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Ethanol fumigation combined with and without nitrogen gas delays potato greening and inhibits glycoalkaloids generation under light

Tiantian Dong[a], Weiqin Meng[a], Jingying Shi[a], Cai-Zhong Jiang[b,c], Qingguo Wang[a,*]

[a] College of Food Science and Engineering, Shandong Agricultural University, Tai'an, 271018, China
[b] Department of Plant Sciences, University of California Davis, One Shields Avenue, Davis, CA 95616, USA
[c] Crops Pathology & Genetic Research Unit, USDA-ARS, One Shields Avenue, Davis, CA 95616, USA

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ABSTRACT

Postharvest potato greening under light accompanied by the generation of toxic glycoalkaloids results in a potential serious health risk and great postharvest losses. This study examined the inhibiting effects of ethanol fumigation and ethanol fumigation combined with N_2 treatment on potato greening and glycoalkaloids generation in potato peel and flesh under light. The results showed that ethanol fumigation with concentrations of 400, 600, and 1000 μL L^{-1} inhibited potato greening but caused decay when the concentration exceeded 600 μL L^{-1}. Ethanol fumigation with 600 μL L^{-1} combined with nitrogen gas (N_2) treatment (600 ET + N_2) enhanced the effect of inhibiting greening and delayed potato greening for 6 ~ 7 d compared with the control. The treatment of 600 ET + N_2 significantly inhibited glycoalkaloids generation in potato peel and flesh and effectively improved the overall visual quality and flavor of potatoes during storage under light. Our results suggested that the ethanol fumigation could provide a useful way to delay potato greening and to prevent glycoalkaloids accumulation for prolonging the shelf-life of potatoes.

1. Introduction

Potato is the fourth-largest major grain crop in China after wheat, corn and rice as grain (Zhang et al., 2016). Postharvest potato is prone to green under the light in some of potato varieties. Potato greening is associated closely with generation of toxic glycoalkaloids (Bamberg et al., 2015), resulting in a potential serious health risk (Smith, 2013) and great postharvest losses (French-Brooks, 2012; Grunenfelder et al., 2006). Glycoalkaloids accumulation and chlorophyll biosynthesis are often concurrent processes in response to illumination although both are physiologically unrelated and have independent genetic variation (Dao and Friedman, 1994). More than 95% of glycoalkaloids found in potato was consisted of bitter and toxic α-solanine and α-chaconine (Percival, 1999). Glycoalkaloids concentration above 200 mg kg^{-1} fresh weight of potato tissue is considered to exceed the limits recommended for food safety (Sinden et al., 1976). Consuming beyond the upper limits of glycoalkaloids can cause gastroenteric symptoms, coma and even death (Gregory, 1984). So greening of potatoes could serve as a warning signal that potatoes may have accumulated higher glycoalkaloids content and are inedible (Bamberg et al., 2015), as greened potatoes contain more glycoalkaloids and taste bitter (Grunenfelder et al., 2006; Haase, 2010; Salunkhe and Salunkhe, 1974).

A common practice to inhibit greening is to keep potatoes away from light by using opaque packaging materials (Martin and Sheppard, 1983), but avoiding all traces of greening during storage or at the market before potatoes reach consumers is seemingly impossible (Akeley et al., 1962). Other practices or techniques including endogenous carbon dioxide (Poapst and Forsyth, 1974), controlled atmosphere storage (Banks, 1985), oil dipping (Malik et al., 1979), detergents and other surfactants (Poapst et al., 1978; Wu and Salunkhe, 2002) have been shown to inhibit potato greening to some extent under light. To date, however, there is no report that any of these techniques have been used commercially.

Poapst (1979) reported that endogenous and exogenous acetaldehyde and ethanol prevented potato greening as the toxicity of acetaldehyde and ethanol could cause cell membrane fluidization (Kiyosawa, 1975). Ethanol, a liposoluble substance (Mukohata et al., 1971), possibly causes the destruction of thylakoid membranes of chloroplasts and thus influences photosynthesis. Previous studies in our laboratory have also demonstrated that ethanol treatment could inhibit potato greening, but high concentrations induced tuber decay (Meng and Wang, 2012). In addition, a high concentration of nitrogen gas (N_2)

* Corresponding author at: College of Food Science and Engineering, Shandong Agricultural University, Room 1101, Xueshi Building, 61 Daizong Street, Tai'an 271018, Shandong Province, China.
E-mail address: wqgyyy@126.com (Q. Wang).

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treatment delayed potato greening (Meng et al., 2012), but treatment for a short time could not achieve ideal effects.

The objectives of this study were to determine the proper ethanol fumigation concentration that would effectively inhibit potato greening and cause less potato decay, and to investigate the effects of ethanol fumigation combined with and without N_2 on potato greening and glycoalkaloids generation under light at a storage temperature of 20 °C.

2. Materials and methods

2.1. Raw material and treatment process

The greening-susceptible potato variety "*Netherlands 15*" (*Solanum tuberosum*) was used in this study. For the first experiment for testing optimal ethanol concentrations, potatoes were newly-harvested and purchased from a local wholesale market. In the subsequent experiments, potatoes were purchased from a local potato store and stored at 2–4 °C for three months. The purchased potatoes for the study were transported to our postharvest laboratory in Shandong Agricultural University and placed in a cold storage room at 2–4 °C until used. Medium size (125–150 g) potato tubers without injury, sprouts, greening or abnormality in shape were selected for the experiments. The potato tubers were washed with tap water, rinsed in 200 μL L^{-1} sodium hypochlorite solution (NaClO) for 3–5 min to sterilize, and then dried by blotting with cheesecloth prior to the experiments.

2.1.1. Ethanol fumigation treatment

In the first experiment to test optimal ethanol concentration, freshly harvested potatoes were placed into sealed opaque plastic containers (30 L). In each of the sealed containers, there was a small fan for air circulation and a piece of gauze containing different amounts of absolute ethanol. The ethanol fumigation concentrations (0, 0, 400, 600, 1000 μL L^{-1}) were calculated according to the net air volume (container volume minus the potato volume). The gauze was placed away from the potatoes to avoid direct contact of potatoes and ethanol. After fumigation for 24 h at 20 °C, the potatoes were removed from the containers and placed one by one in evaluation trays under a light intensity of 400 Lux and 24 h/d illumination at 20 °C for evaluation and sampling. Each container had 30 potatoes and two containers of potatoes (60 potatoes) were as one replicate, one container of which served for greening and decay evaluation and the other one was used for sampling at 0, 3, 6 and 9 d to assess the influence of fumigation with different ethanol concentrations on chlorophyll generation of potato peel. For sampling, peel of six potatoes selected randomly was cut with thickness of 1 ~ 2 mm and flesh was sampled at 1/3, 1/2 and 2/3 of potatoes and then mixed. The samples of peel and flesh were froze with liquid nitrogen respectively, and stored at −80 °C until used. Three replicates were set up for each ethanol concentration.

2.1.2. Ethanol fumigation combined with N_2 treatment

To examine the efficacy of ethanol fumigation combined with N_2 treatment on potato greening and glycoalkaloid generation, potatoes stored for three months were randomly divided into five groups: control under light (control L), control in the dark (control D), 600 μL L^{-1} ethanol fumigation only (600 ET), 99% N_2 treatment only (N_2), 600 μL L^{-1} ethanol fumigation combined with 99% nitrogen treatment (600 ET + N_2). Prior to ethanol fumigation, potatoes subjected to N_2 treatments in the N_2 only or 600 ET + N_2 groups were packed into the sealed opaque polyethylene (PE) plastic bags with a thickness of 0.12 mm which were kept inflated with 99% nitrogen for 3 days. Potatoes in the other three groups (control L, control D and 600 ET only) were packed into the same PE bags which were kept inflated with air for three days. Afterward, potatoes were fumigated with absolute ethanol for 24 h in the 600 ET + N_2 and 600 ET groups or without absolute ethanol treatments in other three groups (control L, control D, and N_2), as described above. Then all the potatoes except control D were placed one by one in the evaluation trays under light to evaluate the effects of treatments on inhibiting greening while potatoes in the control D were placed under the same environmental condition without light. All the treatments were conducted at 20 °C. Potatoes in each group were divided and placed in six containers with 30 potatoes in each container. Each group had three replicates and each replicate had two containers of potatoes (60 potatoes), one container of which served for greening and decay evaluation and the other one was used for sampling at 0, 3, 6 and 9 d to assess physiological parameters. Sampling method was the same with above.

2.2. Evaluation of greening, decay and overall visual quality

Visual greening degree of potatoes can be divided into six grades (0–5): scale 0 means no greening (excellent); 1 = greening area below 5% of whole potato surface area (good); 2 = fair, with greening area between 5%–10%; 3 = poor, with greening area between 10%–30%; 4 = bad, with greening area between 30%–50%; and 5 = no commercial value, with greening area above 50%.

The grading standard for the degree of decay was as follows: 0 = excellent, with no rot; 1 = good, with rot area below 1% of whole potato surface area; 2 = fair, with rot area between 1%–5% of whole potato surface area; 3 = poor, with rot area between 5%–10% of whole potato surface area; 4 = no commercial value, with rot area above 10% of whole potato surface area.

To evaluate overall visual quality, including greening and decay, six potatoes were randomly selected from each replicate. The evaluation standard for overall visual quality was: 9 = excellent, with no greening and no decay; 7 = good, with greening area below 5% and decay area below 1%; 5 = fair, with greening area between 5% ~ 10% and decay area between 1%–5%; 3 = poor, with greening area between 10%–30% and decay area between 5%–10%; 1 = no commercial value, with greening area above 30% and decay area above 10%. A score of 6 was defined to be the limit of salability.

To evaluate effects of treatments on potato flavor, potatoes of the five groups were peeled and shredded, dipped in water to avoiding browning, and stir-fried as Chinese food. Eight people were invited to judge in a blind tasting to score the taste quality according to the following standard: 9 means excellent, with normal aroma and lustre, good taste and no bitterness; 7 means good, with no off-flavor and no bitterness, general taste; 5 tasted a little bad, with minor flavor defects, no off-flavor and no bitterness but with poor lustre; 3 tasted poor, with off-flavor and poor lustre but no bitterness; 1 tasted bad, with off-flavor, bitterness and poor lustre. A score of 6 was considered as the limit of inedible.

2.3. Evaluation for potato skin color

The skin colors of potatoes were evaluated by the L^*, a^* and b^* values of the CIELAB colorimetric system, especially a^* (reddish-greenish) reflecting the greening degree of potato skin. Six potatoes selected randomly from each replicate for physiological studies were measured at four different locations on each potato. Peels with thickness of about 0.1–0.2 mm were removed and then a^* value measurement was performed.

The chlorophyll concentration of potato peels were measured according to the method of Knudson et al. (1977) with some modifications. Four gram samples of peel were mixed with 15 mL of 95% ethanol and 0.1 g of calcium carbonate, and then they were ground into homogenate and incubated for 12 h at 4 °C in the dark. Afterward, the mixture was filtered into 25 mL brown volumetric flask and the residue was washed repeatedly with further 10 mL of 95% ethanol to ensure a complete extraction. The extracts were finally made up to volumes of 25 mL with 95% ethanol. The absorbances at 665 and 649 nm were measured with UV-spectrophotometer to calculate the concentrations of chlorophylls a and b in the extracts.