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Postharvest chitosan treatment affects quality, antioxidant capacity, antioxidant compounds and enzymes activities of 'El-Bayadi' table grapes after storage

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ABSTRACT

The effect of postharvest chitosan treatments at 1, 1.5 and 2% on quality, antioxidant capacity, antioxidant compounds and some related enzymes activities of 'El-Bayadi' table grapes after 30 days of storage plus 2 days of shelf life were investigated. Chitosan treatments decreased decay after storage (ranged from 2.95 to 4.42%) compared to control (16.25%). However, weight loss was lower only at 1% chitosan (0.39%) than other rates and control (1.66). Quality characteristics TSS, acidity and pH of berries slightly changed after storage compared to initial and were not affected by chitosan treatments. However, berry firmness decreased after storage, but chitosan treatments retained higher firmness than control. Chitosan treatments, especially at 1%, maintained higher total phenols and flavonoids concentration than control. Total phenols concentration decreased as chitosan rate increased, in contrast to total flavonoids. However, vitamin C concentration showed higher values after storage than initial and was not affected by chitosan treatments. Peroxidase (POD) showed higher activity after storage, in all treatments, than initial and was higher in chitosan treatments than control. POD activity increased as chitosan rate increased. Polyphenoloxidase (PPO) showed lower activity after storage in control compared to initial. While, chitosan treatments maintained higher PPO activities than control. Both polygalacturonase (PG) and xylanase activities slightly changed after storage in most treatments compared to initial. However, chitosan treatments maintained higher PG and xylanase activities than control. Both PG and xylanase activities increased as chitosan rate increased. Antioxidant capacity measured by the DPPH method decreased after storage in control compared with initial. Chitosan treatments showed higher antioxidant capacity than control. However, antioxidant capacity measured by the ABTS method was not affected by chitosan treatments. It is concluded that chitosan treatments enhanced antioxidant systems, retained and improved quality of 'El-Bayadi' table grapes after storage.

1. Introduction

Grapes (*Vitis vinifera* L.) are common worldwide fruit that represent the world's second most cultivated fruit crop (Zhou and Raffoul, 2012). Grapes contain several classes of antioxidant compounds such as vitamins, carotenoids, phenolics that benefit human health (Zhou and Raffoul, 2012; Fahmi et al., 2013). In the Kingdom of Saudi Arabia (KSA), the cultivated area has greatly increased during the last decades and mainly concentrated in Medinah, Hael, Tabouk and Taif regions. In Taif region, 'El-Bayadi' is the main table grape cultivar, representing about 90% of total table grapes production in this region. This cultivar is a mid season and highly productive with white color, large size berry and high sensory quality (Al-Qurashi and Awad, 2013). However, berry skin is relatively thin that increase susceptibility to water loss and decay both by cracking and fungus following harvest (Al-Qurashi and Awad, 2013). However, the cold storage life of table grapes is limited by pathogens attack, softening and weight loss (Zahavi et al., 2000; Droby and Lichter, 2004). Pre and/or postharvest application of synthetic fungicides may effectively decrease decay and enhance the efficacy of cold storage. However, in most grape producing

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countries, there are growing public concerns against the use of synthetic fungicides. SO_2 is currently used as an alternative that effectively decrease fungal growth and decay. However, SO_2 has some negative impacts that limit its use, especially with colored grapes (Luvisi et al., 1992; Romanazzi et al., 2002; Droby and Lichter, 2004) and also is not approved in organic grapes production (Mlikota Gabler and Smilanick, 2001). The concept of using natural edible coatings to control decay is a worldwide active area of research (Romanazzi et al., 2012, 2013; Bautista-Banos et al., 2003, 2006; Shiekh et al., 2013). Chitosan, a natural biopolymer with antifungal and eliciting properties is considered an ideal coating to decrease decay of fresh fruit and vegetables due to its biocompatibility, biodegradability and bioactivity properties (Wu et al., 2005; Bautista-Banos et al., 2003, 2006; Xu et al., 2007; Romanazzi et al., 2012, 2013; Shiekh et al., 2013). Chitosan acetate, as the most effective form, has been reported to prolong storage life and effectively reduce decay of grapes and strawberries (Bautista-Banos et al., 2006; Romanazzi et al., 2009). Romanazzi et al. (2002) reported that pre and postharvest chitosan treatments at 0.1, 0.5 or 1% decreased the incidence and severity of grey mold, especially at the high rate, than control in 'Itlia' table grapes. In this respect, pre-harvest spray of 1% chitosan reduced decay and increased phenylalanine ammonialyase (PAL) activity, suggesting that chitosan has not only a direct effect on *Botrytis cinerea* but also induce the resistant mechanism of fruit against decay (Romanazzi et al., 2002). Pre-storage chitosan treatment of 'Shahroudi' grapes at either 0.5 or 1% decreased weight loss, decay, shattering, and cracking and improved quality after 60 days of cold storage compared to control (Shiri et al., 2013). These treatments also delayed the decrease in total phenols and antioxidant capacity of berry extract, but decreased quercetins compared to control. However, the inhibitory effects of chitosan on fungus growth and decay of strawberries during storage were attributed to its fungistatic properties rather than to defense enzymes induction (El Ghaouth et al., 1991, 1992a; Romanazzi et al., 2012). A synergistic effect was observed between UV-C radiation and chitosan on phytoalexins biosynthesis (Romanazzi et al., 2006, 2012). Combining 0.5% chitosan with ethanol was more effective in controlling gray mold of table grapes than each of them alone (Romanazzi et al., 2007). Also, combining 0.5% chitosan with hot water for 5 and 10 min was more effective in reducing decay of sweet cherry than each of them alone (Chailoo and Asghari, 2011). Jinasena et al. (2011) reported that postharvest dipping of banana fingers in 1% chtitosan decreased the incidence of anthracnose during cold storage and shelf life. Bautista-Banos et al. (2003) found that chitosan treatment at 2 and 3% of papayas had a fungicidal effect on *Colletotrichum gloeosporioides* with no impact on TSS and weight loss during papayas storage. Similar inhibitory effects of chitosan treatments on fungus growth and decay have been reported in litchi fruit (Zhang and Quantick, 1997), strawberry and raspberry (Zhang and Quantick, 1998) and peach, Japanese pear and kiwi fruit (Du et al., 1997). Accordingly, this study aim to evaluate the response of 'El-Bayadi' table grape to postharvest chitosan treatment at different doses to control decay, maintain and assure quality during cold storage and shelf life. The effect of chitosan on antioxidant capacity, antioxidant compounds and some related enzymes activities of berries will be investigated.

2. Materials and methods

2.1. Plant materials and experimental procedure

A commercial drip irrigated vineyard of 'El-Bayadi' table grape was selected in Taif region during 2014 growing season. Uniform samples of bunches were picked at commercial maturation and directly transferred to the horticulture laboratory at King Abdulaziz University, Jeddah. A completely randomized experimental design with four replicates (four bunches of each) was established. Bunches of each treatment/replicate were weighted and dipped either in chitosan (100,000–300,000 MW) (Acros Organic, New Jersey, USA) solution at 1, 1.5 or 2% for 10 s. A surfactant (Tween 20 at 1 ml/L) was added to all solutions. A control treatment in which bunches were dipped only in water plus Tween 20 surfactant was included. All treatments were stored in perforated plastic bags inside cardboard cartons with air holes for 30 days at $0\,^{\circ}C \pm 1$ and 90–95% relative humidity plus 2 days of shelf life at $20\,^{\circ}C$. Samples of 30 berries from each treatment/replicate were collected for initial quality measurements and also after 30 days of cold storage plus 2 days of shelf life. Additional samples of berries were collected and kept at $-80\,^{\circ}C$ for later biochemical determinations.

2.2. Decay incidence and weight loss determination

The decay incidence by storage rot was recorded. The total loss in weight was calculated on initial weight basis.

2.3. Firmness, TSS, acidity and vitamin C measurements

Berry firmness was recorded independently in each of the 30 berries per replicate by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter that measure the force required just to break the berry and the results expressed as Newton. A homogeneous sample was prepared from these 20 berries per replicate for measuring total soluble solids (TSS), acidity, vitamin C, total phenols, and soluble tannins. Total soluble solids (TSS) was measured as Brix% in fruit juice with a digital refractometer (Pocket Refractometer PAL 3, ATAGO, Japan). Titratable acidity was determined in distilled water diluted berry juice (1: 2) by titrating with 0.1N sodium hydroxide up to pH 8.2, using automatic titrator (HI 902, HANNA Instrument, USA) and the results expressed as a percentage of tartaric acid (g of tartaric acid per 100 ml grape juice). Ascorbic acid (vitamin C) was measured by the oxidation of ascorbic acid with 2,6-dichlorophenol endophenol dye and the results expressed as mg/100 ml grape juice (Ranganna, 1979).

2.4. Preparation of the methanol extract

Two grams of berries skin tissue were extracted by shaking at 150 rpm for 12 h with 20 ml methanol (80%) and filtered through filter paper No. 1. The filtrate designated as methanol extract.

2.4.1. Estimation of total phenols by the Folin–Ciocalteu test

Total phenols concentration was measured according to Velioglu et al. (1998). Fifty μL of the methanol extract was mixed with 100 μL Folin–Ciocalteu reagent, 850 μL of methanol and allowed to stand for 5 min at ambient temperature. A 500 μl of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as mg gallic acid equivalent/g tissues.

2.4.2. Estimation of total flavonoids

Total flavonoids concentration was determined using a modified colorimetric method described previously by Zhishen et al. (1999). Methanol extract or standard solution (250 μL) was mixed with distilled water (1.25 ml) and 5 % $NaNO_2$ solution (75 μL). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150 μL), 1 M NaOH (0.5 ml) and distilled water (275 μL) were added to the mixture 5 min later. The absorbance of the solutions at

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