Food Chemistry 244 (2018) 93–101

Contents lists available at ScienceDirect

Food Chemistry

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

Lasiodiplodia theobromae (Pat.) Griff. & Maubl.-induced disease development and pericarp browning of harvested longan fruit in association with membrane lipids metabolism

Shen Zhang[a,1], Yuzhao Lin[a,1], Hetong Lin[a,*], Yixiong Lin[a], Yihui Chen[a], Hui Wang[a], John Shi[b], Yifen Lin[a,*]

[a] Institute of Postharvest Technology of Agricultural Products, College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[b] Guelph Food Research Center, Agriculture and Agri-Food Canada, Guelph, Ontario N1G 5C9, Canada

ARTICLE INFO

Keywords:
Longan (*Dimocarpus longan* Lour.) fruit
Postharvest disease
Pericarp browning
Lasiodiplodia theobromae (Pat.) Griff. & Maubl.
Cellular membrane permeability
Membrane lipids metabolism
Membrane phospholipids
Membrane lipids-degrading enzymes

ABSTRACT

Effects of *Lasiodiplodia theobromae* inoculation on disease development, pericarp browning and membrane lipids metabolism of harvested "Fuyan" longan fruit were studied. Compared with control fruit, *L. theobromae*-inoculated longans showed higher fruit disease index, pericarp browning index and cell membrane permeability, as well as higher activities of phospholipase D, lipase and lipoxygenase. Additionally, there were lower contents of membrane phospholipids but higher content of phosphatidic acid, and lower level of unsaturated fatty acids but higher level of saturated ones with lower ratio of unsaturated fatty acid to saturated fatty acid and lower index of unsaturated fatty acids in pericarp of *L. theobromae*-inoculated longans. These results suggested that *L. theobromae*-induced disease development and pericarp browning of harvested longans might be attributed to the damaged cellular membrane structural integrity, induced by the activated membrane lipids-degrading enzymes increasing the degradation of membrane phospholipids and unsaturated fatty acids in pericarp of harvested longan fruit.

1. Introduction

Longan (*Dimocarpus longan* Lour.), the favored and valued subtropical fruit in China, is consumed and cultivated globally (Lin et al., 2013; Lin, Lin, Lin, Ritenour, et al., 2017). The mature and harvest period in high temperature season with high humidity leads to vigorous physiological and pathological activities of harvested longan fruit, facilitating the senescence and fruit rot characterized by the developments of pericarp browning and disease, which greatly affects its storability, obstacle to transport, and shelf life (Jiang, Zhang, Joyce, & Ketsa, 2002; Lin et al., 2014, 2018).

The compartmentalization of cell membrane system was believed to be a considerable factor in plant cell preventing browning under regular circumstance, for its prevention on polyphenol oxidase and phenolic substrates from contact triggering enzymatic browning (Duan et al., 2007; Holcroft, Lin, & Ketsa, 2005; Lin et al., 2016). Literature revealed that increasing the degradation and peroxidation of membrane lipids during ripening and senescence or under stress could damage the structural integrity of cell membrane system, featured by the rise of cell membrane permeability, the degradation of membrane phospholipids, the decrease of unsaturated fatty acids (USFAs) and the increase of saturated fatty acids (SFAs) in cell membrane (Lin et al., 2016; Yi et al., 2008, 2009; Zheng & Tian, 2006). These alterations of membrane lipids could be attributed to the actions of membrane lipids-degrading enzymes such as phospholipase D (PLD), lipase, and lipoxygenase (LOX) (Liu et al., 2011; Yi et al., 2008, 2009). PLD was found capable of directly catalyzing the hydrolysis of phospholipids to start their degradation, and the hydrolysates could be further degraded to free fatty acids (FFAs) under the action of lipase (Wang et al., 2013). LOX could initiate the peroxidation of cell membrane lipids via exclusively catalyzing the oxygenation of polyunsaturated fatty acids (PUFAs) generating peroxidation products (Lin et al., 2016; Lin, Lin, Lin, Shi, et al., 2017; Yi et al., 2008). Those peroxidation products might attack the cell membrane in turn, resulting in the loss of compartmentalization of cell membrane system and consequential enzymatic browning (Cai et al., 2015; Lin et al., 2016; Zhang et al., 2010). It had been found that the activated PLD and LOX damaging cellular membrane structural integrity might account for the accelerated developments of pericarp

* Corresponding authors.
E-mail addresses: hetonglin@163.com, hetonglin@126.com (H. Lin), yifenlin@126.com (Y. Lin).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2017.10.020
Received 15 July 2017; Received in revised form 4 October 2017; Accepted 6 October 2017
Available online 07 October 2017
0308-8146/

browning and disease in *Peronophythora litchii*-infected litchis (Yi et al., 2008). Furthermore, the delayed developments of pericarp browning and disease of *P. litchii*-infected litchi fruit by exogenous supply of ATP might be associated with better membrane integrity due to suppressing the activities of PLD and LOX, and maintaining higher levels of USFAs (Yi et al., 2008, 2009). Therefore, pathogen infection could be regarded as a key factor causing the damage of cellular membrane structure, and then promoting the development of browning in harvested fruit.

In addition, *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. was reported to be a primary pathogenic fungus causing disease development and fruit rot of China's longan fruit during postharvest storage (Zhang et al., 2017). However, rare effort to date has been put into *L. theobromae*-induced structural change in cellular membrane with regard to the degradation of cellular membrane lipids and its relation to the developments of pericarp browning and fruit disease of harvested longans. Therefore, this work focused on the effects of *L. theobromae*-infection on cell membrane permeability, activities of PLD, lipase and LOX, and corresponding changes in membrane phospholipids and compositions of fatty acids (FAs) in pericarp of harvested longans, in relation to disease development and pericarp browning. This study aims to clarify the mechanism of *L. theobromae*-infection inducing disease development and pericarp browning of stored longans in a perspective of cellular membrane lipids metabolism.

2. Materials and methods

2.1. Materials and treatments

Commercially mature fruit of "Fuyan" longan (*Dimocarpus longan* Lour. cv. Fuyan) were harvested from Nan'an City, Fujian province, China. The fruits were transported to the laboratory under ambient temperature at 25 °C within 3 h. The fruits selected for test were free of blemishes or disease, and in uniform maturity, color, shape, and size. Fruits were sterilized with 0.5% sodium hypochlorite solution for 10 s and air-dried at 25 °C for about 1 h before the following *L. theobromae* inoculation.

The preparation and inoculation of *L. theobromae* were performed according to our previously published work (Zhang et al., 2017). In detail, *L. theobromae* was cultured on oats bran medium at 28 °C, 90% relative humidity, under continuous illumination for 21 days to obtain mature spores. The spore suspension was prepared at concentration of 1×10^5 spores mL^{-1}, which was calculated with hemacytometer under a light microscope. For inoculation, half of the fruits were immersed into the *L. theobromae* spore suspension for 5 min, and another half were immersed into distilled water for 5 min and defined as control.

After inoculation, 30 polyethylene film bags (0.015 mm thickness, 50 fruits for each bag) were used in packing the fruits of each treatment. The fruits were then stored at constant temperature of 28 °C and relative humidity at 90%. Samples of 3 bags (150 fruits) per treatment were employed randomly on daily basis for evaluation of disease development and pericarp browning, and analysis of cell membrane lipids metabolism. Triple assessments were performed on all the indices.

2.2. Assessments of fruit disease index and pericarp browning index

Fruit disease development was measured according to the method of Chen et al. (2014). The fungal growth area on the lesion proportion on surface of 50 individual longan fruits was measured and defined to five disease scales. The calculation of fruit disease index was conducted according to the method of Chen et al. (2014).

Pericarp browning development was evaluated with the method of Lin et al. (2015). The total browning area on inner pericarp of 50 individual longan fruits was measured and defined to six scales. The calculation of pericarp browning index was conducted according to the method of Lin et al. (2015).

2.3. Determination of pericarp cell membrane permeability

Cell membrane permeability was determined with the method of Chen et al. (2015). The conductivities of watery extract of longan pericarp tissue before complete cell membrane damage (C_1) and after that (C_2) were measured at 28 °C with a conductometer (Model 3173, Shanghai Electronics Co., Ltd., China). Relative leakage rate was employed to represent cell membrane permeability, expressed as (C_1/C_2) × 100%.

2.4. Assays of activities of PLD, lipase and LOX in pericarp

PLD activity was determined with the method of Liu et al. (2011). Briefly, PLD was extracted from pericarp tissue (2 g) of 10 longan fruits. The enzymatic reaction was carried out for 1 h at 28 °C with phosphatidyl choline as substrate. The product was combined with 1% (*w/v*) Reinecke's Salt. The generated sediment was dissolved in acetone for absorbance monitoring at 28 °C and 520 nm with a spectrophotometer (Model T6, Beijing Purkinje General Instrument Co., Ltd., China). One unit of PLD activity was defined as the amount of the enzyme that caused 0.1 of the absorbance change per hour.

Lipase activity was determined following the method described by Liu et al. (2011). Briefly, lipase was extracted from pericarp tissue (2 g) of 10 longan fruits. The enzymatic reaction was conducted for 30 min at 25 °C with α-naphthyl acetate as substrate, followed by adding 0.15% (*w/v*) Fast Blue B Salt as chromogenic agent. The absorbance of the solution was recorded at 25 °C and 520 nm with the spectrophotometer. One unit of lipase activity was defined as the amount of the enzyme that caused 0.001 of the absorbance change per minute.

The method of Lin et al. (2016) was employed for assaying LOX activity. Briefly, after LOX extraction from pericarp tissue (2 g) of 10 longan fruits, the enzymatic reaction was performed at 30 °C with sodium linoleate as substrate. The absorbance of the solution after reaction at 30 °C and 234 nm was recorded with the spectrophotometer. One unit of LOX activity was defined as the amount of the enzyme that caused 0.1 of the absorbance change per minute.

The method of Bradford (1976) was employed to measure the protein content. The unit of $U \cdot mg^{-1}$ protein represented the activities of PLD, lipase and LOX.

2.5. Determination of membrane phospholipids in pericarp

The method of Xu et al. (2016) was applied for the extraction and content determination of the phospholipids, with some modifications. 5 g of pericarp tissue from 30 fruits was ground within liquid nitrogen and then mixed with 15 mL of 2:1 (*v/v*) chloroform/methanol solvent for ultrasonic extraction at 4 °C for 1 h. The mixture was then centrifuged at 10,000 × *g* and 4 °C for 20 min. The chloroform extract was collected and combined with 1 mL of acetone for 2 min of vortex shaking, which was repeated thrice. After dried with nitrogen gas, 1 mL of chloroform/methanol solvent was added to the extract for Shimadzu-2030C high performance liquid chromatography (HPLC, Shimadzu Corporation, Japan) analysis with a Shimadzu LT II evaporative light scattering detector (ELSD, Shimadzu Corporation, Japan). An Inersil SIL 100A column (4.6 × 250 mm) was used for the assay at 30 °C and 254 nm, with mobile phase consisted of *n*-hexane/isopropanol/methanol/1% acetic acid (4:9:5:2, *v/v/v/v*). The mixture of phosphatidylcholine (PC), phosphatidylinositol (PI) and phosphatidic acid (PA) (Larodan Fine Chemicals, Sweden) was used as external standard for identification and content calculation of individual phospholipids.

2.6. Determination of membrane fatty acid composition and contents in pericarp

FAs extraction, methyl esterification and determination were performed according to the method of Lin et al. (2016). Briefly, FAs from