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RESEARCH ARTICLE

Chromatic changes in the broccoli stored under modified packaging

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SUMMARY

Broccoli was stored under modified atmosphere packaging (MAP) at 15°C in perforated and non perforated low density polyethylene (LDPE) and polypropylene (PP) films packages having different head space and in un-sealed packages for a storage period of 10 days to evaluate the effect of modified atmospheres on chromatic changes. At the end of storage, MAP resulted in differential changes in the original green color of broccoli under different packaging treatments. Instead of conventional CIELAB color space system utilizing L*, a* and b* value, the chromatic changes were analyzed as per L*C*h* colour space system using lightness, chroma, and hue angle values to evaluate final hue (color) along with its associated attributes. On 10^{th} day the result showed that LDPE retained better color than PP films. In non- perforated, the sample become anaerobic after 24 hr. Overall at the end of storage, perforated LDPE package having more head space controlled the colour change within human acceptable limits. In comparison, the unsealed packages turned orange yellow compared to MAP. Also the results of sensory evaluation and visual analysis confirmed the results obtained from L*C*h* color space diagram, and indicated that the modified atmosphere (6.0% O_2 and 7.8% CO_2) generated inside the LDPE perforated packages having 2 holes and 0.8 head space was suitable in maintaining the chromatic quality of the broccoli heads.

Key Words : Broccoli, Storage, Modified packaging, Perforated films

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Broccoli (*Brassica oleracea* italica) is important source of vitamin and anti carcinogenic substances (Nestle, 1998). It has shorter shelf-life and high respiration due immaturity of the texture at the moment of harvest and due to other physiological factors (Vamos, 1981). Storing broccoli at low checks its shelf-life, but the senescence can be controlled effectively through proper and optimized packaging to maintain its physic chemical constituents.

Degradation of chlorophyll, opening of flower buds, development of off-odors, loss of turgidity and loss of nutritional value and the major changes that take place during senescence (Hansen *et al.*, 2001). Chlorophyll is

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degraded by light and enzymatic reactions. Degradation of chlorophyll results in chromatic changes in broccoli heads, leading to rapid yellowing under normal atmospheric conditions. Modified atmosphere packaging in combination with cool tem storage, has been reported to delay its senescence and maintenance of its physico-chemical constituents and extension of shelf-life (Hirata *et al.*, 1995 and Toivonen, 1997). Selection and application of appropriate packaging film for broccoli is an important criteria for its storage as improper film selection can lead to development of improper modified atmosphere resulting in development of off-flavours, and chromatic changes in very short period, leading to loss in its marketability. In general for high respiring produce like broccoli, micro perforated film having high O_2 and CO_2 gas transmission rates can be used to extend their shelf life (Saito and Rai, 2005 and Rai and Paul, 2007).

But micro-perforated films are special films are costly and not easily available everywhere. Moreover, the loss in major pigment *i.e.* chlorophyll during storage can be quantified objectively and their comparison with fresh broccoli cannot be pinpointed easily.

In the present study, the influence of different head space gaseous concentrations on physicochemical constituents particularly chromatic changes was studied during the storage under MAP at 15°C in perforated polypropylene (PP) and low density poly ethylene (LDPE) film packages; to assess the most suitable headspace gaseous condition, to maintain the color in broccoli as well as to point out the exact hue attained by the stored broccoli, its saturation and its differences with the originally stored fresh broccoli.

MATERIAL AND METHODS

Broccoli (*Brassica Oleracea* italica) was obtained from the fields of the farmer, located at village, Bargari, during 2015, of district Faridkot. Broccoli heads of size 90-100 mm with unopened florets were harvested and stored safely for further handling. Broccoli heads were trimmed, hydro cooled (water containing 0.5% citric acid used at 3l / kg of broccoli for 1 min.), broccoli heads were shaked carefully to remove the surface water and then placed inside temperature regulated storage room maintained at 15°C for 2 h for air cooling as well as to prepare them for subsequent storage temperature. Packaging of broccoli heads was carried inside the cold room. The broccoli florets were packaged in perforated (total of 2 perforations per package on both LDPE and PP package, perforation diameter of 0.3 mm each) packages having bag area of 0.14m^2 made from LDPE and PP films. The sample size was 250g and 350g resulting in head space (HS) of 0.8 and 0.7, respectively. The package use for packaging were procured form the market of same guage (150 guage), but on actually measuring the thickness in laboratory with the help of thickness tester (Labthink, model CHY-C2, China)it was found that thickness of LDPE film was 40µm and that of PP film was 36µm. The gas permeability of co-efficients of LDPE packaging film for O_2 and CO_2 at 15°C, measured by gas permeability tester (labthink, model BTY-B1P, China) were observed to be 5.96 x 10^{-5} and 2.52x10^{-5} ml m m^{-2} h^{-1} kPa^{-1}, respectively. Effective permeability of gases was water vapour through perforations was estimated by the following relationship given by Techavises and Hikida (2008) which is valid for temperature range of 5-25°C and film thickness of less than 0.025 mm. The effective permeability of the perforation was 0.986 ml.h^{-1}kPa^{-1}, taken to be equal for both O_2 and CO_2. The packages containing fresh broccoli heads were then heat sealed and kept for storage in an environmental control chamber, the gaseous concentration of O_2 and CO_2 in all the packages was assumed to be 21.6 per cent and 0.03 per cent and at the beginning of the experiment.

Colour evaluation :

The colour of broccoli is one of the important quality parameters, which indicates the freshness. It also affects the commercial value of the product. The basic purpose of colour measurement was to get an idea of comparative change in colour in different treatments with storage time. Conventionally, all conceivable colours can be located using Commission Internatinale de I'Enclairage (CIE) L*, a*, b* colour space system abbreviated as CIELAB (Mc Guire, 1992), which is specified by three perpendicular axes. The L* indicates intensity of colour *i.e.* lightness which varies from L*=100 for perfect white to L*=0 for black. 'a*' and 'b*' are chromaticity dimensions which give understandable designations of colour *i.e.* the value of 'a*' measured redness when positive, grey when zero and greenness when negative and the value of 'b*' measured yellowness when positive, grey when zero and blueness when negative (Fig. A). These co-ordinates pinpoint the measured colour in a three-dimensional colour space. In this system, although lightness is correctly pointed out using L*, the other two parameters *i.e.* a* and b* are merely chromatically co-ordinates, which needs to be further manipulated to arrive at the appropriate terms namely, hue and chroma. The hue is used for classification of colour as red, yellow, blue etc. and the chroma refer to an index somewhat analogous to saturation or intensity of a particular hue (Mc Guire, 1992). In the present study, on each day of observation, for different packaging treatments, L*, a* and b* values were determined at four places over the entire surface of the broccoli; using a Hunter Labscan (Model Miniscan XE plus, Hunter associated, USA) (Fig. B) in such a way that the sample was placed in close contact with the lens of the instrument so that the light which falls on the sample should reflect back and no light is transmitted to the surroundings and values obtained were averaged. The instrument was first calibrated with a standard white plate and black plates provided along with equipment before taking observations. The instrument data was analysed to evaluate the colour changes during the entire storage period as per L* C* h* colour space system (Konica Minolta Sensing Inc. 1998), which uses cylindrical co-ordinates as compared to rectangular co-ordinate system of CIELAB colour space. In this system, L* indicate lightness and is similar to L* of L* a* b* colour space system, C* is chroma and h* is the hue angle (Fig. C). The chroma, hue angle and yellowing index are represented in terms of a* and b* as per the equations:

$$\text{Chroma } (C^*) = \sqrt{a^{*2} + b^{*2}}$$

$$\text{Hue angle } (h^*) = \tan^{-1}(b^*/a^*)$$

$$\text{Yellowing index} = 142.86^*(b^*/L^*)$$

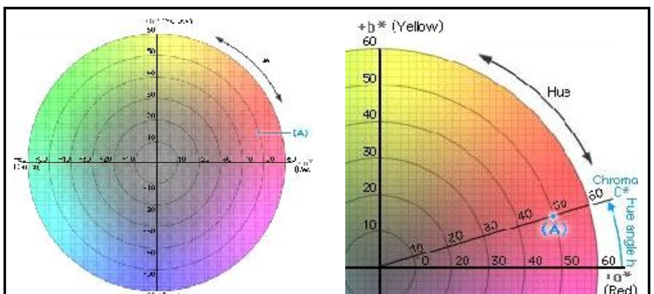

Fig. A : Colour space system describing chroma and hue angle

In L*, C* h* colour space system, a*, b* and C* vary between 0 and 60. While a* and b* increase along the axes of the system, C* varies along the depicting hue. On the other hand, hue angle (h*) starts at +a*axis and is expressed as degrees; where 0°C, 90°C, 180°C and 270°C refer to +a* (red), +b* (yellow), -a* (green) and –b* (blue). The intermediate values of hue angle *i.e.* between 0°C, 90°C, 180°C and 270°C represent the other in-between hues. While a particular hue is identified by a corresponding hue angle, its saturation is denoted by C*. The colour comparison between the reference and sample objects in this system is evaluated in terms of hue and chroma difference *i.e.* ΔH* and ΔC*. The ΔC* is chroma difference between the reference and sample objects, ΔH* is evaluated from the following relationship:

$$\Delta H^* = \sqrt{\Delta E^{*2} - \Delta L^{*2} - \Delta C^{*2}}$$

where ΔE*, ΔL* and ΔC* refer to the size of the colour difference, lightness difference and chroma difference, respectively between the reference and sample objects.

Fig. B : Hunter Labscan

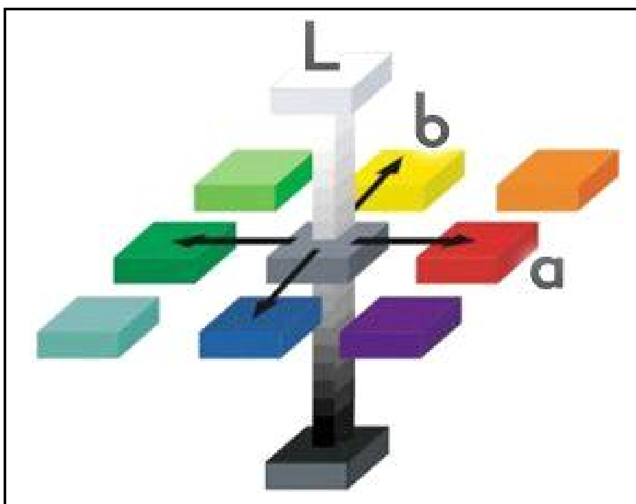

Fig. C : Colour space system

The ΔE^* can be calculated as per the equation as under:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

where Δa^*, Δb^* refer to the change in the chromatically coordinates for reference and sample objects

The above analysis can identify the hue of an object and its saturation, its difference from the reference object can be expressed in terms of ΔH^* and ΔC^*. Further, the colour difference between reference and sample can be expressed in term of certain term such as vivid, light, pale, dull dark and deep using the differences in $\Delta L^*$and ΔC^* (Fig. D). The further precise degree of colour difference for each direction can be expressed in term of certain modifiers in order of increasing intensity as very light, slightly, medium etc. In the present study the samples stored were compared with the fresh broccoli florets at the start of experiment to evaluate the chromatic changes during the storage.

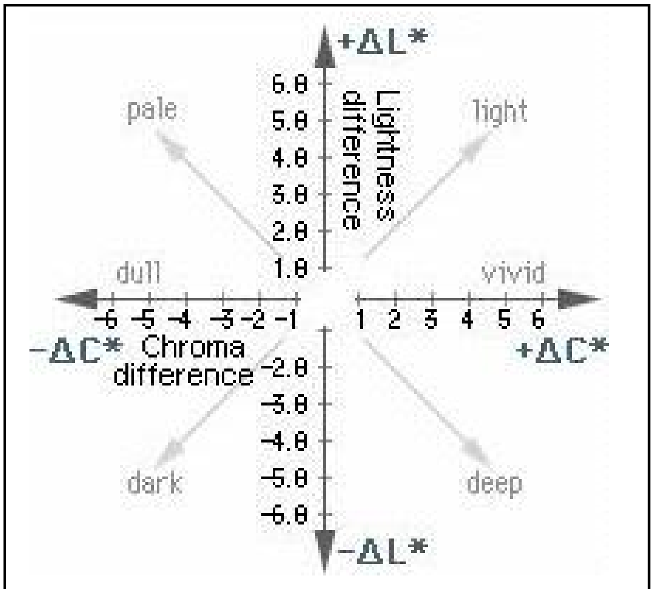

Fig. D : Colour space system terms for describing differences in chroma and lightness

Sensory evaluation :

The stored samples were analysed for sensory evaluation by a three member panel on each day of observation. The selected panel was briefed with the sensory characteristics that were to be judged and also with the available scales according to which the samples were to be rated. The panel members were requested to assemble at one place prior to evaluation, as the samples were required to be judged immediately when opened. Each member was provided with the sensory evaluation rating scales based on which the rating was given to various samples. The average values of the ratings given by all the members were then calculated and used for further analysis.

The sensory evaluation scale (Table A) for rating the sensory quality of fresh broccoli was developed on the basis of three main parameters (*i.e.* Visual appearance, odour and water accumulation), visual appearance was examined by the rating scales proposed by Ying *et al.* (1999), odour was evaluated on the basis of the rating scales proposed by Carvalho and Clemente (2004), and water accumulation inside the package was determined as per the nine point scale proposed by Rai *et al.* (1999), respectively. Visual colour was rated to the samples according to the yellowness of the floret as observed by the eyes. The odour was rated by smelling the package environment immediately after making a small opening in the package. The water accumulation conditions inside packages were judged visually to provide them rating according to the scale. During scoring, the intermediate scores were also given to the samples depending upon the perceived condition of the samples.

Table A : Sensory rating scale

Scale	Condition	Rating
Visual appearance	Yellowness not detected by eyes	0
	Slightly yellow 1-3 flower buds are yellow	1
	5% flower buds became yellow	3
	50% flower buds became yellow	5
	75% flower buds became yellow	7
	100% area became yellow	9
Odour rating	No off odour	5
	Very light off odour	4
	Light off odour	3
	Medium off odour	2
	Strong off odour	1
Water accumulation	No water accumulation	9
	Produce slightly wetty	7
	Produce and film slightly wetty	5
	Produce moderately wetty	3
	Produce and film moderately wetty	1
	Produce completely wetty and dripping of water	0

Statistical analysis :

Entire data obtained during the experiment was expressed as means ± standard deviation. One-way analysis of variance (ANOVA) and multiple comparisons (Fisher's least significant-difference (LSD) test) were used to evaluate the significant difference among different treatments at $p < 0.05$ (Sun *et al.*, 2007) using a statistical package (Statgraphics Plus, Statpoint Inc., USA).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Chromatic changes :

The chromatic (colour) changes in the stored broccoli heads during the entire period storage period were expressed in terms of chroma difference (ΔC*), hue difference (ΔH*), lightness difference (ΔL*) taking their corresponding value at the start of experiment as zero. These changes are affected by gaseous concentrations as is clear from the Fig. 1, 2 and 5 that the modified atmosphere created in the packaged packages was effective in controlling the chromatic changes in the produce till tenth day. The difference in the colour change was not significant between the different head space in both the films, but the colour change was slightly more in the 0.8 HS packages. The change in colour was not significant among the films, but it was more in the PP film. The chromatic change showed a significant difference among the non perforated and perforated packages in both the films. On the other hand the changes in the control packages (unsealed) remained nearly equal to that of the packaged packages upto first day of storage. After that the colour change parameters (ΔC*, ΔH*) in the control samples increased significantly which indicated a substantial increase in the saturation of the hue for control samples.

The total changes at the end of tenth day of storage with references to fresh broccoli florets used were also evaluated by representing ΔC*, ΔH* in the first and second quadrants of the L*C* h* colour space diagram [Fig. 3 (a-e) and Fig. 4 (a-e)]. It is clear from the [Fig. 3 (a, b) and Fig. 4 (a, b)] that increase in ΔC* which was towards the right side of the reference (*i.e.* toward

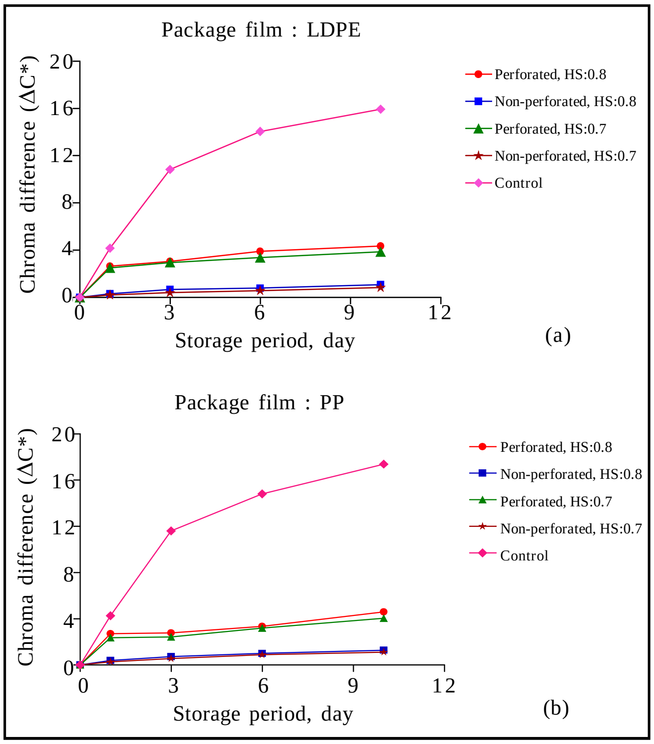

Fig. 1 : Changes in chroma (ΔC*) of broccoli florets at different levels of package head space stored under modified atmosphere. The plotted values are means of three replications per treatment along with their standard deviations

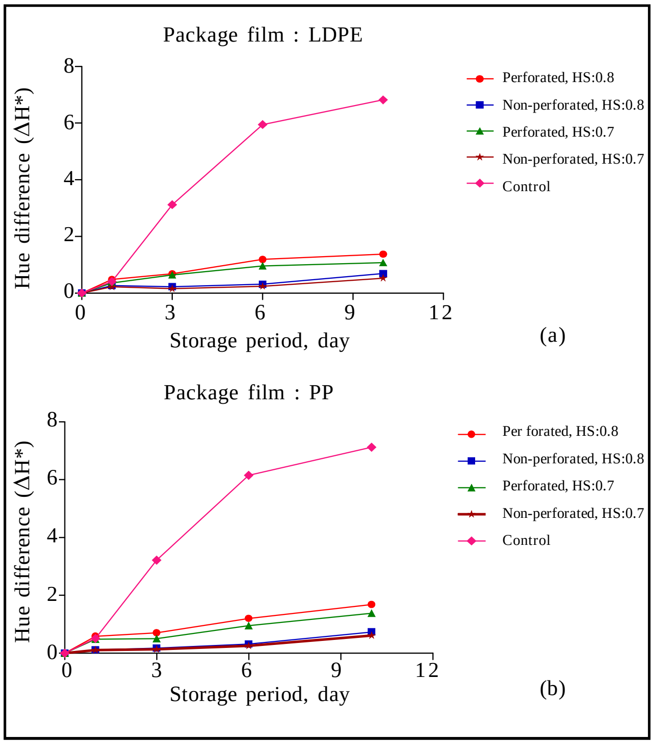

Fig. 2 : Changes in hue difference (aH*) of broccoli florets at different levels of package head space stored under modified atmosphere. The plotted values are means of three replications per treatment along with their standard deviations

yellow line) was more in perforated PP films packages than the LDPE film. But in non perforated packages the change in ΔC* which is towards the left side of the reference (*i.e.* toward green line) was more in LDPE packaged samples than the PP packaged samples. The increase in ΔH* remain confined to second quadrant (Fig. 3(a-d) and Fig. 4 (a-d)), which meant that towards the end of storage, the broccoli samples kept under different modified atmospheres after losing their original colour remained between yellow-green [Fig. 3 (a, b) and Fig. 4 (a, b)] for perforated packaged samples for both the films. The non perforated packaged samples remain in the green colour range as shown in Fig. 3 (c, d) and Fig. 4 (c, d) for both the films. On the other hand, the ΔH* for control samples showed much larger increase which corresponds to first quadrant of L* C* h* colour space diagram and pointed towards the orange-yellow colour at the end of storage (Fig. 3e and Fig. 4e). The increase in the saturation of a particular colour (*i.e.* colour intensity) on tenth day for all the perforated packages was less in LDPE films and for all the non perforated packages was less in PP films.

The lightness of particular colour computed from the direction of colour difference for different treatments

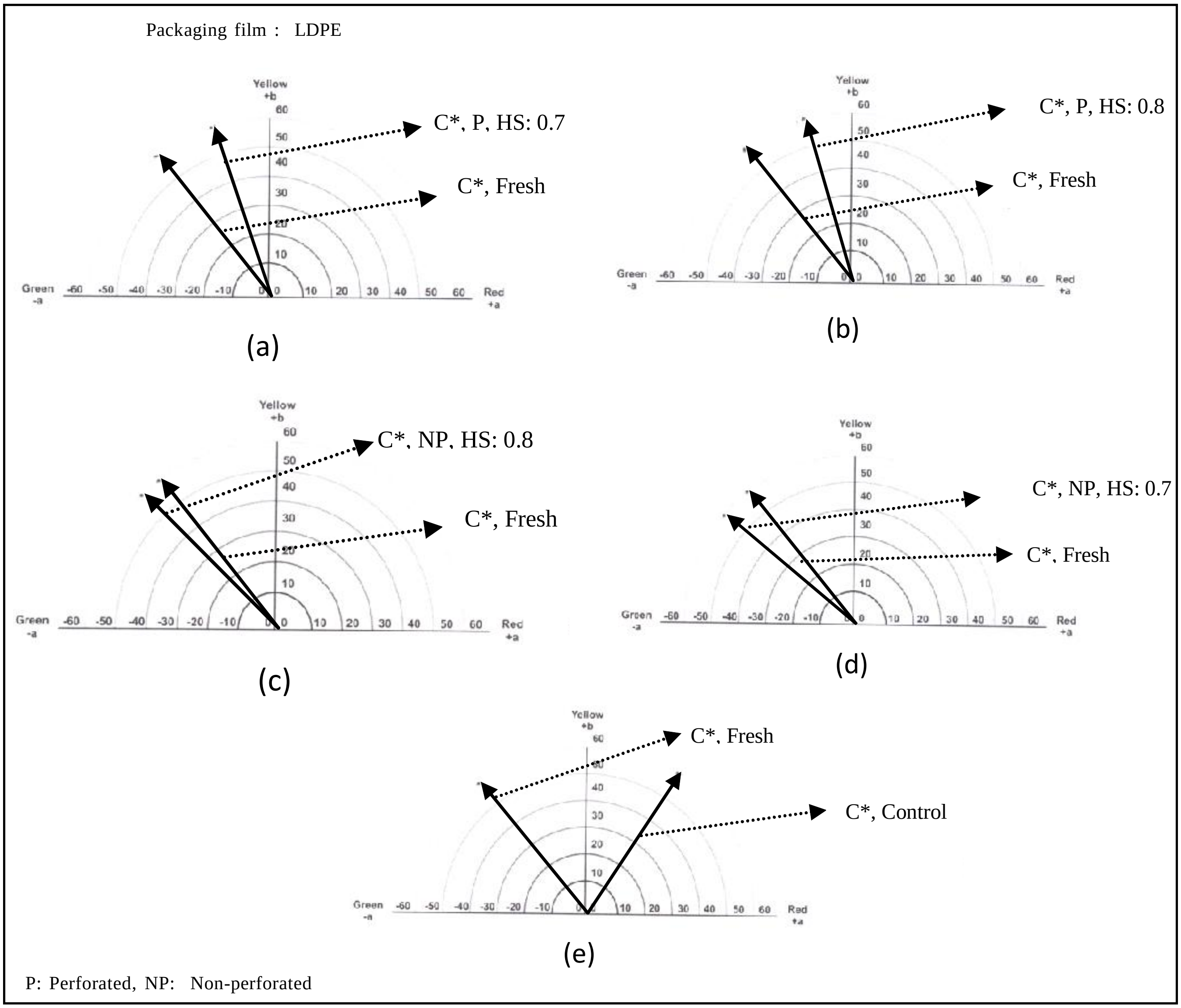

Fig. 3 : Depiction of chroma and hue differences on the portion of a*, b* chromaticity diagram of L* C* h* colour space system to determine the hue and its saturation after 10 days of storage of broccoli florets at different levels of head space stored under modified atmosphere

with respect to fresh broccoli florets was evaluated by means of ΔC*, ΔL* (Fig. 4). For different treatments the direction of colour was observed to light for perforated and deep for non-perforated samples. The difference among the lightness intensities in both the films for perforated and non perforated treatments was observed to be significant. Among all the perforated treatments for both the films it was found to be non-significant and similar results were obtained among all non-perforated treatments for both films. The degree of colour difference was represented by means of additional modifiers such as slightly light for perforated packages and slightly deep for non perforated packages for both the films. The control could be described as highly light.

The L* C* h* colour space diagram analysis of instrumental data obtained during ten days storage period could easily identify the final hue with all its attributes. The result showed that LDPE film packages retained better colour compared to PP films packages for all the treatments. The analysis of the result showed that non perforated packages in both the films had slightly green hue which was slightly darker than fresh broccoli florets, but the sample become anaerobic (rotten) after 24 hr and become unfit for human consumption. The perforated packages having 0.7 HS in both the films had slightly saturated yellow-green hue which was slightly lighter than fresh broccoli florets. The 0.8 HS perorated packages were slightly lighter than 0.7 HS for both the films. But after ten days of storage, the 0.7 HS packages showed change in odour which is not within acceptable

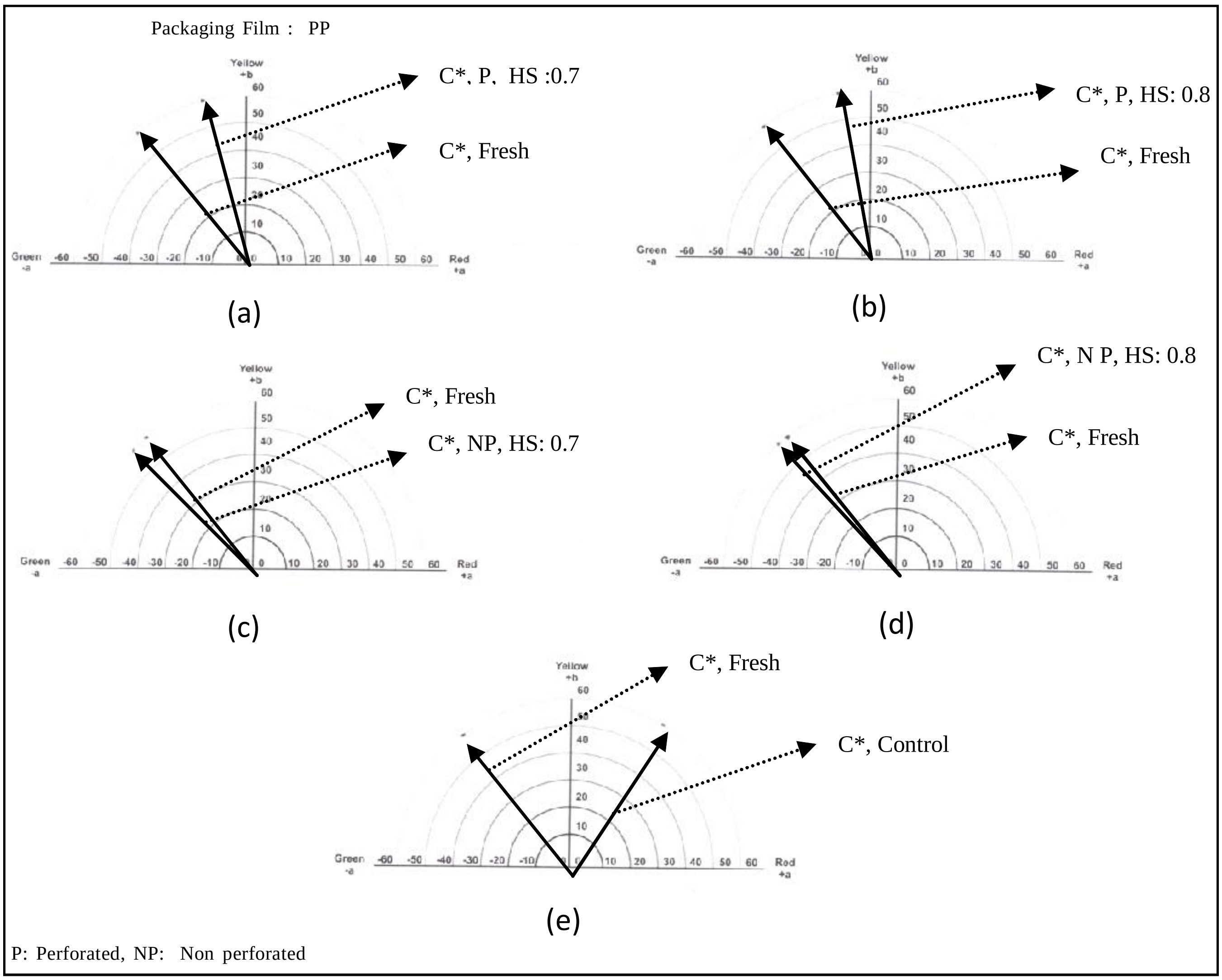

Fig. 4 : Depiction of chroma and hue differences on the portion of a*, b* chromaticity diagram of L* C* h* colour space system to determine the hue and its saturation after 10 days of storage of broccoli florets at different levels of package head space stored under modified atmosphere

limits, in comparison to 0.8 HS packages which have odour within acceptable limit. The LDPE film package having 0.8 HS could effectively control the chromatic changes and other quality parameters of packaged broccoli florets during storage period within the human acceptable limits. The results are in agreement with the earlier reported work (Rai *et al.*, 2009) that produce packaged in suitable packages remained fresh with acceptable quality for longer period in comparison to unpackaged samples.

Changes in odour, visual colour and water accumulation :

The consumer selects and accepts the fresh produce based essentially on the perception and sensory evaluation of colour, smell, skin appearance, crispness, wrinkles on the surface etc.

The consumer is not influenced by the instrumental quality indicators. As such, three parameters were selected: odour, visual appearance and the resultant decay (rot) due to water accumulation. Generally synthesis of sulphurous compounds is responsible for accumulation of off-odour in packaged broccoli (Carvalho and Clemente, 2004). The results of the study suggest that the odour in the perforated 0.8HS packages in both the films changed to light off-odour, while for 0.7 HS packages it changed to nearly strong off-odour at the end of storage period Kasmire *et al.* (1974). The non perforated packages become anaerobic (rotten) and unfit for consumption after one day of storage. No off-odour was observed in perforated 0.8 HS packages till first day in LDPE, till third day in PP; and till first day in 0.7 HS perforated PP packages (Fig.6a and 6b). Very light off-odour was observed in the PP packages having 0.8 HS on sixth day and for 0.7 HS packages on third day. In LDPE perforated packages traces of off-odour were observed at the end of third day of storage in 0.8 HS packages; whereas for 0.7 HS packages it was by the

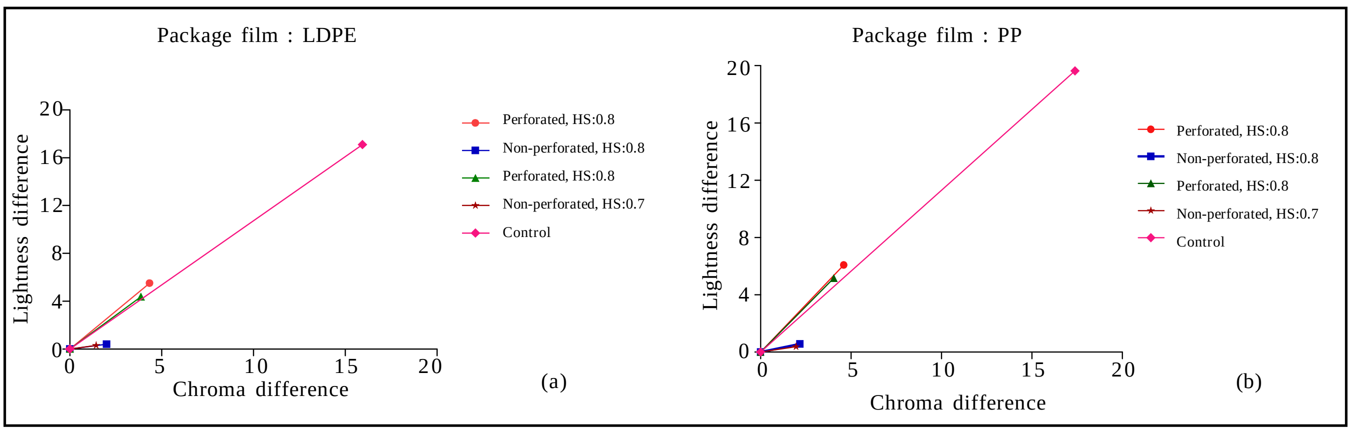

Fig. 5 : Difference in chroma and lightness of broccoli florets at different levels of package head space stored under modified atmosphere. The plotted values are means of three replications per treatment

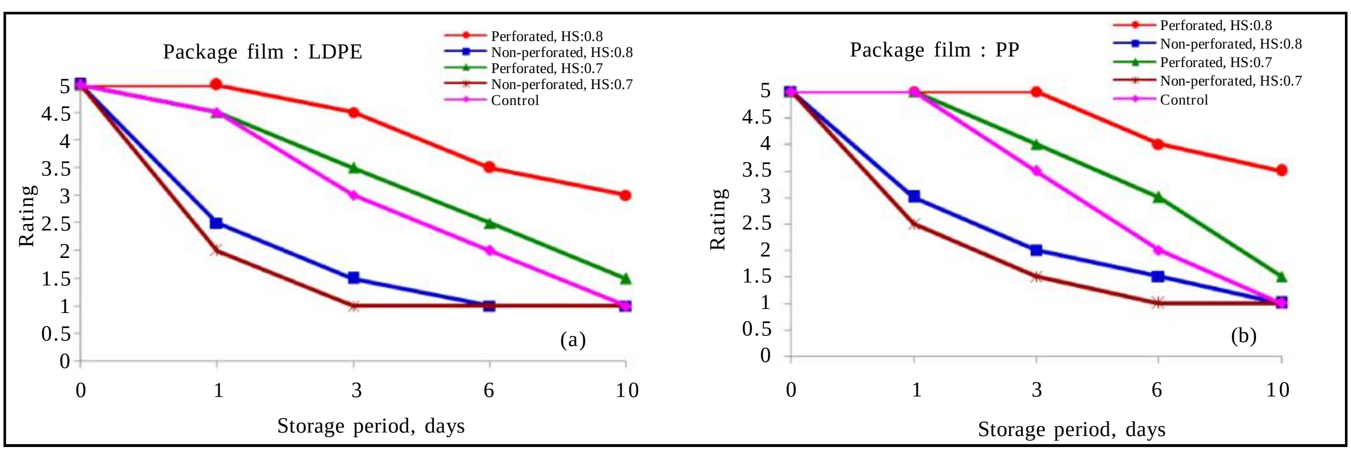

Fig. 6 : Odour rating of broccoli florets at different levels of package head space stored under modified atmosphere

end of first day. The traces of off-odour were observed in control packages after first day of storage which changed to very strong off-odour after ten days of storage.

As expected, it was observed that water accumulation was less in perforated packages than in non perforated packages. In LDPE packages having 0.7 HS slight moisture accumulation on the film was observed in non-perforated packages, but negligible moisture was observed in perforated packages at the end of third day of storage. At the end of sixth day slightly wetty produce and film was observed in perforated and non-perforated 0.7 HS LDPE packages. In LDPE perforated packages having 0.8 HS no water accumulation was observed in at the end of third day and slightly wetty produce was observed in non-perforated packages at the end of sixth day (Fig.7a). In PP packages, no water accumulation was observed in all the samples till first day of storage (Fig. 7b). At the end of third day in non perforated and perforated PP packages slightly wetty produce was observed having 0.8HS; and wetty film and produce was observed in 0.7 HS packages. In general, the water accumulation was more in the packages which have less void volume (head space). However, for the same head space, the LDPE film helps in less accumulation of water. In the present study number of perorations and the film permeability were not enough to diffuse the moisture produced in the package. That is why, some moisture accumulation was observed for all the treatments. The water accumulation results in vapour condensation within the packages resulting in the deterioration of the produce. In control samples (unsealed packages) no water accumulation was observed, but was rotten, smelling badly, florets got dried, wrinkled and turned yellowish- - orange making the produce unfit for human consumption and consumer acceptance.

The visual appearance of LDPE packaged produce was better in comparison the PP samples at the end of

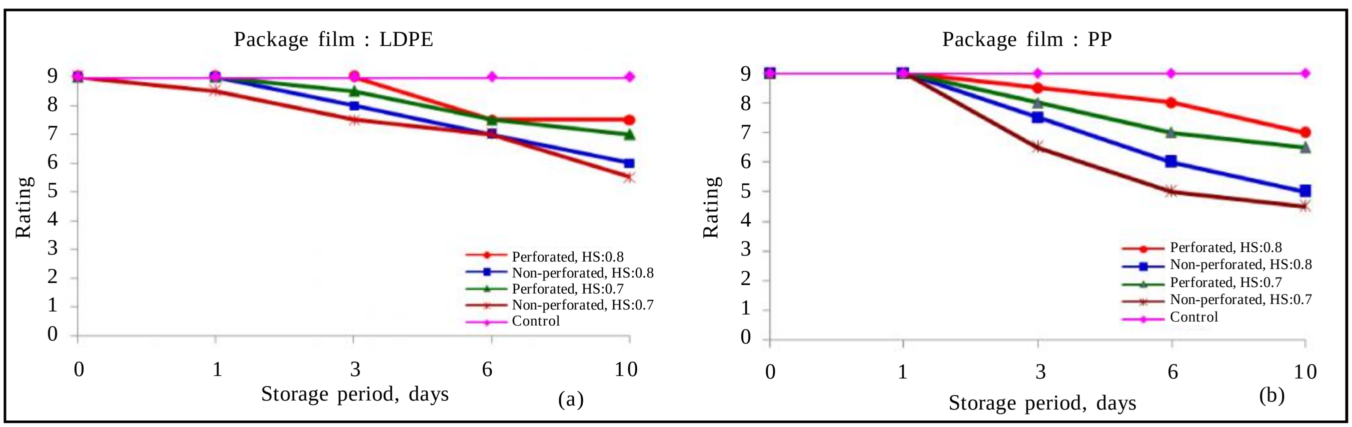

Fig. 7 : Water accumulation rating of broccoli florets at different levels of package head space stored under modified atmosphere

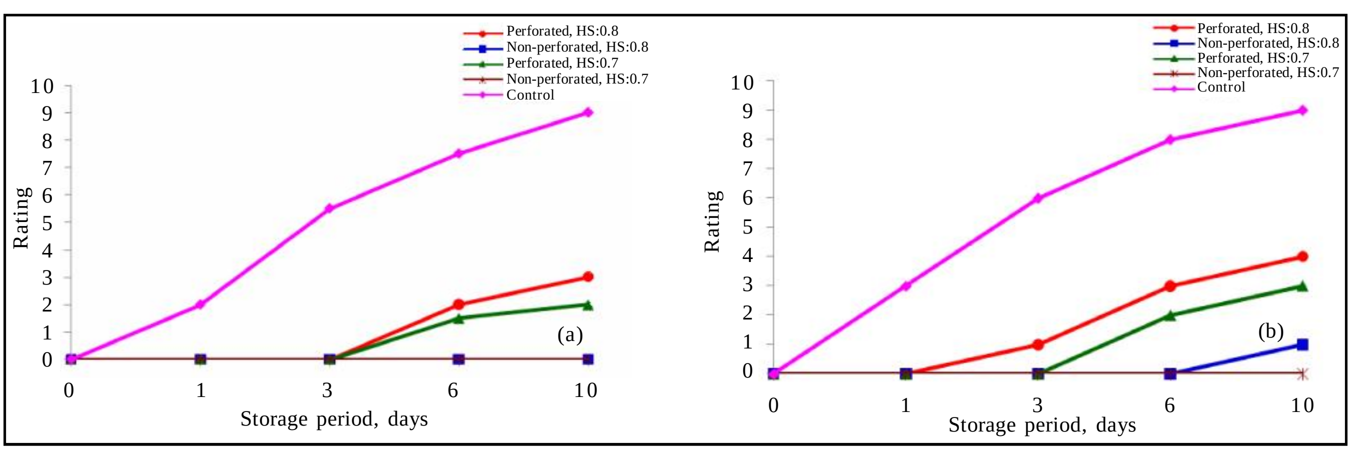

Fig. 8 : Visual appearance rating of broccoli florets at different levels of package head space stored under modified atmosphere

storage (Fig. 8). The non perforated packaged samples in both the films showed no visual colour change during the storage, but the florets appeared rotten/decay due to anaerobic conditions. The perforated LDPE packages having 0.8 HS showed yellowness such that 5 per cent of flower buds became yellow but in 0.7 HS it was less than 5 per cent (Fig. 8 a); and in PP packages having 0.8 HS 5-50 per cent flower buds became yellow and 0.7 HS it was 5 per cent (Fig. 8 b), respectively. The visual quality of control samples fell below the acceptable limits after three days of storage. Similar results were reported by Ren *et al.* (2006) using the same visual rating scale that packaged broccoli florets gave better colour than unpackaged. In the present study the quality of packaged samples taking into account all the three sensory parameters was found to be better in LDPE packages having 0.8 HS.

Overall, for the broccoli florets stored under different packaging treatment for ten days, the headspace gaseous concentration of O_2 remained above anaerobic level in all perforated packages except for non-perforated packages in both the films. The difference in chroma and hue was less in perforated LDPE packages as compared to perforated PP packages. It was also observed less in 0.7 HS than the 0.8HS packages for both the films. It may be concluded that increase in in-pack CO_2 concentration (7-9 %) along with decrease in in-pack O_2 concentration (3-8 %) might have enhanced the retention of antioxidant *i.e.* β-carotene, phenol and ascorbic acid for longer period, which in turn have resulted in chlorophyll retention and maintenance of green colour of packaged produce. The experimental results correlated by the sensory evaluation indicated that broccoli florets stored in perforated packages (LDPE, 150 guage) having 80 per cent void volume could safely be stored upto 10 days at 15°C and 75 per cent relative humidity without adversely affecting the quality.

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