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ANTIHYPERTENSIVE ACTIVITY OF QUINOA (*Chenopodium quinoa* Willd.) PROTEIN HYDROLYSATES

Sergio Moreno-Limón[1], Rodrigo González-Luna[1*]

[1]Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Departamento de Botánica San Nicolás de los Garza, Nuevo León, México

Corresponding Author's E-mail: rodrigomx.uanl@gmail.com

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Abstract

Background: Nowadays, there has been an increase in the number of studies focused on the search for bioactive compounds produced by hydrolytic reactions from natural sources, such as the Quinoa grain, which represents an interesting agro-alimentary source that can have a beneficial influence on health, specifically antihypertensive potential. For this reason, the aim of the present study was to evaluate the antihypertensive activity of the protein hydrolysates obtained of Quinoa, the results of which are important at that time, to consider the incorporation of such peptides in the design of functional foods.
Materials and Methods: Quinoa (*Chenopodium quinoa* Willd.) seeds were ground and the obtained flour was degreased and the protein isolate was obtained by isoelectric precipitation. The protein isolate was enzymatically hydrolyzed with Alcalase® and Flavourzyme® and the antihypertensive effect of peptides against angiotensin converting enzyme was evaluate using a mixture of 50 µL of sample, 50 µL of ACE working solution, 200 µL of substrate working solution and the fluorescence was determined with a microplate fluorometer following these characteristics: λ (excitation) = 355-375 nm; λ (emission) = 400-430 nm.
Results: Peptides obtained using Alcalase® (protein content= 72.13%; DH= 31.22%) showed the highest inhibitory activity against the angiotensin converting enzyme (ACE), close to 88%.
Conclusion: The Quinoa protein hydrolysates can be considered as a new agri-food source to be incorporated in the elaboration of functional foods with antihypertensive potential.

Keywords: Quinoa grain, Protein hydrolysates, Antihypertensive potential.

Abbreviations: ACE: Angiotensin converting enzyme, AU: Anson unit, LAPU: Leucine aminopeptidase unit, DH: Degree of hydrolysis, TNBS: 2,4,6-trinitrobenzenesulfonic acid, SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis, ANOVA: Analysis of variance.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) from the Chenopodiaceae family is a pseudocereal that has been cultivated for thousands of years in the andean region of South America (Jancurová *et al*., 2009; Galvez-Ranilla *et al.*, 2009). This grain counts with an exceptional nutritional value and is considered one of the best vegetable protein sources, as its protein levels are higher than those present in cereals. Besides, it contains all essential amino acids and is gluten-free by nature (González-Martín *et al*., 2014; Vega-Gálvez *et al*., 2010).

In recent years, there has been an increase in the number of studies focused on the search for bioactive compounds from natural sources that can be usable and incorporated as an alimentary substitute, as well as the elaboration of functional

foods (Rastogi and Shukla, 2013). A wide range of agro resources, provide a rich source of bioactive peptides after gastrointestinal digestion. It has been demonstrated that these peptides present biological activity, such as the inhibition of the activity of the angiotensin converting enzyme (ACE), the principal causing agent of the hypertension, which is one of the most widespread chronic diseases in developed countries (Matsui *et al*., 1993; Kim *et al*., 2001; Wu and Ding, 2001). For this reason, Quinoa grain represents an interesting agro-alimentary source that can be modified chemically to provide improvements in its biofunctional properties, specifically for the treatment of hypertension.

Therefore, considering the degree of hydrolysis influences the bio- and techno- functional properties of the peptides, the aim of the present study was to evaluate the antihypertensive activity (inhibition of the angiotensin I converting enzyme) of the protein hydrolysates obtained of Quinoa, the results of which are important at the time, to consider the incorporation of such peptides in the design of functional foods.

Materials and Methods

Quinoa (*Chenopodium quinoa* Willd.) seeds were obtained from Vizana Nutrition Company (Monclova, Coahuila, México). The seeds were ground and the obtained flour was degreased by extraction with hexane for 9 h in a Soxhlet extraction unit. The defatted meal (0.25% ± 0.03 crude fat, dry matter basis) was stored in hermetically sealed containers at 4°C until use.

Obtention and Enzymatic Hydrolysis of Protein Isolates

The protein isolate was prepared as previously described by Vioque *et al*. (1999). It was produced by suspending 1000 mg of quinoa defatted meal in water (1:10, w/v) with Na_2SO_3 (0.25 %, w/v) at pH 10.5. The suspension was stirred for 1 h at 25°C then centrifuged for 25 min at 8500 rpm at 4°C; the protein in the supernatant was precipitated at pH 4.5. The protein isolate was hydrolyzed with Alcalase® and Flavourzyme® under the following parameters: substrate concentration, 5%; enzyme/substrate ratio, 0.3 AU g^{-1} for Alcalase® and 50 LAPU g^{-1} for Flavourzyme®; pH, 7 for Alcalase® and 8 for Flavourzyme®; temperature, 50 °C; hydrolysis time, 60 min. Degree of hydrolysis (DH) was calculated by determining free amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) following Adler-Nissen (1979). These enzymatic treatments will allow the obtaining of hypoallergenic hydrolysates.

Protein quantification

Total nitrogen was determined by combustion according to Dumas (1831) procedure and converted to crude protein considering that all the nitrogen is in raw using a conversion factor (AOAC, 1997).

SDS-PAGE of Quinoa Protein Fractions

Protein profile of Quinoa was carried out by SDS-PAGE with 5% stacking gel and 15% of separating gel and a standard protein marker (250-10 kDa) was used as molecular weight standard.

Determination of Molecular Weight Distribution of Bioactive Peptides

Molecular weights were analyzed by gel filtration chromatography using a molecular exclusion column monitoring at an absorbance of 280 nm.

ACE inhibitory activity

The inhibitory effect of peptides against angiotensin converting enzyme, were evaluated according to Sentandreu and Toldra (2006). The angiotensin converting enzyme inhibition was calculated using the following equation:

Inhibitory peptides concentration (%)

$$= 100\ [(\text{Fluorescence}_{control} - \text{Fluorescence}_{blank}) - (\text{Fluorescence}_{B\ sample} - \text{Fluorescence}_{sample}) / \text{Fluorescence}_{control} - \text{Fluorescence}_{blank})]$$

Statistical analysis

The collected data were analyzed using the statistical software IBM SPSS Statistics 20®. Statistical comparisons of mean values for each experiment were performed using a single ANOVA, followed by a Tukey test ($P < 0.05$).

Results and Discussion

The hydrolysates obtained from *Chenopodium quinoa* Willd. showed a 72.13±0.44% protein content, with a degree of hydrolysis of 31.22%, and the electrophoresis patterns are shown in Figure 1.

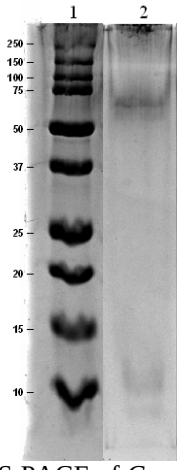

Figure 1: SDS-PAGE of *C. quinoa* Willd.

As a consequence of the hydrolysis, fractions of 15-10 kDa begin to be perceived (lane 2). Extensive hydrolysis may result in the formation of shorter peptides that can make their detection difficult on SDS-PAGE, hence the reason why the molecular weight of the protein hydrolysate was determined by gel filtration chromatography (Figure 2).

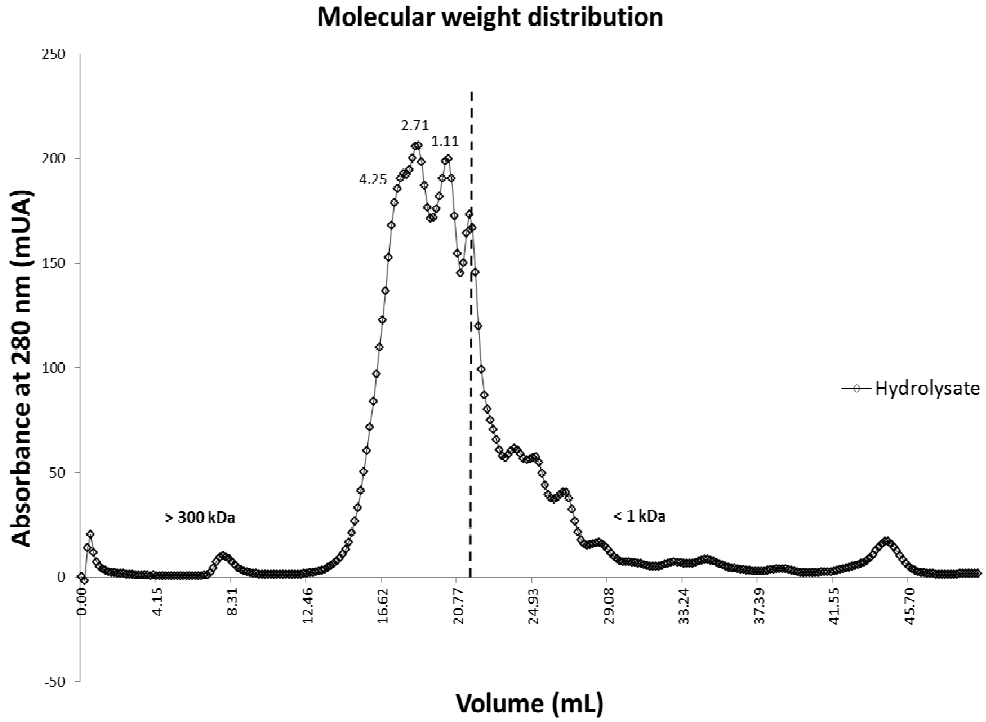

Figure 2: Molecular weight distribution of *C. quinoa* Willd.

The chromatogram showed three chromatographic peaks with molecular weights of 4.25 kDa, 2.71 kDa and 1.11 kDa. These results anticipating the presence of peptides in which the inhibitory activity of the angiotensin converting enzyme can be evaluated (Figure 3).

The influence exerted by the type of enzyme used during the hydrolysis on the inhibitory capacity of the angiotensin converting enzyme can be clearly seen. The action of Alcalase® and Flavourzyme® differed significantly ($p<0.01$) due to the different times of hydrolysis in the inhibition of ACE. The highest values are those shown the hydrolysates obtained with Alcalase®, which are around 80-90%, while the most deficient values are those exhibited with Flavourzyme®, ranging from 40-50%.

These results are very similar to those described by Aluko and Monu (2003), who observed that the reduction of peptide size significantly increases the ability of the hydrolysate to inhibit ACE activity, reporting values ranging from 15 to 10% of residual activity using Alcalase®. This suggests that the low molecular weight peptides of Quinoa could be easily absorbed by the body, which results in a promising effect that helps to counteract health problems and the possibility of being incorporated as a food substitute in the formulation of functional foods.

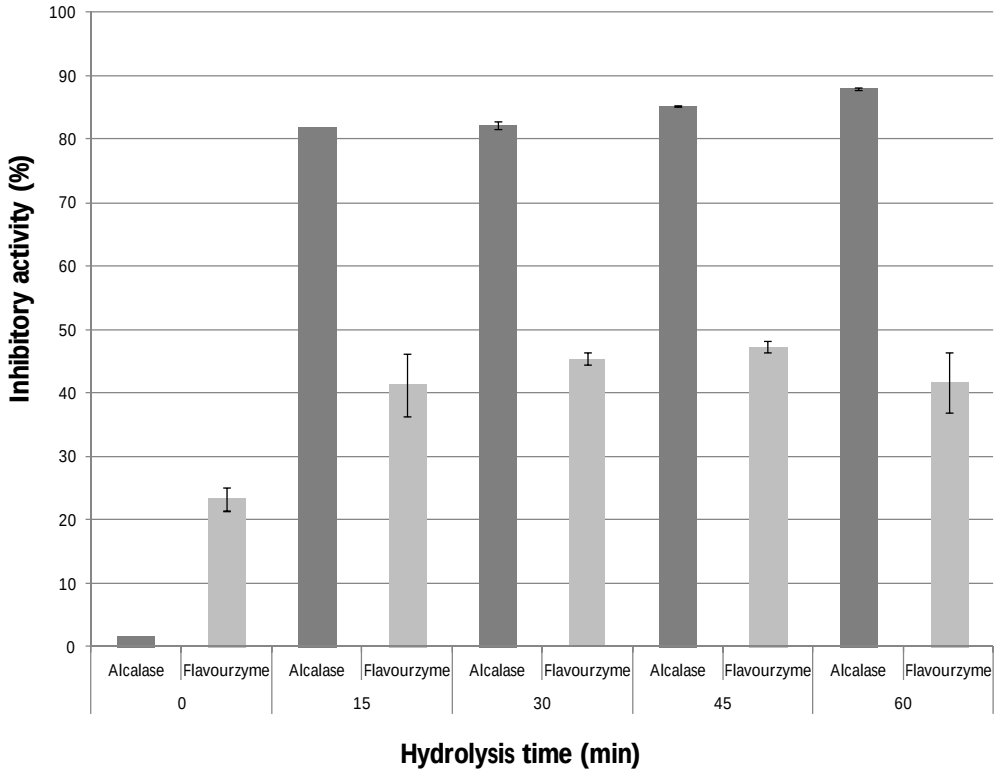

Figure 3: ACE inhibitory activity of Quinoa hydrolysates

Conclusions

Quinoa bioactive peptides with molecular weights between 1 and 5 kDa obtained with Alcalase® showed a high potential to act as angiotensin converting enzyme inhibitors, besides to the advantage of its hypoallergenic character, which suggests that these peptides can be considered to be incorporated in the elaboration of functional foods.

Conflict of Interest Declaration: Authors declare that this research presents no conflict of interest.

Acknowledgements

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