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# Assessment and comparison of nutritional qualities of thirty quinoa (*Chenopodium quinoa* Willd.) seed varieties

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Keywords: Quinoa seed Nutrition quality Antioxidant activity Weighted average score	Quinoa ( <i>Chenopodium quinoa</i> Willd.) is an ancient crop with perfect nutritional composition and antioxidants substances. However, the current research on the nutritional quality of quinoa is limited to a small number of varieties or a single origin. In this study, we aimed at providing a detailed evaluation of abundant nutrients of quinoa seeds from thirty varieties with different color in different origins, including soluble protein, soluble sugar, amino acid, vitamin, fatty acid and saponin. Results showed that there were significant differences in the contents of $\gamma$ -aminobutyric acid (6.67–78.67 mg/100 g DW) and vitamin C (11.675–105.135 mg/100 g DW) in quinoa seeds. Here, we scored thirty quinoa seeds using a weighted average score system first time and identified four varieties, black quinoa JQ-00145, red quinoa JQ-00125 and two white quinoa JQ-00005/JQ-00077, with superior nutritional quality and oxidation resistance. The results of this study will provide theoretical guidance for consumption of quinoa.

# 1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) seed, a staple human pseudocereal from Andean origin, has received more scientific and commercial attention in recent years (Carciochi et al., 2016). Although quinoa is mainly cultivated in the Andean region of South America, more and more places such as the USA, Europe, Canada, Australia, China and India also cultivated it over resent years (Jacobsen, 2003; Stikic et al., 2012). The quinoa seeds are usually round, small and flat. Most quinoa seeds are white in color, but there are also a few varieties that appear red or darker black (Nowak et al., 2016). In Peru, as a native crop, quinoa always consumed as breakfast or served as a garnish in salads to enhance texture (Repo-Carrasco-Valencia et al., 2010). Quinoa owns an enormous adaption and plasticity to different environmental conditions with its broad genetic diversity, which can be tolerant to frost, salinity and drought, and have the ability to grow on marginal soils (Jacobsen, 2003; Stikic et al., 2012).

As a healthy grain marketed as a "superfood" with great nutritional value, many of its nutritional qualities are significantly higher than the daily consumption crops such as wheat, rice and corn etc. (Graf et al., 2015; Nowak et al., 2016). Quinoa seed has a fine quality of its nutritions, as regards essential amino acids content (especially lysine), essential fatty acids with relative suitable  $\omega$ -6/ $\omega$ -3 ratio, vitamins and

high antioxidants such as polyphenols and flavonoids compounds. Interestingly, quinoa seed coat color appears to contribute to antioxidant activity due to the content of flavonoids (Pedrali et al., 2023). Moreover, the content of each substance is similar to the normal nutrient demand intake of the human bodies (Repo-Carrasco-Valencia et al., 2010; Tang et al., 2015; Verza et al., 2012). Quinoa is also considered as a gluten-free food suitable for patients with celiac disease and people with wheat allergy (Nowak et al., 2016). Besides nutrient compositions, quinoa contains a kind of bitter component, saponins, which really affect taste of its seed, especially in the hull (Gil-Ramirez et al., 2018). Although saponins have a strong antioxidant capacity, considering that quinoa is consumed as a food crop, many scientific literatures use various means to remove it (Verza et al., 2012).

The planting areas of quinoa are very wide, spanning different latitudes and altitudes, which leads to different planting conditions of quinoa, including various factors such as light, temperature and pH. And relevant research shows that, these factors could affect the nutrient composition and secondary metabolite content of plants, which means that quinoa from different places of origin may have different nutritional qualities. Quinoa varieties selected from different regions of Chile showed significant quality differences, which may be caused by different climates (Miranda et al., 2012). The same quality has also been reported in Spain and the Andean region, and the results show that the quality

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difference is due to variety rather than geographical factors (Pedrali et al., 2023). However, such reports just selected limited quinoa varieties and places of origin, which is relatively less representative.

Many articles have characterized the nutritional quality of quinoa, but most of them focused on a single variety of quinoa, in addition, monotonous nutritional qualities or indicators, such as protein, antioxidant capacity, etc. (Abugoch et al., 2008; Hemalatha et al., 2016). In this study, we intended to show the detail nutritional composition of thirty quinoa cultivars with different color (white, red and black) and different origins (Bolivia, the USA, Chile, Holland and Agentina). The relationship between antioxidant activity, seed coat color and origin of quinoa will be also explored. Furthermore, we assessed them with a weighted average score system, which is the first time been applied to compare the nutritional quality of quinoa. The purpose is to screen out quinoa varieties with good nutritional quality through such comparison, and provide a theoretical basis for quinoa as a functional health food, which has good market development prospects and application value.

# 2. Materials and methods

#### 2.1. Raw material

All quinoa (*Chenopodium quinoa* Willd.) seed samples were obtained from a Cooperative of Producers (Jiaqi Agricultural Science and Technology co. LTD, Shanxi, China). Morphology and basic information about thirty quinoa varieties, including common name, origins, color, athousand-seed weight, and diameter were showed in Figure S1 and Table S1.

#### 2.2. The soluble protein and soluble sugar content measurement

The soluble protein and soluble sugar content of quinoa seeds were determined with a Synergy HT MultiMode Microplate Reader (Biotek, Rochester, VT, USA).

For Soluble protein assay, 0.2 g quinoa powder were dissolved with 5 mL deionized water and centrifuged at 10 °C, 3000 rpm for 10 min. Then 10  $\mu$ L supernatnat was added into 190  $\mu$ L Coomassie Brilliant Blue G-250 solution for 2 min and at last, the absorbance of all samples was read at 740 nm. The same procedure was repeated for the standard solution of bovine serum albumin and a calibration curve was constructed.

For soluble sugar assay, soluble sugar content was analyzed by anthrone colorimetry method. Briefly,  $150 \,\mu\text{L}$  extract with 3 mL 150 mg/mL anthrone solution were incubated in 90 °C water for 20 min. Then the solution was cooled to the room temperature and the absorbance was determined at 620 nm.

#### 2.3. The amino acid content measurement

All kinds of amino acids and GABA were assessed by L-8900 automatic amino acid analyzer, as previously described with some modification (Zhang et al., 2021). Adding 0.1 g dry sample powder into 5 mL 6 M hydrochloric acid at 110 °C for 24 h. The volume was adjusted to 100 mL with double-distilled water. Then 1 mL extraction was used for rotary evaporation and then reconstituted with 1 mL double-distilled water. The solution to be detected was then filtered through a 0.22 µm filter. The peak time and peak area of the standard were used to calculate the various amino acid contents.

# 2.4. The $VB_1$ , $VB_2$ , $VB_6$ and VC content measurement by HPLC

The contents of VB<sub>1</sub>, VB<sub>2</sub>, VB<sub>6</sub> and VC were assessed by HPLC. Samples were extracted by an acid and enzymatic hydrolysis. The separation was carried out using a Supelco column C18 ( $250 \times 4.8 \text{ mm}$ , 5  $\mu$ m) with 2% metaphosphoric acid (A) and methanol (B) as mobile phase with a flow rate of 0.8 mL/min in HPLC. Detection was performed by fluorescence using 354 nm and 450 nm as excitation and emission

wavelengths, respectively. Gradient elution procedure is as follows: isocratic elution 98% A, 0–3.5 min; linear gradient from 98% A to 60% A, 3.5–4 min; isocratic elution 60% A, 4–10 min; post-time 2 min before the next injection; total run time was 12 min. All vitamins were quantified based on the corresponding calibration curves (0–50  $\mu$ g/mL).

#### 2.5. The fatty acids content measurement by GC

A variety of fatty acid content in the samples were determined using gas chromatography (GC). Fatty acid methyl esters were purchased from Shanghai Anpu Experimental Technology Co., Ltd. as a standard compound. For each sample, 2 g sample was accurately weighed and immersed into 8 mL water and 10 mL chromatographic pure grade hydrochloric acid. Then the mixture was incubated by water bath method at 60 °C for 50 min. During the incubation, the mixture was shaken for 20 s every 10 min. After cooling, 10 mL ethanol, 50 mL diethyl ether and petroleum ether (30-60 °C) mixture (1:1) were added. After 3-minshaking, the pipette was used to remove most of the supernatant in 100 mL pear shape in a bottle, and the organic solvent was rotary evaporated at 180 kPa at 40 °C until the remaining yellow oil remained. Then 4 mL isooctane was added into 60 mg oil, and 0.2 mL 2 M KOHmethanol solution was added after 30 s shaking, and 1 g sulfuric acid monohydrate was added after 2 min of vigorous shaking. The column (Agilent db23. column) was operated at 50 °C for 1 min, then temperature-programmed at 10 °C/min to 175 °C, held for 12 min and kept at 175 °C for 6 min. After heat preservation, the column was programmed at 3 °C/min to 230 °C, and finally held for 17 min. Total time of whole program was 36 min.

#### 2.6. The total phenolics content measurement

Total phenolics were assessed by the Folin Ciocalteu (FC) micromethod adapted to a microplate reader. Briefly, 0.1 g sample was put in each tube and reacted with 6 mL extract (acetone: water: acetic acid = 70: 29.5: 0.5, v/v), under ultrasonic extraction at 20 °C for 30 min. Thereafter, samples were centrifuged at 10 °C, 10000 rpm for 10 min. The resulting supernatant was the total phenolics extract. 0.5 mL FC reagent and 0.5 mL 7.5% (w/v) sodium carbonate were added to the 0.5 mL extract. The same operation was carried out with different concentration gallic acid standard solution (0 – 200 µg/mL) as the control. The reaction was carried out in the dark for 30 min. 200 µL mixture was injected into the 96-well microplate and the absorbance was read at 740 nm in a Synergy HT MultiMode Microplate Reader (Biotek, Rochester, VT, USA).

# 2.7. The saponins content measurement

Saponins content was analysed using a spectrophotometric method. 0.5 g dry sample powder was.mixed with 15 mL of 70% ethanol and dealt with sonication for 90 min. Then samples were centrifuged at 20 °C, 4000 rpm for 5 min. Supernatant was evaporated in a rotary evaporator added 10 mL methanol to redissolve, which was the saponin extract. After 200  $\mu$ L extract was evaporated to dryness in a water bath at 70 °C, 0.2 mL 5% vanillin-glacial acetic acid solution and 0.8 mL perchloric acid solution were added and kept at 70° C for 15 min. After 5 min of ice bath, 4 mL glacial acetic acid was added and absorbance was measured at 545 nm using a UV–vis spectrophotometer (Genesis 10UV, Thermo Scientific) against the control containing methanol. Saponins content was calculated from a standard curve of Oleanolic acid (0–20  $\mu$ g/mL).

## 2.8. The antioxidant activities measurement

For each sample, 0.1 g were accurately weighed and added 10 mL 70% (v/v) ethanol, sonicated for 10 min. Then samples were centrifuged at 10 °C, 11000 rpm for 10 min, the supernatant were prepared for later

use.

#### 2.8.1. DPPH free radical scavenging activity

Free radical scavenging activity was determined using the 2,2,diphenyl-2-picrylhydrazyl (DPPH) method. Briefly, 100 uL supernatant was mixtured with 3.9 mL 0.1 mM DPPH methanol solution, vortexmixed for 30 s and left stand at room temperature in the dark for 20 min. Then 200 µL mixture was injected into the 96-well microplate and the absorbance was measured at 517 nm, with methanol used as a blank, using a Synergy HT MultiMode Microplate Reader (Biotek, Rochester, VT, USA). Control samples were prepared without adding extract but 100 µL 70% (v/v) ethanol. The percentage inhibition of the DPPH radical scavenging activity was determined by Eq. (1):

DPPH scavenging activity (%) = 
$$(1 - A_{sample} / A_{control}) * 100$$
 (1)

#### 2.8.2. FRAP activity

For ferric reducing antioxidant power (FRAP) assay, 100 µL supernatant were mixtured with 3 mL FRAP and reacted for 20 min. Absorbance at 593 nm was recorded using a Synergy HT MultiMode Microplate Reader (Biotek, Rochester, VT, USA). The standard curve was acquired with FeSO<sub>4</sub> as the standard. The antioxidant capacity of the sample is expressed by the FRAP value, and the antioxidant capacity of the samples were equivalent to the mmol/L of FeSO<sub>4</sub>.

# 2.8.3. ABTS radical scavenging activity

JQ-02256

JQ-00304

JQ-00303 JQ-00302

JQ-00301

JQ-00300

JQ-00145

JQ-00129

JQ-00125

JQ-00106

JQ-00101

JQ-00100

JQ-00099

JQ-00098

JQ-00097

JQ-00085

JQ-00084

JQ-00080

JQ-00079

JQ-00077

JQ-00055

JQ-00028

JQ-00021

JQ-00016

JQ-00014

JQ-00011

JQ-00009

JQ-00005 JQ-00003

50

100

L C C C

tein

F

Sol

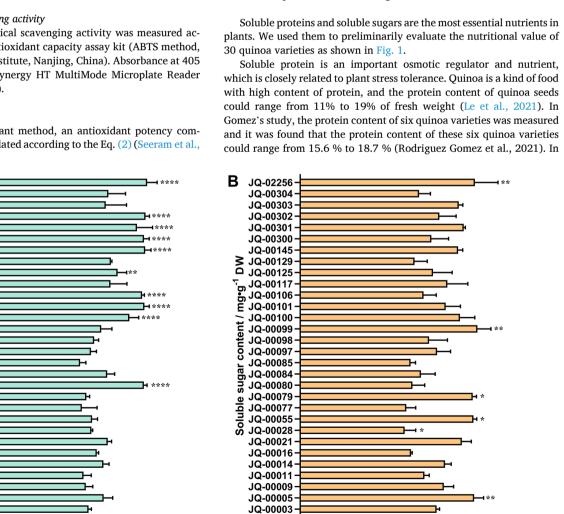
່ວງ JQ-00117

ABTS (2,2'-azino-bis) radical scavenging activity was measured according procotol in Total antioxidant capacity assay kit (ABTS method, Jiancheng Bioengineering Institute, Nanjing, China). Absorbance at 405 nm was recorded using a Synergy HT MultiMode Microplate Reader (Biotek, Rochester, VT, USA).

# 2.8.4. APC index

Α

For each of the antioxidant method, an antioxidant potency composite (APC) index was calculated according to the Eq. (2) (Seeram et al.,



#### Antioxidant index score = [(sample score/best score) $\times$ 100] (2)

And the APC index was calculated as the average of the antioxidant index score of each method.

#### 2.9. Statistical analysis

2008):

Results were expressed as mean value  $\pm$  standard deviation of three independent extractions. One-way analysis of variance (ANOVA) was used to compare the means by Tukey's test. Comparison of assays was made by correlation and linear regression analysis. Differences were considered as significant at  $p \leq 0.05$ . Data analysis and processing were performed using SPSS software (version 18.0, Chicago, IL). The results were calculated using Origin 9.0 software.

The nutritional qualities of 30 quinoa varieties were compared using the weighted average score system, according to the Eq. (3):

Weighted average score = 10 \* first 10% group score + 10/3 \* first 30% score +2 \* first 50% score(3)

10

15

20

25

#### 3. Results and discussion

# 3.1. Soluble proteins and soluble sugars

Fig. 1. The content of soluble protein (A) and soluble sugar (B) of thirty quinoa seed varieties. Value are expressed as mean ± SD, n = 3. Significantly higher: \*P < 0.05 \*\*P < 0.01 \*\*\*P < 0.001 \*\*\*\*P < 0.0001 (Tukey's test).

250

150

200

our study, the range of soluble protein content were 114.24–190.58 mg/g DW with an average of 149.33 mg/g DW. Among them, JQ-00085 contains the lowest soluble protein content (114.24 mg/g DW) and JQ-02256 contains the highest soluble protein content (190.58 mg/g DW) (Fig. 1A).

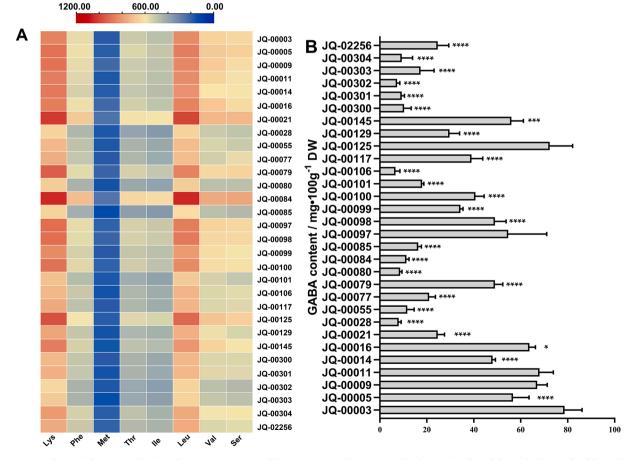
Soluble sugars include glucose, fructose, maltose and sucrose. Carbohydrates play an important role in seed energy storage and seedling development (Pereira et al., 2019). But from the perspective of consumers, quinoa is a low-sugar grain, which is more suitable for people with special needs, such as diabetics and people in diet. The content of soluble sugar in different varieties of quinoa seeds was varied significantly. The soluble sugar content of 30 quinoa varieties ranged from 12.363 to 20.70 mg/g DW (Fig. 1B). In Gomez's study (Rodriguez Gomez et al., 2021), soluble sugar levels ranged from 10.9 g/100 g FW to 40.3 g/100 g FW. Pereira et al. (2019) reported total sugar concentrations in quinoa seeds from 23.5 g/100 g FW to 27.9 g/100 g FW. As reported by Repo-Carrasco-Valencia and Valdez Arana (2017), different extraction methods or origins of quinoa and cultivation affect sugar concentration as well.

#### 3.2. Essential amino acids and GABA

Compared to major grains such as rice, corn, and wheat, quinoa seeds contain higher amino acid and have all essential amino acids such as methionine, valine, and leucine (Pathan and Siddiqui, 2022). The essential amino acids and non-essential amino acid composition of thirty quinoa seeds varieties are presented in Fig. 2A and Figure S2. In our data, quinoa proteins processed high content in Lys (647–1056 mg/100

g DW), Leu (630–1060 mg/100 g DW) and relatively low content in Met (142–255 mg/100 g DW). Other essential amino acids are roughly equivalent in their content. These amino acids we measured were similar to the results reported by Mota et al. (2016). We observed a moderate negative correlation between Phe and antioxidant activity (r = -0.527, Table 3). High levels of Phe can lead to decreased activity of antioxidant enzymes, which is consistent with our results (Mazzola et al., 2013). Lys is the first limiting essential amino acid. Recently, we screened the candidate genes participated in lysine accumulation during quinoa germination and explored the factors affected lysine biosynthesis by multi-omics analysis (Niu et al., 2023).

GABA (y-aminobutyric acid) is a naturally occurring non-protein organic amino acid which acts as an inhibitory neurotransmitter in the mammalian central nervous system. It has many functional properties to help human health such as regulating blood pressure and heart rate or relieving pain and anxiety (Yin et al., 2014). So numerous studies have been carried out to enrich plants with GABA. In our results, there was a huge difference in GABA content of thirty quinoa varieties, ranged from 6.67 to 78.67 mg/100 g DW (Fig. 2B). JO-00003 had the highest GABA content of 78.67 mg/100 g DW, followed by JQ-00125 of 72.33 mg/100 g DW. This may be due to differences in quinoa varieties. Recently, our research group selected JQ-00003, JQ-00125 and JQ-00145 with high GABA content and JQ-00077, JQ-00106 and JQ-00303 with low GABA content, and then analyzed the molecular mechanism of increasing GABA content during quinoa germination by RNA-seq. Eight GAD genes, two GABA-T genes, one SSADH gene, nine polyamine oxidase (PAO) genes, five diamine oxidase (DAO) genes, four 4-aminobutyraldehyde dehydrogenase (BADH) genes, and three thermospermine synthase



**Fig. 2.** Heatmap of essential amino acid (A) and GABA content (B) of thirty quinoa seed varieties. The change in color of the scale from red to blue indicated the amino acid content from high value to low value. Value are expressed as mean  $\pm$  SD, n = 3. Significantly higher: \*P < 0.05 \*\*P < 0.01 \*\*\*P < 0.001 \*\*\*\*P < 0.0001 (Tukey's test). Lys, lysine; Phe, phenylalanine; Met, methionine; Thr, threonine; Ile, isoleucine; Leu, leucine; Val, valine; Ser, serine; GABA,  $\gamma$ -aminobutyric acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

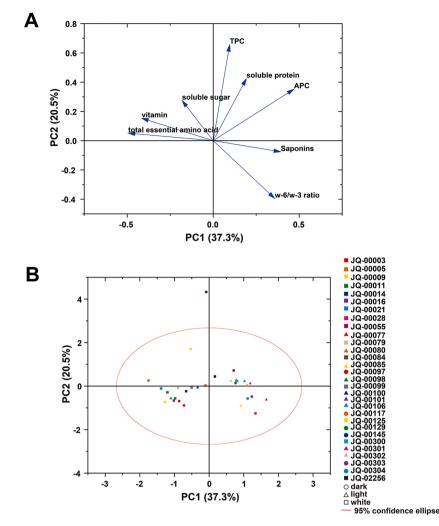


Fig. 3. Loading plot (A) and score plot (B) of principal component analysis (PCA) from nutrition and antioxidant capacity of 30 quinoa varieties. TPC, total phenol content; APC, antioxidant potency composite.

ACAULIS5 (*ACL5*) genes were identified (Zhang et al., 2021). Perhaps due to the differential expression of these genes, the difference in GABA content in quinoa is caused.

# 3.3. Vitamin analysis

Vitamin is a kind of trace organic substance that human must obtain from food in order to maintain normal physiological functions. It plays an important role in the process of human growth, metabolism and development. Table 1 showed the amount of certain vitamins. Vitamin B<sub>1</sub> (VB<sub>1</sub>), also known as thiamine, is involved in carbohydrate metabolism (Miranda et al., 2012). The thiamine content ranged from 0.060 to 0.679 mg/100 g DW. Among 30 varieties, the content of VB<sub>1</sub> in most quinoa samples ranged from 0.2 to 0.5 mg/100 g DW. This result was similar to Miranda et al. (2012) where VB<sub>1</sub> value was between 0.349 and 0.648 mg/100 g DW. JQ-00125 (0.679 mg/100 g DW) and JQ-00145 (0.578 mg/100 g DW) had the highest value of VB<sub>1</sub> which probably related to seed coat color.

Vitamin B<sub>2</sub> (VB<sub>2</sub>), also known as riboflavin, mainly functions as a coenzyme to promote metabolism. VB<sub>2</sub> (0.034-0.253 mg/100 g DW) value is similar to the results reported by Miranda et al. (2011) and Granda et al. (2018), but lower that reported by Koziol (1991) of 0.39 mg/100 g DW. The riboflavin has the highest value on JQ-00028 (0.253 mg/100 g DW), followed by JQ-00106 (0.184 mg/100 g DW), which are greater than most of other quinoa samples ranging from 0.03 to 0.09 mg/100 g DW. This result was significantly higher than that of barley

(about 0.9 mg/kg) and wheat (about 0.7 mg/kg) (Granda et al., 2018). This is consistent with Navruz-Varli and Sanlier's conclusion (Navruz-Varli and Sanlier, 2016).

Vitamin  $B_6$  (pyridoxine, VB<sub>6</sub>) is synthesized mainly in the leaves of plants, so it is low in the edible parts (Mohsin et al., 2022). Quinoa has higher levels of VB<sub>6</sub> than most other grains, such as wheat, oats, and rice (Navruz-Varli and Sanlier, 2016). VB<sub>6</sub> content ranged from 0.102 to 0.947 mg/100 g DW which was higher than the report data (Granda et al., 2018). The highest value of pyridoxine is JQ-00003 (0.947 mg/100 g DW), followed by JQ-00005 (0.713 mg/100 g DW), and both of them were white varieties. Compared to other crops, our results are similar to those of Granda et al. (2018), but much higher than that of wheat at VB<sub>6</sub> content (0.01 mg/100 g) (Mohsin et al., 2022). Notably, for VB<sub>1</sub>, the darker the seed coat color, the higher the VB<sub>1</sub> content in quinoa, while for VB<sub>2</sub> and VB<sub>6</sub>, the lighter varieties had higher contents.

Vitamin C is an important nutrient with strong antioxidant activity. In this study, the ascorbic acid content of 30 quinoa seeds varieties ranged from 11.675 (JQ-00106) to 105.135 (JQ-00011) mg/100 g DW, but Miranda et al. (2011) reported that quinoa contains only 12.402 – 23.065 mg/100 g ascorbic acid. This may be due to geographical variety differences, as the Chilean varieties in this study also had low ascorbic acid levels. In addition, the content of ascorbic acid in germinated quinoa seeds was between 36.63 mg/100 g and 69.48 mg/100 g (Le et al., 2021), indicating that 30 quinoa seeds varieties selected in this study were more representative and had research significance.

#### Table 1

Vitamin content of 30 quinoa seed varieties.

Sample	VB <sub>1</sub> (mg/100 g DW)	VB <sub>2</sub> (mg/100 g DW)	VB <sub>6</sub> (mg/100 g DW)	VC (mg/100 g DW)
JQ- 00003	$0.130{\pm}0.018$	$0.060 \pm 0.006$	$0.947 \pm 0.086$	84.212 ±10.169
JQ- 00005	$0.386 \ {\pm} 0.008$	$0.044 \pm 0.001$	$0.713 \pm 0.017$	95.415 ±3.032
JQ-	$0.301 \ {\pm} 0.045$	$0.049 \pm 0.006$	$0.640 \ {\pm} 0.013$	79.088
00009 JQ-	$0.326 \ {\pm} 0.022$	$0.090 \ {\pm} 0.002$	$0.573 \pm 0.082$	$\pm 9.097$ 105.135
00011 JQ-	$0.311 \pm 0.003$	$0.085 \ {\pm} 0.002$	$0.570 \ {\pm} 0.086$	±4.098 75.609
00014 JQ-	$0.335 \ {\pm} 0.025$	$0.070 \pm 0.016$	$0.545 \pm 0.018$	$\pm 2.344$ 94.410
00016 JQ-	$0.297 \pm 0.062$	$0.045 \pm 0.002$	$0.533 \pm 0.014$	$\pm 8.512$ 22.416
00021 JQ-	$0.275 \pm 0.005$	$0.253 \pm 0.004$	$0.502 \pm 0.023$	±2.630 24.874
00028 JQ-	0.267 ±0.009	0.100 ±0.009	$0.442 \pm 0.028$	±1.711 18.490
00055 JQ-	0.324 ±0.012	$0.112 \pm 0.006$	$0.436 \pm 0.013$	±2.179 15.617
00077 JQ-	0.296 ±0.005	0.064 ±0.001	$0.423 \pm 0.027$	$\pm 1.443$ 60.304
00079				$\pm 0.120$
JQ- 00080	$0.327 \pm 0.003$	$0.119 \pm 0.002$	$0.418 \pm 0.017$	$17.692 \pm 0.138$
JQ- 00084	$0.473 \pm 0.015$	$0.078 \pm 0.008$	$0.408 \pm 0.013$	$25.001 \pm 2.709$
JQ- 00085	$0.226 \ {\pm} 0.006$	$0.054 \ {\pm} 0.005$	$0.386 \pm 0.007$	$19.016 \pm 2.239$
JQ- 00097	$0.455 \ {\pm} 0.011$	$0.053 \ {\pm} 0.001$	$0.385 \pm 0.016$	$86.749 \pm 2.108$
JQ- 00098	$0.247 \ \pm 0.060$	ND	$0.373 \pm 0.021$	57.655 ±5.533
JQ-	$0.461 \ {\pm} 0.010$	$0.073 \ {\pm} 0.006$	$0.351 \pm 0.016$	71.811
00099 JQ-	$0.275 \pm 0.046$	$0.085 \pm 0.012$	$0.325 \pm 0.011$	$\pm 0.784$ 63.017
00100 JQ-	$0.060 \pm 0.004$	$0.059 \pm 0.004$	$0.322 \pm 0.025$	$\pm 8.083$ 15.888
00101 JQ-	0.394 ±0.009	$0.184 \pm 0.002$	$0.310 \pm 0.003$	±1.484 11.675
00106 JQ-	0.143 ±0.011	$0.034 \pm 0.001$	$0.294 \pm 0.012$	$\pm 0.533$ 56.569
00117			$0.288 \pm 0.020$	$\pm 1.202$
JQ- 00125	0.679 ±0.064	$0.078 \pm 0.009$		$\begin{array}{c} 100.857 \\ \pm 10.424 \end{array}$
JQ- 00129	0.388 ±0.009	$0.055 \pm 0.010$	$0.277 \pm 0.037$	48.877 ±0.866
JQ- 00145	$0.578 \ {\pm} 0.023$	$0.082 \pm 0.005$	$0.276 \ {\pm} 0.018$	$94.936 \pm 6.368$
JQ- 00300	ND	$0.092 \pm 0.003$	$0.257 \ {\pm} 0.019$	$32.119 \pm 6.662$
JQ-	$0.480 \ {\pm} 0.006$	$0.097 \pm 0.001$	$0.253 \pm 0.011$	16.638
00301 JQ-	$0.255 \pm 0.008$	$0.106 \pm 0.005$	$\textbf{0.212} \pm \textbf{0.004}$	$\pm 1.430$ 13.590
00302 JQ-	$0.306 \pm 0.003$	$0.139 \pm 0.007$	$0.195 \pm 0.027$	$\pm 0.887$ 20.245
00303 JQ-	$0.421 \pm 0.070$	ND	$0.102 \pm 0.017$	$\pm 3.141$ 20.628
00304 JQ-	$0.362 \pm 0.005$	$0.082 \pm 0.006$	$0.365 \pm 0.021$	$\pm 3.516$ 16.462
02256				±1.201

Value are expressed as mean  $\pm$  SD, n = 3. ND = not detected.

#### 3.4. Fatty acids analysis

The composition of the major fatty acids obtained from thirty quinoa seeds are shown in Table 2 and Figure S3. The fatty acid composition was similar to the data reported by Tang et al. (2015). Among the ten fatty acids measured by GC, the main fatty acids detected were linoleic acid (39.68–58.15%) > oleic acid (13.57–25.98%) > palmitic acid (6.47–13.73%) >  $\alpha$ -linolenic acid (6.57–12.95%) (Fig. S3A). These results had been strongly agreed by a number of writers (Peiretti et al.,

Table 2
Relative content of major fatty acid of 30 quinoa seed varieties.

Sample	SFA (%)	MUFA (%)	PUFA (%)	ω-6/ω-3 ratio
JQ-00003	$12.50 {\pm} 0.19$	20.28±5.12	65.43±10.70	6.52
JQ-00005	$12.98 {\pm} 0.66$	$24.18{\pm}2.51$	$61.16{\pm}6.84$	4.25
JQ-00009	$11.31{\pm}5.30$	$23.79 \pm 3.47$	$63.13 {\pm} 9.28$	5.31
JQ-00011	$10.70{\pm}2.08$	$23.56{\pm}2.97$	$64.37 {\pm} 2.87$	5.33
JQ-00014	$11.97 {\pm} 3.11$	$17.96 {\pm} 3.74$	$68.26 {\pm} 9.23$	5.28
JQ-00016	$11.21 {\pm} 4.64$	$24.79 {\pm} 1.77$	$62.23{\pm}2.88$	5.16
JQ-00021	$14.41 {\pm} 0.73$	$15.32{\pm}3.66$	$68.91 {\pm} 8.29$	4.93
JQ-00028	$11.24{\pm}3.90$	$20.31 {\pm} 3.37$	$66.96 {\pm} 3.26$	7.97
JQ-00055	$8.87 {\pm} 2.17$	$21.22{\pm}2.87$	$68.40 {\pm} 5.95$	4.18
JQ-00077	$11.45 {\pm} 3.16$	$19.69 {\pm} 5.66$	$66.84{\pm}8.26$	7.71
JQ-00079	$10.48 {\pm} 2.45$	$23.31 {\pm} 4.54$	$64.76 {\pm} 8.70$	4.30
JQ-00080	$9.32{\pm}4.34$	$24.07{\pm}2.11$	$64.33{\pm}10.38$	5.89
JQ-00084	$11.74{\pm}0.78$	$17.31 {\pm} 0.86$	$69.02{\pm}6.57$	4.37
JQ-00085	$10.36{\pm}2.78$	$23.13{\pm}1.94$	$64.54{\pm}2.21$	5.62
JQ-00097	$10.84{\pm}5.50$	$21.31{\pm}5.71$	$65.63 {\pm} 9.28$	6.35
JQ-00098	$11.78 {\pm} 3.09$	$23.67 {\pm} 4.11$	$62.64{\pm}10.55$	4.44
JQ-00099	$11.18{\pm}5.34$	$22.17{\pm}2.86$	$64.81{\pm}10.82$	5.52
JQ-00100	$10.08 {\pm} 2.37$	$23.05 {\pm} 4.11$	$64.97 {\pm} 3.70$	5.75
JQ-00101	$10.05 {\pm} 1.12$	$22.35 {\pm} 2.74$	$65.57 {\pm} 9.71$	5.92
JQ-00106	$9.81{\pm}1.17$	$19.06 {\pm} 3.91$	$68.81{\pm}6.91$	6.40
JQ-00117	$10.14{\pm}5.16$	$26.23 {\pm} 1.86$	$63.18{\pm}4.51$	5.32
JQ-00125	$13.29 {\pm} 4.71$	$20.40{\pm}6.08$	$61.40{\pm}10.89$	5.41
JQ-00129	$12.54{\pm}4.76$	$25.85{\pm}1.08$	$60.00{\pm}10.46$	5.87
JQ-00145	$15.30{\pm}3.95$	$27.24{\pm}4.80$	$54.77 {\pm} 8.16$	3.18
JQ-00300	$12.51 {\pm} 3.26$	$17.21 \pm 3.77$	$68.02{\pm}10.73$	5.58
JQ-00301	$12.09 {\pm} 2.92$	$21.10{\pm}6.72$	$64.57 {\pm} 6.06$	7.60
JQ-00302	$9.14{\pm}1.83$	$23.42{\pm}2.40$	$66.02 {\pm} 7.60$	5.55
JQ-00303	$13.13{\pm}3.50$	$21.09{\pm}1.81$	$64.18{\pm}8.27$	8.49
JQ-00304	$9.26{\pm}4.01$	$21.37{\pm}5.18$	$67.34{\pm}5.46$	6.84
JQ-02256	$12.99{\pm}4.49$	$14.56{\pm}0.99$	$71.08{\pm}1.26$	4.37

Value are expressed as mean  $\pm$  SD, n = 3. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

2013; Pellegrini et al., 2018). There are two types of fatty acids, saturated and unsaturated, and the majority of the fatty acids were unsaturated fatty acids (UFA) (81.80–89.62%) in quinoa seeds. Approximately one third of them were monounsaturated fatty acids (MUFA) (14.56–27.24%) and two thirds were polyunsaturated fatty acids (PUFA) (54.77–71.08%) (Table 2). According to the data demonstrated by (Nowak et al., 2016), quinoa contains over 20 times more unsaturated fatty in comparison to rice, especially linoleic acid, but approximately half of unsaturated fatty acids and 10 times less saturated fatty acids compared to that of soybean.

PUFA were mainly from linoleic acid (an  $\omega$ -6 fatty acid) and  $\alpha$ -linolenic acid (an  $\omega$ -3 fatty acid) which are essential fatty acids for human bodies. The  $\omega$ -3 and  $\omega$ -6 fatty acids not only serve as a source of energy, they also provide the basic biological functions that the human body needs. A large number of recent scientific studies had shown that increased intake of  $\omega$ -3 fatty acids was associated with a reduced risk of cardiovascular disease, which may inhibit inflammation and benefit other chronically ill patients (Simopoulos, 2008). Among 30 quinoa seeds in this study, JQ-00145, JQ-00084, JQ-00055, JQ-00079 and JQ-02256 contained relatively higher  $\omega$ -3 (Fig. S3B). It is also highly important in health risk reduction between the balance of  $\omega$ -6 and  $\omega$ -3. According to our results, the  $\omega$ -6/ $\omega$ -3 ratio in quinoa seeds is about 6/1 (3.18/1-8.48/1). Among them, JQ-00084, JQ-00005, JQ-00021, JQ-00055 and JQ-00079 were very close to ideal ratio (4/1), while other samples were slightly inferior to the ideal ratio. Nevertheless, this result is much better than the typical Western diets (15.0/1-16.7/1) (Simopoulos, 2008).

#### 3.5. Saponins analysis

The content of saponins was shown in Table 3. Although their high nutritional value, saponins of quinoa seed have an intensely bitter flavour, which can impede its taste and sales volume as commercial cereals. It will also be potentially toxic if consumed in large quantities,

Table 3

Saponins content, total phenolic content (TPC) and antioxidant activity of thirty different quinoa seeds.

Sample	Saponins content (mg/100g DW)	TPC (mg GAE/100g DW)	DPPH (%)	FRAP (mmol Fe <sup>2+</sup> equivalents/100g)	ABTS (mM TEAC/g DW)	APC
JQ-00003	$286.36 \pm 36.36$	$109.52 \pm 19.05$	$10.90 \pm 1.38$	$2.11 \pm 0.12$	$5867.30 \pm \!$	48.48
JQ-00005	$222.73 \pm 39.39$	$65.48 \pm 14.29$	$9.62 \pm 2.00$	$1.41 \pm 0.37$	$6866.74 \pm 237.33$	44.93
JQ-00009	$271.21 \pm 30.30$	$53.57 \pm 21.43$	$10.28 \pm 1.64$	$1.59 \pm 0.24$	$5723.83 \pm 116.02$	43.88
JQ-00011	$366.67 \pm 24.24$	$100.00 \pm 16.67$	$10.26 \ {\pm} 0.82$	$1.63 \pm 0.43$	$7565.74 \pm 151.51$	49.41
JQ-00014	$371.21 \pm 63.64$	$80.95 \pm 9.52$	$10.44 \pm 0.92$	$1.88 \pm 0.35$	$7699.10 \pm 372.95$	51.57
JQ-00016	$303.03 \pm 93.94$	$52.38 \pm 14.29$	$10.92 \pm 0.62$	$1.69 \pm 0.57$	$7311.76 \pm 226.74$	50.19
JQ-00021	$136.36 \pm 9.09$	$45.24 \pm 0.71$	$8.90 \ {\pm} 0.72$	$2.16 \pm 0.41$	$7847.44 \pm 612.41$	51.05
JQ-00028	457.58 ±57.58	$107.14 \pm 14.29$	$13.54 \pm 2.62$	$2.67 \pm 0.31$	$10273.72 \pm 71.52$	69.15
JQ-00055	$556.06 \pm 30.30$	$148.81 \pm 9.52$	$18.13 \pm 2.05$	$3.43 \pm 0.22$	$9207.99 \pm 525.53$	78.53
JQ-00077	$539.39 \pm 3.03$	$190.48 \pm 0.24$	$18.00 \ {\pm} 0.87$	$3.89 \pm 0.14$	$10412.70 \pm \! 608.17$	84.55
JQ-00079	454.55 ±45.45	$75.00 \pm 11.90$	$12.44 \pm 0.56$	$1.69 \pm 0.14$	$6864.49 \pm 542.48$	51.51
JQ-00080	$378.79 \pm 69.70$	$80.95 \pm 0.71$	$14.33 \pm 1.28$	$3.39 \pm 0.51$	$8736.37 \pm 550.43$	70.36
JQ-00084	$213.64 \pm 48.48$	$47.62 \pm 19.05$	$9.82 \pm 0.97$	$2.15 \pm 0.08$	$8503.37 \pm 470.96$	54.49
JQ-00085	$412.12 \pm 1.52$	$67.86 \pm 7.14$	$12.36 \pm 2.67$	$3.55 \pm 0.55$	$10045.59 \pm 309.38$	71.72
JQ-00097	416.67 ±33.33	$68.21 \pm 0.71$	$10.79 \pm 0.46$	$1.55 \pm 0.16$	6661.46 ±79.46	47.23
JQ-00098	$262.12 \pm 24.24$	$39.29 \pm 7.14$	$10.74 \pm 0.92$	$1.40 \pm 0.09$	$7503.18 \pm 249.52$	48.69
JQ-00099	443.94 ±9.09	$72.62 \pm 21.43$	$12.54 \pm 0.87$	$1.86 \pm 0.43$	$7311.39 \pm 441.29$	53.96
JQ-00100	469.70 ±33.33	$64.29 \pm 7.14$	$12.97 \pm 0.77$	$1.28 \pm 0.33$	$6750.61 \pm 56.16$	49.61
JQ-00101	$369.70 \pm 63.64$	$84.52 \pm 7.14$	$12.49 \pm 0.62$	$3.17 \pm 0.18$	$8821.78 \pm \! 586.45$	66.13
JQ-00106	$381.82 \pm 15.15$	$128.57 \pm 88.10$	$15.69 \pm 4.10$	$1.95 \pm 0.29$	$11188.50 \pm \!$	71.18
JQ-00117	$374.24 \pm 66.67$	$107.14 \pm 40.48$	$12.54 \pm 1.03$	$2.07 \pm 0.20$	$8106.29 \pm 141.45$	57.55
JQ-00125	$313.64 \pm 27.27$	$350.00 \pm 7.14$	$18.05 \pm 1.44$	$2.59 \pm 0.41$	$8091.68 \pm 16.95$	70.15
JQ-00129	468.18 ±75.76	$159.52 \pm 30.95$	$16.97 \pm 3.03$	$3.60 \pm 0.22$	$8071.83 \pm 74.70$	74.29
JQ-00145	$307.58 \pm 33.33$	$571.43 \pm 14.29$	$19.28 \pm 1.90$	$5.56 \pm 1.04$	$7469.09 \pm 108.07$	88.28
JQ-00300	$509.09 \pm 45.45$	$100.00 \pm 16.67$	$16.08 \ {\pm} 0.92$	$1.51 \pm 0.14$	$9252.94 \pm 621.94$	63.62
JQ-00301	478.79 ±51.52	$117.86 \pm 40.48$	$18.56 \ {\pm} 0.72$	$1.43 \pm 0.24$	$10471.51 \pm 108.60$	70.95
JQ-00302	$260.61 \pm 3.03$	$64.29 \pm 47.62$	$13.44 \pm 1.28$	$2.64 \pm 0.43$	9070.88 $\pm 154.69$	65.31
JQ-00303	$401.52 \pm \! 18.18$	$128.57 \pm 21.43$	$14.95 \pm 0.92$	$3.56 \pm 0.14$	$8607.88 \pm 953.58$	72.09
JQ-00304	$548.48 \pm 36.36$	$100.00 \pm 26.19$	$16.10 \pm 1.74$	$1.86 \pm 0.27$	$11520.39 \pm \! 1853.65$	72.30
JQ-02256	$484.85 \pm 15.15$	$34.52 \pm 16.67$	$11.18 \pm 0.97$	$1.85 \pm 0.12$	$8365.14 \pm \! 118.67$	54.60

Value are expressed as mean  $\pm$  SD, n = 3. TPC, total phenolic content; DPPH, 2,2,-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ABTS, 2,2'-azino-bis.

as saponins reduce the absorption of vitamins and sterols (Chaudhary et al., 2023). Therefore, screening quinoa varieties with high comprehensive nutritional value and relatively low saponins content is our goal. As the results we obtained, the saponins content of thirty quinoa seeds were ranged from 135.75 to 550.96 mg/100 g DW, which was similar to the data reported by Gomez-Caravaca et al. (2014) but some samples partly higher. JQ-00021 (135.75 mg/100 g DW) had the lowest saponins content and was very closed to the reported content of "sweet quinoa" containing 110 mg/100 g saponins or less of saponins, whose level is lower than the threshold for detecting bitterness in quinoa flour (Koziol, 1991). Moreover, JQ-00084 and JQ-00005 had relatively lower saponins. The content of saponins in JQ-00055, JQ-00077 and JQ-00304 were higher than 500 mg/100 g DW, which were not suitable as commercial cereals, but can provide materials for anti-inflammatory and anti-cancer activities (Escribano et al., 2017).

# 3.6. TPC and antioxidant activities

The total phenol content (TPC) and the antioxidant activities of the lipophilic compounds in quinoa seeds which were assessed using 2,2diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis (ABTS) methods were given in Table 3. As regarded to TPC, a black quinoa JQ-00145 (569.25 mg GAE/100 g) and a red quinoa JQ-00125 (348.08 mg GAE/100 g) showed the highest TPC content. This result agreed with those presented by Tang et al. (2015), which found a higher TPC in black quinoa, followed by red quinoa. But of the three varieties reported by Pereira et al. (2020), the black variety had the highest total phenol content, followed by the white variety and the red variety with the lowest. In several reports, the black variety was the highest in total phenol, but there was a difference in the content of red and white. In this study, 30 varieties were selected, and the results were more representative. The total phenol content of other 28 quinoa varieties were between 34.78 and 189.28 mg GAE/100 g, which were comparable to those found from Brazil quinoa grains (97.67 mg GAE/

100 g) and seeds from Bolivian (71.7 mg GAE/100 g), but relatively lower than that colored quinoa seeds (3.27–7.92 mg GAE/100 g) (Abderrahim et al., 2015; Alvarez-Jubete et al., 2010; Nickel et al., 2016).

The radical scavenging activities and reducing power of thirty quinoa seeds were shown in Table 3. The higher DPPH radical scavenging activity is associated with a higher percentage value. The comparison of the different quinoa seeds results showed that the best antioxidant activities in all assays were obtained for the black quinoa JQ-00145, and the red varieties also had relatively high antioxidant capacities compared with most of the white quinoa. As regarded to FRAP, JQ-00145 (5.52 mmol Fe<sup>2+</sup> equivalents/100 g) had significantly stronger reducing activity than other quinoa seeds (1.27-3.53 mmol Fe<sup>2+</sup> equivalents/100 g), while JQ-00100 had the weakest reducing power among all the varieties. ABTS values varied from 5723.83 to 11520.39 mM TEAC/g DW among the 30 quinoa varieties. In order to comprehensively compare the antioxidant capacity of various varieties, we calculated the antioxidant potency composite (APC) index according to the method of Seeram et al (2008). The results in Table 3 showed that the highest antioxidant activity was JO-00145 (88.28) and the lowest was JQ-00009 (43.88). Correlation analysis showed that the overall APC was significantly positive correlated with the TPC (r = 0.633), indicating that phenolic compounds were the main contributor of antioxidant capacity in quinoa.

The varieties with higher APC indices were mostly pink, red or black, which is consistent with previous reports (Abderrahim et al., 2015; Hemalatha et al., 2016; Pedrali et al., 2023). We noted that varieties from Chile, the United States, and Argentina all show darker colors and higher antioxidant activity, which is consistent with the results of Pedrali et al. (2023). Overall, we supposed that the total phenol content and antioxidant activities of quinoa seeds are related to seed color.

## 3.7. Principal component analysis

Principal component analysis (PCA) was applied to analyzed all quinoa samples, and it was performed nutritional components including saponins, TPC, antioxidant capacity and nutritional components (Fig. 3). The first two principal components could explain 55.4% of total variance (PC1 = 32.9 % and PC2 = 22.5 %, respectively). PCA result showed that the quinoa varieties with high nutrition were located to the left in the score plot, whereas seed samples with strong antioxidant capacity were situated at the right in the graph.

We found a positive correlation between essential amino acids and vitamins (r = 0.551). This may be because amino acids act as precursor substances for vitamin biosynthesis (Miret and Munné-Bosch, 2014).

#### 3.8. Weighted average score system analysis

Based on the above analysis, we obtained the nutritional qualities of thirty quinoa varieties. In order to screen for quinoa varieties with good taste, high and comprehensive nutritional value, we assessed these nutritional indicators with scoring method. We chose soluble sugars; soluble proteins; total essential amino acids and GABA; sum of VB<sub>1</sub>, VB<sub>2</sub>, VB<sub>6</sub> and VC;  $\omega$ -6/ $\omega$ -3 ratio; saponins; TPC as evaluation indexes. Due to health and taste, the content of soluble sugar,  $\omega$ -6/ $\omega$ -3 ratio and saponins was ranked from low to high, while the content of other nutrients was ranked from high to low. The first 50%, the first 30% and the first 10% of the nutritional quality content were selected for the result analysis (Table S2).

According to the analysis, we used the above nine indicators as the scoring basis to compare the nutritional qualities of thirty quinoa varieties. One point was added when each index in first 50%, first 30%, or first 10% once appeared. The highest would get 8 points and the lowest just get 0 point, and the varieties which got 0 point would not be shown in the table. Table S3 was drawn based on the frequency of the first 50%, first 30% and first 10% of the nine nutrients. In order to understand their ranking more intuitively, we introduced the weighted average score system. We gave 10/5 points to the first 50% varieties, 10/3 points to the first 30% varieties, and 10 points to the first 10% varieties. Then, using the following Eq. (3) to get the final score.

As shown in Figure S4, we found that the black quinoa JQ-00145 had the highest score, followed by the red quinoa JQ-00125, and the two white quinoa JQ-00005/JQ-00077 also had a relatively high and comprehensive nutritional quality. Obviously, JQ-00145 and JQ-00125 were the best choices as they contained almost all nutrients we measured and were relatively high in weighted average score.

#### 4. Conclusion

Quinoa is an pseudo-cereal with great environmental tolerance and nutritional value. This study showed that quinoa has higher nutritional value than other staple foods. And we visualize interrelationships of the investigated nutrition and antioxidant capacity parameters of quinoa sprouts using PCA. In this study, the nutritional quality and oxidation resistance of thirty quinoa varieties were first evaluated through a weighted average score system. The black quinoa JQ-00145, the red quinoa JQ-00125, and two white quinoa JQ-00005, JQ-00077 were finally selected. They have sufficient amino acids, vitamins and reasonable  $\omega$ -6/ $\omega$ -3 ratio which can meet the nutritional needs of human beings. Moreover, the black quinoa JQ-00145 and the red quinoa JQ-00125 had a very high antioxidant capacity compared to other varieties and cereals, which showed a positive correlation with seed coat color to some extent. Overall, results of this study provide valuable information to the knowledge of nutritional composition of quinoa and add a new method for evaluating nutritional quality, meanwhile, help establish a functional quinoa varieties system suitable for consumption and application.

# **Declaration of Competing Interest**

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

#### Data availability

The data that has been used is confidential.

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#### Author contributions

Zhenguo Shen contributed to the conception of the study. Nana Su designed the experiments and critically reviewed the manuscript. Xuan Chen and Yueyue Zhang wrote the manuscript and performed the experiments. Beier Cao and Xiaonan Wei helped with graphical edit. Nana Su and Yueyue Zhang modified the grammatical and format errors.

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# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100808.

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