Enhancing Antioxidant Bioaccessibility in *Rosa rugosa* through *Lactobacillus plantarum* Fermentation

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Abstract: This study explores the biotransformation of phenolic compounds in *Rosa rugosa* through *Lactobacillus plantarum* fermentation, enhancing their bioaccessibility and antioxidant capacity. We developed a sensitive and reproducible analytical method using ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-QqQ-MS/MS), enabling the analysis of 17 phenolic compounds from Rosa (R) and fermented Rosa (FR). Additionally, we conducted a density functional theory (DFT) study to correlate the structure of key phenolic compounds from R and FR with their antioxidant activity. Our findings revealed that both R and FR mitigate oxidative stress in tert-butyl-hydrogen peroxide (TBHP)-induced Caco-2 and HT-29 cells by elevating the activities of crucial antioxidative enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR). Furthermore, fermented Rosa significantly upregulated *Nrf2*, γ-GCS, HO-1, and NOQ-1 mRNA expression in TBHP-induced cells with Quantitative and real-time PCR technology, emphasizing its protective function primarily through the *Nrf2* signaling pathway. This study is the first to demonstrate the link between the enhanced antioxidant potential in fermented Rosa and the biotransformation of its phenolic compounds. It paves the way for augmenting the antioxidant capacity of plant foods through *Lactobacillus plantarum* fermentation, offering a novel approach to reinforce their health benefits.

Keywords: *Rosa*; *Lactobacillus plantarum* fermentation; phenolic compounds; UHPLC-QqQ-MS/MS; oxidative stress; antioxidative enzymes

1. Introduction

Oxidative stress is characterized as the imbalance between the occurrence of reactive oxygen/nitrogen species (ROS/RNS) and the protective antioxidant defense system, which can lead to various chronic health problems [1], including Parkinson’s, Alzheimer’s disease [2], neurodegenerative diseases [3] as well as atherosclerosis [4]. Epidemiological studies have substantiated that antioxidants can mitigate the effects of ROS and reduce the incidence of cancer and other degenerative diseases. Nevertheless, the use of synthetic antioxidants remains controversial due to associated toxicity issues [5–7]. Conversely, the exploration of natural antioxidants is gaining prominence due to their cost-effectiveness, compatibility with dietary intake, and absence of harmful effects on the human body [8]. It has been confirmed that exogenous antioxidants present in natural foods, such as vitamins C and E, carotenoids, and various phenolics (stilbenes, phenolic acids, flavonoids-flavonols, anthocyanidins) [9–11], can enhance the organism’s ability to counteract oxidative stress.
Rosa rugosa, a member of the Rosaceae family widely distributed across Asia, Europe, and North America, holds significant edible and medicinal value. Rich in various compounds like flavonoids, terpenes, polysaccharides, phenolic acids, fatty acids, organic acids, carotenoids, and vitamins [12], rosa has been extensively studied for its pharmacological effects, including antioxidant, antimicrobial [13], anti-inflammatory [14], anti-tumor [15], anti-diabetic [16], anti-fatigue [17], cardiovascular disease prevention [18]. Despite this potential, the clinical development of these natural foods faces challenges, such as the recalcitrance of plant cell walls and the absorption of macromolecular substances.

Concurrently, fermentation, a biotechnological technique, holds promise for enhancing the health-promoting effects of medicinal herbs. Fermentation, especially with lactic acid bacteria (LAB), has been shown to break down cell walls, potentially improving the nutritional and organoleptic quality of Chinese herbs [19]. However, there is limited literature on the bioactivity of rosa during Lactobacillus fermentation. Therefore, this study aims to assess the ability of rosa and fermented rosa to scavenge reactive radicals resulting from deleterious processes. The findings contribute to our understanding of oxidative stress-induced toxicity and the impact of dietary antioxidants, providing insights for future research in this field.

The study developed a precise analytical method using UHPLC-QqQ-MS/MS to measure characteristic phenolic substances from R and FR. Recognizing fermented rosa as a potential natural antioxidant, our research applied DFT to explore the structural aspects of selected phenolic compounds and their antiradical properties. The study also evaluated the protective effects of fermented rosa against oxidative damage induced by TBHP, using Caco-2 and HT-29 cells. The paper delved into the potential mechanism of protection, focusing on FR-mediated Nrf2 activation to mitigate oxidative stress. In summary, these findings contribute to developing postbiotic products from foods and enhance our understanding of the molecular mechanisms driving the antioxidant activities of fermented rosa.

2. Materials and Methods

2.1. Materials and Chemical Reagents

Rosebud was provided by Gansu Dongmei Yuchi Biomedical Technology (company) in Lanzhou, China. Lactobacillus plantarum LZU-J-TSL6 and LZU-S-ZCJ were isolated from JiangShui and yogurt. The strains were identified by 16S rRNA gene sequence analysis and were deposited in the Guangdong Microbial Culture Collection Center (GDMCC), (Guangzhou, China) under accession numbers GDMCC 61242 and GDMCC 61402. Due to the fact that both strains of Lactobacillus plantarum have the property of metabolizing plants well, the previous work demonstrated the attenuation of D-gal-induced liver aging by fermented Angelica sinensis [20]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay Kit, the scavenging capacity Assay Kit of hydroxyl free radical (·OH) and superoxide anion (·O₂⁻), SOD and MDA Assay Kits were purchased from Nanjing Jiancheng bioengineering institute (Nanjing, China, 2023). GR Assay Kit, GSH-Px Assay Kit, and CAT Assay Kit were obtained from the Beyotime Institute of Biotechnology (Haimen, China, 2023). Chromatographic grade methanol was obtained from Macklin Biochemical Technology Company (Shanghai, China, 2023). The proanthocyanidin B2 (PB2), isorhamnetin, hyperoside, epicatechin gallate, chlorogenic acid (CGA), cyanidin-3-O-glucoside (C3G) were purchased from Macklin Biochemical Technology Company (Shanghai, China, 2023). The other standards like catechin (CATE), epicatechin, kaempferol, quercetin, gallic acid (GA), syringic acid (SA), vanillic acid (VA), protocatechuic acid (PA), caffeic acid (CA), 4-hydroxybenzoic acid (4-HBA), ferulic acid (FA) were acquired from Shanghai Standard Technology Company (Shanghai, China, 2023).

2.2. Preparation of Rosa and Rosa Fermentation

The following are the preparation details:
R: Rosa powder was obtained from rosebud by grinding and filtering using 100 mesh sieves. The rosa powder was mixed with distilled water (w/v) at the proportion of 1:20 and sterilized at 90 °C for 60 min.

FR: *Lactobacillus plantarum* LZU-J-TSL6 and LZU-S-ZCJ were cultured with MRS broths from Qingdao Hi-tech Industrial Park Hope Bio-technology Company (Qingdao, China, 2022) at 37 °C for 18 h. The final concentration of *Lactobacillus plantarum* LZU-J-TSL6 and LZU-S-ZCJ in the culture was approximately 10^8 CFU/mL.

*Lactobacillus plantarum* LZU-J-TSL6 and LZU-S-ZCJ were mixed in a ratio of 1:1 (mL/mL) and added into the R with 3% initial inoculum (10^8 CFU/mL) and cultured at 35 °C for 12 h. Next, the mixture was centrifuged and sterilized at 90 °C for 60 min to obtain FR. As a functional fermented beverage, the activity of FR needs to be further explored.

### 2.3. Measurement of Antioxidant Activity In Vitro

DPHH free-radical scavenging activities of the R and FR were measured according to Gupta et al. [21]. The absorbance was measured at 517 nm using a microplate reader (Thermo Scientific, Vantaa, Finland, 2010). The Fenton method of Luo et al. [22] was referenced to evaluate the scavenging rate of R and FR on hydroxyl radicals. Meanwhile, to appraise the scavenging rate of R and FR on superoxide anion radicals, the pyrogallol method of Zhao et al. [23] was used as a reference.

### 2.4. Contents of Total Flavonoids, Total Phenols, and Total Anthocyanins

The contents of total phenols and total flavonoids of R and FR were examined following the previously reported method with minor modifications [24]. The rosa and fermented rosa were extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. The content of total anthocyanins analysis was performed according to the pH differential method [25]. Meanwhile, the contents of total phenols, total flavonoids, and total anthocyanins were expressed as mg of gallic acid equivalent/mL, rutin equivalents/mL, and cyanidin-3-O-glucoside chloride equivalents/mL, respectively.

### 2.5. Quantitative Analysis of the Phenolic Compounds from FR and R by UHPLC with Triple-Quadrupole Tandem Mass

#### 2.5.1. Preparation of Sample and Standard Solutions

For analysis, a 100 mg sample of freeze-dried was using 100 mL of 70% methanol/water in an ultrasonic bath for 60 min, centrifuged at 12,000 × g at 4 °C. The supernatant was collected and filtered through 0.45 μm membrane filters. The stock solution of proanthocyanidin B2, catechin, epicatechin, kaempferol, isorhamnetin, hyperoside, quercetin, epicatechin gallate, gallic acid, syringic acid, vanillic acid, protocatechuic acid, caffeic acid, 4-hydroxybenzoic acid, ferulic acid, chlorogenic acid and cyanidin-3-O-glucoside were prepared in 70% methanol/water (v/v) which were stored at −4 °C in the dark. For preparing the calibration dilution series, working solutions were diluted to 7 concentration levels with stock solutions. All standard solutions were filtered through a 0.22 μm membrane before being loaded onto a UHPLC column.

#### 2.5.2. UHPLC-QqQ-MS/MS Method

UHPLC-QqQ-MS/MS system consists of the Agilent 1290 Infinity system coupled with the Agilent 6470 triple quadrupole tandem mass system (Agilent, Santa Clara, CA, USA, 2021). The UHPLC chromatographic separations were performed on an EclipsePlus C18 column (50 mm × 2.1 mm, 1.8 μm). Column oven temperature and injection volume were maintained at 30 °C and 0.5 μL with a flow rate of 0.3 mL/min, separately. The DAD detection wavelength was 280~520 nm. The binary mobile phase included 0.1% formic acid in water (A) and acetonitrile (B). The gradient program was carried out as follows: 0 min, 5% B; 1.5 min, 5% B; 3 min, 15% B; 5 min, 35% B; 7 min, 50% B; 9 min, 99% B; 9.5 min, 99% B; 10 min, 5% B; 11 min, 5% B. Mass spectra of QqQ were acquired using AJ ESI under the positive or negative ionization mode. The capillary voltage was set to 3500 V, the gas...
temperature was 300 °C with a flow rate of 10 L/min, and the nebulizer pressure of 45 psi. The multiple reaction monitoring (MRM) mode of UHPLC-QqQ-MS was used to detect the 17 phenolic compounds according to Supplementary Table S1.

2.5.3. Accuracy and Precision

We assessed method recovery and precision by analyzing three replicates of quality control samples at two concentration levels, low-quality control (LQC) and high-quality control (HQC). Recovery was quantified as the relative error (RE, %), and precision was evaluated through the relative standard deviation (RSD, %) of the measured concentrations across replicates. These metrics provide insights into the accuracy and consistency of the analytical method employed.

2.6. Computational Method

In this study, all calculations were performed using Gaussian 09D01 software (Wallingford, CT, USA, 2009) [26]. The B3LYP functional was selected as the method, and the 6-31 + G (d, p) basis set was employed to fully optimize the molecules in their ground state [27,28]. Frequency analysis was conducted to determine the minimum point of the optimized conformation and to obtain its energy at 298 K. Solvent effects were accounted for using the implicit solvent model, specifically the SMD-H2O model, to represent the influence of the surrounding environment [29].

To better understand the relevance of the structural differences of the investigated phenolic compounds for their antioxidant activity, we also analyzed the energy of the highest occupied molecular orbital (HOMO)/lowest unoccupied molecular orbital (LUMO). Using the global description parameters of the molecule, it is possible to understand the relationship between its chemical reactivity, and response to changes in external conditions [30]. The global descriptive parameters include the ionisation potential, electron affinity chemical potential, electronegativity, electrophilicity, hardness, softness, etc [31]. Global reactive descriptors can be determined through two distinct methods. The first method relies on computing the variance in the total electronic energy between a neutral molecule and its corresponding anion and cation. This involves extracting the geometrical information from the neutral molecule while maintaining a constant external potential. This approach is commonly referred to as ‘energy vertical’ and was introduced by Sadasivam and Kumaresan [32]. The global properties are computed by using the following equations.

\[
\text{Ionisation Potential (IP)} = -\text{EHOMO}
\]
\[
\text{Electron Affinity (EA)} = -\text{ELUMO}
\]
\[
\Delta E_{\text{(LUMO-HOMO)}} = \text{ELUMO} - \text{EHOMO}
\]
\[
\text{Chemical potential (µ)} \approx \frac{\text{EHOMO} + \text{ELUMO}}{2}
\]
\[
\text{Electronegativity (χ)} \approx \frac{-\text{EHOMO} + -\text{ELUMO}}{2}
\]
\[
\text{Electrophilicity (ω)} \approx \frac{\mu^2}{2\eta}
\]
\[
\text{Chemical hardness (η)} \approx \frac{\text{ELUMO} - \text{EHUMO}}{2}
\]
\[
\text{Chemical softness (ζ)} \approx \frac{1}{2\eta}
\]

2.7. Cell Culture

Thus, in this study, the antioxidative efficiency of fermented rosa-rich phenolic compounds was investigated in the human colon tumor cell lines Caco-2 and HT-29. The
Caco-2 and HT-29 cells obtained from the Shanghai Institutes for Biological Science, Chinese Academy of Sciences (Shanghai, China, 2022) were cultivated in RMPI1640 with 15% fetal bovine serum (FBS) and 1% penicillin-streptomycin, respectively. The cells were incubated in a 37 °C Heracell CO² incubator (Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA, 2020) at 5% CO², which were subcultured every 2 days until the cells reached 70–80% confluent monolayer cells. Passages 5–10 were used in all experiments.

2.8. Cell Viability

Using the MTT assay, the FR and R under different concentrations (5, 10, 20, 40, and 80 µg/mL in 0.1% dimethyl sulfoxide (DMSO)) were tested for measuring cell viability. First, the cells (1 × 10⁵ cells/mL) were seeded onto 96-well plates. After 12 h, the culture medium was replaced with the R and FR in sample wells, respectively, and the plates were kept at 37 °C in a 5% CO²-humidified atmosphere for 24 h. Then 10 µL of MTT solution (5 mg/mL) was added to each well, and the cells were incubated for 4 h at 37 °C. The absorbance at 490 nm was measured using a microplate reader after adding the DMSO. Results are expressed as a percentage of cell viability referred to as the absorbance measured in control cells.

2.9. Induction of Oxidative Stress

To evaluate the protective effects against oxidative stress, the different concentrations of R and FR (10, 20, and 40 µg/mL) were added to the cells for 24 h. Then, the incubation period was followed by a 3 h treatment with culture oxidant chemicals TBHP (300 µM). The cells treated with TBHP only were taken as the positive control (TBH). The untreated cells were taken as the negative control (Control).

2.10. Determination of MDA and Antioxidant Enzyme Activity

Caco-2 and HT-29 cells with the same density were seeded in flat-bottom six-well plates and then pretreated with RIPA to lyse them. The supernatant was harvested by centrifuging at 12,000 × g for 10 min at 4 °C. The activities of SOD, CAT, GR, and GSH-Px and the content of MDA were determined according to the instructions of the kits. The cell lysate protein concentration was measured using the bicinchoninic acid Protein Assay Kit from Beyotime Institute of Biotechnology (Haimen, China, 2023).

2.11. Quantitative and Real-Time PCR

Total RNA was extracted using the Total RNA Extraction Kit and complementary DNA was prepared using the First Strand cDNA Synthesis Kit (with dsDNase) from Seven Biotech Company (Beijing, China, 2023). Quantitative real-time PCR (qRT-PCR) was conducted using the 2 × SYBR Green qPCR MasterMixII (Universal) from Seven Biotech Company (Beijing, China, 2023) under the conditions of 95 °C for 3 min for the initial heat activation, followed by 40 cycles of 95 °C for 20 s for denaturation, 55 °C for 20 s for combined annealing and 72 °C for 30 s for extension. According to the gene sequence obtained from PrimerBank, the National Center for Biotechnology Information (NCBI) was used to blast the specificity of different primers. Nrf2 is regarded as the master regulator of the cellular defense mechanism against oxidative stress [33]. The primer sequences for the genes related to oxidative stress and the reference gene, β-actin, are listed as follows: Nrf2 (forward primer 5'-TCAGCGACGGAAGAGATATGA-3' and reverse primer 5'-CCACTTGTTTCTGTGATGAC-3'); HO-1 (forward primer 5'-AAACTGCTTCATCTGCTCA-3' and reverse primer 5'-AAAGCCTTACACACT-3' and reverse primer 5'-AAAGGCTTACACACT-3'); γ-GCS (forward primer 5'-GGCACAAAGCGCTCTCAAAGT-3' and reverse primer 5'-CAGACAGGACACACCCCGAC-3'); NQO-1 (forward primer 5'-GGCAGATGGGAAGGAGA-3' and reverse primer 5'-AAAAACCCAGAGGAGGATC-3') and β-actin (forward primer 5'-GACCTCTATGCAGCT-3' and reverse primer 5'-AGTACTTGCAGGA-3').
2.12. Statistical Analysis

All the experiments were conducted at least in triplicate. ("n" denoted the number of biological replicates, n = 3). Results are expressed as mean ± standard deviation (SD). Differences were determined by One-way analysis of variance (ANOVA), Dunnett’s test, Multiple correlations, and Bonferroni’s correction, using GraphPad Prism 9 (GraphPad Software, Inc, San Diego, CA, USA, 2020). A value of p < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Contents of Total Flavonoids, Total Phenols, and Total Anthocyanins

A significant increase in the content of total flavonoids was observed in FR (Figure 1A). The content of total phenols increased during fermentation as shown in Figure 1B. As shown in Figure 1C, the content of total anthocyanins was decreased from 12.1 ± 0.2 to 6.8 ± 0.2 mg/mL.

![Figure 1. (A) Content of total flavonoids. (B) Content of total phenols. (C) Content of total anthocyanins. (D) Effects of R and FR on scavenging free radicals, n = 3. Data are presented as mean ± SD; * p < 0.05, and **** p < 0.0001: significantly different compared with the R. ns: not significant.](image)

3.2. Effects of R and FR on Scavenging Free Radicals

Furthermore, studies of the enhancement of antioxidant activities in tomato [34], cabbage [35], and apple juices [36] by LAB fermentation have recently been reported. Thus, the DPPH, ·O⁡_2\(^{-}\) and ·OH radical scavenging abilities of R and FR are shown in Figure 1D. Compared with the R, the DPPH· and ·O_2\(^{-}\) radical scavenging abilities of FR increased 13.6 ± 0.5% to 22.5 ± 1.2% and 15.8 ± 0.9% to 23.9 ± 1.6% during the 12 h of incubation, whereas the ·OH radical scavenging abilities of R and FR had almost insignificant difference. For the FR group, *Lactobacillus plantarum* fermentation enhanced the antioxidative ability of rosa.

3.3. UHPLC-QqQ-MS/MS Method Development and Optimization

The rosa is rich in various biologically active compounds, including anthocyanins, flavonols, flavan-3-ols, proanthocyanidins, ellagitannins, phenolic acids, and essential oils. Furthermore, the substantial content of these compounds is considered crucial for health reasons [37]. The analysis of major phenolic compounds originating from R and FR was carried out using the UHPLC-QqQ-MS/MS method. Optimization of the method
was achieved by fine-tuning multiple key parameters closely related to chromatographic behaviors such as mobile phase, flow rate, and column temperature. Additionally, the establishment of an accurate method contributes to the stabilization of the peaks of the standards. The previous study investigated that the positive ion mode was more selective and sensitive for anthocyanins in blueberry and the negative ion mode was more selective and sensitive for flavonols, proanthocyanidins, and phenolic acids in blueberry [38]. Thus, the positive ion mode and negative ion mode were used in QqQ-MS/MS analysis. Structural information of 17 phenolic compounds corresponding retention time, the parameters of fragment voltage and collision voltage (CE), and precursor-product ion pair transitions specific to individual analytes are shown in Supplementary Table S1.

3.4. Validation of UHPLC with Triple-Quadrupole Tandem Mass Method

Following method optimization, the proposed LC-MS method was validated in terms of selectivity, linearity, limit of detection/quantification (LOD/LOQ), recovery, and precision. The reliability and reproducibility of the developed method were assessed by examining the correlation coefficients for each calibration curve based on the peak integration of the corresponding concentration. As shown in Table 1, all 17 reference standards exhibited excellent linearity with correlation coefficients above 0.99. Regarding sensitivity, LOD, and LOQ were determined for each analyte among the 17 compounds tested. Notably, this method demonstrated significantly improved sensitivity compared to previously reported HPLC and LC-MS methods.

Table 1. Calibration curve, Linear correlation, LOD and LOQ, recovery, and precision of 17 standards. (n = 3. Data are presented as mean ± SD).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Standard Curves</th>
<th>Linear Correlation (r²)</th>
<th>LOD (µg/L)</th>
<th>LOQ (µg/L)</th>
<th>Recovery (%) (n = 3)</th>
<th>Precision (%) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epicatechin</td>
<td>y = 9933.9 * x + 1813.8</td>
<td>0.9944</td>
<td>13.0</td>
<td>40.0</td>
<td>98.5 ± 2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Catechin</td>
<td>y = 3046.2 * x + 1979.1</td>
<td>0.9920</td>
<td>20.0</td>
<td>60.0</td>
<td>98.9 ± 2.4</td>
<td>1.1</td>
</tr>
<tr>
<td>ProcyanidinB2</td>
<td>y = 32.62 * x + 17,260.0</td>
<td>0.9990</td>
<td>1.1</td>
<td>5.0</td>
<td>99.9 ± 2.9</td>
<td>12.1</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>y = 31.7 * x + 9797.9</td>
<td>0.9998</td>
<td>81</td>
<td>243</td>
<td>98.4 ± 2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>y = 123.9 * x - 16,219.2</td>
<td>0.9888</td>
<td>14.1</td>
<td>45.0</td>
<td>100.5 ± 2.1</td>
<td>9.4</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>y = 18.1 * x + 98,205.5</td>
<td>0.9941</td>
<td>21.0</td>
<td>63.8</td>
<td>98.3 ± 3.8</td>
<td>8.6</td>
</tr>
<tr>
<td>Quercetin</td>
<td>y = 45.0 * x + 3048.0</td>
<td>0.9999</td>
<td>0.7</td>
<td>2.0</td>
<td>98.9 ± 1.2</td>
<td>14.6</td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>y = 1.7 * x + 5038.4</td>
<td>0.9918</td>
<td>78.0</td>
<td>210.0</td>
<td>99.9 ± 1.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>y = 0.03 * x + 209.9</td>
<td>0.9998</td>
<td>6000.0</td>
<td>15,000.0</td>
<td>99.8 ± 4.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>y = 134.4 * x + 37,077.2</td>
<td>0.9976</td>
<td>0.9</td>
<td>3.0</td>
<td>99.5 ± 1.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>y = 0.4 * x + 149.1</td>
<td>0.9991</td>
<td>100.0</td>
<td>330.0</td>
<td>99.3 ± 0.79</td>
<td>3.5</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>y = 0.2 * x + 124.3</td>
<td>0.9999</td>
<td>613.0</td>
<td>1840.0</td>
<td>100.6 ± 2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>y = 443,933.2 * x + 345,006.1</td>
<td>0.9914</td>
<td>20.0</td>
<td>60.0</td>
<td>101.9 ± 3.9</td>
<td>18.0</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>y = 13.6 * x + 151.1</td>
<td>0.9999</td>
<td>25.0</td>
<td>75.0</td>
<td>99.9 ± 2.4</td>
<td>4.3</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>y = 0.3 * x + 923.8</td>
<td>0.9997</td>
<td>1.0</td>
<td>5.0</td>
<td>99.9 ± 4.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>y = 70.4 * x - 53.8</td>
<td>0.9999</td>
<td>1.0</td>
<td>5.0</td>
<td>99.2 ± 2.4</td>
<td>12.9</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>y = 1.2 * x - 543.1</td>
<td>0.9996</td>
<td>310.4</td>
<td>1000.0</td>
<td>100.2 ± 4.2</td>
<td>15.4</td>
</tr>
</tbody>
</table>

3.5. Quantifying Phenolic Compounds in Rosa: Utilizing UHPLC QqQ-MS/MS and Exploring Microbial Metabolic Transformations

Additionally, the compounds identified in this study can be categorized into two groups: flavonoids, and phenolic acids. Anthocyanins in rosa, such as cyanidin-3-O-glucoside, were identified using positive ion mode MS. Furthermore, the other phenolic compounds including proanthocyanidin B2, catechin, epicatechin, kaempferol, isorhamnetin, hyperoside, quercetin, epicatechin gallate, gallic acid, syringic acid, vanillic acid, protocatechuic acid, caffeic acid, 4-hydroxybenzoic acid, ferulic acid and chlorogenic acid were identified from FR and R using negative ion mode MS.

The results observed a significant decrease in procyanidinB2 and epicatechin gallate and a concurrent increase in catechin and epicatechin concentrations in FR when treated with the Lactobacillus strain, respectively, as shown in Table 2.
### Table 2. Content (µg g⁻¹ DW) of 17 phenolic compounds in rosa and fermented rosa. (n = 3. Data are presented as mean ± SD).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Content (µg g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
</tr>
<tr>
<td>Epicatechin</td>
<td>116.5 ± 1.6</td>
</tr>
<tr>
<td>Catechin</td>
<td>172.0 ± 11.2</td>
</tr>
<tr>
<td>ProcyanidinB2</td>
<td>829.0 ± 34.0</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>289.0 ± 10.3</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>192.9 ± 0.3</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>588.9 ± 6.4</td>
</tr>
<tr>
<td>Quercetin</td>
<td>229.2 ± 3.2</td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>2085.3 ± 20.1</td>
</tr>
<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>757.2 ± 12.43</td>
</tr>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1153.1 ± 9.2</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>181.3 ± 23.48</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>179.7 ± 7.8</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>279.9 ± 6.4</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>252.8 ± 5.8</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>15.4 ± 3.8</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>13.0 ± 1.6</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>458.2 ± 2.0</td>
</tr>
</tbody>
</table>

Values indicate the content of rosa difference significantly during the fermentation (** p < 0.01, *** p < 0.001 and **** p < 0.0001).

Flavan-3-ols, present in monomeric forms such as catechin and epicatechin, as well as oligomeric and polymeric forms known as condensed tannins or proanthocyanidins, are among the most abundant and bioactive dietary polyphenols [39]. As depicted in Figure 2, the degradation of flavan-3-ols encompasses a multitude of reactions. On one hand, catechin and epicatechin would be implicated in the microbial catabolism of proanthocyanidin B2 and epicatechin gallate, which exhibited significant reduction during fermentation. On the other hand, the intricate catabolism of catechin and epicatechin involved a C-ring opening, followed by lactonization, decarboxylation, dehydroxylation, and oxidation reactions among others [40]. An increase in the response was observed for the Gallic acid, 4-hydroxybenzoic acid as well as protocatechuic acid concentrations. Henceforth, it is noteworthy to mention that low molecular weight phenolics might be generated from flavan-3-ols by microbial processes with high bioavailability potential.

Notably, among the flavonoids examined, hyperoside and epicatechin gallate exhibited a decline in content following the fermentation process. In contrast, the concentrations of other identified flavonoids, such as kaempferol, isorhamnetin and quercetin experienced an upward trend.

In the majority of situations, rosa was regarded as an abundant source of anthocyanin compounds. Meanwhile, recent studies have shown that anthocyanins enhance the growth of the beneficial bacterial *Lactobacillus* spp. and *Bifidobacterium* spp. [41]. We found that cyanidin-3-O-glucoside was decreased from 757.2 ± 12.43 to 435.2 ± 15.26 µg/g during fermentation. Furthermore, the *Lactobacillus* could secrete the β-glucosidases for anthocyanin degradation, which resulted in more energy for the growth of the bacteria. Due to the numerous reactions, such as oxidation, dehydroxylation, α-oxidation, β-oxidation, rearrangement reactions, and methylation [42], the C3G was further degraded into phenolic acids as shown in Figure 2. The vanillic acid, PA, and 4-HBA were detected to the accumulation in bacterial metabolites. Finally, the metabolism of the rich in anthocyanin-contained plant foods may be beneficial to the growth of bacterial and resultant metabolites exert health-promoting effects.
3.6. Evaluating the Antioxidant Potential of Compounds Exhibiting Substantial Content Changes during Fermentation Using the DFT Method

3.6.1. Molecular Orbital Analysis

The structures of the investigated molecules, which represent the major compounds undergoing significant changes in content during fermentation, are illustrated in Supplementary Figure S1. The most stable conformation with the lowest energy predominantly contributes to the system properties depicted in Figure 3. Frontier molecular orbitals HOMO/LUMO can be utilized to map the antioxidant activity of these compounds. The HOMO indicates a higher electron instability and a greater tendency for donation, while LUMO signifies enhanced electronic acceptance capacity [43]. Therefore, Figure 3 displays the HOMO and LUMO molecular orbitals of the examined phenolic compound structures. The energy gap $\Delta E_{\text{LUMO-HOMO}}$ reflects the energy required for electron transition from HOMO to LUMO, with lower values indicating easier excitation and occurrence of related reactions. As shown in Supplementary Table S4 CATE exhibits the highest $\Delta E_{\text{LUMO-HOMO}}$ value (5.7207 eV), whereas C3G has the lowest $\Delta E_{\text{LUMO-HOMO}}$ value (2.9769 eV), aligning with previous findings on high anthocyanin content and antioxidant activity correlation [44].

As shown in Table 2, the other phenolic acids were quantitatively analyzed in the R and FR samples, including syringic acid, caffeic acid, ferulic acid, and chlorogenic acid. Notably, the concentrations of SA and GA in the FR group were threefold and twofold higher, respectively, compared to those in the R group.
3.6.2. Global Reactive Descriptors

The computed global descriptors of five phenolic compounds are given in Supplementary Table S4. The high ionization potential suggests the challenging removal of electrons from these compounds, while their relatively small electron affinity indicates their ability to gain electrons. Electronegativity is employed to explain the tendency of a chemical species to attract electrons, whereas electrophilicity refers to its capacity for accepting electrons [45]. Additionally, chemical potential describes the inclination of electrons to flow from regions with higher potential to those with lower potential until equilibrium is achieved throughout. Hardness and softness are defined by the HOMO-LUMO energy gap and serve as crucial indicators of a compound’s reactivity.

As shown in Supplementary Table S4, PA exhibits a high ionization potential and relatively low electron affinity, suggesting that it is slightly more difficult to remove an electron from PA than it is for it to accept one. GA and PA display comparatively low hardness values and high softness values compared to C3G, PB2, and CATE, making them more reactive than the others due to their lower energy gaps. The C3G possesses a high electronegativity enabling it to retain some charge within itself. In comparison with other phenolic compounds, when there is an opportunity for electron transfer, C3G will accept electrons from free radicals and neutralize them. The reactivity is increased in polar media so that C3G, GA, and PA can be used as a radical scavenger in a biological system.

3.6.3. Antioxidant Property

The scavenging of a reactive species (ROS/RNS) by an antioxidant molecule can proceed via different pathways involving hydrogen atom abstraction by the reactive species from the neutral form (HAT, SET-PT, and SPLET) [46]. In this work, we have studied three pathways for the antioxidant behavior of 5 compounds in aqueous solution. Moreover, the mechanisms are given in Supplementary Figure S2 and the parameters can be calculated as shown in the equation (Supplementary Text S1). The calculated bond dissociation enthalpies (BDE), ionization potentials (IP), proton dissociation enthalpies (PDE), proton affinities (PA1), and electron transfer enthalpies (ETE) values of each hydroxyl group present in compounds in aqueous solution are listed in Table 3.
Table 3. Solubility, thermochemical, and reactivity properties computed in the aqueous phase for the major polyphenols of R and FR.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Position</th>
<th>BDE</th>
<th>IP</th>
<th>PDE</th>
<th>PA1</th>
<th>ETE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3G</td>
<td>4'-OH</td>
<td>90</td>
<td>101</td>
<td>2</td>
<td>24</td>
<td>79</td>
</tr>
<tr>
<td>PB2</td>
<td>4'-OH</td>
<td>84</td>
<td>85</td>
<td>12</td>
<td>85</td>
<td>12</td>
</tr>
<tr>
<td>CATE</td>
<td>4'-OH</td>
<td>84</td>
<td>92</td>
<td>5</td>
<td>32</td>
<td>65</td>
</tr>
<tr>
<td>3-OH</td>
<td></td>
<td>82</td>
<td></td>
<td>7</td>
<td>46</td>
<td>80</td>
</tr>
<tr>
<td>GA</td>
<td>4-OH</td>
<td>76</td>
<td>119</td>
<td>1</td>
<td>37</td>
<td>83</td>
</tr>
<tr>
<td>5-OH</td>
<td></td>
<td>79</td>
<td></td>
<td>4</td>
<td>40</td>
<td>84</td>
</tr>
<tr>
<td>PA</td>
<td>4'-OH</td>
<td>80.6</td>
<td>95.3</td>
<td>7.62</td>
<td>31.4</td>
<td>71.5</td>
</tr>
</tbody>
</table>

Several studies based on thermochemical properties have demonstrated that the 4'-OH site is the preferred binding site for radicals of the investigated polyphenols and related compounds in this study [47]. Previous research has shown that both catechin and procyanidin B-2 exhibit excellent hydrogen atom donation ability through the HAT mechanism, in both gas and aqueous phases. Cyanidin-3-glucoside exhibits a low ability to donate hydrogen atoms through HAT and SET-PT mechanisms, but it demonstrates the highest electron transfer ability through the SPLET mechanism (possibly due to its positive charge), in both gas and aqueous phases [48]. It should be noted that GA possesses the lowest BDE values, ranging from 76–82 kcal/mol, suggesting that GA could compete with other compounds as the best donor of hydrogen atoms via the HAT mechanism. The radical scavenging activity of GA has been extensively studied by evaluating various antioxidant mechanisms, leading to the identification of the most suitable mechanism [49]. Furthermore, from a thermodynamic perspective, SPLET should be considered as the preferable action mechanism for protocatechuic acid since its PA1 values determining occurrence are relatively low compared to all calculated descriptors [50]. Overall, the computational analysis revealed that the antioxidant capacity of these five compounds depended on molecular orbital analysis as well as BDE, IP, PDE values, etc.

3.7. Effects of FR and R on Caco2 and HT-29 Cells

The cytotoxicity test was performed to determine the toxic effect of FR and R on Caco2 and HT-29 cells. As shown in Figure 4A,B, the toxic effect of rosa after fermentation was slightly reduced. The statistical analysis results indicated a significant difference between the control and sample groups. Since the three concentrations are inhibitory to cell proliferation but not lethal, 40, 20, and 10 µg/mL were selected to perform the subsequent experiments.

3.8. The Effects of FR and R on Antioxidant Enzymes and Lipid Peroxidation in TBHP-Induced Caco2 and HT-29 Cells

To investigate the potential mechanism underlying the protective effects of bioactive fermented rosa, the study initially quantified the levels of antioxidant enzymes and MDA content. Free radical reactions in the body and lipid peroxidation play a critical role in metabolism. As shown in Figure 4C,D, the experiments on lipid peroxidation detection revealed an elevation in MDA levels in TBHP-damaged Caco2 and HT-29 cells compared to the Control group, suggesting that the induction of oxidative cellular damage was successful. Notably, in Caco2 cells, the MDA content was significantly reduced in the FR group compared with the R group, indicating that the FR might exert a protective effect by inhibiting lipid peroxidation. Moreover, all three concentrations of fermented rosa effectively reduced the MDA content compared to the TBH group as shown in Figure 4D in HT-29 cells.
Oxygen-free radical scavengers, like SOD, have been demonstrated to eliminate harmful substances produced during organismic metabolism [51]. Furthermore, as shown in Figure 4E, the SOD activity in the Caco2 cell line was found to be significantly higher than those in the TBH control group under the effect of all three sets of concentrations of FR, suggesting that the FR has the potential to scavenge the harmful substances such as reactive oxygen species; whereas, in Figure 4I, the analytical assay of the HT-29 cells revealed that both FR and R significantly increased the SOD activity, for example, at 40 μg/mL concentration treatment, the measured SOD enzyme activities were 7.24 ± 0.06 U/mg and 6.71 ± 0.03 U/mg, respectively.

Additionally, the catalase enzyme participates in reducing the H₂O₂ to H₂O and O₂ [52], with our data suggesting that fermented rosa induces the activity of CAT to prevent accumulation of excess H₂O₂ and thus reduce damage from oxidative stress. As shown in Figure 4F, when Caco2 cells were given 10, 20, and 40 μg/mL of FR, the activity of CAT was increased by 4.71, 7.64, and 12.08 U/mg, respectively.

The stimulatory impact of FR on Glutathione Reductase (GR) activity in HT-29, depicted in Figure 4K, surpassed that in Caco2. Importantly, the fermentation of rosa during oxidative stress significantly enhanced GSH-Px activity compared to the TBHP group across both cell lines. It is worth noting that the maximum GSH-Px activity observed in Caco2 was 92.13 ± 1.54 mU/mg, whereas in HT-29, it reached 112.56 ± 4.87 mU/mg, as illustrated in Figure 4H. These findings imply that both FR and R play significant roles in safeguarding Caco2 and HT-29 cells from TBHP-induced damage, serving pivotal functions in the antioxidant enzymes against oxidative stress. Notably, FR exhibits a more pronounced protective effect compared to R.
3.9. Regulation of Nrf2 Signaling Pathway

Due to its involvement in the xenobiotic response against a wide range of substances, Nrf2 is widely recognized as the pivotal regulator of cellular defense mechanisms against oxidative stress [53]. As shown in Figure 5, the relative expression levels of Nrf2, γ-GCS, HO-1, and NOQ-1 mRNA were significantly upregulated in Caco2 (Figure 5A–D) and HT-29 (Figure 5E–H) cells treated with 40 µg/mL FR. Notably, 40 µg/mL FR exhibited the most significant effect on NOQ-1 expression in both cell types. The relative expression of Nrf2 in FR-treated Caco2 cells showed extremely significant differences. Moreover, compared to the TBHP-induced control group, certain concentrations of samples demonstrated different beneficial effects on these indicators in HT-29 cells, particularly at 20 and 40 µg/mL. In conclusion, FR mitigates the negative effects of TBHP on Caco2 and HT-29 cells to varying degrees and exhibits evident protective effects against gene downregulation.

![Figure 5](image-url)

Figure 5. Effects of FR on Nrf2 and Nrf2-upstream target genes in Caco2 (A–D) and HT-29 (E–H) cells, n = 3. Data are presented as mean ± SD.; * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001: significantly different compared with the TBH model group. ns: not significant.

4. Discussion

Antioxidants play a crucial role in neutralizing reactive oxygen species and preventing oxidative stress-induced damage to essential molecules. As the perception of food as a source of therapeutic value gains traction among consumers, it becomes increasingly important to identify effective antioxidants that can protect the body against oxidative stress [54]. In this study, we employed microbial fermentation technology to ferment rosa, aiming to enhance its efficacy in combating oxidative stress. By leveraging its abundant enzyme systems, Lactobacillus plantarum can utilize polysaccharides and other substances present in traditional Chinese medicines (TCMs) as carbon and energy sources. This capability enables the breakdown of the complex compounds present in TCMs, facilitating the release of their active ingredients [55]. Furthermore, the microbial metabolism involved in TCM fermentation can lead to the transformation of macromolecular substances into smaller molecules. This enzymatic hydrolysis process results in the generation of small, bioactive compounds that can be more easily absorbed by the body. Consequently, the increased bioavailability and improved efficacy of TCMs can be achieved through microbial fermentation [56].

During fermentation, microbial enzymes like glucosidases, amylases, cellulases, and chitinases catalyze the hydrolysis of glucosides and the degradation of plant cell walls or starch [57]. This process liberates active components, such as flavonoids, which serve as antioxidants by neutralizing free radicals, chelating metals, quenching singlet oxygen, or donating hydrogen to radicals [58].

It has been shown that Lactobacillus plantarum has a greater ability to increase total polyphenolic compounds compared to other LAB, which may be due to the fact that Lactobacillus plantarum induces de-glycosylation of more glycosylated phenolics from the mulberry juice during the fermentation process that helps to release the phenolic com-
pounds that are soluble or insolubilised bound in the plant cell wall [59]. Glycosylated phenolic compounds, including tannins, ellagic acid, lignans, isoflavones, flavonols, and anthocyanins, prevalent in plant foods, undergo extensive deglycosylation upon absorption or by the colonic microbiota. *Lactobacillus plantarum* CECT 748T has been proven to efficiently convert various food aryl glycosides (quercetin glucoside, phloridzin, esculetin, daidzin, and salicin) into their corresponding aryl aglycones (quercetin, phloretin, esculetin, daidzein, and saligenin) [60]. Thus, enzymatic hydrolysis of complex polyphenols by microorganisms during fermentation into simpler flavonol compounds may be responsible for the increase in total flavonoid content. This is in line with the findings of Svensson et al. who reported an increase in flavonol content during lactic acid fermentation [61]. Some microorganisms are capable of hydrolysing complex compounds to produce biologically active flavonoids [62]. In particular, it has been suggested that specific microorganisms can synthesise isoflavones and glycoside isoflavones [63]. It has been found that the major groups of bacteria that can metabolize anthocyanin are the *Bifidobacterium* spp. and *Lactobacillus* spp. [64].

Nonetheless, the existing research has established connections between oxidative stress and certain compounds [65]. After fermentation, total flavonoids and total polyphenols increased and total anthocyanins decreased. This result is consistent with the earlier conclusion of an increase in the bioactive compounds content [66,67].

Interestingly, previous studies have demonstrated that fermentation using specific microbial strains can enhance the antioxidant properties of various substances. For instance, the fermentation of apple pomace using *Lactobacillus rhamnosus* L08 increased its antioxidant properties significantly [68]. Free radical scavenging activities of rosa on DPPH, hydroxyl free radicals, and superoxide were investigated [69]. In addition, the antioxidative activity of FR increased, associated with the accumulation of phenolic compounds.

The screening and characterization of phenolic compounds in rosa were conducted based on previous research [37,70,71]. Based on previous studies and commercially available standard compounds, the study explored changes in phenolic content in rosa during fermentation. The current research delves into the antioxidant components found in rosa, emphasizing the need for quantitative analytical techniques to closely monitor how the levels of these antioxidant agents shift throughout fermentation. Phenolic compounds, which encompass flavonoids, phenolic acids, and anthocyanins, stand out as superior antioxidants. In this study, we focused on analyzing the relative changes in the antioxidant content of an and R components to identify the main components that showed increased levels after fermentation (Supplementary Table S2).

The conversion of phenolics from various classes during fermentation often yields compounds with enhanced bioactivity compared to their parent compounds [72]. Possibility of speculating on the metabolism of phenolic compounds during in vitro fermentation of *Lactobacillus plantarum* based on changes in content and reports in the literature. Phenolic compounds are called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species, such as singlet oxygen, superoxide free radicals, and hydroxyl radicals [73].

Based on the changes in the relative content of intermediate metabolites (Supplementary Table S3), it can be surmised that the following metabolic pathways may exist for rosa polyphenol compounds during *Lactobacillus plantarum* fermentation.

The significant decrease in procyanidinB2 and epicatechin gallate and a concurrent increase in catechin and epicatechin concentrations could be attributed to the possible metabolic processes involving flavan-3-ols [74]. However, the bioavailability of flavan-3-ols is predominantly influenced by their degree of polymerization; while monomers are readily absorbed, oligomers and polymers require biotransformation by colonic microbiota prior to absorption [75]. Consequently, it is likely that phenolic metabolites rather than the original high molecular weight compounds present in foods are responsible for the health effects associated with flavan-3-ols consumption. Currently, the anti-inflammatory properties of GA have received increasing attention [76]. Furthermore, mounting evidence
suggests that PA may emerge as an efficacious and safe substance for safeguarding against neurodegenerative disorders [77].

Previously, significant attention has been devoted to investigating the antioxidative properties of flavonoids in vitro, owing to their capacity to mitigate free radical formation and scavenge existing free radicals [78]. Research indicates that bacterial fermentation not only generates flavonoids as bioactive components but also modifies their structure. Specifically, it can deglycosylate, sulfate, or methylate flavonoids, influencing their absorption and metabolism by the liver [79]. Firstly, it causes the structural breakdown of plant cell walls, which results in the release or synthesis of compounds with antioxidant activity. Secondly, fermentation enhances the absorption and bioavailability of these extracts. This enhancement occurs through the production of active ingredients or their conversion into metabolites and by breaking down glycosides to generate smaller molecules, such as ligands for glycosides [80]. During fermentation, some flavonoid glycoside components are transformed into glycosides or small molecule phenolic acids. Many of these products retain the ability to neutralize free radicals, thereby increasing the antioxidant capacity of the fermented samples.

This intricate interplay between flavonoid transformations and the generation of phenolic acids underscores the multifaceted nature of the antioxidative mechanisms involved in the fermentation process. The metabolism of phenolic acids is primarily facilitated by enzymes such as reductases, decarboxylases, glycosidases, and esterases [81]. Further illustrating this process, Ripari et al. observed the metabolism of ferulic acid by Lactobacillus plantarum and Lactobacillus hammersii into vinyl guaiacol, dihydroferulic acid, and ethyl guaiacol. The conversion was driven by the action of several enzymes, including phenolic acid esterase, hydroxycinnamic acid decarboxylase, hydroxycinnamic acid reductase, and vinyl phenol reductase [82]. Previous studies revealed that a high intake of flavonoids from fruits and vegetables may protect against oxidation, inflammation, and chronic disease [83].

Gathering information from in vitro, peonidin-based anthocyanin monomers and purple sweet potato anthocyanins have been reported to induce a significant increase in the numbers of Lactobacillus acidophilus [84]. Furthermore, it is noteworthy that the fermentation process could result in the extensive degradation of most anthocyanins into various phenolic acids. Compared to the intact anthocyanins, the anthocyanin metabolites, which are highly bioavailable, are known to display high anti-oxidative potential. Based on the previous works of literature, it can be surmised that anthocyanins could have been degraded into phenolic acids during LAB fermentation [85]. Intriguingly, some of these phenolic acids retained radical-scavenging capabilities, thereby contributing to the overall antioxidative potential observed in the fermented samples. It has been reported that VA might have a variety of biological activities, such as cardiovascular diseases, anti-inflammatory, and antioxidant [86]. Several pieces of evidence indicated that CGA demonstrates very valuable actions involving anti-inflammation and anti-oxidants [87]. It has found that syringic acid was O-demethylated step by step to gallic acid followed by ring cleavage [88]. Moreover, SA possesses antioxidant, antimicrobial, and anti-inflammatory properties, serving as an effective scavenger of free radicals and mitigating oxidative stress markers. Hence, there is potential for applying metabolic engineering to enhance the content of diverse phenolic classes and bioactivities in food. Although this study has quantified representative 17 compounds in rosa and fermented rosa, the variety of bioactive substances detected from rosa and fermented rosa was limited because some minor or less common phenolic compounds are commercially unavailable or difficult to isolate in large quantities. Hence, future research should be expanded to encompass a wider array of studies, concentrating on the variations in numerous phenolic compounds from rosa and the dynamic observation of intermediate metabolites throughout the metabolism process of in vitro fermentation. Therefore, fermented rosa with various phenolic compounds holds great promise for potential free radical scavenging activities; however further investigations are needed to explore underlying mechanisms.
Further research into the specific interactions and molecular mechanisms by which rosa phenolics exert their antioxidant effects can help in the development of therapeutic strategies for managing oxidative stress and its associated disorders. In this work, the antioxidant properties of five different phenolic compounds from fermented rosa were evaluated using density functional theory at the B3LYP/6-31 + G (d, p) level in aqueous solution. The results of this study revealed that all analyzed compounds display significant antioxidant capabilities. This indicates their potential role in mitigating oxidative stress by neutralizing harmful radicals, thereby contributing to the preservation of cellular integrity and function.

The impact of blueberry phytochemicals on oxidative stress has been assessed across various cell models, encompassing neurons, fibroblasts, and enterocytes [89–91]. The toxicity of the samples to both cell lines is evaluated by MTT and oxidative stress induction conditions were determined. We verified the antioxidant effects of FR using TBHP induced oxidative stress injury model. This emphasis is reflected in our discussion of the distinct concentrations and their corresponding antioxidant activities, highlighting the significant differences observed among the groups in Figure 4. This assay is similar to the Wei et al., used to analyze the difference in activity before and after fermentation [92].

It is believed that oxidative stress ends up causing free radicals to attack lipids, resulting in the generation of toxic lipid aldehydes, such as malondialdehyde and 4-hydroxynonenal [93]. Not surprisingly, the high MDA content was observed in TBHP-damaged Caco2 and HT-29 cells, suggesting that the induction of oxidative cellular damage was successful. In addition, both SOD and CAT are important antioxidant enzymes that play a key role in maintaining the balance of oxidative stress within organisms and protecting cells from free radical damage. Glutathione (GSH) plays a vital role by providing antioxidant protection, through glutathione peroxidase-catalyzed reactions involving reductive removal of ·OH, organic peroxides like lipid hydroperoxides, and peroxynitrite [94]. Glutathione peroxidases detoxify peroxides via a reaction coupled with GSH oxidation into glutathione disulfide (GSSG), while reduced GSH is regenerated by glutathione reductase along with cofactor NADPH [95]. The results also revealed that FR and R were able to improve the antioxidant capacity of the Caco2 and HT-29 cells by increasing the activities of SOD, CAT, GSH-Px, and GR. It was similar to the finding of Mehdi et al., which showed that solid-state fermentation with Lactobacillus reuteri and Lactobacillus plantarum improved the phenolic compounds and antioxidant activity of corn bran [96].

Recent investigations have also considered the therapeutic potential of targeting the Nrf2 pathway in diseases characterized by oxidative stress and inflammation. To investigate whether Nrf2 transcriptionally modulates the antioxidant activity of fermented rosa, total RNA was extracted from Caco2 and HT-29 cells to detect changes in Nrf2 mRNA expression levels using qRT-PCR. It was observed that TBHP stimulation primarily downregulated the antioxidant pathway mediated by Nrf2, which serves as compelling evidence for demonstrating the protective mechanism of FR on Caco2 and HT-29 cells. Indeed, deeper in vivo studies should be conducted to explore the antioxidant activity of fermented rosa and to elucidate its antioxidant mechanism.

5. Conclusions

In conclusion, the present study demonstrated that fermented rosa exhibited protective effects against oxidative damage. Moreover, the altered levels of flavonoids, total phenols, and anthocyanins indicated their degradation and consumption by Lactobacillus plantarum, resulting in enhanced abilities of rosa to scavenge reactive free radicals. Notably, high concentrations of phenolic acids were observed during fermentation, with gallic acid, 4-hydroxybenzoic acid, protocatechuic acid, and vanillic acid being the predominant metabolites. A DFT-based evaluation of antioxidant properties was conducted for several compounds in rosa that exhibited significant variability in content during Lactobacillus plantarum fermentation. These findings suggested that rosa enhanced antioxidant enzyme activity during fermentation to bolster cellular defense against oxidative stress. Addition-
ally, fermented rosa exerted its protective activity on cells through activation of the Nrf2 signaling pathway. Nevertheless, more extensive studies should be conducted to explain the relationship between phenolic compounds and antioxidant properties. Collectively, these results highlighted the potential use of fermented rosa as a source of antioxidants in functional foods while providing insights into *Lactobacillus plantarum* metabolism and facilitating the development of novel postbiotic products.

**Supplementary Materials:** The following supporting information can be downloaded at: [https://www.mdpi.com/article/10.3390/fermentation10070368/s1](https://www.mdpi.com/article/10.3390/fermentation10070368/s1), Table S1: Tentative identification of the chemical constituents of Rosa by UHPLC-Q-qQ-MS/MS under negative and positive ionization; Table S2: The relative quantification of differential metabolites in rosa and fermented rosa (n = 3. Data are presented as mean ± SD); Table S3: The relative quantification of intermediate in microbial metabolite (n = 3. Data are presented as mean ± SD); Table S4: Computed global chemical reactivity descriptors (eV) of the studied C3G, PB2, CATE, GA, PA (water); Figure S1: Molecular structure of the main phenolic compounds; Figure S2: Probable routes associated with different antioxidant mechanisms; Text S1: The relevant equation of the mechanisms.

**Author Contributions:** Data curation, Z.H.; Formal analysis, J.L. (Junxiang Li); Methodology, H.Y., Y.M. and Y.L.; Project administration, C.Z.; Software, S.W.; Writing—original draft, J.L. (Jiaru Li). All authors have read and agreed to the published version of the manuscript.

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**Abbreviation**

LAB, lactic acid bacteria; R, rosa; FR, fermented rosa; UHPLC-QqQ-MS/MS, triple quadrupole tandem mass spectrometry; DFT, density function theory; ROS, reactive oxygen species; RNS, reactive nitrogen species; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ·OH, hydroxyl free radical; ·O₂−, superoxide anion; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; GR, glutathione reductase; TBHP, tert-butyl-hydrogen peroxide; LQC, low-quality control; HQC, high-quality control; LOD, limit of detection; LOQ, limit of quantification; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; DMSO, dimethyl sulfoxide; C3G, Cyanidin-3-O-glucoside; GA, Gallic acid; 4-HBA, 4-hydroxybenzoic acid; PA, protocatechuic acid; VA, vanillic acid; SA, syringic acid; CA, caffeic acid; FA, ferulic acid; CGA, chlorogenic acid; BDE, bond dissociation enthalpies; IP, ionization potentials; PDE, proton dissociation enthalpies; PA1, proton affinities; ETE, electron transfer enthalpies; GSH, Glutathione; traditional Chinese medicines, TCMs.

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