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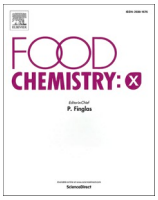

# Dynamic migration of phenolics in microwaved combined cooked sorghum: Focus on the polyphenols interact with starch/protein

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ABSTRACT

Based on the perspective of whole sorghum food, the polyphenols migration process was analyzed during microwave-combined cooking treatment utilizing wide metabolomics, simulated reactions, and molecular docking. Microstructure confirmed that microwave broke the grain cells, resulting in the elevated polyphenols contents. Flavonoids were significantly released by microwave (e.g. arbutin, eriodictyol-7-o-glucoside, narirutin, and naringenin-7-o-glucoside), which regulated the antioxidant activity of sorghum. Simulated *co*-gelatinization reaction revealed that polyphenols interacted non-covalently with starch, resulting in higher levels of polyphenols being retained during cooking (711.12 mg GAE/100 g). Molecular docking results exhibited that 6 flavonoids could also bind to the kafirin via hydrogen bonds and hydrophobic interaction during cooking. Meanwhile, the γ-mangostin also possessed stabilized root-mean-square deviation and outstanding binding free energies. The effective retention of bioactive components under synergetic microwave and cooking treatment highlights the potential of natural ingredients in food processing, promoting a more natural approach to modern cereal nutrition.

## 1. Introduction

Based on the balanced nutrition and diversity of diet structure, sorghum gets attention due to its various biological functionalities and nutritional quality (Svensson et al., 2010). Summing up the results from various studies, there is an association between the consumption of sorghum and glycolipid metabolism, insulin resistance, and systemic inflammation (Girard & Awika, 2018; Hajira & Khan, 2022; Hithamani & Srinivasan, 2014; Paes et al., 2024), These advantages are mainly attributed to their polyphenolic compounds with antioxidant capacity (Louise et al., 2019). Sorghum phenolic compounds, including phenolic acids, procyanidins, and flavonoids with varying biological activities such as antimicrobial and anti-inflammatory (Solomon et al., 2015). Unlike other grains, the mature sorghum contains the abundant 3-deoxyanthocyanins as the dominant secondary plant pigments, including apigeninidin or luteolinidin and their *o*-methylated and conjugated derivatives (Tanwar et al., 2023). Research confirmed that 3-deoxyanthocyanidin molecules were considerably superior to their anthocyanidin analogs at cellular antioxidant activity in vitro (Xiong et al., 2021). The evidence suggested potential unique applications of sorghum in health and disease prevention (Kang et al., 2016). However, most phenolics combined with cell substances such as proteins, carbohydrates, and organic acids via ester bonds, glycoside bonds, and ether glycoside bonds to form insoluble bound phenolics in sorghum grains. These bound phenolics as glycosides reduce their function and health-promoting characteristics (Al-Juhaimi et al., 2018). Therefore, by liberating these bound phenolics using some pretreatments, the antioxidant-rich sorghum can be preferably introduced to the human diet.

A frequently used approach to improve the nutritional and functionality of coarse cereals is physical modifications using microwaves (Yan et al., 2014). In fact, low-power microwave is a fairly fast process for food, resulting in a more efficient and less costly method to modify the starch/protein in a raw matrix (Vicente et al., 2024), and its application at an exposure time of 120–150 s in production of popped sorghum (Barba et al., 2020). Beyond that, previous studies revealed that

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microwave treatments can promote the release of bound phenolic substances and procyanidins in sorghum hulls, and significantly enhance the antioxidant capacity (Luo et al., 2020). This was attributed to the violent collision between the polyphenol molecules and the interrupted phenol hydroxyl group caused by microwaves, which further leads to the molecular structure rearrangement and polymerization effect (Hayat et al., 2010). However, from the perspective of food processing, microwave processing difficult to eliminate interference from other components, to act directly on a single active component (Mirzapour-Kouhdasht et al., 2024). Exploring comprehensive polyphenol profiles under the premise of including macroscopic components is particularly essential for a deeper understanding of health-promoting effects.

In reality, sorghum is usually heated or boiled before intake, which has a large influence on nutrition and functionality. The presence of water during hydrothermal treatment, including cooking and steaming, usually intensifies the degradation of polyphenols at high temperatures (Osuna-Gallardo et al., 2023; Siah et al., 2014). However, the cell rupture caused by superheated cooking will increase the content and antioxidant capacity of extractable polyphenols (Lafarga et al., 2019), and similar results were obtained in pumpkin seed hydrothermal treatment (Chao & Fan, 2023). The fact that the reciprocal effects between polyphenols and macro-elements are complicated under cooking conditions, adequate research is needed to establish types and specific compositions of polyphenols in microwaved sorghum, and if the potential functional enhancement of microwave-treated sorghum raw results in an improvement in the nutritional value of corresponding food. However, changes in macroscopic properties almost always involve microscopic processes, especially interactions of endogenous components in food. Molecular docking technology, as a computer-aided drug design method, can predict potential ligand-receptors binding based on the substance structure, and reveal the variously interacted ways (hydrogen bond, hydrophobic interaction, ionic bond, etc.). It has been widely used in the fields of screening active peptides derived from foods (Zhuang et al., 2024), targeted drug design, and molecular interaction analysis (Taghizadeh et al., 2024). Moreover, the docked stability can be further verified by molecular dynamics simulation. Molecular dynamics simulation can achieve the closest approximation of the real situation for various complex systems by using different procedures and force fields (Kalin et al., 2025). The parameters commonly used to evaluate the results of dynamical models included root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of rotation (Rog) and number of hydrogen bonds, etc. At present, it has been revealed that polyphenols can bind to zein through hydrogen bonding and hydrophobic interaction, which affects the secondary structure of the protein (Wang et al., 2022). However, the natural food substrates are pretty complex, it is difficult to isolate a monomer for experiments under the cooking circumstance, and the interaction mechanism of proteins and phenols at the molecular level also needs more research.

Therefore, the present study performed the component identification of microwave sorghum and corresponding cooked sorghum, analyzed the differences in composition and contents of free and bound polyphenols, and elucidated their antioxidant capacity. Based on the perspective of microstructure, the effect mechanism of microwave on cereal polyphenols was elaborated with a primary focus on the interaction between polyphenols and macromolecules during the cooking process involving polyphenol transform by simulation reaction, spectral technique, and molecular docking. This study aimed to elucidate the change characteristics of sorghum polyphenols from raw grains to food and to provide a theoretical framework for improving the function of grain.

## 2. Material and methods

### 2.1. Materials and chemical reagents

Sorghum (Hongnuo 16#) was purchased at Luozhuang District, Linyi City, Shandong Province (34°51′N, 118°06′E). The raw seeds were sifted and cleaned, full and plump seeds were selected and stored at 4 °C. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonicacid) (ABTS), 2,2 diphenyl-1-picrylhydrazyl (DPPH), α-amylase (30,000 U/g), folin–ciocalteu reagent were purchased from Yuanye Reagent Co., Ltd. (Shanghai, China). The *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG) and acarbose, were purchased from Maclin Biochemical Technology Co., Ltd. (Shanghai, China). All chemical reagents used were of analytical grade.

### 2.2. Microwave treatment of raw sorghum

The microwave treatment was performed according to the method described by Li et al., 2021 with a slight modification. Raw sorghum (20 g) was placed in a glass dish and microwaved at 350 W for 240 s (MR). During the treatments, the sample plate was rotated at 10 rpm.

### 2.3. Cooking treatment of raw sorghum

Normal raw (NR) and microwaved raw (MR) sorghum (20 g) with 40 mL distilled water were soaked for 50 min (solid:liquid = 1:2). After steaming for 40 min, cooked sorghum was dried to a constant weight with lyophilizer (Scientz-18 N; Xinzhi Biotechnology Co., Ltd., Ningbo City, China), named MRC and NRC.

### 2.4. Extraction and analysis of sorghum polyphenols

The extraction and determination of free polyphenols and flavonoids was performed according to the method described by Kukhtenko et al. (2024) with slight modifications. The results were expressed as mg gallic acid (mg GAE/100 g DW) and rutin (mg RE/100 g DW) equivalent per 100 g of sample. The precipitation was collected for extracting bound polyphenols via alkaline hydrolysis. The detailed group information was as follows: Free phenols of microwaved raw sorghum (FMR); Free phenols of normal raw sorghum (FNR); Bound phenols of microwaved raw sorghum (BMR); Bound phenols of normal raw sorghum (BNR); Free phenols of microwaved cooked sorghum (FMRC); Free phenols of normal cooked sorghum (FNRC); Bound phenols of microwaved cooked sorghum (BMRC); Bound phenols of normal cooked sorghum (BNRC) (Table S1).

The DPPH• and ABTS+• scavenging capacity and total antioxidant capacity (T-AOC) were determined by the test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Moreover, the inhibitory activity of sorghum polyphenol on α-amylase and α-glucosidase was determined according to the Mphahlele, et al.'s method with a little change (Mphahlele et al., 2021).

### 2.5. Metabolomics analysis of polyphenols based on UHPLC-QE-MS/MS

100 μL of the sample was transferred to an EP tube. After the addition of 300 μL of extract solution (methanol, containing isotopically-labeled internal standard mixture), the sample was vortexed for 30 s, sonicated for 10 min in an ice-water bath, and incubated for 1 h at −40 °C to precipitate proteins. Then the sample was centrifuged at 12000 rpm (RCF = 13,800 (×g), $R$ = 8.6 cm) for 15 min at 4 °C. The supernatant was carefully filtered through a 0.22 μm microporous membrane and transferred to a fresh glass vial for analysis. The quality control (QC) sample was prepared by mixing an equal aliquot of the supernatants from all of the samples.

LC-MS/MS analyses were performed using a UHPLC system (Vanquish, Thermo Fisher Scientific) with a UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm) coupled to Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo). The mobile phase consisted of 5 mmol/L ammonium acetate and 5 mmol/L acetic acid in water (A) and acetonitrile (B). The auto-sampler temperature was 4 °C, and the injection volume was 2 μL. Gradient elution program: 0–0.7 min, 99 % A

and 1 % B, the flow rate was 0.35 mL/min; 0.7–9.5 min, 1 % A, 99 % B, the flow rate was 0.35 mL/min; 9.5–11.8 min, 1 % A, 99 % B, the flow rate was 0.5 mL/min; 11.8–14.6 min, 99 % A, 1 % B, the flow rate was 0.5 mL/min; 14.6–15 min 99 % A, 1 % B, the flow rate was 0.35 mL/min. The running time of each sample was 15 min.

The Orbitrap Exploris 120 mass spectrometer was used for its ability to acquire MS/MS spectra on information-dependent acquisition (IDA) mode in the control of the acquisition software (Xcalibur, Thermo). In this mode, the acquisition software continuously evaluates the full scan MS spectrum. The ESI source conditions were set as follows: sheath gas flow rate as 50 Arb, Aux gas flow rate as 15 Arb, capillary temperature 320 °C, full MS resolution as 60,000, MS/MS resolution as 15,000 collision energy as 10/30/60 in NCE mode, spray Voltage as 3.8 kV (positive)/−3.4 kV (negative), respectively. The raw data were converted to the mzXML format and processed with an in-house program, which was developed using R and based on XCMS, for peak detection, extraction, alignment, and integration. Then an in-house MS2 database and HMDB (https://www.hmdb.ca) were applied to metabolites annotation. The cutoff for annotation was set at 0.3.

### 2.6. Fundamental ingredient and starch characteristics of sorghum

Basic components (fat, protein, total fiber, and moisture) of sorghum were determined according to the method stated by Li et al., 2022. Meanwhile, the starch digestion assay was adapted from previous research with some modifications (Englyst et al., 1992). The rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) contents were obtained using the following Eqs:

$$\text{RDS}\ (\%) = \frac{(\text{G20} - \text{FG}) \times 0.9}{\text{TS}} \times 100 \quad (1)$$

$$\text{SDS}\ (\%) = \frac{(\text{G120} - \text{G20}) \times 0.9}{\text{TS}} \times 100 \quad (2)$$

$$\text{RS}\ (\%) = \frac{[\text{TS} - (\text{RDS} + \text{SDS})] \times 0.9}{\text{TS}} \times 100 \quad (3)$$

where FG G20 is the content of free glucose before digestion, G20 is the produced content of glucose at time 20 min; G120 is the produced content of glucose at time 120 min; TS is the total starch content.

### 2.7. Physical characterization of sorghum

#### 2.7.1. Light microscopy and scanning electron microscopy (SEM) of sorghum

Scanning electron microscopy of raw sorghum and cooked sorghum were observed using the method, as described elsewhere (Tamura et al., 2018). Sections (3–5 μm) were stained with toluidine blue staining solution, viewed using a Nikon Eclipse E100 Light microscope and images were captured using a Nikon DS-U3 imaging system. The transverse section micromorphology of raw sorghum (MR, NR) was visualized with an accelerating voltage of 5.0 keV in low vacuum mode using a secondary electron detector at magnifications of 300 ×, 600 ×, and 1000 × .

#### 2.7.2. Particle size distribution and specific surface area analysis

The sample size distribution was characterized by dry methods using Malvern Mastersizer (MS3000, Dandong Better Instrument Co., Ltd., Liaoning, China). The specific surface area of the MR and NR samples (250 mg) was measured using a micromeritics surface area and porosity analyzer (ASAP 2460, Micromeritics Instrument Co., Ltd., Shanghai, China). The $N_2$ was an adsorption gas, and the degassing time was 8 h.

### 2.8. Simulated co-gelatinization reaction of starch and polyphenol

Starch (94 g/100 g extract) and polyphenols of sorghum were extracted respectively. A simulated co-gelatinization process was performed according to the cooking method of sorghum. According to the content of starch and polyphenol in raw sorghum after microwave, the substance proportion of the co-gelatinization system was determined. Specifically, 10 g of sorghum starch and polyphenols that containing 1.6 % (*w*/w) of the total weight were added to 30 mL deionized water, followed by steaming for 40 min for gelatinization. The resulting precipitate was vacuum freeze-dried and ground into powder. The conjugation behavior of polyphenols onto the starch was analyzed via a FT-IR spectrometer (Thermo Nicolet is5, Thermo Fisher Scientific, USA) and X-ray diffractometer (Bruker, Germany).

### 2.9. Molecular docking

The molecular docking methods of 6 types of flavonoids: genistein, (PubChem ID: 5280961), melanoxetin, (PubChem CID: 15560442), rhodioloside (PubChem CID: 159278), daidzein (PubChem CID: 5281708), gallocatechin (PubChem CID: 65084), γ-mangostin (PubChem CID: 5464078) and kafirin (sorghum alcohol-soluble protein) were referred to a method described by Eberhardt et al. (2021). The 3D structure of the small molecule was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)and minimized energy under the MMFF94 force field. The 3D structural models of kafirin were obtained from the UniProt database (https://www.uniprot.org). The molecular docking was performed using the AutoDock Vina tool (versions 1.2.3, Scripps Research, USA). Before the initiation of docking, the receptor protein was treated with PyMol 2.5.5[2] (versions 2.5.5, DeLano Scientific LLC, USA), including the removal of water molecules, salt ions, and small molecules. The docking box is then set up to enclose the entire protein structure. Setting the global search detail to 32 and keeping other parameters at their default settings. The docking conformation with the highest output score was considered to be the connective conformation. Finally, PyMol 2.5.5 docking results were used for visual analysis. The inhibition constant (Ki) was calculated based on the binding energy (Shahraki et al., 2024).

### 2.10. ADMET properties of 6 flavonoids

Flavonoids were assessed for their pharmacokinetics and drug-likeness using the SwissADME server (http://www.swissadme.ch/). The SMILES representation of flavonoids was entered to complete the assessment. The contents of the assessment included solubility, lipophilicity, bioavailability score, blood-brain barrier permeability, skin permeability (Log$K_P$), gastrointestinal absorption, and compliance with the Lipinski, Ghose, Veber, Egan, and Muegge rules (Lotfi et al., 2025).

### 2.11. Molecular dynamics simulation

Based on the ligand-protein complex obtained by docking as the initial structure, the whole atom molecular dynamics simulation was carried out using AMBER 22 software (University of California, California, USA) (Salomon-Ferrer et al., 2013). The ligand and protein topology files were generated utilizing the GAFF2 forcefield and the ff14SB forcefield (Wang et al., 2004), respectively. LEaP module was used in each system to add hydrogen atoms to the system, a truncated octahedral TIP3P solvent box was added at the distance of 10 A of the system, and $Na^+/Cl^-$ was added to the system to balance the system charge. Subsequently, the systems underwent energy optimization with the steepest descent method over 2500 steps and equilibration under a pressure of 1 bar and at a temperature of 368.15 K. A 100 ns NPT (constant-pressure, constant-temperature) simulation was performed under periodic boundary conditions. Structural properties of the molecular dynamic trajectories (interval 10 ps), including root mean square deviation (RMSD), root mean square fluctuation (RMSF), surface accessible solvent area (SASA), radius of gyration (Rog) and Hydrogen bond number, were analyzed using AMBER 22 software. Finally, the

molecular mechanics-generalized born surface area (MM-GBSA) was utilized to calculate the free binding energy (0–100 ns) according to the following equation:

$$\Delta G_{bind} = \Delta G_{complex} - (\Delta G_{receptor} + \Delta G_{ligand}) \quad (4)$$

where $\Delta G_{binding}$ represents the binding free energy, $\Delta G_{complex}$ denotes the total complex energy, $\Delta G_{receptor}$ refers to the protein solution free energy, and ΔG ligand represents the ligand solution free energy. Meanwhile, the free energy of polar solvation, free energy of non-polar solvation, electrostatic energy, and van der Waals energy of protein-ligand complex were calculated by this program.

### 2.12. Statistical analysis

Filtered and standardized data were submitted to R software for multivariate statistical analysis including Principal Component Analysis (PCA) and hierarchical cluster analysis (HCA). The variable importance in projection (VIP) ≥ 1, *P* value <0.05 was set for screening differentially accumulated metabolites. SIMCA software (V16.0.2, Sartorius Stedim Data Analytics AB, Umea, Sweden) was used for LOG conversion and UV formatting of the data, and OPLS-DA modeling analysis of the first principal component was carried out. Data were analyzed by a one-way analysis of variance (ANOVA) using the statistical package SPSS 22.0 (IBMCorp., Armonk, NY, USA), where differences were considered significant at $P < 0.05$. All results were expressed as the mean ± standard deviation (SD).

## 3. Results and discussion

### 3.1. Content and antioxidant activity of sorghum polyphenols and flavonoids

For raw sorghum (FMR, FNR, BMR, and BNR), the contents of polyphenol were significantly increased after microwave treatment ($P < 0.05$) (Fig. 1), which may be associated with the release of phenolic

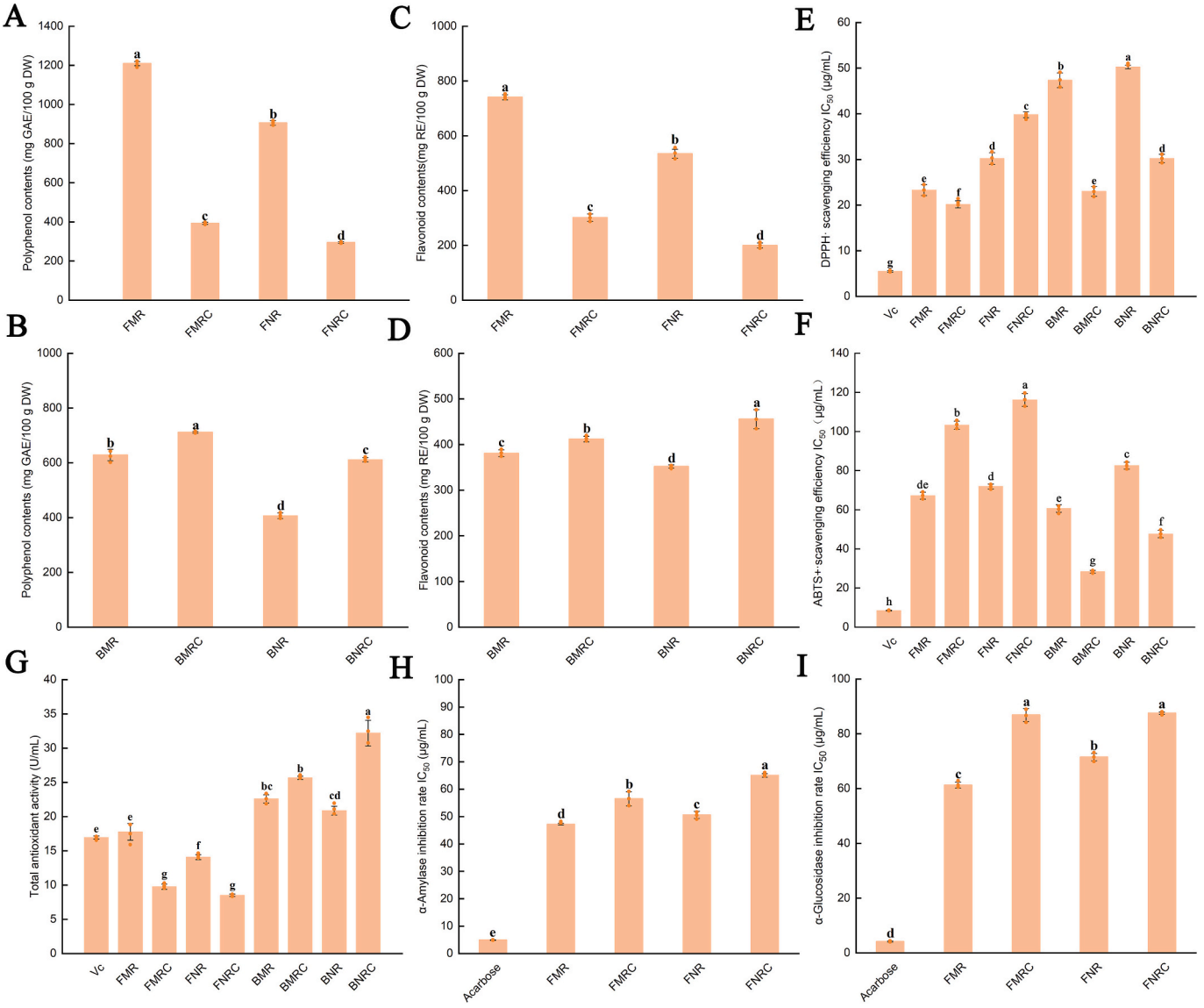


**Fig. 1.** The content of free polyphenols in raw sorghum and cooed sorghum (A); The content of bound polyphenols in raw sorghum and cooked sorghum (B); The contents of free flavonoids in raw sorghum and cooked sorghum (C); The contents of bound flavonoids in raw sorghum and cooked sorghum (D). The scavenging rate of DPPH• (E) and ABTS+• (F) was expressed by half scavenging concentration ($IC_{50}$). Total antioxidant capacity of sorghum polyphenols(G); α-amylase inhibition rate ($IC_{50}$) of sorghum polyphenols (H); α-glucosidase inhibition rate ($IC_{50}$) of sorghum polyphenols (I). MR: microwaved raw sorghum; MRC: microwaved raw sorghum was cooked; NR: normal sorghum; NRC: normal sorghum was cooked; F: free polyphenols; B: bound polyphenols.

compounds from sorghum fiber as an effect of the microwave energy. Similar results were observed in microwave-treated black quinoa (Zhang et al., 2022). After cooking, the free polyphenol and flavonoid contents were significantly reduced in comparison with raw sorghum ($P < 0.05$) (Fig. 1A, C). Unexpectedly, bound polyphenols and flavonoids contents were significantly higher in cooked sorghum, which were 711.12 mg GAE/100 g, 611.16 mg GAE/100 g, 411.81 mg RE/100 g, 455.53 mg RE/100 g for BMRC and BNRC (Fig. 1B, D), respectively. Although heat treatment could destroy the heat-sensitive polyphenols, the free polyphenols and flavones probably were bound to the large molecules such as starch and protein during cooking (Chi et al., 2017), which resulted in lower levels of compounds that could be extracted.

The DPPH• scavenging activity of free and bound polyphenols of sorghum was significantly ($P < 0.05$) affected by microwave (Fig. 1E). Significantly lower $IC_{50}$ value (30.18 μg/mL and 50.26 μg/mL, $P < 0.05$) represented an increase in antioxidant capacity of in FMR and BMR. Meanwhile, BMR also represented higher ABTS+• scavenging activity (Fig. 1F), compared with BNR ($P < 0.05$). The difference between FMR and FNR was not significant ($P > 0.05$). The antioxidant activity increased with the rise of polyphenol contents. The research of chickpeas powder and sorghum has presented similar results (Almaiman et al., 2021; Praveen et al., 2017), suggesting that microwave can become an efficient technique to increase the antioxidant capacity of sorghum. Following microwaves, the T-AOC in FMR and BMR were 17.76 U/mL and 22.57 U/mL, respectively, which were significantly higher than those of in FNR and BNR (Fig. 1G). This result was consistent with the free radical scavenging rate. Therefore, the increase of antioxidant activity was attributed to the enhancement of polyphenols

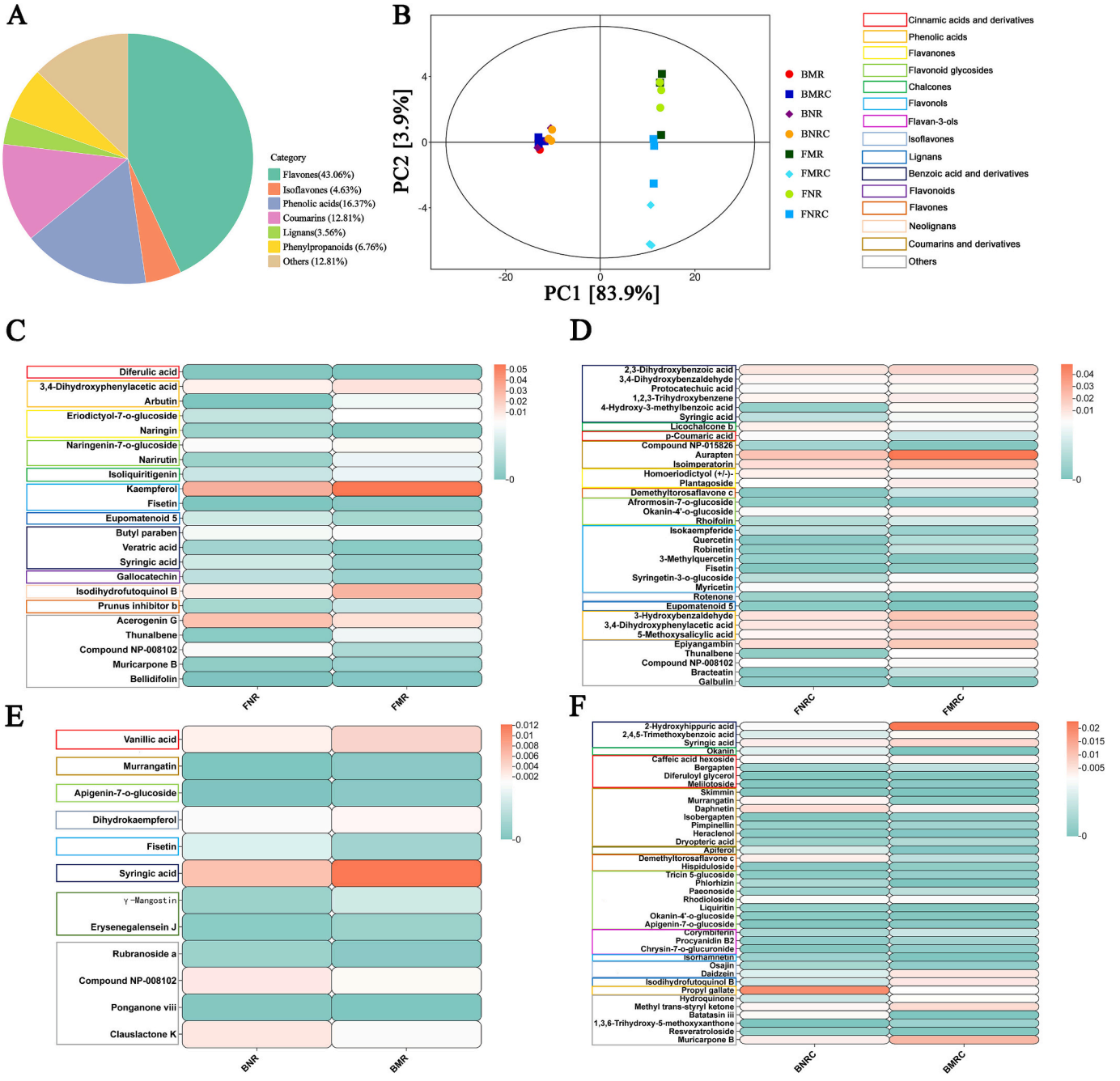


**Fig. 2.** Classification of the 280 metabolites of sorghum samples (A); principal component analysis (PCA) of metabolic profiles in eight samples (B); free polyphenols of raw sorghum (C); free polyphenols of cooked sorghum (D); bound polyphenols in raw sorghum (E); bound polyphenols in cooked sorghum (F).

during microwave.

Moreover, the ABTS+• scavenging ability of corresponding cooked sorghum polyphenols was significantly affected by microwave (Fig. 1F). Especially the bound polyphenols, the $IC_{50}$ value of BMRC (28.32 μg/mL) and BNRC (47.54 μg/mL) were significantly decreased ($P < 0.05$). Similar results were obtained for the determination of DPPH• scavenging activity (Fig. 1E). However, the DPPH• and ABTS+• scavenging activity in FMRC and FNRC were remarkably lower than those of in FMR and FNR ($P < 0.05$). The free polyphenols might be gradually converted to the bound state during the cooking. The results of T-AOC further illustrated that phenolic compounds have been altered by microwaving and cooking. Moreover, the obtained results revealed that the inhibitory effect in FMRC on α-amylase and α-glucosidase was significantly higher than that of in FNRC ($P < 0.05$), although both were lower than that in raw sorghums (FMR and FNR) (Fig. 1H, I).

### 3.2. Widely targeted metabolomics analysis

#### 3.2.1. Metabolic profiling of sorghum raw and cooked sorghums

Under microwave and cooking, the content and antioxidant changes were closely related to the polyphenol composition. In positive and negative patterns, a total of 280 phenolic compounds were annotated among all the analyzed samples (Table S2), pointing out a broad diversity that included flavones (121), isoflavones (13), phenolic acids (46), coumarins (36), lignans (10) and other polyphenols, such as phenylpropanoids and xanthones, etc. Phenolic acids (16.37 %) and flavonoids (43.06 %) accounted for a substantial proportion of all samples (Fig. 2A).

#### 3.2.2. Multivariate statistical analysis

For obtaining an overview of sample discrepancy and similarity, the PCA of an unsupervised model was used, while the highly concentrated features illustrate better repeatability for each group (Fig. 2B). The PCA displayed the degree of variance in the overall metabolic differences between each group. The cumulative contribution rate of two principal components (PC1 83.9 % and PC2 3.9 %) reached 87.8 % in the PCA score plot (Fig. 2B). In addition, all sample points were within a 95 % confidence interval. The three biological repeats of BMR, BNR, BMRC, and BNRC were pretty close, which revealed the bound polyphenols were similar in metabolite composition regardless of cooked. Due to the role of covalent and non-covalent, the stability of polyphenols was improved to avoid the degradation of polyphenols caused by light and heat (Sun et al., 2017).

Effects of microwave and cooking on sorghum were subsequently analyzed on the base of PCA results. A supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed to identify the metabolites responsible for the observed differences (Supplementary Fig. S1 A-D, Supplementary Fig. S1 I-L). The 200 replacement tests were executed in pairwise comparison groups. Consequently, all OPLS-DA models exhibited robust values for the goodness-of-fit ($R^2Y$) and prediction ability ($Q^2$) parameters, which implies that the predicted results confer reliable modeling of the metabolic differences of all the pairwise comparisons. OPLS-DA score plots also indicated that the microwave had significant effects on both free and bound polyphenol components of raw sorghum. It is important to note that the difference between MRC and NRC was more significant compared with raw sorghum (MR and NR). This means that the effect of microwaves on polyphenols after cooking was further amplified. As for the contrast between the free and the bound polyphenols, regardless of cooking or not, the difference was quite significant.

#### 3.2.3. Characteristic metabolites in microwaved raw sorghum

Based on VIP value>1 and global differential metabolites (Supplementary Fig. S2), polyphenol metabolites were further screened with PC value>1.5 and < 0.5 as the standard, which was the characteristic differential metabolites of raw sorghum by microwave treatment (Fig. 2). For the free components, flavonoids (flavanones, flavonoid glycosides, flavonols, and flavones, etc.) were the main components of sorghum polyphenols, which were significantly affected by microwave. Spearman correlation analysis also showed that flavonoids (arbutin, eriodictyol-7-o-glucoside, narirutin) have made outstanding contributions to the improvement of antioxidant ability (Supplementary Fig. S3). Moreover, benzoic acid /cinnamic acids (derivatives) such as veratric acid, syringic acid, and diferulic acid, showed a significant downregulation. Dielectric heating of microwave might lead to the loss of some phenolic acids. For bound polyphenols, apigenin-7-o-glucoside, γ-mangostin and dihydrokaempferol, etc., flavonoids were significantly upregulated (Fig. 2E). According to the FC value of fisetin (0.38 for BMR, 3.24 for FMR), microwave energy possibly promotes the conversion of bound polyphenols into free states. Theoretically, electromagnetic waves produced by microwaves cause a certain amount of pressure on the tissues and cell walls of the plant or food substrate, causing structural changes, that further promote increased mass transfer of solutes due to the rupture of cells (Herrman et al., 2020). Moreover, dihydroquercetin and dihydrokaempferol were significantly upregulated in the BMR compared with BNR. Previous studies have also shown that high-temperature drying can promote the increase of dihydroflavonoid components (Wang et al., 2024). During the heat treatment period, due to the enzyme being deactivated, the biosynthetic pathway is also impeded, and the conversion mainly occurs via a thermochemical reaction. Therefore, the up-regulation of benzoic acid derivatives may come from the automatic degradation of flavonoid-o-glycoside under microwave conditions (Lin et al., 2023). However, it was also observed that flavonoids were mainly up-regulated in FMR and BMR, the newly synthesized benzoic acid derivative may only be produced in small or trace amounts. Hence, the destruction and lysis of sorghum cells by microwave may be the main reason for the changes of polyphenol components and antioxidant properties.

#### 3.2.4. Characteristic metabolites in cooked sorghum

Compared to microwave treatment, the role of hydrothermal treatment was highlighted. The number of benzoic acid (derivates), flavonols, and flavones (e.g. quercetin, robinetin, and demethyltorosaflavone C) with differences were increased (Fig. 2D). Hydrothermal treatment may loosen the plant matrix, and induce the migration and transformation of phenolic acids by altering their molecular structure, which further influences the changes in phenolic acid content (Chao & Fan, 2023; Huma et al., 2019), and the pretreatment raw sorghum by microwave power promoted the further release of phenolic acid. However, phenolic acids did not effectively improve the antioxidant capacity of free polyphenols in cooked sorghum, flavonols (syringetin-3-o-glucoside and myricetin), flavanones and coumarins (derivatives) significantly improved antioxidant capacity (Supplementary Fig. S3B) ($P < 0.05$). Therefore, the content of some polyphenol components does not completely correspond to their functional strength. Similar to the free phenolic acids, the bound phenolic acids (2-hydroxyhippuric acid, 2,4,5-trimethoxybenzoic acid, and syringic acid) were significantly upregulated in the microwave sorghum (Fig. 2F) ($P < 0.05$). Moreover, more differential flavonoid glycosides (such as okanin-4′-o-glucoside, apigenin-7-o-glucoside, and rhodioloside) have appeared in BMRC and BNRC. However, these components did not cause significant differences between the BMR and BNR, which indicated that the influence of cooking on sorghum polyphenols was more obvious than that of microwaves.

#### 3.2.5. Behavioral analysis of characteristic metabolites

Based on the contribution of sorghum polyphenols to antioxidant capacity from raw material to food, flavonoids with greater variability were screened, and both the free and the bound discrepancies were considered (Supplementary Fig. S2). Noteworthy, upregulated flavonoids including genistein, melanoxetin, rhodioloside, daidzein, gallocatechin, and γ-mangostin in BMRC vs FMRC (Supplementary Fig. S2J)

comparative group also demonstrated upregulated trend in BMRC vs BNRC. However, the levels of genistein, melanoxetin, and rhodioloside were downregulated in the FMRC vs FNRC, which testified that the cooking could promote the flow of free polyphenols to the bound state. The effectiveness of the microwave was highlighted when the differentiation between the free and the bound flavonoids was taken into account. Bound genistein, melanoxetin, rhodioloside, gallocatechin, and daidzein were drastically exceeded 184.90-fold, 11.62-fold, 1002.76-fold, 325.31-fold and 728.72-fold in comparison with the FMR, respectively. After cooking, difference multiples of these flavonoids were enlarged by 2835.48-fold, 1465.04-fold, 1086.86-fold, 364.39-fold, and 779.90-fold in BMRC vs FMRC. Nevertheless, the normal sorghum, from raw to cooking, was present an opposite trend. Therefore, these six flavonoids can represent the overall change of sorghum polyphenols (Fig. 3).

Upregulated flavonoids via microwave might combine with

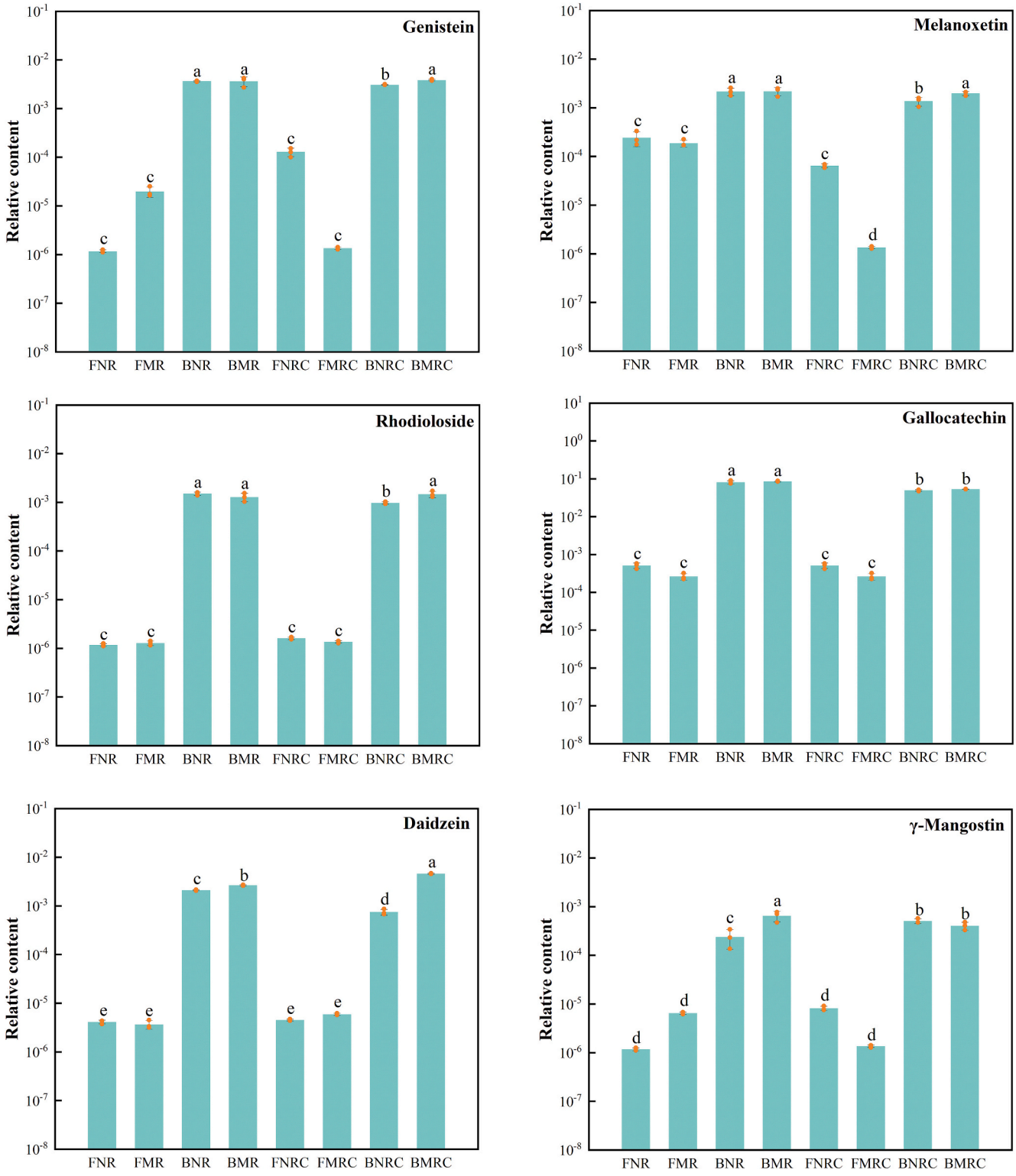


**Fig. 3.** Key flavonoids screened based on differences between free and bound polyphenols.

macromolecules such as starch or protein during cooking. However, macronutrients such as starch and protein were not significantly changed compared with NRC (Table 1). If the reduction in RDS content was attributed to the inhibition of digestive enzymes by polyphenols, the increase of SDS and RS indirectly indicated that there may be a complex system that affects the digestion of starch in the MRC, which affected the overall composition of cooked sorghum polyphenols. Accordingly, the behavior of polyphenols from the perspective of intact sorghum was closely related to the microstructure and major elements. Therefore, the polyphenol transport mechanism was further analyzed.

### 3.3. Physical characteristics of microwaved raw sorghum

Compared to NR, the cross-section of the microwaved sorghum became rough, and a large number of micropores appeared, with cracks, the cross-section became rough (Fig. 4A). Usually, thermal treatment may induce starch gelatinization and cell wall breakage (Wan et al., 2022). The normal raw sorghum cross-section was dense, and the starch particles and cell walls were intact (Fig. 4B). The movement of ions and dipoles causes sample heating in a shortened time and enhances pressure build-up in the plant cells, which allows cellular constituent releases bound bioactive compounds during microwave treatment (Feki et al., 2021). Starch-based grains treated at high temperatures will cause liquid water “flash evaporation”, and the instantaneous release of cooking will lead to raw expansion and the formation of porous structures (Tushar & Datta, 2016). Optical microstructure displayed that the internal cell damage was more severe with some ruptured cells evident at the particle in the microwave sorghum (Fig. 4C). Moreover, cracks appeared in the raw cortex after microwave treatment. Normal sorghum grains were predominantly comprised of intact starch-rich endosperm tissues with a denser structure (Fig. 4D).

Furthermore, the particle size of the microwave-treated raw sorghum was increased slightly after passing through the 150 μm micron screen (Fig. 4E). Generally speaking, B-type starch (<10 μm) is more susceptible to damage than A-type starch (<10 μm) during microwave, and B-type starch is easily converted to A-type after microwave treatment. Moreover, microwave treatment could cause heat energy to be gathered in the interior of grains, which led to the expansion of amylopectin and hydrogen bond breaking, forming certain short and medium amylose (Trung et al., 2017). Polyphenols can bind to the amylose molecules through hydrogen bonds, promoting the aggregation of amylose molecules, resulting in an increase in the molecular hydrodynamic radius (Chai et al., 2013), and revealing the increase of specific surface area (Fig. 4F). Generally speaking, B-type starch is more susceptible to damage than A-type starch after microwave treatment, and B-type starch is easier to convert to A-type after microwave treatment. The cohesion of polyphenols and starch under microwave treatment influenced the content of bound polyphenols (Jiang et al., 2023).

### 3.4. Simulated reaction to verify changes in polyphenol during cooking

Significant starch dissolution was observed in cooking sorghum (Fig. 5D), and the solubility of starch in the MRC was more obvious. Therefore, under the same conditions as the cooking treatments, co-gelatinization simulation reactions were carried out using sorghum polyphenol extract and sorghum starch. Compared with starch-polyphenol (SP), microwaved starch-polyphenol (MSP) can save more polyphenols after gelatinization ($P < 0.05$) (Fig. 5C). High temperature can promote the mobility of starch chains and orientated rearrangement of starch, thus enhancing intermolecular interaction (Luo et al., 2024). Further, FT-IR results showed typical absorption information of starch samples (Fig. 5A). Peaks at 930 $cm^{-1}$ were the absorption peak of the 1,4-glucoside bond of starch, and at 1155 and 1081 $cm^{-1}$ were caused by C-O-C, respectively. The peak around 2929 $cm^{-1}$ was due to the stretching vibration of C—H, and the peak around 1641 $cm^{-1}$ was a feature of C-O-O stretching vibration in a carbohydrate group (Li, Shen, et al., 2023). Meanwhile, no new peak appeared in the starch-polyphenol co-gelatinization system, suggesting that there was no chemical bond modification or covalent bond formation. Compared with gelatinized starch (S), the absorption peak intensity of SP and MSP at 3378 $cm^{-1}$ was weakened, indicating that the number of hydrogen bonds was reduced, which suggested that polyphenols and starch could interact through hydrogen bonding. It was found that the peak strength of starch ferulate ester near 3400 $cm^{-1}$ was also reduced (Mathew & Abraham, 2007), mainly due to the reduced number of hydrogen bonds. Moreover, the absorption peak of SP at 930 $cm^{-1}$ was higher than that of MSP, indicating that the number of 1,4-glucoside bonds in MSP was less than that of in SP, which further demonstrated that sorghum polyphenols hinder the aggregation of amylose molecules through hydrogen bonds. In addition, adding polyphenols caused this hydroxyl group peak to shift towards lower wavenumbers, suggesting an increased density and strength of hydrogen bonds between starch and polyphenols. Moreover, the results of XRD revealed that SP and MSP groups displayed a prominent characteristic peak around 20° (Fig. 5B), indicating a *V*-type crystal structure (Gao et al., 2024), which confirmed the presence of starch-polyphenol complexes under co-gelatinization circumstance. The embedding of polyphenol micromolecules within the helical cavity of starch could result in the formation of similar complexes, as evidenced by the observed V-type diffraction peaks (Liu et al., 2019). Meanwhile, polyphenols could directly bind to amylose or amylopectin chains via electrostatic interactions, hydrogen bonds, or CH-π bonds to generate non-inclusion complexes.

Moreover, the optical microstructure of cooked sorghum showed that starch gelatinization and dissolution were more obvious in MRC (Fig. 5D), suggesting that the complex degree of starch-polyphenols was higher. Overall, raw sorghum starch interacts with polyphenols during gelatinization, which influences the composition and content of bound polyphenols in cooked sorghum. The simulation reaction of co-gelatinization confirmed the result. However, the biggest difference between the simulated reactions and the real cooking process was that the simulated reactions were monomer reactions, while the reaction in the cooking process also involved the chemical transformations and spatial distributions of metabolites. Account flavonoids are not only distributed in the vacuoles of plants but also in various subcells (Bozzo & Unterlander, 2021), and intact cell and grain structure can effectively avoid the loss of polyphenols in the actual cooking of sorghum.

### 3.5. Molecular docking of 6 flavonoids with sorghum protein

In addition to starch, sorghum protein may also interact with

**Table 1**
The basic components of sorghum.

| Samples | RDS (%) | SDS (%) | RS (%) | Starch (%) | Protein (%) | Fat (%) | Moisture (%) | TDF (%) |
|---|---|---|---|---|---|---|---|---|
| MR | $22.82 \pm 0.14^{b}$ | $15.19 \pm 0.40^{c}$ | $62.14 \pm 0.73^{b}$ | $75.42 \pm 0.82^{a}$ | $8.54 \pm 0.49^{a}$ | $3.05 \pm 0.35^{ab}$ | $9.77 \pm 0.75^{d}$ | $7.33 \pm 2.53^{ab}$ |
| NR | $21.75 \pm 0.38^{b}$ | $14.63 \pm 0.07^{d}$ | $63.53 \pm 0.66^{a}$ | $76.25 \pm 1.25^{a}$ | $8.49 \pm 0.33^{a}$ | $3.18 \pm 0.06^{a}$ | $11.54 \pm 1.13^{c}$ | $7.67 \pm 1.75^{a}$ |
| MRC | $43.54 \pm 0.75^{b}$ | $27.41 \pm 0.20^{a}$ | $28.19 \pm 0.05^{c}$ | $75.84 \pm 0.39^{a}$ | $8.08 \pm 0.86^{a}$ | $2.52 \pm 0.17^{c}$ | $61.28 \pm 0.69^{a}$ | $7.04 \pm 1.94^{b}$ |
| NRC | $49.47 \pm 0.93^{a}$ | $24.86 \pm 0.93^{b}$ | $23.95 \pm 0.42^{d}$ | $75.67 \pm 1.53^{a}$ | $8.37 \pm 1.20^{a}$ | $2.67 \pm 0.39^{bc}$ | $60.63 \pm 1.84^{b}$ | $7.83 \pm 1.38^{a}$ |

Note: All values are shown as mean ± SD. Distinct letters (a-d) within the identical column signify significant variations among the four samples, as established by the Duncan test ($P < 0.05$).

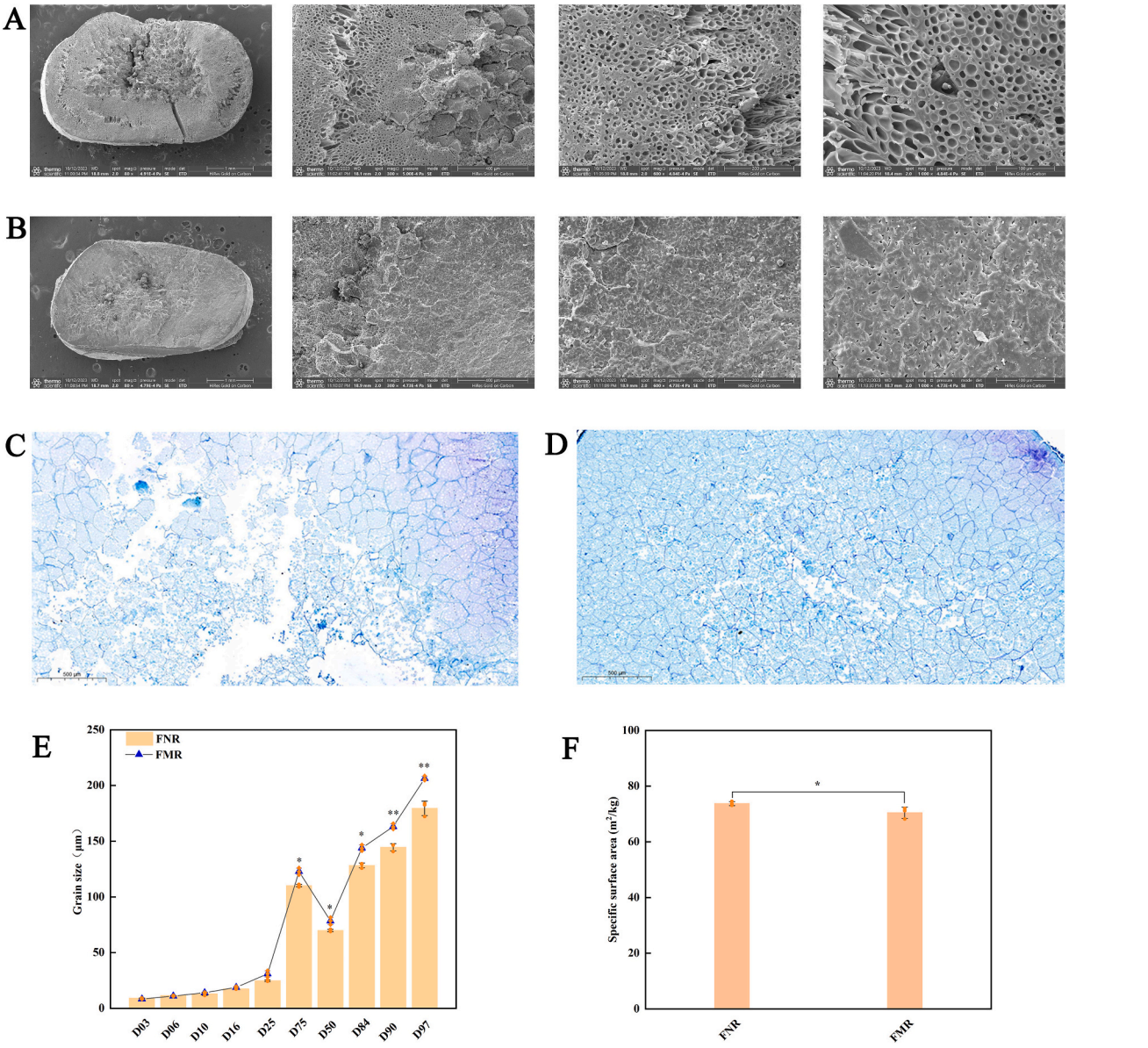


**Fig. 4.** Microcosmic characteristics of raw sorghum under microwave influence. Scanning electron microscopy of cross-sections of microwave raw sorghum (A); normal raw sorghum (B); optical microstructure of microwave raw sorghum (C); optical microstructure of normal raw sorghum (D); particle size distribution (E); specific surface area (F).

polyphenol molecules. Therefore, molecular docking could be used to verify that the interaction of sorghum protein and polyphenols during cooking. A lower binding energy (BEs) indicates a stronger affinity between the ligand and the receptor and a more stable binding structure (Wang et al., 2023). Six flavonoids could effectively bind to the amino acid residue of kafirin, mainly through hydrogen bonds and hydrophobic interactions (Fig. 6), and their BEs ranged from −7.31 to −8.85 kcal/mo1 (Table 2). Notably, the ligand, γ-mangostin formed hydrogen bonds with GLN-101, GLN-75, GLN-98, and GLN-96, and had the lowest binding efficiency (−8.85 kcal/mol) with kafirin, accompanied by an inhibition constant of 0.32 μM. In contrast, the ligands rhodioloside demonstrated a binding energy of −7.28 kcal/mol when bound to the kafirin, along with an inhibition constant of 4.55 μM (Table 2). The active sites of kafirin were predicted via the Site Finder module in MOE (Yamaguchi et al., 2012), mainly comprised follow residues: GLN96, GLN98, GLN101, GLN102, PHE103, MET190, ASN192, TYR196, GLN102, PHE103, GLN75, SER77, LEU73, GLN98. Molecular docking analysis revealed that all ligands with a binding energy below −7.0 kcal/mol are effectively bound to the active site of the kafirin (Table 2). This verified the conclusion that flavonoids can be bound to the sorghum protein during cooking treatment, which further affected the composition of polyphenols. Hydrophobic interactions and hydrogen bonds were important non-covalent interactions between polyphenols and proteins (Zhao et al., 2022), which can prompt proteins to bind more tightly to small molecules. Moreover, γ-mangostin had hydrophobic interactions with LEU-73, TYR-196, PHE-103, and MET-190 that provided strong van der Waals forces for the complex. The strong hydrophobicity and self-assembly capability of kafirin resulted in the polyphenols being easily bound to kafirin in the hydrothermal environment through hydrogen bonding and hydrophobic interactions (Xiao et al., 2015).

In the subsequent phase, the pharmacokinetics and drug-likeness properties of all selected ligands were assessed. This analysis provided valuable insights into the physicochemical properties and in vivo behavior of these compounds. The ADMET results revealed that out of the 6 flavonoids exhibited high gastrointestinal absorption potential. Moreover, all of these ligands were capable of passing the blood-brain

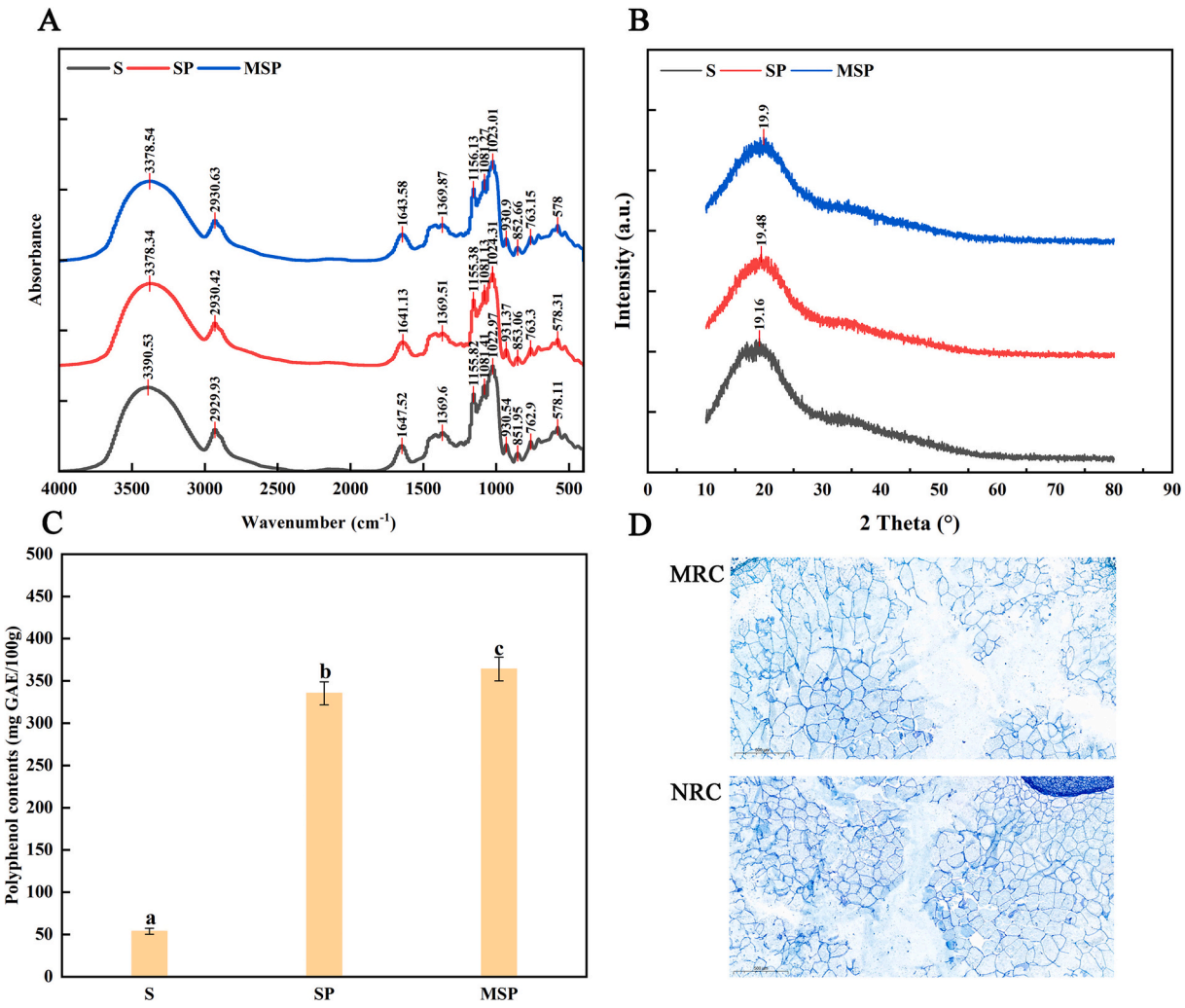


**Fig. 5.** Characterization of starch-polyphenol co-gelatinization results. FT-IR spectra of starches and starch-polyphenol complexes (A); X-ray diffractogram of starches and starch-polyphenol complexes (B); polyphenol contents in the co-gelatinized complexes (C); Optical microstructure of cooking sorghum (D). S: starch; SP: starch-polyphenol; MSP: microwaved starch-polyphenol.

barrier (Table 3). Additionally, all ligands complied with the rules of drug-likeness, except for the Muegge rule, with a bioavailability score of 0.55. Compounds with molecular weight ≤ 500 Da, logP ≤5, hydrogen bond donor ≤5, and hydrogen bond acceptor ≤10 meet Lipinski's rule and are more likely to have favorable oral bioavailability. The results showed that the 6 flavonoids all conform to the above conditions. Additionally, Veber's rule revealed that compounds should possess no more than 10 rotatable bonds and a polar surface area (PSA) of less than 140 $Å^2$ to have a higher likelihood of good oral absorption and permeability (Yadav et al., 2021). Finally, Egan's rule displayed that the molecule should meet the following criteria: molecular weight ranging from 200 to 600 Da, LogP value between −0.4 and 5.0, no more than six hydrogen bond donors, no more than five hydrogen bond acceptors, and no more than 12 hydrogen bond acceptors, for favorable oral absorption. Based on the above findings, all flavonoids adhered to the guidelines set by Veber's and Egan's rules, which indicated that the natural flavonoids in sorghum still had good gastrointestinal absorption and biological activity after cooking.

### 3.6. Molecular dynamics analysis and free binding energy

To evaluate the stability of kafirin-flavonoids, the molecular dynamics simulation of γ-mangostin and kafirin was carried out (Fig. 7). The results indicated that no large root-mean-square deviation (RMSD) occurred during the protein simulation, which meant that the protein structure did not disintegrate, providing the basis for the binding of small molecules (Fig. 7A). RMSD value of γ-mangostin reached stable fluctuation in the early stage of simulation (fluctuation range < 2 Å), which showed that γ-mangostin was pretty closely bound to the kafirin and had good stability.

To track these changes over time, an analysis of root-mean-square fluctuation (RMSF) was conducted (Fig. 7B). In the kafirin-γ-mangostin complex, notable peaks with heightened fluctuations were observed in the regions of 25–30 and 260–265. Especially, these regions revealed potentially leading to partial structural instability, but were not binding sites. However, at some specific residues, such as residues numbering about 350, the fluctuation of the kafirin-γ-mangostin was significantly higher than that of the individual kafirin, which may indicate that these sites have undergone a large conformational adjustment after binding the ligand. Except for the end region and the low mass region, the fluctuation range of most amino acid residues was less than 3.0 Å, indicating that the flexibility of the protein was within a reasonable range during the whole simulation process. In general, after combining γ-mangostin, the overall flexibility of kafirin showed a certain change, which was reflected in the reduction of volatility in specific regions. The analysis of Rog, which serves as an indicator of protein compactness and stability (Kianipour et al., 2022). After binding, the Rog value of kafirin was consistently between 35 Å and 39 Å,

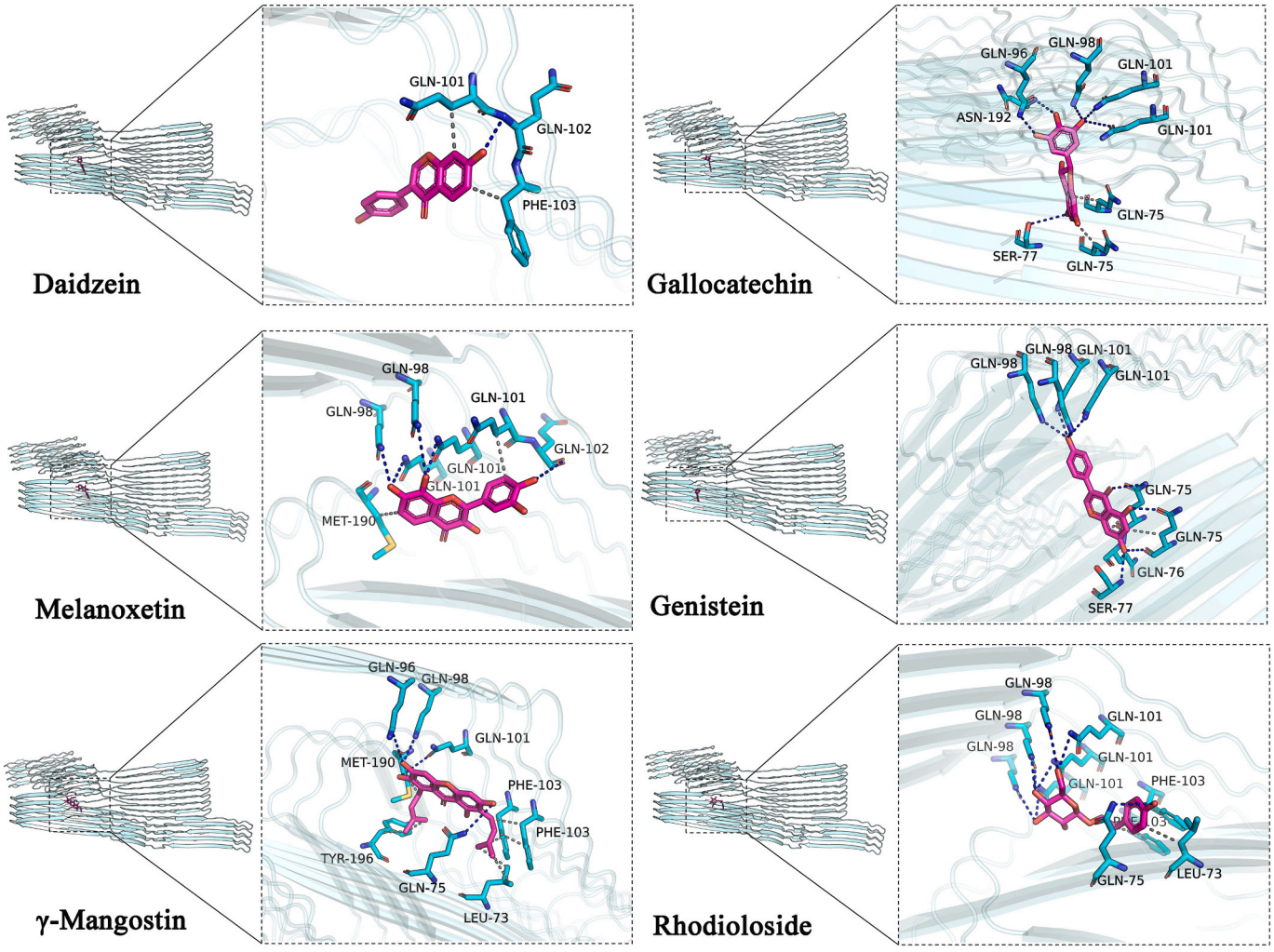


**Fig. 6.** Molecular docking models of flavonoids and kafirin.

**Table 2**
The interactions between kafirin and six flavonoids.

| Receptor | Ligand | Binding energy (kcal/ mol) | Inhibition constant (μM) | Interacted residues with ligand |
|---|---|---|---|---|
| Kafirin | Genistein | −7.370 | 3.906 | GLN-101, SER-77, GLN-98, GLN-76, GLN-75, GLN-75 |
| | Melanoxetin | −7.667 | 2.365 | GLN-101, GLN-98, GLN-102, GLN-101, MET-190 |
| | Rhodioloside | −7.280 | 4.548 | GLN-101, GLN-75, GLN-98, LEU-73, PHE-103 |
| | Daidzein | −7.405 | 3.682 | GLN-102, GLN-101, PHE-103 |
| | Gallocatechin | −7.307 | 4.345 | GLN-96, GLN-101, SER-77, GLN-98, ASN-192, GLN-75 |
| | γ-Mangostin | −8.851 | 0.320 | GLN-101, GLN-75, GLN-98, GLN-96, LEU-73, TYR-196, PHE-103, MET-190 |

Note: The interacted residues through H-bond formation were indicated in bold.

indicating that the overall structure of the protein did not expand or collapse significantly, but revealed slight dynamic fluctuations (Fig. 7C). This result suggested that γ-mangostin binding had a limited effect on the overall compactness of kafirin, possibly maintaining the original conformational stability of the protein while providing a dynamic basis for structural adjustment in local regions.

The increase of SASA value indicated that the protein had a more obvious conformational adjustment and exposed more surface areas at the beginning of the simulation (Fig. 7D). Subsequently, the SASA value gradually leveled off and fluctuated around 48,000 Å$^2$ to 52,000 Å$^2$, manifested that the protein reached a relatively stable conformational state after binding the γ-mangostin. This fluctuation reflects the slight dynamic changes that may exist in the protein after binding the ligand, while maintaining the overall structural stability. Moreover, based on the trajectory of molecular dynamics simulation, the binding energy of the whole duration was calculated by the method of MM-GBSA (Table S3). The BE value of kafirin-γ-mangostin was −33.05 ± 2.53 kcal/mol, manifesting the excellent combination ability. Through residue decomposition, the main contributing forces were van der Waals force (−39.18 kcal/mol), electrostatic attraction (−248.48 kcal/mol), and non-polar solvation free energy (−5.10 kcal/mol). These interactions will provide higher thermal stability and lower thermal decomposition for flavonoids (Li, Wang, et al., 2023). Further, hydrogen bonding is one of the strongest non-covalent interactions, so we monitored the number of hydrogen bonds between ligands and proteins during 100 ns molecular dynamics simulations (Fig. 7E). The number of hydrogen bonds formed by γ-mangostin and kafirin during the simulation ranged from 0 to 7, mainly focusing on 1–2, indicating that hydrogen bonds contribute to the stable binding of small molecules and proteins.

**Table 3**
Evaluating pharmacokinetics and drug-likeness properties of potentially effective ligands using the SwissADME server.

| Compound | Solubility* | Lipophilicity# | Pharmacokinetics | | | Drug-likeness | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | GI absorption | BBB permeant | Log$K_P$ | Lipinski | Ghose | Veber | Egan | Muegge | Bioavailable score |
| Genistein | M | 2.04 | High | No | −6.05 | Yes | Yes | Yes | Yes | Yes | 0.55 |
| Melanoxetin | S | 1.19 | High | No | −7.00 | Yes | Yes | Yes | Yes | Yes | 0.55 |
| Rhodioloside | S | −0.59 | High | No | −8.88 | Yes | No | Yes | Yes | Yes | 0.55 |
| Daidzein | M | 2.24 | High | Yes | −6.1 | Yes | Yes | Yes | Yes | Yes | 0.55 |
| Gallocatechin | S | 0.52 | High | No | −8.17 | Yes | Yes | Yes | Yes | No | 0.55 |
| γ-Mangostin | M | 4.25 | High | No | −4.49 | Yes | Yes | Yes | Yes | No | 0.55 |

Note: GI: gastrointestinal; BBB: Blood-brain barrier; Log$K_P$: indicates skin permeation of compounds (cm/s). *S: Soluble; M: Moderately soluble. # Lipophilicity was represented according to the average of all five Log P o/w predictions.
Note: $\Delta E_{vdw}$: van der Waals energy; $\Delta E_{elec}$: electrostatic energy; $\Delta G_{GB}$: electrostatic contribution to solvation; $\Delta G_{SA}$: non-polar contribution to solvation; $\Delta G_{bind}$: binding free energy.

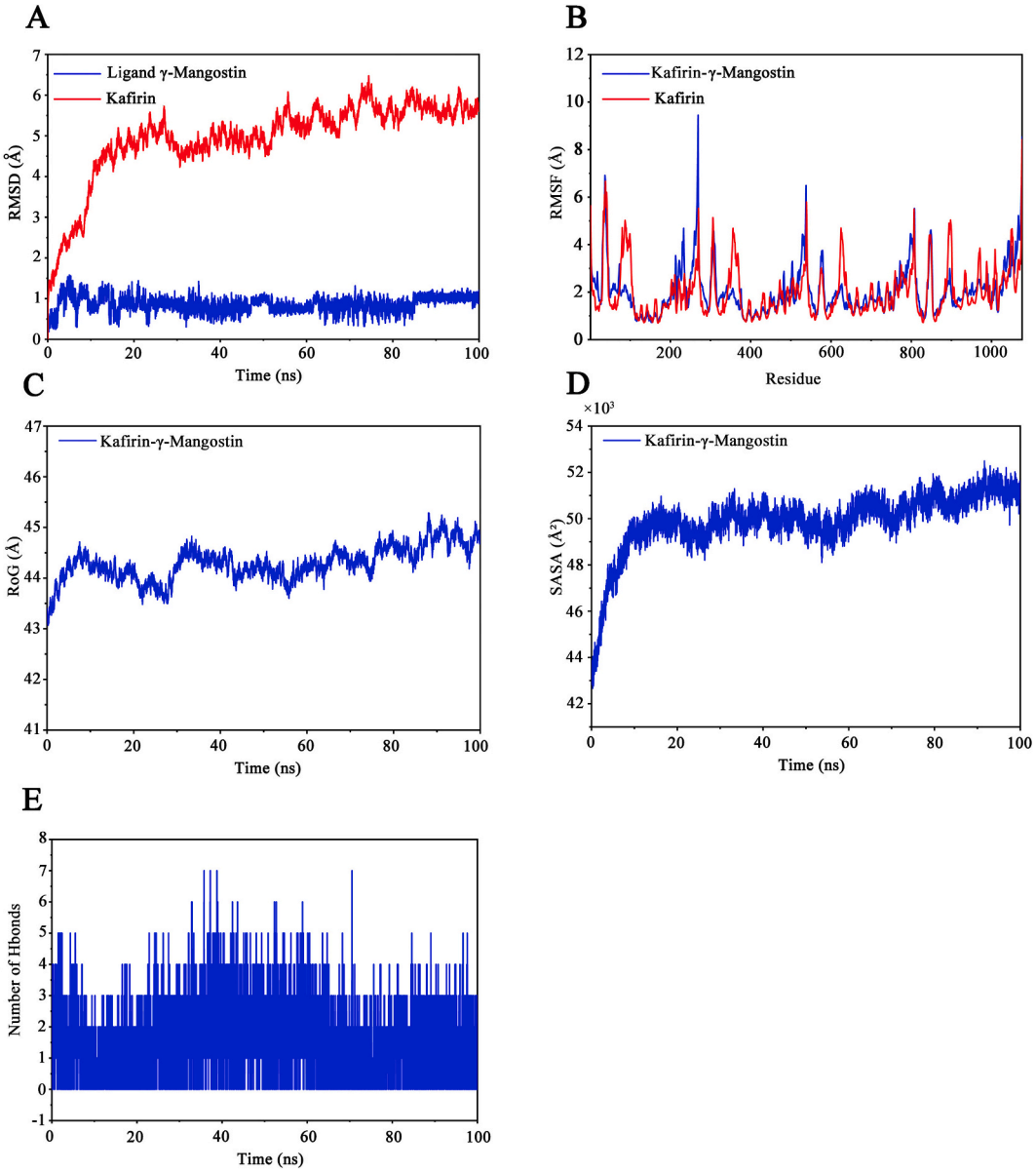


**Fig. 7.** Molecular dynamics simulation of kafirin and γ-mangostin based on the RMSD (A), RMSF (B), Rog (C), SASA (D), and hydrogen bond number (E).

In accordance with the above results, the transport mechanism of sorghum polyphenols can be divided into three categories (Fig. 8): (1) Microwave energy destroyed the cell structure of raw sorghum and promoted the release of polyphenols. (2) These polyphenols interacted with starch molecules mainly through hydrogen bonds during cooking, which affected the composition and antioxidant capacity of the bound polyphenols. (3) Meanwhile, genistein, melanoxetin, and rhodioloside et al., combined with kafirin via a hydrogen bond, van der Waals force,

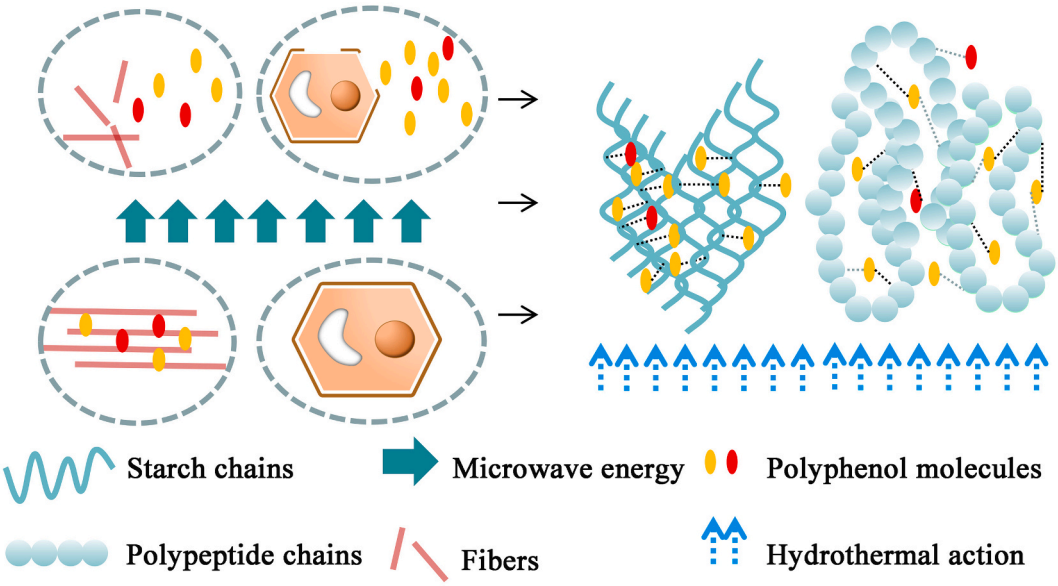


**Fig. 8.** Interaction between sorghum polyphenols and macroelements from raw grain to cooking process.

and hydrophobic interactions. From the research perspective, microwaves had a favorable adaptability to the hot processing of agricultural products, and were superior to conventional hot processing methods in efficiency and quality. This study proved that microwave sorghum has potential improvement in polyphenol components and antioxidant activity, which may provide a theoretical basis for the processing of functional cereals. However, the industrial application advantage may not be as significant as expected, mainly due to the attenuation of microwaves due to material thickness. Therefore, according to the type of grain, diffusion capacity and critical water content, variable power input can be used to meet the energy demand of different states and improve the energy utilization rate in industrial applications.

## 4. Conclusion

In conclusion, microwave was an effective strategy to promote sorghum polyphenol functional effects. Widely targeting metabolomic results revealed that the flavonoid components were significantly affected by microwaves, which regulated the antioxidant activity of sorghum polyphenols. These were mainly attributed to the breaking of raw grain cells by microwave, which was confirmed with morphological characteristics of raw sorghum grain and particle size distribution of powder. Moreover, after cooking, the bound flavonoids in the microwave group were significantly upregulated, primarily including genistein, melanoxetin, rhodioloside, gallocatechin, daidzein, and γ-mangostin. The simulated reaction confirmed that the *co*-gelatinization of polyphenols and starch led to the formation of *V*-type complexes. Further molecular docking and dynamics simulation results represented that these upregulated flavonoids also stably bound to kafirin during cooking and highlighted the key roles of hydrophobic interaction, van der Waals force, and hydrogen bonds. Therefore, the microwave-upregulated polyphenols were complexed with large molecules such as starch and protein during cooking, thus affecting the content and function of bound metabolites. This research is valuable for exploring strategies that analyze functional components from the perspective of macromolecules in foods. Meanwhile, it emphasizes the importance of non-synthetic polyphenol-macromolecule coexistence systems in food nutrition and processing.

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## CRediT authorship contribution statement

**Lei Xu:** Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Xuejian Song:** Visualization, Supervision, Software, Methodology. **Di Yao:** Writing – review & editing, Supervision. **Changyuan Wang:** Methodology, Conceptualization. **Xinmiao Yao:** Supervision, Funding acquisition. **Zhijiang Li:** Writing – review & editing, Visualization, Supervision, Data curation.

## 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.

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## Data availability

Data will be made available on request.

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