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Research Article

Screening and extraction process optimization for potential α-glucosidase inhibitors from quinoa seeds

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Abstract: To explore the potential factors of quinoa to control blood sugar, five active components, polyphenols, flavonoids, saponins, alkaloids and polysaccharides, were extracted from three kinds of quinoa by using ten different solvents, then *in vitro* α-glucosidase activity inhibitory experiment these 30 kinds of extracts were determined. Results showed that the total polyphenols from black quinoa had a strong correlation on the inhibition of α-glucosidase (r = 0.91, P < 0.001). Thus, the extraction process of black quinoa crude polyphenols (BQCP) was carried out, and the most efficient extraction conditions were: material-liquid ratio was 1:70 g/mL, ultrasonic temperature was 45 °C, ultrasound time was 30 min, ultrasonic power was 400 W, the optimal extraction amount was 5.148 ± 0.038 mg/g. Moreover, the black quinoa polyphenols (BQP), purified from BQCP, had stronger inhibitory ability on α-glucosidase (IC_{50} = 0.59 mg/mL) and α-amylase (IC_{50} = 2.85 mg/mL) and its inhibition effect was greatly to BQCP. This study demonstrates that BQP can be a good potential source of natural hypoglycemic drugs or functional foods.

Keywords: quinoa; active ingredients; α-glucosidase; ultrasonic extraction; polyphenols; α-amylase

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1 Introduction

Diabetes is the third major non-communicable disease that threatens human health after tumors and cardiovascular diseases, is becoming more prevalent globally. According to the International Diabetes Federation's Global Diabetes Map 2021, 537 million adults are currently living with diabetes, which is 10.5% of the global population in that age group, and the number of diabetic individuals is predicted to increase to 783 million (12.2%) in 2045[1]. Diabetic patients with chronic hyperglycemia can suffer a range of diabetic complications, including diabetic nephropathy, diabetic cardiovascular complications, neuropathy, eye and liver complications[2], which are the main causes of their death. Currently, the main drugs used clinically for the treatment of type 2 diabetes are sulfonylureas, biguanides, thiazolidines, meglitinide, dipeptidyl peptidase 4 inhibitors, sodium-glucose cotransporter inhibitors and α-glucosidase inhibitors[3]. Compared to other oral hypoglycemic drugs, α-glucosidase inhibitors are milder and only act on part of the intestinal tract rather than regulating certain biochemical processes in the body. Moreover, α-glucosidase inhibitors can also lower the level of plasma triglyceride and decrease the risk of cardiovascular disease and hypertension. As a result, they are considered the first-line drugs for the prevention and treatment of type 2 diabetes. The α-glucosidase inhibitors can be extracted from natural plants and through chemical synthesis. Acarbose and miglitol are frequently utilized in the treatment of diabetes. However, drug resistance and other side effects, such as gastrointestinal discomfort, hypoglycemia, and weight gain, can arise from long-term use of these drugs[4]. Therefore, it is of great significance to developing safer, more effective anti-diabetic functional foods and drugs with fewer side effects. Bioactive components like flavonoids, polyphenols, polysaccharides, alkaloids, saponins and terpenoids were found in numerous natural products, which have been proven that they have certain hypoglycemic effects[5]. Hence, it might be the potential source to explore hypoglycemic foods or drugs.

Quinoa (*Chenopodium quinoa* Willd) is an annual Chenopodium herbaceous crop, which is native to the Andes Mountains of South America. There are many varieties of quinoa, and during their physiological maturity, quinoa grains will display a distinct variety of colors, including white, cream, yellow, orange, pink, red, purple, light coffee, dark coffee, green coffee, and black. After removing the husk, however, the seeds usually only exhibit three main colors: white, red, and black. The color of quinoa seeds affects their commercial value largely due to their different levels of nutrients and potential health effects. Quinoa is not only abundant in dietary fiber, vitamins and minerals, but also containing all the fatty acids and amino acids, as well as substance

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beneficial to the human body such as polyphenols, flavonoids, saponins, beets, and phytosterols, which can help prevent cancer, inflammation, hyperglycemia, and hyperlipidemia[6]. Up to date, several studies have shown that quinoa has beneficial effects on glycemic control. Zhang *et al.*[7] reported a significant reduction in glycemic index in mice that consumed quinoa flour. Erfidan *et al.*[8] found a therapeutic effect of quinoa in insulin-resistant rats. Lappi *et al.*[9] reported that products with added fermented quinoa were more effective in lowering postprandial glycaemia and insulinaemia.

Currently, most of the studies on the hypoglycemic effects of quinoa have focused on the hypoglycemic capacity of quinoa itself and its potential use as a functional food to prevent the onset of diabetes in humans, so the hypothesis of this study was to uncover the potential hypoglycemic functional components of quinoa. In this study, *α*-glucosidase activity inhibitory capacity was chosen as a reference standard for screening *α*-glucosidase activity inhibition factors in three types of quinoas. After knowing the active ingredients that exerted the main effects, the optimal extraction process was also investigated. The purpose of this study is to provide information for the development and application of quinoa in functional food and anti-diabetes drugs, as well as to provide some reference for researchers to choose different types of quinoa materials.

2 Material and methods

2.1 Materials and reagents

The black quinoa (BQ), red quinoa (RQ), and white quinoa (WQ) used in this study were all produced in Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, China. Acarbose (purity ≥ 95.0%) was purchased from Beijing Solarbio Science and Technology Co. (Beijing, China). *α*-Glucosidase (≥ 50 units/mg), Porcine pancreatic *α*-amylase (≥ 5 units/mg), gallic acid (purity ≥ 98%), rutin (purity ≥ 98%), oleanolic acid (purity ≥ 98%), berberine hydrochloride (purity ≥ 98%), and standard glucose solution were provided by Shanghai Yuanye Biotechnology Co., LTD. (Shanghai, China). *P*-nitrophenyl-*α*-*D*-glucopyranoside (PNPG) (purity ≥ 99%) was obtained from Shanghai McLean Biochemical Technology Co., LTD. (Shanghai, China). AB-8 macroporous resin (AR) is supplied by Shanghai McLean Biochemical Technology Co., LTD. (Shanghai, China). All other reagents including methanol, ethanol, acetone, ethyl acetate, ether and chloroform analytical grade chemicals were purchased from Aladdin Co., LTD. (Shanghai, China).

2.2 Sample preparation and extraction procedures

The dried BQ, RQ and WQ seeds were crushed separately using a DFY-500 high speed traditional Chinese medicine mill (Linda Machinery Co., LTD., Wenling, China) and passed through an 80-mesh screen. An appropriate amount of quinoa powder was taken at a solid/solvent ratio of 1:50 g/mL and mixed with the extractant (methanol, ethanol, acetone, water, ethyl acetate, chloroform, ether, 70% methanol, 70% ethanol, 70% acetone). The extraction was carried out under ultrasonic conditions (JY-92IIN ultrasonic cell morcellator, Ningbo Xinzhi Biotechnology Co., LTD., Ningbo, China) for 30 min at a power of 500 W and a temperature of 50 °C. After filtration and centrifugation at 4 000 r/min, extracts were concentrated under vacuum using a rotary evaporator to obtain 30 samples.

2.3 Determination of active ingredients in different solvent extracts of quinoa

2.3.1 *Total polyphenols content (TPC)*

The TPC was determined using Folin-Ciocalteau reagent[10]. TPC assay was conducted by mixing 4.0 mL of water, 1.0 mL of extracts, 0.5 mL Folin-Ciocalteu reagent and 1.0 mL 10% (*m*/*v*) Na_2CO_3. Absorbance of mixture was measured at 740 nm in a TU-1901 dual-beam UV-visible spectrophotometer (Beijing Puz General Instruments Co., LTD., Beijing, China). A standard curve was prepared with gallic acid ($y = 15.619\ 9x + 0.032\ 4$, $R^2 = 0.999\ 5$). The TPC were expressed as mg gallic acid equivalent (GAE)/g DW.

2.3.2 *Total flavonoids content (TFC)*

The TFC was measured according to a previous method[11]. A 1.0 mL aliquot of extracts was mixed with 1.7 mL of water and then reacted with 0.3 mL of 5% (*m*/*v*) $NaNO_2$ solution for 5 min. The mixture was incubated for 5 min at the room temperature after 0.3 mL of 10% (*m*/*v*) Al $(NO_3)_3$ solution was added. Then, the mixture was reacted with 2.0 mL of 4% (*m*/*v*) NaOH solution and the water was then added to bring the final volume to 10 mL. After the mixture was reacted for 10 min, the absorbance was detected at 510 nm. A standard curve was prepared with rutin ($y = 25.328\ 6x–0.026\ 7$, $R^2 = 0.999\ 9$). The results were expressed as mg rutin equivalent (RE)/g DW.

2.3.3 *Total saponins content (TSC)*

The TSC was measured based on a previously described method with some modifications[12]. Briefly, 0.1 mL of the aliquot of extracts was put in a 70 °C water bath to remove the solvent. Then, the cooled residue was mixed with 0.2 mL of 5% (*m*/*v*) vanillin-acetic acid solution and 0.8 mL of perchloric acid. The mixture was incubated in a water bath at 70 °C for 15 min. After cooling, the mixture was incubated for an additional 10 min at room temperature after 4.0 mL of acetic acid was added. Absorbance was determined at 560 nm. A standard curve was prepared with oleanolic acid ($y = 8.865\ 7x–0.025\ 1$, $R^2 = 0.999\ 5$). The TSC were expressed as mg oleanolic acid equivalent (OAE)/ g DW.

2.3.4 *Total alkaloids content (TAC)*

The TAC was determined through the implementation of the methods mentioned in the literature, with modifications[13]. Absorbance was measured at 416 nm. The TAC was expressed as mg of berberine hydrochloride equivalents (BHE) per g DW of quinoa. The standard curve ($y = 4.811\ 4x + 0.003\ 1$, $R^2 = 0.999\ 8$) was obtained when berberine hydrochloride was used as the standard. The TAC was expressed as mg Berberine hydrochloride equivalent (BHE)/ g DW.

2.3.5 *Total polysaccharides content (TPSC)*

The TPSC was determined by the phenol sulfuric acid method[14]. A 1.0 mL aliquot of extracts was mixed with 1.0 mL of 5% phenol and 5.0 mL concentrated sulfuric acid. The mixture was incubated for 30 min at 37 °C. The absorbance was determined at 490 nm. A standard curve was prepared with glucose solution ($y = 8.878\ 6x–0.152\ 3$, $R^2 = 0.999\ 8$). The TPSC in the sample was calculated according to the standard curve.

2.4 Inhibition assay of *α*-glucosidase

The inhibitory activities of *α*-glucosidase were measured as

previously described with a few modifications[15]. The samples, substrates and enzymes solutions were dissolved in 0.1 mol/L phosphate buffer saline (PBS) (pH 6.8). 40 μL of test samples were mixed with the enzyme solution in microplate wells and incubated for10 min at 37 °C. Subsequently, 40 μL PNPG solution was added. After incubation at 37 °C for 30 min, the reaction was terminated by the addition of 200 μL of 1.0 mol/L Na_2CO_3. α-Glucosidase activity was measured spectrophotometrically at 405 nm on 96-well microplate reader by measuring the amount of p-nitrophenol released. Acarbose was used as a reference inhibitor for enzymes. The α-glucosidase inhibitory activity was expressed as the inhibition rate. The inhibition rate was calculated using the following equation:

$$Inhibitionrate\,(\%) = [1-(A_1-A_2)/(A_3-A_4)]\times 100\% \quad (1)$$

Where A_1, A_2, A_3, and A_4 represent the absorbance values of the buffer containing the enzyme, the buffer without the enzyme, the sample solution containing the enzyme and the sample solution without the enzyme, respectively.

2.5 Single factor experiment for ultrasound-assisted extraction of polyphenols from BQ

2.5.1 *The effect of solid/solvent ratio*

BQ powder was precisely weighed and mixed with water at five different solid/solvent ratios (1:30, 1:40, 1:50, 1:60, 1:70 and 1:80 g/mL). Extract polyphenols at a fixed extraction temperature of 50 °C, ultrasound assisted extraction time of 30 min, and ultrasound power of 500 W.

2.5.2 *Effect of extraction temperature*

Polyphenols were extracted at different temperatures (35, 40, 45, 50, 55 and 60 °C), according to the optimal solid/solvent ratio conditions obtained by the above experiments. In addition, the ultrasonic power was 500 W and the ultrasonic time was 30 min.

2.5.3 *Effect of ultrasound time*

Under the conditions of optimal extraction temperature and solid/solvent ratio obtained above, the BQ polyphenols were extracted with 500 W ultrasonic power for 20, 25, 30, 35, 40 and 45 min.

2.5.4 *Effect of ultrasound power*

Under the conditions of fixed optimal solid/solvent ratio, ultrasonic temperature and ultrasonic time, the effects of different ultrasonic powers (200, 300, 400, 500, 600 and 700 W) on the extraction of BQ polyphenols were investigated.

2.6 Optimization of the extraction process of polyphenols using orthogonal method

According to the results of the above single-factor experiment, four influencing factors of solid/solvent ratio, ultrasonic temperature, ultrasonic time and ultrasonic power were selected, and the extraction amount of polyphenols was taken as the investigation target, and the BQ polyphenol extraction process conditions were optimized according to the orthogonal table of $L_9(3^4)$ (Table 1).

Table 1 Orthogonal experimental factors and level tables

Factors	Levels		
	1	2	3
A. Solid/solvent ratio (g/mL)	1:50	1:60	1:70
B. Extraction temperature (°C)	45	50	55
C. Ultrasonic time (min)	25	30	35
D. Ultrasound power (W)	400	500	600

2.7 Purification and enrichment of quinoa polyphenols

The BQ crude polyphenols (BQCP) was extracted with optimal ultrasonic extraction conditions, then be purified and enriched by AB-8 macroporous resin. Briefly, BQCP was dissolved in deionized water to prepare 3 mg/mL loading solution, and the pH was adjusted to 3.0. The loading flow rate was 1 mL/min. Elution solution was collected and TPC was measured after every 10 mL solution. The resin is defined to be in adsorption equilibrium when the TPC of the eluent is one tenth of the initial sample solution. After the adsorption equilibrium was determined, the resin column was first rinsed with 3 BV deionized water to remove impurities such as polysaccharide and inorganic salts. The sample was then eluted with 50% ethanol at a flow rate of 1 mL/min. Eluent solution was collected and TPC was measured after every 10 mL of solution flow. The resin was defined as reaching desorption equilibrium when the TPC of the eluted solution was at its lowest level and did not substantially change. The eluent collected was evaporated and concentrated at 45 °C in a rotary evaporator, and then freeze-dried to obtain BQ polyphenols (BQP).

2.8 Inhibition assays of α-amylase

The samples were determined with α-amylase activity inhibition according to the previous method[16] with slight modifications. Different concentrations of BQCP, BQP and acarbose were mixed with 40 μL α-amylase (7.0 U/mL) and incubated at 37 °C for 10 min. The mixture was incubated for an additional 10 min at 37 °C after 40 μL of soluble starch solution was added.

Subsequently, the reaction was terminated by the addition of 80 μL of DNS reagent while the mixture was placed in a boiling water bath for 5 min to destroy the activity of enzymes. After cooling to room temperature, the absorbance was measured at 540 nm. The inhibition rate can be calculated by the following formula:

$$Inhibitionability\,(\%) = [(A_b-A_s)/A_b]\times 100\% \quad (2)$$

Here, A_b and A_s represent the control group minus the absorbance value of the control blank group and the sample group minus the absorbance value of the sample blank group, respectively.

The inhibitory activities of α-glucosidase and α-amylase were calculated using Eqs. (1) and (2). The IC_{50} values, namely the concentration giving 50% inhibition of α-glucosidase/α-amylase activity, were expressed as mg/mL.

2.9 Statistical analyses

Extraction and analysis were performed uniformly in triplicate, and experimental results were expressed as the mean ± SD of triplicate samples. SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Spearman correlation coefficient was used in the correlation analysis. Correlation analysis, principal component and cluster analysis were performed by OriginPro 2022 Learning Edition (Origin Lab, Northampton, MA, USA). The value of $P < 0.05$ was considered significant correlation and $r > 0$ was considered positive correlation.

3 Results and discussion

3.1 TPC, TFC, TSC, TAC and TPSC of quinoa

The TPC was calculated through using gallic acid as the standard and the results are shown in Fig. 1A. When water and 70% acetone were used as extraction solvent, the highest TPC was found in the extract of BQ, which was 3.60 mg GAE/g and 3.34 mg GAE/g, respectively. There was no significant difference in the amount of polyphenol between the water (WE) and 70% acetone extracts (AE) ($P < 0.05$). For both RQ and WQ, the highest TPC was obtained when water was used as the extraction solvent (4.29 mg GAE/g and 4.81 mg GAE/g, respectively), followed by 70% acetone (4.13 mg GAE/g and 3.03 mg GAE/g, respectively). From the solvent point of view, water and 70% acetone were used as extraction solvent for quinoa polyphenols extraction. However, considering the environmental protection, cost and extraction efficiency, water was the best extraction solvent for quinoa polyphenols.

Using rutin as the standard, the TFC was calculated, and the results were shown in Fig. 1B. When 70% methanol, 70% ethanol and 70% acetone were used as extraction solvents for BQ, the extraction effect of flavonoid was better, and the extraction amounts were 1.95 mg RE/g, 2.00 mg RE/g and 1.93 mg RE/g, respectively. There was no significant difference in flavonoid content between 70% methanol extract (ME), 70% ethanol extract (EE) and 70% AE of BQ ($P < 0.05$). For RQ, the highest flavonoid content was obtained from 70% AE (3.06 mg RE/g), followed by

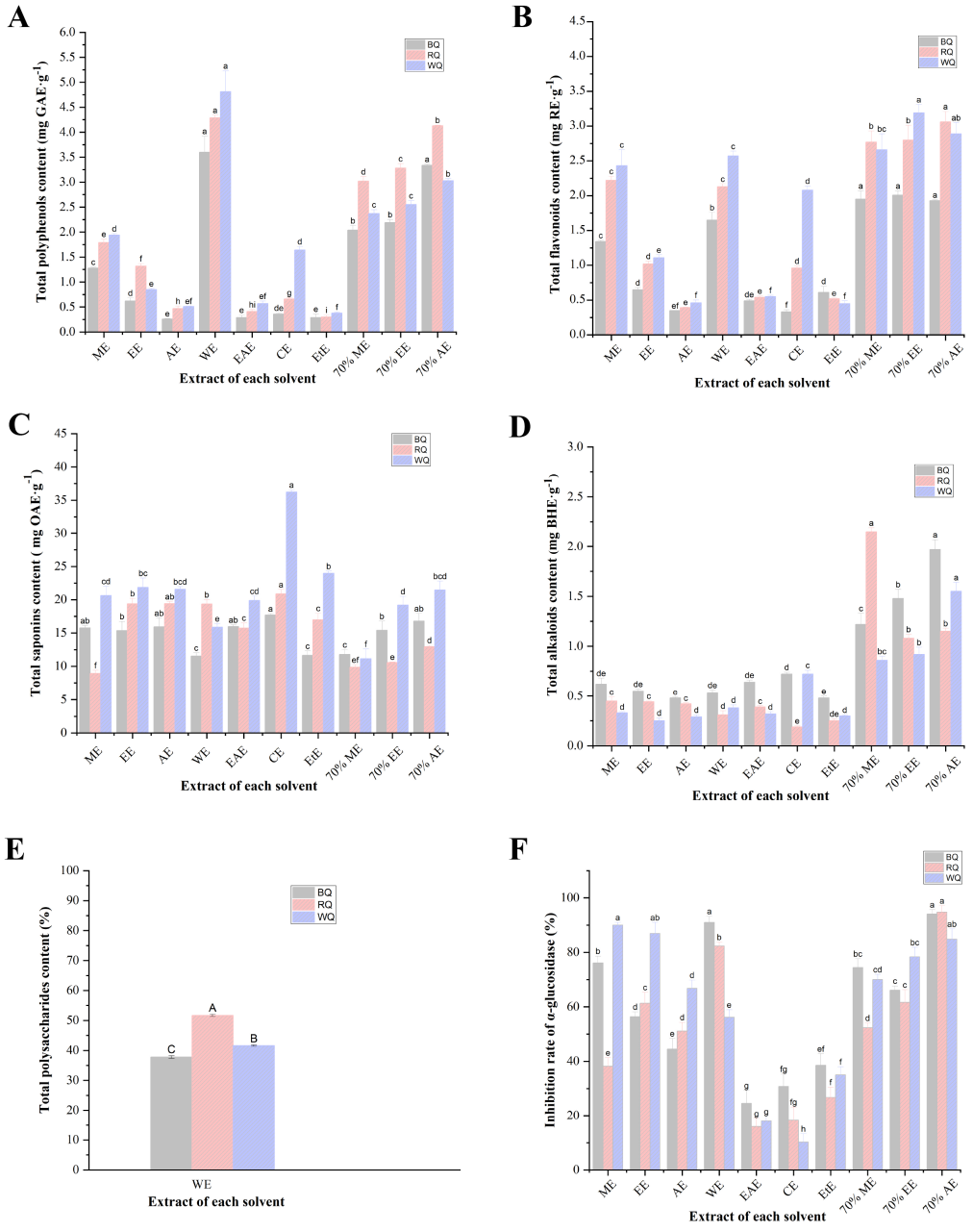

Figure 1 Total polyphenols (A), flavonoids (B), saponins (C), alkaloids (D) and polysaccharides (E) content and the inhibition rate of α-glucosidase (F) in each solvent extract of three varieties quinoa. Mean ± SD, $n = 3$. Different letters indicate ($P < 0.05$) differences in the amount between the samples. (BQ: black quinoa, WQ: white quinoa, RQ: red quinoa, ME: methanol extract, EE: ethanol extract, AE: acetone extract, WE: water extract, EAE: ethyl acetate extract, CE: chloroform extract, EtE: ether extract).

70% ME (2.77 mg RE/g) and 70% EE (2.80 mg RE/g). For WQ, the highest flavonoid content was found in 70% EE (3.19 mg RE/g), followed by 70% AE (2.89 mg RE/g), with no significant difference ($P < 0.05$). In general, 70% methanol, 70% ethanol and 70% acetone were more effective in the extraction of flavonoid from quinoa compared to the remaining seven extractants.

The TSC was calculated with oleanolic acid as the standard, and the results were shown in Fig. 1C. For RQ, the TSC of chloroform extract (CE) (20.88 mg OAE/g) and AE (19.4 mg OAE/g) was higher, followed by WE (19.37 mg OAE/g). Compared with other two kinds of quinoa extracts, the TSC of WQ was higher. The CE of WQ had the significantly highest TSC of 36.24 mg OAE/g.

Berberine hydrochloride was used as the standard to calculate TAC, and the results were shown in Fig. 1D. For alkaloids, 70% methanol, 70% ethanol and 70% acetone showed that the extraction performance is significantly better than the other solvents. Both BQ and WQ had the highest TAC in 70% AE (1.97 mg BHE/g and 1.55 mg BHE/g, respectively). In the case of RQ, 70% methanol gave the greatest extraction of the alkaloids it contained (2.15 mg BHE/g).

The TPSC was calculated using glucose as a standard, and the results were shown in Fig. 1E. Because polysaccharides can only be soluble in water, only water was used to extract the three types of quinoa in this experiment, and their polysaccharide content is illustrated in Fig. 1E. The polysaccharide content in the WE of RQ was found to be higher after comparing the differences among groups.

3.2 Inhibition rate of α-glucosidase

The inhibitory effect of 30 samples on α-glucosidase was determined separately (Fig. 1F). Among the BQ extracts, WE and 70% AE showed the strongest inhibitory effects on α-glucosidase, followed by ME and 70% ME. RQ was 70% AE with the highest enzyme inhibition rate, followed by WE. For WQ, ME, EE and 70% AE had the best α-glucosidase inhibition, and there was no significant difference in the inhibitory effect of α-glucosidase among these three extracts.

The different inhibitory effects of quinoa extracts on the α-glucosidase by different solvents may be primarily due to the different types and contents of active constituents in each sample, which also related to the polarity of the extraction solvent. It can be seen from Fig. 1F, all the three quinoa extracts exhibited varying degrees of α-glucosidase inhibition. No matter what kind of quinoa, the α-glucosidase inhibitory ability of its WE, 70% ME, 70% EE, 70% AE, ME, EE and AE was generally stronger than that of CE, ether extract (EtE) and ethyl acetate extract (EAE), it suggested that quinoa components extracted by strong polar solvent had a greater contribution to the inhibition of α-glucosidase. Similar to quinoa, soybean, as a legume food, also occupies an influential position in the diet of our country. Lee *et al.* [17] reported that 70% ME of soybean had the highest α-glucosidase inhibition rate (81%), which was significantly higher than that of acetone (24%) and dichloromethane extract (17%). As strong polar solvents, methanol and water are typically more favorable for the extraction of active components such as polyphenols and flavonoids. In this study, WE of quinoa has the highest TPC, but the correlation between quinoa polyphenol content and its α-glucosidase inhibition needs to be further analyzed.

3.3 Correlation analysis of α-glucosidase activity inhibition

In order to identify the functional active factors in quinoa that play the main anti-hyperglycemic effect, the correlation analysis between each component and the inhibitory ability of α-glucosidase were performed. As shown in Figs. 2 (A-C), the closer the correlation coefficient is to 1, the redder the color is. On the contrary, the closer the correlation coefficient is to −1, the bluer the color is. In addition, the closer the absolute value of the correlation coefficient is to 1, the closer the shape of the graph is to an ellipse. From this, we could find that the TPC had a strong positive correlation with the TFC in BQ ($r = 0.88$, $P < 0.05$). The components that were highly correlated with the α-glucosidase inhibition ability of BQ extract were polyphenols ($r = 0.91$, $P < 0.001$), followed by flavonoids ($r = 0.84$, $P < 0.001$) (Fig. 2A). Meanwhile, TAC in BQ was also correlated with its α-glucosidase inhibition ability ($r = 0.55$, $P < 0.05$). For RQ (Fig. 2B), polyphenols were the major contributors to α-glucosidase inhibition ($r= 0.86$, $P < 0.001$), followed by flavonoids ($r = 0.68$, $P < 0.05$). Interestingly, unlike with BQ and RQ, no significant correlation was found between TPC in WQ and its α-glucosidase inhibitory capacity ($P > 0.05$) (Fig. 2C). Although TPC in WQ was higher than that in BQ and RQ, the α-glucosidase inhibitory ability was not related to its TPC. It suggested that polyphenol compounds in WQ might be quite different from those of BQ and RQ, or polyphenol compounds of great content in WQ might have a weak α-glucosidase inhibition. Hemalatha *et al.* [18] pointed out that the α-glucosidase inhibition of polyphenol extracts from quinoa bran was stronger than other milled fractions including hulls, dehulled grain and milled grain, this might owe to the high contents of gallic acid, ferulic acid, rutin and quercetin in this fraction. Ferulic acid[19] and gallic acid[20] have been confirmed to have stronger α-glucosidase inhibition with lower IC_{50} value. In addition, gallic acid inhibited α-glucosidase with non-competitive manner[20]. Hence, we supposed that the contents of these polyphenol compounds with strong α-glucosidase inhibitory effect in BQ might be higher than that in WQ. However, the identification and quantitation of polyphenol compounds in BQ, RQ and WQ need further being performed, respectively.

In a similar study, polyphenol extracts from a variety of Canadian lentil cultivars were found to have stronger α-glucosidase inhibition ability, which may help to control blood glucose level and obesity[21]. In the functional activity evaluation of millet polyphenols, it is known that millet shows effective inhibition of α-glucosidase before and after processing, and it is a strong natural inhibitor source of α-glucosidase[22]. In a study on red rice, polyphenol compounds were found to be the main components that inhibited α-glucosidase[23].

3.4 Principal component and cluster analysis

The relationship between TPC, TFC, TSC, TAC, TPSC and α-glucosidase inhibition ability were analyzed by PCA and cluster analysis. The PCA biplot showed that the first principal component PC1 accounted for 50.3% of the total variance, and the second principal component PC2 accounted for 21.4% of the total variance (Fig. 2D). These two components could explain 71.7% of the variance. The PCA biplot showed that the correlation between PC1 and variables was better than PC2, among which PC1 had strong positive correlations with α-glucosidase inhibitory activity, TFC and TPC. In the PCA plot, when the sample points overlap or are close to each other, it indicates that their compositional similarity is high. In Fig. 2D, some sample points overlap, indicating that although quinoa extracted by different solvents are different in general, a considerable part of them are similar, which may be related to the polarity of the extraction solvent. When the polarity of the solvent is similar, the active ingredients extracted

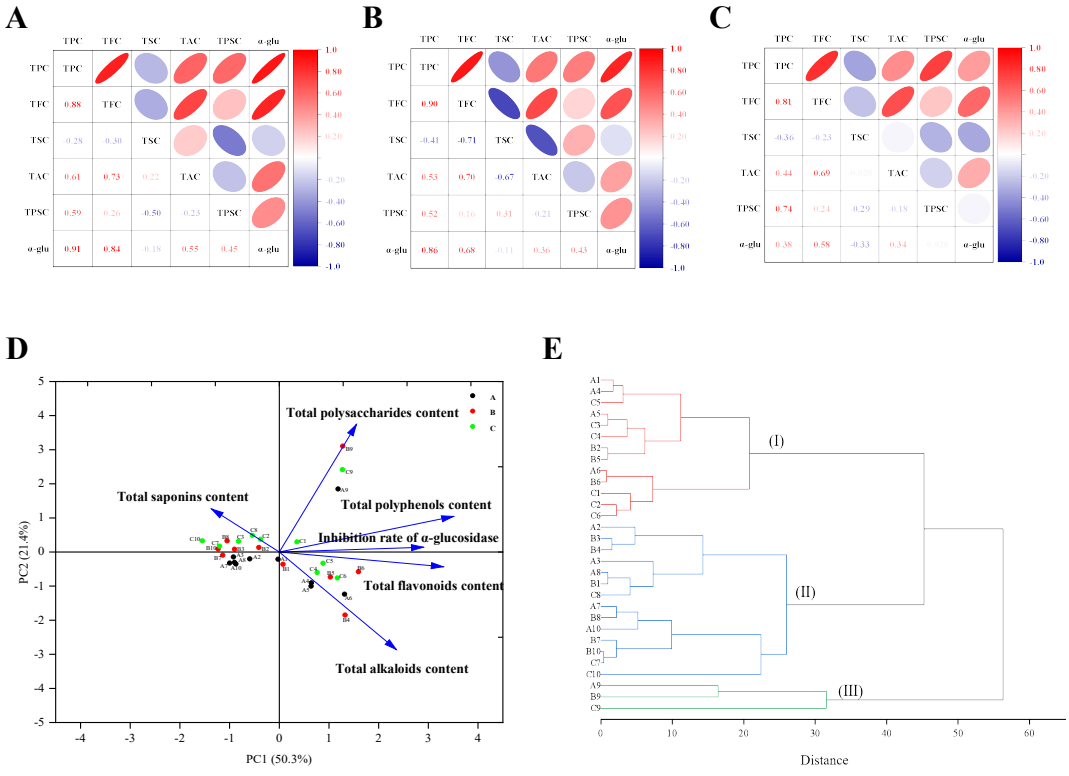

Figure 2 Correlation analysis among the total content of polyphenols (TPC), flavonoids (TFC), saponins (TSC), alkaloids (TAC), polysaccharides (TPSC), and α-glucosidase inhibition capacity (α-glu) in black quinoa (A), red quinoa (B) and white quinoa (C), respectively. Principal components (D) and cluster analysis results (E). (1: methanol extract, 2: ethanol extract, 3: acetone extract, 4: 70% methanol extract, 5: 70% ethanol extract, 6: 70% acetone extract, 7: ethyl acetate extract, 8: chloroform extract, 9: water extract, 10: ether extract).

are similar (similar phase solution principle). The dendrogram of cluster analysis (Fig. 2E) confirmed our view.

As shown in Fig. 2E, at a Euclidean distance of 35, the different solvent extracts were divided into three main groups based on their ability to inhibit α-glucosidase and the TPC, TFC, TSC, TAC, TPSC of the extracts. The first group was mainly composed of extracts from more polar solvents, including ME, EE, 70% ME, 70% EE and 70% AE, which had higher TFC and TAC. The second group consisted of the extracts of medium polarity solvents, including AE, CE, EtE and EAE, which had the highest TSC and low content of other active ingredients. Group 3 was the WE group with higher α-glucosidase inhibition ability and TPC.

Combined with the previous correlation analysis results, it was found that the polyphenols of BQ had the strongest correlation with the inhibition ability of α-glucosidase, which was also verified in the subsequent PCA and cluster analysis. In addition, by comparing the results of 10 solvent extracts, it was found that water is the best extraction solvent for BQ polyphenols. Therefore, water was selected as the extraction solvent for BQ polyphenols for the next experiment.

3.5 Results of ultrasonic extraction process optimization of polyphenols

Among the myriad variables, the solid/solvent ratio was particularly crucial, and the change of the solid/solvent ratio had a greater impact on the amount of polyphenols extracted. The lower the solid/solvent ratio, the better the solvent extraction effect on the solid. The relationship between extraction amount and solid/solvent ratio in this study was shown in Fig. 3A. The results showed that the solid/solvent ratio in the range of 1:30 g/mL to 1:60 g/mL, with the increase of solvent amount, the amount of polyphenols extracted also increased significantly. That was because at a lower solid/solvent ratio, the solvent had a higher adequate dissolution of the polyphenols. At the same time, according to the mass transfer principle, the larger the concentration gradient between solid and liquid in the mass transfer process, the stronger the driving force wound be, which was conducive to the dissolution of polyphenols[24]. Pinelo *et al.* [25] also have reported similar results on the effect of the solid/solvent ratio on the extraction amount of polyphenol compounds. However, when the solid/solvent ratio continued to decrease, the amount of polyphenols dissolved began to decrease. This may be because some impurities (such as polysaccharides) begin to dissolve, reducing the leaching of polyphenols, or because the amount of extractable has reached dissolution equilibrium, and leading to no increase.

When the extraction temperature was increased from 35 °C to 50 °C, the extraction amount increased from 4.47 to 5.03 mg GAE/g, after which the temperature was increased again and the extraction amount began to decrease (Fig. 3B). The increase of temperature enhanced the solubility of the solvent and accelerated the penetration and diffusion of the polyphenols. However, when the temperature continued to rise, the polyphenols were prone to oxidation, and some of them wound decompose, which led to the decline of the extractable amount[26].

The effect of ultrasound time from 20 min to 45 min on the amount of polyphenols extraction was shown in Fig. 3C. At the beginning of ultrasonic treatment, ultrasonic extraction was not efficient, because it took more time to destroy the cell wall. When the ultrasound time was extended from 20 min to 30 min, polyphenols were continuously released from the cells, so the extraction amount increased significantly, reaching the peak at 30 min. After prolonging the ultrasonic time, the extraction amount

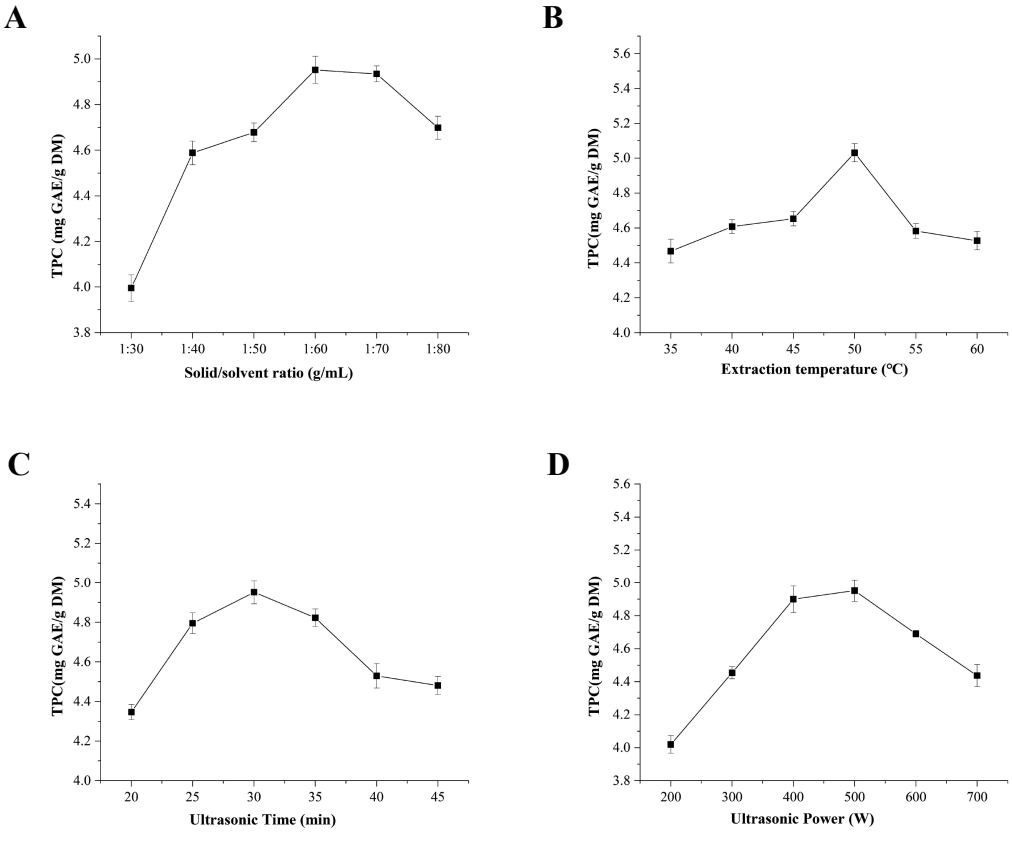

Figure 3 Results of single factor experiments on polyphenol extraction.

of polyphenols began to decrease, because the polyphenols were essentially dissolved within 30 min of the ultrasound time. If the ultrasound time was continued, it would lead to the degradation or transformation of certain polyphenols[27].

In addition, it can be seen that with the increase of ultrasound power, the extraction amount of polyphenols first increased and then decreased (Fig. 3D). This is mostly because within a certain range, ultrasonic cavitation and thermal effects can promote the dissolution of polyphenol compounds in the cell wall of cell plants, while excessive power can destroy the molecular structure of polyphenol compounds[28]. Therefore, the ultrasonic power should be maintained between 400 W and 500 W.

The optimal levels of the four factors was determined by the previous univariate experiment, which laid for the foundation for the subsequent orthogonal optimization experiments of polyphenol extraction. It can be seen that from the data analysis (Tables 2 and 3), the solid/solvent ratio was the most influential factor affecting the amount of polyphenols extracted from BQ, and the extraction temperature had a minimal effect on the amount of polyphenols extracted from BQ. The influence of each factor was $A > C > D > B$. According to the results of visual analysis, the optimal extraction process of polyphenols was $A_3B_1C_2D_2$, that was, the solid/solvent ratio was 1:70 g/mL, the extraction temperature was 45 °C, the ultrasonic time was 30 min,

Table 2 Orthogonal experimental design and results

Number	Factors				TPC (mg/g)
	Solid/solvent ratio (g/mL) *A*	Temperature (°C) *B*	Ultrasound time (min) *C*	Ultrasound power (W) *D*	
1	1	1	1	1	4.609±0.087
2	1	2	3	2	4.592±0.022
3	1	3	2	3	4.561±0.089
4	2	1	3	3	4.867±0.083
5	2	2	2	1	4.954±0.106
6	2	3	1	2	4.776±0.157
7	3	1	2	2	5.148±0.038
8	3	2	1	3	5.068±0.136
9	3	3	3	1	5.120±0.156
K_1	4.588	4.875	4.812	4.895	
K_2	4.865	4.871	4.888	4.839	
K_3	5.112	4.819	4.860	4.832	
R	0.524	0.056	0.076	0.063	

Note: TPC, total polyphenols content

Table 3 Analysis of Variance

Factors	SS	DF	MS	F	P
A	1.241	2	0.620	36.061	0.000
B	0.017	2	0.009	0.509	0.610
C	0.022	2	0.011	0.650	0.534
D	0.021	2	0.011	0.615	0.552
Error	0.310	18	0.017	\	\

and the ultrasonic power was 500 W. According to the optimal extraction conditions, the experiment was repeated, and the highest extraction amount was (5.148 ± 0.038) mg GAE/g.

3.6 Inhibition of α-glucosidase and α-amylase by BQP

Compared with BQCP, the TPC of BQP increased from 17.06 mg/g to 132.98 mg GAE/g, and the polyphenol content increased by 7.79 times. In order to further demonstrate the hypoglycemic effect of BQ polyphenols, the inhibitory ability of BQP on α-glucosidase and α-amylase was determined and compared with BQCP and acarbose (Table 4).

Table 4 IC_{50} values of BQCP, BQP, and acarbose (mg/mL)

	BQCP	BQP	Acarbose
α-glucosidase	18.25	2.12	0.11
α-amylase	30.52	18.18	0.13

Note: BQCP, black quinoa crude polyphenols; BQP, black quinoa purified polyphenols.

The inhibitory effect of BQP on α-glucosidase was measured and its IC_{50} value was 2.12 mg/mL. The inhibitory effect of BQP on α-glucosidase was not as favorable as acarbose on the market (IC_{50} = 0.11 mg/mL), but it was a considerable improvement compared with the BQCP (IC_{50} = 18.25 mg/mL). These results suggested that BQP has inhibitory effects on α-glucosidase and α-amylase and is an excellent source of natural hypoglycemic active ingredients.

As a traditional diet of Asian people, cereals have been one of the indispensable foods on the tables of common people for thousands of years. It also occupies an important position in the diet of our country and is regarded as the traditional staple food. Although numerous studies have been conducted on the hypoglycemic effects of cereals, less research has been done on the hypoglycemic effects of quinoa, especially as an α-glucosidase inhibitor. Based on studies conducted on patients with cardiovascular disease, it was found that patients had lower blood sugar levels than control groups after eating bread rich in quinoa[29]. It revealed the hypoglycemic effect of quinoa , but the active constituents of it were not yet clear. Dong *et al.*[30] identified eight flavonoids that may bind to α-glucosidase and demonstrated their α-glucosidase inhibitory ability to α-glucosidase by using molecular docking techniques. In this study, the hypoglycemic activity of quinoa extract was evaluated using α-glucosidase inhibition as an evaluation index. Correlation analysis and PCA showed that significant correlation existed between the α-glucosidase inhibitory capacity of BQ and its TPC, this finding also confirmed the report by Hemalatha *et al.* [31]. Then, according to the results of the inhibitory ability of BQP on α-glucosidase measured above, we could see that, although the IC_{50} value of BQP was higher than that reported previously for ME of red rice (IC_{50} value was 20.4 μg/mL)[23] and several sorghum 70% EEs (IC_{50} value ranged from 1.1 μg/mL to 102.7 μg/mL)[32]. However, the inhibitory effect was still stronger than that of some naturally-derived α-glucosidase inhibitors previously reported. For example, Ranilla *et al.*[33] previously reported the inhibition effect of WEs of RQ and other grains on α-glucosidase, that is, when the sample weight was 5 mg, the inhibition rate of quinoa on α-glucosidase was 30%, and the inhibition rate of corn and beans on α-glucosidase was only between 11% and 51%. Sreerama *et al.* [34] studied the effects of polyphenol extracts of four legumes on α-glucosidase inhibition, the result showed that the effects of polyphenol extracts on α-glucosidase inhibition were in a dose-dependent. The IC_{50} value of broad bean was 50.42 mg/mL, the IC_{50} values of the black and red varieties of adzuki bean were 26.28 and 319.22 mg/mL, respectively[34]. Vadivelan *et al.*[35] reported that the WE of asparagus inhibited α-glucosidase and α-amylase in a dose-dependent manner, with IC_{50} values of 62.65 mg/mL and 63.28 mg/mL, respectively. In contrast, BQ polyphenols are potential natural α-glucosidase inhibitors.

In addition to evaluating the ability of BQP to inhibit α-glucosidase, we also evaluated its ability to inhibit α-amylase. According to the results in Table 4, the IC_{50} value of BQP inhibition of α-amylase activity was 18.18 mg/mL, which was 1.68 times higher than that of BQCP (IC_{50} = 30.52 mg/mL), and the inhibitory ability was also inferior to that of commercial drug acarbose (IC_{50} = 0.13 mg/mL). Compared with the inhibitory ability of BQ polyphenols on α-glucosidase, the inhibitory effect of BQ polyphenols on α-amylase was weaker. Similar findings by Ranilla *et al.* [33], in their health-related functional assessment of Peruvian Andean cereals, which showed that several Andean cereals, including quinoa, had α-glucosidase inhibition, but none showed a significant inhibitory on α-amylase. In the study of Hemalatha *et al.*[18], quinoa also showed strong α-glucosidase inhibition (The IC_{50} value of ground grain was 182.01 μg/mL, and the IC_{50} value of acarbose was 83.65 μg/mL, which was approximately 2.18 times that of acarbose). In contrast, the inhibition of α-amylase was weaker (The IC_{50} value of ground grain was 241.36 μg/mL, and the IC_{50} value of acarbose was 7.21 μg/mL, which was roughly 33.48 times that of acarbose.)[18]. In a study on blueberry leaf polyphenols, it was noted that blueberry leaf polyphenols were able to compete with the substrate and preferentially bind to the enzyme to form a complexes, thereby inactivating the enzyme[36]. Polyphenols could bind stronger to α-glucosidase than that to α-amylase, therefore, the inhibitory ability of polyphenols to α-glucosidase is stronger than that of α-amylase. From this, we could speculate whether quinoa polyphenols would be more advantageous in competition with enzyme-inhibiting substrates, or whether quinoa polyphenols would be more stable when bound to α-glucosidase to produce a complex. These inferences still need to be followed up with further studies and research.

4 Conclusion

This work demonstrated that quinoa is a potential natural source of α-glucosidase inhibitor, and its polyphenols and flavonoids had certain inhibitory effects on α-glucosidase activity. In this study, comparative analysis revealed that the polyphenols in the WE of BQ were the main contributing component to the inhibition of α-glucosidase activity, and the polyphenols in the WE of BQ also had some inhibitory effect on α-amylase activity. The results of this study might provide a reference for the development of quinoa as a functional food and nutritional supplement to help people control postprandial blood glucose

level and prevent the occurrence of diabetes. As a natural product, quinoa had fewer side effects and higher safety, offering advantages over synthetic drugs such as acarbose.

Author contribution statement

Ruili Zheng and Jie Wang: Conceptualization, Methodology, Software, Date curation, Writing-Original draft preparation. Siyi Liu: Writing-Review and Editing. Zhipeng Sun: Investigation. Liyan Zhao and Guitang Chen: Conceptualization, Supervision, Project administration, Resources.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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