

## Optimization of Nutritional Composition, Bioactive Compounds and Antioxidant Activity in Broccoli (*Brassica oleracea*) Microgreen Sprinkler using Alternate Drying Techniques

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#### **ABSTRACT**

**Background:** Fresh microgreens are nutrient dense crops with high moisture content, thus have a short life span. Application of different drying techniques enhanced its shelf life but leads to nutrient losses. There is permanent need to enhance the nutrient retention in dried microgreen.

**Methods:** The research study of microgreens investigated during 2019-2020 at Punjab Agricultural University. Broccoli (*Brassica oleracea* var. *Palam Samridhi*) seeds were soaked overnight and grown in outdoor setting and harvested at 10<sup>th</sup> day after germination. The harvested broccoli microgreens were dried using shade drying, tray drying and microwave drying. Dried samples were analyzed for nutritional parameters.

Result: Microgreens contain significantly higher nutrients (Ca, Fe, Zn, Mg, K, ascorbic acid and  $\beta$  carotene), bioactive compounds (chlorophyll, total flavanoids and total phenols) and antioxidant activity as compared to their sprouts and mature counterparts. These are excellent sources of Fe, Ca, Zn, Mg, Vitamin C and beta carotene (pro-vitamin A), meeting per cent estimated average requirements (EAR) of 536%, 373%, 264%, 228%, 38%, 20% for Indian adult. To address the problem of perishability, there is a need to prolong their shelf life while retaining the nutritional quality. The present research has identified the optimum drying technique, on basis of nutrient retention, from a set of alternate techniques. Significant higher content of ascorbic acid (16.80 mg/100 g), chlorophyll (3.63 g/100 g), antioxidant activity (67.55%), Zn (35.23 mg/100 g), Fe (57.66 mg/100 g), Ca (2857 mg/100 g), K (334.23 mg/100 g) and Mg (706.43 mg/100 g) were observed in microwave dried microgreens. Incorporation of dried microgreen powder in food sprinkler resulted in a significant increase in vitamins, minerals, bioactive compounds and antioxidant activity as compared to the control.

Key words: Antioxidant activity, Bioactive compounds, Broccoli microgreens, Drying techniques, Nutrient retention.

#### INTRODUCTION

Microgreens also known as 'vegetable confetti', are tenuous immature greens grown from the seeds of pulses, herbs and vegetables (Xiao et al., 2012). Microgreens are categorized in the class of 'super foods' due to high content of bioactive compounds (Sharma et al., 2012). Along with high nutrient content, they also possess a variety of texture, colours and intense flavors (Michell et al., 2020). Microgreens contain several health promoting and disease preventing micronutrients in higher concentration as compared to their mature counterparts (Renna et al., 2018). Broccoli is among the most popular variety of microgreens owing to its fast germination, intense aromatic flavor and high nutritional content (Weber 2017).

Microgreens, are high in moisture content which aids in quick deterioration (Paradiso *et al.*, 2018), also high respiration rate affects their shelf life. Dehydration is the simplest technology for preserving green leafy vegetables. Also, dehydration reduces microbiological activity, increases the storage period (Henriquez *et al.*, 2013). Processing of microgreens into powder form will make them available throughout the year for better marketability.

There are limited comparative studies on suitable methods of drying of microgreens. The effect of these methods on nutritional content of microgreens still remains

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relatively unexplored domain. The present study aims to evaluate the effect of different drying techniques on nutritional composition of broccoli microgreens and to develop microgreen sprinkler with maximum nutrient retention and health promoting properties.

#### **MATERIALS AND METHODS**

#### Cultivation and drying of broccoli microgreens

Broccoli (Brassica oleracea var. Palam Samridhi) seeds were soaked overnight and grown in outdoor setting and

harvested at 10<sup>th</sup> day after germination. The experiment was conducted at PAU, Ludhiana in 2019-20. The harvested broccoli microgreens were dried using shade drying, tray drying and microwave drying. Three hundred grams of broccoli microgreens were divided into three parts, 100 g of broccoli microgreens were dried under shade at room temperature till the desire moisture content of less than 9% was achieved. The second portion (100g) was dried to a constant weight at 50°C for 10 hours by spreading microgreens over a tray lined with butter paper. The third part (100 g) was microwave dried using a commercial microwave at 1020 Watt for 10 minutes. The broccoli microgreen samples dried by three methods were powdered and packed in high density polythene pouches (HDPE) then stored at -18°C in deep freezer until further analysis.

#### Moisture content (AOAC 2010)

Five gram of microgreen were weighed in the Petri plate and later dried in a hot air oven for 8 hours at 105°C.

#### Ascorbic acid

We assessed ascorbic acid by AOAC (2012), in which 5 g of a dried sample of microgreens was used.

#### Beta-carotene

The beta-carotene content was assessed by Ranganna (2002) method using a column of calcium hydroxide and determined spectrophotometrically.

#### Chlorophyll

Chlorophyll content was estimated by the procedure given by Asimovic *et al.* (2016).

#### **Flavonoids**

We estimated flavonoid content by aluminium chloride colorimetric method using quercetin as a standard (Mathur and Vijayvergia, 2017).

#### Total phenolic content

For estimation of TPC methanol extract of samples were used. Estimation of TPC was conducted by Folin-Ciocalteu method (Mathur and Vijayvergia, 2017).

#### Minerals

Minerals namely, iron; magnesium, calcium, zinc and potassium were evaluated by using AOAC (2012), after wet digestion.

#### Antioxidant activity (DPPH assay)

Extraction: About two gram of dried microgreen was extracted with 25 ml of 80% methanol. The DPPH assay by Dehshahri *et al* (2012) was used to estimate the free radical scavenging activity of microgreens samples.

#### Estimation of percent adequacy of microgreens

The per cent adequacy was calculated using Estimated Average Requirement (ICMR, NIN 2020) of the nutrients

against nutrient content of broccoli microgreens determined in the present study.

Per cent Adequacy (%) =

Nutrient content of microgreen (mg/100 g)
Ear of the nutrient 
$$\left(\frac{mg}{day}\right)$$

#### Nutrient quality score (NQS)

The NQS 7.1 was computed based on the sum of percent adequacy for 7 nutrients to encourage (Ca, Mg, K, Fe, Zn, ascorbic acid, pro vitamin A) minus the sum of percent adequacy of sodium (the nutrient to be limited). The calculation of NQS 7.1 was done on the basis of per cent adequacy of various nutrients: Estimated Average Requirements (EAR) for adult Indians with some modifications according to Ghoora *et al.* (2020). The percent adequacy of each nutrient, per serving size (100 g), was calculated by referring to the data from ICMR-NIN (2020). The equation used to calculate NQS is reported below:

NQS =  $\Sigma_{1-7}$  (per cent adequacy) - per cent adequacy Na

#### Sensory evaluation

The developed food sprinkler using different propositions of dried microgreen powder was evaluated for the sensory properties by a panel of semi trained judges (n=10). The product was evaluated on a nine-point hedonic scale (Wichchukit and Mahony 2015).

#### Statistical analysis

Analysis of Variance along with post hoc analysis was employed to study the variation in vitamins, minerals, bioactive content and antioxidant activity across different drying techniques on broccoli microgreens using computer software JMP 10.0.1. Paired t-test was applied to nutritional estimation of food sprinkler of broccoli microgreen powder. Data is reported as mean± SD for at least three triplicates for each sample.

#### **RESULTS AND DISCUSSION**

## Nutritional and biochemical composition of broccoli microgreens vis a vis sprouts and mature leaves

The growth stages of plant affect the nutrient content of the plant. In order to examine the effect of different growth stages, microgreens were compared with sprouts and mature leaves of broccoli in terms of their nutritional and biochemical composition. The results indicated that â-carotene, ascorbic acid, chlorophyll, total phenolic content, flavonoid content, minerals (Ca, Mg, Zn, Fe and K) and antioxidant activity of broccoli microgreens was significantly higher than the broccoli sprouts and mature leaves (Fig 1). Previous research studies in the literature elucidated similar findings (Ghoora *et al.*, 2020). Further, percentage adequacy was estimated for each nutrient based on Estimated Average requirements (EAR) for Indian adults against the nutrient

present in broccoli microgreens and their mature counterparts. The percent adequacy of nutrient composition of microgreens in comparison to mature is represented in Fig 2. The vitamins and mineral content were significantly lower in mature broccoli leaves as compared to the broccoli microgreens. Just 100 g of broccoli microgreens fulfilled the estimated average requirements (EAR) of more than 100% for essential minerals namely calcium, magnesium, iron and

zinc (Fig 2). 100 g of broccoli microgreens fulfilled 38% of EAR for vitamin C and 20.07% for  $\beta$ -carotene as compared to 15.38% and 9% by mature leaves, respectively. Broccoli microgreens are good source of iron, calcium, zinc and magnesium contributing to more than 200% of the EAR. Renna *et al.*, (2020) reported the contribution of  $\beta$ -carotene of three *Brassica* microgreens ranged from 31 to 86% of the daily reference value.

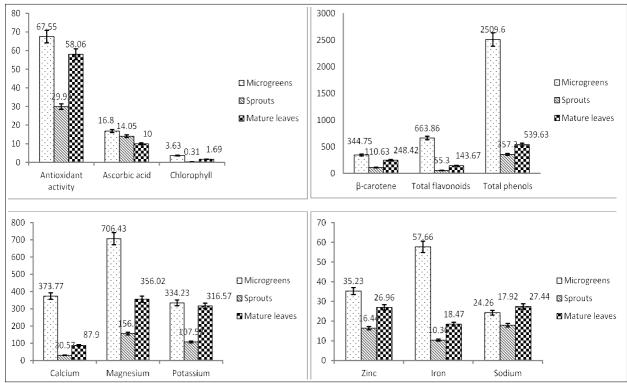


Fig 1: Nutrient content of broccoli microgreens in comparison to sprouts and mature leaves.

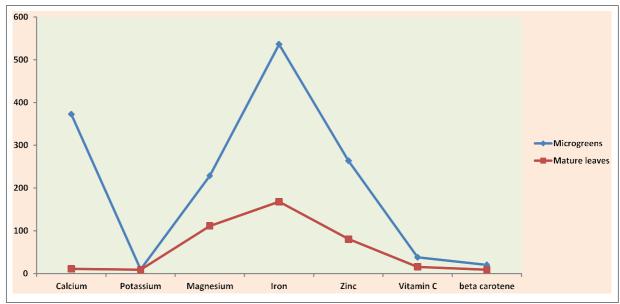


Fig 2: Comparison of per cent adequacy of broccoli microgreens and mature broccoli leaves.

# Vitamins, bioactive compounds and antioxidant activity of dried broccoli microgreens using different drying techniques in comparison to fresh microgreens

Fresh microgreens are concentrated source of nutrients but are highly perishable due to their high moisture content. The fresh broccoli microgreens had significantly (p<0.0001) high moisture content (95.01±0.59) as compared to the powdered broccoli microgreen (8.55±0.33).

Table 1 presents the comparison of different drying techniques in terms of vitamins and bioactive compounds of broccoli microgreens. F value 66730.68 (p<0.0001) indicates a significant variation in β-carotene content across different drying techniques. Further it was seen that β-carotene content in shade dried broccoli microgreens was significantly (p<0.05) higher as compared to tray and microwave drying methods. This is on account of a significant positive correlation between high temperature and β-carotene degradation (Park 1987). Many studies have shown the dependence of the carotene losses on the method of drying. The degradation of β-carotene in GLV dried in oven (6-25.5%) was significantly higher as compared to sun drying (24-40%) (Aletor and Adeogun 1995). The β-carotene content of fresh broccoli microgreens was 554.95 µg/100 g that significantly reduced after drying. The percentage retention in β-carotene was 72, 71 and 62 per cent for shade, oven and microwave dried microgreens (Fig 3). Low-temperature drying of leafy vegetables retained more β-carotene content than high drying temperature i.e. sun, solar or cabinet drying (Negi and Roy 2000). At high temperature and oxidation, β-carotene lead to degradation and more precisely to isomerization (Coşkun et al. 2013) which explains the higher β-carotene content of broccoli microgreens in shade drying as compared to oven and microwave drying in the present study .

For ascorbic acid, the F value 217.24 (p<0.0001) indicates a significant variation in ascorbic acid content

across different drying techniques. Ascorbic acid content in microwave dried broccoli microgreens was significantly (p<0.05) higher followed by shade and tray dried broccoli microgreens (Table 1). The ascorbic acid content was significantly higher in fresh broccoli microgreens (25.16 mg/ 100 g) as compared to microwave dried microgreens (16.80 mg/100 g). Microwave drying retained maximum ascorbic acid with the percentage retention of 86 (Fig 3). Ascorbic acid is highly sensitive to most assessable atmospheric constitutes like oxygen, light and temperature. The primary reason of reduction in vitamin C is the degradation of ascorbic acid to diketoglulonic acid on the application of heat treatment. The vitamin may also degrade due to prolonged duration of drying and presence of oxygen (Kiremire et al., 2010) that elucidate higher deterioration of ascorbic acid in shade drying as compared to tray drying in the present study. Similar results were reported by Managa et al., (2020), on the contrary Khatoniar et al., (2019) reported that the shade dried amaranth leaves had higher ascorbic acid content.

The fresh broccoli microgreens had high chlorophyll content (10.85 g/100 g) that significantly decreased with the heat treatment. The F value 5267.4 (p<0.0001) indicates a significant variation in chlorophyll content across different drying techniques. The chlorophyll content of microwave dried broccoli microgreens was significantly (p<0.05) higher than shade and tray dried microgreens (Table 1). Microwave drying enables to shorten dehydration time and controls undesirable biological transformations (Bondaruk *et al.*, 2007). Chlorophyll is sensitive to heat treatment and is dependent on temperature and duration of heat treatment (Negi and Roy 2000). Maximum retention of chlorophyll was observed in microwave drying (33.4%), followed by shade (14%) and tray (4.42%) drying techniques (Fig 3). Higher losses in shade drying could be due to longer duration of

**Table 1:** Vitamins, bioactive compounds, antioxidant activity and mineral content of broccoli microgreens under different drying techniques as compared to the fresh microgreens.

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Parameters	Fresh microgreens	;	Drying techniques		F value (p value)
T dramotoro		Shade	Tray	Microwave	i varao (p varao)
β-carotene (µg/100 g)	554.95d±6.30	400.54°±0.76	392.22b±0.20	372.20°±0.37	66730.69 (<0.0001)
Ascorbic acid (mg/100 g)	25.16d±0.03	10.88°±0.56	13.16b±13.16	16.80°±1.21	217.24 (<0.0001)
Chlorophyll (g/100 g)	10.85d±0.57	1.47 <sup>b</sup> ±0.17	0.48°±0.01	3.63°±0.12	5267.411 (<0.0001)
Total phenolic content	2346.46°±0.36	2645.88b±112.41	2538.22b±67.36	2509.60b±115.80	6.017 (0.019)
(mg GAE/100 g)					
Flavonoid (mg QE/100 g)	820.24°±0.78	673.82b±0.81	671.46b±1.14	663.86°±4.64	2702.55 (<0.0001)
Antioxidant activity (%)	90.68d±0.99	41.54b±1.42	33.03°±1.06	67.55°±2.18	903.34 (<0.0001)
Zinc (mg/100 g)	37.89 <sup>d</sup> ±0.09	30.40°a±0.20	32.87b±0.71	35.23°±0.30	97.95 (<0.0001)
Iron (mg/100 g)	59.97d±0.11	56.32b±0.35	51.55°±0.50	57.66°±0.11	350.45 (<0.0001)
Calcium (mg/100 g)	2990.16d±0.04	2231.72°±35.39	2538.00b±17.65	2857.90°±0.03	883.66 (<0.0001)
Potassium (mg/100 g)	341.53d±0.54	249.02°±0.90	201.73b±1.12	334.23°±0.12	14384.10 (<0.0001)
Magnesium (mg/100 g)	713.81 <sup>d</sup> ±1.54	664.88°±0.34	687.74b±0.42	706.43°±0.41	1647.09 (<0.0001)
Sodium (mg/100 g)	25.46d±0.08	19.79°±0.52	18.16°±0.57	24.26b±1.70	39.71 (<0.0001)

Values are expressed as Mean±SD.

For antioxidant, beta carotene, TPC, Vitamin C and Fe the values of Brown-Forsythe statistic are been presented.

drying. High retention of chlorophyll at a lower temperature drying has been previously reported (Zheng-Wei *et al.*, 2004; Cesare *et al.*, 2003). Microwave drying evaporates the moisture quickly during a shorter time span that reduces

the oxidation reaction thus, retaining the nutrients and bioactive compounds.

A significant increase in the total phenolic content was seen after different drying techniques as compared to fresh

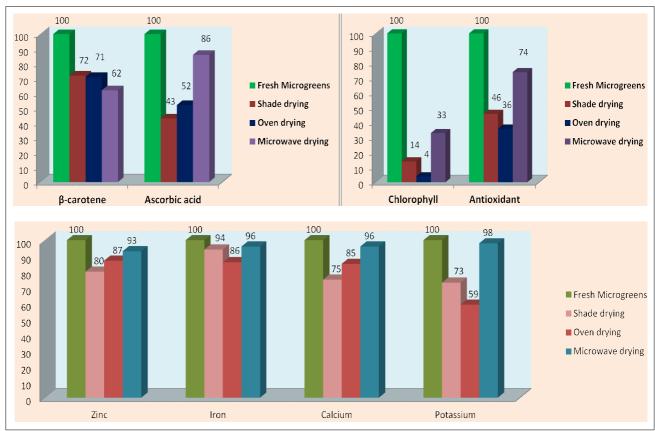


Fig 3: Retention of nutrients by shade, tray and microwave dried broccoli microgreens as compared to fresh microgreens.

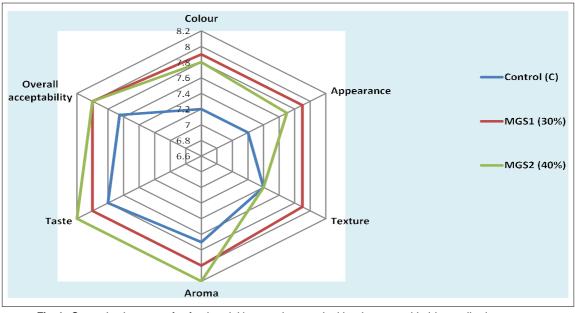


Fig 4: Organoleptic scores for food sprinkler supplemented with microwave dried broccoli microgreens.

microgreens (2346.46 mg GAE/100 g). This increase in phenolic content is attributed to the liberation of phenolic compounds from the matrix during the drying process (Chang et al., 2006). The total phenolic content of shade dried broccoli microgreens was non significantly higher as compared to the tray and microwave dried sample. This is in agreement with previous studies (Kumar et al., 2014). The heat generation by the different drying techniques produces a high vapor pressure and temperature inside plant tissue, resulting in the disruption of plant cell wall polymers. Subsequently, in certain cases, cell wall phenolics or bond phenolics can be released, hence triggering more phenolics to be extracted (Inchuen et al., 2010). Total flavonoids significantly decreased with the use of drying techniques as compared to the fresh one (820.24 mg QE/100 g). The statistical analysis showed that the flavonoid content of shade dried broccoli microgreens was significantly (p<0.0001) higher than the microwave dried broccoli microgreens. Similar findings have been reported by Kumar et al., (2014).

The antioxidant activity of fresh microgreens (90.68%) significantly decreased with the use of different drying techniques. The F value 903.34 (p<0.0001) indicates a significant variation in antioxidant content across different drying techniques. The antioxidant activity of microwave dried broccoli microgreens was significantly (p<0.05) higher than the shade and tray dried broccoli microgreens (Table 1). These results are in agreement with the previous studies (Managa et al., 2020; Kumar et al., 2014). The retention in antioxidant activity was 74.4, 45.8 and 36.4 per cent in microwave, shade and tray drying respectively (Fig 3) thus clearly indicating that microwave drying retained the maximum antioxidants. Retention of antioxidant activity decreases with increase in temperature and duration of drying (Ozcan et al., 2020).

#### Mineral content of dried broccoli microgreens using different drying techniques in comparison to fresh microgreens

Fresh microgreens are rich source of minerals (iron, zinc, potassium, sodium, calcium and magnesium), that significantly decreased with the use of various drying techniques. The mineral content like zinc, iron, calcium, potassium, magnesium and sodium were significantly (p<0.05) higher in microwave drying as compared to shade and tray drying (Table 1). The maximum retention of minerals was observed in microwave drying technique as Zn, Fe, Ca and K were retained at 92.97%, 96.14%, 95.57% and 97.86% respectively in microwave dried microgreen samples (Fig 3). These results are in agreement with the previously reported studies, articulating the better retention of minerals at low temperature drying (Khatoniar and Barooah 2019).

Overall, microwave drying techniques was the best method for the drying of broccoli microgreens. Short period of heat treatment by microwave drying led to higher nutrient retention. Microwave dried broccoli microgreens are nutrient dense and an excellent sources of Fe, Ca, Zn, Mg, Vitamin

	lable 2: Nuti	ritional evaluati	ion of tood spr	inkler suppleme	ented with drie	lable 2: Nutritional evaluation of food sprinkler supplemented with dried broccoli microgreen.	green.						
	Trootmont	Vitamin C	Vitamin C β-carotene	Calcium	Iron	Potassium	Potas sium Magnesium Sodium Zinc Chlorophyll TPC (mg Antioxidant	Sodium	Zinc	Chlorophyll	TPC (mg	TFC(mg ,	Antioxidant
		(mg/100 g)	(mg/100 g) (µg/100 g) (mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g) (mg/100 g) (mg/100 g) (mg/100 g) (mg/100 g) (g/100 g) GAE/100 g) QE/100 g) activity (%)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(g/100 g)	GAE/100 g)	QE/100 g)	activity (%)
	Control	4.25±0.5	9.41±0.04	$1.06\pm0.05$	1.20±0.01	10.26±0.041	10.26±0.041 18.30±0.02 15.31±0.02 2.23±0.2 0.15±0.3 45±0.10	15.31±0.02	2.23±0.2	$0.15\pm0.3$	45±0.10	28.21±0.01 5.61±0.12	5.61±0.12
Asi	(Spice mix)												
an J	Experimental	10.10±0.04	10.10±0.04 221.83±0.28 1196.06±0.12	$1196.06\pm0.12$	$23.99\pm0.50$	$136.61\pm0.04$	285.51±0.01	$10.18\pm0.00$	15.17±0.02	1.55±0.14 1	285.51±0.01 10.18±0.00 15.17±0.02 1.55±0.14 1015.84±0.02 273.54±0.03 28.02±0.04	$273.54\pm0.03$	28.02±0.04
lour	(Microgreen												
nal	sprinkler)												
of E	t -value	152.81	1146.23	15453.06	748.45	3523.48	18126.02	316.49	618.10	139.54	2569.27	1017.23	680.38
airy	(p-value)	(< 0.0001)	(< 0.0001) (< 0.0001)	(< 0.0001)	(< 0.0001)	(< 0.0001)	$(<0.0001) \qquad (<0.0001) \qquad (<0.$	(< 0.0001)	(< 0.0001)	(< 0.0001)	(< 0.0001)	(<0.0001)	(< 0.0001)
/ an	Values are ex	Values are expressed as Mean±SD.	ean±SD.										

Experimental: Sprinkler with 40% supplementation t-values are absolute values

C and beta carotene, meeting 524%, 357%, 252%, 220%, 25%, 13% of adequacy of Estimated Average Requirements (EAR) in Indian adult.

### Organoleptic and nutritional evaluation of food sprinkler supplemented with dried broccoli microgreens

Dried broccoli microgreens are dense source of vitamin, minerals, bioactive compounds and antioxidant activity. Microgreen sprinkler (MGS) was prepared by supplementing different levels of dried broccoli microgreens prepared by microwave drying technique at 30 and 40 per cent level of incorporation to the regular spice mix (without microgreen powder) treated as control. Mean sensory score of food sprinkler is presented in Fig 4. The regular spice mix supplemented with 40 per cent of broccoli microgreens powder was well accepted organoleptically by sensory panelists and was further evaluated for its nutritional composition.

Comparison of microgreen sprinkler supplemented with 40 per cent of dried broccoli microgreens and control spice mix, on basis of nutritional evaluation, is presented in Table 2. There was a significant (p<0.0001) increase in vitamin C,  $\beta$ -carotene content, minerals (calcium, iron, potassium, magnesium) bioactive compounds and antioxidant activity as compared to the control except for the sodium content. The high sodium content in the control was due to the presence of high salt in the spice mix which subdued with the addition of microgreens. Thus, for the fulfillment of the nutrient requirements, microgreen sprinkler can be used on daily basis.

#### **CONCLUSION**

Fresh microgreens are nutrient dense crops with high content of vitamins, minerals, bioactive compounds and antioxidant activity. Highest nutrient retention was found in microgreens dried by microwave drying techniques for antioxidant, chlorophyll and ascorbic acid. The mineral (Ca, Zn, Fe, K, Mg) content was also found to be the highest in microwave dried microgreens. Microwave dried microgreens are an excellent sources of Fe, Ca, Zn, Mg, Vitamin C and beta carotene, meeting percent adequacy of 524%, 357%, 252%, 220%, 25%, 13% of EAR for Indian adult. The use of dried microgreen powder in food sprinkler led to a significant enhancement in the nutritional composition as compared to the control. Microgreen sprinkler can be used on regular basis in daily life for fulfilling the nutrient requirements. This study, thus, provides a useful insight for the maximum retention of nutritional potential of microgreens in dried form with the use of appropriate drying technique.

Conflict of Interest: None.

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