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Original Article

Protein Potential of Cushuro (*Nostoc sphaericum*) Gelatinous Cianobacteria Powder: An Analysis of Its Protein Quality

Potencial proteico de la cianobacteria gelatinosa en polvo cushuro (Nostoc sphaericum): un análisis de su calidad proteica

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ABSTRACT

Cushuro (*Nostoc sphaericum*) is a cyanobacteria whose most notable characteristic is the ability to fix nitrogen from the environment and convert it into proteins, through biochemical processes. Its consumption is promoted mainly in the Andean regions, where its growth is favored by environmental conditions. The objective of this study was to determine the protein quality, moisture content and amino acid profile of cushuro, comparing the latter with the scoring pattern established by WHO/FAO/UN in 2017 for children, adolescents and adults. Cushuro powder samples were obtained from 51 kg of fresh cushuro, through drying and grinding processes. Proximate analysis of the components and determination of the amino acid profile were performed using HPLC UV-VIS. The results showed that cushuro contains 30.48 % protein and 1.62 % moisture. Additionally, the amino acid profile revealed that cushuro partially met the amino acid intake recommendations set by the WHO/FAO/UNU in 2017, with the exception of cysteine, methionine and valine. The cyanobacterium cushuro (*Nostoc sphaericum*) powder cannot be considered as a high protein quality food due to the presence of limiting amino acids. However, its practicality and versatility in fresh and/or dry present many opportunities for its use in hospital gastronomy, vegan enteral formulas, protein modules, and more as needed.

Keywords: Nostoc sphaericum, cyanobacterium, proteins, amino acid profile, protein quality.

RESUMEN

El cushuro (*Nostoc sphaericum*) es una cianobacteria que tiene como característica resaltante, la capacidad de fijar nitrógeno del ambiente y convertirlo en proteínas, mediante procesos bioquímicos. Su consumo se promueve principalmente en las regiones andinas, en donde su crecimiento se ve favorecido por las condiciones medio ambientales. El

objetivo de este estudio fue determinar la calidad proteica, el contenido de humedad y el perfil de aminoácidos del cushuro, comparando este último con el patrón de ingesta aminoacídica establecido por la WHO/FAO/ONU en 2017 para niños, adolescentes y adultos. Se obtuvieron muestras de cushuro en polvo a partir de 51 kg de cushuro fresco, mediante procesos de secado y molienda/pulverización. Se realizó el análisis proximal de los componentes y la determinación del perfil de aminoácidos mediante HLPC UV-VIS. Los resultados mostraron que el cushuro contiene 30,48 % de proteínas y 1,62 % de humedad. Por otro lado, el perfil de aminoácidos reveló, que el cushuro cubría parcialmente las recomendaciones de ingesta aminoacídica establecidas por la WHO/FAO/ONU en 2017, con excepción de la cisteína, metionina y valina. La cianobacteria cushuro (*Nostoc sphaericum*) en polvo no puede considerarse como un alimento de alta calidad proteica debido a la presencia de aminoácidos limitantes (cisteína y metionina). Sin embargo, su practicidad y versatilidad en fresco y/o seco, representa muchas oportunidades para su uso en la gastronomía hospitalaria, fórmulas enterales veganas, módulos proteicos, entre otros, según necesidad.

Palabras clave: Nostoc sphaericum, cianobacteria, proteínas, perfil de aminoácidos, calidad proteica.

INTRODUCTION

Nostoc is a cyanobacterium that forms colonies in shades of blue-green, olive green, or brown and can survive extreme climatic conditions (Maquera, 2022). Its optimal development occurs at altitudes between 3,000 and 5,000 meters above sea level. The colonies of Nostoc resemble "translucent, gelatinous, spherical grapes, with a diameter ranging from 10 to 25 mm," and they have the ability to fix nitrogen from the air and other elements to produce amino acids, thus enhancing their nutritional value (Ponce, 2014). Specifically, the species Nostoc sphaericum has been identified in South America, where it is known by different common names such as cushuro, murmunta, and llayta. Traditionally, it has been used as a food source in the Andean regions (Ponce, 2014).

One of the most remarkable characteristics of Nostoc sphaericum or cushuro is its high protein content (Maquera, 2022). Proteins are essential macronutrients, which vary in their structure, size and function. These molecules are essential and necessary components for the maintenance of muscle mass in the human body, since they are structural and functional constituents of cells; they also fulfill enzymatic, hormonal, defense and transport functions, among others. Protein requirements are calculated based on the individual characteristics of each person, due to their crucial role in the health and function of the organism (Guillamón *et al.*, 2021).

The protein sources consumed to meet these requirements come from both animal and plant-based foods, differing in the quality of proteins offered. Animal-based proteins are considered high biological value due to their abundance in essential amino acids, whereas most plant-based proteins lack certain essential amino acids, which limits their nutritional value (Guillamón *et al.*, 2021).

Amino acids are the building blocks of proteins, and their functionality depends

on their sequence (Morales *et al.*, 2017). There are 20 amino acids in total, classified based on the body's ability to synthesize them. Of these, nine are considered essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), while the others are non-essential because the body can synthesize them, making their intake unnecessary to meet requirements (López, 2014; Morales *et al.*, 2017). The amino acid profile is used as a parameter to determine the protein quality of foods by measuring the amount of essential amino acids per gram of protein, following WHO/FAO/UNU 2017 recommendations to ensure growth in children and adolescents and tissue maintenance in adults (FINUT, 2017).

Previous research has evaluated the total protein content (Fernández & Suyón, 2018; Maquera, 2022) and amino acid profile (Galetovic *et al.*, 2017) in samples of cushuro powder to record its contribution as a macronutrient in the diet and consider it a protein consumption alternative. However, given the aforementioned limitations, there is an interest in analyzing protein quality more precisely to confidently recommend its use as a protein source, ensuring it meets amino acid requirements according to the recommendations set by the aforementioned organizations.

MATERIALS AND METHODS

Moisture Determination

For the analysis of cushuro powder samples, 3–4 g were accurately weighed onto a Petri dish (Czaja *et al.*, 2020). Drying was then performed for 12–18 hours at 100–102 °C or 4 hours at 125 °C (Silva *et al.*, 2020). After drying, the cushuro powder sample was removed from the oven and placed in a desiccator for 30 minutes before weighing (Zumbado, 2022).

Fat Determination

For this procedure, 3–4 g of cushuro powder were placed in an extraction thimble with a filter paper circle (Zumbado, 2022). The thimble and its contents were then transferred to the extraction apparatus, ensuring the beaker was rinsed with ethyl ether to avoid sample loss. In Soxhlet extraction equipment, the sample is extracted with ethyl ether for 6-8 hours at a condensation rate of 3-6 drops per second (Hewavitharana *et al.*, 2020). Once extraction was complete, the ethereal extract was transferred to an evaporation dish in a fume hood, and the ethyl ether was evaporated until no odor was detected (Zumbado, 2022). The dish and its contents were dried in a mechanical convection oven for 30 minutes at 100 °C, cooled in a desiccator, and weighed (Zumbado, 2022).

Ash Determination

Five grams of cushuro powder were weighed into a pre-weighed porcelain crucible (Czaja *et al.*, 2020). The sample was dried at 100 °C for 3–4 hours in a mechanical convection oven. After drying, the crucible was removed, and the sample was initially carbonized by placing the crucible over a Bunsen burner until it turned black (Zumbado, 2022). The crucible with the sample was then transferred to a muffle furnace and incinerated at 500–600 °C until the sample turned grayish or white (approximately 8 hours) (Ayensu *et al.*, 2019; Zumbado, 2022). Subsequently, the crucible was removed from the muffle furnace, and the ash was moistened with a few drops of water. The sample was dried again in the oven at 100 °C for 3–4 hours and re-incinerated in the muffle furnace

at 500–600 °C for an additional hour (Zumbado, 2022). Finally, the crucible was removed from the furnace, allowed to cool briefly, and placed in a desiccator until it reached room temperature before being weighed (Bayata, 2019; Zumbado, 2022).

Protein Determination

The Kjeldahl digestion method was used to determine protein content, with ~1 g of cushuro powder placed into a digestion flask. Subsequently, 25 ml of sulphuric acid and 10 g of catalyst was added with caution. In a fume hood, the digestion is performed slowly at first, in order to avoid excessive foaming, and is extended for at least 45 min after the solution has turned a light pale green (Zenteno, 2019). It was important to allow the solution to cool completely before quickly adding 100-200 ml of water and mixing. It was recommended to rinse the digestion flask 2-3 times and add the rinses to the volume. Then, 80-85 ml of saturated sodium hydroxide solution measured with a measuring cylinder were added in order to avoid ammonia losses.

If after stirring, the solution does not turn blue due to copper hydroxide, it indicates that insufficient alkali has been added (Zenteno, 2019 and Zumbado, 2022). As for distillation, it is suggested in 25 ml of 0.1 N hydrochloric acid with a few drops of red methylene indicator. Alternatively, it can be distilled in 50 ml of 2 % boric acid with the same indicator. Boric acid is neutral to the indicator and the alkaline ammonium borate formed can be titrated directly with 0.01 N HCL (Sáez-Plaza and García, 2019 and Zumbado, 2022).

Determination of the Amino Acid Profile by HPLC UV-VIS

High-performance liquid chromatography (HPLC) serves as a technique for the separation of various components within a sample. Due to the structural diversity and physicochemical properties of these components, they are divided into two phases: the stationary phase and the mobile phase (Sucasaca and Ramírez, 2021). The stationary phase may consist of a solid, a liquid supported by a solid, or a gel, and can be contained within a column arranged either as a layered structure or as a film. Conversely, the mobile phase may be either gaseous or liquid, contingent upon the state of matter being analyzed (Legaz, 2011). This technique can be classified from multiple perspectives, including the nature of the phases involved—whether for the mobile phase (gas or liquid) or the stationary phase (liquid or solid)—the type of support utilized in the analysis, such as columns, paper, or plates, the separation mechanisms applied, including absorption, partition, ion exchange, or gel permeation, and the nature of the solute being analyzed, encompassing ions, proteins, and polymers, among others (Legaz, 2011; Sucasaca and Ramírez, 2021). Furthermore, when considering the partition mechanism as a method of separation in liquid chromatography, a stationary phase that exhibits lower polarity compared to the mobile phase is classified as a reverse phase. The disparity in polarity significantly affects polar analytes, resulting in a reduced affinity for the stationary phase in comparison to nonpolar analytes. In this context, the stationary phase is characterized as apolar, while the mobile phase is moderately aqueous and polar (Legaz, 2011; Sacristán *et al*., 2019).

Therefore, it is imperative to consider the polarity of the components present in the sample, as previously noted by Sacristán *et al*. (2019), given its substantial

influence on the analytical results. These authors establish an ascending order of the polarity of various functional groups of the analyte as follows: "hydrocarbons < ethers < esters < ketones < aldehydes < amides < amines < alcohols."

Procedure:

The assay employed a reverse phase methodology, executed through pre-column derivatization of amino acids utilizing o-phthalaldehyde (OPA). The reaction between amino acids and the OPA reagent, facilitated by a strong reducing agent (2-mercaptoethanol) under alkaline conditions, yields fluorescent isoindoline derivatives. This strategy represents a sensitive and selective detection method for amino acids, encompassing the initial amino acids in diverse samples (Abdo-de la Parra *et al.*, 2017; Benítez *et al.*, 2002).

RESULTS AND DISCUSSION

Proximate Analysis of Cushuro

The findings from the proximate analysis of the cushuro (*Nostoc sphaericum*) powder sample are illustrated in Table 1. This table delineates the values obtained for various constituents within the sample, including total protein, total carbohydrates, ash, fat, moisture, and total energy. The results presented in Table 1 reveal that, when arranged in descending order, the sample composition predominantly consists of moisture, followed by carbohydrates, proteins, and ash.

The moisture content found in the product in fresh base is 98.38 %, which is similar to the results obtained by Chili *et al.* (2010) with a value of 98.61 % and what was recorded in the research work of Fernandez and Suyon (2018) with 98.41 %. Regarding total carbohydrates, a value of 60.08 % was found in the analyzed powder sample, being similar to the values reported by Chili *et al.* (2010) with a value of 55.15 %, Galetovic *et al.* (8) with a value of 60.80 % and Maquera (2022) with 62.07 percentage points; however, the result obtained by Fernández and Suyon (2018) differs from the aforementioned authors, reporting a value of 34.86 %. In relation to protein, a value of 30.48 % was obtained, which is similar to the value reported by Chávez (2014) in his research with a value of 32.36 %, while Maquera (2022) found a value of 28.18 % and Fernández and Suyón (2018) obtained a result of 44.48 % of the contribution of this macronutrient. However, other authors found results more similar to those obtained in this research, with values of 30.54 % by Chili and Terrazas (2010) and 30.40 % by Galetovic *et al.* (2017). Finally, the ashes of the sample present a 6.98 % of its composition, which is comparable with the values obtained by Chili and Terrazas (2010) with a value of 6.81 %, Galetovic *et al.* (2017) with a value of 6.40 %, Maquera (2022) with 7.68 percentage points and Fernandez and Suyon (2018) with 10.62 %.

Table 1.
Proximal analysis of cushuro (Nostoc sphaericum) powder

Parameter	Result
Total protein	30.48%
Total carbohydrate	60.08%
Ash	6.98%
Fat	0.84%
Moisture	1.62%
Total energy	369.8 kcal/100 g

Source: Delgado (2022)

Amino Acid Profile of Cushuro

The results of the evaluation of the amino acid profile of the cushuro (*Nostoc sphaericum*) powder sample obtained by HPLC are presented in Table 2. The amino acids are ordered according to their need for dietary intake, i.e., essential amino acids that the human body does not synthesize and require daily intake, and non-essential amino acids that the human body has the capacity to synthesize. Likewise, in Table 3 a comparison is made with the results obtained by Galetovic *et al.* (2017), who analyzed the amino acid profile of the same species of *Nostoc.*

Table 2.
Amino Acid content of Cushuro Powder

Amino acids (mg/g)			
Essential		**Non-essential**	
Histidine	1.3	Alanine	7.9
Isoleucine	18.7	Aspartic acid	44.3
Leucine	28.2	Glycine	13.8
Lysine	26.9	Glutamic acid	10.5
Methionine	23.3	Serine	40.4
Phenylalanine	6.2	Glutamine	12.8
Threonine	0.3	Proline	5.2
Tryptophan	0.3	Asparagine	ND **
Valine	34.8		
Cysteine*	10.8		
Tyrosine*	5.6		
Arginine*	47.6		

* Essential conditional amino acids
** ND: Not determined
Source: Delgado (2022)

In addition, the results of the comparison of the essential amino acids found in the cushuro powder sample with the WHO/FAO/ UNU 2017 reference standard for children, adolescents and adults (FINUT, 2017), indicated in Table 5, show a similar behavior to the reference standard for children aged 6 months to 3 years.

Evaluation of the Amino Acid Profile of Cushuro Compared to WHO/FAO/UNU 2017 Recommendations for Children Aged 6 Months to 3 Years

Table 4 presents the results of the comparison of essential amino acids in the cushuro powder sample with the WHO/ FAO/UNU 2017 reference pattern for children aged 6 months to 3 years. This table also includes the score, which indicates the percentage by which the sample meets the recommended intake levels for essential amino acids.

In comparing the essential amino acids found in cushuro powder with the WHO/FAO/UNU 2017 reference pattern for children aged 6 months to 3 years (FINUT, 2017), differences between the two data sets are evident, as shown in Table 4. Specifically, most of the essential amino acids in the cushuro powder sample do not meet the recommendations for this age group. The limiting amino acids in this protein source are histidine, isoleucine, leucine, lysine, aromatic amino acids (phenylalanine and tyrosine), threonine, tryptophan, and valine. However, the sulfur-containing amino acids (cysteine and methionine) exceed the established recommendations

Table 3.

Comparison of Amino Acid profiles of cushuro (Nostoc sphaericum)

Essential Amino Acids			Non-Essential Amino Acids		
	Delgado (mg/g)	Galetovic *et al.* (mg/g)		Delgado (mg/g)	Galetovic *et al.* (mg/g)
Histidine	1.3	1.3	Alanine	7.9	8.3
Isoleucine	18.7	19.2	Aspartic acid	44.3	46.2
Leucine	28.2	26.4	Glycine	13.8	14.5
Lysine	26.9	26.5	Glutamic acid	10.5	20.3
Methionine	23.3	26.8	Serine	40.4	40.9
Phenylalanine	6.2	5.2	Glutamine	12.8	ND **
Threonine	0.3	0.07	Proline	5.2	ND **
Tryptophan	0.3	ND **	Asparagine	ND **	ND **
Valine	34.8	35.1			
Cysteine*	10.8	0.5			
Tyrosine*	5.6	6.2			
Arginine*	47.6	45.6			

* Essential conditional amino acids
** ND: Not determined
Source: Delgado (2023)

Table 4.

Comparison of Amino Acid content in cushuro with the WHO/FAO/UNU reference for children aged 6 months to 3 years

Amino Acids	Cushuro Powder (mg)	WHO/FAO/UNU reference (mg/g)	Score (%)
Histidine	1.3	20	6.5
Isoleucine	18.7	32	58.4
Leucine	28.2	66	42.7
Lysine	26.9	57	47.2
Sulfur (Cys* and Met)	34.1	27	126.3
Aromatics (Fen and Tir*)	11.8	52	22.7
Threonine	0.3	31	1
Tryptophan	0.3	8.5	3.5
Valine	34.8	43	80.9

* Essential conditional amino acids
Source: Delgado (2022)

Analysis of Cushuro Amino Acid Profile Compared to WHO/FAO/UNU 2017 Recommendations for Children, Adolescents, and Adults

Table 5 shows the comparative results of essential amino acids in cushuro powder with the WHO/FAO/UNU 2017 reference pattern for children, adolescents, and adults. The same comparison criteria used in Table 4 were applied to assess the sample's coverage of the reference recommendations.

Table 5.

Differences in Amino Acids profile between cushuro and WHO/FAO/UNU reference for children, adolescents and adults

Amino Acids	Cushuro Powder (mg)	WHO/FAO/UNU Reference (mg/g)	Score (%)
Histidine	1.3	16	8.1
Isoleucine	18.7	30	62.3
Leucine	28.2	61	46.2
Lysine	26.9	48	56
Sulfur (Cys* and Met)	34.1	23	148.3
Aromatics (Fen and Tir*)	11.8	41	28.8
Threonine	0.3	25	1.2
Tryptophan	0.3	6.6	4.5
Valine	34.8	40	87

* Essential conditional amino acids
** ND: Not determined
Source: Delgado (2023)

CONCLUSIONS

Cushuro powder contains 30.48% total protein and partially meets the amino acid intake recommendations established by WHO/FAO/UNU in 2017 for children, adolescents, and adults. Notably, the sulfur-containing amino acids (cysteine and methionine) exceed the recommended levels, contributing to a higher score for this category. Additionally, cushuro powder contains glutamine, which is considered a non-essential amino acid.

Cushuro can be integrated into various nutritional strategies, including: Dietary therapeutic treatments, requiring specific protein supplementation with controlled nitrogen load and biological value. Hospital gastronomy, as the transformation of fresh cushuro into powder allows for protein fortification Vegan enteral formulas, protein modules, and other applications tailored to specific nutritional needs.

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