INTRODUCTION

Pericarpium Citri Reticulatae (PCR), the mature fruit peel of Citrus reticulata Blanco and its different cultivars, is an important citrus by-product used by the pharmaceutical and food industries (Yu, Sun, et al., 2018). Phytochemical investigations indicated that flavonoids, volatile components, and alkaloids are the major bioactive components of PCR (Yu, Zhang, et al., 2018). Because of its unique aroma and health benefits, it is popular and used as a food, tea, and seasoning. However, due to the lack of high value-added methods for its development and utilization, a large amount of PCR is discarded or wasted.

Microbial biotransformation of flavonoids has been studied because of the diversity of the enzyme systems, easy production of high-value products, and high selectivity. The processes involved are environmentally friendly and nonpolluting (Cao et al., 2015; Kumar & Pandey, 2013). Flavonoids are common plant secondary metabolites that have been developed into many food, medicine, and health products.

Microbial biotransformation of Pericarpium Citri Reticulatae (PCR) by Aspergillus niger and effects on antioxidant activity

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Abstract
Pericarpium Citri Reticulatae (PCR), the mature fruit peel of Citrus reticulata Blanco and its different cultivars, is an important citrus by-product with beneficial health and nutritive properties. However, due to the lack of value-added methods for its development and utilization, a large amount of PCR is discarded or wasted. To explore a possibly more effective method to utilize PCR, we compared the chemical and biological differences before (CK) and after (CP) microbial transformation of PCR by Aspergillus niger. UPLC-ESI-MS/MS, HPLC, and LC-MS methods were used to compare the chemical profiles of CK and CP. The results demonstrated that microbial biotransformation by A. niger could transform flavonoid compounds by utilizing the carbohydrate and amino acid nutrients in PCR. This could also promote the accumulation of polyhydroxyflavones compounds in CP. The antioxidant assay demonstrated that CP had significantly greater free radical-scavenging activity than CK. The higher antioxidant activity of CP may result from the high level of flavonoids with associated phenolic hydroxyl groups. Microbial biotransformation is an effective method for improving the antioxidant capacity of PCR and may be effective and useful in other natural product situations.

KEYWORDS
antioxidant activity, Aspergillus niger, Microbial transformation, Pericarpium Citri Reticulatae, UPLC-ESI-MS/MS
products (Gonzales et al., 2015). Flavonoids have a variety of pharmacological activities, anti-inflammatory (Gil-Cardoso et al., 2016), antioxidant (Jia et al., 2012), antithrombosis (Chen et al., 2012), antitumor (Jiang, Zhu, et al., 2019), antithrombosis (Vazhappilly et al., 2019), antibacterial (Xie et al., 2015), antiviral (Zakaryan et al., 2017), hypolipidemic (Bao et al., 2016), antituberculosis (Tao et al., 2019), antiinflammatory (Basu et al., 2016), anti-Alzheimer’s disease (Shahinozzaman et al., 2018), and liver protection (Jiang, Yan, et al., 2019). Aspergillus niger is a fungus commonly used in the microbial biotransformation of chemical components (Kang et al., 2019). Consumption of A. niger is safe, and it is widely used in the food industry (Schuster et al., 2002). Aspergillus niger has also been used in the biotransformation of flavonoids such as flavone (Parshikov and Sutherland, 2015), flavonol (Kostrzewa-Suslow et al., 2014), flavonol glycoside (Cao et al., 2015), flavanone (Kostrzewa-Suslow and Janeczko, 2012), polymethoxy flavonoids (Sanchez-Gonzalez & Rosazza, 2004), chalcone, and isoflavonoids (Abdella et al., 2018). The biotransformation mechanism may involve the formation of transformation products through hydroxylation, methylation, dehydrogenation, and other processes with the participation of enzymes produced by A. niger (Caspani et al., 2019; Bianchini et al., 2015). However, little is known about the specific enzymes involved in the biotransformation. The same A. niger strain may produce different conversion products in response to different flavonoids, indicating that the microbial transformation can be highly specific (Das & Rosazza, 2006). Present research involves screening the metabolic enzymes of A. niger and the biotransformation of chemical components.

**Pericarpium Citri Reticulatae** contains large amounts of flavonoids, with hesperidin identified as the major compound. To date, only a small number of constituents, such as naringenin (Xu et al., 2012), rutin (You et al., 2010), tangeretin (Mahmoud et al., 2008), and nobiletin (Okuno & Miyazawa, 2004), have been reported to be transformed by A. niger. No studies have analyzed the chemical profiles and the biological differences before and after microbial biotransformation of PCR by A. niger.

We selected PCR as the experimental material and transformed it with a strain of A. niger. We compared the chemical and biological differences before (CK) and after (CP) microbial transformation by A. niger. UPLC-ESI-MS/MS techniques were used to compare the chemical profiles of CP and CK. HPLC coupled with diode array detector (HPLC–DAD) method and LC-MS were used to determine the major constituents in CP and CK. Three different methods (DPPH, FRAP, and ABTS) were used to evaluate the antioxidant activity. The objective of this study was to provide a practical method for the additional development and use of PCR.

**MATERIALS AND METHODS**

**Microbial transformation**

The strain of A. niger (3.13901) was isolated from soil and preserved in the China General Microbiological Culture Collection Center. The methods refer to the reported literature (Stankov-Jovanović et al., 2015). The details are as follows: an 8 g sample was weighed and spread in a petri dish. It was sterilized by ultraviolet irradiation on an ultra-clean workbench for 30 min, then turned over, and sterilized by irradiation for an additional 30 min. The sterilized samples were divided into the reverse inoculation group (CP) and the control group (CK). The spore suspension was obtained by eluting A. niger culture dish with sterile normal saline and filtered with absorbent cotton; then, 1 ml spore suspension was diluted 1,000 times with normal saline to obtain the standard spore suspension. CP: 1 ml standard A. niger spore suspension (10⁶ cfu/ml) was added to each petri dish (n = 6). CK: We added 1 ml of sterile water per petri dish (n = 6). The two groups of samples were cultured in an artificial climate chamber at 30°C with 95% RH. Samples were removed for detection after 5 days.

**Metabolites extraction**

A 50 mg sample was added to an EP tube and 1,000 μl of extraction solution (acetonitrile: methanol: water = 2:2:1) containing internal standard (L-2-chlorophenylalanine, 2 μg/ml) was added. After a 30 s vortex, the samples were homogenized at 35 Hz for 4 min and sonicated for 5 min in an ice-water bath. The homogenization and sonication cycle was repeated two times. Then the samples were incubated at −40°C for 1 hr and centrifuged at 11,180 g for 15 min at 4°C. A 250 μl sample of the supernatant was transferred to a fresh tube and dried in a vacuum concentrator at 37°C. The dried samples were reconstituted in 400 μl of 50% acetonitrile by sonication on ice for 10 min. The solution was then centrifuged at 18,894.2 g for 15 min at 4°C, and 75 μl of the supernatant was transferred to a fresh glass vial for LC/MS analysis. The quality control (QC) sample was prepared by mixing an equal aliquot of the supernatants from all of the samples (Wu, Jiao, et al., 2018).

**LC-MS/MS analysis**

UHPLC separation was carried out using a 1,290 Infinity series UHPLC System (Agilent Technologies), equipped with a UPLC BEH Amide column (2.1 x 100 mm, 1.7 μm, Waters). The mobile phase consisted of 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water (pH = 9.75) (A) and acetonitrile (B). The analysis was conducted with an elution gradient as follows: 0 – 0.5 min, 95% B; 0.5–7.0 min, 95%–65% B; 7.0 – 8.0 min, 65%–40% B; 8.0–9.0 min, 40% B; 9.0–9.1 min, 40%–95% B; and 9.1–12.0 min, 95% B. The column temperature was 25°C. The auto-sampler temperature was 4°C, and the injection volume was 1 μl (pos) or 1 μl (neg), respectively. The TripleTOF 6,600 mass spectrometry (AB Sciex) was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of...
MS/MS spectra depending on preselected criteria. In each cycle, the most intensive 12 precursor ions with intensity above 100 were chosen for MS/MS at collision energy (CE) of 30 eV. The cycle time was 0.5 s. ESI source conditions were set as following: Gas 1 at 60 psi, Gas 2 at 60 psi, Curtain Gas at 35 psi, Source Temperature at 600°C, Declustering potential at 60 V, and Ion Spray Voltage Floating (ISVF) at 5,000 V in positive mode (Shimizu et al., 2018).

Data preprocessing and annotation

MS raw data (.wiff) files were converted to the mzXML format by ProteoWizard and processed by R package XCMS (version 3.2). The process included peak deconvolution, alignment, and integration. Minfrac and cutoff were set as 0.5 and 0.3, respectively. In-house MS2 database was used for metabolite identification.

Determination of total antioxidant capacity

Approximately 2.0 g of citrus peels from each sample were freeze-dried using a vacuum freeze dryer and then ground into powder using a mortar. The antioxidant activities of citrus peels were evaluated by the DPPH radical scavenging activity assay. Briefly, 0.02 g of citrus peels was mixed with 180 µl of a DPPH working solution. The mixture was incubated at room temperature for 30 min in darkness. The absorbance was measured at 517 nm with a microplate reader (Sirivibulkovit et al., 2018). The ABTS radical scavenging capacities of citrus peels were conducted with a Total Antioxidant Capacity Assay Kit with ABTS method (Beyotime Biotechnology Co., Ltd.). Trolox was used as a standard compound. A calibration curve was prepared with different concentrations of Trolox in solution, and the results were expressed as mmol TEAC/L of citrus peels where TEAC is defined as the Trolox equivalent antioxidant capacity (Polak & Bartoszik, 2018). The reducing abilities of citrus peels were measured by a Total Antioxidant Capacity Assay Kit with the FRAP method (Beyotime Biotechnology Co., Ltd., Shanghai, China). The standard curve was constructed using FeSO4 solution, and the results were expressed as µM Fe(II)/g dry weight of the citrus peels (Mozaffari et al., 2018).

Targeted verification of hesperidin and vitex

Hesperidin and vitex were analyzed as described previously with some modifications (Miura et al., 2020). A 200 mg sample of powder was weighed and extracted overnight at 4°C in 1.0 ml of 70% aqueous methanol. Following centrifugation at 10,000 x g for 10 min, the extracts were absorbed (CNWBOND Carbon-GCB SPE Cartridge, 250 mg, 3 ml; ANPEL) and filtered (SCAA-104, 0.22 µm pore size; ANPEL, Shanghai, China) before LC-MS analysis. The HPLC conditions were as follows: HPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 µm, 2.1 mm × 100 mm), solvent system, water: acetonitrile, gradient program, 90:10 v/v at 0 min, 90:10 v/v at 1.0 min, 10:90 v/v at 3 min, 10:90 v/v at 5 min, 10:90 v/v at 6 min; flow rate, 0.42 ml/min; temperature, 40°C; and injection volume: 2 µl. The TripleTOF 6,600 mass spectrometry (AB Sciex) was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 500°C; ion spray voltage (IS) 5,500 V; ion source gas I (GS1), gas II (GS2), and curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively.

Targeted verification of quentin

Quentin was analyzed by HPLC, as described previously with some modifications (Xiao et al., 2020). The mobile phase, a mixture of buffer (0.4% phosphoric acid), and methanol (50:50 v/v) were filtered through a 0.45 µm membrane filter and degassed by sonication. HPLC analysis was performed at 30°C with a flow rate of 0.5 ml/min, and the samples were injected into an ODS C18 (4.6 mm × 250 mm) column (Beckman Coulter Inc.). The column effluent was monitored at 360 nm. Quantification was performed by comparing the peak areas obtained from the samples with those of standards.

Targeted verification of narirutin and naringenin

Narirutin and naringenin were determined according to the method described by Wang (Wang et al., 2018). We used 0.2 g powder and added 25 ml of methanol. The mixture was refluxed for 1 hr in a 75°C water bath. Following centrifugation at 10,000 x g for 5 min, the extracts were absorbed (CNWBOND Carbon-GCB SPE Cartridge, 250 mg, 3 ml; ANPEL, Shanghai, China) and filtrated (SCAA-104, 0.22 µm pore size; ANPEL, Shanghai, China) before use. Chromatographic Conditions were as follows: chromatographic column was a Hypersil BDS C18 (4.6 × 200 mm, 5 µm); solvent system: 0.05% phosphoric acid water and acetonitrile. The gradient elution program was time: 0–5 min, acetonitrile: 20%–25%; time: 5–10 min, acetonitrile: 25%–30%; time: 10–20 min, acetonitrile: 30%–50%; time: 20–30 min, acetonitrile: 50%–60%; time: 30–35 min, acetonitrile: 60%–90%; time: 35–40 min, acetonitrile: 90%–100%; column temperature: 30°C; flow rate: 0.7 ml/min; wavelength: 283 nm and 335 nm; injection volume: 5.0 µl.

RESULTS

Metabolic profiling

The metabolites of the citrus peels from CK and CP were investigated based on UPLC-ESI-MS/MS and relevant databases. In this study, 2,136 metabolites were detected (Table S1), and 767 of the metabolites were identified (Table S2). All of the metabolites were putatively identified according to the retention time, accurate mass,
MS2 and searches of the Human Metabolome Database (HMDB), MassBank, a comprehensive species-metabolite relationship database (KNAPSAck), and LIPID MAPS Structure Database (LMSD) metabolomics databases. A heat-map was applied to visualize the variations of the different metabolites in CK and CP (Figure 1a). The content of metabolites in CP greatly contrasted with that of the CK. This showed that the metabolic transformation caused by A. niger had substantial effects on the metabolic components in the citrus peels. Figure 1b shows that the correlation coefficient $R^2$ within the group were all greater than 0.9, indicating good repeatability between samples. This finding was demonstrated by clustering analysis of the two samples and showed that they could be clearly distinguished from each other.

**PCA and OPLS-DA analyses of differential metabolites**

Principal component analysis is an unsupervised pattern recognition method used for analyzing, classifying, and reducing the dimensionality of numerical datasets in multivariate problems (Ardila et al., 2015). This approach has been widely used for quality control of herbal medicines. Similarly, OPLS-DA analysis maximizes the variations between groups and is commonly used to screen differential metabolites (Triba et al., 2015). In this study, PCA was carried out to provide additional insight into the chemical differences between CK and CP. As shown in Figure 2a, the cumulative contribution rate of PC1 and PC2 was 88.64%, with 82.80% attributed to PC1 and 5.84% attributed to PC2 (Figure 2a,b). The classification results of PCA show noticeable differences between the CP and CK. Of the differential metabolites, they were used to establish an OPLS-DA model. The parameters of log2FC, p-value, and VIP values are shown in Table S3. The results presented in Figure 2c demonstrate that the $R^2X$, $R^2Y$, and $Q^2$ values determined using this model are 0.809, 0.999, and 0.986, respectively. Considering that $Q^2$ exceeds 0.9 and the red and green dot did not exceed the corresponding line (Figure 2d), the OPLS-DA model is stable and reliable and it can be used to identify the differential metabolites.

**Differential metabolite screening analysis**

The differential metabolites of CK and CP were screened based on the fold change and variable importance in project (VIP) values of the OPLS-DA model. Specifically, the metabolites having fold change values ≥2 or ≤0.5 and VIP values ≥1 were identified as differential (Table S3). The fold change of metabolites of the two samples was compared and analyzed, and the metabolites with greater changes are shown in Figure 3a after log2 treatment. The volcano plots of different metabolites (Figure 3b) show that the number of compounds upregulated and downregulated was 558 and 541, respectively. The number of compounds remaining unchanged was 1,037. From these large datasets, we focused on the changes of flavonoids because of the metabolic transformation of flavonoids by A. niger. Figure 3c shows that 244
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Flavonoid metabolites were identified, including 118 flavones, 39 flavonols, 25 flavonoids, 23 anthocyanins, 5 isoflavones, and 13 polyphenols. There were 52 significantly different flavonoid metabolites between CP and CK (Figure 3d), including 26 flavones (11 downregulated, 15 upregulated), 6 flavonols (2 downregulated, 4 upregulated), 3 flavonoids (1 downregulated, 2 upregulated), 3 flavanones (2 downregulated, 1 upregulated), 6 anthocyanins (3 downregulated, 3 upregulated), 4 isoflavones (0 downregulated, 4 upregulated), and 4 polyphenols (2 downregulated, 2 upregulated). The free radical scavenging ability of flavonoids is proportional to the phenolic hydroxyl groups of the A and B rings. The scavenging ability of phenolic hydroxyl groups on the B ring is stronger than that of the A ring. The position of the 3′ phenolic hydroxyl is the most important (Chen et al., 2002; Bagchi et al., 2014). All the upregulated differential flavonoids metabolites between CP and CK are shown in Table 1. The upregulated flavonoids have abundant phenolic hydroxyl groups in the A and B rings. Some compounds, such as keracyanin, 6-C-hexosyl-luteolin-5-O-pentoside, C-hexosyl-luteolin-5-O-feruloylpentoside, and hesperetin, have 3′ phenolic hydroxyl on the B ring.

In addition, we analyze the enrichment of the differential metabolites and interestingly found that some primary metabolites such as amino acids and sugars changed greatly (Figure 4), which suggest...
that the mechanism of metabolite transformation by *A. niger* may also be closely related to the changes of energy substances, such as amino acids or sugars.

### Antioxidant and targeted quantitative analysis

#### Antioxidant analysis

The antioxidant activities of CP and CK were determined by DPPH, ABTS, and FRAP assays. The DPPH, ABTS, and FRAP values of CK were $12.2 \pm 1.43$ TE mmol/g, $5.34 \pm 3.42$ TE mmol/g, and $2.63 \pm 2.29$ FeII mmol/g, respectively, while the DPPH, ABTS, and FRAP values were $7.48 \pm 1.52$ TE mmol/g, $3.92 \pm 2.38$ TE mmol/g, and $1.38 \pm 1.91$ FeII mmol/g, respectively (Table 2). CP exhibited higher antioxidant activity than CK (Figure 5a).

#### Targeted quantitative analysis

To verify the absolute content of upregulated flavonoids, five up-regulated differential flavonoids—quercetin, naringenin, hesperetin,
The accumulation of phenolic hydroxyl compounds. The higher composition of metabolites, transform flavonoids, and promote antioxidant activity of CP may be attributed to the presence of a higher level of flavonoids with rich phenolic hydroxyl groups.

**TABLE 1** A list of upregulated differential flavonoids metabolites between *Pericarpium Citri Reticulatae* (CP) and *Pericarpium Citri Reticulatae* (CK)

| No. | Molecular weight | Compounds | Class | Hydroxyl group and its position |
|-----|------------------|-----------|-------|--------------------------------|
| 1   | 2.86E+02         | Orobol (5,7,3',4'-tetrahydroxyisoflavone) | Isoflavone | 5-OH; 7-OH; 3'-OH; 4'-OH |
| 2   | 4.30E+02         | Formononetin -7-O-glucoside (Ononin) | Isoflavone | 4'-OH |
| 3   | 2.86E+02         | 2'-Hydroxygenistein | Isoflavone | 5-OH; 7-OH; 2'-OH; 4'-OH |
| 4   | 2.84E+02         | Prunetin | Isoflavone | 7-OH; 4'-OH |
| 5   | 5.95E+02         | Cyanidin-3-O-rutinoside (Keracyanin) | Anthocyanins | 4-OH; 6-OH; 3'-OH; 4'-OH |
| 6   | 6.11E+02         | Cyanidin-3,5-O-diglucoside (Cyanin) | Anthocyanins | – |
| 7   | 4.33E+02         | Pelargonidin-3-O-beta-D-glucoside | Anthocyanins | 5-OH; 4'-OH |
| 8   | 4.64E+02         | Isoquercitrin | Flavonoid | 4-OH; 6-OH; 3'-OH; 4'-OH |
| 9   | 3.44E+02         | 5,7-Dihydroxy-3',4',5'-trimethoxyflavone | Flavonoid | 5-OH; 7-OH |
| 10  | 3.14E+02         | Velutin | Flavone | 7-OH; 4'-OH |
| 11  | 6.24E+02         | 3',4',5'-Tricetin-5-O-rutinoside | Flavone | 3'-OH; 4'-OH; 5'-OH |
| 12  | 6.24E+02         | Chrysoeriol-5-O-hexosyl-3-O-hexoside | Flavone | 7-OH; 4'-OH |
| 13  | 7.28E+02         | C-hexosyl-apigenin-5-O-hexosyl-3-O-pentoside | Flavone | 7-OH; 4'-OH |
| 14  | 7.56E+02         | 6-C-hexosyl-apigenin-5-O-hexosyl-6-O-hexoside | Flavone | 7-OH; 4'-OH |
| 15  | 5.80E+02         | 6-C-hexosyl lutelenin-5-O-pentoside | Flavone | 7-OH; 3'-OH; 4'-OH |
| 16  | 5.94E+02         | Vitex | Flavone | 5-OH; 7-OH; 4'-OH |
| 17  | 7.56E+02         | C-hexosyl-luteolin-5-O-feruloylpentoside | Flavone | 7-OH; 3'-OH; 4'-OH |
| 18  | 3.02E+02         | Hesperetin | Flavone | 5-OH; 7-OH; 3'-OH |
| 19  | 5.64E+02         | C-hexosyl-apigenin C-pentoside | Flavone | 5-OH; 7-OH; 4'-OH |
| 20  | 4.76E+02         | Chrysoeriol-5-O-hexoside | Flavone | 7-OH; 4'-OH |
| 21  | 6.08E+02         | Chrysoeriol-7-O-rutinoside | Flavone | 5-OH; 4'-OH |
| 22  | 2.86E+02         | Luteolin | Flavone | 4-OH; 6-OH; 3'-OH; 4'-OH |
| 23  | 3.02E+02         | Tricetin | Flavone | 5-OH; 7-OH; 3'-OH; 4'-OH; 5'-OH |
| 24  | 5.81E+02         | Narirutin | Flavone | 7-OH; 4'-OH |
| 25  | 3.02E+02         | Ellagic acid | Polyphenol | 3-OH; 4-OH; 4'-OH; 5'-OH |
| 26  | 4.58E+02         | Gallocatechin gallate | Polyphenol | 5-OH; 7-OH; 3'-OH; 4'-OH; 5'-OH |
| 27  | 4.34E+02         | Naringenin | Flavanone | 5-OH; 7-OH; 4'-OH |
| 28  | 3.02E+02         | Quercetin | Flavonol | 5-OH; 7-OH; 3'-OH; 4'-OH |
| 29  | 4.64E+02         | Quercetin-3-O-glucoside (Iso trifoliin) | Flavonol | 5-OH; 7-OH; 3'-OH; 4'-OH |
| 30  | 3.04E+02         | Dihydroquercetin (Taxifolin) | Flavonol | 5-OH; 7-OH; 3'-OH; 4'-OH |

vite, and narirutin—were quantitatively analyzed by HPLC or LC-MS. The contents of quercetin, naringenin, hesperetin, vitex, and narirutin in CP were 15.11 ± 3.23 μg/g, 14.83 ± 2.02 μg/g, 47.13 ± 2.11 μg/g, 48.80 ± 1.47 ng/g, and 2.26 ± 1.33 mg/g, respectively (Table 2). The contents of quercetin, naringenin, hesperetin, vitex, and narirutin in CK were 12.11 ± 2.66 μg/g, 12.33 ± 2.36 μg/g, 21.33 ± 2.01 μg/g, 29.33 ± 1.22 ng/g, and 1.33 ± 1.25 mg/g, respectively. CP exhibited higher contents than CK (Figure 5b-d). The absolute quantitative results were consistent with the relative quantitative results. Previous studies have reported a linear positive correlation between flavonoid compounds rich in phenolic hydroxyl groups with their antioxidant capacities (Wang et al., 2017). Therefore, microbial transformation of PCR by *A. niger* can change the composition of metabolites, transform flavonoids, and promote the accumulation of phenolic hydroxyl compounds. The higher antioxidant activity of CP may be attributed to the presence of a higher level of flavonoids with rich phenolic hydroxyl groups.

**DISCUSSION**

Bioconversion technology has attracted attention because of its potential for producing novel active chemicals (Hidalgo et al., 2018). *A. niger* is a eukaryote that is considered safe in biomicrobial transformation (Schuster et al., 2002). The metabolites of *A. niger* contain abundant metabolic enzymes, which can efficiently transform different types of compounds (Cao et al., 2015; Kumar & Pandey, 2013). *A. niger* transformation of monomer compounds has been a focus because of its great potential for producing novel active components. However, the conversion of complex mixtures has rarely been
The purpose of this study is to provide a practical method for the additional development and use of PCR using microbial transformation. In this study, microbial biotransformation of PCR by A. niger was analyzed based on the combination of UPLC-ESI-MS/MS, HPLC, and LC-MS methods, and the antioxidant activity was also compared using DPPH, FRAP, and ABTS methods. The results demonstrated detection of 2,136 metabolites among which 767 metabolites were reported. The purpose of this study is to provide a practical method for the additional development and use of PCR using microbial transformation.
identified in CP and CK. Heat-map analysis, principal component analysis (PCA), and orthogonal signal correction and partial least squares-discriminant analysis (OPLS-DA) were used to clearly discriminate between CP and CK. The results revealed that *A. niger* participated in the transformation of metabolites and had a great impact on the metabolism of PCR. The changes of these metabolites are often closely related to the corresponding changes in biological activity. Ma et al. reported that the biological activity of plant extracts were strongly positively correlated with their changes in active ingredients after microbial biotransformation (Ma et al., 2019).

To understand the difference of antioxidant activity between CP and CK, 109 differential flavonoids metabolites were screened out and the number of upregulated and downregulated differential flavonoids was analyzed. The number of upregulated flavonoids was significantly greater than the downregulated flavonoids, and the upregulated flavonoids contained abundant polyhydroxy groups. In general, flavonoids often contain polyhydroxy groups which serve as antioxidants due to their free radical scavenging activity (Gil-Cardoso et al., 2016). In particular, 3′ phenolic hydroxyl on the B ring can greatly increase its ability to scavenge free radicals. We concluded that the metabolic transformation produced by *A. niger* can significantly improve the antioxidant activity of PCR. The antioxidant activity assay demonstrated that CP had significantly higher free radical-scavenging activity than CK, and the higher antioxidant activity of CP may be attributed to the presence of a high level of flavonoids with rich phenolic hydroxyl groups. These results provide useful information related to the mechanism of flavonoid transformation by *A. niger* (Abdella et al., 2018).

**CONCLUSION**

We analyzed the changes of metabolites before (CK) and after (CP) microbial transformation of *Pericarpium Citri Reticulatae* (PCR) by *Aspergillus niger* using UPLC-ESI-MS/MS, HPLC, and LC-MS methods, and we compared the antioxidant activity using three different methods (DPPH, FRAP, and ABTS). Qualitative and quantitative analyses showed that microbial transformation of PCR by *A. niger* could transform flavonoids and also promote the accumulation of phenolic hydroxyl compounds. The antioxidant assay demonstrated that CP had significantly higher free radical scavenging activity than CK, and the higher antioxidant activity of CP may be attributed to the presence of a high level of flavonoids with rich phenolic hydroxyl groups. These results provide useful information related to the mechanism of flavonoid transformation by *A. niger*. The strain of *A. niger* may also have a great influence on the high efficiency extraction of flavonoids, the conversion of active ingredients, and the improvement of PCR quality.
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CONFLICT OF INTEREST
All the authors declare that there are no conflicts of interest.

ETHICAL APPROVAL
This study does not involve any human or animal testing.

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This study does not involve any human or animal testing.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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