Red and Gray Bean (Phaseolus vulgaris L.) Protein Hydrolysates: Food Prototypes with Pota (Dosidicus gigas) by-Product Meal †

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† Presented at the IV Conference la VaSe-Food CYTED and VII Symposium Chia-Link, La Plata and Jujuy, Argentina, 14–18 November 2022.

Abstract: The bean (Phaseolus vulgaris L.) known as the ñuña, numia, or Andean popping bean, native to the central Andes of Peru, is often consumed as a snack food after a quick toasting process. The characterization of two of its varieties, red and gray ñuña beans, was performed to determine their proximate composition, total phenolics, and antioxidant activity. Moisture content ranged from 12.67% (red ñuña bean) to 11.94% (gray ñuña bean); fat content varied from 1.77% (red ñuña bean) to 1.44% (gray ñuña bean); protein content was high, with a content range from 23.90% (red ñuña bean) to 26.81% (gray ñuña bean); ash content ranged from 4.04% (red ñuña bean) to 3.88% (gray ñuña bean); and a high content of carbohydrates was also found (from 57.60 to 55.94%). The phenolic compounds were consistently higher according to particle size, and the total phenolic content varied from 8589 µg of gallic acid equivalent (GAE)/g powder (red ñuña bean) to 3478 µg GAE/g powder (gray ñuña bean), with antioxidant activity varying from 9879 µg trolox/g powder (red ñuña bean) to 5539 µg trolox/g powder (gray ñuña bean). Food prototypes were then developed with the hydrolyzed proteins from ñuña beans, mashua (Tropaeolum tuberosum) tuber flour, purple corn (Zea mays L.) flour, and pota (Dosidicus gigas) by-product meal with a high content of protein and omega-3 acids (~50% eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) on total fat).

Keywords: Phaseolus vulgaris; DPPH; degree of hydrolysis; protein; polyphenols; prototypes; ñuña beans

1. Introduction

Ñuña beans are an important crop and a typical food for the Andean population, especially in the regions from Cajamarca, Peru to Chuquisaca, Bolivia [1]. These beans have a high protein content of around 20%, with a low foaming and viscosity capacity, where the predominant protein is phase Olin, a kind of globulin [2]. The most important world organizations have expressed the constraint of having not enough protein production for an increasing population. The lack of water and farmable land as well as the contribution to climate change due to the cattle industry create an unsustainable situation. For these reasons, is important to find new sources of protein alternatives. This work aimed to develop a food prototype using the hydrolyzed proteins from ñuña beans, mashua (Tropaeolum tuberosum) tuber flour, purple corn (Zea mays L.) flour, and pota (Dosidicus gigas) by-product meal with a high content of protein and omega-3 acids (~50% eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) on total fat).
2. Materials and Methods

2.1. Raw Material

Ñuña beans (*Phaseolus vulgaris* L.), mashua (*Tropaeolum tuberosum*) tuber, and purple corn (*Zea mays* L.) were obtained from the local market in Lima city and pota (*Dosidicus gigas*) by-products were collected by Mi Cautivo de Ayabaca company from Piura Department, Peru. The beans were ground in a disc mill in the Functional Foods Laboratory from the Universidad de Lima-Peru and sieved with 125, 250, and 500 µm mesh to obtain four sieved fractions of ñuña bean flour. Pota by-products were washed, dried at 60 °C × 12 h by an infrared dryer (Irconfort IRC D18, Sevilla, Spain), and ground in a disc mill to obtain pota by-product meal. Mashua tuber and purple corn were washed, dried at 40 °C, and ground in a food shredder (Grindomix GM200, Restch, Haan, Germany). All the samples were kept at −5 °C in polyethylene bags for later analysis. Alcalase 2.4L was purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Proximal Composition

The moisture content of the samples was determined at 110 °C to a constant weight. The ash content was determined by the ignition method (550 °C for 72 h). The fat content was determined with hexane for 9 h. The total protein content was determined as % nitrogen × 6.25 using a Kjeldahl analyzer (UDK 139, VELP, Usmate Velate. Italy) with official methods.

2.3. Total Polyphenolic Content

The total phenolic content (TPC) of the samples was determined by the Folin-Ciocalteau method [3]. A 15 mg of sample was dissolved in 4.5 mL of methanol and 2.5 mL of Folin-Ciocalteau reagent 2 N, and the mixture was stirred using a vortex for 1 min. After 5 min, 2.5 mL of sodium carbonate solution (20%) was added, and the mixture was left at 40 °C for 30 min. The mixture was cooled and filtered through Whatman filter paper N° 2 (Whatman International Ltd., Maidstone, UK). The absorbance of the solution was measured at 760 nm using a spectrophotometer (UV 1280 Vis Spectrophotometer, Shimadzu, Kyoto, Japan). Ultrapure water was used as a control blank. The results were expressed as µg of gallic acid equivalent (GAE)/gram (powder). All analyses were done in triplicate and the results are expressed as mean values.

2.4. Antioxidant Activity

A total of 15 mg of samples were resuspended in 4.5 mL of methanol/acetic acid/water (50:8:42, v/v/v), stirred using a vortex for 1 min, and left in a water bath for 20 min at 80 °C [3]. Then, 3.9 mL of 25 ppm 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical solution (2.5 mg DPPH in 100 mL MeOH) was added, and the samples were left in the dark at 25 °C. The mixture was stirred using a vortex for 1 min and then filtered through Whatman filter paper N° 2. The absorbance of the solution was measured at 517 nm by spectrometry (UV 1280 Vis Spectrophotometer, Shimadzu, Kyoto, Japan). For a control blank, 500 µL of methanol with 3.9 mL of 25 ppm DPPH radical solution was used. All analyses were carried out in triplicate and the results are expressed as mean values.

2.5. Protein Solubility Curve

The sieved fraction of the ñuña bean (red and gray) flour was dissolved in ultrapure water (10% w/v) and the pH was adjusted to 12 with NaOH 1 N or HCl 1 N for 1 h at room temperature and centrifuged for 15 min at 10,000 × g rpm [4]. Six aliquots of the supernatant were taken and the pH was adjusted from 2 to 12 with NaOH 1 N or HCl 1 N. They were then centrifuged for 15 min at 10,000 × g rpm. The supernatant was measured in protein content according to the Lowry method [5]. The absorbance of the solution was measured at 750 nm using a spectrophotometer (UV 1280 Vis Spectrophotometer, Shimadzu, Kyoto, Japan). The results were expressed as % solubilized protein regarding the total protein content.
2.6. Ñuña Protein Extraction

The ñuña bean (red and gray) sieved fraction flour was dissolved in ultrapure water (10% w/v) and the pH was adjusted to 10 with NaOH 1 N or HCl 1 N for 1 h at room temperature. The mixture was then centrifuged for 10 min at 10,000 × g rpm. The supernatant obtained was adjusted to the isoelectric point (pI) of ñuña proteins (pH 4) and centrifuged at 10,000 rpm × g for 6 min. The precipitate was washed with ultrapure water [4].

2.7. Hydrolysis of Ñuña Protein

The hydrolysis of ñuña beans protein was performed according to Paz et al. [4] with some modifications, using ultrapure water (10% w/v, g protein/mL water), pH 8 with NaOH 1 N or HCl 1 N, and 800 rpm for 1 h at 50 °C, using a bioreactor (TEC-BIO-FLEX-II, Tecnal, São Paulo, Brazil). Alcalase 2.4L was added at a ratio enzyme/substrate = 0.32 Anson Unit (AU)/g protein. The inactivation of the enzyme was performed at 85 °C for 15 min. The supernatant obtained was spray-dried in a Büchi B-290 (Büchi Labortechnik AG, Flawil, Switzerland) with a nozzle atomization system (0.7 mm nozzle diameter), air flow rate of 55 m³/h, compressor air pressure of 50 mbar, inlet and outlet air temperatures 180 °C and 90 °C, respectively, and flow rate feed of 55 mL/min. The dried powders collected were stored in opaque hermetic bags at 5 °C for further analysis.

Degree of Hydrolysis

The degree of hydrolysis was calculated by the determination of free amino groups by reaction with 2,4,6-trinitrobenzenesulphonic acid (TNBS) [6]. The absorbance of the supernatant was measured at 340 nm using a spectrophotometer (UV 1280 Vis Spectrophotometer, Shimadzu, Kyoto, Japan). A calibration curve was constructed using L-Leucine (0.10–2.5 mM) solution.

2.8. Development of Food Prototype

The food prototype (porridge powder) was developed by mixing hydrolyzed protein from gray ñuña (Phaseolus vulgaris L.) bean with pota (Dosidicus gigas) by-product meal, mashua (Tropaeolum tuberosum) tuber flour, and purple corn (Zea mays L.) flour. The formulation was kept at −5 °C in a polyethylene bag.

3. Results and Discussions

According to Table 1, the proximate composition was evaluated in regard to their particle size. The gray ñuña bean retained less moisture content (11.46 ± 0.04% to 11.94 ± 0.06%) than red ñuña bean (12.02 ± 0.02% to 12.67 ± 0.02%). The gray ñuña bean had the highest protein content (21.06 ± 2.12% to 30.11 ± 0.82%, fractions between 0–500 µm), followed by the red ñuña bean (18.82 ± 0.37% to 23.9 ± 0.15%, fractions between 0–500 µm). The fat content presented values between 0.43 ± 0.04% to 1.89 ± 0.03% (red ñuña bean) and 0.42 ± 0.03% to 2.26 ± 0.03% (gray ñuña bean). The ash content ranged from 4.04 ± 0.07% to 5.92 ± 0.05% (red ñuña bean) and 3.88 ± 0.09% to 5.37 ± 0.03% (gray ñuña bean), and a high carbohydrate content was discovered (75.32 ± 0% to 75.81 ± 0.03%) for both red and gray ñuña beans.

The results of TPC and DPPH are shown in Table 2. The red ñuña bean was found to have a higher TPC (8589 ± 110 µg GAE/g powder) compared with the gray ñuña bean (3478 ± 117 µg GAE/g powder). The antioxidant activity of the red ñuña bean ranged from 478 ± 17 to 9879 ± 24 µg trolox/g powder (red ñuña bean) and 148 ± 20 to 5539 ± 11 µg trolox/g powder for gray ñuña bean. According to Xu and Diosady [7], phenolic compounds could interact with isolate proteins, which could create a synergetic effect with hydrolysate proteins from ñuña beans.
Table 1. The proximal composition of ñuña (*Phaseolus vulgaris* L.) bean flour.

| Particle Size (µm) | Moisture (g/100 g) | Protein (g/100 g) | Lipids (g/100 g) | Ash (g/100 g) | Carbohydrates (g/100 g) |
|---------------------|---------------------|-------------------|------------------|--------------|-------------------------|
|                     | Red Ñuña Bean        | Gray Ñuña Bean     | Red Ñuña Bean     | Gray Ñuña Bean | Red Ñuña Bean            | Gray Ñuña Bean            | Red Ñuña Bean            | Gray Ñuña Bean            |
| >500                | 12.02 ± 0.02         | 11.79 ± 0.02      | 6.27 ± 0         | 6.63 ± 0.01   | 0.43 ± 0.04              | 0.42 ± 0.03               | 5.92 ± 0.05              | 5.37 ± 0.03               | 75.32 ± 0                | 75.81 ± 0.03              |
| 250–500             | 12.54 ± 0.03         | 11.82 ± 0.01      | 23.44 ± 1.84     | 21.06 ± 2.12  | 1.74 ± 0.07              | 1.24 ± 0.01               | 4.41 ± 0.02              | 4.37 ± 0.24               | 57.86 ± 1.93              | 61.38 ± 2.25              |
| 125–250             | 12.63 ± 0.03         | 11.46 ± 0.04      | 18.82 ± 0.37     | 30.11 ± 0.82  | 1.89 ± 0.03              | 2.26 ± 0.03               | 4.23 ± 0.01              | 4.23 ± 0.02               | 62.44 ± 0.35              | 51.95 ± 0.74              |
| 0–125               | 12.67 ± 0.02         | 11.94 ± 0.06      | 23.9 ± 0.15      | 26.86 ± 3.23  | 1.77 ± 0.1              | 1.44 ± 0.06               | 4.04 ± 0.07              | 3.88 ± 0.09               | 57.6 ± 0.15               | 55.94 ± 3.25              |

Results are expressed as means ± SD ($n = 2$).
Table 2. The total polyphenolic content (TPC) (µg GAE/g powder) and DPPH (µg Trolox/g powder) from ñuña (Phaseolus vulgaris L.) bean flour.

| Particle Size (µm) | Total Phenolic Content (µg GAE/g Powder) | DPPH (µg Trolox/g Powder) |
|-------------------|-----------------------------------------|----------------------------|
|                    | Red Ñuña Bean                           | Gray Ñuña Bean             |
| >500               | 8589 ± 110                              | 3478 ± 117                 |
| 250–500            | 2252 ± 103                              | 1397 ± 17                  |
| 125–250            | 411 ± 83                                | 350 ± 15                   |
| 0–125              | 159 ± 16                                | 255 ± 86                   |

Results are expressed as the means ± SD (n = 3).

The protein solubility curve from ñuña beans showed an isoelectric point between pH 4 to 4.5. A greater solubility was obtained between pH 8 to 12. For this reason, the protein extraction from ñuña beans was settled at pH 10 with an isoelectric point at pH 4.

The characterization of protein hydrolysates (PH) from ñuña beans is reported in Table 3. The PH from red ñuña bean retained less moisture content (5.38 ± 0.09%). The PH from gray ñuña bean was found to have a higher protein content (69.78 ± 0.82%), high degree of hydrolysis (26.11 ± 0.57%), and ash content (11.62 ± 0.12%). The degree of hydrolysis (26.11 ± 0.57%) of grey ñuña protein hydrolysate was higher than 5%, which indicates that it could be scaled up to industrial steps [8]. For these reasons, the food prototype was formulated with grey ñuña protein hydrolysate. The PH from red ñuña bean showed a higher TPC (55,157 ± 72 µg GAE/g powder) and antioxidant activity (4588 ± 49 µg Trolox/g powder).

Table 3. The characterization of protein hydrolysates from ñuña (Phaseolus vulgaris L.) beans.

|                   | Moisture (g/100 g) | Protein (g/100 g) | Degree of Hydrolysis (%) | Ash (g/100 g) | Total Phenolic Content (µg GAE/g) | DPPH (µg Trolox/g) |
|-------------------|--------------------|-------------------|--------------------------|--------------|----------------------------------|-------------------|
| Red ñuña bean     | 5.38 ± 0.09        | 58.42 ± 0.16      | 15.06 ± 0.15             | 11.47 ± 0.07 | 55,157 ± 724                     | 4588 ± 49         |
| Gray ñuña bean    | 5.96 ± 0.26        | 69.78 ± 0.82      | 26.11 ± 0.57             | 11.62 ± 0.12 | 12,323 ± 76                      | 740 ± 25          |

Results are expressed as means ± SD (n = 3).

The purple porridge showed a high protein content (25.47 ± 0.34%), a low percentage of moisture content (7.46 ± 0.09%), and a high number of phenolic compounds (42,429 ± 202 µg GAE/g powder) and antioxidant activity (14,938 ± 25 µg Trolox/g powder), with an acceptable taste and a deep purple color. These results indicate that purple porridge could be an important source of protein and polyphenols for children with malnutrition states.

4. Conclusions

Ñuña beans are a promising source of plant-based protein (around 20%). In addition, the ñuña protein hydrolysates show higher phenolic content (12,323 ± 76 µg GAE/g powder) and antioxidant activity (740 ± 25 µg Trolox/g powder), indicating the developed food prototype would be an important source of protein (25.47 ± 0.34%), polyphenols (42,429 ± 202 µg GAE/g powder), and antioxidant activity (14,938 ± 25 µg Trolox/g powder), all while having an acceptable taste and deep purple color.

Author Contributions: All authors have contributed equally to this manuscript. Conceptualization, N.C., R.A., B.F.G., A.S., M.T., B.G., and M.C.P.-C.; Methodology, N.C., R.A., B.F.G., A.S., B.G., and M.C.P.-C.; Investigation and Data analysis, N.C., R.A., B.F.G., A.S., M.T., P.J., B.G. and M.C.P.-C.; Writing—original draft preparation, N.C., B.F.G., and M.C.P.-C.; Writing—review and editing, N.C., B.F.G., and M.C.P.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grant La ValSe-Food-CYTED (119RT0567) and project SP-2021-01581—Programa Nacional de Investigación en Pesca y Acuicultura (PNIPA), belonging to “the Ministry of Production-Peru”, and “Universidad de Lima-Peru”.

Table 2. The total polyphenolic content (TPC) (µg GAE/g powder) and DPPH (µg Trolox/g powder) from ñuña (Phaseolus vulgaris L.) bean flour.
Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the manuscript.

Acknowledgments: The authors thank the Instituto de la Grasa-CSIC, Sevilla-España, and Universidad de Lima-Perú.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Santa Cruz Padilla, A.E.; Vásquez Orrillo, J.L. Catálogo de Ñuña (Phaseolus Vulgaris L.) Del Banco de Germoplasma Del INIA; Instituto Nacional de Innovación Agraria, Ed.; Instituto Nacional de Innovación Agraria: Lima, Peru, 2021.
2. Vargas-Salazar, T.A.; Wilkinson, K.A.; Urquiaga Zavaleta, J.M.; Rodriguez-Zevallos, A.R. Physicochemical Charaacterization of Ñuña Bean (Phaseolus vulgaris L.) Protein Extract. Vitae 2020, 27, 1–9. [CrossRef]
3. Luo, Q.; Zhang, J.R.; Li, H.B.; Wu, D.T.; Geng, F.; Corke, H.; Wei, X.L.; Gan, R.Y. Green Extraction of Antioxidant Polyphenols from Green Tea (Camellia Sinensis). Antioxidants 2020, 9, 785. [CrossRef] [PubMed]
4. Paz, S.M.D.l.; Martinez-Lopez, A.; Villanueva-Lazo, A.; Pedroche, J.; Millan, F.; Millan-Linares, M.C. Identification and Characterization of Novel Antioxidant Protein Hydrolysates from Kiwiche (Amaranthus caudatus L.). Antioxidants 2021, 10, 645. [CrossRef] [PubMed]
5. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 1951, 193, 265–275. [CrossRef]
6. Adler-Nissen, J. Determination of the Degree of Hydrolysis of Food Protein Hydrolysates by Trinitrobenzenesulfonic Acid. J. Agric. Food Chem. 1979, 27, 1256–1262. [CrossRef] [PubMed]
7. Xu, L.; Diosady, L.L. Interactions between Canola Proteins and Phenolic Compounds in Aqueous Media. Food Res. Int. 2000, 33, 725–731. [CrossRef]
8. Bui, X.D.; Vo, C.T.; Bui, V.C.; Pham, T.M.; Bui, T.T.H.; Nguyen-Sy, T.; Nguyen, T.D.P.; Chew, K.W.; Mukatova, M.D.; Show, P.L. Optimization of Production Parameters of Fish Protein Hydrolysate from Sarda Orientalis Black Muscle (by-Product) Using Protease Enzyme. Clean Technol. Environ. Policy 2020, 23, 31–40. [CrossRef]