




In vitro antihypertensive activity by bioactive components of Andean grains
Actividad antihipertensiva *in vitro* de componentes bioactivos de granos andinos

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Abstract

Andean grains (quinua, cañahua and tarwi) have recognized health benefits. The aim of the present study was to evaluate the inhibitory activity of phenolic extracts (PE), hydrolyzed proteins, peptide fractions of royal white quinoa (QRB), royal black quinoa (QRN), royal red quinoa (QRR), J'acha grain quinoa (QJG), wild Ajara quinoa (QA), Phisanqalla quinoa (QP), Kurmi quinoa (QK), cañahua (C) and tarwi (T), quinoa Phisanqalla (QP), quinoa Kurmi (QK), cañahua (C) and tarwi (T) were evaluated on angiotensin I converting enzyme (ACE) *in vitro*, also the flours hydrolyzed with α amylase/alkalase (AMY/ALC) and α amylase/flavourzyme (AMY/FLA) of QRB, QA, C, T were evaluated. In addition, flavonoid content, protein concentration, degree of hydrolysis (GH) and starches were evaluated. The flavonoid content of the quinoa ecotypes ranged from 63 to 92 mg/mL, of C (43 mg/mL) and T (91 mg/mL). Of the RCT inhibitory activity, the T and C EFs exhibited 54.25±2.2 and 56.38±2.4 % inhibition, the quinoa EFs exhibited an average of 24 % inhibition. Protein hydrolysates and peptide fractions obtained by biological digestion by 4 enzymes: ALC, FLA and PAN, revealed ACE inhibitory activity higher than 60 %, with PEP it was lower. From the AMY/ALC hydrolyzed flours, a water-soluble product (PHS) and a non-water-soluble product (PNHS) were separated. The IC₅₀ of PHS obtained by AMY/ALC for QRB (0.68 mg/mL), QA (0.38 mg/mL), C (0.74 mg/mL) and T (0.67 mg/mL). Of the AMY/FLA-treated flours, the IC₅₀ of PHS were QRB (0.52 mg/mL), QA (0.49 mg/mL), C (0.48 mg/mL) and of T (0.72 mg/mL). The results suggest the possibility of the development of modified foods with antihypertensive activity.

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Resumen

Los granos andinos (quinua, cañahua y tarwi) poseen reconocidas cualidades beneficiosas para la salud. El objetivo del presente estudio fue evaluar la actividad inhibitoria de extractos fenólicos (EF), proteínas hidrolizadas, fracciones peptídicas de quinua real blanca (QRB), quinua real negra (QRN), quinua real roja (QRR), quinua J'acha grano (QJG), quinua Ajara (QA) silvestre, quinua Phisanqalla (QP), quinua Kurmi (QK), cañahua (C) y tarwi (T) en la enzima convertidora de angiotensina I (ECA) *in vitro*, también se evaluó las harinas hidrolizadas con α amilasa/alcalasa (AMY/ALC) y α amilasa/flavourzyme (AMY/FLA) de QRB, QA, C, T. En adición, se evaluó contenido de flavonoides, concentración de proteínas, grado de hidrolisis (GH) y almidones. El contenido de flavonoides de los ecotipos de quinua presento una concentración entre 63 y 92 mg/mL, de C (43 mg/mL) y T (91 mg/mL). De la actividad inhibitoria de ECA, los EF de T y C expusieron 54.25±2.2 y 56.38±2.4 % de inhibición, los EF de las quinuas expusieron un promedio de 24 % de inhibición. Los hidrolizados proteicos y fracciones peptídicas obtenidas por digestión biológica por 4 enzimas: ALC, FLA y PAN, revelaron actividad inhibitoria de ECA mayor al 60 %, con PEP fue menor. De las harinas hidrolizadas con AMY/ALC, se separó un producto hidrosoluble (PHS) y no hidrosoluble (PNHS). Los IC₅₀ de PHS obtenido por AMY/ALC para QRB (0.68 mg/mL), QA (0.38 mg/mL), C (0.74 mg/mL) y T (0.67 mg/mL). De las harinas tratadas con AMY/FLA, los IC₅₀ de PHS fueron QRB (0.52 mg/mL), QA (0.49 mg/mL), C (0.48 mg/mL) y de T (0.72 mg/mL). Los resultados sugieren la posibilidad del desarrollo de alimentos modificados con actividad antihipertensiva.

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Introducción

Arterial hypertension is a multifactorial disease, whose treatment is based on pharmacological inhibition of the renin-angiotensin system at 3 points: angiotensin I converting enzyme (ACE), direct action of angiotensin II and renin, many drugs synthetics are used to inhibit ACE¹. A current alternative is to resort to foods with functional properties. Functional foods (FF) are defined as those and their food components that, taken as part of the diet, provide benefits beyond their traditional nutritional values, improving a function in the body or reducing disease risk².

Since 1979, bioactive peptides with different biological activities were described, one of these activities obtained by hydrolysis with greater significance was antihypertensive. FF containing them may represent a new strategy for the prevention and/or treatment of hypertension. Antihypertensive peptides (AHP) were isolated from proteins of different foods of animal and plant origin³.

Bolivia is characterized by having different ecological floors, with high production of Andean grains (AG) by tradition, particularly in the Andean Altiplano. The AG, quinoa (Q), cañahua (C) and tarwi (T) (*Chenopodium quinoa* Willd, *Chenopodium pallidicaule* Aellen, *Lupinus mutabilis* Sweet), rustic crops, with resistance to drought, frost and salinity, considered today as food for high quality⁴.

The Q, is characterized by containing unsaturated and polyunsaturated fatty acids, type omega 3 and omega 6, high quality proteins, minerals, vitamins in a higher concentration than cereals, phytosterols, phenolic compounds that give them antioxidant properties, betalains, betaine-glycine. There is clinical

evidence of its health benefits, which reveals improvements in the performance of athletes, recommended for people with anemia, dyslipidemia and

lactose intolerance, as well as for people with celiac-disease⁵.

Of C (*C. pallidicaule*), a higher fiber content is reported, protein in quantity and quality similar to Q, contains phenolic compounds with high antioxidant properties, high mineral content^{6,7}, high iron and zinc⁸ content, low saponins, that do not give it the characteristic bitter taste⁹, being a high-quality food alternative that does not need to be processed like Q and T.

Of the T (*L. mutabilis* Sweet), the highlight is the high protein content, even in relation to lupinus subspecies, high fatty acid content¹⁰, it has a high potassium, phosphorus and iron content¹¹.

AHP are the most studied of the FF, with ACE inhibitory activity, related to the regulation of blood pressure by modulation of the renin-angiotensin system¹².

Arterial hypertension is one of the pathologies with the highest prevalence worldwide, treated with various synthetic drugs that usually cause side effects, hence the search for natural compounds has increased, there are several peptides that are in markets in Europe, North America and Asia, various products that contain elucidated peptides, have the ability to reduce blood pressure¹³.

The objective of this study was to evaluate the inhibition capacity, in vitro, of phenolic extracts, protein hydrolysates, peptide fractions, and AG hydrolyzed flours on angiotensin I converting enzyme (ACE).

Materials and methods

Raw material. The AG, white royal quinoa (WRQ), black royal quinoa (BRQ), cañahua (C) and tarwi (T) were provided by the food company Irupana Andean S.A. The varieties of quinoa J'acha Grano (JGQ), wild Ajara quinoa (AQ), Kurmi quinoa (KQ) and Phisanqalla quinoa (PQ) from the Altiplano of La Paz were donated by the Umala Jurisdiction Quinoa Producers Association (ASPROQUIJU). JGQ and KQ are genetically improved by hybridization, promising for export¹⁴.

Obtaining phenolic extracts (PE). 100 g of each grain, Q, C and T, were weighed, ground and defatted with petroleum ether 2 times for 1 h, the phenolic compounds were extracted with methanol/water (85:15), then rotary evaporated, frozen and lyophilized, a second extraction was carried out with n-butanol/water (50:50), liquid/liquid separation, the organic phase was dried in a Buchi rotary evaporator.

Determination of total flavonoids (TF). TF (compounds containing keto groups at C4, C3 or C5 hydroxyl groups, and orthodi-hydroxyl groups in the B cycle, such as flavones and flavonols) were determined using the aluminum ion colorimetric method¹⁵ using quercetin (QE) as standard. The absorbance was measured at 510 nm in the Thermo Scientific genesys 10S UV/Visible spectrophotometer and the results were expressed in mg of quercetin equivalents per g of sample, dry weight¹⁵.

Proteins. Its quantification was carried out by the AOAC method established in the Bolivian Standard (N x 6.25)¹⁶.

Starches. Its determination was made by the AOAC 996.11¹⁷ method, using glucose standard curve.

Obtaining proteins. The isoelectric pH method was used, 50 g of flour from each grain (the grains were ground in a Bosch mill and sieved to 60-mesh), defatted in Soxhlet, the defatted flour was washed with distilled water at 200 mL to proceed to extract with

0.1 N sodium hydroxide until pH 9 and then precipitate with 0.1 N hydrochloric acid. The precipitate (proteins) was centrifuged and dried by lyophilization.

In vitro enzymatic digestion of AG proteins. Protein hydrolysates (PH) were obtained by enzymatic digestion, with alcalase (ALC) in borate buffer (pH 8.0), with flavorzyme (FLA) and pancreatin (PAN) in phosphate buffer (pH 7.0), with pepsin (PEP) in citrate buffer (pH 3.0) separately. In each treatment, 1.0 g of protein (substrate) was reconstituted with 50 mL of the corresponding buffer. An enzyme/substrate (E/S) ratio of 1.5 was worked for 90 min in each case.

Degree of hydrolysis (DH). The protein extracted from the WRQ, JGQ and AQ varieties, the first refers to the typical export Q of the salar zone, the second modified by hybridization and the third wild, C and T, by the pH-stat¹⁸ method, work was carried out at 4% at 50° C and pH of 8.5 with borate buffer for the commercial enzyme ALC® 2.4 L and at pH of 7.0 at 50° C for FLA®, with phosphate buffer, at an E/S ratio of 2.0, monitoring was performed with a Thermo Scientific Orion A112 pH-meter using 0.1 N sodium hydroxide. Each analysis was performed in triplicate.

Using the following formula: $DH = \frac{h}{h_{tot}} * 100$

Where: DH = Degree of hydrolysis in %, h = Number of peptide bonds hydrolyzed in meq/g, h_{tot} = Total number of peptide bonds present in the protein in meq/g.

Peptide fractionation. The PH were dried in the laboratory lyophilizer (Telstar). For fractionation, Sephadex G-25 exclusion chromatography was used, using methanol as eluent. 5 mL aliquots were taken, each aliquot was dried in an oven at 37° C in Petri dishes. Fractionation monitoring was performed us-

ing thin layer chromatography (TLC) with eluent, butanol: acetic acid: water (3:1:1). It was developed with ninhydrin to join the equal fractions.

In vitro AG amylase/alcalase digestion of flour (AMY/ALC) and amylase/flavourzyme (AMY/FLA).

The flour of each grain was obtained in a Bosch grain grinder, to then be sieved in 60 mesh steel sieves, ASTM E 1116 standard, a suspension was prepared with WRQ, AQ, C flour at a concentration of 10 % with boiled water, without modifying the pH, which in all cases was between pH 5.5-6.0, suitable for AMY.

Enzyme Termamyl Sc. (AMY). At an E/S ratio of 0.6 considering the starch content in each case, at $82\pm 1^\circ\text{C}$ for 1 h in a stirring bioreactor and adjustable temperature with a built-in pH-meter, after boiling for 5 min.

Alcalase® Enzyme 2.4 L (ALC). After treatment with AMY, it was conditioned at 50°C , conditioning the pH to 8.5, with ALC in an E/S ratio of 2.5, considering the protein content in each case with continuous agitation for 6 h. It was brought to a boil to complete the hydrolysis. In the case of T, it was treated directly with the protease previously defatted.

Flavourzyme® enzyme (FLA). After treatment with AMY, it was conditioned at 50°C , conditioning the pH to 7, with FLA in an E/S ratio of 1.5, considering the protein content in each case, under continuous agitation for 6 h. It was brought to a boil to complete the hydrolysis. In the case of T, it was treated directly with the protease, previously defatted due to the high fat content and almost no starch presence¹⁹.

Obtaining water-soluble and non-water-soluble product. Once the WRQ, QA, C and T flour hydrolysates were obtained, they were filtered in a 304

stainless steel basket filter-centrifuge, drum-type support, with removable filter cloth, with a 0.5 hp 220-380 VAC motor at 100 revolutions per minute (RPM) to obtain water-insoluble product (WIP) and water-soluble product (WSP). Both were dried by lyophilization.

Angiotensin I converting enzyme (ACE) inhibitory activity. It was measured according to the method of Parris *et al.*²⁰ with a slight modification. Enalapril was used as a positive control, sample, ACE and hippuryl-histidyl-leucine (HHL) solutions were prepared using 0.1 M sodium borate buffer (pH 8.3) containing 0.1 M sodium chloride. reaction mixture containing 200 μL of 5.83 M HHL, 80 μL of sample solution (2 mg/mL) and 20 μL of ACE solution (50 mU/mL) was incubated at 37°C for 60 min. The reaction was stopped by the addition of 250 μl of 1 M hydrochloric acid followed by the addition of ethyl acetate. After vigorous shaking, the mixture was centrifuged for 5 min at 3500 rpm the top layer was placed in a tube. After evaporating the ethyl acetate in an oven at 80°C , the residue was dissolved in distilled H_2O to measure the absorbance at 228 nm on a Thermo Scientific genesys 10S UV/Visible spectrophotometer. The ACE inhibition activity was expressed as $100(1-X/C)\%$, where X and C were the absorbances of the sample and the control, respectively¹⁸. To obtain the IC₅₀, samples were prepared at different concentrations in relation to their inhibition percentages²¹.

In the case of the hydrolyzed flours (HF) of the pseudocereals (WRQ, AQ, C), the sample was prepared at 0.75 mg/mL of protein contained in WSP and WIP.

Del T was prepared at 2.0 mg/ml protein contained in WSP and WIP.

Statistic analysis. Each analysis was performed in triplicate and the results are expressed as mean and standard deviation (SD). Data were analyzed using analysis of variance and Dunn's²² post hoc test (significance of differences $p < 0.05$) was used to observe significant differences between data.

Results

Effect of FE on ACE inhibitory activity. T and C revealed a high activity, the FE of WRQ, BRQ, QJG, AQ, PQ and QK exhibited an average activity of 24.3% (Table 1), which represents a moderate activity, according to Dunn's test there are no significant differences each other ($p < 0.05$).

Table 1 Total flavonoids and ACE inhibitory activity of phenolic extracts from Andean grains

Sample	Total flavonoids (mg QE/g) a	% ACE inhibition	Dunn's post hoc test*
Enalapril		91.20±3.1	
Tarwi	91.25±0.8	54.25±2.2	A
Cañahua	42.78±1.2	56.38±2.2	A
White royal quinoa (WRQ)	91.75±0.8	26.75±1.8	B
Blak royal quinoa (BRQ)	72.38±1.8	22.38±1.8	B
Quinoa J'acha Grain (QJG)	84.8±1.2	24.8±1.2	B
Ajara quinoa (AQ)	63.95±0.6	25.5±2.0	B
Quinoa Phisanqalla (QP)	92.86±0.8	24.2±2.2	B
Quinoa Kurmi (QK)	77.82±0.06	22.4±1.2	B

a Values are the average of three determinations. * Similar letters do not show significant differences ($p < 0.05$).

Tabla 2 Concentración de proteínas y almidones de granos andinos

Analyte	T a	C a	QRB a	QRN a	QJG a	QA a
Proteins %*	48.5±0.4B	14.3±0.5A	15.4±0.3A	14.7±0.2A	13.8±0.3A	14.4±0.1A
Starches %*	1.8±0.2B	54.3±0.3A	53.1±0.4A	50.7±0.6A	55.3±0.8A	53.5±0.2A

a Values are average of 3 determinations. * Similar letters do not show significant differences ($p < 0.05$), T Tarwi, C Cañahua, WRQ white royal quinoa, BRQ black royal quinoa, JGQ J'acha grain quinoa, AQ Ajara quinoa

Table 3 Degree of hydrolysis in % of Andean grain proteins

Enzyme treatment	Degree of hydrolysis (DH) %					
	QRB a	QRN a	QJG a	QA a	T a	C a
ALC	25.2±0.2	24.6±0.4	25.6±0.2	24.4±0.3	16.8±0.4	19.4±0.3
FLA	24.1±0.3	23.8±0.2	24.8±0.4	23.4±0.2	14.8±0.2	12.8±0.5

a. The values are average of three determinations, ALC Alcalase, FLA Flavourzyme, QRB royal red quinoa, QRN royal black quinoa, QJG quinoa J'acha grano, QA quinoa Ajara, T tarwi, C cañahua.

Table 4 Antihypertensive activity in % of protein hydrolysates from Andean grains with 4 proteolytic enzymes, IC₅₀ (mg/mL)

Proteolytic enzymes	Enalapril	QRB a	IC ₅₀	QA a	IC ₅₀	C a	IC ₅₀
Pepsina	91.2 ±3.1	43.7±1.8	1.52±0.08	33.4±3.1	2.06±0.12	46.2±1.6	1.82±0.08
Pancreatina		72.2±2.4	.90±0.12	62.4±1.4	1.04±0.11	62.2±3.1	1.04±0.14
Alcalase		67.2±2.1	.74±0.04	83.4±1.6	.68±0.08	49.3±1.1	1.42±0.08
Flavourzyme		74.1±2.2	.98±0.05	75.4±2.2	.88±0.08	75.6±2.2	.88±0.12
	T a	IC ₅₀	QRN a	IC ₅₀	QJG a	IC ₅₀	
Pepsina	54.6±2.2	1.72±0.02	34.9±2.5	Nd	29.4±2.6	Nd	

Pancreatina	64.3±3.2	1.40±0.08	61.5±2.1	Nd	63.3±2.4	Nd
Alcalasa	64.7±2.1	1.34±0.12	61.2±2.2	Nd	60.9±1.1	Nd
Flavourzyme	71.6±1.8	1.12±0.12	69.8±1.8	Nd	73.5±2.9	Nd

Analysis of proteins and starches. The concentration of proteins and starches of the AG is within expected ranges, similar in the 4 varieties WRQ, BRQ, QJG, QA and in C.

DH from Andean grain proteins (AG). In all cases, a DH greater than 10 % was obtained (Table 3). The different varieties WRQ, BRQ, QJG, QA, by application of ALC and FLA. From T and C lower protein DH is observed.

ACE inhibitory activity by AG protein hydrolysates.

The treatment of hydrolyzed proteins with 4 enzymatic systems: PEP, PAN, ALC and FLA on the antihypertensive activity of ACE, the hydrolysates treated with ALC, FLA and PAN exhibited an inhibitory activity greater than 60%, in most cases. (Table 4), the hydrolysates treated with PEP were those with the lowest inhibitory activity (<54.6 %), the one with the highest activity was the hydrolyzate of the wild QA treated with ALC (83.4).

ACE inhibitory activity by AG peptide fractions.

Of the peptide fractions obtained by exclusion chromatography after carrying out the hydrolysis of each protein obtained from AG (WRQ, QJG, QA, T, C), with the enzymes (ALC, FLA, PAN and PEP), up to fraction 6 exposed of moderate to high activity (Table 5), the follow-up of obtaining peptide fractions was performed by TLC, as observed in Figure. The peptide fractions by application of ALC and FLA were those that exhibited the highest inhibitory activity in ACE up to fraction 6, reaching more than 80%. The treatments with PEP, exhibited an activity inhibitory between 50 and 70% in ACE. The treatment with ALC in proteins of the different varieties WRQ,

QJG and QA exposed peptide fractions of similar inhibitory activity in ACE.

ACE inhibitory activity by hydrolyzed flours (HF) by AMY/ALC and AMY/FLA. Of the WRQ, QA, C and T flours treated with AMY/ALC and AMY/FLA enzymes sequentially. The WSP presented a high ACE inhibitory activity in relation to the WIP, which presented a considerably lower activity (Figure 2).

As can be seen in Table 6, the highest antihypertensive activity exhibited the WSP of QA with the AMY/ALC treatment showing an IC₅₀ of 0.38 mg/mL, in the AMY/FLA treatment the WSP that showed the lowest IC₅₀ were QA and C with 0.49 and 0.48 mg/mL. As can be seen in Figure 3, of the HF of WRQ, QA and C, the WSP obtained by the AMY/FLA treatment expresses an activity process at different concentrations similar in all cases, to inhibit 50 % of inhibitory activity of ECA with values close to 0.5 mg/mL. In the treatment with AMY/ALC, a higher concentration of the WRQ hydrolysates was needed, and C (Table 6), highlights the activity of the QA hydrolyzate, which presents a concentration close to half that of WRQ and C to inhibit 50 % of ACE activity. In the case of T flour hydrolysates, it is observed that a higher concentration was needed to reach 50 % anti ACE activity.

Figure 1 Chromatographic runs on TLC of peptide fractions post hydrolysis by Alcalase



Table 5 Antihypertensive activity of peptide fractions from Andean grains obtained by exclusion chromatography

Fraction	QRB				QJG*	QA*	T			C				
	ALC	FLA	PAN	PEP	ALC	ALC	ALC	FLA	PAN	PEP	ALC	FLA	PAN	PEP
F1	82.5±3.2	81.2±1.2	74.4±2.4	68.8±3.1	72.2±2.4	80.2±2.5	85.1±2.2	84.4±2.8	76.7±2.8	67.2±1.1	61.1±2.2	70.2±2.5	62.2±3.1	64.1±3.4
F2	80.4±3.4	76.6±3.7	71.2±2.2	60.2±2.2	68.6±4.1	78.0±2.2	82.2±3.2	81.2±3.2	72.0±2.2	65.4±2.2	72.2±1.8	61.1±2.1	61.7±2.8	66.7±3.1
F3	70.8±3.8	78.6±2.6	73.2±1.6	61.1±1.8	70.6±2.6	75.6±1.6	78.4±2.4	82.7±3.4	68.4±3.4	70.4±3.2	58.3±2.1	78.5±2.4	66.2±2.1	52.4±2.2
F4	72.6±4.2	75.4±4.1	70.2±1.2	54.6±1.2	57.4±4.5	78.8±3.2	77.1±3.2	73.2±2.4	70.1±2.8	58.2±4.1	71±4.2	70.2±1.8	58.0±4.0	55.2±2.6
F5	70.4±4.2	64.3±3.5	67.4±1.8	26.8±2.4	66.5±3.5	76.2±1.8	70.0±2.1	72.0±2.8	61.5±2.1	62.6±2.8	54±2.1	51.2±4.1	55.2±3.2	50.4±2.1
F6	65.4±3.1	68.1±4.6	56.5±2.2	38.4±2.2	61.2±4.6	72.1±2.2	71.1±2.4	66.1±2.2	56.2±2.1	52.2±3.2	73±2.4	58.3±3.0	52.5±2.1	56.1±3.4
F7	55.4±3.2	60.4±2.2	50.2±2.8	16.2±1.6	46.4±2.8	66.4±2.6	44.1±1.1	62.5±2.4	54.0±2.6	56.1±2.1	54±4.1	33.1±1.6	44.6±2.4	52.2±2.1
F8	60.3±4.2	68.2±3.3	32.2±1.8	18.1±1.8	38.4±2.2	61.2±1.8	63.2±2.2	58.1±0.8	56.6±1.8	48.8±2.4	48±3.1	45.1±2.1	36.8±1.6	40.5±2.4
F9	23.2±1.3	54.4±3.8	27.2±1.6	16.1±2.2	40.3±3.3	51.1±2.4	33.6±1.4	55.1±3.1	48.2±3.1	49.2±2.1	35±2.8	18.2±1.5	28.4±2.3	36.6±1.1
F10	27.4±2.2	47.4±4.5	42.7±1.2	40.3±1.2	26.1±1.2	49.2±2.1	52.1±1.1	55.0±1.1	52.2±2.2	44.4±2.4	22±2.1	29.5±2.6	26.4±2.8	30.6±1.8
F11	15.8±1.2	34.9±7.2	16.4±1.2	8.2±2.2	13.0±1.4	44.5±2.4	19.4±2.1	58.2±2.6	39.4±2.1	18.2±2.1	20±2.4	12.8±2.0	14.8±1.8	18.1±1.6
F12	16.3±3.1	28.4±6.8	11.2±0.8	17.6±0.8	-	38.4±2.4	21.2±0.9	48.4±2.2	29.2±1.8	38.2±1.1	24±3.1	26.4±1.1	18.4±2.1	20.0±1.8
F13	24.5±3.2	27.4±3.2	25.4±1.9	52.5±1.8	-	28.0±1.7	27.7±1.2	41.6±1.8	16.1±2.2	41.1±0.8	10±1.1	18.5±0.8	16.2±0.9	20.2±2.2
F14	16.2±4.6	16.5±4.7	16.8±1.4	17.2±1.6	-	-	13.3±1.4	37.1±1.1	16.1±1.4	18.6±3.2	12±0.8	20.4±1.2	14.8±2.0	17.1±1.1
F15	12.3±3.7	12.6±3.4	4.6±0.2	6.6±0.8	-	-	11.2±1.1	15.2±0.8	12.8±1.1	10.8±2.4	14±2.1	16.0±1.8	8.2±2.2	2.4±0.8

*Treated only with Alcalase, Values are average of three determinations, ALC Alcalase, FLA Flavourzyme, PAN Pancreatina, PEP Pepsina, White Royal quinoa, QJG quinoa J'acha grane, QA quinoa Ajara, T tarwi, C cañahua

Figure 2 ACE inhibitory activity (%) by hydrolyzed flours from Andean grains (n=3) a) AMY/ALC, b) AMY/FLA, PHS: water-soluble product, PNHS: water-insoluble product White Royal Quinoa (QRB), quinoa Ajara (QA), tarwi (T), cañahua (C)

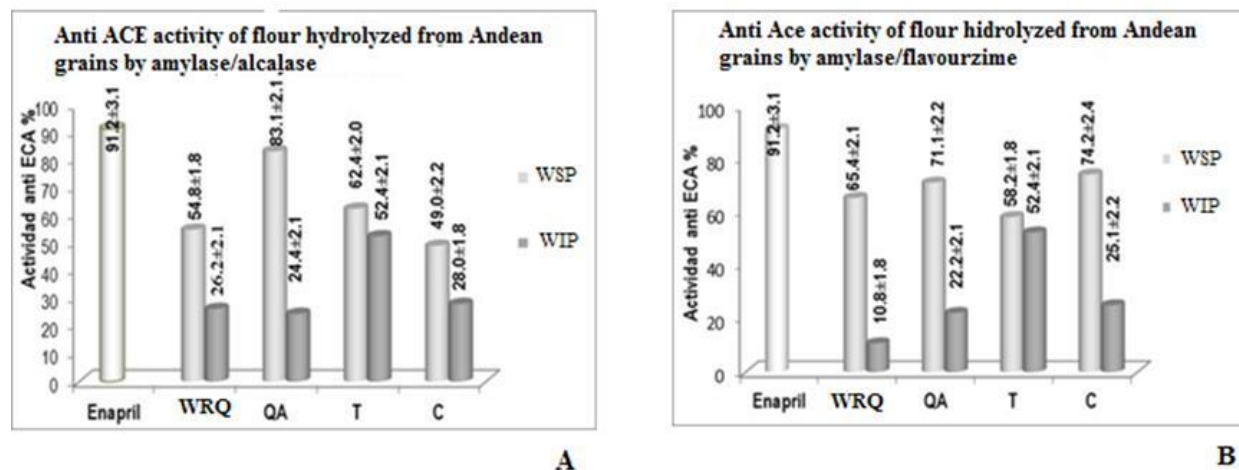
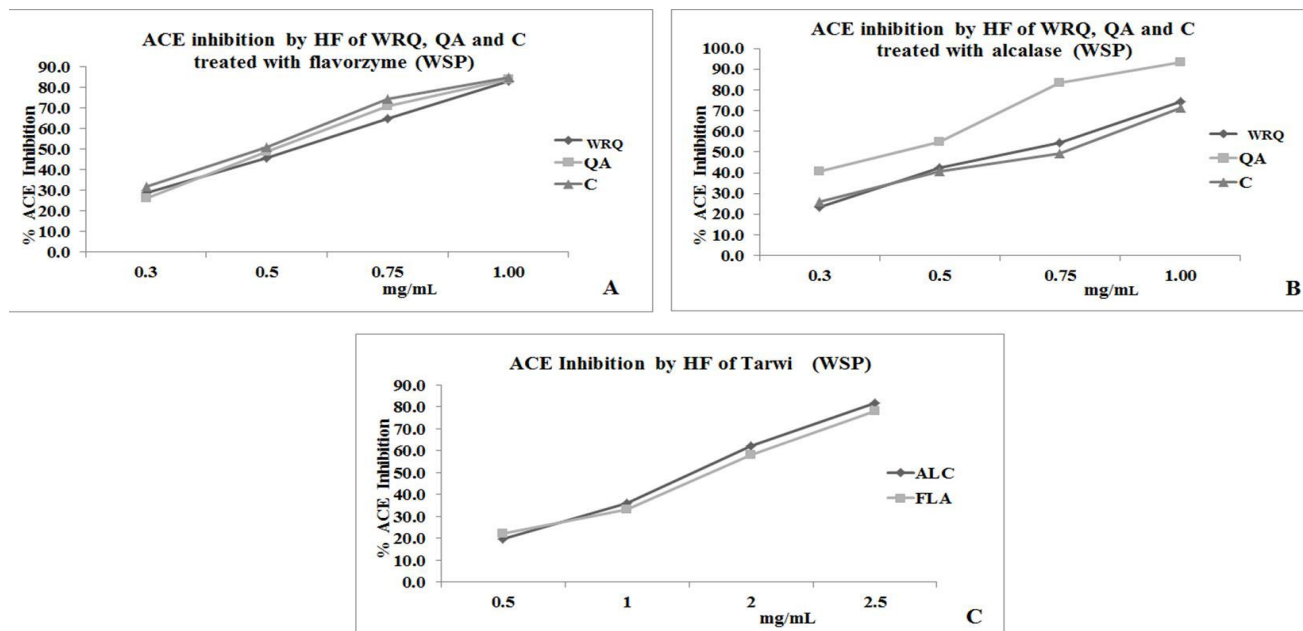


Table 6 IC₅₀ mg/mL of WSP of hydrolyzed flours of Andean grains in anti ACE activity

IC ₅₀ (PHS)	QRB		QA		C		T	
	ALC	FLA	ALC	FLA	ALC	FLA	ALC	FLA
	.68±0.18	.52±0.08	.38±0.08	.49±0.06	.74±0.18	.48±0.08	.67±0.03	.72±0.04

The values are average of three determinations, WRQ white royal quinoa, QA Ajara quinoa, ALC alcalase, FLA flavourzyme, T tarwi, C cañahua

Figure 3 ACE inhibition process at different WSP concentrations of hydrolyzed flour (HF) from Andean grains: A. WRQ, QA and C by AMY/FLA treatment, B. WRQ, QA and C by AMY/ALC treatment, C. T for treatment with AMY/ALC and AMY/FLA



Discusión

Of the AG, quinoa, C and T, the grain that obtained the highest reference in its functional qualities was Q, which has exhibited different health benefits, such as celiac disease, weight loss and/or metabolic parameters associated with diabetes, obesity, hypertension, hyperlipidemia, postmenopausal disorders, improvement of physical condition⁵, royal quinoa is the most widely commercialized, which is why it is exported to different countries, has different ecotypes, WRQ, BRQ, RRQ. Among the quinoas of the present study, quinoas were also considered improved by hybridization, QJG and QK that are promising for ex-

port, particularly QJG¹⁴, because it is resistant to mildew and has a short production period. QA is wild and is not consumed, it is used in anti-inflammatory processes as plasters in traditional medicine²³.

The Q, C and T, are characterized by having bioactive compounds recognized as antioxidants^{7,24,25} this activity is attributed to flavonoids, this type of compound also reported anti-ACE activity in other natural sources²⁶, Hettihewa et al.²⁷ & Guerrero et al.²⁸ identified several types of flavonoids from natural sources with inhibitory activity on ECA^{27,28}, several flavonoids reported, are also found in Q and C, the reported study confirms this activity is found in AG, such as kaempferol and quercetin found in Q²⁹, rutin

phenolic compounds, chlorogenic acid and gallic acid in Q and C³⁰.

According to the report by Repo-Carrasco-Valencia *et al.*³¹, the flavonoid content of Q varies from 36.2 to 72.6 mg/100 g, of C from 24.2 to 41.9 mg/100 g³¹, in the present study, all the varieties, WRQ, BRQ, QJG, QA, QP, QK presented a flavonoid content of 63.95 to 92.86 mg/100 g, being the wild QA the one with the lowest content, from C 42.78±1.2 mg/100 g was obtained. From *lupinus mutabilis* (T), a recent study of 33 ecotypes obtained the total flavonoid content from 30 to 135 mg/100g³², in our study 91.2 mg/100 g was obtained.

Asao & Watanabe³³ reported the anti ACE activity of the extract boiled and treated with ethanol of Q with high antioxidant activity and an ACE inhibition of 23.3 %³³. Our group evaluated the PE rich in flavonoids of the 6 Q varieties that presented ACE inhibitory activity with an average of 24.3±2.4 %. The flavonoids detected in the Q are glycosylated, 4 derived from kaempferol and 2 derived from quercetin³⁴. This characteristic of these compounds could influence the ACE inhibitory activity. In the study carried out by Ranilla *et al.*³⁵ of extracts obtained by boiling Q, C, and T, in antihypertensive activity, only T presented anti-ACE activity, with an activity of 52% inhibition³⁵, in the present study, we worked with PE, obtaining antihypertensive activity of EF of C (56.4 %) and T (54.2 %) of inhibitory activity in ACE. The flavonoid content does not correlate with the anti-ACE activity (Table 1), which gives rise to 2 options: i) flavonoid glycosylation interferes to a different degree with ACE activity, ii) there are compounds other than flavonoids that present this activity. According to Moreno-Limon & González-Luna³⁶, the Q hydrolyzed proteins treated with ALC exhibited ACE inhibitory activity greater than 80 % with a DH of 32

%, and with FLA up to 48 % inhibition³⁶, in the present study, the BRQ and QJG varieties exhibited 61 % inhibition, WRQ (67 %) and QA (83 %) with ALC with an average DH of 25.0±0.6, data similar to those reported, with FLA the hydrolyzed proteins of all grains presented 70 to 75 % of ACE inhibitory activity with a DH of 24.1±0.7, the proteins treated with PAN and PEP also presented a high ACE inhibitory activity as observed in Table 4, indicating the probability that peptide sequences of different sizes exist in the proteins. AG proteins with this activity, which coincides with what was reported by Alexandre *et al.*³. The work of Shi *et al.*³⁷ reported high inhibitory activity of Q protein hydrolysates in ACE, treated for 120 min with PEP with an IC50 of 0.78 mg/mL³⁷. In our study, the protein hydrolyzate by PEP was for 90 min with a IC50 of 1.52±0.08 mg/mL, which indicates that the greater the hydrolysis with PEP, the greater the anti ACE activity.

The C protein hydrolysates, obtained by sequential treatment with Neutrase/ALC enzymes for 180 min at 50° C, presented an anti ACE activity of 69.8% as reported by Chirinos *et al.*³⁸, our group obtained 44.3±1.1 % of inhibitory activity of the C protein hydrolyzate with the ALC treatment. In relation to the treatments with FLA, PAN and PEP our results indicate a greater or equal percentage of inhibition to the treatments with ALC.

From T, our study reports an ACE inhibitory activity greater than 50 % of the protein hydrolysates due to the enzymatic application of the 4 enzymes (ALC, FLA, PAN and PEP), as observed in Table 4.

Of the peptide fractions obtained by exclusion chromatography and bound by TLC, in the cases treated with ALC, FLA and PAN up to fraction 6 with high anti-ACE activity, even reaching 80 % of ACE inhibitory activity of WRQ, QJG, QA, C and T, similar

studies indicate that peptides between 1 and 5 kDa have greater activity than those of 10 kDa^{36,39}. From T, purified gamma-conglutin peptides have been identified that have references with ACE³⁹ inhibitory activity. In short, peptides with this activity have been reported in other lupine species, proposing their clinical use in hypertension⁴⁰.

ALC and FLA enzymes are the ones that are usually used to obtain protein hydrolysates in the determination of ACE inhibitory activity, mainly in proteins isolated from vegetable grains, cereals, and pseudocereals^{41,42}. Our group not only worked with ALC and FLA, in addition, was also used with pepsin and pancreatin, obtaining highly significant anti-ACE activity in each case.

The WSP of FH of WRQ, QA, C, T obtained by ALC and FLA presented in all cases greater inhibitory activity (>50 %) of ACE in relation to the WIP. The WIP presented less activity, between 24 and 28 % for the pseudocereals (WRQ, QA, C), the exception was the T that presented an activity of 52 %, however, we must consider that the protein content in the T flour was higher, so the IC₅₀ of the WSP of T by ALC and FLA were the highest of 1.49 and 1.52 mg/mL corresponding.

A greater antihypertensive activity of HH treated with ALC of QA was confirmed (83.1% IC₅₀ 0.38 mg/mL), which correlates with the activity of protein hydrolysates (83.4 % IC₅₀ 0.68 mg/mL), considering that the protein content in the Samples of FH subjected to anti ACE activity was lower, so that greater activity of the WSP could be attributed to the presence of glycosylated phenolic compounds together with the peptides obtained by enzymatic hydrolysis. The PE rich in flavonoid compounds, protein hydrolysates and peptide fractions of different ecotypes of Q, C (pseudocereals) and T (legumes), presented a

moderate to high inhibitory activity in ACE. Protein hydrolysates, peptide fractions obtained by treatment with ALC, FLA, PAN and PEP, as well as water-soluble products of flours hydrolyzed by AMY/ALC and AMY/FLA exhibited high ACE inhibitory activity. The present work proposes to develop clinical studies and dietary strategies to control hypertension from Q, C and T hydrolysates as a natural alternative.

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Conflicts of interest

The authors declare that they have no conflicts of interest with respect to the research, authorship and/or publication of this article.

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Ethical considerations

The present work was carried out in vitro, the research did not involve living beings (humans and animals), therefore it is not subject to an ethical analysis.

Research limitations

The time established for the completion of the project is a limitation that was presented, however the common work by the members helped a lot to meet the goals and objectives.

Authors' contribution to the article

The authors *Gigliola Ormachea Peggy Brenda* and *Salcedo Ortiz Lily*, carried out the conception and design of the study. *Nina Mollisaca Gastón Luis*, *Navia Coarite Nancy Alejandra* and *Mena Gallardo Evelin Paty*, carried out the data collection and the study of the techniques used. *Hurtado Ulloa Rosember*, carried out the statistics and interpretation of data. The approval of the final version to be published was approved by all the authors, which is ratified with the letter of originality.

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