Postharvest Biology and Technology 140 (2018) 85–92

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Applicability of a colorimetric indicator label for monitoring freshness of fresh-cut green bell pepper

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ARTICLE INFO

Keywords:
Intelligent packaging
Color change
pH dye
Food spoilage indicator
Fresh-cut green bell pepper

ABSTRACT

Freshness is one of the main considerations for consumption and storage of fresh-cut products. In this study, a freshness indicator label of packaged fresh-cut green bell pepper has been constructed based on pH-sensitive indicators. Compared to indicator labels made by bromothymol blue alone, indicator label made by mixing methyl red and bromothymol blue solutions (at 3:2 proportion) with a concentration of 70 mL L^{-1} in indicator film solution (MB2 formula) could more clearly monitor pepper decay, where indicator label of MB2 type changed from yellow-green to orange. The label accurately responded to the pepper freshness by significant color change, due to the increased carbon dioxide concentrations in the package as a result of deterioration of pepper at chill temperature. Similarly the other parameters, such as aerobic plate count, weight loss, chlorophyll content, malondialdehyde content, membrane permeability and sensory scores, were also evaluated. The levels of these parameters reached the threshold of spoilage at days 7 at 7 ± 1 °C. Thus, the results showed that label made with a mixture of methyl red and bromothymol blue can be applied as an easy-to-use and promising indicator for freshness monitoring of packaged fresh-cut green bell pepper.

1. Introduction

In recent years, the market of fresh-cut fruit and vegetables has shown a significant growth, largely stimulated by increasing consumer demand for convenient, fresh, and healthy foods. Green bell pepper is a popular vegetable that is produced throughout the world for fresh market consumption (Singh et al., 2014). Fresh-cut fruit and vegetables are highly perishable and need appropriate handling and storage condition to maintain quality and extend shelf-life. One of the major issues of fresh-cut products is the potential microbial spoilage (Gorni et al., 2015). Traditional quality criteria based on microbiology (Alexopoulos et al., 2013), chemistry (Chen et al., 2016), physics (Benítez et al., 2012) and sensory evaluation (Albertini et al., 2016) are time-consuming and lengthy procedures. Thus, it is necessary to mention the increasing importance of real-time monitoring the spoilage or freshness of fresh-cut products, which can conveniently evaluate quality deterioration of fresh-cut products and ensure their safety during the storage and retail.

Intelligent packaging can communicate with the consumer or food manufacturer and provide early warning to the user by sensing the internal or external environment of the package (Ghaani et al., 2016). As one type of intelligent packagings, freshness indicator is a small device that is printed on the packaging film or in the form of a package label. A typical type of freshness indicators can indicate the spoilage or freshness of packaged goods via color change detected directly using the naked eye. It is based on comprehensive knowledge of quality-related metabolites closely associated with the type of product, microbial growth, packaging material and storage condition (Fang et al., 2017).

Color based pH-sensitive indicators have been reported to be promising intelligent packaging for the determination of the microbial metabolites for freshness of packaged fish (Pacquit et al., 2007; Pacquit et al., 2006), shrimp (Kuswandi et al., 2011), broiler chicken cut (Kuswandi et al., 2014) and beef (Kuswandi and Nurfawaidi, 2017). These indicators are bromocresol green, natural dye of curcumin, methyl red, and bromocresol purple, which shift from acidic form to basic form because of the pH increase inside package headspace caused by volatile amines produced during meat and aquatic products spoilage. The works using similar principle have also been reported by Nopwinyuwong et al. (2010), where a mixed pH dye-based indicator has been proposed for real-time detection of intermediate-moisture

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https://doi.org/10.1016/j.postharvbio.2018.02.011
Received 22 November 2017; Received in revised form 21 February 2018; Accepted 23 February 2018
0925-5214/

dessert spoilage, which shifts from basic form to acidic form as the pH decrease due to increased carbon dioxide (CO_2) during the spoilage. However, no prior research report has developed a freshness indicator for real-time detection of fresh-cut fruit and vegetables.

Considering both respiration and microbial spoilage of fresh-cut fruit and vegetables that may cause increased CO_2 in the headspace composition of package during the storage, the aim of this work was to design, apply and evaluate the effectiveness of a pH-sensitive indicator as an on-package indicator label for real-time monitoring freshness of fresh-cut green bell pepper (capsicum) in a chill temperature condition.

2. Materials and methods

2.1. Vegetable preparation

Green bell peppers (*Capsicum annuum* var. *grossum*) were purchased in located market, Wuxi, China. Peppers without damage and defects were washed, cut in pieces of 2.2 cm × 0.3 cm ± 8.0 cm × 0.5ncm, dipped in 0.1 mL L^{-1} sodium hypochlorite solution for 5 min, washed again with water, and weighted within 150 ± 10 g. Then they were packaged in circular polyethylene terephthalate (PET) trays and capped (15.3 cm × 5.3 cm). Eighty trays containing samples were stored at 7 ± 1 °C.

2.2. Indicator label fabrication

The indicators were formulated specifically with potential for monitoring the freshness of fresh-cut green bell pepper. The selection of dyes (bromothymol blue (BB) and methyl red (MR)) was based on previously reported by various researchers (Nopwinyuwong et al., 2010; Rukchon et al., 2014) and our experience in designing freshness indicators. Two groups of pH-sensitive dyes were prepared for indicator solutions. One was 1 g L^{-1} BB in solvent of ethanol (B formula) and the other was a mixture of 1 g L^{-1} MR and 1 g L^{-1} BB in solvent of ethanol in a ratio of 3:2 (MB formula). The final indicator label materials were made by mixing a suspension of 30 g L^{-1} methylcellulose (as a binder), 10 g L^{-1} polyethylene glycol-6000 (as a plasticizer), distilled water and indicator solution. The indicator solution for each formula, including (1) B1 formula (50 mL L^{-1} B formula in mixture), (2) B2 formula (70 mL L^{-1} B formula in mixture), (3) MB1 formula (50 mL L^{-1} MB formula in mixture), and (4) MB2 formula (70 mL L^{-1} MB formula in mixture). Then, the mixtures were adjusted for pH at 7.60 ± 0.05 for B1 and B2 formulas and adjusted for pH at 7.10 ± 0.05 for MB1 and MB2 formulas, and stirred for 2 h. Then, the solvent was removed using hot air drying at 40 °C for 6–10 h resulting in an indicator film. This film was cut into 1 cm × 1 cm. Lastly, the indicator film and filter paper (as background) were wrapped with the food-grade polyethylene (PE) cling wrap film (water vapor permeability of 150 g m^{-2} 24 h^{-1}).

2.3. Determinations of physicochemical indices

Samples were analyzed for traditional physicochemical indices of green bell pepper samples during 9 d-storage periods. Eight repeat trays with samples were prepared for each sampling day.

2.3.1. In-package CO_2 concentrations

Concentrations of CO_2 were measured by using a gas detector (RION Technology Co., Shanghai, China). Results were expressed as % (v/v).

2.3.2. Aerobic plate counts

Microbiological examination was determined by aerobic plate counts according to National Standard of the People's Republic of China GB 4789.2. (2010). The data were recorded as colony-forming units (CFU) and expressed as $\log_{10}$ CFU g^{-1}.

2.3.3. Sensory evaluation

A panel of ten trained judges was used for sensory test of the bell pepper according to the method of Meng et al. (2012). Panelists evaluated the samples on a 9-point scale (9 = excellent and fresh appearance, 7 = good quality, 5 = fair quality (limit of marketability), 3 = not saleable condition, and 1 = extremely poor quality).

2.3.4. Weight loss

Weight loss during the storage period was measured by a laboratory level weighting scale (Precision Balance XPE303S, Mettler-Toledo International Inc., Switzerland) and expressed on a wet weight basis (g kg^{-1}) by the difference in initial and final weights of the sample (Singh et al., 2014).

2.3.5. Chlorophyll content

Determination of chlorophyll content was adapted from the method of Sgroppo and Pereyra (2009) and the absorbance was determined using spectrophotometer (UV2600, TECHCOMP, China) at 645 nm and 663 nm. Results were expressed as g kg^{-1}.

2.3.6. Malondialdehyde (MDA) content

The MDA content was detected based on the method of Hodges et al. (1999) and the absorbance was measured using spectrophotometer (UV2600, TECHCOMP, China) at 450 nm, 532 nm and 600 nm. Results were expressed as μmol kg^{-1}.

2.3.7. Membrane permeability

Membrane permeability of the bell pepper was measured by electric conductivity value using a conductivity meter (DS-11AT, Shanghai Precision Science Instrument Co., Ltd, China) according to the method of Meng et al. (2012). Results were expressed as %.

2.4. Color changes of indicator label responded to different CO_2 concentrations

Color changes of indicator labels as a response to different CO_2 concentrations were investigated by enclosing indicator labels in gas-tight vials (50 mL). CO_2 was mixed with nitrogen (N_2) and injected into the vials with a gas-tight syringe, obtaining CO_2 concentrations of 0–5% (v/v). Images of the indicator labels were captured by a camera in a color controller light box (T60(5), TILO Colorcontroller Co., Ltd, China) with constant lighting conditions. Colorimetric data of indicator labels were extracted from the photos using MATLAB R2016a (MathWorks Inc., USA) to isolate CIELab (L^*, a^*, b^* values) coordinates. The total color difference (ΔE) of indicator label was calculated from the differences of L^* (lightness), a^* (deviation towards green to red), and b^* (deviation towards blue to yellow) using the following equation (Ohta, 1977):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

L_0^*, a_0^*, and b_0^* values represented the L^*, a^*, and b^* values of the initial samples with CO_2concentrations of 0% (v/v).

2.5. Color changes of indicator labels for spoilage trial of fresh-cut green bell pepper

Indicator labels were stuck inside the cap of PET trays without directly contacting the peppers stored at 7 ± 1 °C. Colorimetric data of indicator labels were extracted from the photos using MATLAB R2016a to isolate RGB (red, green and blue values) and CIELab (L^*, a^*, b^* values) coordinates. L_0^*, a_0^*, and b_0^* values represented the L^*, a^*, and b^* values of the initial samples on day 0.