Abstract: The pre-treatment of seeds prior to processing is gaining attention as alternative ways of modifying properties of foods. In this work, the extrusion and germination of quinoa seeds were evaluated for their effect on protein profile, alcalase hydrolysis and the antioxidant capacity (AOC) of peptide fractions (>10, 3–10 and <3 kDa). The proteins in extruded (EQ), germinated (GQ) and unprocessed (UQ) seeds were extracted, hydrolysed and fractionated by ultrafiltration. An SDS-PAGE protein profile showed that the pre-treatments partially hydrolysed high-molecular-weight proteins (75–100 kDa) into low-molecular-weight polypeptides, and chenopodin was unaltered. The hydrolysis degree in hydrolysates reached 38.38% for UQ seeds, 31.85% for EQ seeds and 30.09% for GQ seeds. Compared to the UQ hydrolysate, the extrusion and germination significantly improved \((p < 0.05)\) the AOC of the <3 kDa fraction by 61.49% (EQ) and 38.11% (GQ) and the 3–10 kDa fraction by 130.98% (EQ) and 57.71% (GQ). The pre-treatment of seeds before protein extraction and hydrolysis modified the peptide profile with improved antiradical activity after alcalase hydrolysis. This study highlights the use of mild pre-treatments applied to quinoa seeds as a way to modify proteins and obtain hydrolysates with enhanced bioactivity.

Keywords: antioxidant; extrusion; hydrolysate; germination; quinoa; whole seeds

1. Introduction

The interest in environmentally safe processes such as extrusion, germination, enzymatic hydrolysis, etc., has increased because of their reduced negative impact on the physical, chemical and biological properties of foodstuffs [1]. Extrusion denatures proteins, improves enzymatic hydrolysis and modifies amino acid profiles [2]. With germination, storage proteins break down, and the profile of essential and non-essential amino acids changes [3]. Food processing technologies such as extrusion and germination can improve the properties of protein hydrolysates. An improved AOC was reported for the hydrolysates of extruded [4] and germinated seeds [5]. However, Montoya-Rodríguez et al. [6] reported that the AOC diminished after the extrusion of amaranth seeds. Hydrolysate production follows the general sequence of protein extraction, enzymatic hydrolysis, fractionation and testing of bio-functional properties; however, few reports describe the effect of pre-treatments applied to seeds before protein extraction. The worldwide-recognised Andean seed quinoa provides all the essentials and a balanced amino acid profile, making it a unique protein among its counterparts and other cereals [7]. In this work, the extrusion and germination processes were evaluated as pre-treatments of quinoa seeds for their potential effect on the protein profile, the hydrolysis performance, and the AOC of peptides.
2. Materials and Methods

2.1. Quinoa Seeds Origin

Desaponified Quinoa (*Chenopodium quinoa* Wild) seeds (var. Hornillos-Gob Jujuy INTA) were purchased at the National Institute of Agricultural Technologies (INTA-IPAF) (Maimará, Jujuy, Northwest Argentina).

2.2. Quinoa Seeds Processing

The extrusion of seeds was carried out using a twin-screw extruder (INCALFER, DT65, Buenos Aires, Argentina). The extrusion conditions were humidity of 28% (w/w), temperature of three sections extruder barrel at 45, 90, and 125 °C and frequency of 8.7 (screw), 12.1 (feeding), and 17.1 (cutter) Hertz. The final products were aired and stored at 4 °C in sealed polyethylene bags. The germination of disinfected (0.5 kg; sodium hypochlorite 100 ppm; 10 min) and washed seeds (3 times, distilled water) was conducted over tissue paper, and incubation was carried out (30 °C, 24 h, humidity >90%), in the dark (Memmert Radiant Warmer Model A52200-35-Vac 230, Büchenbach, Germany). Germinated seeds (max. 2.5 mm radicle) were dried (~10% moisture content) in an air-drying oven (40 °C).

2.3. Protein Concentrate Preparation

Milled flour from germinated, extruded and unprocessed seeds was defatted (1:5; in petroleum ether), and protein concentrates (PCs) were prepared by alkaline solubilization followed by centrifugation and acid precipitation [8]. Polyphenols were removed with anhydrous ethanol, and the PC was neutralised to a pH of 7 (NaOH) and dried at 30 °C. Total nitrogen was determined via the Kjeldahl method.

2.4. Electrophoretic Profile by SDS-PAGE

The samples of protein concentrates were solubilised (pH 8) and boiled (3 min) in running buffer (2% SDS), glycerol (10%), bromophenol blue (0.01%), Tris-HCl buffer (pH 6.8) and mercaptoethanol (5%), and 5 µL of them were put in Laemmli buffer and stacking (60 V) and running gels (120 V) (Bio-Rad Laboratories, Hercules, CA, USA). Gels were dyed (Coomassie R-250) and decoloured (methanol/acetic acid/water (50/20/30)) [8]. Bands were identified (GelAnalizer 19.1. 2010) with a standard protein (6.5–200 kDa, Sigma-Aldrich, Steinheim, Germany).

2.5. Protein Hydrolysis, Fractionation and Quantification of Peptides

Each protein (1% w/v; 300 mL) was hydrolysed with alcalase (E:S 1:10) at a constant pH of 9, 50 °C and a NaOH concentration of 0.3 M. Enzyme inactivation was at 95 °C for 10 min. The hydrolysis degree (HD%) was calculated via the pH-stat method. The peptide fractions (Amicon® 10 and 3 MWCO centrifugal filters, Millipore, Burlington, MA, USA) were lyophilised, mixed with phosphate buffer (pH 8.2, 3.4 mL, 0.2 M) and TNBS (0.5 mL, 5% v/v), incubated (dark, 50 °C, 60 min, 200 rpm) for peptide quantification at 420 nm and plotted against a standard (L-leucine, mM/mL) [8].

2.6. Antiradical Activity of Peptide Fractions

Radical ABTS [2,2′-azo-bis (3-ethylbenzothiazoline-6-sulfonic acid)] was formed for 12–16 h in the dark (7 mM in water and ammonium persulphate 2.45 mM). Working radical solutions (0.7 absorbance, 734 nm) were blended with peptide fractions (10 µL, 1 mg/mL), and the absorbance was measured after 6 min. Ascorbic acid was plotted as the standard [8].

2.7. Statistics Analysis

Data are expressed as mean ± standard deviation (SD) of three replicates. Means were compared by two-way analysis of variance (ANOVA), and differences were compared
with the Tukey test \( p < 0.05 \). GraphPad Prism® V5.03 software (GraphPad Software Inc., San Diego, CA, USA) was used for graph plotting.

3. Results and Discussion

3.1. Electrophoretic Protein Profile of Extruded, Gerninated and Control Quinoa Proteins

The electrophoretic profile of the protein concentrates of unprocessed (UQ), extruded (EQ), and germinated (GQ) seeds is show in Figure 1. The lanes UQ, EQ and GQ displayed bands of MW (kDa) of 45.70 (A), 29.70–25.10 (B) and 15.93–20.00 (C). Bands B and C are comparable to chenopodin, which is found in quinoa [9]. After processing, the intensity in the 93–79 kDa band (lane UQ) was reduced by 70.11 ± 15\% (EQ) and by 42.74 ± 19\% (GQ). This reduced band intensity may be due to denaturation, proteolysis or insoluble aggregates. Furthermore, the EQ and GQ lanes augmented their intensity by 140.76 ± 12\% (EQ) and 107.21 ± 14\% (GQ) for the <14.2 kDa band, suggesting a higher concentration of polypeptides. Similar results were also described in sprouted quinoa, amaranth [9,10] and extruded amaranth seeds [6]. This evidence demonstrates that extrusion and germination partially hydrolysed high-MW proteins and formed low-MW polypeptides. Each protein concentrate showed different protein patterns and polypeptide profiles.

![Figure 1. Electrophoretic profile (SDS-PAGE) of alkaline soluble quinoa proteins. Unprocessed (UQ), extruded (EQ) and germinated (GQ) seeds protein concentrate. Molecular weight standard (MW std). MW of chenopodin 45.70 (A), 29.70–25.10 acid (B) and 15.93–12.54 basic (C), subunits. Black arrows: high molecular weight proteins (HMW proteins).](image-url)
3.2. Impact of Processing on the Degree of Hydrolysis

Table 1 shows that the HD% increased noticeably (30 min), reaching a significantly higher value for the UQPH (28.45%) compared to the GPH (23.98%) ($p < 0.05$) and the EPH (25.04%). At the end of the hydrolysis (150 min), the HD% was 35.38 (UQPH), 31.85 (EPH) and 30.09% (GPH). The differences in the HD% could be attributed to the presence of more proteins susceptible to hydrolysis in the UQPH than in the EPH and the GPH, as shown in the electrophoretic run lane UQ (Figure 1). The low HD% in EPH could also be attributed to less soluble proteins or aggregates being formed during extrusion. Similar conclusions were reported by Montoya-Rodríguez et al. [6] for extruded amaranth seeds. However, an increase in the HD% was reported for pea (*Pisum sativum*) extruded protein [4]. According to Nor Afizah and Rizvi [11], an extrusion at <75 °C forms aggregates susceptible to hydrolysis, but at 90 °C forms aggregates resistant to hydrolysis. In the case of germination, the internal proteolysis caused the degradation of high-MW proteins and the formation of short polypeptides (Figure 1, lane GQ). Similar results have been reported for germinated quinoa [12].

### Table 1. Hydrolysis degree kinetics (HD%) of protein concentrates from unprocessed, extruded and germinated quinoa seeds.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>UQPH</th>
<th>GPH</th>
<th>EPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td>30</td>
<td>28.45 ± 1.00</td>
<td>23.98 ± 3.32</td>
<td>25.04 ± 0.83</td>
</tr>
<tr>
<td>60</td>
<td>31.27 ± 1.00</td>
<td>27.74 ± 2.66</td>
<td>28.80 ± 0.83</td>
</tr>
<tr>
<td>120</td>
<td>34.56 ± 1.00</td>
<td>27.51 ± 1.00</td>
<td>30.91 ± 0.83</td>
</tr>
<tr>
<td>150</td>
<td>35.38 ± 0.83</td>
<td>30.09 ± 3.32</td>
<td>31.85 ± 0.83</td>
</tr>
</tbody>
</table>

UQPH: unprocessed seed protein hydrolysate; GPH: germinated seed protein hydrolysate; EPH: extruded seed protein hydrolysate. Data are represented as mean ± standard deviation (n = 3); different letters in the same row indicate a statistically significant difference ($p < 0.05$). n.a: not applicable.

3.3. Peptide Quantification in Protein Hydrolysates

Generally, bioactive peptides are composed for up to 20 amino acids. Figure 2 shows the distribution of peptides in the >10, 3–10 and <3 kDa fractions. The largest abundance of peptides was found in the <3 kDa fraction for the UQPH, EQPH and GQPH, followed by the 3–10 and >10 kDa fractions. The extrusion significantly augmented ($p < 0.05$) the concentration of peptides in all fractions, which may be attributed to the formation of polypeptides, as shown by the SDS-PAGE profile (Figure 1 lane EQ). Similar findings were reported for amaranth seeds [6]. These data suggest that a two-step hydrolysis occurred: the first one during the extrusion, followed by the enzymatic hydrolysis per se.

3.4. AOC of Peptide Fractions

The contribution of each peptide fraction to the total AOC of the hydrolysate was studied. Figure 3 shows that all fractions displayed antiradical activity. The <3 kDa fraction had the highest AOC in the UQPH, EPH and GPH. The <3 kDa fraction in the EPH and GPH displayed increases ($p < 0.05$) of 61.49% and 38.11%. The 3–10 kDa fraction in the EPH and GPH showed improvements ($p < 0.05$) by 130.98% and 57.71%, compared to UQPH. Although the UQPH and GPH had similar concentrations of peptides in the 3–10 kDa fraction (Figure 2), the GPH had a higher AOC, which suggests the presence of different antiradical peptides. The EPH and GPH displayed a similar HD%, but this was lower than that of the UQPH (Table 1), which suggests that the better AOC could also be related to the presence of large peptides. Overall, the presented data suggest that not only short peptides (<3 kDa) exhibited AOC but also polypeptides between 10 and 3 kDa, due to extrusion and germination. The improvement in the AOC could be attributed to changes in the amino acid composition, peptide profile and steric factors, as well as the production of new, larger peptides due to the extrusion and germination of seeds. Other authors have reported similar results for extruded proteins and peptides [4,13]. Additionally, the type
of enzyme and the hydrolysis time was also reported to influence the AOC [6]. Similarly, Montoya-Rodríguez et al. [6] found an enhanced AOC of hydrolysates from extruded amaranth seeds and for germinated amaranth seeds [10].

![Figure 2](image2.png)

**Figure 2.** Abundance of peptides (leucine mM/mL) for different MW fractions in protein hydrolysates from unprocessed quinoa seed protein hydrolysate (UQPH), extruded seed protein hydrolysate (EPH) and germinated seed protein hydrolysate (GPH) after 180 min of hydrolysis. Data are shown as mean ± standard deviation (n = 3). Different letters in the same molecular weight fraction indicate statistically significant difference (p < 0.05).

![Figure 3](image3.png)

**Figure 3.** Antioxidant activity (ascorbic acid equivalents µg/mL) of hydrolysate fractions from unprocessed quinoa seed protein hydrolysate (UQPH), extruded seed protein hydrolysate (EPH) and germinated seed protein hydrolysate (GPH) after 180 min hydrolysis. Data shown as mean ± standard deviation (n = 3). Different letters in same molecular weight fraction indicate statistically significant difference (p < 0.05).

### 4. Conclusions

The novel Andean Argentine quinoa variety, Hornillos-Gob Jujuy INTA, was suitable for extrusion and germination for producing PCs with considerable high-protein purity (61–66%). The processing of the seeds did not alter the chenopodin, but the HMW proteins were partially hydrolysed. Distinctive protein hydrolysates were prepared, and the proteolysis kinetics were modulated by reducing the HD. The pre-treatments that were applied enhanced the AOC of the long (3–10 kDa) and short (<3 kDa) peptides. The improvement may be due to the synergistic effects of different peptides and the new peptides formed due to processing. These results provide an insight into how affordable, green and controlled pre-treatments of quinoa seeds may change and modulate the hydrolysis products with enhanced bio-functional properties.
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