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Development of smart film labels for storage quality assessment of yellow bell pepper (*Capsicum annuum* L.) under active packaging

Entwicklung von intelligenten Folienetiketten zur Beurteilung der Lagerqualität von gelbem Paprika (Capsicum annuum L.) unter aktiver Verpackung

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Summary

Yellow bell pepper is a popular high value crop rich in vitamins, antioxidants and flavonoids. It is highly sensitive to high levels of carbon dioxide, so this property was capitalized to develop and optimize smart film labels for monitoring headspace CO_2 concentration of packaged bell pepper, indicating their quality. Strips of smart film, obtained at optimized conditions were attached inside the packages followed by low temperature storage. With progress in storage, the concentration of CO_2 increased which deteriorated the quality of the packaged bell pepper. A correlation was established between headspace CO_2 concentration and colour change of smart label. High value of Pearson correlation coefficient r (0.9605) and coefficient of determination R^2 (0.957) depicted that CO_2 concentration and colour difference of smart label have good correlation and the films can be used to monitor headspace carbon dioxide concentration and quality of packaged bell pepper in a non-destructive and real time manner.

Keywords: smart films, bell pepper, active packaging, characterization, headspace gas concentration

Introduction

Yellow bell pepper (*Capsicum annuum* L.) is an important crop grown worldwide, can be green (unripe), red, yellow, orange, or brown when ripe. Yellow bell peppers are rich in carotenoids and vitamins (A, B-6, C) and folate and are low in calories (Devgan et al 2019; Howard et al, 1994). Bell pepper's consumption is increasing exponentially now-a-days. The demand from the urban consumers is everlasting owing to the associated health benefits of bell pepper as it's a rich source of antioxidants and has anti-inflammatory attributes (Devgan et al 2019). It can be consumed raw in the form of salads or can be used in the preparation of pizza, sandwich and other food items.There is also a high demand for export. The export market requires fresh produce with longer shelf life. On the contrary, bell pepper is highly perishable crop due to high water activity, thereby emitting high moisture when packed and stored. It is also sensitive to the high levels of oxygen and carbon dioxide. High water activity facilitates the growth of molds and bacteria which leads to deterioration of product and reduction in shelf life and ultimately market price. The answer to this problem is shelf life modelling, which includes measurement of metabolic rate of the fresh produce and use it in designing suitable packages according to the characteristics of fresh produce. The passive packaging technique can slow down the negative impact of environmental conditions on the packaged produce but in practical this process is inadequate and not effectual and efficient in maintaining the quality attributes of fresh and sensitive food products with an enhanced shelf life (Kumar et al 2017; Lopez et al 2004; Malathi et al 2017; Mwaurah et al 2020). Modern packaging industry always looks for auxiliary functions in response to the consumer demands for minimally processed foods free from preservatives, raised regulatory requirements, market globalisation, increasing concern for the food safety and recent menace of food biological terrorism (Yam et al 2005). The field of smart packaging is being researched so as to account for the needs of ever-growing consumers and challenges faced by the packaging industries in order to meet these needs.

Active packaging may be defined as packaging in which a supplementary constituent has been intentionally incorporated either in the packaging headspace or to the packaging material so as to modify the packaging system (Robertson 2006). This system interacts with the atmosphere inside the food package, helps in maintaining the quality and prolonging of shelf life of the packaged food. These active packaging systems generally involve absorption or removal of an undesirable compound (Wilson 2007). Gas absorbing/ releasing packaging (Smith et al 1995) consists of the use of packaging films or sachet in order to absorb the undesirable gases (such as oxygen, water vapour, and ethylene) from the food package headspace so that a desirable optimum internal environment is established and an extension in shelf life is achieved (Yam et al 2005; Kirandeep et al 2018). Intelligent packaging system has the ability to communicate the conditions of the packaged product (Biji et al 2015, Pavelková 2012). Different types of intelligent packaging systems are available like time temperature indicators, gas leakage indicators, bio sensors and freshness sensor/indicators which gives indication of the quality of the packaged product by change in color of the sensors. Most of them monitor the oxygen and carbon dioxide concentrations. The concentrations of these gases often correlate closely with the advance of spoilage (Meng et al 2014). The functionality of most devices is based on redox dyes, a reducing compound and an alkaline component (Ghaani et al 2016).

Vegetables like bell pepper are highly perishable and need adequate handling and care to enhance the shelf-life and quality characteristics. Its storage life is restricted by pathological spoilage (Ceponis et al 1987; Kumar et al 2020; Kumar 2018), water loss during long term storage (Diaz-Perez et al 2007), and being prone to chilling injury (Paull 1990). Commonly found decay microorganisms are botrytis or gray mold, alternaria, and soft rots of fungal and bacterial origin. Botrytis can thrive at the recommended storage temperatures. Higher concentration of CO_2 (> 10%) can inhibit botrytis, but it can also damage bell peppers (Kader 1997). Chilling injury leads to alternaria black rot on the stem end of bell peppers. Physical and pathological degradation causes tissue damage, thereby enhancing the susceptibility of bacterial soft rot. Thus, low temperature (7–10°C and high humidity of 80–85%) may prove beneficial for extending the shelf life of bell peppers (Devgan et al 2019). As bell pepper fruits are sensitive to the headspace gas concentration so an indicator is required which can indicate the increase in level of carbon dioxide. Bell pepper go through a continuous natural senescence during storage and distribution, their ripeness or over ripeness cannot be detected by general testing tools without destroying the packaging materials. Nowadays, novel methods are required to reliably and readily detect the ripeness of packaged bell pepper in a non-destructive manner are needed.

In today's era, fairly inexpensive instruments are readily available for measuring the temperature and humidity changes during storage. On the contrary, the equipment for measuring O_2 and CO_2 concentrations are costly and are not handy. Thus, handy and cheap colour labels for monitoring the gas concentration are required. In the present study, a colour changing indicating label comprising a CO_2 absorber and a chemical dye has been manufactured, optimized and evaluated for active packaging of yellow bell pepper. The objective of this study was to develop and evaluate the colour changing indicator labels to monitor the headspace gas concentration of packaged yellow bell pepper samples.

Materials and methods

A smart film was developed using methyl cellulose, polyethylene glycol and a dye solution. Optimization of smart film composition was done using response surface methodology. The smart film obtained at optimized conditions was cut in to small labels which were attached to the LDPE packages. Further evaluation of smart films involved packaging of yellow bell pepper in the smart packages under low temperature storage (10°C; 80±5% RH), and monitoring of package headspace, produce quality and label colour at regular intervals.

Preparation of smart films

A colour changing indicator film (smart film) was developed as per experimental design (Table 1). The experiments were designed using design expert 8.0.7.1 (Statease Inc, Minneapolis, USA,). A 3-level Box-Behnken experimental design was selected. The levels of independent variables namely methyl cellulose, polyethylene glycol and indicator solution concentration were varied from

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TABLE 1: *Experimental values of colour changing indicator film properties.*

Cellulose (%)	Ethylene Glycol (%)	Indicator solution (%)	Thickness (mm)	Tensile strength (N/mm²)	Chroma	Hue angle	WVTR
3	1	2	0.043±0.007	0.374±0.072	24.899±0.04	34.191±0.01	57.312±0.006
3	2	1	0.045±0.001	0.378±0.073	27.820±0.08	36.890±0.01	56.211±0.001
3	2	3	0.046±0.002	0.379±0.083	27.061±0.05	35.427±0.08	55.871±0.001
3	3	2	0.049±0.004	0.383±0.033	22.413±0.07	32.983±0.06	58.411±0.003
5	1	1	0.060±0.003	0.461±0.043	22.161±0.05	32.867±0.03	57.735±0.005
5	1	3	0.059±0.004	0.458±0.061	34.830±0.01	45.031±0.04	56.981±0.001
5	2	2	0.061±0.001	0.466±0.018	20.639±0.08	27.937±0.07	55.970±0.004
5	2	2	0.062±0.004	0.467±0.011	20.572±0.06	26.251±0.09	56.974±0.004
5	2	2	0.062±0.002	0.466±0.021	20.572±0.02	26.251±0.05	55.210±0.009
5	3	1	0.066±0.06	0.471±0.049	21.277±0.04	31.674±0.02	54.933±0.002
5	3	3	0.065±0.003	0.469±0.028	29.180±0.01	39.306±0.05	55.611±0.008
7	1	2	0.090±0.009	0.584±0.093	20.998±0.09	30.826±0.02	53.622±0.009
7	2	1	0.093±0.003	0.585±0.042	21.737±0.02	29.050±0.03	50.635±0.008
7	2	3	0.094±0.006	0.589±0.022	32.683±0.03	43.906±0.05	51.420±0.004
7	3	2	0.098±0.006	0.594±0.047	25.369±0.02	34.640±0.07	50.962±0.008

TABLE 2: *Analysis of variance of process parameters.*

Sources	Responses F values			
	Thickness	Tensile strength	Chroma	Hue angle
A-Cellulose	(+) 7275.79*	(+) 28015.05*	(–) 0.055	(–) 0.037
B-Glycol	(+) 133.42*	(+) 63.83*	(–) 0.60	(–) 0.61
C-Indicator	(+) 0.001	(+) 0.004	(+) 26.43*	(+) 36.14*
AB	(+) 1.58	(+) 0.080	(+) 2.63	(+) 1.66
AC	(+) 0.004	(+) 0.72	(+) 7.65*	(+) 17.48*
BC	(+) 0.001	(+) 0.080	(–) 1.27	(–) 1.35
A^2	(+) 342.67*	(+) 369.53*	(+) 2.23	(+) 7.19
B^2	(+) 2.59	(–) 0.10	(+) 1.15	(+) 12.73
C^2	(+) 0.16	(–) 1.97	(+) 21.35*	(+) 44.59*
CV (%)	1.20	0.37	2.12	5.77
S D	7.958E-004	1.770E-003	8.52	1.95

Note: *Significant at 5% level, (+)/(–) sign shows positive/negative effect

3–7%, 1–3% and 1–3% respectively. The smart film was fabricated using methyl cellulose (S.D. Fine-Chem Ltd, Boisar, India) as a binder, polyethylene glycol-400 (Loba chemie, Thane, India) as a plasticizer, bromothymol blue (S.D. Fine-Chem Ltd, Boisar, India) as a dye and calcium hydroxide (S.D. Fine-Chem Ltd, Mumbai, India) as a carbon dioxide absorber. Smart film was prepared by modifying the method given by Nopwinyuwong et al (2010) and Hong and Park 1999. Indicator dye solution was prepared by mixing bromothymol blue in ethanol. The ingredients for film solution i.e. methyl cellulose (5% in distilled water), polyethylene glycol-400 (1% in distilled water) and calcium hydroxide (1% in distilled water) were thoroughly mixed. This mixture was kept in hot water bath at 80oC for proper dissolving of methyl cellulose. Indicator dye solution was then mixed with film solution. The mixture of all these ingredients i.e. indicator dye solution and film solution were homogenized using a homogenizer until complete dissolution was attained. This solution was again kept in hot water bath at 80°C for 10 min for degassing. To cast the film, the mixture obtained was poured on to the teflon moulds and kept in the incubator at 35°C for 24 hours.

Characterization of the smart film

The developed smart film was evaluated for its physical, mechanical and optical properties in terms of thickness, tensile strength, colour and water vapour transmission rate.

Film thickness

The thickness of the smart film was measured using a digital micrometer (Model: IP-65, Mitutoya Corp., Japan) having a sensitivity of ± 0.1 µm. The final thickness of developed smart film was taken as average of three replications measured at different points of the film.

Water vapour transmission rate

The water vapour transmission rate of the developed indicator films was measured using gravimetric techniques (ASTM E96-80, 1987). The desiccant material (anhydrous $CaCl_2$) was placed in a glass petri dish and the film was sealed properly over the petri dish with the help of vacuum grease. The initial weight of the petri dish with contents was measured and then it was placed in the desiccator containing saturated sodium chloride solution to maintain a constant RH of 76%. The desiccator was placed in an incubator at a temperature of 38°C (Solak and Dyankova 2014) until the constant change in weight was attained. The weight of the petri dishes was measured after every one hour to determine the gain or loss of moisture. The water vapour transmission rate (WVTR) was evaluated using the equation given below:

$$\text{WVTR } (\text{g m}^{-2}\text{ d}^{-1}) = \frac{24 * M_v}{t * A}$$

Where Mv is mass loss or gain in g; t is the time in hours; A is the film surface area in m².
Area (πr^2, r being radius of petri dish which was 0.045 m) in m².

Tensile strength (TS)

The tensile strength of the developed smart film was measured by using double column texture analyzer (Stable Micro Systems, Model: TA.TXT. Plus). Tension test was performed to evaluate the strength of the smart film. The value of strain was set to 50% and the return distance was fixed to 60 mm (Rhim 2004). Tensile strength was evaluated using the equation below:

$$\text{Tensile Strength} = \frac{F_{max}}{A}$$

Where F_{max} is the maximum load used for breaking the film (N) and A is area of cross section of the film sample (mm²).

Colour, Chroma and Hue angle

Colour of the smart film was measured using Colour Reader CR-10 (Konica Minolta Sensing Inc. Tokyo, Japan) in terms of 'L', 'a' and 'b' values and then these values were used for calculating chroma and hue angle. The chroma and hue angle were computed by the formulae reported by Gnanasekharan et al 1992.

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\text{Hue angle} = Tan^{-1}\frac{b}{a}$$

Analysis of smart labels

Freshly harvested bell pepper fruits (*Capsicum annuum* L. var. *Cv. Oribelli*) were procured from farms of Punjab Agricultural University, Ludhiana. Samples were washed with water mixed with 100 ppm potassium meta-bisulphite so as to remove the dirt and reduce the bacterial load. Afterwards the surface of bell peppers was dried and selection of bell peppers was done on the basis of their shape, weight and size. Labels were prepared by cutting smart film in to the strips of 5 x 3cm. Smart rectangular packages were developed by attaching these labels inside the packages (20 x 25 cm) made from low density polyethylene of thickness 37.5 microns. The thickness of the package was measured by a micrometer (Model: IP 65, Mitsuya corp., Japan) having a sensitivity of 0.1 µm. The oxygen absorbing inserts (O-Busters, Sorbead India, Gujarat, India) were placed inside the packages. Amount of oxygen absorbers was used based upon the previous experiments conducted (Devgan et al 2019). Each package consisted of two bell pepper fruits weighing approximately 300±10 g, and sealed using impact sealer (Marvel Portable Seal©, India), leaving a headspace of 10 cm. These packages were stored at 10±1°C and 80±5% RH. The package headspace, label colour and overall acceptability of packaged fruits were assessed every 4th day. Gas samples from the package headspace gas concentration were drawn from the packages through septum placed on the packages using a gas analyser with a needle probe (Systech Instruments; Model: Gaspace Advance, UK). The overall acceptability was recorded using a 9 point hedonic scale by selected committee members (Professors). The samples were accordingly perceived and rated by the members from "dislike extremely" to "like extremely."

Statistical analysis

All the experiments were conducted in triplicates and results are presented as the average ±standard deviation. Response surface methodology (RSM) was used and a three variable (three levels of each variable) Box-Behnken experimental design was used for designing the experiments. Independent variables for smart film were the concentration of cellulose, polyethylene glycol and indicator solution. The response parameters were tensile strength (T), water vapour transmission rate (WVTR), thickness, chroma and hue angle. Various models were fitted and goodness of fit of models was evaluated respectively. A correlation was established between colour change of smart la-

TABLE 3: *Optimum values of process parameters.*

	Target	Experimental Range Min	Max	Optimum value	Experimental	Desirability
Cellulose (%)	range	3	7	5.56	5.6	
Polyethylene glycol (%)	range	1	3	1	1	
Indicator solution (%)	range	1	3	3	3	
Responses				**Predicted values**		
Thickness (mm)	maximize	0.043	0.09	0.070	0.069	**0.79**
Tensile strength (N)	maximize	0.374	0.59	0.506	0.502	
Chroma	maximize	20.57	34.8	33.18	33.73	
Hue angle (°)	maximize	26.25	45.0	44.60	44.15	

TABLE 4: *Experimental values of CO2 and total colour difference of the smart label during storage.*

Storage period (days)	CO_2	L	a	b	TCD	Chroma	Hue	OA (%)	Label Colour	Label Colour code
0	0.03±0.01	55.4±0.13	–24.2±0.02	–23.5±0.03	0.00±0.12	33.73±0.11	44.15±0.23	100		1
4	2.6±0.02	58.9±0.11	–22.2±0.04	–20.4±0.01	5.08±0.27	30.14±0.25	42.58±0.27	100		2
8	4.8±0.01	60.4±0.24	–29.3±0.07	–9.3±0.03	15.86±0.10	30.64±0.22	17.66±0.31	100		3
12	5.5±0.02	62.6±0.18	–31.2±0.08	10.2±0.04	34.97±0.32	31.87±0.28	18.67±0.14	93.33		4
16	5.9±0.03	64.8±0.32	–20.8±0.02	15.4±0.01	40.16±0.17	25.88±0.11	36.51±0.11	86.66		5
20	6.8±0.01	65.7±0.11	–16.5±0.04	25.4±0.06	50.56±0.15	30.28±0.19	56.99±0.18	80		6
24	8±0.01	66.3±0.35	–10.6±0.08	35.7±0.09	61.71±0.11	37.24±0.30	73.46±0.09	73.33		7

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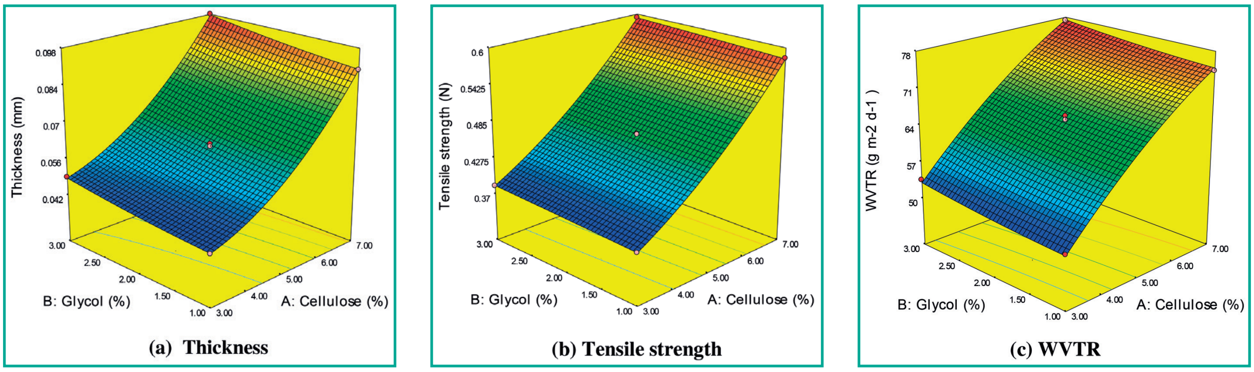

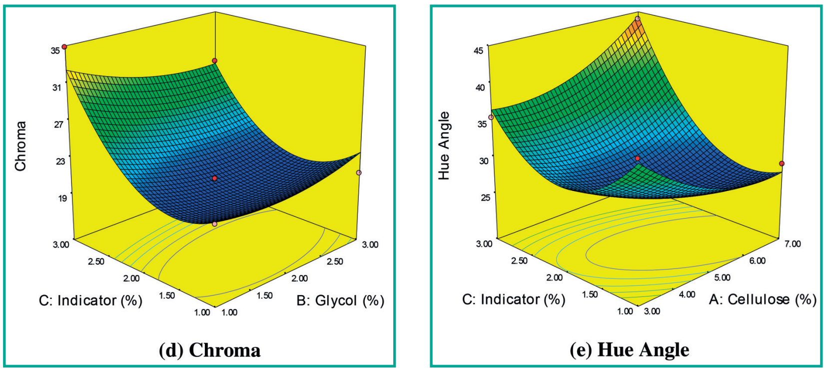

FIGURE 1: *Response surface plots showing the effect of process parameters on indicator film properties.*

bel and headspace gas concentration. Analysis of the data was done using linear regression analysis.

Results and discussion

Smart films were developed at varying levels of methyl cellulose, glycol and indicator solution. The films were tested for their thickness, chroma, hue angle, tensile strength and WVTR. The results obtained were optimized using response surface methodology. The film obtained at optimized conditions was used for preparing the smart labels for smart packages. The results obtained from analysis have been discussed below:

Fabrication and Characterization of the developed smart film

Thickness

The thickness of the colour changing indicator film varied from 0.043 to 0.098 mm. Maximum thickness of 0.098 mm was observed for film made with cellulose concentration of 7% and polyethylene glycol concentration of 2% whereas minimum film thickness 0.043mm was for cellulose concentration of 3% and polyethylene glycol concentration of 1% (Table 1). The correlation and significance of the independent parameters on response variables is as provided in Table 2. It is evident that thickness of the film increased with the increase in cellulose level and polyethylene glycol level whereas indicator solution level had a non-significant effect (Fig 1a). Similar trends were observed by Paul and Kumar (2003).

Tensile strength

Tensile strength, which is ability to resist breakage is an important mechanical property of packaging films. Tensile strength of colour changing indicator film varied from 0.374 to 0.594 N/mm^2 with different process conditions (Table 1). The concentration of cellulose and polyethylene glycol has a significant effect on various response variables whereas indicator solution had positive non-significant effect (Table 2). Tensile strength of the film increased with increase in cellulose concentration. Maximum tensile strength of 0.594 N/mm2 was observed for film made with cellulose concentration of 7% and ethylene glycol concentration of 3%. The effect of significant terms on tensile strength is shown in Fig 1b. Similar trends were observed by Rhim (2004) for alginate films.

Chroma

Chroma value is used to describe the purity, intensity and saturation of the colour. Chroma values for smart film varied from 20.57 to 34.83 (Table 1). With increase in indicator solution concentration, chroma value of film increased significantly as the indicator solution had a bluish chroma; while it decreased insignificantly with increase in cellulose and ethylene glycol solution. The reason attributed to this is that the solution of cellulose and ethylene glycol was colourless to white which could've effected only L value. The chroma value is dependent on a (greenness to redness) and b (blueness to yellowness) values. This is the reason why it rapidly spiked on increasing the indicator solution concentration (Fig 1d). The results are in accordance with the results presented by Kumar et al, 2020 and Meng et al, 2014. Maximum chroma value was observed for film made with 5% cellulose concentration, 1% ethylene glycol and 3% indicator solution.

Hue angle

Hue angle describes the quality of colour on the basis of their dominant wavelength. Hue angle for smart film varied from 26.25 to 45.03 (Table 1). The increase in indicator solution concentration showed a significant increase in hue angle values whereas increase in cellulose concentration and polyethylene glycol concentration had a negative non-significant effect on response variables (Fig 1e). Akin the chroma, hue value is also dependent on a (greenness to redness) and b (blueness to yellowness) values. Maximum hue angle was observed for film made with cellulose concentration 5%, ethylene glycol 1% and indicator solution 3%. Similar results were also reported by Kumar et al, 2020 and Meng et al, 2014.

Optimization of inputs for Smart films using Response Surface Methodology

Numerical optimization technique was used for optimization of the conditions for development of smart label. The aim of the optimization process was to determine the levels of independent parameters viz. cellulose (3–7%), polyethylene glycol (1–3%) and indicator solution (1–3%) so as to maximize tensile strength, chroma, hue angle, and minimize thickness (Table 3). The optimized values of the process parameters obtained during development of colour changing indicator film are provided in Table4. The actual and predicted values of the responses corresponding to these optimized conditions are also given in Table 4. The desirability, which varied from 0 (outside limits) to 1 (at the goal) was 0.79. To validate the optimized parameters, smart film was developed using optimum values of the ingredients. The values obtained by conducting experiments were in close correlation with predicted values.

Evaluation and validation of developed smart packages

For validation of labels, yellow bell peppers were packaged in smart packages. Label colour and overall acceptability of the packaged fruits and headspace O_2 and CO_2 were measured at regular intervals during storage.

Headspace gas concentration and colour change of indicator label

It is evident from the perusal of Table 4 that 'L' and 'b' values increased gradually with storage time, while 'a' value decreased (Fig 1). The 'b' values changed from negative to positive and 'a' value changed from being more negative to less negative indicating change of colour from greenish blue to greenish yellow range. Colour change of the smart label during storage within the packages may be attributed to in-pack increase in CO_2 levels as a result of product respiration and O_2 absorption by the O_2 absorbing inserts. The colour change of the indicator was caused by the combined function of its components i.e. carbon dioxide absorber and chemical dye (Hong & Park, 1999; Pavelková 2012). Bromothymol blue is yellow at pH 5.8 (and lower) and changes to blue at about pH 7.6. At the initial stage the dye was mixed with calcium hydroxide, making the label basic in nature and the initial colour was blue. As the senescence progressed, more carbon dioxide was produced, which permeated into the smart label within the packages and chemically reacted with calcium hydroxide to form water in the smart film. More carbon dioxide molecules and some other organic acid vapours (as minor contributors) permeated into the labels and dissolved in the water, resulting in neutralization of the relative basic mixture. Thus change in the pH caused to colour changes in the label form reddish blue to greenish yellow.

The hunter L, a, b and total colour difference of the film was dependent upon carbon dioxide level (Fig 2). For the validation of labels yellow bell pepper correlation was established between label code, overall acceptability and carbon dioxide concentration (Fig 3). Colour of the smart label can indicate about the overall acceptability and head space carbon dioxide concentration. Linear regression analysis of the data obtained during storage showed a highly significant effect of CO_2 concentration on colour of the label. Analysis of the data indicated a p value of 0.0006, showing that it is significant at 0.1% level of significance. The coefficient of determination, R^2 was 0.957 indicating goodness of fit of the model. Further correlation between carbon dioxide concentration and total colour difference of the indicator film was established using Karl Pearson correlation coefficient. Pearson correlation coefficient, r was found to be 0.9605. High value of Pearson correlation coefficient r and R^2 shows that carbon dioxide concentration and total colour difference of indicator film have good correlation (Fig. 4).

Conclusion

Colour is an important property of fresh produce which informs the consumers about the quality, deterioration

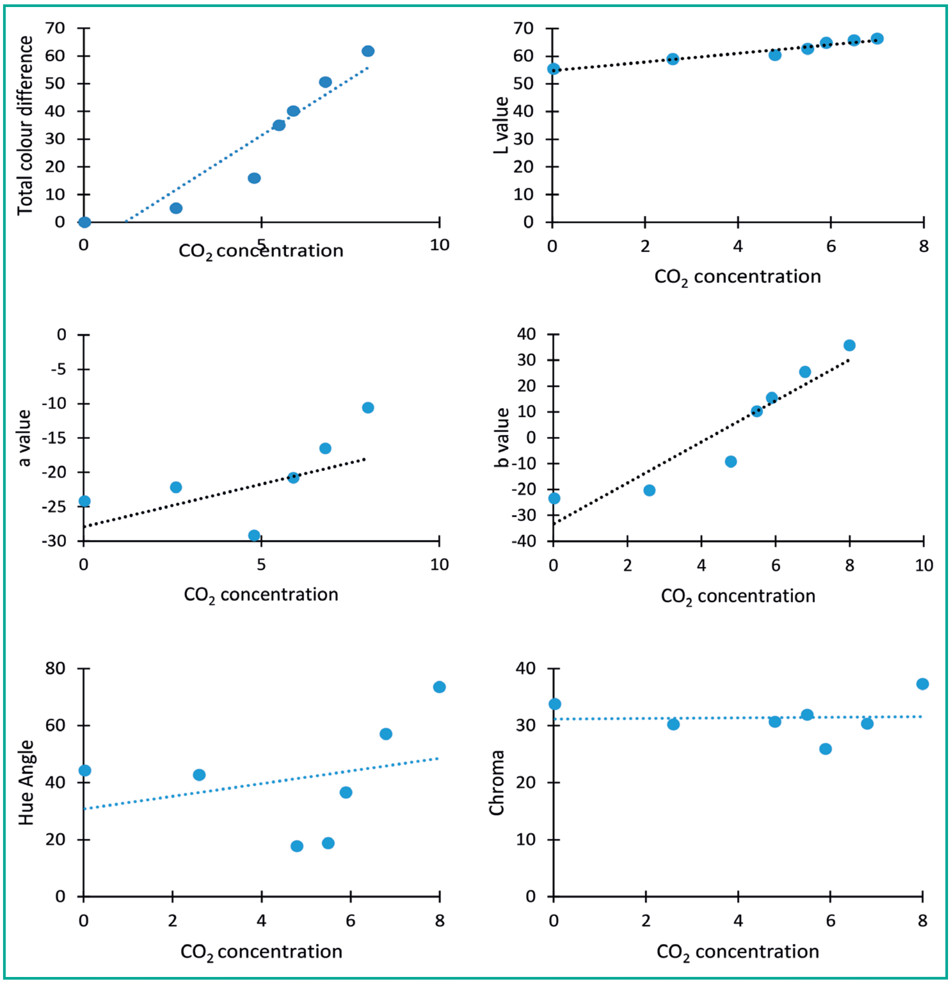

FIGURE 2: *Correlation between CO_2 concentration and colour change of the indicator label during storage.*

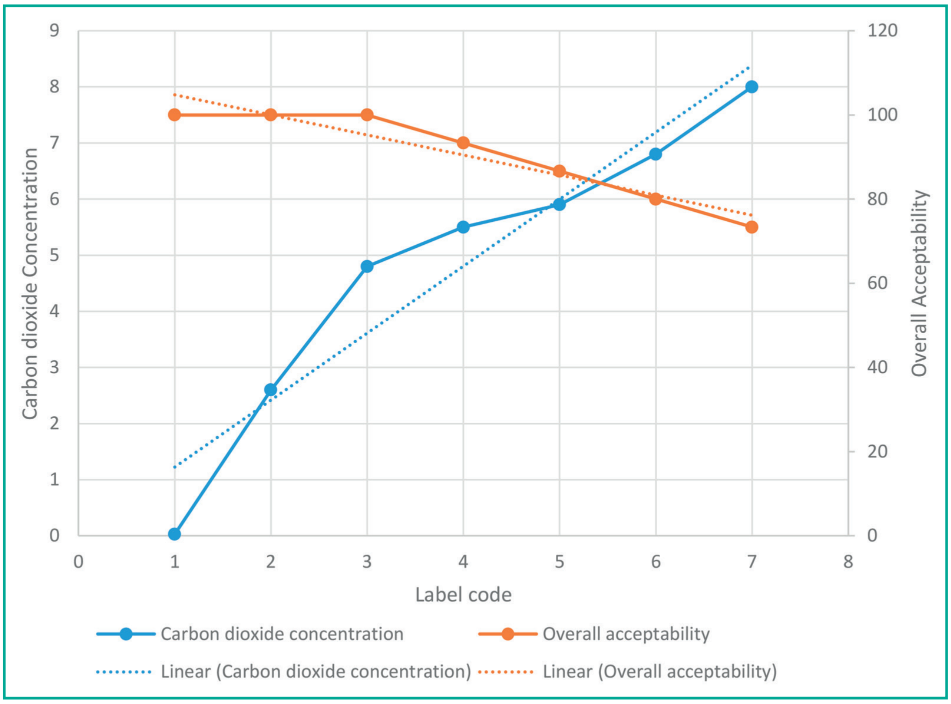

FIGURE 3: *Correlation of Label code, Carbon dioxide concentration and Overall acceptability.*

the water, resulting in neutralization of the relative basic mixture. Thus change in the pH caused to colour changes in the label form reddish blue to greenish yellow. Such colour indicators could be employed as an effective active packaging technology for evaluating the ripeness of bell pepper fruits in a non-destructive and real time manner. The developed film has the potential which can be applied to other fruits and vegetables. So, further meticulous research is required to understand the correlation of other factors peculiar to a particular product.

Conflict of interest

The authors declare no conflicts of interest.

and adulteration characteristics. The colour changes of the developed smart labels indicated the level of carbon dioxide inside the packages of bell peppers which was further related with degree of senescence of bell pepper. As the senescence progressed, more carbon dioxide was produced, which permeated into the smart label within the packages and chemically reacted with calcium hydroxide to form water in the smart film. More carbon dioxide molecules and some other organic acid vapours (as minor contributors) permeated into the labels and dissolved in

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FIGURE 4: *Change in colour of the indicator label during storage.*

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