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Gamma radiation control quality and lignification of bamboo shoots (*Phyllostachys praecox* f. *prevernalis.*) stored at low temperature

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ABSTRACT

Bamboo shoots are highly nutritious but possess poor shelf life after harvest due to rapid decline of tenderness mostly result from lignification processes. Present investigation was performed to evaluate the potential of gamma radiation as a postharvest technology to preserve bamboo shoots. The effects of 0.5 kGy gamma radiation on quality and lignification of bamboo shoots during storage at 2 °C were investigated. The results showed that gamma radiation reduced the decay rate of bamboo shoots by 71% at the end of storage. It also significantly retarded ethylene production through controlling the activities of ACC synthase (ACS) and ACC oxidase (ACO), which may contribute to inhibition of lignin synthesis. Meanwhile, gamma radiation decreased the activities of phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD), which are associated with lignin accumulation, thus reduced lignin content by 12.5% than that of the control after twenty-eight days of storage. These results demonstrated the potential of the gamma radiation as a promising postharvest technique to maintain the quality and inhibit the lignification of bamboo shoots.

1. Introduction

Bamboo shoots are of considerable nutritional value to human health since they are rich in functional nutrients like dietary fiber, high-quality protein, vitamins, bioactive compounds and low in fat and sugar contents (Nirmala et al., 2014; Choudhury et al., 2012). So the consumption of bamboo shoots benefits in improving digestion, relieving sweating and hypertension, preventing cardiovascular diseases and cancer (Nirmala et al., 2014). Apart from the Asian countries such as China, Japan, and Philippines, where it is frequently used as common food material, bamboo shoots have become very popular in many other countries where bamboo shoots are mostly imported (Nirmala et al., 2007). Now processed bamboo shoots account for a large proportion in the market, while fresh bamboo shoots have much better taste and higher nutritional value. On the other hand, various detrimental changes was induced during postharvest handling (Kleinhenz et al., 2000), leading to lignification that mainly contributes to the subsequent rapid deterioration (Cheng, 2006), while the deterioration of bamboo shoots is characterized by an unusual increase in firmness and stiffness of the edible parts of shoots. There was other report showing that the unusual firmness increase in bamboo shoots may be the result of tissue lignification from the harvest wound (Xi et al., 2001; Luo et al., 2008). Actually, lignification, the biochemistry process of sealing a plant cell wall by lignin deposition, often occurs during the course of normal tissue development (Bubna et al., 2011). Phenylalanine ammonia-lyase (PAL) is the first enzyme involved in lignin cell wall deposition, cinnamyl alcohol dehydrogenase (CAD) is the enzyme catalyzing the conversion of coniferaldehyde into coniferyl alcohol, while peroxidase (POD) is involved in the last step for the polymerisation of cinnamyl alcohols to form lignin in loquat fruit during cold storage (Cai et al., 2006). Therefore, delaying the lignification should be an effective way to reduce quality loss of bamboo shoots during transportation.

In local markets around Southeast and East Asia, postharvest bamboo shoots are usually stored and sold at ambient temperatures and unpackaged though shelf life of fresh bamboo shoots are usually limited to less than three days. Therefore, proper measures need to be taken to conserve the quality of harvested bamboo shoots and delay its lignification urgently. Some methods have been employed to prevent lignification and maintain the quality of harvested bamboo shoots for longer time. Modified atmosphere packaging (Shen et al., 2006), for example, can extend shoots' storage life in some degree, but anaerobic respiration tends to be promoted in this way. Antimicrobial and enzymatic antibrowning film coating (Badwaik et al., 2014), nitric oxide treating (Yang et al., 2010), 1-methylcyclopropene (1-MCP) treating (Luo et al., 2007)

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and heat treatment (Luo et al., 2012) are also effective means for freshness-keeping and lignification-postponing. But due to high cost and technical problems, they are still not commercially practical.

Irradiation has been approved for many years by the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) for use on fruits and vegetables at doses up to 1.0 kGy for quality control and safety improvement (Fan and Mattheis, 2001). Gamma radiation alone and in combination with refrigeration as a hurdle technology has been proven an efficient way to increase storability and extend the shelf life of numerous fruits and vegetables, such as blueberry (Perkins-Veazie et al., 2008), wild edible mushroom (Fernandes et al., 2013), and ash gourd (Tripathi et al., 2013). As a technique steadily gaining worldwide acceptance, gamma radiation will become a versatile postharvest tool before long (Mahto and Das, 2014). To the best of our knowledge, the study of bamboo shoots treated with gamma radiation has not been reported so far. To evaluate its ability as a postharvest technique to control quality loss and postpone lignification of harvested bamboo shoots and to clearly understand how gamma radiation affects the lignification process, effects of 0.5 kGy gamma radiation on firmness, decay rate, total sugar (TS) and reducing sugar (RS) content, respiration rate and ethylene production, lignin and cellulose content, and the activities of ACC synthase (ACS), ACC oxidase (ACO), PAL, CAD and POD were examined during storage at 2 °C for twenty-eight days.

2. Materials and methods

2.1. Bamboo shoots, treatment, and storage

Bamboo shoots (*Phyllostachys praecox* f. *prevernalis.*) were harvested by cutting 10 cm section above the ground from a plantation in Linan, Zhejiang Province of China. The shoots were then packed in fiberboard cartons, and transferred to the laboratory in three hours, and 360 shoots of uniform size (diameter: 3–4 cm) and free from blemishes were selected. 360 shoots were divided into six sets of 60. Three sets were used for each of the following treatments: control and irradiation treatment with doses 0.5 kGy (this gamma radiation treatment was chosen as being optimal from preliminary experiments from 0.25, 0.5, 0.75, 1.0 kGy). The irradiation process was accomplished at room temperature with a dose rate of 0.75 kGy/h for 40 min. Bamboo shoots were then cool air-dried for about 1 h and stored at 2 °C and 90% RH for twenty-eight days. Shoots firmness, decay rate, respiration and ethylene production were assessed every seven days. Shoots flesh samples (about 50 g each) were frozen in liquid nitrogen and stored at −70 °C until used for the measurement of TS, RS, lignin and cellulose content, ACS, ACO, PAL, CAD and POD activity.

2.2. Decay rate evaluation

Based on the shoots surface visible fungal growth or bacterial lesions area, it was graded as 5 decay levels: level 0, no infection; level 1, decay area ≤10% fruit surface, similarly, level 2, ≤30%; level 3, ≤50%; level 4, ≤100%.

$$\text{Decay rate}(\%) = \sum \left(\frac{\text{decay level} \times \text{shoot count}}{\text{decay level}_{\max} \times \text{total shoot count}} \times 100\% \right).$$

2.3. Texture measurement

Texture measurements were conducted using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., UK) incorporating a 5 mm diameter probe. Shoots were cut in half longitudinally. Firmness was measured on the middle (about 5 cm from the tip) of the shoots. Eight shoots from each treatment were compressed 1 mm at a rate of 0.5 mm s^{-1} and the maximum force developed during the test was recorded and expressed in Newton (N).

2.4. Determination of respiration

Respiration was measured by CO_2 production. Three pairs of bamboo shoots (two bamboo shoots per chamber) from each treatment were enclosed in a chamber and air was passed through the chamber at 101.325 kPa and 20 °C. The effluent air was connected to a GXH-3051 (Institute of Junfang Scientific Instrument of Beijing, China) Infrared Gas Analyzer (IRGA) and respiratory rate was measured. The IRGA was earlier calibrated with standard CO_2. The results were expressed as mg CO_2 h^{-1} kg^{-1} fresh weight.

2.5. Determination of TS and RS content

Frozen shoots samples (5 g) were ground and extracted for 30 min with 50 mL of ethanol. The mixture was centrifuged at 14,000 × *g* for 15 min and 5 mL of the supernatant was brought to 50 mL with H_2O, then 3 mL solution was mixed with 2 mL 27.6 mM dinitrosalicylic acid and boiled for 5 min. The content of RS was determined spectrophotometrically at 520 nm according to Miller (1959). For TS determination, the samples were first hydrolyzed with 0.1 M HCl for 10 min and then processed as described above. Results were expressed as g of glucose per 100 g fresh weight.

2.6. Lignin and cellulose determination

About 5 g of frozen samples were extracted four times with 50 mL methanol containing 110 mM HCl at 20 °C for 1 h each time under continuous stirring and centrifuged at 14,000 × *g* for 10 min. The final residue was used for analysis of lignin. Lignin (Klason-lignin) content was determined gravimetrically after acid hydrolysis of the insoluble-alcohol residue under the method described by Saura-Calixto et al. (1991) with modifications. This residue was mixed with 50 mL 12 M H_2SO_4 and hydrolyzed for 3 h at 20 °C with stirring. The solution was then diluted with 550 mL distilled water up to 1 M H_2SO_4, and heated for 2.5 h at 100 °C with continuous shaking, cooled, vacuum filtered through an acid-treated 0.45 μm Millipore HVLP filter, and rinsed with 100 mL 100 °C distilled water. The filter containing Klason-lignin was air-dried at 60 °C overnight and weighed. Results were expressed as g lignin per 100 g fresh weight. Three independent replicates were conducted in each treatment.

Cellulose was extracted and measured by the method described by Oomena et al. (2004) with modifications. For the isolation of cell wall material (CWM), 10 g of frozen tissue powder was extracted in 50 mL 50 mM Tris–HCl, pH 7.2 solution containing 35 mM SDS for 3 h at room temperature with continuous shaking. The CWM was pelleted by centrifugation at 14,000 × *g* for 15 min. Subsequently, the residue was washed with water, ethanol, and acetone and air-dried. Fifty milligrams of CWM was incubated for 90 min at 120 °C in 5 mL 2 M trifluoroacetic acid. The remaining cellulose was pelleted and washed with water and ethanol. The pellet was solubilized in 5 mL 12 M H_2SO_4 at 37 °C for 60 min and diluted appropriately (the absorbance of the solution between 0.4 and 0.8) to determine the cellulose content colorimetrically using anthrone as a coloring agent. Quantification was determined using glucose as a standard. Three independent replicates were conducted in each treatment.