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RESEARCH ARTICLE

# *Penicillium* and patulin distribution in pears contaminated with *Penicillium expansum*. Determination of patulin in pears by UHPLC-MS/MS

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**Abstract**

The danger of mycotoxin contamination entering the food supply through post-harvest infection is of perennial concern to food safety experts. To explore the distribution of *Penicillium expansum* and diffusion of its mycotoxin, patulin, in blue mold-damaged pears, *Pyrus bretschneideri* Rehd. *cv*. Yali obtained from markets and orchards in China were artificially inoculated with *P. expansum* and assayed for patulin accumulation and degree of fungal colonization. The inoculated pears were incubated until the lesions were 5, 10, 20, or 30 mm in diameter. We sampled tissue at a range of distances from the lesion, measured the spread of *Penicillium* by plate colony-counting methods, and used UHPLC-MS/MS to detect and quantify the patulin concentration. More *P. expansum* colony-forming units were isolated from pears with a higher degree of decay. Farther from the lesion, the fewer *P. expansum* colonies were observed, and the lower the patulin content detected. We found a significant difference in the patulin content between samples due to lesion size, and also in tissue sampled 10 mm away from the lesion. In consideration of this finding, to ensure food safety, we recommend that when a blue mold rot lesion on pear is 5, 10, or 20 mm in diameter, 20, 30, and 40 mm beyond the lesion should be removed, respectively. If a lesion surpasses 30 mm in diameter, the whole pear should be thrown away.

**Keywords:** pear, *Penicillium expansum*, patulin, food safety, UHPLC-MS/MS

## 1. Introduction

The pear is one of the most popular fruits in China, and the cultivated area ranks the third, after apple and citrus. The pear is sweet, crisp and juicy, it can be eaten fresh or processed into pear cream, dried pears, pear juice, and other products. Pears can also be used to make wine and vinegar. The storage of pear is important for prolonging shelf life in order to meet the needs of people's consumption habits, exploiting markets that require long-distance transportation, and increasing the benefit of fruit production by eliminating

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waste. During storage, many post-harvest diseases can cause pears to rot, shortening the storage time, reducing fruit quality, and often incurring huge economic losses to producers and distributors. The biggest post-harvest disease of pears during storage period is blue mold rot, caused by *Penicillium expansum* (Moss 2008). *P. expansum* is a destructive pathogen on pomme fruits (Lai *et al.* 2015), which produces patulin (Elhariry *et al.* 2011), a toxic secondary metabolite. Patulin is potentially carcinogenic, mutagenic, teratogenic, and immunotoxic. Moreover, it can cause adverse effects to the gastrointestinal tract and the developing fetus (Moake *et al.* 2005; Puel *et al.* 2010).

*P. expansum* infects pears, causing obvious macerations and decay. When infected pears are stored for a long time or are seriously decayed, the pathogenic fungi and mycotoxins migrate from the diseased tissue to healthy tissue. Apparently sound tissue may contain pathogenic fungi and mycotoxins. Molds in raw fruit increase consumer risk because they may produce patulin during storage, transport, and processing. Patulin is stable during the machining processes, because of its low molecular weight, low volatility and heat resistance (Harrison 1988). Diseased fruits that are processed into food are a major source of patulin. Removal of the rotten part of a pear does not necessarily guarantee effective elimination of the pathogen or the mycotoxin (Harwig *et al.* 1973; Seppo and Aimo 1978).

The diffusion behavior of patulin is important for the safety of fruit products (Baert *et al.* 2012). Fruit cultivars may differ in their susceptibility to *P. expansum* rot, and to contamination with patulin. The diffusion of patulin in apples extends no more than 2 cm from infected tissue (Taniwaki *et al.* 1992; Rychlik and Schieberle 2001). Marin *et al.* (2006) assessed the diffusion of patulin in infected apples of different varieties and degrees of ripeness. Laidou *et al.* (2001) reported the penetration of *P. expansum* and three other post-harvest pathogens to different depths in the flesh of pears, but only analyzed the diffusion of patulin in pears with lesions 10 mm in diameter. In these pears, patulin diffused to a depth of 0.6 cm. In comparison, when patulin was inoculated to tomatoes at a concentration of 52.9 mg $kg^{-1}$, 450 μg $kg^{-1}$ of patulin was detected 4 cm from the inoculated tissue (Rychlik and Schieberle 2001). Patulin penetrates the entire fruit. Many methods have been established for quantification of patulin in a wide range of fruit matrices, especially apple and apple products, based on colorimetry (Subramanian 1982), thin layer chromatography (Harwig *et al.* 1973), gas chromatography mass spectrometry (Cunha *et al.* 2014), and high performance liquid chromatography with ultra-violet light detection or diode array detector (DAD) (Baert *et al.* 2007; Katerere *et al.* 2008; Funes and Resnik 2009). In recent years, UHPLC MS/MS has also been used for patulin detection and quantification in fruit (Beltrán *et al.* 2014; Fowler and Seymour 2014), including pear (Desmarchelier *et al.* 2011; Vaclavikova *et al.* 2015).

In the present study, we concurrently measured the diffusion of *P. expansum* and the distribution of patulin in infected pears with a range of lesion sizes. *Penicillium* was isolated by plate colony-counting methods and quantified by GB4789.15-2010 (2010). Patulin extraction and purification was based on a modified QuEChERS method, and patulin was measured by UHPLC-MS/MS. We examined the degree of colonization by *P. expansum*, as well as the diffusion distance of patulin in the flesh of pears, in order to test the safety of the remaining pear after removal of a visible infected lesion. In this work, we established a method for patulin detection in pears, and analyzed representative pears obtained from different provinces in China. We also provide evidence-based recommendations for safe consumption of pears.

## 2. Materials and methods

### 2.1. Reagents and chemicals

Standard patulin (98.8% purity) was obtained from Pribolab Biological Engineering Limited (Singapore). Streptomycin sulfate and HPLC grade acetonitrile (MeCN) were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical grade anhydrous sodium sulfate ($Na_2SO_4$) and ethyl acetate were purchased from Beihua Fine Chemicals (Beijing, China). Ammonium acetate was purchased from Yili Fine Chemicals (Beijing, China). Primary secondary amine (PSA, 40 μm) and nylon syringes filter (0.22 μm) were purchased from Agela Technologies, Inc. (Tianjin, China). Ultrapure water was prepared using a Milli-Q Reagent Water System (Bedford, MA, USA).

### 2.2. Instrumentation

Mass spectrometry was performed with a triple-quadrupole (TQD) mass spectrometer (Waters Crop, Milford, MA, USA) equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed on a Waters Acquity UHPLC binary solvent manager equipped with a Waters Acquity UHPLC BEH HILIC column (100 mm×2.1 mm, 1.7 μm particle size, Milford, MA, USA).

### 2.3. Methods

**Pear inoculation and fungi isolation** Healthy pears (*Pyrus bretschneideri* Rehd. *cv*. Yali) of consistent size were purchased from supermarkets in Beijing, China. Pears were surface disinfected by immersion in sterile water for 3 min, rinsed with sterile water, and wiped with 75% alcohol.