Influence of Antibacterial Packaging on Shelf Life of Fresh Grape

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Abstract: Grey moulds caused by Botrytis cinerea are the main problem for grape in the storage time. Fungicide treatment is proved to be necessary to retard the growth of mould spores and delay spoilage. One effective fungicide widely used in grape preservation is sulfur dioxide (SO_2). A kind of antimicrobial packaging for controlled release of SO_2 was developed in this paper. This mixed matrix film with three-layer structure can release SO_2 gas into package, and the release amount of SO_2 can be controlled by adjusting inner layer. The effect of the application of this controlled release packaging in Kyoho grape preservation has been studied. The result showed that it can continuously release a lower level (10~40 ppm) of SO_2 for about 50 days. Compared with the control group, this controlled release packaging can well maintain the freshness and firmness of grape, and it also can decrease the content of soluble solids and organic acids. Furthermore, it lowered rotting rate and shattering rate of the experimental grape during storage. It was found after analysis that the better preservation was obtained when the proportion of EVA resin in EVA/LDPE blend ingredients is between 5% ~16%. This active antimicrobial packaging is considered as a effective replacement of traditional SO_2 fumigation method for grape preservation

Keywords: antimicrobial packaging; controlled release; sulfur dioxide (SO_2); Kyoho grape

1. Introduction

Worldwide post harvest fruit and vegetables losses are as high as 30 to 40% and even much higher in some developing countries (especially in China). Reducing post harvest losses is very important in today's population explosion and scarce resources. In China, Kyoho grape is one of the most popular table fruit because it is rich in nutritious and delicious juicy. However, it is a highly perishable, non-climacteric fruit. Significant quality losses may happen during post harvest handling, storage and marketing, e.g., peduncle rot, weight loss, softening of berries, and flesh browning. Grey moulds caused by Botrytis cinerea are considered as the main problem for grape decay (Sanchez-Ballesta MT et al., 2007). Thus, in order to obtain a longer shelf life, fungicide treatment is necessary to retard the growth of mould spores and delay spoilage for grape.

Sulfur dioxide (SO_2) is proved to be one of the most effective fungicides widely used in grape preservation. SO_2 has a strong microbial action, with the SO_2 molecule probably penetrating the fungal cells and reacting with various cell components, although the precise mechanism is unknown. Many experiments have confirmed that SO_2 can control growth of Botrytis mould on grape and inhibit the activity of polyphenol oxidase in grape. Thereby, it maintains the original flavors and nutritive ingredients of grape, and then prolong the shelf life (Dan QIN et al., 2006)

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SO_2 fumigation is a conventional treatment measure for medium and long-term storage of table grapes (Carlos H. Crisosto et al., 2002). However, this method can not accurately control the concentration of SO_2, fade and off-odor of grape frequently occur because of local excess fumigation. The influence of SO_2 dose on residues and control of decay of grape has been well researched (Marois et al., 1986, Smilanick et al., 1990, Smilanick and Henson, 1992, Luvisi et al., 1992). If the dose is too small, the effect of prolonging shelf life will lose. On the contrary, it will injure stored fruits (John M.H., et al., 1988, Juan Pablo Zoffoli, et al., 2008, H. Gao, et al., 2003). Some methods of controlling appropriate SO_2 dose were developed. One method of application of SO_2 is by use of pad packaging technology. One pad containing sodium metabisulphite was placed inside the grape packaging (Soylemezoglu et al., 1994). Another alternative method is the controlled release polymer, which can continuously release SO_2 gas for a relatively long time. Some controlled release packaging with double or multi-layer structure was disclosed (Penny Corrigan, 2004).

One controlled release antimicrobial packaging with three-layer structure was prepared and its application in grape preservation was studied in this paper. This laminated film was developed according to the concept of 'Mixed Matrix Membrane'. Firstly, the outer layer is BOPP film, which is used as the barrier layer because BOPP film is an excellent barrier against polar gas and can prevents SO_2 from penetrating to outside. The generation layer of SO_2, consists of sodium metabisulfite ($Na_2S_2O_5$), calcium sulfite ($CaSO_3$) and polyurethane adhesive, was located in the middle of this composite structure. Sodium metabisulfite and calcium sulfite all can release SO_2 gas when in a humid atmosphere. Finally and most important, the core of this structure is controlled release layer. A series of EVA/LDPE blend film with different gas permeability can control the release SO_2 dose from middle layer into bag at a lower level. Furthermore, some quality changes of the application of this controlled release packaging in Kyoho grape preservation were determined. And good preservation effect of experimental grapes in this antimicrobial packaging compared with general packaging was obtained.

2 Materials and Methods

2.1 Materials and Film Preparation

Biaxial-oriented polypropylene film (BOPP film, 25μm, Guangdong Decro Packaging Films Co.,Ltd.,China) was used as the barrier layer, the WVTR (water vapor transmission rate) of BOPP film is 4.188g/m^2·day·0.1MPa. This film has an excellent barrier properties of polar gas and can prevents SO_2 from penetrating to outside. Low density polyethylene resin (LDPE, LD100-AC, MFI=2.0, China Petroleum & Chemical Corporation) blended with Ethylene vinyl acetate resin (EVA5/0.3, MFI=0.3, Beijing Organic Chemical Plant, China) with different proportion (2%, 5%, 10%, 16% and 25%), LDPE resion without adding EVA resion used as a control. The influence of EVA addition amount in LDPE/EVA blend systems on SO_2 release rate was studied specially. LDPE/EVA blend films were blew by film blowing machine and they constitute the controlled release layer. The gas transmission rate of this blend films increase with the addition of EVA resin. Sodium metabisulfite ($Na_2S_2O_5$) and Calcium sulfite ($CaSO_3$) together formed two-component SO_2 release agents, this mixed agents homogeneous dispersed in Polyurethane adhesive (PU,YH501S, Food-Grade, Beijing Comens New Materials Co.,Ltd.,China). Eventually, the controlled release films with three-layer structure were prepared by dry lamination. At last, the laminated films were placed at ambient temperature for 48 hours, and then they were made into experimental bags in size of 300mm×250mm.

2.2 Grape Preservation Experiment

Grapes (Kyoho grape) used in this preservation experiment were purchased from local fruit market in Beijing. Selected samples must be uniform in color, size, firmness and free of damage and disease. Experimental samples were randomly divided into seven groups and were treated separately as follows:

Seven same treatments were set as the parallel test and two replicates per treatment were conducted in this experiment. These groups of grapes were stored in the refrigerator at 0 ºC. Some quality changes (weight loss rate, shattering rate, decay rate, SSC, firmness, good fruit rate, appearance) of experimental grapes were determined every 10 days (5 days one interval for the last two tests)

Table 1. Seven treat method of experimental samples

Treatment	*packaging*	*EVA（wt%）*
Control group1 (CK1)	naked	—
Control group2 (CK2)	general film	—
Treatment group1 (TG1)	CR film*	0
Treatment group2 (TG2)	CR film	5
Treatment group3 (TG3)	CR film	10
Treatment group4 (TG4)	CR film	16
Treatment group5 (TG5)	CR film	25

*CR film is controlled release film prepared above.

3. Results and Discussion

3.1. Cotrolled Release Rate

Water vapor and organic acids generated by respiration of fruits can penetrate into the middle layer through the high gas permeable inner layer. SO_2 gas can be release when water vapor contact with the two-component SO_2 release agents.

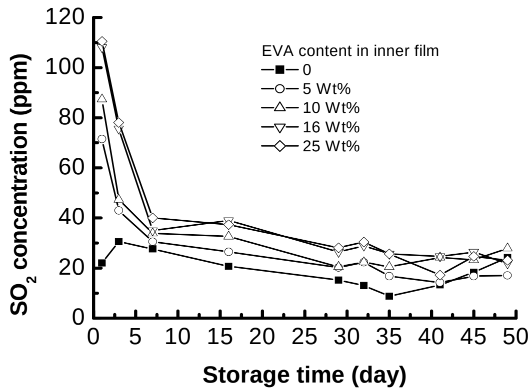

Figure 1. Influence of EVA addition amount in LDPE/EVA blend systems on SO_2 release rate

On the contrary, SO_2 gas enters into the packaging from the release layer. By adjusting addition amount of EVA resion in LDPE/EVA blend systems, different gas permeability of inner blend films can be obtained (Hua Wei, Wencai Xu, et al.,2009). As can be seen from Figure. 1, these antimicrobial packaging can continuously release SO_2 for about 50 days and the amount of SO_2 in packaging can be controlled at a lower level. More detailedly, the SO_2 concentrations are high in the initial 5 days and the release amounts gradually stabilized at around 10~40ppm after 7 days. This concentration range is effective for inhibiting the growth of Botrytis mould on grape. The influence of EVA addition amount in LDPE/EVA blend systems on SO_2 release rate are showed as below. The levels of SO_2 in packaging are associated with the increase of EVA addition amount in LDPE/EVA blend systems.

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3.2. Weight Loss Rate

Weight loss rate reflects the changes of weight of experimental objects during storage, which mainly caused by water loss of fruit tissue.

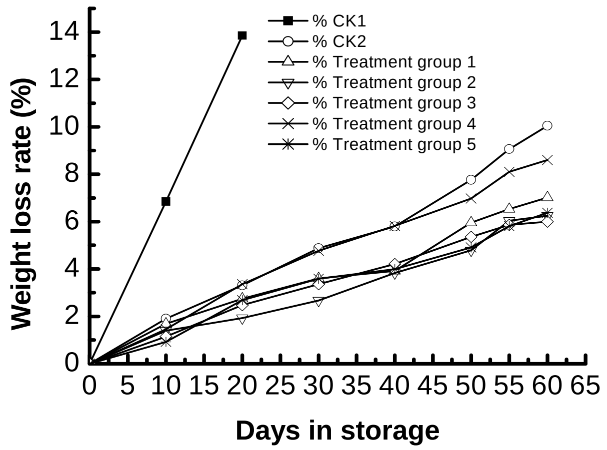

Figure 2. Weight loss rate of experimental grapes during storage.

As can be seen from Figure. 2, weight loss of grape fruits without packaging bags (CK1) is particularly serious and the weight loss rate reach 14% when stored for 20 days. However, the weight loss rates of grapes in packaging bags were less than 10% when stored for 60 days. In particular, the weight loss rates of treatment group 1,2,3,5 were less than 6% when stored for 60 days. Water loss of fruit tissue will lead to changes of fruits in shape, texture, color, luster, flavor, and many other aspects, which can reduce the freshness of the fruit. Thus, these developed controlled release packaging bags can reduce water loss of grapes and maintain freshness of experimental grapes during storage.

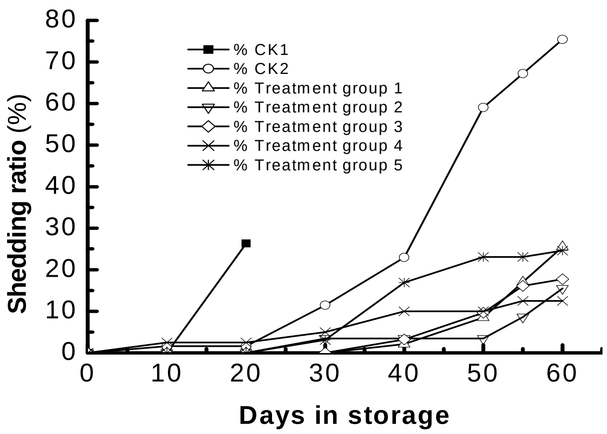

Figure 3. Shattering rate of experimental grapes during storage

3.3. Shattering Rate

The shattering of post harvest grape berry is a common phenomenon during storage, and it seriously affect their commercial value. From Figure 3, as can be seen clearly that the shattering rate of grape berry in CK1 and CK2 is higher. Because the aging of stem caused by water loss of CK1 is particularly serious. After 30 days, the fracture formed at junction between fruit and stem, and then berry drop happened.

The shattering of grape berry in CK2 result from loose panicle and threshing caused by microbial infection and decay of stalks and stems. Grapes stored in controlled release packaging bags have lower shattering rate because these packaging can reduce water loss and inhibit the infection of microbial.

3.4. Decay Rate

As can be seen from Figure.4, decay of grapes stored without SO_2 fungicide is very serious, its decay rate exceeds 35% when stored for 60 days.

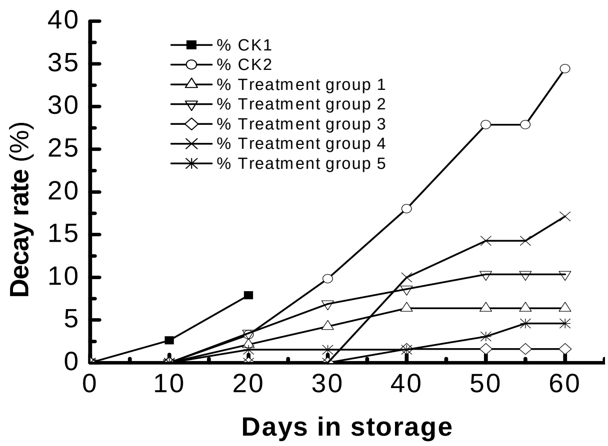

Figure 4. Decay rate of experimental grapes during storage

However, decay rates of grapes stored in controlled release packaging bags are lower than 15%. In identical stored for 60 days, decay rate of treatment group 3 is only 1.6%. Therefore, it can be implied that SO_2 fungicide released from developed antimicrobial packaging bags can inhibit the growth of moulds and other microorganisms on the surface of grapes and prevent grapes from rotting.

3.5. Soluble Solids Content

The effects of different treatments on SSC (Soluble Solids Content) of grapes are shown in Fig.5. The SSC of control groups decreased rapidly in the initial 10 days. The SSC of CK1 increased rapidly and the SSC of CK2 showed a slow downward trend after 10 days. There is small-range fluctuation of SSC of grapes wrapped in antimicrobial packaging bags during the first 10 days, and the SSC declined slowly after stored for 10 days. The levels of SSC in treatment groups show a slower decline compared to CK2.

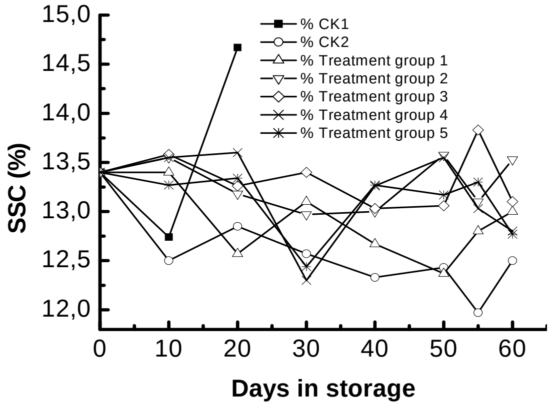

Figure 5. SSC of experimental grapes during storage

The SSC of grapes in treatment groups were higher than CK2 when stored for 60 days because of respiration and water transpiration of fruits. Biochemically, respiration consumes soluble solids, while water transpiration increases SSC. For control groups, respiratory consumption is the main factor causing the decline of SSC in the early storage. During the later storage period, the inspissation generated by transpiration of water is relatively obvious, therefore, SSC of control groups decreased slowly. In particular, water loss of CK1 is very serious and its SSC is significantly increased. Grapes stored in antimicrobial packaging bags can reduce water loss of grapes, meanwhile, SO_2 fungicide released from antimicrobial packaging bags can retard the respiration of fruits and reduce the consumption of soluble solid. Consequently, the SSC of grapes stored in antimicrobial packaging bags remained stable during the whole storage period.

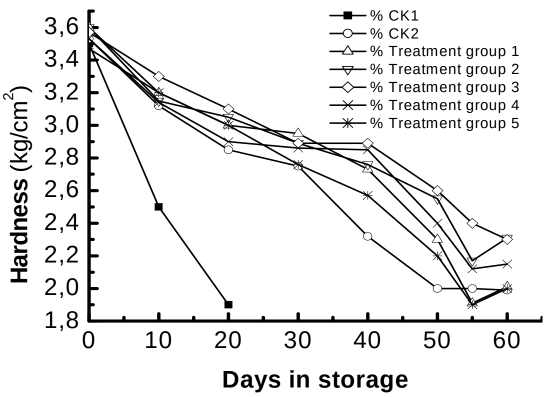

Figure 6. Firmness of experimental grapes during storage

3.6. Firmness of Berry

As you can see in Figure.6, the firmness of grapes show a downward trend over the entire storage period. Firmness of CK1 experienced the sharpest declines, down to about 1.9 kg/cm^2 when storage for 20 days. The firmness of grapes in treatment groups decreased more slowly compared with control group 2 (CK2). Firmness of treatment groups 2, 3 and 4 have the slowest rate of decline, their firmness are still above 2.15kg/cm^2 when stored for 60 days. Nutrient depletion, degradation of pectin substances and water loss are the three main factors causing the decline of grape firmness.

The developed antimicrobial packaging bags, used in the storage of grapes, can effectively lower the respiration rate of grapes, reduce the consumption of nutrients, inhibit the water transpiration of grapes and maintain a high internal turgor pressure.

3.7. Good Fruit Rate

The good fruit rate of control groups and treatment groups were evaluated when stored for 60 days, the results are shown in Figure.7. Grapes stored in antimicrobial packaging bags can still maintain a relatively higher good fruit rate when stored for 60 days, among these treatment groups, treatment group 3 has the highest good fruit rate (82%). However, the good fruit rates of CK1 and CK2 are 0 and 19.6% respectively.

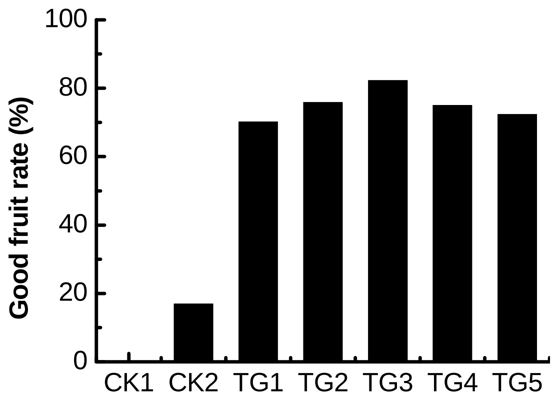

Figure 7. Good fruit rate of experimental grapes during storage

This results show that developed antimicrobial packaging bags can reduce water evaporation of grapes and decrease the occurrence of shattering and decay, and have a remarkable preservation effect.

a. CK1 **b. CK2**

Figure 8. Appearance photos of control groups after 20 days storage

3.8. Appearance Photos

Water loss and grey moulds spot severely presented when CK1 was stored for 20 days. So the observation data of CK1 is only limited to the first 20 days of storage.

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The phenomenon of microbial infection on fruit and stems (shown in Figure.8) appeared when grapes of CK2 stored for 20 days, because that there is no SO_2 fungicide in packaging bag.

As shown in Figure.9, there are significant differences in color, firmness and other sensory quality of grapes stored in antimicrobial packaging bags compared with control groups after stored for 60 days.

CK2 TG1 TG2 TG3 TG4 TG5

Figure 9. Appearances photos of experimental grapes after stored for 60 days

Grapes wrapped in antimicrobial packaging bags have good freshness, basically no fungal infection and decay phenomenon appear. However in CK2, the majority of fruit dropped, the stems were seriously infected by moulds and most of the fruit became softened due to fungal infection and over maturity.

4. Conclusions

SO_2 gas in the range of 20~40ppm can be released continuously for about 50 days in every prepared antimicrobial packaging bags. The preservation effect is better in treatment groups compared with that in the control groups, especially when EVA content in the blend systems is 5%~16%. The good fruit rates of these three groups are over 75%. The water loss of grapes, berry drop and consumption of soluble solids and organic acids were reduced. The firmness of grapes was well maintained and the appearances of grapes were improved during storage.

Respiration, evaporative water loss and microorganism infection are the three main factors influencing the quality of postharvest grapes. There are two advantages for grapes preservation with prepared antimicrobial packaging bags. On the one hand, grapes stored in antimicrobial packaging bags can reduce water loss. On the other hand, SO_2 fungicide released from the middle layer of antimicrobial packaging bags can inhibit the growth of moulds on grapes and reduce respiration rate of grapes by retarding the activity of oxidase in grapes. Therefore, the antimicrobial packaging bags are effective for maintaining the original flavors and nutritive ingredients of grapes and prolonging the shelf life of grapes. In addition, these antimicrobial packaging bags with controlled release SO_2 can also be used for preservation of litchi, longan, actinidia and other fruits. The release rate of SO_2 can be adjusted to suit the requirement by different fruit varieties.

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