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Patulin biodegradation and quality improvement of apple puree fermented with Byssochlamys nivea *FF1-2*

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Ethyl acetate (PubChem CID: 8857)
Acetic acid (PubChem CID: 176)
Trifluoroacetic acid (PubChem CID: 6422)
Sodium chloride (PubChem CID: 5234)
Acetonitrile (PubChem CID: 6342)
3,5-Dinitrosalicylic acid (PubChem CID: 11873)

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ABSTRACT

The filamentous fungus *Byssochlamys nivea FF1-2* is a recently reported strain with excellent patulin biodegrading capability. This study investigated the effects of FF1-2 on the quality of apple puree during the process of patulin degradation. Apple puree artificially contaminated with high-dose patulin (125, 250 and 500 μg/mL) was fermented by FF1-2 for 10 days, and over 97% of patulin was degraded in the apple purees after fermentation, and the maximal degradation of patulin was 98.5%. Physicochemical analysis demonstrated that after 10 days of fermentation, the pH and the amount of reducing sugars in the apple puree decreased significantly. The volatile profile assessment of fermented apple puree obtained from HS-SPME/GC-MS indicated that the amounts of acids, aldehydes and ketones decreased, and the content of aroma-active compounds mainly aliphatic alcohols and esters, were significantly increased after FF1-2 fermentation. In addition, further reports of sensory evaluation also revealed good acceptability of the fermented apple puree. Thus, FF1-2 not only effectively degraded patulin in apple puree, but improved quality of apple puree, especially the aroma profile. These findings indicate that FF1-2 has a promising application for controlling mycotoxin contamination and quality improvement in juice production industry.

1. Introduction

Apples are rich sources of several phytochemicals, including vitamins, minerals, flavonoids and other phenolic compounds (e.g., catechins, quercetin and epicatechin). Many reports have found correlations between the low risks of cardiovascular diseases, diabetes and cancers with high consumption of apples and apple by-products (Boyer & Liu, 2004; Fügel, Carle, & Schieber, 2005; McCann et al., 2007).

Patulin (4-hydroxy-4H-furo[3,2-*c*]pyran-2(6H)-one) is a toxic fungal metabolite produced by certain fungal species (*Aspergillus*, *Penicillium* and *Byssochlamys*) growing on several foods, especially rotting apples and apple-derived products (Moake, Padilla-Zakour, & Worobo, 2005; Sant'Ana, Rosenthal, & Massaguer, 2008). Patulin exhibits a number of potential risks to human health. The danger posed by patulin necessitates its control and removal from food products. Diverse techniques and methods have been proposed to prevent patulin mycotoxin contamination during the various stages of apple juice production (Moake et al., 2005; Sant'Ana et al., 2008). Even though, patulin still can contaminate food during postproduction of food processing. Thus, a number of studies have been devoted to the separation/extraction of patulin through the use of ultraviolet radiation (Tikekar, Anantheswaran, & Laborde, 2014), pulsed light (Funes, Gómez, Resnik, & Alzamora, 2013) and high hydrostatic pressure (Hao, Zhou, Koutchma, Wu, & Warriner, 2016) but these techniques have low commercial applications due to high cost and limited patulin degradation capacities. A biological approach to degrading patulin has emerged as an innovative tool.

In recent years, a biological approach using antagonistic yeasts and lactic acid bacteria (LAB) has emerged as an innovative tool to degrade patulin. Ricelli, Baruzzi, Solfrizzo, Morea, and Fanizzi (2007) found that *Gluconobacter oxydans* can degrade patulin up to a 96% of degradation rate in apple juice contained 800 μg/mL of patulin. By comparing the patulin degrading capacity of 30 different LAB strains, Fuchs et al. (2008) demonstrated that 80% of patulin was degraded by using *Bifidobacterium animalis*. A study by Castoria et al. (2011) showed that a biocontrol yeast *Rhodosporidium kratochvilovae strain LS11* was able to degrade patulin to desoxypatulinic acid.

Our previous work suggested that the FF1-2 strain of *Byssochlamys nivea* has excellent capacity to degrade patulin (Zhang, Guo, Ma, Chai, & Li, 2016). In this study, we investigated the patulin degradation efficacy of the FF1-2 strain in apple puree. In addition, we also analyzed the changes in physicochemical characteristics and aroma substances of apple puree fermented by FF1-2. Furthermore, the sensory scores of apple puree after fermentation were also investigated to

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comprehensively evaluate the quality of fermentation products. The results may be useful in patulin control and food quality improvement in the food industry.

2. Materials and methods

2.1. Materials

Fuji apples were purchased from a local market in Xian, China. Ethyl acetate, acetic acid, trifluoroacetic acid and sodium chloride were of analytical grade and purchased from Tianli Chemical Reagent Co. Ltd. (Tianjin, China). Acetonitrile was of HPLC grade and provided by Fisher Scientific (Fair Lawn, NJ., USA).

2.2. Strain

The strain FF1-2 (accession number CCTCC M2013547) was provided by the Laboratory of Apple By-product Utilization (SNNU, Xi'an, China).

2.3. Patulin degradation experiment

Patulin was purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and dissolved in deionized water, where the pH value was previously adjusted to 4.0 with acetic acid, enabling the final patulin concentration to reach 7.0 mg/mL. Apple puree was prepared by washing, peeling and pulping fresh apples and subsequently sterilized in an autoclave (Shen An, LDZX-50KBS, Shanghai, China) at 121 °C for 20 min. Then, 1.8 mL, 3.7 mL and 7.7 mL of patulin solutions were added into 100.0 g of sterilized apple puree samples with final patulin concentration of 125 μg/mL, 250 μg/mL and 500 μg/mL, respectively. 1.0 mL of a sporal and hyphal suspension of the FF1-2 strain previously cultured on PDA slants was incorporated into the apple puree solutions. 1.0 mL of deionized water was used as a control. All samples were incubated in a digital water bath oscillator (ZBR, THZ-82, Changzhou, China) at 37 ± 1 °C for 10 days. The samples were collected at the 2nd, 5th, 7th and 10th day of storage, for analyzing the concentration of patulin.

The patulin concentrations were determined using the HPLC method described by Castoria et al. (2011), with some modifications. The samples were extracted with ethyl acetate. After collecting and drying the extracts with anhydrous sodium sulfate, ethyl acetate was removed in a rotary evaporator at 40 °C. The dried residues were dissolved in acetic acid solution (pH 4.0) to make a final volume of 15 mL. After filtrating with filter membrane (0.45 μm), the filtrates were stored at −20 °C for further HPLC analysis.

HPLC analyses were performed using the Dionex® HPLC system (P680, California, U.S.A.) equipped with a UVD170U detector and Dikma® C18 column (250 mm × 4.6 mm, 5 μm, Beijing, China) at 30 °C. For recording and data processing, the Dionex Chromeleon Version 6.70 system was used. Eluted products were detected at 276 nm. Elution with solvent A (acetonitrile) and solvent B (water with 0.1% trifluoroacetic acid) with a flow rate of 1.0 mL/min was conducted as follows: from 10% A + 90% B in 12 min, then in 5 min to 50% A + 50% B which was then held for 5 min. Then in 5 min to 10% A + 90% B, which was then held for 5 min as well.

The degradation rate of patulin was calculated using the following Eq. (1):

$$\text{Degradation rate (\%)} = [1- (\text{patulin peak area of sample} / C_0)] \times 100 \quad (1)$$

where "C_0" is the peak area of patulin in the control group.

2.4. Physicochemical analysis

The pH values of the apple purees and fermented counterparts were measured using a digital pH meter (Atago, Tokyo, Japan). The soluble solids content (SSC) was determined at 25 °C with a pocket refractometer (PAL-1, Atago, Tokyo, Japan). Free amino acids in the samples before and after fermentation were analyzed according to the method of Zeng et al. (2015) using the fully automated amino acids analyzer HITACHI L-8900 (Hitachi, Tokyo, Japan). The concentration of free amino acids in the samples were calculated by calibrating with standard amino acids and expressed as g/kg sample. The acidity of the samples was determined titrimetrically according to the AOAC official method 942.15 (AOAC, 1999). The content of total sugars and reducing sugars in the samples were determined according to the method followed by Miller (1959) using the DNSA (3,5-dinitrosalicylic acid) reagent. The absorbance of the samples was measured at 540 nm using a UV-2100 spectrophotometer (Unico, Shanghai, China).

2.5. Analysis of fragrant compositions

The variance of fragrant compositions in the apple puree samples occurred during the process of fermentation by FF1-2 was analyzed by the HS-SPME-GC-MS method. 7.7 mL of patulin solution at the concentration of 7 mg/mL was added in 100.0 g of sterilized apple puree, to make the final patulin concentration up to 500 μg/mL. Then, 1.0 mL of a sporal and hyphal suspension of the FF1-2 strain was added into the mixture and incubated at 37 °C for 10 days. Deionized water was used as a control group. Afterwards, 5.0 g of apple puree from the treated and control groups were transferred into a headspace vial (20 mL) for GC-MS analysis. Fragrant compositions in the samples were extracted using solid-phase micro extraction (SPME) fiber (Supelco, Bellefonte, PA, USA) at 40 °C for 60 min. The desorbed volatile compounds from HS-SPME analysis were separated and analyzed on an Agilent 7890 A gas chromatograph coupled with a 5975 C single-quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The MS capillary columns DB-264 (30 m × 250 μm × 0.25 μm) and DB-wax (30 m × 250 μm × 0.25 μm) (Agilent Technologies, Palo Alto, CA, USA) were programmed at 40 °C for 3 min, increased by 6 °C/min to 250 °C and held for 2 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The transfer line and injector temperatures were maintained at 150 °C and 280 °C, respectively. The quadrupole mass spectrometer was operated in the electron impact mode and source temperature was 230 °C. The mass spectrometer was operated under the following conditions: full scanning mode (Scan) with 1 min solvent delay, electron impact (EI) ionization at 70 eV over the mass range 30–400 amu. Identification of volatile components was achieved by comparing the mass spectra and relevant retention time with the NIST 2008 MS LIB database.

2.6. Sensory evaluation

Sensory characteristics of the apple puree samples and fermented counterparts were evaluated by a 30-member semi-trained panel (15 woman and 15 men, aged between 20 and 45 years old). Panelists scored for sensory characteristics, such as taste, aroma, and overall quality using a nine-point hedonic scale(1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely, Renna et al., 2013). The sensory evaluation was a blind test in a colored glass under low light and all samples were served at room temperature.

2.7. Statistical analysis

Data obtained from physicochemical analysis and sensory evaluation were subject to analysis of variance (ANOVA) to determine the