Scientia Horticulturae 197 (2015) 72–89

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Proteomic analysis of postharvest peach fruit subjected to chilling stress or non-chilling stress temperatures during storage

Fang Yu, Xingfeng Shao*, Lina Yu, Feng Xu, Hongfei Wang

Department of Food Science and Engineering, Ningbo University, Ningbo, Zhejiang 315211, PR China

ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form 23 October 2015
Accepted 30 October 2015
Available online 17 November 2015

Keywords:
Proteomics
2-DE
Peach fruit
Storage temperature
Storage time

ABSTRACT

Proteome patterns from peach fruit subjected to chilling stress (5 °C) and non-chilling stress (10 °C) temperatures were compared by 2-dimensional gel electrophoresis (2-DE) and MALDI-TOF/TOF mass spectrometry. Among the 60 differentially expressed proteins detected by 2-DE, those involved in stress response and defense were the most abundant. Proteins associated with membrane stability, as well as sucrose content were reduced in fruit stored at 5 °C, possibly contributing to the development of chilling injury (CI) seen at 5 °C. In addition, small heat shock proteins and components of the antioxidant defense system were expressed at higher levels under chilling stress. In contrast, storage at non-chilling temperature (10 °C) promoted the softening of peach fruit accompanied by increased levels of expansin and pectate lyase. The expression of pathogenesis-related proteins also increased at 10 °C, in parallel with the relatively higher incidence of decay observed at this temperature.

1. Introduction

Peaches (*Prunus persica* L. Batsch), prized for their rich flavor and nutritional value, are also one of the most economically important fruit crops in the world. Because peaches deteriorate quickly at ambient temperature after harvest, low temperature storage is widely used to maintain quality and extend shelf life. However, peach fruit succumbs to chilling injury (CI) within 1–2 weeks when stored at 2–5 °C (Lurie and Crisosto, 2005). CI is characterized by internal browning, juicelessness, mealiness, failure to ripen normally and other imperfections (Crisosto and Labavitch, 2002; Lurie and Crisosto, 2005), resulting in unmarketable fruit.

Membrane integrity is strongly correlated with CI tolerance in plants (Wongsheree et al., 2009). Crawford and Braendle (Crawford and Braendle, 1996) found that ATP depletion contributes to membrane damage, as evidenced by increased electrolyte leakage under stress. In longan fruit, when high levels of ATP are maintained by treatment with pure oxygen, skin browning is decreased (Su et al., 2005). The enzymes involved in glycolysis and the tricarboxylic acid cycle are responsible for ATP production. Cytoplasmic triosephosphate isomerase has a role in glycolysis for production of ATP and C skeletons in growing potato leaves (Dorion et al., 2005). In addition, Zhang et al. (2010) reported that major allergen Pru p1, a lipid transfer protein, is related to membrane stability.

Enzymatic and metabolic antioxidants, including conventional and reformed antioxidants, also play a vital role in protecting cellular membranes from oxidative injury. Conventional antioxidants are divided into non-enzymatic metabolites and enzymatic scavengers such as glutathione S-transferase (GST) and phospholipid hydroperoxide glutathione peroxidase (PHGPx). Reformed antioxidants are soluble sugars and their associated metabolic enzymes. For example, exogenous γ-aminobutyric acid significantly increases CI tolerance of peaches by enhancing the activities of antioxidant enzymes such as GST, superoxide dismutase (SOD), and catalase (CAT) (Yang et al., 2011). Increased GST abundance may be a response to the increase in oxidative stress during papaya ripening (Nogueira et al., 2012). Expression of the PHGPx gene can be markedly induced by oxidative stress in rice (Li et al., 2000). Among the soluble sugars in peach fruit, sucrose protects membranes more effectively than reducing sugars (Wang et al., 2013). It may serve as an osmoprotectant, protect the cytomembrane, scavenge reactive oxygen species, act as a signaling molecule, and regulate gene expression (Ruelland et al., 2009; Van den Ende and Valluru, 2008) to protect peaches against stress. Regulation of carbon metabolites by the gene silencing of soluble inorganic pyrophosphatase in leaves may decrease drought tolerance in *Nicotiana benthamiana* (George et al., 2010).

Peach fruit is susceptible to attack by pathogens during ripening. Pathogen invasion provokes systemic acquired resistance, result-

* Corresponding author. Fax: +86 574 87608347.
E-mail address: shaoxingfeng@nbu.edu.cn (X. Shao).

http://dx.doi.org/10.1016/j.scienta.2015.10.045
0304-4238/

ing in the activation of pathogenesis-related (PR) proteins. PR proteins are classified into 17 PR-protein families based on their structural and functional features (Zubini et al., 2009). Among them, PR-1 proteins are commonly used as markers for systemic acquired resistance (Van Loon and Van Strien, 1999). Glucan endo-1, 3-beta-glucosidase and chitinase function in plant defense by depolymerizing the cell walls of pathogenic fungi (Saikia et al., 2005). Overexpression of these proteins in transgenic plants results in enhanced resistance to fungal pathogens (Ding et al., 2002). Allergen Pru du 1.01 and major allergen Pru p 1 from peach are members of the PR-10 protein family, and play a key role in defense against abiotic stress (Chadha and Das, 2006). Elicitor-responsive protein is associated with pathogen defense and stress response as well (D'Ambrosio et al., 2013). Transcription of elicitor-responsive genes are specifically elevated in response to the rice blast fungus (Kim et al., 2000).

Fruit ripening is accompanied by softening during prolonged cold temperature storage. Numerous enzymes are implicated in softening, among them, expansin and pectate lyase. Expansin is a key regulator of cell wall extension (Li et al., 2003), allowing cell wall elongation and accumulation of solutes within vacuoles. Pectate lyase is involved in ripening-related pectin degradation. Antisense inhibition of the pectate lyase gene in strawberry results in prolonged fruit firmness (Santiago-Doménech et al., 2008).

Recent advances in proteomic methods have made it feasible to analyze whole-cell protein data and identify individual factors that underlie fruit quality. Several proteomic studies have focused on peach fruit and postharvest treatments involving treatment with heat (Lara et al., 2009; Zhang et al., 2011), 1-methylcyclopropene (1-MCP), and ethephon (Zhang et al., 2012), aimed at improving peach fruit organoleptic and shelf-life features. Storage temperature of peach (0 °C vs. 5 °C) (Zhang et al., 2010) and nectarine (0 °C vs. 20 °C) (Giraldo et al., 2012) have also been investigated to understanding of how fruit responds to chilling stress. In this study, proteomic profiles from peach fruit stored at chilling stress (5 °C) and non-chilling stress (10 °C) temperatures at 0, 14 and 28 days were compared using 2-DE, followed by protein identification using an ABI 5800 MALDI-TOF/TOF plus mass spectrometer. Our results provide a better understanding to reveal proteins potentially involved in the response of peach fruit to low temperature.

2. Materials and methods

2.1. Plant materials and treatments

Peaches (*P. persica* L. Batsch cv. 'Yulu') were hand-harvested at commercial maturity from a plantation located in Fenghua, Zhejiang Province, China, and immediately transported to the laboratory. Fruits were pre-cooled and manually selected for uniform color, size and absence of mechanical damage, then randomly divided into two groups. The control group was stored at 10 °C, and the treatment group was placed at 5 °C to induce development of CI symptoms. Fruit was stored at approximately 95% relative humidity for up to 28 days. Thirty fruits (three replicates, each with 10 fruits) were randomly sampled at 0, 14, and 28 days from the 5 °C and 10 °C groups. Flesh slices without skin were collected from each sample, frozen in liquid nitrogen, and stored at –40 °C for further analysis.

2.2. Determination of fruit quality

CI index was determined according to Meng et al. (2009), using the following scale. 0: no signs of internal browning. 1: browning area less than 25%. 2: browning area between 25% and 50%. 3: browning area more than 50%. CI index was calculated as $\sum$(CI scale × number of corresponding fruit at the CI scale)/(total number of fruit × highest CI scale)

Decay index was measured according to Zheng et al. (2007), using the following scale. 0: no visible decay. 1: decay area less than 1%. 2: decay area between 1% and 20%. 3: decay area between 20% and 50%. 4: decay area more than 50%. Decay index was calculated as $\sum$(decay scale × number of corresponding fruit at the decay scale)/(total number of fruit in the treatment group × highest decay scale).

Fruit firmness was assessed on two paired sides of ten fruits without skin using a TA-XT*plus* texture analyzer (Stable Micro System Ltd., UK) with a 5 mm diameter probe. The test speed was 1 mm/s and the puncture distance was 5 mm. Each reading was taken per fruit and a mean value was calculated.

Sucrose content was measured using the method described by Wang et al. (2013). Five grams of fruit flesh were ground with 0.5 mL of 54.8 g/L zinc acetate and 0.5 mL of 26.5 g/L potassium ferrocyanide. The homogenate was diluted to 25 mL and passed through a 0.22 μm membrane filter. A 20 μL aliquot was injected into a high performance liquid chromatography system (model 2695, Waters, USA) fitted with an amino column (Kromasil® 100A, Sweden) and a refractive index detector (model 2414, Waters, USA) operating at a flow rate of 1.0 mL/min at 35 °C, using acetonitrile/water (75:25, v/v) as mobile phase. Sucrose was identified and quantified by comparison with the retention times and peak areas of a pure sugar standard. The results were expressed as mg/g fresh weight (FW).

Soluble solids contents (SSC) were measured on the expressed juice from peach fruits using a hand-refractometer (WYT-4; Quanzhou Optical Instrument Co. Ltd., Quanzhou, China). Fresh tissue without peel (100 g) from peach fruit was homogenized in a Waring blender (HR2839; Philips, Zhuhai, China) in 100 mL distilled water for 1 min. A 20 g sample of the homogenate was placed in a 250 mL flask and heated at 75 °C for 30 min, then filtered. Titratable acidity (TA) was measured by titrating 50 mL of the filtered liquid to pH 8.1 with 0.1 M NaOH and calculating the result as percent malic acid.

The background color was measured by using a chromameter (CR-200, Minolta, Tokyo, Japan) according to the Commission Internationale de l'Eclairage LAB color parameters: L* indicated luminance, a* indicated mean redness (+) or greenness (−) and b* indicated yellowness of peel. Two readings of L* or a* values were recorded per sample and a mean value was calculated.

2.3. Protein sample extraction

Five grams of frozen mesocarp was ground to power in liquid nitrogen, suspended in 10 mL of 10% w/v TCA/acetone at –20 °C for 1 h, then centrifuged at 15,000 × *g* for 15 min at 4 °C. The pellet was suspended in cold phenol extraction buffer (0.7 M sucrose, 0.1 M KCl, 0.5 M Tris–HCl (pH 7.5) and 50 mM EDTA), an equal volume of phenol saturated with 0.5 M Tris–HCl (pH 7.5) was added, and the mixture was shaken for 30 min at 4 °C, then centrifuged at 5000 × *g* for 30 min. The upper phenolic phase was collected, extracted twice with the cold phenol extraction buffer and precipitated with five volumes of 0.1 M ammonium acetate in methanol at −20 °C for 1 h. Samples were centrifuged for 30 min at 5000 × *g* at 4 °C and pellets washed once with ice-cold methanol and twice with chilled acetone, then air dried and dissolved in lysis solution (9 M urea, 4% CHAPS, 1% DTT, 1% IPG buffer) at 30 °C for 1 h. The solution was then centrifuged at 15,000 × *g* for 15 min at room temperature. Protein concentrations in the extracted protein supernatants were determined by the Bradford method (Bradford, 1976). Samples were stored at −80 °C for further analysis.