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Original Research Paper

Effect of modified atmosphere packaging on quality of minimally processed fenugreek (*Trigonella foenum-graecum* L.) microgreens

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) microgreens is an underutilized vegetable with limited shelf life having good source of antioxidants, carotenoid as well as vitamins. The study deals with nutritional quality and optimization of a suitable passive modified atmosphere packaging (MAP) for improving the shelf life of fenugreek microgreens in its minimally processed form (MPFM) at 8°C Semipermeable plastic films *viz.*, low density polyethylene, polypropylene, Cryovac PD 961® and stretchable PVC cling film with varying thickness were evaluated as packaging materials to obtain different MAP composition inside MPFM packages. Packaging of MPFM in 40 µm thick polypropylene film resulted in development of in-pack equilibrium MA with 10-14% oxygen and 5-8% carbon dioxide during storage. This in-pack MA maintained 'fresh-like' sensory properties, biochemical and nutritional quality in MPFM till 15 days of storage. Significant loss of B vitamins was recorded in all packages during low temperature storage. Packaging in 40 µm thick polypropylene film retained B vitamins significantly better than other semipermeable films. Low temperature storage in modified atmosphere conditions enhanced vitamin E content in MPFM. The outcome of the study will benefit the entrepreneurs and retailers for distant transport and storage of fenugreek microgreens in commercial open chillers maintained in supermarkets in their ready-to-cook form.

Keywords: fenugreek, passive modified atmosphere packaging, vitamin, semi-permeable films

INTRODUCTION

Fenugreek microgreens are tiny green vegetable herbs harvested with two fully developed cotyledon leaves and first pair of true leaves. This stage often coincides with growth stage of 10-15 days of seedling emergence (Kyriacou et al., 2016). Microgreens catch the attention of nutritionists, chefs, and consumers due to nutritional quality and bioactive compounds besides their unique flavours; and are considered nutritional enhancers of diet. Microgreens possess a higher content of minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) and lower nitrate content than mature lettuces (Pinto et al., 2015), and are rich sources of antioxidants and vitamins due to abundance of ascorbic acid, phylloquinone, carotenoids, tocopherols, phenolic compounds etc.

Several microgreens are yet to be evaluated for their food use and nutritional quality (Mir et al., 2017). Fenugreek microgreens (FM) is a commodity with market niche restricted to Western parts of India, where it is locally known as *Choti methi* and *Samudra methi*, due to their characteristic tiny size and cultivation in sandy beaches of Arabian sea. The crop is usually sold in loose bundles in local markets, resulting in limited shelf life of less than a day at ambient temperature.

Passive modified atmosphere packaging (PMAP) is a low-cost packaging strategy for enhancement of shelf life in respiring foods such as fruits, vegetables and leafy greens. The basic technique of PMAP relies on modification of the atmosphere inside package to obtain an increased carbon dioxide and low oxygen levels through respiration of the produce. After a certain period, gas composition in the package of fresh product reaches an equilibrium state of gas composition, which is determined by respiration rate, product weight, temperature and permeability of packaging film (Kader et al., 2001; Soltani et al., 2015). Semipermeable plastics such as polypropylene, low density poly ethylene, PVC stretchable films, and many coextruded films are found useful for creating modified atmosphere in fresh produce packages (Ranjitha et al., 2018).

There is a potential for obtaining an enhanced shelf life of FM through minimal processing and packaging in passive MA packages. This study is a first ever report on the nutritional quality changes and shelf-life enhancement of minimally processed fenugreek microgreens (MPFM) during storage at open chiller temperature recommended in supermarkets.

MATERIALS AND METHODS

Plant material and minimal processing

Seeds of fenugreek were soaked overnight and broadcasted in aluminum trays filled with Arka fermented coco-peat as medium for seed germination (Kotur, 2014). The crop was harvested by uprooting on 14^{th}-15^{th} day of sowing at microgreen stage. The minimal processing operations included trimming out the roots, washing with tap water, dipping in sodium hypochlorite (100 ppm) for 5-minutes followed by rinsing with potable water and surface drying. Then the processed microgreens were used for further packaging studies.

Standardization of modified atmosphere packaging of MPFM

MPFM prepared with the above procedure were packed in different semi-permeable films having different gas and water vapour permeability characteristics. The films used were stretchable PVC Cling film (12 µm thickness), polypropylene films (25 µm and 40 µm), LDPE (40 µm) and Cryovac film (PD-961®), respectively to create different in-pack modified atmospheres. The MPFM packs were sealed with an impulse sealing machine and stored at 8°C. Twelve replicates were maintained for each type of packaging. Permeability characteristics of the packaging films used in the study are presented in Table 1.

In-pack gas composition analysis

Oxygen and carbon dioxide levels within the packs were recorded at periodic interval using auto gas analyzer (model : Checkmate 3-Dansensor, UK). Sufficient care was taken by affixing a septum on the packages to seal the needle puncture during gas measurement. Ethylene concentration within the packs was recorded at periodic interval using ethylene analyzer (model : ETHAN Bioconservacion, Spain).

Visual quality monitoring and sensory analysis

The unopened packages were observed daily for any changes in visual quality such as wilting, rotting and yellowing. This was carried out since the appearance is one key indicator of freshness in greens. Detailed sensory evaluation was carried out after drawing samples at five days interval. The experiment was terminated on 15^{th} day of storage, as sensory deterioration was observed in all packages, including those which were judged as equal to freshly packed MPFM in previous days of evaluation. Sensory quality of MPFM was carried out in a 5-point Hedonic scale for characteristic *viz.*, greenness, fresh-like odor, texture and overall acceptability; where the scores were classified as 5- excellent (exactly similar to freshly harvested), 4- very good, 3- good, 2- average and 1- poor. Score was given to individual quality attributes, and the overall marketability was taken as the most important quality attribute (Ranjitha et al., 2018). The samples were analysed for detailed nutritional quality parameters after 5, 10 and 15 days of storage. The experiment was terminated on 15^{th} day of storage.

Instrumental measures of quality

Table 1 : Permeability characteristics of the packaging films

Film	Monomer	Permeability		
		O_2 (ml mil^{-1} m^{-2} day^{-1} atm^{-1})	CO_2 (ml mil^{-1} m^{-2} day^{-1} atm^{-1})	WVTR (g m^{-2} day^{-1} at 38°C; 90% R.H.)
Polypropylene	Propylene	1300-6400	7700-21,000	16-24
LDPE	Ethylene	3900-13,000	7700-77,000	10-12
PD-961	Polyolefin	7400–8500	21,000–24,000	9-14
PVC Cling	Vinyl chloride	63,555	127,733	140-171

Physiological loss of weight of MPFM was measured gravimetrically. Protein content was measured using modified Lowry's method (Lowry et al., 1951). Parameters *viz.*, total chlorophyll (mg g^{-1}), ferric reducing anti-oxidant potential (mg ascorbic acid equivalent antioxidant capacity, AEAC mg g^{-1}), total phenols (mg gallic acid equivalent g^{-1}, GE mg g^{-1}) and total flavonoids (catechin equivalent mg g^{-1}, CE mg g^{-1}) were measured spectrophotometrically using the standard methods (Benzie & Strain, 1996; Singleton & Rossi, 1965) after 15 days storage. Chlorophyll was measured spectrophotometrically (Haskin, 1942). All biochemical analyses were carried out in triplicates. Water soluble and fat-soluble vitamins were estimated sequentially per Santos et al. (2012).

Statistical analysis

The data obtained on biochemical analysis were statistically analyzed using complete randomized design (CRD) in MS Excel 2007 software. Single factor analysis of Variance (ANOVA) with three replications was carried out to determine the statistical significance and least significant difference (LSD) between treatments on different parameters was found out at 99% confidence level.

RESULTS AND DISCUSSION

Details on the effect of different packaging film on in-pack gas composition of MPFM is presented in Fig. 1. Cling film maintained a nearly normal atmospheric composition throughout storage, with O_2 ranging from 19-20% and CO_2 less than 1% levels. Among the different films, PP 40 µm film packages accumulated carbon dioxide rapidly during initial days of storage, and CO_2 levels in the packages were 12% within 4 days. Thereafter, the EMAP phase began, and CO_2 level balanced in a range of 5-8%. A rapid oxygen depletion was observed concomitant with CO_2 accumulation, reaching O_2 level to 2.5% within 4 days, followed by a further rise to 7.3% on 7th day, marking the EMAP level. The remaining three films, though developed a modified atmosphere, retained a higher oxygen and lower CO_2 than PP 40 µm throughout the storage. Briefly, the equilibrium modified atmosphere formed in the films *viz.*, PP 25 µm, PP 40 µm, LDPE 35 µm, Cryovac PD-961® packs were 11.2-15.1% and 3.7-4.3%, 10.4-14.0% and 5-8%, 13.2-15.3% and 1.7-2.1%, 12.7-14.30% and 2.5-2.7%, respectively. This difference in equilibrium modified atmosphere (EMAP) in these packages is the primary reason for a better shelf life of the MPFM in PP 40 µm packages.

The EMAP development is mainly dependent on respiration of the MPFM and gas permeability characteristics of the packaging film. Microgreens are rapidly respiring fresh produce, and the strategies to reduce respiration gives immense benefit in improving the shelf life (Sharma et al., 2022). It may be inferred that that permeability features of PP 40 µm is suitable for reducing the respiration rate of MPFM to develop a suitable EMAP. Ethylene accumulation inside packages also varied, and was found minimum in PP 40 µm packs (<1-5 mg/L), while, cling film package head space had 20-25 mg/L ethylene during most of the storage period (Fig. 1S). Ethylene is the main senescence hormone in plant tissues; and the accumulation of ethylene is a cause as well as

Table 2 : Effect of MAP on vitamin content of fresh and minimally processed fenugreek micro greens (MFM) stored for fifteen days at 8°C

Packaging film	Vit. B1 (µg g^{-1})	Vit. B2 (µg g^{-1})	Vit. B5 (µg g^{-1})	Vit. B6 (µg g^{-1})	Vit. B9 (µg g^{-1})	Vit. B7 (µg g-1)	Vit. E (µg g^{-1})	Vit. K (µg g^{-1})
Fresh	3.70±0.08	1.42±0.03	2.80±0.01	3.88±0.02	0.0020±0.00	0.07±0.002	700.40±24.50	867.70±28.50
Cling film	3.30±0.10	1.22±0.08	0.10±0.00	3.48±0.03	0.0014±0.000	0.04±0.004	745.10±63.20	604.20±1.60
PP 25 µm	3.30±0.10	1.27±0.05	0.17±0.00	3.52±0.03	0.0013±0.00	0.01±0.003	1005.60±19.1	744.30±17.50
PP 40 µm	3.40±0.10	1.47±0.07	0.19±0.00	3.54±0.03	0.0033±0.000	0.04±0.004	1237.80±6.70	939.50±8.90
LDPE 35 µm	3.00±0.10	1.25±0.01	0.16±0.00	3.44±0.10	0.0046±0.00	0.03±0.006	1170.60±4.90	736.10±8.80
PD-961®	3.40±0.10	1.38±0.08	0.16±0.00	3.39±0.02	0.0030±0.00	0.03±0.009	1147.60±0.30	738.60±17.60
F-test	*	*	*	*	NS	*	*	*
SE (m)	0.04	0.01	0.02	0.02	0.001	0.01	18.50	9.20
CD at 1%	0.16	0.04	0.09	0.11	0.003	0.03	77.80	38.80

Vit. indicates vitamin

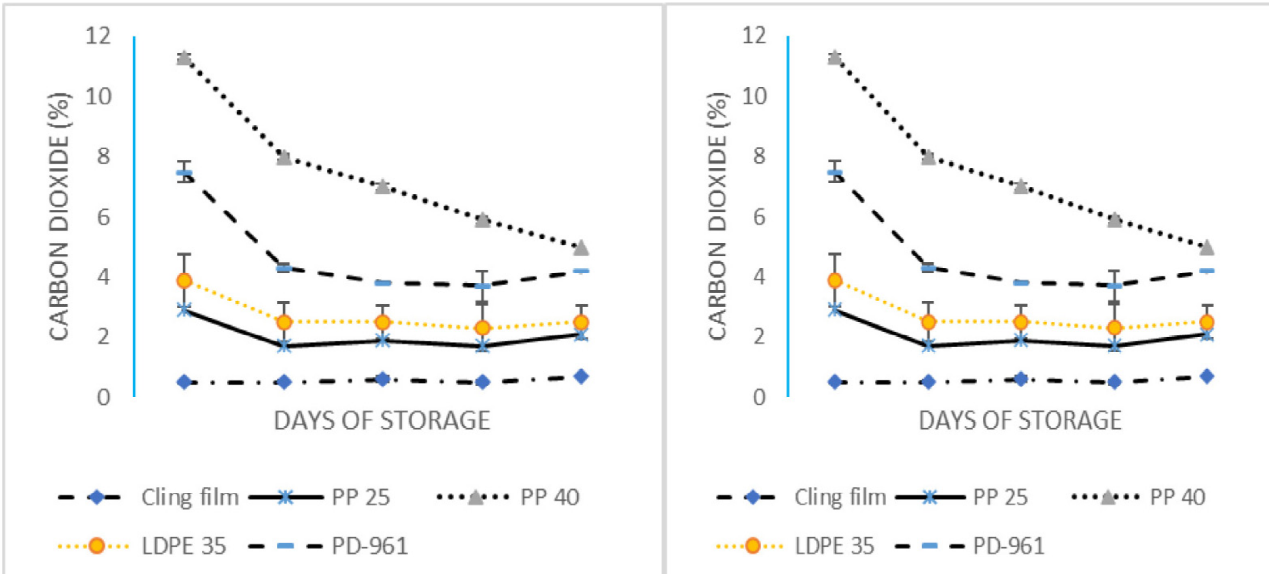

Fig. 1 : In pack O_2 and CO_2 gas composition of the minimally processed fenugreek micro greens packs during storage at 8°C. (Error bars represent standard deviation, n=12

consequence of tissue decay in microgreens (Turner et al., 2020).

Sensory quality of MPFM during storage was influenced by the EMAP conditions in the packaging films. The MPFM stored in PP 40 µm and Cryovac PD-961® possessed acceptable sensory quality till 15 days of storage (Table 3). The change in sensory quality at 5 and 10 days storage is presented in supplementary tables (Table 1S and 3S). In general, cling film wrapped samples scored low due to the poor appearance caused by wilting and yellowing of the produce; while, other packages resulted in slight yellowing of the produce due to extended storage period. This could be correlated well with the difference in chlorophyll levels of different MPFM samples after storage.

Total chlorophyll content was recorded highest in fresh MPFM (4.46 mg g^{-1}) followed by PP 40 µm (3.83 mg g^{-1}) and Cryovac PD-961® (3.27 mg g^{-1}), whereas, lowest was observed in cling film packed MPFM (1.01 mg g^{-1}). The degradative changes began within 10 days of storage in samples drawn from all packages, except PP 40 µm (Table 2S and 4S). Physiological loss of weight (PLW), was recorded highest in MPFM packed in cling film. Colour and turgidity are the main features contributing to the fresh

Table 3 : Effect of MAP on sensory and nutritional quality of fresh and minimally processed fenugreek microgreens (MPFM) stored for fifteen days at 8°C

Packaging film	Sensory acceptability	PLW (%)	Protein (mg g^{-1})	Total Carotenoids (mg g^{-1})	Total chlorophyll (mg g^{-1})	FRAP (mg g^{-1} AEAC)	Total Phenols (mg g^{-1} GAE)	Total flavonoids (mg g^{-1} CE)
Fresh	5.00±0.00	NA.	28.00±0.50	0.41±0.012	4.47±0.23	0.40±0.001	0.54±0.02	0.0011±0.00
Cling film	2.00±0.33	4.53±0.24	25.40±0.50	0.43±0.62	1.01±0.05	0.36±0.010	0.73±0.05	0.0012±0.00
PP 25µm	3.00±0.28	0.24±0.03	26.30±1.40	0.46±0.005	1.18±0.18	0.37±0.001	0.56±0.01	0.0011±0.00
PP 40 µm	4.50±0.19	0.15±0.01	27.20±0.60	0.47±0.006	3.83±0.40	0.42±0.004	0.55±0.07	0.0011±0.00
LDPE 40 µm	3.50±0.22	0.21±0.01	26.30±0.20	0.42±0.01	2.02±0.11	0.39±0.002	0.62±0.00	0.0011±0.00
PD-961	3.70±0.41	0.21±0.01	24.00±1.10	0.44±0.03	3.27±0.51	0.40±0.010	0.62±0.05	0.0011±0.00
F-test		*	NS	NS	*	*	NS	NS
CD at 1%		0.14			1.12	0.037		

All values are expressed as mean of triplicates ± standard deviation; *significant at 1% confidence level; AEAC: ascorbic acid equivalent antioxidant capacity; FRAP: ferric reducing antioxidant potential; GAE: gallic acid equivalent; CE: catechin equivalents; NS: non-significant

Table 4 : Effect of MAP on vitamin content of fresh and minimally processed fenugreek micro greens (MFM) stored for fifteen days at 8°C

Packaging film	Vit. B1 (μg g^{-1})	Vit. B2 (μg g^{-1})	Vit. B5 (μg g^{-1})	Vit. B6 (μg g^{-1})	Vit. B9 (μg g^{-1})	Vit. B7 (μg g-1)	Vit. E (μg g^{-1})	Vit. K (μg g^{-1})
Fresh	3.70±0.08	1.42±0.03	2.80±0.01	3.88±0.02	0.0020±0.00	0.07±0.002	700.40±24.50	867.70±28.50
Cling film	3.30±0.10	1.22±0.08	0.10±0.00	3.48±0.03	0.0014±0.000	0.04±0.004	745.10±63.20	604.20±1.60
PP 25 μm	3.30±0.10	1.27±0.05	0.17±0.00	3.52±0.03	0.0013±0.00	0.01±0.003	1005.60±19.1	744.30±17.50
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LDPE 40 μm	3.00±0.10	1.25±0.01	0.16±0.00	3.44±0.10	0.0046±0.00	0.03±0.006	1170.60±4.90	736.10±8.80
PD-961®	3.40±0.10	1.38±0.08	0.16±0.00	3.39±0.02	0.0030±0.00	0.03±0.009	1147.60±0.30	738.60±17.60
F-test	*	*	*	*	NS	*	*	*
SE (m)	0.04	0.01	0.02	0.02	0.001	0.01	18.50	9.20
CD at 1%	0.16	0.04	0.09	0.11	0.003	0.03	77.80	38.80

Vit. - indicates vitamin

appearance of greens. Greenness and turgid appearance of the plant tissues represents the integrity of vacuoles and other membrane bound organelles in the cell (Martínez-Sánchez et al., 2011). High water permeability of cling film resulted in loss of turgidity and resulted in tissue disintegration at a rapid rate in cling wrapped MPFM samples. Better retention of chlorophyll content and low PLW were the major contributing features of fresh-like appearance and sensory acceptability of MPFM samples packaged in PP 40 μm film.

Nutritional quality of MPFM was also influenced by EMAP conditions in the packages. Ferric reducing antioxidant capacity of the samples packaged in LDPE, PP 40 and PD-961 remained statistically on par with the fresh FM even after 15 days storage, and significantly superior than cling film wrapped samples. This showed that the antioxidants of the microgreens could be well preserved during postharvest storage using a wide range of modified atmosphere packaging. Antioxidant capacity of microgreens is affected by the storage duration, temperature, relative humidity and the in-pack atmosphere (Xiao et al., 2014). Though, the phenolics in cling wrapped MPFM were on par with other packages, the lower total antioxidant capacity level could be attributed to the loss of other antioxidants such as vitamins.

Vitamin profiling of FM showed that fresh FM is a good source of B vitamins such as B1, B2, B5 and B6 and the fat-soluble vitamins E and K (Table 3). Low temperature storage invariably caused the loss of B vitamins, and the highest loss was visible in vitamin B5. Water soluble vitamins are lost rapidly during postharvest storage of leafy greens such as cabbage (Hounsome et al., 2009). At the same time, low temperature storage enhanced vitamin E content in many MPFM samples. Tocopherol is the main form of vitamin E present in leaves. Vitamin E level enhances during senescence of leaves, cold stress and dark stress (Keles & Oncel, 2002; Kobayashi & Della Penna, 2008). In plant tissues, tocopherols act as antioxidants to protect photosystem II from photoinactivation and cell membranes from lipid peroxidation (Havaux et al., 2005). In the present study, storage in walk-in cold room provided the stresses of low temperature, senescence and dark conditions which have probably helped in accumulation of tocopherols in MPFM in most of the EMAP packages. In cling film wrapped MPFM, where the physiological loss of weight and tissue deterioration was substantially high, vitamin E was less, which may be reasonably attributed to tissue dehydration related metabolic responses.

The present study proved that FM is a rich source of vitamin K. Phylloquinone (vitamin K1) is the major form of vitamin K, which ranges from 90 to 410 μg g^{-1} in different microgreens (Xiao et al., 2012). In MPFM, there was a substantial increase in phylloquinone (vitamin K1) in PP 40 μm packed leaves, which was best MAP for maintaining quality. It can be presumed that the suitability of MAP conditions in PP 40 μm helped to retain freshness in the leaves including accumulation of phylloquinone, while, a range of degradative changes in other suboptimal MA packages have contributed to degradation of phylloquinone too.

CONCLUSION

Present investigation has proven the potential dietary use of FM as source of antioxidants, B vitamins, vitamin E and K. The study has also proven the potential of a PMAP method using a commonly available semipermeable film to obtain an economically viable shelf life. Superior results on shelf-life enhancement and nutritional quality retention of MPFM in fenugreek microgreens was obtained by packaging of MPFM in 40 µm thick polypropylene film, which created an equilibrium modified atmosphere of 10.4-14.0% O_2 and 5-8% CO_2. Besides retaining the consumer acceptability features, this packaging has substantially reduced ethylene accumulation, maintained antioxidants and vitamins, and 15 days shelf life during storage at 8°C. Thus, insights from this study reiterates the importance of underutilized microgreens in local food systems, as well as provides an opportunity for its value addition and packaging suitable for distant transport and marketing.

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