Biochemical and physiological changes in Fenugreek (*Trigonella foenum-graecum* L.) leaves during storage under modified atmosphere packaging

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Abstract Fenugreek (*Trigonella foenum-graecum* L.), is a highly respiring leafy vegetable enriched with many nutrients and other active ingredients such as protein, vitamin C, niacin, potassium, alkaloids, lysine and L-tryptophan as well as steroidal saponins which are beneficial for human health. Both, quantitative and qualitative losses in this vegetable can be reduced by appropriate packaging and storage techniques which have not been applied hitherto. This study was taken upon to assess the potential of macro-perforated MAP for storage of fenugreek leaves. Packages (bag area: 0.075 m²) made from polypropylene (PP) film (Thickness: 35 µmm) were selected for the storage studies. The leaves were packaged in macro-perforated (2 perforations, perforation diameter: 0.3 mm each, with and without mustard seeds as natural absorbents) as well as in non-perforated PP film packages with and without mustard seeds. 10 g of mustard seeds were placed inside the packages to check water accumulation. The packaged samples were stored for 6 days at 75% relative humidity (RH) at 15°C to check water accumulation, if any. Different physiological and biochemical characteristics which generally affect the post-harvest life of the produce

were monitored during the storage period. Results of the study suggested that among all the treatments, packaging of fenugreek in two perforation packets with mustard seeds resulted in best maintenance of chlorophyll, ascorbic acid, phenols and aroma. Water accumulation was also consoled due to mustard seeds after 6 days of storage.

Keywords Antioxidants · Fenugreek · Modified atmosphere packaging · Headspace · Pigments

Fenugreek (*Trigonella foenum-graecum* L.), also known as Greek Hay and Fenigreek is a herb commonly found in many Asian, European and African countries. India is the largest producer of fenugreek in the world. Rajasthan, Gujarat, Uttaranchal, Uttar Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states of India. Another variant, popularly known as “Kasuri Methi” grown commonly in Pakistan is known for its appetizing fragrance and is a basic ingredient in many culinary preparations. Seeds and leaves besides being a gourmet’s delight are also used for treatment of a variety of health related problems. It contains many nutrients and other active ingredients such as protein, vitamin C, niacin, potassium, diosgenin, alkaloids, lysine and L-tryptophan as well as steroidal saponins which are beneficial for human health (Hemavathy and Prabhakar 1989; Schryver 2002; Basch et al. 2003; Michael and Kumawat 2003; Jana and Das 2007).

Modified atmosphere packaging (MAP) along with low temperature storage is one of the most effective ways to improve the shelf-life of harvested crops (Church and Parsons 1995; Gorny 1997; Paull 1999; Saito and Rai 2005), through interaction of the natural process of

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respiration of produce with the restricted gaseous exchange across the polymeric film package to control the headspace O_2 and CO_2 (Ishikawa et al. 1992; Hertog et al. 1998). An equilibrium within the package is established when the rate of O_2 and CO_2 transmission through the package equals the rate of respiration of packaged produce (Day 1992a, b). This gaseous equilibrium may or may not be suitable for the packaged produce and thus, requires to be optimized in order to have the nutrition intact for a longer period of time. The design and generation of an optimum MAP requires thorough understanding of the interaction between the various factors such as film characteristics (surface area, gas and water vapour permeability), temperature, free volume inside the package, weight and respiration rate of the produce and initial gaseous composition (Beaudry et al. 1992; Cameron et al. 1994; Beaudry 1999; Salvador et al. 2002).

Among the various issues envisaged and being practiced for a beneficial MAP, selection of suitable packaging material for MAP is a complex issue as different polymeric and biomaterials based packaging materials have a range of gas permeability characteristics. MAP may be accomplished by using packages made from polymeric packaging films like low density polyethylene, high density polyethylene, polypropylene etc. (Ishikawa and Hasegawa 1998; Rai and Paul 2007). However, these materials have been largely designed for non-food applications. Hence, design of MAP require a proper assimilation of produce and packaged characteristics to get beneficial shelf-life extension as well as maintenances of bio-active components of packaged produced.

Assessment of respiratory behaviour of fenugreek during the course of this study showed that high rates of respiration and water vapour production were principal impediments for design of appropriate packaging for its long term storage. These observations also suggested that fenugreek could be stored under MAP utilizing macro-perforated MAP. The preliminary trials also suggested that shelf-life of fenugreek was adversely affected by in-pack moisture. Further, the specific information related to design of MAP for fenugreek to take care of its high rates of respiration and water accumulation is apparently lacking. Hence, this study was taken upon to assess the potential of macro-perforated MAP for storage of fenugreek leaves.

Materials and methods

Freshly harvested fenugreek leaves were first hydro-cooled for 60 s using ice-cooled water containing citric acid (0.5% v/v); centrifuged to remove surface water using a basket centrifuge (CIPHET, India) rotating at 400 RPM for 15 s for removal of excess surface moisture and were then air-

cooled for 2 h at 15°C in a cold room (Sheel Biotech Ltd, India), before the start of the experiment. Air cooling helped in surface drying as well as prepared the leaves for further experimentation. The leafy vegetables were packaged in cold room itself after they were found to be sufficiently acclimatised to the surrounding environment in the cold room.

Packaging and storage Packages (bag area: 0.075 m²) made from polypropylene (PP) film (Thickness: 35 µm, Gas permeability coefficients: 4.15×10^{-14} ml.cm. cm⁻² .s⁻¹ .Pa⁻¹ for O_2 and 1.45×10^{-13} ml.cm. cm⁻² .s⁻¹ .Pa⁻¹ for CO_2 at 15 °C and 75% RH) were selected for the storage studies. The gas transmission rates of the packaging film for O_2 and CO_2 at 15°C, were measured by a gas permeability tester (Labthink, model BTY-B1P, China). During preliminary experimentation with different desiccants such as white silica gel beads and mustard seeds, it was observed that packaging of fenugreek in PP film packages resulted in excessive accumulation of water inside the packages which could be prevented best with the use of mustard seeds. The leaves (250 g each) were packaged in macro-perforated (2 perforations, perforation diameter: 0.3 mm each, with and without mustard seeds as natural absorbents) as well as in non-perforated PP film packages with and without mustard seeds of the same size to vary the headspace volume as well as the in-pack gaseous atmospheres. 10 g of mustard seeds were utilized and were placed inside the packages. The packaged samples were stored for 6 days at 75% relative humidity (RH) at 15°C. Fenugreek kept in the unsealed PP bags was taken as control samples. Weight loss and headspace gaseous concentrations of O_2 and CO_2 were evaluated regularly. Three packages from each type of packaging treatments were analyzed on 2nd, 4th, and 6th day of storage for pigments, ascorbic acid, phenolic content, flavonoids and DPPH radical scavenging activity.

Weight loss and headspace gas analysis During the entire storage period, the weight loss was determined by weighing the individual package on the day of observation using a laboratory level weighing scale having 0.01 g accuracy (Scaltec Instruments, Germany). Package headspace was monitored by means of a portable Gas Analyzer (Model 902 D Dualtrak, Quantek, USA). The instrument evaluated the headspace by means of an electrochemical and an infrared sensor (sensitivity: 0.1% O_2 ; 0.1% CO_2 , accuracy: 0.1% O_2 ; 0.2% CO_2) for O_2 and CO_2 concentrations, respectively. The instrument was calibrated with the standard O_2 and CO_2 gases; before the actual observation. A sampling probe containing a particular filter and a removable needle having dual side-port holes were used to draw the sample from the package headspace with the

help of an electronically controlled miniature pump. The samples so drawn were fed simultaneously to the O₂ and CO₂ sensors and concentrations of O₂ and CO₂ were directly read on the digital display panel of the instrument.

Total chlorophyll Total chlorophyll was determined by homogenizing 1 g of leaves with 10 ml of acetone and n-hexane (4:6); using a tissue homogenizer (Labco, New Delhi, India) over ice to prevent any biochemical changes during extraction. The extraction was carried out till the whole tissue was extracted leaving white precipitate behind. The homogenized solution was allowed to stand for 1 min in an amber colored glass tube placed inside the flaked ice and was protected from direct exposure to the light by covering it from outside by aluminium foil. One ml of the supernatant was taken and was diluted with 9 ml of the extract solution. The resulting solution was analysed spectrophotometrically with the help of a UV-Vis spectrophotometer (Spectroscan 80DV, Biotech Engineering Management Company Limited, UK). Optical density of the solution was measured at different wavelengths namely 663, 645, 505, 453 nm using acetone and n-hexane (4:6) as blank. Total chlorophyll (mg/100 g fresh weight of leaves) was quantified using the relationships as described by Nagata and Yamashita (1992) as follows:

$$\text{Chlorophyll a} = 0.999A_{663} - 0.989A_{645} \quad (1)$$

$$\text{Chlorophyll b} = -0.328A_{663} + 1.77A_{645} \quad (2)$$

Total Chlorophyll content: Chlorophyll a + Chlorophyll b
where, A_{663} and A_{645} are the absorbances at 663 and 645 nm, respectively.

Ascorbic acid Ascorbic acid was determined quantitatively as per the modified 2,6-dichlorophenolindophenol (DIP) method (Klein and Perry 1982). For extraction, 1 g of leaves from different packaging treatments was homogenized using a tissue homogenizer (Labco, New Delhi, India) for 1 min with 10 ml of 1% metaphosphoric acid (v/v) over ice. The extract was centrifuged at 3000 g in a cold centrifuge (MP 400-R, Eltek Limited, India) at 3°C for 15 min. 1 ml of the supernatant was mixed with 9 ml of 0.05 mM of DIP using a vortex shaker (Labco, New Delhi, India) for 15 s and absorbance of solution was measured against the blank at 515 nm with the help of a UV-V spectrophotometer (Spectroscan 80DV, Biotech Engineering Management Company Limited, UK). Standard curve for the ascorbic acid was prepared within the linear range of 0–500 mg ascorbic acid/ml.

Total phenolic content Total phenols were determined as per McDonald et al. (2001), using Folin Ciocalteu reagent.

1 g of fenugreek was extracted with 10 ml of methanol: water (50:50, v/v). 0.5 ml of the diluted (1:10) extract or the standard phenolic compound (Gallic acid) was mixed with 5 ml of Folin Ciocalteu reagent (1:10 diluted with distilled water) and 4 ml of aqueous Na₂CO₃ (1 M). The mixture was allowed to stand for 15 min and optical density of the mixture was determined against the blank at 765 nm with the help of UV-Vis spectrophotometer (Spectroscan 80DV, Biotech Engineering Management Company Limited, UK). Standard curve was prepared using 0, 50, 100, 150, 200, 250 µg solutions of gallic acid per ml of methanol: water (50:50, v/v). Total phenol values were expressed in terms of the standard reference compound as gallic acid equivalent (g/100 g fresh weight of leaves).

Total flavonoids determination Total flavonoid content was determined as per method of Chang et al. (2002). 1 g of leaves were extracted with 10 ml of pure methanol. 0.5 ml of extract solution was mixed with taken 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The solution was then incubated for 30 min. The optical density of the reaction mixture was measured against the blank at 415 nm with the help of UV-Vis spectrophotometer (Spectroscan 80DV, Biotech Engineering Management Company Limited, UK). The standard calibration curve was prepared by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Antioxidant capacity Antioxidant capacity was determined through assessment of free radical-scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (HiMedia Laboratories, India) as per the method of De Ancos et al. (2000). 1 g of leaves was extracted with 5 ml of methanol over ice to prevent any biochemical changes during extraction. The extract solution was centrifuged at 6000 g for 15 min at 4°C, using a cold centrifuge (MP 400-R, Eltek Limited, India). Aliquots of 0.01 ml of supernatant so obtained were mixed with 3.9 ml of methanolic DPPH (0.025 g/l) and 0.090 ml of distilled water. The resulting mixture was shaken on a vortex shaker (Labco, New Delhi, India) and was then incubated in dark for 30 min. Absorbance of the mixture was measured against the blank at 515 nm using UV-Vis spectrophotometer (Spectroscan 80DV, Biotech Engineering Management Company Limited, UK). The results were obtained as the percentage decrease with respect to the absorbance of a reference DPPH solution.

Sensory and visual appearance assessment The sensory and visual assessment for vegetable was carried out by a five member trained panel by suitably modifying the procedure of Carvalho and Clemente (2004). Aroma was evaluated as per the scale: 5 = no off-odour, 4 = very light off-odour, 3 = light

off-odour, 2 = medium off-odour and 1 = strong off-odour. Visual appearance was scored as per Deza et al. (2003) using: 5 = very good, 4 = good, 3 = normal, 2 = limited quality and 1 = not acceptable. Another nine point scale; 0 = vegetable completely wet and water accumulation; 1 = vegetable and film moderately wet; 3 = vegetable moderately wet; 5 = vegetable and film slightly wet; 7 = vegetable slightly wet; 9 = no water accumulation was used to represent the water vapour accumulation inside the film packages (Rai et al. 2009a). During scoring, the intermediate scores were also given to the samples depending upon the perceived condition of the samples.

Statistical analysis The statistical analysis of data obtained was carried out to establish the difference among the samples. All the experiments were performed in triplicate. One-way analysis of variance (ANOVA) and multiple comparisons (Fisher's least significant-difference test) were used to evaluate the significant difference of the data at $p < 0.05$ using a statistical package (Statgraphics Plus, Statpoint Inc., USA) and the means were plotted to draw out inferences.

Results and discussion

Headspace composition Package headspace compositions of O_2 and CO_2 for different packaging treatment are plotted in Fig. 1. Under all the packaging treatments, initially a rapid decrease in O_2 and a corresponding increase in CO_2

Fig. 1 Changes in concentrations of O_2 and CO_2 inside the polypropylene bag containing fenugreek under modified atmosphere packaging at 15°C and 75% RH ($n=3$)

concentrations was observed on the 1st day which could be attributed to the consumption of O_2 and production of CO_2 at a faster rate in the beginning in response to high rates of respiration (109.95 and 95.75 ml/kg.h for O_2 and CO_2 , respectively) of fenugreek under transient atmospheric conditions, as reported by different researchers for certain highly respiring produce (Emond et al. 1991; Rai and Paul 2007; Rai et al. 2009b). Subsequently, as the storage progressed further, respiration rate of the fenugreek decreased and stabilized on 2nd day. The O_2 concentration inside the 2 perforation packages (with seed) arrived at 0.5% and for packages without mustard seeds settled around at 3% on 6th day, respectively indicating that the mustard seeds absorbed the headspace moisture evolved through respiration of leaves (water vapour), sprouted and the sprouting mustard seeds also consumed the available in-pack O_2 . For non-perforated packages (both with mustard seeds and without mustard seeds) the O_2 levels became anaerobic largely due to combined effect of high rates of respiration of fenugreek and mustard seed sprouts. CO_2 concentrations inside the macro-perforated packages were observed to be 16 and 15.5% for perforated packages with seed and without mustard seeds, respectively. On the other hand, CO_2 in non-perforated packages arrived at 10.7 and 15% for with seed and without seed packaging treatments, respectively and stabilized at the same level thereafter during entire storage under the prevailing anaerobic levels of O_2 in these packages.

Biochemical constituents Enhanced respiration, physical abuse, effect of endogenous enzyme and other environmental factors largely attribute to deterioration in quality (Watada et al. 1990; Kim et al. 1993; Chau and Talasila 1994; Wiley 1994; Gil et al. 1998). Greenness, an indicator of freshness and wholesomeness of leafy vegetable product quality, is the result of the chlorophyll. As chlorophyll degrades during senescence, the green leaves become yellow and brown in colour. Antioxidants are related to chlorophyll retention in plants (Ferruzzi and Schwartz 2001; Lanfer-Marquez et al. 2005). Aharoni et al. (1993) reported that packaging coriander, dill, chervil, parsley, chives and water cress in non perforated polyethylene bags resulted in development of moderate modified atmosphere capable of retarding yellowing and decay. The retention of chlorophyll content is largely dependent upon the dynamics of in-pack gaseous atmosphere. Low O_2 retards the degradation rate of chlorophyll in Bok Choy (Shen et al. 1999; Lu 2007) concluded that MAP resulted in slower degradation of chlorophyll in Bok Choy with an average reduction of 32% during 10 days of storage at 10°C. The change in chlorophyll, as observed after 6 days of storage under different packing treatments has been depicted in Fig. 2(a). Mature fenugreek leaves contains large amount of

Fig. 2 Changes in total chlorophyll (a) and phenolic (b) contents of fenugreek inside the polypropylene bag under modified atmosphere packaging at 15°C and 75% RH ($n=3$)

green coloured chloroplast, which are largely responsible for its dark green colour. Present study indicated that different headspace environment under different packaging treatments resulted in differential decrease in the green colour during storage. A slight rise in chlorophyll content on 2nd day was also observed in non-perforated samples (with mustard seeds), but later on as the headspace environment become anaerobic, the total chlorophyll content dropped thereafter. Whereas, the chlorophyll content in the control samples kept on decreasing during entire storage; largely due to onset of senescence and transpiration from the exposed surface area of the leaves.

Ascorbic acid content generally decreased under all the packaging treatments largely due to its oxidation under prevailing package headspace O_2 as well as under anaerobic environment (Table 1). On the 6th day, the ascorbic acid levels finally arrived to 30.2, 10.1, 48.2 and 27.5 % for 2 perforation with mustard seeds, non-perforated with mustard seeds, non-perforated without mustard seeds and control with mustard seeds treatments, respectively. On the other hand, ascorbic acid level stabilized to 84 % in 2 perforations without seeds largely due to combine effect of low O_2 and high CO_2 prevailing in these packages

(Plantenius and Jones Platenius and Jones 1944; Saito et al 2000). A drastic decrease in ascorbic acid content for control samples was observed under both the treatments largely due to oxidation of ascorbic acid under normal atmosphere.

Flavonoids are reported to reduce risks of cancer, heart diseases and other age related degenerative diseases (Law and Morris 1998; Kris-Etherton et al. 2002; Sellappan et al. 2002). They have an anti-oxidative action, and may scavenge reactive oxygen species or quench singlet oxygen. The anti-oxidative activity depends on the chemical structure, such as the number of hydroxyl groups substituted on the B ring. In this study, the initial flavonoid content of fenugreek was observed to be 549 mg/100 g fresh weight of fenugreek. After 6th day of storage under MAP, the increase was prominent in 2 perforations with mustard seeds and non-perforated without mustard seeds to the order of 22.1 and 30%, respectively (Table 1). The wound induced ethylene concentrations are reported (Del Caro et al. 2004) to help increase the flavonoid content; which might have also helped to increase the levels of flavonoids in packaged vegetable. On the other hand, unsealed control samples had only insignificant increase to the order of 4.8 and 8.5% as the

Table 1 Changes in biochemical constituents of fenugreek after storage for 6 days under modified atmosphere packaging at 15°C and 75% RH

	Ascorbic acid mg/100 g fw	Beta-carotene mg/100 g fw	DPPH Inhibition %	Flavonoid mg/100 g fw
At harvest	72.5 ^a	44.4 ^a	25.0 ^{a,h}	549.4 ^a
2 perforations with mustard seeds	21.9 ^b	28.8 ^b	24.4 ^{b,h,i}	671.1 ^b
2 perforations without mustard seeds	61.1 ^c	11.1 ^c	17.6 ^c	418.0 ^c
Non-perforated with mustard seeds	7.3 ^d	22.0 ^{d,h}	11.6 ^d	580.7 ^d
Non-perforated without mustard seeds	34.9 ^e	22.5 ^{e,h}	24.6 ^{e,h,i}	715.0 ^e
Control with mustard seeds	19.9 ^f	32.7 ^f	24.1 ^{f,h,i}	576.1 ^f
Control without mustard seeds	62.9 ^g	17.4 ^g	28.9 ^g	596.2 ^g

Means within the same column with different superscripts are significantly different at $p<0.05$; $n=3$

generated ethylene could easily escape to surrounding environment, leading to dilution in its concentration.

Phenolic compounds constitute a large and ubiquitous group of phytochemicals and protect the vegetables from photosynthetic stress (Tomas-Barberan and Gil 2008). Serrano et al. (2006) indicated the importance of the type of film package on the maintenance of antioxidant capacity and phenol content in broccoli during storage. The total initial phenolic content of fenugreek was observed as 144.12 mg /100 g fresh weight of fenugreek (Fig. 2(b)). MAP resulted in an increase in the phenolic content of the fenugreek during the storage for 2 perforations on 2nd day, which may be due to low levels of O₂ prevailing in these packages and an increase in the activity of the phenyl-propanoid pathway under stressful condition as evident from synthesis and accumulation of phenolics compound. However, it was observed that on 6th day the phenolic content arrived to 9.76, 13.80 and 7.75 mg/100 g fresh weight of fenugreek for non-perforated, 2 perforation and control sample respectively. Pourmorad et al. (2006) also reported higher flavonoid content in leaves in comparison to its phenolic content in same crop for 2 Iranian medicinal plants *Adiantum capillus-veneris* and *Urtica dioica*. Values of phenol and flavonoid contents and antioxidant activity of *Ginkgo biloba* standardized extract obtained for comparison with the values of *T. Montanum* also revealed higher content of flavonoid in comparison to its phenolic content (Stankovic et al. 2011).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is a stable free radical that has been widely utilized to evaluate the free radical scavenging effects of antioxidants contained in fruits, vegetables and the other products of natural origin (Ansari et al. 2005; Jiang et al. 2006; Hasan et al. 2009). Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, poly-phenols, tannins, and phenolic terpenes (Rahman and Moon 2007; Hasan et al. 2008). DPPH inhibition was observed to be 25.05% in freshly harvested fenugreek sample (Table 1). Radical scavenging activity in 2 perforations package with mustard seeds remained unchanged after 6 days. While, it decreased to 17 % for 2 perforation package treatments without mustard seeds. On the other hand, in case of non-perforated packages it decreased to 11.6 and 24 % for with and without mustard seeds package respectively, after 6 days of storage due to the highest level of headspace CO₂ observed in these packages, which might have prevented the action of antioxidants, which clearly shows that the effect of different antioxidants and thus the DPPH free radical scavenging activity is a function of package headspace. In control samples, DPPH inhibition was observed to be more in samples without mustard seeds than those containing mustard seeds.

Carotenoids are widely distributed yellow, orange or red pigments, responsible for the colour of many fruits and vegetables. Several of them are precursors of vitamin A (i.e. β -carotene, γ -carotene, and β -cryptoxanthin). Green leafy vegetables are rich sources of carotenoids (Devadas and Saroja 1980). Pandrangi and LaBorde (2006) observed that carotenoid losses increased with the temperature when fresh spinach was packaged and stored at 4°C, 10°C and 20°C respectively. Rai et al. (2009b) reported that a combination of low in-pack O₂ (4.2%) and high CO₂ (11.8%) concen-

Fig. 3 The variation in sensory and visual ratings for aroma (a); visual appearance (b); and water accumulation (c) inside the packages containing fenugreek for 6 days of storage at 15°C under different packaging treatments ($n=3$)

trations had beneficial effect on β -carotene content of non-washed shredded cabbage samples. At the beginning of the study, Beta-carotene content for fresh fenugreek sample was 44.5 mg/100 g fresh weight of fenugreek (Table 1). In non-perforated packages it decreased marginally to 22.0 and 22.5 mg/100 g fresh weight of fenugreek in with and without mustard seeds package, respectively after 6 days of storage. This could be attributed to the low O_2 and high CO_2 in the film packages, in agreement with the earlier reported observations (Ishikawa and Hasegawa 1998; Saito and Rai 2005). Control samples had higher levels of beta-carotene largely due to senescence of the tissues, and thus increased yellowness was observed in these packages. On the other hand, for perforated packaging treatment beta-carotene was comparatively higher in packages containing mustard seeds than the packages without mustard seeds. It was also observed that under all the package treatments chlorophyll content was lowest after 6th day of storage.

Changes in aroma, texture, visual colour and water accumulation Aroma, texture, visual colour and water accumulation rating are important subjective attributes from consumer's point of view. In this study after 6 days of storage aroma rating for different packaging treatment are shown in Fig. 3(a). Packages with 2 perforations and without mustard seeds had "medium off odour" since the equilibrated headspace gaseous concentration of O_2 and CO_2 did not allow much synthesis and accumulation of off odour. However, for these perforated packages with mustard seeds, anaerobic headspace had a predominant effect as the aroma ratings continued to decline during the entire storage period rapidly. On the other hand, "strong off odour" was observed in non-perforated packages mainly due to fermentative respiration leading to accumulation of alcohols, aldehydes and thus ketones and synthesis of off odour under non-perforation conditions. For control samples, as observed by various other researchers (Toivonen 1997; Carvalho and Clemente 2004; Rai et al 2009a), no off-odour could accumulate inside the packages as they could escape easily to the storage environment.

The control samples started losing their green colour at faster rate and at the end, they became light yellow green (Fig. 3(b)). Chlorophyll content in the control samples also kept on decreasing during the entire storage. On the other hand, for 2 perforated packaging treatments visual colour rating were green. Persual of data for non-perforated packaging treatment revealed that since chlorophyll loss was slight in non-perforated packages, therefore loss of visually observed green colour was also less than the other treatments.

Depending upon water vapour permeability characteristics of packaging film, as the product respire, accumulation of the water vapour inside the film packages take places which either diffuses out of the packages or

condenses, our investigation showed that storage of fenugreek leaves under MAP leads to substantive condensation and thus accumulation of moisture inside the packages. Water accumulation among the packaging treatment and control samples remained within acceptable limits largely due to its absorption by packaged mustard seeds. The packages with mustard seeds were moderately wet as compared to non seed treatments which were completely wet (Fig. 3(c)). This is due to fact that the mustard seeds absorbed the evolved moisture, gained weight and then germinated as sprouts, which could lead to less accumulation of in-pack water.

Conclusion

Fenugreek is an important and highly respiring leafy vegetable. High rates of respiration and water vapour production are the principal impediments for design of appropriate packaging for its long term storage. Fenugreek could be stored under MAP utilizing macro-perforated MAP. Modified atmosphere had a beneficial effect on the shelf-life and biochemical content of otherwise highly perishable fenugreek stored for 6 days at 15°C. Shelf-life of fenugreek was adversely affected by in-pack moisture. Modified atmosphere packaging resulted in better maintenance of chlorophyll as well as antioxidants. Among all the treatments, it was observed that packaging of fenugreek in two perforation packets with mustard seeds resulted in best maintenance of chlorophyll, ascorbic acid and aroma. The phenolic content reduction in 2 perforation packet with mustard seeds was also least as compared to other treatments. Water accumulation was also consoled due to mustard seeds after 6 days of storage. Although mustard seeds helped in controlling water absorption but the germinating sprouts resulted in accumulation of anaerobic conditions. We need to open more perforations if we want to use mustard seeds.

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