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Steeping and Germination as Technological Strategies to Enhance the Biofunctional Properties of Huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) Seeds for Metabolic Health Applications

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ABSTRACT

Despite its phylogenetic relationship to quinoa, one of the most extensively studied pseudocereals worldwide, huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) remains overlooked in terms of its nutritional composition and functional properties. This study demonstrated that cost-effective, scalable processing strategies for huauzontle seeds significantly enhance the biological activity of protein hydrolyzates derived from them. Protein fractions obtained from raw (H1), soaked (H2), and germinated (H3) seeds were extracted by alkaline solubilization and subsequently hydrolyzed with Alcalase®. SDS–PAGE revealed that soaking and germination increased endogenous proteolytic activity in seeds and improved the effectiveness of Alcalase-mediated enzymatic hydrolysis. In particular, germination deeply impacted the biological activity of H3 hydrolyzates, with higher scavenging capacities against DPPH (26.9%) and ABTS (93.1%) radicals and modulating ROS production in FHC and Caco-2 cells. These redox-modulating activities of H3 hydrolyzates were accompanied by a pronounced inhibition of lipid accumulation (60.07%) at 0.5 mg/mL. Likewise, H3 hydrolyzates (0.125 mg/mL) reduced nitric oxide production by 64.01% in RAW-246.7 cells. These findings identify germination as a low-cost strategy to unlock the functional potential of huauzontle proteins, positioning this neglected crop as a promising source of multifunctional bioactive peptides relevant to metabolic health and diversifying the pseudocereal portfolio with nutritional relevance.

1 | Introduction

Currently, 1 in 8 people in the world live with obesity (12.5%), by far exceeding predictions made by international organizations. It was estimated that 1 billion people would be affected by 2030; nonetheless, this quantity was surpassed as of 2022 [1, 2]. In the following years, all countries are expected to experience a significant rise in the number of obese adults. Unfortunately, countries with the highest obesity-increasing rates are those with the lowest health-system preparedness levels [3], signifying lower national investment in healthcare programs and research in obesity treatments and prevention.

Although obesity and overweight are multifactorial conditions [4], overall energy intake, food quality, and nutritional value have been strongly associated with their development and contribute to perpetuating the problem. It is generally accepted that a higher diet quality, rich in macro- and micronutrients, and

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adequate caloric intake are inversely proportional to body mass index (BMI) and fat storage [5–7]. Moreover, the world's rapidly increasing population poses a challenge in producing and distributing food that is both accessible and nutritionally adequate [8]. Identifying high-quality, affordable, and reliable sources of nutrients will be a crucial factor not only in a country's development but also in contributing to the population's food security.

Conventional strategies for weight loss focus mainly on caloric restriction [9] and modifications to macronutrient consumption [10]. However, recent research trends focus primarily on the quality of the food consumed [11, 12], highlighting the relevance of nutrient-dense nutrition high in lean protein and fiber, which promotes insulin sensitivity [13], satiety [14], and weight loss/maintenance [15]. Over the last decade, the search for new and diverse protein sources has been driven to meet this need, without compromising sustainability, leading to the development of plant-based foods that can complement and eventually replace animal proteins [16].

Among the strategies implemented to find accessible and sustainable plant-based protein sources, the adoption of traditional foods and food preparation practices has been highlighted [17]. Multiple studies have shown the relevance of re-evaluating endemic plants as food sources, highlighting the biological attributes available upon treatment and consumption, as well as the potential economic advantage in growing crops native to the area [18–21]. The Mexican endemic huauzontle (*Chenopodium berlandieri* subspecies *nuttalliae*) is a plant whose seed contains 16%–18% protein. This pseudocereal, native to central Mexico, is commonly consumed before its floral stage and has been attributed to multiple nutritional and medicinal properties [22]. Despite being taxonomically related to quinoa (*Chenopodium quinoa*) and possessing similar qualities [23], huauzontle has been consistently overlooked in scientific research.

In addition to their widely recognized nutritional value, plant-based proteins are known to possess high biological activity, attributed to the bioactive peptides they contain or can be obtained through enzymatic hydrolysis during digestion or as a pretreatment. Plant peptides are commonly 4–100 amino acids long [24, 25], as their small size attributes them an effect on multiple systems, including the digestive, nervous, and endocrine systems, as well as exhibiting drug- or hormone-like activities [26].

The difference between dormant and active (rehydrated) seeds has been a research focus in the past decade. Yu et al. [27] concluded that 260 proteins were differentially found in mung bean (*Vigna radiata*) sprouts than in seeds, attributing their presence to seed germination-related metabolism. Moreover, the diversity and concentration of peptides in germinated seeds were higher than those found in raw seeds. In different seeds and grains, the amount of protein and essential amino acids was found to increase during germination [28–30]. During seed germination, internal metabolic pathways promote enzyme activation, resulting in the storage of protein and carbohydrate degradation in the endosperm to meet the needs of the rapidly dividing cells [31]. This hydrolytic activity results in the generation or release of polyphenols and peptides smaller than 10 kDa, which exhibit enhanced bioactive properties [32, 33]. Previous studies have demonstrated the effect of steeping and germination of cereals and legumes on their protein composition, as well as their effect on metabolic diseases such as diabetes and obesity [30, 34, 35]. Therefore, this study will seek to elucidate the impact of steeping and germination of huauzontle seeds (*C. berlandieri* subspecies *nuttalliae*) on the antioxidant, anti-inflammatory, and anti-adipogenic activity of their protein hydrolyzates, obtained through enzymatic hydrolysis with alcalase. The obtained information will help identify plant-treating methodologies that promote the obtention not only of high-quality protein but also of biologically active compounds.

Huauzontle seeds are highly nutritious, serving as a powerful and economical source of plant protein, as well as calcium, potassium, and magnesium. Not only has its protein content been reported to be higher than that of amaranth (*Amaranthus* sp.) and quinoa (*C. quinoa* Willd) at 20.4, 17.3, and 12.4 g/100 g dry weight (DW), respectively [36], but it is also plentiful in essential amino acids. López-Monterrubio et al. [37] report essential amino acid values, except for valine, that are equal to or higher than those of amaranth and quinoa [38], highlighting the abundance of phenylalanine and leucine. Moreover, the protein content of huauzontle is similar to that of common bean (*Phaseolus vulgaris* L.), which contains 21.4 g/100 g DW, while being similarly rich in essential amino acids [39]. Differently, the content of the most important amino acids is significantly higher than that found in soybean (*Glycine max*), despite it having a higher protein concentration (35.8 g/100 g DW), demonstrating an improved nutritional profile for whole sources of protein [40].

In 2024, Mexico produced 7613 tons of huauzontle, representing over 36.2 million Mexican pesos (~2.01 million USD) [41]. Furthermore, huauzontle is known to have agronomic advantages, including adaptability to multiple climates and the ability to grow in harsh conditions, such as poor soil. Similarly, its production has been qualified as economically viable, regardless of the production magnitude [42]. Despite the above-mentioned information, relatively few researchers have explored its composition and developed techno-functional enhancements for human consumption. Evidently, for a food matrix with such a desirable nutritional profile, the amount of research conducted is poor; thus, the need to find economical strategies to enhance its nutritional profile and scientific divulgation to promote human consumption.

2 | Materials and Methods

2.1 | Sample Collection and Treatment

Huauzontle (*C. berlandieri* subsp. *nuttalliae*) seeds were collected from Atlixco, Puebla (8°54′29.3″N 98°26′10.1″W), during the harvest of 2022. Seeds recovered throughout the harvesting period were grouped into a pool after removal of contaminants and broken and diseased grains, as previously reported by Lazo-Vélez et al. [43] and Mejía-Valdez et al. [44]. Experimental groups consisted of raw seeds (H1), seeds soaked for 3 min and rinsed three times in water 1.5×10^{-5} MΩ·cm at 25°C (H2), and seeds germinated for 72 h in a commercial nebulizer germinator (EasyGreen, Canada), with water 1.5×10^{-5} MΩ·cm, 80% relative humidity at 22 ± 1°C (H3). Afterward, seeds were freeze-dried for further analysis.

2.2 | Huauzontle Seed Protein Extraction

Freeze-dried seeds were ground and sifted through a 0.150 mm pore size. The sifted flour was added to water at a 1 : 9 (w/v) ratio and stirred for 30 min at 200 rpm at 20°C. A protein extraction curve was performed with different pH levels (2–12) to identify optimal extraction of soluble proteins. An alkaline extraction was performed at pH 10 ± 0.05 with 1 M NaOH, with readjustments made manually every 5 min for 30 min [45] with 1 M NaOH and the help of a HI 2550 pH Meter (Hanna Instruments, USA). The liquid phase was recovered and centrifuged (10,000 rpm for 20 min at 4°C) with a Labnet Prism R Refrigerated Microcentrifuge (Labnet International Inc., USA). The supernatant was microfiltered using a 0.22 μm pore membrane and then underwent ultrafiltration in a Vivaflow 200 Laboratory Cross Flow Cassette (Sartorius Stedim Biotech, Germany), with a 5 kDa molecular weight (MW) cutoff. The ultrafiltration was carried out between 2.2 and 2.5 bar, with a flow rate of 300 mL/min and a membrane loading capacity of 200 mL, for 2.5 h.

2.3 | Protein Quantification

Protein quantification was performed via bicinchoninic acid (BCA) assay, according to Hueso et al. [46], with minor modifications. A total of 49 parts of BCA stock solution (Sigma-Aldrich, USA) were mixed with 1 part of a 4% $CuSO_4 \cdot 5H_2O$ (Alta Pureza Maquiladora, Mexico) solution to create the working solution. In 96-well plates, 48 μL of sample was added to 208 μL of freshly prepared BCA working solution, and the absorbance was measured at 562 nm after a 30-min incubation at 37°C in a Cytation 5 imaging reader (BioTek-Agilent, USA). Absorbance results were compared to the bovine serum albumin calibration curve expressed as mg protein/mL ($y = 1.26x + 0.207$, $R^2 = 0.996$).

2.4 | Protein Enzymatic Hydrolysis

Protein hydrolysis was carried out with Alcalase (≥ 2.4 U/g) in the form of protease from *Bacillus licheniformis* (Sigma-Aldrich, USA) at a 1 : 90 enzyme : substrate (w/w) ratio, as described by [47]. Alcalase concentration was similarly measured using the BCA assay to determine enzyme mass per unit of volume. The pH was initially adjusted to 8 ± 0.05 with 1 M NaOH, and further pH corrections were made every 10 min, as reported in Section 2.2. The reaction was carried out for 180 min at 60°C, after which the temperature was increased to 100°C for 5 min to inactivate the enzyme. Different analyses were performed for protein extracts of raw (H1P), steeped (H2P), and germinated (H3P) huauzontle, as well as the resulting hydrolyzates: H1h, H2h, and H3h, respectively.

2.5 | Protein Hydrolyzate Desalting and Drying

To minimize impurities in the hydrolyzates, a fast protein liquid chromatography (FPLC) was performed using an ÄKTA Pure (GE Healthcare, USA) system, equipped with a HiTrap™ Desalting column (Sigma-Aldrich, USA). A volume of 1.5 mL of the sample was injected. The absorbance was read at 280 nm to detect fractions abundant in protein, and the conductivity was measured to detect fractions high in salts. Fractions exhibiting high absorbance at 280 nm and low conductivity were collected. After freezing the recovered fractions at −80°C, lyophilization was performed using a FreeZone® 2.5 (Labconco, USA) freeze dryer at 0.045 mbar and −50°C for 72 h.

2.6 | Degree of Hydrolysis (DH)

The ninhydrin method for determining free alpha amino nitrogen (AAN) [48] was used to determine the DH. About 2 mL of a 1 mg/mL sample was mixed with 1 mL of a 0.5% (w/v) ninhydrin solution (Amresco, USA), set in a water bath at 95°C for 16 min, and cooled down at 20°C for 20 min. Afterward, 5 mL of a 0.2% (w/v) potassium iodate (KIO_3, Meyer, Mexico) solution were added, and 200 μL of the resulting solution were transferred to a 96-well plate, in triplicate. Absorbance was read at 570 nm. AAN ((mg)/(gsample)) was calculated as follows:

$$\mathrm{AAN}\left(\frac{\mathrm{mg}}{\mathrm{gsample}}\right) = \frac{\left(\mathrm{Abs}_s \cdot 2 \cdot \mathrm{DF}\right)/\left(\mathrm{Abs}_{\mathrm{gly}}\right)}{\mathrm{Sample\,concentration\,(g/L)}}, \quad (1)$$

where Abs_s is the absorbance of the sample, $\mathrm{Abs}_{\mathrm{gly}}$ is the absorbance of the glycine working solution, and DF is the dilution factor. The DH was calculated as follows [49]:

$$\mathrm{DH}(\%) = \frac{\mathrm{AAN}_h - \mathrm{AAN}_P}{\mathrm{AAN}_P} \cdot 100, \quad (2)$$

where AAN_h is the AAN ((mg)/(gsample)) of the hydrolyzed sample and AAN_P is the AAN ((mg)/(gsample)) of the raw protein.

2.7 | Low MW Electrophoretic Profile

To effectively observe low-molecular-weight proteins, a Tricine Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed as described by Schägger (2006), using the conditions reported by Briceño-Islas et al. [50]. Essentially, a 16% gel was created using a Mini-Protean® Handcast System (BioRad, USA). The gel was loaded with 30 μg of protein or protein hydrolyzate samples, and a Polypeptide SDS-PAGE (161-0326) MW was used (BioRad, USA). Electrophoresis was carried out at 100 V for 10 min, followed by 140 V for 1.5 h. The gel was fixed with a 40% methanol and 10% glacial acetic acid solution for 30 min, followed by 12 h of staining in a solution containing 0.05% Coomassie Blue R250 (Sigma-Aldrich, USA), 40% methanol, and 10% glacial acetic acid.

2.8 | Evaluation of Biologic Activities of Protein Hydrolyzates Obtained From Huauzontle Seeds

2.8.1 | α-Amylase Inhibition Assay

In a test tube, 500 μL of samples (0.1–1 mg/mL), positive control (c+), consisting of 1 mM acarbose (Sigma-Aldrich, Germany), or negative control (c−), consisting of $NaH_2PO_4 \cdot H_2O$ 20 mM and NaCl 6.7 mM, were added to 500 μL of 3 U/mL α-amylase (Sigma-Aldrich #A6255, USA) and incubated 10 min at 37°C in constant shaking at 100 rpm. A 1% starch solution (JT Baker, USA) was added and incubated for an additional 10 min in the same conditions. A 96 mM 3,5-dinitrosalicylic acid (DNS) solution was added to achieve a final volume of 2.5 mL. Afterward, the tubes were placed in water at 95°C for 5 min [51]. After cooling to room temperature, the samples were diluted with distilled water at a 1 : 1 v/v ratio. In a 96-well plate, 200 μL of sample, positive control, or negative control was added. The phosphate buffer was

used as a blank, and the plate was read at 540 nm. Results were expressed as α-amylase inhibition percentage, as

$$\alpha - \text{amylase inhibition}(\%) = \left(\frac{\text{Abs}_{C-} - \text{Abs}_{\text{sample}}}{\text{Abs}_{C-}}\right) * 100, \quad (3)$$

where Abs_{C-} is the mean absorbance of the negative control group, and $\text{Abs}_{\text{sample}}$ is the absorbance of each sample.

2.8.2 | 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging

In a 25 mL volumetric flask, 1.5 mg of DPPH (Sigma-Aldrich, USA) was added and diluted with 96% ethanol. Samples were adjusted to final concentrations of 0.1–1 mg/mL, using distilled water. In a 96-well plate, samples or standards (20 μL) reacted with 200 μL of DPPH solution. Then, the plate was covered with parafilm to prevent solvent volatilization, incubated in dark conditions, and read at an absorbance of 520 nm after 90 min [52].

As a standard, 6-hydroxy-2,5,7,8-tetramethylchromium-2-carboxylic acid (Trolox, Sigma-Aldrich, USA) was used in concentrations ranging from 50 to 800 μM. As blanks, 200 μL of distilled water and 20 μL of methanol were used, whereas the control (c) consisted of 200 μL of DPPH and 20 μL of methanol. Results were reported as inhibition percentage, calculated as

$$\text{DPPH radical scavenging}(\%) = \left(\frac{\text{Abs}_c - \text{Abs}_{\text{sample}}}{\text{Abs}_c}\right) * 100, \quad (4)$$

where Abs_C is the mean absorbance of the control group, and $\text{Abs}_{\text{sample}}$ is the absorbance of each sample.

2.8.3 | 2,2′-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Radical Scavenging

Five milliliters of a 7 mM ABTS (Sigma-Aldrich, USA) solution were mixed with 88 μL of a 140 mM $K_2S_2O_8$ solution (ChemCruz, USA) and stored for 12 h in a dark place (Solution A). Afterward, 0.5 mL of Solution A was added to 20 mL of 96% ethanol and mixed. The absorbance of this solution was measured at 734 nm, and additional ethanol was added until the absorbance fell between 0.7 and 1.0 optical density, therefore obtaining Solution B [21].

In dark conditions, blanks and controls were added to a 96-well plate as follows: Blank: 230 μL ethanol + 20 μL methanol; Control: 230 μL Solution B + 20 μL methanol. A 20 μL volume of samples (0.1–1 mg/mL) or Trolox dilutions (50–800 μM) was mixed with 230 μL of Solution B. The plate was covered with parafilm to avoid solvent volatilization, and it was read at an absorbance of 734 nm at 0 and 6 min. Results were reported as inhibition percentage, calculated as

$$\text{ABTS radical scavenging}(\%) = \left(\frac{\text{Abs}_c - \text{Abs}_{\text{sample}}}{\text{Abs}_c}\right) * 100, \quad (5)$$

where Abs_C is the mean absorbance of the control group and $\text{Abs}_{\text{sample}}$ is the absorbance of each sample.

2.8.4 | Cellular Antioxidant Activity (CAA)

The CAA of the protein hydrolyzates was measured as reported by Serrano-Sandoval et al. [53]. Human epithelial colonic cells (FHC, passages 17–19) and human colorectal adenocarcinoma cells (Caco-2, passages 37–38) (American Type Culture Collection, USA) were seeded at 5×10^4 cells per well in a 96-well plate, using Dulbecco's Modified Eagle's Medium (DMEM)-F12 (Gibco Invitrogen, USA), supplemented with 5% fetal bovine serum (FBS; Gibco Invitrogen, USA) and 1% penicillin/streptomycin (Gibco Invitrogen, USA), and incubated at 37°C and 5% CO_2 for 24 h. Cell viability assays were performed to determine non-cytotoxic treatment concentrations as described by Peña-Vázquez et al. [54].

After washing with sterile phosphate buffer saline (PBS), the cells were treated with 200 μL of protein hydrolyzates at concentrations ranging from 0.0625 to 0.5 mg/mL and 60 μM of dichlorodihydrofluorescein diacetate (DCFH-DA, Sigma-Aldrich, USA) and then incubated at the above-mentioned conditions for 20 min. The treatments (T) were washed twice with sterile PBS, and 100 μL of 500 μM of 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH, Sigma-Aldrich, USA) were added to promote reactive oxygen species (ROS) production. The positive control (c+) was treated with 60 μM DCFH-DA and 500 μM AAPH, whereas the negative control (c−) was treated only with AAPH. Fluorescence was measured at 538 nm after an excitation of 485 nm every 2 min for 2 h at 37°C. The CAA was calculated as

$$\text{CAA}(\%) = 1 - \frac{T}{TC}, \quad (6)$$

where CAA is the CAA, $\int T$ is the integral of the curve created with the treatment measurements throughout 2 h, and $\int C$ is the integral of the curve created with the positive control (c+) measurements.

2.8.5 | Anti-Adipogenic Effects of Huauzontle Seed Protein Hydrolyzates

2.8.5.1 | Cell Viability Assessment of Protein Hydrolyzates on Murine Pre-Adipocytes (3T3-L1). The viability of cells after exposure to protein hydrolyzates was conducted in murine pre-adipocyte cells (3T3-L1, passage 31, American Type Culture Collection, USA), as explained by Peña-Vázquez et al. [54]. Cells were seeded in 96-well plates at a density of 2000 cells/well. The wells were filled with 200 μL of a cell suspension prepared in Dulbecco's Modified Eagle Medium Nutrient Mixture F12 (DMEM/F12, Sigma Aldrich, USA) supplemented with 10% FBS (Gibco Invitrogen, USA) and 1% penicillin–streptomycin (Gibco Invitrogen, USA), containing protein hydrolyzate treatments (0.1–1 mg/mL). Untreated cells were used as a control (C). After incubating for 48 h at 37°C and 5% CO_2, the medium was replaced with 100 μL of prepared DMEM-F12 + 20 μL of CellTiter 96 AQueous One Solution (Promega, USA). Plates were incubated for 45 min, and absorbance was read at 490 nm. Results were expressed as cell viability using the equation as follows:

$$\text{Cell viability}(\%) = 1 - \left(\frac{\text{Abs}_C - \text{Abs}_{\text{sample}}}{\text{Abs}_C}\right) * 100, \quad (7)$$

where Abs_C is the mean absorbance of the control cells (untreated) and $\text{Abs}_{\text{sample}}$ is the absorbance of each sample.

2.8.5.2 | Adipogenesis Attenuation Assay. The adipogenic attenuation potential of huauzontle samples was assessed by measuring inhibition of lipid accumulation using Oil Red O (Sigma-Aldrich, USA). Pre-adipocytes (3T3-L1, passage 31) were seeded as explained in Section 2.8.5.1. Before and during the differentiation process, cells were treated with different concentrations (0.1–1.0 mg/mL) of the huauzontle protein hydrolyzates. Untreated cells were used as the positive control. Cell differentiation was carried out as reported by Luna-Vital et al. [55] with minor modifications. After 48 h of incubation, the cells were grown with Differentiation Medium I (DMI) for an additional 48 h at 37°C and 5% CO_2, along with the respective protein hydrolyzates, for a total of 96 h. DMI consisted of DMEM-F12 supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (Sigma-Aldrich, USA), 0.25 μM dexamethasone (Sigma-Aldrich, USA), 2 μM rosiglitazone (Sigma-Aldrich, USA), and 10 μg/mL insulin (Sigma-Aldrich, USA). Afterward, cells were grown in Differentiation Medium II (DMII) for 48 h in similar conditions. DMII consisted of DMEM and 10 μg/mL insulin. Finally, the cells were grown for 2 days in DMEM-F12 with the treatments, followed by 5 more days in DMEM-F12 (Figure 1).

Cells were fixed with a 10% formalin solution, permeated with a 60% isopropyl alcohol solution, and dyed with Oil Red O (ORO, Fluka Chemicals, UK), as described by Kaczmarek et al. [56]. After removing excess dye four times with distilled water, the absorbance was read at 492 nm, and the lipid accumulation inhibition was calculated as

$$\text{Lipid accumulation inhibition}(\%) = \left(\frac{\text{Abs}_C - \text{Abs}_{\text{sample}}}{\text{Abs}_C}\right) \cdot 100, \quad (8)$$

where Abs_C is the mean absorbance of the control cells (untreated) and $\text{Abs}_{\text{sample}}$ is the absorbance of each sample.

2.8.6 | Anti-Inflammatory Potential Measured by Nitric Oxide (NO) Release

Murine macrophage cells (RAW-246.7, passage 16, American Type Culture Collection, USA) were seeded in 96-well plates at a density of 5×10^4 cells per well and incubated as stated in Section 2.8.5. Cells were treated to achieve a final concentration ranging from 0.03125 to 0.125 mg/mL of huauzontle seed protein hydrolyzates and incubated for 4 h. Afterward, 50 μL of *Escherichia coli* lipopolysaccharide (LPS, L4516, Sigma-Aldrich, USA) at a final concentration of 10 μg/mL was added. Cells treated with nonstimulated LPS samples were used as sample controls to clearly differentiate the specific effect on cell NO production from the impact of LPS stimulation. Likewise, untreated cells stimulated with LPS were used as a positive control (c+) representing the maximum (100%) NO production, while untreated cells nonstimulated with LPS were considered as a negative control (c−). Treated cells were incubated for 18 h, after which 100 μL of the medium was recovered for NO quantification, whereas the cells and remaining medium were used to perform a cell viability assay [57]. The detection of nitrites was performed with Promega's Griess Reagent System (Promega, USA), according to manufacturer instructions. Samples were measured at 550 nm and compared to a calibration curve ranging from 0.78 to 50 μM NO_2^-. The cell viability assay was performed as previously stated in Section 2.8.5.1, and only concentrations with viability ≥ 90% were included in this study.

2.9 | Experimentation and Statistical Analysis

All tests were performed at least in triplicate. After confirming the normality of the data, ANOVA tests and subsequent Tukey's HSD post hoc tests were performed with a 95% confidence level, with the help of the statistical software JMP. Results are expressed as mean ± standard deviation.

3 | Results and Discussion

3.1 | Steeping and Germination Modify the Protein Profile

The soluble protein concentration, measured across multiple pH intervals, is shown in Appendix A. H1 samples had a statistically greater ($p < 0.05$) soluble protein concentration than H2 and H3 samples, which account only for 12.52%–53.05% and 19.68%–46.62% of H1 protein levels. The highest extractable soluble protein levels were detected at pH 12, resulting in 6.78 ± 0.07, 2.30 ± 0.07, and 2.82 ± 0.24 mg/mL for H1, H2, and H3 samples, respectively. Nonetheless, at pH 10, relevant protein levels were extracted: 5.57 ± 0.61, 0.86 ± 0.01, and 1.09 ± 0.03 mg/mL for H1, H2, and H3, respectively. Protein extraction at pH levels higher than 10 can alter the charge, three-dimensional structure, and/or solubility of the desired proteins [58], potentially leading to altered biological activities. A similar soluble-protein extraction tendency was observed by Rueda et al. [59] for *C. quinoa* wild raw seeds.

After hydrolysis and purification, the protein hydrolyzate concentration was measured and reported as a percentage (w/w) (Table 1). H1 seeds had a significantly higher ($p < 0.05$) soluble protein concentration and content than H2 or H3 samples, whereas there was no statistical difference between H2 and H3 protein content. Lower protein concentrations in both samples could be the result not only of protein hydrolysis but also of usage upon steeping and germination [26]. While raw seeds (H1) presented more than the protein content reported in the literature of 15.92 ± 0.12–20.4 ± 0.3% on a DW basis [22, 37], steeped (H2) and germinated (H3) seeds had less than half of the reported content. The DH assay (Table 2) was calculated with the AAN concentration (Appendix B) and demonstrated that H1 samples had a significantly higher ($p < 0.05$) protein hydrolysis, resulting in a larger amount of surface peptide bonds [60]. This explains why a quantification with BCA could overestimate the presence of proteins, as it is based on the reduction of Cu^{2+} ions into Cu^{1+} by peptide bonds [61, 62].

The Tricine–SDS-PAGE gel showed low MW proteins, especially in protein hydrolyzates (Figure 2). In the cases of raw (H1), steeped (H2), and germinated (H3) protein extracts, clear and separated bands can be observed. The DH was consistent with SDS-PAGE results, where an increase in DH was reflected by the decreased expression of intact protein bands in hydrolyzed samples (H1h, H2h, and H3h), accompanied by the presence of low-molecular-weight bands (< 3.5 kDa). This confirms the extensive proteolysis in the hydrolyzates. H1 shows two prominent bands larger than 26.6 kDa, as well as multiple less prominent bands, while also having noticeable bands smaller than 26.6 kDa.

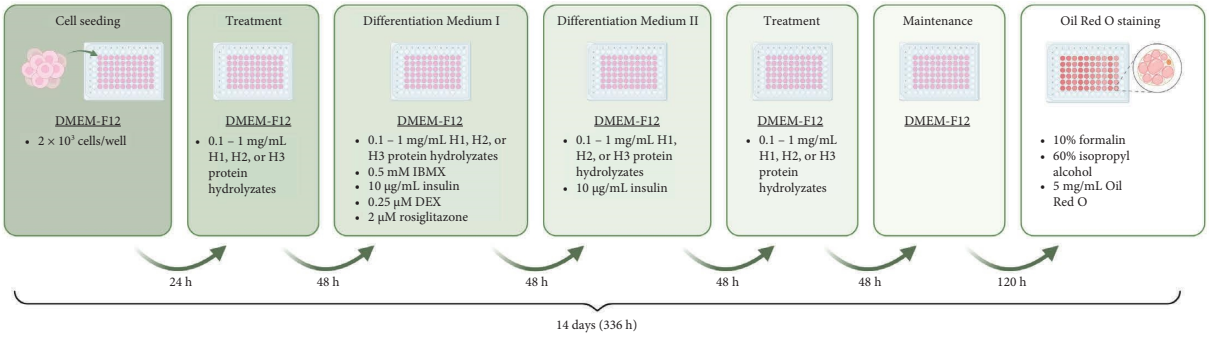

FIGURE 1 | Methodology for murine pre-adipocyte (3T3-L1) differentiation into adipocytes for lipid accumulation inhibition assay, associated media composition, and treatments. DEX = dexamethasone, IBMX = 3-isobutyl-1-methylxanthine.

TABLE 1 | Protein hydrolyzates concentration extracted from raw (H1), steeped (H2), and germinated (H3) huauzontle seeds (*Chenopodium berlandieri* subsp. *nuttalliae*) in a dry base.

Sample	Protein content (% w/w)
H1	22.23 ± 0.44^{a}
H2	7.82 ± 0.44^{b}
H3	7.97 ± 0.44^{b}

Note: Data expressed as mean ± SD ($n = 3$).
[a,b]Different letters indicate statistically significant differences ($p < 0.05$) among treatments using a Tukey test.

TABLE 2 | Alpha amino nitrogen content and degree of hydrolysis per sample of huauzontle seeds (*Chenopodium berlandieri* subsp. *nuttalliae*).

Sample	DH (%)
H1h	97.70 ± 4.64^{a}
H2h	77.10 ± 12.42^{b}
H3h	82.94 ± 3.90^{ab}

Note: Data expressed as mean ± SD ($n = 3$).
Abbreviations: DH = degree of hydrolysis, H1h = raw seed hydrolyzates, H2h = steeped seed hydrolyzates, H3h = germinated seed hydrolyzates.
[a,b]Different letters indicate statistically significant differences ($p < 0.05$) among treatments using a Tukey test.

The findings support the work of López-Monterrubio et al. [37], who reported the presence of five primary bands from huauzontle seed flour (HSF), all of them smaller than 75 kDa, as well as other less noticeable bands. The case is different for H2 and H3 samples, which demonstrate less noticeable bands at similar MWs but highly concentrated bands at MWs smaller than 17 kDa. As they had high degrees of hydrolysis (Table 2), hydrolyzed samples H1h, H2h, and H3h show diffuse hydrolyzate bands (as opposed to defined bands), falling in between 1.4 and 3.5 kDa. The diminished appearance of H3h, followed by H2h, could indicate a higher amount of protein susceptible to hydrolysis in H1 samples as a consequence of no prior treatment [63].

H1P shows one band of approximately 45 kDa and another band of approximately 32 kDa. Multiple sources have attributed these bands to globulins in *C. quinoa* [64], a close member of the *Chenopodium* genus, highlighting the presence of globulin 11S [65, 66], a major storage protein. Moreover, the band observed at 45 kDa has been recognized as chenopodin in quinoa protein electrophoretic profiles [63]. These bands appear similarly on H2P and H3P, although with reduced intensity, likely reflecting the progressive degradation of storage proteins during germination. Moreover, H2P and H3P exhibit two distinct bands between 17 and 14 kDa that are absent in H1P, suggesting the emergence of smaller peptide fragments or the expression of *de novo* proteins associated with germinative stages. Three major bands are noticeable at around 3.5 kDa, which supports the hypothesis proposed by Tang et al. [30], where the process of germination and, consequently, steeping promotes a natural protein hydrolysis, resulting in a concentrated pool of hydrolyzates with smaller MWs. Moreover, the bands in H2P and H3P below 11 kDa are evidently thicker, indicating a higher concentration of protein hydrolyzates. While H2P and H3P have a similar protein profile, it is evident that H3P bands appear to be less noticeable, suggesting that the germination process is more effective in inducing natural protein hydrolysis at the expense of larger storage proteins.

3.2 | Evaluation of Biologic Activities of Protein Hydrolyzates Obtained From Huauzontle Seeds

Alcalase specifically breaks down hydrophobic bonds [67], generating peptides with low MWs and a hydrophobic amino acid composition [68, 69]. In consequence, protein hydrolyzates created via Alcalase hydrolysis can withstand gastrointestinal conditions and/or experience minimal degradation of bioactive domains [70], as there are no further cleavage sites upon which digestive proteases can act. Studies have demonstrated that after in vitro gastrointestinal digestion simulation, alcalase-derived protein hydrolyzates maintained their antioxidant activity [67, 71], α-glucosidase and dipeptidyl peptidase IV (DPP-IV) inhibitory activity [72], and angiotensin-converting enzyme (ACE) inhibitory activity [68–70], among others.

3.2.1 | α-Amylase Inhibition Assay

Results revealed that at the lowest concentration (0.1 mg/mL), huauzontle seed protein hydrolyzates showed the highest inhibitory effect, with significant differences between treatments H1 and H2 and also between H1 and H3 (Figure 3). These results suggested that both steeping (H2) and germination (H3) decrease the α-amylase inhibitory activity of huauzontle seeds. As protein sample concentrations increased, the inhibitory effect decreased.

Suárez-Estrella et al. [73] obtained supporting results in *C. quinoa* Wild, as germinated samples presented a decreased

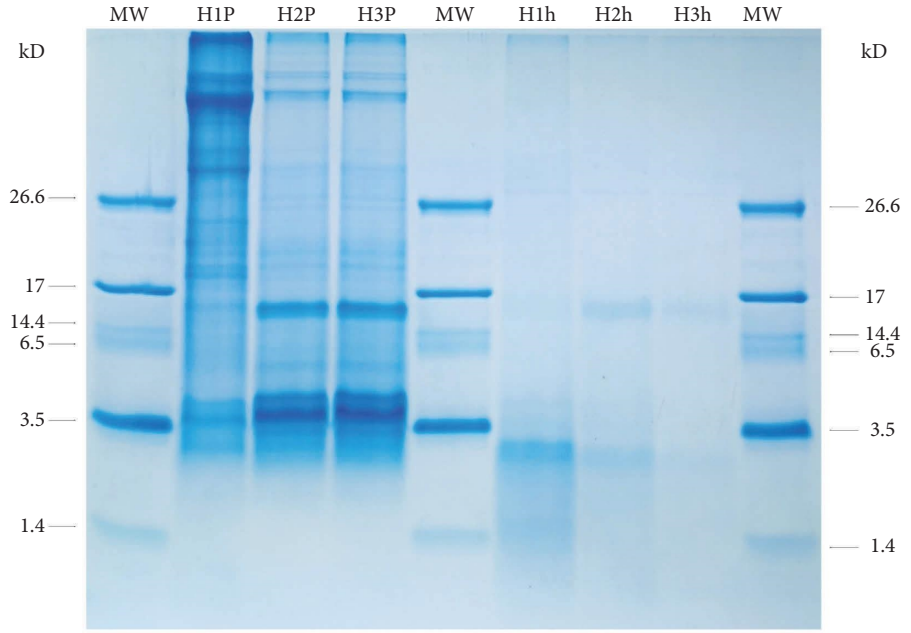

FIGURE 2 | Tricine–SDS-PAGE of protein concentrates of raw (H1P), steeped (H2P), or germinated (H3P) huauzontle seeds, protein hydrolyzates of raw (H1h), steeped (H2h), or germinated (H3h) huauzontle seeds, and molecular weight (M2).

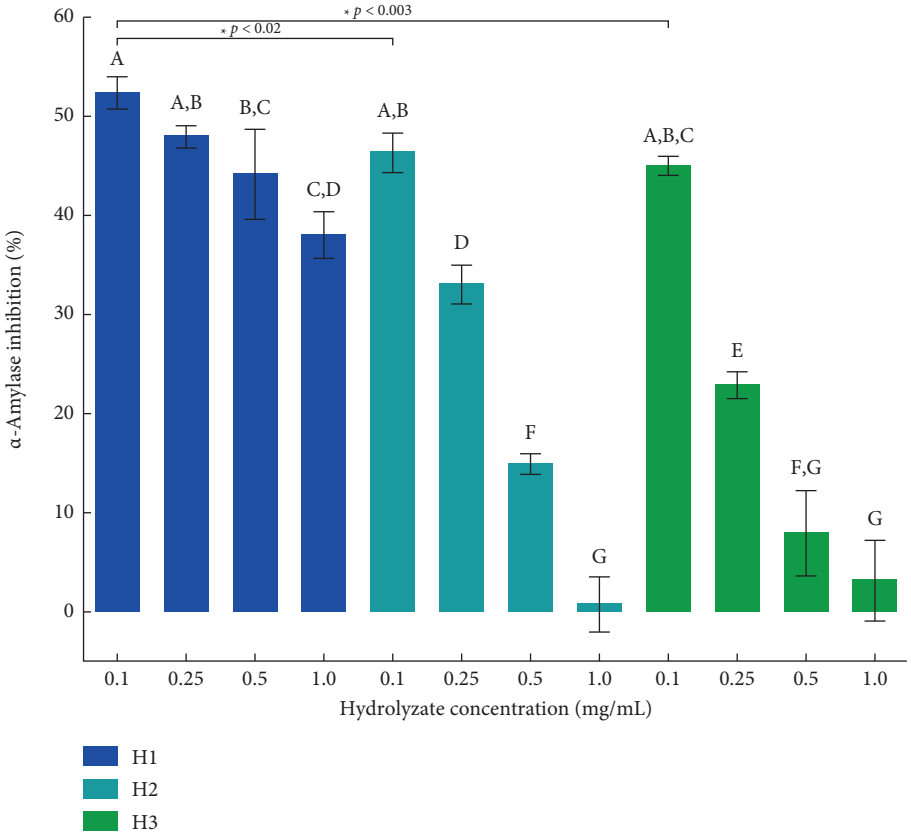

FIGURE 3 | Inhibition of α-amylase activity induced by huauzontle seeds (*Chenopodium berlandieri* subsp. *nuttalliae*) protein isolates from raw seeds (H1), steeped seeds (H2), or germinated seeds (H3) at different concentrations (0.1–1.0 mg/mL). $^{A–G}$Different letters indicate statistically significant differences ($p < 0.05$) among treatments using the Tukey test; *statistically significant differences ($p < 0.05$) between two samples in a two-sample *t*-test ($n = 3$).

α-amylase inhibitory activity, whereas raw and steeped seeds had a statistically significant increase in α-amylase activity inhibition. These results are attributed to the fact that during germination, protein and starch hydrolysis occur to provide the rapidly growing cells with sufficient nutrients. Therefore, it is only evident that α-amylase-inhibiting peptides would be

counterproductive in the seed germination process. In contrast, Zhou et al. [74] not only confirm that hydrolyzed components (< 3 kDa) exhibit significantly higher inhibitory activity but also demonstrate that peptide isolation can lead to an inhibition of up to 66.41% of the enzymatic activity. Thus, future huauzontle peptide isolation could potentially yield better inhibitory yields. While some peptides might have significantly higher inhibitory activity, the process through which they are isolated might not be time- and cost-effective.

3.2.2 | In Vitro Antioxidant Activity of Chickpea Protein Hydrolyzates

The type of sample and the dose influenced both the ABTS and DPPH scavenging potential ($p < 0.001$), in a dose–response manner (Figure 4). Steeping (H2h) and germination (H3h) had a significantly higher DPPH-scavenging capacity than the raw sample (H1h). When compared to H2h and H3h at 1 mg/mL, H1h's activity only accounted for 34.7% and 27% of their activity, respectively. A Tukey test revealed that H3h (1 mg/mL) exhibited the statistically highest inhibitory potential of all samples, at 26.99 ± 1.73%. The case is similar for H2 (1 mg/mL), where, despite having a lower effect than H3 at the same concentration, it is statistically different ($p < 0.05$) from the rest of the samples.

At 1 mg/mL, H1h, H2h, and H3h had ABTS scavenging potentials of 83.6 ± 1.06%, 86.9 ± 1.22%, and 93.1 ± 0.22%, respectively (Figure 5). Even though a Tukey test groups the three samples at 1 mg/mL in the same effect category, a paired *T*-test revealed that H3h had a significantly higher ($p < 0.001$) scavenging potential than H1h or H2h, whereas H2h also had a statistically higher ($p = 0.02$) effect than H1h. These findings demonstrate the improved antioxidant capacity resulting from protein modification and hydrolysis during seed germination and, to a lesser extent, steeping.

Quinoa (*C. quinoa*) protein hydrolyzates were identified to scavenge DPPH free radicals, reaching a maximum scavenging activity of 67.8% [75] and 20.0 ± 4.0% [59], depending on the hydrolysis time. This accounts for more than double the inhibitory activity observed in germinated huauzontle seeds (H3) at 1 mg/mL. Despite it being a different kind of cereal, a study performed by Hernández-García et al. [35] revealed that germinated amaranth (*Amaranthus hypochondriacus* L.) peptides could reach a DPPH scavenging potential of 85.04 ± 1.49% after fermentation with *Lactobacillus*. The type of protease has been reported to affect the restriction site and size of the resulting hydrolyzates, consequently affecting their biological activity [76]. Notably, a higher DPPH scavenging potential can be achieved through various treatment methodologies.

The positive effect of protein hydrolysis in antioxidant activity was proved by Serena-Romero et al. [77] and Chirinos et al. [78],

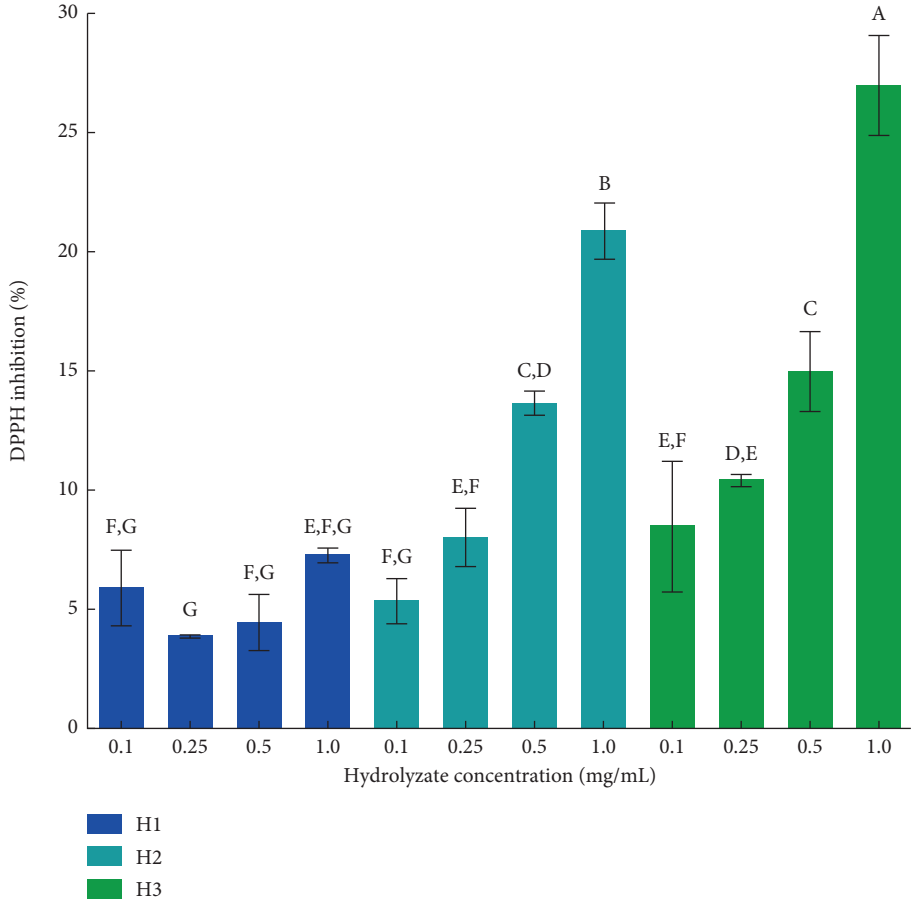

FIGURE 4 | DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenging potential of raw (H1), steeped (H2), or germinated (H3) huauzontle seeds (*Chenopodium berlandieri* subsp. *nuttalliae*) protein hydrolyzates. $^{A–G}$Different letters indicate statistically significant differences ($p < 0.05$) among treatments using the Tukey test ($n = 3$).

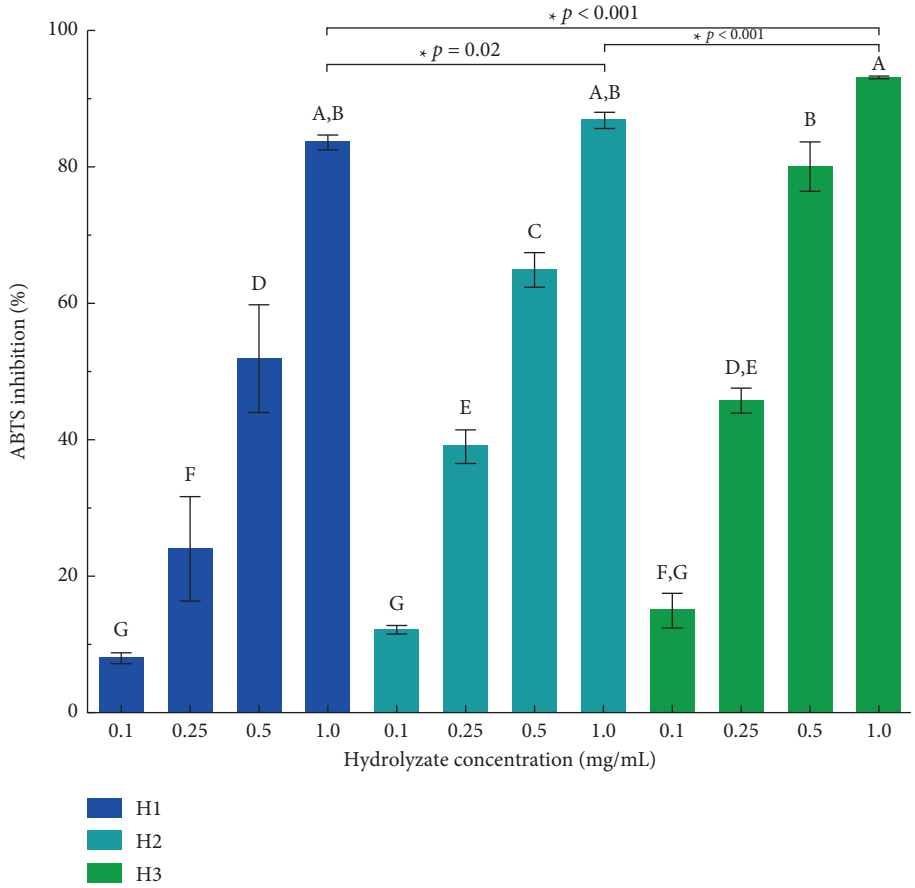

FIGURE 5 | ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging potential of raw (H1), steeped (H2), or germinated (H3) huauzontle seed protein hydrolyzates. [A–G]Different letters indicate statistically significant differences ($p < 0.05$) among treatments using the Tukey test; [*]statistically significant differences ($p < 0.05$) between two samples in a two-sample t-test ($n = 3$).

who performed an in vitro digestion of *Chenopodium pallidicaule* Aellen and *C. quinoa* with alcalase, respectively. Both studies demonstrate that longer digestion times result in improved antioxidant potential due to enhanced release of peptides and amino acids. In comparison, Hernández-García et al. [35] reported a maximum inhibitory potential of amaranth peptides of 76% when exposed to 0.1 mL of 0.28 ± 0.038 mg/mL of soluble protein. The scavenging potential of huauzontle hydrolyzates proves to be higher than that of amaranth peptides.

Seed germination (H3) yields an improved protein hydrolyzate profile compared to steeping (H2) and the control, raw seeds (H1), resulting in a higher antioxidant capacity. Evidently, huauzontle protein hydrolyzates, regardless of their treatment, have a higher scavenging effect for ABTS radicals than for DPPH radicals. Chen et al. [79] have attributed this phenomenon to the media in which inhibitory assays take place. As ABTS is water-soluble, hydrophilic peptides can interact directly with ABTS radicals. In contrast, the DPPH assay is typically performed in an organic solvent, making it suitable for hydrophobic peptides, which tend to be a minority. Moreover, studies have demonstrated that lower MW peptides (< 3 kDa) show a higher scavenging potential for ABTS radicals, as opposed to DPPH radicals [80, 81], potentially indicating the presence of small MW hydrolyzates in the samples.

As chemical assays revealed suitable antioxidant potentials, an evaluation of nonmalignant human colon cells (FHC) and human colorectal adenocarcinoma cells (Caco-2) was performed, considering that the colon would be one of the fundamental contact points in the event of huauzontle protein consumption. In FHC cells (Figure 6(a)) and CaCo-2 cells (Figure 6(b)), germinated seed protein hydrolyzates (H3) at the four different concentrations exhibited the highest inhibitory activity of all samples ($p < 0.05$), demonstrating an improved antioxidant capacity in protein hydrolyzates obtained after seed germination. In contrast, raw seed (H1) protein hydrolyzates exhibited a maximum antioxidant activity in FHC and CaCo-2 cells of 60.91 ± 5.34% at 0.125 mg/mL and 77.18 ± 0.25% at 0.250 mg/mL, only to decrease at higher protein concentrations. This phenomenon can be attributed to intermediate concentrations saturating active-transport mechanisms and/or creating peptide aggregation, which blocks transport pathways and results in reduced effective cellular concentrations. Conversely, higher concentrations promote alternative uptake pathways and passive diffusion, allowing more bioactive peptides to permeate the cell membrane [82], thus leading to an increase in antioxidant activity. Additionally, higher hydrolyzate concentrations may promote the formation of protective nanostructures derived from peptide self-assembly [83].

Upon steeping and germination, proteins undergo structural modifications, including the presence of increased sulfhydryl groups, unfolding, and rearrangement, which enhance their solubility and functional properties [84]. These protein

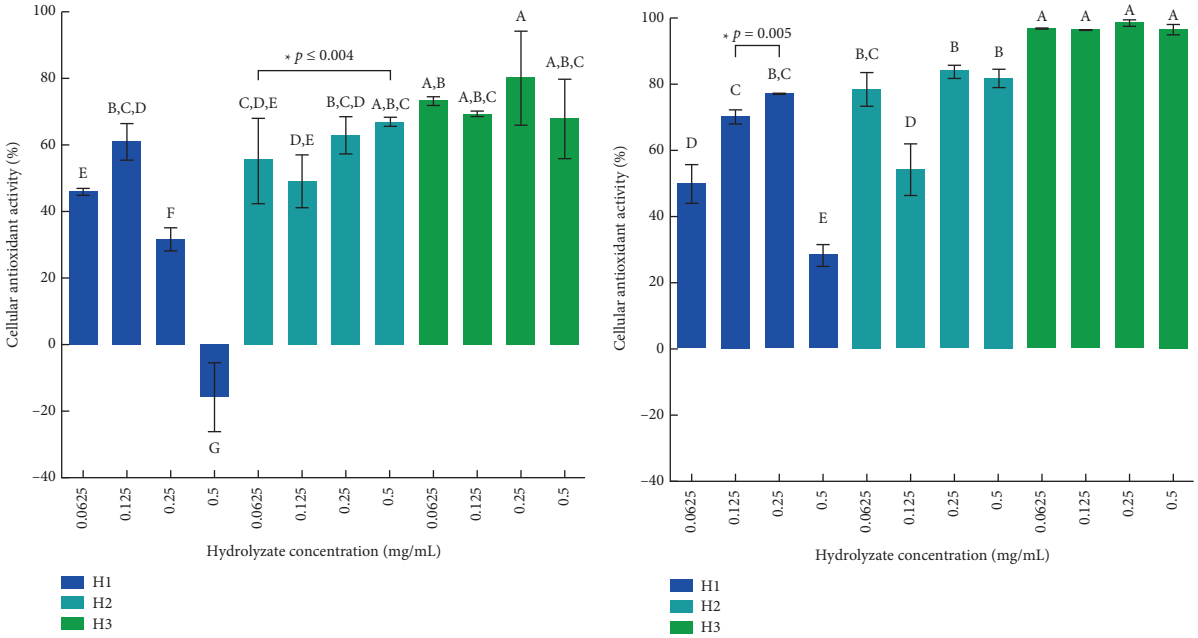

FIGURE 6 | Cellular antioxidant activity (CAA) induced in human colon cells treated for 24 h with different concentrations (0.0625–0.5 mg/mL) of protein hydrolyzates from raw (H1), steeped (H2), or germinated (H3) huauzontle seeds in (a) fetal human colon cells (FHC) and (b) human colorectal adenocarcinoma cells (Caco-2). $^{A–C}$Different letters indicate statistically significant differences ($p < 0.05$) among treatments using a Tukey test; *Statistically significant differences ($p < 0.05$) between two samples in a two-sample *t*-test ($n = 3$).

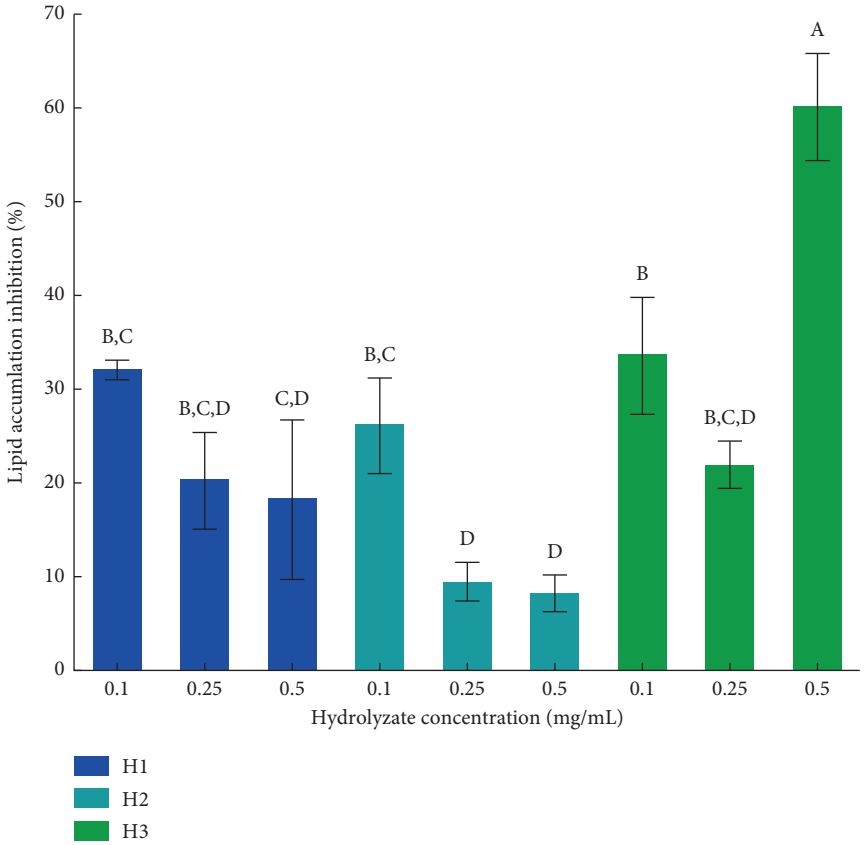

FIGURE 7 | Lipid accumulation inhibition in murine pre-adipocyte (3T3-L1) treated with different concentrations (0.1–1 mg/mL) of raw (H1), steeped (H2), and germinated (H3) huauzontle seeds protein hydrolyzates. ^{ABC}Different letters indicate statistically significant differences ($p < 0.05$) among treatments using the Tukey test; lines indicate statistically significant differences ($p < 0.05$) between two samples in a two-sample *t*-test ($n = 3$).

modifications in germinated seeds result in the upregulation of superoxide dismutase (SOD), glutathione peroxidase (GPx), and other antioxidant enzymes for the sake of protecting the growing cells from oxidative stress [85]. Thus, an enhanced antioxidant profile is present in germinated samples.

In Caco-2 cells stressed with H_2O_2, Zhang et al. [86] proved that isolated peptides from *G. max* L. at 100 μM suppressed 85.9%–96.2% of the ROS generated. Moreover, studies have identified the effectiveness of whey protein, amaranth protein, and other hydrolyzates not only in the reduction of ROS but also in the expression and activity of GPx and/or SOD presence in Caco-2 cells [87–89], enzymes crucial for promoting cellular homeostasis.

3.2.3 | Anti-Adipogenic Effects of Huauzontle Seed Protein Hydrolyzates

At the maximum concentrations (0.5 mg/mL), all treatments had a cell viability of 90% or higher. As seen in Figure 7, the lipid-accumulation inhibition of H1 and H2 treatments was highest at 0.1 mg/mL, the lowest protein hydrolyzate concentration. The case is different for H3 protein hydrolyzates, where the highest potential to inhibit lipid accumulation was observed at 0.5 mg/mL (60.07 ± 5.71%), as demonstrated by a Tukey test ($p < 0.05$). During sprouting, protease activation occurs due to biochemical transformations in response to the presence of water, thus ensuring the availability of nutrients for the plant's uptake [90]. This inherent activity results in the production of biologically active peptides, which have been known to counteract metabolic syndrome-related diseases [91].

Grancieri et al. [92] demonstrated that total protein hydrolyzates from chia seeds had a significantly higher ($p < 0.05$) lipid-accumulation inhibitory activity, as opposed to isolated albumin or glutenin, reaching a 37.2% inhibitory capacity. The authors concluded that this effect was achieved in 3T3-L1 pre-adipocytes through the suppression of the peroxisome proliferator-activated receptor gamma (PPAR-γ) pathway. Christofides et al. [93] described the role of PPAR-γ as a key differentiator of pre-adipocytes into adipocytes while also controlling the expression of genes involved in fatty acid uptake and storage. Potentially, a germinated huauzontle (H3) protein hydrolyzate can suppress PPAR-γ activity in macrophages. This effect cannot be attributed to all legumes and cereals. Ruiz et al. [94] concluded that pea protein hydrolyzates promoted lipid accumulation ($p < 0.01$) when compared to control groups; an advantage of huauzontle protein hydrolyzates, regardless of the treatment, could be inferred.

The use of alcalase as a hydrolytic agent has been extensively studied, as it promotes the obtention of low MW peptides, often associated with improved biological activities [95, 96]. Alcalase is known to have a broad specificity, meaning that it can cleave proteins at multiple amino acid sequences; thus, it is optimal for different substrates. This quality generates complex mixtures of peptides with varying MWs and biological activities [96, 97], making it the enzyme of choice for multiple research purposes.

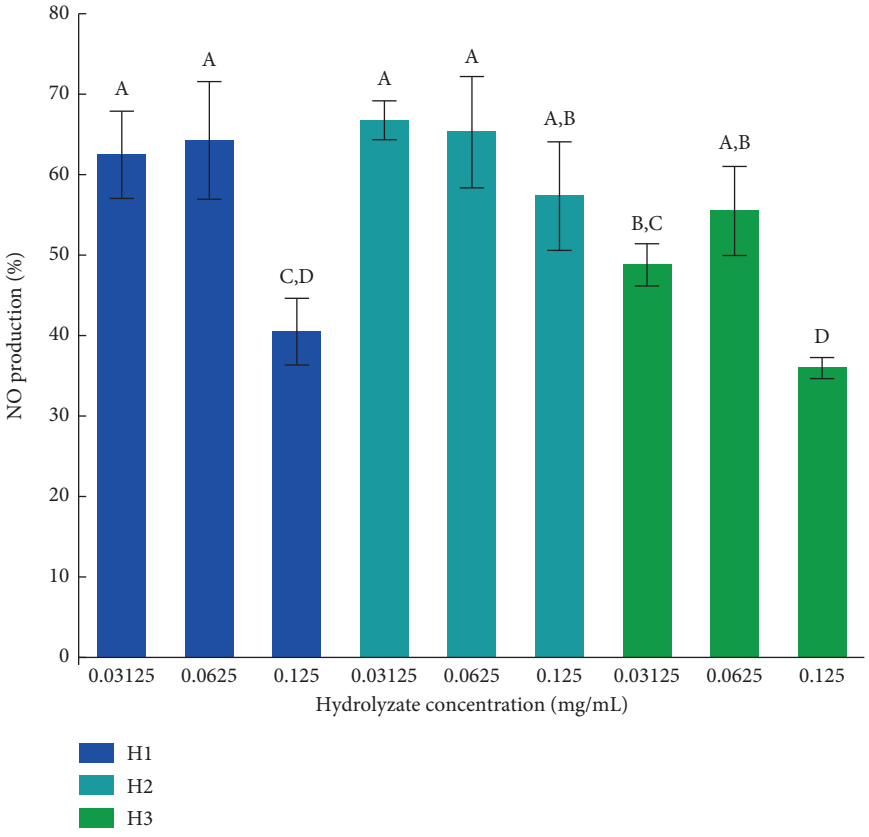

FIGURE 8 | Relative nitric oxide (NO) production in murine macrophages (RAW-264.7) after 4 h of treatment with different concentrations (0.03125–0.125 mg/mL) of protein hydrolyzates from raw (H1), steeped (H2), or germinated (H3) huauzontle seeds. ^{ABC}Different letters indicate statistically significant differences ($p < 0.05$) among treatments using a Tukey test ($n = 3$).

Bioactive peptides extracted from hazelnut (*Corylus heterophylla* Fisch), similarly hydrolyzed with alcalase, were proven to reduce the lipid accumulation in 3T3-L1 murine macrophages to approximately 80% of the positive control at a concentration of 80 mg/mL [98]. In a similar manner, Oseguera Toledo et al. [99] proved the effectiveness of common bean (*P. vulgaris* L.) protein hydrolyzed with alcalase in inhibiting lipid accumulation, ranging from 13% to 28% inhibition.

ROS production has been identified as a mediator for pre-adipocyte differentiation. Excessive mitochondrial metabolism during pre-adipocyte differentiation generates an increase in ROS, promoting a damaging, oxidative environment within the cell, which ultimately leads to cellular senescence [100]. Younce and Kolattukudy [101] have reported that inhibitors of ROS production have attenuated MCP-1-induced protein (MCPIP)-induced adipogenesis, as observable through inhibition of lipid accumulation, among other factors. Consequently, as H3 hydrolyzates have been shown to possess a high cellular antioxidant capacity, their inhibition of adipogenesis may be linked to their capability to inhibit or counteract ROS production.

3.2.4 | Anti-Inflammatory Potential Measured by the NO Release

As chronic inflammation has been associated with overweight, obesity, or other factors related to metabolic syndrome [102, 103], assessing the anti-inflammatory potential of huauzontle seeds seems imperative to determine their effectiveness as a potential functional ingredient. Concentrations higher than 0.125 mg/mL of protein hydrolyzates did not result in desirable (≥ 90%) RAW 264.7 cell viability (results not shown). Overall, the production of NO from treatment groups varied between 56% and 66% of the levels observed in the untreated control (Figure 8). In contrast, at 0.125 mg/mL, raw (H1) and germinated (H3) samples exhibited a statistically higher ($p < 0.05$) inhibitory capacity, with $40.53 \pm 4.13\%$ and $35.99 \pm 1.32\%$ NO production, respectively, thereby emphasizing their potential as anti-inflammatory functional foods. Sandoval-Sicairos et al. [104] evaluated the role of in vitro digested proteins from germinated amaranth flour under similar conditions, demonstrating that the highest concentration and digestion time (3 mg/mL, 90 min) resulted in only 46.9% of the NO released, with respect to the positive control.

In comparison, Ji et al. [105] identified a dose–response effect of foxtail millet (*Setaria italica*) peptides on the inhibition of NO production. It was demonstrated that at the highest peptide concentration (250 μg/mL), NO release could be decreased by approximately 38%, highlighting the importance of peptide identification and isolation for achieving enhanced results upon further testing. With half of said concentration, H3 samples demonstrated a higher capacity to inhibit NO release, without the need for peptide isolation, demonstrating their ability to prevent inflammatory responses through uncomplicated, cost-effective production methods. Similar observations were made in the isolation of a wheat germ peptide, consisting of 8 residues, where the highest tested concentration (80 μM) resulted in the highest NO inhibitory capacity [106].

4 | Conclusion

The comparison between protein hydrolyzates derived from raw (H1), steeped (H2), and germinated (H3) huauzontle seeds revealed that the natural hydrolysis that occurs in the endosperm upon seed stepping or germination enhances the production of biologically active peptides, useful to modulate obesity-related afflictions. Raw (H1) seeds had a higher α-amylase inhibitory activity than steeped (H2) and germinated (H3) protein hydrolyzates. In comparison, H3 protein hydrolyzates exhibited superior antioxidant activity, as evidenced by statistically higher ($p < 0.01$) ABTS and DPPH scavenging potential at 1.0 mg/mL and across all concentrations in the cellular antioxidant assay in healthy and cancerous colon cells. Moreover, this antioxidant activity was also improved in steeped seeds (H2), outperforming ($p < 0.05$) raw seeds (H1). When compared to untreated controls, germinated seeds had the highest inhibitory effect ($p < 0.05$) on NO release and lipid accumulation at the highest tested concentrations (0.125 mg/mL and 0.5 mg/mL, respectively). The findings described highlight the improved biological activity of protein hydrolyzates from huauzontle seeds after germination, observable in *in vitro* overweight- and obesity-related assays. This improved activity can be observed, although in a lesser manner, in protein hydrolyzates from steeped huauzontle seeds. These results warrant further consideration of germinated huauzontle protein hydrolyzates, primarily as a functional ingredient for human consumption in an overweight or obesity-related context.

Author Contributions

Emilio López-Millán: investigation, formal analysis, methodology, writing–original draft, and writing–review and editing. Astrid Domínguez-Uscanga: methodology, supervision, and writing–review and editing. Daniel Guajardo-Flores: methodology, resources, and writing–review and editing. Marilena Antunes-Ricardo: investigation, methodology, project administration, resources, supervision, and writing–review and editing. Diego A. Luna-Vital: conceptualization, investigation, methodology, project administration, project validation, resources, supervision, visualization, and writing–review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available within the article and/or its supporting information.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Appendix A: Protein solubility curve for raw (H1), steeped (H2), and germinated (H3) huauzontle seeds by bicinchoninic acid assay in extractions with water at different pH levels ($n = 3$). Appendix B: Alpha amino nitrogen content per sample of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) seeds.

Graphical abstract: Huauzontle seeds were used raw, soaked, or germinated, and their extracted protein was hydrolyzed. Soaked and germinated samples possessed smaller protein hydrolyzates. Germinated samples performed better in antioxidant, anti-adipogenic, and anti-inflammatory in vitro tests.