



Development of Powdered Beverage with Cushuro (*Nostoc commune*) Concentrated Protein and Quinoa (*Chenopodium quinoa*)[†]

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Abstract: The trend in protein consumption is towards plant-based foods, for this reason a powdered beverage with a balanced profile of essential amino acids was developed, combining two different sources of protein, cushuro (*Nostoc commune*) and quinoa (*Chenopodium quinoa*). The cushuro is an ancient Andean cyanobacteria that grows over 3000 and 4000 m above sea level, and quinoa is an important Andean seed, and they are part of the basic diet of the Andean Peruvian population. The cushuro obtained from the region of Ancash-Peru was dried (60 °C, 18 h) and the flour was dissolved in 0.1 N HCl at 90 °C for 20 min to obtain a protein concentrate with 6.79% moisture, 0.16% fat, 71.03% protein, and good balance of essential amino acids. Quinoa flour presented 8.52% moisture, 4.84% fat, 12.90% protein, and essential amino acids such as phenylalanine, and lysine. Quinoa flour (65.45%), cushuro concentrated protein (4.76%), cocoa (17.85%), panela (11.90%), and stevia (0.05%), were mixed to obtain the powdered beverage. The results showed that powdered beverage presented an adequate balance of amino acids according to FAO/WHO, with 14.36% proteins, 72.53% carbohydrate, 5.19% fat, 716.6 mg/kg potassium, 319.8 mg/kg phosphorus, 139.2 mg/kg magnesium, 82.69 mg/100 g vitamin C, 1.49 µg/100 g vitamin B12, heavy metals below the detection level (<0.050 mg/kg). The powdered beverage complied with the Peruvian legislation of the Healthy Law No. 30021 and with the microbiological requirements.



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1. Introduction

Cushuro belongs to the genus *Nostoc*, a type of algae (cyanobacteria), when they are hydrated, they can form spheres of 10 to 25 mm in diameter, like grapes. Cushuro is found in the Andean foothills over 3000 m above sea level. Its characteristic color is bluish green and is part of the basic diet of the Andean Peruvian population. Cushuro is also known as “murmunta”, “llullucha” or “llayta”. The *Nostoc commune* Vauch. form colonies that in their natural environment resist dryness and can easily restore their metabolism after being rehydrated because cells produce large amounts of protective polysaccharides. Cushuro is source of nutrients such as protein, calcium, and iron as well as bioactive compounds [1].

The quinoa (*Chenopodium quinoa*) seed is a pseudocereal with high protein content and excellent amino acid composition, domesticated 5000 years ago in the Andean region, due to its high nutritional value, and bioactive properties including antioxidant, hypolipidemic, immunomodulatory, weight-regulating, hypoglycemic, hypotensive, probiotic, antitumor, and hormone-regulating effects [2].

Functional powdered beverages with plant-based protein have been developed as a strategy to get a better nutritional balance, however, few plant species have been used

in the industry for this purpose [3]. The present research focuses on the obtention and characterization of a powdered beverage of cushuro (*Nostoc commune*) concentrated protein and quinoa (*Chenopodium quinoa*) flour, with a balanced profile of essential amino acids.

2. Materials and Methods

2.1. Raw Material

The cushuro was obtained from Cotaparaco district, Recuay province, Ancash region-Peru and quinoa was obtained from a local market from the local market in Lima city. The cushuro was washed, dried at 60 °C for 12 h in an infrared dehydrator (IRC D18, Irconfort, Sevilla, Spain), then ground in the food shredder (Grindomix GM200, Restch, Haan, Germany) in the Functional Foods Laboratory from the Universidad de Lima-Peru to obtain cushuro flour. Quinoa was dried at 40 °C and ground in the food shredder. All the samples were stored in aluminized bags at room temperature until use.

2.2. Extraction of Cushuro Concentrated Protein (CCP)

The cushuro flour was pretreated with HCl 0.1 N for 20 min (64.44 mL/g, mL solvent/g sample) by ultrasound-assisted microwave (CW-2000, Nade, Shanghai, China) extraction (500 W, 50 W/60 Hz). The result of the extraction was filtered under vacuum with muslin cloth. The solid residue (CCP) was washed with water to remove excess acid and dehydrated for 12 h in infrared dryer at 60 °C (IRC D18, Irconfort, Sevilla, Spain). The CCP solid was ground (Grindomix GM200, Restch, Haan, Germany) and stored in aluminized bags for later use.

2.3. Development of Powdered Beverage (PB)

The powdered beverage was developed by mixing quinoa flour (27.5 g), cushuro protein flour (2 g), cocoa (7.5 g), cane sugar (5 g) and stevia (0.02 g) in a planetary mixer (FPSTSMPL1-053, Oster, Guangdong, China). The mixture was packaged in aluminized bags with a net weight of 42 g per unit. The formulation was made to obtain a PB with a protein percentage greater than 14%.

2.4. Proximal Composición

The proximal composition was carried out according to official methods. The moisture content was determined at 110 °C to constant weight. The total protein content was determined as % nitrogen $\times$ 6.25 using a Kjeldahl analyzer (UDK 139, VELP, Usmate Velate, Italy). The ash content was determined by incineration at 550 °C for 72 h, in a muffle furnace. The fat content was determined with hexane for 9 h.

2.5. Determination of Mineral Content

The concentrations of Ca, Cu, Fe, K, Mg, Na, P, Zn and heavy metals such as As, Pb, Hg and Cd in the powdered beverage were determined by atomic absorption spectrophotometry (Nexion 350x, Perkin Elmer, MA, USA) according to AOAC Official Method 2015.01 (2015).

2.6. Determination of Vitamins C and Vitamin B12

The concentrations of Vitamin C and Vitamin B12 were determined by AOAC 985.33 (2019) and AOAC 952.20 (2019), respectively.

2.7. Determination of Solubility, Bulk and Compacted Density and Hausner Index

Solubility, bulk and compacted density and Hausner Index were determined according to Chasquibol et al. [4].

2.8. Determination of Amino Acid Profile

The amino acid profile was estimated according to Chasquibol et al. [5], and it was quantified by HPLC (ARC, Waters, Milford, CT, USA) with an inner diameter of 150 mm × 3.9 mm. column C18 reverse phase.

2.9. Determination of Na, Total Sugar, Saturated Fat, and Trans Fat

The concentrations of Na, total sugar, saturated fat, and *trans* fat were determined by AOAC 969.23 (2019), AOAC 968.28 (2019), ISO 12966-1 (2014) and ISO 12966-1 (2014) respectively.

2.10. Microbiological Analysis

The powdered beverage was analyzed microbiologically to establish the microbiological criteria of sanitary quality and safety for food and beverages for human consumption.

2.11. Statistical Analysis

Results were expressed as mean ± standard deviation. All measurements were determined in duplicate or triplicate. Analysis of variance (ANOVA), which was used to analyze data acquired at a 95% significance level with Minitab 19.0 Software (Minitab Inc., State College, Palo Alto, CA, USA).

3. Results and Discussions

Table 1 shows the physicochemical characterization of PB, CCP, and quinoa flour (QF). The powdered beverage had a high protein content ($14.36 \pm 0.33\%$). According to FAO, 42 g of PB rehydrated in 400 mL of water, would cover 20% of the protein recommended daily for children under 5 years old with an average weight of 20 kg. Also, according to Codex Alimentarius CAC/GL 23-1997, the PB could be declared as a high-protein food for children under 5 years of age. Besides, the carbohydrate ($72.53 \pm 0.36\%$), ash ($3.29 \pm 1.15\%$) and fat content ($5.19 \pm 0.01\%$) increased, but moisture ($4.63 \pm 0.11\%$) content decreased.

Table 1. Proximal composition of cushuro concentrated protein (CCP), quinoa flour (QF) and powdered beverage (PB).

Determination	CCP	QF	PB
Moisture (g/100 g)	6.79 ± 0.28	8.52 ± 0.11	4.63 ± 0.11
Ash (g/100 g)	0.94 ± 0.00	2.54 ± 0.08	3.29 ± 1.15
Fat (g/100 g)	0.16 ± 0.03	4.84 ± 0.02	5.19 ± 0.01
Carbohydrates (g/100 g)	21.41 ± 0.10	71.2 ± 0.21	72.53 ± 0.36
Protein (g/100 g)	71.03 ± 0.73	12.9 ± 0.15	14.36 ± 0.33

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

The composition of amino acids profile is summarized in Table 2. The predominant amino acids of PB were aspartic acid + asparagine (120.5 ± 0.58 mg/g protein), glutamic acid + glutamine (140.7 ± 10.29 mg/g protein), serine (55.79 ± 1.63 mg/g protein), glycine (56.66 ± 2.54 mg/g protein), threonine (71.19 ± 2.12 mg/g protein), arginine (63.36 ± 4.36 mg/g protein), alanine (63.30 ± 1.88 mg/g protein), valine (60.39 ± 1.32 mg/g protein) and leucine (64.60 ± 3.95 mg/g protein). The amino acid composition of PB showed a balanced profile, according to the FAO/WHO recommendations. Limited amino acids (leucine 58.95 ± 2.64 mg/g protein, and tryptophan 6.00 ± 0.02 mg/g protein) in quinoa were improved in the CCP (leucine 64.00 ± 0.00 mg/g protein, and tryptophan 8.60 ± 0.00 mg/g protein). Thus, the powdered beverage showed higher content of specific amino acids (leucine 64.6 ± 3.95 mg/g protein, and tryptophan 27.02 ± 4.37 mg/g protein). Bonke et al. [6] showed that beverages formulated with oat, lentil and pea were rich in amino acids, except for tryptophan. In this research, the powdered beverage was rich in tryptophan, three times above the recommendations of the FAO/WHO.

Table 2. Amino acids profile of cushuro concentrated protein (CCP), quinoa flour (QF) and powdered beverage (PB).

Amino Acids	CCP (mg Amino Acid/g Protein)	QF (mg Amino Acid/g Protein)	PB (mg Amino Acid/g Protein)	OMS
Aspartic acid + Asparagine	149.80 ± 0.80	70.27 ± 16.32	120.5 ± 0.58	
Glutamic acid + Glutamine	80.50 ± 3.20	123.83 ± 14.55	140.7 ± 10.29	
Serine	59.20 ± 0.20	43.62 ± 1.98	55.79 ± 1.63	
Histidine	2.20 ± 0.10	27.43 ± 0.11	16.6 ± 0.87	15
Glycine	57.30 ± 0.40	51.02 ± 2.21	56.66 ± 2.54	
Threonine	121.90 ± 0.40	37.06 ± 1.75	71.19 ± 2.12	23
Arginine	44.80 ± 0.40	73.4 ± 2.08	63.36 ± 4.36	
Alanine	87.30 ± 0.40	39.88 ± 3.89	63.6 ± 1.88	
Proline	42.90 ± 2.30	22.1 ± 8.9	22.59 ± 1.07	
Tyrosine ^b	17.80 ± 0.10	22.8 ± 1.95	25.03 ± 1.93	38 ^b
Valine	103.80 ± 0.10	42.34 ± 1.95	60.39 ± 1.32	39
Methionine ^a	1.50 ± 0.10	1.37 ± 0.28	8.44 ± 0.86	22 ^a
Cysteine ^a	1.10 ± 0.00	4.27 ± 0.21	4.69 ± 0.62	
Isoleucine	57.80 ± 0.00	34.81 ± 1.62	43.57 ± 1.5	30
Tryptophan	8.60 ± 0.00	6.00 ± 0.02	27.02 ± 4.37	6
Leucine	64.00 ± 0.00	58.95 ± 2.64	64.6 ± 3.95	59
Phenylalanine ^b	69.30 ± 0.12	37.18 ± 1.09	48.4 ± 1.57	
Lysine	30.10 ± 0.10	52.1 ± 1.63	42.17 ± 3.25	45

Results are expressed as mean ± SD (*n* = 3). Note: According to the Food and Agriculture Organisation, 'Assessment of the quality of dietary proteins in human nutrition' (2013). ^a: Met + Cys; ^b: Phe + Tyr.

The PB had a compacted density of 0.935 g/mL and a Hausner index of 1.03. These values indicate that the powdered beverage has little fluidity and would occupy a smaller volume in its packaging. The solubility values obtained are lower (28.08%) by the higher fiber contain the quinoa, extrusion process would improve the solubility of the beverage.

The PB contained important quantity of minerals (Table 3): calcium (130.6 ± 10.40 mg/kg), potassium (716.6 ± 57.28 mg/kg), magnesium (139.2 ± 11.12 mg/kg), sodium (62.4 ± 4.96 mg/kg), phosphorus (319.8 ± 25.52 mg/kg), copper (0.8 ± 0.06 mg/kg), iron (4.0 ± 0.32 mg/kg) and zinc (2.8 ± 0.22 mg/kg). According to the daily requirements for children under 5 years of the Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes [7] and National Institute of Health [8], the PB would cover 6.8% about requirement daily of calcium, 26.86% phosphorus, 44.97% magnesium, 13.08% potassium and 16.8% iron. So, the PB developed could contribute to the reduction of malnutrition and childhood anemia in our country. The presence of heavy metals (Hg, As, Cd and Pb) was not detected in the PB (<0.050 mg/kg).

Table 3. Minerals, heavy metals and vitamins content of powdered beverage (PB).

Minerals (mg/kg)		Heavy Metal (mg/kg)		Vitamins	
Calcium	130.6 ± 10.40	Arsenic	<0.050 ± 0.001	Vitamin C (mg/100 g)	82.69 ± 0.10
Copper	0.8 ± 0.06	Cadmium	<0.050 ± 0.001	Vitamin B12 (µg/100 g)	1.49 ± 0.01
Iron	4.0 ± 0.32	Mercury	<0.050 ± 0.001		
Potassium	716.6 ± 57.28	Lead	<0.050 ± 0.001		
Magnesium	139.2 ± 11.12				
Sodium	62.4 ± 4.96				
Phosphorus	319.8 ± 25.52				
Zinc	2.8 ± 0.22				

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

The PB had higher vitamin C (82.69 mg/100 g) than commercial brands in Peru and the vitamin B12 (1.49 µg/100 g) content could cover 51.1% of the daily requirement according to the National Institute of Health.

The content of sodium, total sugar, saturated fat, and trans fat of the PB is shown in Table 4. The PB have a low content in sodium (46.02 ± 3.68 mg/100 g), total sugar (9.33 ± 0.72 g/100 g), saturated fat (1.39 ± 0.01 g/100 g) and trans-fat ($<0.01 \pm 0.001$ g/100 g fat), below the limits permissible established in Peruvian legislation.

Table 4. Sodium, total sugars, saturated fat and *trans* fats content of powder beverage (PB).

Determination	PB	Maximum According to Standard *
Sodium mg/100 g	46.02 ± 3.68	400 mg/100 g
Total sugars (g/100 g)	9.33 ± 0.72	10 g/100 g
Saturated fat (g/100 g)	1.39 ± 0.01	4 g/100 g
Trans fats (g /100 g fat)	$<0.01 \pm 0.001$	5 g/100 g

Results are expressed as mean $\pm$ SD ($n = 3$). * Law No. 30021 Law on the Promotion of Healthy Eating for Children and Adolescents, regulated in Supreme Decree No. 017-2017-SA-Peru.

The microbiological analysis of powder beverage (Table 5) showed that the PB complied with the microbiological standard according to the limits permissible, established in Peruvian legislation of sanitary quality and safety for food and beverages for human consumption.

Table 5. Microbiological analysis of powder beverage (PB).

Microorganism	PB	Maximum According to Standard *
<i>Staphylococcus aureus</i> (CFU/100 g)	$<10 \pm 0.01$	10^2
<i>Coliformes</i> (CFU/100 g)	$<10 \pm 0.01$	10^2
<i>Salmonella</i> sp. (presence/absence)	Absence	Absence
<i>Bacillus cereus</i> (CFU/100 g)	$<100 \pm 0.10$	10^3

Results are expressed as mean $\pm$ SD ($n = 3$). * Ministerial Resolution 591-2008-MINSA-Peru. Sanitary standard that establishes the microbiological criteria of sanitary quality and safety for food and beverages for human consumption. CFU: Colony Forming Units.

4. Conclusions

The results showed that the PB of cushuro and quinoa had a balanced profile of essential amino acids according to FAO/WHO, being the majority aspartic acid + asparagine, glutamic acid + glutamine, serine, glycine, threonine, arginine, alanine, valine, and leucine. In addition, PB had important quantity of minerals such as potassium, phosphorus, and iron. The PB accomplishes the microbiology sanitary standards of the Peruvian law and the healthy standards legislation for children. Thus, the powdered beverage could be an important supplement for children to promote a healthy nutrition.

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