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Heat acclimation reduces postharvest loss of table grapes during cold storage – Analysis of possible mechanisms involved through a proteomic approach

Ziming Wu [a,1], Xiaozhuan Yuan [a,1], Huan Li [a], Fei Liu [a], Yadan Wang [a], Jun Li [b], Han Cai [c], Yun Wang [a,*]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, China
[c] School of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China

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ABSTRACT

In this study, a combined biochemical and proteomic approach was used to investigate changes of fruit quality and protein expression profiles of grape berries upon hot water treatment (HWT) during the subsequent 45 days of cold storage. HWT obviously inhibited postharvest loss and chilling damage by reducing weight loss and membrane damage and slowing fruit softening. Proteomic analysis allowed the identification of 64 proteins regulated by heat treatment. Most of the up-regulated proteins in heat-treated grape berries were related to defense response and redox metabolism, suggesting a physiological adaptation to environmental stress. In addition, the increased expression of proteins associated with carbohydrate and energy metabolism immediately after HWT provided a molecular explanation for the rapid decline of soluble sugar content observed in heat-treated grape berries, which might be necessary for activation of the energy-demanding defense response to storage environmental stress, thereby reduced postharvest loss during storage at low temperature.

1. Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit crops worldwide. Grapes are rich in sugars, organic acids, vitamins, and essential minerals. However, table grapes are subject to serious water loss and decay during postharvest handling, possibly due to their soft texture and succulent flesh, which reduce the fruit quality, limits storage and marketing. Attempts have been made to maintain fruit quality and prolong postharvest shelf-life of table grapes, such as controlled- and modified-atmosphere storage (Candir et al., 2012), bio-control (Liu et al., 2010), edible coatings (Romanazzi et al., 2007), exogenous application of growth regulators (Zoffoli et al., 2009) and heat treatment (Sabir and Sabir, 2013).

Among these, heat treatments, such as hot water and hot air, are safe and environmentally friendly procedures with increasing acceptability in commercial operations (Lurie, 1998). Postharvest heat treatments were originally used for insect disinfestations and disease control, which can prevent the development of plant disease by inhibiting pathogen mycelia growth and structurally altering the epicuticular wax (Schirra et al., 2011). It has also been well documented that heat treatment was effective in retaining fruit quality and extending storage life, and alleviating chilling injury in cold-sensitive cultivars (Sivakumar and Fallik, 2013; Aghdam and Bodbodak, 2014). For example, heat treatment not only inhibited decrease in firmness, slowed down respiration and ethylene production and delayed de-polymerization of pectic substances (Luo et al., 2009), but also increased the content of phenolic and total antioxidative activity (Mirdehghan et al., 2006). In the last decade, researchers have paid increasing attention to identify and characterize molecular changes induced by heat treatment, which might be associated to disease control, cold injury protection and maintaining fruit quality (Aghdam and Bodbodak, 2014). Sapitnitskaya et al. (2006) reported that chilling tolerance was activated in heat-treated grapefruit by regulating stress genes and common chilling tolerance genes, such as heat shock protein, dehydrin, universal stress protein, superoxide dismutase, alcohol dehydrogenase and translation initiation factor. Lauxmann et al. (2012) observed that more than one hundred

* Corresponding author. Tel.: +86 511 88797059; fax: +86 511 88797201.
E-mail address: wangy1974@ujs.edu.cn (Y. Wang).
[1] Both authors contributed equally to this work.

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genes were regulated by heat treatment in peach fruit using a differential display technique and some of them showed similar changes to both heat treatment and cold storage, which indicated that heat treatment of peach fruit after harvest induced a cold response involving complex cellular processes.

Two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS) analysis based proteomic approach has been used successfully to improve our understanding of the molecular basis involved in fruit ripening, physiological disorders and fruit response to storage conditions (Hertog et al., 2011; Pedreschi et al., 2013). Perotti et al. (2011) studied the proteomic changes taking place in orange fruit induced by heat treatment during the subsequent cold storage and identified 28 proteins regulated by heat treatment, most of which were involved in defense, metabolism and storage. Recently, a comparative proteomic analysis in hot water treated citrus fruit was performed by Yun et al. (2013), and revealed that resistance associated proteins were up-regulated, but redox metabolism-related proteins were down-regulated by heat treatment. However, there is still a lack of molecular information about heat treatment and its possible role in maintaining fruit quality and protecting against environmental stress during postharvest storage, especially the immediate responses of fruit to heat treatment. Therefore, the aims of this work were: (i) to investigate physiological changes taking place in grapes (cv. Kyoho) induced by heat treatment during the subsequent cold storage, and (ii) to employ a proteomics approach to study the possible molecular mechanisms involved in this process. By combining these different sources of data, some important points of the molecular networks induced by heat treatment in grapes were examined.

2. Materials and methods

2.1. Plant materials and heat treatment

Grapes (*V. labruscana* cv. Kyoho) were harvested at optimum maturity (about 14% soluble solids content and 0.7% tartaric acid) from a vineyard in Jurong, China. Immediately after harvest, clusters were manually selected on the basis of uniform color, size, and firmness, while blemished and diseased fruit were discarded. Hot water treatments (HWT) were performed according to Kou et al. (2007) by dipping in a 45 °C warm water bath for 8 min, and controls were dipped in a 25 °C water bath for 8 min. After treatment, grapes were kept at room temperature with a relative humidity of 95% for 1 day, and then transferred into a temperature-controlled chamber and stored at 2 °C with a relative humidity of 95% for 45 days. Samples were collected at 1 day after HWT (0 day of cold storage) followed by sample collection at 15 and 45 days of cold storage. Three biological replicates (45 grape berries for each replicate) were taken randomly from each treatment for biochemical and proteomic analysis.

2.2. Fruit quality attributes and membrane leakage determination

Flesh firmness was determined using a texture analyzer (TX-XT2i, Stable Micro Systems, UK) with a 5 mm tip and recorded in Newtons (N). Membrane leakage was determined as described previously (Yang et al., 2012) using a digital conductometer (DDB-6200, Shanghai Leici Apparatus, Shanghai, China) and recorded as percentage of the conductivity of total tissue electrolyte. Soluble solids content (SSC) and titratable acidity (TA) were measured according to the method described by Yuan et al. (2014). SSC was determined using a WAY-2S Refractometer (Shanghai Precision & Scientific Instrument Co., Ltd., China) at 20 °C (expressed as %, w/w). TA was measured by titrating with 0.1 M NaOH up to pH 8.1, and the results were presented as percentage of tartaric acid.

2.3. Preparation of total protein extract

Total protein was extracted with phenol, followed by ammonium acetate precipitation as previously described (Yuan et al., 2014). Approximately 10 g of deseeded grape tissue taken from at least 15 fruit were ground to a fine powder in a mortar with liquid nitrogen. Five milliliters of extraction buffer (0.8 M sucrose, 0.1 M Tris–HCl pH 7.5, 10 mM EDTA, 0.1 M KCl, 2 mM PMSF and 2% (v/v) β-mercaptoethanol) were added and the mixture was incubated by shaking for 1 h at 4 °C. Then, an equal volume of Tris-saturated phenol (pH 7.9) was added and the mixture was incubated on a shaker for another 30 min at room temperature. The homogenate was centrifuged at 9000 × *g* for 10 min, and then the upper phenol phase was collected followed by washing twice with extraction buffer. The phenol phase was carefully transferred to a new tube, and the extracted proteins were precipitated by adding five volumes of cold methanol containing 0.1 M ammonium acetate. After centrifugation at 9000 × *g* for 30 min, the pellets were collected and washed three times with cooled methanol containing 0.1 M ammonium acetate and twice with cooled acetone. The resulting pellets were dried under vacuum at room temperature and dissolved in lysis buffer (7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 1% (w/v) DTT, 1% (v/v) CA, pH 4–7). Protein concentration was determined using the Bradford method with bovine serum albumin as standard.

2.4. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis (2-DE) was performed as described by Wang et al. (2013). Briefly, 700 μg of protein were loaded by passive rehydration overnight on immobilized pH gradient strips (pH 4–7, 17 cm). Focusing was performed with a PROTEAN isoelectric focusing system (Bio-Rad) for a total of 60 kVh at 17 °C. Prior to running the second dimensional electrophoresis, strips were equilibrated in 10 mL of SDS equilibration buffer (7 M urea, 2% (w/v) SDS, 0.075 M Tris–HCl pH 8.8, 30% (v/v) glycerol), first with buffer containing 1% (w/v) DTT for 15 min and afterwards with buffer containing 2.5% (w/v) iodoacetamide for 15 min. The second dimensional electrophoresis was run on a 12% polyacrylamide gel in an Ettan DALT System (GE Healthcare). Proteins spots in 2-DE gels were visualized by Coomassie blue staining. Images were analyzed with PDQuest software (Bio-Rad) using the protocol described in Yuan et al. (2014). Spot intensities were normalized as a percentage of the total volume in all the spots present in the whole gel. Normalized spot volumes were compared between the control and treatment samples on each gel, and only those spots with an average fold change greater than 1.5 at least at one time point after cold storage ($p < 0.05$) were considered as differentially expressed proteins.

2.5. In-gel digestion and mass spectrometric analysis

Protein spots of interest were manually excised from the gels and destained with 50 mM ammonium bicarbonate/50% (v/v) acetonitrile solution. The gel pieces were then reduced in 10 mM DTT for 45 min at 55 °C and alkylated in 55 mM iodoacetamide for 45 min at room temperature. After washing with 50% (v/v) acetonitrile in 0.025 mM NH_4HCO_3, the dried gel pieces were incubated overnight at 37 °C in 10 μL of 10 μg/L trypsin (Sigma–Aldrich, USA). The supernatant was collected for mass spectrometry analysis.

Mass spectrometry data were obtained using an Ultraflex matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Bruker, Germany). Database searching was performed using the MASCOT program (http://www.matrixscience.com) with the following parameters: NCBInr Viridiplantae