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Scientific Article

Application of Chickpea Glutelin Hydrolysates in the Green Synthesis of Selenium Nanoparticles

Aplicación de hidrolizados de glutelina de garbanzo en la síntesis verde de nanopartículas de selenio

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Abstract

This study evaluated the potential of total chickpea protein (TP), the glutelin fraction (Glu), and their <10 kDa hydrolysates (TPH and GluH) as functionalizing agents in the green synthesis of selenium nanoparticles (SeNPs). Physicochemical parameters, including particle size, zeta potential, polydispersity index, surface plasmon resonance (SPR), and antioxidant activity, were analyzed using ORAC and ABTS assays. The results showed that the hydrolysates interacted more efficiently with SeNPs than the intact proteins, generating more defined spectral profiles. SeNPs functionalized with TP and Glu did not exhibit higher antioxidant activity than their parent proteins. In contrast, SeNPs functionalized with GluH and TPH displayed significantly greater antioxidant activity (+53 % and +21 % in ORAC and ABTS, respectively) compared to the hydrolysates. GluHSeNPs reached a particle size of 136 nm, while TPHSeNPs formed aggregates larger than 1 μm, demonstrating low colloidal stability. Both systems exhibited low zeta potentials (-9 to -13 mV). These findings indicate that <10 kDa chickpea glutelin hydrolysates are promising materials for the synthesis of

nanostructures with high antioxidant activity, with potential applications in functional foods and biomedicine.

***Keywords*: Selenium nanoparticles, green synthesis, Chickpea proteins, antioxidant activity, glutelin protein**

Resumen

Este estudio evaluó el potencial de la proteína total de garbanzo (TP), la fracción de glutelina (Glu) y sus hidrolizados de <10 kDa (TPH y GluH) como agentes funcionalizantes en la síntesis verde de nanopartículas de selenio (SeNPs). Se analizaron parámetros fisicoquímicos, incluyendo tamaño de partícula, potencial zeta, índice de polidispersidad, resonancia de plasmón superficial (SPR) y actividad antioxidante mediante los ensayos ORAC y ABTS. Los resultados mostraron que los hidrolizados interactúan más eficientemente con las SeNPs que las proteínas intactas, generando perfiles espectrales más definidos. Las SeNPs funcionalizadas con TP y Glu no presentaron mayor actividad antioxidante que sus proteínas de origen. En contraste, las SeNPs funcionalizadas con GluH y TPH exhibieron una actividad antioxidante significativamente superior (+53 % y +21 % en ORAC y ABTS, respectivamente) en comparación con los hidrolizados. GluHSeNPs alcanzaron dimensiones de 136 nm, mientras que TPHSeNPs formaron agregados >1 μm, evidenciando baja estabilidad coloidal. Ambos sistemas presentaron bajos potenciales zeta (-9 a -13 mV). Estos hallazgos indican que los hidrolizados <10 kDa de glutelina de garbanzo son materiales prometedores y novedosos para la síntesis de nanomateriales con elevada actividad antioxidante, con potencial aplicación en alimentos funcionales y medicina.

***Palabras clave*: nanopartículas de selenio, sistesis verde, proteínas de garbanzo, actividad antioxidante, glutelina proteínas**

1. Introduction

Selenium is a vital trace element involved in protecting against oxidative damage, regulating thyroid hormone metabolism, and modulating immune function (Zhou et al., 2022). Dietary Se deficiency has been linked to increased susceptibility to oxidative stress, cardiovascular diseases, cancer, and impaired immune function (Shimada et al., 2021). Inorganic Se salts, such as sodium selenite, are widely used as supplements but present limitations due to narrow safety margins, low bioavailability, and potential toxicity at higher doses (EFSA Panel, 2023). Recently, selenium nanoparticles (SeNPs) have gained attention as promising alternatives because they can improve bioavailability, decrease toxicity, and provide controlled release characteristics (Waqar, 2025).

Nowadays, green synthesis of nanoparticles using biomolecules from plants and food proteins has gained increasing attention as a sustainable and safe approach compared to conventional chemical methods (Osman et al., 2024). Proteins and peptides can act as both reducing and stabilizing agents, conferring colloidal stability and bioactivity to nanoparticles (Spicer et al., 2018). Legume proteins, particularly from chickpea (*Cicer arietinum* L.), are abundant, and rich in bioactive peptides with antioxidant, antihypertensive, and immunomodulatory properties (Kumar et al., 2025). Enzymatic hydrolysis of chickpea proteins releases low molecular weight peptides with enhanced solubility

and radical scavenging activity (Ghribi et al., 2015; Xu et al., 2020), which may improve the stabilization and functionality of SeNPs.

Glutelin from germinated chickpeas in the presence of selenium has been identified as the protein fraction with the greatest capacity for accumulating this micronutrient, surpassing albumin and globulin (Serrano-Sandoval et al., 2019; Hernández-Grijalva et al., 2022). Furthermore, selenized glutelin has demonstrated superior emulsifying and antioxidant capacity, as well as greater colloidal stability compared to other protein fractions (Hernández-Grijalva et al., 2022; Milán-Noris et al., 2025). These properties offer significant advantages for the design of nanostructured systems with applications in functional foods, nutraceuticals, and cosmetics, where the combination of SeNPs with plant proteins can enhance bioactivity and protection against oxidative stress.

Despite the growing interest in legume derived peptides their application in the synthesis and functionalization of SeNPs remains poorly explored. Accordingly, this study aimed to develop SeNPs produced and functionalized through a green approach using chickpea proteins and their hydrolysates, to characterize their physicochemical properties and antioxidant activity. Overall, this research offers novel insights into chickpea derived peptides as sustainable biomaterials for designing functional foods enriched with Se based nanoparticles.

2. Materials and methods

2.1 Chickpea flour production

Kabuli chickpea (*Cicer arietinum* L.) seeds were sourced from Angostura, Sinaloa, Mexico. Seeds were disinfected with 200 mL of a 0.2 % sodium hypochlorite solution, rinsed three times with distilled water, spread on a metal tray and dried in a convection oven (UNOX, Padua, Italy) at 50 ± 1 ℃ for 24 h. Once dried, the seeds were ground using Standard Model No. 5 Wiley Mill (Swedesboro, NJ, USA) and sieved through a No. 60 mesh; the resulting flour was packed in plastic bags and stored at 4 °C until needed (Guardado-Félix et al., 2017).

2.2 Total protein extraction

Total protein was extracted from chickpea flour following Serrano-Sandoval et al. (2019). In short, the flour was defatted with hexane (1:4, w/v) under continuous agitation at 50 °C for 12 h, replacing the solvent after 6 h. Then 20 g of flour were mixed with 200 mL of distilled water, and the pH was raised to 8.5 with 1 M NaOH. The suspension was stirred for 2 h and centrifuged at 10,000 × g for 20 min at 4 °C (Eppendorf AG, Hamburg, Germany). Supernatant and pellet were separated; the supernatant was kept at 3 °C, while the pellet was resuspended in distilled water (1:5, w/v) and centrifuged under the same conditions. The supernatant from this second spin was pooled with the first, the pH was lowered to 4.5 using 1 M HCl, the mixture was stirred for 2 h and centrifuged again. The final pellet was freeze dried and stored for subsequent analyses.

2.3 Glutelin extraction

The glutelin (Glu) fraction was extracted sequentially following Serrano et al. (2019). Defatted chickpea flour was suspended in distilled water (1:4 w/v) and stirred at 3000 rpm for 2 h, then centrifuged at 10,000 × g for 20 min. The pellet was resuspended in 5 % NaCl (1:4 w/v), stirred for 2 h and centrifuged again under the same conditions. The final residue was solubilized in 0.1 M NaOH and stirred for 2 h; precipitation was induced by adjusting the pH to 4.8 with 1 M HCl, followed by centrifugation at 10,000 × g for 20 min. The resulting pellet, corresponding to the Glu fraction, was lyophilized and stored at -20 °C until analysis.

2.4 In vitro enzymatic digestion

The protein extracts were hydrolyzed according to the protocol reported by Serrano-Sandoval et al. (2019). The protein solution (5 % w/v, in distilled and deionized water with 0.02 % sodium azide) was adjusted to pH 2.0 with 1M HCl before pepsin (4 % w/w, protein base) was added. The solution was incubated at 37 °C for 1 h and then the pH was adjusted to 5.3 with 0.9M $NaHCO_3$. Pancreatin (4% w/w, protein base) was added, and the pH was adjusted to 7.5 with 1M NaOH. The solution was further incubated at 37 °C for 2 h and subsequently immersed in boiling water to terminate the digestion. The digested protein was centrifuged at 16,000 × g for 10 min, and the supernatant, containing the peptides of interest, was collected. Finally, the hydrolysates were freeze-dried and stored at 4 °C until further use.

2.5 Preparation of hydrolysates <10 kDa by ultrafiltration

The supernatants from the enzymatic digestion were processed using Amicon® Ultra-15 centrifugal filters (Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 5,000 rpm for 30 min (Eppendorf Centrifuge 5804 R, Germany). Filtrates containing peptides <10 kDa were collected, lyophilized, and stored for later use.

2.6 Quantification of total soluble protein

Total soluble protein in TPH and GluH was measured using the Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol. A standard curve prepared with bovine serum albumin (BSA) was employed, and results were expressed in μg/mL.

2.7 Obtaining methanolic extracts of GluH and TPH (<10 kDa)

Extracts were prepared following Guardado-Félix et al. (2017). Briefly, 50 mg of chickpea hydrolysate were combined with 1 mL of 80 % methanol, stirred for 10 min, and centrifuged at 3,000 rpm for 15 min at 4 °C (Eppendorf Centrifuge 5804 R, Germany). The precipitate was removed and the supernatant was dried to completeness under reduced pressure at 45 °C in a Carbolite PF 200

oven (Thermo Scientific, Waltham, MA, USA). The dried residue was subsequently reconstituted in 200 μL of 80 % methanol for the quantification of total phenolic compounds.

2.8 Quantification of phenolic compounds in GluH and TPH

Total phenolic content in the methanolic extracts was measured by the Folin Ciocalteu colorimetric assay. In brief, 20 μL of each sample were placed into a 96 well plate, and to both the gallic acid standard (Sigma-Aldrich, St. Louis, MO, USA) and the samples were added 180 μL of Folin Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 50 μL of 7 % Na_2CO_3. After incubating the mixtures at 25 °C for 90 min, absorbance was read at 750 nm on a microplate reader (Synergy HT, Biotek Instruments), using methanol as the blank. Total phenolic content was reported as μg of gallic acid equivalents (GAE) per mL of sample.

2.9 Synthesis of Se nanoparticles functionalized with Glu and TP

SeNPs functionalized with either chickpea total protein (TP) or glutelin (Glu) were prepared based on Zhang et al. (2018) with slight modifications. Ascorbic acid was used as the reducing agent and functionalization was performed with GluH or TPH. In brief, 1 mL of a 1 % (w/v) hydrolyzed protein solution was combined with 5 mL of 0.3 M ascorbic acid and 3 mL of deionized water while stirring at 600 rpm. Then 1 mL of 0.06 M sodium selenite (Sigma-Aldrich, St. Louis, MO, USA) was added under 300 rpm stirring to trigger reduction; the development of a red color signaled nanoparticle formation, and the mixture was stirred for another 30 min at 25 °C to homogenize. The pH was adjusted to 3.5. The nanoparticle suspension was filtered through a 0.45 μm membrane, centrifuged at 5,000 rpm for 15 min at 4 °C, and stored at 4 °C until use.

2.10 Synthesis of SeNPs functionalized with Glu and TP hydrolysates (<10 kDa)

GluHSeNPs and TPHSeNPs were synthesized following the method of Ye et al. (2020) with minor modifications. Ascorbic acid served as the reducing agent, while <10 kDa hydrolysates derived from total chickpea protein (TPH) or glutelin (GluH) were employed as stabilizing agents. Briefly, 4 mL of 4 mM ascorbic acid, 4 mL of 1 mM sodium selenite (Sigma-Aldrich, St. Louis, MO, USA), and 1 mL of <10 kDa hydrolysates (600 μg/mL) were combined in a 50 mL Erlenmeyer flask. The mixture was agitated at 150 rpm for 1 h at 55 °C, and pH was adjusted to 8. The change from a clear to an orange solution signaled the formation of nanoparticles. The resulting SeNPs were stored at 4 °C until further use.

2.11 Surface plasmon resonance characterization, droplet size, polydispersity index, and zeta potential of SeNPs

The surface plasmon resonance (SPR) of SeNPs was analyzed using a UV-Vis spectrophotometer (Multiskan GO, Thermo Scientific) over a wavelength range of 200-800 nm. Droplet size, polydispersity index (PDI), and zeta potential of the SeNPs were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature

(25 ± 0.5 °C). Measurements were taken at a scattering angle of 173°. For the analysis, the nanoparticle suspensions were diluted in deionized water (1:20, v/v) and placed into a disposable DTS1070 cell (Malvern Instruments, UK).

2.12 Oxygen Radical Absorbance Capacity (ORAC) assay

The ORAC assay was conducted following Ou et al. (2001) with minor adjustments. Fluorescein was used as the fluorescent probe, AAPH (2,2′-Azobis(2-methylpropionamidine) dihydrochloride) acted as the radical generator, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as the standard. Samples, Trolox standards, and AAPH were diluted in 75 mM sodium phosphate buffer (pH 7.4). Aliquots of 25 μL were loaded into a 96 well plate and measured with a Synergy HT microplate reader (BioTek Instruments). Kinetic readings were taken every 2 min for 60 min with excitation at 485 nm and emission at 538 nm. Antioxidant activity was determined from the difference in area under the curve (AUC) of fluorescein decay between the blank and each sample, and results were reported as μmol Trolox equivalents (TE) per mL.

2.13 Determination of antioxidant activity by ABTS assay

The ABTS radical cation assay (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was carried out according to Re et al. (1999). A stock ABTS+ solution was generated by reacting ABTS with 2.45 mM potassium persulfate and kept in the dark for 12-16 h prior to use. This solution was diluted with PBS to reach an absorbance of 0.70 ± 0.02 at 734 nm. Aliquots of 7.5 μL of blanks, samples, and standards were placed into a 96-well plate, then 292.5 μL of the ABTS+ solution was added to start the reaction. After incubating the plate at room temperature for 10 min, absorbance at 734 nm was recorded using a Multiskan GO spectrophotometer (Thermo Scientific). The decrease in absorbance was calculated against the blank, and antioxidant capacity was reported as μmol Trolox equivalents (TE) per mL of sample.

2.14 Statistical analysis

All experiments were carried out with at least three independent replicates. Results are reported as mean ± standard deviation (SD). Statistical analysis was performed by ANOVA, and mean comparisons were made using Tukey's test with a significance level of $p<0.05$. Analyses were conducted using JMP version 14 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1 Soluble protein, total phenolic content, and antioxidant activity of GluH and TPH

Table 1 displays the soluble protein content, total phenolic content, and antioxidant activities (ORAC and ABTS) for the <10 kDa fractions of GluH and TPH. Although both treatments showed similar amounts of soluble protein, GluH exhibited a 51 % higher concentration of phenolic compounds than TPH, resulting in a greater abundance of bioactive metabolites in the glutelin fraction.

Regarding antioxidant activity, GluH showed higher ORAC values (16.88 %) than TPH, treatments, whereas ABTS values were not statistically different between treatments. This difference between methods may be attributed to the chemical nature of the radicals assessed: ORAC measures the ability to neutralize peroxyl radicals, whereas ABTS measures the ability to act against cationic radicals (Munteanu & Apetrei, 2021). The higher ORAC activity in GluH may be related to the presence of phenolic compounds and low-molecular-weight antioxidant peptides. These findings align with previous studies reporting enhanced antioxidant bioactivity following protein hydrolysis in plant sources such as *Brassica napus* and lentil (Hernández-Jabalera et al., 2015; Rezvankhah et al., 2021).

Table 1. Soluble protein, total phenolic content, and antioxidant activity (ABTS and ORAC) of hydrolysates <10 kDa of GluH and TPH.
Tabla 1. Proteína soluble, contenido fenólico total y actividad antioxidante (ABTS y ORAC) de los hidrolizados <10 kDa de GluH y TPH.

Sample	Soluble protein (μg/mL)	Total phenolic content (μg/mL)	ORAC (μmol TE/mL)	ABTS (μmol TE/mL)
TPH	897.27 ± 34.71[a]	1746.45 ± 82.11[b]	15.4 ± 0.35[b]	1.62 ± 0.05[a]
GluH	953.18 ± 9.64[a]	2636.77± 55.63[a]	18.0 ± 0.2[a]	1.72 ± 0.18[a]

Values are expressed as mean ± standard deviation (SD) of three replicates. Different letters within the column for each type of protein hydrolysate indicate significant differences according to Tukey's HSD test ($p < 0.05$). GluH: hydrolysate of chickpea glutelin; TPH: hydrolysate of chickpea total protein.

3.2 Surface plasmon resonance (SPR) of Se nanoparticles

The UV-Vis spectra presented in Fig. 1 confirms the successful formation of Se nanoparticles (SeNPs) functionalized with chickpea proteins and their hydrolysates, with apparent differences

observed between intact proteins and hydrolyzed fractions. Selenium nanoparticles functionalized with (A) glutelin and (B) total protein displayed broader, less defined absorption bands, which is indicative of a heterogeneous size distribution and partial aggregation; whereas those functionalized with (C) glutelin hydrolysate and (D) total protein hydrolysate showed sharper, and more intense bands. These spectral features are consistent with the formation of smaller, more uniformly dispersed nanoparticles and improved colloidal stability.

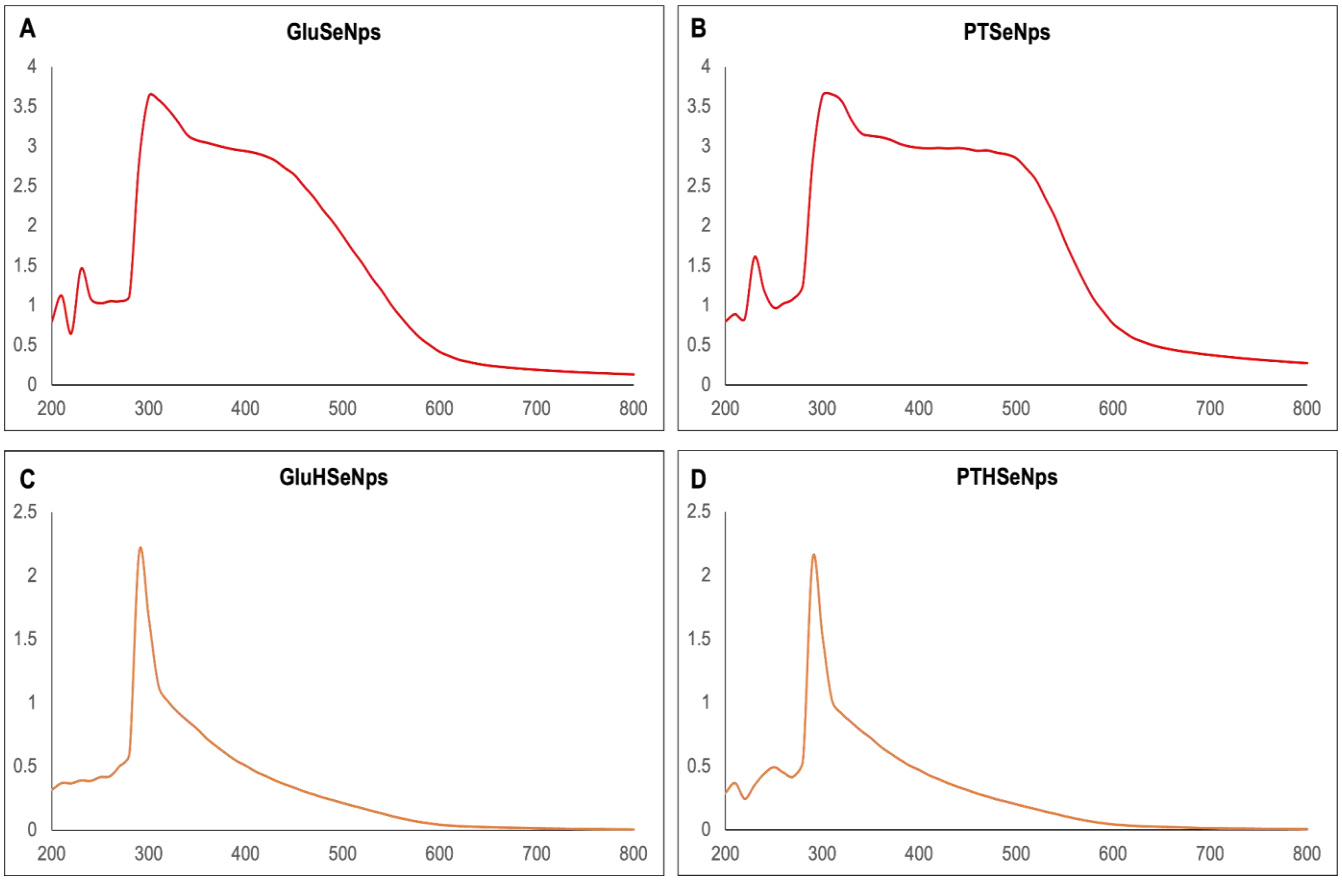

Figure 1. Surface plasmon resonance of Se nanoparticles functionalized with chickpea proteins and hydrolysates <10 kDa. (A) GluSeNPs: Se nanoparticles functionalized with chickpea glutelin, (B) PTSeNPs: Se nanoparticles functionalized with chickpea total protein, (C) GluHSeNPs: Se nanoparticles functionalized with hydrolysate of chickpea glutelin, (D) PTSeNPs: Se nanoparticles functionalized with hydrolysate of chickpea total protein.

Figura 1. Resonancia plasmónica superficial de nanopartículas de selenio funcionalizadas con proteínas y hidrolizados de garbanzo de menos de 10 kDa. (A) GluSeNPs: nanopartículas de selenio funcionalizadas con glutelina de garbanzo, (B) PTSeNPs: nanopartículas de selenio funcionalizadas con proteína total de garbanzo, (C) GluHSeNPs: nanopartículas de selenio funcionalizadas con hidrolizado de glutelina de garbanzo, (D) PTSeNPs: nanopartículas de selenio funcionalizadas con hidrolizado de proteína total de garbanzo.

These variations can be explained by the presence of low molecular weight peptides and phenolic compounds in the hydrolysates, which introduce additional functional groups (-OH, -COOH, amide) capable of coordinating with surfaces. This enhanced interaction reduces nanoparticle aggregation and stabilizes smaller particles. Similar stabilization mechanisms have been reported in green synthesis approaches. For instance, Alhawiti (2022) described SeNPs synthesized with citric acid and alginate, showing a distinct surface plasmon resonance and at 296 nm and high stability due to hydroxyl and carboxyl groups acting as capping agents. Likewise, Alagesan and Venugopal (2019) reported that SeNPs were obtained using a *Withania somnifera* extract, with an SPR maximum at 320 nm, in which flavonoids and tannins served as reducing and stabilizing agents, thereby enhancing antioxidant activity. Taken together, these findings suggest that chickpea hydrolysates act in a manner comparable to plant derived extracts or biopolymers, providing effective stabilization of SeNPs.

3.3 Antioxidant activity of SeNPs functionalized with proteins and hydrolysates <10 kDa

For the synthesis of nanoparticles using intact proteins (PT and Glu) and their respective hydrolysates (PTH and GluH), the concentrations of ascorbic acid, sodium selenite, protein, and pH conditions differed. Although a direct comparison of antioxidant activity between the two systems is not feasible, the activity of each component can be evaluated in relation to the synthesized nanoparticles. Fig. 2, panel A) shows the antioxidant activity of nanoparticles functionalized with <10 kDa PTH and GluH, together with ascorbic acid (4 mM), sodium selenite (1 mM), hydrolysates (600 μg/mL), and their combinations. Panel B) shows the antioxidant activity of nanoparticles functionalized with PT and Glu fraction, as well as their components: ascorbic acid (0.3 mM), sodium selenite (0.06 mM), 1 % (w/v) protein, and respective combinations.

The results showed that in both ORAC and ABTS assays, nanoparticles functionalized with <10 kDa PTH and GluH (PTHSeNPs and GluHSeNPs) exhibited 53 % higher antioxidant activity than ascorbic acid alone or in combination with PTH, GluH, and sodium selenite (Fig. 2, panel A). A similar trend was observed in the ABTS assay, where PTHSeNPs and GluHSeNPs displayed more than 21 % higher antioxidant activity compared to ascorbic acid and its combinations. Sodium selenite, in contrast, showed no antioxidant activity in either assay. PTH and GluH alone exhibited significantly lower activity than both nanoparticles and ascorbic acid (Fig. 2, panel A). These findings indicate that SeNPs functionalized with <10 kDa hydrolysates of PT and Glu generate nanostructures with enhanced antioxidant capacity, surpassing the properties of their hydrolysates and ascorbic acid. Furthermore, no significant difference was observed between the antioxidant activity of PTHSeNPs and GluHSeNPs.

Panel B of Fig. 2 shows the antioxidant activity, measured by ORAC and ABTS, of nanoparticles functionalized with TP and Glu fraction of chickpeas. In the ORAC assay, the highest activity was observed for combinations of ascorbic acid with PT and Glu, exhibiting up to 39.2 % higher activity than PTSeNPs and GluSeNPs. A similar trend was observed in the ABTS assay, where ascorbic acid and its combinations with protein fractions and sodium selenite, as well as PTSeNPs and GluSeNPs, showed the highest activities.

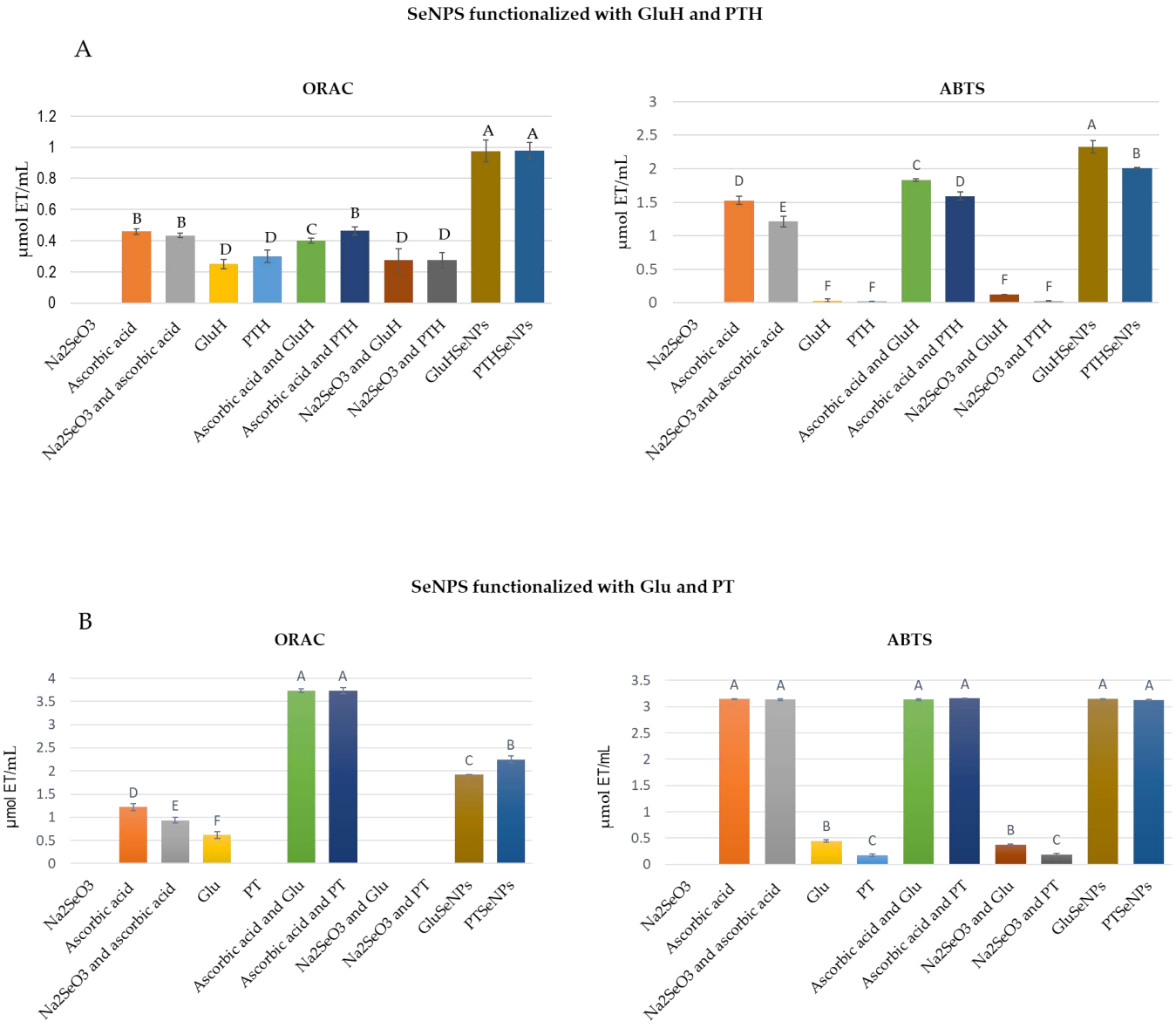

Figure 2. Antioxidant activity of SeNPs functionalized with proteins and hydrolysates <10 kDa. PT: Chickpea total protein, Glu: chickpea glutelin, PTH: Hydrolysate of PT, GluH: Hydrolysate of Glu, PTSeNPs: Se nanoparticle functionalized with PT, PTHSeNPs: Se nanoparticle functionalized with PTH, GluSeNPs: Se nanoparticle functionalized with Glu, GluHSeNPs: Se nanoparticle functionalized with GluH. The concentrations of the components correspond to the conditions used for nanoparticle synthesis: SeNPs with intact protein=Na_2SeO_3 0.06 M, ascorbic 0.3 M and protein 1 % (w/v), SeNPs with hydrolysates = Na_2SeO_3 1 mM, ascorbic acid 4 mM, hydrolysates 600 µg/mL.

Figura 2. Actividad antioxidante de las nanopartículas de selenio (SeNPs) funcionalizadas con proteínas e hidrolizados de menos de 10 kDa. PT: Proteína total de garbanzo, Glu: glutelina de garbanzo, PTH: hidrolizado de PT, GluH: hidrolizado de Glu, PTSeNPs: nanopartículas de Se funcionalizadas con PT, PTHSeNPs: nanopartículas de Se funcionalizadas con PTH, GluSeNPs: nanopartículas de Se funcionalizadas con Glu, GluHSeNPs: nanopartículas de Se funcionalizadas con GluH. Las concentraciones de los componentes

corresponden a las condiciones utilizadas para la síntesis de nanopartículas: SeNPs con proteína intacta = Na_2SeO_3 0,06 M, ácido ascórbico 0,3 M y proteína al 1 % (p/v); SeNPs con hidrolizados = Na_2SeO_3 1 mM, ácido ascórbico 4 mM, hidrolizados 600 μg/mL.

No significant differences were found among the samples. Notably, the concentration of ascorbic acid (0.06 mM) used in the synthesis of these nanoparticles was higher than that employed in the hydrolysate-based system, which may interfere with the measurement of the actual antioxidant activity of each component and nanoparticles. Therefore, to compare hydrolyzed and non-hydrolyzed chickpea proteins, it is necessary to establish a synthesis method under identical concentration conditions, as this study followed previously established protocols for SeNPs synthesis.

The use of peptides for the synthesis of selenium nanoparticles has been proven successful. Tang et al. (2020) developed a novel method for synthesizing selenium nanoparticles (SeNPs) by directly reducing sodium selenite with ascorbic acid and using tilapia polypeptides (TPs) as stabilizing agents. The main bonding forces of TPSeNPs were electrostatic and hydrophobic interactions. Ye et al. (2020) employed peanut flour peptides as stabilizing agents in SeNPs synthesis, obtaining particles of approximately 140 nm, with stabilization primarily attributed to electrostatic and hydrophobic interactions, as well as Se-O and Se-N bonds. In the case of intact proteins used as stabilizing agents, β-lactoglobulin has been successfully applied, yielding particle sizes of 36.8 ± 4.1 nm, with its NH_2 and OH functional groups responsible for binding to SeNPs (Zhang et al., 2018).

The use of proteins and peptides as stabilizing agents for SeNPs has been less studied than the use of polysaccharides (Shi et al., 2021). Chickpea proteins, particularly the glutelin fraction and its peptides, represent a novel alternative for synthesizing nanostructures with high antioxidant potential. However, further studies are needed to elucidate the interactions of chickpea protein and peptide functional groups with selenium, which remain undocumented, as well as to analyze the peptide profile.

3.4 Size, polydispersity index, and zeta potential of GluHSeNPs and TPHSeNPs

Table 2 summarizes the hydrodynamic diameter, polydispersity index (PdI), and zeta potential of SeNPs functionalized with hydrolyzed chickpea proteins. Notable differences were detected between GluH coated SeNPs and those functionalized with total protein (TPH).

The GluHSeNPs exhibited a markedly smaller average size (135.7 nm) compared to TPHSeNPs (1043.7 nm), indicating that glutelin peptides favored the formation of more compact and homogeneous nanoparticles. This finding aligns with previous reports indicating that glutelin fractions from chickpea sprouts accumulate higher levels of Se and yield more stable antioxidant hydrolysates (Serrano-Sandoval et al., 2019; Hernández-Grijalva et al., 2022). In contrast, the larger aggregates observed in TPHSeNPs suggest that the heterogeneous mixture of peptides in the total protein fraction promoted extensive cross linking and agglomeration, a phenomenon also described for biogenic SeNPs synthesized by *Bacillus subtilis* and *Azospirillum* species, where particle size varied widely depending on the biomolecular corona (Tugarova et al., 2018; Ullah et al., 2021).

The PdI values (0.41-0.45) indicate moderate polydispersity in both systems, consistent with other protein or plant mediated SeNPs, which typically exhibit broader distributions than chemically stabilized nanoparticles (Zhang et al., 2018). GluHSeNPs exhibited a more compact size distribution, supporting the idea that glutelin derived peptides act as more effective coating agents.

Regarding surface charge, both nanoparticle system types exhibited negative zeta potentials, with TPHSeNPs showing a higher magnitude (-12.9 mV) than GluHSeNPs (-9.1 mV). These values fall within the range reported for biogenic SeNPs stabilized by proteins or polysaccharides (-18 to -27 mV), which confer relative colloidal stability through electrostatic repulsion (Tugarova et al., 2018; Ullah et al., 2021). However, the less harmful potential of GluHSeNPs suggests that stability is not solely electrostatic but also involves steric hindrance from peptide adsorption, as previously observed in β-lactoglobulin SeNPs and peanut peptide SeNPs (Zhang et al., 2018; Ye et al., 2020).

Overall, these results show that glutelin hydrolysates are superior stabilizers compared to total protein hydrolysates, yielding smaller, more uniform SeNPs with adequate surface charge. This is consistent with the higher antioxidant and emulsifying activities reported for glutelin fractions enriched with Se (Serrano-Sandoval et al., 2019; Hernández-Grijalva et al., 2022).

Table 2. Size, polydispersity index (PdI), and zeta potential of GluHSeNPs and TPHSeNPs
Tabla 2. Tamaño, índice de polidispersidad (PdI) y potencial zeta de las GluHSeNPs y las TPHSeNPs

Sample	Z-average (d.nm)	PdI	Zeta Potential (mV)
GluHSeNPs	135.73 ± 12.21^{b}	0.45 ± 0.05^{a}	-9.12 ± 1.85^{b}
TPHSeNPs	1043.7 ± 44.40^{a}	0.41 ± 0.90^{a}	-12.87 ± 0.31^{a}

Values are expressed as mean ± standard deviation of three repetitions. Different letters within the column indicate significant differences among types of nanoparticles according to the Tukey HSD test ($p < 0.05$). PTHSeNPs: Se nanoparticle functionalized with hydrolysate of chickpea total protein, GluHSeNPs: Se nanoparticle functionalized with hydrolysate of chickpea glutelin.

4. Conclusions

This study demonstrates that the <10 kDa glutelin hydrolysate of chickpea is an effective biomaterial for the green synthesis and functionalization of SeNPs, enhancing their antioxidant activity. GluHSeNPs exhibited more than 50 % higher antioxidant activity compared to glutelin peptides. Nevertheless, several limitations remain. The characterization was restricted to physicochemical and antioxidant properties, without biological validation such as cytotoxicity or bioavailability assays. In addition, structural characterization techniques, including transmission or scanning electron microscopy (TEM/SEM) and FTIR spectroscopy, were not applied, leaving gaps in the understanding of nanoparticle morphology and protein–selenium interactions. Addressing these aspects in future work will be essential to strengthen the translational potential of SeNPs in nutraceutical and biomedical applications.

CRediT authorship contribution statement

M.C.Q.: Investigation, Formal analysis, Writing-original draft. S.N.S.S.: Investigation, Conceptualization, Data curation. A.K.M.N.: Data curation, Methodology. J.M.A.: Methodology, Data curation. D.G.F.: Conceptualization, Writing-original draft, Investigation, Project administration, Supervision, Writing-review & editing.

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Conflict of interest

The authors declare they have no conflicts of interest.

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