Postharvest Biology and Technology 146 (2018) 51–59

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Changes in volatile compounds emitted by fungal pathogen spoilage of apples during decay

Seong Mi Kim[a,1], Sang Mi Lee[a,1], Jeong-Ah Seo[b,*], Young-Suk Kim[a,*]

[a] Department of Food Science and Engineering, Ewha Womans University, Seoul 120-750, Republic of Korea
[b] School of Systems Biomedical Science, Soongsil University, Seoul 06978, Republic of Korea

ARTICLE INFO

Keywords:
Apple
Postharvest disease
Fungal pathogen
Volatile organic compounds

ABSTRACT

Postharvest diseases in apples are mainly caused by pathogenic fungi. Fungal contamination and decay can change some of the emitted volatile organic compounds (VOCs). In this study, three major pathogens isolated from Fuji apples; *Penicillium expansum*, *Botryosphaeria dothidea,* and *Alternaria alternata*, were inoculated onto disease-free Fuji apples. The VOCs released were analyzed using gas chromatography-mass spectrometry combined with solid-phase microextraction to compare the changes in VOCs according to the decay index and pathogen species. After apples were inoculated with pathogens, ethanol, 3-methylbutan-1-ol, benzaldehyde, styrene, limonene and some ethyl esters constituted the main VOCs emitted during decay. The main volatile compounds according to pathogen species were (E)-hex-2-enal, 1-methoxy-3-methylbenzene, methyl heptanoate, diethyl carbonate, ethyl 2-phenylacetate, propyl octanoate, and ethyl decanoate produced in *P. expansum*, (E)-hex-3-enyl acetate, 1-methyl-4-propan-2-ylbenzene, 2-phenylethanol, α-terpinene, and α-terpinolene in *B. dothidea*, and phenylmethanol, 2-ethylhexan-1-ol, and acetophenone in *A. alternata*. The increase of fungal VOCs can be affected by tissue degradation and/or fungal metabolism of apples during decay.

1. Introduction

Many fruits are commercially harvested before their full maturity in order to extend postharvest storage. Among the characteristics of fruit, the aroma, comprising of a complex mixture of volatile compounds, is often considered to be a critical factor of quality of fruit (Goff and Klee, 2006). Several studies have found that apples contain more than 350 volatile compounds of which esters are the most abundant (Dixon and Hewett, 2000; Risticevic et al., 2012). Dixon and Hewett (2000) considered certain esters (e.g., butyl acetate, pentyl acetate, and hexyl acetate), alcohols [butan-1-ol, hexan-1-ol, and (E)-2-hexenol], and aldehydes [acetaldehyde, (E)-2-hexenal, and hexanal] to be the "character impact" compounds of apple. However, these compounds related to sensory attributes can be changed during their postharvest storage, and it can influence the quality of apples (Sutton et al., 2014; Vikram et al., 2004; López et al., 2015). Accordingly, maintaining appropriate aroma profiles during postharvest storage is one of the important issues in agriculture. Apples are highly vulnerable to storage disease, so most postharvest losses are due to diseases during storage (Dixon and Hewett, 2000; Vikram et al., 2004; López et al., 2015). The stored diseased fruit that exhibit changes in many volatile organic compounds (VOCs) usually produce a stench that lowers their value. The storage diseases are mainly caused by pathogenic fungi (Prusky et al., 2013), which play an important role in plant-biomass degradation (van den Brink and de Varies, 2011). This is also a critical issue in exporting apples, which require a relatively long storage time during shipping, because infected fruit can spread fungi to surrounding non-infected fruit in a package. In addition, fungi produce both primary and secondary metabolites in the form of VOCs. Changes in VOCs emitted by fungi can negatively affect the odor quality and human health (Gallois et al., 1990).

More than 90 species of fungi have been found to be responsible for the decay of stored apples (Sutton et al., 2014). Several studies have been performed to determine volatiles changes in fungal infected apples (Sutton et al., 2014; Vikram et al., 2004; Karlshøj et al., 2007). VOCs in McIntosh apples were compared by either not inoculated or inoculated with *Botrytis cinerea* pers, *Penicillium expansum* Link, *Mucor piriformis* Fischer, or *Monilinia* spp. at an early stage of disease progression (i.e., when the lesions resulting from the pathogens were smaller than 3 cm) (Vikram et al., 2004). Each fungus-inoculated apple emitted unique VOCs: fluoroethene and 3,4-dimethyl-1-hexene by *Penicillium expansum* Link; butyl butanoate, 4-methyl-1-hexene, and 2-methyltetrazole by

* Corresponding authors.
E-mail addresses: sja815@ssu.ac.kr (J.-A. Seo), yskim10@ewha.ac.kr (Y.-S. Kim).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.postharvbio.2018.08.003
Received 12 April 2018; Received in revised form 30 July 2018; Accepted 6 August 2018

Mucor piriformis Fischer; methyl acetate by *Botrytis cinerea* pers; and fluoroethane by *Monilinia* spp. (Vikram et al., 2004). Some fungal volatile metabolites, such as styrene, 1-methoxy-3-methylbenzene, 3-methyl-1-butanol, and 3-methyl-1-butyl acetate, as well as methyl propanoate, which is a well-known volatile metabolites of apple, were detetcted in decaying apples inoculated with *Penicillium expansum* (Karlshøj et al., 2007). Moreover, volatile compounds were analyzed after inoculation with *Penicillium expansum* and *Rhizopus stolonifer*, and detected ethyl hexanoate and *Z*-3-hexenyl 2-methylbutanoate as well as a large increase in ethanol (López et al., 2015). While some studies have compared the VOCs of fungus-inoculated and non-inoculated apples, there has been no research into the VOCs produced by apples according to wide specific ranges of decay lesions. In addition, there has been no study to analyze the complicated data sets by multivariate statistical analysis to clarify and determine major marker compounds according to pathogens and decay index. Therefore, the aim of this study was to measure the changes of VOCs of apples inoculated with three apple pathogenic fungi and compare the VOCs according to the decay index during postharvest storage, both qualitatively and quantitatively. Apples were inoculated with the following three pathogens: *Penicillium expansum*, which is responsible for most of the important postharvest diseases of apples, causing blue mold and then soft watery brown spots (Vikram et al., 2004). *Botryosphaeria dothidea*, which is related to one of the most destructive diseases of apples, leading to white rot and, under favorable weather conditions, further progressing to extensive crop loss (Biggs, 2004). *Alternaria alternata*, which produces dry core rot in apples, is the predominant fungal pathogen in apples and exhibits virulence immediately after harvest (Troncoso-Rojas and Tiznado-Hernández, 2014).

2. Materials and methods

2.1. Preparation of samples

Apples (Fuji) were cultivated in Chungju-si, Chungcheong-do in Korea in 2016 and selected based on a uniform shape, size, weight (250 ± 20 g), and ripening degree. For surface-sterilization, each apple surface was washed with distilled water and rinsed with 70% ethanol.

2.2. Pathogens

Three fungal pathogens; *P. expansum* (PE), *B. dothidea* (BD) and *A. alternata* (AA) were single spore-isolated from decayed *Fuji,* which were collected from the orchard. These pathogens were identified as previously reported, and cultured on the potato dextrose agar (PDA) medium for inoculum sources (White et al., 1990).

2.3. Chemicals

All the volatile compounds, which were used for positive identification, were purchased from Sigma-Aldrich (St. Louis, MO, USA), except for acetaldehyde and hexyl acetate, which were obtained from Fluka (Milwaukee, WI, USA). Ethanol was supplied by JT Baker (Philipsburg, NJ, USA), whereas acetic acid, chloroform and benzene, were purchased from Junsei (Chuo-ku, Tokyo, Japan).

2.4. Single strain inoculation and incubation

Each cleaned apple without any bruises was punched by a hole of 6.0 mm diameter on the surface. For inoculation, the PDA blocks containing 5-days' fungal culture of each *P. expansum* (PE), *B. dothidea* (BD) and *A. alternata* (AA) were placed on the punched holes of apples (White et al., 1990). The inoculation was performed as five replicates in each different fungal species. Each inoculated apples were put in airtight 2 L jars and kept at 24 °C and 90% relative humidity in a constant environment chamber (Hanbaek Scientific Technology, Korea) for a while before closed jars with air-tight 11 mm septa on the lid to adjust the initial humidity as well as temperature. They were further kept under the same condition in the chamber during storage.

2.5. Decay index

The percentage of decay was calculated by the ratio of decayed surface area to whole apple surface area. Four state-decay index (DI 0–3) was used to indicate the spoilage scale of apples, where DI 0 = normal state of apples without decay as a control, DI 1 = slight decay (X < 10% decay of apple surface), DI 2 = moderate decay (10 ≤ X < 35% decay of apple surface), DI 3 = severe decay (35% ≤ X decay of apple surface).

2.6. Analysis of volatile compounds

In order to collect volatile components of apples, samples were placed into airtight 2 L jars and 3 μL of 2,3-dimethyl pyrazine (100 mg/L in methanol) were added as an internal standard inside the jars. Then the airtight jar was immediately closed and kept at 23 ± 1 °C for 1 h. After the equilibrium, volatiles were extracted by solid-phase microextraction (SPME) using SPME fiber coated with 50–30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) purchased from Supelco (Bellefonte, PA, USA). SPME fiber was exposed into headspace of the jar for 20 min at 23 ± 1 °C to adsorb volatile components (Song et al., 1997). All experiments were performed in triplicate. The analysis of volatile compounds was carried out using a 6890 N gas chromatograph connected to a 5975 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). After the adsorption procedure, SPME fiber was injected into GC injection port and desorbed at 250 °C for 5 min in the splitless mode. An HP-5MS U.I. capillary column (30 m length × 0.25 mm I.D., 0.15 μm film thickness, J&W Scientific, Folsom, CA, USA) was used with helium gas as carrier gas at a constant flow rate of 0.8 mL min^{-1}. For improved resolutin, cryofocusing was applied by automatically placing front part of GC column in liquid nitrogen during desorption. Then oven temperature programmed was started at 40 °C for 6 min, followed by increase up to 220 °C at 4 °C min^{-1}, and then raised up to 250 °C at 10 °C min^{-1} and held at 250 °C for 2 min. The transfer line temperature was kept at 280 °C and electron impact (EI) ion source mode was operated at 70 eV. Mass scan range was 35–350 atomic mass unites (a.m.u) and scan rate was 4.45 scans/sec.

2.7. Identification and quantification of volatile compounds

Volatile compounds were positively identified by comparing both their mass spectral data and retention index (RI) values with those of authentic standards. In respect of unavailable authentic standards, each volatile compound was tentatively identified by using mass spectral database (Wiley9n.1 and NIST08) and retention index (RI) value in the literatures (Larsen and Frisvad, 1995; Gallori et al., 2001; Azodanlou et al., 2003; Bertrand et al., 2006; Zhao et al., 2006; Fan et al., 2009). RI value of each compound was calculated using n-alkane mixture from C_7–C_{30} as external references, under the same GC/MS conditions. The quantitative analysis of volatile compounds were performed by comparing each peak areas of volatile compounds to that of the internal standard compound, 2,3-dimethyl pyrazine (100 mg/L in methanol). Then the fold changes of each compounds were calculated as the ratio of the average metabolite content in treated samples to that in the DI 0 (control) sample. If the compounds were not detected in DI 0, the next decay index, in which the compounds began to be detected, was used as the control for the fold change.