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Accumulation of lignin in relation to change in activities of lignification enzymes in loquat fruit flesh after harvest

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Abstract

Loquat (*Eriobotrya japonica* Lindl.) fruit are non-climacteric and have a short postharvest life. During postharvest ripening over 8 d at 20 °C, fruit firmness increased and showed a positive correlation with accumulation of lignin in the flesh. Among the enzymes associated with lignin synthesis, phenylalanine ammonia lyase (PAL) activity increased rapidly during the first 3 d after harvest and then declined in the fruit flesh, while cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD) activities showed a persistent rise over the whole 8 d. Accumulation of lignin in flesh tissue was also positively correlated to activities of CAD, guaiacol-POD (G-POD) and syringaldazine-POD (S-POD). Cellulose content in flesh tissue decreased and showed a significant negative correlation with lignin content. Where fruit ripening was enhanced by ethylene treatment, or retarded by low temperature or use of 1-methylcyclopropene (1-MCP), the inhibitor of ethylene reception, firmness, lignification and the enzyme activities were consistently enhanced or retarded accordingly. Our results suggest that increase in firmness of loquat fruit during ripening is a consequence of tissue lignification, a process associated with increases in PAL, CAD and POD activities, and might involve a coordinated regulation of lignin biosynthesis and cellulose hydrolysis.

Keywords: Cellulose; Cinnamyl alcohol dehydrogenase; Ethylene; Lignification; Lignin; Loquat; 1-MCP; Phenylalanine ammonia lyase; Peroxidase; Postharvest; Senescence

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a non-climacteric fruit native to China and its postharvest ripening is characterized by an unusual increase in firmness and toughness of the flesh and a decrease in juiciness (Zheng et al., 2000; Ding et al., 2002; Cai et al., 2006). Ding et al. (2002) showed that the main causes of loquat fruit quality loss after harvest were internal browning, dry pulp tissue and adherence of peel and flesh, and suggested that these disorders might be the result of tissue lignification, although no data on this were provided. Zheng et al. (2000) also observed increases in postharvest firmness at low temperatures, but described this as a chilling injury symptom where phenylalanine ammonia lyase (PAL) activity, lignin and fiber contents all increased at low temperature, along with adherence of peel and flesh, and development of a leathery and juiceless pulp. We have reported that loquat fruit firmness increased steadily after harvest at 20 °C and thus is not a specific low temperature response (Cai et al., 2006).

An increase of lignification in fruit tissue has been found in other fruit, but mainly associated with impact damage. For example, increased firmness of damaged mangosteen fruit was found to be related to enhanced lignin biosynthesis (Ketsa and Atantee, 1998). The characteristics of lignin differ among cell walls, tissues, and plant organs (Grabber et al., 2004). It comprises polyphenolic polymers derived from the oxidative polymerization of different monolignols, including *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol via a side pathway of phenylalanine metabolism leading to lignin

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synthesis (Whetten and Sederoff, 1995). Lignin synthesis involves phenylalanine ammonia lyase, PAL (EC 4.3.1.5), cinnamyl alcohol dehydrogenase, CAD (EC 1.1.1.195) and peroxidase, POD (EC 1.11.1.7) (Whetten and Sederoff, 1995; Boudet, 2000).

PAL, in catalysing deamination of phenylalanine to *trans*-cinnamate, is believed to be the critical enzyme controlling accumulation of lignin in plants (Lewis et al., 1999), although in studies in cherimoya fruit, increases in PAL activity do not necessarily result in an increase in lignin (Assis et al., 2001). CAD catalyzes the last step of the monolignol pathway, while POD catalyzes the polymerization of monolignol to complete the process of lignification (Imberty et al., 1985). Although these enzymes are all part of the lignification pathway, the extent to which they may regulate the pathway is different in different tissues and not yet clear.

Lignin is deposited within the cell wall carbohydrate matrix, and thus cellulose and hemicellulose microfibrils may have a mechanical influence on lignin development (Donaldson, 2001). Atalla and Agarwal (1985) have shown that the aromatic rings of lignin are often oriented within the plane of the cell wall. The incorporated lignin imparts rigidity to cell walls (Hu et al., 1999) providing a close connection between the carbohydrate matrix and the cellulose polymers (Boerjan et al., 2003). This supports a role for lignin in helping maintain cell wall structure. However, the interaction between lignin and cellulose deposition is not known.

With evidence of postharvest firmness increases in loquat fruit, and the suggestion that lignification may be involved, we investigated the changes in lignin content of loquat flesh in ripening fruit and its relation to PAL, CAD and POD activities, cellulose content and cellulase (EC 3.2.1.4) activity under different conditions, using ethylene, 1-MCP and low temperature to modify ripening rates. Our results suggest that the increase in loquat fruit firmness involves a coordinated regulation of lignin biosynthesis and cellulose hydrolysis.

2. Materials and methods

2.1. Fruit and storage

Loquat fruit (*Eriobotrya japonica* Lindl. cv. Luoyangqing) were picked at commercial maturity in Huangyan, Zhejiang Province, China, and transported to the laboratory on the day of harvest. In the laboratory the fruit were screened for uniform size and maturity (based on color) and absence of mechanical damage.

Fruit were divided into three lots each of 240 fruit, and each lot was divided into three replicates of 80 fruit. Each set of three replicates was given one of three treatments: (1) control fruit (no 1-MCP or ethylene), (2) 5 μL/L 1-MCP, and (3) 100 μL/L ethylene applied for 12 h in an airtight 6 L container. The treated fruit were then stored at 92–98% relative humidity at 20 ± 0.5 °C for 8 d. A 1-MCP was supplied as Ethybloc™ (a.i. 0.14%) with the 1-MCP released from the powder by water. The procedure for release of 1-MCP was as described by Jiang et al. (2001), and the concentrations were estimated based on quantitative data from Y. Jiang (personal communication).

For the low temperature experiment, 300 fruit (3 × 100 fruit replicates) were stored at 12, 5 and 0 °C for up to 25 d. Fruit were sampled from the treated replicates for various measurements and assays, at two intervals, as described below. Data are presented for 8 d at low temperature only, to provide a comparison with the 20 °C controls.

2.2. Fruit firmness

Firmness was measured on 10 individual fruit per treatment, combining 3, 3, and 4 fruit from the three replicates, respectively, each day over the 8 d for the 20 °C experiment, and at longer time intervals as indicated in the results, for the low temperature experiment. The measurements were made using a TA-XT2i (Stable Micro Systems, England) texture analyzer with a probe 5 mm in diameter, a penetration depth of 4 mm, and rate of penetration 1 mm/s. Measurements were made on two sides of each fruit after removal of a small piece of peel.

2.3. Lignin determination

To measure lignin, five fruit from each treatment replicate (3 × 5 per treatment) were peeled, cut into small pieces, and the bulked fruit samples (from the five fruit) were frozen in liquid nitrogen and stored at −20 °C until analysis. Samples were taken daily for the 8 d of the 20 °C experiment, and at longer intervals as shown in the results, for the low temperature experiment.

Lignin was extracted and measured by the method of Bruce and West (1989). Three grams of frozen tissue powder was homogenized in 10 mL 99.5% (v/v) ethanol and centrifuged at 20,000 × *g* for 20 min. The pellet was dried overnight at room temperature. Fifty milligrams of dried residue were placed in a screw-cap tube, and then 5 mL of 2 M HCl and 0.5 mL of thioglycolic acid were added. The sample was heated at 100 °C for 8 h, then cooled on ice and centrifuged at 20,000 × *g* for 20 min at 4 °C. The pellet was washed with distilled water, and re-suspended in 5 mL 1 M NaOH. The solution was agitated gently at 25 °C for 18 h, and then centrifuged at 20,000 × *g* for 20 min, and the supernatant then transferred to a test tube. One milliliter of concentrated HCl was added to the test tube and the lignin thioglycolic acid was allowed to precipitate at 4 °C for 4 h. After centrifugation at 20,000 × *g* for 20 min, the pellet was dissolved in 1 mL of 1 M NaOH. The absorbance was measured against a NaOH blank at 280 nm, and data expressed on a fresh weight basis.

2.4. Cellulose determination

The same frozen samples as used for lignin analysis were used for the cellulose assay. The method described by