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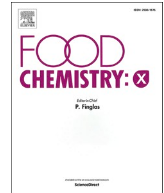

Tailoring the composition, antioxidant activity, and prebiotic potential of apple peel by *Aspergillus oryzae* fermentation

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ABSTRACT

Apple peel is a typical lignocellulosic food by-product rich in functional components. In this work, apple peel was solid-state fermented with *Aspergillus oryzae* with an aim to modulate its composition and bioactivity. The results showed that *A. oryzae* fermentation substantially tailored the composition, improved the antioxidant activity and prebiotic potential of apple peel. Upon the fermentation, 1) free phenolics increased and antioxidant activity improved; 2) the pectin substances degraded significantly, along with a decrease in soluble dietary fiber while an increase in insoluble dietary fiber; 3) the *in vitro* fermentability increased as indicated by the increase in total acid production. The gut microbiota was shaped with more health-promoting potentials, such as higher abundances of *Lactobacillus*, *Bifidobacterium*, *Megamonas* and *Prevotella*-9 as well as lower abundances of *Enterobacter* and *Echerichia-Shigella*. This work is conducive to the modification of apple peel as a potential ingredient in food formulations.

1. Introduction

Apple peel is a major byproduct of the apple processing industry. Its proper disposal is critical for the construction of a sustainable, eco-friendly, and profitable apple processing facility. Apple peel is rich in dietary fiber (40.27–68.8 g/100 g) and phenolics, which are much higher in apple peel (1157–5119 μg/g) than in pulp (423–1534 μg/g) (Kim et al., 2019). Previous studies have revealed that the dietary fiber and phenolics in apple peel are capable of preventing obesity-related metabolic dysfunction, lowering inflammatory markers, and altering gut microbiota (Elkahoui et al., 2019).

Despite its nutritional and bioactive merits, apple peel powder is difficult to be incorporated into various foods, even when ultramicro-pulverized. This is mainly due to the high content of lignocellulosic substances, the poor compatibility with food matrix, and rough texture. Hence, from a traditional perspective, apple peel was mainly consumed as an animal feed, a raw material for extracting pectin and phenolics, or a substrate for producing organic acids, enzymes and exopolysaccharide through fermentation (Perussello et al., 2017). Like other lignocellulosic byproducts from food industry, apple peel is certainly a valuable food resource in terms of the overburden of various metabolic diseases for human beings the demand for the healthy diets. In this scenario, many efforts had been made by food scientists in converting food lignocellulosic byproducts from a waste stream to an income source. These efforts mainly aimed at significantly improving the texture of these byproducts, resulting in their better compatibility with certain food matrix and substantially eradicating their negative impacts on the mouthfeel of incorporated foods. To achieve this, physical, chemical, and biological methods as texture modifying pretreatments of food lignocellulosic byproducts were developed. Among them, biological processes such as enzymatic hydrolysis and microbial fermentation were of excellent outcome, easy operability, high safety, and good economic feasibility. In contrast to enzymatic hydrolysis, microbial fermentation has received a lot of interests in recent years due to its extensive changes made for items. Tosun and Yasar (2020) found that both the fermentations of *Pleurotus ostreatus* and *Phanerochaete chrysosporium* significantly increased crude ash and protein but decreased crude fiber and reducing sugar of apple pomace. The results of Gulsunoglu et al. (2020) showed that the fermentations of four *Aspergillus spp*. (*Aspergillus aculeatus* ZGM6, *Aspergillus japonicus* ZGM4, *Aspergillus niger* ZDM2, *Aspergillus*

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tubingensis ZDM1) could significantly improve the total phenolics, flavonoid, and antioxidant activity of apple peel up to three to five folds, although the increments were strain specific.

Clearly, the selection of microorganism strain is of extreme importance in fermentation pretreatments. Besides the above-mentioned strains, *A. oryzae* is widely used in manufacturing various fermented foods such as miso, koji and soy sauce. The feasibility of *A. oryzae* fermentation to pretreat or activate lignocellulosic biomass, such as cereal brans, has been verified (Shina et al., 2019). Specifically, the fermentation of *A. oryzae* not only increased phenolic acids, especially protocatechuic acid and ferulic acid, in black rice bran but also improved the DPPH radical scavenging capacity and tyrosinase inhibitory activity of its phenolic extract (Shina et al., 2019). However, the feasibility of *A. oryzae* fermentation to modulate the bioactivities and functional properties of lignocellulosic fruit and vegetable wastes remained unknown. To this end, the effects of *A. oryzae* fermentation on the chemical composition, antioxidant activity and prebiotic potential of apple peel were explored in this study. The results obtained herein will certainly favor the value-added and sustainable utilization of apple peel.

2. Materials and methods

2.1. Materials and reagents

Dried apple peel, as a by-product of apple chips, was obtained from Meiqingyuan Food Co. (Sichuan, China). *A. oryzae* (CICC 2339) in lyophilized powder form was acquired from China Center of Industrial Culture Collection (CICC) and it was kept at 4 °C until use. The standards of mannose (Man), rhamnose (Rha), galacturonic acid (GalA), glucose (Glc), galactose (Gal), xylose (Xyl), arabinose (Ara) and fucose (Fuc) and lactose (Lac) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). High-performance liquid chromatograph (HPLC)-grade chlorogenic acid, hyperin, isoquercitrin, ellagic acid, phloridzin, quercitrin, quercetin and Folin-Ciocalteu's reagent was obtained from YuanyeBio-Technology Co., Ltd (Shanghai, China). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was obtained from Adamas Reagent Co., Ltd. (Shanghai, China). Acetonitrile and methanol of chromatography grade and other chemicals of analytical grade were purchased from Kelong Chemical Reagent Co., Ltd (Chengdu, China).

2.2. Fermentation of apple peel with A. oryzae

Dried apple peel was micronized to pass through a 60-mesh sieve resulting in a fine powder. The lyophilized *A. oryzae* was reactivated on a solid medium containing 130 g/L malt powder, 0.1 g/L chloramphenicol, and 15 g/L agar. After incubated at 28 °C for 5 days under a relative humidity (RH) of 80 %, the strain was transferred to the same medium to achieve full restoration. To perform the fermentation, apple peel powder (15.0 g db) was suspended in distilled water (18 mL) in a 250-mL Erlenmeyer flask and autoclaved at 121 °C for 15 min. Being cooled to ambient temperature, the resultant slurry was inoculated with 100 μL of newly restored spore suspension (10^6 spores/mL). Subsequently, the slurry was incubated at 28 °C under a RH of 80 %. The slurry (in triplicate) was sampled at specific intervals (0, 4, 8 and 12 days, the limit of fermentation time (12 d) was determined in a pre-experiment as the time that the strain achieved its highest mycelium amount) and subjected to subsequent analysis after lyophilization.

2.3. Proximate composition analysis

Crude protein and fat were determined using Kjeldahl method and Soxhlet extraction following the methods of AOAC, respectively. Reducing sugar and soluble sugar were estimated by a 3,5-dinitrosalicylic acid method (Zhang et al., 2019). Insoluble (IDF), soluble (SDF) and total (TDF) dietary fiber were determined by enzymatic–gravimetric method (De Simas et al., 2010).

2.4. Identification and quantification of free and bounded phenolics

The levels of free and bounded phenolics were determined according to the method of Bei et al. (2018a) with some modifications. The phenolics monomers in the extract were mainly identified and quantified by a HPLC method with the aid of phenolics standards (Del Pino-García et al., 2017). Briefly, apple peel powder (1.0 g) was extracted twice with 40 mL aqueous acetone (80 % w/v) and the supernatants obtained by centrifugation (4000 r/min, 10 min) were combined and evaporated to dryness at 45 °C under vacuum (0.098 MPa) on a RE-3000B rotary evaporator (Shanghai, China). The dry residue was dissolved in methanol (10 mL) for the determination of free phenolics. The solid residue resulted from the acetone extraction was subjected further determination of bounded phenolics. To perform, the residue was digested in 2 M NaOH (20 mL) at room temperature for 2 h. After neutralized with 12 M HCl, the slurry was extracted with hexane (20 mL) for 10 min with continuous shaking. Upon a centrifugation (4000 r/min, 10 min), the hexane layer was carefully decanted and the remains was extracted twice with ethyl acetate (20 mL) for 10 min with continuous shaking. The supernatants obtained by centrifugation (4000 r/min, 10 min) were further processed as did for free phenolic. The concentrations of total phenolics in both extracts were determined by a Folin-Ciocalteu's method as did elsewhere.

A LC-20A HPLC system (Shimadzu, Kyoto, Japan) equipped with a Thermo BDS-C18 reversed-phase column (250 × 4.6 mm i.d., 5 μm) (Waters Co., Milford, MA, USA) was applied. A binary mobile phase consisted of 0.1 % (v/v) phosphoric acid (A) and absolute methanol (B) was supplied at a flow rate of 0.4 mL/min at 40 °C. The elution was programmed as follows: 15 % B (0 ~ 30 min), 60 % B (30 ~ 35 min), 80 % B (35 ~ 40 min), 90 % B (40 ~ 50 min) and 15 % B (50 ~ 60 min). The sample injection volume was 5 μL and the elute was monitored at 280 nm. The phenolics monomers were identified by their retention times and quantified with their peak areas by an external standard method. For unidentifiable peaks, ultra performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-TOF-MS) was used for their authentication as previously performed by Bourvellec et al. (2011). In the present study, quercetin-3-D-xyloside and quercetin-3-O-α-L-arabinoside were identified and they were quantified by consulting the calibration curve of quercetin.

2.5. Structural characterization of pectin substances

Pectin content in apple peel was determined by a carbazole sulfuric acid method as described previously (Liang et al., 2022). For its structural characterization, the pectin material was extracted from apple peel according to the method of Pasandide et al. (2017) with some modifications. Briefly, apple peel powder (4.0 g) was extracted twice with 0.05 M hydrochloric acid solution (60 mL) at 80 °C for 2 h with continuous stirring. After each extraction, the slurry was centrifuged at 4000 rpm for 20 min. The obtained supernatants were combined and the molecular weight distribution of polysaccharides was determined by a high-performance size exclusion chromatography (HPSEC) equipped with a TSK gel G4000PWXL column (TOSOH, Tokyo, Japan) and a refractive index RID-10A detector (Shimadzu, Tokyo, Japan) (Feng et al., 2019). Moreover, the supernatant (50 mL) was mixed with absolute ethanol (1:1, v/v) for 6 h at ambient temperature and the precipitate was collected by a centrifugation at 4000 rpm for 15 min representing the higher molecular weight fraction of pectin material. Upon lyophilization, its monosaccharide composition was analyzed as described by Feng et al. (2019).

2.6. Evaluation of antioxidant capacity of apple peel powder

To evaluate 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging capacity of apple peel powder, a method for measuring the antioxidant activity of insoluble food components was

adopted (Serpen et al., 2007). The ferric ion reducing power (FRAP) was evaluated according to a previous report with some modifications (Tao et al., 2014). Briefly, the apple peel powder was diluted with microcrystalline cellulose prior to measurement. In the present study, the final weight fraction of apple peel powder was settled in the range of 0.10 ~ 0.35 mg/mg. The diluted apple peel powder (5 mg) was suspended in 1 mL methanol in a 10-mL centrifuge tube. Then 3.5 mL of ABTS solution was added and the suspension was mixed vigorously. Upon a holding at 30 °C for 3 min, the absorbance of the resultant up-layer was immediately recorded at 735 nm. The ABTS radical scavenging rate (%) was calculated as $[(A_0 - A)/A_0] \times 100$, where A_0 and A were the absorbance values of the blank and sample, respectively.

Apple peel powder was weighed into a 10-mL centrifuge tube and then 1 mL methanol and 3.5 mL of fresh FRAP working solution was added. The resultant suspension was shaken and kept in a water bath at 37 °C for 5 min and the absorbance of the up-layer was immediately recorded at 593 nm. The FRAP was expressed as the value of (A-A_0), where A_0 and A referred to the absorbance of the sample and blank.

2.7. Evaluation of the prebiotic potential of apple peel powder

In vitro fermentability of apple peel powder (unfermented and fermented by *A. oryzae*) by fresh human feces was conducted according to the reported method with slight modifications (Zhou et al., 2018). The fresh human feces were obtained from three college students (23 ~ 25 age, two females and one male), who did not receive any antibiotics treatment at least 3 months. The feces from three volunteers were mixed equivalently. The 10 % (w/w) fecal slurry was prepared by suspending the mixed feces in sterilized phosphate buffered saline (0.1 M, pH 7.2). The basal nutrient growth medium was prepared according to the following formulation: 2.0 g/L yeast extract, 2.0 g/L peptone, 0.1 g/L NaCl, 0.04 g/L KH_2PO_4, 0.04 g/L K_2HPO_4, 0.01 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $CaCl_2$, 2 g/L $NaHCO_3$, 0.02 g/L hemin, 0.5 g/L cysteine-HCl, 0.5 g/L bile salt, 2.0 mL/L Tween 80, 1.0 mg/L resazurin, and 10 μL/L vitamin K. In practice, apple peel powder (100 mg) was suspended in 9 mL of autoclaved basal nutrient growth medium and 1 mL of fecal slurry was added. After totally mixed, the mixture (in triplicate) was anaerobically incubated for 48 h at 37 °C on a ZQPL-500 T thermostatic shaker (Tianjin, China) operated at 150 r/min. At specific incubation intervals (0, 6, 12, 24 and 48 h), the mixture was sampled and subjected to a centrifugation at 12,000 rpm for 10 min at 4 °C. The resultant supernatant was used for short chain fatty acids (SCFAs) quantification, while the sediment was applied of microbiota analysis. If these samples were not immediately analyzed, they were stored at − 80 °C prior to the measurements. In contrast, a parallel trial omitting the addition of apple peel powder was used as the blank control.

The SCFAs in the supernatant was determined by our previous method with slight modifications (Zhao et al., 2006). The supernatant (0.5 mL) from fermentation slurry was mixed with acidified 50 % ethanol (pH 2, 0.5 mL) and subjected to ultrasound (40 W) for 20 min. Then, the resulted mixture was centrifuged at 5000 rpm for 20 min and the obtained supernatant (1 μL) was directly injected. The SCFAs were quantified by using an internal standard of 2-ethylbutyric acid. To analyze the microbiota, the total bacterial DNA was extracted from the sediment and sequenced under the help of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). More specifically, the V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′GGACTACHVGGGTWTCTAAT-3′) primers in a Gene Amp 9700 thermocycler PCR system (ABI, USA). The raw sequencing data was analyzed on Majorbio ISanger Cloud Platform (https://www.i-sanger.com/).

2.8. Statistical analysis

All analyses were performed in triplicate, and the results were presented as mean ± standard deviation (SD). The data were evaluated by using SPSS 20.0 and any difference was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Changes in proximate composition

The proximate composition of apple peel at various fermentation stages was summarized in Table 1. Obviously, the dry mass of the substrate gradually decreased as the fermentation process to 12 d (from 13.07 ± 0.32 g to 12.37 ± 0.20 g, by about 5.3 ± 3.6 %). In considering this and to reflect the substantial changes of these ingredients, the data in Table 1 referred to actual mass instead of content used elsewhere. Like the dry mass of substrate, reducing sugar and soluble sugar decreased with the fermentation extended but in greater degrees (by approximate 22.7 ± 4.1 %). As for crude fat, an opposite trend was concluded (from 0.37 ± 0.05 g to 0.76 ± 0.06 g). Interestingly, crude protein increased in

Table 1
The composition changes of apple peel as affected by the fermentation by *A. oryzae*.

Component	Fermentation time (d)			
	0	4	8	12
Wet weight (g)	32.07 ± 0.38^{b}	31.77 ± 0.12^{ab}	31.67 ± 1.00^{ab}	30.87 ± 0.49^{a}
Dry weight (g)	13.07 ± 0.32^{b}	13.52 ± 0.04^{b}	13.14 ± 0.29^{b}	12.37 ± 0.20^{a}
Reducing sugar (g)	0.45 ± 0.01^{c}	0.41 ± 0.03^{bc}	0.40 ± 0.01^{b}	0.34 ± 0.02^{a}
Soluble sugar (g)	0.47 ± 0.03^{c}	0.47 ± 0.01^{c}	0.41 ± 0.01^{b}	0.37 ± 0.01^{a}
Crude fat (g)	0.37 ± 0.05^{a}	0.44 ± 0.01^{a}	0.56 ± 0.04^{b}	0.76 ± 0.06^{c}
Crude protein (g)	0.71 ± 0.08^{a}	0.94 ± 0.08^{b}	0.85 ± 0.09^{ab}	0.71 ± 0.12^{a}
Soluble dietary fiber (g)	2.30 ± 0.23^{c}	1.87 ± 0.05^{b}	1.22 ± 0.07^{a}	1.10 ± 0.07^{a}
Insoluble dietary fiber (g)	4.47 ± 0.16^{a}	4.83 ± 0.21^{b}	4.87 ± 0.22^{b}	4.74 ± 0.08^{ab}
Total dietary fiber (g)	6.77 ± 0.33^{b}	6.70 ± 0.20^{b}	6.10 ± 0.29^{a}	5.83 ± 0.14^{a}
Pectin (g)	1.11 ± 0.15^{c}	0.57 ± 0.04^{b}	0.36 ± 0.02^{a}	0.30 ± 0.03^{a}
Free phenolic (GAE mg)	107.61 ± 7.26^{a}	132.28 ± 13.25^{a}	230.76 ± 18.18^{b}	285.89 ± 21.82^{c}
Bound phenolic (GAE mg)	29.74 ± 4.54^{a}	33.55 ± 3.46^{a}	29.04 ± 2.48^{a}	28.83 ± 0.91^{a}
Total phenolic (GAE mg)	137.35 ± 6.72^{a}	165.82 ± 16.46^{a}	259.79 ± 20.54^{b}	314.71 ± 21.79^{c}
Chlorogenic acid (mg)	2.59 ± 0.08^{c}	1.94 ± 0.19^{b}	0.31 ± 0.01^{a}	0.19 ± 0.00^{a}
Caffeic acid (mg)	0.15 ± 0.02^{a}	0.34 ± 0.04^{b}	0.90 ± 0.02^{d}	0.65 ± 0.01^{c}
Hyperin (mg)	5.88 ± 0.06^{c}	5.54 ± 0.10^{b}	5.61 ± 0.15^{b}	5.26 ± 0.06^{a}
Isoquercitrin (mg)	0.46 ± 0.02^{a}	0.47 ± 0.00^{a}	0.48 ± 0.03^{a}	0.45 ± 0.02^{a}
Ellagic acid (mg)	1.16 ± 0.00^{a}	1.16 ± 0.01^{a}	1.17 ± 0.07^{a}	1.11 ± 0.05^{a}
Quercetin-3-xyloside (mg)	1.19 ± 0.03^{c}	1.14 ± 0.01^{bc}	1.10 ± 0.03^{ab}	1.03 ± 0.07^{a}
Quercetin-3-arabinoside (mg)	0.76 ± 0.04^{c}	0.65 ± 0.03^{b}	0.65 ± 0.01^{b}	0.59 ± 0.01^{a}
Phloridzin (mg)	1.52 ± 0.05^{a}	1.50 ± 0.04^{a}	1.56 ± 0.06^{a}	1.48 ± 0.06^{a}
Quercitrin (mg)	1.26 ± 0.06^{a}	1.39 ± 0.02^{ab}	1.44 ± 0.12^{b}	1.35 ± 0.03^{ab}
Quercetin (mg)	8.09 ± 0.10^{a}	8.41 ± 0.35^{ab}	9.13 ± 0.26^{c}	8.77 ± 0.15^{bc}

[a-c] Values bearing different superscript lower letters in the same line were significantly different ($p < 0$ 0.05).

early fermentation stage and then decreased in following stages. The highest protein mass was found at the fermentation of 4 d (0.94 ± 0.08 g) and the value at 12 d was comparable to that at beginning (0.71 ± 0.12 g). All of these changes were fundamentally based on the growth of *A. oryzae*, its consumption of the chemicals in apple peel, and the composition differences between its mycelia and apple peel (Xu et al., 2016). For reducing sugar and soluble sugar, they acted as the carbon sources of *A. oryzae*, being utilized and thus decreasing in mass. The increase in crude fat was interpreted as a result of the fact that *A. oryzae* mycelia (6 g/100 g) contain a much higher level of fat than apple peel (3 g/100 g) (Xu et al., 2016). For the parabolic trend observed in protein mass, the early increasing part resulted from the growth of A. *oryzae* mycelia, which has a higher protein level (50 g/100 g) than apple peel (5 g/100 g) (Xu et al., 2016). The subsequent decreasing part was a consequence of microbial metabolism. These supported the view of Perussello et al. (2017) that apple peel can provide many vital nutrients necessary for the growth of microorganisms. The fermentation-induced increases in crude protein and/or fat were evidenced for apple pomace, such as the fermentations cases by autochthonous yeasts (Madrera et al., 2017), *Pleurotus ostreatus* and *Phanerochaete chrysosporium* (Tosun & Yasar, 2020).

For dietary fibers, SDF gradually decreased along the fermentation process, while IDF displayed a small but significant rise first and then recovered at the end of fermentation (12 d). It was proposed that the SDF/IDF ratio is a key parameter to evaluate the nutritional quality of a dietary fiber product and a value of 1:2 is generally recommended (Jagelaviciute et al., 2023). In this context, the present method was impossible to improve the nutritional quality of dietary fiber in apple peel by increasing its SDF/IDF ratio. On the other hand, the present results indicated that SDF in apple peel could be utilized by *A. oryzae*. The increase of IDF during fermentation, despite of its minor degree, can be attributed by the accumulation of chitin as a cell wall component of *A. oryzae*. In this case, the decrease of TDF in apple peel with *A. oryzae* fermentation took place in the portion of SDF, whereas IDF increased or remained. Similarly, the crude fiber in apple pomace decreased with the fermentation by *Pleurotus ostreatus* and *Phanerochaete chrysosporium* (Tosun & Yasar, 2020). Like SDF, pectin in apple peel underwent a significant reduction during *A. oryzae* fermentation. Specifically, in contrast to unfermented specimen, the samples upon the fermentation for 4 d and 8 d presented SDF mass reductions of 0.42 ± 0.19 g and 1.07 ± 0.28 g, and pectin mass reductions of 0.54 ± 0.12 g and 0.74 ± 0.16 g, respectively. By comparing these values, it was easy to conclude that pectin is more liable to be utilized than other SDF by *A. oryzae* and the microbiota utilized these ingredients possible in a sequential pattern. It was widely recognized that *A. oryzae* can secrete a serial of extracellular enzymes, such as amylolytic, proteolytic, cellulolytic, xylanolytic and pectinolytic enzymes, which degrade carbohydrates and polypeptides in fermentation matrix. In this way, the microorganism obtained more carbon and nitrogen sources for growth and reproduction.

3.2. Changes in the composition of polyphenols

In terms of free, bound, and total phenolics, the data in Table 1 clearly indicated that the fermentation with *A. oryzae* resulted in significant increases in free (from 107.61 ± 7.26 GAE mg to 285.89 ± 21.82 GAE mg) and total (from 137.35 ± 6.72 GAE mg to 314.71 ± 21.79 GAE mg) phenolics, while bound phenolics did not change substantially. This result also demonstrated that the phenolics in apple peel were mainly in a free form rather than a bound form, which was consent to the findings reported by Pérez-Jiménez and Saura-Calixto (2018). Clearly, the increase in free phenolics was not a consequence of the conversion of bound phenolics as evidenced in other materials (Bei et al., 2018b). For the present work, the increase in free phenolics can be attributed to the enzymes produced during fermentation. First, the fermentation produced some cell wall degrading enzymes, such as α-amylase, xylanase and β-glucosidase, which destroyed the cell wall of apple peel and ultimately increased the extractability of phenolics. This was based on the fact that phenolics are usually found in conjugated forms through hydroxyl groups with sugar and glycosides (Ajila et al., 2011). These enzymes were also evidenced with a moderate capacity to degrade macromolecular phenolic compounds, such as lignin, which certainly led to the increase in free phenolics (Schmidt et al., 2014). Besides these glycosidic enzymes, the presence of esterase or protease in fermentation may also contribute to the increase in free phenolics (Bei et al., 2018b). The increases in phenolics were widely observed in many cases involving the fermentation of lignocellulosic biomass (Bei et al., 2018b; Liu et al., 2021). For example, during the solid-state fermentation by *A. niger* and *Rhizopus oligosporus* for 12 d, the free phenolics in chokeberry pomace increased up to 1.7-fold (Dulf et al., 2018). Similarly, the soluble phenolics in oat powder were significantly increased (281.48 mg/kg) upon the solid-state fermentation by *Monascus anka* (Bei et al., 2018b).

As for the composition of free phenolics in original apple peel (13.07 ± 0.32 g), ten phenolics, including quercetin (8.09 ± 0.10 mg), hyperin (5.88 ± 0.06 mg), chlorogenic acid (2.59 ± 0.08 mg), phloridzin (1.52 ± 0.05 mg), quercitrin (quercetin-3-rhamnoside, 1.26 ± 0.06 mg), quercetin-3-xyloside (1.19 ± 0.03 mg), ellagic acid (1.16 ± 0.00 mg), quercetin-3-arabinoside (0.76 ± 0.04 mg), isoquercitrin (0.46 ± 0.02 mg), and caffeic acid (0.15 ± 0.02 mg), were found as the major constitutes (Table 1). Upon the fermentation, the changes in free phenolics were compound specific. In details, the significant increases were found with quercetin (12.9 ± 4.6 %), quercitrin (14.3 ± 6.2 %), and caffeic acid (504.4 ± 50.0 %), while the substantial decreases were observed with chlorogenic acid (92.7 ± 0.4 %), hyperin (10.6 ± 1.4 %), quercetin-3-xyloside (13.1 ± 8.0 %), and quercetin-3-arabinoside (22.3 ± 5.3 %), leaving isoquercitrin, ellagic acid, and phloridzin unchanged. The altering effects of microbial fermentation on phenolic composition were widely reported. For example, the fermentation by *Lactobacillus rhamnosus* L08 significantly decreased quercitrin and phlorizin, while increased quercetin and phloretin in apple pomace (Liu et al., 2021). In the present study, the increased phenolics (quercetin, quercitrin, and caffeic acid) displayed their maximum values at the fermentation of 8 d, and the further extension of the fermentation clearly resulted in the consumption of these phenolics. It was likely that the degradation or polymerization of free phenolic compounds occured due to enzymatic oxidation, which was activated as a part of the stress response associated with the nutrient depletion during fermentation (Dulf et al., 2023). The increases in quercetin and quercitrin were possibly derived from the hydrolysis of quercetin-3-xyloside, hyperin (quercetin 3-galactoside) and quercetin-3-arabinoside as well as the deglycosylation of these compounds under the actions of the enzymes produced by *A. oryzae*, especially *β*-glucosidase (Liu et al., 2021). A previous study had convinced that *A. niger* and *Rhizopus oligosporus*, promoted the conversion of glycosylated anthocyanins and flavonols into their aglycones (cyanidin and quercetin) by producing a *β*-glucosidase (Dulf et al., 2018). The increase in caffeic acid was assumed to a consequence of chlorogenic acid degradation. The findings of Shen et al. (2018) that a biotransformation of chlorogenic acid to caffeic acid in sweet potato was associated with the fermentation of *Lactobacillus acidophilus*.

3.3. Changes in the mass and structural features of pectin substances

As previously mentioned, the total mass of pectin gradually decreased along with the fermentation by *A. oryzae*, which certainly confirmed the degradation capacity of *A. oryzae* fermentation on pectin substances. The molecular weight data in Fig. 1 clearly revealed that *A. oryzae* fermentation resulted in significant degradation of pectin substances. In Fig. 1(A), the longer the retention time of the peak (1 ~ 4), the smaller the molecular weight. Clearly, as the fermentation time extended, the areas of peak 1 and peak 2 decreased, while those of peak 3 and peak 4 increased, which attested that large pectin molecules were degraded into small pectin molecules upon *A. oryzae* fermentation. More

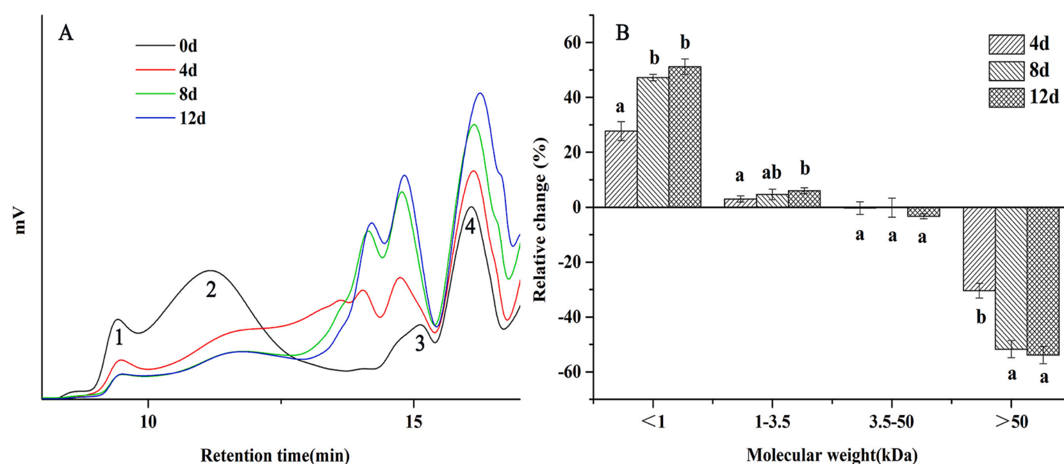

Fig.1. The elution curve of high-performance size exclusion chromatography (HPSEC) (A) and the resultant molecular weight distribution (B) of pectin at different time (0, 4, 8, and 12 d) by *A. oryzae* fermentation. The columns bearing different lowercase letters were significant different ($p < 0.05$).

specifically (Fig. 1B), the fermentation of *A. oryzae* mainly resulted in a transform of high molecular weight pectin fractions (>50 kDa) into low molecular weight pectin fractions (<1 kDa). The pectin degradation degree chiefly occurred in the early stage of the fermentation and peaked at the fermentation of 8 d. The further extension of the fermentation from 8 d to 12 d displayed insignificant effects on the molecular weight of pectin substances.

Usually, pectin is composed of alternating homogalacturonan (HG) and rhamnogalacturonan (RG-I) regions with neutral sugar side chains. HG is commonly defined as the backbone of the pectin molecules and often called as smooth region, while RG-I and its attaching side chains consisted of hairy region. Obviously, the structural discrepancies of these two regions certainly signified their distinct physicochemical properties, including their stability against *A. oryzae* fermentation. To explore this and uncover the degrading mechanism of *A. oryzae* fermentation on apple peel pectin, the monosaccharide composition and the structural parameters of pectin substances were determined (Table 2). Regardless of the fermentation, a total of eight monosaccharaides were found with the original and degraded pectin substances, namely, Galacturonic acid (GalA), Galactose (Gal), Glucose (Glc), Arabinose (Ara), Xylose (Xyl), Rhamnose (Rha), Mannose (Man), and Fucose (Fuc). However, the relatively proportions (mol%) of these monosaccharaides was sharply altered upon the fermentation. In details, the relative proportions of GalA (65.1 ± 3.8 %), Ara (64.6 ± 7.0 %), and Fuc (all degraded at 12d) significantly decreased, while those of Gal (93.8 ± 24.9 %), Glc (56.8 ± 17.3 %), Xyl (113.0 ± 36.8 %), and Man (294.4 ± 14.3 %) increased greatly. Along with the degradation, the branch density of pectin substances increased in 3-fold and their linearity decreased more than 4-fold. As a result, the relative proportion of HG substantially decreased (69.4 ± 4.6 %) while that of RG-I greatly increased (50.5 ± 13.1 %). These findings indicate that the fermentation of *A. oryzae* mainly broke the backbone (HG) of pectin substances but presented limited effects on their side chains. Previous researches had revealed that *A. oryzae* is an efficient producer of extracellular enzymes, which included HG degrading exo- and endo-polygalacturonan hydrolases, pectin and pectate lyases, as well as RG-I degrading rhamnogalacturonan hydrolases and rhamnogalacturonan lyases (Jaramillo et al., 2015). Based on this, it was assumed that, in the present fermentation of *A. oryzae*, the extracellular enzymes secreted by the microbiome absolutely predominated by HG degrading. It was must be noted that the "significant increase" in side chain length is of deceitful nature and it possibly resulted from the diminishing of un-branched Rha originally located in the backbone of pectin molecules. Thus, the ratio of (Ara + Gal)/Rha or the side chain length was nominally elevated.

Table 2
The monosaccharide composition and structural parameters of pectin molecules in apple peel at different fermentation time of *A. oryzae*.

Index	Fermentation time of *A. oryzae* (d)			
	0	4	8	12
Monosaccharide (mol%)				
Mannose (Man)	0.53 ± 0.01^{a}	0.93 ± 0.11^{b}	2.11 ± 0.07^{c}	2.10 ± 0.12^{c}
Rhamnose (Rha)	2.75 ± 0.21^{a}	3.25 ± 0.11^{b}	2.84 ± 0.21^{a}	2.95 ± 0.19^{ab}
Galacturonic acid (GalA)	47.12 ± 1.53^{c}	33.99 ± 2.56^{b}	16.48 ± 2.32^{a}	16.70 ± 1.37^{a}
Glucose (Glc)	16.25 ± 1.54^{a}	19.80 ± 1.53^{b}	25.30 ± 0.84^{c}	24.61 ± 2.07^{c}
Galactose (Gal)	23.53 ± 2.10^{a}	30.42 ± 0.29^{b}	44.83 ± 2.24^{c}	45.27 ± 1.64^{c}
Xylose (Xyl)	2.93 ± 0.59^{a}	5.45 ± 0.48^{b}	6.10 ± 0.40^{b}	6.04 ± 0.34^{b}
Arabinose (Ara)	6.63 ± 1.28^{b}	6.05 ± 1.23^{b}	2.29 ± 0.04^{a}	2.33 ± 0.24^{a}
Fucose (Fuc)	0.27 ± 0.17^{b}	0.12 ± 0.08^{ab}	0.05 ± 0.04^{a}	—
Structural parameters				
Molecular structure[d]	0.06 ± 0.01^{a}	0.10 ± 0.01^{a}	0.18 ± 0.04^{b}	0.18 ± 0.01^{b}
Side chain length[e]	10.97 ± 0.33^{a}	11.22 ± 0.69^{a}	16.60 ± 0.59^{b}	16.15 ± 0.71^{b}
Linearity[f]	1.31 ± 0.13^{c}	0.75 ± 0.08^{b}	0.30 ± 0.06^{a}	0.30 ± 0.03^{a}
HG[g]	44.37 ± 1.67^{c}	30.73 ± 2.51^{b}	13.63 ± 2.49^{a}	13.74 ± 1.30^{a}
RG-I[h]	35.67 ± 2.24^{a}	42.97 ± 1.32^{b}	52.81 ± 2.66^{c}	53.50 ± 1.89^{c}

[a–c] Values bearing different superscript lower letters in the same line were significantly different ($p < 0$ 0.05); [d] Molecular structure of pectin is defined by the value of Rha/GalA; [e] Side chain length of rhamnogalacturonan-I (RG-I) is defined as (Ara + Gal)/Rha; [f] Linearity of pectin molecules is defined as the ratio of (GalA)/(Fuc + Rha + Ara + Gal + Xyl); [g] HG refers to homogalacturonan and its fraction was calculated by GalA-Rha; [h] RG-I refers to rhamnogalacturonan-I and its fraction was caculated as 2Rha + Ara + Gal.

3.4. Changes in the antioxidant activity of apple peel

The effects of *A. oryzae* fermentation on the ABTS radical-scavenging activity and FRAP of apple peel were shown in Fig. 2 and expressed in terms of half maximal inhibitory concentration (IC_{50}). A lower value in IC_{50} suggested a higher antioxidant capacity. In terms of ABTS radical scavenging activity, the fermented peels consistently demonstrated

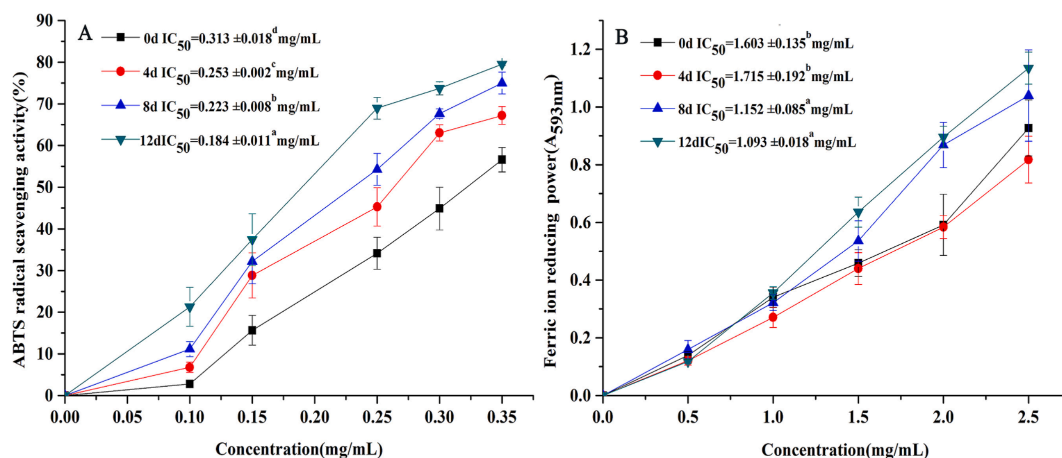

Fig.2. The antioxidant activities of apple peel fermented by *A. oryzae* at different time (0, 4, 8, and 12 d): 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging activity (A) and ferric ion reducing power (B). IC_{50} presents the half maximal inhibitory concentration and its values bearing different superscript lowercase letters were significant different within the same antioxidant activity ($p < 0.05$).

higher values than the unfermented counterpart at any fermentation time and concentration tested. Furthermore, the increase in ABTS radical scavenging activity gradually increased with fermentation time and the IC_{50} values of fermented peels observed the same trend. In terms of FRAP, the apple peel fermented for 4 d presented a proximate value to the unfermented peel, while the ones fermented for 8 d and 12 d were observed with higher values than the unfermented peel. As seen from the IC50 values of FRAP, the 8 d- and 12 d-fermented samples were concluded with the similar values, which were significantly higher than the values of 4 d-fermented and unfermented samples. The improvement in these antioxidant activities of apple peel could be attributed to the increase in free phenolics and the transformation of glycosylated phenolics into their aglycones, especially the increase in quercetin. It was widely acknowledged that the aglycone is more bioactive than its glycosylated form (Madrera et al., 2017; Gulsunoglu et al., 2020). For example, the solid-state fermentation of *Phanerocheate chrysosporium* for 14 d significantly increased the extractable phenolics and improved the antioxidant activity of apple pomace (Ajila et al., 2011). Moreover, a previous study had evidenced that the fermentations with four *Aspergillus* spp. species highly increased phenolic content of apple peel (the highest level of total phenolic: 1440 ± 37 mg GAE/100 g db in *A. niger* ZDM2 case; flavonoid: 495 ± 19 mg CE/100 g db in *A. tubingensis* ZDM1 case) and enhanced its antioxidant activity (up to 5 folds in *A. tubingensis* ZDM1 case) (Gulsunoglu et al., 2020).

3.5. Changes in the prebiotic activity of apple peel

To assess the effects of *A. oryzae* fermentation on the prebiotic activity of apple peel, the *in vitro* fermentability and the impacts on gut microbiota were examined. To do these, an *in vitro* anaerobic fermentation trial was conducted and the pH, SCFA production and microbiota composition were monitored. As for pH, the data in Table 3 clearly showed that, the blank broth displayed higher pH values in the whole anaerobic fermentation than apple peel added broths. This certainly applauded the fermentability of apple peel with or without *A. oryzae* fermentation. For these broths with apple peel, they displayed significant pH values at the beginning of *in vitro* anaerobic fermentation and their pH values decreased with the time of *A. oryzae* fermentation. This indicated that organic acids were produced in the fermentation of *A. oryzae* and their content increased with the fermentation time. Upon the *in vitro* anaerobic fermentation up to 48 h, only a decrease of 0.51 ± 0.15 was observed in the pH of blank broth, which was much less than the decreases (2.70 ± 0.29 ~ 2.32 ± 0.36) observed in the broths with apple peel. This again asserted the fermentability of apple peel. However, the unfermented apple peel showed a greater decrease in pH (2.70 ± 0.29) compared to its fermented counterparts (2.32 ± 0.36 ~ 2.60 ± 0.27), suggesting that unfermented apple peel has higher fermentability than *A. oryzae* fermented ones. This was probably caused by the fact as mentioned above that *A. oryzae* consumed some soluble and fermentable carbohydrates in apple peel.

SCFAs, mainly acetic acid, propionic acid, and butyric acid, are the major intestinal microbial metabolites of indigestible carbohydrate. Hence, their productivities are good indicators to the fermentability of indigestible carbohydrate. As showed in Table 3, the blank broth presented significant lower total acid production than the apple peel incorporated ones. This was certainly a joint consequence of the benefit of apple peel on the growth of gut microbiota as well as the fermentation, metabolism, and conversion of the indigestible carbohydrates in apple peel by gut microbiota. In terms of the extent of *A. oryzae* fermentation, it was clearly showed that the total acid production at 48 h *in vitro* anaerobic fermentation increased as the *A. oryzae* fermentation extended from 0 d to 8 d (from 3.78 ± 0.34 mM to 6.47 ± 0.44 mM). However, the further extension of *A. oryzae* fermentation to 12 d resulted in a sharp decrease in total acid production at 48 h *in vitro* anaerobic fermentation (1.84 ± 0.27 mM). In terms of production-fermentation time profile, the major SCFA monomers differed each other. Specifically, butyric acid production shared a similar profile with the total acid production, while both productions of acetic acid and propionic acid in *A. oryzae* fermentation at 0 d, 4 d, and 8 d were insignificantly differed. In their relative abundance, the acid productions were in the order of acetic acid > butyric acid > propionic acid. This was in line with the findings obtained by Bazzocco et al. (2008) that acetic acid, butyric acid, and propionic acid presented the highest, intermediary, and lowest relative proportions in the fermentation of apple samples by human fecal microbiota, including apples powders, apple proanthocyanidin extract, cell-wall polysaccharides and ciders.

SCFAs have a positive impact on host health, they can diffuse through the intestinal wall and enter the body to provide energy and participate in metabolism. Moreover, SCFAs are the main energy source for gut bacteria and have been reported to create an acidic environment that could inhibit pathogenic bacterial growth and selectively promote beneficial bacterial growth (Ríos-Covian et al., 2016). Butyric acid has many physiological functions, such as maintaining intestinal barrier morphology and function. It has been demonstrated to serve as an important colonocytes energy source and play pivotal roles in the prevention of inflammatory bowel diseases by regulating colonocyte proliferation and apoptosis, gastrointestinal tract motility and antiinflammatory activity (Załęski et al., 2013).

To uncover the changes in gut microbiota during *in vitro* anaerobic

Table 3
Effects of *A. oryzae* fermentation on the *in vitro* anaerobic fermentability of apple peel as indicated by the concentration of short chain fatty acids (SCFAs) and pH upon *in vitro* anaerobic fermentation.

SCFA	Sample[a]	SCFA concentration at different *in vitro* anaerobic fermentation time (mM)				
		0 h	6 h	12 h	24 h	48 h
Acetic acid	B	0.18 ± 0.01^{bA}	0.44 ± 0.05^{bBC}	0.56 ± 0.10^{bC}	0.29 ± 0.01^{bAB}	0.77 ± 0.17^{bD}
	0-d	0.36 ± 0.02^{cA}	1.75 ± 0.05^{fB}	2.74 ± 0.08^{dC}	3.55 ± 0.31^{eD}	3.12 ± 0.53^{cCD}
	4-d	0.33 ± 0.07^{cA}	1.54 ± 0.03^{eB}	2.20 ± 0.07^{cB}	3.70 ± 0.85^{eC}	3.90 ± 0.59^{cC}
	8-d	0.33 ± 0.02^{cA}	1.13 ± 0.07^{dB}	2.06 ± 0.38^{cC}	2.27 ± 0.19^{dC}	3.61 ± 0.98^{cD}
	12-d	0.33 ± 0.01^{cA}	0.61 ± 0.08^{cB}	0.93 ± 0.01^{bC}	1.45 ± 0.10^{cD}	1.31 ± 0.31^{bD}
Propionic acid	B	0.04 ± 0.00^{bA}	0.06 ± 0.01^{bAB}	0.06 ± 0.01^{bcAB}	0.09 ± 0.03^{bcB}	0.54 ± 0.01^{cC}
	0-d	0.05 ± 0.01^{cA}	0.06 ± 0.01^{bA}	0.12 ± 0.02^{dA}	0.63 ± 0.04^{eC}	0.50 ± 0.13^{cB}
	4-d	0.05 ± 0.00^{cdA}	0.07 ± 0.01^{cA}	0.08 ± 0.01^{cA}	0.23 ± 0.12^{dB}	0.39 ± 0.03^{cC}
	8-d	0.05 ± 0.00^{dA}	0.05 ± 0.01^{bA}	0.05 ± 0.02^{bA}	0.18 ± 0.02^{cdA}	0.35 ± 0.15^{bcB}
	12-d	0.05 ± 0.00^{cdA}	0.05 ± 0.01^{bA}	0.04 ± 0.00^{bA}	0.06 ± 0.01^{bA}	0.17 ± 0.07^{bB}
Butyric acid	B	0.04 ± 0.01^{cA}	0.04 ± 0.01^{bA}	0.03 ± 0.02^{bA}	0.04 ± 0.00^{bA}	0.15 ± 0.04^{bB}
	0-d	0.03 ± 0.00^{bA}	0.09 ± 0.04^{cAB}	0.20 ± 0.00^{cB}	0.38 ± 0.12^{cC}	0.17 ± 0.01^{bB}
	4-d	0.03 ± 0.00^{bA}	0.09 ± 0.01^{cA}	0.17 ± 0.02^{cA}	0.67 ± 0.18^{dB}	1.41 ± 0.32^{cC}
	8-d	0.04 ± 0.00^{cA}	0.10 ± 0.03^{cA}	0.42 ± 0.10^{dA}	0.97 ± 0.27^{eB}	2.42 ± 0.62^{dC}
	12-d	0.04 ± 0.00^{cA}	0.08 ± 0.01^{bcA}	0.19 ± 0.08^{cB}	0.29 ± 0.00^{bcC}	0.37 ± 0.04^{bD}
Total acid	B	0.25 ± 0.01^{bA}	0.54 ± 0.04^{bBC}	0.64 ± 0.14^{bC}	0.42 ± 0.02^{bAB}	1.45 ± 0.20^{bD}
	0-d	0.44 ± 0.02^{cA}	1.90 ± 0.08^{fB}	3.06 ± 0.05^{eC}	4.56 ± 0.39^{eE}	3.78 ± 0.34^{cD}
	4-d	0.41 ± 0.07^{cA}	1.71 ± 0.04^{eB}	2.45 ± 0.07^{dB}	4.59 ± 0.92^{eC}	5.70 ± 0.93^{dD}
	8-d	0.43 ± 0.02^{cA}	1.29 ± 0.10^{dB}	2.52 ± 0.44^{dC}	3.42 ± 0.42^{dD}	6.47 ± 0.44^{dE}
	12-d	0.41 ± 0.01^{cA}	0.74 ± 0.10^{cB}	1.16 ± 0.08^{cC}	1.79 ± 0.11^{cD}	1.84 ± 0.27^{bD}
pH	B	7.72 ± 0.01^{eC}	7.53 ± 0.01^{fBC}	7.46 ± 0.17^{dB}	7.23 ± 0.02^{dA}	7.21 ± 0.16^{cA}
	0-d	7.28 ± 0.01^{dC}	5.24 ± 0.01^{eB}	4.65 ± 0.03^{bA}	4.75 ± 0.05^{bA}	4.58 ± 0.28^{bA}
	4-d	7.29 ± 0.01^{dC}	5.12 ± 0.02^{dB}	4.69 ± 0.04^{bA}	4.97 ± 0.05^{cB}	4.69 ± 0.27^{bA}
	8-d	7.25 ± 0.01^{cB}	4.98 ± 0.07^{cA}	4.91 ± 0.06^{cA}	5.04 ± 0.07^{cA}	4.93 ± 0.35^{bA}
	12-d	7.21 ± 0.01^{bC}	4.91 ± 0.03^{bB}	4.76 ± 0.04^{bA}	4.97 ± 0.03^{bA}	4.72 ± 0.13^{bA}

[a] B refers to the blank without apple peel and 0-d, 4-d, 8-d, and 12-d represent the trails with apple peel fermented by *A. oryzae* for 0 d, 4 d, 8 d, and 12 d, respectively. [b-f] Data for each SCFA bearing different superscript lowercase letters within the same *in vitro* anaerobic fermentation time were significantly different ($p < 0.05$). [A–E] Data bearing different superscript capital letters in the same row were significantly different ($p < 0.05$).

fermentation, a high throughput sequencing analysis was performed. Totally, 2,928,239 usable raw reads were obtained and they were filtered and clustered with 97 % similarity, resulting in 132 ± 21 Operational Taxonomic Units (OTUs) per sample. The shape of Rarefaction curve and Shannon curve (first increased and then gradually reached to a platform) (Supplementary material 1) indicated that the data size obtained was big enough to reflect mostly biological information in samples (Zhou et al., 2018). As shown in Fig. 3A, the microbiota in all tests, including the blank and fermented apple peel trials, mainly consisted of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria at a phylum level. In contrast to the blank, all apple peel involved broths presented higher relative abundances of Bacteroidetes (except the *in vitro* anaerobic fermentation at 48 h), Firmicutes, and Actinobacteria. However, the broths with fermented apple peel gave a lower relative abundance of Proteobacteria than the blank. In this context, the original apple peel was a bioactive food ingredient and the *A. oryzae* fermentation was somewhat harmful in this aspect. This was assumed to highly relate the rich presence of pectin materials in innate apple peel and its degradation and decrease during *A. oryzae* fermentation. The findings of Tian et al. (2016) indicated that the pectin supplementation certainly increased the relative abundance of Bacteroidetes in the colonic digesta and feces of weaning pigs. For the biological significance of present genus changes, on the one hand, Bacteroidetes-rich microbiota was believed to be correlated with a reduced risk of obesity in humans and both Firmicutes and Actinobacteria played important roles in obesity and diabetes (Kumar et al., 2021). On the other hand, the reduction in the relative abundance in Proteobacteria was a positive event due to the fact that Proteobacteria consists of many pathogenic bacteria, such as *Salmonella*, *Shigella*, *Campylobacter*, and *Escherichia*, who often play a crucial role in intestinal microbiota imbalance and chronic colitis (Cao et al., 2022).

At a genus level, a total of 50 genera were found in this experiment (Supplementary material 2) and 19 of them were observed with relative abundances higher than 1 % (Fig. 3B) (Detailed data was provided Supplementary material 3). However, regardless of substrate and fermentation time, *Escherichia-Shigella*, *Lactobacillus*, *Enterobacter*, *Megamonas*, *Prevotella-9*, *Bifidobacterium* were the major genera and accounted for about 80–90 % in terms of genus community. Clearly, *Escherichia-Shigella* and *Enterobacter* predominated in blank broth, especially at the early stage of the *in vitro* anaerobic fermentation. In contrast to the blank, the broths with apple peel presented higher relative abundances of *Megamonas*, *Lactobacillus* (especially at latter *in vitro* anaerobic fermentation stages at 24 h and 48 h), *Bifidobacterium*, and *Prevotella-9*, but a lower relative abundance of *Enterobacter*. Regarding *Escherichia-Shigella*, the blank presented a higher abundance than the apple peel involved broths at the *in vitro* anaerobic fermentation of 6 h. However, at the other *in vitro* anaerobic fermentation stages, the latter displayed higher values than the former. Linear discriminant analysis effect size (LEfSe) analysis identified the microbes whose abundances significantly differed between the all groups. The findings with regards to genus are shown in the plot (Linear discriminant analysis (LDA) score > 3.5, $p < 0.05$) (Fig. 3C). It could be clearly seen that *Escherichia-Shigella* and *Enterobacter* were significantly present at 6 h and 24 h in the blank group, respectively. However, *Megamonas* and *Prevotella-9* were significantly enriched in 0 d-fermented apple peel (12 h and 24 h), *Lactobacillus* significantly grew in 8 d-fermented apple peel (48 h), and *Bifidobacterium* significantly grew in 12 d-fermented apple peel (6 h).

Obviously, the increases of *Lactobacillus* and *Bifidobacterium* were highly desired due to their health-promoting effects by regulating gut microbiota and host physiology. They were very effective in controlling serum cholesterol, preventing intestinal diseases, improving obesity, and modulating immune system (Nicholson et al., 2012). The increase of *Prevotella-9* was also an exciting event because *Prevotella* was a kind of beneficial gut microbiota, who could regulate blood glucose and reduce the risk of diabetes by protecting against Bacteroides-induced glucose intolerance (Kovatcheva-Datchary et al., 2015). Moreover, *Megamonas* always promoted human health by producing SCFAs and vitamins and it was found in the gut microbiota of long-lived elderly people (Yu et al., 2015). On the other hand, the reductions of *Enterobacter* and *Escherichia-Shigella* were of positive health significance because they were generally defined as opportunistic pathogens, possibly initiating microbiota dysbiosis and gave rise to various diseases such as pneumonia, urinary tract infection, septicemia, colorectal cancer (Gao et al., 2015).

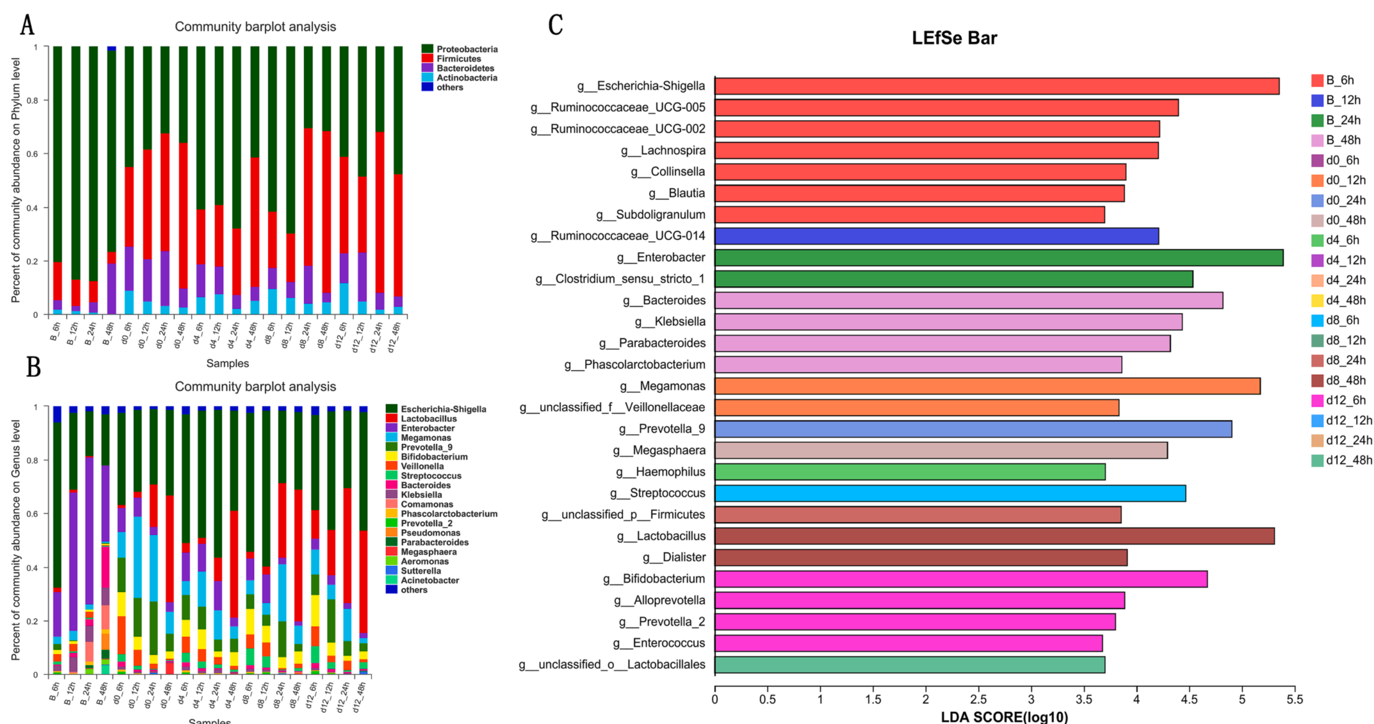

Fig.3. The effects of *A. oryzae* fermented apple peel on the microbial composition at phylum (A) and genus (B) levels in *in vitro* fermentation as well as the significantly differed (LDA > 3.5, $p < 0.05$) strains from Linear discriminant analysis (LDA) effect size (LEfSe) analysis (C). For the samples, B presented the blank without apple peel and others were denoted by composing (_) the fermentation time of apple peel by *A. oryzae* (d0, d4, d8, and d12) and the time of *in vitro* fermentation (6 h, 12 h, 24 h, and 48 h).

4. Conclusions

The effects of *A. oryzae* fermentation on the composition, antioxidant activity and prebiotic potential of apple peel were reported at the first time. The obtained data herein clearly applauded that *A. oryzae* fermentation was an effective way to modulate the physicochemical properties and *in vitro* fermentability of apple peel. Moreover, this work opened a window to the value-added utilization of lignocellulosic food by-products and ultimately favored the sustainability of plant food industry. In the future, the *in vivo* bioactivities and the actual application of fermented apple peel should be evaluated systematically to specify its potential application scene.

CRediT authorship contribution statement

Jianting Li: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Fayin Ye:** Supervision, Project administration, Methodology, Investigation. **Yun Zhou:** Validation, Supervision. **Lin Lei:** Supervision, Methodology, Investigation. **Jia Chen:** Validation, Supervision. **Sheng Li:** Supervision, Methodology, Funding acquisition. **Guohua Zhao:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101134.

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