Abstract: The present study extracted total saponins from quinoa husks with pressurized hot water extraction and optimized the extraction conditions. The response surface methodology (RSM) with a Box–Behnken design (BBD) was employed to investigate the effects of extraction flow rate, extraction temperature and extraction time on the extraction yield of total saponins. A maximal yield of 23.06 mg/g was obtained at conditions of 2 mL/min, 210°C and 50 min. The constituents of the extracts were analyzed by liquid chromatography–mass spectrometry (LC-MS). A total of twenty-three compounds were identified, including five flavonoids, seventeen triterpenoid saponins and a phenolic acid. Moreover, we performed an in vitro assay for the α-glucosidase activity and found a stronger inhibitory effect of the quinoa husk extracts than acarbose, suggesting its potential to be developed into functional products with hypoglycemic effect. Finally, our molecular docking analyses indicated triterpenoid saponins as the main bioactive components.

Keywords: Chenopodium quinoa Willd; pressurized hot water extraction; response surface methodology; saponins; α-glucosidase; molecular docking

1. Introduction

Quinoa (Chenopodium quinoa Willd.) is an annual crop of the genus Chenopodiaceae [1], originating from the Andes of South America. It may help solve food shortage problems due to its strong tolerance and growth adaptability to cold, drought, saline–alkali, barren and other severe soil and climate conditions [2]. As one of the top healthy foods globally, quinoa has high nutritional and biological values and is known as the “golden grain” [3]. In addition to the large number of nutrients it contains [4], quinoa is also rich in saponins, phenols, polysaccharides and other phytochemicals [5–7] that exert excellent health-protecting effects, including anti-inflammatory, antibacterial, antioxidant and antidiabetic properties [8].

As food, quinoa grains are usually dehulled. Quinoa husks are mostly discarded [9], which causes a waste of resources and pollution. Interestingly, studies have shown that quinoa husks are a good source of saponins [10]. In fact, the majority of saponins in quinoa husks are triterpenoid glycosides, which are derived from β-vanillin and comprise sugar chains and sapogenins [11]. Quinoa saponins possess a variety of biological and physiological properties, such as hypoglycemia, antioxidation, anti-cancer and anti-inflammation [12–15]. Moreover, studies also suggest that the hypoglycemic effect of triterpenoid saponins is mainly due to their inhibition of α-glucosidase and improved β-cell function and insulin resistance [16,17]. Therefore, it is significant to explore a safe and efficient extraction method for quinoa saponins and develop the related health-benefiting functional products to promote the maximal utilization of quinoa husks.

Pressurized hot water extraction (PHWE) is an environmentally friendly and efficient extraction technology using water as the extractant. The water remains in a liquid state when the temperature rises to 100–374 °C under the critical pressure (1–22.1 MPa) [18,19].
Under such state, the dielectric constant ($\varepsilon$) of water significantly decreases with increased temperature, which makes the properties of the water similar to those of organic solvents and thus capable of extracting compounds of low polarity [20]. Compared with traditional extraction methods, pressurized hot water extraction has the advantages of short extraction time, high extraction efficiency, safety and environmental friendliness [21], and it has been used in the extraction of polyphenols [22–24], polysaccharides [25,26] and other natural products. However, few studies have been conducted on the effects of pressurized hot water extraction on the yield of total saponins from quinoa husks.

The present study investigated the effects of PHWE extraction parameters (extraction flow rate, extraction temperature and extraction time) on the yield of total saponins using the response surface methodology (RSM) with a central composite design. Meanwhile, we also analyzed the chemical constituents of the extracts (by LC-MS), their potential inhibition on $\alpha$-glucosidase (using an in vitro glycosidase activity assay) and the molecular interactions (by molecular docking).

2. Materials and Methods

2.1. Materials

The powder of the husks of Chenopodium quinoa was provided by Qinghai Boji Biotechnology Co., Ltd. (Xining, China). Saccharomyces cerevisiae-derived $\alpha$-glucosidase (G5003) was purchased from Sigma-Aldrich (Shanghai, China). p-Nitrophenyl-$\alpha$-D-glucopyranoside (pNPG) was purchased from Aladdin Reagents (Shanghai, China). Acarbose was purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). The other reagents and solvents used were of analytical grade.

2.2. Optimization of the Extraction of Total Saponins

2.2.1. Experimental Design

A three-level–three-factor Box–Behnken design response surface methodology (BBD-RSM) [27] was utilized to optimize the conditions to maximize the yield of total saponins from quinoa husks. The extraction flow rate (A, mL/min), extraction temperature (B, °C) and extraction time (C, min) were set as factors. Three levels were considered for each factor (Table 1).

| Factors               | Symbols | Levels |
|-----------------------|---------|--------|
| Extraction Flow Rate  | A       | −1  0  1 |
| (mL/min)              |         |  2  4  6 |
| Extraction Temperature | B       | 150 180 210 |
| (°C)                 |         |       |
| Extraction Time (min) | C       | 30  45  60 |

2.2.2. Pressurized Hot Water Extraction of Total Saponins from Quinoa Husks

The pressurized hot water extraction was performed with a laboratory-built system (constructed by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, China). The extraction system consisted of a liquid chromatography infusion pump (LC-20AT, SHIMADZU, Kyoto, Japan) delivering ultra-pure water, a gas chromatography column oven (GC-14B, Shanghai Kechuang Chromatography Instruments, Shanghai, China) controlling the extraction temperature, a back pressure regulator (TESCOM, Elk River, MN, USA), SS-0006WT-B-P micro-channel heat exchanger (Hangzhou Shenshi Energy Conservation Technology Co., Ltd., Hangzhou, China), stainless coil preheater (0.3 mm in inner diameter and 2.0 m in length) and a 100 cm$^3$ stainless steel reaction vessel (2.0 cm in diameter and 8.0 cm in height, internal dimensions). The powder of quinoa husks (1.000 g) mixed with 8–16 mesh quartz sand was placed into the reaction vessel. The ultra-pure water was delivered into the stainless coil preheater in the column oven by the pump and
previously heated, and when the temperature reached the set point, the ultra-pure water was pumped into the extraction vessel at a constant flow rate. The extraction procedure was carried out according to the conditions in Table 2. The extraction liquid was filtered, concentrated and dried.

Table 2. Box–Behnken experimental design and results.

| Run Order | Extraction Flow Rate (mL/min) A | Extraction Temperature (°C) B | Extraction Time (min) C | The Yield of Total Saponins (mg/g) Y |
|-----------|-------------------------------|-------------------------------|------------------------|----------------------------------|
| 1         | 2                             | 180                           | 60                     | 16.77 ± 0.56                    |
| 2         | 4                             | 180                           | 45                     | 18.67 ± 1.47                    |
| 3         | 2                             | 150                           | 45                     | 19.16 ± 0.38                    |
| 4         | 4                             | 210                           | 60                     | 21.89 ± 1.81                    |
| 5         | 4                             | 180                           | 45                     | 19.61 ± 1.05                    |
| 6         | 6                             | 180                           | 30                     | 17.02 ± 0.30                    |
| 7         | 6                             | 180                           | 60                     | 18.81 ± 0.18                    |
| 8         | 6                             | 150                           | 45                     | 22.54 ± 0.67                    |
| 9         | 6                             | 210                           | 45                     | 22.90 ± 1.29                    |
| 10        | 4                             | 180                           | 45                     | 19.01 ± 0.75                    |
| 11        | 2                             | 210                           | 45                     | 23.57 ± 0.69                    |
| 12        | 2                             | 180                           | 30                     | 17.88 ± 0.83                    |
| 13        | 4                             | 180                           | 45                     | 19.31 ± 1.28                    |
| 14        | 4                             | 150                           | 30                     | 19.74 ± 0.64                    |
| 15        | 4                             | 150                           | 60                     | 18.01 ± 0.29                    |
| 16        | 4                             | 180                           | 45                     | 18.91 ± 1.53                    |
| 17        | 4                             | 210                           | 30                     | 20.34 ± 1.58                    |

2.2.3. Determination of the Content of Total Saponins

The extraction yield of total saponins was measured following a previously described method, with modifications [28]. Firstly, 0.2 mL of quinoa husk extracts was evaporated to dryness in a 70 °C water bath and cooled down. Amounts of 0.4 mL of 5% vanillin-acetic acid solution and 1.6 mL of 72% perchloric acid were added in sequence and incubated in a 70 °C water bath for 10 min, followed by cooling with ice-cold water for another 10 min. Then, the mixture was mixed with 10 mL of 17 M acetic acid and incubated at 25 °C for 15 min. The absorbance was measured at 560 nm using a Cary 300 Bio spectrometer (Agilent Corporation, Santa Clara, CA, USA), with the oleanolic acid solution as standard and the sample solution as a blank control.

2.2.4. Optimization of RSM

RSM was used to determine the optimal extraction conditions for total saponins from quinoa husks. The extraction flow rate, extraction temperature and extraction time were set as the investigation factors, and the yield of total saponins was set as the response value. The Box–Behnken center combination design was carried out by RSM, with the data being analyzed with Design Expert 8.0.

2.3. LC-MS Analysis of Quinoa Husk Extracts at Optimal Extraction Conditions

The chemical constituents of the extracts at the optimal extraction conditions were analyzed by LC-MS. The extracts (1 mg) added to 1 mL of 80% methanol were sonicated and centrifuged to obtain the supernatant. The UPLC-Triple-TOF-MS system (Waters Corp., Milford, USA) was employed for the analysis of components. The ultra HPLC system was equipped with a HSS T3 column (1.7 µm, 2.1 mm × 150 mm), which was used at the column temperature of 50 °C. The mobile phase was composed of solvent A (0.1% formic acid) and solvent B (0.1% formic acid acetonitrile), and gradient elution (0–10 min, 95–75% A; 10–25 min, 75–40% A; 25–32 min, 40–5% A) was performed. The flow rate was 0.3 mL/min, the volume loaded was 2 µL, and the detection wavelength was 230 nm. The mass spectrometry analysis was performed with a scan range of 100–1500 m/z in the negative ion scan mode. The mass spectrometry data were acquired under the following conditions: nebulizer gas (GS1): 55 psi; nebulizer gas (GS2): 55 psi; curtain gas (CUR): 35 psi; ion source temperature (TEM): 550 °C (negative), 600 °C (positive); ion source
voltage (IS): −4500 V (negative), 5500 V (positive); primary scan: declustering voltage (DP): 100 V; focusing voltage (CE): 10 V; secondary scan: TOF MS~Product Ion~IDA mode, the CID energy was 20, 40 and 60 V. The mass axis was calibrated with a CDS pump with an error <2 ppm.

2.4. Measurement of α-Glucosidase Activity of Quinoa Husk Extracts at Optimal Extraction Conditions

In vitro measurement of the α-glucosidase activity was performed as previously described, with modifications [29]. The test included a blank group, a sample group and a sample control group. Amounts of 20 µL of the sample (quinoa husk extracts solution) of different concentrations and 80 µL of 0.1 mM phosphate-buffered solution (PBS, pH 6.8) were added to a 96-well plate. The sample control group was added with 50 µL of 0.1 mM PBS (pH 6.8), and the other groups were added with 50 µL of α-glucosidase solution (1 U/mL). After incubating for 30 min at 37 °C, 50 µL of 0.5 mM pNPG solution was added and mixed well and incubated at 37 °C for 30 min. Finally, the reaction was stopped by adding 50 µL of 0.1 mol/L Na₂CO₃ solution. The absorbance value was measured at 405 nm (EPOCH2 microplate reader, BioTek, Inc., Vermont, USA). Acarbose was used as the positive control. The inhibition rate of each sample was calculated according to the following formula.

\[
\text{Inhibition (\%)} = \frac{A - (A_1 - A_2)}{A} \times 100
\]

A: Absorbance value of blank group; A₁: Absorbance value of sample group; A₂: Absorbance value of sample control group.

2.5. Molecular Docking

Molecular docking was performed to determine the interaction between the α-glucosidase and the constituents in the quinoa husk extracts. As the 3D structure of α-glucosidase was unavailable, the isomaltose from Saccharomyces cerevisiae with high sequence homology to α-glucosidase was used for the docking analysis. The crystal structure of isomaltose was downloaded from the Protein databank (PDB ID: 3A4A). A stable receptor was created with AutoDock by adding hydrogen atoms and removing water and ligands to modify the protein [30]. ChemBio 3D Ultra 19.0 was used to create the 3D structures of the compounds and standard acarbose, and the energy minimization was performed to obtain a reasonable conformation. Then, the AutoDock Vina [31] software was used to dock the modified molecular structures and the protein crystal structure. Compounds with lower binding energy were considered to have stronger α-glucosidase inhibitory activity, and some compounds with better docking scores were selected to analyze the docking interactions.

2.6. Statistical Analysis

Experiments were performed in triplicate. Data were expressed as mean ± standard deviation. The results of response surface methodology were analyzed and graphed using Design Expert 8.0.

3. Results and Discussion

3.1. Optimization of the Extraction Conditions for Total Saponins with RSM

According to the BBD-RSM with three levels and three factors, the factors and levels of the response surface are shown in Table 1. Taking the extraction yield of total saponins (Y) as the response value, the Box–Behnken experimental design and results are shown in Table 2. Regression analysis was performed on the data in Table 2 using Design Expert 8.0, and the response surface variance analysis and results obtained are shown in Table S1.

Based on statistical analysis of the experimental data, the relationships between the yield of total saponins and extraction parameters were described by the following equation:
\[ Y = 19.10 + 0.49 \times A + 1.16 \times B + 0.063 \times C - 1.01 \times AB + 0.73 \times AC + 0.82 \times BC + 0.29 \times A^2 + 2.66 \times B^2 - 1.76 \times C^2 \]

The response surface analysis of variance (ANOVA) showed a high significance of the regression \((p < 0.01)\), whereas the lack of fit was not significant \((p > 0.05)\), implying a high accuracy and reliability of our model (Table S1). The F value represented the effects of extraction flow rate, extraction temperature and extraction time on the yield of total saponins, with a higher F value representing a greater effect [32]. Within the range of the selected factors, their F-value-based ranking is listed in Table S1, showing an order of extraction temperature > extraction flow rate > extraction time. Therefore, the effect of extraction temperature on the yield of total saponins from quinoa husks is most significant.

The interaction diagram of the extraction flow rate, extraction temperature and extraction time was plotted according to the regression equation (Figure 1). A steep interaction curve meant a very significant effect of the interaction [33]. Combined with the results of the ANOVA, the interactions of two independent variables had significant effects on the yield of total saponins from quinoa husks, while extraction temperature was the most influential variable. The equation was solved by the regression model, and the optimal extraction conditions were as follows: extraction flow rate of 2.03 mL/min, extraction temperature of 209.94 °C and extraction time of 48.40 min. For convenience in practical operation, the conditions were modified to be 2.00 mL/min, 210 °C and 50 min. With this optimal condition, the yield of total saponins averaged from three independent experiments was 23.06 mg/g. The relative error for the predicted value (23.64 mg/g) was 2.5%, suggesting a high reliability of the regression model obtained by RSM.

![Figure 1](image-url)

**Figure 1.** Interaction effects of factors on total saponins extraction yield in quinoa husks. (A) the interaction of flow rate and temperature; (B) the interaction of time and flow rate; (C) the interaction of time and temperature.

It should be noted that the extraction temperature was a key parameter for the extraction yield of total saponins in this study. The effect might be attributed to the dielectric constant and polarity of the solvent, which could decrease along with the rising temperature and promote the dissolution of the saponins component, improving the yield. Additionally, with increased temperature, the movement and material exchange between saponin molecules accelerate; the viscosity and surface tension of the solvent reduce and thereby improve the wetting, penetrating and dissolving ability of the solvent [34]. However, more studies are required to determine whether more mechanisms are involved in the effect of temperature on extraction yield.

Previous studies with ultrasonic-assisted extraction, microwave-assisted extraction, supercritical CO₂ extraction and solvent reflux extraction reported a yield of total saponins as 23.7 mg/g [35], 26.32 mg/g [36], 9.6 mg/g [37] and 16.85 mg/g [38], respectively. The values were comparable to those obtained by pressurized hot water extraction in this study (Table 3). However, compared with previous extraction methods, the pressurized hot water extraction method was more environmentally friendly and safer. For instance, the successful extraction of hydrophobic saponins with water reduces the usage of organic solvents.
Table 3. Comparison of total saponins extraction technology in quinoa husks.

| Extraction Technique                  | Solvent Used | Extraction Temperature (° C) | Extraction Time (min) | Solid/Solvent | Other Parameters | The Yield of Total Saponins (mg/g) | Ref  |
|---------------------------------------|--------------|------------------------------|-----------------------|---------------|-----------------|-----------------------------------|------|
| Ultrasonic-assisted extraction        | 75% EtOH     | 45                           | 90                    | 1:15          | -               | 23.7                              | [35] |
| Microwave-assisted extraction         | 68% EtOH     | -                            | 10                    | 1:32          | Power 455 W     | 26.32                             | [35] |
| Supercritical CO₂ extraction          | 74% EtOH     | 60                           | 96                    | -             | Pressure 37 MPa | 9.6                               | [37] |
| Solvent reflux extraction             | 72% EtOH     | 72                           | 147                   | 1:20.8        | -               | 16.85                             | [38] |
| Pressurized hot water extraction      | Water        | 210                          | 50                    | -             | Flow rate 2 mL./min | 23.06                        |      |

3.2. Identification of Chemical Constituents in Quinoa Husk Extracts

The total ion flow diagram of chemical constituents in quinoa husk extracts by LC-MS is shown in Figure 2. Based on the molecular ion, main fragments and retention time in the MS spectrum, a total of twenty-three compounds were identified (Figures 3 and 4), including five flavonoids, seventeen triterpenoid saponins and a phenolic acid, the main information on which is listed in Table 4.

Figure 2. Total ion flow diagram of chemical constituents in quinoa husk extracts in negative ion mode by LC-MS.

Figure 3. The structures of Compounds 1–5 and 8.
Table 4. Twenty-three compounds identified from the extracts of quinoa husks by LC-MS.

| NO. | RT (min) | [M-H] (m/z) | MS/MS Fragments | Formula | Compound | Ref |
|-----|----------|-------------|-----------------|---------|----------|-----|
| 1   | 8.04     | 755.214     |                 |         | C₃₀H₄₆O₂₀ | Quercetin 3-O-(2,6-di-α-L-rhamnopyranosyl)-β-d-galactopyranoside | [39] |
| 2   | 8.77     | 739.2213    |                 |         | C₂₀H₁₉O₇ | kaempferol 3-O-(2,6-di-α-L-rhamnopyranosyl)β-β-glucopyranoside | [39] |
| 3   | 9.95     | 477.067     |                 |         | C₂₀H₁₉O₇ | quercetin 3-O-(β-D-glucopyranosyl)β-D-glucopyranoside | [39] |
| 4   | 10.11    | 479.3041    |                 |         | C₂₀H₁₉O₇ | Myricetin-3-O-β-D-galactopyranoside | [40] |
| 5   | 11.30    | 187.0096    |                 |         | C₇H₁₈O₃ | p-Coumaric acid | [41] |
| 6   | 12.76    | 957.4882    |                 |         | C₃₀H₄₆O₂₀ | Serjania 3-O-[β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl]β-D-glucopyranoside | [42] |
| 7   | 13.36    | 827.4482    | [M+COOH]        |         | C₂₀H₁₉O₇ | 28-O-a-L-arabinopyranosyl-(1-3)-β-D-glucopyranosyl ester | [43] |
| 8   | 14.05    | 301.0366    |                 |         | C₁₁H₁₂O₂ | Quercetin | [44] |
| 9   | 15.34    | 1017.4968   |                 |         | C₃₀H₄₆O₂₀ | 3-O-β-D-glucopyranosyl-(1-3)-O-a-L-arabinopyranosyl phytolaccagenic acid | [45] |
| 10  | 16.42    | 855.4429    | [M+COOH]        |         | C₂₀H₁₉O₇ | 3-O-[β-D-glucopyranosyl-(1-3)-O-a-L-arabinopyranosyl phytolaccagenic acid | [39] |
| 11  | 17.10    | 809.4465    |                 |         | C₂₀H₁₉O₇ | Hederagenin 3-O-[β-D-glucopyranosyl-(1-3)-α-a-L-arabinopyranosyl]β-D-glucopyranosyl ester | [42] |
| 12  | 17.63    | 973.5057    | [M+COOH]        |         | C₂₀H₁₉O₇ | basellasin A | [39] |
| 13  | 18.49    | 969.4519    |                 |         | C₂₀H₁₉O₇ | 3-O-a-L-arabinopyranosyl oleanolic acid | [42] |
| 14  | 19.41    | 793.4486    |                 |         | C₂₀H₁₉O₇ | 3-O-a-L-arabinopyranosyl phytolaccagenic acid | [45] |
| 15  | 19.85    | 693.3514    |                 |         | C₁₁H₁₂O₂ | Quercetin | [46] |
| 16  | 20.51    | 953.4559    |                 |         | C₃₀H₄₆O₂₀ | O-β-D-glucopyranosyl-(1-3)-β-D-glucopyranosyl oleanolic acid | [47] |
| 17  | 20.99    | 851.4580    |                 |         | C₂₀H₁₉O₇ | 28-O-β-D-glucopyranosyl ester | [42] |
| 18  | 22.04    | 647.3820    |                 |         | C₂₀H₁₉O₇ | Hederagenin 3-O-β-D-glucopyranosyl phytolaccagenic acid | [42] |
| 19  | 25.32    | 633.3916    |                 |         | C₂₀H₁₉O₇ | 3-O-β-D-glucopyranosyl oleanolic acid | [44] |
| 20  | 26.65    | 775.3988    |                 |         | C₂₀H₁₉O₇ | 28-O-β-D-glucopyranosyl-(1-3)-β-D-glucopyranoside | [42] |
| 21  | 27.51    | 791.3892    |                 |         | C₂₀H₁₉O₇ | 14 isomer | [42] |
| 22  | 29.97    | 763.4042    |                 |         | C₂₀H₁₉O₇ | oleanolic acid 3-O-β-D-xylopyranosyl-(1-3)-β-D-glucopyranoside | [44] |
| 23  | 30.79    | 763.4042    |                 |         | C₂₀H₁₉O₇ | 22 isomer | [44] |

Compounds 1–5 and 8 are phenols, mainly flavonoids. In positive and negative ion modes, flavonoid glycosides are prone to cleavage, resulting in loss of the neutral fragments and cracking of the glycosidic bonds, generating the corresponding aglycone [45]. The sugar groups that make up flavonoid glycosides are mainly glucose, rhamnose, rutinoside and galactose, with the corresponding main fragment ions of [M–H–162]⁻, [M–H–146]⁻, [M–H–308]⁻ and [M–H–162]⁻. Compounds 1, 3 and 8 should contain quercitin nucleus because of the existence of fragment ion at m/z 301 [46]. Compound 1, with ion [M–H]⁻ m/z 755.2144 and the secondary fragment ion m/z 300.0274, was speculated to be a quercetin derivative. Compared with the aglycone ion [Y₀]⁻ m/z 301.0353, the relative abundance of the radical aglycone ion [Y₀–H]⁻ m/z 300.0274 was higher, indicating that it may be quercitin 3-O-glycoside compound aglycone [40]. Overall, it was very likely that the molecular ion m/z 755.2144 lost two pentose molecules and a hexose molecule to produce the aglycone ion m/z 300.0274. In sum, Compound 1 should be quercitin 3-O-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside. Compound 3 should be quercerin 3-O-β-D-glucuronopyranoside due to the existence of fragment [M–H–179]⁻ generated by missing one molecule of glucuronic acid. Compound 8 was identified as quercerin because of the [M–H]⁻ ion at m/z 301.0366 and the fragment ion m/z 151.0033 generated by retro-Diels–Alder cleavage after losing the neutral fragment CO [47]. The presence of the kaempferol parent nucleus fragments ion at m/z 285 suggests Compound 2 is a kaempferol derivative. Its ion [M–H]⁻ m/z 739.2213, by the loss of two rhamnose molecules and one glucose molecule to obtain aglycone, further suggests it is kaempferol 3-O-(2,6-di-α-L-
rhamnopyranosyl)-β-D-galactopyranoside. Compound 4 was preliminarily identified as myricetin-3-O-β-D-galactopyranoside [40], with the ion [M−H]− at m/z 479.3041; it could lose a hexoside to produce the secondary fragment ion at m/z 319.1914, which corresponded to myricetin aglycones. The deduced structures of Compounds 1–5 and 8 are shown in Figure 3. Compound 4 was identified for the first time from quinoa, while the other compounds were identified from Chenopodium quinoa [39,41].

![Figure 3. The structures of Compounds 1–5 and 8.](image1)

![Figure 4. The structures of Compounds 6–7 and 9–23.](image2)

Compounds 6–7 and 9–23 are triterpenoid saponins, in which the aglycones (oleanolic acid, hederagenin, phytolaccagenic acid, serjanic acid) are linked with glycosides (glucose, galactose, arabinose, glucuronic acid, xylose) at C-3 or C-28 [10]. The triterpenoid saponins in quinoa mainly exist in the form of oxyglycosides, which undergo glycosidic bond cleavage in the positive or negative ion modes and generate glycosyl fragment ions ([M−H−162]−, [M−H−132]−, [M−H−179]−) and aglycone ions [48]. The existence of
ions [M−H]− at m/z 455 suggests Compounds 14, 16, 19, 21, 22 and 23 contain oleanolic aglycone and were characterized as oleanolic acid saponins. Compounds 14 and 19 were assigned as 3-O-β-D-glucuronopyranosyl oleanolic acid 28-O-β-glucopyranosyl ester ([M−H]− ion at m/z 793.4496) and 3-O-β-D-glucuronopyranosyl oleanolic acid ([M−H]− ion at m/z 631.3916), respectively. Both compounds had the glucuronic acid representative fragment [M−H−179]−. Compound 20 with deprotonated molecule ion [M−H]− at m/z 471, indicating the presence of hederagenin aglycone. Compound 21 exhibited the deprotonated molecule ion [M−H]− at m/z 809.4465, the ion m/z 647.3887 generated by its loss of a hexose and the ion m/z 471.3520 by a further loss of a glucuronic acid, whose fragmentation pattern corresponded to the previously reported 3-O-β-D-glucuronopyranosyl hederagenin 28-O-β-D-glucopyranosyl ester saponin [42]. It is reported that a compound of large molecular size could lose its acidic group to generate the fragment ion [M+HCOOH−H]− [39], which is the case for the carboxyl-groups-containing Compounds 12 and 13. Compound 12, with ion m/z 973.5057, lost fragment [M−H−44−162]− and generated fragment ion m/z 765.4548, which further generated hederagenin aglycone ion m/z 471.3520 by losing a glucose molecule and a xylose molecule; it could be identified as hederagenin 3-O-[β-D-glucopyranosyl-(1,3)-α-L-arabinopyranosyl]-28-O-β-D-glucopyranoside by comparison with previous studies [39]. Compound 13 was characterized as basellasaponin A ([M−H]− ion at m/z 969.4519), and the fragment ion at m/z 809.4471 represented the [M−H−44−116]−; the aglycone ion was generated by the loss of a glucose molecule and a glucuronic acid molecule. Compounds 9, 10, 15 and 17 were assigned as phytolaccagenic acid saponins because of the aglycone fragment ions at m/z 515; they all contained carboxyl groups, with the difference in positions where glycosides were attached. Compound 10 was speculated as 3-O-β-D-glucopyranosyl-(1-3)-O-α-L-arabinopyranosyl phytolaccagenic acid. It showed an ion at m/z 855.4429 and a fragment ion m/z 809.4522 due to losing a carboxyl group, with the latter further generating the aglycone fragment ion at 515.3412 by the loss of a glucose molecule and an arabinose molecule. Compound 15 was identified as 3-O-[α-L-arabinopyranosyl phytolaccagenic acid ([M−H]− ion at m/z 693.3514), which also produced a phytolaccagenic acid aglycone ion by decarboxylation and the removal of a pentose molecule. Compound 6 exhibited deprotonated molecule ion [M−H]− at m/z 957.4882; the fragment ion at m/z 633.3719 suggested the loss of multiple glucuronic molecules, whereas the ion m/z 501.3251 indicated the missing of an arabinose molecule. Therefore, Compound 6 was deducted to be serjanic acid 3-O-[β-D-glucopyranosyl-(1-3)-α-L-arabinopyranosyl]-28-O-β-D-glucopyranoside, as previously reported in the analysis of seed hulls of quinoa [42]. The fragment ion at m/z 487 suggests Compound 7 was a pentacyclic triterpenoid saponin-containing entagentic acid. The ion m/z 781.4515 suggested the missing of the carboxyl fragment [M−H−44]− from ion [M−H]− ion of m/z 827.4482, while the fragment ion m/z 619.3924 and 487.3436 corresponded to the losses of a glucose molecule and an arabinose molecule, respectively. By referring to the previously reported structure [43], Compound 7 was deducted to be 3β,15α,16α-trihydroxy-18β-olean-12-en-28-oic acid 28-O-α-L-arabinopyranosyl-(1-3)-β-D-glucopyranosyl ester. The structures of Compounds 7, 9, 16, 17, 18 and 20 were firstly identified from Chenopodium quinoa and were not found in other natural products. The speculated structures of Compounds 6–7 and 9–23 are presented in Figure 4.
and preparation of active ingredients from quinoa husks in the following studies. However, we realize the existence of limitations for this analytical method in determining the linkage positions of sugar moieties, and more methods will be employed to confirm the structures in our future studies.

3.3. Inhibitory Effect of Quinoa Husk Extracts on α-Glucosidase

3.3.1. In Vitro Inhibition of α-Glucosidase Activity by Quinoa Husk Extracts

The potential inhibition of α-glucosidase by quinoa husk extracts at the optimal extraction conditions was measured using pNPG as substrate. The commercially available α-glucosidase inhibitor acarbose served as a positive control and was frequently used as a reference standard for evaluating the effect of candidate α-glucosidase inhibitors [49].

Figure 5 shows the inhibition rate of different concentrations of quinoa husk extracts and acarbose on α-glucosidase. The results illustrated that along with the increase in their concentrations, the inhibition rate of α-glucosidase increased in a concentration-dependent manner. The quinoa husk extracts showed a lower IC₅₀ value of 32.62 mg/mL compared to that of acarbose (64.71 mg/mL), suggesting a stronger inhibition on α-glucosidase (p < 0.05). Our finding is consistent with the previous reports of the strong effect of triterpenoid saponins and flavonoids in inhibiting the activity of α-glucosidase [50,51]. Moreover, studies also suggest the functions of key groups in the structure of flavonoids and triterpenoid saponins in their α-glucosidase inhibiting activity, such as the number and position of hydroxyl groups and the carbonyl group in flavonoids [52] and the position of sugar moieties in the aglycon in triterpenoid saponins [53]. Therefore, quinoa husk extracts possess potential inhibitory effects on α-glucosidase, the main active ingredients of which are probably flavonoids and triterpenoid saponins. Nevertheless, most α-glucosidase inhibitor screening studies select enzyme preparations from nonmammalian sources (usually Saccharomyces cerevisiae), which could present some limitations due to the difficulty in assessing the actual in vivo hypoglycemic potential [54]. Accordingly, in future research work, we will further verify the potential hypoglycemic activity of the extracts through in vivo assays.

![Figure 5](image-url)

**Figure 5.** Inhibitory effect of the quinoa husk extracts and acarbose on α-glucosidase at different concentrations.

3.3.2. Interaction between α-Glucosidase and Components in Quinoa Husk Extracts

Molecular docking was performed on the 23 compounds identified to explore the mechanism of the α-glucosidase inhibiting effects by the quinoa husk extracts. The docking affinity values between the compounds and α-glucosidase are shown in Tables 5 and S2. Remarkably, most compounds showed lower affinity values (within the range of −12.7 to −7.7 kcal/mol) than acarbose, suggesting greater binding with α-glucosidase. The strong binding found here is in agreement with previous reports on the analysis of flavonoids and triterpenoid saponins interacting with α-glucosidase [55,56].
The interactions of some flavonoids and triterpenoid saponins with better affinity values are shown in Figures 6 and 7. The binding site on α-glucosidase by flavonoids 1, 2, 3, 8 was similar to that of acarbose [57]. They were stabilized at the active site of α-glucosidase and interacted with amino acid residues by hydrogen bonds, salt bridge, hydrophobic interaction and other types of forces, via residues, such as His280, Asp325, Asp307, Arg315, Leu313, Pro312, Thr310, Ser311, Asp242, Glu332 and Ala281. It was reported that a flavonoid with two catechol groups in rings A and B and a 3-OH group in the C ring was very likely to have stronger α-glucosidase inhibitory activity than acarbose [58], a notion, which is supported by our findings. For instance, the 5-OH, 7-OH and 5′-OH of Compound 3 could interact with amino acid residues Asp352, Gln353 and Asp242 through hydrogen bonds, which showed a better affinity value (−9.7 kcal/mol) compared to acarbose (−8.5 kcal/mol).

![Figure 6](image_url)

**Figure 6.** Molecular docking analysis and 3D modeling for the interaction between the compounds (Table 5) and α-glucosidase. Compounds 1, 2, 3, 8 are flavonoids; Compounds 9, 14, 16, 19 are triterpenoid saponins.

| Compound Number | Flavonoids | Triterpenoid Saponins | Acarbose |
|-----------------|------------|-----------------------|----------|
| Affinity (kcal/mol) | -8.6 | -8.1 | -9.7 | -8.9 | -11.6 | -12.6 | -12.2 | -12.7 | -8.5 |

**Table 5.** Affinity values of major compounds with α-glucosidase.

Triterpenoid saponins 9, 14, 16, 19 exerted excellent binding affinity toward α-glucosidase. They could bind with amino acid residues in the catalytic active site of α-glucosidase through hydrogen bonds and hydrophobic interactions via amino acid residues, such as His280, Gly309, Asn 247, Asp325, Asp307, Pro312, Leu246, Val319, Val308 and Ala329. Both Compounds 14 and 19 contained glucuronic acid groups at the C-3 position, which formed hydrogen bonds with the amino acid residue Gly309 and Asn 247, respectively. Considering the higher affinity value of Compounds 14 and 19, we speculate that hydrogen bonds serve as the main force to inhibit the catalytic activity. Moreover, the carboxyl group at the C-28 position may establish hydrogen bond to induce conformational change of α-
glucosidase and thereby could reduce the catalytic activity [59]. Furthermore, the aglycones at C-28 connected to the glucose moiety could also effectively inhibit the α-glucosidase activity [60]. Taken together, the inhibitory effect on α-glucosidase is largely determined by the hydrogen bonds and hydrophobic interactions, which mainly depends on the nature of the interaction-related residues and that of the compounds themselves [61]. These results could expand our knowledge on the interaction mechanisms between α-glucosidase and triterpenoid saponins and facilitate the development of potential α-glucosidase inhibitors.

Figure 7. Conformations of the compounds (Table 5) interacting with amino acid residues at the active site of α-glucosidase. Compounds 1, 2, 3, 8 are flavonoids; Compounds 9, 14, 16, 19 are triterpenoid saponins.

4. Conclusions

The present study utilized pressurized hot water extraction to extract total saponins from quinoa husks and optimized the extraction conditions to obtain a maximal yield of 23.06 mg/g. Meanwhile, twenty-three compounds, mainly triterpenoid saponins and flavonoids, were identified from the extracts by LC-MS. The in vitro glycosidase activity assay showed that the quinoa husk extracts possessed stronger inhibiting activity on α-glucosidase than acarbose, while molecular-docking-based interaction analyses further suggested the main bioactive components to be triterpenoid saponins. In conclusion, pressurized hot water extraction is an environmentally friendly, safe and efficient extraction method for quinoa husk saponins. Quinoa husk saponins are promising food supplements to control postprandial hyperglycemia and candidates to be developed into α-glucosidase inhibitors. In addition, the mechanism of the effects of quinoa husk saponins on hyperglycemia requires more studies to be elucidated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods11193026/s1, Table S1: The response surface analysis of variance (ANOVA); Table S2: Affinity values of compounds 1–23 and acarbose with α-glucosidase.

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