Innovatively processed quinoa (Chenopodium quinoa Willd.) food: chemistry, structure and end-use characteristics

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Abstract

BACKGROUND: Quinoa (Chenopodium quinoa Willd.) flour and processed traditional Peruvian quinoa breakfast foods were studied to evaluate the effect of extrusion and post-processing on protein properties, morphology and nutritional characteristics (amino acids and dietary fibers).

RESULTS: The extrusion increased quinoa protein crosslinking and aggregation observed by size exclusion high-performance liquid chromatography and the amount of soluble fibers, as well as decreasing the amounts of insoluble fibers in the processed foods. The post-processing drying resulted in additional crosslinking of large protein fractions in the quinoa products. The microstructure of the extruded quinoa breakfast flakes and heat-post-processed samples studied by scanning electron microscopy and X-ray tomography differed greatly; post-drying induced formation of aerated protein microstructures in the heat-treated samples. Nanostructures revealed by small-angle and wide-angle X-ray scattering indicated that extrusion imparted morphological changes in the quinoa protein and starch (dominance of V-type). Overall, extrusion processing only reduced the content of most of the essential amino acids to a minor extent; the content of valine and methionine was reduced to a slightly greater extent, but the final products met the requirements of the Food and Drug Organization.

CONCLUSION: This study presents innovative examples on how extrusion processing and post-processing heat treatment can be used to produce attractive future food alternatives, such as breakfast cereal flakes and porridge powder, from quinoa grains. Extrusion of quinoa flour into Peruvian foods was shown to be mostly impacted by the processing temperature and processing conditions used. Protein crosslinking increased due to extrusion and post-processing heating. Starch crystallinity decreased most when the product was dried after processing.

Supporting information may be found in the online version of this article.

Keywords: pseudo-cereal; protein morphology; quinoa starch structure; microstructure; polymerization; processing; functional properties

INTRODUCTION

Quinoa, Chenopodium quinoa Willd., is a pseudo-cereal originating from the Andean region, where it used to be the key food crop for the Incas and other ancient Andean cultures, dating back to 5000 BC. Recently, quinoa has gained increased attention because of its highly nutritional food characteristics. Quinoa seeds are known to contain compounds of high nutritional value, such as proteins, vitamins and minerals.1,2 The high nutritional value of quinoa seed is primarily due to its relatively high content of protein content (varying between 13.8% and 16.5%)3,4 and quality (well-balanced amino acid composition equivalent to that of casein in milk).1 In addition to the highly nutritious proteins, quinoa seeds contain dietary fiber, starch and bioactive

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compounds, making this crop attractive for a wide range of food products. In the two main quinoa-producing countries, Peru and Bolivia, as well as in other countries in the Andean region, whole quinoa seeds are traditionally used in soups, bread, cookies and, if milled into flours, in pasta, crisps and tortilla. Quinoa can also be processed into breakfast cereals and snacks, including coating, by temperature processing such as extrusion puffing. During extrusion of several quinoa types, the best extrudates in terms of maximum expansion, minimum density, high degree of gelatinization and low water solubility were obtained at 15–16% moisture, 130–140 °C die temperature and high mechanical shear, and were dependent on quinoa modification and variety characteristics. The high mechanical stress used in durum wheat processing was found to rupture the polysaccharide glycosidic bonds and lead to an increase in soluble dietary fiber. Other quinoa seed processing methods, such as roasting and extrusion, have been found to affect quinoa seed components and chemistry. However, to our knowledge no studies have thoroughly evaluated effects of heat treatment on quinoa protein properties in processed quinoa foods.

Extrusion cooking is an important food processing technique, allowing production of a whole range of cereal products, including beverages, instant soups, porridges, breakfast cereals and snacks. However, this processing method has so far been extensively explored to a lesser extent for quinoa-based products. Extrusion is known to eliminate antinutritional factors of quinoa, while, for other cereals, extrusion is known to impact gelatinization of starch, increase solubility of dietary fibers and reduce lipid oxidation. Furthermore, extrusion and pressure cooking are considered suitable methods to reduce the activity of inhibitors (α-amylase, trypsin/chymotrypsin, hemagglutinin, etc.) and antinutrients (phytic acid and oxalates) and increase the solubility of phenolic compounds at temperatures of 140–175 °C. Extrusion processing is also known to improve digestibility and increase the bulk density of proteins, which are important parameters for enriching children’s foods with proteins. Thus there is significant potential in producing attractive future food alternatives from quinoa, but greater knowledge is needed on how quinoa protein chemistry, protein interactions and structure are impacted by extrusion processing towards better tuning nutritional and functional characteristics of processed quinoa foods.

In this study, quinoa protein chemical and structural characteristics of processed quinoa flour are assessed in the context of enhancing the value for traditional Peruvian foods such as breakfast cereals, ready-to-eat snacks, porridge and instant quinoa products. The aim of the study was also to investigate the effect of post-processing heat treatment on protein properties and morphology of processed quinoa foods.

MATERIALS AND METHODS

Materials
Commercially desaponified (pearled) quinoa seeds (INIA Salcedo variety) were purchased from Kumara Foods EIRL in Lima, Peru. The seeds were ground into flour using a laboratory hammer mill (Milliflour GBS Group SpA) (Fig. 1 (A, B)). Flour characteristics such as moisture, protein content (N × 6.25), fat and ash were determined according to standard AOAC methods and AACC methods. All measurements were done in triplicate. The carbohydrate content (CCarb) was calculated using the formula: CCarb, g kg⁻¹ = 100 – (fat amount (g) + protein amount (g) + ash amount (g) + moisture (g)). The milled quinoa seed (Q grain) was designated as quinoa flour (Qfl) and was further used in making processed quinoa breakfast cereals and porridge powder.

Sample preparation and extrusion
Qfl was mixed with water (50 g water for every 1 kg Qfl) before going into the extruder barrel and a further 90 g water for every 1 kg Qfl was added during extrusion with the aim of gelatinizing the starch. A commercial simple-screw extruder (Buhler) was used with the following parameters: feeding velocity 50 rpm, extruding temperature 69–95 °C and screw velocity 149 rpm. The puff-extrudate obtained from this process was in the form of round-shaped breakfast cereals (Fig. 1(C)) and the sample was designated QextD. The QextD sample was further ground into powder using a hammer mill (Milliflour GBS Group SpA) to achieve a final powder-like product (for use in instant foods, beverages or porridge, for example) and designated QextG.

Particle size distribution
The particle size distribution of Qfl and QextG samples was determined using a light scattering (LS) laser diffraction particle size analyzer (Malvern Mastersizer 3000, Malvern Panalytical Ltd, Malvern, UK) equipped with AeroS standard venturi dry powder disperser. Results were analyzed with Mastersizer v3.81 software using a Mie scattering model incorporated into the instrument. The experiments were performed at RISE Research Institutes of Sweden AB, Stockholm, Sweden.

Size-exclusion high-performance liquid chromatography (SE-HPLC)
The amount and size distribution of proteins were analyzed by SE-HPLC, adopting a procedure commonly applied to wheat, but also found useful for peas. Thus sodium dodecyl sulfate (SDS)-extractable and SDS-unextractable proteins were analyzed following the two-step method described in Muneer et al., with some modifications. For the first extraction, 1.4 mL SDS-phosphate buffer (0.5% SDS, 0.05 mol L⁻¹ NaH₂PO₄, pH 6.9) was added to each sample. The samples were thereafter vortexed for 10 s by Whirli Vib 2 (Labassco, Sweden), shaken for 5 min using IKA-VIBRAX VXR (IKA, Germany) at 2000 rpm and centrifuged for 30 min at 10 000 rpm. The supernatant was collected and designated as SDS-extractable protein fraction. For the second extraction, an additional 1.3 mL buffer was applied to the remaining pellet and this sample was sonicated for 45 s and centrifuged; the supernatant was collected and designated SDS-unextractable protein. Both SDS-extractable and SDS-unextractable samples were analyzed using the Waters 2690 separation module connected to a Waters 996 photodiode array detector (Waters, Milford, MA, USA), equipped with a pre-filter (Security Guard GFC 4000, Phenomenex, Torrance, CA, USA) and a main column (Biosep-SEC-S 4000, 300 × 4.5 mm², Phenomenex). All samples were analyzed as three technical replicates. Protein extractability was normalized on the basis of mean total extractable protein measured by SE-HPLC of a whole grain flour of milled (using an IKA-WERKE grinder type A10, from SKAFTE Medlab, Mindelheim, Germany) quinoa grains. The same grinder was used for the Qext and QextD samples to prepare them for protein extraction and SE-HPLC analyses. For comparisons of samples, chromatogram areas of total SDS-extractable
and total SDS-unextractable proteins were used. Furthermore, the chromatograms were divided according to time intervals into eight sections, in which the areas were used for calculations and comparisons.

**Scanning electron microscopy (SEM)**
The microstructure of the cross-sections of Qext and QextD samples was studied by SEM (SEM) (LEO 435VP, Cambridge, UK) coupled with a secondary electron detector; where an acceleration voltage of 10 kV was used. An Au/Pd (3:2) coating (JFC-1100, JEOL, Tokyo, Japan) was used for the selected samples prior to SEM analysis according to Muneer *et al.*

**X-ray microtomography**
The microstructure of Qext and QextD was studied using X-ray microtomography, which was performed at the 4D imaging lab at Lund University with a Zeiss Xradia Versa XRM520. The X-ray source parameters used were 60 kV and 5 W. A total of 1601 radiographs were acquired over a 360° rotation of the samples with exposure times of either 1 or 1.5 s (depending on the sample). A 0.4× optic was used with the camera set to 2 × 2 binning,
A wavelength of 0.91 Å was used and the sample-to-detector distance was 1904 mm, covering the scattering vector \( q \), which was in the range 0.0082–0.47 Å\(^{-1}\), where \( q = 4\pi/\lambda \sin(\theta) \), and where \( 2\theta \) is the scattering angle. Exposure times were 30 s and 5 min. The detector employed was a bidimensional hybrid pixel detector (Pilatus 1M, Dectris). The SAXS data were azimuthally averaged using the software bil0114,\(^{20}\) normalized with respect to integrated intensity incident on the sample during the exposure time and corrected for sample absorption and background.

**Small-angle X-ray scattering (SAXS)**

All the quinoa samples and Qfl were analyzed by small-angle X-ray scattering (SAXS) at the beamline I911-4 of MAX IV Laboratory (Lund, Sweden).\(^{20}\) A wavelength of \( \lambda = 0.91 \text{ Å} \) was used and the sample-to-detector distance was 1904 mm, covering the scattering vector \( q \), in the range 0.0082–0.47 Å\(^{-1}\), where \( q = 4\pi/\lambda \sin(\theta) \), and where \( 2\theta \) is the scattering angle. Exposure times were 30 s and 5 min. The detector employed was a bidimensional hybrid pixel detector (Pilatus 1M, Dectris). The SAXS data were azimuthally averaged using the software bil0114,\(^{20}\) normalized with respect to integrated intensity incident on the sample during the exposure time and corrected for sample absorption and background.

**Wide-angle X-ray scattering**

Wide-angle X-ray scattering measurements for all the samples from this study were carried out at the beamline I911-2 of MAX IV Laboratory (Lund, Sweden). For the experiment, a wavelength of 1.0384 Å and a sample-to-detector distance of 150 mm were used. The detector used was a bidimensional CCD of 165 mm (Rayonix). The sample exposure time was 1 min. A silicon powder was used as a standard sample for calibration. The collected data were analyzed using the software FIT2D.\(^{21}\) A degree of crystallinity \( X_c \), in volume percentage, was calculated according to the equation:

\[
X_c = \frac{A_I}{A_I + A\_amorphous},
\]

where \( A_I \) is the total area related to the crystalline peaks and \( A\_amorphous \) is the total area of the diffracted signal (including amorphous and crystalline phases).

**Dietary fiber**

The total, soluble and insoluble dietary fiber were analyzed by an enzymatic–gravimetric method according to the Official Method of AOAC (1995) using the K-TDFR kit from Megazyme International Ireland Ltd.\(^{17}\) One gram of dried flour samples (in duplicate) was subjected to sequential enzymatic digestion by heat-stable \( \alpha \)-amylase, protease and amyloglucosidase.

For soluble/insoluble dietary fiber determination, insoluble dietary fiber (IDF) was filtered, and the residue was washed with warm distilled water. A combined solution of filtrate and water washings was precipitated with 4 volumes of 95% ethanol (EtOH) for soluble dietary fiber (SDF) determination. The precipitate was then filtered and dried. Both SDF and IDF residues were corrected for protein, ash and blank, for the final calculation of SDF and IDF values.

For total dietary fiber determination, SDF was precipitated with EtOH, and the residue was then filtered, dried and weighed. The total dietary fiber (TDF) value was corrected for protein and ash content.

**Determination of amino acids**

Amino acids were determined using reversed-phase (RP) HPLC according to the procedure by Heinrikson and Meredith.\(^{22}\) The amino acids were separated on a Waters system equipped with a column (Microbondapak C18, 300 × 4.5 mm\(^2\)). Identification and quantification of amino acids was done comparing their retention times and UV–visible spectral data with known previously injected standards. The content of amino acids in grains was expressed in grams of amino acid per 100 g protein. The samples were analyzed in duplicate.

**Statistics**

All experiments were conducted in either duplicate or triplicate and reported as mean ± standard deviation. Statistical analysis software (SAS, version 9.2) was used on SE-HPLC data to perform analysis of variance (ANOVA), from which the mean was calculated and compared by applying Tukey’s test (\( P < 0.05 \)). Minitab Express 1.5.1 was used by applying Tukey’s test (\( P < 0.05 \)) and Microsoft Excel was used to calculate mean and standard deviation values on flour characteristics, dietary fiber and amino acid data.

**RESULTS AND DISCUSSION**

**Protein extractability evaluated by SE-HPLC**

Puff-extruded Qfl samples, shown in Fig. 1, indicated some differences in particle size distribution between Qfl and QextG (Fig. 1(F) and Table 1). The smallest particle sizes (15.5 μm) were observed for Qfl protein sample (Table 1), whereas the greater volume density of large particles was observed for QextG (Fig. 1 and Table 1). Quinoa protein extractability was compared in the studied samples by SE-HPLC as shown by percentage of SDS-extractable protein (black bar) and SDS-unextractable protein (white bar); percentage values were obtained from normalization of the obtained SE-HPLC data with the mean of total extractable protein measured by SE-HPLC of a whole grain milled Qfl (Fig. 2(B)). A decreased protein extractability compared to the levels of protein extracted from the flour samples (Fig. 2) was observed in the processed samples Qext, QextD and QextG, indicating crosslinking of proteins at extrusion. The decrease in protein extractability was primarily detected in protein fractions of larger size among the SDS-extractable protein (areas I–IV indicated by the dashed line in Fig. 2(A)), showing that these proteins contribute to the largest polymerization of the quinoa proteins. No significant differences in the total protein extraction (SDS-extractable + SDS-unextractable protein) were detected in between grain and flour samples. Also, total protein extractability did not differ between the different types of extruded/extruded dried/extruded grounded samples (Fig. 2(B)). Values of total protein extractability correlated

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**Table 1. Volume and particle size distribution of Qfl and QextG samples**

| Sample | Mean (μm) | Particle size < 10% (μm) | <50% (μm) | <90% (μm) |
|--------|-----------|------------------------|-----------|-----------|
| Qfl    | 182.0 (± 7.5) | 15.5 (± 0.1) | 119.5 (± 3.1) | 447.4 (± 20.3) |
| QextG  | 204.5 (± 8.1) | 44.1 (± 0.5)  | 163.1 (± 2.8) | 420.0 (± 18.8) |

Values in parentheses indicate standard deviation.
well with the amount of SDS-extractable proteins; the SDS-extractable protein fraction of the total protein extractability was around 88% and 79%, respectively, for grain/flour samples versus puff-extruded samples. A significant decrease in SDS-unextractable proteins were seen in the Qfl sample as compared to the quinoa seeds (I–VII areas of SDS-unextractable protein chromatogram compared for all the studied samples); the protein fractions of larger size in particular, presented in I–IV areas of the chromatogram, were responsible for this decrease (Fig. 2 A (indicated areas of the chromatogram) and C (variation between the

Figure 2. Amount and size distribution of quinoa proteins studied by SE-HPLC: (A) an example of a chromatogram for the sodium dodecyl sulfate (SDS)-extractable protein fractions of quinoa flour (green line) and QextD sample (red line); the dashed green line indicates areas I–IV; (B) relative amount of extractable quinoa protein divided into SDS-extractable and SDS-unextractable fractions. Letters indicate significant differences (Tukey P < 0.05) among total amount of proteins (above staples) and SDS-extractable and -unextractable proteins (inside staples); (C) relative amount of SDS-unextractable proteins divided into sizes according to areas under the chromatogram (compare part B) for the different samples.
studied samples). This decrease in SDS-unextractable proteins for QextD was most likely due to the major type of proteins; that is, the 2S albumin (29%; with molecular weight (MW) 12 kDa and 2 SS-linked subunits) and 11S globulin (chenopodin, 34%; 320 kDa and 6 SS bonds), connecting via SS bridges to larger sizes. It can be inferred that milling of quinoa samples gave rise to some variation in particle sizes between Qfl and QextG, as was observed in this study (Table 1 and Fig. 1), due to differences in protein crosslinking. The drying of the puff-extruded samples resulted in a decrease in the medium-size protein fractions of the SDS-unextractable proteins as compared to samples only being puff extruded (areas III–VII, Fig. 1(A, C)). Thus the drying procedure resulted in additional crosslinking of large and unextractable protein fractions in quinoa. Indeed, in another study on quinoa, it was observed that slightly elevated temperatures as (an increase in temperature from 40 to 80 °C) during air-drying processing decreased the amount of protein (to 10%) and negatively affected fibers.24 In our study, the extrusion processing temperatures used (69–95 °C) were slightly higher than in Miranda et al.,24 but much lower than in most other studies, which commonly used 130–170 °C,25 affecting both protein quality and, to a minor extent, protein quantity (Table 2). In our study, extrusion appears to have induced primarily crosslinking, but also denatured some of the quinoa globulin protein at the extrusion temperature used (e.g. 69 °C), while previous studies have shown denaturation of the major 11S globulin at higher temperatures (>95 °C).26 Furthermore, in our study, some types of proteins (probably medium size) were further crosslinked at post-drying treatment (10 min at 130 °C). It is possible that the more crosslinked particles (difficult to disrupt by grinding) were the reason for the slightly larger particle sizes observed in QextG versus Qfl samples (Fig. 1(F) and Table 1).

### Chemical composition of quinoa before and after processing

The protein content of raw quinoa was 11.8 ± 0.2 g 100 g⁻¹ and there was a slight decrease after milling to 11.3 ± 0.2 g 100 g⁻¹ and, after extrusion, to 10.6 ± 0.1 g 100 g⁻¹, indicating a mild reducing impact of extrusion on the protein concentration (Table 2). Similar behavior has been observed in previous studies, where the content of both ash and fat was reduced, the latter being the most common. Nowak et al.33 reported a protein content of 13.1 ± 0.2 g 100 g⁻¹ and a total fat content between 4.0 and 7.6 ± 0.1 g 100 g⁻¹ of raw quinoa, Blanca de Juli variety from Puno, Peru. The protein value was in accordance with the value found in this study for the INIA Salcedo variety (Table 2).

Our study showed that no significant difference in IDF and TDF was observed when Qext was compared with the raw quinoa samples (Table 3). Nowak et al.33 found a TDF content of between 8.8 and 14.1 ± 0.1 g 100 g⁻¹ for Bolivian quinoa – values similar to those observed in this study. In other study, a reduction in IDF and TDF and an increase in SDF after extrusion were previously reported by Repo-Carrasco-Valencia and Serna.27 During the extrusion process mechanical stress may cause breakdown of polysaccharide glycosidic bonds, releasing oligosaccharides and therefore ending up with an increase in SDF.28

### Composition of amino acids in quinoa before and after processing

The composition of essential amino acids of raw quinoa grain and processed quinoa in our study (Supporting Information Table S1) corresponded well to findings by Stikic et al.,29 but are lower than in other studies.30,31 Quinoa is known for being very rich in the essential amino acid lysine. Escuredo et al.31 analyzed the amino acid profile of three genotypes of quinoa and found a lysine content between 1.32 and 3.70 g 100 g⁻¹ protein, while in our study in raw quinoa seeds we observed 4.03 ± 0.1 g 100 g⁻¹ protein. During the processing of quinoa, there was a small reduction in content of all essential amino acids, with the exception of valine, and the largest losses were observed for methionine (Table S1). It is assumed that these lower amino acid values are due to the pearling process for the elimination of saponins.32 Singh et al.33 studied the effect of extrusion on the retention of essential amino acids. They found that lysine showed the lowest retention, followed by tryptophan, threonine and methionine.

The essential amino acid composition of the quinoa used in the present study was also compared with the FAO reference (Supporting Information Table S1). Only threonine, isoleucine and leucine met the requirements of the FAO. Repo-Carrasco et al.34 found that the content of essential amino acids in Amarilla de Marangani variety met the FAO requirements for all essential amino acids. Thus there seem to be major differences in amino acid composition between quinoa varieties. Despite the nutritional damage (e.g. loss of essential amino acids such as lysine) that occur during the extrusion process, the protein nutritional value is favorable in extruded products compared in products obtained by other processing methods (boiling, roasting, microwave processing).34 A general suggestion to minimize lysine degradation would be not to use temperatures higher than 180 °C and feed moisture above 15%.34 In addition, high temperature and shear conditions in the processing of cereals produce significant structural modifications in starch molecules, with the destruction of polymer chains, allowing their release, as was observed for Qext in comparison to Qfl and grain (Fig. 5(B)).

### Microstructure of processed quinoa food

**Scanning electron microscopy**

Color and shape differences were observed between our samples, with Qext sample being dark grey, with an oval shape of around

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**Table 2.** Composition of raw quinoa grain, flour and processed quinoa foods

| Sample | Moisture (g 100 g⁻¹) | Protein (g 100 g⁻¹) | Crude fat (g 100 g⁻¹) | Ash (g 100 g⁻¹) | Carbohydrates (by difference) |
|--------|---------------------|-------------------|---------------------|----------------|-------------------------------|
| 1. Qgrain | 10.22 ± 0.05c | 11.80 ± 0.28b | 3.66 ± 0.01b | 2.19 ± 0.04b | 72.15 ± 0.37b |
| 2. Qfl | 8.87 ± 0.01b | 11.30 ± 0.08ab | 4.02 ± 0.18b | 2.15 ± 0.01b | 73.67 ± 0.08c |
| 3. Qext | 15.85 ± 0.05d | 10.62 ± 0.11a | 1.31 ± 0.01a | 1.96 ± 0.04a | 70.27 ± 0.13a |
| 4. QextD | 4.56 ± 0.08a | 11.80 ± 0.28b | 1.41 ± 0.01a | 2.16 ± 0.06b | 80.09 ± 0.43d |
| 5. QextG | 4.41 ± 0.24a | 11.90 ± 0.02b | 1.71 ± 0.25a | 2.14 ± 0.03b | 79.85 ± 0.06d |

Values in parentheses indicate standard deviation. Different letters in the same column indicate significant difference.
Table 3. Dietary fiber content in raw quinoa grain and extruded quinoa foods

| Sample  | IDF (g 100 g⁻¹) | SDF (g 100 g⁻¹) | TDF (g 100 g⁻¹) |
|---------|-----------------|-----------------|-----------------|
| 1. Qgrain | 12.20 ± 1.56a  | 1.43 ± 0.04a    | 13.63 ± 1.60a   |
| 2. Qext  | 8.50 ± 0.80a    | 2.11 ± 0.57a    | 10.61 ± 1.36a   |

Different letters in the same column indicate significant differences. DF, dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

Figure 3. Microstructure of the processed quinoa samples, Qext and QextD, studied by SEM: (A–C) extruded quinoa breakfast cereals (Qext) at various magnifications; (D, E) QextD samples; broken green line in the main image indicates a porous microstructure, which is enlarged in the top image on the right; yellow line points at the inner area of a pore. Broken bars at the bottom of each picture indicate scale.
4–5 mm (in a longitude direction) compared to the QextD extruded-dried particles, which are light brown and >5 mm (in a longitude direction) (Fig. 1(C, D)). The microstructure of the inner cross-section of Qext and QextD indicates clear variation between the samples (3D movies shown in Supporting Information Figs S2 and S3 for Qext and QextD, respectively). Qext was rather compact, with a dense matrix, and had few large air bubbles (Fig. 3(A, C)). The matrix consisted of proteins and starch granules (some of them embedded in a seed coat; Fig. 3(C)) of rounded shape, in which the quinoa starch granules differed from those observed in Figure 4.

Figure 4. X-ray tomography images performed on Qext (A) and QextD (B) samples; (A) pixel size 7.5 μm and (B) pixel size 9.5 μm. Both samples were exposed to 60 kV and 5 W power for an exposure time of 1 s, and 1601 projections were taken.
Rayner et al. The matrix of components holding the bubbles for QextD was aerated, clearly indicating changes that occurred during post-processing heat treatment, as shown in Fig. 3(D, E). The development of a textural pattern of QextD samples such as porous structure around the edges of the sample with the central part being non-aerated (Fig. 3(E)) might be due to the evaporation of water in this sample (4.6% vs. 15.8% of moisture in QextD vs. Qext, respectively; Tables 2, 3 and 4). The impact of water content is known to affect mechanical properties such as compression force, which is related to the microstructure of cereal-based snacks. On the other hand, hardness and breaking strength in extrudates is known to be affected by the strength of cell walls and was found to be influenced by protein denaturation and highly correlating starch gelatinization. From the micrographs observed in this study, the more porous QextD samples (Fig. 3(D, E)) compared to Qext (Fig. 3(A–C)) may suggest that protein denaturation and crosslinking were to some extent more severe when more water was present during both processing and post-heat treatments.

**X-ray microtomography**

Complementary to SEM analysis, 3D microstructures of whole sample were analyzed for Qext versus QextD samples studied by X-ray microtomography (Fig. 4.). Even though the outer surfaces of the compared samples were visually very similar (despite the color differences), the inner structures are clearly very different: Qext can be seen to be compact and continuous (Fig. 4(A)), whereas QextD can be seen to be porous and discontinuous throughout its inner structure (Fig. 4(B)). Interestingly, QextD structure appeared different in horizontal cross-section (Fig. 4(B), top image, left) compared to the vertical cross-section (Fig. 4(B), top image, right, and lower image; video movies on microstructure for both samples are included in the Supporting Information). The structure observed was anisotropic, suggesting differences in bubble growth in the matrix, most likely due to the differences in water amounts in the samples. Thus the plasticization effect of water in QextD and its adsorption by flour components during post-processing drying can be assumed to be rather critical and leads to a pronounced effect on the microstructure of the sample. These different internal structures might lead to different liquid absorption properties of the quinoa extrudates, for example for breakfast cereals.

**Nanostructure of processed quinoa samples**

**Molecular morphology from SAXS**

The SAXS patterns of the unprocessed quinoa samples (grain/flour) were compared with those for the extruded samples, which revealed that the extruded-dried and extruded-dried ground sample morphology differed as a result of the processing (milling, extruding and temperature drying) (Fig. 5(A)). Two characteristic broad peaks were observed for Qf (Fig. 5(A); indicated by dotted arrows) and Qgrain (Supporting Information Fig. S1), whereas no such peaks were found in the processed samples (Fig. 5(A)). The peaks observed most likely originated from the extended semi-crystalline lamellar structure of starch (first and second order). From the fitting of the first peak, the periodicity of the lamellar structure was estimated as 84 and 82 Å, for Qgrain and Qf, respectively (Fig. 5(A)). The fact that no such peaks were present in the SAXS signals for the processed samples suggests that the extrusion processing decreased the processed samples’ crystallinity (e.g. starch crystallinity). This difference in morphology can be related to the starch partial gelatinization and protein

![Figure 5. Structural characterization of the quinoa grain, Qf, QextD and QextG studied by SAXS (A); arrows indicate the starch semicrystalline structure; by WAXS (B), and (C–E) Xc determination for Qf, Qext and QextD (fitting, red line; crystalline peaks, green line; amorphous structure, blue line).](image-url)
aggregation, which was observed by SE-HPLC results and by SEM images in this study (Figs 2 and 3). Decrease in starch crystallization and a more intense starch gelatinization can be seen in this study in QextD and QextG compared to the grain and flour samples, due to the drying process at high temperature. This suggests an increase in starch fragmentation, which was also observed in the WAXS data, and lowered absorption of water, as was observed by Gutkoski and El-Dash.40

The SAXS data also indicate that the protein structure seemed to be further unfolded and degraded in the processed samples. Protein extractability evaluation by SE-HPLC showed a similar total amount of the extracted protein, although significantly lower amounts of the SDS-unextractable protein were found for QextD compared to Qext. This suggests that post-processing heat treatment even further denatured the protein. The weakening of the protein structure due to heat has been previously observed in several studies.31–33 However, a dedicated study on individually separated Qfl components (starch and protein) would provide a better picture of the temperature effect on Qfl component morphology.

**Atomic structure from WAXS**

The crystallinity of Qfl (and grain) and all the processed samples was evaluated by WAXS, and it was found that extrusion processing and post-processing changed the crystalline structure of starch, from A-type to V-type (Fig. 5(B, C)). Qgrain and Qfl showed typical scattering patterns with the main peaks at \( q = 10.62, 11.95, 12.65, 16.13 \) and 17.00, corresponding to \( d \)-values varying from 5.92 to 3.70 Å, as shown in Table 4 (highlighted area). These values represent a typical A-type polymorph starch.39,44 The degree of crystallinity \( X_c \) was 33.2% and 29% for Qgrain and Qfl, respectively (Table 4).

The degree of crystallinity in our samples was slightly higher than those reported for diverse quinoa genotypes (21.5–43%) in another study.35 Quinoa starch has been reported to have significant amounts of short unit chains (Fingerprint-A chains, \( A_{fi} \), of amylopectin, as well as a lack of long chains, which contributes to a lower crystallinity,39 compared to other cereal starches. In this study, the broader WAXS peaks observed in quinoa grain/flour indicate that the crystallinity of starch was significantly decreased in the quinoa processed samples Qext, QextD and QextG. This behavior was observed in both the scattering patterns (Fig. 5(B)) with decreasing \( X_c \) values, from 8.3% to 4.9%, for Qext and QextD/QextG, respectively (Table 4) (the fittings for the selected samples are shown in Fig. 5(B, C)). It is important to point out that the WAXS data show that extrusion processing had a clear impact on the atomic-scale structure and gelatinization of the quinoa starch, changing its type from A-type to V-type 3537 for the samples, Qext, QextD and QextG (Fig. 5(B, C)). A possible explanation is that extrusion processing induced morphological transformation and drastically decreased crystallinity from 30% in Qfl (and grain) to 8% in Qext, and 4% in QextD and QextG. The gelatinization temperature for quinoa starch is known to be around 56.2–65.0 °C, and is highly dependent on the fine structure of amyllopectin.39 Also, quinoa starch could potentially form aggregations, which was indicated by the spherically shaped aggregates that could be seen by SEM. Formation of such aggregates might be largely influenced by the presence of protein. Therefore, we can assume that in our study extrusion processing induced both starch gelatinization observed by SEM (Fig. 3) and protein denaturation/aggregation observed by SE-HPLC (Fig. 2). Furthermore, the nanostructural amorphous pattern in the WAXS signal is likely the result of the protein crosslinking. The main protein fractions in quinoa grain are albumins and globulins, and these proteins form molecular structures stabilized by disulfide-type bridges.46 These bridges appear, from this study, to be severely affected primarily during extrusion, and to a lesser extent during the temperature post-processing step.

**CONCLUSIONS**

According to the results from this study, we conclude that Qfl, due to its nutritional characteristics and processing behavior, can be easily processed into traditional Peruvian foods such as puffed or milled breakfast cereals and become a very attractive superior breakfast food of high value worldwide. Qfl processing was mostly impacted by the extrusion temperature (69–95 °C) and processing conditions used, but less impacted by milling and drying temperature. Extrusion induced protein crosslinking and aggregation, decreased protein solubility, as observed by SE-HPLC, but also decreased the amounts of total dietary fibers and essential amino acids (in particular, methionine and cystine). Insoluble dietary fibers decreased, while the soluble fibers increased after extrusion processing. Post-extrusion drying was favorable for crosslinking some larger quinoa proteins, such as 11S globulin.

Microstructure analysis using SEM and X-ray microtomography indicated that the extruded samples had a dense and compact protein matrix stabilized by disulfide bridges. However, the post-dried sample was very aerated due to water plasticization, which is assumed will deliver different liquid absorption if these
products are used as breakfast cereals. The analysis also highlights how microstructure evaluation of processed breakfast cereals by X-ray microtomography can be a useful tool for industrial structure–quality evaluation.

Quinoa protein aggregation and denaturation, and starch partial gelatinization, as observed by SAXS, are important factors that can essentially impact functional properties and structure of the processed products.

The extrusion and post-extrusion heat treatment was seen, by WAXS analysis, to impact the nanomorphology of the samples by decreasing starch crystallinity and changing the starch type from the A-type to V-type. The degree of crystallinity was around 30% for quinoa grain and flour, around 8% for extruded processed samples and around 5% for the post-extrusion heated samples, indicating a significant impact on morphology of temperature and drying–milling processing.

The extrusion processing and post-extrusion heat treatment decreased nutritional value of the quinoa products. This study has shown that the traditional Peruvian foods considered – for example, breakfast cereal flakes (Qext and QextD) and porridge powder (QextG) – could be favorably compared with products processed by more traditional means – for example, roasting – and can provide attractive nutritious foods with novel functionalities.

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

SUPPORTING INFORMATION
Supporting information may be found in the online version of this article.

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