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ORIGINAL ARTICLE

Effect of pretreatments and modified atmosphere packaging on the shelf life and quality of fresh- cut green bell pepper

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Abstract Present study was aimed at understanding the effect of pretreatments and modified atmosphere packaging on the quality of fresh-cut green bell pepper (Capsicum annuum L.) during low temperature storage. Dip treatment of freshly cut green bell pepper pieces in 2 % calcium propionate followed by surface drying and subsequent packing in cryovac PD961 film which maintained an equilibrium modified atmosphere of 13–14 % O2 and 7 % CO2 helped to extend the marketability till 9 days storage at 8 °C. The microbiological quality was at the best level up to 6 days of storage, as evidenced by a surge in aerobic plate count, pectinolysers and pseudomonads on subsequent days. Head space volatile analysis of the produce at regular intervals showed a reduction in monoterpenoids and simultaneous increase of aldehydes and ketones, sesquiterpenoids, esters, furans and pyrazines during storage. Principal component analysis of the head space volatiles identified, cis - ocimene, 1,3, 8-paramenthatriene, trans 3- caren 2-ol, bergamotene, 2-hexenal, ethyl 1- decanol, (E)-3- hexenol and heptane thiol as the markers of freshness in minimally processed green bell pepper.

Keywords Minimal processing · Capsicum · Volatiles · Modified atmosphere · Calcium propionate · Pectinolysers

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Introduction

Minimally processed vegetables (MPV or fresh-cut vegetables) have gained consumer popularity due to their ready- to – use (RTU) or ready - to -eat (RTE) nature, nutritional quality and taste equivalent to the fresh vegetables (Ragaert et al. 2004). Unit operations for their preparation such as slicing, shredding etc., result in high respiration, microbial susceptibility, and in turn faster browning and other spoilage (Allende et al. 2006). Chlorine, the traditionally used sanitizer and an antibrowning agent, poses health concern due to its residual activity, thus stricter regulations are likely to come in future on the use of chlorine in fresh-cut industry. Organic acids, vitamin C, plant essential oils, chelators, calcium salts, polyphosphates etc. are cheap, safe and promising alternatives for fresh-cut industry (Rico et al. 2007). Equilibrium modified atmosphere packaging (EMAP) is the most commonly used packaging technology for fresh- cut vegetables. It involves packaging vegetables and fruits the gas atmosphere of package is not air (O_2 – 21 %; CO_2 – 0.01 %; N_2 – 78 %) but a lowered level of O_2 and a heightened level of CO_2. This kind of package slows down the normal respiration of the product and so prolongs the shelf life of the product (Sandhya 2010). Minimal processing technology is to be tailor made for each fresh produce; and the quality deterioration in minimally processed vegetables is manifested as change in colour, flavour, firmness, odour and nutritional quality mainly brought about by physiological and microbiological factors (Ragaert et al. 2007). Post - harvest metabolomic analysis has the potential for detecting and understanding food spoilage, and is rapidly progressing field as reviewed by Kushalappa et al. (2008). The present paper describes the standardization of a low cost pretreatment and packaging material for extending the shelf life and maintaining the quality of minimally processed green bell pepper, as well as the associated nutritional, microbiological and flavour changes during storage.

Materials and methods

Plant material

Green bell peppers var. Indra grown in polyhouse of Indian Institute of Horticultural Research, Bangalore, India, was used for preparation of fresh-cut vegetables in the experiments. In all treatments, the freshly harvested produce was washed in potable water and surface dried. These were cut into two halves, seeds and the white placenta were removed, and approximately 1″ × 1″ sized pieces were made using sharp stainless steel knives.

Experiments to study the effect of pretreatments and modified atmosphere suitable for extending the shelf life

In the first experiment, effect of pretreatments on shelf life and quality of fresh-cut green bell pepper was studied. The freshly cut bell pepper pieces were dipped for 5 min in solutions of different pretreatment chemicals viz., 100 ppm sodium hypochlorite, 2 % calcium chloride, 2 % calcium lactate, 2 % calcium propionate, 5000 ppm H_2O_2, 5000 ppm ascorbic acid or 2 % citric acid. Green bell pepper pieces dipped in water and without any dip treatment served as the control for comparison. The slices were then surface dried and 200 g of samples were packed in plastic trays over wrapped with cling film. All samples were stored at 8 °C for 6 days. Ten replications were maintained for each treatment.

In the second experiment, effect of modified atmosphere packaging (MAP) on shelf life and quality of fresh-cut green bell pepper was studied. 200 g freshly cut green bell pepper pieces were dipped in the best identified pretreatment, i.e., 2 % calcium propionate for 5 min, subsequently surface dried and packed in 17 × 17 cm semi permeable plastic bags with different O_2 and CO_2 permeabilities viz., 31.25 μm thick Cryovac PD961® (7400–8500 O_2& 21,000–24,000 CO_2 mL m^{-2} day^{-1} atm^{-1}, 25 μm thick polypropylene (1300–6400 O_2 & 7700–21,000 CO_2 mL m^{-2} day^{-1} atm^{-1}), 25 μm thick LDPE(3900-13,000 CO_2 mL m^{-2} day^{-1} atm^{-1}) and 12 μm thick PVC cling film (63,555 O_2 & 127,733 CO_2 mL m^{-2} day^{-1} atm^{-1}) to serve as control. Ten replications were maintained in each treatment. All samples were sealed and stored at 8 °C for 9 days i.e., the day of sensory deterioration in best package.

Samples in the above experiments were analyzed for sensory, colour, firmness, nutritional quality and microbiological quality at the end of storage period. Sensory properties viz., color, firmness, odour and overall marketability of the stored samples in the above experiments were measured using a 5 point Hedonic scale (5 = excellent, 4 = very good, 3 = good, 2 = average 1 = poor) in regular intervals, and the packs were sampled for biochemical and microbiological analysis when the best treatment as judged by sensory evaluation also showed signs of deterioration. In-pack oxygen and carbon dioxide levels during storage of the packages were analyzed using a Gas analyzer (Checkmate-9900, PBI Dansensor, Denmark) at regular intervals. Colour readings (L, a, b values) of the bell pepper pieces were measured using a Colour reader (CR-10, Minolta Co. Ltd., Osaka, Japan). The total colour difference between stored and fresh sample were calculated as per standard formulae (Mohammadi et al. 2008). Firmness of the cut pieces were analyzed using firmness analyzer (Instron-4201 Universal testing machine, Instron Corporation, USA) with a 3 mm probe and the values were expressed as kg/cm^2. Vitamin C was measured colorimetrically (Davies and Masten 1991). Microbiological quality was assessed by enumerating total aerobes (plate count agar) and coliforms (violet red bile agar) by standard plating method. Data obtained were subjected to statistical analysis for comparison of means (Gomez and Gomez 1984).

Studies on microbial dynamics and volatile flavour changes during storage

Freshly cut capsicum pieces were dipped in calcium propionate and packed in Cryovac PD961® bags (the best MA packaging film identified in the previous experiment) or cling film (control) as described above and the packs were stored at 8 °C for 10 days. The dynamics of different bacterial groups using selective and differential media viz., lactic acid bacteria (Mann Rogosa Sharpe agar, Hi Media), pectinolytic bacteria (Pectate agar, Hi Media), and pseudomonads (Pseudomonas agar, Hi Media), coliforms (Violet red bile agar, Hi Media) as well as aerobic plate count (Plate count Agar, Hi Media) was analyzed at regular intervals during storage.

The flavour changes were also analyzed at frequent intervals by headspace-solid phase micro-extraction (HS-SPME) technique using capillary GC and GC–MS/MS. For the analysis, 10 g samples were macerated to slurry by using a prechilled homogenizer, and transferred to the 150 mL vials having screw caps with silicon rubber septum containing a magnetic stirrer, and 0.5 g NaCl was added after three minutes incubation (Pawliszyn 1997). The samples were stirred, and equilibriated for 20 min. After closing the cap, the sample was stirred continuously to increase the rate of transfer of the analytes and was allowed to equilibrate with the headspace for 20 min, and sampled using a pre-conditioned SPME fiber (highly crossed linked DVB/CAR/PDMS −50/30 μm fiber conditioned by inserting it into the GC injector port at 250 °C for 3 h) (Mazida et al. 2005). Subsequently, the

SPME fiber was introduced in the injector port for gas chromatographic analysis and was allowed to remain in the inlet for 10 min. The GC-FID analysis was carried out using a Varian-3800 Gas Chromatograph, equipped with a FID detector. Nitrogen (1 mL/min) was used as the carrier gas. The volatile components were separated on VF^{-5}, (factor Four) capillary column from Varian, USA, 30 m × 0.25 mm i.d., 0.25 μm film thickness. The injector temperature was set at 250 °C and all injections were made initially in split (1:20) mode for 0.5 min followed by split-less. The detector temperature was 270 °C, and the temperature programmes for column was as follows: 40 °C for 3 min at an increment 3 °C/min to 190 °C, hold for 1 min, then 5 °C/min to 220 °C and maintaining the constant temperature for 5 min. The MS column VF-5MS(Factor four) (Varian, USA) fused-silica capillary column of 30 m × 0.25 μm i.d., 0.25 mm film thickness was used for the analysis. The mass spectrometer was operated in the external electron ionization mode with the carrier gas helium 1 mL/min; injector temperature, 250 °C; trap temperature 200 °C, ion source-heating at 210 °C, transfer line temperature 230 °C, EI-mode was 70 eV, with full scan-range 50–450 amu was used. Temperature programs for column was used as same as described for GC-FID analysis. The total volatile production was estimated by the sum of all GC-FID peak areas in the chromatogram and individual compounds were quantified as relative percent area. Volatile compounds were identified by comparing the retention index which was determined by using homologous series of n-alkanes (C_5 to C_{32}) as standard (Kovats 1965) and comparing the spectra using two spectral libraries available as Wiley and NIST-2007. Principal component analysis of the head space volatile data was performed using excel add-in program Multibase 2013.

Results and discussion

Standardization of pretreatment

The data on sensory qualities, colour values and firmness of fresh-cut green bell pepper stored at 8 °C for 5 days are given in Table 1. These chemicals and their concentrations were chosen based on earlier scientific reports of their utility in minimal processing of other vegetables and fruits (Rico et al. 2007). Among the pretreatments tested, dipping in 2 % calcium propionate was found to be the best for maintaining the marketability till 6th day of storage at 8 °C. Calcium propionate treated samples possessed firmness and Hunter's a and b colour values on par with the freshly cut green bell pepper. Hunter's 'L' value was significantly low in samples dipped in calcium propionate as compared to fresh produce. Total color difference during storage was also low in these samples in comparison with other samples. Second best treatment identified was chlorine, where in the major difference with fresh produce was observed in terms of odour, browning, and firmness of the produce. Citric acid, calcium chloride and calcium lactate treatment significantly lowered the marketability of the treated produce, primarily due to whitening of cut ends. Present study proved thatt calcium propionate resulted in a better retention of sensory properties of fresh-cut green bell pepper as compared to calcium lactate and calcium chloride, two other widely used calcium sources for pretreatment in fresh- cut vegetables.

Calcium salts such as calcium gluconate, calcium carbonate, calcium chloride, calcium propionate, calcium lactate etc. serve as source of calcium for the plant cells. Calcium ions cross link with middle lamella pectins of cell walls, stabilize cell membranes, affect cell turgor potential by preventing membrane leakage and inhibit browning related enzymes (Martın-Diana et al. 2007). Effectiveness of different calcium sources in freshness maintenance can vary according to the produce (Aguayo et al. 2008; Luna-Guzman and Barrett 2000), and the current study also reflected similar observations.

The effect of pretreatments on antioxidant and microbiological quality of stored fresh-cut green bell peppers are given in Table 2. Vitamin C content in the treated samples after 5 days storage was significantly lower than the fresh produce. As expected, ascorbic acid treatment resulted in a higher level of vitamin C compared to all treatments. Calcium propionate and chlorine treated samples ranked next and were on par with each other. Microbiological quality as evidenced by aerobic plate count, lactic acid bacteria and coliforms population were excellent in freshly cut pieces, but deteriorated at the end of storage period. Unlike bacteria, mould population was lesser than 0.7 log cfu/g in all samples, and no significant difference was observed among treatments (data not shown). Major microbiological concern in fresh-cut vegetables are bacteria; while moulds are less important due to the intrinsic properties such as slightly acid to neutral pH favouring bacteria which will overgrow moulds (Tournas 2005).

Apart from acting as calcium source, calcium propionate and calcium lactate are antimicrobial compounds. Calcium propionate possess antibacterial and antifungal activity by interfering cellular respiration steps (Luna-Guzman and Barrett 2000). It is a food additive designated as GRAS (Generally Recognized As Safe) by the U.S. Food and Drug Administration (SCOGS report No.79, 1979). This study showed that calcium propionate treatment and subsequent storage in trays overwrapped with cling wrap was not effective in retaining vitamin C and microbiological quality of fresh-cut bell pepper till 6th day of storage. This observation indicates that microbiological proliferation occurred in fresh-cut green bell pepper earlier to sensory quality deterioration.

Table 1 Effect of pretreatments on sensory appeal, colour and texture of minimally processed green bell pepper stored for 5 days at 8 °C

Sample	Sensory appeal				Colour				Firmness (kg/cm^2)
	Absence of browning	Absence of white cut ends	Odour	Marketability	L	a	b	Total colour difference	
Fresh	5.0^a	5.0^a	5.0a	5.0^a	36.3^{bc}	-11.7^a	20.0a	–	19.52^b
Calcium propionate	4.5^{ab}	4.0^b	4.5a	4.5^{ab}	34.0^c	-10.4^{ab}	18.8^a	3.7^a	22.6^{bc}
Chlorine	3.8^b	4.0^b	4.0^b	4.0^b	38.0^{ab}	-11.7^a	20.3^a	4.1^{ab}	26.3^{de}
Hydrogen peroxide	3.0^c	3.0^c	4.5^a	3.0^c	37.6^{ab}	-11.7^a	20.7^a	5.8^c	29.3^{cd}
Ascorbic acid	3.0^c	2.5^{cd}	4.5^a	3.0^c	37.0^{ab}	-10.2^{ab}	19.4^a	4.5^b	24.3^d
Calcium lactate	2.8^c	2.5^{cd}	$3.5b^c$	2.0^d	38.9a	-9.8^b	19.9^a	4.7^b	20.8^c
Calcium chloride	2.8^c	2.0^d	3.9^b	2.5^{cd}	36.3^{abc}	-9.9^b	19.8^a	5.4^c	20.8^c
Citric acid	2.0^c	2.0^d	3.0^c	2.0^d	39.0^a	-8.9^c	19.0^a	5.1^{bc}	15.3^e
Water	2.5^c	2.5^{cd}	3.4^{bc}	3.3^{bc}	37.9^{ab}	-10.0^{ab}	20.6^a	4.6^b	23.9^{cd}
Undipped	2.8c	3.5^{bc}	3.0^c	2.8^c	38.7^{ab}	-9.0^b	19.0^a	5.4^c	20.44^c

The numbers preceded by same superscripts indicate same ranks by DMRT ($p = 0.01$)

Standardization of modified atmosphere packaging

Apart from pretreatment, modified atmosphere packaging also play important role in maintaining the quality of minimally processed vegetables during storage. An experiment on modified atmosphere packaging was carried out with the best pretreatment selected from above experiment. In- pack gas composition of the green bell pepper packages during storage is given in Fig. 1, which shows that Cryovac PD961® packs maintained a high CO_2 and low O_2 atmosphere and reached an equilibrium modified atmosphere of approximately 13–14 % O_2 and 7 % CO_2. This was followed by LDPE (15 % O_2 and 5 % CO_2 and polypropylene (17–18 % O_2 and 3.6–4.6 % CO_2); while cling film maintained a near normal atmosphere 19–20 % O_2 and 0.5–1.5 % CO_2).

Sensory characteristics as well as L, a and b values of the produce packed in Cryovac PD961® ranked similar to fresh green bell pepper (Table 3). Firmness values were significantly higher in stored samples, compared to freshly cut pieces. The produce packed in polypropylene and LDPE were similar in quality, while cling wrapping deteriorated the quality further. Storage of green bell pepper pieces in modified atmosphere had a great impact on preservation of antioxidant nutrients (Table 4). Vitamin C in Cryovac PD 961® packs were on par with freshly cut green bell pepper, while other types of packing resulted a severe reduction in vitamin C during storage. Vitamin C is susceptible to oxidation by ascorbic acid oxidase, and losses can be minimized by modified atmosphere packaging of the produce after harvest (Howard and Hernandez-brenes 1998; Barth et al. 1993). The

Table 2 Effect of pretreatments on vitamin C and microbiological qualities of minimally processed green bell pepper stored for 5 days at 8 °C

	Vitamin C (μg ascorbic acid/100 g)	APC (log cfu/g)	LAB (log cfu/g)	Coliforms (log cfu/g)
Fresh	41.0^a	1.20^a	1.3^a	0.30^a
Calcium propionate	21^c	8.50^b	7.23^d	5.10^b
Chlorine	22^{bc}	8.30^b	4.30^b	4.93^b
Hydrogen peroxide	10.1^d	8.12^b	5.69^c	5.69^b
Ascorbic acid	28.6^b	9.19^b	4.20^b	6.69^c
Calcium lactate	10.3^d	8.56^b	4.03^b	6.59^c
Calcium chloride	16.0^{cd}	8.01^b	5.31^c	5.25^b
Citric acid	10.6^d	8.02^b	4.68^b	5.59^b
Water	12.6^d	8.69^b	6.95^d	7.45^d
Undipped	10.3^d	9.12^b	5.32^c	6.35^c

The numbers preceded by same superscripts indicate same ranks by DMRT ($p = 0.01$)

APC aerobic plate count, *LAB* lactic acid bacteria

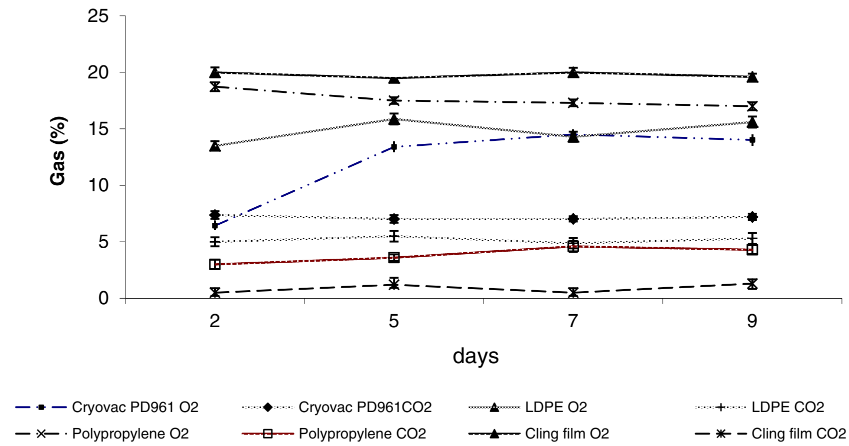

Fig. 1 Gas composition of minimally processed green bell pepper during storage at 8 °C

microbiological quality was significantly lower in all packs, in comparison with fresh produce. However, a superior microbiological quality among the treatments was observed in Cryovac PD961® films at the end of storage period, likely due to creation of unfavorable gaseous environment for the multiplication of spoilage organisms.

Microbial dynamics during the storage of fresh-cut bell pepper

Above results explain that the combined effect of pretreatment and modified atmosphere packaging had well preserved the visual marketability, colour, firmness, antioxidants etc., but the microbiological quality deterioration took place before the end of storage period. Modified atmosphere packaging can result in fermentative and spoilage microbes as well as off odours during storage, than causing visual spoilage signs in packed fresh produce (Ragaert et al. 2007). Therefore, population dynamics of the indicator group of spoilage organisms was analyzed in MA and the non MA packed produce at frequent intervals during the storage period (Fig. 2). Total aerobic plate count showed a drastic surge from fourth day onwards in non MA packed produce, while these were suppressed much and the growth was gradual in produce stored after optimized treatment, with a level of less than log 6 cfu/g till 8 days of storage. The lactic acid bacterial population surge occurred in the MA packs on the 6th day of storage, and their levels reached to 5.2 log cfu/g on 8th day and remained static till 10th day. It is worthwhile to mention that in non MA Packed samples, the pectinolysers began to grow at high rate on the 2nd day of storage (3.1 log cfu/g), while the standardized packs has delayed their proliferation till 6 days. The pseudomonads also showed a similar trend, and the maximum population reached to 4.1 log cfu/g. Coliforms population reached to >3 log cfu/g levels on 4th day and 8th day in non MA and MA packs respectively. Lactic acid bacteria are GRAS organisms but can cause spoilage of vegetables and their multiplication is favoured in low oxygen environments; and are spoilage organisms in foods. This study shows that lactic acid bacteria till a level of approximately 5 log cfu/g do not cause a visual quality reduction of MA packed fresh-cut green bell pepper, while pectinolysers, pseudomonads and coliforms are the major organisms relating to the quality of fresh- cut green bell pepper during low temperature storage similar observation was made by García-Gimeno and Zurera-Cosano (1997) in carrot, where it was reported that when the spoilage becomes observable, the count of lactic acid bacteria in fresh- cut carrots exceeded 6 log cfu/g. Present findings

Table 3 Effect of modified atmosphere packing on sensory appeal, colour and texture of fresh- cut green bell pepper stored for 9 days at 8 °C

Sample	Sensory appeal				Colour				Firmness (kg/cm^2)
	Absence of browning	Absence of white cut ends	Odour	Marketability	L	a	b	Total colour difference	
Fresh	5.0^a	5.0^a	5.0^a	5.0^a	36.8^{ab}	-11.0^a	20.0^a	–	18.69^a
Cryovac PD961®	4.7^a	5.0^a	4.5^{ab}	4.5^{ab}	36.9^{ab}	-11.51^a	21.3^a	3.4^b	27.27^b
Polypropylene	3.0^b	3.3^b	3.1^c	2.8^c	38.5^a	-11^{ab}	19.83^{ab}	4.4^{ab}	26.08^b
LDPE	3.0^b	3.2^b	3.1^c	2.5^c	36.4^{ab}	-10.79^{ab}	18.41^{ab}	6.0^{ab}	19.51^a
Cling	2.5^b	2.0^c	3.2^c	2.0^c	34.3^b	-9.33^b	14.3^b	11.3^a	17.5^a

The numbers preceded by same superscripts indicate same ranks by DMRT ($p = 0.01$)

Table 4 Effect of modified atmosphere packaging on the vitamin C and microbiological quality of minimally processed green bell pepper stored for 9 days at 8 °C

	Vitamin C (μg ascorbic acid/100 g)	APC (log cfu/g)	LAB (log cfu/g)	Coliforms (log cfu/g)
Fresh	41.3^{a}	1.2^{a}	1.3^{a}	0.3^{a}
Cryovac PD961®	41.4^{a}	6.4^{b}	6.5^{c}	2.0^{b}
Polypropylene	15.7^{b}	8.5^{c}	4.1^{b}	5.3^{c}
LDPE	14.0^{b}	8.3^{c}	4.5^{b}	5.6^{c}
Cling	10.4^{b}	8.3^{c}	4.3^{b}	5.3^{c}

The numbers preceded by same superscripts indicate same ranks by DMRT ($p = 0.01$)

APC Aerobic plate count, *LAB* lactic acid bacteria

show that nearly 2–4 days metabolic activity by pectinolysers results in a noticeable quality deterioration, while proliferation of pseudomonads can result immediate quality reduction of the produce. Earlier scientific reports suggest that in general, total counts of microbiological population on minimally processed vegetables after processing range from 3.0 to 6.0 log cfu/g., and dominating bacterial population during low temperature storage mainly consists of species belonging to the *Pseudomonadaceae* and *Enterobacteriaceae*, besides some species belonging to the lactic acid bacteria. (Ragaert et al. 2007). Microbiological quality studies on ready to use vegetables show that aerobic plate count of 6.0 log cfu/g is preferred specification in fresh-cut vegetables, and the signs of spoilage begins when the total aerobic plate count is >8 log cfu/g (Gomez- Lopez et al. 2008). At present, the microbiological specifications of fresh-cut vegetables given by various countries mainly emphasize on the qualitative or quantative levels of food borne pathogens. The spoilage microorganisms, even though may not be directly important in causing food borne diseases, are overlooked. The present study showed that deterioration in microbiological quality sets in well before the sensorial and nutritional quality changes in minimally processed green capsicum. The fact that the microbial changes in MAP showed a slow increase during storage confirmed the suppression of these organisms during storage. Though there was no perceived change in sensorial quality due to MA packaging till 9 days; by 8th day, deterioration in microbiological quality was noticed in MA packed samples. It can be concluded that the microbiological quality remains at its best during first 6 days, and are acceptable till 8 days.

Changes in volatile metabolites of fresh-cut green bell pepper during storage

Apart from tissue softening, browning and proliferation of microorganisms, the loss of original flavour and development of off flavour is an important spoilage symptom in fresh-cut produce, and these changes sets in much early to the other signs. Some times MA packaging results in high off flavour production, even as other types of spoilages are bare minimum. There fore, the knowledge on volatile emissions from fresh vegetables can help to develop and optimize a rapid

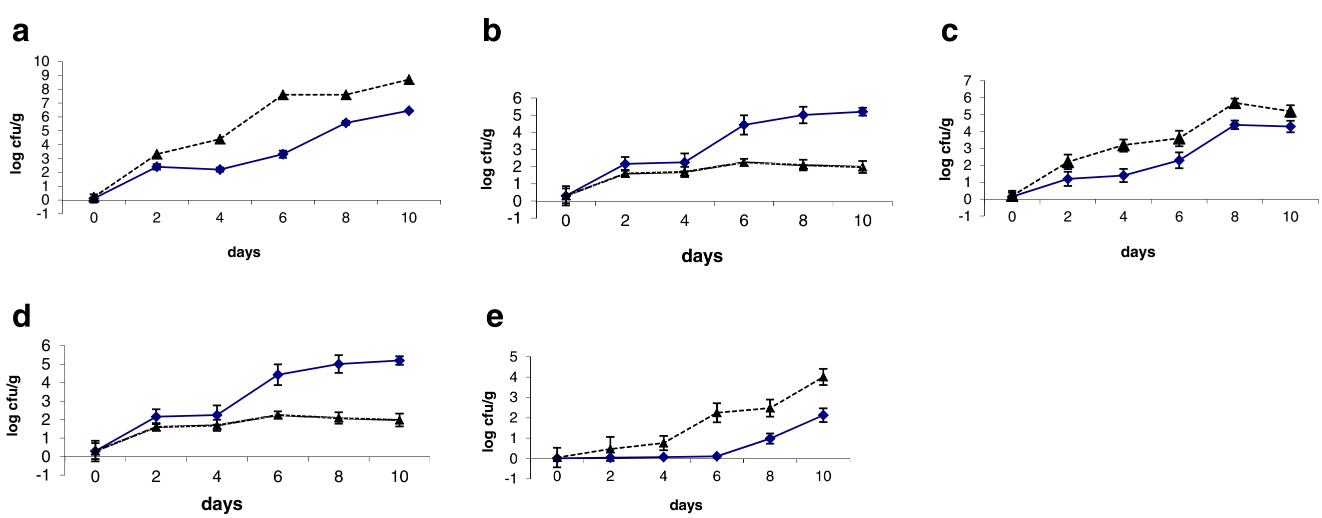

Fig. 2 Changes in the population of spoilage bacterial groups during storage of minimally processed green bell pepper at 8 °C **a** total aerobic plate count **b** lactic acid bacteria **c** pectinolytic bacteria **d** coliforms **e** pseudomonads —◆— MAP (Cryovac PD961;---▲---: non MAP(Cling film)

quality-monitoring method as well as to understand metabolic basis of volatile emission changes in MPV during storage (Lonchamp 2006). Keeping this in mind, the volatile emissions of minimally processed green bell pepper during storage was analyzed (Table 5). Major group of volatile compounds in fresh green bell pepper was monoterpenoids; with *cis*-ocimene as the most predominant one, occupying 60.26 % of the total head space. This was followed by nitrogenated compounds (6.7 %) and alcohols (4.5 %) respectively. 2-Methoxy 3-isobutyl pyrazine (4.7 %) and methyl salicylate (2.48 %), 1,3,8 paramenthatriene (2.0 %), hexanol (1.33 %), 1,3,5,5-tetramethyl −1,3- cyclohexadiene (1.58 %) were also present in high proportions. The results are in agreement with majority of the earlier findings which identified capsicum volatiles as C6 volatile compounds viz., hexanal, (*Z*)-3-hexenal, (*E*)-2- hexenal, hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenol; terpenoids viz., limonene, linalool, (*E*)-β- ocimene, and δ-3-carene; the aldehydes (*E*,*Z*)- 2,6-nonadienal and (*E*,*E*)-2,4-decadienal; the esters methyl salicylate and ethyl acetate; the ketones 1-penten-3-one and 2,3-butanedione as well as the pyrazine viz., 2-methoxy-3-isobutylpyrazine (El-Ghorab et al. 2013). The volatile profile change in minimally processed green pepper initiated in the early stages of storage, which was evident by a reduction in monoterpenoids and simultaneous increase in sesquiterpenoids, alcohols, esters, aldehydes and ketones, oxygenated compounds etc. Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and they constitute a very large group of secondary metabolites, and their synthesis is induced by stress factors such as disease, and other tissue injuries etc. Cheng et al. (2007). During the non MA packed storage, sesquiterpenes accumulation was high; indicating the elevated metabolic stress in produce as a result of injury by cutting in aerobic environments. Also oxidative pathway initiated by the lipoxygenase (LOX) enzyme results in aldehydes and alcohols synthesis from C18 polyunsaturated fatty acids in various fruits and vegetables upon tissue disruption (Niinemets et al. 2013). The observed changes in the volatiles in the present study also largely support these findings.

Principal component analysis (PCA) was carried out to determine major volatiles differing in freshly cut and stored capsicum pieces at aerobic (non MAP) and optimal modified atmospheres. The PCA can compress data based on similarities and differences, by reducing the number of dimensions without much loss of information and define the number of "principal components". In the present case, PC1 and PC2 explained 39 and 26 % of days' variance during storage, accounting for an aggregate of 66 % of variance.

Loading and Scores plots of volatiles based on PC1 and PC2 (Fig. 3a, b) clearly indicated the clustering of three diverse groups of volatiles. Group 1 volatile compounds are mostly present in fresh and MAP- 3 days storage. The group I compounds consisted of *cis* - ocimene, 1,3,8-*para*menthatriene, *trans* 3- caren - 2-ol, bergamotene, 2-hexenal, ethyl 1- decanol, (*E*)-3- hexenol and heptane thiol. Therefore, these may be considered as potential biomarker volatiles of fresh capsicum. The above analysis also explains the role of storage atmosphere in retaining the original flavour and generation of off flavour compounds. Group II volatiles are present in stored non MA packed produce, and this consisted of compounds viz., myrtenal, ferulene, γ- selinene, β-selinene, linalool, 1-(2-*tert*-butylcyclopropyl)-2,2-dimethyl-1-propanone, valencene, 2,3- dimethyl pyrazine and ethyl furan. This shows the high abundance of sesquiterpenoids and furan compounds in non MA packed samples stored for 8–10 days, beyond the marketable period.

Group III volatiles formed a distinct cluster mainly found in MA packed samples stored for the marketable period, i.e., 10 days; and are most distantly placed from Group I. The group is composed of compounds viz., *cis*-6-nonenal, 3,3,6 -trimethyl - 4,5-heptadien-2-one, 4,5-dimethyl-3H-1,2-dithiole-3-thione, norbornanaone, α-campholenal, 2-(3′-methyl-2′-butenyl)-3-methylfuran, and pinene. This group is a representative spoilage markers of fresh - cut capsicum stored at modified atmospheres with high CO_2 and low O_2 atmospheres. It is clear that these mainly belong to carbonyl groups, the compounds mainly synthesized in anaerobic environment by various plants and microbes (Lopez-Galvez et al. 1997). Also, the study showed that 2-isobutyl-3-methoxy pyrazine, an important aroma finger print compound of bell pepper mentioned in several studies, can not be used as a biomarker volatile for freshness of minimally processed green bell pepper, as it did not cluster with above mentioned groups.

Little information is known about the relationship between the outgrowth of spoilage microorganisms, their production of metabolites, including volatiles, and the perception of the decay of minimally processed vegetables by consumers (Jacxsens et al. 2003). The present studies suggest that Group II compounds would have been generated due to the activity of the plant enzymes and primarily by bacteria such as pseudomonads and pectinolysers. Coliforms and pseudomonads are known to be pectinolytic, and accounted for over 65 % of bacterial rot of fruits and vegetables in storage and transit (Wakil and Oyinlola 2011). Microorganisms have been reported to synthesize high levels of volatiles on fresh or spoiled vegetables (Ibrahim et al. 2011). In the present study, it is hard to attribute the volatile emission to a single factor, as diverse group of enzymes exists in plant and microbes for such bioconversions. Apart from the contribution by plant enzymes, lactic acid bacteria also would have a major role in the production of fermentative metabolites of group III compounds.

Table 5 Relative abundance of head space volatile compounds in minimally processed green bell pepper during storage at 8 °C

Name of the compound	Relative area percentage								
	Fresh	MA packed				Non MA packed			
		3 days	5 days	8 days	10 days	3 days	5 days	8 days	10 days
Hydrocarbons									
1,2,3-Trimethylbenzene	0.067	0.134	0.265	0.313	0.566	0.141	0.247	0.290	0.383
1,2,3,4-Tetramethylbenzene	0.127	0.205	0.351	0.315	0.309	0.113	0.172	0.218	0.297
2-(1-Methyl-2-propenyl) icycle[2.2.1]heptane	0.023	0.029	0.058	0.047	0.057	0.435	0.188	0.140	0.174
(+)-Nootkatane	0.000	0.000	0.000	0.000	0.000	0.031	0.044	0.121	0.334
1,3,5,5-Tetramethyl-1,3-cyclohexadiene	1.553	1.366	1.046	0.699	0.782	1.407	0.725	0.831	0.095
Total hydrocarbons	1.760	1.728	1.720	1.374	1.714	2.117	1.376	1.600	1.293
Monoterpenoids									
α-Pinene	2.165	2.700	2.600	3.176	3.517	1.564	2.867	2.704	2.022
cis-Ocimene	60.265	37.956	40.291	27.965	17.449	39.254	37.155	29.149	10.158
3-Carene	0.213	0.156	0.154	0.132	0.185	0.169	0.170	0.136	0.067
E,E-2,6-Dimethyl-1,3,5,7-octatetraene	0.371	0.392	0.240	0.155	0.181	0.323	0.221	0.107	0.085
Allo-Ocimene	1.025	0.918	0.766	0.881	0.907	1.278	0.700	0.772	0.622
1,3,8-para-menthatriene	2.007	2.703	0.971	0.875	1.013	2.898	0.804	0.898	0.411
Total monoterpenoids	66.046	44.825	45.022	33.184	23.252	45.486	41.917	33.766	13.365
Sesquiterpenoids									
α-Copaene	0.156	0.409	0.343	0.528	0.541	0.294	0.325	0.213	0.638
β-Elemene	0.017	0.031	0.032	0.102	0.071	0.174	0.163	0.556	0.364
β-Caryophyllene	0.026	0.056	0.028	0.040	0.036	0.133	0.059	0.094	0.160
γ-Gurjunene	0.000	0.000	0.000	0.000	0.000	0.042	0.040	0.087	0.293
Bergamotene	0.387	0.702	0.218	0.205	0.266	0.694	0.281	0.128	0.213
Valencene	0.017	0.104	0.016	0.025	0.028	0.141	0.032	0.081	0.415
α-Muurolene	0.030	0.072	0.093	0.898	0.390	0.030	0.023	0.063	0.297
γ-Selinene	0.000	0.000	0.000	0.000	0.000	0.170	0.200	0.439	1.298
α-Selinene	0.004	0.014	0.008	0.109	0.038	0.203	0.255	0.687	0.251
α-Ferulene	0.000	0.000	0.000	0.000	0.000	1.015	0.655	1.403	4.283
Valencene	0.000	0.000	0.005	0.240	0.049	0.327	0.165	0.367	1.619
β-Selinene	0.000	0.000	0.000	0.114	0.086	0.257	0.084	0.231	0.897
Total sesquiterpenoids	0.637	1.388	0.743	2.261	1.505	3.480	2.28	4.35	10.73
Alcohols									
1-Octene-3-ol	0.312	0.289	0.274	0.298	0.301	0.305	0.225	0.219	0.218
Hexanol	1.326	1.029	1.159	1.256	1.022	0.985	1.235	1.331	1.451
(E)-3-Hexenol	0.285	0.279	0.254	0.209	0.212	0.315	0.219	0.225	0.218
(Z)-3-Hexenol	0.189	0.211	0.195	0.201	0.202	0.169	0.179	0.168	0.198
Linalool	0.681	1.306	1.673	0.984	2.258	4.195	2.501	5.041	4.802
trans-3-caren-2-ol	0.572	0.919	0.268	0.215	0.256	1.029	0.221	0.229	0.071
2-Ethyl-1-decanol	0.516	0.721	0.122	0.080	0.150	0.598	0.140	0.157	0.139
Perilla alcohol	0.000	0.000	0.104	0.116	0.611	0.000	0.000	0.000	0.000
Total alcohols	3.881	4.754	4.049	3.359	5.012	7.596	4.720	7.370	7.09
Aldehydes and ketones									
3-Buten-2-one	0.315	0.216	0.189	0.316	0.215	0.352	0.451	0.551	0.485
3-Pentanone	0.251	0.335	0.656	0.452	0.325	0.125	0.268	0.267	0.351
2-methyl pentanal	0.556	0.458	0.569	0.259	0.225	0.458	0.559	0.569	0.505
Hexanal	1.235	1.156	1.005	1.112	0.989	1.253	1.136	1.002	1.012
(Z)-2-pentenal	0.615	0.885	0.895	0.884	1.231	0.954	1.312	1.215	1.319

Table 5 (continued)

Name of the compound	Relative area percentage								
	Fresh	MA packed				Non MA packed			
		3 days	5 days	8 days	10 days	3 days	5 days	8 days	10 days
(Z)-3-Hexenal	1.542	1.361	1.335	1.285	1.215	1.356	1.251	1.224	1.316
(E)-2-Octenal	0.087	0.297	0.124	0.031	0.025	0.101	2.124	0.276	0.863
2-Norbornanone	0.138	0.394	0.262	2.406	1.428	0.000	0.000	0.000	0.000
Myrtenal	0.000	0.000	0.000	0.000	0.000	0.072	0.145	0.286	0.220
β-Cyclocitral	0.060	0.125	0.411	0.486	0.213	0.220	1.162	0.598	0.487
Safranal	0.027	0.166	0.021	0.025	0.036	0.310	0.104	0.044	0.099
1-(2-tert-Butylcyclopropyl)-2,2-dimethyl-1-propanone	0.024	0.034	0.021	0.078	0.046	0.048	0.142	0.071	0.181
5,5-Dimethyl-1,3-dithian-2-one	0.040	0.060	0.075	0.097	0.143	0.032	0.074	0.095	0.113
(E,E)-2,4-Decadienal	0.263	0.695	0.052	0.280	0.464	0.180	0.673	0.224	0.547
(2E,3Z)-2-Ethylidene-6-methyl-3,5-heptadienal	0.005	0.024	0.031	0.070	0.139	0.020	0.088	0.067	0.015
2,4-Decadienal	0.034	0.145	0.000	0.000	0.000	0.011	0.342	0.186	0.174
α-Campholenal	0.025	0.195	0.381	0.224	0.823	0.104	0.213	0.117	0.103
cis-6-Nonenal	0.164	0.695	1.876	1.747	4.695	0.433	0.796	1.478	1.276
3,3,6-Trimethyl-4,5-heptadien-2-one	0.187	0.424	0.819	0.556	0.918	0.408	0.355	0.443	0.571
β-Ionone	0.110	0.296	0.714	0.675	0.263	0.547	1.225	0.961	0.686
α-Sinensal	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.429
Total aldehydes and ketones	5.678	7.961	9.436	10.983	13.393	6.984	12.42	9.674	10.752
Esters									
Methyl salicylate	2.476	3.320	3.586	6.456	6.972	4.924	2.702	5.521	7.925
Methyl hexadecanoate	0.025	0.026	0.097	0.128	0.106	0.098	0.083	0.061	0.269
Z-3-hexenyl tiglate	0.119	0.090	0.031	0.386	0.330	0.181	0.035	0.074	0.119
Total esters	2.62	3.436	3.714	6.970	7.408	5.208	2.820	5.656	8.313
Nitrogenated compounds									
2-Methoxy-3-isobutylpyrazine	4.648	6.447	10.771	10.461	18.354	10.209	10.672	11.241	16.473
2,3-Diethylpyrazine	1.562	1.998	2.451	2.896	3.215	1.451	2.338	2.459	2.889
2,3-Dimethylpyrazine	0.512	0.956	1.321	0.989	1.225	1.268	1.889	1.851	2.101
Indole	0.014	0.121	0.388	0.126	0.151	0.042	0.013	0.005	0.034
Total nitrogenated compounds	6.736	9.522	14.931	14.472	22.945	12.97	14.91	15.56	21.497
Furan compounds									
2-Ethyl furan	1.325	1.625	1.854	1.889	1.956	1.998	2.548	2.649	2.759
2-Pentylfuran	1.257	2.229	3.953	4.400	6.720	1.384	7.841	7.376	6.125
2-[(2Z)-2-Pentenyl]furan	0.116	0.109	0.219	0.338	0.409	0.130	0.965	0.620	0.673
2-(3′-methyl-2′-butenyl)-3-methylfuran	1.292	1.688	2.763	2.937	3.541	1.462	1.517	1.759	2.136
Total furan compunds	3.99	5.651	8.789	9.564	12.625	4.974	12.871	12.404	11.693
Others									
1-Heptanethiol	0.187	0.162	0.026	0.024	0.048	0.132	0.035	0.032	0.118
2-Pentylthiophene	0.202	0.279	0.390	1.183	0.366	0.244	0.358	0.418	0.562
4,5-Dimethyl-3H-1,2-dithiole-3-thione	0.209	0.124	0.485	0.441	0.715	0.143	0.215	0.536	0.217
1,3-Dichlorobenzene	0.175	0.390	0.543	0.384	0.459	0.368	0.294	0.452	0.516
Total other compounds	0.773	0.955	1.444	2.032	1.588	0.887	0.902	1.438	1.413

Conclusion

To summarize, the present study showed that dipping of freshly cut green bell pepper pieces with 2 % calcium propionate followed by surface drying and subsequent packing in Cryovac PD961 film that maintained equilibrium modified atmosphere of 13–14 % O_2 and 7 % CO_2 helped to maintain the visual marketability as judged by sensory analysis, and

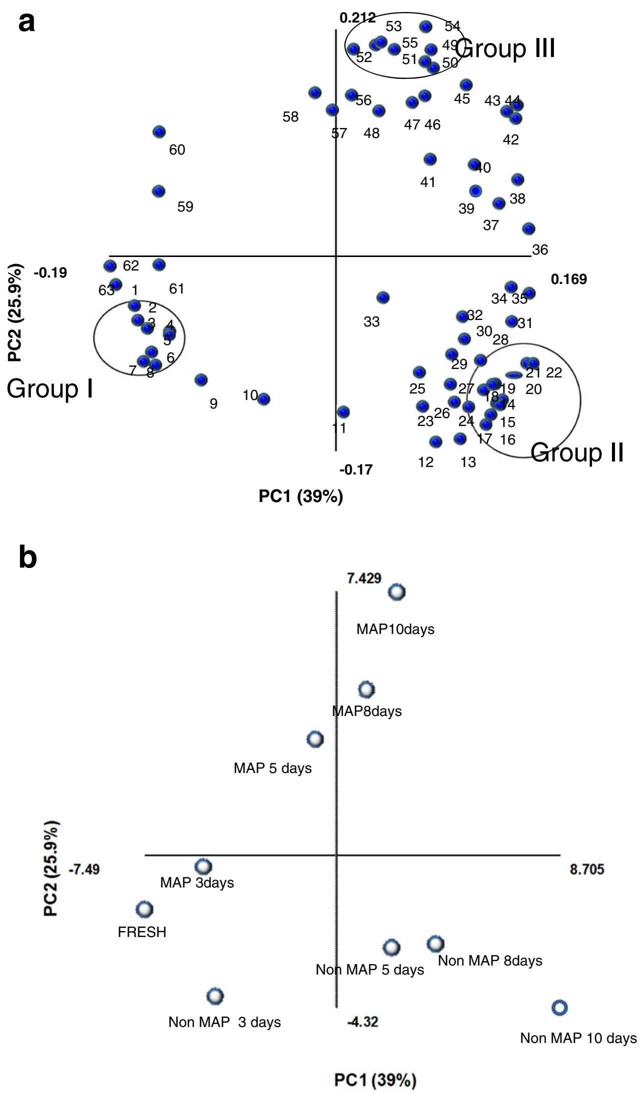

Fig. 3 **a** Loading plot for head space volatiles of minimally processed green bell pepper during storage at 8 °C. ***Group I*** **(1–8):** *cis*- ocimene, 1,3,8-paramenthatriene, Trans 3- caren 2-ol, bergamotene, 2-hexenal, Ethyl 1- decanol, (E)-3- hexenol, heptane thiol. ***Group II*** **(14–22):** Myrtenal, Ferulene, γ- selinene, β- selinene, Linalool, 1-(2-tert-butylcyclopropyl)-2,2-dimethyl-1-propanone, Valencene, 2,3- dimethyl pyrazine, Ethyl furan, ***Group III*** **(49–55):** cis-6-Nonenal, 3,3,6-Trimethyl-4,5-heptadien-2-one, 4,5-Dimethyl-3H-1,2-dithiole-3-thione, Norbornanaone, α-Campholenal, 2-(3′-methyl-2′-butenyl)-3-methylfuran, Pinene. **b** Score plot from minimally processed green bell pepper pieces during storage at 8 °C

vitamin C till 9 days storage at 8 °C. The microbiological quality of the stored produce was at its best till 6 days, and then started deteriorating, as evidenced by a surge in aerobic plate count, pectinolysers and pseudomonads during of storage. Head space volatile analysis of the produce at regular intervals showed a reduction in monoterpenoids and simultaneous increase of aldehydes and ketones, sesquiterpenoids, esters, furans and pyrazines during storage of the produce. Principal component analysis of the head space volatiles identified, *cis* - ocimene, 1,3,8-paramenthatriene, trans- 3- caren 2-ol, bergamotene, 2-hexenal, ethyl - 1- decanol, (*E*)-3- hexenol and heptane thiol as the markers of freshness in minimally processed green bell pepper.

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