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Artículo de investigación

Production of a protein isolate from boyacense quinoa flour *(chenopodium quinoa)* by combined methods

Obtención de un aislado proteico, a partir de harina de quinua boyacense *(chenopodium quinoa)*, mediante métodos combinados

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Abstract

Quinoa is a pseudocereal known for its high content of plant protein. The aim of this study was to obtain a high-purity protein isolate using different extraction methodologies. Initial characterization of the quinoa flour on a dry basis (QF) showed protein content of 16% and starch content of 53%. Optimal pH values were determined through solubility curves, and extractions were performed using 0% and 1% NaCl solutions, resulting in a protein yield of 38%. Fractionated extraction was then used, increasing the protein yield to 42%. Finally, enzymatic hydrolysis of the starch, which acts as an interferent in protein extraction, was performed. The combined use of enzymatic hydrolysis and fractionated extraction resulted in a protein isolate with a high degree of purity (~55%). This study demonstrates that the combination of different extraction methodologies can significantly improve the yield and purity of the final product.

Keywords: α-amylase; protein isolate; fractional extraction; enzymatic hydrolysis; quinoa

Resumen

La quinua es un pseudocereal que es conocido por su alto contenido de proteína vegetal. El presente estudio se enfocó en obtener un aislado proteico, utilizando diferentes metodologías de extracción. La caracterización inicial de la harina de quinua en base seca (QF) mostró un contenido de proteína del 16 % y de almidón del 53 %. Se establecieron los mejores valores de pH, a través de curvas de solubilidad, y se llevaron a cabo extracciones en soluciones 0 % y 1 % de NaCl, obteniendo un rendimiento del 38% de proteína. Posteriormente, se empleó una extracción fraccionada, lo que permitió aumentar el rendimiento proteico al 42 %. Por último, se aplicó una hidrólisis enzimática del almidón presente en la harina, que actúa como interferente en la extracción proteica. El uso combinado de la hidrólisis enzimática y la extracción fraccionada permitió obtener un aislado proteico con un alto porcentaje de pureza (~55 %). Este estudio demostró que la combinación de diferentes metodologías de extracción puede mejorar significativamente el rendimiento y pureza del producto final.

Palabras Clave: α-amilasa; aislado proteico; extracción fraccionada; hidrólisis enzimática; quinoa.

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1 Introduction

Quinoa (Chenopodium quinoa) is a crop native to Peru with a rich archaeological and historical background, dating back approximately 7,000 years in the Andean region [1]. In Colombia, the Chibcha tribe of the Cundiboyacense highlands cultivated this plant and later migrated to San Agustín (Huila), contributing to the spread of quinoa to the south of the country, in the department of Nariño [2]. In Boyacá, it has been stated that the area dedicated to quinoa cultivation spans 250 hectares with a production of 375 tons [3]. According to the morphological shape of its grain, quinoa is classified as a pseudocereal [4]. Due to its high nutritional quality, low production cost, genetic variability, and easy adaptability, quinoa has become a food with great nutritional potential and a strategic crop that contributes to food security and sovereignty [1]. Its protein content ranges from 13.8% to 21.9%, depending on the variety type. Quinoa is also a potential source for oil extraction, as it contains high amounts of ω-3, ω-6, and ω-9 fatty acids. In addition, it contains 68.0% carbohydrates (starch), micronutrients (P, Mg, K, Fe, Zn, Ca, and Mn), and natural vitamins [5].

Different studies, such as electrophoresis methods, have revealed that the two main protein contents are albumin and globulin, with a special secondary structure and interactions between their subunits, which influence their structural and physicochemical properties [6, 7]. Quinoa protein has the particularity of containing an excellent amino acid profile, including the essential ones that the human body cannot produce. The importance of this protein lies in its quality, as it has a balanced composition of essential amino acids, approximating its composition to that of casein (milk protein) [1]. The chemical composition of quinoa makes this pseudocereal potentially suitable for obtaining a protein isolate. There are different extraction methods, and the process will depend on how beneficial it is for obtaining the final product, improving extraction efficiency without drastically altering the characteristics of the isolated protein, and which, in turn, is easy to apply and low-cost with respect to energy consumption. Proteins are highly sensitive to pH changes, and the extraction buffer is an important factor that allows for better reproducibility of results[8, 9]. For this reason, it must be evaluated from different points of view, considering possible interactions with other components.

Regarding purification methods for one or more proteins, they are based on properties such as size or charge, where the extract is subjected to separation processes called fractionation [10, 11]. The stages that initiate the process use differences in solubility, dependent on the aforementioned factors (pH, temperature, ionic strength, among others). In general, these processes involve a mechanical disruption [12, 13]. At an industrial level, quinoa in Colombia does not present much potential beyond the food sector. However, the quality of its protein content makes it a plant of interest for research towards its application in other fields such as materials science, chemical industry, and pharmaceuticals, which can enhance the global potential of this pseudocereal, its products, and by-products [2].

Therefore, this work presents an eco-efficient alternative for obtaining protein isolates from quinoa flour, while also providing further information and identifying potential uses in the regional, departmental, and national industries, with the premise of obtaining compounds that have a particular use.

2 Materials and methods

Sampling and protein characterization of quinoa flour.
Quinoa flour was randomly sampled from the production centers in Soracá, Boyacá (5°30′2" N, 73°20′0" W), to obtain a representative characterization. The protein value of the quinoa flour (HQ) was quantified using the Bradford and Kjeldahl methods, following standard quality norms 955.39 [14, 15]. Additionally, reducing sugars were determined through the DNS spectrophotometric method [16, 17].

Degreasing of Quinoa Flour.
The quinoa flour (QF) was degreased using the methodology proposed by TAPPI 204 cm-97 standards [18]. Hexane was used as the solvent for approximately 7 hours at a temperature of 50 °C. The entire procedure was carried out using a soxhlet equipment. The percentage of fats was quantified by Eq. 1.

$$\frac{\text{Final weight of the extract}}{\text{Initial weight of the extract}} * 100\% = \%\text{Fat} \tag{1}$$

Starch determination.
The extraction of starch was performed using perchloric acid. Initially, 100 mg of the sample was taken for prior quantification, and iodinated complexes were precipitated using gravimetric techniques [19]. The procedure was carried out in duplicate, and the percentage of starch was quantified by Eq. 2.

$$\frac{\text{Final weight of the extract}}{\text{Initial weight of the extract}} * 100\% = \%\text{Starch} \tag{2}$$

Evaluation of Protein Solubility as a Function of pH.
Eleven assays were prepared with de-fatted quinoa flour (DQF), at a 1:10 w/v ratio, with pH adjusted to values ranging from 2 to 12. The samples were then subjected to constant agitation at 150 rpm for 1 hour at a temperature of 30 °C. The entire process was carried out in an orbital shaker with incubation. The process was then repeated in saline solution (NaCl) at a concentration of 1 M. After the reaction time was complete, the samples were centrifuged for 20 minutes at 4000 rpm, the supernatant was taken, and readings were taken using a UV-Vis spectrophotometer with the Bradford method.

Effect of NaCl Concentration.
After determining the optimal extraction and precipitation pH for the protein isolate, a set of test tubes was prepared at a 1:10 w/v ratio with varying concentrations of NaCl (0.50%, 0.75%, and 1.00%). The procedure described in the previous section was then carried out at reaction times of 1 hour and 2 hours.

Protein Isolation.
To extract the protein, two solutions were prepared using DQF in a 1:10 w/v ratio with either water or 1% saline solution (NaCl). The pH of the mixture was then adjusted to 11 using 0.1 M NaOH and allowed to react for approximately 2 hours. Afterward, the mixture was centrifuged at 4000 rpm for 20 minutes to collect the supernatant. The pH of the supernatant was then adjusted to 3.5 using 0.1 M HCl, and the mixture was centrifuged again at 4000 rpm for 20 minutes to obtain the solid residue. Finally, the protein yield percentage was determined using Eq. 3.

$$\text{Yield} = \frac{weight_{isolate}}{\%_{protein} * weight_{sample}} * 100 \tag{3}$$

Enzymatic treatment.
Three solutions were prepared using a 1:10 w/v ratio of DQF in citrate buffer at pH 5.8. Commercial α-amylase was added at concentrations of 0.3 mg/mL, 0.6 mg/mL, and 0.9 mg/mL and allowed to react for 10 h at temperatures of 30°C and 50°C. The reaction was monitored by measuring protein and reducing sugar concentrations at specific times between 0 and 10 hours. After hydrolysis was complete, the solid was filtered, and fractionated extraction was carried out. To extract the protein isolate, variables were selected based on the solubility curve of the protein determined using the Bradford method. Optimal solubility values were established at pH values between 8 and 11. The effect of salt concentration on protein solubility was also observed. Solutions were prepared at a 1:10 w/v ratio using water and 1% NaCl as solvents. Each variable was combined in triplicate, and protein and reducing sugar concentrations were measured at different time points, compared to the respective blank.

After obtaining the results, the best pH and time were selected, and a fractionated extraction was performed on the same solid by combining the previously analyzed solvents and adding acetic acid in the order shown in Figure 1.

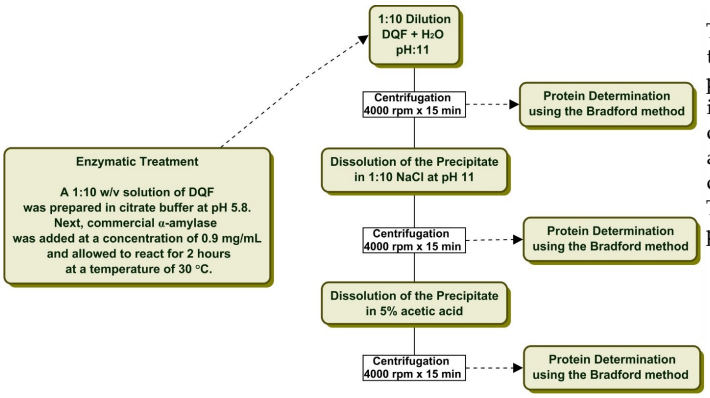

Figure 1: Fractional protein extraction diagram.

3 Results and discussion

Analysis of protein

Table 1 displays the percentages of protein, fat, and starch obtained in the degreasing process. The values obtained are consistent with those reported in the literature and fall within the range found in studies conducted by the FAO [1].

Table 1: Protein, fat, and starch content of natural and degreased quinoa.

Analysis\sample	Natural Quinoa (QF)	De-fatted quinoa flour (DQF)
Raw protein (%)	13.86	16.07
Fat (%)	1.31	0.00
Starch (%)	54.31	53.00

Preparation of solubility curve and determination of pI

Figure 2 shows the concentration of quinoa protein extracted at different pH ranges. The obtained data demonstrate that the protein solubility gradually increases as pH values increase. Optimal solubility values were observed within pH 7 to 12, with pH 11 identified as the best solubilization reference. These findings align with previous studies reported in the literature [20, 21].

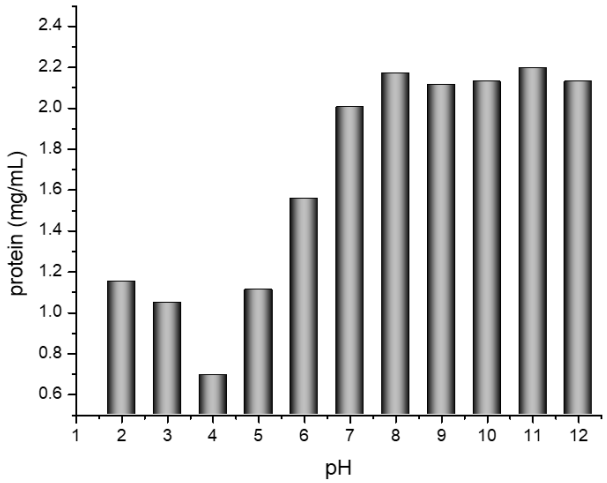

Figure 2: Solubility curve.

Various physicochemical properties of molecules influence the solubility of proteins, which can be altered by different processing methods. When proteins are subjected to alkaline conditions, their conformation can be altered, resulting in enhanced interaction with water and increased solubility [22].

The solubility curve presented in Figure 2 allowed us to identify the pH ranges where the pI was found. The pI corresponds to the pH value at which the protein conformation is least altered and its interaction with water is minimized. Furthermore, the solubility of quinoa protein was found to be lower at pH values between 2.0 and 5.0 (Figure 3). The lowest concentration of soluble protein was observed at a pH of 3.5, which corresponds to the specific pI value. This result agrees with previous studies that have reported lower protein solubility in acidic conditions [22].

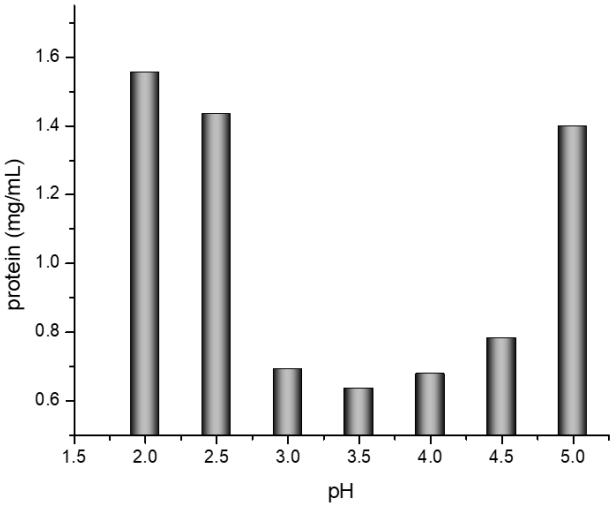

Figure 3: Determination of the Isoelectric Point.

Impact of NaCl Concentrations

The addition of NaCl at varying concentrations improved the isolation of protein from quinoa. Increasing the salt concentration resulted in a higher protein yield. Additionally, reaction time played a crucial role in the isolation process, with a reaction time of 2 hours yielding better results compared to 1 hour (see Figure 4). Notably, a reaction time of 1 hour and a NaCl concentration of 0.75 M resulted in ambiguous protein solubility, suggesting only partial solubilization. This was confirmed in the second trial where a longer reaction time improved the yield. These findings are consistent with previous studies that have demonstrated the impact of NaCl concentration on protein solubility [23, 24].

Obtention of protein isolates

The obtained protein isolates for each reaction showed a yield percentage of 44% for NaCl and 85% for H_2O. Although the NaCl yield was lower, it presented a better purity percentage compared to the H_2O isolate, as shown in Table 2. This is because the saline solution binds to the hydrophilic domain of the protein, with counter ions efficiently coating the ionic charges of the molecule, thus improving protein solubility by increasing its charge [25, 26].

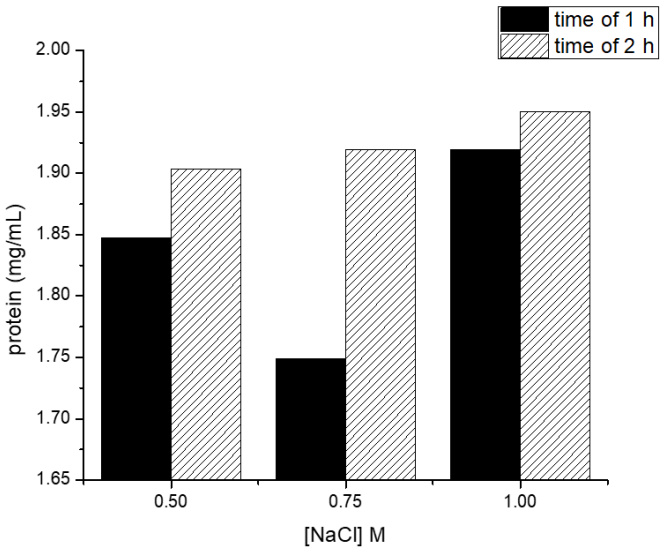

Figure 4: Effect of NaCl concentration at reaction times of 1 hour and 2 hours.

Table 2: Crude protein analysis of treated samples

Analysis\sample	Protein isolated with H_2O	Protein isolated with NaCl
Raw protein (%)	32.270	38.685

Enzymatic treatment

Figure 5 shows the results of α-amylase hydrolysis of quinoa flour. It was observed that the maximum concentration of produced glucose was achieved at a temperature of 30°C in 2 and 4 hours for enzyme concentrations of 0.6 mg/mL and 0.9 mg/mL, respectively. In contrast, for an enzyme concentration of 0.3 mg/mL, the best glucose production times ranged from 4 to 6 hours, which can be related to the activation energy of enzyme. It is understood that there is a direct proportional relationship between enzyme concentration and reaction rate. Additionally, protein analyses were performed to determine if there was protein solubilization during this process. However, no significant increases were observed during the reaction [27].

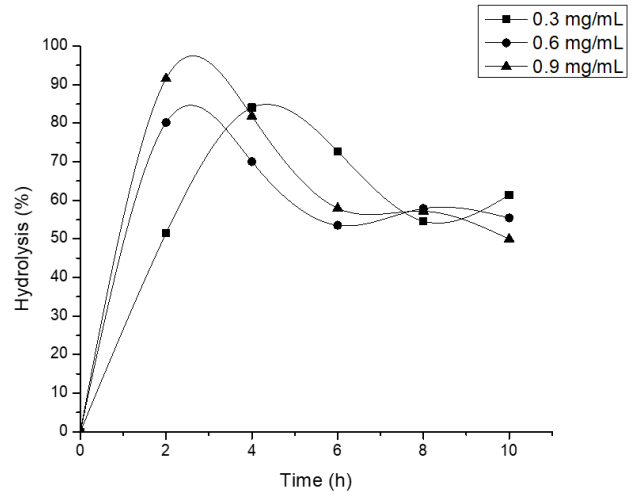

Figure 5: Hydrolysis of defatted quinoa flour with α-amylase vs. time.

The obtained results allowed us to define that the optimal enzyme concentration to use is 0.9 mg/mL, for a reaction time of 2 h, as after this time the maximum values of reducing sugar production are reached, with a yield percentage of 92%. Likewise, it is confirmed that at this concentration, the enzyme presents better enzymatic activity, as it hydrolyzes almost all the sugars present in the de-fatted quinoa flour [27].

Once the enzyme concentration and reaction time were defined, the optimal temperature for the reaction was established (Figure 6). It was found that the optimum hydrolysis temperature is 30 °C. After 100 minutes at a temperature of 50 °C, there is a decrease in product formation due to enzyme denaturation. The enzyme loses its catalytic activity, and the substrate can no longer bind to the active site [28, 29]

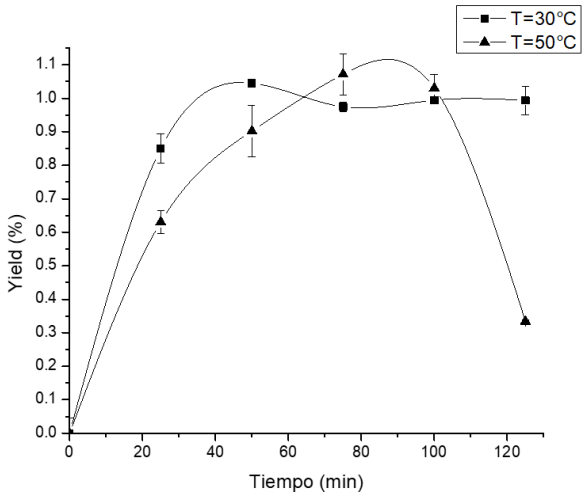

Figure 6: Hydrolysis of de-fatted quinoa flour with 0.9 mg/mL of α-amylase at 30 °C and 50 °C.

Fractional extraction

The protein values are reported in Table 3. It was observed that the percentage of protein increased considerably after fractionation, which can be attributed to the action of α-amylase that hydrolyzed the starch present, refining the de-fatted quinoa flour (DQF). Moreover, the solubility of the proteins present in DQF is not only dependent on the variables analyzed but also on the type of solvents used for extraction. Based on the results obtained from each of the solutions, it can be inferred that DQF contains a higher percentage of albumin-type proteins, which are soluble in water, and globulin-type proteins, which are soluble in saline solutions. Additionally, it contains a lower percentage of glutenin-type proteins, which are soluble in alkaline or acidic solutions.

Table 3: Protein quantification by fractional extraction

Solvent	Protein (mg/mL)	%protein isolated
α-amylase	2.16	13.50
H_2O	3.05	19.07
NaCl 1%	2.83	17.70
CH_3COOH 5%	0.75	4.65
Total	8.79	54.93

In Figure 7, the FTIR-ATR spectra of quinoa protein obtained through fractionation and hydrolysis with α-amylase (PDQ-A) and quinoa protein without hydrolysis with α-amylase (PDQ-B) are shown. Four major bands attributed to vibrations produced by primary amide (~1641 cm^{-1}), secondary amide (~1533 cm^{-1}), and tertiary amide (~1075 cm^{-1} and ~1149 cm^{-1}) bonds were observed, which allowed

for the verification of the chemical nature of the quinoa protein. The band at ~930 cm^{-1} suggests the presence of α-1-4 glycosidic bonds (C-O-C) characteristic of amylose present in starch, as reported in the literature [30]. Additionally, a decrease in the intensity of the band related to glycosidic bonds was observed for PDQ-A due to the loss of molecular order of starch by α-amylase action. Finally, the band at ~1006 cm^{-1} is characteristic of the C(1)-H bond flexion of the alpha carbon of the amino acid structures that make up the protein [19].

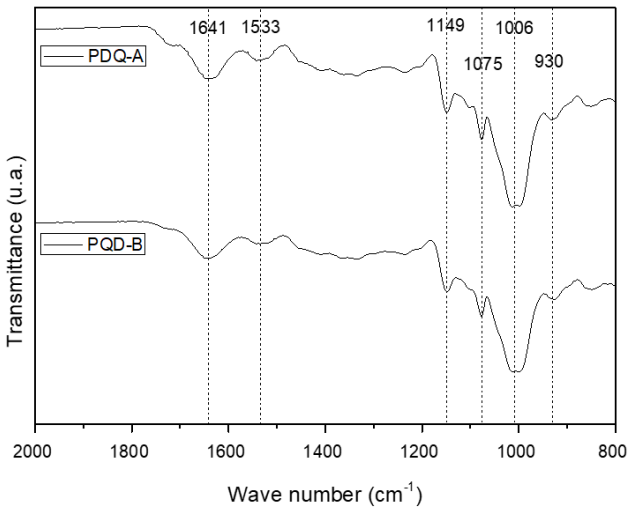

Figure 7: FTIR spectrum of quinoa protein isolate, obtained by fractional and hydrolyzed (PDQ-A) and non-hydrolyzed (PDQ-B) extraction.

4 Conclusions

The solid yield percentages for water and saline dissolution were determined to be 85% and 44%, respectively. However, the protein percentage in the solid did not show significant values for the isolate, indicating the presence of interferents that prevented complete solubilization. To address this issue, alternative methods were explored, including fractionated extraction using specified solvents to enhance protein solubility. This approach led to a higher percentage of protein isolate and significantly improved purification, revealing that starch is the major interferent in quinoa flour.

Additionally, enzymatic treatment resulted in yield percentages between 80% and 90% for starch hydrolysis. These findings suggest that the enzymatic treatment can effectively eliminate interferents and a combined extraction method involving enzymatic hydrolysis and fractionated extraction processes is recommended for quinoa protein extraction.

The protein isolation process from quinoa requires careful consideration of NaCl concentration and reaction time. Higher NaCl concentrations and longer reaction times were found to improve protein yield, while certain combinations led to partial solubilization. These findings provide valuable insights for refining protein isolation methods and achieving more efficient outcomes.

Additionally, a significant variation in protein solubility was observed based on pH levels. Solubility decreased in acidic pH and increased in alkaline pH, emphasizing the critical influence of pH on protein stability and solubility. Understanding this relationship is crucial for designing formulations and biochemical processes involving proteins, with potential far-reaching implications in industries such as pharmaceuticals, food, and biotechnology, where protein solubility plays a fundamental role in their functionality and applications.

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Ethical Implications

The methodology outlined for this research proposal adheres to the criteria and guidelines established by Resolution 8430 of 1993 from the Ministry of Health, concerning biosafety standards and conditions. Additionally, it follows the statement issued by the Academic Vice-Rectory, the Research Directorate (DIN), and the Ethics Committee for Scientific Research of the Universidad Pedagógica y Tecnológica de Colombia (UPTC) dated June 27, 2014.

Authors' Contribution Statement

The authors confirm their contributions to the article as follows: study conception and design: Ivan Dario Niño Bernal, Gerardo Andrés Caicedo Pineda; data collection: Gerardo Andrés Caicedo Pineda; analysis and interpretation of results: Ivan Dario Niño Bernal, Gerardo Andrés Caicedo Pineda; manuscript draft preparation: Gerardo Andrés Caicedo Pineda. All authors reviewed the results and approved the final version of the manuscript.

Declaration of Source of Funds

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article.

References

[1] W. Rojas, G. Alandia, J. Irigoyen, J. Blajos, and T. Santivañez, "La Quinua: Cultivo milenario para contribuir a la seguridad alimentaria mundial," 2011. doi: 10.1016/j.jaridenv.2009.03.010.

[2] A. Guerrero-López, "Impacto del cultivo de la quinua (Chenopodium quinoa Willd) como alternativa productiva y socioeconómica en la comunidad indígena Yanacona de La Vega, Cauca, Colombia," 2018.

[3] Ministerio de agricultura y desarrollo rural, "En los últimos 4 años, la quinua ha tenido un crecimiento de más del 150% en áreas de producción," 2018.

[4] J. P. Rodríguez, H. Rahman, S. Thushar, and R. K. Singh, "Healthy and Resilient Cereals and Pseudo-Cereals for Marginal Agriculture: Molecular Advances for Improving Nutrient Bioavailability," Front Genet, vol. 11, no. February, pp. 1–29, 2020, doi: 10.3389/fgene.2020.00049.

[5] L. C. A. Rivera and a. K. H. Hernández, "calidad y germinación de semillas de quinua chenopodium quinoa willd. Almacenadas artesanalmente por productores.," universidad de ciencias aplicadas y ambientales u.d.c.a, 2017. Doi: 10.1038/132817a0.

[6] L. E. Abugoch James, Quinoa (Chenopodium quinoa Willd.): Composition, chemistry, nutritional, and functional properties, 1st ed., vol. 58, no. 09. Elsevier Inc., 2009. doi: 10.1016/S1043-4526(09)58001-1.

[7] F. M. Montes, j. P. P. Vázquez, and h. R. Rosas, bioquímica de laguna y piña. 2018.

[8] M. Amor Lluch, "Influencia de la germinación en la obtención de aislados proteicos de altramuz," Sep. 2022, Accessed: Jul. 23, 2023. [Online]. Available: https://riunet.upv.es:443/handle/10251/186564

[9] C. A. Gamboa Hipolito and B. Llanos Caballero, "Métodos de extracción de proteína y sus aplicaciones en el mejoramiento nutricional de productos Agroindustriales," Journal of Agro-industry Sciences, vol. 4, no. 3, pp. 133–140, Dec. 2022, doi: 10.17268/jais.2022.016.

[10] Z. Gao et al., "Effect of alkaline extraction pH on structure properties, solubility, and beany flavor of yellow pea protein isolate," Food Research International, vol. 131, no. January, p. 109045, 2020, doi: 10.1016/j.foodres.2020.109045.

[11] M. L. Sosa-Flores, D. G. García-Hernández, C. A. Amaya-Guerra, M. Bautista-Villarreal, and A. R. González-Luna, "Obtención de aislados e hidrolizados proteicos de grillo (Acheta domesticus) y evaluación de su actividad antioxidante," 2023.

[12] L. D. López Quimbayo, "Extracciòn a partir de hojas y semillas de Pentacalia nítida y evaluaciòn de la actividad antimicrobiana del extracto proteico acuoso," 2012.

[13] C. Gutiérrez Paz Nutricionista et al., "extracción de aislado proteico de quinua (chenopodium quinua: variedad blanca junín) como alternativa para el uso en suplementos altos en proteína," 2022.

[14] C. L. Kielkopf, W. Bauer, and I. L. Urbatsch, "Bradford assay for determining protein concentration," Cold Spring Harb Protoc, vol. 2020, no. 4, pp. 136–138, 2020, doi: 10.1101/pdb.prot102269.

[15] N. J. Kruger, "The Bradford Method For Protein Quantitation," Basic Protein and Peptide Protocols, pp. 17–24, 2009.

[16] G. L. Miller, "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar," Anal Chem, vol. 31, no. 3, pp. 426–428, 1959, doi: 10.1021/ac60147a030.

[17] I. P. Wood, A. Elliston, P. Ryden, I. Bancroft, I. N. Roberts, and K. W. Waldron, "Rapid quantification of reducing sugars in biomass hydrolysates: Improving the speed and precision of the dinitrosalicylic acid assay," Biomass Bioenergy, vol. 44, pp. 117–121, Sep. 2012, doi: 10.1016/j.biombioe.2012.05.003.

[18] M. Buchanan, "TAPPI 204 cm-97," Nanofibers - Production, Properties and Functional Applications, 2007, doi: 10.5772/916.

[19] C. R. Bernal Bustos, L. F. Ramírez, P. D. Duarte, A. M. Guzmán, and J. Acero, "Quinua, Chenopodium quinua (Willd.) en Colombia Caracterización de granulos de almidón nativo de quinua por IR-ATR, MEB, DRX," Rev Invest (Guadalajara), vol. 8, no. 2, pp. 122–131, 2015, doi: 10.29097/2011-639x.31.

[20] S. A. Elsohaimy, T. M. Refaay, and M. A. M. Zaytoun, "Physicochemical and functional properties of quinoa protein isolate," Annals of Agricultural Sciences, vol. 60, no. 2, pp. 297–305, 2015, doi: 10.1016/j.aoas.2015.10.007.

[21] José J. Martínez, Oscar J. Medina, and Rocio Zambrano, "Estudio fisicoquímico funcional de los aislados proteicos en semillas de maracuya(Passiflora eduli s f).," vol. 9, 2011.

[22] M. Föste, D. Elgeti, A. K. Brunner, M. Jekle, and T. Becker, "Isolation of quinoa protein by milling fractionation and solvent extraction," Food and Bioproducts Processing, vol. 96, pp. 20–26, 2015, doi: 10.1016/j.fbp.2015.06.003.

[23] S. A. Elsohaimy, T. M. Refaay, and M. A. M. Zaytoun, "Physicochemical and functional properties of quinoa protein isolate," Annals of Agricultural Sciences, vol. 60, no. 2, pp. 297–305, 2015, doi: 10.1016/j.aoas.2015.10.007.

[24] C. Valenzuela, L. Abugoch, C. Tapia, and A. Gamboa, "Effect of alkaline extraction on the structure of the protein of quinoa (Chenopodium quinoa Willd.) and its influence on film formation," Int J Food Sci Technol, vol. 48, no. 4, pp. 843–849, 2013, doi: 10.1111/ijfs.12035.

[25] B. Eab and S. Mpc, "efectos de la sal sobre la solubilidad y las propiedades emulsionantes de la caseína," 2006.

[26] D. Voet and J. G. Voet, Bioquimica Voet 3ed.

[27] R. Lodeiro, Catálisis enzimática Fundamentos químicos de la vida, vol. 1. 2017.

[28] R. c. Bohinski, bioquímica. 1967.

[29] M. N. Perović, Z. D. Knežević Jugović, and M. G. Antov, "Improved recovery of protein from soy grit by enzyme-assisted alkaline extraction," J Food Eng, vol. 276, no. December 2019, 2020, doi: 10.1016/j.jfoodeng.2019.109894.

[30] A. Vera, M. A. Valenzuela, M. Yazdani-Pedram, C. Tapia, and L. Abugoch, "Conformational and physicochemical properties of quinoa proteins affected by different conditions of high-intensity ultrasound treatments," Ultrason Sonochem, vol. 51, no. October 2018, pp. 186–196, 2019, doi: 10.1016/j.ultsonch.2018.10.026.