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Internal browning disorder of eight pear cultivars affected by bioactive constituents and enzyme activity

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ABSTRACT

Internal browning (IB) is a disorder in pears that is frequently observed in some cultivars. The present research was carried out to study biochemical changes and IB disorder of pear fruit during storage and ripening. Eight pear cultivars harvested and stored at 1 °C up to 90 days. IB incidence, some bioactive compounds, polyphenol oxidase (PPO), peroxidase (POX), and superoxide dismutase (SOD) enzymes activities were measured during storage. IB increased during storage time but the susceptibility of cultivars was different. The ascorbic acid (AA), antioxidant capacity (AC) and SOD activity decreased while POX activity increased during storage but the rate of changes were different in studied cultivars. Total phenol (TP) and total flavonoid (TF) average content varied among pear cultivars and the highest TP and TF were observed in 'Bakhi' cultivars during storage. Fruit IB had positive correlation with the PPO activity, but negative correlation with TP, AC and AA.

1. Introduction

A number of physiological disorders limit pears quality during storage, including external CO_2 injury, flesh browning and core browning (Larrigaudière, Lentheric, Puy, & Pintó, 2004). Browning is an important disorder of pear fruit which can lead to considerable economic losses as the symptoms are internal and cannot be observed visually without cutting the fruit in half (Franck et al., 2007). Internal browning (IB) of the core and/or flesh usually occurs during handling, processing and storage in apple (Jang, Sanada, Ushio, Tanaka, & Ohshima, 2002) and pear (Crisosto, Garner, Crisosto, Sibbett, & Day, 1994). Various names have been applied to the disorder in pears since it has been difficult to establish clear differences on the basis of similar symptoms. The main factor influencing disorder development appears to be a combination of low temperature and excessive CO_2 concentrations during storage.

Browning is initiated in the core of the fruit and expands concentrically to the cortex tissue, but, except for extreme cases, the outer appearance of affected fruits is normal. In severe cases the affected tissue loses water to the adjacent tissue, and eventually cavities are formed. The browning phenomenon usually impairs the sensory properties of products because of the associated changes in color, flavor and softening (Kim, Kim, & Park, 2005). The important biochemical factors involved in tissue browning are total phenol (TP) concentration, polyphenol oxidase (PPO) and peroxidase (POX) enzymes, ascorbic acid (Amiot, Aubert, Nicolas, Goupy, & Aparicio, 1992; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994) and probably other bioactive compounds. A proposed mechanism for browning is the result of an enzymatic oxidation of polyphenol substances to o-quinones, a reaction catalyzed by PPO (Veltman et al., 1999).

In a fruit, the range and abundance of biochemical compounds can vary according to the growth period (Jiang et al., 2006), the year of harvest (Van der Sluis, Dekker, De Jager, & Jongen, 2001), the geographic location (McGhie, Hunt, & Barnett, 2005), the storage conditions (Rossle, Wijngaard, Gormley, Butler, & Brunton, 2010), and most importantly, genetic variation (Wojdylo, Oszmianski, & Laskowski, 2008). In other side, the susceptibility of pears to IB depends on geographical factors and orchard, storage conditions, cultivar (Amiot, Tacchini, Aubert, & Oleszek, 1995), growth year (Chen, Borgic, Sugar, & Mellenthin, 1986) and harvest date.

Food production showing considerable interest to develop products with protected health benefit compounds, such as strong antioxidant capacity, to handle the growing interest of consumers in the relationship between diet and health (Kevers, Tabart, Defraigne, & Dommes, 2011). The objective of the current study was to evaluate some pears fruit bioactive constituent changes during cold storage. In addition, the incidence of IB disorder in 8 pear cultivars were monitored and the involvement of PPO, POX,

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superoxide dismutase (SOD) enzymes and ascorbic acid (AA), antioxidant capacity (AC), TP and total flavonoids (TF) in IB development were investigated.

2. Materials and methods

2.1. Plant sources and storage condition

Eight pear fruit cultivars including: 'Siv harmah', 'Sorah', 'Bakhi', 'Sibri Shahini', 'Beleshti', 'Hamrobah', 'Sibri Sarchi', and 'Shah miveh' cultivar were harvested at commercial maturity stage. After harvest, fruit immediately transferred to postharvest lab and graded to ensure that fruit were of uniform size and free of blemishes. Fruit of each cultivar were divided into four groups each contain 30 fruit, packed in boxes and stored in cold storage at 1 ± 1 °C with 80–90% relative humidity.

A bag from each cultivar were sampled randomly at harvest and after 30, 60, and 90 days and kept at 20 °C for 72 h. Fruit of each bag randomly divided into 3 groups of each 10 fruit, each group assumed as a replication. Approximately 1/16th segments from two opposite sides of the fruit were immediately frozen in liquid nitrogen and stored at −80 °C until used for extraction and analysis.

2.2. IB evaluation

Each fruit were then sliced at least three times to reveal the presence or absence of internal disorders, flesh breakdown and browning (Larrigaudière et al., 2004). The severity index of disorders were determined as follow: ((% fruit with slight disorder × 1) + (% fruit with medium disorder × 2) + (% fruit with severe disorder × 4))/4.

2.3. Enzymes extraction and assay

The frozen samples were ground in a pre-chilled mortar in the presence of liquid nitrogen, and 1 g of the resulting powder was then homogenized with 3 mL of extraction medium consisting of 50 mM phosphate buffer (pH 7), triton X-100 and polyvinylpyrrolidone (PVP). After centrifugation of the homogenate at 10000*g* for 30 min, the supernatant was used for enzyme assay. All steps to obtain enzyme preparations were carried out at 4 °C. PPO activity was assayed using the method of Kahn, 1975. Aliquots of supernatant (100 μL) were added to 1.4 mL assay solution (100 mM citrate, 200 mM phosphate buffer pH 5.0) containing catechol at a final concentration of 0.05 M. The increase in absorbance was monitored at 420 nm for 2 min at 24 °C on a spectrophotometer and activity of enzyme was expressed as Unit mg^{-1} protein of the homogenate. The protein concentration in each sample was determined by the method described in the literature using bovine serum albumin (BSA) as a standard (Bradford, 1976).

Antioxidant enzymes (POX and SOD) were extracted using 1 g of frozen sample homogenized in 6 mL of freshly prepared 25 mM phosphate buffer (pH 7). The homogenate was centrifuged at 18000*g* for 15 min, and the supernatant was used as a source of crude enzyme. All steps to obtain enzyme preparations were carried out at 4 °C. POX activity was determined by the rate of guaiacol oxidation in the presence of hydrogen peroxide at 470 nm for 1 min, as described previously (Ghanati, Morita, & Yokota, 2002). The reaction mixture contained 25 mM phosphate buffer (pH 7), 28 mM guaiacol, and enzyme extract in a 3 mL assay volume, and the reaction was initiated by adding hydrogen peroxide at a final concentration of 5 mM. The enzyme activity was expressed as units of enzyme per milligram of protein. SOD activity was determined by measuring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm after exposure to a light for 30 min. The assay mixture contained 25 mM phosphate buffer (pH 7), 12 mM L-methionine, 75 μM NBT, 1 μM riboflavin, 50 mM Na-carbonate (pH 10.2), and enzyme extract in a total volume of 3 mL. The blank mixture had the same composition but was kept in the dark. A total of 1 unit of SOD activity is defined as the amount of enzyme that inhibits the NBT photoreduction by 50% under the assay conditions.

2.4. TP and TF assay

TP concentrations was measured by homogenizing 0.7 g of frozen tissue from each replicate with 2 mL of ice cold 1% HCl–methanol solution and then centrifuged at 4 °C for 10 min at 12000*g*. The supernatant was collected and used for phenol determination (Koushesh Saba, Arzani, & Barzegar, 2012). The TP content in the extracts was determined according to the Folin–Ciocalteu procedure, using gallic acid for the standard curve. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of FW.

TF content was measured as described previously (Kevers et al., 2007). Briefly 1 mL diluted methanol extract was mixed with 1 mL of reagent (2% $AlCl_3$ $6H_2O$ in methanol) and the absorbance was measured at 430 nm after 10 min. Quercetin (Sigma) was used as standard, and results were expressed as mg of quercetin equivalents per 100 g of FW.

2.5. AA measurement

Diluted samples in 5% metaphosphoric acid or ascorbic acid calibration solutions (600 μL) were mixed with 500 μL of 10% metaphosphoric acid, 300 μL of citrate buffer (pH 4.2), and 300 μL of the 2,6-dichloroindophenol (DCIP) (0.1 mg mL^{-1}). The optical density blanching was used; for each sample, the blank value was determined after the addition of 60 μL of ascorbic acid (1 mg mL^{-1}) with the aim to measure the interference due to the sample color. A standard curve was prepared using a series of known ascorbic acid (AA) concentrations. The results were expressed as mg of AA per 100 g of fresh weight (FW) (Kevers et al., 2007).

2.6. AC measurement

Antioxidants were extracted from the pear samples using the same protocol as that described above for phenol extraction. AC was determined by the 2,2-diphenyl-1-picryl-hidrazyl (DPPH) radical-scavenging method according to Kevers et al. (2007). The absorbance was measured at 517 nm, using a spectrophotometer (UV-S2100). Total antioxidant activity was expressed as the percentage inhibition of the DPPH radical and was determined by following equation.

$$AC = \frac{\text{ABS Sample} - \text{ABS Control}}{\text{ABS Control}} \times 100$$

where AC is the antioxidant capacity and ABS is the absorbance.

2.7. Statistical analysis

Data for the analytical determinations were subjected to analysis of variance (ANOVA) using MSTATC software. Sources of variation were storage duration (days) and cultivars. Least significant difference (LSD) test at $P = 0.05$ was used to compare means between genotypes or cultivars at each sampling stage. Pearson correlation was used to evaluate relationship between measured parameters. Regression within graphs and model fits were calculated using SigmaPlot 12.0 (Systat Software, San Jose, CA).