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Full Length Research Paper

A new combined treatment and effects on reducing decay in stored honey peaches: A study from a multi-year experiment

Hui LI¹, Hai-hong Wang², Jian-long LI^{*1,2}, Zhi-kuan Cui¹, Yang LI¹, Cheng-Cheng Gang¹

¹College of Life Science, Nanjing University, Nanjing, Jiangsu 210000, China;
²Nanjing Communications Institute of Technology, Nanjing, Jiangsu 211188, China
^{*}Corresponding author Email::<u>jlli2008@nju.edu.cn</u>, lijianlongnju@163.com,
Tel: +86-25-86214644

ABSTRACT

Peach is a popular fruit worldwide because of its nutritional valueand pleasant flavor ,and it is difficult to keep fresh under high temperature and high humidity. The compositional use of multi-materials is a trend for the peach post-harvest preservation. In order to investigate the effect of combined treatment of UV-C+pullulancoating, CaCl₂+pullulancoating and UV-C+ modified atmosphere packaging in ambient temperature, the Fenghuang honey peaches were selected as the experiment materials. Based on one year's preliminary experiments and four years' repeated tests, the effects of different treatments about weight loss, firmness, total soluble solids (TSS), membrane permeability, respiration rate and soluble sugar, malondialdehyde (MDA) content, polyphenol oxidase (PPO) were examined each day during the storage period. After 7 days, the sound fruit rate of the control group decreased to 20%, while the part treated with 2%CaCl₂+ 2%Pullulan coating kept at 80%. The results suggested that the 0.25kJ/m⁻²UV-C+2% Pullulan coating combination treatment was a useful technique for Fenghuang peach preservation. The results suggested that this combination could influence the degradation of pectin molecules, as well as the ability to induce antioxidant systems. Based on the results, the compositionof 0.25kJ/m⁻²UV-C+2%Pullulan coating have a prospect in future commercial production.

Keywords: Fenghuang Honey Peach; UV-C, CaCl_{2;} Pullulan Coating; Modified Atmosphere Packaging; Storage Life; Bio-safety

INTRODUCTION

fenghuang town (jiangsu, china) is one of the most famous growing areas for peaches, a popular fruit in china.

native peaches from fenghuang town are famous for their rich nutrition and suitable ratio of sugar to acid as well as

their strong flavor. however, peaches have a very short shelf life of less than 3 days at ambient temperature due to their rapid metabolism after harvest(akhtaret al., 2010; asgharia and aghdam, 2010). this is due to their higher respiration rate and high susceptibility to chilling injury and fungal decay (robertson et al., 1990). their shelf life is limited by weight loss and physiological disorders, as well as internal browning and changes in texture (fishman et al., 1993). therefore, cold storage has always been used as the main method to slow these processes to delay the peaches decay, but chilling injury (ci) limits its peach storage life at low temperatures, such as apples (lurie et al., 1994), mango (ketsa et al., 1998), and strawberry (garcia et al., 1995). But for consumers' increasing awareness of health and environment consideration, alternative solutionsto control browning that meet food safety policy and easy topromote are needed the single treatments of calcium chloride (cacl₂), ultraviolet(uv-c), pullulan film and map have been extensively studied for commercial applications (li et al., 2009; luoet al., 2011; manganaris et al., 2007).Calcium affects fruit guality and preservation by playing a fundamental role in maintaining the stabilization of cell wall (vicente et al., 2005). the post-harvest application of cacl₂ was also reported to enhance the storage life of apricots (antunes et al., 2003), peaches (manganaris et al., 2007) and strawberries (verdini et al., 2008), mainly by reducing pectin solubility (i. lara et al., 2004), strengthening cell wall(vicente et al., 2007), delaying fruit ripening and decreasing decay rate(i. lara et al., 2004) and maintaining firmness (manganaris et al.,2007). the appropriate use of cacl₂ can close the passage of ca_2 + in the cell membrane stable cell wall and cell membrane (mo and wang, 1994). ultraviolet light has mainly been used for surface disinfestation (el-gaouth and wilson, 1995). the effect of ultraviolet light (uv-c, λ =254nm) could beattributed to two underling mechanisms. first, there is the direct effect on the pathogens because of dna damage. second, uv-c can induce resistance mechanisms in different fruits against pathogens (liu et al., 1993; nigroet al., 1998). uv-c was used for harvested fruits in controlling microbial activity and deterioration (erkanet al., 2001). post-harvest application of uv-c was also reported

to enhance the storage life of pepper (vicente et al., 2005), tomato (barkaet al., 2000), citrus fruits (ben-yehoshua et al., 1992), grapefruit (d'hallewin et al., 2000), zucchini squash (erkan et al., 2000) mainly by fungal decaying and keeping firmer (vicente et al., 2005), delaying the cell wall degradation and retarding softening of the fruits tissues (barka et al., 2000), inducing the appearance of phytomalexins (d'hallewin et al., 2000). Modified atmosphere packaging is an alternative way to package respiring products, respiring products must have O₂ to produce enough energy to maintain their integrity and quality through the process of aerobic respiration, in which carbohydrates are converted to co2 and water at the expense of O₂(gorrisetal., 1992). post-harvest application of map was reported to enhance the shelf-life of suli pear (jiang et al., 2011), european plum (zhanget al., 2005) mainly by decreasing the rates of respiration and ethylene production (jiang et al., 2011), restraining decline of deoxidized sugar and reducing weightlessness and rotting of fruits (zhang et al., 2005).Pullulan is a polysaccharide polymer consisting of maltotriose units, also known $as\alpha$ -1,4(6)-glucan(fujii and shinohare 1986; kimoto et al. 1997). pullulan films are prepared by drying a pullulan solution on a smooth surface and it can be as thin as 5-60um (yuen 1974).Our research target is to characterize the physiological and biochemical responses of the 'fenghuang' honey peaches to 0.25kj/muv-c+2% pullulan coating ,2% cacl₂ + 2% pullulan coating, 0.25kj/muv-c+ modified atmosphere packaging in mixed treatments.based on 1 year's preliminary study and 4-year continuous tests, the effects of treatments on weight loss rate, firmness, soluble solids content (ssc), electrolyte leakage, soluble sugar, malondialde-hyde(mda)content, respiration rate and polyphenol oxidase (PPO) were examined each day during 7 days ambient temperature storage(25±1°c) so as to evaluate its potential use for browning control and quality maintenance of peach fruits, in addition, fruit quality was evaluated from a consumer perspective. this study was also to determine the impact of this three combined methods on fruit quality during the storage period of 'fenghuang' honey peach and intended to serve

as a practical guideline for fruit producers and consumers as well.

MATERIALS AND METHODS

Material and pretreatments

The "Fenghuang" water peach in Zhangjiagang City is the earliest cultivated peach in the southern China with more than 100 years' history. The subtropical humid monsoon climate with four distinct seasons and sufficient light provide a suitable natural condition for peaches growing. The annual average temperature is about 15.7°C and total rainfall 1167mm, and the deep and loose yellow–brown soil in this area is rich of soil organic matter. The tree of "Fenghuang" water peach is about 2-3 meters high with wide and deep root system. Most of fruits ripened in mid-late July and August with about 105 days growth period, the average weight of fruit is 250 grams.

Peaches (Prunuspersica Batsch cv. Fenghuang) were hand-harvested towards the end of the commercial harvest from a commercial orchard in Nanjing, China. Fruits were transported to the experimental laboratory at the Nanjing University on the same day and were selected for uniform size, color and absence of mechanical damage after excluding the ones inappropriately matured and with defects. 2%CaCl₂ solution0.25kJ/m-2UV-C, 2%Pullulan coating, 0.02mm MAP bag were chosen as the optimal concentration for our experiment based on 1 year preliminary experiments and numerous repeated tests. Peaches were divided into four lots, part one was control group, part two was irradiated with 25W UV-C lights(The peaches were placed individually on opposite rotating rods, -25cm from the irradiation source, and turned continuously during UV-C treatment to provide uniform irradiation over the whole fruit surface) for 3 minutes and then coated with 2% Pullulan solution for 3 minutes, after that then air dried for 3 hours; part three was treated with 2%CaCl₂ + 2%Pullulan mix solution for 3 minutes then air dried for 3 hours and the last lot were irradiated with 25W UV-C lightsfor 3 minutes and then packaged with MAP. All treatments were applied at 90-95% RH. After treatment, fruit were transferred to 25±1°C for 7 days storage. A fruit sample from each condition was removed for ripening assessment (three replicates of six peaches each) every day. The same fruit used for ripening measurements was used for the sensory panels.

Mass loss, fruit firmness and TSS

The mass was measured by an MP2000-2 balance $(\pm 0.05g)$ (Shanghai Balance Instruments, China). Mass loss was calculated as: mass loss = Wi being the initial sample weight and Wf the final sample weight. Results are reported as percentage weight loss.

The firmness of peaches was recorded during storage to determine the shelf life. Firmness values of each individual peach without skin were measured at three points of the equatorial region by using GY-3 firmness tester (Zhejiang Tuopu, China) with an 11 mm diameter flat probe. The firmness of each peach was measured three times on different sides. Three replicates were used per treatment and the result is the mean of the replicates.

Measurement of TSS was conducted on juice samples collected from five fruits per treatment, using a Refractometer (WZS-I, Shanghai Optical Instruments, China) and expressed as Brix.

Membrane integrity

Membrane permeability, expressed by relative electrolyte leakage rate, was determined using a conductivity meter (Model DDS-11A, Shanghai Scientific Instruments, China). Determination of membrane integrity was carried out according to a modified method of Jiang et al (Jiang YM. and Chen F., 1995). These were then incubated at 25°C for 180min. The resulting solution was then placed in a water bath (100°C) for 30min and cooled to 25°C before the final conductivity (total electrolyte leakage) was measured.

The percentage membrane integrity was calculated as: Membrane integrity% =[1-(electrolyte leakage after 180 min submersion/total electrolyte leakage)] × 100.

Rate of respiration

Respiration was measured by the rate of CO2 production. In doing this 30 fruits (ten fruit per chamber) from each treatment were sealed in a chamber and air was passed through the chamber. The effluent air was connected to a GXH-3051 (Institute of Junfang Scientific Instrument; Beijing, China) Infrared Gas Analyser (IRGA) and the respiration rates was expressed as mg•h⁻¹•kg⁻¹. Three independent replicates were conducted under each treatment.

Soluble sugar and membrane oxidation

MDA concentration was measured according to Dhindsa (Dhindsa RS et al., 1981) with modification. This involved grinding of tissue samples (2.0g) in liquid nitrogen and an extract was obtained in 5 mL 10% (w/v) trichloroacetic acid (TCA). After centrifugation at 10,000 ×g for 15 min, 2 mL aliquot of the supernatant was mixed with 2mL 10% (w/v) TCA containing 0.6% (w/v) thiobarbituric acid (TBA). The mixture was heated to 100°C for 20 min, quickly cooled and centrifuged at 10,000 x g for 10 min. The supernatant was collected and absorbances at 532,600 and 450 nm were then measured in spectrophotometer (Shanghai Jinghua, 756MC). The MDA concentration was calculated according to the formulaand the soluble sugar =11.71 * A_{450} . Three independent replicates were conducted in each treatment.

Determination of the PPO activity

To measure PPO activities, 2-g tissue each from 10 fruits was homogenized in 5 ml of 0.1 M phosphate buffer (pH 6.4) and the supernatant was collected, after centrifugation (10,000 rpm 15 min at 4 °C), as enzyme extracts. The increase in absorbance at 410 nm against blank (prepared in the absence of enzyme) was automatically recorded at every 30 sec up to 3 min, using a spectrophotometer (756MC). One unit (u) of enzyme activity was defined as the amount that caused a change of 0.001 in absorbance per minute.

Data processing and statistical design

For each combined treatment, the experiment was conducted as a completely randomized design with all the treatments. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA) and Microsoft Excel. Data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Where significant differences were detected, mean comparisons were done by the L.S.D. test (P < 0.05).

RESULTSAND ANALYSIS

Figure1. Effects of 0.25kJ/m-2UV-C+2%Pul, 2%CaCl₂+2%Pul, 0.25kJ/m-2 UV-C+ MAP treatment after harvest on weight loss rate (A), firmness (B), soluble solids content (C). electrolyte leakage (D), malondialdehyde content (E), respiration rate (F) and polyphenol oxidase (PPO)(G) of 'Fenghuang' peach fruit at the end of 7 days ambient temperature storage. Bars represent the standard errors of the means of triplicate assays.

Effects of UV-C+Pul, Pul+CaCl₂ and UV-C+MAP on weight loss rate during storage

As shown in Figure1A, the weight loss rate of fruit treated with CaCl2+Pul reduced by 1.384% after 7 days of storage and was the lowest compared to the others (Figure 1). The CK set recorded maximum weight loss rate of 3.232%. The other two combined methods (i.e., 0.25kJ/m-2UV-C+2%Pullulan coating and 0.25kJ/m-2UV-C+modified atmosphere packaging) showed the maximum weight loss rate of 1.534% and 1.978% after 7 days of ambient temperature. There was no significance difference among all the treatments (P>0.05), while there were significance difference between the CK and all treatments (P < 0.05). Calcium ions increase the stability of cell walls by binding non-sterifiedpectins. Although plant cell walls are permeable to water, reduced disassembly of this structure



might result in a higher resistance to water flux. Similarly, Navjot et al (Navjot et al., 2010) found that a dip of 6% CaCl₂ proved very effective in reducing loss in weight of "Earli Grande" peach over control. Schirraet al (Schirra et al., 1997) demonstrated that 2% CaCl₂ treatment decreased the rate of fruit weight loss during simulated marketing period; On the other hand, Pullulan solution films have excellent mechanical properties and the ability of oxygen resistance, which couldeffectively protect the fruits from transpiration.

Effects of UV-C+Pul, Pul+CaCl₂ and UV-C+MAP on fruit firmness

Fruit firmness is an important indicator of postharvest physiology and quality of fruits during storage. The major changes of softening in peaches involved the catabolism of cell walls and the development of an intercellular matrix containing pectins (Harker and Maindonald 1994). Fruit firmness decreased gradually during storage most likely due to hydrolysis of metabolites. Higher firmness (4.56 kg/cm²) was observed in fruits treated with 2% CaCl₂+Pul after 7 days storage whereas minimum firmness was under control after same storage period (Figure1B). The experimental results showed that the firmness of control with an initial firmness of 7 kg/cm² decreased to 1.97 kg/cm² after 7 days storage, while the firmness of 0.25kJ/m-2UV-C+2%Pullulan coating treatment was 3.61 kg/cm² and 0.25kJ/m-2UV-C+ modified atmosphere packaging treatment was 3.95 kg/cm². Those three combined treatment effectively reduced the firmness by 32.7%, 50.2% and 39.3% throughout the storage period separately. It was shown that combined of CaCl₂ and Pul treatment was more effective than the application of either treatment. It was indicated that the softening of fruit texture had a close relationship with the deploymerisation and solubility of flesh cell wall polysaccharides. Reasonable contents of calcium can retain high levels of ionically-bound pectins and retard the textural degradation (Vicente et al., 2007). Previous studies showed that Ca₂+-treated fruits exhibited higher values of flesh firmness during storage (Akhtar et al., 2010; Manganaris et al., 2007) and GA3 significantly affected (p < 0.05) the fruit firmness of '0900 Ziraat' sweet cherry cultivar(Li et al., 2009).

Effects of UV-C+Pul, Pul+CaCl₂ and UV-C+MAP on soluble solids contents (SSC)

Soluble solids content (SSC) is an important parameter for fruit quality evaluation during the storage periods. Sugars are the main soluble metabolites, and include glucose, fructose and sucrose comprising 99% of total sugar content. Figure1C shows the results of different combined treatments on soluble solids contents (SSC), indicating that treatment with 2% CaCl₂+Pulwas most effective (p<0.05) in maintaining the SSC than other treatments. The SSC of fruits treated with UV-C +Pul (p<0.05), UV-C + MAP reduced to 9.39%, 10.94%, while control grope recorded to 8.05% after 7d storage. It was



indicated that 2% CaCl₂+Pul treatment was more effective in maintaining the SSC than other treatments. It is possible that high CO₂ concentrations inside packages activated hydrolysis and glycolysis that resulted in consumption of sugars (Bodelón et al., 2010). Similar findings that CaCl₂ inhibited the decrease of soluble solid content of Fraiar plum fruit is reported by Zhang et al., ((Zhang et al., 2009).

Effects of UV-C+Pul, $Pul+CaCl_2$ and UV-C+MAP on electrolyte leakage

Electrolyte leakage, which is a measure of loss of semi-permeability of cell membranes, can be used as an indicator of membrane damage; it is also widely used as an indicator of chilling injury (Zhang et al., 2011). As shown in Figure.1D, electrolyte leakage increased during the 7 days storage. Fruit treated with 2% CaCl₂+Pul treatment was most effective in inhibiting the leakage increase, increasing to 13.53% on the 7th day of storage, while the control was 21.23% (P<0.05).The electrolyte leakage of fruits treated with 0.25kJ/m-2UV-C+2%Pullulan coating and 0.25kJ/m-2UV-C+modified atmosphere packa -geing were recorded 15.85% and 16.54%. It was reported that calcium plays an important role in the stabilization of cell membranes, as fruits softened more with the increase in free calcium in flesh fruits

(Manganaris et al., 2007). Based on the treatment of 2% CaCl₂+Pul the cell wall of honey peach becomes more strengthened which limits the rise in electrolyte leakage effectively, suggesting greater membrane integrity. Similar effect of CaCl₂ on fruit quality was also reported by Antunes in fresh-cut kiwifruit (Antunes et al., 2010).

Effects of UV-C+Pul, Pul+CaCl₂ and UV-C+MAP on respiration rate

Respiration is a major factor contributing to postharvest loss, as it converts stored sugar to energy in the presence of an oxygen substrate, leading to premature senescence (Amnon et al., 1998). The rate of CO₂ production of control fruit increased guickly and reached a maximum value on 2th day and decreased for a while, and in the last 4 days it kept a rising trend. (Figure 1E). The respiration rate of fruits treated with those three combined treatment reached their peaks at 96.92mg(kg•h)-1, 103.845 mg(kg •h) -1 and 83.36 mg • (kg • h) -1 separately. The respiration rate of 2% CaCl₂+Pul treatment fruit was considerably lower(P < 0.05) than that in control fruit, indicating that 2% CaCl₂+Pul treatment inhibited most effectively. These results demonstrated that 2% CaCl₂+Pul treatment suppressed the rate of respiration production. It has been demonstrated that CaCl₂ markedly inhibited ethylene production and respiration rate in Qinwang peach (Yu et



al., 2004) during storage and 'Hongjiang' orange at ambient temperature (Xia et al., 2009).

Effects of UV-C+Pul, $Pul+CaCl_2$ and UV-C+MAP on MDA

Long time storage can alter the structure of the membranes due to lipid peroxidation. MDA, a secondary end product of polyunsaturated fatty acid oxidation, is thus widely used as an indicator of lipid peroxidation. Changes in MDA levels in a tissue can be a good indicator of the structural integrity of the membranes of plants subjected to decrepit. As shown in Figure 1F, MDA content of control increased gradually within 7 days storage. Highest MDA (2.74 μ mol·L-1) was observed in control fruits after 7 days of storage and minimum MDA(p<0.05) (1.51 μ mol·L-1) was observed under three combined treatment after same period. At the end of storage, MDA content in treated fruit was nearly 45% lower than that in control fruit. These data clearly indicate that damage caused by high temperature stress could be

alleviated by three combined treatment. This suppression in MDA content by CaCl₂ treatment has also been reported by Xia (Xia et al., 2009).

Effects of UV-C+Pul, $Pul+CaCl_2$ and UV-C+MAP on PPO

The major concern with regard to quality deterioration of peach fruits is flesh browning, which is associated with phenol-related metabolic enzymes POD and PPO (Loaiza –Vealarde and Saltveit, 2001). PPO is a key enzyme for enzymatic browning in many fruits. The latent form of PPO is often activated during ripening, senescence or stress condition when the membrane is damaged, which results in an increase of PPO activity (Mayer, 1987). As shown in Figure.1G, PPO activity decreased inconstantly during the storage period. It has also been demonstrated that three combined treatment can inhibit PPO effectively, that PPO had a sudden increase over the first 2 days, reached the first peak, then it decreased on the third day, after that. it will increased gradually and reached maximum values on the 7th day, showing about a 118% PPO increase. in the fruits treated with 0.25kJ/m-2UV-C+2%Pullulan coating and 0.25kJ/m-2UV-C+modified atmosphere packaging increased within 2 days and lowered down afterwards. This change may be related to the stress at the first 2 days of storage, and the inhibition effect appeared later on. The recorded of 0.25kJ/m-2UV-C+2%Pullulan coating was the lowest 408.7U/g. Meanwhile, the PPO activities of all treatments were lower than that of the control. Previous work demonstrated that CaCl₂ markedly inhibited the activities of polyphenol oxidase of Qinwang peach (Yu et al., 2004), Pul coating treatment delayed activities of PPO of 'Yinhualu' honey peach (Dong et al., 2011)(Dong et al., 2011).

DISCUSSIONS

Mechanism analysis of different treatments on Fenghuang honey peach

The weight lossrate of fruit treated with 2% CaCl₂ + Pul ranged from 0 to 1.38% during 7 days of storage and was lowest compared to others (Figure1A). The control fruits recorded maximum.Fruit firmness decreased gradually during storage, but the rate of decrease in firmness was slower in 2% CaCl₂+Pul treated fruits (Figure1B), that may because the calcium have the ability to stable the membrane and cell walls' structure strongly, help to maintain the firmness of flesh, and at the same time calcium and pullulan may decrease the activity of cellulose pectinase. The fruits treated with 2% CaCl₂+Pul retained higher (p≤0.05). SSC of 2% CaCl₂+Pul treated group decreased throughout the storage period as compared to control. Those two factors make the result that 2% CaCl₂+Pul is better than the others. Long time storage can alter the structure of the membranes due to lipid peroxidation. The MDA levels after Pul+CaCl₂ treatment increased slowly (1.69 µmol+L-1) compared with other treatments during storage. At the end of storage, MDA content in treated fruit was nearly 45% lower than that in control fruit. This suppression in MDA content by

CaCl₂ treatment has also been reported by Xia et al., (Xia et al., 2009). There was a progressive increase in the respiration of fruits rate during storage(107.89mg*kg-1*h-1). The lowest PPO was recorded by in fruits(408.7Ug-1) treated by UV-C+Pul during storage might be due to an increased concentration of organic solutes as a consequence of water loss, UV-C treatment caused inactivation of hydrolyzing enzymes responsible for conversion of starch into sugars. From the skin color, flesh color, aroma and flavor we found that there are better than the control, and there is a significant different between the treated and the control group. Under current dose, no negative influence of UV-C treatment on fruit quality or fungal development on peaches could be found, external damage of fruit due to ultraviolet exposure, such as peel browning or off-flavors.

Security analysis of the treatments on Fenghuang honey peach

Calcium is an important element to build up creature body, in agricultural production this element is often used as fertilizer, so it clearly safe to human and animals. The effect of ultraviolet light (UV-C, λ =254nm) can be explained in two ways. First, there is the direct effect on the pathogens because of DNA damage. Second, UV-C can induce resistance mechanisms in different fruits against pathogens. It is well known that plant tissues produce increased amount of phytohormone when subjected to various biotic and abiotic stresses. Exposure to UV-C irradiation has been shown to induce resistance against pathogens in a number of species. At the same time, we can see that the ultraviolet light works in a physical way and meet the food safety requirements well.We use the MAP package to provide an isolated circumstance with low level of O₂ or high slevel of CO₂ to inhibit the intensity of aerobic respiration. Meanwhile the MAP materials we used were stable enough to keep in good condition and safe to human and animals.Pullulan is non-toxic, non-mutagenic, odorless, tasteless, and edible (Fujii and shinohare 1986; Kimoto et al. 1997). For it

non-toxic, non-mutagenic, odorless, tasteless, and edible, it is safe in the food industry.

CONCLUSIONS

First, similar to previous studies, we found that the mixture applications of UV-C+Pul, CaCl₂+Pul and UV-C+MAP could alleviate high temperature injury and prolong the storage life of peach fruit. This suggested that the mixture treatments activate higher temperature stress defensive capacity. All the results here indicated that the combined treatment might alleviate oxidative stress in peach fruit.

Second, the combination of CaCl₂+Pullulan treatment enhanced most of the indicators which might be attributed to its ability to enhance defense systems against high temperature stress and pathogen, induce antioxidant systems, affect the levels of polyamines and provide an isolated environment. The combined treatment could result in a significant enhanced control of blue mold rot in peach fruit, which suggested that the two treatments in combination activate more defensive capacity against blue mold in peach fruit.

Finally, the results of this work support the use of UV-C+ Pul, CaCl₂+Pul and UV-C+MAP treatment as a possible strategy to alleviate the softening and protect the peaches from pathogen effectively and prolong the storage life. Due to its easy availability and operation, meet the food safety policy and no harm to environment, 2% CaCl₂ +Pullulan coating treatment could be a reliable solution to control postharvest decay of peach fruit and much more suitable for promotion in the farmers. All these 2% results suggested that the combination of potential CaCl2+Pullulan coating for may have comercialization.

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