Application of chlorine dioxide microcapsule sustained-release antibacterial films for preservation of mangos

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Abstract In this study, fresh mangos were packed using a custom-made antimicrobial film coated with sustained-release chlorine dioxide microcapsules. We then compared physical and chemical indexes, such as weight loss rate, firmness, chromatic aberrations, soluble solids, vitamin C, titratable acid, and other nutritional indicators, to examine changes in the mango and film during storage. Our findings revealed that control mango showed loss of edible value and commercial value after 21 days of storage, and the chlorine dioxide microcapsule antibacterial film group still retains food value and commercial value. Cross-sectional scanning electron microscopy images of the used film showed that the polylactic acid film was smooth and flat, whereas cross-sections of the antimicrobial film showed that the film was covered with voids due to deliberate release of chlorine dioxide gas during the packaging process. Thus, the antibacterial film exhibited erosion and degradation. These findings provided important insights into the use of antimicrobial films for the packaging of fruits during storage, which is essential for promoting the application of solid chlorine dioxide antimicrobial film in packaging preservation.

Keywords Chlorine dioxide · Microcapsule · Antibacterial film · Mango · Preservation

Introduction

Mangos, which grow in both tropical and subtropical regions of the world, are full of dietary fiber and vitamin C. However, mangos are also a climacteric fruit; after picking, they exhibit yellowing and softening due to various metabolic processes. Additionally, mangos are susceptible to microbial invasion and cold storage, and therefore, chilling of mangos can lead to spoilage. These factors affect the edible value and market value of these fruits; accordingly, novel methods for safe mango preservation are needed.

Owing to its strong oxidizing capacity and reactivity, chlorine dioxide (ClO_2) has been identified as an A1-class high-efficiency antibacterial agent by the World Health Organization. Thus, ClO_2 is widely used as a food preservative, including during food processing sterilization and food preservation. Processed foods prepared using ClO_2 maintain the original flavor and appearance (Gião et al. 2013). ClO_2 is also a nontoxic bactericide and can be used to sterilize, disinfect, and preserve fruits, vegetables, and meats directly, usually requiring low concentrations. During fresh food storage and transportation, the metabolic processes occurring in fruit and vegetables, such as protein metabolism, result in oxidization and decomposition into ethylene, carbon dioxide, and other degradation products. Ethylene affects ripening and promotes the aging of fruit and vegetables, whereas ClO_2 prevents the metabolism of proteins and other substances (Du et al. 2003). Various organizations, including the United States Environmental Protection Agency, the Food and Drug Administration, and the Departments of Agriculture in the United States, Western Europe, Canada, Japan, and other countries and regions, have approved and recommended the use of ClO_2 in foods (Guo et al. 2014), food processing (Tomás-Callejas et al. 2012), drinking water disinfection (Al-

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Otoun et al. 2016; Van Haute et al. 2017), and food preservation (Arango et al. 2016). Around 2006, China also began to promote the application of ClO_2 products. On June 1, 2006, the national standard of the People's Republic of China was established as a stable ClO_2 solution (Singh et al. 2008). The Ministry of Health of China has also approved ClO_2 as a disinfectant and a new food additive (Shao et al. 2018; Diab et al. 2001).

ClO_2 gas has unique advantages in reducing foodborne diseases, reducing microbial spoilage, preserving food freshness, and maintaining the nutritional quality of food. Sun et al. studied the effects of ClO_2 on the safety of tomatoes and the antibacterial activity of ClO_2 on blueberries (Sun et al. 2014, 2017). Additionally, Wang et al. considered the effects of controlled-release ClO_2 on the freshness of strawberries. ClO_2 treatment was found to promote stomatal closure, decrease weight loss rate, and slow down softening and decay (Wang et al. 2014). Jiang et al. (2015) found that ClO_2 also reduced the respiratory rate of green peas, thereby inhibiting the production of ethylene, postponing fruit senescence, and improving shelf-life. Chen et al. found that ClO_2 treatment effectively delayed the loss of food nutrients; they showed that flavonoid and vitamin C contents in mulberry fruits were lower than those in control fruits during early storage following ClO_2 treatment. Moreover, ClO_2 treatment has been shown to slow the loss of flavonoids and vitamin C if stored for an extended period of time (Chen 2015; Chen et al. 2011).

Thus, although ClO_2 has been shown to act as a strong preservative for fruits, the application of ClO_2 in mango preservation has rarely been reported. In this paper, a custom-made antimicrobial film coated with sustained-release ClO_2 microcapsules was used to wrap fresh mangos, and physiochemical indexes were examined to determine changes in the mangos and film during storage.

Materials and methods

Materials and reagents

The following materials and reagents were used in this study: fresh mangos, green mangos (Tian Yang County, Baise City, Guangxi Province, China), oxalic acid (analytical grade; Tianjin Beichen Founder Reagent Factory; Tianjin, China), 2,6-dichloro-indophenol (analytical grade; Tianjin Guangfu Fine Chemical Research Institute), ascorbic acid (Tianjin Bodie Chemical Co., Ltd.), sodium hydroxide (analytical pure; USA Aladdin Industrial Company), phenolphthalein indicator (Tianjin Zhiyuan Chemical Reagent Co., Ltd.), kaolin (Tianjin Institute of Fine Chemical Recovery), anhydrous ethanol (analytical grade;

Tianjin Zhiyuan Chemical Reagent Co., Ltd.), polylactic acid (PLA) film (custom-made; oxygen transmission rate: $3500 \text{ cm}^3/\text{m}^2/24 \text{ h}$; water vapor transmission rate: $31.32 \text{ g}/\text{m}^2/24 \text{ h}$), ClO_2 microcapsule slow-release antimicrobial film (homemade; oxygen transmission rate: $2800 \text{ cm}^3/\text{m}^2/24 \text{ h}$; water vapor transmission rate: $20.00 \text{ g}/\text{m}^2/24 \text{ h}$).

Instruments and equipment

The following instruments and equipment were used in this study: spectrophotometer (CM-3600d; Japan Konica Minolta Company), fruit firmness meter (GY-1 type; Zhejiang Top Instrument Co., Ltd.), electronic balance (PL601-L; METTLER TOLEDO Instrument Co., Ltd.), digital Abbe refractometer (WYA-2S; Shanghai Shen Guang Instrument Co., Ltd.), plastic film sealing machine (SF-300; Wenzhou City Industrial Machinery Co., Ltd.), electric hot water bath (HHS-type; Shanghai Boxun Industrial Co., Ltd.), artificial climate chamber (CLIMACELL404; Germany MMM Company), clean bench (SW-CJ-2F; Suzhou Antai Air Technology Co., Ltd.), refrigerators (HYC-260; Haier Group), juicers (JYL-C020E; Nine Yang Co., Ltd.), and scanning electron microscope (Feiner Phenom; Philips Electron Microscope Technology).

Preservation of mangos

Preparation of ClO_2 microcapsule sustained-release antibacterial films

ClO_2 microcapsules were prepared in the presence of a wall material PLA concentration of 40 g/L, gelatin concentration of 20 g/L, and internal water phase of 1:2 compared with the oil and with a stirring speed of 1200 rpm. The embedding rate of ClO_2 microcapsules was 37.04%. The prepared microcapsules were added to the film at a ratio of 20% by mass of the PLA and the activator tartaric acid and formed into ClO_2 microcapsule sustained-release antibacterial films using the solvent casting method. Details were reported previously (Huang et al. 2018).

Preservation of mangos

Mangos were selected to be uniform in size, free from pests and diseases, without mechanical damage, and roughly the same color and maturity. The fruits were trimmed to leave about 1–2 cm of stalk. We first cleaned the mangos again with tap water, rinsed with deionized water, placed on a sterile bench to blow dry, and subjected to ultraviolet (UV) disinfection for 5 min. The prepared PLA original film and ClO_2 microcapsule antibacterial film were placed on a sterile surface and subjected to sterilization with UV for 5 min.

The samples were divided into three groups ($n = 2$ per sample): ClO_2 microcapsule antibacterial film group, PLA original film group, and blank control group. The mangos in the blank control group were placed directly in an environment with a temperature of 25°C and a humidity of 50% without any other treatment. Samples were placed in a climatic chamber at a temperature of 25°C and a relative humidity of 50%. On days 0, 3, 6, 9, 12, 15, 18, and 21, samples were removed to measure weight loss rates, color, firmness, soluble solids, vitamin C, titratable acids, and other indicators. Packaging film was then used for cross-sectional scanning electron microscopy (SEM) after 21 days. A schematic diagram of mango preservation is shown in Fig. 1.

Index tests

Weight loss rate

Using the direct weighing method, before samples were packaged, samples were weighed using an electronic balance. Before each sampling, the samples were weighed. Each operation was repeated three times, and the results were averaged. The weight loss rate was calculated as follows:

$$R_w(\%) = 100 \times (W_i - W_0)/W_0$$

where R_w is the mango weight loss rate (%), W_i is the weight of the mango on day i , and W_0 is the weight of the mango on day 0.

Firmness

According to a previously described approach (Jongsri et al. 2016), the mangos were peeled, and a fruit firmness meter was used to measure the firmness of the fruit by penetrating the instrument to a depth of 1 cm at three different locations in the fruit (proximal, distal, and

middle). Each group of samples was measured three times, and the results were averaged.

Chromatic aberration

A spectrophotometer was used to measure the color of mango pulp. Each group was measured three times, and the results were averaged. Whiteboard and blackboard calibrations were performed before each measurement. The chromatic aberration was calculated as follows:

$$\Delta E_{ab} = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

where ΔE is the chromatism value of mango pulp; L is the lightness index ($L = 0$ is used to represent black, $L = 100$ is used to represent white), a represents the red/green value (the value of $+a$ is the red direction, the value of $-a$ is the green direction), b represents the yellow/blue value ($+b$ is the yellow direction, $-b$ is the blue direction), L_0 is the lightness on day 0, L_i is the lightness on day i , a_0 is the red/green value on day 0, a_i is the red/green value on day i , b_0 is the yellow/blue value on day 0, and b_i is the yellow/blue value on day 0.

Soluble solids

According to a previously described method (Jongsri et al. 2016), soluble solids were measured using an Abbe refractometer. First, 25 g of the edible part of the sample was measured and mashed in a juicer. Juice was then extruded using gauze, and Abbe refractometer data were recorded. Each group of samples was measured three times, and the results were averaged.

Vitamin C

According to a previously described method (Cissé et al. 2015), 25 g of the edible part of the sample was measured and mashed in a juicer. Next, 200 mL extractant was

Fig. 1 Schematic diagram of mango preservation

added, and the sample was quickly pounded into a homogenate. The homogenate and extractant were then added into a 200-mL volumetric flask and shaken. The filtrate plus kaolin decolorizer were mixed and filtered. Next, 10 mL of the filtrate was titrated with a solution of 2,6-dichloroindophenol. Each group of samples was measured three times, the results were averaged. Vitamin C content was calculated using the following formula:

$$\text{Vitamin C (mg/100 g)} = \frac{(V - V_0) \cdot T \cdot A}{W} \times 100$$

where V is the titration sample consumption 2,6-dichloroindophenol solution volume (mL), T is 2,6-dichloroindophenol solution titer (mg/mL), A is the dilution factor, and W is the sample weight (g).

Titrateable acid

As previously described (Yuan Hui et al. 2017), take 25 g of mango pulp into the juicer, add 250 mL of distilled water, and knead into a homogenate. Transfer the homogenate into a 250 mL volumetric flask, place the volumetric flask on a 75–80 °C water bath for 30 min, then cool to room temperature, dilute to the mark, shake and filter. Take 50 mL of the filtrate, add 5–10 drops of phenolphthalein indicator, and titrate with 0.1 mol/L sodium hydroxide standard solution. Each group of samples was measured three times, and the results were averaged. Titrateable acid content was calculated according to the following formula:

$$\text{Acid content (\%)} = \frac{V \times N \times K \times B}{b \times A} \times 100$$

where V is the volume of NaOH solution consumed by titration of the filtrate (mL), N is NaOH solution concentration (M), K is the conversion factor (0.064) calculated by citric acid, B is the sample volume (mL), b is the filtrate volume used for titration (mL), and A is the sample weight (g).

Section analysis

The PLA film and antibacterial film wrapped in mangos for 3 weeks were cut in liquid nitrogen, and cross-sections of the films were attached to the sample stage, sputter coated with gold for 60 s, and observed by SEM (Feiner Phenom, Philips Electron Microscope Technology; 500 ×, 10 kV).

Statistical analysis

All experiments were repeated three times in triplicate. Data were analyzed using IBM SPSS Statistics 19. All data points were expressed as means ± standard deviations. Origin 9.1 Professional was used for drawing of experimental graphics.

Results and discussion

Changes in weight loss rate

As storage time increases, the consumption of nutrients and transpiration of water lead to loss of quality (Valero et al. 2013; Krüger et al. 2011); in mangos, such manifestations include wilting, folds in the epidermis, tissue aging, and freshness reduction.

Figure 2a shows weight loss in mangos during storage. All samples suffered weight loss during storage, and the weight loss increased linearly with storage time. The blank control group showed the greatest weight loss. On day 21, the weight loss rate in the blank control group was about twice that of the PLA film group ($P < 0.05$), and the weight loss of the PLA film group was about 1.5 times that of the antimicrobial film group ($P > 0.05$). Moreover, the weight loss rate of mangos coated with the antibacterial film was obviously lower than that of the blank control because the film reduced the transpiration of water and blocked the loss of water vapor. The weight loss rate in the antibacterial film group was significantly lower than that in the PLA group; this may be due to release of ClO_2 from the antimicrobial film, which could have some inhibitory effects and reduce the intensity of respiration (Wang et al. 2014), combined with the gas barrier properties of the antibacterial film.

Changes in firmness

Firmness is an important factor affecting the value of fruits and vegetables (Leiva-Valenzuela et al. 2013). As storage time increase, mango pectin substances produced by various enzymes and starches produced by enzyme hydrolysis are altered, the cell wall composition changes, and the number of cells between adhesions decreases, resulting in decreased firmness of mangos (Atkinson et al. 2012).

Figure 2b shows changes in the firmness of mangos during storage. The firmness of all samples tended to decrease during storage. The firmness of the blank control group decreased rapidly after day 6 due to water loss and loss of support in cells. Similarly, the firmness of mangos coated with PLA film also decreased rapidly after day 12. Moisture dramatically affected the firmness of the mangos. Mangos treated with the antimicrobial film showed greater firmness than the other two groups ($P < 0.05$); the firmness value of the antimicrobial film group was 185.71% higher than that of the PLA group and 566.67% higher than the blank control group on day 21. This may be because the antibacterial film barrier was better at blocking the loss of water vapor, and the slow release of ClO_2 gas may play a role in inhibiting enzymes, reducing metabolism, and

Fig. 2 **a** Loss of quality during mango storage; **b** changes in firmness during mango storage; **c** variations in chromaticity values (**b** values) during mango storage; **d** changes in color during mango storage

suppressing pectin production and starch hydrolysis, thereby maintaining the integrity of the cell wall and the firmness of the mangos, similar to the results of a previous study (Tomás-Callejas et al. 2012).

Changes in color

Color is an important indicator of the maturity of mangos and the value of commodities. With increased storage time, chlorophyll is hydrolyzed by enzymes to produce water-soluble substances, such as phytol and chlorophyll; coupled with photo-oxidation, the chlorophyll levels decrease or disappear, along with changes in green color.

Figure 2c reflects changes in *b* values in mangos during storage. As the storage time increased, the *b* values of mangos increased, and the color of mango pulp changed to yellow. In the blank control group, the *b* value of mangos increased, and the color of flesh gradually turned yellow, reaching the maximum value on day 15; these data indicated that the mangos were completely mature. Before day 12, the *b* value did not change substantially in the PLA film

and antimicrobial film groups. However, after day 12, the *b* value increased rapidly. On day 21, the *b* value of mangos in the antimicrobial film group was 31.25% lower than that in the blank control and PLA film groups ($P < 0.05$), effectively delaying the time of mango yellowing. This may be the PLA film created a low O_2 condition on the surface of the mango, inhibiting the oxidative decomposition of chlorophyll. The antimicrobial properties of ClO_2 may also inhibit the activity of chlorophyllase, resulting in less changes in the *b* value of mango in the antimicrobial film.

Figure 2d shows color changes in mangos during storage. In general, the color differences in mangos tended to increase gradually during storage. The color of the blank control group changed greatly during storage. From days 6 to 15, the color difference value increased rapidly, and the changes were obvious. On days 15–21, the color difference value reaches a maximum value and remained basically unchanged. This is because the mangos gradually matured as the storage time increased, and the flesh turned yellow. In the PLA film group, the mango color difference

quickly increased after day 9. On day 21, the color difference value reached a maximum, with the flesh showing yellow decay and the peel having dark spots, indicating loss of commercial value. The color difference in the antibacterial film group was minor, the color was stable during storage, and the color quality was higher. On the day 21, the color differences in the antibacterial film group were 72.26% and 72.65% lower than those of the PLA group and blank control group, respectively ($P < 0.05$). The slow release of ClO_2 gas from the antimicrobial film resulted in reduced enzyme activity, protected the chlorophyll from hydrolysis and oxidation, and maintained the color of the mangos.

Figure 3 shows changes in the color of the peel and pulp after 21 days of storage. On day 21, the mango peel in the blank control group had turned yellow, and many dark spots appeared on the surface of the peel, indicating loss of commercial value due to spoilage of the mangos. Moreover, the pulp inside the mangos had also turned from white to yellow or black, indicating loss of edible value due to microorganism growth. In the PLA film group, mango peels had also turned yellow, with some black spots. Most of the flesh had turned yellow. However, mangos in the ClO_2 microcapsule antibacterial film group showed more green skin, darker skin, flesh-colored pulp, and slight yellowing of some flesh, indicating that these mangos had retained commercial and consumption value.

Soluble solids

Soluble solids refer to all compounds dissolved in water, including sugar, acids, vitamins, and minerals. In fruits and vegetables, soluble solids and sugar contents are proportional to fruit and vegetable weight as an important indicator of quality. The content of soluble solids has a direct impact on the taste of mango. During the storage period, as metabolism progresses, mangos become larger, and the content of soluble solids increases.

Figure 4a shows changes in soluble solids contents during storage. The overall contents of soluble solids increased, with increases in sugar contents over time. In the blank control group and PLA group, the soluble solids contents sharply increased after day 6 and reached a maximum on day 21. This may be due to the fact that most of the organic acids in the fruit were converted to soluble sugars as the storage time was extended, resulting in increased soluble solids content. The content of soluble solids in the antimicrobial film group remained relatively stable until day 21, at which time content of soluble solids was 28.05% lower than that in the PLA group ($P < 0.05$) and 40.40% lower than that in the blank control group ($P < 0.05$). These results could be explained by the observation that ClO_2 can inhibit acyl-CoA synthetase and acyl-CoA oxidase synthesis, which regulate the production of ethylene, thereby postponing the ripening of mangos and inhibiting the conversion of some organic acids in fruit to soluble sugars (Guo et al. 2014).

Vitamin C

Vitamin C is one of the most important vitamins in human nutrition; lack of this vitamin can cause diseases and affect metabolism. Notably, water-soluble vitamin C is not stable under normal conditions and is easily oxidized by oxygen and metal ions. Vitamin C is also susceptible to temperature, pH, and light. Vitamin C content is high in fresh fruits and vegetables and gradually decreases as the freshness of these foods decreases (Naidu 2003).

As shown in Fig. 4b, as the storage time increased, the vitamin C content of the three sample groups increased first and then decreased. This may be due to the effects of respiration and enzymes leading to the ripening of mangos and resulting in a gradual increase in the content of vitamin C. The decrease in vitamin C content is due to the consumption of vitamin C during breathing. The vitamin C content peaked on days 6 and 9 in the control and PLA

Fig. 3 Changes in pericarp and pulp color after 21 days of storage. **a** Blank control (CK) group; **b** PLA film group; **c** antibacterial film group

Fig. 4 **a** Changes in soluble solids contents during storage; **b** changes in vitamin C contents during storage; **c** changes in titratable acid contents during storage

groups, respectively, but then reached the lowest value on day 21. Vitamin C content changed slowly in mango fruits packed with antibacterial film, reaching 182.30% higher than PLA film on day 21 ($P > 0.05$) and 520.74% higher than the control ($P < 0.05$). Therefore, the antibacterial film had a significant effect on the maintenance of vitamin C content because the release of ClO_2 by the antibacterial film inhibited respiration and ethylene production, thereby reducing the oxidation of vitamin C. These results were consistent with previous studies (Chen 2015; Chen et al. 2011).

Changes in titratable acid contents

Titratable acid content is a significant indicator of mango maturity and is related to mango respiration and metabolism. Titratable acid content increases briefly during the early storage and then decreases because of respiration and metabolism.

As shown in Fig. 4c, titratable acid contents decreased as the storage time increased. Organic acid contents

increased because of the process of respiration. Indeed, as storage time increased, some of the organic acids were converted to soluble sugars, and some were consumed by respiration, resulting in a decrease in the titratable acid content of mango fruit. Titratable acid content peaked at day 12 and constantly decreased in PLA-packaged mango fruit. Moreover, titratable acid content dramatically decreased after day 9 and reached a minimum on day 21 in the control. In the antibacterial film group, titratable acid content also varied, reaching 125% higher than that of the PLA film ($P > 0.05$) and 718.18% higher than that of the control on day 21 ($P < 0.05$). Thus, the antimicrobial film effectively maintained the titratable acid content, possibly by blocking conversion of organic acids to soluble sugars and reduced respiration following ClO_2 release by the antibacterial film.

Cross-sectional SEM images of the films

As shown in Fig. 5, cross-sections of PLA films were relatively smooth, with few gaps. In contrast, the cross-

Fig. 5 Film sections. **A** PLA film; **B** antibacterial film; **a** PLA film after 3 weeks; **b** antimicrobial film after 3 weeks

sections of antimicrobial films were rough and filled with microcapsules covered with voids. A possible explanation for this result may be that the moisture generated by the respiration and transpiration of the mangos gradually diffused and penetrated into the antimicrobial film during storage; the microcapsules and tartaric acid were activated by water and slowly released ClO_2 gas. During this process, erosion and degradation of the antibacterial film occurred, resulting in a gap.

Conclusion

In this study, our results showed that untreated mangos were perishable and suffered significant weight loss and color changes. Moreover, the soluble solids content obviously increased, whereas firmness, vitamin C, and titratable acid contents decreased. Notably, these above indicators were significantly improved in mangos treated with the antimicrobial film. Thus, our ClO_2 microcapsule-coated antibacterial film effectively delayed the decay of mangos and extended the shelf-life of the fruit, indicating

obvious advantages in the preservation of mango nutrition and in maintaining various physical and chemical indicators in mangos. After 21 days of storage, the control had lost its edible and commercial value, whereas mangos treated with ClO_2 microcapsule-coated antibacterial films maintained food and commercial value. Thus, our findings indicated that ClO_2 microcapsule-coated antibacterial films may be effective alternatives to improving the quality of mangos. Furthermore, the ClO_2 microcapsule-coated antibacterial films may have wide applications in the food industry.

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