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Study of Essential Amino Acids Bioaccessibility in a Quinoa (*Chenopodium quinoa willd*) and Amaranth (*Amaranthus caudatus*) Supplement for Ecuadorian Adolescents,

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Abstract:

The consumption of food supplements in Latin America represents 7% of the world, as reported by the Latin American Alliance of Responsible Nutrition (ALANUR) in 2021. Developing high-quality Andean grains supplements could be interesting for enhancing the country's food security.

A supplement has been developed that contains high-quality protein and carbohydrates sourced from a blend of precooked quinoa and amaranth flours. Additionally, it includes omega-3 and omega-6 fatty acids derived from microencapsulated sacha inchi and chia oils, along with vitamins and minerals. The process for obtaining the precooked flours involved cooking at 75 C for 12min, followed by drying in a tray dryer at 70 C for 8-9 hours, grinding in a disk mill, and sieved to achieve a particle size of 150 μm. Pasta tests were conducted using RVA and DSC to check their gelatinization. The supplement´s composition adheres to the mandatory nutrient requirements specified by the Ecuadorian standard NTE INEN1334-2, 2011. Moreover, the supplement satisfies sensory criteria related to taste and consistency.

To evaluate the impact of the processing on nutrient attributes, assessing their bioaccessibility becomes significant. To accomplish this, the static in vitro digestion method was employed, both before and after the digestion process. The digestion protocol involves the following steps: oral phase with amylase, gastric phase with pepsin, and intestinal phase with pancreatin. The resulting digest was subsequently centrifuged and filtered. The apparatus utilized consisted of a reactor equipped with precise controls for temperature, pH, and agitation.

The in vitro digestibility percent for the supplement shake was determined to be 96.7 % (IVD). Essential amino acids were quantified through HPLC analysis with a fluorescence detector. As a result, lysine and histidine exhibited the highest bioaccessibility values of 97% and 79%, respectively, while methionine had the lowest value of 32%. The remaining amino acids showed intermediate values.

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Keywords: Quinoa; amaranth; precooked flours; in vitro digestion; bioaccessibility; essential amino acids

1. Introduction

Quinoa (Chenopodium quinoa Willd.) is an ancient crop dating back to 5000 B.C. Its cultivation was highly developed within the Inca Empire and served as the foundation of indigenous nutrition. Following the Spanish conquest, it was replaced by wheat and barley. This high-nutrition crop originates from the Andean region of South America, with a history dating from 5000 BC to 3000 BC [1]. Ecuador has ranked as the third-largest producer of quinoa since 2005, following Peru and Bolivia [2].

Amaranth (*Amaranthus caudatus*) belongs to the Amaranthaceae family within the Amaranthus genus, comprising approximately 70 species. Forty of these species are native to the Americas, while the remainder are distributed in Australia, Africa, Asia, and Europe. It thrives in the Andean and coastal regions and enjoyed significant popularity during the pre-Columbian era before fading into obscurity [3]. Both quinoa and amaranth have been recognized as sources of complete proteins [4].

The World Health Organization (WHO) identifies malnutrition as a leading global cause of death. In Ecuador, malnutrition, obesity, and overweight conditions account for 4.3% of the Gross Domestic Product (GDP), equivalent to 4,344 million dollars, according to research conducted by the Economic Commission for Latin America and the Caribbean and the World Food Programme [5].

In terms of sales, Latin America has experienced remarkable growth, increasing from a 3% share of global supplement sales in 1999 to 7% in 2017. This demonstrates that the region's sales more than doubled in less than two decades (ALANUR) [6]. The development of high-quality Andean grain supplements holds promise for boosting Ecuador's economic potential and enhancing the country's food security.

The supplement's composition complies with the mandatory nutrient requirements specified by the Ecuadorian standard NTE INEN1334-2, 2011 [7]. Furthermore, the supplement meets sensory criteria related to taste and consistency.

To confirm the high quality of Quinoa and Amaranth protein in the supplement, it was proposed to investigate the accessibility of the essential amino acids present and their digestibility during the gastrointestinal process.

2. Materials and Methods

A supplement aimed at adolescents was developed, taking into account the Ecuadorian Technical Standard NTE INEN1334-2, 2011. This standard establishes mandatory nutrient declarations and daily values for children over 4 years old and adults. To create the supplement, a combination of pre-cooked quinoa (Tunkahuan) and amaranth (Alegría) flours was prepared. Additionally, a microencapsulation of a blend of sacha inchi and chia oils, along with a mixture of vitamins and minerals, was included.

The pseudocereal seeds were sourced from the local market in Ambato, Ecuador, and the extra virgin Sacha inchi and chia oils were acquired from Inpalca and Novachem respectively, both in Ecuador.

To obtain the pre-cooked flours, the quinoa underwent a washing process to remove the saponins present in its husk. Impurities accompanying the seeds were then removed. The quinoa was cooked for 12 minutes at 75°C, followed by drying in an electric tray dryer (CT-C-II) at 70°C for 8 hours. It was then milled using an industrial pulverizer (Zion) until achieving a particle size of 150 μm. Tests for pasta and calorimetry were conducted to verify the gelatinization of the flour.

For the production of oil microcapsules, an initial emulsion was prepared by mixing maltodextrin and arabic gum (1:1) with water at 42°C. A mixture of sacha inchi and chia oils (67:33) was added, homogenized at 2400 rpm, and subjected to spray drying in a mini Spray Dry Büchi B-290 with an inlet temperature of 150°C and an outlet temperature of 90°C. Tests for the quantification of free oil were carried out to verify the efficiency of microencapsulation2.1 In vitro digestion

The supplement beverage (water:supplement; 8:1) was analyzed using an in vitro digestion method following the standardized protocol established for food (COST INFOGEST network). This involved subjecting the sample to the following steps: an oral phase with amylase, simulated salivary fluid (SSF) (1:1) and CaCl2 at a pH of 7, 37°C for 2 minutes; a gastric phase with pepsin and simulated gastric fluid (SGF) (1:1) and CaCl2 at a pH of 3, 37°C for 2 hours; an intestinal phase with simulated intestinal fluid (SIF) (1:1) and CaCl2 at a pH of 7, 37°C for 2 hours. The sample was then centrifuged at 4500 rpm for 30 minutes and filtered through a 1µm membrane. The simulated fluids were prepared according to the methodology outlined by Minekus, et al. [8]. After, the samples were combined and lyophilized using a protease inhibitor, in accordance with the procedures described by Minekus, et al. [8]. The in vitro digestibility value of the sample (IVD%) was calculated as the difference between the initial and undigested samples; the obtained result was then divided by the initial mass of the sample and multiplied by 100, following the methodology of Igual, et al. [9], with some modifications

2.1. Amino acids (AA) determination

The free amino acids were determined following the methodology reported by Kerkaert, et al. [10]. In this process, 0.5 g of the digested and lyophilized sample underwent acid hydrolysis (using 4 mL of 6N HCL), while another 0.5 g of the sample underwent alkaline hydrolysis (with 4 mL of 6N NaOH). Both were manually agitated separately for 1 minute and then dried at 105°C for 24 hours. The resulting extracts were combined with 20 mL of HPLC-grade water, neutralized using a pH meter with a 1N NaOH or HCL solution, and filtered through a 0.45 µm membrane.

The filtered sample was derivatized with ortho-phthalaldehyde (OPA) for primary amino acids, and with 9-fluorenylmethylchloroformate (FMOC) for secondary amino acids in the injector of an HPLC system (Agilent Technologies). The derivatized amino acids were separated on a Zorbax Eclipse AAA column (4.6 x 150 mm, 3.5µm, Agilent Tech) at a flow rate of 2 mL/min, at a temperature of 40°C, using a solvent gradient of A: 45% methanol, 45% acetonitrile, and 10% water, and B: 45 mM NaH2PO4 H2O, 0.02% NaN3, pH 7.8. The detector employed was a fluorescence detector at 340/450 nm and 266/305 nm. Norvaline and sarcosine were used as internal standards.

3. Results y Discussion

The pasting properties are presented in Table 1. The absence of the gelatinization peak in the DSC analysis, along with the reduction in the pasting temperature (Table 1), confirms the thorough gelatinization of the flours.

Table 1. Pasting properties.

Sample	Peak 1	Breakdown	Final Visc	Setback	Peak time	Pasting Temp
Raw amaranth flour	1235.00	71.00	1564.00	400.00	6.07	59.80
Cooked amaranth flour	1614.00	608.00	1390.00	384.00	3.20	50.10
Raw quinua flour	378.00		430.00	50.00	10.00	69.85
Cooked quinua flour	1605.00	608.00	1397.00	400.00	3.33	50.10

[1] The experiments were conducted in triplicate.

Regarding the developed microencapsulation, an efficiency of 68.20% was achieved, a value considered adequate according to reports by Bae, et al. [11] and Castejón, et al. [12].

The content of free amino acids, analyzed from the lyophilized sample obtained after static in vitro digestion and determined using the methodology of Kerkaert, et al. [10], is presented in Table 2. The percentage of in vitro digestibility of the supplement shake (IVD%) was 96.7%. The bioaccessibility of the amino acids ranges from 32% to 97%, with methionine having the lowest value and lysine the highest, as shown in Table 2.

As indicated by Igual, et al. [9], Igual, et al. [13] and Uribe-Wandurraga, et al. [14], in vitro digestion is useful for estimating pre-absorptive events, such as nutrient bioaccessibility from a food matrix. The bioaccessibility results obtained (Table 2) demonstrate a high bioaccessibility of most essential amino acids present in quinoa and amaranth, making the development of new products without nutrient loss during the transformation process an interesting prospect.

Table 2. Essential Amino Acids and Bioaccessibility of the Developed Supplement.

Essential amino acids	mg/100g supplement	% bioaccessibility
Histidine	0.0099 ± 0.0004	79 ± 6
Arginine	0.027 ± 0.008	90 ± 3
Threonine	0.0077 ± 0.0007	48 ± 5
Valine	0.038 ± 0.003	68 ± 8
Methionine	0.0393 ± 0.007	32 ± 4
Lysine	0.160 ± 0.006	97 ± 4
Isoleucine	0.0252 ± 0.0013	78 ± 11
Leucine	0.139 ± 0.004	73 ± 6
Phenylalanine	0.070 ± 0.002	68 ± 6

* The experiments were conducted in triplicate and analyzed by taking the mean of the triplicate.

4. Conclusions

This study assessed the release of essential amino acids from a supplement crafted from a blend of precooked quinoa and amaranth flours, a microencapsulation of sacha inchi and chia oils, as well as vitamins and minerals. A high in vitro digestibility value of the supplement shake (96.7 %) was determined, with bioaccessibility ranging from 32 to 97%. The cooking and drying process of quinoa and amaranth flours proved suitable for achieving gelatinization and high digestibility of protein and carbohydrates. The supplement meets the nutritional requirements of the population, along with acceptable sensory characteristics.

Author Contributions:

Conceptualization: Purificación García-Segovia, Javier Martínez-Monzó, and Mayra Paredes-Escobar

Methodology: Purificación García-Segovia, Javier Martínez-Monzó, and Marta Igual

Formal Analysis: Marta Igual, Xavier Carrera, Johana Ortiz, and Mayra Paredes-Escobar

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

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