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Proceeding Paper

Development and Characterization of Andean Pseudocereal Bars Enriched with Native Collagen from Pota (*Dosidicus gigas*) By-Products †

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Abstract: In recent years, consumers have been increasingly concerned about their health. Therefore, the snack market is rapidly developing more innovative and functional products such as cereal bars. Quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*) are Andean pseudocereals with protein (10.90–11.35%) content and other functional components that reduce the risk of cardiovascular diseases and inflammatory illnesses. Peru is the world's second largest exporter of Pota (*Dosidicus gigas*), with 476.5 million metric tons in 2023; however, only between 50% and 70% of it has been taken advantage of. Pota by-products such as skins, viscera, and necks have significant protein content (70%) and are discarded. In this investigation, cereal bar formulations with Pota by-products and Andean pseudocereals were optimized and characterized using a five-run simplex centroid mixture design. The effects of two independent variables were examined, namely collagen (2–8%) and binders (22–28%), on the sugar (%), protein (%), and antioxidant (µg Trolox/g dry weight, dw) content as response variables. The optimized cereal bar (M6) showed high protein (21.27 ± 1.51%) content, moisture (10.37 ± 0.04%), ash (2.57 ± 0.03%), fat (15.12 ± 0.15%), carbohydrates (53.67 ± 1.70%), total polyphenol (1570 ± 267 µg Gallic acid equivalent/g dw) content, antioxidant activity (1656 ± 77 µg Trolox/g dw), essential amino acid–leucine (15.65 ± 1.83 mg/g protein) content, and higher in vitro digestibility (78.78 ± 1.40%) than the control sample. The cereal bar had a positive sensory acceptability (88.89%) and complied with Peruvian standards. The functional bar emerges as a nutritious alternative in the food industry and proposes a sustainable solution using Pota by-products, fostering a circular economy.

Keywords: circular economy; functional bars; native collagen; non-communicable diseases; Pota by-products; pseudocereals

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1. Introduction

The reuse of fishery waste and by-products could be valuable for developing products with high nutritional content, offering various potential applications across industries and contributing to economic growth. These by-products are rich in proteins, with collagen being the most prevalent, constituting about 30% of the total protein content in animals and found in tissues such as skin, tendons, bones, cartilage, and ligaments [1,2].

Consumer habits are evolving, with more people opting for healthier snacks like nutrient-rich bars that offer real health benefits. Cereal bars have become a popular choice worldwide because they are not only nutritious and low in calories but also help satisfy hunger quickly and support better overall health [3].

This study focuses on the optimization and characterization of the first Andean pseudo-cereal bar formulated with quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*), enriched with collagen extracted from by-products of Pota (*Dosidicus gigas*).

2. Materials and Methods

2.1. Raw Material

Flakes of Andean pseudocereals, quinoa (*Chenopodium pallidicaule Aellen*) and kiwicha (*Amaranthus caudatus linnaeus*), along with raisins and pecans, were obtained from a local market in Lima, Peru. Tarwi legume (*Lupinus mutabilis*) was washed and dehydrated in an infrared dryer (IRC D18, Irconfort, Sevilla, Spain) at 60 °C for 12 h in the Functional Foods Laboratory at the Universidad de Lima, Peru. The dehydrated tarwi was then grounded using a food shredder (Grindomix GM200, Restch, Haan, Germany) to obtain tarwi flour. By-products of Pota (*Dosidicus gigas*) were collected from the Piura Department, Peru. The process for extracting collagen from these by-products was determined following the method described by [4] with 5 N NaOH for 30 h, followed by washing, dehydration, grinding in the food shredder, and sieving to 212 µm. The binder was obtained by extracting pineapple juice and mixing it in a 1:20 ratio with flaxseed at 40 °C for 2 h. All samples were stored in aluminized bags at room temperature for subsequent analysis.

2.2. Formulation Optimization

A simplex-centroid mixture design (SCMD) based on response surface methodology (RSM) was implemented. The design included 5 treatments and 2 central point replicates with two components, which were collagen (2%, 3.5%, 5%, 6.5%, 8%) and the pineapple binder (28%, 26.5%, 25%, 23.5%, 22%). This design was based on a cereal bar formulation to evaluate the impact of native collagen derived from Pota by-products. The dependent variables assessed were protein content (%), antioxidant activity (%), and sugar content (%). The highest compound desirability was used as a statistical indicator to validate the product design. The analysis used Minitab 19.0 statistical software (Minitab Inc., State College, Palo Alto, PA, USA).

2.3. Sample Preparation

Five samples were prepared with different amounts of Pota nape collagen (2–8%) and binder (22–28%) while maintaining proportions of quinoa (17%), kiwicha (17%), tarwi flour (20%), raisins (7%), pecans (7%), and pineapple fiber (1%). All the dry ingredients were mixed, including the flakes roasted at 60 °C for 10 min and the crushed nuts. The binder was incorporated into the mixture at room temperature and mixed until homogeneous. The mixture was poured into a mold and refrigerated for 15 min. Afterwards, it was wrapped in butter paper and baked at 180 °C for 12 min, obtaining sticks of 20 to 25 g and stored in aluminized bags for later use.

2.4. Physicochemical Characterization

2.4.1. Proximal Composition

The moisture content of the cereal bars was determined at 110 °C at a constant weight. The ash content was determined by an ignition method (550 °C for 72 h). The fat content was determined with hexane for 9 h, and the total protein content was determined as % nitrogen × 6.25 using a Kjeldahl analyzer (UDK 139, VELP, Usmate Velate, Italy) by official methods. Each analysis was conducted in triplicate, and the results were presented as mean values.

2.4.2. Total Phenolic Content (TPC)

The total phenolic content (TPC) of the Andean pseudocereal bars was measured using the Folin–Ciocalteau method [5]. A total of 15 mg of the 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 absorbance was measured at 760 nm with a spectrophotometer (UV 1280 Vis Spectrophotometer Shimadzu, Kyoto, Japan). The results were expressed as µg of Gallic acid equivalents (GAEs) per gram of dry sample. Each analysis was conducted in duplicate as mean values.

2.4.3. Antioxidant Activity

The antioxidant activity of the samples was evaluated using the DPPH method [5] with certain modifications; a total of 15 mg of the samples was 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. 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 the absorbance was measured at 517 nm by spectrometry (UV 1280 Vis Spectrophotometer Shimadzu, Kyoto, Japan). The results were reported as µg Trolox/g of the dry sample. Each analysis was performed in triplicate and presented as mean values.

2.4.4. In Vitro Protein Digestibility (IVPD)

In vitro protein digestibility was assessed using the method of Tinus et al. (2012) [6] with slight modifications. The results are reported as

$$\text{IVPD (Digestibility (\%)} = 65.66 + 18.10 \times (\text{pH 0 min} - \text{pH 10 min}))$$

2.5. Determination of Amino Acid Profile

The amino acid profile was determined following the method described by Chasquibol et al. [4]. Acid hydrolysis was conducted for all amino acids except tryptophan, which underwent basic hydrolysis. Protein samples (4 mg) were incubated in 4 mL of 6 N HCl at 110 °C for 24 h in nitrogen-sealed tubes. The hydrolyzed samples were then reconstituted in 1 M sodium borate with 0.02% sodium azide at pH 9.0. The samples were then quantified using HPLC (ARC, Waters, Milford, CT, USA) equipped with a C18 reverse phase column with an internal diameter of 150 mm × 3.9 mm. The calibration curves for each amino acid were developed using a mix of the amino acid standards at the same hydrolysis conditions of the samples. Each analysis was performed in triplicate and presented as mean values.

2.6. Sensory Analysis

A panel of 30 individuals (aged 20 to 51) evaluated the sensory attributes of appearance, color, aroma, taste, flavor, texture, and overall acceptability. The evaluation was conducted using a 9-point hedonic scale (1 = extreme dislike, 9 = extreme liking) [7].

3. Results and Discussion

3.1. Formulation Optimization

The results obtained for all samples are presented in Table 1. With a composite desirability of 66.78%, the optimal formulation of the cereal bar features a collagen concentration of 6% and a pineapple binder concentration of 24%. According to the response variables estimated using Minitab 0.19 software, the enriched cereal bar displays a theorical protein content of 21.77%, a theorical antioxidant activity of 1800.10 µg Trolox/g dw, and a theorical sugar content of 5.95%.

Table 1. Results of the response variables for the optimal formulation.

Sample	Protein (%)	Sugar (%)	DPPH (µg Trolox/g dw)
M1 (2% CPBP)	18.10 ± 0.68	8.03 ± 0.72	1420.84 ± 712.78
M2 (3.5% CPBP)	19.88 ± 1.48	5.88 ± 0.28	1557.24 ± 488.51
M3 (5% CPBP)	20.73 ± 1.46	6.10 ± 0.76	1713.52 ± 520.54
M4 (6.5% CPBP)	24.28 ± 0.04	7.16 ± 0.57	1744.08 ± 482.09
M5 (8% CPBP)	24.31 ± 1.28	5.52 ± 0.49	1870.80 ± 449.99

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

3.2. Physicochemical Characterization

Table 2 presents the physicochemical characterization of the optimal bar (M6), control sample bar (C), and commercial cereal bars (CCB). The M6 bar exhibited a high protein content (21 ± 2%), significantly exceeding the 10% threshold required to be classified as a high-protein food according to the FAO and the 8.5% minimum standard for cereal-based products according to NTP-PNAEQW [8]. Regarding sugar content, M6 recorded a value of 3.94 ± 0.03%, which is lower than the control value of 9.5% and complies with the maximum allowable level of 10% established by Law No. 30021 [9]. Additionally, the carbohydrate content (53 ± 2%), ash content (2.57 ± 0.03%), and fat content (15.1 ± 0.2%) in M6 were all superior to those of C and CCB [10].

Table 2. Proximate composition of the optimized Andean pseudocereal bar.

Sample	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)
M6	10.37 ± 0.04	15.12 ± 0.15	21.27 ± 1.51	2.57 ± 0.03	53.67 ± 1.70
C	7	16.5	7.5	1.5	68
CCB	7.9 ± 2.1	13.1 ± 3.8	5.5 ± 1.3	1.1 ± 0.3	66.0 ± 7

Results are expressed as means ± SD (n = 3). Data available from Oliveira-Carrion et al. [10].

3.2.1. Total Phenolic Content (TPC) and Antioxidant Activity

The phenolic content found in M6 was 1569 ± 268 μg GAE/g dw. Additionally, the antioxidant activity measured by DPPH was 1656 ± 77 μg Trolox/g dw. These results were lower than the commercial bars because our primary antioxidant activity is believed to come from the action of serine protease derived from collagen, used in waste valorization processes and the extraction of naphthol esters, acetates, and triacylglycerols. Unlike commercial cereal bars that rely on various sources of antioxidants, these phenolic compounds are particularly noteworthy for their potent antioxidant properties and anti-inflammatory effects [11].

3.2.2. In Vitro Protein Digestibility (IVPD)

The cereal bar presented high in vitro digestibility IVPD (78.78 ± 1.40%), which was higher than the IVPD extruded snack (74.40 ± 0.51%) [7]. These results indicate that the cereal bar could be an important source of protein and polyphenols for consumers.

3.3. Determination of Amino Acid Profile

The amino acid profile composition showed histidine (25.1 ± 0.9 mg/g protein), isoleucine (35 ± 1 mg/g protein), phenylalanine + tyrosine (56.78 ± 2.6 mg/g protein), threonine (42 ± 2 mg/g protein), tryptophan (10.8 ± 2 mg/g protein), and valine (41 ± 3 mg/g protein). However, leucine (57.60 ± 0.52 mg/g protein), lysine (31.6 ± 0.3 mg/g protein), and methionine + cysteine (18.0 ± 0.5 mg/g protein) are limited components. According to the FAO/WHO recommendations for healthy nutrition, the amino acid composition of the M6 bar displays a well-balanced profile.

3.4. Sensory Analysis

It is concluded that both the optimized bar (M6) and the control sample (C) were well received by the panelists. The M6 bar achieved scores above six in all evaluated attributes, with appearance and color standing out with a rating of eight and an overall acceptability of 88.89%.

4. Conclusions

The incorporation of pseudocereals, such as quinoa and kiwicha, and collagen extracted from Pota by-products, significantly enhanced the physical properties of the (M6) cereal bar, including increased protein and antioxidant content and lower sugar and carbohydrate levels, compared to the control sample (C) and commercial cereal bars (CCB). This development, positively evaluated by a sensory panel and compliant with NTP-PNAEQW (2022) and Law No. 30021 (2013), represents an innovative and sustainable approach that enhances market acceptance.

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