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Metabolic, biochemical, mineral and fatty acid profiles of edible *Brassicaceae* microgreens establish them as promising functional food

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ABSTRACT

Hidden hunger due to micronutrient deficiencies affecting one in three people is a global concern. Identifying functional foods which provide vital health beneficial components in addition to the nutrients is of immense health relevance. Microgreens are edible seedlings enriched with concentrated minerals and phytochemicals whose dietary potential as functional foods needs evaluation. In this study, comprehensive biochemical, mineral, metabolic, and fatty acid profiles of four *Brassicaceae* microgreens- mustard, pak choi, radish pink, and radish white has been investigated. The biochemical profiling confirms their promising nutritional and antioxidant nature. Mineral profiling using ICP-MS exhibited promising levels of Fe, Mn, Mg, K, and Ca in microgreens indicating them as excellent sources of minerals. GC–MS based metabolite profiling highlighted a range of phytochemicals- sugars, amino acids, organic acids, amines, fatty acids, phenols, and other molecules. Fatty acid profiling established promising levels of health beneficial oleic acid and linoleic acids. It is estimated that fresh microgreens can meet about 20 % to 50 % recommended dietary allowance of macro/micro-minerals along with providing useful fatty acids and antioxidants. Overall, the study highlighted *Brassicaceae* microgreens as an excellent nutrient source that can act as functional foods with promising potential to overcome "hidden hunger".

Introduction

Despite innovation in global food production, the demand is expected to continuously increase by 35–56 % to feed an approximately 10 billion world population by 2050 (Dijk et al., 2021). According to the Food and Agriculture Organization (FAO), 25.8 % of the world's population could not have regular access to a healthy diet in 2019 (FAO, 2020). This includes the deficiency in multiple micronutrients while consuming an energy-efficient diet or a minimum number of calories, termed "hidden hunger." Identifying and adopting micronutrient-rich diets to existing foods will immensely benefit global health. Young edible seedlings, now termed microgreens, are being promoted as nutritionally promising candidates to address the problems of hidden hunger and malnutrition (Bhaswant et al., 2023).

Microgreens are young seedlings grown in soil or *in-vitro*-controlled environmental conditions from the seeds of vegetables and herbs, having two fully developed cotyledon leaves. Their harvesting stage depends upon the growing conditions and species type, varying from 7 to 21 days after germination (Xiao et al., 2012). The major families contributing to microgreens include *Brassicaceae, Asteraceae, Apiaceae, Amaryllidaceae, Amaranthaceae, Cucurbitaceae, Fabaceae, Poaceae,* and *Lamiaceae*. They are known for both flavoring and nutrition, and numerous studies have established their dietary importance in terms of mineral contents, vitamins, antioxidants, etc. (Lester et al., 2010). Studies on twenty-five different varieties of microgreens, such as arugula, celery, cilantro, radish, and amaranth, showed higher concentrations (4 to 40 times) of nutrients, antioxidants, and vitamins than mature plants (Xiao et al., 2012).

The *Brassicaceae* family is reported to be rich in antioxidants, phenolics, vitamins, minerals, and other phytochemicals like glucosinolates with anti-inflammatory and anticarcinogenic activity (Bell et al., 2017). It is shown that microgreens could fulfill children's dietary requirements for minerals such as Ca, Mg, Fe, Mn, Zn, Se, and Mo (Pinto et al., 2015). Furthermore, low potassium-containing microgreens were recommended for patients with reduced kidney function (Renna et al., 2018). Studies in mice suggest that when microgreens are supplemented with a high-fat diet, they can modulate weight gain and cholesterol metabolism and may protect against cardiovascular diseases by preventing hypercholesterolemia (Huang et al., 2016). These statements nominate microgreens to be considered a "Superfood." However, there is still a

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need for a comprehensive study to evaluate the antioxidant potential, phytochemical, and mineral (ionomics) profiling of *Brassicaceae* microgreens. In addition, it will be interesting to investigate fatty acids in *Brassicaceae* microgreens; being a rich source of oils, the unsaturated fatty acids available in the microgreens will be immensely relevant to health benefits.

This study is focused on biochemical analysis and comprehensive profiling of metabolites, minerals, and fatty acids of four *Brassicaceae* microgreens - mustard (*Brassica juncea*), pak choi (*Brassica rapa* subsp. *chinensis*), radish pink (*Raphanus sativus*), and radish white (*Raphanus ruphanistrum*). The optimal *in-vitro* cultivation of microgreens under controlled conditions was achieved for the study. The biochemical content of total proteins, carbohydrates, lipids, phenols, and antioxidants is evaluated. The mineral concentrations, metabolic and lipid profiles are investigated using sensitive analytical platforms of inductively coupled plasma-mass-spectrometry (ICP-MS), Gas chromatography–mass spectrometry (GC–MS), and GC-FID-FAME, respectively. Finally, we compared these profiles with nutritional significance, establishing the *Brassicaceae* microgreens as a promising functional food.

Material and methods

All the chemicals and reagents were purchased from Sigma Aldrich, unless mentioned in the methods.

Plant materials and growth conditions for microgreens

Four commonly consumed microgreens from the family *Brassicaceae* were selected in this study. The microgreen seeds of *Brassica juncea* (Mustard), *Brassica rapa* subsp. *chinensis* (Pak Choi), *Raphanus sativus* (Radish Pink), and *Raphanus ruphanistrum* (Radish white) were purchased from the local company "AllThatGrows" (https://www.allthatgrows.in/). Further substrate, seed density, and germination parameters were investigated for the efficient growth of these microgreens. In general, the seeds were sown in different combinations of coco-peat and vermiculite in the ratios 1:0, 3:1, 1:1, and 1:3 in a square petri plate (144 cm^2, HiMedia-PW050) with adequate moisture content. These were kept in dark for three days at 22 ± 2 °C temperature, 60 ± 5 % relative humidity (RH), and the germination percentage was calculated (Supplementary Table S1). Finally, the emerged seedlings were transferred to light with a 16/8-h light-dark cycle, after which the shoots of microgreens (6–9 cm height) were harvested at the fully grown, two-leaf stage (Day 7). The samples were quenched using liquid nitrogen and stored at −80 °C for further analysis.

Nutritional analysis in microgreens

Determination of total proteins

Total proteins were estimated according to the standard DC protein assay (Bio-Rad, catalog number 500–0116). For protein extraction, 20 mg of fresh sample was weighed and crushed in liquid nitrogen. Further, 200 μL of extraction buffer (40 $mmolL^{-1}$Tris–HCl, 250 $mmolL^{-1}$ Sucrose, 10 $mmolL^{-1}$ EDTA) was added to each vial (Wu et al., 2014). The content was then vortexed for 20 s and incubated in ice for 5 min. This step was repeated thrice for 15 min, followed by centrifugation at 12, 600 *g* for 20 min at 4°C. Next, the supernatant was collected and used for the assay. Next, 5 μL of this aliquot was taken, and 25 μL Reagent A' (an alkaline copper tartrate solution) was added. Further, 200 μL of Reagent B (a dilute Folin Reagent) was added, and the content was mixed slowly. After 15 min of incubation in the dark, the absorbance of the sample was taken at 750 nm in a microplate reader. Total proteins were estimated against BSA (Bovine serum albumin) standard curve.

Determination of total lipid

Lipid extraction and quantification were done using the gravimetric approach (Bligh & Dyer, 1959). Freshly harvested microgreens (1 g) were crushed with liquid nitrogen, and 3 ml of chloroform: methanol (1:2) was added. The samples were homogenized and centrifuged at 1000 *g* for 5 min. The supernatant was collected separately, and 3 ml of chloroform: methanol (1:2) with 0.8 mL of 1 % KCl was added to the pellet. The above centrifugation step was repeated, and both supernatants were pooled together. Further, 2 mL of chloroform and 1.2 mL of 1 % KCl was added to it and vortexed, followed by centrifugation at 1000 *g* for 5 min. Finally, the bottom layer was collected from the pooled lipid extract sample into a previously weighed centrifuge tube and subjected to solvent evaporation. The final weight of the tubes was recorded, and total lipids were calculated as an increase in weight.

Determination of total carbohydrates

20 mg of fresh microgreens sample were ground in a pestle and mortar using liquid nitrogen. Carbohydrate extraction was performed in 80 % ethanol (1 mL) (Bauer et al., 2022). The samples were vortexed and centrifuged at 12,600 *g* for 10 min. The supernatant was collected, and 4 mL of anthrone reagent (2 mg/mL in H_2SO_4) was added. The mixture was again vortexed and placed in a heat block at 100°C for 10 min. The tubes were allowed to cool at room temperature, and absorbance was recorded at 630 nm. Total carbohydrates were calculated using glucose as standard.

Determination of total phenolic compounds

Fresh microgreens (100 mg) were crushed in liquid nitrogen and mixed with 70 % acetone (2 mL) for phenols extraction. The samples were vortexed and centrifuged at 5600 *g* for 10 min at 4 °C. The procedure was repeated with 1 mL of 70 % acetone, and the supernatants were pooled. Next, 100 μL of this sample was mixed with 500 μL Folin-Ciocalteau reagent (10 % v/v in MiliQ water) and incubated for 5 min (Ainsworth and Gillespie 2007). The reaction was initiated by adding 400 μL sodium carbonate (5 % w/v in water) and setting it for 20 min in the dark at room temperature. Total soluble phenols were calibrated using Gallic acid as a standard (concentration ranging from 20 to 100 μg/mL) after absorbance was measured at 765 nm with a spectrophotometer against water as blank.

Determination of DPPH radical-scavenging activity

500 mg of fresh-weight of samples were taken and crushed in liquid nitrogen. 10 mL of 80 % ethanol was added, and the content was centrifuged at 5600 *g* for 15 min. The supernatant was collected in a separate vial. 900 μL of DPPH solution (0.1 $mmolL^{-1}$ DPPH in 80 % ethanol) was mixed with 100 μL of different concentrations of sample extract (five concentrations- 0.04 % to 0.2 %). The reaction mixture was vortexed and kept at room temperature in the dark for 30 min. The decrease in absorbance was recorded at 515 nm (Lingwan et al., 2021). Inhibition percent (I %) of the free radical DPPH• in microgreens samples was expressed as-

$$I\% = \left(A_{control} - A_{sample}\right) \times 100 / A_{control}$$

where: $A_{control}$ is the absorbance of blank DPPH solution; A_{sample} is the absorbance of samples. Finally, IC50 (50 % inhibitory concentration) was calculated, and values were compared to a positive ascorbic acid standard. The less the IC50 value to ascorbic acid (0.12 μg/mL), the more it is antioxidant.

Elemental analysis through ICP-MS

Powdered microgreens (100 mg dry weight) were subjected to acid digestion with 3.2 mL nitric acid and 800 μL of hydrogen peroxide (Zou et al., 2021). The samples were filtered using a 0.2- μm filter and diluted five times with MilliQ water. Finally, the samples were subjected to ICP-MS along with standards for quantification of the following elements- Na, Mg, K, Ca, Mn, Fe, Cu, and Zn. The quantities were expressed

in milligrams per gram dry weight (mg/g DW) for macro minerals and microgram per gram dry weight for micro minerals (μg/g DW). Further, the recommended dietary allowance (RDA) % was calculated as the percent contribution of minerals present in microgreens to the recommended levels by *FSSAI*.

Gas chromatography-mass spectrometry (GC–MS) based profiling of metabolites

Metabolite extraction was performed in lyophilized microgreens samples (25 mg) with 1.2 mL of 80 % methanol (Lisec et al., 2006). 20 μL of Ribitol (0.01 % w/v) was added to each sample as an internal standard for relative quantification. These were incubated in a Eppendorf SmartBlocks thermomixer at 70 °C with 1 *g* vortexing for 5 min and centrifuged at 9500 *g* for 15 min at 25 °C. Next, 50 μL of supernatant was dried in a speedvac (ThermoFisher-DNA SpeedVac System) for further derivatization step. 35 μL of pyridine containing methoxyamine hydrochloride (20 mg/mL) was added to each dried sample and incubated at 37 °C by vortexing for 2 h at 1 g. Later, 49 μL of MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide) was added to the tubes and incubated for 30 min. The sample was finally centrifuged at 9500 *g* for 10 min, and the supernatant was transferred to new inserts (0.2 mL volume) for GC–MS data acquisition (Masakapalli et al., 2014; Lingwan & Masakapalli, 2022).

GC–MS-based analysis was performed using Agilent Technology GC, model no. 7890B with a run time of 60 min in splitless mode using helium as carrier gas at a flow rate of 0.6 mL/min. The program was set initially at 50 °C temperature for 1 min, increasing to 200 °C for 4 min at the rate of 10 °C and finally to 300 °C at 5 °C/min for 10 min (Shree et al. 2019). The mass spectra were processed through Metalign software for baseline correction. Further, analyzing retention time and fragmentation patterns, metabolites corresponding to the peaks were identified through MassHunter Qualitative Navigator software using NIST version 2.3, 2017, and Fiehn Metabolomics 2013 libraries with an identity score of $\geq$ 70 %.

Lipid extraction and FAME-based profiling

For lipid extraction and transesterification, fresh microgreens samples (50 mg) were crushed in liquid nitrogen and saponified with 1 mL of saturated methanolic KOH at 100 °C for 30 min. After 2 min incubation at room temperature, 2 mL of 5 % HCl prepared in methanol was added to the extract and subjected to 80 °C for 10 min. Additionally, 1.25 mL solution of 1:1 n-hexane and methyl tertiary-butyl ether was added, and the mixture was gently mixed. The tubes were positioned upright for phase separation, and the top layer was collected and washed with 3 mL of 1.2 % KOH solution. Finally, a saturated NaCl solution was added to completely separate the n-hexane phase containing Fatty acid methyl esters (FAMEs) (Woo et al., 2012). 1 μL of these extracts were directly injected into the GC–MS equipped with HP-5 (30 m, 0.32 mm i.d., 0.25 μm) column. The method parameters include a run time of 40 min in splitless mode using helium as carrier gas at a flow rate of 1 mL/min. The program was set initially at 50 °C temperature for 1 min, increasing to 200 °C at 25 °C/min for 5 min and finally to 230 °C at 3 °C/min for 18 min.

Statistical and multivariate data analysis

The identified compounds and their respective abundances were subjected to multivariate statistical analysis using MetaboAnalyst 5.0 online tool. Data pre-processing was performed by normalization by the median, log transformation, and Pareto scaling. Finally, PCA (Principal component analysis), PLS-DA (Partial least square discriminant analysis) plots, and Heat maps were generated (Chong et al., 2019). The area of the internal standard, Ribitol, is used to obtain the relative proportions of the peaks, leading to the calculation of fold changes of metabolite/ peak levels among the treatments. Fundamental statistical methods were used to determine the significance or non-significance of data using GraphPad Prism8 software.

Results and discussion

Optimal growth of Brassicaceae microgreens

Substrate, Seed density, and germination percentage were optimized for the efficient growth of microgreens**.** Different ratios of Coco-peat: vermiculite (1:0, 3:1, 1:1, and 1:3) were tested as substrates for microgreens. Based on the maximum number of seeds germinated, the 1:1 ratio of coco-peat and vermiculite mixture was observed to be the best composition. The seeds of selected microgreen seeds were categorized under three seed densities (low, medium, and high) based on their relative sizes. Seed density of 2.5 g and 3 g for pakchoi and mustard, and 3.5 g for radish pink and radish white per 144 cm^2 (grown in square petri plate) was found to be optimum. The germination percentage of four selected microgreens was between 86.5 $\pm$ 9 % and 98 $\pm$ 4 %. There were no significant differences among the microgreens since all germinated efficiently on the cocopeat-vermiculite (1:1) substrate (Fig. 1A). Microgreens can grow on multiple substrates, jute mats, potting mixes, and hydroponically. Therefore, optimal substrate combinations are vital for producing safe and high-yield microgreens, and a coco-peat and vermiculite mixture could be considered.

Biochemical analysis show nutritional relevance and higher antioxidant nature of Brassicaceae microgreens

The study of biochemical analysis of *Brassicaceae* microgreens shed light on their promising nutritional and antioxidative potential. The total carbohydrates ranged between 32 and 86 mg/g of fresh weight (FW) in the microgreens (Fig. 1B). No apparent differences were found among mustard, radish pink, and radish white microgreens and are similar to the levels present in mature mustard leaves (U.S. Department of Agriculture, 2019). Pak choi microgreens showed 2 to 4-fold higher carbohydrate levels than the others (U.S. Department of Agriculture, 2019; Goyeneche et al., 2015). The total proteins were observed to be ranging from 7 mg/g FW to 17 mg/g FW in microgreens which were less than the total proteins reported in mature leaves of mustard, pak choi, and radish, i.e., 15 to 40 mg/g FW (Goyeneche et al., 2015; U.S. Department of Agriculture, 2019). The total lipids ranged between 3.9 mg/g FW and 7.5 mg/g FW. No significant differences were observed between mustard and pak choi microgreens. Also, both radish pink and radish white varieties had similar lipid content. However, the lipids were less in mustard and pak choi than in radish greens (Fig. 1B). Furthermore, these levels were equivalent to the mature *Brassicaceae* leaves (Goyeneche et al., 2015; U.S. Department of Agriculture, 2019).

The total polyphenols and DPPH scavenging activity of *Brassicaceae* microgreens established their promising antioxidant potential (Fig. 1B). The total polyphenol content (TPC) in microgreens ranged from 1.85 mg to 3.33 mg GAE/g FW which were higher compared to the whole mustard plant (3.5 mg/g dry weight) and mature leaves (17.7 mg/g dry weight) reported in the literature (Sun et al., 2018). We observed promising antioxidant potential of *Brassicaceae* microgreens as evidenced by DPPH radicle scavenging abilities with IC50 of 76.5 μg/mL to 161.5 μg/mL compared to the positive control ascorbic acid (IC50: 119.6 g/mL). Comparatively, similar values were noted in other microgreens previously reported (Ghoora et al., 2020). An antioxidant-rich diet reduces the risk of cardiovascular diseases, hypertension, and diabetes (Alissa & Ferns, 2017). In addition, polyphenols and other antioxidants function as scavengers and reduce oxidative stress, which helps manage chronic non-communicable diseases (Urquiaga & Leighton, 2000). Overall, TPC was higher in microgreens than in mature plants, which can directly correlate to healthy human nutrition.

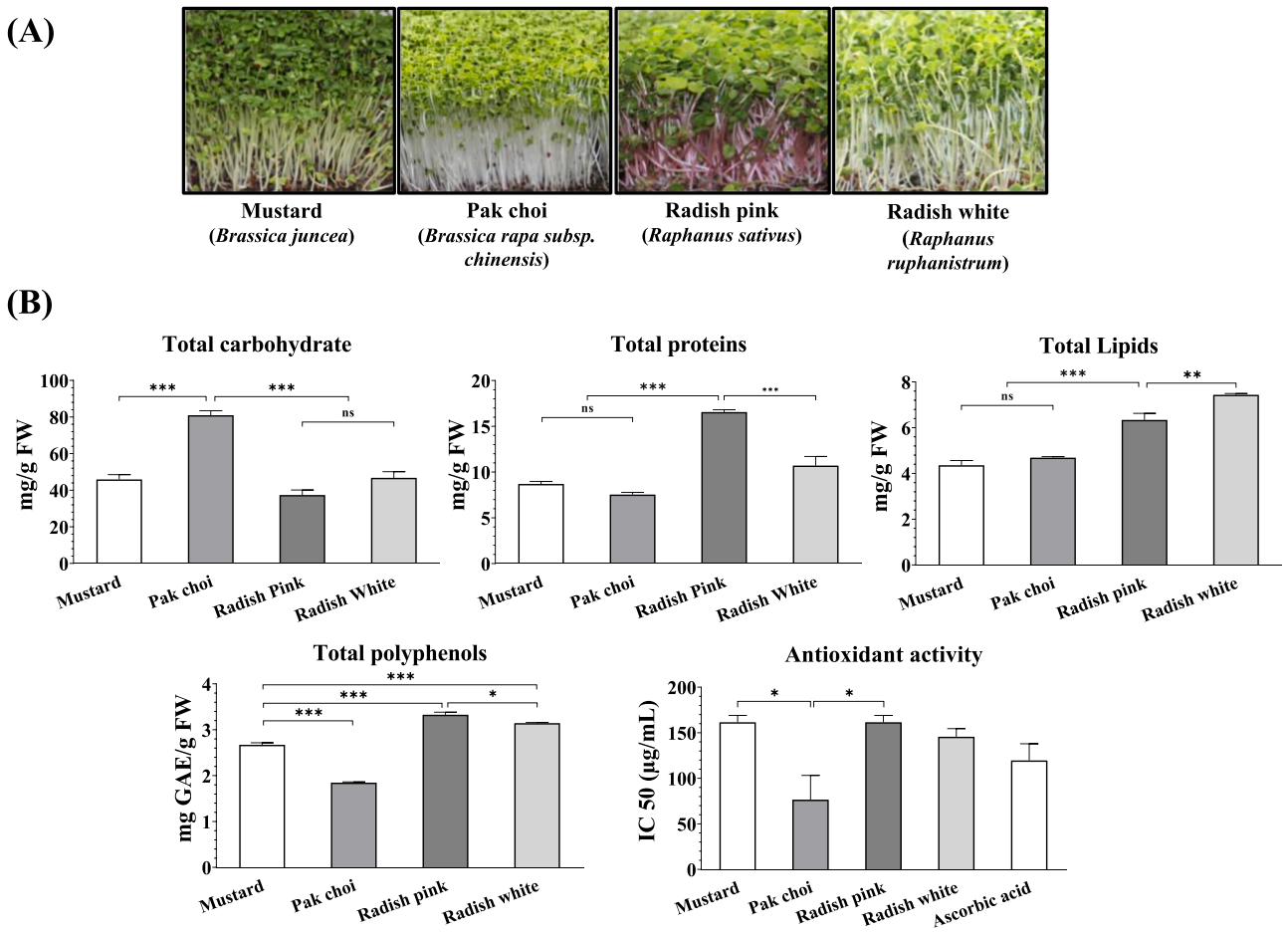

Fig. 1. (A) 7 days old microgreens of Mustard, Pak choi, Radish Pink, and Radish white grown on 1:1 cocopeat-vermiculite substrate. (B) Comparison of total carbohydrates, proteins, lipids, polyphenols, and antioxidant activity in *Brassicaceae* microgreens expressed in mg g^{-1} fresh weight (FW). Error bars represent the standard deviation for $n = 3$.

Brassicaceae microgreens are an excellent source of minerals

Minerals are necessary for any organism to function correctly and are vital to the human diet. They regulate physiological and biochemical activities, metabolism, and homeostatic balance (Kyriacou et al., 2021). Here we evaluated the *Brassicaceae* microgreens as a potential source of minerals (macro and micro) measured through ICP-MS analysis (Table 1). Macro minerals Na and K were found in concentrations ranging from 17 to 27 mg/g DW and 36 to 61 mg/g DW respectively. In comparison, Ca and Mg were present at 9 to 17 mg/g DW and 6 to 9 mg/g DW. Among the microminerals, Fe has the highest concentration (277 to 1092 µg/g DW), while Cu has the lowest concentration (7.5 to 10.4 µg/g DW). Additionally, Mn and Zn are present in the range between 53.5 µg/g and 206.5 µg/g DW of microgreens. The data was compared to the *Food Safety and Standards Authority of India* (FSSAI) 2020 recommendations for recommended dietary allowance (RDA). It is known that fruit and vegetable intake supply approximately 11 % Na, 24 % Mg, 35 % K, 7 % Ca, 21 % Mn, 16 % Fe, 30 % Cu, and 11 % Zn of recommended RDA to the human body (Levander, 1990). From the elemental profile, it is clear that all the selected *Brassicaceae*

Table 1
Different macro and micro minerals concentrations in *Brassicaceae* microgreens measured using ICP-MS. The estimated RDA% of minerals met via dietary source on consuming 10 g DW of microgreens daily (equivalent to 100 g FW) is tabulated and described as the percent contribution of minerals present in *Brassicaceae* microgreens to recommended dietary allowance (RDA) values approved by *FSSAI* 2020.

Macro-minerals (mg/g DW)	RDA (mg per day), by *FSSAI*	Mustard	RDA %[a]	Pak choi	RDA %[a]	Radish pink	RDA %[a]	Radish white	RDA %[a]
Na	2000	20.7 ± 1.44	10	26.3 ± 1.33	13	20.9 ± 1.99	10	18.2 ± 1.03	9
Mg	385	7.3 ± 0.52	19	8.6 ± 0.38	22	6.6 ± 0.70	17	6.9 ± 0.43	18
K	3500	56.3 ± 4.0	16	58.3 ± 3.1	17	52.9 ± 5.3	15	38.7 ± 2.5	11
Ca	1000	14.4 ± 1.5	14	16.9 ± 0.64	17	10.4 ± 0.98	10	9.4 ± 0.56	9
Micro-minerals (µg/g DW)	RDA (µg per day)								
Mn	4000	115.7 ± 8.8	29	88.5 ± 4.2	22	51.2 ± 5.1	13	50.4 ± 3.1	13
Fe	19,000	1021±71.9	51	266.4 ± 11.5	13	624.6 ± 66.1	31	293.6 ± 18.8	15
Cu	2000	7.4 ± 0.9	4	9.6 ± 0.8	5	8.0 ± 0.6	4	6.7 ± 0.8	3
Zn	17,000	135.9 ± 10.3	8	196.2 ± 10.3	12	136.3 ± 13.9	8	118.9 ± 6.9	7

[a] Percent RDA (%) met after consuming 10 g DW of Brassicaceae microgreens [compared with RDA 2020; FSSAI].

microgreens are excellent sources of macro and micro minerals. Consuming mustard microgreens (about 10 g DW) can fulfill 50 % RDA of iron (Table 1). Overall, these *Brassicaceae* microgreens can provide 10–20 % of the RDA for macronutrients and 4–50 % of the requirement for micronutrients, depending on the mineral type and species. The analysis confirmed that the levels of Fe, Mn, Mg, K, Ca, and Na in *Brassicaceae* microgreens were higher when compared with the mineral content of mature wild edible parts of mustard, pak choi, and radish (Filho et al., 2018; Kyriacou et al., 2021; Mezeyova et al., 2022). High levels of minerals have also been reported in other *Brassicaceae* microgreens, such as arugula, broccoli, and red cabbage, compared with their mature leaves (Johnson et al., 2021; Supplementary table S3). In addition, calcium and magnesium, critical elements in the human diet, were higher in all the *Brassicaceae* microgreens studied (Armesto et al., 2019).

Many of these macro and micro minerals are commonly deficient in the population of both developed and developing countries, the symptoms of which are not always immediately visible. Hence, including *Brassicaceae* microgreens in the diet which are observed to be dense in minerals, can assist in meeting daily needs and overcoming "hidden hunger."

Metabolite profiling shows microgreens are a rich source of bioactive compounds

The aqueous methanol soluble metabolite profiles of *Brassicaceae* microgreens using GC–MS captured sugars, amino acids, fatty acids, organic acids, and bioactive compounds, including various polyphenols, sugar alcohols, and amines (Supplementary Figure S1, Supplementary Table S2). Among the sugars and sugar alcohols, fructose, glucose, meso-erythritol, and threitol were predominant (Supplementary Figure S2). Other sugars identified are mannose, arabinose, glycerol, erythritol, glucopyranoside, and myoinositol. The amino acids; alanine, valine, leucine, isoleucine, glycine, serine, threonine, aspartic acid, glutamic acid, phenylalanine, asparagine, glutamine, lysine, and tyrosine were identified. These include essential amino acids, which can be of nutritional relevance. In addition, the branched-chain amino acids valine, leucine, and isoleucine, generally recommended in protein supplements for athletes, could be interesting. Myristic acid, palmitic acid, linolenic acid, and stearic acid are among the identified fatty acids. Organic acids found in microgreens, such as lactic acid, glycolic acid, citric acid, malic acid, amino-butanoic acid, glyceric acid, and butenedioic acid, may aid in human digestion (Nguyen & Kim, 2020). The

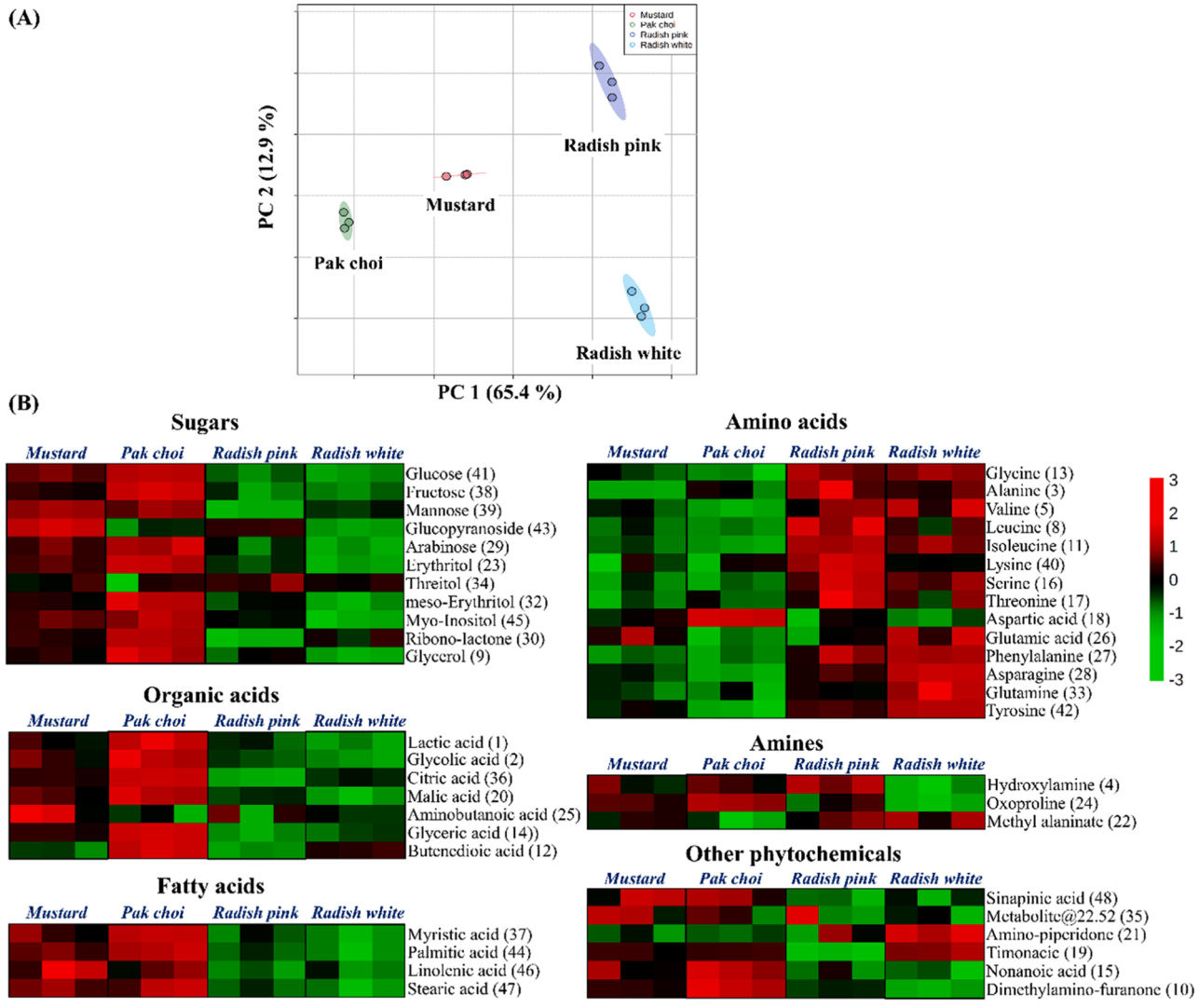

Fig. 2. Microgreens from the *Brassicaceae* family have distinct metabolic profiles. A) Principal Component Analysis (PCA) plots depict the variation among the *Brassicaceae* microgreens. B) The heat map shows the metabolic response to microgreens, characterized as sugars, amino acids, organic acids, amines, fatty acids, and other phytochemicals. For each of the observed metabolites, the average relative abundance ($n = 4$) is shown as a set of color-coded groups, from red (+3, high) to green (−3, low). The numbers against each metabolite correspond to the designated peaks observed in the GC–MS spectra depicted in Supplementary figure S1.

metabolite profiles also revealed the presence of polyphenols and amines of nutritional relevance in all the *Brassicaceae* microgreens. These include sinapinic acid, oxoproline, and hydroxylamine. In addition, amino furanone and an unknown metabolite at a retention 22.52 min were also reported. Overall, the metabolite profiles of microgreens showed several small molecules belonging to sugars, amino acids, fatty acids, organic acids, polyphenols, sugar alcohols, and amines that have nutritional significance.

Multivariate statistical analysis showed variations among the microgreen species

The variations in the metabolite profiles among the *Brassicaceae* microgreens were further captured via Multivariate statistical analysis. Principal component analysis (PCA) showed distinct clusters of microgreens represented by the first two principal components (PCs), where PC1 and PC2 explained 65.4 % and 12.9 % of the variance in metabolite profiles, respectively (Fig. 2A). Additionally, the relative abundances of identified metabolites among the microgreens were presented in bar graphs (Supplementary figure S3). This confirms that the composition of soluble metabolites among the microgreens is distinct, which could contribute to different attributes such as taste, color, smell, texture, etc., along with other parameters.

The heat map exhibited the response of microgreens' metabolites, classified as sugars, amino acids, organic acids, amines, fatty acids, and other phytochemicals. Results indicate that the metabolite profiles of mustard and pak choi are comparable with higher levels of identified sugars, organic acids, and fatty acids. Additionally, radish pink and radish white responded nearly similar with higher levels of all amino acids except aspartic and glutamic acids. The amines, hydroxylamine, oxoproline, and methyl alaninate were detected in relatively high amounts in mustard, pak choi, and radish pink, whereas radish white had low levels in their microgreens.

Lastly, metabolite profiling also detected significant levels of polyphenol sinapinic acid in the microgreens, with elevated levels in mustard and pak choi, followed by radish white and radish pink.

The fatty acid profile of Brassicaceae microgreens showed beneficial lipids

The FAMEs (fatty acid methyl esters) based profiling in *Brassicaceae* microgreens identified six primary fatty acids (saturated and unsaturated). Saturated fatty acids include palmitic acid, and stearic acid, whereas unsaturated fatty acids include oleic acid, linoleic acid, eicosenoic acid, and erucic acid (Fig. 3). Additionally, two terpenes, neophytadiene, and phytol, were also observed. The relative abundances of palmitic acid, stearic acid, oleic acid, and linoleic acid were similar among the microgreens. However, differences in the levels of erucic acid and phytol were observed. Erucic acid was less in radish pink and highest in radish white.

The presence of essential fatty acids and terpenes identified through GC–MS FAMEs, i.e., oleic acid, linoleic acid, neophytadiene, and phytol makes *Brassicaceae* microgreens nutritionally rich (Figs. 3,4). These unsaturated fatty acids are not synthesized in the human body and should be involved in diet. They play several roles in human nutrition, including the building block of the cell, cell membrane, hormone production, blood pressure regulation, inflammatory responses, etc. (Chen & Liu, 2020). Besides the essential fatty acids, average erucic acid levels were less than 5 % in all the *Brassicaceae* microgreens samples, which are considered safe for human ingestion (Fig. 4). Overall, the fatty acid profiles in microgreens are encouraging, given that many are nutritionally essential.

Conclusion

Microgreens are the edible young seedlings of various vegetables, herbs, and flowers consumed at the two-leaf stage. These are reported to be rich in essential nutrients and other health beneficial components for being considered as functional food. The current study evaluates the dietary potential through biochemical, minerals, metabolite, and fatty acid profiles of *Brassicaceae* microgreens- mustard, pak choi, radish pink, and radish white. Biochemical analysis showed promising antioxidant nature of *Brassicaceae* microgreens as evidenced by DPPH radicle scavenging abilities. The elemental profiles of mustard, pak choi, and radish microgreens established them to be an excellent source of Fe, Mn, Mg, K, Ca, and Na that can meet a decent proportion of Recommended dietary allowance. The metabolite profiles of microgreens showed variations

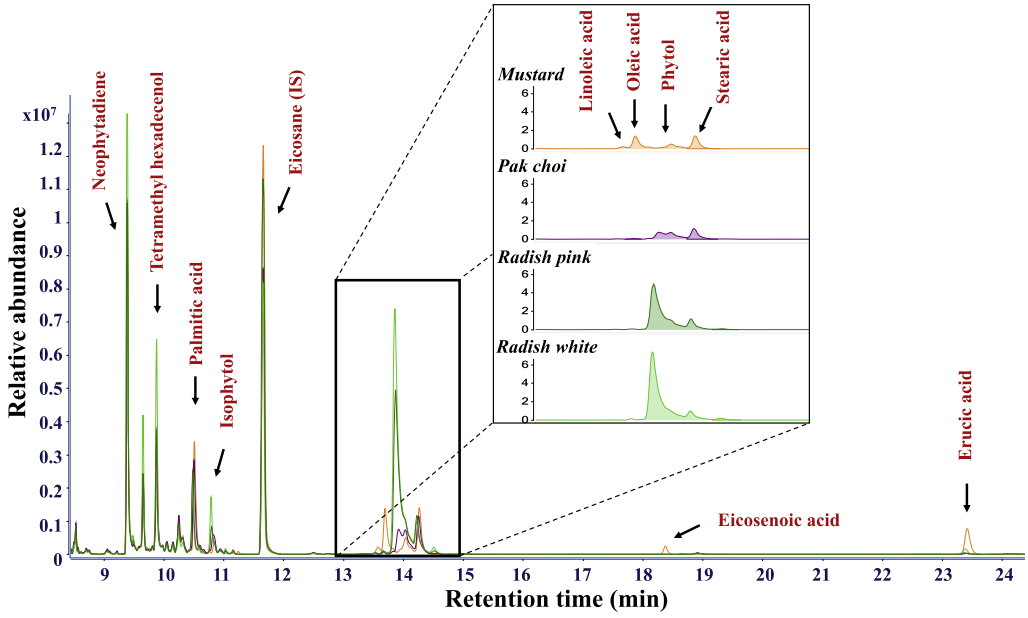

Fig. 3. *Brassicaceae* microgreens have a beneficial lipid composition in their fatty acid profile. The profiling using FAMEs (fatty acid methyl esters) revealed the presence of saturated and unsaturated fatty acids. The orange, purple, dark green, and light green GC-FAMES spectra of mustard, pak choi, radish pink, and radish white, respectively. Eicosane (IS) was used as an internal standard at a concentration of 0.01 %.

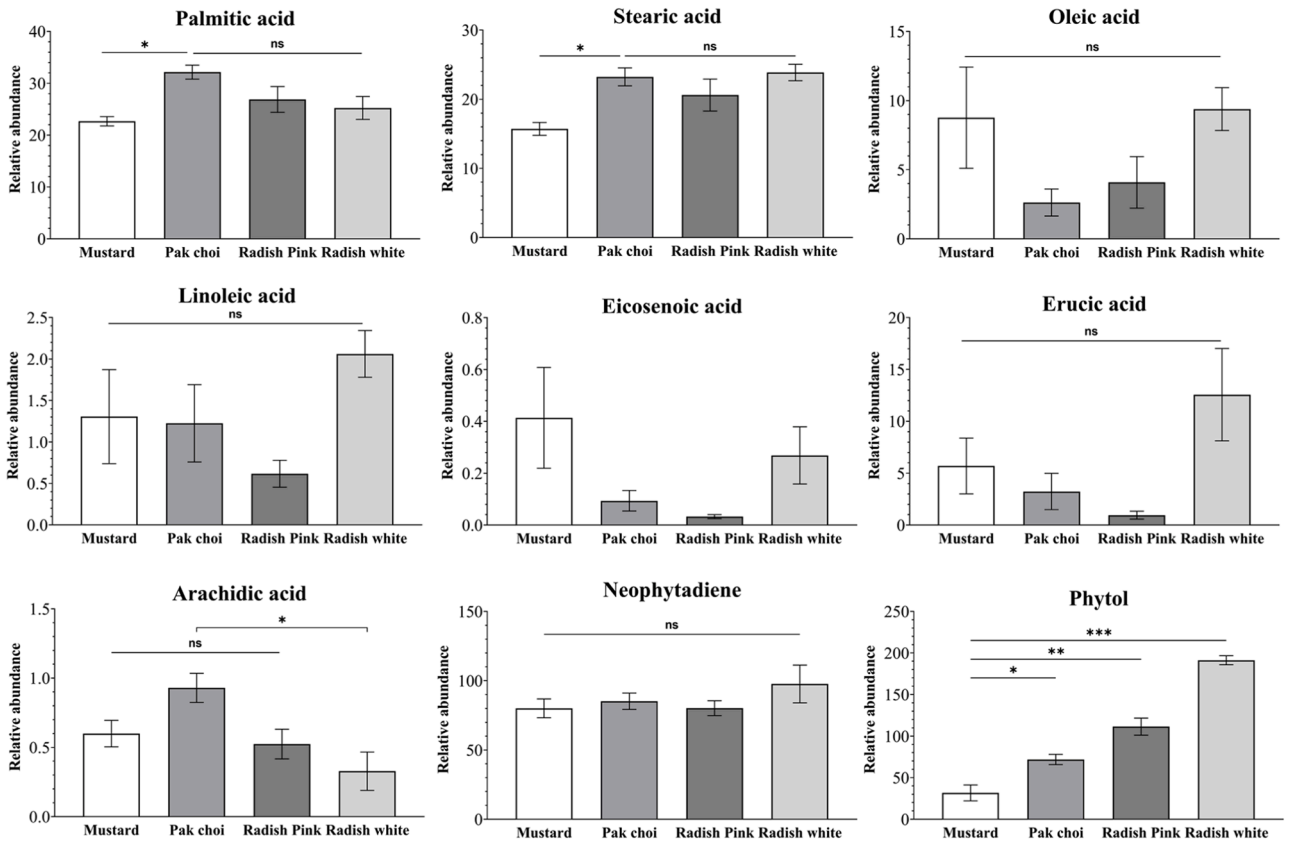

Fig. 4. Qualitative levels of primary fatty acids and terpenes in *Brassicaceae* microgreens identified through GC–MS. The relative peak area abundance was normalized with the internal standard Eicosane and expressed as mean ± standard deviation ($n = 3$). The abbreviation 'ns' denotes the absence of a statistically significant difference between the replicates.

among the sugars, amino acids, fatty acids, organic acids, polyphenols, sugar alcohols, and amines that have nutritional significance. The presence of essential fatty acids (oleic acid, linoleic acid) in mustard, pak choi, and radish microgreens makes them nutritionally relevant. These unsaturated fatty acids are not synthesized in the human body and should be involved in diet. All these nutritional parameters show that the *Brassicaceae* microgreens have health-beneficial role and can be used as excellent nutritive sources as functional food. It can be emphasised that microgreens can assist in overcoming "hidden hunger". Recent studies have shown that UV-B irradiation in plants can enhance their polyphenol levels. In future studies, such conditions can be optimized in microgreens to develop biofortified food.

Credit author statement

Yogesh Pant: performed experiments, writing original draft and conceptualization. Maneesh Lingwan and Shyam Kumar Masakapalli: review and editing, conceptualization and supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100461.

References

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature protocols, 2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Alissa, E. M., & Ferns, G. A. (2017). Dietary fruits and vegetables and cardiovascular diseases risk. *Critical reviews in food science and nutrition, 57*(9), 1950–1962. https://doi.org/10.1080/10408398.2015.1040487

Armesto, J., Gómez-Limia, L., Carballo, J., & Martinez, S. (2019). Effects of different cooking methods on the antioxidant capacity and flavonoid, organic acid and mineral contents of Galega Kale (Brassica oleracea var. acephala cv. Galega). *International journal of food sciences and nutrition, 70*(2), 136–149. https://doi.org/10.1080/09637486.2018.1482530

Bauer, N., Tkalec, M., Major, N., Talanga Vasari, A., Tokic, M., Vitko, S., Ban, D., Ban, S. G., & Salopek-Sondi, B. (2022). Mechanisms of Kale (*Brassica oleracea var. acephala*) tolerance to individual and combined stresses of drought and elevated temperature. *International Journal of Molecular Sciences, 23*(19), 11494. https://doi.org/10.3390/ijms231911494

Bell, L., Methven, L., Signore, A., Oruna-Concha, M. J., & Wagstaff, C. (2017). Analysis of seven salad rocket (*Eruca sativa*) accessions: The relationships between sensory attributes and volatile and non-volatile compounds. *Food chemistry, 218*, 181–191. https://doi.org/10.1016/j.foodchem.2016.09.076

Bhaswant, M., Shanmugam, D. K., Miyazawa, T., Abe, C., & Miyazawa, T. (2023). Microgreens- a comprehensive review of bioactive molecules and health benefits.

Molecules (Basel, Switzerland), 28(2), 867. https://doi.org/10.3390/molecules28020867

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology, 37*(8), 911–917. https://doi.org/10.1139/o59-099

Chen, J., & Liu, H. (2020). Nutritional indices for assessing fatty acids: A mini-review. *International journal of molecular sciences, 21*(16), 5695. https://doi.org/10.3390/ijms21165695

Chong, J., Wishart, D. S., & Xia, J. (2019). Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. *Current protocols in bioinformatics, 68*(1), e86. https://doi.org/10.1002/cpbi.86

FAO, IFAD, UNICEF, WFP and WHO. (2020). *The state of food security and nutrition in the world 2020. transforming food systems for affordable healthy diets*. Rome: FAO. https://doi.org/10.4060/ca9692en

Ghoora, M. D., Haldipur, A. C., & Srividya, N. (2020). Comparative evaluation of phytochemical content, antioxidant capacities and overall antioxidant potential of select culinary microgreens. *Journal of Agriculture and Food Research, 2*, Article 100046. https://doi.org/10.1016/j.jafr.2020.100046

Goyeneche, R., Roura, S., Ponce, A., Vega-Gálvez, A., Quispe-Fuentes, I., Uribe, E., & Di Scala, K. (2015). Chemical characterization and antioxidant capacity of red radish (Raphanus sativus L.) leaves and roots. *Journal of functional foods, 16*, 256–264. https://doi.org/10.1016/j.jff.2015.04.049

Huang, H., Jiang, X., Xiao, Z., Yu, L., Pham, Q., Sun, J., & Wang, T. T. (2016). Red cabbage microgreens lower circulating low-density lipoprotein (ldl), liver cholesterol, and inflammatory cytokines in mice fed a high-fat diet. *Journal of agricultural and food chemistry, 64*(48), 9161–9171. https://doi.org/10.1021/acs.jafc.6b03805

Johnson, S. A., Prenni, J. E., Heuberger, A. L., Isweiri, H., Chaparro, J. M., Newman, S. E., Uchanski, M. E., Omerigic, H. M., Michell, K. A., Bunning, M., Foster, M. T., & Weir, T. L. (2021). Comprehensive evaluation of metabolites and minerals in 6 microgreen species and the influence of maturity. *Current developments in nutrition, 5*(2), nzaa180. https://doi.org/10.1093/cdn/nzaa180

Kyriacou, M. C., El-Nakhel, C., Pannico, A., Graziani, G., Zarrelli, A., Soteriou, G. A., Kyratzis, A., Antoniou, C., Pizzolongo, F., Romano, R., Ritieni, A., De Pascale, S., & Rouphael, Y. (2021). Ontogenetic variation in the mineral, phytochemical and yield attributes of brassicaceous microgreens. *Foods (Basel, Switzerland), 10*(5), 1032. https://doi.org/10.3390/foods10051032

Lester, G. E., Hallman, G. J., & Perez, J. A. (2010). γ-Irradiation dose: Effects on baby-leaf spinach ascorbic acid, carotenoids, folate, α-tocopherol, and phylloquinone concentrations. *Journal of agricultural and food chemistry, 58*(8), 4901–4906. https://doi.org/10.1021/jf100146m

Levander, O. A. (1990). Fruit and vegetable contributions to dietary mineral intake in human health and disease. *HortScience : a publication of the American Society for Horticultural Science, 25*(12), 1486–1488. https://doi.org/10.21273/HORTSCI.25.12.1486

Lingwan, M., & Masakapalli, S. K. (2022). A robust method of extraction and GC-MS analysis of Monophenols exhibited UV-B mediated accumulation in Arabidopsis. *Physiology and Molecular Biology of Plants, 28*(2), 533–543. https://doi.org/10.1007/s12298-022-01150-2

Lingwan, M., Shagun, S., Pahwa, F., Kumar, A., Verma, D. K., Pant, Y., Kamatam, L. V. K., Kumari, B., Nanda, R. K., Sunil, S., & Masakapalli, S. K. (2021). Phytochemical rich Himalayan *Rhododendron arboreum* petals inhibit SARS-CoV-2 infection in vitro. *Journal of Biomolecular Structure and Dynamics*, 1–11. https://doi.org/10.1080/07391102.2021.2021287

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry–based metabolite profiling in plants. *Nature protocols, 1*(1), 387–396. https://doi.org/10.1038/nprot.2006.59

Masakapalli, S. K., Bryant, F. M., Kruger, N. J., & Ratcliffe, R. G. (2014). The metabolic flux phenotype of heterotrophic Arabidopsis cells reveals a flexible balance between the cytosolic and plastidic contributions to carbohydrate oxidation in response to phosphate limitation. *The Plant Journal, 78*(6), 964–977. https://doi.org/10.1111/tpj.12522

Mezeyova, I., Hegedűsova, A., Golian, M., Andrejiova, A., Slosar, M., & Mezey, J. (2022). Influence of microgreens biofortification with selenium on their quantitative and qualitative parameters. *Agronomy, 12*(5), 1096. https://doi.org/10.3390/agronomy12051096

Nguyen, D. H., & Kim, I. H. (2020). Protected organic acids improved growth performance, nutrient digestibility, and decreased gas emission in broilers. *Animals, 10*(3), 416. https://doi.org/10.3390/ani10030416

Paula Filho, G. X., Barreira, T. F., Santos, R. H., Priore, S. E., Della Lucia, C. M., & Pinheiro-Sant'Ana, H. M (2018). Chemical composition, carotenoids, vitamins and minerals in wild mustard collected in native areas. *Horticultura Brasileira, 36*, 59–65. https://doi.org/10.1590/S0102-053620180110

Pinto, E., Almeida, A. A., Aguiar, A. A., & Ferreira, I. M. (2015). Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. *Journal of Food Composition and Analysis, 37*, 38–43. https://doi.org/10.1016/j.jfca.2014.06.018

Renna, M., Castellino, M., Leoni, B., Paradiso, V. M., & Santamaria, P. (2018). Microgreens production with low potassium content for patients with impaired kidney function. *Nutrients, 10*(6), 675. https://doi.org/10.3390/nu10060675

Shree, M., Lingwan, M., & Masakapalli, S. K. (2019). Metabolite profiling and metabolomics of plant systems using 1H NMR and GC-MS. *OMICS-based approaches in plant biotechnology, 129*, 129–144. https://doi.org/10.1002/9781119509967.CH7

Sun, B., Tian, Y. X., Jiang, M., Yuan, Q., Chen, Q., Zhang, Y., Luo, Y., Zhang, F., & Tang, H. R. (2018). Variation in the main health-promoting compounds and antioxidant activity of whole and individual edible parts of baby mustard (*Brassica juncea var. gemmifera*). *RSC advances, 8*(59), 33845–33854. https://doi.org/10.1039/C8RA05504A

USDA. U.S. department of agriculture, agricultural research service. FoodData Central, 2019. fdc.nal.usda.gov.

Urquiaga, I., & Leighton, F. (2000). Plant polyphenol antioxidants and oxidative stress. *Biological research, 33*(2), 55–64. https://doi.org/10.4067/s0716-97602000000200004

van Dijk, M., Morley, T., Rau, M. L., & Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food, 2*(7), 494–501. https://doi.org/10.1038/s43016-021-00322-9

Woo, S. G., Yoo, K., Lee, J., Bang, S., Lee, M., On, K., & Park, J. (2012). Comparison of fatty acid analysis methods for assessing biorefinery applicability of wastewater cultivated microalgae. *Talanta, 97*, 103–110. https://doi.org/10.1016/j.talanta.2012.04.002

Wu, X., Gong, F., & Wang, W. (2014). Protein extraction from plant tissues for 2DE and its application in proteomic analysis. *Proteomics, 14*(6), 645–658. https://doi.org/10.1002/pmic.201300239

Xiao, Z., Lester, G. E., Luo, Y., & Wang, Q. (2012). Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. *Journal of Agricultural and Food Chemistry, 60*, 7644–7651. https://doi.org/10.1021/jf300459b

Zou, L., Tan, W. K., Du, Y., Lee, H. W., Liang, X., Lei, J., Striegel, L., Weber, N., Rychlik, M., & Ong, C. N. (2021). Nutritional metabolites in *Brassica rapa subsp. chinensis var. parachinensis* (choy sum) at three different growth stages: Microgreen, seedling and adult plant. *Food Chemistry, 357*, Article 129535. https://doi.org/10.1016/j.foodchem.2021.129535