Current Research in Food Science 7 (2023) 100556

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Effects of quinoa flour (*Chenopodium Quinoa* Willd) substitution on wheat flour characteristics

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ARTICLE INFO

Handling Editor: Dr. Xing Chen

Keywords:
Quinoa flour
Quality characteristics
Tensile property
Pasting property
Secondary structure

ABSTRACT

Quinoa is a pseudo-cereal with great nutritional and functional qualities, serving as an excellent substitution to develop quinoa-containing foods. This study aimed to explore the influence of quinoa flour substitution on quality characteristics of wheat flour (WF). WF was substituted with different level of quinoa core flour, ground quinoa whole flour and recombined quinoa whole flour. Increasing levels of quinoa flour in WF declined dough swelling index, while increased falling number of composite flours. Besides, quinoa flour substitution considerably decreased the chemical forces of gluten in composite flours. The proportions α-helix and β-sheets reduced, while the random coil proportion increased in gluten secondary structure. SEM images revealed that the gluten network structure was severely damaged. Our findings indicated that substitution of WF with quinoa flours was promising to be developed as an ingredient for food products.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd) is a pseudo-cereal and has recently become popular in human diets, owing to its high nutritional and nutraceutical value (Xu et al., 2019). It can be employed as an ingredient in the development of functional food products owing to its functional and rheological qualities, sensory features, and nutritional profiles. Quinoa has attracted considerable attention in recent years for its nutrient-dense profiles and remarkable adaptability to challenging growing conditions as it is considered to be a whole plant protein and whole grain carbohydrate with high quality oils and minerals, numerous vitamins, polyunsaturated fatty acids, bioactive compounds and dietary fiber (Abugoch James, 2009).

Wheat (*Triticum aestivum*) is the most commonly utilized and best-studied cereals, partially due to its rheological and baking qualities of gluten. Over the past several years, a growing number of studies on potential applications of quinoa in food product creation and evaluation have emerged (Gostin, 2019; Tiga et al., 2021; Wang et al., 2021). The suitability of quinoa flour and wheat flour (WF) blend for bakery products was studied. Alizadehbahaabadi et al. (2021) incorporated 49% (w/w) quinoa flour into white flour (100 g). Their results showed that quinoa appeared to be able to formula breads with high loaf volume and softness, and acceptable sensory characteristics. Czekus et al. (2019) reported also that adding 20% quinoa flour increased protein level of bread by 16%, when compared with those of WF-bread. These documented clearly that quinoa flour addition improved the nutritional qualities of wheat-based products. Besides, studies have reported that the quinoa addition could obviously affect the physicochemical characteristics of WF-bread (Azizi et al., 2020; Ballester-Sánchez et al., 2019; Romano et al., 2018). A desired consuming quality and acceptable physicochemical attributes is vital for quinoa application in creating the unique food products. Consequently, products made with quinoa flour should either have good sensory qualities or be equivalent to products made with WF.

Quinoa is known as a super grain nutritionally and could be utilized in the bakery sector to improve the quality of final products or to produce unique goods, and the starch in quinoa seeds has similar qualities to wheat starch. Several studies on the replacement of WF with quinoa

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https://doi.org/10.1016/j.crfs.2023.100556
Received 19 February 2023; Received in revised form 29 June 2023; Accepted 26 July 2023
Available online 29 July 2023

flour have been conducted. However, no previous studies, if any, concerning the effects of different processing ways (e.g., grinding mill and flour mill) of quinoa flour on qualities, rheological properties, and chemical structures of WF, have been done.

The objectives of this study were: 1) to produce three types of quinoa flours using grinding mill and flour mill facilities separately to obtain WF-quinoa flour composite flours with different levels; 2) to investigate the influence of quinoa flour substitution on qualities and gluten structures of composite flours. The findings of this study would be useful for further food industrial research, and for the development of quinoa flour as an ingredient for such products as bakery and noodle with improved nutritional profiles and quality characteristics.

2. Materials and methods

2.1. Materials and reagents

All-purpose wheat flours were bought from Jinmailang Food Co., LTD in Baoding, China. Quinoa seeds were obtained by a plantation in Zhangjiakou, China. Total starch (AA/AMG) assay kit was bought from Megazyme International Co., Ltd (Bray, Wicklow, Ireland). Grinding mill 3100 and flour mill GLU-202 were bought from Penten Co., LTD (Germany) and Buller Machinery Manufacturing Co., LTD (Wuxi, China), respectively.

2.2. Preparation of composite flours

According to Fig. 1, in order to produce quinoa whole flour (GQWF), quinoa seeds were pulverized with a grinding mill and passed through a 100-mesh sieve. In addition, quinoa seeds were tempered for 24 h and stored in a moist warehouse for 12 h before being ground with a flour mill to produce quinoa shorts, quinoa core flour (QCF) that was passed via a 100-mesh sieve, and quinoa bran. These three were mixed (2.5:1.5:1) to produce the recombined quinoa whole flour (RQWF). QCF (10, 20, 30 and 40%), GQWF and RQWF (10, 20, and 30%) were substituted WF with different levels separately. The obtained composite flours, including QCF, GQWF and RQWF, were stored in a refrigerator at 4 °C prior to analysis.

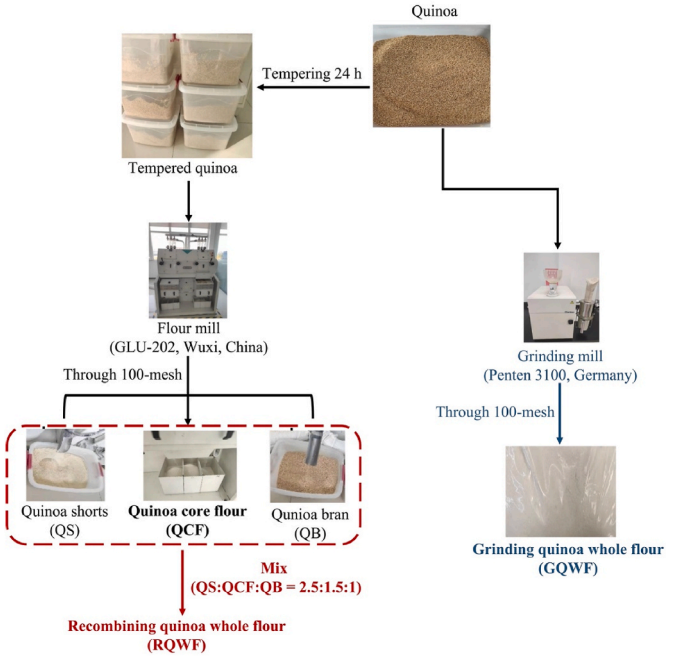

Fig. 1. Preparation of composite flours.

2.3. Proximate analysis and protein compositions of flours

According to AACC method (Method 56–81.04), the moisture, protein, fat, dietary fiber and ash content in flours were evaluated (AOAC, 2017). The protein level was determined by applying a factor of 6.25. The total starch level was determined by the total starch (AA/AMG) assay kit (Gu et al., 2019). The protein compositions of flours were extracted and quantified. **Albumin:** Five grams of sample was added to 10 mL of distilled water. The mixture was shaken in a water bath at 50 °C for 0.5 h. After cooling to ambient temperature, the mixture was centrifuged at 21,000×*g* for 15 min at 4 °C. The residue was twice rinsed. The supernatant was combined to reach a total volume of 50 mL. **Salt-soluble protein:** 10 mL of 10% NaCl was added to the above residue to wash, then centrifuged at 17,000×*g* for 15 min at 4 °C. The supernatant was collected to make the volume up to 50 mL. **Prolamin**: 10 mL of 75% ethanol was added to the above residue. The mixture was shaken at a 50 °C water bath for 5 min, and then reacted at room temperature for 30 min. Afterwards, the extraction method was carried out as followed by the extraction method of albumin. **Gluten:** 10 mL of 0.2% NaOH was added to the above residue to extract gluten. The method was the same as albumin. The protein concentration was determined by Kjeldahl method.

2.4. Determination of swelling index of gluten (SIG) and falling number of flours

Using the procedure described from a previous study (Wang and Kovacs, 2002), SIG value was calculated. Each flour sample (0.4 g, dry basis) was added to 0.8 mL of distilled water to mix for 5 s to create suspension, and then placed in the thermomixer at 4000×*g* for 10 min at room temperature. Following that, 0.4 mL of an isopropanol-lactic acid stock solution was added. Suspension was vortexed for 5 s before being put in a thermomixer at 4000×*g* for 10 min at 25 °C. The suspended sample was centrifuged at 100×*g* for 5 min. Supernatant was poured out, and the tube was drained. Th weight of the tube weight was evaluated (W_2). SIG value was computed as a percentage of the initial flour weight on the basis of the moisture content. The falling number test was evaluated to be in accordance with the procedure 56–81.04 (AACC, 2000).

$$\text{SIG}\ (\%) = \frac{W_1 - W_2}{W_F} \times 100\%$$

Where W_1 depicts the tube weight after draining; W_2 refers to the empty tube weight; W_F is the original flour sample.

2.5. Pasting properties of flours

Pasting properties of samples were determined (standard code: GB/T 14490–2008) using a Brabender Viscograph-E (Brabender, Germany). The pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), breakdown value (BV), final viscosity (FV) and setback value (SV═FV-TV) were recorded.

2.6. Determination of chemical forces of gluten

Following preparation in the farinograph, 16 g of dough samples were washed, centrifuged, and dried with Glutomatic 2200, Centrifuge 2015; Glutork 2020 (Perten Instruments, USA), respectively. Gluten was extracted from the dough by passing it through a fine sieve for 2 min and then a coarse sieve for 3 min. Gluten was freeze-dried for 72 h prior to further analysis.

Chemical forces experiments were carried out according to the methods of Gómez-Guillén et al., (1997). The lyophilized gluten was treated as following chemicals: SA:NaCl (0.05 mol/L), SB:NaCl (0.6 mol/L), SC:NaCl (0.6 mol/L) + urea (1.5 mol/L), SD:NaCl (0.6 mol/L) + urea (8 mol/L). 0.2 g of the sample was weighed to mix well with 10 mL

of the solution above separately. The mixture was stood at 4 °C for 1 h, and then centrifuged at 10,000×*g* for 10 min to obtain the supernatant. The protein concentration was calculated using an Automatic Kjeldahl nitrogen analyzer K1100 (Haineng Instrument Co., LTD, Jinan, China). Ionic bond contribution = SB-SA; Hydrogen bond contribution = SC-SB; Hydrophobic interaction contribution = SD-SC. The level of sulfhydryl-groups was measured as described by Chang et al. (2021).

2.7. Fourier transform infrared spectroscopy (FT-IR) analysis

Protein secondary structures of the flour samples were estimated using FT-IR spectrophotometer (Ocean Optics, Inc, FL, USA) following the method of a previous study (Mollakhalili Meybodi et al., 2019). Spectra were the averages of 64 scans. The wavenumber resolution was 4 cm^{-1} for range of 400–4000 cm^{-1}. Peakfit software was used to analysis data. The secondary structures were quantified using the ratio between a specific region and the entire area of the amide I band.

2.8. Scanning electron microscopy (SEM)

The gluten was made into thin slices and then coated with gold particles in an automated critical point dryer (model SCD 050, Leica Vienna). Gluten samples were analyzed by SEM (LEO EVO 40, Zeiss, Germany) at 20 kV acceleration voltage and 2000× magnification for microstructure.

2.9. Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) of gluten samples

SDS-PAGE analysis of gluten samples was conducted as described by Jideani et al. (1994). Gluten sample (10 mg) was added to 1 mL of Tris-HCl buffer (pH = 7.0) in a 1.5 mL of centrifuge tube for 2 min. Afterwards, sample was mixed well with the loading buffer at a ratio of 4:1 in 100 °C water bath for 10 min. The mixture was centrifuged at 8000×*g* for 5 min. Vertical plate electrophoresis was used. 7 μL of sample and marker was added to the sample well separately. The voltage (constant voltage mode) was set to 130 V. 50 mL of Foto SDS-PAGE dye was used to stain for 15 min and was then put into deionized water for decolorization. Gels were analyzed using AlphaEase software.

2.10. Statistical analysis

Values were mean ± standard deviation (mean ± SD, n = 3). Data was processed through one-way analysis of variance with Minitab Statistical Software 14.12.0 (MINITAB, State College, PA, USA). Statistical significance was set at a probability level of $p < 0.05$.

3. Results and discussion

3.1. The basic compositions and protein components in composite flours

The basic compositions of WF, QCF, GQWF, and RQWF are depicted in Table 1. The fat, ash, and starch content of QCF were considerably greater ($p < 0.05$) as compared with those of WF. The moisture content of the flours ranged from 11.50 ± 0.02 to 14.34 ± 0.26%. The low moisture observed in GQWF and RQWF was a good indicator of their potential for longer shelf life (Gautam et al., 2021). The ash contents ranged between 0.39 ± 0.04 and 2.30 ± 0.01%. The higher values were observed in GQWF (2.29 ± 0.07%) and RQWF (2.30 ± 0.01%). The ash content is closely related to the morphological structure. Concentrations of minerals found in quinoa are greater than those reported for the majority of grain crops (Wieme et al., 2020). High ash content, due to the substitution of WF with QCF, may include an increasing amount of minerals in the composite flours. The crude protein levels in WF and QCF differed considerably ($p < 0.05$) from one another. The greatest crude protein content was found in GQWF (13.42 ± 0.67%) and RQWF (14.20 ± 0.66%). This observation indicated that substituting WF with quinoa flour enhanced the protein level of the final products. The fat content of composite flours varied between 0.51 ± 0.07 and 5.96 ± 0.12%. Quinoa flour substitution in WF dramatically enhanced its fat content. Besides, the starch content varied between 52.35 ± 0.52 and 80.01 ± 0.52%. When the protein and ash content in the flours rose, their starch content in the flour samples decreased.

Four component parts in flour protein, including albumin, salt-soluble protein, prolamin, and gluten, were determined, and the results are depicted in Table 1. Albumin level in GQWF and RQWF were 5.81 ± 0.55% and 6.84 ± 0.23%, respectively, which were significantly higher than that in WF ($p < 0.05$). Albumin level in QCF was the least among four sample flours (1.77 ± 0.22%, $p < 0.05$). Similarly, the content of salt-soluble protein in GQWF and RQWF was dramatically greater than that in WF ($p < 0.05$), but no obvious difference between the content of salt-soluble protein in WF and QCF was found ($p > 0.05$). Prolamin and gluten are the main components of gluten, among which prolamin plays an essential role in the ductility, viscosity, and foaming properties of dough (Kan et al., 2021). According to the results, the prolamin content of WF was 3.61 ± 0.14%, while the prolamin content of composite flours ranged from 0.12 ± 0.11% to 0.88 ± 0.12%, which was considerably lower than that of WF ($p < 0.05$). Gluten is responsible for the elasticity and strength of dough by forming its linear structures through intermolecular disulfide bonds (Guardianelli et al., 2021). The content of gluten in WF was considerably higher than that in composite flours ($p < 0.05$). Albumin and salt-soluble protein in quinoa are the main storage proteins, accounting for approximately 70–80%, while the content of prolamin and gluten is relatively low. Therefore, grains, such as WF that rich in prolamin and gluten, are usually accompanied in those QCF-enriched food products, such as QCF-noodles.

Table 1
The basic compositions and protein components of wheat flour, quinoa flour and composite flours.

Samples	Moisture (%)	Fat (%)	Ash (%)	Starch (%)	Protein (%)	Dietary fiber (%)	Albumin (%)	Salt-soluble protein (%)	Prolamin (%)	Gluten (%)
WF	14.26 ± 0.03^{a}	0.51 ± 0.07^{c}	0.39 ± 0.04^{c}	70.66 ± 1.39^{b}	10.75 ± 0.23^{b}	3.43 ± 0.01^{c}	3.35 ± 0.29^{c}	0.58 ± 0.07^{b}	3.61 ± 0.14^{a}	3.86 ± 0.16^{a}
QCF	14.34 ± 0.26^{a}	1.08 ± 0.08^{b}	0.48 ± 0.02^{b}	80.01 ± 0.52^{a}	4.36 ± 0.13^{c}	0.00 ± 0.00^{d}	1.77 ± 0.22^{d}	0.96 ± 0.34^{b}	0.12 ± 0.11^{c}	1.20 ± 0.00^{c}
GQWF	11.50 ± 0.02^{c}	5.96 ± 0.12^{a}	2.29 ± 0.07^{a}	52.79 ± 1.19^{c}	13.42 ± 0.67^{a}	14.04 ± 0.67^{a}	5.81 ± 0.55^{b}	3.88 ± 0.15^{a}	0.88 ± 0.12^{b}	2.01 ± 0.19^{b}
RQWF	12.77 ± 0.04^{b}	5.78 ± 0.09^{a}	2.30 ± 0.01^{a}	52.35 ± 0.52^{c}	14.20 ± 0.66^{a}	12.60 ± 0.36^{b}	6.84 ± 0.23^{a}	4.61 ± 0.17^{a}	0.54 ± 0.18^{b}	2.05 ± 0.20^{b}

Values are mean ± standard deviation. Values with different letters in the same column represent statistical difference between each other ($p < 0.05$). WF = wheat flour; QCF = quinoa core flour; GQWF = quinoa whole flour obtained using a grinding mill; RQWF = quinoa flour obtained by recombining method using a flour mill.

3.2. Falling number and SIG of composite flours

The falling number of composite flours has shown in Fig. 2a. The falling number is negatively correlated with α-amylase activity and damage starch content of flours (Delatte et al., 2019). Herein, the falling number of WF was 445 s. Increasing substitutional amount of quinoa flours increased the falling number of QCF, GQWF and RQWF, rising to 705, 558 and 644 s, respectively. This test was done by combining ground wheat flour and water to create a slurry. Sound grain with a high falling number always forms a thicky slurry since the starches are still intact, whereas the sprout damaged grain may generate a flour/water combination that is "thin" or non-viscous as the starches started to break down owing to α-amylase activity (Kiszonas et al., 2018). Hence, shortened time in the falling number test might reduce the viscosity of the mixture, enhance α-amylase activity or cause sprout damage. Herein, the increased falling number suggested that quinoa flour substitution could reduce the α-amylase activity and damaged starch level in these composite flours.

The effect of quinoa flours on SIG was also investigated, and the result is depicted in Fig. 2b. SIG is normally used to evaluate baking and processing quality of flour products (Vanin et al., 2018). Herein, no considerable difference in SIG between WF and QCF10-20 was observed. When the substitutional level of QCF was >20%, SIG was significantly decreased ($p < 0.05$). SIG of GQWF20-30 and RQWF20-30 was significantly lower than that of WF ($p < 0.05$), mainly owing to the low gluten level in composite flours. Increasing substitution amount of quinoa flours declined the gluten content and swelling capacity of the composite flours, thus leading to the decreased SIG of composite flours.

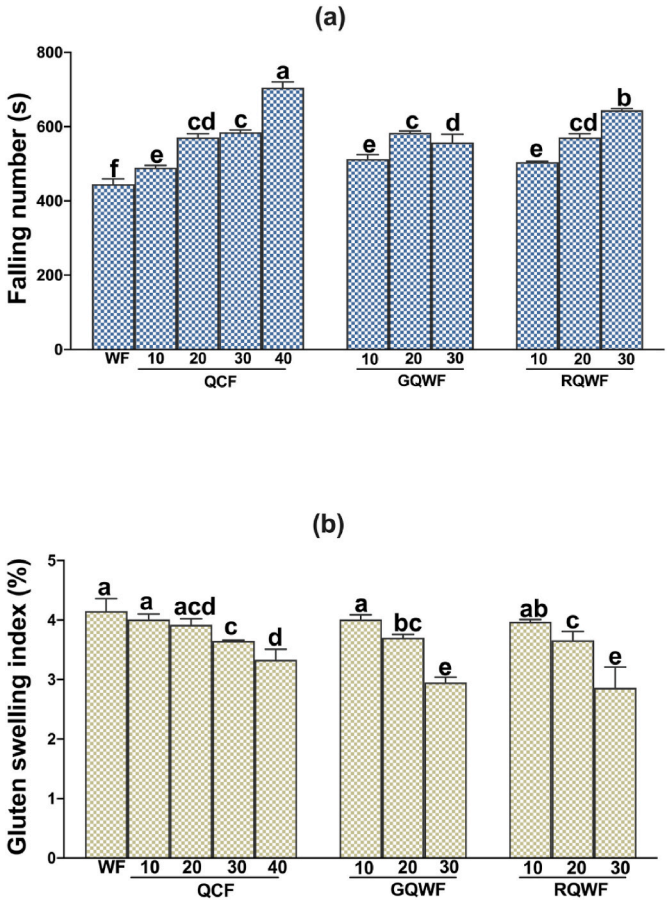

Fig. 2. The effects of quinoa flour substitution on composite flours towards (a) falling number; (b) gluten swelling index. Values are mean ± standard deviation. Bars with different letters represent statistical difference between each other ($p < 0.05$). WF = wheat flour; QCF10-40 = substitution of wheat flour with quinoa core flour at 10, 20, 30, and 40% levels, respectively; GQWF10-30 = substitution of wheat flour with quinoa whole flour using a grinding mill at 10, 20, and 30% levels, respectively; RQWF10-30 = substitution of wheat flour with quinoa whole flour by recombining method using a flour mill at 10, 20, and 30% levels, respectively.

3.3. Pasting properties of composite flours

Pasting property affects both texture and appearance of noodle products (Pasqualone et al., 2021). The pasting properties of QCF, GQWF, and RQWF are depicted in Table 2. Peak viscosity (PV), minimum viscosity (MV) and final viscosity (FV) of QCF were considerably higher than those of WF due to its high starch content ($p < 0.05$), while the PV, MV and FV of GQWF and RQWF were dramatically lower than those of WF due to their low starch contents ($p < 0.05$). Pasting temperature (PT) of GQWF and RQWF had no significant difference with WF, while PT of QCF was lower than that of WF ($p < 0.05$). Substitution with different proportions of QCF significantly changed the pasting properties of composite flours. Increasing QCF substitutional level obviously improved the PV and MV when compared with that of WF ($p < 0.05$), while the FV of QCF was lower than that of WF. Substitution with quinoa flour significantly decreased the breakdown and backup values of flours ($p < 0.05$), and the stability of starch became worse, indicating that QCF substitution delayed the aging of starch. When the amount of GQWF and RQWF was 30%, the PT was considerably greater than that of WF ($p < 0.05$). GQWF and RQWF substitutions significantly decreased PV, MV, and FV ($p < 0.05$). High dietary fiber content reduced the starch concentration in composite flours, and superabsorbent of the dietary fiber prevented the combination of starch with moisture, reducing the ability of starch to bind to water, thus affecting the starch pasting properties. The breakdown values of GQWF20 and RQWF20 were 296 ± 10.61 and 373 ± 5.66 BU, respectively, which were dramatically lower than the that of WF ($p < 0.05$), indicating that they had a better thermal stability than WF. The PV and setback values of GQWF and RQWF were lower than that of WF ($p < 0.05$), indicating that the aging degree of these composite flours was considerably lower than that of WF. In conclusion, the substitution of quinoa flours had an obvious effect on the pasting characteristics of flours ($p < 0.05$), which made the worse stability of starch particles in flours.

3.4. The effects of quinoa flour substitution on chemical forces of composite flours

Sulfhydryl (-SH) plays a vital role in gluten secondary structure. Its quantity and distribution are related to the quality of dough (Cappelli et al., 2020). The content of gluten sulfhydryl group in flours has shown in Fig. 3a. Substitution with different proportions of quinoa flours decreased the –SH group content in gluten of composite flours ($p < 0.05$), from 5.32 ± 0.05 to 1.15 ± 0.10 μmol/g. The –SH group contents in GQWF30 and RQWF30 decreased by 69.5 and 64.5%, respectively, as compared to WF. The reason is that the gluten level in composite flours is relatively low. Hence, the –SH group contents decreased as the substitution level of composite flours increased, thus leading to a negative impact on processing of noodle products.

Non-covalent forces, including hydrogen bond, ionic bond and hydrophobic interaction, make great contributions in dough formation and viscoelasticity, and are represented by the difference of protein solubility in different reducing solvents (Zhang et al., 2022). The gluten strength has a correlation with ionic bond. As can be seen from Fig. 3b, the contribution of ionic bond in QCF-40, GQWF-30 and RQWF-30 decreased significantly to 42.9%, 34.7% and 22.4%, respectively, as compared with WF ($p < 0.05$). The result suggested that the presence of quinoa flours interfered with electrostatic interaction between gluten molecules, thus reducing the contribution of ionic bonds to stable protein molecular structures. When the amount of quinoa flour was too high, the gluten network structure was easier to collapse, which could

Table 2
The effect of quinoa flour on pasting properties of flours.

Samples	Pasting temperature/°C	Peak viscosity/BU	Minimum viscosity/BU	Final viscosity/BU	Breakdown value/BU	Setback value/BU
WF	61.3 ± 0.28^{c}	1416 ± 6.36^{d}	996 ± 6.36a^{b}	2065 ± 6.36^{b}	420 ± 0.00^{d}	988 ± 7.07^{a}
QCF	60.5 ± 0.07^{d}	2291 ± 3.54^{a}	1242 ± 6.36^{ab}	2190 ± 14.85^{a}	1049 ± 9.90^{a}	880 ± 12.73^{b}
GQWF	61.3 ± 0.00^{c}	554 ± 4.24^{i}	461 ± 4.24^{b}	680 ± 12.02^{j}	93 ± 0.00^{h}	234 ± 7.78^{j}
RQWF	61.5 ± 0.07^{c}	924 ± 9.19^{h}	729 ± 7.78^{ab}	1066 ± 7.07^{c}	195 ± 1.41^{g}	338 ± 0.00^{h}
QCF-10	62.0 ± 0.28^{b}	1467 ± 30.41^{c}	984 ± 9.19^{a}	1917 ± 5.66^{c}	483 ± 21.21^{c}	883 ± 8.49^{b}
QCF-20	61.7 ± 0.07^{bc}	1540 ± 5.66^{b}	992 ± 7.07^{a}	1860 ± 3.54^{de}	548 ± 12.73^{b}	840 ± 11.31^{d}
QCF-30	61.4 ± 0.14^{c}	1545 ± 31.82^{b}	977 ± 15.56^{a}	1835 ± 29.70^{e}	568 ± 16.26^{b}	851 ± 3.54^{cd}
QCF-40	61.8 ± 0.07^{c}	1572 ± 12.73^{b}	1010 ± 3.54^{a}	1885 ± 7.07^{d}	563 ± 9.19^{b}	866 ± 2.83^{bc}
GQWF-10	61.7 ± 0.35^{bc}	1342 ± 21.92^{e}	865 ± 9.19^{ab}	1769 ± 1.41^{f}	477 ± 12.73^{c}	859 ± 7.07^{cd}
GQWF-20	63.0 ± 0.00^{a}	1170 ± 9.19^{g}	874 ± 1.41^{ab}	1472 ± 14.14^{i}	296 ± 10.61^{f}	577 ± 14.14^{g}
GQWF-30	62.6 ± 0.42^{a}	1202 ± 14.85^{g}	768 ± 2.83^{ab}	1479 ± 2.12i	434 ± 17.68^{d}	710 ± 4.24^{f}
RQWF-10	61.6 ± 0.21^{bc}	1286 ± 15.56^{f}	860 ± 8.49^{ab}	1761 ± 14.14^{f}	426 ± 7.07^{d}	856 ± 7.78^{cd}
RQWF-20	61.8 ± 0.14^{bc}	1191 ± 12.73^{g}	818 ± 7.07^{ab}	1628 ± 14.85^{g}	373 ± 5.66^{e}	778 ± 9.90^{e}
RQWF-30	62.7 ± 0.21^{a}	1294 ± 20.51^{f}	823 ± 15.56^{ab}	1588 ± 29.70^{h}	471 ± 4.95^{c}	761 ± 10.61^{e}

Values are mean ± standard deviation. Values in the same column with different letters represent statistical difference between each other ($p < 0.05$). WF = wheat flour; QCF10-40 = substitution of wheat flour with quinoa core flour at 10, 20, 30, and 40% levels, respectively; GQWF10-30 = substitution of wheat flour with quinoa whole flour using a grinding mill at 10, 20, and 30% levels, respectively; RQWF10-30 = substitution of wheat flour with quinoa whole flour by recombining method using a flour mill at 10, 20, and 30% levels, respectively.

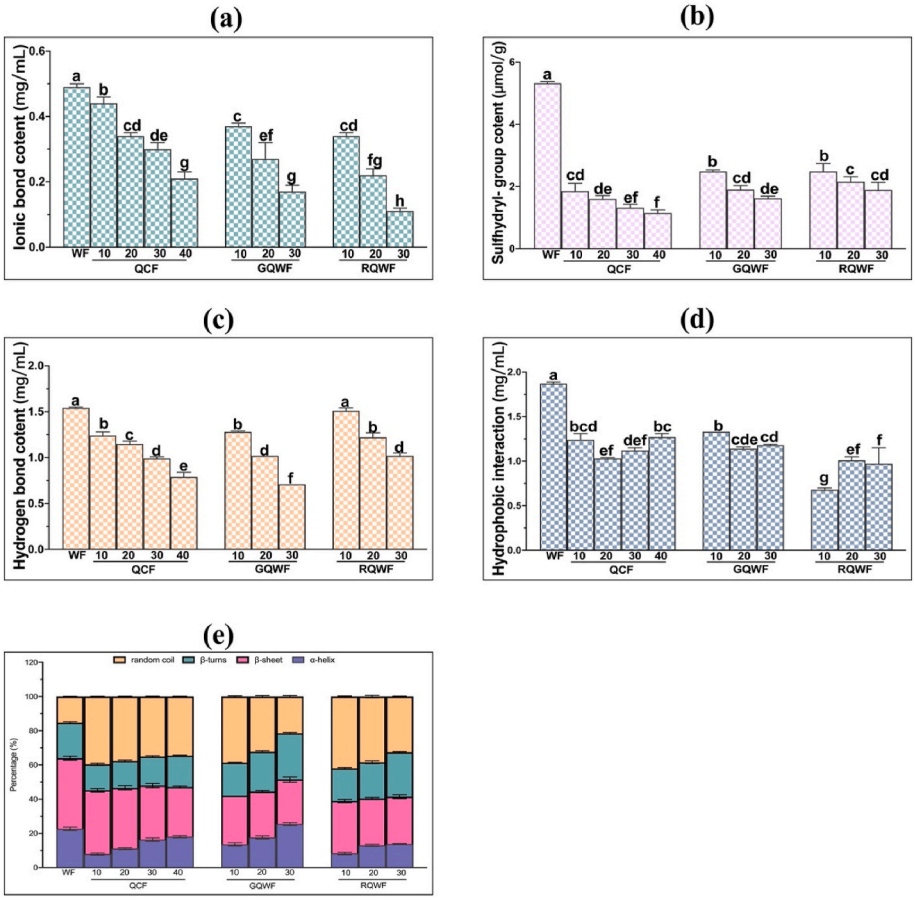

Fig. 3. The effects of different amount of quinoa flour substitution on chemical forces and secondary structure in gluten. (a) Distribution of –SH group (μmol/g); (b) Distribution of ionic group (mg/mL); (c) Distribution of hydrogen bond (mg/mL); (d) Distribution of hydrophobic interaction (mg/mL); (e) Proportion of secondary structures of α-helix β-sheets, β-turns and random coil (%) by FT-IR analysis. Values are mean ± standard deviation. Bars with different letters represent statistical difference between each other ($p < 0.05$). WF = wheat flour; QCF10-40 = substitution of wheat flour with quinoa core flour at 10, 20, 30, and 40% levels, respectively; GQWF10-30 = substitution of wheat flour with quinoa whole flour using a grinding mill at 10, 20, and 30% levels, respectively; RQWF10-30 = substitution of wheat flour with quinoa whole flour by recombining method using a flour mill at 10, 20, and 30% levels, respectively.

have a negative impact on dough and was not conducive to the production of flour products.

Gluten contains a large amount of glutamine, forming intermolecular and intramolecular hydrogen bonds between glutamines. Hydrogen bonds can make gluten structure more stable and have a favorable effect on dough rheology (Liu et al., 2021). As can be seen from Fig. 3c, compared to WF, the hydrogen bond contributions of QCF-40, GQWF-30 and RQWF-30 decreased from 1.54 ± 0.01 to 0.79 ± 0.05, 0.71 ± 0.00 and 1.02 ± 0.03 mg/mL, respectively, indicating that the structural stability of gluten network deteriorated in composite flours. The hydrophobic interaction is related to the oxidation of –SH groups. A relatively low hydrophobic interaction can stabilize protein, thus improving the gluten strength and elasticity (Taylor et al., 2016). This is the main reason for improving the dough toughness and elasticity. According to Fig. 3d, the contribution of hydrophobic interaction to gluten in flours was significantly decreased due to QCF, GQWF and RQWF substitution ($p < 0.05$), and contribution of hydrophobic interaction to protein of QCF-40, GQWF-30 and RQWF-30 was decreased by 0.6, 0.69 and 0.9 mg/mL, respectively, when compared with that of WF.

3.5. FT-IR of gluten in composite flours

On the basis of the distinctive infrared absorption of certain functional groups, FT-IR analysis is frequently used to examine the secondary structure and conformation of food biopolymers (Hosseini and Jafari, 2020). The stretching vibration of the C═O bond is the main source of the vibration frequency of the amide I band in FT-IR spectrum, which is the most valuable for the analysis of protein secondary structure. There are four main types of protein secondary structures, namely β-sheet, random coil, α-helix and β-turns. The bands of their corresponding amide I after crimping are 1615–1637 and 1682-1700 cm^{-1}, 1637–1645, 1646–1664 and 1661-1681 cm^{-1}, respectively, among which α-helix and β-turns have the greatest influences on gluten network structure formation as well as increase the dough elasticity. The impact of quinoa on secondary structures of gluten has shown in Fig. 3e. Substitution of WF with quinoa flours decreased the α-helix and β-turns of gluten, whereas the proportion of random coil was higher than that of WF, and the β-sheet did not change significantly. β-Sheet and α-helix contain more hydrogen bonds, which make the protein have a certain rigidity and a more regular and compact structure. α-Helix is closely associated with gluten network structure formation. This high starch level in quinoa flours competed with protein for water, inhibiting gluten network structure formation, while the dietary fiber in GQWF and RQWF bran competed with gluten for moisture, decreasing the proportion of α-helix, and weaken the hydrogen bonding between peptide chains. β-Sheet has an influence on the viscosity and homogeneity of dough through hydrogen bonds on the hydrophilic side chains. Herein, the decreased α-helix and β-turns proportions indicated that quinoa flours adversely affected the gluten network structure of dough. The absence of hydrogen bond in random coil and β-turns makes the protein molecule more flexible and loose structure (Cai et al., 2021). Substitution of WF with quinoa flours partially increased the proportion of random coil, mainly owing to the low gluten level in quinoa flour. Additionally, a serious shearing effect on gluten could occur due to the substitution of QCF, formatting more random coil structure and preventing gluten development.

3.6. Microstructures of gluten with different substitution amount of quinoa flours

Fig. 4 shows the representative SEM images. The continuous fibrous aggregates of gluten form the "skeleton" of the network, which is filled with gliadin. The gluten of WF exhibited smooth pore boundaries, and had a relatively uniform size distribution, which may be due to the fact that during the gluten formation, the peptide chain expanded and then gathered via hydrophobic interactions and disulfide bonds to form a tight three-dimensional network structure (Zhang et al., 2022). Quinoa flours substitutions destroyed the smoothness and uniformity of the gluten hole. The morphological structure of gluten was damaged and presented a honeycomb structure, and it gradually became chaotic. The gluten network structure was severely damaged as observed in GQWF30 and RQWF30. The holes became larger and more irregular than that in WF. This destruction might be due to the interaction of dietary fiber with gluten. The dietary fiber competed with gluten for the moisture, resulting in the rearrangement of water in the dough. This made gluten not fully react with water, and thus leading to the dehydration. Consequently, the gluten network structure was damaged, among of which would collapse.

3.7. Gel electrophoresis of gluten in flours with different substitution levels of quinoa

In Figs. 5 and 6, the electrophoretic profiles of gluten extracted from WF, QCF, GQWF, and RQWF were reported. Fig. 5 shows the electrophoretic bands of gluten in the non-reduced state. No considerable difference in the location and color of the electrophoretic bands of each sample was found, and the molecular weight (MW) of the samples was mainly between 10 and 35 kDa, among which the bands were more

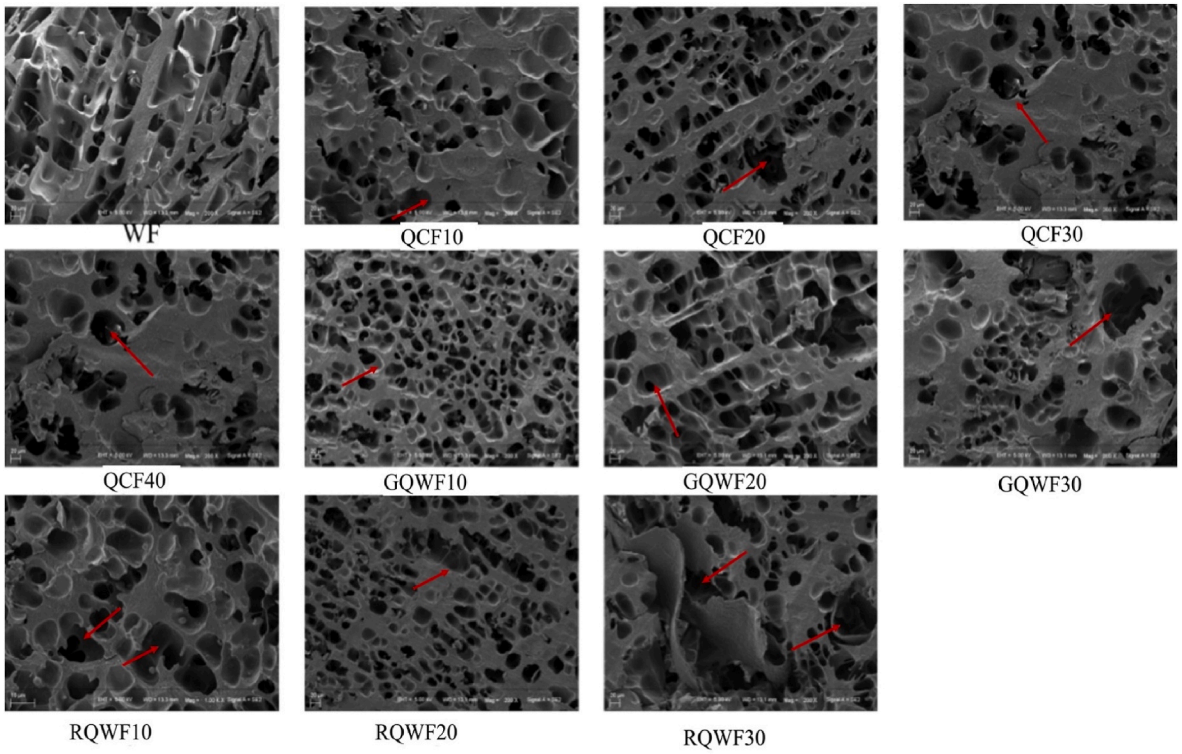

Fig. 4. Microstructures of gluten in composite flours by SEM analysis. WF = wheat flour; QCF10-40 = substitution of wheat flour with quinoa core flour at 10, 20, 30, and 40% levels, respectively; GQWF10-30 = substitution of wheat flour with quinoa whole flour using a grinding mill at 10, 20, and 30% levels, respectively; RQWF10-30 = substitution of wheat flour with quinoa whole flour by recombining method using a flour mill at 10, 20, and 30% levels, respectively.

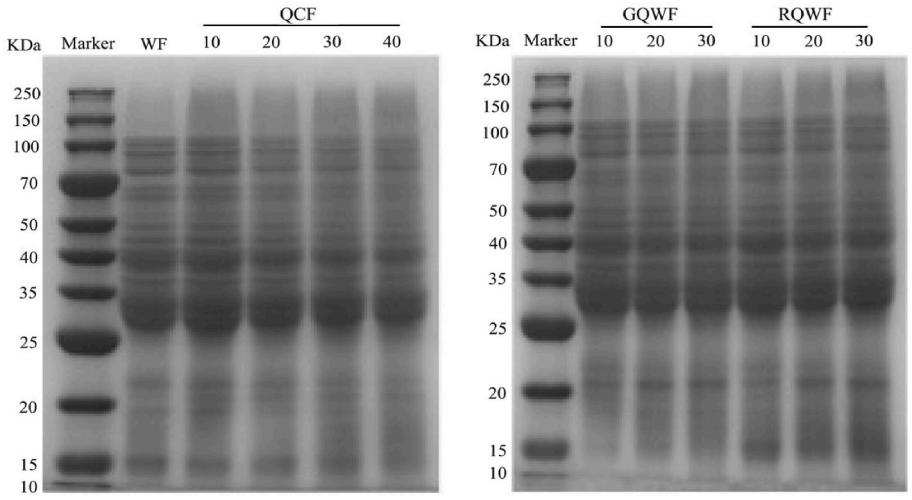

Fig. 5. SDS-PAGE patterns of non-reduction of gluten in composite flours. WF = wheat flour; QCF10-40 = substitution of wheat flour with quinoa core flour at 10, 20, 30, and 40% levels, respectively; GQWF10-30 = substitution of wheat flour with quinoa whole flour using a grinding mill at 10, 20, and 30% levels, respectively; RQWF10-30 = substitution of wheat flour with quinoa whole flour by recombining method using a flour mill at 10, 20, and 30% levels, respectively.

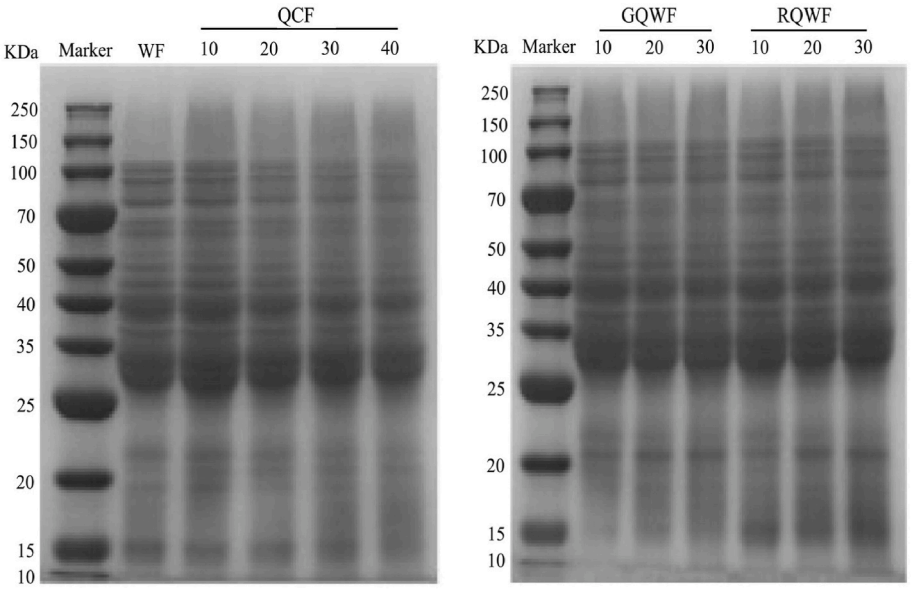

Fig. 6. SDS-PAGE patterns of reduction of gluten in composite flours. WF = wheat flour; QCF10-40 = substitution of wheat flour with quinoa core flour at 10, 20, 30, and 40% levels, respectively; GQWF10-30 = substitution of wheat flour with quinoa whole flour using a grinding mill at 10, 20, and 30% levels, respectively; RQWF10-30 = substitution of wheat flour with quinoa whole flour by recombining method using a flour mill at 10, 20, and 30% levels, respectively.

intense between 25 and 35 kDa. The bands between 10 and 15 kDa became shallow or even disappeared when the quinoa powder was added. The band at around 30 kDa became more intense. Fig. 6 depicts the bands of reduced gluten. The MW of gluten was mainly distributed in the range of 25–40 kDa. The bands between 10 and 25 kDa were less intense. The bands at 15 kDa became lighter when substitution of WF with quinoa flour. Compared with non-reduced gluten, the bands with high MW (about 100 kDa) increased after addition of β-mercaptoethanol, indicating that addition of β-mercaptoethanol resulted in the reduction of macromolecule proteins. The bands of gluten did not change significantly after substitution of quinoa flour, suggesting that quinoa did not change protein conformation via covalent bond with gluten.

4. Conclusion

This study explored the effect of quinoa flour substitution on quality properties of wheat dough. The results showed that quinoa flour substitution significantly disrupted the gluten network structure. 10% and 20% were the ideal substitution levels to maintain the network structures of composite flours. On the whole, quinoa flour at certain levels had a positive effect on dough quality, not only improved thermal stability (indicated in pasting properties), but also yielded promising results on healthy food (indicated in the increased contents of dietary fibers and proteins, as well as the decreased starch content). These findings would provide valuable guidance for the further development of quinoa-based products in food industries, such as quinoa-based muffin and instant noodles.

CRediT authorship contribution statement

Jianlou Mu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing – original draft. **Yiwen Qi:** Conceptualization, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **Kexin Gong:** Validation, Methodology, Formal analysis, Investigation. **zhizhou Chen:** Validation, Data curation, Writing – review & editing. **Margaret A. Brennan:** Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Qianyun Ma:** Validation, Data curation, Writing – review & editing. **Jie Wang:** Writing – review & editing. **Charles S. Brennan:** Conceptualization, Supervision, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – review & editing.

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

Data will be made available on request.

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