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Pre-harvest zinc spray impact on enzymatic browning and fruit flesh color changes in two apple cultivars

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ABSTRACT

Enzymatic browning (EB) in apple occurred during processing or fresh consumption as fresh cut. EB in response to pre-harvest Zn spray at two apple cultivars was investigated. Trees of apple (*Malus domestica* cultivar 'Delbard Estival' and 'Kohanz') were sprayed with water (control), 0.13% of Chelated Zn (C-Zn) or 0.13% of Nano-Chelated Zn (NC-Zn) three or four weeks before harvest. Assessments were carried at spray time, three or four weeks (H1 and H2) after spray and also after 3 weeks of storage (H1 + 3 W and H2 + 3 W) at 4 °C with 95% (RH). The browning index (BI) and total color changes (ΔE) decreased from spray time until harvest time. BI and ΔE in treated fruit were lower than control at all time points except in H1. Starch index was delayed in fruit treated with C-Zn or NC-Zn. The highest firmness of 'Delbard Estival' and 'Kohanz' cultivars was observed in NC-Zn than other treatments at all sampling times. Titratable acidity, soluble solids content and total phenol showed no distinct changes during time. The ascorbic acid, total antioxidant activity and superoxide dismutase in C-Zn or NC-Zn treatments were higher than control in both cultivars except superoxide dismutase activity at H1 for NC-Zn. The polyphenol oxidase activity in both cultivars treated with C-Zn or NC-Zn was lower than control at all sampling time except at H1 in 'Kohanz' cultivar. These findings suggest that the pre-harvest spray with Zn and especially NC-Zn can improve flesh color changes and quality parameters in apples.

1. Introduction

Browning or fruit flesh color changes is one of the most important undesirable reactions that occur in apple fruit. Two kind of browning observed in apple fruit including flesh browning and EB (Du et al., 2012). Flesh browning observed in whole apple at harvest time or during cold storage and in some fruit cultivars it is related to chilling damage (Hatoum et al., 2016). EB is a result of cell disruption which occurs due to cut, peeled, juiced, or exposed to any abnormal conditions during postharvest processing. It is an important cause of quality losses and could decrease consumer acceptance (Mesquita and Queiroz, 2013). This issue not only reduces the visual quality but also results in undesirable changes in flavor and nutritional value of apple products (Hemachandran et al., 2017).

EB in apple occurs via the reaction of polyphenol oxidase (PPO) with phenolic compounds and is facilitated by diffusion of O_2 into the cut tissue (Gil et al., 1998). PPO catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to highly reactive o-quinones which may give rise to heterogeneous black, brown or red pigments commonly called melanins (Tomas-Barberan and Espin, 2001; Caballero et al., 2015). In past decade, most studies have been focused on the use of anti-browning agents on apple including: Citric acid, ascorbic acid, calcium chloride (Chiabrando and Giacalone, 2012; Koushesh Saba and Sogvar, 2016) and carboxymethyl cellulose coatings (Koushesh Saba and Sogvar, 2016). However, little information is available about the role of nutrients application relation with flesh browning or EB.

Zinc (Zn) is an essential micronutrient for plants and is known to have an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a wide number of enzymes (Hotz et al., 2004). Also, the role of Zn in photosynthesis, carbon metabolism and respiration can increase the yield and quality of fruit (Swietlik, 1999). The spray of Zn on the aerial parts is an effective method to improve plant Zn content (Swietlik, 2001), furthermore, Cakmak et al. (2010) reported that Zn spray has advantages than any other applications forms because of high effectiveness, rapid plant response and economic aspect. Spray with chelated forms of Zn is mainly used at fruit set to improve tree yield and fruit quality (Alloway, 2008). C-Zn compounds have been used in spray form, because they were more effective than other forms of Zn (Brennan, 1991). Moreover, recently, application of nanotechnology increased in chelated fertilizers such as C-Zn. Nanoparticles have proportionally larger surface area and

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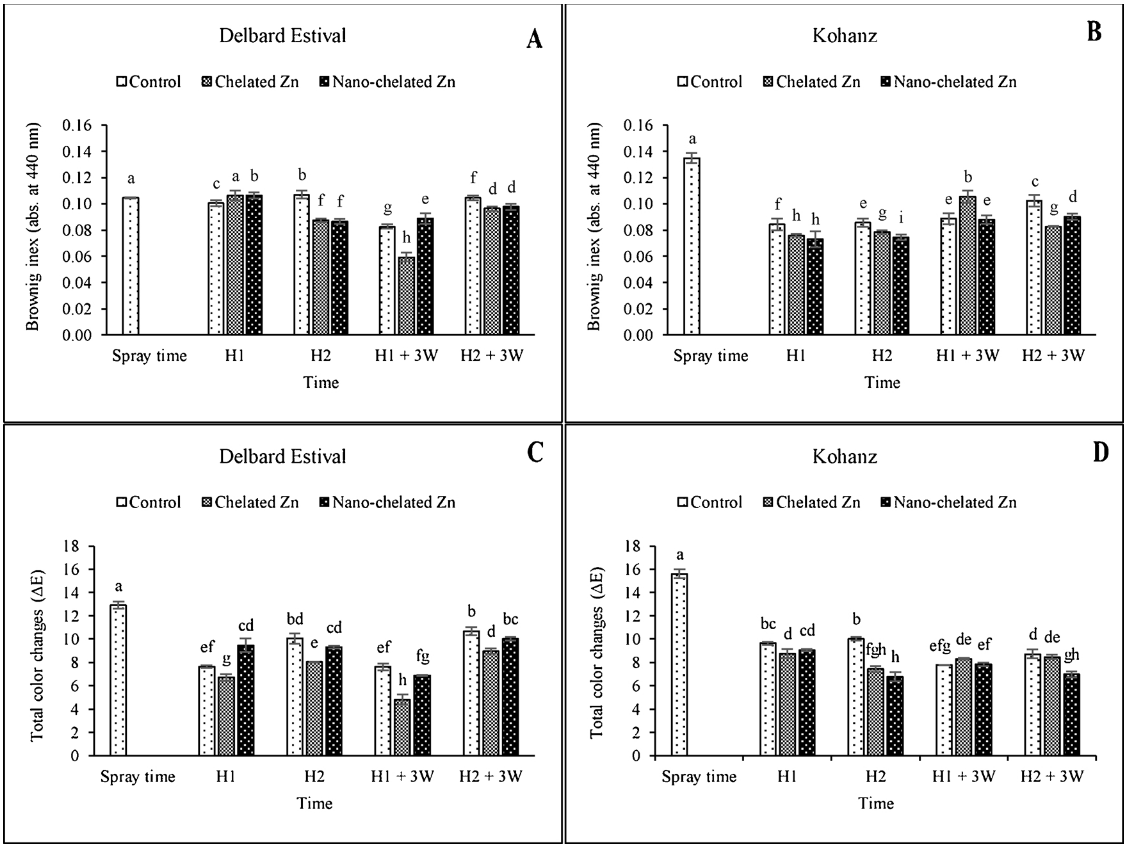

Fig. 1. Browning index (A and B) and total color changes (C and D) of 'Delbard Estival' and 'Kohanz' cultivars at different time sprayed by chelated and nano-chelated Zn. Spray time, first harvest (H1), second harvest (H2), 3 weeks after the first harvest (H1 + 3 W) and 3 weeks after the second harvest (H2 + 3 W). Means with different letters are significantly different ($P \leq 0.05$) according to the LSD test.

consequently have more surface atoms than their micro scale counterpart (Rai et al., 2015).

In addition to the forms and the methods of Zn fertilization, application time also may affect its effectiveness (Swietlik, 2001). The application of Zn in apple trees mainly used at the start of new growth, the swelling of flower buds, after flowering, and the formation of fruit (Peryea, 2005). In recent years, the effects of pre-harvest Zn spray on apple fruit quality raised researcher's attentions (Zhang et al., 2013; Aglar et al., 2016), but little is known about the role of pre-harvest Zn spray in reducing the browning of apples.

Apple cv. 'Delbard Estival' and 'Kohanz' are the early ripening apples with a unique flavor and crisp flesh that are commonly used as fresh and have a short postharvest life. The color changes of flesh after the cut or juiced are among the main problems that complained by consumers. The objective of the current study was to survey the effects of pre-harvest Zn spray in two forms of chelated and nano-chelated on EB, fruit flesh color changes and bioactive constituents in 'Delbard Estival' and 'Kohanz'apple cultivars.

2. Materials and methods

2.1. Plant selection and treatments

This study was conducted in a commercial orchard located at Sanandaj (35° 24´ 17.5´N, 46° 59´ 39.32´E and 1460 m above sea level), Iran. Eleven-year-old trees of apple (*Malus domestica* cultivars 'Delbard Estival' and 'Kohanz') were chosen based on the uniformity of tree size and no symptoms of Zn deficiency (Alloway, 2008). Trees of both apple cultivars were sprayed at July 18, 2016, with 0.13% of Chelated Zn ((C-Zn), Oligo Zn-EDTA, Eurosolids® Company, Germany) or 0.13% of Nano-Chelated Zn ((NC-Zn), Sepehr Parmis Company, Iran) about 1 month before harvest calendar. Control trees of each cultivar were sprayed with water. For all treatments, 0.2% Tween-20 (v/v) was added to the solutions as a surfactant. The leaves and fruits were sprayed on the abaxial/adaxial surfaces with machines backpack up to runoff at early morning (7 a.m.) when the weather was cool. Each treatment consisted of 3 replicate with 2 trees in each replicate. Fruit assessments were carried out in 5 stages; spray time, first harvest (H1): 3 weeks after spray time, second harvest (H2): 4 weeks after spray time. After harvest, fruit was transferred to physiology laboratory at department of horticultural Science, University of Kurdistan, Sanandaj, Iran. Fruit of first and second harvest divided to tow lots, one lot was measured at harvest time (H1 and H2) and the others were stored for 3 weeks at 4 °C with 95% relative humidity (H1 + 3 W and H2 + 3 W). Eight fruit were used in each replication at each sampling time.

2.2. Browning index (BI)

The BI in apples was determined according to Coseteng and Lee (1987). Briefly, two wedge-shaped slices from two opposite sides of each fruit of each replication were taken and pooled for BI assessment. Fifty grams of described sample were weighed into a 250 mL beaker containing 100 mL distilled water. The sample was homogenized in a blender for 1 min. The sample left at room temperature for 1 h, then 7.5 mL 96% ethanol was added to 5 mL of the sample then was homogenized, and centrifuged at 1000*g* for 15 min. The degree of browning of the supernatant was determined by measuring absorbance at 440 nm using a spectrophotometer (Unico UV-2100, USA).

2.3. Total color changes (ΔE)

The color characteristics of fruit were obtained using a color meter