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Detection of NEO in muskmelon fruits inoculated with *Fusarium sulphureum* and its control by postharvest ozone treatment

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ABSTRACT

Fusarium rot of muskmelon, caused by *Fusarium* spp., is one of the most important postharvest decays, that not only causes economic losses but leads to trichothecenes contamination. A rapid and sensitive method was developed for neosolaniol (NEO) analysis in muskmelon inoculated with *F. sulphureum,* utilizing acetonitrile/water (84:16, v/v) extraction and PriboFast M270 columns purification and UPLC-MS/MS detection. Method validation was evaluated by linearity ($R \geq 0.9990$), recovery (88.1–136.9%), precision (RSD ≤ 3.97%) and sensitivity (LOD, 0.5 μg/kg; LOQ, 1.5 μg/kg). The effect of ozone treatment on *Fusarium* rot development and NEO accumulation in inoculated muskmelon was also evaluated. The results showed that UPLC-MS/MS method was suitable for analyzing NEO in inoculated muskmelon, and 1.10 mg/l ozone treatment for 120 min significantly controlled *Fusarium* rot development and NEO accumulation in fruits after 5, 8 and 11 days. *In vivo* tests showed that ozone at 1.10 mg/l effectively degraded NEO in acetonitrile.

1. Introduction

Muskmelon (*Cucumis melo* L.) is one of the most widely cultivated and economically important fruit crops. However, it has a very short postharvest storage and shelf life due to its high susceptibility to fungal decay (Li et al., 2012; Wang et al., 2014). Many pathogens result in the decay of harvested muskmelon; among them, *Fusarium sulphureum* is a typical muskmelon *Fusarium* rot pathogen, after harvest, in northwest areas of China, which drastically affects yield and fruit quality by producing mycotoxins (Wang, Bi, Zhang, Zhang & Ge, 2011).

It was reported that trichothecin was detected by two-dimensional TLC in pink mold rot of muskmelon caused by *Trichothecium roseum* (Takahashi et al., 1995). Trichothecin is one of the type C trichothecenes with the function of anti-tumor activity. Tang et al. (2015) detected neosolaniol (NEO) in apple fruit inoculated with *T. roseum* by UPLC-MS/MS. However, no information was reported on the presence of NEO in infected muskmelon. NEO is one of the type A trichothecenes associated with strong phytotoxicity and a variety of mycotoxicoses in human and livestock health (Nicholson, Simpson, Wilson, Chandler, & Thomsett, 2004). NEO also causes an immunosuppressive effect due to its multiple inhibitory effects on eukaryotic cells, including suppression of protein biosynthesis, DNA transcription and RNA translation function, restraining mitochondrial function, cell division and membrane function (He, Zhou, Young, Boland, & Scott, 2010). Therefore, it is necessary to develop an analytical method to monitor NEO accumulation in muskmelon fruit so as to meet regulatory requirements.

Generally, the application of synthetic fungicides is the main strategy for management of postharvest plant disease (Niu et al., 2015; Wang, Duan, & Zhou, 2016). However, due to a series of increased concerns related to fungicide residue, development of fungicide resistance against pathogens, potential harmful effects on human and animal health, as well as environmental pollution, researchers have been prompted to develop new strategies to control postharvest diseases (Xue et al., 2017).

Ozone is a powerful oxidant with good, broad-spectrum germicidal efficacy. Due to its potential oxidizing capacity, it is widely used as an antimicrobial agent in both gaseous and aqueous phases to control fungal growth and for mycotoxin detoxification. It has been approved by the US FDA according to Federal Register provisions (FDA, 2001). Postharvest ozone application has gained increasing commercial interest for controlling postharvest decay in fruits and vegetables, especially because ozone does not leave any residues on the treated produce (Horvitz & Cantalejo, 2014). Postharvest treatment with electrolyzed oxidizing (EO) water, in combination with ozone, significantly

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controlled green mold in tangerine fruits caused by *Penicillium digitatum* (Whangchai, Saengnil, Singkamanee, & Uthaibutra, 2010) and gray mold in table grapes caused by *Botrytis cinerea Pers* (Gabler, Smilanick, Mansour, & Karaca, 2010). Yaseen, Ricelli, Turan, Albanese, and D'onghia (2015) also suggested that ozone treatment post-harvest reduced fungal populations and patulin production in apple fruits inoculated with *Penicillium expansum*. Ozone was also widely applied to degrade and detoxicate mycotoxins, such as aflatoxins (McKenzie et al., 1997), and deoxynivalenol of trichothecenes in contaminated grains (Wang et al., 2016). However, studies on ozone treatment to control postharvest muskmelon decay and reduce accumulation of NEO in *Fusarium* rot of muskmelon are very limited.

The present study aims (1) to develop a rapid and reliable method of UPLC-MS/MS for NEO determination in muskmelon fruits inoculated with *F. sulphureum*; (2) to investigate the effect of postharvest ozone treatment on control of development of *Fusarium* rot; (3) to analyze the effect of ozone application on NEO accumulation in *Fusarium* rot of muskmelon. The study not only provides occurrence data on its presence in muskmelon fruits to protect consumers from the risk of exposure to trichothecene, but also provides scientific information on the maximum limit standard introduction for NEO.

2. Materials and methods

2.1. Chemical reagents

NEO (analytical standard) and acetonitrile (HPLC-grade) used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained from a Milli-Q water-purification system (Millipore, Bedford, MA). All reagents were used as obtained, without further purification. The 0.22 μm poly-tetrafluoroethylene filter was from Millipore (Bedford, MA). The PriboFast M270 column was obtained from Pribolab (Singapore).

2.2. Muskmelon fruits

Muskmelon (*Cucumis melo* L. cv. Manao) fruits were harvested at commercial maturity in Minqin County, Gansu Province, China, in June 2015. Fruits of uniform size and free of obvious injuries were chosen and packed individually, and then put in standard melon-shipping boxes (six melons/box), transported to the laboratory within 24 h, and stored at room temperature (22 ± 2 °C, 55–60%).

Prior to the experiment, the fruits were disinfected with a 2% sodium hypochlorite solution for 2 min and rinsed with sterile distilled water, then air-dried (Li et al., 2012).

2.3. Pathogen

An isolate *of Fusarium sulphureum* was obtained from the Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou, China. The pathogen was cultured on potato dextrose agar (PDA) medium for 7 days. Conidia were collected from PDA by adding 10 ml sterile water containing 0.02% aqueous solution Tween 80. Spores were filtered through four layers of cheesecloth, and then the spore suspension concentration was adjusted to 1×10^6 CFU/mL for inoculation.

2.4. Sample preparation

2.4.1. Sample inoculation

The sterilized muskmelon fruits were inoculated with *F. sulphureum* according to the method of Li et al. (2012), with some modifications. Four wounds (3 mm in depth and 3 mm in diameter) were made on the equator of each fruit with a cork borer after disinfection with 70% ethanol. A portion of 20 μl spore suspension was inoculated into each wound; the inoculated fruits were kept in plastic bags with a sterile, moist paper towel (22 ± 2 °C, 75–80%). Subsequently, the rotten part was excised from each wound and immediately stored at −80 °C until analysis.

2.4.2. Sample extraction and clean-up

Sample extraction and clean-up were carried out by the method according to Xue et al. (2013) and Xue, Bi, Tang, Zhao, & Wang, 2014), with some modifications. In brief, 5.0 g of homogenized sample in liquid nitrogen was suspended in a 10 ml mixture of acetonitrile/water (84:16, v/v), followed by vortexing for 3 min, then centrifuging at 10 × 000*g* for 5 min at 4 °C. The extracted solution was filtered and transferred to a clean and dry distillation flask, and the pellet was extracted twice as described above. The three portions of supernatants were collected and mixed, then evaporated to the volume of 3–4 mL at 60 °C. The resulting solution was purified with a PriboFast M270 column; the eluate was evaporated to dryness under nitrogen stream, then the residue was re-suspended in 1 ml of mobile phase solvent, then passed through a 0.22 μm filter and kept at 4 °C for analysis (Xue et al., 2013).

2.4.3. UPLC-MS/MS

NEO was analyzed with UPLC-MS/MS (Waters Acquity Ultra Performance LC system, Waters, Milford, MA). The column used was a 50 × 2.1 mm inner diameter, 1.7 μm of ACQUITY UPLC BEH C_{18}, with a 4 × 4 mm inner diameter guard column of the same material (Waters, Milford, MA). The column was maintained at 25 °C, the linear gradient elution was used by starting with 35% of mobile phase A (acetonitrile) and 65% of mobile-phase B (0.1% formic acid and 10 mM ammonium acetate in water), and then ramped to 90% of mobile phase A and 10% of mobile-phase B in 4.8 min, subsequently shifted to 35% of mobile phase A within 0.2 min and maintained for 1 min. The flow rate was 0.3 ml/min, and the injection volume was 5.0 μl (Tang et al., 2015).

All samples were analyzed in MS/MS mode, which was carried out on an Acquity Quattro Premier XE triple quadrupole mass spectrometer equipped with an electrospray source in positive ionization mode (ESI^+) sources (Waters). The parameter of ionization source conditions followed as: 3.2 kV of capillary voltage, 110 °C of source temperature, 350 °C of desolvation temperature, 50 L/h of cone gas flows, and 550 L/h of desolvation gas flows, respectively. Quantitation was used via MRM mode, the parameters of the analysis of NEO were that qualitative and quantitative ion pairs were 400.0/185.0 and 400.0/215.0, respectively, and collision energy was 10.20 E/eV, and dwell time was 0.2 s, and cone voltage was 25 U/v (Tang et al., 2015). Data acquisition and processing were performed with MassLynx, version 4.1, and QuanLynx (Waters).

2.5. Method validation

The method was evaluated by the following criteria: selectivity, linearity, sensitivity, accuracy and precision (Intra- and inter-day variability) (Brabcová, Hlaváčková, Šatínský, & Solich, 2013).

2.5.1. Selectivity

Selectivity was performed by analyzing the retention time of NEO in the sample to determine if it matched with the retention time in the matrix standards, and there were no other interfering peaks (Reuveni, Sheglov, Sheglov, Ben-Arie, & Prusky, 2002).

2.5.2. Linearity

Linearity was assessed by the matrix standard working solutions with concentrations of 1.00, 0.50, 0.20, 0.10 and 0.05 μg/ml for NEO. The standard curve was calculated by the linear regression method for the peak area of different concentrations of NEO (Berger, Oehme, & Kuhn, 1999). Linearity was analyzed from the correlation coefficients (R^2) obtained with the linear regression analysis of the concentration versus peak area for different concentrations of NEO (Berger et al., 1999). Standard concentration was back-calculated with the standard

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