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STORAGE EFFECTS ON PHYTOCHEMICALS, ANTIOXIDANT ACTIVITY AND SENSORY QUALITY OF FENUGREEK (TRIGONELLA FOENUM-GRAECUM L.) MICROGREENS AND MATURE LEAVES

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Microgreens are tiny and delicate form of edible leafy greens gaining increasing popularity amongst consumers. Microgreens (FMG) and mature leaves (FML) of fenugreek were compared for their phytochemical content, antioxidant activity and sensory quality during a storage period of 14 days at 10 °C. The greens were evaluated for the following phytochemicals-ascorbic acid (AsA), total polyphenols (TPP) and lutein (LUT) using standard procedures on 0D, 7D and 14D of storage. The antioxidant activity was determined using the DPPH radical scavenging activity (DPPH RSA) and FRAP assays at the same intervals during storage. Sensory quality was evaluated in terms of freshness, colour, wiltness, typical aroma and tenderness of the greens. The organoleptic acceptability of microgreens was tested through incorporation in common recipes. Comparatively higher initial levels of AsA, TPP, DPPH, RSA and FRAP were recorded in FMG and exhibited greater retention throughout the storage period as compared to FML. FMG also maintained better sensory quality than FML on storage. All microgreens incorporated recipes were found to have good acceptability. Thus, fenugreek microgreens represent a good source of phytochemicals with high antioxidant potential and sensory quality. These could be used in conjunction with regular green leafy vegetables to enrich the health benefits of common diets.

Keywords: Microgreens, Fenugreek leaves, Phytochemical, Antioxidant, Sensory, Storage

INTRODUCTION

Green Leafy Vegetables (GLVs) form an important component of healthy diets world-over (FAO, 2014) and their consumption is reported to be on the rise due to increased consumer awareness regarding health benefits of GLVs in their diets (Rai *et al*., 2006; and Rocha and Morais, 2007). Leafy vegetables are rich sources of phytochemicals, the bioactive compounds which confer protection against diseases (Slattery *et al*., 2000; Nakahara *et al*., 2002; Islam, 2006; and Pollock, 2016). The bioactive compounds present in abundance in GLVs include ascorbic acid, carotenoids, chlorophyll, polyphenols and other phytochemicals (Rao, 2003; Thu *et al*., 2004; Ekman and Patterson, 2005; and Rodriguez-Amaya *et al*., 2007) which apart from other health benefits also contribute to their antioxidant properties.

Ascorbic acid is an effective antioxidant which protects against free radical species (Smirnoff, 1996) and also serves as an enzyme cofactor in many metabolic reactions in the human body (Davies *et al*., 1991). Leafy vegetables are generally good sources of phenolic compounds which assume antioxidant functions by donating electrons, chelating metal ions and quenching active oxygen species (Rice-Evans *et al*., 1997). Greens are also known to be enriched with various carotenoids such as lutein, beta-

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carotene and zeaxanthin (Seddon *et al*., 1994). Lutein found in the retina and lens of the eye is known to prevent macular degeneration disease by quenching singlet oxygen and scavenging free radicals (Kirschfeld, 1982).

Leafy vegetables, however, are highly perishable in nature and their deterioration during storage is accompanied by several changes including variations in their phytochemical content. Compositional variations in bioactive compounds during storage in fresh and minimally processed leaves have been investigated by Negi and Roy (2004) and Kobori *et al*. (2011) respectively.

In addition to the phytochemical content, sensory quality is one of the important parameter which affects consumer acceptability. It can be related to several sensory quality attributes such as appearance, color, texture and flavor (Barrette *et al*., 2010). Sensory quality of leafy greens is optimal right after harvest, but deteriorates as the processes of senescence are initiated. These changes can be noticed at the physical or physiological level (Løkke, 2012).

A significant portion of leafy vegetables in the developed countries are now available in the fresh-cut format such as ready-to-eat packaged salads due to their convenience factor (Hedges and Lister, 2005). Another format of leafy vegetables gaining increased popularity is the microgreens. They form the target of high-end markets for fresh-produce as specialty greens due to their nutritional concentrations, special health benefits and strong flavors, colours along with tender textures (Xiao *et al.,* 2012; and Aggarwal and Aggarwal, 2013). The demand for such greens is also gaining impetus in the developing world where there is a need to adapt indigenous and traditional GLVs as part of health-promoting strategies to ensure good nutrition and wellbeing of the population (AVRDC, 2004).

One of the common indigenous GLVs widely used is fenugreek (*Trigonella foenum-graecum* L.). It is a self-pollinating leguminous plant of the Fabaceae family found extensively in the semi-arid regions of India, Mediterranean region, North Africa and Europe. It is commonly available throughout the year and its trifoliate leaves find widespread use for culinary and medicinal purposes.

Few studies have reported the phytochemical content of fresh fenugreek leaves (Simopoulos and Gopalan, 2003; Pasricha and Gupta, 2014; and Saini *et al*., 2016) and limited investigations are available on the changes in the contents of selected phytochemicals in fenugreek during storage (Yadav and Sehgal, 1997; and Negi and Roy, 2004). Some works have also focused on the antioxidant activity of *T. foenum-graecum* (Jha and Srivastava, 2012). However, there is no comparative study carried out so far to investigate the phytochemical changes and antioxidant activity of microgreens and their mature counterparts, in particular, fenugreek, during storage. Thus, the present work is set to explore this area.

MATERIALS AND METHODS

Sample Growth and Preparation

Fenugreek (*Trigonella foenum-graecum* L.) microgreens (FMG) were grown organically using vermicompost-enriched soil under ambient conditions in a polyshade house. A 25 g sample of fenugreek seeds (local market, Anantapur, India) was soaked in potable water for 8 hours and sown in furrows of even depth (1 inch) in soil filled plastic trays. Trays were kept in darkness in a high moisture (Relative humidity - 90 ± 5%) tunnel located within the polyshade house at a temperature of 30 ± 5 ºC during the first 48 hours of germination. After germination, the trays were exposed to ambient light and watered thrice daily. Microgreens were harvested after 7-8 days at a height of 2-3 inches by cutting them at the soil surface using a sharp sterile scissor. They were inspected for defects and unusable stems and leaves were discarded. Fenugreek Mature Leaves (FML), grown under the same conditions as microgreens, were harvested after 20 days of growth. The roots were excised, defected parts removed and the remaining stems and leaves were cleaned from any contaminating soil particles. Bundles of FMG and FML (20 g) were packaged separately in 5 x 6 inches macroperforated (4.5 mm diameter) low-density polyethylene (LDPE) re-sealable bags and stored at 10 ± 2 ºC for 14 days.

Phytochemical Analysis

Periodic analysis of FMG and FML was carried out on the initial day (0D), seventh day (7D) and fourteenth day (14D) of storage for the following phytochemicals – ascorbic acid (AsA), total polyphenols (TPP) and lutein (LUT).

Ascorbic Acid: The AsA content of fenugreek greens was determined by direct colorimetric method as given by Ranganna (1986) with minor modifications. Weighed samples (1 g) of leaves and stems were macerated with 3% metaphosphoric acid (10 ml) and centrifuged at 2000 rpm for 3 min. The supernatant was filtered through activated charcoal and the volume made up to 10 ml. Two millilitre of

the fresh extract was mixed with 2 ml of acetate buffer (pH 4) and 2 ml of distilled water. Dye solution of 2, 6 – dichlorophenol indophenol (3 ml) was added followed by 10 ml of isoamyl alcohol. The tubes were vortexed for 15 s and were allowed to stand for the separation of the alcohol layer. The lowermost water layer formed was discarded. Few crystals of anhydrous sodium sulphate were added to the alcohol layer and the tubes were shaken. The upper clear alcohol layer was analysed for ascorbic acid at 520 nm. Concentrations of AsA were estimated from an L – ascorbic acid calibration curve at concentrations 0.5 to 2 mg/ml.

Total Polyphenols: The concentration of TPP was determined by the Folin – Ciocalteau method (Kähkönen *et al.,* 1999) with some modifications. Dried samples of fenugreek leaves (0.1 g) were extracted with 80% methanol (10 ml). Four millilitre of hexane was added to the extraction mixture to remove the total chlorophyll and the tubes were vortexed for 30 s. The contents were centrifuged at 3000 rpm for 5 min at 4 °C. The hexane layer was discarded. This was repeated 2-3 times until the filtercake was devoid of chlorophyll. The supernatants were collected and volume was made up to 10 ml with 80 % methanol. To 0.1 ml of the sample extract, 0.5 ml of aqueous methanol (50% v/v) and 0.5 ml of freshly prepared Folin-Ciocalteau reagent (50% v/v) were added. Two millilitres of 7.5% Na_2CO_3 was added after 3 min. The tubes were mixed and allowed to stand for 15 minutes in darkness. Absorbance was measured at 750 nm. A calibration curve was used to estimate the TPP concentration against gallic acid at concentrations of 10 to 50 µg/ml and results were expressed as milligram of Gallic Acid Equivalents (GAE) per 100 grams of dried weight sample.

Lutein: Spectroscopic estimation of LUT was done using the procedure of Nurhidayati and Irianty (2012). Fenugreek leaves and stems (1 g) were macerated with 30 ml of acetone. Extraction was performed for 16 h in a flask containing 0.1 mg of calcium carbonate. After 16 h the residue was remacerated with 20 ml of acetone until the sample residue was clear. The extract was filtered and to the filtrate, 50% KOH (10 ml) was added and heated for 10 min. The solution was transferred to a separating funnel and diethyl ether (30 ml) was added followed by distilled water (20 ml). It was shaken vigorously for 2 min and allowed to stand. The water phase was discarded and the organic phase was washed 2-3 times with distilled water. The diethyl ether phase was collected and volume made up to 100 ml. Absorbance was read at 445 nm. Lutein content was estimated using the following formula:

$$\text{Lutein content (mg/kg)} = 0.0421 + 0.1246\,X$$

(X = Absorbance at 445 nm)

Determination of Antioxidant Activity

The antioxidant activity of FMG and FML was analysed on 0D, 7D and 14D of the storage period using 2,2-diphenyl picrylhydrazyl radical scavenging activity (DPPH RSA) and Ferric Reducing Antioxidant Power (FRAP).

DPPH RSA: The DPPH RSA was performed using the procedure of Yu *et al.* (2002). To methanolic extracts of the sample (0.1 ml), 2.9 ml of DPPH reagent (0.1 mM in methanol) were added and vortexed vigorously. The tubes were then incubated in darkness for 30 minutes at room temperature. The discoloration of DPPH was measured against a blank at 517 nm after 30 minutes. Ascorbic acid (0-50 µg/L concentration) was used as a positive control.

FRAP: The FRAP procedure was adapted from Benzie and Strain (1999). Briefly, to 0.1 ml of methanolic sample extract, 3 ml of FRAP reagent (300 mM acetate buffer at pH 3.6; 10 mM 2,4,6-tripyridyl-s-triazine; 20 mM ferric chloride in the ratio of 10:1:1 respectively) was added. It was incubated for 6 minutes at room temperature and absorbance of the reaction mixture was measured at 593 nm. Ferrous sulphate was used as the standard and BHT as the positive control.

Sensory Quality

Sensory quality of freshly harvested FMG and FML was assessed by a 10-member semi-trained panel on 0D, 5D, 10D and 14D. The sensory attributes of freshness, colour, wiltness, typical aroma, and tenderness were clearly defined and were rated on a 5-point hedonic scale. Briefly, freshness was defined as having a fresh-cut appearance, where a score of 5 = high, 3 = moderate and 1 = low freshness. Colour was rated based on the degree of greenness of the adaxial surface of the leaves, where a rating of 5 = dark green, 3 = light green and 1 = light green with noticeable yellowing of leaves. Wiltness was evaluated as the degree of shrinkage of the leaves with the following scores - 5 = no wiltness, 3= acceptable wiltness and 1 = severe wiltness. Typical aroma, defined as the characteristic leafy smell of fenugreek, was evaluated by crushing a sample of the leaves and smelling at a close proximity, where a score of 5 = maximum aroma, 3 = moderate aroma and 1 = no aroma. Tenderness was examined by the ease of tearing the leaves on a 5 to 1 scale, where 5 = high tenderness, 3 = moderate tenderness and 1 = low tenderness. The panellists were apprised of the different quality parameters before the evaluation. The samples were

labelled with a random 3-digit number and were served to the panel members in a random order.

Organoleptic Acceptability: The acceptability of fenugreek microgreens in the diet was tested through their incorporation in salad and common traditional recipes such as fenugreek pilaf (methi pulao), fenugreek flatbread (methi roti), fenugreek leaves dal (methi dal) and potato and fenugreek curry (aloo methi subzi). The recipes were scored on a 9 – point hedonic scale where 9 and 1 represents the highest and lowest acceptability scores**.**

Statistical Analysis

The samples were run for a total of 4 replicates. The data was averaged and expressed with the standard deviation. Student's *t*-test was used to analyse the data at a significance level of $P \leq 0.05$ using MS-Excel Data Analysis ToolPak package.

RESULTS

Phytochemical Analysis

The variations in the phytochemical content of FMG and FML during storage is shown in Table 1.

The initial ascorbic acid content of the samples was 154.8 mg/100 g FW for FMG and 87.5 mg/100 g FW for FML. With increasing storage period, significant reductions ($P < 0.05$) in the ascorbic acid content were observed in both FMG and FML. While the percent loss in ascorbic acid content of FMG was 17% and 25 % on the 7th and 14th day of storage, significantly higher reductions of 35% and 72% were noted on the 7th and 14th day of storage, respectively.

A significantly higher ($P < 0.05$) initial total polyphenol content was found in FMG (136 mg gallic acid equivalents/ g DW) as compared to FML (109 mg gallic acid equivalents/ g DW). Loss of total polyphenols was rapid during storage with 51% and 68% in FMG and 47% and 71% in FML on the 7th and 14th day of storage, respectively.

The lutein content of fenugreek greens was 15 mg/100 g FW in FMG and 28 mg/100 g FW in FML at the beginning of the storage period. A loss of 31% was recorded in FMG and 38% in FML after 14 days of storage.

Antioxidant Activity

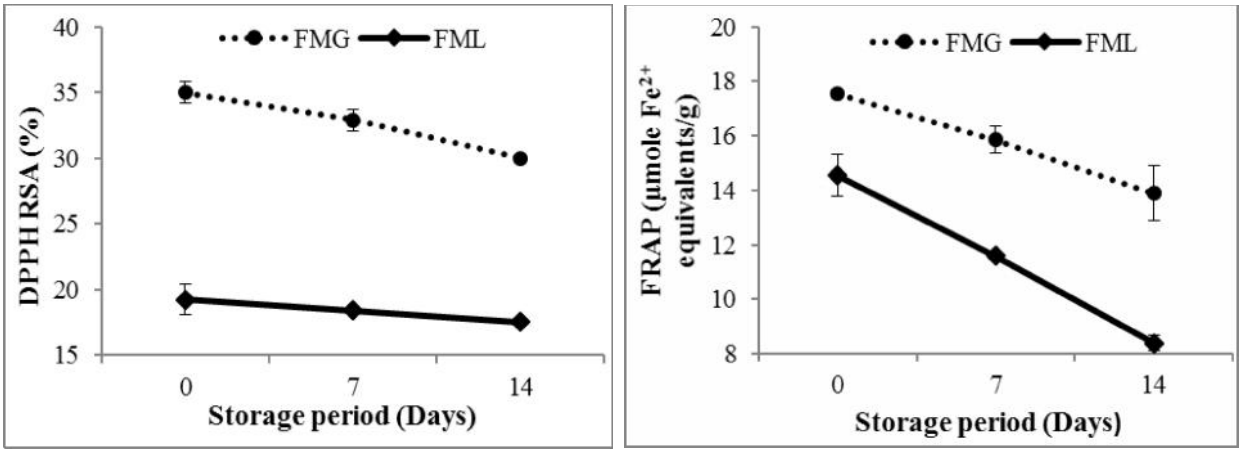
The antioxidant activity of FMG and FML of fenugreek during storage is illustrated in Figure 1.

Table 1: Effect of Storage on the Phytochemical Content of Fenugreek Microgreens (FMG) and Mature Leaves (FML)

Phytochemical	Sample	Storage Period		
		0D	7D	14D
Ascorbic acid (mg/100 g FW)	FMG	154.82^{a} ± 5.05	128.46^{b} ± 2.26	116.75^{c} ± 1.92
		(0.0)*	(17.0)*	(24.5)*
	FML	87.49^{a} ± 2.93	56.81^{b} ± 3.71	24.54^{c} ± 4.78
		0.0	(35.0)	(71.9)
Total polyphenols (mg GAE/g DW)	FMG	136.35^{a} ± 2.70	66.54^{b} ± 2.10	43.62^{c} ± 2.04
		(0.0)*	(51.1)*	(68.0)*
	FML	109.09^{a} ± 0.93	58.13^{b} ± 3.06	31.44^{c} ± 2.10
		0.0	(46.7)	(71.1)
Lutein (mg/100 g FW)	FMG	14.79^{a} ± 0.14	10.56^{b} ± 0.09	10.14^{c} ± 0.06
		(0.0)*	(28.6)*	(31.4)*
	FML	28.93^{a} ± 0.19	22.93^{b} ± 0.10	17.9^{c} ± 0.18
		0.0	(20.7)	(37.8)

Note: Results are expressed as mean ± standard deviation (n = 4). Values in parentheses indicate percentage loss of the bioactive compounds during storage. Means with different superscripts (a,b,c) within the respective row indicate significant difference at $P < 0.05$. *indicates significant difference at $P < 0.05$ between FMG and FML for the respective storage period. FW: Fresh weight basis; DW: Dry weight basis.

Figure 1: Effect of Storage on the Antioxidant Activity (A) DPPH Radical Scavenging Activity (%), (B) FRAP (µmol Fe^{2+} Equivalents/g) of Fenugreek Microgreens (FMG) and Mature Leaves (FML)



Both DPPH RSA and FRAP assays showed higher antioxidant activity in fenugreek microgreens than mature leaves throughout storage. The initial DPPH RSA was 35% in FMG and only 19.3% in FML. With increasing days of storage, there was a reduction in the DPPH RSA with a value of 30% in FMG while FML recorded a DPPH RSA of 17.6%, at the end of storage. Similar trend was observed in the FRAP assay. The initial FRAP value of FMG was 17.6 µmol Fe^{2+} equivalents/g while that of FML was 14.6 µmol Fe^{2+} equivalents/g. In the case of FML, major losses of 20% and 42% were registered in the FRAP value on the 7^{th} and 14^{th} day of storage resulting in a FRAP value of 8.4 µmol Fe^{2+} equivalents/g at the end of the storage while in FMG, reductions were only 10% and 20% on the respective days of storage ending with a much higher FRAP value (14 µmol Fe^{2+} equivalents/g) on the 14^{th} day of storage.

Sensory Quality

The sensory quality of FMG and FML across storage is depicted in Figure 2.

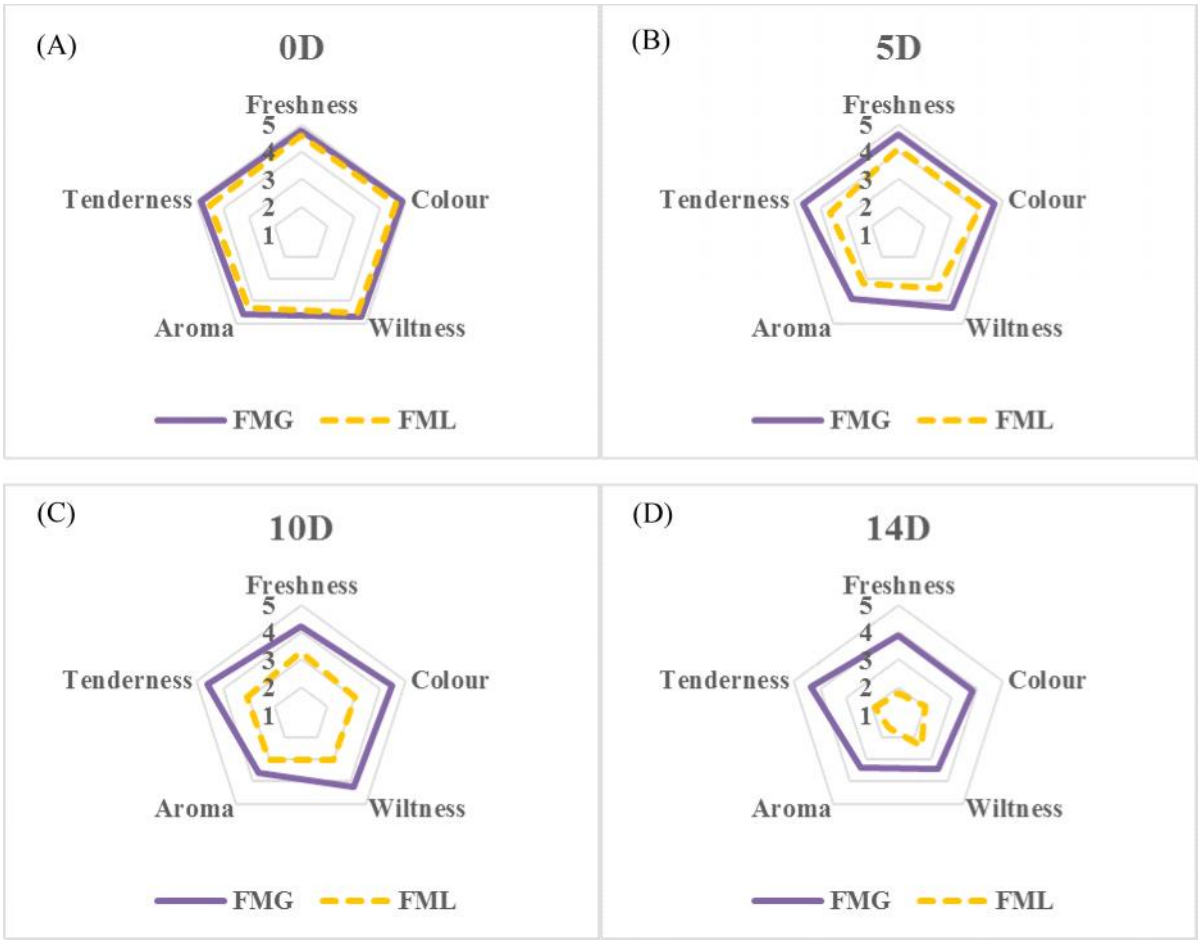
Microgreens were evaluated with a mean freshness score of 4.75, with a relatively dark green leaf colour (score 4.85), having negligible wiltness with a score of 4.7, strong fenugreek leaves aroma (score 4.6) and having very tender leaves with a tenderness score of 4.85 on 0D. In the case of mature fenugreek leaves, the initial mean sensory scores were 4.7, 4.5, 4.7, 4.3 and 4.5 for freshness, colour, wiltness, aroma and tenderness, respectively. At the end of the 14D storage period, better retention of the sensory quality was demonstrated in FMG with the following scores-freshness – 3.9, colour – 3.85, wiltness – 3.45, aroma – 3.35, and tenderness – 4.35, than when compared to FML (freshness – 1.8; colour – 2; wiltness – 2.4; aroma – 1.6; and tenderness – 1.9).

Incorporation of fenugreek microgreens in salad and traditional recipes were found to have a very good to excellent overall acceptability (Figure 3) with the following scores – *Methi Aloo* Curry – 8.64, *Methi Pulao* – 8.54, *Methi* Salad – 8.08, *Methi Dhal* – 7.92, and *Methi Roti* – 7.81.

DISCUSSION

Our study clearly reveals a difference in the concentrations of phytochemicals between the microgreens and the mature leaf stages. Khan *et al*. (2009) claimed that growth stages of plants have significant impacts on the concentrations of phytochemicals due to their different bioactivities. The higher levels of ascorbic acid, total polyphenols and total chlorophyll obtained in fenugreek microgreens in this study coincide with previous literature reports which also recorded higher levels of phytochemicals in the younger leaves than mature ones (Bergquist, 2006; Lester and Hallman, 2010; and Xiao *et al*., 2012). Raya *et al*. (2015) revealed that younger plant parts of *Clinacanthus nutans* contained higher amounts of the phytochemicals, ascorbic acid and chlorophyll, than its mature parts, which confirmed that phytochemical content tend to decrease with increasing maturity of the plant. Lutein is one of the carotenoids which play a key role in the photoprotection of the photosynthetic apparatus in plants (Huang *et al*., 2010). The higher lutein content observed in the present study in FML with respect

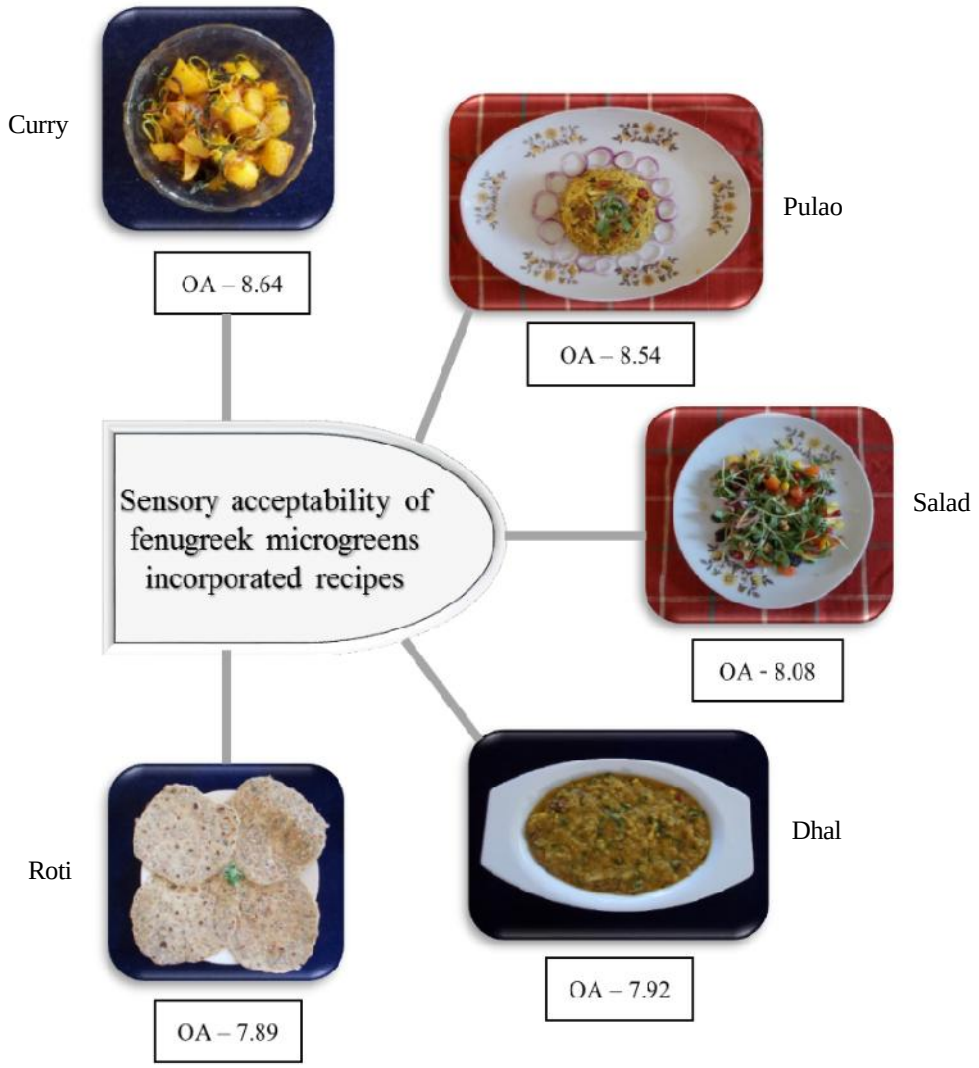
Figure 2: Sensory Quality of Fenugreek Microgreens (FMG) and Mature Leaves (FML) During (A) 0D, (B) 5D, (C) 10D and (D) 14D of Storage, Data are Means of 10 Replicates



to FMG may be attributed to the longer period of exposure to light during their growth.

Another factor which affects the phytochemical content of plants is the storage duration (Raya *et al*., 2015). Ascorbic acid is highly labile that it is often the first phytochemical to show decline during storage (Toivonen and Hodges, 2011), as observed in the present study. Negi and Roy (2004) also reported 65% loss in ascorbic acid on the 6th day of storage in mature fenugreek leaves packaged in polyethylene bags and stored in cool chamber (13-17 ºC and 86-92% relative humidity). Rapid reduction of vitamin C content (50%) on storage by also reported by Gil *et al*. (1998) in minimally processed Swiss chard after eight days under modified atmosphere. Bergquist (2006) observed loss of ascorbic acid in baby spinach leaves during storage at 2 ºC and 10 ºC, with more pronounced decrease at 10 ºC. The lower content of ascorbic acid corresponded to a higher dehydroascorbic acid/vitamin C ratio in these leaves, suggesting the oxidation of ascorbic acid to dehydroascorbic acid on storage. The advancement of storage period also caused reduction in the contents of other phytochemicals, such as total polyphenols and lutein. Similar loss of phenolic content in *C. nutans* (Raya *et al.*, 2015) and reduction in content of xanthophyll including lutein in parsley leaves (Yamauchi and Watada, 1993) has been reported on storage. Considering ascorbic acid as the most sensitive phytochemical compound, the nutrient contribution of a portion size (50 g expressed as percent RDA) of FMG for was 145% at the end of 14-day storage period, While FML contributed only 70% and 30% of the RDA on 7D and 14D of the storage period, respectively. Thus, the phytochemical quality of FMG was high enough even at the end of 14 days

Figure 3: Organoleptic Acceptability of Fenugreek Microgreens Incorporated Recipes



to meet the dietary requirement. Mature fenugreek leaves require cooking prior to consumption, which is likely to further reduce the phytochemical quality.

The three studied phytochemical compounds have antioxidant roles in the plants. The higher contents of most of these phytochemicals in FMG corresponded to the higher antioxidant activity observed in these tiny greens. Raya *et al.* (2015) also reported higher antioxidant activity in younger plants than older plants of *C. nutans*. This difference in their antioxidant function with growth stages may have physiological implications. As antioxidants, these phytochemicals participate in the scavenging of reactive oxygen species. The present study indicates fenugreek microgreens to have greater ability to protect against oxidative damage for a longer period of time as compared to mature leaves. In addition, antioxidant activity is known to reduce during storage, as observed in both greens. Reduction in the radical scavenging activity of greens during storage has also been reported by Supapvanich *et al.* (2012), who noted decrease in the DPPH RSA in fresh-cut sweet leaf bush on the 8th day of storage. Similarly, Zhan *et al.* (2012) observed reduction in the antioxidant capacity of fresh-cut romaine lettuce when stored at 4 ^{o}C for 7 days.

A decrease in the quality of the sensory attributes was observed during the storage period in both the greens due to initiation of senescence of the leaves. However, at the

end of the storage period, FMG showed considerably lower degree of senescence based on sensory quality compared to FML. In a previous study too shelf life of fenugreek mature leaves stored at 10 ºC was limited to 7 days as confirmed by the senescence indicators such as respiration rate, physiological loss in weight, decay percentage and sensory quality (Srividya and Ghoora, 2016). On an average, more than 80% of FMG retained better freshness, colour, wiltness, aroma and tenderness, whereas only about 40% of FML retained acceptable sensory properties.

Organoleptic evaluation indicated that fenugreek microgreens could be used both in the fresh and cooked form with good acceptability

CONCLUSION

In conclusion, fenugreek microgreens were found to have higher ascorbic acid, and total polyphenol contents than mature leaves. During storage, microgreens were found to have better retention of the phytochemical compounds. For optimal health benefits from the bioactive functional compounds present in fenugreek greens, it is recommended to utilize the mature leaves within one week of storage and the microgreens within a fortnight under refrigerated conditions and appropriate packaging.

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