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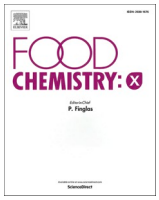

# The profiles of free, esterified and insoluble-bound phenolics in peach juice after high pressure homogenization and evaluation of their antioxidant capacities, cytoprotective effect, and inhibitory effects on α-glucosidase and dipeptidyl peptidase-IV

Shenke Bie [a,b,c,d], Shuai Zhao [a,b,c,d], Shengbao Cai [a,b,c,d], Junjie Yi [a,b,c,d], Linyan Zhou [a,b,c,d,*]

[a] Faculty of Food Science and Engineering, Kunming University of Science and Technology, Kunming, Yunnan Province 650500, China
[b] Yunnan Engineering Research Center for Fruit & Vegetable Products, Kunming, Yunnan Province 650500, China
[c] International Green Food Processing Research and Development Center of Kunming City, Kunming, Yunnan Province 650500, China
[d] Yunnan Key Laboratory for Food Advanced Manufacturing, 650500 Kunming, China

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ABSTRACT

The phenolic profiles, antioxidant capacities, cytoprotective effect, and α-glucosidase and DPP-IV inhibitory capacity of free (FP), esterified (EP) and insoluble-bound (IBP) phenolic fractions in 'Lijiang snow' peach juice after high pressure homogenization (HPH) were investigated, and the molecular docking was used to explore the enzyme inhibition mechanism. HPH increased total phenolic and total flavonoid contents in three fractions without changing compositions. The $IC_{50}$ of radicals scavenged by three fractions were all reduced by HPH. The best inhibition on intracellular ROS production were found for phenolic fractions after HPH at 300 MPa, with ROS levels ranged within 95.26–119.16 %. HPH at 300 MPa reduced the apoptosis rates of FP and EP by 16.52 % and 9.33 %, respectively. All phenolic fractions showed effective inhibition on α-glucosidase and DPP-IV by formation of hydrogen bonding and van der Waals forces. This study explored the feasibility of HPH to enhance the phenolics and bioactivity of peach juice.

## 1. Introduction

Peach (*Prunus persica* L.) originated in China, which has been grown for over 4000 years (Liao, Greenspan, & Pegg, 2019). The 'Lijiang Snow' peach is a late-maturing type, and attracts customers due to its rich nutrient, beautiful appearance, delicate flesh, and aromatic flavor (Wang et al., 2021). The 'Lijiang Snow' peach contains a variety of beneficial substances, of which phenolics and flavonoids are the most important. Phenolics and flavonoids are produced as secondary metabolites of plant, which are widely distributed in plant and plant-based food products (Prakash et al., 2019). Phenolics and flavonoids have been proved to have severally biological functions such as antioxidant, antiviral, hypoglycemic and lipid lowering (Gao et al., 2022), as well as many potential health benefits. Moreover, the multiple biological activities of dietary flavonoids contributed to human's health are associated to the bidirectional interactions with the commensal gut flora. On the one hand, flavonoids are metabolized by the intestinal flora to increase their bioavailability, and on the other hand, flavonoids modulate the population structure of intestinal microbial (Baky, Elshahed, Wessjohann, & Farag, 2022). Generally, peaches are rich in phenolic compounds, whose contents and composition are varied depended on varieties, ripeness, regions and extraction methods. Guo et al. (2020) reported that the total phenolic contents (TPCs) in 7 varieties of peaches from China ranged from 34.01 to 82.03 mg gallic acid equivalents (GAE)/100 g fresh weight (f.w.), and total flavonoid contents (TFCs) ranged from 11.43 to 30.01 mg rutin equivalents (RE)/100 g f.w. In addition, the TPCs in 6 varieties of peaches from orchards in South Georgia ranged from 97.44 to 208.50 mg chlorogenic acid equivalents

* Corresponding author at: Faculty of Food Science and Engineering, Kunming University of Science and Technology, Kunming, Yunnan Province 650500, China.
*E-mail address:* zhoulinyan916@hotmail.com (L. Zhou).

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(CHAE)/100 g f.w. (Liao et al., 2019). It is generally believed that there were three phenolic compounds existed in plant, including free (FP), esterified (EP), and insoluble-bound (IBP) states (Zhou et al., 2019). The FP fraction is located within the vacuole, while the EP fraction is linked to the sugar and other substances in the plant cell by covalent or ester bonds, IBP fraction is linked to insoluble macromolecular substrates of the cell wall by covalent bonds, hydrophobic interactions and hydrogen bonds (Zhang, Zhang, Li, Deng, & Tsao, 2020). The biological activities and bioavailability of three phenolic fractions vary greatly according to their resource and amounts, as well as the content and composition of monomeric phenolics. Only 5–10 % of the FP and EP in plant-based food matrix are absorbed in the small intestine; the unabsorbed FP, EP and IBP are transported to the colon to be metabolized and degraded by microorganisms of the intestinal flora (Zhang, Zhang, Li, Deng, & Tsao, 2020). Zhou et al. (2019) reported that the IBP from oil palm fruits had the higher TPC and TFC than those of EP and FP, while the antioxidant activity and cytoprotective effect of IBP were also higher. Zhang et al. (2022) reported that IBP and FP extracted from water caltrop husk have the stronger radical scavenging ability for ABTS and DPPH, respectively, meanwhile FP showed the best inhibition of α-glucosidase.

Although phenolic compounds are beneficial to the human health, they are unstable during the process of processing and digestion (Fang & Bhandari, 2011). In general, fruit product usually require thermal or non-thermal processing for sterilization, enzyme passivation and other purposes, and different processing methods will cause different levels of effects on the phenolic compounds in fruit product. Thermal processing showed different effects on phenolic compounds in fruit product, and it might increase or decrease their contents and bioaccessibility. For instance, the TPC of kutkura juice significantly decreased by 5.07 % after treated by hot water bath at 90℃ for 60 s (Krishnan Kesavan, Gogoi, & Nayak, 2023). Conversely, the TPC of apple juice increased by around 5-fold after by pasteurized at 71.7℃ for 0.4 min (Alongi, Verardo, Gorassini, & Anese, 2018).

Non-thermal technologies are generally considered to be better at maintaining or even enhancing the amount of bioactive substances, and improving their biological activities, in comparison of thermal processing. For example, ultra-high pressure treatment (UHP) at 500 MPa for 10 min boosted significantly the TPCs in three phenolic fractions in oil palm fruit by around 64 %, 64 % and 110 %, respectively, and their antioxidant activity and cytoprotective effects were also significantly enhanced (Zhou et al., 2019).

High pressure homogenization (HPH) is a non-thermal processing technology, which can improve the juice's cloudy stability (Liu et al., 2019). With the advancement of pertinent technology and apparatus in recent years, the homogenizer's maximum pressure can now approach 450 MPa. The liquid food subjected to HPH experiences significant shear, cavitation, and turbulence effects, and in this process achieves the purpose of sterilization and enzyme deactivation (Donsì, Ferrari, Lenza, & Maresca, 2009). HPH can also make the sample particle size up to the nanometer level, greatly broken plant cell tissue structure, and more conducive to the dissolution of active ingredients (Liu et al., 2019). However, previous studies also showed that phenolics compounds contents in fruit juice and their antioxidant capacity were greatly altered by HPH. For example, the TPC of mango juice significantly increased by 21.42 % after HPH at 190 MPa and 60℃ as compared to non-treated sample (Guan et al., 2016), and that value of pomegranate juice also significantly increased by around 21 % after HPH at 150 MPa and 55℃ (Benjamin & Gamrasni, 2020). However, HPH at 100, 200 and 300 MPa showed no effect on the TPC of both clear apple juice (Velázquez-Estrada, Hernández-Herrero, Rüfer, Guamis-López, & Roig-Sagués, 2013) and orange juice (Suárez-Jacobo et al., 2011). In contrast, the TPCs of strawberry cloudy juice decreased by about 17 % and 19 % treated by HPH at 50 and 100 MPa, respectively (Joubran, Katz, Okun, Davidovich-Pinhas, & Shpigelman, 2019). Nowadays, metabolomics is a key factor in linking many different molecules, which has been used to study the changes in phenolic compounds after different treatments in fruit juices, such as apple cloudy juice (Szczepańska, Barba, Skąpska, & Marszałek, 2022), orange juice (Velázquez-Estrada, Hernández-Herrero, Rüfer, Guamis-López, & Roig-Sagués, 2013). Metabolomics involves the comprehensive identification and quantification of metabolites, either individually or in combination. Thus, it is an effective tool for analysis of small molecules in fresh and processed fruit, and the obtained composition information is help to uncovering quality-attribute of fresh and processed fruit (Wang et al., 2023).

Thus, it was deduced that HPH will bring different effect on different states of phenolic fractions in fruit juices, as well as changing their biological activities. At present, there was no related research reported. Therefore, to clarify the effects of HPH on the phenolic profiles and their biological activities of 'Lijiang Snow' peach juice, three phenolic fractions (FP, EP and IBP) of 'Lijiang snow' peach juice before and after HPH were identified and quantified by metabolomics, and their antioxidant activities and inhibitory effects on α-glucosidase and DPP-IV were evaluated comprehensively. Furthermore, the protective effects on oxidative damage in Caco-2 cells by different phenolic fractions were further evaluated by cellular experiments. The inhibition mechanisms of α-glucosidase and DPP-IV by the predominant compounds were further elucidated by molecular docking and molecular dynamics. The aim of this study was to investigate the feasibility of HPH to improve the phenolic compounds of different phenolic fractions in 'Lijiang snow' peach juice, and to elucidate the relationship between phenolic compound and biological activity.

## 2. Materials and methods

### 2.1. Materials and reagents

Peaches were purchased from an orchard in Lijiang, Yunnan Province, China, transported to laboratory and stored at 4℃. Then the peaches were washed, cored, sliced and juiced, filtered through a layer of gauze, and then frozen with liquid nitrogen immediately, and stored at − 40℃ before using.

Formic acid, Folin–Ciocalteu reagent, methanol, and acetonitrile were obtained from Darmstadt Merck, Germany. Standards, including quinic acid, gallocatechin, chlorogenic acid, salicylic acid, procyanidin B1, (+)-catechin, methyl chlorogenic acid, caffeic acid, (-)-epicatechin, taxifolin and protocatechuic acid, and α-glucosidase (EC: 3.2.1.20, ≥26.5 units/mg protein) were provided by Shanghai Yuanye Biological Technology. Standards, including ferulic acid, rutin, cyanidin-3- *O* -glucoside, kaempferol, syringic acid, protocatechuic acid and *p*-coumaric acid were obtained from Chengdu Must Bio-Technology Co., Ltd. (Chengdu, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were obtained from Sigma-Aldrich (Shanghai, China). Penicillin, streptomycin, fetal bovine serum (FBS), and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (Grand Island, NY). DPP-IV inhibitor screening kit was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Caco-2 cells were purchased from Kunming Cell Bank. Annexin V-FITC/PI kit was purchased from Beijing 4A Biotech Co., Ltd. (Beijing, China).

### 2.2. High pressure homogenization (HPH) processing

The frozen peach juice completely thawed at 4℃, and mixed thoroughly before using. Peach juice was processed using HPH equipment (FPG12805, Standard Fluid Power Ltd., Essex, UK) equipped with a cooling circulation system at 4℃. The HPH treatments at 50, 200 and 300 MPa for 1 pass were applied for juice processing, respectively. Peach juice (400 mL) was subjected to HPH processing with a flow rate of 40–50 mL/min. Then all samples, including non-treated and HPH-treated peach juice, were frozen with liquid nitrogen immediately after HPH processing and then lyophilized by a freeze-drier (Scientz–10

N Freeze dryer, Ningbo Scientz Biotechnology Co., Ltd., China). The lyophilized peach powder was then vacuum-packed (DZD-600/2SC, Jiangsu Tengtong Packaging Machinery Co., LTD, China) and placed in a Vacuum dryer before using.

### 2.3. Extraction and partial purification of different phenolic fractions

There phenolic fractions, including free (FP), esterified (EP), and insoluble-bound phenolic (IBP) fractions, were sequentially extracted from peach powder according to Zhou et al. (2019) and Xu et al. (2023). Firstly, peach powder (approximately 50.0 g) was defatted twice with petroleum ether and ethyl ether to remove fat-soluble impurities (1:1, v/v) (1:5, w/v), and then the defatted powder extracted by 250 mL of extraction solution consisted of 70 % aqueous acetone and 70 % aqueous methanol (1:1, v/v) by 200 W ultrasonication at 20℃ for 30 min (SB-5200DTDG, Ningbo Scientz Biotechnology Co., Ltd., China). Then, the slurry was filtered with filter paper, and the residue was repeatedly extracted twice. After that, the filtrate was collected and concentrated using a rotary evaporation rotational (HeiVAP, Heidolph, Schwabach, Germany). The aqueous phase obtained after rotary evaporation was applied to obtain FP and EP, and filtered residue was used to extract IBP. The above aqueous phase was extracted five times with 100 mL of a mixture of ethyl acetate and ether with volume ratio of 1:1. FP was obtained by freeze-drying the upper organic phase after rotary evaporation. After the lower aqueous phase treated with 4 mol/L NaOH (1:10, v/v) for 4 h, the solution was readjusted to pH 2 with 6 mol /L HCl, and then extraction was performed for five times with 100 mL of a mixture of ether and ethyl acetate with volume ratio of 1:1. The EP was obtained by freeze-drying the upper organic phase of the above extraction. The filtered residue for IBP extraction was treated by 4 mol/L NaOH (1:10 w/v) for 4 h, the sediment was then removed by filtration, and the subsequent operation was the same as the extraction of EP. Finally, all extracted samples were stored at −20℃ before using.

### 2.4. Determination of total phenolic content (TPC) and total flavonoid content (TFC)

Folin-Ciocalteu reagent was used to determine total phenolic contents (TPCs) in different phenolic fractions as a previous study (Gao et al., 2022). All phenolic fractions (1 mg) were dissolved by 2 mL of 80 % methanol. Then, 50 μL of methanol extract was added by 500 μL of 10-fold diluted Folin-Ciocalteu reagent firstly. After 5 min, 450 μL of 7.5 % (w/v) $Na_2CO_3$ was added. Then, the mixture reacted for 1 h in the dark at room temperature and then determined at 760 nm using a microplate reader (EPOCH2, BioTek 199 Instrument Co., Ltd., Vinooski, USA). Gallic acid with a range of concentrations were used to obtained the calibration curve, and the TPC was presented as milligrams of gallic acid equivalents (GAE) per gram of extract.

Total flavonoid content (TFCs) in different phenolic fractions were determined by the aluminum trichloride colorimetric method (Zhou et al., 2019). One milligram of phenolic fractions were diluted by 2 mL 80 % methanol. Firstly, 30 μL 5 % $NaNO_2$ was added into 200 μL methanol extract, and 5 min later 30 μL 5 % $AlCl_3$ was added. After another 6 min reaction, 200 μL 1 M NaOH was added. After the reaction standing in the dark at ambient temperature for 30 min, the absorbance were determined at 510 nm with a microplate reader (EPOCH2, BioTek Instrument 208 Co., Ltd, Vinooski, USA). The calibration curve was obtained using rutin with different concentrations, and the TFC was represented as milligrams of rutin equivalents (RE) per gram of extract.

### 2.5. Identification and quantification of phenolics by UHPLC-ESI-HRMS/MS

A Thermo Fisher Ultimate 3000 UHPLC system (Merck, Darmstadt, Germany) were used for the qualitative and quantitative analysis of the phenolic compounds in FP, EP and IBP, and an Agilent C18 column (2.1 × 100 mm, 1.9 μm, Agilent, USA) was used. The phase A was acidified ultrapure water with 0.1 % of formic acid, and the phase B was pure acetonitrile. The elution gradient was 0–2 min, 5 % B; 2–5 min, 5 %–10 % B; 5–9 min, 10 %–20 % B; 9–16 min, 20 %–30 % B; 16–18 min, 30 %–40 % B; 18–19 min, 40 %–5% B; 19–21 min, 5 % B. The column temperature was 30℃, the injection volume was 2.0 μL, and the flow rate was 0.2 mL/min.

Negative mode mass spectrometry data were collected for analysis of phenolic compounds. The chromatographic separation was performed at the following parameters: negative ion scanning mode with a *m/z* of 100–1500; capillary and heater temperatures of 320℃ and 350℃, respectively; spray voltage of 3.3 kV; scanning gas flow rate of 4.0 L/min; auxiliary gas flow rate and intrathecal gas flow rate of 8.0 L/min and 32.0 L/min, respectively; and 50 % S-lens radio frequency level. The phenolics were initially identified though comparing the mass spectrometry data with the references or data from databases, then the identified phenolic compounds were quantified or semi-quantified according to curves of their corresponding standard.

### 2.6. Evaluation of antioxidant activity

#### 2.6.1. Scavenging activity of the DPPH radical

The DPPH radical scavenging activities were determined as previously method (Zhou et al., 2019). All phenolic samples were diluted in 80 % methanol with the concentrations of 2, 4, 6, 8, 10, 12 and 14 μg/mL. $V_C$ was also dissolved in 80 % methanol with concentrations of 1, 2, 3, 4, 5, 6, and 7 μg/mL. DPPH working solution with a concentration of 0.1 mM was obtained by dissolved in 100 % methanol. Fifty microliters of diluted phenolic sample and 200 μL of 0.1 mM DPPH working solution were mixed, wrapped in tinfoil to avoid light, and shaken for 30 min in a shaker. The absorbances of mixtures were then measured at 517 nm using a microplate reader with Vc as the positive control. The concentration of the sample when the free radical scavenging rate reached 50 % was denoted as the half maximal inhibitory concentration ($IC_{50}$) value.

#### 2.6.2. Scavenging activity of ABTS radical

The ABTS radical scavenging activities were measured as a previously method (Zhang et al., 2022). All phenolic samples were diluted in 70 % ethanol with concentrations of 2, 3, 4, 5, 6, 7 and 8 μg/mL. $V_C$ was also dissolved in 70 % ethanol and concentrations were diluted with 1, 2, 3, 4, 5, 6, and 7 μg/mL. Five milliliters of 7 mM ABTS solution and 88 μL of 0.14 M $K_2S_2O_8$ were mixed and stored for 14 h, and the mixture was 35-fold diluted with anhydrous ethanol to obtain ABTS working solution. Then, 50 μL of diluted phenolic sample and 400 μL of ABTS free radical working solution were mixed and standing in the dark at 30℃ for 6 min. The absorbances of mixtures were then measured at 734 nm using a microplate reader with $V_C$ as the positive control. The $IC_{50}$ was the concentration of the sample when the free radical scavenging rate reached 50 %.

#### 2.6.3. Cell culture, cell viability assay and effects on the reactive oxygen species (ROS) generation

Cell model was constructed by referring to a previous study (Luo et al., 2018). Caco-2 cells were incubated in DMEM with 15 % FBS and 1 % penicillin–streptomycin with condition of 37 °C and 5 % $CO_2$ by a cell culture incubator (CCL-170B-8, ESCO, Singapore).

The cytotoxicity of three phenolic fractions from non-treated and HPH-treated juices on Caco-2 cells was determined by MTT assay. The cells were inoculated in a 96-well plate with 200 μL per well for 24 h with a density of $1 \times 10^5$ cells/mL. After 24 h, the sample solution (100–500 μg/mL) after removing the cell medium, was added to the wells and incubated for 20 h. Then, the medium containing the samples was removed and MTT (0.5 mg/mL) was added. Two hundred microliters of DMSO was added to each well after 4 h, and the absorbances at 570 nm were measured by a SpectraMax M5 microplate reader (Molecular Device, San Jose, CA). When the number of cells cultured in the

sample solution showed no significant difference with the blank (cell culture medium), the sample was proven to be non-cytotoxic at that concentration.

The intracellular ROS in Caco-2 induced by $H_2O_2$ was measured as Urade et al. (2023). In brief, Caco-2 cells were seeded in 6-well plates, and then each well was added with 2.0 mL of cell suspension at a density of $1.5 \times 10^5$ /mL and incubated for 24 h. Then, 2.0 mL of 300.0 μg/mL of phenolic solution or 10 μg/mL of Vc was added and incubated for another 24 h. After removing the medium, all groups were subjected to 1.0 mmol/L $H_2O_2$ for 6 h, except the negative control group. The cells were cleaned 3 times with PBS to remove dead cells, and labeled with 10.0 mol/L DCFH-DA. Finally, the cells were cleaned twice and examined by a flow cytometer promptly (Millipore, Billerica, MA).

#### 2.6.4. Cytoprotective activity against $H_2O_2$-induced cell apoptosis

Annexin V-FITC/PI Kit was used to measure Caco-2 cell apoptosis rate by flow cytometry. Briefly, cell culture, phenolics samples and $H_2O_2$ induction were performed in the same way as for ROS. Cells after digested with 200 μL EDTA-free trypsin were washed twice with cold PBS, then centrifugated at 1000 g and 4℃ for 5 min (H1650R, Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Hunan, China), and then resuspended with 1 × binding buffer (395 μL). Next, cells was added with 5.0 μL Annexin VFITC and mixed well, and incubated in dark for 5 min at ambient temperature. Thereafter, 10.0 μL PI was added, and incubated for 5 min on ice and in dark. Finally, 400 μL PBS was added, mixed well and determined by the flow assay immediately.

### 2.7. α-Glucosidase and dipeptidyl peptidase-IV (DPP-IV) inhibitory activity

The α-glucosidase inhibitory activity was assessed accorded to a previous method (Tan, Chang, & Zhang, 2017). In brief, 10–100 μg/mL of the phenolic samples, 50–500 μg/mL of the phenolic standards, 4 mM of *p*NPG solution and 1 U/mL of α-glucosidase solution were all dissolved by 1 M phosphate buffer at pH 6.8. Eighty microliters of the phenolic solution was combined with 100 μL *p*NPG solution and 20 μL α-glucosidase solution, and then reacted for 10 min in a 37 °C water bath. As negative and positive controls, the phenolic sample solution was replaced with phosphate buffer and acarbose solution (0.05–1 μg/mL), respectively. Then, the absorbances of solutions at 405 nm were detected using a microplate reader. The formula for calculating α-glucosidase inhibition was as follows.

$$\alpha-\text{glucosidase}\ \ \text{inhibitory}\,(\%) = \left(1-\frac{OD_{\text{sample}}}{OD_{\text{control}}}\right)\times 100 \quad (1)$$

where $OD_{control}$ and $OD_{sample}$ are the absorbance of the control and sample groups, respectively. The $IC_{50}$ value is the sample concentration when the DPP-IV inhibition is 50 %.

The inhibition of DPP-IV by different phenolic fractions and standards were determined using the Cayman DPP-IV inhibitor screening assay kit. All reagents for activity inhibition assay are provided by the kit. Firstly, 1 × diluted buffer solution with 20 mM Tris and pH 8.0 was obtained by diluting assay buffer solution 10-fold with HPLC-grade water. Then, 20–500 μg/mL of the phenolic sample solutions and 20–200 μg/mL of the phenolic standards were obtained by dissolving in 1 × diluted buffer solution. The substrate solution was obtained by diluting 120 μL H-Gly-Pro conjugated with aminomethylcoumarin (5 mM) by 2.88 mL of 1 × diluted buffer. A total of 30 μL diluted buffer, 10 μL diluted DPP-IV and 10 μL diluted phenolics sample solution were added to a 200 μL PCR tube and mixed, followed by adding 50 μL substrate solution, and then incubated for 30 min at 37℃. The phenolic sample was replaced with 1 × diluted buffer solution and sitagliptin solution for the negative control and positive control, respectively. The absorbances were detected by a microplate reader with excitation and emission wavelengths of 355 nm and 455 nm, respectively. The inhibition of DPP-IV was calculated as follows.

$$\text{DPP (IV)}\ \ \text{inhibitory}\,(\%) = \left(1-\frac{A_{\text{sample}}}{A_{\text{control}}}\right)\times 100 \quad (2)$$

where $A_{control}$ and $A_{sample}$ are the absorbance of the control and sample groups, respectively. The $IC_{50}$ value is the sample concentration when the DPP-IV inhibition is 50 %.

### 2.8. Molecular docking

The 3D crystal structures of α-glucosidase (PDB ID: 3A4A) and human DPP-IV (PDB ID: 1X70) were both got from the Protein Data Bank (https://www.pdbus.org/), and unnecessary water molecules, ligands and ions were removed. The structures of the phenolics were sourced from the PubChem molecular library (https://pubchem.ncbi.nlm.nih.gov/). Before docking, AutoDock Tools (ADT, version 1.5.7) was used to optimize the protein and ligand by adding non-polar hydrogen and gasteiger charges. Ligands were set to flexible and proteins were set to rigid. In addition, the docking box information for α-glucosidase and human DPP-IV were: center coordinates: x = 25.38, y = -2.75 and z = 18.19, size: 52.00 × 68.00 × 62.00 Å; center coordinates: x = 18.20, y = 3.20 and z = 53.40, dimensions: 28.40 × 26.90 × 33.00 Å. Semi-flexible molecular docking was carried by using AutoDock Vina software (1.1.2) (Trott & Olson, 2010). The value of exhaustiveness was set to 100, and the docking model was set to 20. After docking, PyMol and *BIOVIA* Discovery Studio 2016 Client were used to analyze the binding site and intermolecular interaction forces of the ligand and protein.

### 2.9. Statistical analysis

All experiment results were carried three times expressed as mean (n = 3) values ± standard deviation (SD). Data were analyzed by one-way ANOVA. The significance of the difference at $p < 0.05$ level was evaluated via Tukey's procedure. Figures were plotted using Origin Pro 2021 (OriginLab, Northampton, MA, USA). Principal component analysis (PCA) was plotted by https://www.bioinformatics.com.cn.

## 3. Results and discussion

### 3.1. Total phenolic content (TPC) and total flavonoid content (TFC)

As shown in Fig. 1a, Fig. 1b and Fig. 1c, in the non-treated peach juice, the free phenolic fraction (FP) possessed the highest values of TPC and TFC with 548.24 mg GAE/g extract and 525.24 mg RE/g extract, respectively, followed by esterified phenolic fraction (EP), and lowest value was found for insoluble-bound phenolic fraction (IBP). The amounts of TPC and TFC in different phenolic fractions from different plant-based food varies greatly depended on resource and extraction methods. Generally, vegetables and fruits contained more FP or EP, while IBP only accounted for an average of 24 % of the TPCs (Sun, Chu, Wu, & Liu, 2002). For example, EP accounted for the highest content of TPCs in medlar fruit with the value of 64 %, while the contents of FP and IBP were 16 % and 20 %, respectively (Gruz, Ayaz, Torun, & Strnad, 2011). Similarly, the highest content of 69.95 % was found for FP of orange TPCs, while the contents of EP and IBP were 5.67 % and 24.38 %, respectively (Sun et al., 2002). In contrast, most of the phenolic compounds in cereal grains were present in the state of IBP, accounting for 54.6 %-88.9 % of the TPCs (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014).

HPH significantly increased the TPCs in FP and EP, and increasing HPH pressure promoted the effect. Interestingly, HPH at 50 MPa and 200 MPa decreased the TPC in IBP, but the value was significantly increased when boosted the pressure to 300 MPa. The TPC in FP, EP, and IBP were increased by 21.04 %, 48.33 % and 29.53 % treated by HPH at 300 MPa, respectively. The changes of TFC treated by HPH showed

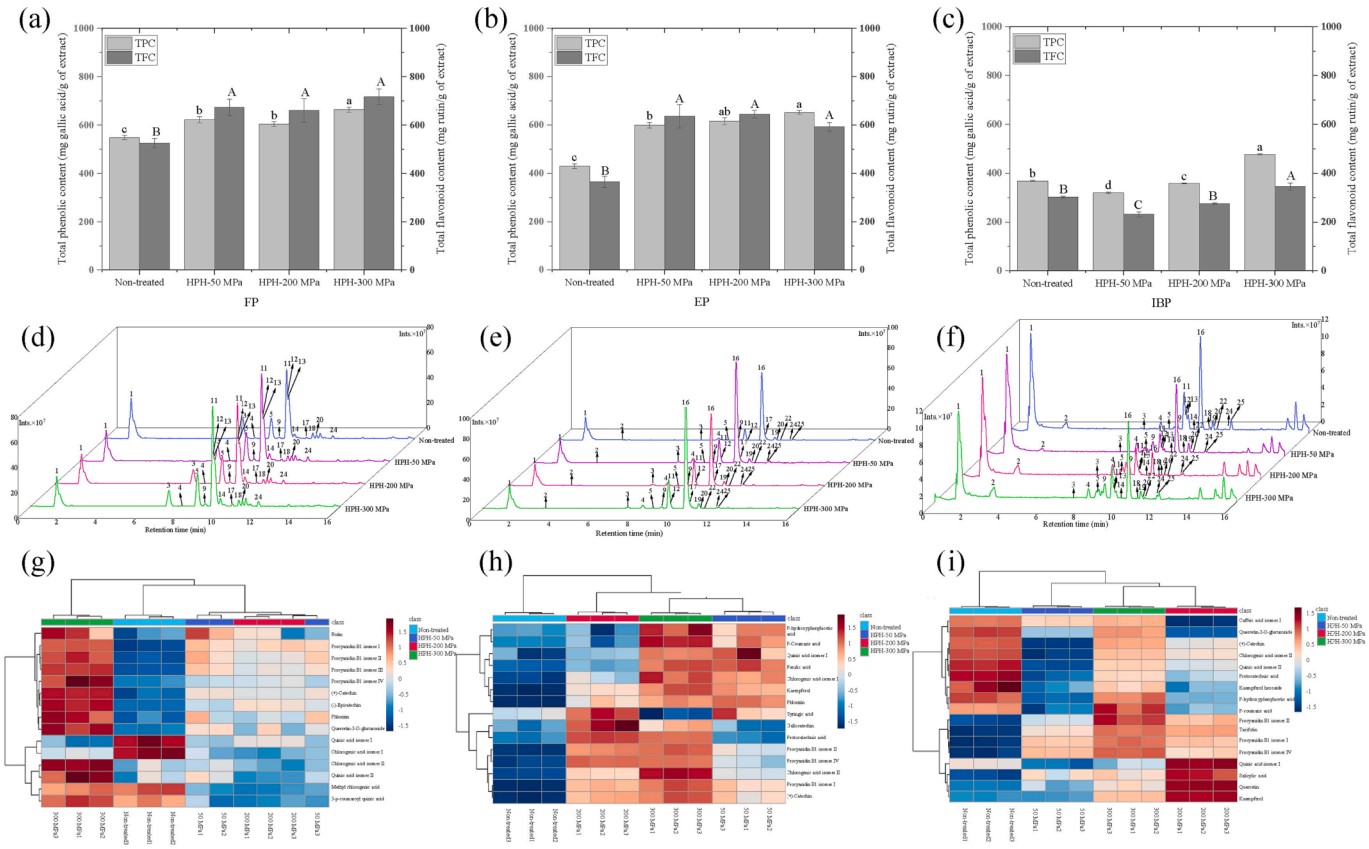


**Fig. 1.** The content of total phenolic and total flavonoid (a, b, c), negative ion current chromatograms (d, e, f), and thermogram analysis of phenolic compounds (g, h, i) in the free, esterified and insoluble-bound phenolic fractions from non-treated and high pressure homogenization treated 'Lijiang snow' peach juice, respectively. Peaks identification and their MS data are shown in Table S1. All values are expressed as mean ± SD (n = 3). "A-C" represent the significant differences between the TFC of the same fractions from the non-treated and high pressure homogenization treated 'Lijiang snow' peach juice, "a-d" represent the significant differences between the TPC of the same fractions from the non-treated and high pressure homogenization treated 'Lijiang snow' peach juice, respectively ($p < 0.05$). "FP" represents free phenolic fraction; "EP" represents esterified phenolic fraction; "IBP" represents insoluble-bound phenolic fraction.

similar trends, and the values was increased by 36.48 %, 62.57 % and 14.41 % treated by HPH at 300 MPa, respectively. The increase of phenolics content in FP might be due to the fact that HPH could destroy the structure of cell wall, accelerate the penetration and diffusion of soluble substances, and thus improve the yield of phenolic substances (Liu et al., 2019). The presence of esterified and insoluble phenolics in the fruit juice could make it possible to consider the juice as an natural oil-in-water emulsion system, which encapsulated the EP and IBP in the macromolecules such as pectin and cellulose (Priyadarshani, 2017). Therefore, the mechanical strength of HPH could destroy the encapsulated layer of EP and IBP, causing the release of EP and IBP. In addition, those values of both TPC and TFC in IBP firstly decreased at 50 MPa and 200 MPa, and then significantly increased as compared to non-treated samples, which could be partly explained by that the conjugated state of phenolic fraction could be converted into the free state by processing, such as fermentation and thermoplastic extrusion, resulting in improving their bioactivity and bioavailability (Septembre-Malaterre, Remize, & Poucheret, 2018; Rochín-Medina et al., 2012). As we know, HPH processing has a strong mechanical effect on food. Thus, we speculated that HPH played 2 different roles in the changes of phenolic in peach juice processing. On the one hand, HPH might convert the IBP to the free state by destroying the structure of phenolic complexes, which covalently bounded to cell wall components of peach tissue, and therefore the amount of IBP reduced when low pressure applied. On the other hand, HPH might release the non-extractable EP and IBP with the increase of HPH pressure, due to the breaking of the encapsulated layer of phenolic (Acosta-Estrada et al., 2014). Thus, as the HPH pressure increased to 300 MPa, the released IBP was exceeded the converted IBP and therefore showed a remarkably increase in content of IBP.

### 3.2. *Identification and quantification of phenolic compounds*

Fig. 1d, Fig. 1e and Fig. 1f showed that phenolic compounds in non-treated and HPH-treated FP, EP and IBP from 'Lijiang snow' peach juice were analyzed by UHPLC-ESI-HRMS/MS in the negative mode. In Table S1, 31 phenolic compounds were identified in all phenolic fractions, including 18 phenolic acids, 9 flavonoids and 4 tannins. Previous studies also reported that there were more than 40 phenolic compounds have been isolated and identified from peach fruit (Guo et al., 2020). The most dominant phenolics in FP, EP and IBP of peach juice were chlorogenic acid isomer II, caffeic acid isomer I, and chlorogenic acid isomer II, which accounted for 43.58 %, 36.75 % and 30.56 % of their respective fractions, respectively. Catechin and procyanidin B1 were the main phenolic compounds in 3 phenolic fractions followed by phenolic compounds mentioned above. Hydroxycinnamates were reported to be the dominant phenolic compounds in peach fruit, including neochlorogenic acid and chlorogenic acid (Mokrani et al., 2016). It was also reported that neochlorogenic acid, catechin and chlorogenic acid accounted for 70.18–84.56 % of TPCs in peach fruit (Guo et al., 2020). In this study, IBP had the largest number of phenolic compounds, with 27 phenolic compounds. Similarly, Zhou et al. (2019) reported that 14 phenolic compounds were detected in oil palm fruit, of which 13 were present in IBP.

As shown in Fig. 1g, Fig. 1h and Fig. 1i, those changes in contents of phenolic compounds in different fractions of peach juice treated by HPH at different pressures were investigated by the clustering thermogram.

Fig. 1g showed that a total of 15 main phenolic compounds identified in FP of peach juice, of which 9 phenolics were clustered together and their contents were significantly increased after HPH regardless of pressure applied, namely rutin, procyanidin B1 isomer I, procyanidin B1 isomer II, procyanidin B1 isomer III, procyanidin B1 isomer IV, (+)-catechin, (-)-epicatechin, phlorizin, and quercetin-3-*O*-glucuronide. Moreover, their contents all reached the highest content after treated by HPH at 300 MPa. The decreases of quinic acid isomer I and chlorogenic acid isomer I contents after all HPH treatments might be due to their instability during processing. Compared to the FP from non-treated peach juice, the contents of chlorogenic acid isomer II and quinic acid isomer II were not changed treated by HPH at 50 MPa and 200 MPa, but their contents were increased by 10.53 % and 11.18 % after increased the pressure to 300 MPa, respectively. The contents of methyl chlorogenic acid and 3-p-coumaroyl quinic acid were both decreased after HPH at 50 MPa and 200 MPa, and then increased with increased pressure to 300 MPa. Fig. 1h also showed that all phenolic compounds in EP were increased after 300 MPa HPH except syringic acid. The contents of protocatechuic acid, procyanidin B1 isomer I, procyanidin B1 isomer II, chlorogenic acid isomer II, procyanidin B1 isomer IV and (+)-catechin in EP were all increased with the increasing HPH pressure. Overall, the disruption of plant cell structure by HPH at 300 MPa could cause the release of phenolic compounds in FP and EP. As shown in Fig. 1i, 9 of 17 phenolic compounds in IBP were reduced after HPH-treated at 50 MPa and 200 MPa, and then increased with increasing HPH pressure to 300 MPa, which was consistent with the trends of TPCs and TFCs in IBP treated by HPH. In contrast, the contents of quinic acid isomer I, salicylic acid, quercetin and kaempferol showed the highest contents after treated by HPH at 200 MPa.

In general, HPH did not alter the composition of phenolic compounds in three phenolic fractions, but significantly changed their contents. Since each phenolic had different structure and property, which therefore led to different changes treated by HPH, but their changes trends were generally consistent with those of TPCs and TFCs. The process of HPH on different phenolic fractions of peach juice could be regarded as a complex dynamic process. Phenolic compounds with different chemical structures could transform and degrade during the treatment process. The mechanical effect of HPP might also alter the interaction of phenolics and flavonoids with other food matrices as well as self-incorporation, thus changing their contents (Eran Nagar et al., 2021). Previous studies have reported that non-thermal processing had less or positive effects on phenolic compounds in fruit and vegetable products. Do date, few studies have investigated on the effect of HPH on phenolics in different phenolic fractions, but the researches regarding on the other non-thermal processes on phenolic compounds have been reported. For example, most phenolic compounds in passion fruit purée after treated by UHP at 600 MPa for 5 min were unchanged in terms of composition and content, but their contents were greatly reduced by the high-temperature short-term treatment at 110 °C for 8.6 s (Niu et al., 2022). Moreover, UHP at 500 MPa for 10 min also significantly increased the contents of all phenolic compounds in FP, EP and IBP from oil palm fruit (Zhou et al., 2019).

### 3.3. Antioxidant activities

#### 3.3.1. DPPH and ABTS radical scavenging activity

As shown in Table 1, all phenolic fractions in non-treated and HPH-treated peach juice showed good DPPH radical scavenging ability with $IC_{50}$ values ranged from 4.78 to 14.26 μg/mL. The $IC_{50}$ values of phenolics from fruits and vegetables were generally within the range from 1 to 200 μg/mL (Zhang et al., 2022; Prakash et al., 2019). In the non-treated peach juice, FP had the strongest scavenging activity for DPPH radical (8.53 μg/mL), followed by the IBP (9.12 μg/mL), and the lowest value was found for EP (14.26 μg/mL). The $IC_{50}$ values of DPPH radical scavenging by FP and EP were both significantly decreased after HPH. Especially, the $IC_{50}$ values of DPPH radicals scavenged by FP, EP and IBP were reduced by 28.96 %, 66.48 % and 47.04 % after HPH at 300 MPa, respectively. It is worth noting that the initial $IC_{50}$ value of DPPH radicals scavenged by IBP in non-treated peach juice was higher than that value of FP, but it was decreased to 4.83 μg/mL after HPH at 300 MPa, which was even 20.30 % lower than that value of FP. That was to say, IBP in peach juice after HPH at 300 MPa had the best scavenging activity for DPPH radical among all samples. The change trend in the scavenging ability for ABTS radical of the three phenolic fractions after HPH was similar to those for DPPH radical, and their changes both well corresponded to changes of TPCs and TFCs. Similar result was found that the DPPH radical scavenging activity of pomegranate juice was increased by 30 % after HPH at 150 MPa (Benjamin et al., 2020). The DPPH and ABTS radical scavenging activities of mango juice were also increased by 42.86 % and 174.49 % after HPH at 190 MPa, respectively (Guan et al., 2016).

**Table 1**
$IC_{50}$ values of different phenolic fractions against DPPH, ABTS radical scavenging capacity, α-glucosidase and dipeptidyl peptidase-IV (μg/mL).

| | Fractions | Non-treated | HPH-50 MPa | HPH-200 MPa | HPH-300 MPa | Positive controls |
|---|---|---|---|---|---|---|
| DPPH | FP | $8.53 \pm 0.14^{Ca}$ | $6.45 \pm 0.03^{Cb}$ | $6.63 \pm 0.03^{Cb}$ | $6.06 \pm 0.02^{Ac}$ | $1.93 \pm 0.01$ |
| | EP | $14.26 \pm 0.26^{Aa}$ | $8.17 \pm 0.11^{Bb}$ | $8.05 \pm 0.36^{Bb}$ | $4.78 \pm 0.73^{Bc}$ | |
| | IBP | $9.12 \pm 0.07^{Bb}$ | $10.62 \pm 0.06^{Aa}$ | $9.24 \pm 0.13^{Ab}$ | $4.83 \pm 0.21^{Bc}$ | |
| ABTS | FP | $8.79 \pm 0.20^{Ba}$ | $7.78 \pm 0.13^{Bb}$ | $7.64 \pm 0.03^{Bb}$ | $6.78 \pm 0.09^{Ac}$ | $3.58 \pm 0.03$ |
| | EP | $10.89 \pm 0.19^{Aa}$ | $6.57 \pm 0.01^{Cb}$ | $6.77 \pm 0.02^{Cb}$ | $6.11 \pm 0.05^{Bc}$ | |
| | IBP | $8.19 \pm 0.10^{Cb}$ | $10.54 \pm 0.09^{Aa}$ | $8.42 \pm 0.04^{Ab}$ | $6.79 \pm 0.10^{Ac}$ | |
| α-Glu | FP | $44.12 \pm 1.04^{Aa}$ | $26.90 \pm 0.38^{Ab}$ | $29.68 \pm 0.35^{Ab}$ | $16.14 \pm 0.64^{Ac}$ | $0.02 \pm 0.00$ |
| | EP | $7.27 \pm 0.02^{Ca}$ | $5.90 \pm 0.35^{Cb}$ | $3.88 \pm 0.10^{Cc}$ | $3.39 \pm 0.68^{Cc}$ | |
| | IBP | $12.59 \pm 0.40^{Bb}$ | $15.27 \pm 0.81^{Ba}$ | $12.81 \pm 1.12^{Cb}$ | $7.90 \pm 0.76^{Bc}$ | |
| DPP-IV | FP | $312.22 \pm 5.85^{Aa}$ | $262.30 \pm 4.44^{Ab}$ | $243.63 \pm 0.91^{Ac}$ | $204.49 \pm 4.49^{Ad}$ | $0.13 \pm 0.01$ |
| | EP | $96.72 \pm 2.13^{Ba}$ | $46.30 \pm 1.51^{Cb}$ | $38.44 \pm 0.89^{Cc}$ | $36.19 \pm 3.06^{Bc}$ | |
| | IBP | $50.96 \pm 0.24^{Cb}$ | $72.02 \pm 2.87^{Ca}$ | $46.29 \pm 1.77^{Bb}$ | $35.25 \pm 2.87^{Bc}$ | |

All the values are presented as mean ± SD (n = 3). Positive controls for free radical scavenging, α-glucosidase and DPP-IV were Vc, acarbose, and sitagliptin, respectively. "A-C" represent the significant differences between the different fractions of the same treatment 'Lijiang snow' peach juice, "a-d" represent the significant differences between the same fractions from the non-treated and high pressure homogenization treated 'Lijiang snow' peach juice, respectively ($p < 0.05$).

#### 3.3.2. Inhibitory effect on intracellular reactive oxygen species (ROS) generation

ROS have a strong oxidative capacity, and excessive accumulation can cause cellular oxidative stress, which can lead to cell damage, aging and apoptosis (Urade et al., 2023). Numerous studies have suggested that phenolic compounds, as natural antioxidants in the human diet, could restore redox homeostasis by activating key transcription factors of antioxidant enzymes, which in turn activate endogenous antioxidant action (Zhang & Tsao, 2016). As shown in Fig. 2b, phenolics samples with a concentration of 300 μg/mL based on cytotoxicity assay, were used to study their inhibitory effect on intracellular ROS production in cells. The intracellular ROS content was rose dramatically to 181.61 % by $H_2O_2$ treatment as compared to control sample, and both Vc and phenolic samples significantly reduced its level. The intracellular ROS content was significantly decreased to 110.92 % in Vc-treated group. In the non-treated peach juice, IBP showed the strongest inhibition of ROS production with an intracellular ROS level of 121.21 %, followed by EP

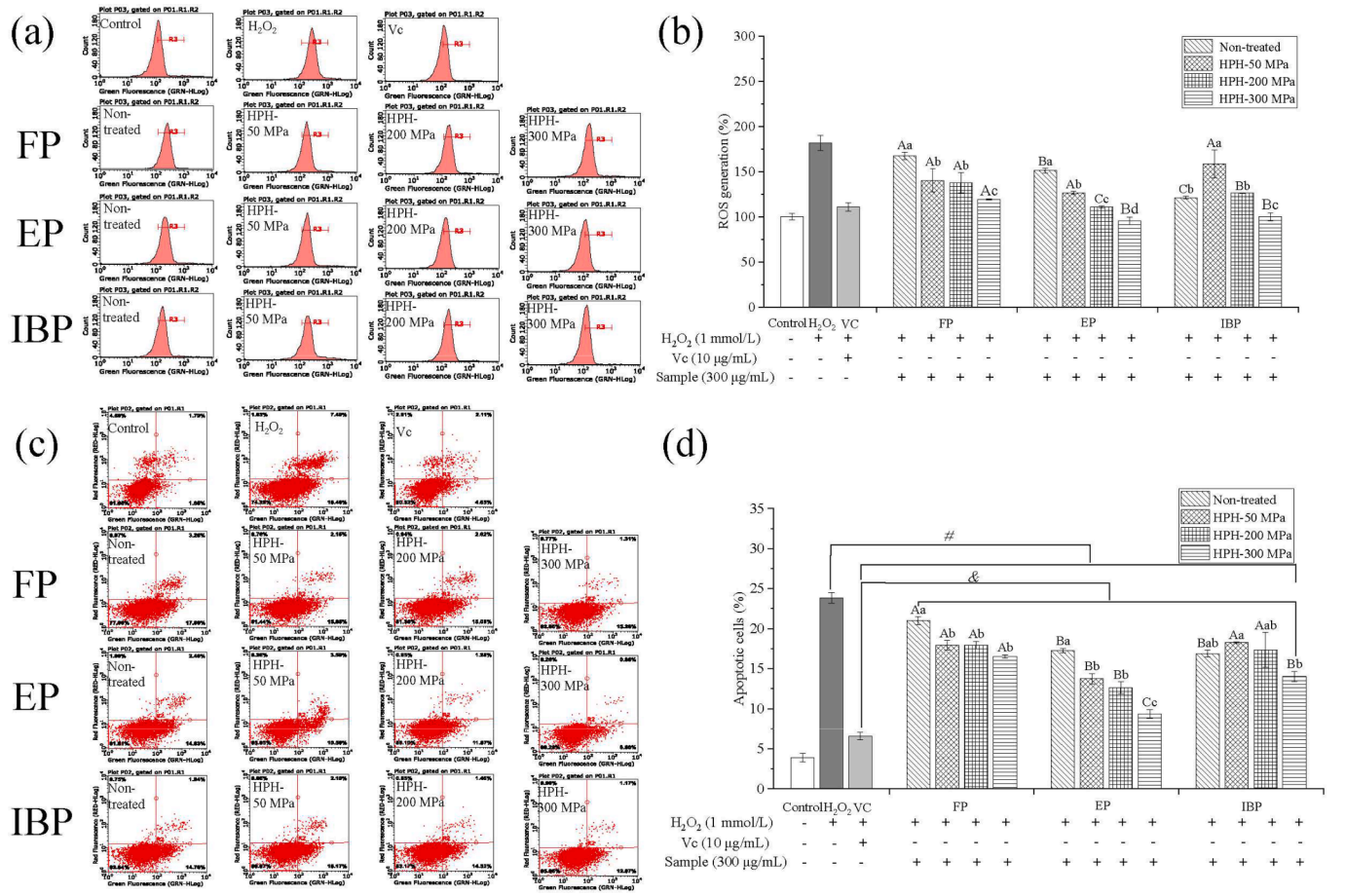


**Fig. 2.** Inhibition of $H_2O_2$-induced ROS production in Caco-2 cells by free, esterified and insoluble-bound phenolic fractions from non-treated and high pressure homogenization treated 'Lijiang' snow peach juice, (a) flow cytogram; (b) relative levels of intracellular ROS. Protective effects of $H_2O_2$-induced apoptosis in Caco-2 cells by free, esterified and insoluble-bound phenolic fractions from non-treated and high pressure homogenization treated 'Lijiang' snow peach juice., (c) flow cytometry; (d) percentage of apoptosis in each group. All the values are expressed as mean ± SD (n = 3). "#" represents the significant difference between the $H_2O_2$ group and the Vc and sample groups ($p < 0.05$). "&" represents the significant differences between the Vc group and the sample groups ($p < 0.05$). "A-C" represent the significant differences between the different fractions of the same treatment 'Lijiang snow' peach juice, "a-d" represent the significant differences between the same fractions from the non-treated and high pressure homogenization treated 'Lijiang snow' peach juice, respectively ($p < 0.05$).

of 151.03 % and FP of 167.28 %. HPH significantly boosted the inhibition effects of FP an EP on intracellular ROS production, which could be increased by increasing pressure. In addition, the intracellular ROS levels in the cells increased when treated by IBP from peach juice after HPH at 50 MPa and then decreased with increased pressure. In each phenolic fraction, the best inhibition effects on intracellular ROS production in Caco-2 cells were all found for FP, EP and IBP from peach juice after HPH at 300 MPa, with intracellular ROS levels of 119.16 %, 95.26 % and 100.00 %, respectively. It was also found that EP and IBP in oil palm fruit were more cytoprotective than FP, with reductions in intracellular ROS production in HepG2 cells of around 25 % and 50 %, respectively (Zhou et al., 2019). Similarly, the methanol extract of phenolic compounds (100 μg/mL) from durian shell also exhibited a good inhibitory effect on intracellular ROS production in HepG2 cells, which reduced ROS level by around 40 % (He et al., 2023). It has been proved that phenolic compounds could inhibit ROS production by scavenging free radicals or chelating metal ions, or indirectly eliminate ROS by enhancing the activity of antioxidant enzymes, thus therefore reducing apoptosis (Zhang & Tsao, 2016).

### 3.3.3. *Cytoprotective activity against $H_2O_2$-induced cell apoptosis*

Fig. 2d showed that the apoptosis rate of control group was 3.92 %, and its value was significantly increased to 23.84 % in the $H_2O_2$-treated group. Both Vc and phenolic samples significantly reduced apoptosis, and Vc showed the strongest cytoprotective effect on cells with the apoptosis rate of 6.58 %. In the non-treated peach juice, IBP and EP with apoptosis rates of around 17 %, showed stronger cytoprotective effects than FP (21.00 %). The cytoprotective effects of FP and EP from peach juice significantly improved with increasing HPH pressure, and their apoptosis rates of cells were reduced to 16.52 % and 9.33 % after treated by HPH at 300 MPa, respectively. As compared to IBP from non-treated peach juice, HPH did not significantly change its cytoprotective effect on cells apoptosis. The inhibition effects of phenolic compounds from peach juice on intracellular ROS production were comparable to Vc, but their cytoprotective effects on cells apoptosis were weaker. This was possibly because that there were several reasons for apoptosis, such as DNA damage, chromosome doubling, ROS production, etc. (Argyris et al., 2023), as well as insufficient antioxidants and antioxidant enzymes (Zhang et al., 2022). Free radicals and ROS were not scavenged completely, and then their excessive accumulation could lead to apoptosis, resulting deleterious effects on organisms. Similar result was found that the ethanolic extracts of phenolic compounds (200 μg/mL) from *Vaccinium dunalianum* flower buds exhibited better inhibition effect on ROS production than its cytoprotective against apoptosis of PC12 cells (Gao et al., 2022).

### 3.4. Inhibition of α-glucosidase and dipeptidyl peptidase-IV (DPP-IV)

As a natural secondary metabolite, phenolic compounds are effective in regulating the type 2 diabetes. Firstly, phenolic compounds can slow down the digestion of carbohydrates by inhibiting α-glucosidase activity, thus reducing postprandial blood glucose (Zhang et al., 2015). Secondly, phenolic compounds may extend the half-life of glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polyptide (GIP) by binding to DPP-IV and increase insulin biosynthesis, thus indirectly regulating blood glucose concentration (Li et al., 2018). Table 1 showed the effects of α-glucosidase and DPP-IV inhibited by three phenolic fractions expressed as $IC_{50}$. All phenolic fractions showed effective inhibition on α-glucosidase with $IC_{50}$ ranged from 3.39 to 44.12 μg/mL. Previous studies have reported that the phenolic compounds from fruit and vegetable have good inhibition effects on α-glucosidase. Studies have shown that some phenolics could bind to α-glucosidase, which affected the elongation of the polypeptide chain, thereby inducing changes in the microenvironment around the tryptophan residues or tyrosine residues in α-glucosidase and secondary structural rearrangements, leading to enzyme inactivation (Qin et al., 2023; Yang, Zhang, Huang, & Zhao, 2023). In the non-treated peach juice, EP showed the highest inhibitory effect for α-glucosidase (7.27 μg/mL), followed by the IBP (12.59 μg/mL), and the lowest value was found for FP (44.12 μg/mL). Differently, different phenolic fractions from water caltrop husk had the inhibitory effects on α-glucosidase was in the order of FP > IBP > EP (Zhang et al., 2022), which might because of the changes in phenolic compounds in different phenolic fractions from different resource. The $IC_{50}$ values of FP and EP on α-glucosidase inhibition were significantly decreased with increasing HPH pressure, and their values were decreased by 63.42 % and 53.37 % after HPH at 300 MPa, respectively. It was also observed that the $IC_{50}$ values of α-glucosidase inhibition by IBP from peach juice after HPH at 50 MPa was 21.29 % higher than that for non-treated peach juice, but the value was then decreased by 37.25 % after increased the pressure to 300 MPa.

Table 2 showed that the inhibitory effects of the 11 major phenolic compounds identified in peach juice against α-glucosidase were investigated to explore the major contributors to α-glucosidase inhibition. Seven of the 11 phenolic standards showed $IC_{50}$ values lower than 1500 μM for α-glucosidase inhibition, including gallocatechin, chlorogenic acid, (+) - catechin, (-) - epicatechin, caffeic acid, taxifolin and procyanidin B1. Among them, (+) - catechin showed the strongest inhibition on α-glucosidase with the lowest $IC_{50}$ value of 244.36 μM, followed by procyanidin B1 and gallocatechin. Tan et al. (2017) compared the inhibition effects of α-glucosidase by 17 phenolic standards, and the $IC_{50}$ value of catechin was 13.40 μM, which was also higher than those values for chlorogenic acid and caffeic acid. It was suggested that the inhibitory effects of the 11 phenolics on α-glucosidase were all lower than those values of three phenolic fractions from peach juice. This might be due to the synergistic effect between multiple phenolic compounds. It has been shown that mixtures of gallic acid and resveratrol with a volume ratio of 1:1 and mixtures of catechins and resveratrol with a volume ratio of 1:1 both exhibited high synergistic effects for antioxidant activities (Skroza, Mekinić, Svilović, Šimat, & Katalinić, 2015).

**Table 2**
$IC_{50}$ values of different phenolic standards against α-glucosidase and dipeptidyl peptidase-IV (μM).

| | Phenolic standards | α-Glu | DPP-IV |
|---|---|---|---|
| 1 | Quinic acid | >1500.00 | >200.00 |
| 2 | Gallocatechin | $323.20 \pm 13.96^{e}$ | $81.87 \pm 2.54^{e}$ |
| 3 | Chlorogenic acid | $421.84 \pm 1.27^{d}$ | $31.20 \pm 2.58^{f}$ |
| 4 | Salicylic acid | >1500.00 | >200.00 |
| 5 | (+) - Catechin | $244.36 \pm 2.66^{g}$ | $132.82 \pm 0.99^{c}$ |
| 6 | (-) - Epicatechin | $1466.18 \pm 96.79^{a}$ | $104.27 \pm 0.95^{d}$ |
| 7 | Methyl chlorogenic acid | >1500.00 | $192.29 \pm 1.93^{a}$ |
| 8 | Caffeic acid | $936.83 \pm 4.67^{c}$ | $162.48 \pm 0.16^{b}$ |
| 9 | Taxifolin | $1006.86 \pm 16.91^{b}$ | $78.92 \pm 0.62^{e}$ |
| 10 | Protocatechuic acid | >1500.00 | >200.00 |
| 11 | Procyanidin B1 | $279.63 \pm 3.43^{f}$ | >200.00 |

All the values are expressed as mean ± SD (n = 3). "a-g" represent the significant differences between different phenolic standards for α-glucosidase or dipeptidyl peptidase-IV.

All phenolic fractions also showed good inhibition effects on DPP-IV with $IC_{50}$ values ranged from 35.25 to 312.22 μg/mL. In contrast to the results of inhibition of α-glucosidase by phenolic fractions in the non-treated peach juice, IBP showed the highest inhibitory activity for DPP-IV with $IC_{50}$ value of 50.96 μg/mL, followed by EP and FP. HPH showed similar effects on the DPP-IV inhibition by FP, EP and IBP to those for α-glucosidase. Finally, the $IC_{50}$ values of inhibition DPP-IV by FP, EP and IBP from peach juice were reduced by 34.50 %, 62.58 % and 30.83 % after treated by HPH at 300 MPa, respectively. Phenolic compounds, including chlorogenic acid, taxifolin and gallocatechin, showed high inhibitory effects against DPP-IV. Among them, chlorogenic acid showed the strongest inhibitory effect on DPP-IV with the lowest $IC_{50}$ value of 31.20 μM. Similarly, binding of phenolics to DPP-IV caused conformational changes of DPP-IV, thereby inhibiting its activity. The binding site in DPP-IV for flavonoids might be closer to the tryptophan residue, and the environment around the tryptophan residue was became hydrophobic after binding (Pan et al., 2022). A previous study also found that DPP-IV was strongly inhibited by chlorogenic acid with the $IC_{50}$ value of 78.85 μM (Li et al., 2022). The inhibitory effects of different phenolic fractions of 'Lijiang snow' peach juice on DPP-IV were within the inhibition range of the major phenolic standards, suggesting that 7 major phenolic compounds played major roles in the inhibition of DPP-IV.

### 3.5. Molecular docking

Fig. 3 showed the molecular docking results of the major phenolic compounds in 'Lijiang snow' peach juice with α-glucosidase and DPP-IV. According to the virtual screening of phenolic standards based on their inhibitory effects on two enzymes in vitro, 7 phenolic compounds were finally selected for molecular docking analysis, including chlorogenic acid, procyanidin B1, gallocatechin, taxifolin, (+) - catechin, (-) - epicatechin and caffeic acid (Fig. 3A). Fig. 3B and 3C showed that all 7 phenolic compounds entered the active pocket of both enzymes, and then bound to the active amino acid of enzyme. Fig. 3D and 3E showed 2D diagrams of the intermolecular interactions of 7 phenolic compounds with two enzyme. The ligand formed a complex with the active amino acid of α-glucosidase through hydrogen bonding, thereby hindering the substrate entry and inhibiting its activity (Li et al., 2022). Numerous studies have confirmed that Asp69, Asp215, Asp352, Gln353, Arg442, and Glu411 were the key catalytic active sites for α-glucosidase (Cai, Wu, Lin, Hu, & Wang, 2020; Hua et al., 2018). As shown in Table 3, 7 phenolic compounds all bound to the key active amino acid residues of α-glucosidase through hydrogen bonds, corresponding to their good inhibition effects on enzyme. In addition, hydrophobic interactions also drove the association between phenolics and proteins (Ye et al., 2022). Among the 7 complexes, procyanidin B1-α-glucosidase formed the van der Waals forces with the highest numbers (15) of amino acid residues, followed by taxifolin (14) and (+) - catechin (13). It could be observed that there were 4 amino acid residues all involved in the formation of van der Waals forces in the 3 complexes of α-glucosidase with procyanidin B1, taxifolin and (+) - catechin, namely Tyr158, Gln279, His280, and Phe303. Thus, the strong inhibitory effect of some phenolic compounds on α-glucosidase activity could be contributed by both the high number of amino acid residues for the formation of van der Waals force and the binding of phenolics to key amino acid residues through hydrogen bonds. In this study, flavonoids showed better α-glucosidase inhibition, which was closely related to their molecular structure. The hydroxylation of C3′ on the B-ring enhanced the inhibitory capacity of flavonols, whereas diglycosylation of C3 on the C-ring weakened the inhibitory capacity of flavonols (Qin et al., 2023).

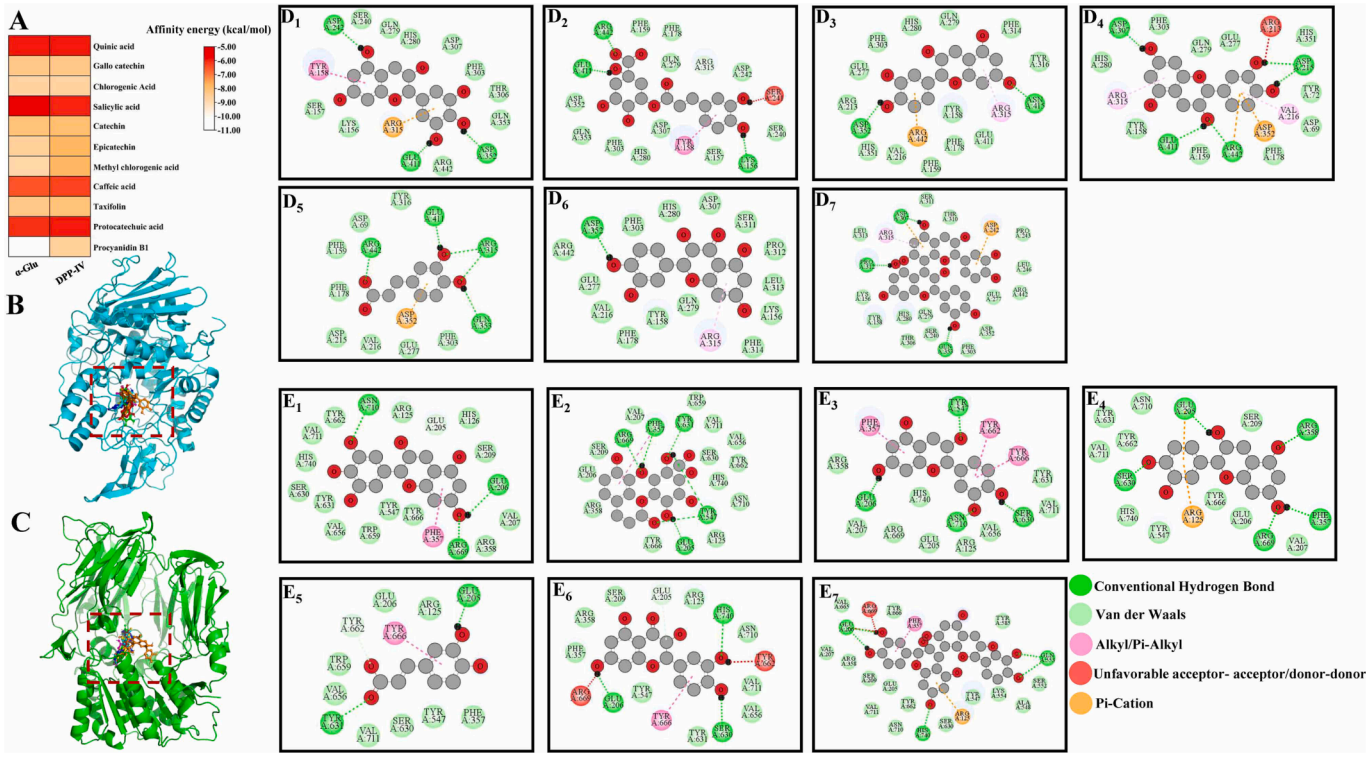


**Fig. 3.** Molecular docking results of phenolic compounds with α-glucosidase and dipeptidyl peptidase-IV, respectively.(A) binding affinity energy of phenolic compounds with α-glucosidase and dipeptidyl peptidase-IV, (B) and (C) binding sites of 7 phenolic compounds in α-glucosidase and dipeptidyl peptidase-IV (gallo catechin: red; chlorogenic acid: green; (+) - catechin: blue; (-) - epicatechin: yellow; caffeic acid: magentas; taxifolin: cyan; procyanidin B1: oranges.), 2D diagram of intermolecular interactions between 7 phenolic compounds bound to α-glucosidase (D) and dipeptidyl peptidase-IV (E), respectively. Where the numbers 1–7 are gallocatechin, chlorogenic acid, (+) - catechin, (-) - epicatechin, caffeic acid, taxifolin and procyanidin B1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 3**
The molecular docking results of procyanidin B1, chlorogenic acid, (-) - epicatechin, gallocatechin, taxifolin (+) - catechin and caffeic acid with α-glucosidase and dipeptidyl peptidase-IV.

| | Compounds | Pubchem ID | Docking Energy (kcal/mol) | | The Number of Amino Acid Residues Forming Van der Waals Force | | Number of hydrogen bonds | | Amino Acid Residues Forming Hydrogen Bonds (Bond Length) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | α-Glu | DPP-IV | α-Glu | DPP-IV | α-Glu | DPP-IV | α-Glu | DPP-IV |
| 1 | Gallocatechin | 65,084 | −8.3 | −8.3 | 10 | 15 | 3 | 3 | Asp-242 (2.1 Å), Glu-411 (2.6 Å), Asp-352 (3.0 Å) | Asn-710 (2.8 Å), Arg-669 (2.2 Å), Glu-206 (1.7 Å) |
| 2 | Chlorogenic acid | 1,794,427 | −8.7 | −8.7 | 12 | 13 | 3 | 6 | Arg-442 (2.2 Å), Glu-411 (2.5 Å), Lys-156 (2.5 Å) | Arg-669 (2.2 Å), Phe-357 (2.4 Å), Tyr-631 (2.3 Å), Tyr-547 (2.9 Å, 3.2 Å), Glu-205 (3.1 Å) |
| 3 | (+)- Catechin | 9064 | −8.1 | −8 | 13 | 9 | 2 | 4 | Asp-352 (2.3 Å), Asn-415 (2.9 Å) | Glu-206 (2.1 Å), Tyr-547 (2.6 Å), Ser-630 (1.9 Å), Asn-710 (2.6 Å) |
| 4 | (-)-Epicatechin | 72,276 | −8.6 | −7.8 | 10 | 10 | 5 | 5 | Asp-307 (2.4 Å), Glu-411 (2.6 Å), Arg-442 (3.1 Å), Asp-215 (2.7 Å, 2.6 Å) | Ser-630 (2.5 Å), Glu-205 (2.2 Å), Arg-358 (3.2 Å), Arg-669 (2.4 Å), Phe-357 (3.3 Å) |
| 5 | Caffeic acid | 689,043 | −6.5 | −6.3 | 8 | 9 | 5 | 2 | Arg-442 (2.0 Å), Glu-411 (2.3 Å), Arg-315 (2.9 Å, 2.3 Å), Gln-353 (3.0 Å) | Glu-205 (2.2 Å), Tyr-631 (1.9 Å) |
| 6 | Taxifolin | 439,533 | −8.3 | −8.1 | 14 | 10 | 1 | 3 | Asp-352 (2.5 Å) | Glu-206 (3.1 Å), His-740 (2.3 Å), Ser-630 (2.2 Å) |
| 7 | Procyanidin B1 | 11,250,133 | −10.1 | −8.6 | 15 | 15 | 3 | 5 | Asp-307 (2.9 Å), Pro-312 (3.4 Å), Gln-353 (2.4 Å) | Glu-206 (2.9 Å, 2.7 Å), Gln-553 (2.3 Å, 2.2 Å), His-740 (2.2 Å) |

It was reported that Arg669, Glu206, Ser209 and His126 played important roles in the forming the DPP-IV-ligand complexes (Li et al., 2018; Fu, Liu, Ma, Yi, & Cai, 2022). Similarly, the molecular docking results showed that 6 phenolic compounds all formed hydrogen bonds to the crucial amino acid residues of DPP-IV as mentioned above, except caffeic acid. Among which, chlorogenic acid formed the highest number of 6 hydrogen bonds with Arg669, Phe357, Tyr631, Tyr547 and Glu205 of DPP-IV, corresponding to it's the highest inhibition effect on DPP-IV.

In contrast, caffeic acid-DPP-IV complex formed only 2 hydrogen bonds involving the residues Glu205 and Tyr631. Among the 7 complexes, procyanidin B1 and gallocatechin both had the highest numbers of amino acid residues to form van der Waals forces in the complexes with DPP-IV with the number of 15, followed by chlorogenic acid with 13, and taxifolin with 10. Since procyanidin B1 showed a weak inhibitory effect on DPP-IV, it might be deduced that the formation of van der Waals forces contributed less to inhibition of DPP-IV. It was reported that the introduction of hydroxyl groups at the 6-position of the A-ring and the 3′ and 4′ positions of the B-ring in the flavonoid structure, as well as the 2,3-double bond and the 4-carbonyl group on the C-ring were beneficial to the enhancement of their inhibitory effect on DPP-IV (Pan et al., 2022). This results showed that the inhibitory effects of phenolic compounds on α-glucosidase and DPP-IV activities were closely related to the molecular structure of phenolic, which further affect their binding forces, such as hydrogen bonding and van der Waals forces.

### 3.6. Multivariate analysis

Principal component analysis (PCA) was performed to explain the variation in the TPC, TFC, DPPH, ABTS, ROS, apoptotic, α-glucosidase and DPP-IV inhibition in three phenolic fractions from non-treated and HPH-treated 'Lijiang snow' peach juice. As shown in Fig. S1, total variation explained 87.0 %, of which first principal component (PC1) and second principal component (PC2) explained for 56.0 % and 31.0 %, respectively. The results showed that PC1 distinguished non-treated and 50 MPa HPH-treated samples from 200 MPa and 300 MPa HPH-treated samples, while PC2 distinguished FP from EP and IBP samples. The lower left quadrant of PC1 included EP by HPH at 50 MPa, 200 MPa, and 300 MPa and IBP by HPH at 300 MPa, indicating higher cytoprotective, α-glucosidase, and DPP-IV inhibitory effects of EP and IBP treated by certain pressures of HPH. The non-treated, HPH at 50 MPa and 200 MPa treated FP were all located in the upper right quadrant of PC1, but were split into two clusters, while the FP of HPH at 300 MPa treated was located in the upper left quadrant alone. This indicated that the TPC, TFC, and free radical scavenging activities of FP increased significantly with increasing pressure, and their values reached the highest at 300 MPa. The lower right quadrant of PC1 included non-treated EP and non-treated and 50 MPa and 200 MPa HPH-treated IBP, which had lower TPC and TFC and weaker biologically activities. The results also suggested that TPC and TFC of three phenolic fractions in 'Lijiang snow' peach juice were positively correlated with their antioxidant activity, cytoprotective effect, and inhibitory effects on α-glucosidase and DPP-IV.

## 4. Conclusion

Peach juice showed the highest values of TPC and TFC in FP, followed by EP and IBP. Overall, the TPC of FP, EP and IBP all increased significantly by HPH, especially their contents were increased by 21.04 %, 48.33 % and 29.53 % after HPH at 300 MPa, respectively. TFC in three phenolic fractions showed similar changes to those of TPC. In total, 31 phenolic compounds were identified by UHPLC-ESI-MS/MS in three phenolic fractions, with chlorogenic acid isomer II being the highest both in the FP and IBP, and caffeic acid isomer I being the highest in the EP. The biological activities of FP, EP and IBP showed similar change trends to TPC and TFC after HPH, including free radical scavenging activity, cytoprotection, α-glucosidase and DPP-IV inhibition. HPH showed the most significant effects on the $IC_{50}$ values of scavenging DPPH and ABTS radicals by EP, which were decreased from 14.26 μg/mL and 10.89 μg/mL to 4.78 μg/mL and 6.11 μg/mL after HPH at 300 MPa, respectively. Moreover, EP exhibited stronger cellular antioxidant activity and cytoprotective effects than FP and IBP, and the intracellular ROS level and apoptosis rate of Caco-2 cells treated by EP after HPH at 300 MPa were reduced by 36.93 % and 46.01 % as compared to the EP from non-treated peach juice. All phenolic fractions showed good inhibition on α-glucosidase and DPP-IV, suggesting they might be effective in regulating the type 2 diabetes. Through in vitro experiments combined with molecular docking, we found that 7 phenolics, including gallocatechin, chlorogenic acid, (+) - catechin, (-) - epicatechin, caffeic acid, taxifolin and procyanidin B1, played major roles in inhibitory of enzyme activities, and they stably bound to the receptor mainly by hydrogen bonding and van der Waals forces. PCA also confirmed the strongest cytoprotective effect of EP after HPH at 300 MPa, also with good inhibitory effects on α-glucosidase and DPP-IV. This study explored the potential utilization of phenolic compounds in 'Lijiang Snow' peach in the field of healthy foods, and provided the theoretical and experimental foundation for the application of HPH technology in the non-thermal processing of fruits and vegetables.

## CRediT authorship contribution statement

**Shenke Bie:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Shuai Zhao:** Methodology, Data curation, Conceptualization. **Shengbao Cai:** Resources, Funding acquisition. **Junjie Yi:** Resources, Funding acquisition. **Linyan Zhou:** Writing – review & editing, Supervision, Resources, 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

The data that has been used is confidential.

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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.2023.101092.

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