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Effectiveness salicylic acid blending in chitosan/PVP biopolymer coating on antioxidant enzyme activities under low storage temperature stress of 'Banati' guava fruit

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ABSTRACT

The performance of chitosan/poly-vinyl-pyrrolidine (CS/PVP) blending in salicylic acid (SA) to increase chilling injury tolerance of 'Banati' guava fruit under cold otherwise the impacts of CS/PVP-SA on antioxidant enzymes were assessed. The guava fruits were coated with CS/PVP biopolymer with SA at different concentration (0,1, and 2 mM) and stored at low temperature (6 °C and 90.95% RH) for fifteen days. Fruit samples were picked every three days interval. The physical measurements weight loss%, chilling injury index (CI-index), and fruit skin color hue angle (h°) were assessed. The chemical analysis for instance soluble solid content (SSC%), titratable acidity (TA%), SSC/AT-ration, total chlorophyll content, fruit firmness. The antioxidant enzyme activities were evaluated such as catalase (CAT, EC: 1.11.1.6), peroxidase (POD, EC:1.11.1.7), ascorbate peroxidase (APX, EC: 1.11.1.11), and superoxide dismutase (SOD, EC: 1.15.1.1). Also, plasma cell membrane compartments such as lipid peroxidation (malondialdehyde, MDA), protein oxidation (protein carbonyl group, PCG), cell membrane ion leakage (IL%), $O_2 \cdot^-$ and H_2O_2 production rate were measured. CS/PVP-SA $_{2mM}$ significantly reduced water loss, CI-index, h°, maintained fruit skin chlorophyll content, firmness, and delayed the qualitative changes SSC%, TA% and SSC/TA-ratio due to increase the CS/PVA and SA at 2 mM. Moreover, changes in the antioxidant capacity of CS/PVP-SA $_{2mM}$ coated guava fruits were activated. The activation of antioxidant enzymes, alleviating CI-index and reducing plasma cell membrane damage. SA bending in Chitosan/PVP biopolymer coating and storage fruit under cold temperature was obtained using parameters to detect the effect of CS/PVP-SA treatments. CS/PVP-SA $_{2mM}$ exhibited a reduction of chilling injury incidences compared to uncoated fruit in all in overall measurements.

1. Introduction

Guava (*Psidium gujava* L. cv 'Banati') is viewed and a primary subtropical fruit. it is grown extensively in tropical and subtropical regions of the world (Silva et al., 2018). There are several guava varieties planted in Egypt. The cultivated area and the crop yield of guava are according to the latest statistics about 20.6 thousand hectares producing 350 thousand tons annually according to the Egyptian Ministry of Agriculture, (E. M. A., 2016). Nutritionally, It contains highly nutritional compounds such as vitamin C, a wide range of types of carotenoids (Uddin et al., 2002). However, the main problem of guava fruit has shown rapid ripening processes after harvesting. It exhibits a high respiration rate and ethylene production (Hong et al., 2012). Since guava fruit is classified a climacteric fruit (Kumar et al., 2015). Consequently, the fast ripening forced fruit to enhance deterioration which leads to short shelf-life, limits transportation and storage period (Hong et al., 2012). Numerous investigations were led to keeping fruit quality characteristics. Many different procedures were implemented to control shelf life or storage. These techniques are cold storage, controlled atmosphere, pre and postharvest chemical substances, and biopolymer coating treatment (Anggarwulan et al., 2015). Therefore, it is essential to make sense of a probable answer for limit fruit quality decay and enhance fruit quality after harvesting.

Fundamentally, the cold storage is one of the main which is used to slow down the physiological processes such as respiration and ethylene production after harvesting. Despite, the low cold storage temperature generates active oxygen species (AOS) which are produced in different organs in plant cells. These are mitochondria, plasma cell membrane, cytoplasm, and cell wall (Lo'ay, 2005). Moreover, AOS generates under different stresses, for instance, drought, salinity, reoxygenation and low

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temperature (Chan et al., 2016). So, enhancing AOS generation leads to cell compartments damage and the death (Foyer et al., 2017), by which it reduces fruit quality (Cheng et al., 2010). Accordingly, the alternative technique that use to reduce AOS formation and increase/improve fruit quality after harvesting can be important for both producer and consumer (Silva et al., 2018). The implemented technique has been used to increase storage ability or shelf life of fruit such biopolymer coating (chitosan) has been affected by fruit quality during shelf life. Chitosan is highly carbohydrate molecular weight, soluble in an organic acid, and polysaccharide (Lo'ay and Dawood, 2017a). Also, it is considered a natural biodegradable polymer and non-toxic material (Drevinskas et al., 2017). Plus, it could be leading in chitosan with another polymer such poly-vinyl-pyrrolidine or organic acid (Lo'ay and Dawood, 2017b). Additionally, it becomes profitable on protection thickness, absorbency, solubilization, and crystallization. Moreover, it has low toxicity and it is utilized in board rage of areas such as medical, food cosmetic, and health-related domains (Drevinskas et al., 2017). Despite, salicylic acid (SA) is considered an endogenous phenolic nature synthesis that it plays as the plant growth regulator. Mainly, SA has optimistically impacted on fruit respiration and ethylene biosynthesis rates (Srivastava and Dwivedi, 2000). It is integrating fruit water loss, microbial infection, and maintaining fruit firmness during fruit handling or marketing (Lo'ay, 2017).

The target of this our study evaluated the capability of chitosan/PVP blend with salicylic acid as a coating treatment, on the increase of capacity storage life of 'Banati' guava fruit. Also, the effect of various concentrations of SA mixing in biopolymer chitosan/PVP on post-harvest life and fruit quality properties during low-temperature stress.

2. Material and methods

2.1. Fruit materials

The study was done on guava (Psidium guajava L. cv 'Banati') planted in topsoil soil in a commercial orchard close to Damietta Gov., Egypt. The investigation was conducted during tow seasons 2016–2017. Fruit samples were picked at yellow-green development arrange (fruits have more yellow than green color) as indicated by color (hue angle) estimation (Lo'ay and EL-Khateeb, 2011). The fruit was transported in the cooler van at 10 °C for three hours. Upon arriving at Lab, fruit samples (480 natural products) were separated into two major groups. The first batch contains 240 fruits that distributed on four treatments. Every treatment contains 60 fruits which are distributed on three replicates (20 fruits), for estimating water loss rate, chilling injury index and fruit skin color hue angle. Be that as it may, the second batch 240 fruits additionally organized as represented previously general treatments for chemical estimations.

2.2. Biopolymer chitosan/PVP leading to salicylic acid preparation

The chemical materials were utilized as a part of this examination: PVP (K-30 polymer; Ashland organization, China), chitosan (CS) (MW 71.3 = kDa, the level of deacetylation = 94%; Merck, Darmstadt Germany), salicylic acid was the explanatory review. chitosan (CS) solution (750 ml, 1% w/v) was prepared by dissolving 7.5 g of CS in 750 ml of 2% (v/v) aqueous CH3COOH anhydrous solution for 8 h under magnetic stirring. The arrangements of PVP and CS were deliberately blended at a proportion of (1:1) and mixed for 2 h. At last, the resultant arrangement (1500 mL) was similarly subdivided into three sets (500 mL in everyone). The control set was not supplemented with any added substance. Salicylic acid was disintegrated and blended into the mixed arrangements of set 2 and 3 under magnetic stirring for 4 h at 25 °C at two SA concentrations (2 and 4 mM), separately. The mixed arrangements were put in funnel-shaped carafes and stored at 4 °C until further examination.

2.3. Treatments protocol

The treatments were prepared for the following control, CS/PVP-SA $_{0mM}$, CS/PVP-SA $_{1\ mM}$, and CS/PVP-SA $_{2\ mM}$. Fruits were immersed in CS/PVA-SA coating for 5 min, and they were stored at cold temperature (6 ± 1 °C and air humidity percentage average during the storage period 90.95% RH) for 15 days

2.4. Non-distractive measurements

Quality elements were proposed, guava fruits were randomly picked from every treatment and were isolated into three replicates to gauge weight loss rate based on underlying the initial weight of fruit at harvest time and it exhibits in rate. Chilling injury symptoms was tried by judging the degree the chilling damage of fruit peel as per the accompanying scale: 1 = no injury; 2 = light injury; 3 = direct injury (25% surface influenced); 4 = extreme injury (26-half surface influenced) and 5 = exceptionally serious harm (> 50% surface influenced) as depicted (Lo'ay, 2005).The chilling damage file is then figured utilizing the accompanying formula:

$$\text{Chilling injury index} = \sum_{1\rightarrow 5}^{5} \frac{(\text{Chilling injury index})*(\text{Number of fruits at the level})}{\text{Total number of fruit}}$$

Fruit skin color profile was recorded (Lo'ay and EL-Khateeb, 2011), from that point, all pictures were investigated by utilizing programming ImageJ Ver.1.43 u the USA to get RGB signs to determine fruit skin color hue angle (Khojastehnazhand et al., 2010). Fruit firmness was recorded on fruit using Effegi-penetrometer supplemented with a plunger 8 mm diameter penetrator and it was presented an N unit (Samaan et al., 2012).

2.5. Chemical measurements

Total soluble solid content (SSC%) was measured utilizing Carlzeiss hand refractometer and total acidity percentage (TA) was dictated by titration with 0.1 N NaOH (A.O.A.C., 1995). So, the SSC/TA ratio was computed as a characterized development record. In term of aggregate chlorophyll was extricated by drenched 2 g of fruit skin in 20 mL of *N*, *N*-dimethylformamide (DMF). Samples were stored at 4 °C for 16 h to permit the DMF to separate the chlorophyll. At long last, samples were centrifuged for 5 min at 15,000 rpm, at that point an clear supernatant example was proposed chlorophyll A at 663 nm and B at 646 nm wavelength utilizing spectrophotometer (UV–vis auto). It exhibited in mg 100 g^{-1} FW. As to carotene pigment was recorded at 452 nm and it exhibited in mg 100 g^{-1} FW (Lo'ay, 2005).

2.6. Antioxidants enzyme activities assessment

Fruit sample (10 g) from every treatment was picked and homogenized in 25 mL of ice-cold extraction buffer and 0.5 g poly-vinyl-poly-pyrrolidone (PVPP) with a Kinematica tissue processor (Crl-6010, Kriens-LU, Switzerland). For CAT and SOD examines, the extraction buffer was 50 mM sodium phosphate (pH 7.8). For POD, 100 mM sodium phosphate cradle (pH 6.4) was utilized. The homogenate was centrifuged at 27,000 rpm for 50 min at 4 °C and the subsequent supernatants were utilized shortly for examining of CAT and SOD enzyme activities (Wang et al., 2005).

In term of, CAT, the response blend comprised of 2.8 mL H_2O_2 (40 mM, in 50 mM sodium phosphate buffer, pH 7.0) and 0.2 mL enzyme extract. The deterioration of H_2O_2 was estimated by the decrease in absorbance at 240 nm amid 120 s. The activity was reported as Unit g^{-1} FW, where one unit of catalase changes over 1 moL of H_2O_2 per mass of fruit per min at 30 °C.

For SOD, the response blend (3 mL) contained 65 mM sodium