Antioxidant Activity of *Vitis davidii* Foex Seed and Its Effects on Gut Microbiota during Colonic Fermentation after In Vitro Simulated Digestion

Huiqin Ma 1, Aixiang Hou 1,2, Jiaojiao Tang 1, Aiai Zhong 1, Ke Li 1,2, Yu Xiao 1,3,* and Zongjun Li 1,2,*

1 College of Food Science and Technology, Hunan Agricultural University, Changsha 410128, China  
2 Hunan Province Key Laboratory of Food Science and Biotechnology, Changsha 410128, China  
3 Key Laboratory of Ministry of Education for Tea Science, College of Horticulture, Hunan Agricultural University, Changsha 410128, China  
* Correspondence: yuxiao_89@163.com (Y.X.); hnlizongjun@163.com (Z.L.); Tel.: +86-731-8461-7007 (Z.L.)

**Abstract:** *Vitis davidii* Foex whole seed (VWS) is a by-product during the processing of grape products, which is rich in bioactive compounds that have great potential in the food industry. In this study, the bioactive compounds and antioxidant activity of VWS were determined, and their dynamic changes during in vitro colonic fermentation were also investigated after VWS subjected to in vitro simulated digestion. Results showed that VWS were rich in polyphenols (23.67 ± 0.52 mg GAE/g), flavonoids (13.13 ± 1.22 mg RE/g), and proanthocyanidins (8.36 ± 0.14 mg CE/g). It also had good DPPH and ABTS radical scavenging activity, which reached 82.10% and 76.10% at 1000 µg/mL. The alteration trend of the antioxidant activity during in vitro fermentation for 24 h was consistent with that of the content of bioactive substances, such as polyphenols, with the extension of fermentation time. The bioactive compounds and antioxidant activity showed a trend of increasing and then decreasing, reaching the highest value at 8 h. The high-throughput sequencing analysis of the regulatory effect of VWS on intestinal micro-organisms revealed that VWS influenced intestinal microbiota diversity. The relative abundance of beneficial microbiota, such as *Blautia* and *Parabacteroides*, increased by 4.1- and 1.65-fold after 24 h of fermentation compared with that of the control group. It also reduced *Escherichia-Shigella* by 11.23% and effectively reduced host inflammation, while increasing the contents of acetic acid, propionic acid, and other metabolites. Taken together, these results reveal the value of VWS utilization and provide new insights into the nutritional and microbiota modulation effects of VWS, which could therefore serve as a nutraceutical ingredient in health promotion.

**Keywords:** *Vitis davidii* Foex seed; bioactive substances; antioxidants; in vitro digestion; in vitro fermentation; short-chain fatty acids

1. Introduction

*Vitis davidii* Foex is a grape species belonging to Vitaceae, mainly distributed in Shaanxi Province, Gansu Province, and Central, Southern, and Southwestern China. *Vitis davidii* Foex has more juices and flavors than other grape varieties; for this reason, they are more suitable for juice processing, with a juice yield of up to 62%. They have many seeds that account for 4.2% of the total weight of fruits, which is higher than that in ordinary grapes (1.2% of the weight of the seeds) [1]. During winemaking, each kilogram of crushed grapes produces more than 0.2 kg of pomace, which is a major by-product of wineries [2]. However, nutrients in grape pomace, which are also rich in phenolic compounds, are not completely extracted during winemaking [3]. Grape seeds make up approximately 25% of pomace and are generally used as an animal feed additive, but it is not an optimal feed because of its low protein content and seasonal restrictions [4]. Moreover, most of the pomace is discarded, contributing to environmental pollution. Therefore, the rational use of grape seeds can avoid the wastage of resources and reduce environmental pollution.
Prebiotics are defined as substrates selectively utilized by the host’s micro-organisms resulting in benefits for metabolic health, the gastrointestinal system, and mental health [5]. Polyphenols are a group of the most extensive metabolites, which have phenolic structural features in nature [6], but because of the low bioavailability of nutrients, such as polyphenols, only a small fraction is directly absorbed by the small intestine, and up to 90% of these compounds are retained in the colon and metabolized by intestinal bacteria [7]. The role of polyphenols in health largely depends on their metabolism, absorption, and bioavailability processes, which are in turn related to the gut microbiota modulation in terms of composition and functionality [8]. Although polyphenols are currently considered to be modulators of gut microbiota composition, the prebiotic effect of each polyphenol may be influenced by the food source and chemical structure of the compound, as well as individual differences in the composition of the gut microbiota [9]. Gut micro-organisms are diverse and numerous; they can communicate not only amongst themselves to transmit information and substances but also with their hosts and can participate in host metabolic processes; thus, they regulate the conversion of important host substances and profoundly influence the host’s immune system and metabolism [10]. Age and body build affect intestinal microbiota, but diet is the main factor influencing changes in intestinal microbiota [11]. Moreover, in vitro models for gastrointestinal digestion and colonic fermentation are becoming more widely used because of the practical, economic, and ethical limitations of in vivo intervention trials [12,13]. For example, the SDS-III monogastric animal bionic digestive system is used to simulate human digestion. This dynamic system can better reflect actual digestion and reduce operational errors than traditional static simulated digestive systems [14]. In 2014, Minekus [15] proposed a generalized and standardized in vitro digestion method incorporating another proposed in vitro colonic fractionated fermentation model, which simulates human digestion and absorption; through this method, food metabolism and bioavailability can be comprehensively studied [16]. This in vitro model has been successfully applied to evaluate changes in the functional properties of chickpeas, oranges, tomatoes, and peanuts before and after in vitro fermentation; it has also been used to explore the effects of different foods on the structure of intestinal microbiota [17].

Few studies have investigated the interaction of various components of VWS with intestinal microbiota. Therefore, in this experiment, in vitro gastrointestinal digestion and colonic fermentation models were used to simulate the digestion and absorption of VWS in humans, explore the interaction between VWS and intestinal microbiota, and elucidate the potential effects of the components in VWS on intestinal health through their joint action. This study was also performed to evaluate their potential effects on human health and provide a reference for the utilization of wine by-products.

2. Materials and Methods

2.1. Material and Chemicals

The compounds 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tris (2-pyridyl)-S-triazine (TPTZ), 2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid (vitamin C, Vc), Gallic acid, rutin, catechins, Folin–Ciocalteu’s reagent were purchased from RYON Biotechnology Co. Ltd. (Shanghai, China), other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

V. davidii Foex seeds were taken from a wine factory in Hunan Province in China.

2.2. Sample Preparation and Extraction

The V. davidii Foex seeds were dried and crushed to 80-mesh powder. An appropriate amount of VWS was then weighed in an Erlenmeyer flask, and ethanol: water (40:60, v/v) at a ratio of 1:12 was added, then extracted with ultrasound at a frequency of 40 kHz at 45 °C for 55 min, and the filtrate was obtained by filtration, repeated five times. The obtained filtrate was concentrated with a rotary vacuum evaporator at 40 °C to eliminate the solvent, posteriorly freeze-dried, and stored at −20 °C until use.
2.3. Determination of Total Phenolics, Flavonoids and Oligomeric Proanthocyanidins Content

The lyophilized powder was dissolved in ethanol aqueous (60%, v/v) as a compound solution with a concentration of 1 mg/mL. Total phenolic content (TPC) was determined via the Folin–Ciocalteu method [18,19]. A standard curve was established by preparing a concentration gradient of gallic acid, and results were expressed as gallic acid equivalents per gram VWS (mg GE/g).

Total flavonoid content (TFC) was determined via the color development method using aluminum nitrate [20]. The standard curve was established by preparing a concentration gradient of rutin, and results were expressed as rutin equivalents per gram VWS (mg RE/g).

Oligomeric proanthocyanidin content (OPC) was determined via the vanillin-sulphuric acid method [21]. First, 2.5 mL of 1% vanillin-methanol solution and 2.5 mL of 32% concentrated sulphuric acid methanol solution were added to 1 mL of the compound solution, mixed well, and left at 32 °C for 20 min. Then, absorbance was measured at the wavelength of 500 nm. A catechin concentration gradient was prepared to establish a standard curve, and test results were expressed as catechin equivalents per gram VWS (mg CE/g).

2.4. Antioxidant Capacity Assay

The in vitro antioxidant capacity of the samples was determined using the method of Liu et al. [22,23], and grape seed extracts were prepared at concentrations of 200, 400, 600, 800, and 1000 µg/mL. Equal amounts of DPPH solution were added to the extracts and the scavenging activity of DPPH radicals was measured at 517 nm; ABTS stock solution was prepared and added to the samples in a 4:1 ratio to obtain ABTS radical cation scavenging activity at 734 nm; hydroxyl radical scavenging activity was measured by salicylic acid method; ferric reducing antioxidant power was measured by reducing Fe^{3+}-TPTZ. Positive control was established by preparing ascorbic acid solution with the same concentration gradient.

2.5. In Vitro Digestion and In Vitro Colonic Fermentation

2.5.1. In Vitro Digestion

The digestion of the oral cavity, stomach, and small intestine was simulated according to the optimized method of Brodkorb et al. [24]. Digestion was carried out in an oral-gastric-small intestine simulator with automatic enzyme addition and automatic cleaning to reduce systematic errors caused by manual operation.

First, the VWS were mixed with simulated saliva (75 U/mL salivary amylases) at 1:1 and shaken at pH 7 for 2 min. The oral digest was then diluted with simulated gastric fluid (2000 U/mL pepsin) at 1:1 (v/v), and digestion was continued at pH 3 for 2 h. The gastric digest was diluted with simulated intestinal fluid at 1:1 (v/v) and incubated with bile salts and trypsin (at 100 U/mL of trypsin) at pH 7 for 2 h. At the end of in vitro digestion, the supernatant and precipitate of the digested samples were separated. The precipitate was lyophilised and stored separately from the supernatant in a refrigerator at −20 °C to obtain the test group digest. Because it is known that (on average) 10% of the supposedly absorbable fraction in the large intestine is actually not absorbed, 10% of all the fermentation supernatant is taken and mixed with all the precipitated lyophilised material to obtain a mixed sub-strate for in vitro fermentation [23]. Under the same conditions, digests without VWS were prepared as blank group digests (Figure 1).
In vitro colonic fermentation was simulated in accordance with previously described methods [25] with slight modifications. Fresh whole stools were collected from subjects (two females and one male, who had not taken antibiotics within 3 months before collection of fecal samples), processed, and fermented in an anaerobic incubator.

A sufficient amount of basal growth medium was prepared (peptone water 2 g/L, yeast extract 1 g/L, NaCl 0.1 g/L, K2HPO4 0.04 g/L, KH2PO4 0.04 g/L, MgSO4⋅7H2O 0.01 g/L, CaCl2⋅2H2O 0.01 g/L, NaHCO3 2 g/L, bile salts 0.5 g/L, L-cysteine hydrochloride 0.5 g/L, hemin 50 mg/L, vitamin K1 10 μL/L, and Tween 80 2 mL/L). The fecal samples of the three volunteers were mixed equally, weighed, vortexed, shaken in sterile PBS buffer for 3 min, mixed again, and filtered through four gauze layers to obtain a 10% fecal suspension. Next, 100 mL of PBS mixture was added to a reagent bottle containing 900 mL of sterile nitrogen-containing basal medium. After the resulting medium was vortex-shaken for 3 min and mixed, the mixed medium containing the intestinal microbiota was obtained. The medium was divided into two portions; one was added with 5 mL of the digest of the control group (group C) and the other was added with 5 mL of the digest mixture prepared in Section 2.5.1 and vortex-shaken for 3 min. After they were thoroughly mixed, the fermentation samples of the control group (group C) and the test group (group GS) were obtained. Groups C and GS were divided into 50 tubes of 10 mL/tube under anaerobic conditions and placed in an incubator at a constant 37 °C for static anaerobic fermentation. Subsequently, 10 tubes of fermentation broth were taken and stored at −80 °C at 0.5, 4, 8, 12, and 24 h of incubation, for the next experiments. In vitro fermentation samples of 0.5, 4, 8, 12, and 24 h were taken from groups C and GS. For group C, these samples were labeled as control 0.5, control 4, control 8, control 12, and control 24 (C0.5, C4, C8, C12, and C24, respectively). For group GS, they were named grape seed 0.5, grape Seed 4, grape seed 8, grape seed 12, and grape seed 24 (GS0.5, GS4, GS8, GS12, and GS24, respectively). Thus, the samples were prepared to determine their TPC, TFC, OPC, and antioxidant properties via the same method as outlined in Sections 2.3 and 2.4.
2.6. DNA Extraction and Sequencing

The genomic DNA of the microbial community was extracted from samples by using an EZNA® Soil DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) in accordance with the manufacturer’s instructions. The quality of the DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

The V3–V4 hypervariable region of the bacterial 16S rRNA gene were amplified with the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and 1737F(5′-GGAAGTAAAAGTCGTAACAAGG-3′) and 2043R (5′-GCTGCGTTCTTCATCGATGC-3′) by using an ABI Gene Amp® 9700 PCR thermos cycler (Applied Biosystems, Foster City, CA, USA). The PCR amplification was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, single extension at 72 °C for 10 min, and final incubation at 4 °C. The PCR mixtures were composed of 4 µL of 5 × TransStart FastPfu buffer, 2 µL of 2.5 mM dNTPs, 5 µM of each primer (0.8 µL), 0.4 µL of TransStart FastPfu DNA Polymerase, 10 ng of template DNA, and 20 µL of ddH2O. PCR was performed in triplicate, and PCR products were extracted from 2% agarose gel, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer’s instructions and quantified using a Quantus™ fluorometer (Promega, Madison, WI, USA).

The purified PCR products were pooled at equimolar ratios and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, CA, USA) in accordance with the standard protocols supplied by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0, and merged using FLASH version 1.2.7. Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed using RDP Classifier (version 2.2) against the 16S rRNA database (Silva v138) at a confidence threshold of 0.7.

2.7. Analysis of pH Values and SCFAs Production

The fermentation broth of groups C and GS removed at 0.5, 4, 8, 12, and 24 h of fermentation and its pH was measured with a pH meter.

The SCFAs content of the samples at different fermentation times was determined via gas chromatography-mass spectrometry (GC-MS). The supernatant of colonic fermentation was mixed with 25% metaphosphoric acid solution at 4:1, vortexed for 2 min, centrifuged at 10,000 rpm for 5 min and filtered through a 0.22 µm microporous membrane.

The chromatographic conditions were as follows: chromatographic column, DB-FFAP gas chromatographic column (30 m × 250 µm × 5 µm); carrier gas, 99.99% high-purity nitrogen at a flow rate of 0.8 mL/min; and auxiliary gas, 99.99% high-purity hydrogen. The FID detector temperature, inlet temperature, splitting ratio, and injection volume were 280 °C, 250 °C, 5:1, and 1 µL, respectively. For the programmed temperature increased, initial temperature was set at 60 °C. Then, this temperature was increased to 220 °C at a rate of 20 °C/min and maintained for 1 min. Mass spectrometry conditions were set as follows: voltage of 70 eV, ion source temperature of 230 °C, mass scan range of m/z 28–300, and electron ionization (EI) mode. The mass spectra of the compounds were compared with the NIST17.L mass spectrometry database, and compounds with a match greater than 80% were extracted for analysis.

2.8. Statistical Analysis

Experimental data were the means of three replicates, and results were expressed as mean ± standard deviation. SPSS 26.0 software was used for statistical analysis, Duncan’s method was used for multiple comparisons, and Origin 2018 and GraphPad Prism 6.01 were used for graphing.
3. Results and Discussion
3.1. TPC, TFC, OPC, and Antioxidant Activity of VWS

The TPC, TFC, and OPC in VWS were $23.67 \pm 0.52$ mg GAE/g, $13.13 \pm 1.22$ mg RE/g, and $8.36 \pm 0.14$ mg CE/g, respectively (Figure 2). The polyphenol content in grape seeds varies widely from 12 mg/g–113 mg/g amongst grape seed varieties [26,27]. Song [28] also analyzed the TPC of different varieties of grape seeds and found that European grapes have the highest polyphenol content (103 mg/g), whereas V. davidii Foex and Vitis quinquangularis Rehd have the lowest polyphenol contents (37.1 and 35.6 mg/g, respectively). Makris extracted proanthocyanidins from wine-produced grape seeds and found that the OPC of fresh and wine-produced grapes are 27.0–43.3 and <10 mg/g, respectively. Similar to OPC, TPC and TFC are also reduced by winemaking [29]. In the present study, because the samples were obtained from V. davidii Foex winery by-products, the TPC, TFC, and OPC were lower than reported in the literature.

![Active substance content of VWS.](image)

Figure 2. Active substance content of VWS.

The antioxidant activity of VWS is shown in Figure 3A–D. VWS extract showed a good scavenging ability of DPPH radicals, reaching more than 50% at a sample concentration of 400 µg/mL. When the concentration was 800 µg/mL, the DPPH radicals scavenging ability of VWS was $80.70 \pm 0.35\%$, which was similar to Vc at the same concentration ($88.64 \pm 1.53\%$). This change gradually leveled off, and the scavenging ability was no longer enhanced. In Figure 3B, the scavenging power of VWS for ABTS radical cation increased as the mass concentration increased and showed a good linear relationship. The scavenging rate was $76.10 \pm 0.57\%$ when the concentration was 1000 µg/mL, and the scavenging power of ABTS radical cation likely increased by increasing the concentration of the sample. The trend of the reducing power of ferrous ions is presented in Figure 3C. In the test concentration range, VWS had a weak reducing power of up to $266.19 \pm 5.37$ µmol/L only for ferrous ions, and the reducing power of Vc at the same concentration was six times higher than $266.19 \pm 5.37$ µmol/L. In Figure 3D, the VWS extract did not elicit a strong scavenging effect on hydroxyl radicals in the tested concentration range. The scavenging ability of hydroxyl radicals was also $14.27 \pm 1.15\%$ only at the maximum sample concentration of 1000 µg/mL. Some studies have shown that the scavenging ability of hydroxyl radicals depends on polyphenol type rather than its content [30]. Gülçin et al. [31] found that the position and number of hydroxyl and carbonyl groups in a polyphenol molecule affect its iron-chelating ability; furthermore, addition of hydroxyl and carbonyl groups to the 3′-, 4′-, and 5′-positions of the B-ring enhances its antioxidant capacity compared with that of single hydroxyl groups. Yıldırım et al. [32] found that most of the phenolic compounds found in grapes can act as antioxidants and that the contents of catechins, epicatechin, and proanthocyanidin B2 have a highly significant positive correlation with in vitro antioxidant capacity ($p < 0.01$) [33]. Lv et al. [34] reported the antioxidant activities of 32 varieties of litchi and found that the higher the OPC, the
stronger the free radical scavenging activity; therefore, the mechanism of antioxidants in litchi may involve free radical scavenging by catechins, epicatechin, and proanthocyanidins, the aggregates formed by catechin and epicatechin. Moreover, flavonoids have a significant free radical scavenging ability and can exert antioxidant activity by chelating variable metal ions [35]. These findings suggested that the antioxidant capacity of VWS is high possible because they are rich in phenolics, flavonoids, and proanthocyanidins.

![Figure 3. In vitro antioxidant activity of VWS.](image)

Although grape seeds, as a by-product of winemaking, are not as rich in bioactive substances as fresh grape seeds, their TPC and TFC are in the middle to upper range compared with fruits, such as popcorn, wolfberry, and banana [36,37]. Babbar [38] compared the TPC of six fruit by-products and found that grape seeds contain 37.4 mg GAE/g of total phenols, which is 10 times more than in citrus seeds and banana peels. Overall, VWS are simple to obtain as an oenological by-product of wine, and their functional activity is not inferior to that of some fresh fruits. Moreover, their bioavailability and true benefits in vivo can be further determined through in vitro and in vivo tests.

### 3.2. Effect of In Vitro Fecal Fermentation on the Dynamic Change of TPC, TFC, and OPC

Phytochemicals undergo great alteration during gastrointestinal digestion. To exert their health effects, the bioactive compounds of VWS must be available in target tissues; thus, the biological activities of VWS may depend on their absorption and bioavailability in the intestinal tract. Thus, the effects of in vitro colonic fermentation on bioactive compounds and the antioxidant capacity of digested VWS were investigated. During in vitro fermentation, the TPC, TFC, and OPC initially decreased and then increased with the extension of fermentation time; after 8 h of in vitro fermentation, their highest values were obtained and showed a 45.45%, 42.61%, and 165.51% increase compared with those at the initial stage, respectively (Table 1). Then, these contents gradually decreased. Dur-
ing the fermentation stage for 0.5–8 h, TPC, TFC, and OPC showed an overall increasing trend probably because covalent, hydrogen, and hydrophobic bonds between the bound polyphenols and cell wall components in VWS are broken due to microbial action during microbial fermentation and released in their free states; as a result, these contents significantly increase [39]. At 8 h of colonic fermentation, all polyphenols were released and possibly decomposed by colonic micro-organisms as the fermentation time was extended; consequently, their content decreased. When the fermentation time reached 24 h, all the active substances decreased and reached the lowest value. Research by de Almeid [40] found that the free phenol content initially increases significantly \( (p < 0.05) \) after 4 h of fermentation and decreases significantly \( (p < 0.05) \) after 24 h of fermentation compared with those in the pre-fermentation period. Gowd [41] investigated the effect of in vitro fermentation on waxberry and found that the TPC and TFC initially decrease with the extension of fermentation time, subsequently increase to their maximum values, and gradually decrease; the antioxidant capacities of the samples changed with the active substances and decreased to the lowest values after 24 h of fermentation, similar to the results of this experimental study. Furthermore, the TPC of plant extracts from different sources gradually decreases after 24 h of in vitro fermentation [42].

Table 1. Changes in the total phenol, total flavonoid, and procyanidin contents during in vitro fermentation.

| Fermentation Time/h | TPC µg GAE/mL  | TFC µg RE/mL  | OPC µg C/mL  |
|---------------------|----------------|---------------|---------------|
| 0.5                 | 69.44 ± 0.22 b | 254.60 ± 1.33 b | 19.05 ± 0.39 c |
| 4                   | 47.56 ± 1.74 c | 94.81 ± 0.69 e | 7.67 ± 0.40 e |
| 8                   | 101.06 ± 0.62 a | 363.09 ± 1.23 a | 50.58 ± 1.68 a |
| 12                  | 15.31 ± 0.49 d | 230.86 ± 1.43 c | 37.14 ± 2.16 b |
| 24                  | 13.11 ± 0.31 d | 134.20 ± 0.97 d | 12.99 ± 1.56 d |

Data expressed as mean ± standard deviation \( (n = 3) \). Different letters in the same column of data indicate significant differences between groups \( (p < 0.05) \).

3.3. Effect of In Vitro Fermentation on the Change of Antioxidant Activity

The activity of each antioxidant changed significantly during 24 h of in vitro fermentation (Figure 4). In particular, the activity of DPPH was much higher than the rest of the indicators. The result was consistent with the antioxidant capacity of VWS when they were undigested and fermented. At 8 h of fermentation, the DPPH radical scavenging power, ABTS radical cation scavenging power, ferrous ion reducing power, and hydroxyl radical scavenging power of the samples peaked at 12.68 mg Vc/mL, 0.50 mg Vc/mL, 1.96 mmol Fe\(^{2+}\)/mL, and 0.47 mg Vc/mL. Moreover, the antioxidant capacity of the samples decreased and gradually stabilized at 12 h of fermentation. Combined with Table 1, TPC, TFC, and OPC peaked at 8 h of colonic fermentation, indicating that these active ingredients were related to antioxidant activity as the phenolic compounds in the samples started to be released under the action of colonic micro-organisms at the early stage of fermentation; then, the antioxidant capacity increased. With the extension of fermentation time, the polyphenolic substances were decomposed or transformed into small molecule metabolites, leading to a decrease in antioxidant activity. Correa [43] investigated Merlot grape seeds subjected to gastrointestinal digestion and in vitro fermentation and revealed that the antioxidant function of grape seeds and the bioactivity of nutrients, such as polyphenols are closely correlated at different fermentation stages. Del Pino-Garcia [44] confirmed this result and found red wine pomace positively affects the total antioxidant capacity after in vitro gastrointestinal digestion and colonic fermentation. Other studies have also shown that polyphenols and flavonoids are strongly correlated with antioxidant activity [45,46], which is consistent with the pattern of variation identified in the present study.
These findings indicated that VWS increased the richness and diversity of microbiota in the intestine. However, this decrease in richness and diversity can lead to intestinal microbiota dysbiosis, which can cause low inflammation and metabolic diseases; conversely, a rich and diverse intestinal microbiota is more beneficial to health [49].
Table 2. Alpha-diversity values of samples.

| Sample | OTUs  | Chao1    | ACE      | Shannon | Simpson |
|--------|-------|----------|----------|---------|---------|
| C0.5   | 489   | 612.645  | 622.445  | 5.728   | 0.958   |
| C4     | 495   | 609.328  | 608.372  | 5.311   | 0.938   |
| C8     | 321   | 384.683  | 373.15   | 1.764   | 0.318   |
| C12    | 359   | 395.212  | 405.988  | 2.415   | 0.448   |
| C24    | 288   | 337.752  | 334.395  | 1.857   | 0.384   |
| GS0.5  | 532   | 696.779  | 731.194  | 4.961   | 0.912   |
| GS4    | 569   | 710.306  | 745.204  | 4.782   | 0.877   |
| GS8    | 265   | 301.14   | 304.607  | 2.153   | 0.422   |
| GS12   | 272   | 313.288  | 319.05   | 2.072   | 0.39    |
| GS24   | 303   | 345.193  | 346.17   | 3.035   | 0.618   |

Evolutionary clustering was analyzed in terms of LDA Effect Size (LEfSe) to identify statistically significant dominant micro-organisms. Figure 5 showed that the bacterial groups in groups C and GS differed after 24 h of in vitro fermentation; some clusters increased, whereas others decreased. At the phylum level, the relative abundance of Firmicutes and Bacteroidetes was significantly higher in group GS24 than in group C24, conversely, the relative abundance of Proteobacteria was significantly lower. At the genus level, Parabacteroides was more abundant in group GS than in group C, whilst Escherichia-Shigella was more abundant in the group C than in group GS.

Figure 5. LEfSe analysis (A) LEfSe evolutionary branching diagram (red nodes indicate enrichment in C24, while green nodes indicate enrichment in G24) (B) Histogram of LDA value distribution (LDA > 4).

Figure 6A shows the gates with relative abundance greater than 1%. At 0.5 h, Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were the four major clades, with Firmicutes and Proteobacteria accounting for more than 90% of the total sequence reads, consistent with the results of related studies [50]. Figure 6C shows that after 24 h of in vitro fermentation, groups C and GS differed significantly in the gate level for Firmicutes and Proteobacteria. Firmicutes dominated at the early fermentation stage, and its abundance gradually decreased with time. After 24 h of fermentation, only 5.19% and 16.05% relative abundance remained in groups C and GS, respectively, and the value in group GS was three times higher than in group C. Most of the beneficial bacteria in the intestinal microbiota belonged to Firmicutes [51]. The correlation heat map (Figure 7) showed that the genera, such as Butyricoccus, Faecalibacterium, Blautia, and Roseburia, belonging to Firmicutes were positively correlated with TPC and OPC, indicating that grapes have a promotional effect...
on the growth and reproduction of these beneficial bacteria. Gil-Sánchez [52] studied the effect of grape seed polyphenols on human intestinal microbiota and found an increase in the main bacteria during fermentation compared with that in the control group; these findings were consistent with the correlation analysis of the present study. During fermentation, the abundance of Proteobacteria increased and then decreased in groups C and GS; specifically, it increased to 83.59% at 8 h and then decreased to 79.28% at 24 h in group C. Group GS showed the same trend as group C but decreased to 64.28% at 24 h, which was slightly lower than the abundance in group C. TPC and OPC were negatively correlated with Proteobacteria and Bacteroidetes, indicating that the active substances in VWS inhibited the growth of Proteobacteria and Bacteroidetes. The increased abundance of Proteobacteria reflects the unstable structure of the intestinal microbial community and serves as a potential diagnostic criterion for diseases that may trigger inflammation [53]. Aura [54] also found that polyphenols elicit a significant inhibitory effect on the growth of Proteobacteria, and the intake of moderate amounts of polyphenols may reduce the risk of colon cancer. In the present study, the abundance of Proteobacteria in group GS was significantly lower than in group C. Therefore, VWS could reduce the increase in the abundance of Proteobacteria and alleviate host intestinal inflammation.

Figure 6. Changes in the relative abundance of intestinal microbiota. (A) Species distribution map at the phylum level; (B) species distribution map at the genus level; (C) analysis of differences in microbial composition at the phylum level for 24 h in vitro fermentation; (D) analysis of differences in microbial composition at the genus level for 24 h in vitro fermentation (p < 0.05).

Figure 6B shows the distribution of the relative abundance greater than 1% at the genus level. Specifically, the intestinal microbiota was mainly composed of Escherichia-Shigella, Faecalibacterium, Romboutsia, Blautia, and other genera. Amongst them, Escherichia-Shigella dominated in the middle and late fermentation stages, peaked at 82.49% in group C8, and decreased to 72.28% after 24 h. Its abundance in group GS was significantly lower than in group C (Figure 6D). After 24 h of in vitro fermentation, its relative abundance in group GS was 61.05%, which was 11.23% lower than in group C. Pasqua [55] found that plant polyphenols can increase the permeability of bacterial cell membranes, causing intracellular ATP efflux and consequently acting as an antibacterial agent. Figure 7 TPC and OPC are negatively correlated with Escherichia-Shigella, indicating that grape seeds inhibit the growth of Escherichia-Shigella and attenuate inflammatory responses, ulcers, and hemorrhagic or mucus diarrhea [56]. The potential prebiotic effect of VWS on fecal bacteria was mainly reflected in the promotion of the growth of Faecalibacterium [57], in the present study, its percentage was low in all fermentation stages in group C, and its maximum
value was only 8.32%; however, this percentage significantly increased in group GS, and the maximum value was 29.98%. In addition to Escherichia-Shigella and Faecalibacterium, Romboutsia, Blautia, and Parabacteroides exhibited more obvious variations. Romboutsia in both groups C and GS decreased, i.e., from 13.43% at 0.5 h to 1.22% at 24 h in group C. In group GS, the percentage decreased from 16.67% to 2.90% after 24 h of fermentation; this trend in group GS was almost similar to group C but with a slightly higher overall content. Blautia and Parabacteroides were effective weight-loss bacteria [58]; after 24 h of fermentation, their relative abundance in group GS increased by 4.1- and 1.65-fold, respectively, compared with group C. These results suggested that VWS might play a role in obesity symptoms. In summary, VWS have a more obvious effect on the growth of beneficial bacteria in the intestinal tract, and their active substances, such as polyphenols and proanthocyanidins, can regulate the balance of intestinal microbiota and maintain colon health.

Figure 7. Heatmap of the correlation between TPC, TFC, and OPC in group GS and changes in intestinal micro-organisms during fermentation.

3.5. Effect of VWS Digest on pH and SCFAs Production during In Vitro Fermentation

Acidic substances, such as SCFAs, produced through the fermentation of human intestinal micro-organisms decrease intestinal pH; therefore, the fermentation of intestinal micro-organisms and changes in their components can be indirectly reflected by changes in intestinal pH [17]. In Figure 8, during 24 h of in vitro fermentation, the pH in groups GS and C decreased and then increased; at 12 h, the lowest pH was obtained. The decrease in the pH of the fermentation broth was mainly the result of acid production from substrate fermentation; during fermentation, intestinal micro-organisms grew rapidly in the first 12 h and accumulated a large number of metabolites, which caused the decrease in pH. Conversely, the nutrients were gradually consumed at the later fermentation stage, and microbial growth was restricted; conversely, pH rebounded at the later stage, but the changes did not fluctuate greatly. Human intestinal pH in the range of 6–7 is beneficial to health [59]. The pH in group GS significantly decreased at all stages compared with that in group C, probably because the nutrients in VWS promoted the growth of intestinal micro-organisms and increased acid production from metabolism; thus, pH was affected. Consistent with the present results, previous findings on extracted ginger polyphenols for
in vitro fermentation showed that pH decreases as fermentation time is prolonged [60]. Active ingredients, such as polyphenols and flavonoids, undergo microbial fermentation in the intestine to produce SCFAs, such as acetic acid and propionic acid, thus decreasing pH [61].

The main metabolites of carbohydrates fermented by intestinal micro-organisms during in vitro fermentation are SCFAs, whose production is related to the abundance and structure of intestinal microbiota [62]. SCFAs mainly include formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, and valeric acid; amongst them, the most abundant are acetic acid, propionic acid, and butyric acid, which can effectively inhibit the production of pro-inflammatory cytokines, enhance mucin expression, and maintain the immune function of the intestinal barrier [63]. At each fermentation stage, the contents of formic acid, acetic acid, propionic acid, and butyric acid increased substantially in group GS compared with those in group C (Figure 9); after 24 h of fermentation, SCFAs in group GS were 78.33%, 14.16%, 19.2%, and 139.31% higher than those in group C, respectively. Figure 9A–C demonstrate that the contents of formic acid, acetic acid, and propionic acid in the fermentation broth were low at 0.5–4 h of fermentation; at this time point, formic acid was not detected because of its low content. With the extension of fermentation time, formic acid, acetic acid, and propionic acid were produced in large quantities in each group, and their contents increased significantly, which was consistent with the trend shown by pH at different times. Butyric acid concentration gradually decreased (Figure 9D). Butyric acid is mainly produced by thick-walled bacteria, Faecalibacterium and Romboutsia detected in this study are typical butyric acid-producing bacteria [64]; therefore, a decrease in butyric acid content may be related to the substantial decrease in the relative abundance of such bacteria at the late fermentation stage. Amongst the individual SCFAs produced, acetic acid had the highest content, followed by propionic acid, which was closely related to certain strains producing SCFAs; carbohydrate-metabolizing bacteria, such as Bacteroidetes, produce acetic acid, which is consistent with the findings of Wang et al. [60]. Furthermore, polyphenols can induce an increase in SCFAs [65]. Martin [66] fed grape pomace to rats and found that it produces higher levels of acetic acid, propionic acid, and butyric acid than in the control group after 24 h. The growth and reproduction of numerous beneficial bacteria largely increase the concentration of SCFAs, which contribute to the acidification of the in vitro fermentation environment; this observation is also consistent with the changes in pH. In turn, the acidification of the colonic environment can promote the proliferation of
beneficial bacteria and inhibit the growth of pathogenic bacteria. These processes play a key role in VWS-induced improvement of human intestinal health.

![Image](image_url)

**Figure 9.** Change of SCFAs concentration during in vitro fermentation. (A) Formic acid concentration; (B) Acetic acid concentration; (C) Propionic acid concentration; (D) Butyric acid concentration. Means with different letters in figures were significantly different at $p < 0.05$, *$p < 0.05$.

4. Conclusions

In this study, the effects of in vitro colonic fermentation on bioactive compounds and the antioxidant capacity of digested VWS were investigated. Furthermore, the effect of digested VWS on modulation of microbial community structure and SCFAs content were also studied. The results showed that the TPC, TFC, OPC, and antioxidant activity of in vitro digested VWS changed greatly during the colonic fermentation process. At 8 h, the TPC, TFC, and OPC increased by 45.45%, 42.61%, and 165.51%, respectively, compared with those at the initial fermentation stage. Thereafter, they gradually decreased as the fermentation progressed. The change of antioxidant activity is in accordance with the bioactive compounds. Furthermore, VWS have great effect on intestinal micro-organisms and modulates the structure of microbiota. The abundance of Escherichia-Shigella in group GS decreased by 11.23% compared with group C after 24 h of fermentation. VWS inhibited the growth and reproduction of harmful microbiota and increased the relative abundance of beneficial bacteria, such as *Faecalibacterium*, *Romboutsia*, and *Blautia*, to further adjust intestinal pH and increase SCFAs concentration. In conclusion, this study suggests that VWS holds potential prebiotic properties for human health and provides insights into the potential benefits of grape processing by-products on gastrointestinal and colonic health. It also presents a feasible scientific basis for improving the utilization of VWS in functional foods to increase the use of wine by-products and to reduce environmental pollution caused by their indiscriminate disposal.

**Author Contributions:** Methodology, investigation, writing—original draft, writing—reviewing and editing H.M.; conceptualization, investigation, writing—original draft, writing—reviewing and editing A.H.; visualization, data curation J.T.; formal analysis, visualization A.Z.; software, formal analysis K.L.; supervision, investigation, data curation, writing—original draft, writing—reviewing and editing Y.X.; supervision, writing—reviewing and editing, project administration resources, funding acquisition Z.L. All authors have read and agreed to the published version of the manuscript.
**Funding:** This research was funded by the Hunan Province Natural Science Foundation (No. 2020JJ5243 and No. 2020JJ5233) and the Hunan Province Science and Technology Innovation Program (No. 2021NK4260).

**Institutional Review Board Statement:** Not applicable to this study.

**Informed Consent Statement:** Not applicable to this study.

**Data Availability Statement:** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest and the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

**References**

1. Liang, N.N.; Pan, Q.H.; He, F.; Wang, J.; Reeves, M.J.; Duan, C.Q. Phenolic profiles of *Vitis davidii* and *Vitis quinquangularis* species native to China. *J. Agric. Food Chem.* 2013, 61, 6016–6027. [CrossRef] [PubMed]

2. Duba, K.S.; Fiori, L. Supercritical CO₂ extraction of grape seed oil: Effect of process parameters on the extraction kinetics. *J. Supercrit. Fluids* 2015, 98, 33–43. [CrossRef]

3. Jara-Palacios, M.J.; Hernanz, D.; Cifuentes-Gomez, T.; Escudero-Gilete, M.L.; Heredia, F.J.; Spencer, J.P. Assessment of white grape pomace from winemaking as source of bioactive compounds, and its antiproliferative activity. *Food Chem.* 2015, 183, 78–82. [CrossRef] [PubMed]

4. Hosseini-Vashan, S.J.; Safdari-Rostamabad, M.; Piray, A.H.; Sarir, H. The growth performance, plasma biochemistry indices, immune system, antioxidant status, and intestinal morphology of heat-stressed broiler chickens fed grape (*Vitis vinifera*) pomace. *Anim. Feed. Sci. Teth.* 2020, 259, 11434. [CrossRef]

5. Amorim, C.; Silvério, S.C.; Cardoso, B.B.; Alves, J.I.; Pereira, M.A.; Rodrigues, L.R. In vitro fermentation of raffinose to unravel its potential as prebiotic ingredient. *Lwt* 2020, 126, 109322. [CrossRef]

6. Dong, R.; Yu, Q.; Liao, W.; Liu, S.; He, Z.; Hu, X.; Chen, Y.; Xie, J.; Nie, S.; Xie, M. Composition of bound polyphenols from carrot dietary fiber and its in vivo and in vitro antioxidant activity. *Food Chem.* 2020, 339, 127879. [CrossRef]

7. Espin, J.C.; Gonzalez-Sarrias, A.; Tomas-Barberan, F.A. The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochem. Pharmacol.* 2017, 87, 82–93. [CrossRef]

8. Yang, L.; Sakandar, H.A.; Sun, Z.; Zhang, H. Recent advances of intestinal microbiota transmission from mother to infant. *J. Funct.Foods* 2021, 87, 104719. [CrossRef]

9. Serreli, G.; Deiana, M. In vivo formed metabolites of polyphenols and their biological efficacy. *Food Funct.* 2019, 10, 6999–7021. [CrossRef]

10. Matamoros, S.; Gras-Leguen, C.; Le Vacon, F.; Potel, G.; de La Cochetiere, M.F. Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol.* 2013, 21, 167–173. [CrossRef]

11. Shankar, V.; Gouda, M.; Moncivaiz, J.; Gordon, A.; Reo, N.V.; Hussein, L.; Paliy, O. Differences in Gut Metabolites and Microbiological Composition and Functions between Egyptian and U.S. Children Are Consistent with Their Diets. *Msystems* 2017, 2, e00169-16. [CrossRef] [PubMed]

12. Xie, J.; Sun, N.; Huang, H.; Xie, J.; Chen, Y.; Hu, X.; Hu, X.; Dong, R.; Yu, Q. Catabolism of polyphenols released from mung bean coat and its effects on gut microbiota during in vitro simulated digestion and colonic fermentation. *Food Chem.* 2022, 396, 133719. [CrossRef] [PubMed]

13. Scrob, T.; Covaci, E.; Hosu, A.; Tanaselia, C.; Casoni, D.; Torok, A.I.; Frentiu, T.; Cimpoiu, C. Effect of in vitro simulated gastrointestinal digestion on some nutritional characteristics of several dried fruits. *Food Chem.* 2022, 385, 132713. [CrossRef]

14. Guerra, A.; Etienne-Mesmin, L.; Livrelli, V.; Denis, S.; Blanquet-Diot, S.; Alric, M. Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends Biotechnol.* 2012, 30, 591–600. [CrossRef] [PubMed]

15. Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carriere, F.; Boutrou, R.; Corredig, M.; Dupont, D.; et al. A standardised static in vitro digestion method suitable for food—An international consensus. *Food Funct.* 2014, 5, 1113–1124. [CrossRef] [PubMed]

16. Perez-Burillo, S.; Pastoriza, S.; Jimenez-Hernandez, N.; D’Auria, G.; Francino, M.P.; Rufian-Henares, J.A. Effect of Food Thermal Processing on the Composition of the Gut Microbiota. *J. Agric. Food Chem.* 2018, 66, 11500–11509. [CrossRef]

17. Perez-Burillo, S.; Rufian-Henares, J.A.; Pastoriza, S. Towards an improved global antioxidant response method (GAR+): Physiological-resembling in vitro digestion-fermentation method. *Food Chem.* 2018, 235, 1253–1262. [CrossRef]

18. Rinaldo, D.; Sotin, H.; Pérot, D.; Le-Bail, G.; Guyot, S. Browning susceptibility of new hybrids of yam (*Dioscorea alata*) as related to their total phenolic content and their phenolic profile determined using LC-UV-MS. *LWT* 2022, 162, 113410. [CrossRef]

19. Chen, Y.; Wang, Y.; Chen, J.; Tang, H.; Wang, C.; Li, Z.; Xiao, Y. Bioprocessing of soybeans (*Glycine max L.*) by solid-state fermentation with *Eurotium cristatum*YL-1 improves total phenolic content, isoflavone aglycones, and antioxidant activity. *RSC Adv.* 2020, 10, 16928–16941. [CrossRef]
20. Khan, Y.; Mulik, S.; ul Haq, I.; Farzana, F.; Abdullah, A.; Mahmood, A.; Alami, S.; Hashemi, M.; Sakhi, S.; Asif, M.; et al. Antioxidant potential in the leaves of grape varieties (Vitis vinifera L.) grown in different soil compositions. Arab. J. Chem. 2021, 14, 103412. [CrossRef]

21. MacKown, C.T.; Carver, B.F.; Edwards, J.T. Occurrence of Condensed Tannins in Wheat and Feasibility for Reducing Pasture Bloat. Crop Sci. 2008, 48, 2470–2480. [CrossRef]

22. Liu, G.; Zhu, W.; Zhang, J.; Song, D.; Zhuang, L.; Ma, Q.; Yang, X.; Liu, X.; Zhang, J.; Zhang, H.; et al. Antioxidant capacity of phenolic compounds separated from tea seed oil in vitro and in vivo. Food Chem. 2022, 371, 131122. [CrossRef] [PubMed]

23. Xiao, Y.; Wu, X.; Yao, X.; Chen, Y.; Ho, C.T.; He, C.; Wang, Y. Metabolite profiling, antioxidant and α-glucosidase inhibitory activities of buckwheat processed by solid-state fermentation with Eurotium cristatum YL-1. Food Res. Int. 2021, 143, 110262. [CrossRef] [PubMed]

24. Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assuncao, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carriere, K.; et al. INFOGEST static in vitro and biological activities. Nat. Protoc. 2019, 14, 991–1014. [CrossRef]

25. Perez-Burillo, S.; Molino, S.; Navajas-Porras, B.; Valverde-Moya, A.J.; Hinojosa-Nogueira, D.; Lopez-Maldonado, A.; Pastoriza, S.; Rufian-Henares, J.A. An in vitro batch fermentation protocol for studying the contribution of food to gut microbiota composition and functionality. Nat. Protoc. 2021, 16, 3186–3209. [CrossRef]

26. Ruberto, G.; Renda, A.; Daquino, C.; Amico, V.; Spatofara, C.; Tringali, C.; Tommasi, N.D. Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. Food Chem. 2007, 100, 203–210. [CrossRef]

27. Yemis, O.; Bakkalbasi, E.; Artik, N. Antioxidative activities of grape (Vitis vinifera) seed extracts obtained from different varieties grown in Turkey. J. Food Sci. Technol. 2008, 43, 154–159. [CrossRef]

28. Song, C.-Z.; Wang, C.; Xie, S.; Zhang, Z.-W. Effects of leaf removal and cluster thinning on berry quality of Vitis vinifera cultivars in the region of Weibei Dryland in China. J. Integr. Agric. 2018, 17, 1620–1630. [CrossRef]

29. Trikas, E.D.; Melidou, M.; Papi, R.M.; Zachariadis, G.A.; Kyriakidis, D.A. Extraction, separation and identification of anthocyanins from red wine by-product and their correlation with phenolic content. J. Funct. Foods 2016, 25, 548–555. [CrossRef]

30. Zhu, M.; Huang, Y.; Wang, Y.; Shi, T.; Zhang, L.; Chen, Y.; Xie, M. Comparison of (poly)phenolic compounds and antioxidant properties of pomace extracts from kiwi and grape juice. Food Chem. 2019, 271, 425–432. [CrossRef]

31. Gulcin, I. Antioxidant activity of grape fruit constituents: An overview. Arch. Toxicol. 2012, 86, 345–391. [CrossRef] [PubMed]

32. Yildirim, H.K.; Akcay, Y.D.; Guvenc, U.; Altindisli, A.; Sozmen, E.Y. Antioxidant activities of organic grape, pomace, juice, must, wine and their correlation with phenolic content. J. Food Sci. Technol. 2005, 40, 133–142. [CrossRef]

33. Sochorova, L.; Prusova, B.; Jurikova, T.; Micek, J.; Adamkova, A.; Baron, M.; Sochor, J. The Study of Antioxidant Components in Grape Seeds. Molecules 2020, 25, 3736. [CrossRef] [PubMed]

34. Lv, Q.; Luo, F.; Zhao, X.; Liu, Y.; Hu, G.; Sun, C.; Li, X.; Chen, K. Identification of proanthocyanidins from litchi (Litchi chinensis Sonn.) pulp by LC-ESI-Q-TOF-MS and their antioxidant activity. PLoS ONE 2015, 10, e0120480.

35. Škerget, M.; Kotnik, P.; Hadolin, M.; Hraš, A.R.; Simonč, M.; Knez, Ž. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem. 2005, 89, 191–198. [CrossRef]

36. Happi Emaga, T.; Robert, C.; Ronkart, M.N.; Wathelet, B.; Paquot, M. Dietary fibre components and pectin chemical features of peels during ripening in banana and plantain varieties. Bioresour. Technol. 2008, 99, 4346–4354. [CrossRef]

37. Shui, G.; Leong, L.P. Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. Food Chem. 2006, 96, 277–284. [CrossRef]

38. Babbar, N.; Oneroi, H.S.; Uppal, D.S.; Patil, R.T. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Res. Int. 2011, 44, 391–396. [CrossRef]

39. Quatrin, A.; Rampelotto, C.; Pauletto, R.; Maurer, L.H.; Nichelle, S.M.; Klein, B.; Rodrigues, R.F.; Moraes, O.Maira; de Menezes, C.R.; et al. Bioaccessibility and catabolism of phenolic compounds from jaboticaba (Myrciaria trunciflora) fruit peel during in vitro gastrointestinal digestion and colonic fermentation. J. Funct. Foods 2020, 65, 103714. [CrossRef]

40. de Almeida, S.S.; da Costa, G.B.M.; Barreto, M.S.; Freire, D.M.G.; Lobo, L.A.; Domingues, R.; Moura-Nunes, N.; Monteiro, M.; Perrone, D. Bioaccessibility and gut metabolism of phenolic compounds of breads added with green coffee infusion and enzymatically bioprocessed. Food Chem. 2020, 333, 127473. [CrossRef]

41. Gowd, V.; Xie, L.H.; Sun, C.D.; Chen, W. Phenolic profile of bayberry followed by simulated gastrointestinal digestion and gut microbiota fermentation and its antioxidant potential in HepG2 cells. J. Funct. Foods 2020, 70, 103987.

42. Celep, E.; Charehsaz, M.; Akuyuz, S.; Acar, E.T.; Yesilada, E. Effect of in vitro gastrointestinal digestion on the bioavailability of phenolic compounds and the antioxidant potentials of some Turkish fruit wines. Food Res. Int. 2015, 78, 209–215. [PubMed]

43. Corrêa, R.C.G.; Haminiuk, C.W.I.; Barros, L.; Dias, M.I.; Calhelha, R.C.; Kato, C.G.; Correa, V.G.; Peralta, R.M.; Ferreira, I.C.F.R. Stability and biological activity of Merlot (Vitis vinifera) grape pomace phytochemicals after simulated in vitro gastrointestinal digestion and colonic fermentation. J. Funct. Foods 2017, 36, 410–417.

44. Del Pino-Garcia, R.; Gonzalez-Sanjose, M.L.; Rivero-Perez, M.D.; Garcia-Lomillo, J.; Muniz, P. Total antioxidant capacity of new natural powdered seasonings after gastrointestinal and colonic digestion. Food Chem. 2016, 211, 707–714. [PubMed]

45. Carmona-Jimenez, Y.; Garcia-Moreno, M.V.; Garcia-Barroso, C. Effect of Drying on the Phenolic Content and Antioxidant Activity of Red Grape Pomace. Plant Foods Hum. Nutr. 2018, 73, 74–81. [PubMed]
47. Sanchez-Patan, F.; Barroso, E.; van de Wiele, T.; Jimenez-Giron, A.; Martin-Alvarez, P.J.; Moreno-Arribas, M.V.; Martinez-Cuesta, M.C.; Pelaez, C.; Requena, T.; Bartolome, B. Comparative in vitro fermentations of cranberry and grape seed polyphenols with colonic food microbiota. *Food Chem.* 2015, 183, 273–282.

48. Cueva, C.; Sanchez-Patan, F.; Monagas, M.; Walton, G.E.; Gibson, G.R.; Martin-Alvarez, P.J.; Bartolome, B.; Moreno-Arribas, M.V. In vitro fermentation of grape seed flavan-3-ol fractions by human faecal microbiota: Changes in microbial groups and phenolic metabolites. *FEMS Microbiol. Ecol.* 2013, 83, 792–805.

49. Conlon, M.A.; Bird, A.R. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 2014, 7, 17–44.

50. Moon, J.S.; Li, L.; Bang, J.; Han, N.S. Application of in vitro gut fermentation models to food components: A review. *Food Sci. Biotechnol.* 2016, 25, 1–7.

51. Dong, R.; Liu, S.; Zheng, Y.; Zhang, X.; He, Z.; Wang, Z.; Wang, Y.; Xie, J.; Chen, Y.; Yu, Q. Release and metabolism of bound polyphenols from carrot dietary fiber and their potential activity in in vitro digestion and colonic fermentation. *Food Funct.* 2020, 11, 6652–6665.

52. Gil-Sanchez, J.; Ferrer, M.; Sanz-Buenhombre, M.; Guadarrama, A.; Moreno-Arribas, M.V.; Bartolome, B. Dynamic gastrointestinal digestion of grape pomace extracts: Bioaccessible phenolic metabolites and impact on human gut microbiota. *J. Food Compos. Anal.* 2018, 68, 41–52.

53. Shin, N.R.; Whon, T.W.; Bae, J.W. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* 2015, 33, 496–503. [PubMed]

54. Aura, A.-M. Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry* 2008, 7, 407–429.

55. Di Pasqua, R.; Mamone, G.; Ferranti, P.; Ercole, D.; Mauriello, G. Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics* 2010, 10, 1040–1049.

56. Dan, Z.; Mao, X.; Liu, Q.; Guo, M.; Zhuang, Y.; Liu, Z.; Chen, K.; Chen, J.; Xu, R.; Tang, J.; et al. Altered gut microbial profile is associated with abnormal metabolism activity of Autism Spectrum Disorder. *Gut Microbes* 2020, 11, 1246–1267. [PubMed]

57. Gibson, G.R.; Hutkins, R.; Sanders, M.E.; Prescott, S.L.; Salminen, S.J.; Scott, K.; Stanton, C.; Swanson, K.S.; Cani, P.D.; et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* 2017, 14, 491–502.

58. Liu, X.; Mao, B.; Gu, J.; Wu, J.; Cui, S.; Wang, G.; Zhao, J.; Zhang, H.; Chen, W. Blautia—A new functional genus with potential probiotic properties? *Gut Microbes* 2021, 13, 1–21.

59. Zhou, W.; Yang, T.; Xu, W.; Huang, Y.; Ran, L.; Yan, Y.; Mi, J.; Lu, L.; Sun, Y.; Zeng, X.; et al. The polysaccharides from the fruits of *Lycium barbarum* L. confer anti-diabetic effect by regulating gut microbiota and intestinal barrier. *Carbohydr. Polym.* 2022, 291, 119626.

60. Wang, J.; Chen, Y.; Hu, X.; Feng, F.; Cai, L.; Chen, F. Assessing the Effects of Ginger Extract on Polyphenol Profiles and the Subsequent Impact on the Fecal Microbiota by Simulating Digestion and Fermentation In Vitro. *Nutrients* 2020, 12, 3194.

61. Erickson, J.M.; Carlson, J.L.; Stewart, M.L.; Slavin, J.L. Fermentability of Novel Type-4 Resistant Starches in In Vitro System. *Foods* 2018, 7, 18. [CrossRef] [PubMed]

62. Dobrowolska Iwanek, J.; Zagrodzki, P.; Wozniakiewicz, M.; Wozniakiewicz, A.; Winnicka, D.; Pasko, P. Procedure optimization for extracting short-chain fatty acids from human faeces. *J. Pharm. Biomed. Anal.* 2016, 124, 337–340. [PubMed]

63. Li, S.; Heng, X.; Guo, L.; Lessing, D.J.; Chu, W. SCFAs improve disease resistance via modulate gut microbiota, enhance immune response and increase antioxidative capacity in the host. *Fish Shellfish Immunol.* 2022, 120, 560–568. [PubMed]

64. Russell, J.T.; Roesch, L.F.W.; Ordberg, M.; Ionen, J.; Atkinson, M.A.; Schatz, D.A.; Triplett, E.W.; Ludvigsson, J. Genetic risk for autoimmunity is associated with distinct changes in the human gut microbiome. *Nat. Commun.* 2019, 10, 3621.

65. Sadeghi Ekkatan, S.; Slono, L.; Sabally, K.; Khairallah, J.; Azadi, B.; Rodes, L.; Prakash, S.; Donnelly, D.J.; Kubow, S. Biotransformation of polyphenols in a dynamic multistage gastrointestinal model. *Food Chem.* 2016, 204, 453–462.

66. Martin-Carrón, N.; Góñi, I.; Larrauri, J.A.; García-Alonso, A.; Saura-Calixto, F. Reduction in serum total and LDL cholesterol concentrations by a dietary fiber and polyphenol-rich grape product in hypercholesterolemic rats. *Food Nutr. Res.* 1999, 19, 1371–1381.