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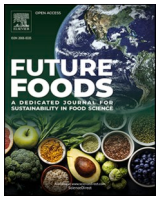

Compositional and techno-functional properties of quinoa-based ingredients for gluten-free applications

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ABSTRACT

Quinoa (Chenopodium quinoa), a pseudocereal with a complete amino acid profile, offers a promising alternative to wheat, barley and oat in plant-based and gluten-free foods, yet its functional properties and processing side-streams remain underutilised. This study characterised and compared the composition and techno-functional properties of a quinoa protein concentrate (QPC) and its side-stream (SS), produced from a single quinoa flour (QF) fractionation process, to evaluate how processing alters their suitability for food applications. QPC showed markedly higher protein content (39.40 ± 1.60 *g*·100 g^{-1}) than QF (15.28 ± 0.92 *g*·100 g^{-1}) and SS (14.54 ± 0.80 *g*·100 g^{-1}), alongside increased fat (9.20 ± 1.10 *g*·100 g^{-1}) and ash (2.65 ± 0.18 *g*·100 g^{-1}), indicating effective nutritional enrichment. Scanning electron microscopy (SEM) highlighted structural changes induced by processing, supporting observed differences in functionality. Techno-functional analyses revealed that QPC had the lowest emulsion separation rate (0.18%/min, significantly lower than SS $p < 0.05$) and intermediate protein solubility at pH 7 (26.92 ± 0.83%), water-holding capacity (148.76 ± 0.59%, significantly higher than QF $p < 0.05$) and oil-holding capacity (63.17 ± 2.99%), positioning it as a suitable protein fortifier for plant-based and gluten-free systems requiring improved emulsion stability and gelation. In parallel, the carbohydrate-rich SS demonstrated potential as a thickening or bulking ingredient, for example in soups, sauces, or other structured foods, supporting more circular use of quinoa processing streams. Overall, this work provides an integrated ingredient and process-level evaluation of quinoa flour fractionation, clarifies the distinct application potentials of QPC and its SS, contributing to the development of sustainable, nutritionally enhanced plant-based and gluten-free food products.

1. Introduction

The adoption of gluten-free and plant-based diets has increased markedly in recent years, driven by rising diagnoses of coeliac disease and non-coeliac gluten sensitivity, as well as broader interest in perceived health and sustainability benefits of reducing wheat and animal-based foods (Melini and Melini, 2019; Willett et al., 2019). At the same time, several nutritional assessments have shown that many gluten-free foods and gluten-free dietary patterns remain highly refined and nutritionally suboptimal, often providing less protein, fibre, iron, B vitamins, and other micronutrients than comparable gluten-containing products, thereby contributing to risk of deficiencies in long-term gluten-free consumers (Gobbetti et al., 2018; Myhrstad et al., 2021). These trends underline the need for alternative gluten-free ingredients that support both plant-based eating patterns and improved nutritional quality.

Quinoa (Chenopodium quinoa), a gluten-free pseudocereal, offers a promising solution to nutritional and functional limitations commonly observed in gluten-free foods. Quinoa seeds provide high-quality protein containing all nine essential amino acids in proportions that exceed

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those of common cereals, which is particularly important in gluten-free diets where reliance on rice- and maize-based formulations can lead to low protein density and suboptimal amino acid balance (Lerner et al., 2019; Manzanilla-Valdez et al., 2024; Vega-Gálvez et al., 2010). Alongside this complete amino acid profile, quinoa delivers dietary fibre, vitamins, minerals, and diverse phytochemicals that contribute to antioxidant and other bioactive properties (Dakhili et al., 2019; Nowak et al., 2016; Touil et al., 2024). Nevertheless, many commercially available gluten-free products, such as bread and cakes, still rely heavily on refined starches and lack sufficient protein, micronutrients, and bioactive compounds, contributing to nutritional inadequacies in consumers who depend on these foods (Allen and Orfila, 2018; Myhrstad et al., 2021).

Recent work on gluten-free cakes and other bakery products has demonstrated that careful selection and formulation of alternative ingredients can improve technological quality, bioactive features, and glycaemic response, underscoring the importance of both nutritional and functional optimisation in this product category (Cakir et al., 2024; Foschia et al., 2016). However, compared with more established gluten-free grains such as rice and corn, quinoa remains relatively underexplored as a plant-based protein source in bakery systems, partly due to its higher cost, variability in composition among cultivars, and the presence of saponins and other surface-active compounds that can affect flavour, bitterness, and processing if not properly managed (Navruz-Varli and Sanlier, 2016; Song et al., 2024; Vega-Gálvez et al., 2010). There is therefore substantial scope to better understand how quinoa-derived protein ingredients can be exploited to address both sensory and nutritional limitations of current gluten-free formulations.

The gluten-free market has expanded rapidly over the past decade, with global sales showing sustained growth and further increases projected, driven not only by medically diagnosed gluten-related disorders but also by a wider consumer segment adopting gluten avoidance and plant-forward diets ("Gluten-free Products Market Size | Industry Report, 2030," n.d.; Melini and Melini, 2019). This growth has intensified efforts to reformulate gluten-free foods with improved nutritional density and functional performance, particularly through the use of alternative protein sources and structurally active ingredients (Foschia et al., 2016; Gobbetti et al., 2018). Quinoa is especially attractive in this context because its proteins exhibit favourable emulsifying, gelation, and water-binding behaviours, making them suitable for structure formation in gluten-free bakery and other complex food matrices (James, 2009; Elsohaimy et al., 2015). Recent reviews and experimental studies have highlighted quinoa proteins as promising plant-based emulsifiers and functional ingredients in a range of food systems, further supporting their potential role in next-generation gluten-free products (Qu et al., 2025; Vilcacundo and Hernández-Ledesma, 2017).

Extensive research has characterised the nutritional composition and functional behaviour of quinoa flour, revealing variability in protein (9.1–15.7 $g{\cdot}100\ g^{-1}$), fat (4.0–7.6 $g{\cdot}100\ g^{-1}$), dietary fibre (8.8–14.1 $g{\cdot}100\ g^{-1}$), and associated bioactive compounds across varieties and growing conditions (Guo et al., 2025; Li and Zhu, 2017; Nowak et al., 2016). Similarly, quinoa protein concentrates (QPCs) have been reported to retain a complete amino acid profile and to display favourable hydration and emulsification properties compared with some cereal proteins, supporting their use as high-quality fortifiers in plant-based foods (Elsohaimy et al., 2015; Qu et al., 2025). However, the wet fractionation and concentration of quinoa protein generate substantial side-streams that may be rich in carbohydrates, fibre, minerals, and residual protein. These materials are often underutilised or discarded, despite increasing interest in circular processing, valorisation of by-products, and the design of sustainable ingredient systems for gluten-free and plant-based foods (Food Waste Recovery, 2018; Li et al., 2025; Sharma et al., 2025). To date, only limited work has systematically examined the composition, structural features, and techno-functional behaviour of such processing side-streams or compared them directly with the originating raw material and the enriched protein fraction. Given these gaps, there is a clear need to characterise quinoa-derived ingredients at multiple levels, composition, structure, and functionality and to relate these characteristics to their potential use in gluten-free and plant-based applications. Understanding how processing steps such as wet fractionation and enzymatic treatment reshape protein structure, microstructure, and techno-functional properties is critical for the rational design of ingredients that improve not only nutrition but also technological quality, texture, and stability in gluten-free foods, in line with recent advances reported for other gluten-free baked food matrices (Bozdogan et al., 2019; Cakir et al., 2024; Foschia et al., 2016). In addition, documenting and comparing the structural and functional properties of both the protein-rich concentrate and its associated carbohydrate-rich side-stream can support more integrated utilisation strategies and reduce waste in quinoa processing, aligning with wider sustainability and circular economy goals for modern gluten-free and plant-based diets (Food Waste Recovery, 2018; Li et al., 2025). . In this context, techno-functional properties are defined as the physical and chemical characteristics that determine how an ingredient performs during processing and in the final product, while side-stream utilisation refers to the valorisation of nutrient-rich processing residues into food ingredients rather than waste

In this study, quinoa flour (QF) was processed via a wet fractionation approach combined with enzymatic treatment to produce a quinoa protein concentrate (QPC) and a corresponding process side-stream (SS). The composition, protein biochemistry, structural features (including microscopy-based observations), and key techno-functional properties (solubility, water- and oil-holding capacity, and emulsion stability) of QF, QPC, and SS were analysed and directly compared. By linking these properties to current knowledge and recent advances in quinoa and gluten-free product research, the work aims to clarify how processing modifies the nutritional and structural attributes of quinoa-derived fractions, identify distinct application potentials for QPC and SS in gluten-free and plant-based food systems, and contribute to more sustainable, circular ingredient strategies in the development of structurally and nutritionally improved gluten-free products.

2. Materials and methods

2.1. Raw materials

After lab trials a pilot scale production method for producing a quinoa protein concentrate and a side-stream product from quinoa flour was developed and is depicted in Fig. 1. The production of the quinoa protein concentrate was carried out at the facilities of Fraunhofer IVV. Quinoa flour was sourced from Quinoa Marche (Quinoa Italia), Italy. The flour was milled to a particle size of $\leq$250 μm using stone-grinding and sieving. The quinoa flour was then put through a wet fractionation process using a 1:8 flour-to-water ratio. Enzyme α-amylase (220,000–286,000 u/g, issue date 04/07/2017) was sourced from Kerry Food Ingredients (Cork) Limited and integrated into the extraction process to break down starch and improve protein isolation and purity. The α-amylase was added at 1 ml per kg of flour during protein extraction under optimised conditions for protein yield which were determined by pre-trials: initial activation of α-amylase at pH 6.0 and 55 °C for 60 min, followed by adjustment to pH 8.0 and continuous stirring for 30 min to solubilise the quinoa proteins. The mixture was centrifuged by means of a decanter to separate the high-protein supernatant from insoluble components, including starch and fibre, which formed a sediment. This was recovered and subsequently freeze-dried for one week, (Telstar LyoQuest, Barcelona, Spain) and ground by hand using a pestle and mortar to produce a side stream product. The decanter used was a 2-phase decanter, type CB 300, manufacturer = GEA Westfalia; separation conditions were as follows; revolution: 4400 rpm, difference speed: 5.5 rpm, weir height: 170 mm, back pressure: 3 bar, the centrifugation time was estimated as it depends on the volume throughput and is not set directly. The volume throughput was 1100 $L{\cdot}h^{-1}$ and the time

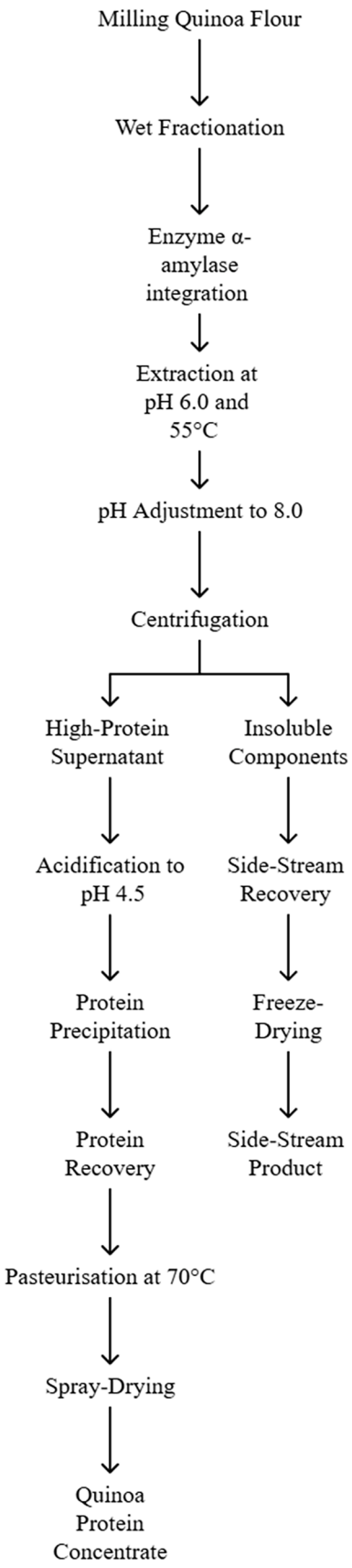

Fig. 1. Production process of quinoa protein concentrate (QPC) and quinoa side-stream (SS).

for separation of the whole batch was 100 min. The supernatant was acidified to pH 4.5 to precipitate proteins, which were then recovered by separation. The separated proteins were then pasteurised at 70 °C and consequentially spray-dried to produce the final protein concentrate. Spray-dryer: APV Anhydro, Modell PSD 58. The nozzle is an internal mixing nozzle manufactured by Delavan (Delavan Spray LLC, 4334 Main Highway, Bamberg, South Carolina 29,003–8456, USA). The minimal gap is 0.64 mm. The inlet temperature was 180 °C and the outlet 80 °C. The supernatant after protein precipitation were poured in 1 L flasks and frozen at −20 °C without further neutralization/treatment. Protein solution remained stable (without further precipitation) before spray drying. The protein-yield of the QPC process was 20.60 $g{\cdot}100\ g^{-1}$ of starting material.

2.2. Compositional analysis

Compositional analysis on QF, QPC and SS was performed externally by Chelab S.r.l. (Resana, Italy) using standardised methodologies. Moisture content was determined by a gravimetric method (AOAC Official Method 950.46 B, Moisture in Meat, AOAC International, 1991). Protein content was analysed using the Dumas method (AOAC Official Method 992.23, Crude Protein in Cereal Grains and Oilseed Products, 17th Ed., 2000) with a nitrogen-to-protein conversion factor of 6.25. Total starch was measured using the Megazyme kit K-RAPRS (Bray, Ireland). Fat content was quantified via the Soxhlet extraction method (AACC Method 30–25.01), the fatty acid composition was carried out using capillary gas chromatography according to method (ISO 16,958:2015); while ash content was assessed gravimetrically (AOAC 945.46). Individual minerals were determined on a method using ICP-OES based on AOAC 2011.14. Dietary fibre was determined using the Rapid Integrated Procedure of Enzymatic-Gravimetric-Liquid Chromatography (AOAC 2017.16). Sugar composition was measured by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) according to ISO 22,184 I IDF 244:2021, and total carbohydrates were calculated by difference (AOAC 986.25). All results are reported on a fresh weight (fw) basis. Resistant, digestible, and total starch were determined using the Megazyme kit K-RAPRS (Megazyme, Bray, Ireland), following the manufacturer's instructions and were reported as a percentage of sample dry weight.

2.3. Protein characteristics

2.3.1. Amino acid profile

Total amino acids were measured as described by Halm et al. (2025).

2.3.2. Protein profile (SDS-PAGE)

For SDS-PAGE analysis, QF, QPC and SS were prepared as described by Gautheron et al. (2024) with slight modifications by suspending 2 % (w/v) protein in 15 mL of chaotropic buffer (5 M urea, 2 M thiourea, 2 % SDS, 0.001 M EDTA, 0.1 M Tris base, pH adjusted to 8.8 with 3 M HCl) for detergent-assisted denaturation and solubilisation of proteins. After transferring 1 mL aliquots to 2 mL screw-cap tubes, 17.5 μL β-mercaptoethanol (BME) was added under a fume hood, and samples were incubated with shaking at room temperature for 16 h. The resulting extracts were clarified by centrifugation at 14,680 rpm for 20 min. For SDS-PAGE, samples were mixed with NuPAGE™ LDS sample buffer (4X) and the NuPAGE™ Sample Reducing Agent (10X), heated at 70 °C for 10 min, and briefly centrifuged. Approximately 20 μL of each sample and the protein ladder (Precision Plus Protein™ Dual Xtra Pre-stained Protein Standard, 2–250 kDa) (BioRad, CA, USA)), was loaded onto NuPAGE™ 4–12 %, Bis-Tris, 1.0–1.5 mm mini protein gels assembled in a chamber containing NuPAGE™ MES SDS running buffer (20X) with 1 mL of NuPAGE™ Antioxidant per chamber. Electrophoresis was performed at 200 V for ~35 min until the dye front reached the gel bottom. Gels were rinsed with water, fixed in 40 % methanol/10 % acetic acid for 30 min, stained with InstantBlue Coomassie Protein stain (Abcam,

Cambridge, UK) for 20 min, and destained in water with frequent solution changes until background was clear. Gels were stored in distilled water at 4 °C until analysis.

2.4. Physical and techno-functional characteristics

2.4.1. Colour

The sample colour was assessed using a handheld Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Tokyo, Japan) following the method of Jaeger et al. (2023), with modifications. Powders were placed in flat glass petri dishes and levelled to create a uniform surface. Three readings were taken per dish, with each dish emptied, refilled, and measured in triplicate. Colour measurements were initially recorded in the CIE D65 colour system (XYZ values) and subsequently converted and reported in the Hunter Lab* colour space.

2.4.2. Ultrastructure (SEM)

The ultrastructure of QF, QPC and SS was examined using scanning electron microscopy (SEM) following the protocol described by Atzler et al. (2021). Samples were mounted on stubs (G 306; 10 mm × 10 mm diameter; Agar Scientific, Essex, UK) and secured with carbon adhesive tabs (G3357N; 9 mm; Agar Scientific). They were then sputter-coated with an 80/20 gold–palladium alloy using a Polaron E5150 sputter coater. Imaging was performed with a JEOL JSM-5510 scanning electron microscope (Jeol Ltd., Tokyo, Japan) at an accelerating voltage of 5 kV, a working distance of 20 mm, and magnifications of 50× and 1500×.

2.4.3. Particle size

Particle size distribution was assessed according to Vogelsang-O'Dwyer et al. (2021) via laser diffraction analysis (Mastersizer 3000, Malvern Instruments Ltd., UK) with a measurable range of 0.01–3000 μm. Measurements were conducted using a dry dispersion method, with a particle refractive index of 1.45 and dispersant (air) refractive index set to 1.00. Methodology of Vogelsang-O'Dwyer et al. (2021) was modified for dry dispersion method. Results are expressed as volume-weighted mean diameter ($D_{4,3}$), and volume percentiles (Dv (10), Dv(50), Dv(90)).

2.4. 4 pH and total titratable acidity (TTA)

The pH and total titratable acidity (TTA) were assessed following the procedure of Waters et al. (2013), with modifications as outlined by Neylon et al. (2023). A 10 g sample was combined with 95 mL of distilled water and 5 mL of acetone, then thoroughly mixed to achieve a uniform dispersion. The pH was measured using a pH meter (Mettler Toledo, Columbus, OH, USA). Titration was carried out with 0.1 M NaOH until the pH reached 8.5. After a 3-minute equilibration period, the pH was checked and, if necessary, readjusted to 8.5. TTA is expressed as the volume (in millilitres) of 0.1 M NaOH required per 10 g sample to reach the endpoint.

2.4.5. Protein solubility

Protein solubility was assessed across pH 3, 5, 7, and 9 using the method of Jaeger et al. (2023). Dispersions containing 1 % (w/w) protein in distilled water were prepared, adjusted to the target pH using HCl or NaOH, and hydrated by shaking overnight at 4 °C. After verifying and readjusting the pH if necessary, 10 mL aliquots were collected from each triplicate sample prior to centrifugation to represent the whole sample. The remaining dispersions were centrifuged at 4000 rpm for 30 min. Protein content in the supernatants was quantified via the Kjeldahl method (nitrogen × 6.25). Solubility was calculated as the percentage of protein in the supernatant relative to the total protein in the whole aliquot.

2.4.6. Foaming capacity and stability

The foaming capacity and stability was analysed using the method of Jaeger et al. (2023). Suspensions with a concentration of 2 % (w/w) were prepared using distilled water. The pH was adjusted to pH 7 with HCl or NaOH (Sigma-Aldrich/ Fisher Scientific, St. Louis, MO, USA) and samples hydrated overnight at 4 °C. After room temperature adjustment, the sediment was redispersed, and the pH was readjusted. The initial height of the samples was recorded, and the samples were frothed using an Ultra-Turrax with a S10N-10 G dispersing element (IKA Labortechnik, Janke and Kunkel GmbH, Staufen, Germany) at maximum speed for 30 s. The foam phase height was recorded immediately and after one hour. The foaming capacity (Formula (1)) was calculated as the percentage of foam development immediately after mixing (0 min), while foam stability (Formula (2)) was determined as the percentage of foam development after one hour compared to immediately after foaming, using the following equations:

$$\textit{Foaming capacity}\ (\%) = \left(\frac{\textit{Foam height immediately after foaming}}{\textit{Initial sample height}}\right)\cdot 100 \tag{1}$$

$$\textit{Foam stability}\ (\%) = \left(\frac{\textit{Foam height after 1 hour}}{\textit{Foam height immediately after foaming}}\right)\cdot 100 \tag{2}$$

2.4.7. Water- and oil-holding capacity

Water-holding capacity (WHC) and oil-holding capacity (OHC) were assessed by placing 1 g of sample into a pre-weighed tube, then adding 6 g of either water (for WHC) or sunflower oil (for OHC) at native pH. The mixtures were vortexed for 3 min, allowed to stand at room temperature for 1 hour, and then centrifuged at 4000 rcf for 30 min at 20 °C. Following centrifugation, the supernatant was discarded, and the tubes were inverted onto a paper towel for 30 min to drain any excess liquid. The final weight of the tubes was recorded, and WHC/OHC (Formula (3)) values were calculated using the equation provided below.

$$WHC \Big/ OHC\ (\%) = \left(\frac{Weight_{tube+pellet} - Weight_{tube} - Weight_{sample}}{Weight_{sample}}\right)\cdot 100 \tag{3}$$

2.4.8. Emulsifying characteristics

The emulsifying characteristics were determined using the method of Vogelsang-O'Dwyer et al. (2022). Aqueous dispersions containing 1 % (w/w) protein were prepared with distilled water, the pH was then adjusted to 7 using either HCl or NaOH, and the mixtures were hydrated by shaking overnight at 4 °C. Emulsions were prepared by blending protein dispersions with sunflower oil at a 90:10 ratio, followed by homogenisation using an Ultra-Turrax fitted with an S10N-10 G dispersing element (IKA Labortechnik, Janke and Kunkel GmbH, Staufen, Germany) at maximum speed setting 5 for 2 min. Emulsion stability was assessed with an analytical centrifuge (LUMiSizer, LUM GmbH, Berlin, Germany) under conditions of 100 rcf at 15 °C. Results are presented as separation rate (%/min) and transmission profiles across the full measurement range. During the centrifugation, the entire sample cell was illuminated with near-infrared light to measure the intensity of transmitted light as a function of time and position over the sample length. The evolution of the transmission profiles is visualized using a time-dependent colour gradient, where red curves correspond to initial measurements and green curves represent the final measurement. Lumisizer software measures the separation rate (%/min) using the following equation:

$$\textit{Separation rate}\ (\%/\textit{min}) = \left(\frac{\Delta T_{\%}}{\Delta t}\right) \tag{4}$$

Where ΔT % is the percentage of transmitted light (either at a chosen position or integrated/averaged over the cell), and Δt is the corresponding time interval during your centrifugal protocol.

2.4.9. Minimum gelling concentration

The minimum gelling concentration was assessed following the method described by Vogelsang-O'Dwyer et al. (2020), with slight adjustments. Protein dispersions (6–23 % w/w) were prepared in 15 mL of distilled water. The pH of each sample was adjusted to 7 using HCl or NaOH at varying concentrations (0.01 M to 2 M), followed by static overnight hydration at 4 °C. Samples were then heated to 90 °C in a water bath for 30 min, rapidly cooled on ice for 10 min, and stored at 4 °C overnight. After inversion, the minimum protein concentration at which the sample remained stable (no flow for ≥30 s) was recorded as the minimum gelling concentration.

2.5. Statistical analysis

All analyses were conducted in triplicate. For data exhibiting normal distribution, one-way analysis of variance (ANOVA) with Tukey's post hoc test ($p \leq 0.05$) was performed using IBM SPSS Statistics (version 28.0.1.1, IBM Corporation, Armonk, NY, USA). When the assumption of equal variances was not met, Welch's test with Games-Howell post hoc analysis ($p < 0.05$) was applied. If the data were not normally distributed, the Kruskal-Wallis test ($p < 0.05$) was used. Regression and Pearson correlation analyses to assess relationships between the functional properties of the quinoa ingredients were carried out in Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1. Compositional analysis

The compositional data of the quinoa flour (QF), quinoa protein concentrate (QPC) and side-stream (SS) are presented in Table 1.

The protein content of the QF used in the production of the QPC and subsequent SS was found to be 15.28 ± 0.92 *g*·100 g^{-1} while the protein content in the SS was found to be slightly lower at 14.54 ± 0.80 *g*·100 g^{-1}. A protein content of 39.40 ± 0.20 *g*·100 g^{-1} in QPC was obtained during the concentration of protein from the QF. Nitrogen conversion factor of 6.25 is commonly used in literature and therefore was also applied in this study (Manzanilla-Valdez et al., 2024; Mu et al., 2023; Tavano et al., 2022).

The fat content in the QF and SS samples were similar, while the QPC was found to have a higher fat content (9.20 ± 1.1 *g*·100 g^{-1}). The fat composition of the quinoa samples is presented in Table 1. The fat was composed largely of polyunsaturated fatty acids, comprising 3.46 ± 0.33, 5.38 ± 0.46 and 3.94 ± 0.04 *g*·100 g^{-1} of the QF, QPC and SS samples, respectively. All samples contained lower levels of saturated fatty acids were than polyunsaturated fatty acids. QF and SS had similar levels of saturated fats with 0.76 ± 0.08 and 0.92 ± 0.1 *g*·100 g^{-1} respectively. The QPC had higher levels with 1.28 ± 0.12 *g*·100 g^{-1}. QPC had the highest concentration of both polyunsaturated and saturated fatty acids when compared to the other two samples, which aligns with higher total fat content of QPC.

The carbohydrate content varied between the samples. The SS sample had the highest carbohydrate content at 64.31 ± 2.85 *g*·100 g^{-1}. The QF had a carbohydrate content of 60.19 ± 1.63 *g*·100 g^{-1}, and the QPC had the lowest with 37.65 ± 2.22 *g*·100 g^{-1}. The fibre content analysis is represented in Table 1. SS, 10.80 ± 2.60 *g*·100 g^{-1}, had the highest total dietary fibre while the values of QPC and QF ranged from 4.94 - 5.69 *g*·100 g^{-1}. Most of the fibre present in QF, QPC and SS samples was high molecular weight dietary fibre (HMWDF; sum of insoluble dietary fibre (IDF) and high-molecular-weight soluble dietary fibre (SDFP), comprising 77.33 %, 85.02 %, and 92.60 % respectively. Low-molecular-weight soluble dietary fibre (SDFS) was present in low concentrations in the QF (1.29 ± 0.31 *g*·100 g^{-1}) and QPC (0.74 ± 0.18 *g*·100 g^{-1}) and was not detected in the SS. The sugar contents analysed are represented in Table 1. Sugar contents of QPC 12.56 ± 1.61 *g*·100 g^{-1}) was found to be higher than those of the QF (3.12 *g*·100 g^{-1}), and the SS (3.57 ± 0.58 *g*·100 g^{-1}). Glucose was found in the highest concentrations in the QPC and SS (10.7 ± 1.60 – 3 ± 0.58 *g*·100 g^{-1}), while sucrose was the most abundant in the QF sample (1.79 ± 0.28 *g*·100 g^{-1}). The starch composition of QF, QPC, and SS displayed distinct profiles (Table 1). QF and SS exhibited comparable total starch contents with no significant difference found, with values of 56.75 ± 0.66 % and 59.81 ± 2.57 % determined, respectively. In contrast, QPC showed a significantly lower total starch content (34.97 ± 1.07 %). The digestible starch values closely mirrored the total starch values across all samples (QF: 56.56 ± 0.64 %, SS: 59.79 ± 2.57 %, and QPC: 34.22 ± 1.12 %), indicating minimal amounts of resistant starch in the ingredients (0.55 – 0.75 %).

The ash contents ranged from 1.16 *g*·100 g^{-1} (QF) and 2.65 *g*·100 g^{-1} (QPC). The mineral composition of the quinoa samples is presented in Table 2. Potassium was the dominant mineral in quinoa flour (QF; 9000 ± 1700 mg/kg) but decreased in the protein concentrate (QPC; 3360 ± 630 mg/kg), where sodium (7100 ± 1300 mg/kg) and chlorides (8220 ± 630 mg/kg) became predominant. QPC also showed higher levels of calcium, iron, and copper compared to QF, indicating that the concentration process selectively enriched certain minerals while depleting others such as potassium, magnesium, and phosphorus. In contrast, SS

Table 1
Compositional analysis of quinoa flour (QF), quinoa protein concentrate (QPC) and quinoa side-stream (SS).

[g·100 g^{-1}]	QF	QPC	SS
Moisture	11.44 ± 0.82	6.16 ± 0.30	2.05 ± 0.30
Protein (N x 6.25)	15.28 ± 0.92	39.40 ± 1.60	14.54 ± 0.80
Fat	6.24 ± 0.38	9.20 ± 1.10	6.77 ± 0.79
Saturated Fatty acids	0.76 ± 0.08	1.28 ± 0.12	0.92 ± 0.1
Monounsaturated Fatty acids	1.34 ± 0.15	1.97 ± 0.45	1.51 ± 0.15
Polyunsaturated Fatty Acids	3.46 ± 0.33	5.38 ± 0.46	3.94 ± 0.04
Total Carbohydrate	60.19 ± 1.63	37.65 ± 2.22	64.31 ± 2.85
Total sugars	3.12 ± 0.34	12.56 ± 1.61	3.57 ± 0.58
Glucose	1.22 ± 0.19	10.7 ± 1.60	3 ± 0.58
Fructose	0.03 ± 0.01	0.26 ± 0.05	< LoQ
Lactose	< LoQ	< LoQ	< LoQ
Sucrose	1.79 ± 0.28	0.94 ± 0.18	0.27 ± 0.05
Maltose	0.08 ± 0.01	0.66 ± 0.05	0.3 ± 0.02
Total dietary fibre	5.69 ± 1.14	4.94 ± 1.02	10.80 ± 2.60
High Molecular Weight Dietary Fibre HMWDF (IDF + SDFP)	4.40 ± 1.10	4.20 ± 1.0	10.80 ± 2.60
SOLUBLE DIETARY Fibre (SDFS)	1.29 ± 0.31	0.74 ± 0.18	< LoQ
Total Starch	64.08 ± 0.74[a]	37.26 ± 1.21[b]	61.07 ± 2.90[a]
Digestible Starch	63.87 ± 0.72[a]	36.47 ± 1.19[b]	61.04 ± 2.63[a]
Resistant Starch	0.67 ± 0.09[a]	0.79 ± 0.06[b]	0.56 ± 0.02[a]
Ash	1.16 ± 0.08	2.65 ± 0.18	1.53 ± 0.11

Values are presented as the mean ± standard deviation, on a fresh weight basis. LoQ indicates that levels were below of the level of quantification., IDF = insoluble dietary fibre, SDFP = soluble dietary fibre that precipitates in alcohol, SDFS = soluble dietary fibre soluble in alcohol. * Data reported as mean values provided by an accredited external laboratory (Chelab S.r.l., Resana, Italy); replicate-level data were not available; therefore, no statistical comparisons were performed.

Table 2
Mineral composition of quinoa flour (QF), quinoa protein concentrate (QPC), quinoa side-stream (SS).

[mg/kg]	QF	QPC	SS
Calcium	519.00 ± 74	900.00 ± 130	1620.00 ± 220
Iron	50.90 ± 10	101.00 ± 20	59.00 ± 12
Phosphorous	3360.00 ± 620	1590.00 ± 290	1700.00 ± 310
Magnesium	1480.00 ± 280	660.00 ± 130	1430.00 ± 270
Manganese	14.70 ± 3.3	2.71 ± 0.61	22.50 ± 5
Potassium	9000.00 ± 1700	3360.00 ± 630	2390.00 ± 450
Copper	6.50 ± 1.4	17.10 ± 3.6	4.17 ± 0.88
Zinc	28.00 ± 5.4	24.70 ± 4.8	33.60 ± 6.5
Sodium	< LoQ	7100.00 ± 1300	1210.00 ± 220
Chlorides	2610.00 ± 200	8220.00 ± 63	618.00 ± 47

Data are presented as mean ± standard deviation. on a fresh weight basis. LoQ indicates that levels were below of the level of quantification. * Data reported as mean values provided by an accredited external laboratory (Chelab S.r.l., Resana, Italy); replicate-level data were not available; therefore, no statistical comparisons were performed.

exhibited a composition more similar to QF, with potassium (2390 ± 450 mg/kg) and magnesium (1430 ± 270 mg/kg) being major contributors. SS contained the highest levels of manganese and zinc, suggesting that the processing caused the retention of these minerals. Overall, the data indicates that mineral distribution in quinoa is strongly influenced by processing, with protein concentration markedly altering the mineral profile.

Moisture content in the QF was the highest at 11.44 ± 0.82 *g*·100 g^{-1}, whereas the moisture content in the SS sample was the lowest at 2.05 ± 0.30 *g*·100 g^{-1}.

3.2. *Protein characteristics*

Leucine and lysine were found to be the most abundantly present essential amino acids in all the three samples analysed. Of all amino acids, glutamic acid was present in the highest concentrations across all the samples. Lysine and cysteine levels decreased from QF to QPC, while dramatic increases in arginine, leucine and phenylalanine from QF to QPC were observed. Fig. 2B displays the percentage of the recommended daily essential amino acid content per gram of protein for older children (>3 years old), adolescents and adults outlined by the FAO (2013 for the ingredients. In the three ingredients, most amino acids exceeded the required levels, although a few fell short of the requirements (Fig. 2B). In QF leucine and valine were limiting and did not reach the FAO requirements, reaching 90.8 % and 93.83 % of the requirement, respectively. QPC exceeded the requirements in all the essential amino acids with the exception of Lysine (90.42 %). In SS, no limiting amino acids were observed and all essential amino acid requirements were exceeded.

SDS-PAGE was used to analyse the protein profile of the samples

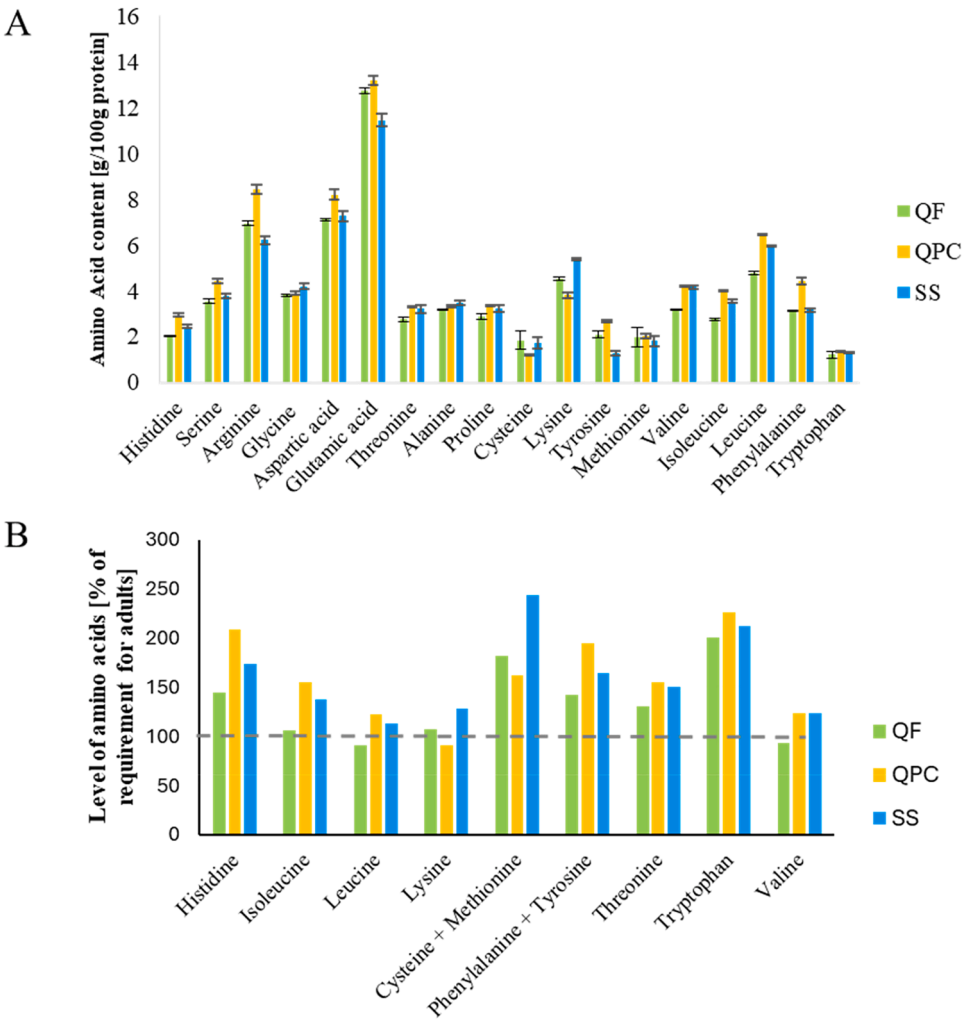

Fig. 2. (A) Amino acid composition of quinoa flour (QF), quinoa protein concentrate (QPC), quinoa side-stream (SS). Data are expressed as mean ± standard deviation. (B) Levels of essential amino acids in quinoa flour (QF), quinoa protein concentrate (QPC) and quinoa side-stream (SS) as a percentage of the FAO (2013) adult requirement for each amino acid.

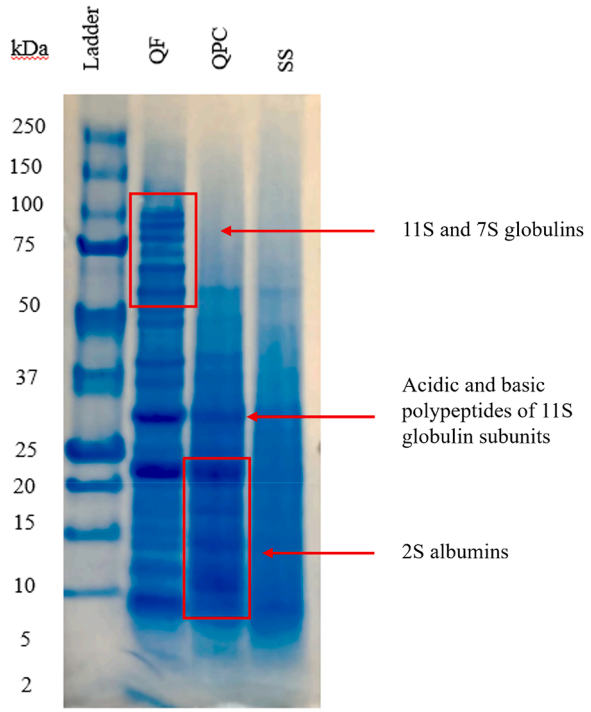

Fig. 3. SDS-Page of protein profiles of different quinoa ingredients: Lane 1 – Ladder, Lane 2 – QF, Lane 3 – QPC, Lane 4 – SS.

(Fig. 3). QF (Lane 2) exhibited a complex protein profile with multiple bands distributed across a broad molecular weight range. Two major band clusters were observed among the ingredients, corresponding to the QF and QPC samples, as highlighted by the red boxes in the figure. The high-molecular-weight region (75–100 kDa) showed several intense bands in QF representing 11S globulins, highlighted by the red box on Lane 2. Prominent bands were also observed at approximately 50–60 kDa, corresponding to subunits of 11S globulin or 7S globulins (Fan et al., 2023; Tavano et al., 2022). For QPC (Lane 3), multiple distinct bands were observed within the low-molecular-weight region (10–25 kDa), representing smaller 2S albumins, highlighted by the red box on Lane 3, (de Carvalho Oliveira et al., 2024a). Additionally, distinct bands at 30–36 kDa in QF and QPC were identified representing the acidic and basic polypeptides of 11S globulin subunits (de Carvalho Oliveira et al., 2024a). The SS (Lane 4) displayed a notably different protein distribution pattern compared to both QF and protein QPC. While still containing proteins across various molecular weight ranges, the SS showed reduced intensity of the major storage protein bands (50–60 kDa) but retained considerable amounts of low-molecular-weight proteins (<25 kDa). These likely represent 2S albumins.

3.3. Physical and techno-functional characteristics

3.3.1. Colour and ultrastructure (SEM)

Ingredient colour (L*a*b*) values are displayed in Table 3. QF had the highest L value (82.08 ± 0.17), indicating the greatest lightness among the samples. However, SS exhibited a comparable lightness, as its L value was not significantly different from that of QF. In contrast, QPC was the least light of the samples (75.97 ± 0.44). The a* value represents the degree of red to green of the samples. All the samples had similar a* values, with measurements of 1.43 ± 0.07, 1.00 ± 0.05, and 1.50 ± 0.05 determined for the QF, QPC and SS respectively. The b* values indicate the degree of yellow to blue, with results showing that all samples had a low degree of yellowness (10.55 ± 0.12 – 14.65 ± 0.40).

The images from the scanning electron microscope are displayed in Fig. 4. In QF, small polygonal shaped starch granules can be observed in large aggregates banded together by proteins. In QPC, the small starch granules were no longer aggregated to the same degree as in the flour and a higher concentration of relatively smooth spherical particles, likely representing aggregated proteins, are seen. SS displayed a matrix, likely consisting of protein and fibre, covered by numerous polygonal starch granules.

Table 3
Techno-functional Properties of quinoa flour (QF), quinoa protein concentrate (QPC), quinoa side-stream (SS).

	QF	QPC	SS
Protein Solubility (%)			
pH 9	85.88 ± 1.64^{a}	33.5 ± 2.09^{b}	19.04 ± 3.67^{c}
pH 7	87.91 ± 2.16^{a}	26.92 ± 0.83^{b}	18.34 ± 6.17^{b}
pH 5	44.85 ± 0.88^{a}	6.24 ± 0.35^{c}	15.11 ± 2.75^{b}
pH 3	32.96 ± 2.45^{a}	21.99 ± 1.26^{b}	17.12 ± 4.54^{b}
WHC (%)	67.93 ± 0.92^{c}	148.76 ± 0.59^{b}	161.55 ± 1.17^{a}
OHC (%)	93.23 ± 5.60^{a}	63.17 ± 2.99^{b}	16.27 ± 2.66^{c}
Separation Rate (%/min)	0.28 ± 0.07^{a,b}	0.18 ± 0.01^{b}	0.41 ± 0.08^{a}
Foaming Capacity (%)	10.00 ± 2.00^{b}	16.67 ± 2.31^{a}	5.43 ± 1.10^{b}
Foam Stability (%)	87.78 ± 10.72^{a}	73.02 ± 11.00^{a}	23.89 ± 16.36^{b}
Minimum gelling concentration (%)	18.00	12.00	23.00
pH	6.49 ± 0.02^{c}	6.94 ± 0.01^{b}	8.21 ± 0.01^{a}
TTA (ml NaOH/10 *g*)	9.56 ± 0.09^{b}	11.73 ± 0.22^{a}	0.765 ± 0.06^{c}
Colour			
L*	82.08 ± 0.17^{a}	75.97 ± 0.44^{b}	81.34 ± 0.41^{a}
a*	1.43 ± 0.07^{a}	1 ± 0.05^{b}	1.5 ± 0.05^{a}
b*	14.65 ± 0.4^{a}	12.01 ± 0.07^{b}	10.55 ± 0.12^{c}
Chroma (C*)	14.72 ± 0.40	12.05 ± 0.07	10.65 ± 0.11
Hue angle (h*)	84.44 ± 0.14	85.24 ± 0.24	81.91 ± 0.23
Particle Size (μm)			
Dv (10)	25.9 ± 0.15	4.45 ± 0.01	45.1 ± 1.04
Dv (50)	198 ± 0.58	11.7 ± 0.15	232 ± 1.41
Dv (90)	436 ± 0.42	38.17 ± 3.17	454 ± 0.99
D [4,3]	216 ± 0.43	20 ± 4.40	245 ± 1.00

Data are presented as mean ± standard deviation. Values within the same row which share the same uppercase letter do not differ significantly.

3.3.2. Particle size

The particle size data measured using a Mastersizer are reported in Table 3. Particle size distribution, as determined by laser diffraction (Mastersizer), was expressed in terms of the volume-weighted mean diameter D [4,3] and percentile diameters (Dv (10), Dv (50), Dv (90)). The D [4,3], which reflects the average particle diameter weighted by volume, was greatest in SS (245 μm), followed by QF (216 ± 0.43 μm), whereas QPC exhibited a substantially smaller mean diameter (20 ± 4.40 μm). The Dv (90), representing the particle diameter below which 90 % of the distribution lies, showed a similar trend with SS (454 ± 0.99 μm) and QF (436 ± 0.42 μm) both exhibiting coarse particle sizes compared to QPC (38.17 ± 3.17 μm). The median diameter, Dv (50), which corresponds to the particle size at the 50th percentile of the distribution, was also highest in SS (232 ± 1.41 μm), intermediate in QF (198 ± 0.58 μm), and lowest in QPC (11.7 ± 0.15 μm). Finally, the Dv (10), denoting the diameter below which 10 % of the particles are found, further emphasised the same pattern, with SS exhibiting the highest fine-particle threshold (45.1 ± 1.04 μm), followed by QF (25.9 ± 0.15 μm), and QPC presenting the smallest value (4.45 ± 0.01 μm).

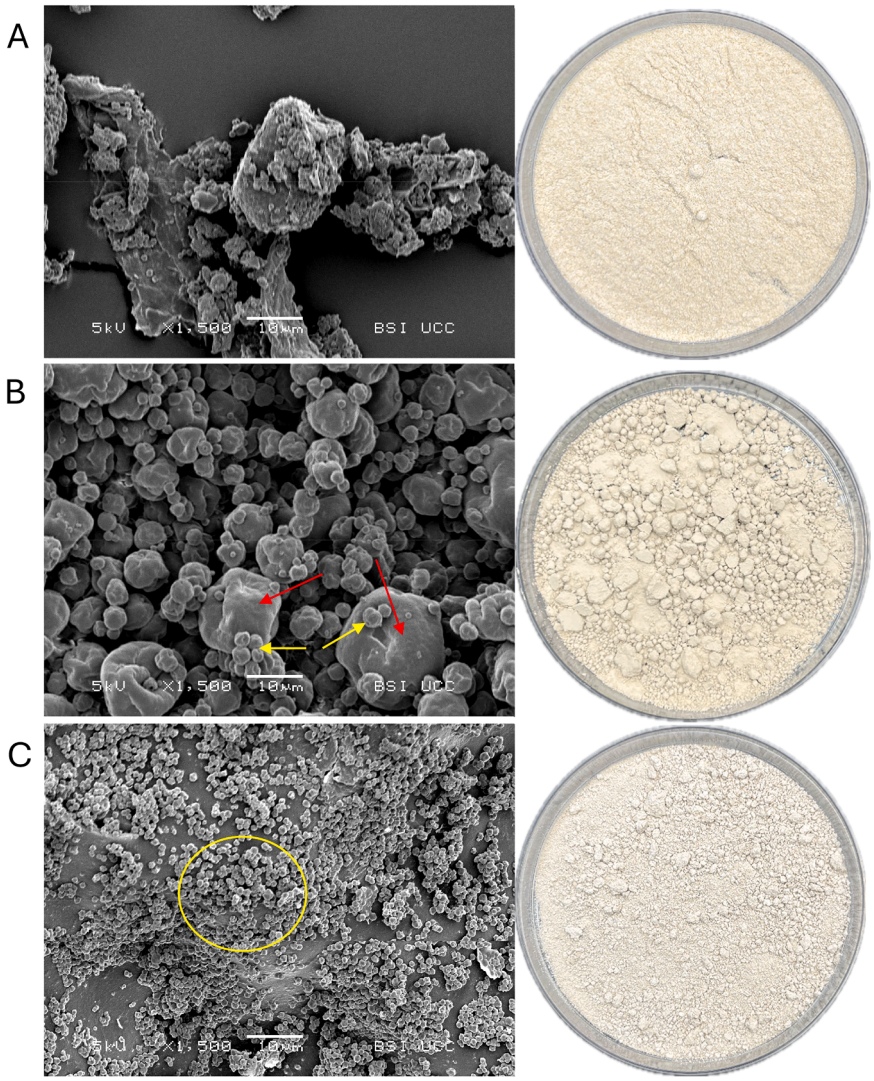

Fig. 4. Images of (A) quinoa flour (QF); (B) quinoa protein concentrate (QPC); (C) quinoa side-stream (SS). Left side pictures are SEM micrographs at 1500 x magnification. Yellow circle and arrows indicate starch granules and red arrows indicate proteins. Right side pictures show the appearance of the ingredient.

3.3.3. *Total titratable acidity and pH*

The pH and total titratable acidity (TTA) are presented in Table 3. Among the samples analysed, SS exhibited an alkaline pH (8.21 ± 0.01), QPC was nearly neutral (6.94 ± 0.01), and QF showed the lowest value (6.49 ± 0.02) QPC had the highest total titratable acidity (TTA), 11.73 ± 0.22 TTA/10 g. The SS sample had the lowest TTA, 0.765 ± 0.06 TTA/10 g. QF had a TTA with 9.56 ± 0.09 TTA/10 g.

3.3.4. *Protein solubility*

The protein solubility results are presented in Table 3. The protein solubility of QF, QPC, and SS varied significantly across pH conditions (3, 5, 7, and 9). QF exhibited the highest solubility of the ingredients, with lower at acidic pH values (3 and 5), but significantly increased solubility at neutral and alkaline pH, ranging from 32.96 ± 2.45 % (pH 3) to 87.91 ± 2.16 % (pH 7) and 85.88 ± 1.64 % (pH 9). QPC showed markedly lower solubility, particularly at pH 5 (6.24 ± 0.35 %), while its highest solubility level was observed at pH 9 (33.50 ± 2.09 %). SS displayed consistently low solubility across all pH levels, ranging from 15.11 ± 2.75 % (pH 5) to 19.04 ± 3.67 % (pH 9).

3.3.5. *Foaming capacity and stability*

Foaming capacity and stability are presented in Table 3, with images provided in supplementary Figure S1. All ingredients showed low foaming capacities, ranging from 5.43 % (SS) to 16.67 % (QPC). The highest foaming stability was determined for QF (87.78 %), while foam stabilities of 73 % and 23 % were measured for QPC and SS, respectively.

3.3.6. *Water and oil-holding capacity*

Water holding and oil holding capacity are presented in Table 3. Soluble components in the supernatant were not considered in the method. The reported WHC values represent apparent water holding capacity and may be affected by the different protein solubilities of the ingredients; however, they remain suitable for comparative assessment of functional water retention under standardised conditions. SS had the highest water-holding capacity (161.55 ± 1.17 %), followed by QPC (148.76 ± 0.59 %). QF had a significantly lower water-holding capacity (67.93 ± 0.92 %). compared to the two other samples.

In contrast, an inverse trend was observed for the OHC of the ingredients. QF was found to have the highest oil-holding capacity at 93.23 ± 5.60 %, while the. OHC of QPC and SS were determined to be 63.17 ± 2.99 % and 16.27 ± 2.66 %, respectively.

3.3.7. *Emulsion stability*

The emulsion stability is presented in Table 3 as separation rate stated in %/min. QPC had the lowest separation rate with 0.18 ± 0.01 %/min and was followed by QF (0.28 ± 0.07 %/min) and SS (0.41

%/min). Images of emulsions and transmission profiles can be found in Supplementary Figure S2.

3.3.8. Minimum gelling concentration

The minimum gelling concentration is presented in Table 3 and images of the gels can be found in Supplementary Figure S3. QPC had the lowest minimum gelling concentration (12 %), while QF (18 %) and SS (23 %) showed higher values.

4. Discussion

Limited research has been conducted on the production and characterisation of quinoa protein concentrates and their by-products. As a result, the aim of this study was the characterisation of the nutritional and techno-functional properties a QPC in comparison to the initial raw material (QF) and a side-stream produced as a process by-product (SS).

During the production of the QPC, proteins in the QF were isolated using a wet fractionation process which included an α-amylase enzymatic treatment during protein extraction before isoelectric precipitation and spray drying. The SS product was produced as a side-stream of this wet fractionation process and was freeze dried. Therefore, it is expected that the QPC had a higher protein content and lower carbohydrate content compared to both the QF and SS. Along with the increased protein content the QPC had a higher fat, ash and sugar content, indicating that these components were also concentrated by the wet fractionation processing. It is common for non-protein impurities to remain in the protein fraction following wet fractionation (Barozzi et al., 2025; Kumar et al., 2021). In the present study, although acidification and protein precipitation would be expected to remove most of the fat and sugars, complete separation was not achieved. The exact reason for this is currently unknown; however, residual fat and sugars may have remained due to co-precipitation with proteins during acidification and became concentrated in the QPC as other components such as starch and fibre were removed. The higher sugar content in the QPC can also be attributed to the enzymatic step induced during processing which was used to break down the starch granules into sugars (Edwards et al., 2021). The carbohydrate content was expectedly highest in the SS as it was a by-product of the QPC production, where the carbohydrates were removed in order to increase the protein content.

Scanning electron microscopy (SEM) images of quinoa flour and two fractions reveal that starch is present as very small granules, typically ranging from 1 to 3 μm in diameter with polygonal and irregular shape (Junejo et al., 2022). Due to their small size, these granules are difficult to separate from surrounding protein, which made enzymatic treatment necessary. The SEM micrographs further show that starch granules tend to aggregate with protein molecules, forming large agglomerates within the matrix. This structural association complicates protein isolation and reflects the intricate organisation of quinoa's macromolecular components. The starch in quinoa consists primarily of amylopectin (77.5 %–96.5 %), while amylose is present in lower amounts (3.5 %–22.5 %) (James, 2009; Valdez-Arana et al., 2020). In QPC, smooth particles can be seen and likely represent agglomeration of proteins as a result of the protein concentration process and the particle shape is associated to spray-drying. Quinoa starch granules have a distinct shape compared to other starches from different sources. Wheat starches has been described as disk shaped or spherical (Zhang et al., 2023), while quinoa starches can be described as polygonal and angular in shape making quinoa starch easily distinguishable.

SDS-PAGE protein profiling highlighted prominent bands for QF at approximately 50–60 kDa, corresponding to subunits of 11S globulin or 7S globulins (Fan et al., 2023; Tavano et al., 2022). Additionally in QF and QPC, distinct bands at 30–36 kDa and 10–20 kDa were identified, representing the acidic and basic polypeptides of 11S globulin subunits, respectively (de Carvalho Oliveira et al., 2024a). These findings are consistent with previous reports indicating that 11S globulins constitute approximately 35 % of total quinoa seed proteins (Dakhili et al., 2019). The increased intensity of lower molecular weight bands in the QPC compared to the QF displays the effect of the protein concentration process on cleaving larger globulins into lower molecular weight subunits while also displaying that the albumin protein fraction was maintained and concentrated in the protein concentration process. At low molecular weights, particularly in SS, clarity between bands was reduced likely due to mineral interactions causing some aggregation and interference with protein denaturation and this is supported by the higher calcium level in SS (1620 ± 220 mg/kg) compared to QF (519 ± 74 mg/kg) and QPC (900 ± 130 mg/kg). Aggregation of proteins is also represented by the spherical particles in the SEM images of QPC. The lower solubility of the SS may also have played a role in the reduced band clarity observed; lower protein in the SDS-PAGE extracts would reduce clarity of bands making the bands appear faint and less defined on the gel. The SS (Lane 4) displayed a notably different protein distribution pattern compared to both QF and QPC displaying the effects of the production process on the protein profile. While still containing proteins across various molecular weight ranges, the SS showed reduced intensity of the major storage protein bands (50–60 kDa) but retained considerable amounts of low-molecular-weight proteins (<25 kDa). These likely represent 2S albumins, which have been reported to constitute approximately 37 % of total quinoa seed proteins (Dakhili et al., 2019). Quinoa has been shown to be rich in essential amino acids which is supported by literature, (James, 2009; Escuredo et al., 2014), and all three samples were found to exceed the majority of FAO essential amino acid daily requirement reference intake for older children and adults > 3 years old. The requirements for Lysine, a common limiting amino acid in cereal grains (Poutanen et al., 2022), were exceeded in both QF and SS and 90 % of the requirements were reached in QPC indicating that the quinoa ingredients are unique among cereal grains and can potentially be utilised to increase lysine levels in food products. Leucine and Lysine were found to be the most abundant essential amino acids present in all the three samples analysed. Glutamic acid was present in the highest concentrations across all the samples. The amino acids analysis of the samples showed that hydrophilic amino acids were present in highest concentrations, with glutamic acid, arginine, aspartic acid and lysine all present in high concentrations. Glutamic acid is also present in high concentrations in wheat, barley and oat (Alemayehu et al., 2023; Jaeger et al., 2021; Siddiqi et al., 2020). Some trends in amino acid profile between the ingredients was observed, lysine and cysteine levels decreased from QF to QPC, while dramatic increases in arginine, leucine and phenylalanine from QF to QPC were observed. The reduction in lysine and cysteine during processing from QF to QPC may be due to protein denaturation at alkaline pH. This causes exposure of hydrophobic and sulfhydryl groups, leading to new protein-protein interactions (Cui et al., 2023). As a result, lower levels of lysine and cysteine are observed in QPC. This is supported by the higher levels of lysine and cysteine found in SS compared to QPC.

The protein content was shown to play a key role in influencing some of the techno-functional properties of the samples, with a Pearson correlation analysis highlighting the link between minimum gelling concentration, emulsion stability, foaming capacity and particle size and protein content. Minimum gelling concentration was lowest in the QPC (12 %), similar values to other studies where a gelling concentration for a quinoa protein isolate was found to be between 10–15 % (Kaspchak et al., 2017). It was also shown that minimum gelling concentration had a negative correlation to protein content across the three samples (R-value (r) = 0.89; $p < 0.3$). Despite the p-value being above the statistical threshold, the R-value is high which indicates a strong relationship. This showed that higher protein content negatively correlated with a lower minimum gelling concentration, i.e. the higher the protein level, the less ingredient required for gel formation. This indicates that the gelling in the quinoa ingredients can be largely attributed to the formation of a protein network within the food system as a result of heat induced denaturation of proteins leading to aggregation and cross-linking. The 11S globulin proteins and its subunits which were

present in high concentrations, shown in the SDS-PAGE, are the primary protein responsible for gel network formations in quinoa ingredients (Van de Vondel et al., 2022). Gelling in food systems is not only influenced by protein. The higher ash content in the QPC likely also contributed to the lower gelling concentration due to high concentrations of minerals such as calcium (900 ± 130 mg/kg) and sodium (7100 ± 1300 mg/kg) promoting cross-linking of proteins, thereby strengthening the gel network (Cui et al., 2023; Kaspchak et al., 2017). Components such as fat and carbohydrates, in particular fibre, have been shown to inhibit protein network formation and would therefore increase the minimum gelling concentration. Fat can coat proteins and consequently interfere with protein-protein interactions reducing aggregation of proteins, therefore increasing the minimum gelling concentration (Sanders et al., 2024). While the fat content in the QPC was the highest of the three samples, because of the high protein concentration in the QPC, this negative effect of gelling inhibition was likely reduced. Carbohydrates compete with proteins for water, inhibiting protein hydration required for protein gel network formations to occur (Lyu et al., 2022). Carbohydrate content showed a strong positive correlation with minimum gelling concentration ($r = 0.98$; $p < 0.13$), supporting that samples with higher carbohydrate content also displayed a higher minimum gelling concentration. The QF had a slightly lower minimum gelling concentration (18 %) compared to the SS (23 %) which is likely due to the lower carbohydrate level, and in particular, the lower dietary fibre content. The fibre likely inhibited gelling but to a lower degree as seen in SS. Minimum gelling concentration is an important property in baked foods, meat and dairy analogues and functional foods. A low gelling concentration in ingredients for baked foods contributes to improved texture by acting as thickening and binding agents which improves structure of products, particularly in gluten-free applications where gluten networks are replaced (Yuan et al., 2021). In meat and dairy analogues plant protein gels can serve as a substitute for fat to improve texture (Guo et al., 2025).

Similarly to the minimum gelling concentration, a negative correlation between protein content and separation rate, ($r = 0.83$; $p < 0.38$), and a positive corelation between carbohydrate content and separation rate ($r = 0.95$; $p < 0.21$) were identified after emulsification. The QPC displayed the lowest separation indicating higher protein content may lead to increased emulsion stability which has been reported by Ma et al. (2023) and Olsmats and Rennie (2024), although lower protein concentrations were used in these studies. Higher levels of both hydrophilic and hydrophobic amino acids in the QPC can explain the improved emulsification properties by allowing emulsification between the lipid and water phase (Zhang et al., 2022). Emulsification properties have been shown to play an important role in baking applications by improving the specific volume of pound cake and the low separation rate of the QPC (0.18 ± 0.01 %/min) is very similar to that of whole egg powder (0.17 ± 0.03 %/min) indicating the potential future use of QPC as a emulsifying agent (Halm et al., 2025). The separation profiles of the samples varied, QPC showed a very uniform separation profile with uniform light transmission % across the sample, displaying that the light was passing the sample at a uniform level throughout with no spikes in light transmission. The SS and QF had a higher transmission % across the midsection, indicating a higher degree of separation of particles within the emulsion, due to the lower protein content and higher levels of fibres and starches present. It is important to note that the analysis was carried out by weighing out the samples based on protein content which meant that more sample was required for the QF and SS which would result in a less uniform phase separation profile due to a higher level of sample used for analysis. Particle size can also influence the stability of emulsions, smaller particle size increases contact surface area and therefore increases the stability of the emulsion (de Paiva Gouvêa et al., 2023). However, as particle size was only measured for dry ingredients, and not the emulsions, it is unclear as to whether particle size did impact emulsion stability in this study. Protein solubility is expected to directly influence emulsification properties (Tang et al., 2020), however in this study no correlation between the two was observed with Etzbach et al., 2024, also finding no correlation.

The protein solubility analysis reveals distinctive pH-dependent patterns among the quinoa samples. QF was over two-fold more soluble at pH 7–9 than pH 3–5. This is consistent with the isoelectric point of quinoa 11S globulin (pH 4.5–5.0), whereby the ingredient is at its least soluble state (Elsohaimy et al., 2015). Notably, despite containing substantially higher protein content (32.01 %), the QPC exhibited significantly lower solubility than QF across all pH values, which can be attributed to structural modifications during the wet-fractionation and spray-drying processes leading to protein denaturation, higher lipid content and the influence of starch-protein interactions (de Carvalho Oliveira et al., 2024). The concentration of protein during the production of QPC from QF may have caused protein aggregation by increasing protein-protein interactions and reducing the carbohydrate content which would inhibit such interactions while the intact soluble albumin proteins present in the QF in combination with the higher starch content allowed for higher solubility. In a study by Vogelsang-O'Dwyer et al. (2020), similar findings were reported, a protein rich faba bean flour produced by dry-fractionation also had a higher protein solubility compared to a faba bean protein isolate which had undergone wet-fractionation and spray-drying processes. This was attributed to denaturation during the processing of the faba bean protein isolate. Compared to an oat protein isolate analysed in a study by Li and Xiong. (2021), the oat protein isolate was reported to have a solubility of 41.5 % at pH 7, higher than what was found in the QPC in this study. The higher starch content of QF and SS compared to the QPC suggests that starch-protein interactions play a crucial role in maintaining protein solubility, as highly soluble proteins are more likely to interact with starch due to its hydrophilic nature, potentially preventing excessive protein aggregation even at unfavourable pH conditions by disrupting protein-protein interactions (de Carvalho Oliveira et al., 2024). This combined with the intact albumin proteins explains why QF maintained relatively high solubility at pH 5 compared to QPC, despite containing significantly less protein. The SS while having similar starch content to the QF, was negatively impacted by the higher fibre content, particularly because the fibre present was almost exclusively HMWDF which is largely insoluble. During the protein concentration process, albumins are more likely to be isolated with the soluble protein fraction while insoluble globulin proteins likely remained in the insoluble side-stream fraction. This lowers albumin content in the SS, resulting in low protein solubility in SS as a consequence. Additionally, the higher lipid content present in the QPC may increase hydrophobic interactions and promote protein–protein aggregation, thereby decreasing protein solubility (Kurtz et al., 2026).

Foaming capacity, similar to minimum gelling concentration and emulsion stability, is highly influenced by proteins and carbohydrates. Correlation analysis showed that higher protein content had a strong positive correlation to a higher foaming capacity, ($r = 0.92$; $p < 0.27$), and also showed that higher carbohydrate content displayed a negative correlation with foaming capacity, ($r = 0.99$; $p < 0.09$). QPC had the highest foaming capacity indicating that the higher protein concentration allowed for an increased amount of foam production. The amphiphilic nature of proteins allows for the formation and stabilisation of gas cells by adsorbing at the air–water interface, unfolding, and forming viscoelastic films around gas bubbles (Amagliani et al., 2021; Han et al., 2023; Yang et al., 2023). Foaming capacity is an important property in baking applications as formation of protective films around air bubbles is essential in the structure of breads, cakes and other baked goods. For applications such as high protein beverages, low foaming ability may be desired. QF had the highest foaming stability, potentially due to its lower fat content, which can inhibit foam stability by coating proteins resulting in a weakening of protein stabilisation (Salt et al., 2018). Carbohydrates and fat interrupt the formation of the viscoelastic films, which may be why the SS, which was high in carbohydrates, showed the lowest foaming capacity and foam stability.

WHC plays important roles in many food applications such as gluten-free baking, meat analogues and thickening agents. In gluten-free baking, flours will typically require higher hydration levels to achieve correct dough consistency in the absence of gluten therefore highlighting the importance of WHC. In meat analogues, WHC is a key factor in determining texture and juiciness which is critical for consumer acceptance. In thickening agent applications for products such as soups or sauces, WHC is vital for increasing viscosity by water absorption. The amphiphilic nature of proteins bind water and fat but carbohydrates also play a major role for functional properties such as WHC, which is directly increased by presence of fibre (Shen et al., 2024; Zhang et al., 2023). The SS had the highest WHC of the three samples which can be attributed to the high fibre and starch content compared to QF and QPC. The fibre content in the samples consisted of mostly HMWDF, which includes both insoluble and high molecular weight soluble fibres that contain hydrophilic hydroxyl groups (Etale et al., 2023) and has a substantial molecular size that allows it to physically trap and bind large amounts of water within its matrix. Hydroxyl groups found in cellulose and hemicellulose form hydrogen bonds with water thereby enhancing WHC (Elleuch et al., 2011). As a result, HMWDF creates a network that can retain water either as free or bound water, increasing the overall WHC (Różańska et al., 2025). QPC and QF had similar fibre contents, however the QPC displayed a significantly higher WHC, highlighting the effect of a higher protein content on the ability to bind water. Starch is another key contributing element to WHC. Starches such as amylopectin, which is the primary starch present in quinoa, contain short chains (8–12 glucose units and very long branches >36 glucose units), which form hydrogen bonds with water molecules, this in turn increases the WHC (Li and Zhu, 2017; Valdez-Arana et al., 2020). The small starch granule size provides a large surface area promoting rapid water penetration, further aiding in increasing WHC. While the QF had a similar level of starch with no statistical significance, native starch granules have been shown to not absorb water as effectively as damaged or gelatinised starch (Zhang et al., 2024). Some starch damage was likely caused during the production of the SS both mechanically and by the enzymatic treatment and therefore along with the higher fibre content, SS was able to absorb more water further increasing the WHC compared to the QF. Although different methods of analysing WHC are used, when comparing the WHC of QF with other flours, the QF was lower than what has been reported in literature for wheat flour (140–164 %), barley flour (190 %) and oat flour (120 %) (Chandra et al., 2015; Rani et al., 2021 and Jokinen et al., 2022). The OHC results were found to have an inverted trend compared to the results found for WHC, where the QF was found to have the highest oil holding capacity (93.23 ± 5.6 %) but was lower than the OHC of wheat flour and barley flour reported by (Rani et al., 2021). The OHC of QPC was lower and the lowest OHC of the three samples was determined for SS. The QF's greater OHC and lower WHC compared to QPC and SS suggests that QF's components have a hydrophobic nature, and this could be due to its diverse composition that includes fibre, starch, and proteins, its higher structural porosity and the lack of extensive processing that could reduce oil-binding sites. The proteins contain both hydrophilic and hydrophobic regions, explaining why the QPC had moderate WHC and OHC compared to the two other samples. Typically it is expected for a protein concentrate to have a higher OHC than a flour, because of the presence of hydrophobic regions in the protein, however in the analysis the protein content in the QPC was relatively low and a spray drying process was applied which has been shown to decrease OHC (Naik et al., 2022). The higher WHC of the SS indicates its hydrophilic nature, potentially resulting in the poor ability of the sample to retain oil.

The quinoa ingredients examined in this study demonstrate several distinctive nutritional and functional advantages that position them as superior alternatives to conventional gluten-free grains for food applications. From a nutritional standpoint, quinoa provides a complete amino acid profile with all nine essential amino acids, a characteristic that distinguishes it from rice, maize, barley, and oat (Craine and Murphy, 2020). Notably, quinoa contains substantially higher lysine levels than the grains most commonly used in gluten-free formulations, with QPC exceeding FAO requirements for all essential amino acids except for a slight lysine limitation (90.42 % of requirement). The QF and SS exceeded the FAO requirements for lysine and this superior lysine content is particularly significant, as conventional maize contains only approximately 1.99 g lysine·100 g^{-1} protein (Sethi et al., 2020), rice exhibits lysine content ranging from 0.25–0.54 g·100 g^{-1} protein (Huang et al., 2023), barley ranging from 0.98–1.89 g lysine·100 g^{-1} protein and oat from 1.35–3.29 g lysine·100 g^{-1} (Batîr Rusu et al., 2024). In comparison, the robust lysine levels observed in the ingredients in the present study, provide a significant nutritional advantage over these common cereal grains. The balanced amino acid composition, combined with the high concentrations of leucine and glutamic acid across all three quinoa ingredients, directly addresses the protein quality deficiencies commonly observed in gluten-free diets that rely heavily on rice and maize (Lerner et al., 2019; Manzanilla-Valdez et al., 2024). Beyond nutritional composition, the QPC exhibited exceptional techno-functional properties that rival animal-based ingredients, most notably an emulsion separation rate of 0.18 ± 0.01 %/min, which is remarkably similar to values reported for whole egg powder (0.17 ± 0.03 %/min), while also being substantially lower than faba bean protein concentrate (1.55 ± 0.21 %/min) (Halm et al., 2025), and significantly lower than both QF and SS. This exceptional emulsion stability, combined with the lowest minimum gelling concentration (12 %) among the quinoa ingredients, positions QPC as a potential egg replacer in bakery applications where emulsification and structure formation are critical. Furthermore, the high water-holding capacity observed in both QPC (148.76 %) and SS (161.55 %), alongside favourable protein solubility profiles at neutral to alkaline pH ranges, demonstrates functional versatility that exceeds many legume-based protein concentrates. The gluten-free status of quinoa ingredients, coupled with their naturally occurring bioactive compounds, superior amino acid balance, and animal product-comparable functional properties, establishes them as uniquely positioned ingredients for addressing both the nutritional inadequacies and technological challenges inherent in gluten-free food product development.

5. Conclusion

This work provides the first integrated characterisation and comparison of ingredients produced from a quinoa flour. The comprehensive investigation of the composition and techno-functional properties of quinoa flour (QF), protein concentrate (QPC), and side-stream (SS) reveals distinct characteristics that position each ingredient for specific nutritional and functional applications in food systems which supports the development of sustainable food processing.

The QPC demonstrated significantly enhanced protein content with over two-fold higher protein content (39.40 ± 1.60 g·100 g^{-1}) than QF (15.28 ± 0.92 g·100 g^{-1}) and SS (14.54 ± 0.80 g·100 g^{-1}), along with enriched fat (9.20 ± 1.10 compared with 6.24 ± 0.38 g·100 g^{-1} and 6.77 ± 0.79 g·100 g^{-1} for QF and SS, respectively) and mineral content (2.65 ± 0.18 g·100 g^{-1} compared with 1.16 ± 0.08 g·100 g^{-1} and 1.53 ± 0.11 g·100 g^{-1} for QF and SS, respectively), confirming successful protein concentration and co-enrichment of lipids and minerals through processing. All three ingredients exceeded FAO essential amino acid requirements for most amino acids, importantly QF and SS surpassed and QPC reached 90.42% of lysine requirements. This positions quinoa-derived ingredients as rare plant-based sources capable of addressing lysine deficiency, a persistent limitation in cereal-based gluten-free formulations.

QPC also showed a favourable techno-functional profile, combining superior emulsion stability (0.18 ± 0.01 %/min), similar to whole egg powder (0.17 ± 0.03 %/min), foaming capacity (16.67 ± 2.31 %) and the lowest minimum gelling concentration (12 %), making it particularly suitable for protein fortification in gluten-free and plant-based food

applications. In contrast, SS emerged as a promising carbohydrate-rich functional ingredient, characterised by the highest total carbohydrate content (64.31 ± 2.85 g·100 g^{-1}), the greatest total dietary fibre (10.80 ± 2.60 g·100 g^{-1}) and an exceptional water-holding capacity (161.55 ± 1.17%), suggesting applications as a thickening and fibre-enriching agent in systems where texture and water retention are critical. These findings could therefore address critical nutritional challenges in gluten-free and plant-based food formulations by identifying QPC as a viable protein fortifier and SS as a fibre fortifier while simultaneously demonstrating possible valuable functional applications for the QPC as an egg replacer and the carbohydrate-rich SS as a thickening agent.

The complementary properties of QF, QPC and SS support a circular approach to quinoa processing, in which each fraction are valorised for specific functional roles rather than generating low value side-streams. By investigating the compositional and functional relationships between these ingredients, this work advances efforts to valorise agricultural by-products, reduce food waste and enhance the economic viability of quinoa processing while broadening the availability of nutrient-rich, gluten-free, plant-based foods. Future optimisation of extraction processes could further enhance protein concentration levels while preserving functional properties, potentially expanding applications across a range of diverse food matrices.

Future studies should address current limitations by undertaking more in-depth analysis of chemical structures and functional groups in the ingredients and by assessing in vitro or in vivo digestibility to clarify nutritional effects in real food applications. As only one quinoa cultivar was studied, further work should examine similar ingredients produced from different cultivars and evaluate whether processing parameters need to be adjusted accordingly.

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Data availability statement

All data is contained within the article or in the supplementary material provided.

Ethical statement - studies in humans and animals

Animals and humans were not involved in this study.

CRediT authorship contribution statement

Patrik Keyes: Writing – original draft, Methodology, Formal analysis, Data curation. **Laura Nyhan:** Writing – review & editing. **Juliane Halm:** Writing – review & editing. **Ute Weisz:** Writing – review & editing, Methodology. **Stephanie Bader-Mittermaier:** Writing – review & editing, Methodology. **Emanuele Zannini:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Elke K. Arendt:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

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Supplementary materials

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Data availability

Data will be made available on request.

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Further reading

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