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Patterns of flesh reddening, translucency, ethylene production and storability of 'Friar' plum fruit harvested at three maturity stages as affected by the storage temperature

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ABSTRACT

Flesh reddening and translucency are two predominant physiological disorders of 'Friar' plum (*Prunus salicina* Lindl.) during cold storage. In order to investigate the occurrence of these disorders, the fruit were harvested at early-, mid- and late-maturity stages and stored at 0, 2, 5, 15 or 25 °C, 85–95% relative humidity. Three concomitant patterns for flesh reddening, translucency and abnormal softening (melting) of 'Friar' plums were evident: rapid increases in these disorders at 5 and 15 °C, a delayed/suppressed of these disorders at 0 and 2 °C, and normal ripening at 25 °C, irrespective of fruit maturity stage. Three similar temperature-mediated patterns of anthocyanin accumulation related to flesh reddening, pectin solubilization related to translucency, and decline in firmness related to abnormal softening were observed. Three patterns of ethylene production were also identified: early and dramatically high ethylene production at 5 and 15 °C, suppressed ethylene production at 0 and 2 °C, and almost no ethylene production at 25 °C. These results suggest that ethylene may be involved in the onset and development of flesh disorders of 'Friar' plums during cold storage. Respiration greatly increased, water-soluble pectin content increased early during storage and remained high, and insoluble pectin (i.e., protopectin) decreased, in plums held at 5 and 15 °C. Overall, the biochemical and physiological behavior of plums were greatly affected by storage at 5 and 15 °C compared with 0, 2 and 25 °C, and late-maturity plums were more susceptible to storage disorders.

1. Introduction

Plums are commonly harvested at the early-maturity stage and stored at 0–5 °C for a period before being transferred to market. However, plums are very sensitive to low temperature and the benefits of cold storage may be limited by the development of various physiological disorders, such as internal browning, flesh translucency (gel breakdown), reddening or bleeding, loss of flavor, and delayed softening or retarded ripening after prolonged cold storage (Taylor et al., 1995; Crisosto et al., 2004; Minas et al., 2013). These physiological disorders are also chilling injury (CI) symptoms, and usually reduce the marketability of the cold-stored plums.

Flesh translucency, one of the most frequently observed CI symptoms, manifests itself as a translucent gelatinous breakdown in the mesocarp tissue and is related to the presence of water-soluble pectin (WSP) (Taylor et al., 1995; Manganaris et al., 2008a; Candan et al., 2011). Flesh reddening/bleeding is usually considered the result of anthocyanin accumulation in the mesocarp tissue. The occurrence of flesh translucency was often accompanied by the development of flesh reddening in many Japanese plum cultivars, including 'Friar' (Crisosto et al., 1999; Abu-Kpawoh et al., 2002; Cantín et al., 2008; Candan et al., 2011) and others (Manganaris et al., 2008b; Minas et al., 2013; Pan et al., 2016).

The onset of flesh reddening, translucency and other CI symptoms in plums is strongly associated with storage temperature. Most plum cultivars are susceptible to CI and the flesh reddening and translucency develop faster and more severely after exposure to 5 °C (Crisosto et al., 2004; Khan et al., 2011; Manganaris et al., 2008b) and even 10 °C (Minas et al., 2013) compared with 0 °C, when fruit are subsequently ripened at 20 °C. Nevertheless, to date, little information is available regarding onset and development of flesh reddening and translucency of plums at various storage temperatures. Fruit maturity stage at harvest is a critical factor determining the susceptibility of plums to internal

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breakdown and flesh reddening, with more mature fruit being more susceptible to CI (Taylor et al., 1995; Abdi et al., 1997; Singh and Singh, 2013). However, a study utilizing 'Blackamber' plums suggested that late harvested fruit were more likely to develop flesh translucency when stored at 5 °C, whereas early harvested fruit were more prone to develop flesh bleeding/browning during storage at 0 or 5 °C (Crisosto et al., 2004). However, there is little literature on the precise maturity stage of plum which will minimize CI.

Previous studies have suggested that ethylene may play an important role in the onset of flesh reddening and other CI symptoms of plums, as the occurrence of CI is usually stimulated or accompanied by rapid ethylene biosynthesis in the fruit (Candan et al., 2008; Manganaris et al., 2008b; Khan et al., 2011; Pan et al., 2016). Inhibiting ethylene action may therefore delay the onset of CI symptoms in plums (Candan et al., 2011; Singh and Singh, 2012; Minas et al., 2013). So far, the relationship between flesh reddening, translucency and ethylene production in plums as affected by storage temperature and harvest maturity is unknown.

'Friar' plum is cultivated world-wide, having purplish-black skin and light yellow flesh when mature, and dark black skin and orange flesh when ripe. Flesh reddening and translucency were the primary CI symptoms in 'Friar' plums during cold storage (Cantín et al., 2008; Candan et al., 2011). The present study was undertaken to investigate the patterns of flesh reddening and translucency in 'Friar' plums as affected by storage temperature and fruit maturity. Patterns of ethylene biosynthesis in 'Friar' plums were also investigated to explore the possible role of ethylene in the occurrence of these disorders. Firmness and soluble solids content related to fruit quality, anthocyanins related to reddening, and pectin related to flesh texture were also measured during storage.

2. Materials and methods

2.1. Fruit harvest and storage

'Friar' plums (*Prunus salicina* Lindl.) were harvested on the 15th (early), 21 st (mid) and 28th (late) of August 2014, from a commercial orchard located in Yanqing County, Beijing, China. The experimental design was a randomized complete block using 9 sets of 10 trees. The plum fruit were harvested from trees in one block only for one maturity stage and three replicates of 10 trees were employed. Plums were picked by hand and sorted according to uniformity of shape, color and size. Those with physical injuries or infections were discarded.

Plums were placed in a plastic basket and then packed with a 30-μm-thick perforated low density polyethylene (LDPE) bag. Some packed fruit were directly stored at 15 or 25 °C, 85–95% relative humidity (RH). Others were pre-cooled in air to 5 °C overnight and then stored at 0, 2 or 5 °C, 85–95% RH. Examination and sampling were conducted each week for 5, 15 or 25 °C, and every two weeks for 0 or 2 °C. Approximately 480 fruit were stored per replicate for each storage temperature: fruit maturity combination.

2.2. Measurements of fruit firmness and soluble solids content (SSC)

Measurements (three replicates of 30 fruit) of plum flesh firmness were performed on three separated but equidistant peeled sites on the equator of each fruit using a penetrometer (GY-B, Mudanjiang Mechanical Institute, Heilongjiang, China) equipped with a flat probe (3 mm diameter). Firmness was expressed as the maximum force (N) attained during the penetration. Afterwards, a longitudinal wedge (from stem end to calyx end) of plum flesh was removed from each fruit and pressed through cheesecloth. The juice from the fruit was pooled. The SSC of the juice was measured using a digital refractometer (PAL-1, Atago Co., Tokyo, Japan).

2.3. Evaluation of flesh reddening and translucency

Plums (three replicates of 40 fruit) were longitudinally cut into halves for the evaluation of flesh reddening (Manganaris et al., 2008b; Candan et al., 2011) and flesh translucency (Navarro-Tarazaga et al., 2008; Khan et al., 2011), with some modification of the cited methods.

Flesh reddening was estimated visually as the percentage of color-changed area compared to the total surface area of each section on a scale where: 0 = no change; 1 = less than 10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; and 5 = more than 75%. The flesh reddening index was calculated using the following formula: reddening index = [$\sum$ (number of the fruit within the scale × each scale)/(total number of fruit × the highest scale)] × 100. Flesh translucency was estimated visually as the percentage of the affected area compared with the total surface area of each section on the same scale as described above. The translucency index was also calculated using the same formula.

2.4. Measurement of total anthocyanin content (TAC)

Flesh tissue (1 g) was homogenized on ice and extracted using 10 mL pre-cooled 1:99 HCl-methanol (v/v) for 2 h at 4 °C in the dark. After centrifuging at 10,000 × *g* at 4 °C for 15 min, the supernatant was collected for TAC determination according to the pH-differential method (Sharma and Sharma, 2015). The TAC was calculated using cyanidin-3-glucoside as the standard and expressed as mg kg^{-1} of fresh flesh.

2.5. Water-soluble pectin (WSP) and insoluble pectin (protopectin) determination

Flesh tissue (20 g) from plums harvested at mid and late maturity was powdered in liquid nitrogen, homogenized in 100 mL of 80% ethanol (v/v) and boiled for 30 min to extract low molecular weight solutes and to prevent autolytic activity. The insoluble material was vacuum-filtered at room temperature. The residue was re-extracted with 100 mL of 80% ethanol three more times as described above. The residue was allowed to dry at 40 °C, yielding the alcohol insoluble residue (AIR) of the cell walls. The AIR was used for the extraction of WSP and insoluble pectin (i.e., protopectin) according to Taylor et al. (1995) and Manganaris et al. (2008a).

The galacturonic acid content of each pectic fraction was assayed using the carbazole colorimetric method (Bitter and Muir, 1962), using a standard curve of galacturonic acid. The pectin content was calculated and expressed as grams of galacturonic acid equivalent per kg fresh flesh. The total pectin content of the fruit was obtained by adding the pectin content of the WSP with that of protopectin.

2.6. Measurements of ethylene production and respiration rate

Plums were removed from storage to 25 °C for 2 h. Eight plums representing a replicate were placed in a 2-L hermetically-sealable glass container and sealed with a rubber stopper for 60 min. One mL of headspace gas was injected into a gas chromatograph (7890F, Tianmei Co., Shanghai, China), equipped with a flame ionization detector (FID) and stainless steel column (inner diameter 2 mm × length 3 m) packed with activated alumina (80/100 mesh), to measure the concentration of ethylene (Pan et al., 2016). Ethylene production was calculated using a calibration curve and expressed as ng kg^{-1} s^{-1}.