post-harvest application in further experimental analysis.

Fresh weight retention of chickpea microgreens with respect to day 0 showed a declining trend during storage under both distilled water and ascorbic acid post-harvest application. The cumulative mean showed maximum fresh-weight retention when microgreens were raised under Fe-EDTA-amended nutrient conditions and subjected to ascorbic acid post-harvest application. Our findings are consistent with reports on mustard microgreens stored up to the 14th day (31). The findings are also in agreement with reports on broccoli



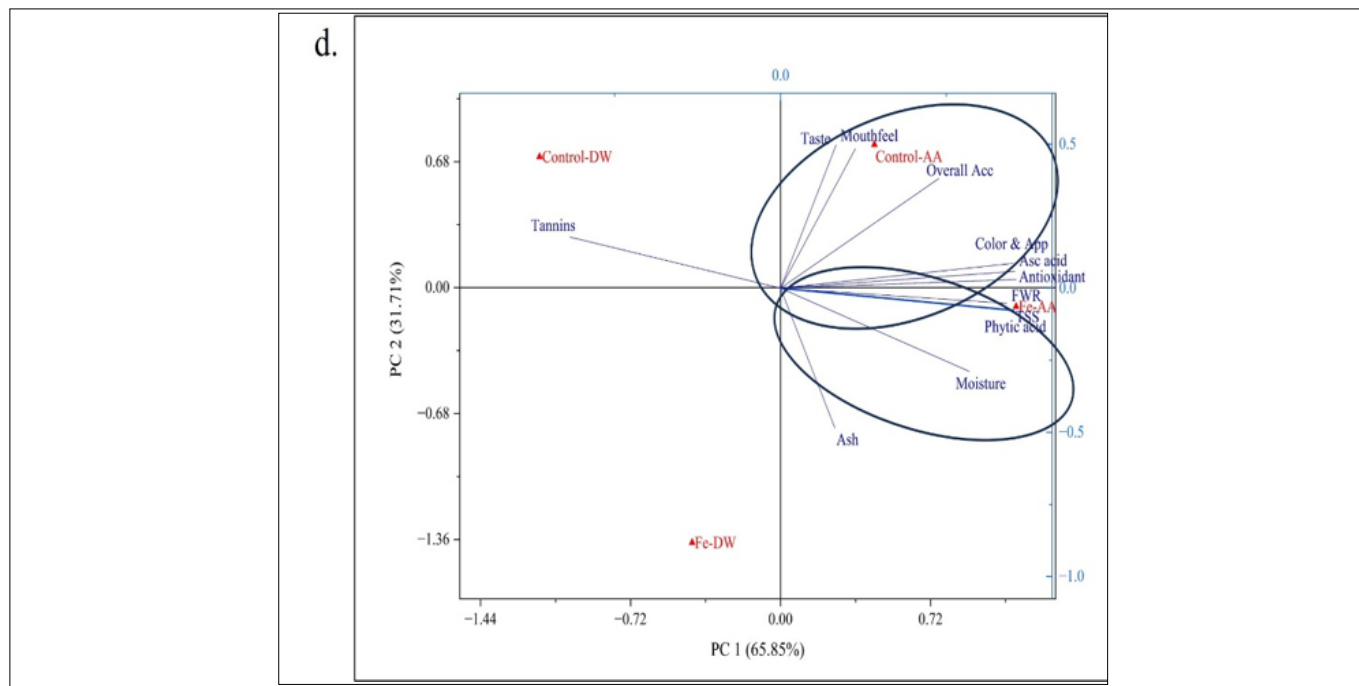


Fig. 8. Biplots showing the association among different quality parameters, growing conditions and post-harvest applications in chickpea microgreens during storage: (a) 0 day, (b) 5th day, (c) 10th day and (d) 15th day of storage.

Fe: iron; DW: distilled water, AA: ascorbic acid; FWR: fresh weight retention; Asc acid: ascorbic acid; TSS: total soluble solids; Colour & App: colour and appearance; Overall Acc: overall acceptability.

microgreens treated with citric and ascorbic acids and stored under modified atmosphere packaging (14).

Moisture content is a crucial factor influencing freshness and consumer perception. This parameter (%) exhibited a linear decline under both control and post-harvest ascorbic acid application conditions. The cumulative means were statistically similar in distilled water-grown plants and those grown under Fe-EDTA-amended conditions. Post-harvest application of ascorbic acid under Fe-EDTA growth conditions showed the highest moisture content, as it facilitates the maintenance of intracellular redox homeostasis under refrigerated storage conditions. Post-harvest application of ascorbic acid helped retain higher moisture in stored microgreens by creating a barrier to water loss and improving membrane stability in leafy vegetables and mustard microgreens (31, 35). Our findings are consistent with those reported in these studies. After harvest, delicate plant tissues experience reactive oxygen species (ROS) generation and undergo metabolic changes through transpiration and respiration, resulting in senescence and membrane disintegration, leading to moisture loss (3). Exogenous ascorbic acid application preserves structural integrity of membrane by suppressing ROS induced lipid peroxidation, thereby maintaining the organisation of membrane phospholipids and selective permeability (36). This reduces electrolyte leakage and water loss through transpiration, thereby improving moisture retention within cell for a prolonged duration.

Ash concentration (%) showed a gradual and variable increase under control conditions with increasing days of storage. With ascorbic acid application, ash concentration increased initially, followed by a slight decrease. The cumulative means were more or less similar under all treatments. A more prominent increase was observed in untreated samples compared with ascorbic acid-treated samples, which may be attributed to the delayed degradation or senescence processes reported in other studies (37).

Ascorbic acid content (mg/100 g) showed a decrease with post-harvest application of distilled water and ascorbic acid. Post-harvest application with ascorbic acid resulted in the highest ascorbic acid content under Fe-EDTA grown conditions, whereas it was lowest under control storage conditions in distilled water-grown plants. Application of ascorbic acid, being an antioxidant, may slow down the degradation of tissue ascorbic acid by counteracting reactive oxygen species and the results are consistent with reports on cabbage, radish, mustard and sunflower microgreens (38, 39). Elevated respiration after harvest, ascorbate oxidase and peroxidase enzyme activities could be responsible for the rapid oxidation of ascorbic acid to dehydroascorbic acid under physiological stress and senescence conditions during storage (40, 41).

Antioxidant activity (% DPPH scavenging potential) showed a pronounced reduction with increasing days of storage, irrespective of post-harvest application. The cumulative mean was highest with 0.5 % ascorbic acid application in Fe-EDTA-grown plants, whereas the lowest antioxidant activity was observed with distilled water application in control-grown plants. Low temperature storage and post-harvest ascorbic acid application might have resulted in reduced accumulation of reactive oxygen species and a lower rate of respiration. Post-harvest application with organic acid and storage at low temperature also retained the highest antioxidant activity in radish sango microgreens (17). Similarly, a combination of citric acid and ascorbic acid as post-harvest applications, along with storage at 5 °C under modified atmospheric packaging (15 % CO₂ + 5 % O₂ + N₂) maintained the highest antioxidant activity in broccoli microgreens up to the 12th day (14). This could be attributed to the application of ascorbic acid alone or in combination with citric acid, which resulted in slower depletion of antioxidant activity in sunflower microgreens (38), while low-temperature storage (4 °C) retained maximum antioxidant activity in mustard microgreens (31). These findings are comparable with the results of the present study.

Ascorbic acid as a post-harvest protectant lowers ascorbate oxidase-mediated oxidation of internal ascorbic acid reserves to dehydroascorbic acid by saturating enzymes active sites (42). Furthermore, applied ascorbic acid scavenges the ROS in coordination with the generation of antioxidant molecules through the ascorbate-glutathione cycle (43). It also collaborates with the internal antioxidant defence system by donating electrons to ascorbate peroxidase, which catalyses the reduction of hydrogen peroxide to water and regenerates itself through the coordinated action of enzymes involved in the ascorbate-glutathione cycle. Being a weak organic acid, it lowers the pH of the surface microenvironment, thereby reducing the activity of cellular degradative enzymes such as polyphenol oxidase and preventing oxidative damage of microgreens tissue (44). The combined antioxidant network minimises oxidative stress-induced disintegration of membrane lipids, proteins and other cellular components, thus delaying senescence-associated deterioration. Therefore, comparatively higher contents of ascorbic acid and overall antioxidant activity were maintained under ascorbic acid protectant application.

The Fe-EDTA nutrient supplementation in the growth substrate would have enhanced the synthesis of Fe-dependent antioxidant enzymes and photosynthetic proteins during growth, resulting in superior cellular reserves compared with control-grown microgreens. Preharvest intervention in combination with ascorbic acid-mediated antioxidant protection, could have improved cellular resilience to oxidative stress after harvest and during storage.

The TSS (%) exhibited a gradual reduction during storage under post-harvest application with distilled water versus ascorbic acid. The cumulative mean showed the best effect of ascorbic acid in Fe-EDTA grown microgreens, whereas the lowest effect was observed under distilled water application in control-grown microgreens. Differential metabolic rates and utilisation of stored soluble metabolites for physiological growth during storage could have contributed to this observation. Similar changes during storage have been reported in mung bean sprouts and sunflower microgreens (45, 46). In contrast, soluble solids in *Eruca sativa* Mill. microgreens increased initially during storage, followed by a decrease (47) which could be attributed to the permeability of the packaging material used. These results are contradictory to the findings of the present study. The elevated metabolic activity and respiration rates after harvest demand increased consumption of stored soluble carbohydrates in the tissues. As a protectant, ascorbic acid alleviates oxidative stress, moderates the cellular respiratory efficiency and preserves mitochondrial membrane integrity, consequently reducing the depletion of metabolic reserves such as sugars (48, 49). The subsequent reduction in the utilisation of respiratory substrate delays senescence, reduces moisture loss and results in prolonged freshness and improved post-harvest quality of microgreens.

Tannins exhibited an increase with storage under both post-harvest applications and the increase was more pronounced under distilled water. These secondary metabolites deter attack by pests, insects and herbivores in plants by imparting them bitter and astringent taste and inactivating their enzymes (50). In humans, they form complex with proteins and certain minerals, thereby reducing their absorption and utilisation (51). The increase in tannins may not be due to *de novo* synthesis but rather enhanced extraction. The cumulative mean showed the highest tannin content under post-

harvest distilled water application in control-grown microgreens, whereas it was lowest with 0.5 % ascorbic acid application when microgreens were grown with Fe-EDTA nutrient supplementation. Post-harvest application with ascorbic acid maintains phenols in reduced forms by limiting chain of oxidative reactions, thus minimising the extractability and detection of tannins. After harvest, plant tissue undergoes softening due to moisture loss and loss of cellular integrity, thereby releasing bound and insoluble tannins, which are a class of phenolic compounds (52). Low temperature and sealed packaging are known to suppress peroxidases and polyphenol oxidases, which may reduce tannin oxidation and the release of phenolic compounds, resulting in their spectrophotometric detection (53).

Concentration of phytic acid decreased under control and ascorbic acid post-harvest applications during storage. It is a major reservoir of phosphorus in plants which is a source of energy and nutrients during early growth stages and simultaneously acts as signalling molecule and natural antioxidant (54). Although it is known to bind with Fe, Zn, Mg and Ca making them unavailable in the human digestive tract, studies have reported their potential benefits for promoting cardiovascular health and as anti-cancer and anti-diabetic compounds (55). A progressive decline in phytic acid has been reported during germination in cereals and legumes, and it also decreases during prolonged storage of chickpea, green gram and soybean (56, 57). The cumulative mean was highest with ascorbic acid application in Fe-EDTA grown plants, whereas the effect of other treatments was more or less similar. The results are consistent with the findings in sprouted and germinated quinoa seeds and were attributed to an increase in phytase activity during storage (58).

Scores for colour and appearance and taste showed a gradual decline during storage. A slow deterioration in freshness, green appearance and quality of microgreens was also observed. Cumulative means were more or less similar for colour and appearance. Taste was maximum with ascorbic acid application and minimum in control grown plants. The decline in taste could be attributed to increased tannins which impart astringent and off-flavour to stored microgreens. Mouthfeel and overall acceptability scores also depicted a gradual decline under both post-harvest applications and cumulative means were more or less similar with post-harvest applications. Post-harvest application with ascorbic acid retained colour, appearance and overall acceptability till 4th day, for taste, texture and aroma till 8th day of storage in sunflower microgreens (38). Storage at low temperature (5 °C) maintained best sensory quality of mustard microgreens up to the 14th day of storage (31); however, deterioration in colour and appearance at low temperature can be due to degradation of chlorophyll (34, 59). During storage, ethylene-induced senescence results in chlorophyll degradation, leading to reduced visual appeal (60, 61).

Conclusion

The findings of the present study helped to elucidate that the post-harvest application of 0.5 % ascorbic acid performed the best compared to other protectants for enhancing the shelf-life of chickpea microgreens. The combination of Fe-EDTA supplementation during cultivation and post-harvest application of 0.5 % ascorbic acid improved fresh-weight retention, preserved antioxidant capacity and ascorbic acid content, maintained TSS and

sensory quality and moderated changes in anti-nutritional compounds during refrigerated storage. Thus, post-harvest application of 0.5 % ascorbic acid and growth under Fe-EDTA amended nutrient supplemented conditions can maintain nutritional and organoleptic quality during storage in chickpea microgreens. Future research may focus on designing appropriate packaging strategies including biodegradable nano packaging, application of chitosan based nano coatings or nanoparticles and light treatments for shelf-life improvement of chickpea microgreens under similar growth conditions.

Future scope

Future studies can be formulated to provide a framework or guidelines for the consumption of microgreens by humans to meet dietary requirements, along with evaluating the bioavailability of micronutrients (Fe and Zn) in chickpea microgreens on the 0th, 10th and 15th day of storage. Furthermore, comprehensive analyses to evaluate the effects of excessive consumption may also be undertaken.

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Authors' contributions

TS conducted the experiments, finalised and analysed the data, prepared the tables, aligned the figures and plates and wrote the original draft of the manuscript. AG supervised and coordinated the study. DWD planned the research, curated the data, reviewed and edited the manuscript and finalised the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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