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Bioactive Composition and Nutritional Profile of Microgreens Cultivated in Thailand

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Abstract: Microgreens are young and tender leafy vegetables that have gained wider consumer acceptance. This is attributed to their low caloric composition and rich micronutrient and antioxidant composition. The present study investigated the bioactive composition and proximate analysis of fourteen microgreens belonging to Brassicaceae, Fabaceae, Pedaliaceae, Polygonaceae, Convolvulaceae, and Malvaceae. All the microgreens showed low calories (20.22 to 53.43 kcal 100 g^{-1}) and fat (0.15 to 0.66 g 100 g^{-1}), whilst mung bean and lentil microgreens showed considerable amounts of carbohydrate (7.16 g 100 g^{-1}) and protein (6.47 g 100 g^{-1}), respectively. Lentil microgreens had the highest total chlorophyll (112.62 mg 100 g^{-1}) and carotenoid (28.37 mg 100 g^{-1}) contents, whilst buckwheat microgreens showed the highest total phenolic content (268.99 mg GAE 100 g^{-1}) and DPPH• scavenging activity (90.83 mM TEAC g^{-1}). The lentil microgreens also presented high ascorbic acid content (128.70 mg 100 g^{-1}) along with broccoli, Chinese kale, purple radish, and red cabbage microgreens (79.11, 81.33, 82.58, and 89.49 mg 100 g^{-1}, respectively). Anthocyanin content was only detected in purple radish (0.148 mg CGE 100 g^{-1}) and red cabbage (0.246 mg CGE 100 g^{-1}). The results provide basic information and highlight the benefits of utilizing genetic biodiversity to obtain microgreens with the desired nutrients and antioxidants.

Keywords: antioxidants; bioactive compounds; proximate composition; microgreens

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1. Introduction

In recent years, the attention towards microgreens is increasing due to the rise in public awareness of healthy eating worldwide. Microgreens are tender immature greens produced from the seeds of vegetables, herbs, or grains, inclusive of the wild relatives [1]. Microgreens can be cultivated in loose and soilless germination media and harvested 7–21 days after germination (species-dependent) when the cotyledon leaves have fully developed and the first true leaves have emerged [2]. These miniature greens are valued as nutraceuticals and functional foods owing to their health-promoting and disease-preventing properties in addition to their nutritional value. For instance, broccoli sprouts and microgreens have higher contents of bioactive compounds and potential antioxidants and exhibit higher anti-inflammatory and anticancer activities compared to their corresponding adult plants [3,4]. Microgreens are low in energy but rich in nutrients, bioactive compounds, and antioxidants [5]. During seed germination, biochemical changes occur within the seed, causing the activation of various enzymes related to the degradation of macromolecules into smaller molecules that the body can absorb easily. The changes also include the synthesis of biochemical compounds that affect germination, thus causing the amount of nutrients such as vitamins and antioxidants to increase rapidly. These nutrients and antioxidants all are beneficial to the human body as they can be absorbed quickly [6].

Crops that are commonly used for microgreen production are mung beans, soybeans, broccoli, alfalfa, lentils, mustard, radishes, red clover, and sunflowers [7–9]. The bioactive values obtained from wheat, lentils, radishes, and sunflowers show the microgreens tend to be richer in saturated fatty acids such as palmitic acid than unsaturated fatty acids [7]. On the other hand, alfalfa microgreens have significantly higher amounts of unsaturated fatty acids such as oleic and linoleic acids than those of other plants. For radish sprouts, it was found that the content of glucosinolates increased after germination. Glucosinolates are beneficial to humans in cancer prevention [10].

The popularity of microgreens also comes from the attractiveness of their shapes, colors, crispy texture, and unique flavor to both children and adults. Some microgreens also have a strong aroma. Moreover, their short production cycle has attracted greenhouse growers and small-scale farmers, thus, generating income for the farmers. The farmers are able to produce multiple cycles of microgreens compared to mature vegetables. Although a wide variety of microgreens are currently being produced, scientific data on their basic nutritional information and functional food potential are not widely available [2]. Most of the information is from temperate regions with little representation for tropical microgreens. Therefore, the main objectives of this study were to investigate the bioactive compounds and proximate composition of microgreens of tropical and subtropical origins, including from the family Brassicaceae, Fabaceae, and others. This information would provide a database and scientific evidence that is useful to farmers and consumers, both local and international. Furthermore, the data produced could add value to agricultural products, expand export opportunities as well as reduce the import of health food supplements, which are expensive but very popular today.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

In this study, 14 microgreens belonging to the family of Brassicaceae (broccoli, Chinese kale, purple radish, radish, rat-tailed radish, and red cabbage), Fabaceae (fenugreek, green pea, lentil, and mung bean), Pedaliaceae (black sesame), Polygonaceae (buckwheat), Convolvulaceae (morning glory), and Malvaceae (roselle) were selected. The scientific nomenclature of the selected microgreens is shown in Table 1. The experiment was conducted using a completely randomized design (CRD).

Table 1. Common names and scientific nomenclature of the studied microgreens.

Common Name	Scientific Name	Family
Broccoli	*Brassica oleracea* L.var. *italica*	Brassicaceae
Chinese kale	*Brassica oleracea* L.var. *alboglabra*	Brassicaceae
Purple radish	*Raphanus sativus* L. var. *longipinnatus*	Brassicaceae
Radish	*Raphanus sativus* L.	Brassicaceae
Rat-tailed radish	*Raphanus caudatus* L. var. *caudatus* Alef	Brassicaceae
Red cabbage	*Brassica oleracea* L.var. *capitata f. rubra*	Brassicaceae
Fenugreek	*Trigonella foenum-graecum* L.	Fabaceae
Green pea	*Pisum sativum* L.	Fabaceae
Lentil	*Lens culinaris* Medicus	Fabaceae
Mung bean	*Vigna radiata* (L.) R. Wilczek	Fabaceae
Black sesame	*Sesamum indicum* L.	Pedaliaceae
Buckwheat	*Fagopyrum esculentum* Moench	Polygonaceae
Morning glory	*Ipomea reptans* L.	Convolvulaceae
Red roselle	*Hibiscus sabdariffa* L.	Malvaceae

2.2. Growth and Cultivation

The seeds for the cultivation of these microgreens were rehydrated and sterilized prior to germination at 21 ± 1 °C. Subsequently, the germinated seeds were sown in a tray (30 cm × 60 cm × 3 cm) and grown until the cotyledons were fully developed with 1–2 real leaves under a controlled environment at 23 ± 1 °C, 65 ± 5% relative humidity, and

800–1000 mg L^{-1} carbon dioxide concentration. The cultivation details of the 14 studied microgreens are shown in Table 2. The seeds mostly required 5–6 h of soaking. Imbibition is crucial before germination as water uptake triggers a progressive cellular process needed for germination. Additionally, the water softens the seed coat and eases radical protrusion [11]. The seeds were surface-sterilized with commercial sodium hypochlorite (200 mg L^{-1}) and left to germinate overnight at 24 ± 1 °C, following techniques practiced by local commercial farmers. All of these microgreens were grown using soilless culture, and the growing materials used are found in Table 2. The germinated seeds were let to sprout in the dark until a hypocotyl length of 5–10 cm depending on the species. Hence the different required periods of sprouting in the dark. After that, the microgreens were exposed to white fluorescent lights and harvested after the first true leave emerged, at varying durations depending on the species (Table 2). The microgreens were harvested from duplicate trays with scissors and used for proximate and bioactive compounds analyses.

Table 2. Microgreen species, soaking time, germination time, growing material, and durations for sprouting in the dark, light exposure, and harvesting.

Microgreens	Soaking Time (h)	Germination Time	Growing Material	Sprouting in the Dark Time (Day)	Light Exposure Time (Day)	Harvesting Time (Day)
			Brassicaceae			
Broccoli	6 *	1 night	Kinocloth®	5	4	9
Chinese kale	6 *	1 night	Sponge	3	4	7
Purple radish	6 *	1 night	Sponge	4	3	7
Radish	6 *	1 night	Sponge	4	3	7
Rat-tailed radish	6 **	1 night	Sponge	4	6	10
Red cabbage	6 **	1 night	Kinocloth®	5	5	10
			Fabaceae			
Fenugreek	6 **	1 night	Sponge	3	4	7
Green pea	6 **	1 night	Peat Moss	4	3	7
Lentil	6 **	1 night	Sponge	4	3	7
Mung bean	12 *	1 night	Sponge	3	1	4
			Others			
Black sesame	6 *	1 night	Kinocloth®	3	5	8
Buck wheat	6 *	1 night	Sponge	4	6	10
Morning glory	6 *	1 night	Sponge	4	6	10
Red roselle	6 *	1 night	Sponge	4	6	10

* 200 mg L^{-1} NaOCl was added in the 6th h of seed soaking; ** 200 mg L^{-1} NaOCl was added in the 5th h of seed soaking.

2.3. Proximate Analysis

Ash, total protein, moisture, total fat, and total calories were determined according to methods of the Association of Official Analytical Chemists [12]. The total carbohydrate content was estimated by subtracting the other proximate parameters. All the parameters were assessed in duplicate.

2.4. Phytochemical Analysis

2.4.1. Chlorophyll and Carotenoids

Freshly chopped microgreens weighing 0.5 g were mixed with 20 mL of N, *N*-dimethylformamide and kept in the dark at 4 °C for 24 h. After that, the mixture was filtered using Whatman filter paper No. 1, and the absorbance was read at 440, 647, and 664 nm [13,14]. Total chlorophyll was calculated as the sum of chlorophyll a (Chla) and chlorophyll b (Chlb) using the following formulas:

$$\text{Chla (mg L}^{-1}) = 12.64OD_{664} - 2.99OD_{647}$$

$$\text{Chlb (mg L}^{-1}) = -0.56OD_{664} + 23.26OD_{647}$$

The carotenoid content was calculated using the following formula:

$$\frac{1000OD_{440} - 0.89Chla - 52.02Chlb}{245}$$

The total chlorophyll and carotenoid contents were expressed as mg 100 g^{-1} of fresh weight.

2.4.2. Anthocyanin

The hypocotyls of purple radish and red cabbage weighing 1 g were sonicated with 10 mL of methanol containing 0.1% hydrochloric acid for 30 min at 40 °C. Subsequently, 0.5 mL of the obtained solution was mixed with two different buffer solutions (2.5 mL of potassium chloride buffer (pH 1.0) and 2.5 mL sodium chloride buffer (pH 4.5)). The mixtures were then incubated at room temperature (RT) for 15 min prior to absorbance measurement by the scanning method λ, by reading the λvis-max and λ700 values [15]. The anthocyanin concentration was calculated according to the following equation:

$$\text{Anthocyanin pigment} = A \times \text{MW} \times \text{DF} \times 10^3/\varepsilon \times \text{L}$$

where, A = ($A\lambda$ vis-max—$A700$)pH 1.0 − ($A\lambda$ vis-max—$A700$)pH 4.5; MW = molecular weight for cyanidin-3-glucoside = 449.2 g/mol; DF = dilution factor; ε = 26,900 molar extinction coefficient; L = path length in cm. The results were expressed as mg cyanidin-3-glucoside equivalent (CGE) 100 g^{-1} of fresh weight.

2.4.3. Total Phenolic Content

The total phenolic content was determined according to the method by ethanolic extraction following Singleton and Rossi (1965) [16]. Briefly, 1 g of sample was homogenized with 15 mL of 80% ethanol and centrifuged at 12,000× g for 20 min. Then, 0.5 mL of filtrate was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent solution and 2 mL of 7.5% sodium carbonate. The mixture was incubated at RT for 90 min, and absorbance was read at 760 nm. The result was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} of fresh weight.

2.4.4. Total Antioxidant Activity

The total antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was adapted from the method by Shimada et al. (1992) [17]. One gram of the samples was homogenized with 15 mL of 80% ethanol and centrifuged for 20 min at 12,000× g. An aliquot of filtrate (0.1 mL) was then mixed with 2.9 mL of 0.1 mM DPPH working solution. The mixture was vortexed and incubated in the dark for 30 min at RT. The absorbance was read at 515 nm, and the result was expressed as mM Trolox equivalent antioxidant capacity (TEAC) g^{-1} of fresh weight.

2.4.5. Ascorbic Acid

The ascorbic content was estimated based on Roe et al. (1948) [18]. A fresh sample weighing 2.5 g was homogenized with 10 mL of 5% metaphosphoric acid and filtered using Whatman No. 1 filter paper. The obtained filtrate of 0.4 mL was mixed with 0.2 mL of 0.02% 2,6-dichlorophenol-indophenol, 0.4 mL of 2% thiourea and 0.2 mL of 2% 2,4-DNP and incubated at 50 °C for 1 h. Subsequently, 1 mL of 85% sulfuric acid was added into the mixture to stop the reaction. The absorbance was read at 540 nm, and the result was expressed as mg 100 g^{-1} of fresh weight.

2.5. Data Analysis

Statistical analysis was performed using SPSS software (IBM SPPSS Statistic 21, Armonk, NY, USA). The data were analyzed using analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was performed to measure specific differences between means when F-test was significant for bioactive compound analysis.

3. Results and Discussion

3.1. Proximate Analysis

Results of the proximate analysis of the fourteen microgreens are shown in Table 3. The total carbohydrate content of the Brassicaceae, black sesame, morning glory, and roselle microgreens was 3.02 g 100 g^{-1} on average. On the other hand, Fabaceae microgreens (except green pea) showed a relatively higher carbohydrate content, with mung bean the highest at 7.16 g 100 g^{-1} followed by buckwheat at 4.90 g 100 g^{-1}. Grain legumes are known to have a high carbohydrate content of up to 65% [19].

Table 3. Proximate analysis of the studied microgreens grown under controlled environment.

Microgreens	Ash	Total Carbohydrate	Total Protein	Moisture	Total Fat	Total Calories
			(g 100 g^{-1})			(kcal 100 g^{-1})
Brassicaceae						
Broccoli	0.51 ± 0.02	2.70 ± 0.20	2.23 ± 0.11	94.07 ± 3.11	0.49 ± 0.01	24.13 ± 0.20
Chinese kale	0.65 ± 0.00	3.13 ± 0.11	2.23 ± 0.00	93.63 ± 2.12	0.36 ± 0.02	24.68 ± 0.22
Purple radish	0.52 ± 0.05	3.70 ± 0.10	3.41 ± 0.05	91.88 ± 1.25	0.49 ± 0.01	32.85 ± 0.15
Radish	0.44 ± 0.02	3.29 ± 0.09	2.58 ± 0.01	93.19 ± 1.23	0.50 ± 0.01	27.98 ± 0.20
Rat-tailed radish	0.43 ± 0.00	2.91 ± 0.03	2.50 ± 0.03	93.50 ± 2.13	0.66 ± 0.03	27.58 ± 0.13
Red cabbage	0.75 ± 0.05	2.32 ± 0.01	1.88 ± 0.02	94.67 ± 3.11	0.38 ± 0.02	20.22 ± 0.22
Fabaceae						
Fenugreek	0.50 ± 0.00	5.12 ± 0.02	4.03 ± 0.06	90.17 ± 1.22	0.18 ± 0.00	38.22 ± 0.27
Green pea	0.36 ± 0.02	3.39 ± 0.04	3.73 ± 0.02	92.37 ± 2.38	0.15 ± 0.00	29.83 ± 0.15
Lentil	0.61 ± 0.00	5.92 ± 0.01	6.47 ± 0.11	86.57 ± 1.12	0.43 ± 0.02	53.43 ± 0.19
Mung bean	0.64 ± 0.04	7.16 ± 0.02	4.55 ± 0.05	87.29 ± 2.28	0.36 ± 0.01	50.08 ± 0.11
Others						
Black sesame	0.34 ± 0.00	3.58 ± 0.03	1.92 ± 0.02	93.75 ± 2.27	0.41 ± 0.02	25.69 ± 0.10
Buckwheat	0.34 ± 0.00	4.90 ± 0.01	1.75 ± 0.03	92.74 ± 1.11	0.27 ± 0.01	29.03 ± 0.14
Morning glory	0.54 ± 0.01	3.08 ± 0.03	1.76 ± 0.00	94.26 ± 1.21	0.36 ± 0.01	22.60 ± 0.12
Red roselle	0.64 ± 0.00	2.51 ± 0.05	4.10 ± 0.07	92.51 ± 1.19	0.24 ± 0.01	28.60 ± 0.07

Mean ± SE, $n = 2$.

The total protein content of the studied microgreens ranged from 1.75 and 1.76 g 100 g^{-1} (buckwheat and morning glory microgreens, respectively) to 6.47 g 100 g^{-1} (lentil microgreens). Lentils are rich in protein, approximately 16% albumins, 70% globulins, 11% glutelins, and 3% prolamins, and contain low levels of sulfur-containing amino acids [20,21]. A similar range of protein was found in fenugreek microgreens by Ghoora et al. (2020) [5], but our study obtained a higher protein content in radish and roselle microgreens than the one published.

As expected, the lentil and mung bean microgreens had higher total calories (53.43 and 50.08 g 100 g^{-1}, respectively) due to their high carbohydrate and protein contents (Table 3). On the other hand, the other studied microgreens had a relatively low caloric content of 27.62 g 100 g^{-1} on average.

Ash and total fat contents of the microgreens were low. A study on six genotypes of microgreens including *Brassica oleracea* L. var *italica* also reported that the lipid content of the microgreens is insignificant [22]. The microgreens contained a high moisture content ranging from 86.57 g 100 g^{-1} to 94.67 g 100 g^{-1}.

3.2. Bioactive Compounds

The coloration of microgreens is one of the main attributes that affect customers' choice of microgreens and their economic value. Chlorophyll and carotenoids are major photosynthetic pigments responsible for the specific coloration of microgreens [23]. These pigments are found to be richer in microgreens than sprouts [24]. In the present study,

the microgreens showed a total chlorophyll content range of 12.35 to 112.62 mg 100 g^{-1}. The smallest concentration was found in green pea, while the highest was detected in lentil microgreens. Our results bear a close resemblance to a previous study of radish and fenugreek microgreens [25]. The content of carotenoids had a similar pattern as the content of the total chlorophyll, ranging from 4.40 to 28.37 mg 100 g^{-1}. Higher carotenoid contents were detected in the Brassicaceae microgreens: broccoli, Chinese kale, radish, and red cabbage, than previously reported (11.9, 10.6, 11.4, and 10.4 mg 100 g^{-1}, respectively) by Xiao et al. (2019) [9]. A similar carotenoid concentration (13.8 mg 100 g^{-1}) had been reported for purple radish [9]. Anthocyanin pigments give the attractive red, orange, blue, or purple coloration to plant tissues [26]. Red cabbage and purple radish exhibit purplish-red hypocotyls owing to the accumulation of anthocyanins. The total anthocyanin content detected in red cabbage was higher than purple radish as shown in Table 4. These pigments not only contribute to the visual quality of microgreens but also their biological activity, making them beneficial to human health [5,23,27].

Table 4. Total chlorophyll, carotenoid, anthocyanin, phenolic, and ascorbic acid contents and total antioxidant activity of the microgreens grown under controlled environment.

Common Name	Total Chlorophyll (mg 100 g^{-1})		Carotenoid (mg 100 g^{-1})		Anthocyanin (mg CGE 100 g^{-1})		Total Phenolic (mg GAE 100 g^{-1})		Ascorbic Acid (mg 100 g^{-1})		DPPH• Scavenging Activity (mM TEAC g^{-1})	
Broccoli	52.26	bc	13.47	bc	NA		87.56	cde	79.11	a	35.56	cd
Chinese kale	58.44	b	15.00	b	NA		130.59	bc	81.33	a	41.90	c
Purple radish	49.80	bcd	13.12	bcd	0.148	b	132.78	bc	82.58	a	38.50	g
Radish	59.21	b	15.61	b	NA		145.04	b	56.49	b	38.39	cd
Rat-tailed radish	36.61	de	9.34	efg	NA		143.11	b	48.24	b	37.63	cd
Red cabbage	39.79	cde	12.08	bcde	0.246	a	112.29	bcd	89.49	a	55.45	b
Fenugreek	57.10	b	14.28	b	NA		59.72	cde	36.18	c	61.48	b
Green pea	12.35	f	4.40	h	NA		38.14	ef	42.45	c	34.82	cd
Lentil	112.62	a	28.37	a	NA		89.05	bc	128.70	a	36.34	cd
Mung bean	26.13	ef	5.86	gh	NA		59.95	def	25.37	c	25.50	e
Black sesame	37.85	de	9.56	def	NA		49.03	ef	6.84	d	33.44	de
Buckwheat	34.65	e	10.42	cdef	NA		268.99	a	62.90	b	90.83	a
Morning glory	28.29	e	6.92	fgh	NA		9.22	f	16.78	cd	10.11	f
Roselle	36.37	de	8.87	efg	NA		57.06	ef	22.23	cd	42.08	c

Means within columns with the same letter are not significantly different, using DMRT test at $p \leq 0.05$. NA: not available.

The intake of dietary antioxidants is commonly linked with lower risks of certain serious illnesses, including cardiovascular diseases, hypertension, and diabetes [28]. Data from biochemical, clinical, and epidemiological research have recommended a dietary ascorbic acid intake of 90–100 mg day^{-1} to lower the risks of these diseases [25,29]. In the present study, the ascorbic acid detected in the microgreens ranged from 6.48 to 128.70 mg 100 g^{-1}. Broccoli, Chinese kale, purple radish, red cabbage, and lentil microgreens exhibited significantly higher ascorbic acid compared to the other microgreens, whereas black sesame microgreens had the least.

Phenolic compounds, products of the phenylpropanoid pathway, are one of the largest secondary metabolites primarily found in fruits and vegetables [30]. These compounds comprise cinnamic acid, benzoic acid, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignin [31]. A significant variation in the total phenolic content of the micro-

greens was observed, with a range of 9.22 to 268.99 mg GAE 100 g^{-1}. The highest content was found in buckwheat, and the least amount was in morning glory microgreens. The detected values for radish, fenugreek, and roselle microgreens were higher than values reported by Ghoora et al. (2020) [25] but lower than values for broccoli, red cabbage, Chinese kale, radish, and purple radish microgreens reported by another study [9]. These variations could be due to various intrinsic and extrinsic factors, such as species, growth conditions, maturity at harvest, and postharvest conditions [32,33]. The phenolic compounds exhibit direct and indirect antioxidant actions that are beneficial to human health. Their strong antioxidant power lies in their ability to donate electrons to oxidant species, scavenge free radicals, chelate metal ions, and indirectly attenuate the accumulation of reactive oxygen species (ROS) by either improving the activity of antioxidant enzymes or inhibiting enzymes that stimulate pro-oxidant effects [31].

Here, the antioxidant activity of the microgreens was estimated by using DPPH• scavenging activity. Antioxidants in the microgreen extract scavenge the DPPH• through donating a hydrogen atom and converting the radical to a reduced form [25]. The radical scavenging potential is signified by the degree of discoloration of the purple DPPH working solution. Buckwheat microgreens showed remarkably high antioxidant activity compared to others. In contrast, morning glory microgreens registered the lowest DPPH• scavenging activity. Buckwheat microgreens exhibited about 9-fold higher DPPH• scavenging activity than morning glory microgreens. It should also be noted that buckwheat and morning glory recorded the highest and lowest TPC, respectively. Other studies have also demonstrated a strong positive correlation between TPC and DPPH• scavenging activity [9,25].

4. Conclusions

In general, the microgreens investigated in this study were low in calories and fat but high in moisture content. Additionally, the microgreens contained relatively low carbohydrate and protein with the exception of mung beans and lentil microgreens, which had high carbohydrate (7.16 and 5.92 g 100 g^{-1}) and protein (4.55 and 6.47 g 100 g^{-1}, respectively) contents. Lentil microgreens had the highest total chlorophyll (112.62 mg 100 g^{-1}), carotenoid (28.37 mg 100 g^{-1},), and ascorbic acid (128.70 mg 100 g^{-1}) contents. Buckwheat microgreens showed the highest TPC and maximum DPPH• scavenging activity. Only red cabbage and purple radish exhibited anthocyanin content, with the higher content found in red cabbage. The data provided in this study on microgreens of temperate and tropical origins will help farmers to select, expand, and add value to their business with the inclusion of microgreens.

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