

# *Hydrolysis monitoring of Quinoa, Soy and Rice Proteins by using spectroscopy FT-IR technique\**

## *Seguimiento de la hidrolisis de las proteínas de Quinoa, Arroz y Soya mediante espectroscopia FT-IR*

ROA-ACOSTA, DIEGO-FERNANDO<sup>1</sup>; HOYOS-CONCHA, JOSÉ-LUIS<sup>2</sup>; BRAVO- GOMEZ, JESÚS-EDUARDO<sup>3</sup>

### Historial del artículo

Recibido para evaluación: 5 de Junio 2019

Aprobado para publicación: 11 de Septiembre 2019

\* Proyecto de investigación de origen: "Obtención y caracterización de aislado de proteína de quinua y sus hidrolizados para su inclusión en un alimento funcional". Financiación: Universidad del Cauca. Culminación: 9 de Octubre de 2021.

1 Universidad del Cauca, Facultad de Ciencias Agrarias, Grupo de investigación en Ingeniería de producto y Procesos Agroindustriales (GIPA). Ph.D. Química Industrial. Popayán, Colombia. <https://orcid.org/0000-0002-7198-9827>

2 Universidad del Cauca, Facultad de Ciencias Agrarias, Grupo de investigación Aprovechamiento de Subproductos y Residuos Agroindustriales (ASUBAGROIN). Ph.D. Química Industrial. Popayán, Colombia. <https://orcid.org/0000-0001-9025-9734>

3 Universidad del Cauca, Facultad de Ciencias Agrarias, Grupo de investigación en Ingeniería de producto y Procesos Agroindustriales (GIPA). MBA. Popayán, Colombia. <https://orcid.org/0000-0001-6462-3512>

**Correspondencia:** droa@unicauca.edu.co

**Cómo citar este artículo:**ROA-ACOSTA, DIEGO-FERNANDO; HOYOS-CONCHA, JOSÉ-LUIS; BRAVO- GOMEZ, JESÚS-EDUARDO. Hydrolysis monitoring of Quinoa, Soy and Rice Proteins by using spectroscopy FT-IR technique. Revista Biotecnología en el Sector Agropecuario y Agroindustrial, v. 20, n. 2, 2022, p. 76-86. Doi: <https://doi.org/10.18684/rbsaa.v20.n2.2022.1941>

## ABSTRACT

Leguminous and cereals are an important source of proteins actually having a large presence in the food industry; although, in recent years, there has been an increment in the use of pseudo cereals like quinoa. These types of proteins had shown physical properties could be taken as an advantage in developing new products. Hydrolysis is a process that enhance bio-active and functional attributes and that is why present research worked monitoring the hydrolysis process on proteins using infrared mid-range spectroscopy, to determine such structural changes. Enzymatic hydrolysis with endo or exo-protease, was made on three isolate proteins using Alcalasa® 2,4 L and Flavorzyme® from Sigma Laboratories. Correlation between enzyme/substrate was 5 %. Degree of Hydrolysis (DH) of 46 and 38,4 % were obtained for quinoa and rice isolate, respectively. Both, quinoa and soy hydrolysis monitoring took 60minutes with Alcalasa® 2,5 L, and 120 minutes con Flavorzima®. There were no significant differences of hydrolysis degree at 80 minutes after procedure. As a comparative view protein isolates and their hydrolysis were studied under  $4000\text{ cm}^{-1}$  to  $600\text{ cm}^{-1}$  infrared range to obtain information about the protein structure. Spectre deconvolution technique was applied through Fourier transform. Results showed significant differences on the secondary protein structure when compared to each configuration area. More relevant changes were observed on structural formation of  $\beta$ -sheet and  $\beta$ -turns structures. For an appropriate control of hydrolysis process will have repercussions over a steady protein, that might be used for developing new products.

## RESUMEN

Las leguminosas y los cereales son una fuente importante de proteínas que actualmente tienen gran presencia en el mercado de alimentos. Sin embargo, en los últimos años se ha incrementado el uso de proteínas provenientes de pseudocereales, como es el caso de la quinua. Estas proteínas han mostrado propiedades físicas que pueden ser aprovechadas en el desarrollo de nuevos productos. El proceso de hidrólisis aumenta las propiedades bioactivas y funcionales, por esta razón, en este trabajo realizó el seguimiento de la hidrólisis de proteínas mediante espectroscopia infrarroja de rango medio con el fin de determinar tales cambios estructurales. La hidrólisis enzimática con endo o exoproteasas se llevó a cabo en los tres aislados proteicos con las enzimas Alcalasa 2,4 L y Flavorzima® del laboratorio Sigma. La relación enzima / sustrato fue del 5 %. Se obtuvieron grados de hidrólisis (GH) entre 46 y 38,4 % para el aislado de proteína de quinua y el aislado de proteína de arroz, respectivamente. Tanto el seguimiento de la hidrólisis enzimática del aislado de proteína de quinua como del aislado de proteína de soja fue de 60 minutos con Alcalasa® 2,5 L y 120 minutos con Flavorzima®. Con respecto al aislado proteico de Arroz, los tiempos de hidrólisis establecidos fueron de 60 minutos con Alcalasa 2,4 L y 20 minutos con Flavorzima®. No se observaron diferencias significativas en el grado de hidrólisis

## KEYWORDS:

Firmness; Quinoa Protein; Rice protein; Soy protein; Hydrolysates;  $\beta$ -sheet structure;  $\beta$ -turns structure; Alcalase Enzyme; Flavorzyme Enzyme; Infrared spectroscopy; Fourier transform.

## PALABRAS CLAVE:

Firmeza; Proteína de Quinoa; Proteína de Arroz; Proteína de Soya; Hidrolizados; Estructura  $\beta$ -sheet; estructura  $\beta$ -turns; Enzima Alcalasa; Enzima Flavorzima; Espectroscopia infrarroja; Función de Fourier.

a 80 minutos. A modo de comparación, los aislados proteicos y sus hidrolizados se estudiaron en el rango del infrarrojo medio ( $4000\text{ cm}^{-1}$  a  $600\text{ cm}^{-1}$ ) para obtener información sobre la estructura de la proteína. Para ello se utilizó una técnica de deconvolución del espectro mediante la función de transformada de Fourier. Estos espectros mostraron diferencias significativas en la estructura secundaria de la proteína, cuando se compararon las áreas de cada estructura. Los cambios más significativos se observaron en la formación de estructuras  $\beta$ -sheet y  $\beta$ -turns. Por lo tanto, un correcto control de la hidrólisis repercutirá en la obtención de una proteína estable, la cual podría ser utilizada en el desarrollo de nuevos productos.

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) belongs to the family Amaranthaceae and is mainly grown in the Altiplano and arid areas of Andean region of South America although is currently cultivated on all continents, it also displays wide adaptability to altitudes ranging from 0 to 4000 m, i.e. cold, temperate and warm environments (García *et al.*, 2020). In the case of Colombia, there are two predominant cultivated varieties, Blanca de Jerico and Tunkahuan, which usually have a yield between 1 and 2 t ha<sup>-1</sup>. Generally, studies on quinoa are focused on genetically improving the seed or cultivars adaptations under different agronomical conditions (Wu *et al.*, 2020).

Quinoa, *Chenopodium quinoa* Willd, is a pseudocereal that recently, has been under the eye because its high nutritional value, low glycemic index, and its gluten free value (Sun *et al.*, 2021). The use of the grain as a raw material in manufacturing food products, researches are mostly focused on its nutritional, physical and chemical characteristics of the grain. Quinoa protein with great functional properties has been considered a potential source for food fortification or the production of functional foods (Daliri *et al.*, 2021).

Results have revealed that quinoa protein has high amino-acids profile thanks to its balanced blend of lysine, methionine, tryptophane and threonine (Le *et al.*, 2021; Rodríguez *et al.*, 2021) for a better digestibility (Ghuman *et al.*, 2021). Its starch is polymorphic with gelatinization enthalpy between 7,2 J/g and 10,3 J/g; low amylose and highly resistant starch (Roa *et al.*, 2019; Xing *et al.*, 2021). In different quinoa varieties, fats can vary between 3,9 g and 5,2 g (Rodríguez *et al.*, 2021). Additionally, FAO and OMS had declared as a promising cultivar, because its gluten free virtue that could be ingest by many people especially those with coeliac disease (Saavedra and Valdez, 2021). On the other hand, Soy protein beverages are considered safe and can enrich the varied diet of its consumers, as long as, they are considered an additional liquid portion of the diet. They can be ingested by adults and children above two years of age that present with cow's milk protein allergy or lactose intolerance (Vazquez-Frias *et al.*, 2020). While that, rice protein has higher nutritional quality, true digestibility (TD), and biological value (BV) than soy protein isolate (Han, Chee & Cho, 2015) and other cereals. Since rice protein contains lysine with high-quality amino acid composition, it is hypoallergenic and, therefore, favorable for human consumption (Pakkawat Detchewa *et al.*, 2022).

From a technological point of view, proteins have become a necessary ingredient to be included on new products (Muchekeza *et al.*, 2021). This would be possible thanks to the proteins capacity to make foams and emulsions (Wouters and Delcour, 2019). Consequently, chemical process had been developed to obtain protein isolates and protein concentrates, which are different from each other from its percentage of extracted protein. Preliminary testing allowed to notice that quinoa protein isolate might preserve some of its technical and functional attributes; its efficiency to make emulsions as well as its stability on thermal procedures after extraction. Therefore, the interest in using quinoa protein is not only because its nutritional value, but also on its technical and functionality (Roa *et al.*, 2019).

Some of the strategies to improve functionality and bioactivity of proteins are enzymatic hydrolysis. It consists on, adding specific enzymes to a protein to break peptic chemical bonds to obtain peptides (Deleu *et al.*, 2019).

The hydrolysis degree is determined because of reaction conditions like substrate concentration; enzyme/substrate affinity; incubation timing, pH and temperature. Other factor is, the nature of such applied enzyme as in type of enzyme and its specificity. Then, the nature of the used enzyme influences over the hydrolysis degree as well as the resulting peptide (Daliri *et al.*, 2021).

Because of previous explanation, peptides can be classified in: low molecular mass hydrolysates

between 1 and 10 % of DH, and high molecular hydrolysates with DH over 10 % (Villanueva *et al.*, 1999). Low mass hydrolysates are used on food industry as emulsifiers and foaming agents. While high mass hydrolysates are used on specialized food industry because its bioactivity and antioxidant properties (Gómez *et al.*, 2021). This explains why this research worked monitoring the enzymatic hydrolysis from three protein isolates seeds: rice, soy and quinoa. A comparative structure analysis between the native protein and hydrolysates through infrared spectroscopy to determine the conformation changes and connect them with some of the functional properties.

## METHOD

This study was made at the Faculty of Agricultural Sciences laboratory at Universidad del Cauca. In Vereda Las Guacas (Popayán-Cauca) 02°45'08.91" North and 76°55'10.86" West. Altitude of 1.737 m.a.s.l; average temperature of 19 °C; and annual precipitation of 1.800 mm. Quinoa (*Chenopodium quinoa* Willd), rice (*Oryza sativa*), and soy (*Glycine max*) grains were carefully selected to dispose of contaminants, defective samples, foreign particles and those with phytosanitary damages. Grains were stored on high density propylene bags at room temperature.

### Extraction of Protein isolates

Quinoa, rice and soy flours were degrease using ethylic ether as a dissolvent on a Soxhelt system for about 6 hours. Protein isolate extract was the methodology described by Mir *et al.* (2021) with just a few modifications.

Flour previously degreased were suspended on distilled water 1:10 w/v for 10 minutes. Then, NaOH (2 N) at 9 pH, was added keeping a constant stirring for 30 minutes. Next, sample passed for centrifugation at 8000 rpm for 10 minutes, using a Hermle Z306 (Germany) equipment. Obtained supernatant was adjusted to Isoelectric point at pH 5, using HCl (2 N). Once again centrifugated at 8000 rpm for 10 minutes. Precipitated recovered and stored in freezer at -18 °C for 24hours. To complete process, it was later dried up at 40 °C for 24 hours.

### Hydrolysis degree determination

Hydrolysis degree in proteins were calculated following "TNBS instructions" method provided by Biotechnology lab (Galante *et al.*, 2020). After getting a calibration curve with Leucine, hydrolysis measurements were taken. This consists of adding: 128 µL of hydrolysate sample, plus 2 mL buffer at pH 8,2 and 1 ml more of TNBS. Reaction was left on a thermostated bath at 50 °C for 30 min. Once time passed, reaction is stopped adding 2 mL of Sodium sulfite for 15 minutes. After that, visible-ultraviolet spectroscopy was used to read at a wavelength of 420 nm.

Total hydrolysis followed this procedure: 1 g of protein isolate was put into a test tube to then add 5 mL of hydrochloric acid (6 N). Next, mixture was placed into a sand bath at 110 °C for 24 hours to finally be neutralized with Sodium hydroxide (6 N). Hydrolysis degree was determined by TBNS.

Work schedule consisted in monitoring hydrolysis timing to which a total acid hydrolysis was performed to all three protein isolates. Enzymatic hydrolysis happened using method proposed by Villanueva *et al.* (1999a) and Villanueva *et al.* (1999b) with few modifications. To obtain DH greater than 35 %, partial hydrolysis (type 1) consisted in adding 1 g of isolate protein on a 50 mL Falcon test tube. Later, 10 mL at pH 8 buffer was added and mixed on Shaker equipment at 150 rpm y 55 °C. Subsequently, 2,4 L of Alcalase enzyme was pour in and left to react under selected timing for each sample.

Partial hydrolysis (type 2) consisted in neutralizing previous reaction to a pH 7, using (2N) HCl. Afterward, Flavorzyme® was added. Then, reaction was stopped 120 minutes later by heating it up to 85 °C for 15 minutes. Samples were centrifugated; and filtered on 110 mm Whatman paper. Lastly, hydrolysis degree was determined by TNBS (Kristoffersen *et al.*, 2020).

### Mid-range infrared spectroscopy

IR spectrum were obtained using IRAFFINITY-1S equipment (Shimadzu Corp., Kyoto, Japan). Spectrum was performed by reflection mode between 400  $\text{cm}^{-1}$  and 4.000  $\text{cm}^{-1}$  and proportional to over 32 scans with 4  $\text{cm}^{-1}$  and 25 °C temperature. Previous to measurement, each cultivar had a blank background spectrum (Roa *et al.*, 2019). Spectrum analysis was made by using an OriginPro, 7th version. Measurements were fixed and standardized over a base line between 0 and 1 (shown on figures) to display highest transmittance peaks. Deconvolution was performed on spectrum using Fourier Transform to determine protein secondary structure changes (band 1.600  $\text{cm}^{-1}$  to 1.700  $\text{cm}^{-1}$ ). Later, a modeling of the Gaussian function spectrum was applied (Perczel *et al.*, 1992). Starch concentration was studied from its formation on short range between 875  $\text{cm}^{-1}$  to 1175  $\text{cm}^{-1}$  band (Roa *et al.*, 2020). Lipids present on 2800  $\text{cm}^{-1}$  and 2900  $\text{cm}^{-1}$  range. All samples were analyzed by triplicate.

### Statistical analysis

All experiments were conducted in triplicate and results were expressed as means and standard deviations. Data were evaluated by analysis of variance (ANOVA) using SPSS (version 16.0 for Windows). Tukey Test was used for multiple comparison tests at statistical significance of  $p < 0,05$ .

## RESULTS

### Degree of Hydrolysis on Quinoa, Soy and Rice protein isolates

Preliminary testing allowed to determine that hydrolysis degree (DH) to both Enzyme/Substrate [E/S] had a 5 % ratio. Hydrolysis time of 180 minutes was adjusted according to authors like: (Villanueva *et al.*, 1999a; Montoya *et al.*, 2019; Mahdavi *et al.*, 2019; Leni *et al.*, 2020). Alcalase hydrolysis reaction in rice (RPI) up to 60 minutes did not show significant differences: 0,54 % DH (t = 0 min) to 29,1 % DH (t = 60 min). When adding Flavorzyme®, DH rose to 38 % at 80 min. After such time, no differences were relevant to include on DH (Fig 1). Reaction stopped at 180 minutes after. SPI hydrolysis (soy) ran for 180 minutes as well. Meaningful differences found under Alcalase treatment: from 2,4 % DH (t = 0 min) up to 37,1% DH (t = 60 min). Upon adding Flavourszyme, DH reached 40,3 % at 80 minutes and then to 42,5 % when reached 180 minutes (Figure 1).

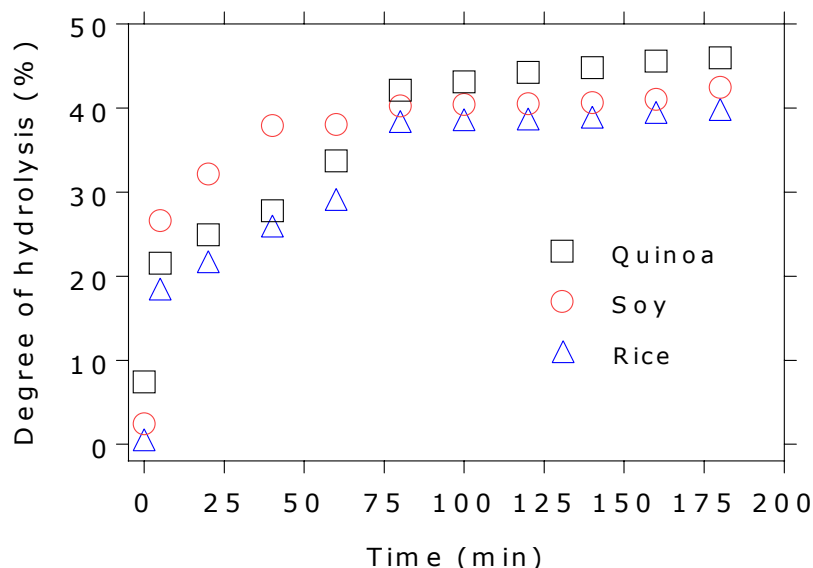


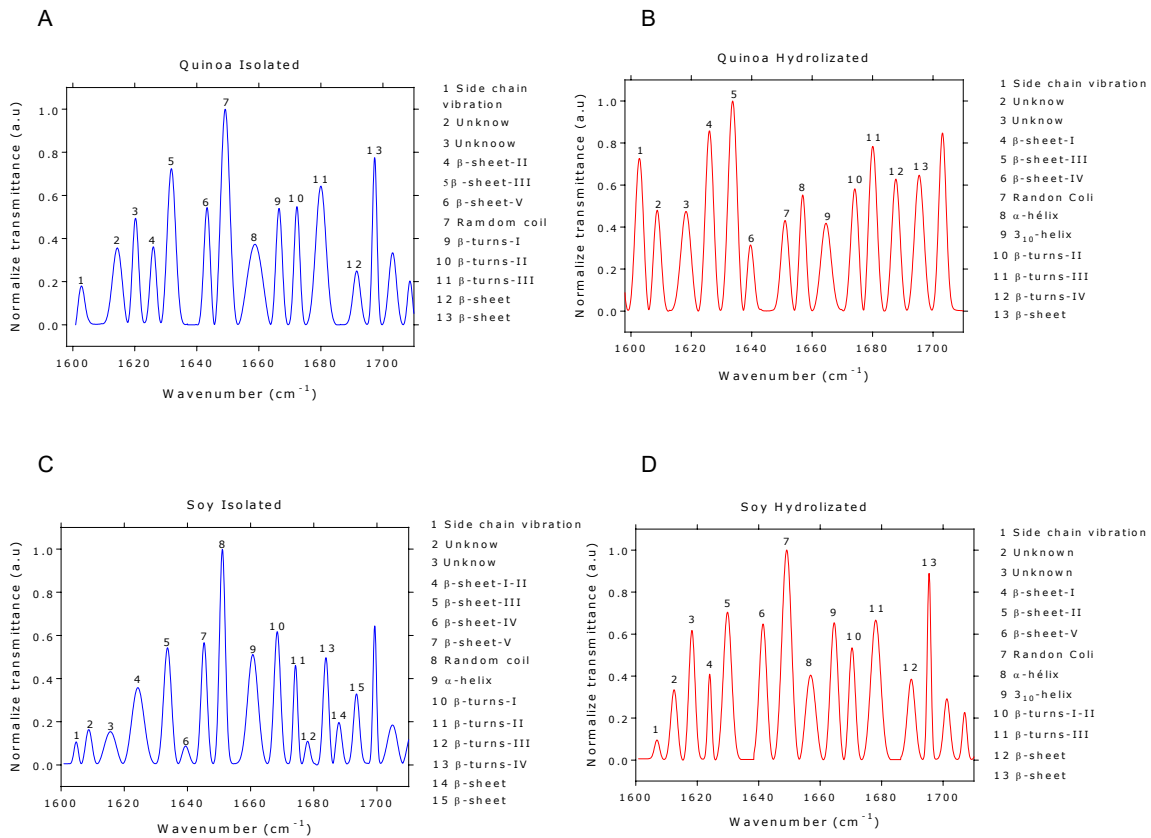
Figure 1. Degree of hydrolysis of quinoa, soy, rice proteins.

Quinoa exhibited a different behavior. At the beginning of the phase 1, changes happened from 7,40 % DH (t = 0 min) to 33,7 % (t = 60 min). Second phase with a DH of 42,1 % (t = 80 min) and ended in 46,0 %. Values on first stage of hydrolysis process on Quinoa Protein Isolate (QPI) and Rice Protein Isolate (RPI) are slightly lower (DH 34,7 %), to those reported by Villanueva *et al.* (1999b), although they are higher to the obtained by Xiaoxue *et al.* (2021).

On the other hand, Soy Protein Isolate (SPI) with a 40,3 % surpasses the values found for the authors. Its conduct on first stage one hydrolysis (0 time) is attributed to the initial extraction technique of protein isolates. In quinoa's situation, acidic procedures boosted an initial hydrolysis greater on the other two commercial protein isolates. Alcalase' digestion, managed to rise the number of N-terminal to give room to locations with exo-proteins activity to, therefore, move to second stage of enzymatic treatment.

Increment of the DH on hydrolysates on first stage happened thanks to Flavorzyme. Result of a DH 54,3 % are lower to the one reported by Villanueva *et al.* (1999). However, degrees of hydrolysis in quinoa and soy are minor to those reported by Hesam *et al.* (2021) (GH: 26,3 %). It is essential to mention that the final hydrolysis degree is determined by substrate concentration; correlation between enzyme/substrate; incubation time; and physical and chemical conditions like temperature and pH. Once the enzymatic hydrolysis of quinoa, rice and soy is finished at 180 minutes, samples were taken to mid-range spectroscopy equipment to review any changes on their secondary structural organization of protein isolates.

Figure 2 shows spectra of such secondary structures of protein isolates and their hydrolysate. Secondary structures allocation was based on previous publish protocols by Yang *et al.* (2015).  $\beta$ -sheets were identified between  $1620\text{ cm}^{-1}$  to  $1640\text{ cm}^{-1}$ . Increase of structural intensity was observed because of hydrolysis process (Figure 2). Hydrolysis makes possible that some structures change and become new structures on  $\beta$ -sheet. It is important to mention that between  $1640\text{ cm}^{-1}$  a  $1660\text{ cm}^{-1}$  range, random spiral structure intensity was reduced for the quinoa's protein isolate. It is possible that such structures change into more organized ones.



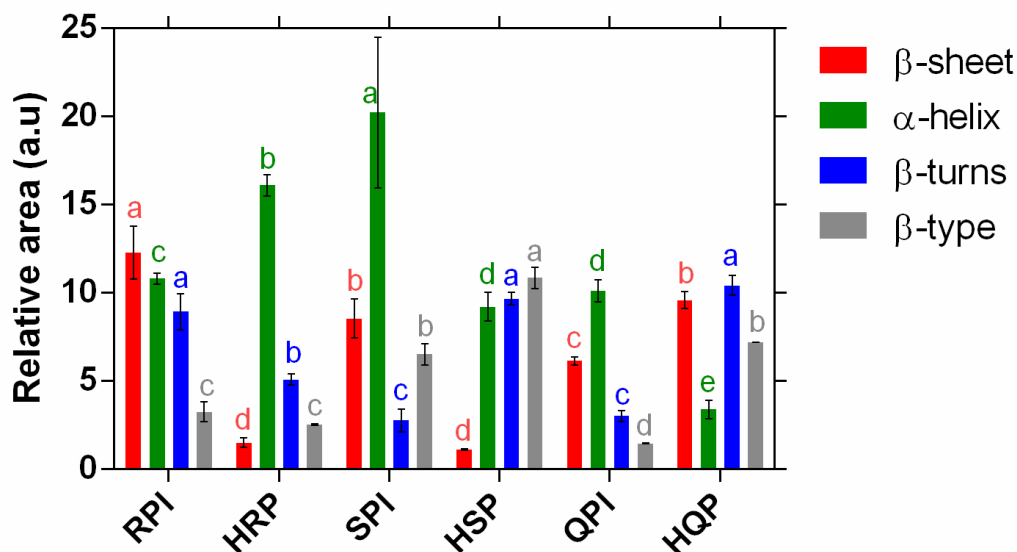
**Figure 2.** Spectrum of quinoa protein isolate (A), Spectrum of soy quinoa protein hydrolyzed (B), Spectrum of soy protein isolate (C), Spectrum of soy quinoa protein hydrolyzed (D), with their secondary structure.

Figure 2B, details that D structure of  $3_{10}$  helix on both hydrolyze, quinoa and soy, structures not observed on hydrolysates (Figure 2A, C), demonstrating that  $\beta$ -turns structures between  $660\text{ cm}^{-1}$  and  $1680\text{ cm}^{-1}$  were more sensitive to enzymes effects. None specific tendency was identified. Also, some of the functional phenolic compounds may vibrate on this specter range, as the aromatic chemical bond on C - H (curve out of plane) at  $1850\text{ cm}^{-1}$  is characteristic on poly-phenolic compounds. However vibrational frequencies more representatives were between  $1563\text{ cm}^{-1}$ ,  $1570\text{ cm}^{-1}$  and  $1577\text{ cm}^{-1}$  assigned to the vibration C = CO aromatic groups.

Figure 3 displays a comparative analysis for  $\beta$ -sheet,  $\beta$ -turns,  $\alpha$ -helix and  $\beta$ -type to each one of isolates. For soy and rice isolates, a diminishing tendency is observed on  $\beta$ -sheet bands during hydrolysis process. However,  $\beta$ -sheet structures behavior in quinoa protein, is different (Figure 3:  $\beta$ -sheet) (Haque *et al.*, 2016). It is interesting to make note that hydrolysis treatment did not affect  $\beta$ -type structure on rice protein (Figure 3:  $\beta$ -type) (Zang *et al.*, 2019).

Analysis over this correlation made evident a substantial link between the structure's behavior  $\beta$ -turns and  $\alpha$ -helix. Hydrolysis process causes and increment on  $\beta$ -turns for soy hydrolysate structures (Klompong *et al.*, 2017) as well as in quinoa; although, simultaneously, there is a reduction on  $\alpha$ -helix structures on same hydrolyzed samples. This might be possible because the structure adjustment of  $\alpha$ -helix to  $\beta$ -turns (Pal Shing *et al.*, 2016). On the contrary, the opposite effect was detected on rice protein.





**Figure 3.** Comparative analysis of each secondary structure ( $\beta$ -sheet III,  $\beta$ -turns III,  $\alpha$ -helix and  $\beta$ -type) of the protein isolate and its hydrolyzed, RPI: rice protein isolate, HRP: rice protein hydrolyzed, SPI: soy protein isolate, HSP: soy protein hydrolyzed, QPI: quinoa protein isolate, HQP: : quinoa protein hydrolyzed . The different letters (a,e) represent statistical differences at  $p < 0.05$ .

## CONCLUSIONS

Enzymatic alkaline action had a greater effect over soy protein, while Flavorizime action is greater over rice and quinoa. This could be due to the similarities on protein structure, because rice is a cereal and quinoa a pseudo-cereal. Secondary structures more influenced were  $\beta$ -sheet and  $\alpha$ -helix. Finally, it is relevant to mention that agro-industrial potential of this hydrolysate proteins is linked to its functionality and may offer benefits as natural antioxidants; anti-hypertensive; and anti-cholesterolemic properties, as well as improving some products' texture of broad use in food industry; since hydrolysates expose their hydrophobic or hydrophilic residues, these give them greater solubility and stability at the interfaces.

## ACKNOWLEDGEMENTS

This research was supported by the Universidad Del Cauca

## REFERENCES

- DALIRI, HESAM; AHMADI, RAMAN; PEZESHKI, AKRAM; HAMISHEHKAR, HAMED; MOHAMMADI, MARYAM; BEYRAMI, HOSSEIN; KHAKBAZ-HESHMATI, MARYAM; GHORBANI, MARJAN. Quinoa bioactive protein hydrolysate produced by pancreatin enzyme- functional and antioxidant properties. *LWT- Food Science and Technology*, v. 150, 2021. <https://doi.org/10.1016/j.lwt.2021.111853>



- DELEU, LOMME; LAMBRECHT, MARLIES; VAN DE VONDEL, JULIE; DELCOUR, JAN. The impact of alkaline conditions on storage proteins of cereals and pseudo-cereals. *Current Opinion in Food Science*, v. 25, 2019, p. 98-103.  
<https://doi.org/10.1016/j.cofs.2019.02.017>
- GALANTE, MICAELA; DE FLAVIIS, RICCARDO; BOERIS, VALERIA; SPELZINI, DARIO. Effects of the enzymatic hydrolysis treatment on functional and antioxidant properties of quinoa protein acid-induced gels. *LWT-Food Science and Technology*, 118, 2020, 108845.  
<https://doi.org/10.1016/j.lwt.2019.108845>
- GARCIA, MIGUEL; ZURITA, ANDRES; STENCHAUER, ROMAN; ROA, DIEGO; JACOBSEN, SVEN. Quinoa (*Chenopodium quinoa* Willd.) and its relationship with agroclimatic characteristics: A Colombian perspective. *Chilean journal agriculture research*, v. 80, n.2, 2020  
<http://dx.doi.org/10.4067/S0718-58392020000200290>
- GHUMMAN, ATINDER; MUDGAL, SWASTI; SINGH, NARPINDER; RANJAN, BRAJESH; KAUR, AMRITPAL; CHAND-RANA, JAI. Physicochemical, functional and structural characteristics of grains, flour and protein isolates of Indian quinoa lines. *Food Research International*, v. 140, 2021.  
<https://doi.org/10.1016/j.foodres.2020.109982>
- GÓMEZ, ANDREA; GAY, CAROLINA; TIRONI, VALERIA; AVANZA, MARIA-VICTORIA. Structural and antioxidant properties of cowpea protein hydrolysates. *Food Bioscience*, v. 41, 2021.  
<https://doi.org/10.1016/j.fbio.2021.101074>
- HAQUE, M. AMDADUL; PUTRANTO, ADITYA; ADHIKARI, BENU. Predictions of Drying Kinetics of Aqueous droplets Containing WPI-Lactose and WPI-Trehalose by application of composite reaction engineering approach (REA). *Journal of Food Engineering*, v. 189, 2016, p. 29-36.  
<https://doi.org/10.1016/j.jfoodeng.2016.05.019>
- HESAM, DALIRIA; RAMAN, AHMADIAB; AKRAM, PEZESHKI; HAMED, HAMISHEHKAR; MARYAM, MOHAMMADI; HOSSEIN, BEYRAMI; MARYAM, HESHMATI; MARJAN GHORBANI. Quinoa bioactive protein hydrolysate produced by pancreatin enzyme- functional and antioxidant properties. *LWT- Food Science and Technology*, v. 150, 2021.  
<https://doi-org.acceso.unicauca.edu.co/10.1016/j.lwt.2021.111853>
- KRISTOFFERSEN, KENNETH-AASE; AFSETH, NILS-KRISTIAN; BÖCKER, ULRIKE; LINDBERG, DIANA; DE VOGEL-VAN DEN BOSCH, HELEEN; RUUD, MARI-LINNÉA; WUBSHET, SILESHI. Average molecular weight, degree of hydrolysis and dry-film FTIR fingerprint of milk protein hydrolysates: Intercorrelation and application in process monitoring. *Food Chemistry*, v. 310, 2020.  
<https://doi.org/10.1016/j.foodchem.2019.125800>
- LE, LIQING; GONG, XUXIAO; AN, QI; XIANG, DABING; ZOU, LIANG; PENG, LIANXIN; WU, XIAOYONG; TAN, MAOLING; NIE, ZHONGLI; WU, QI; ZHAO, GANG; WAN, YAN. Quinoa sprouts as potential vegetable source: Nutrient composition and functional contents of different quinoa sprout varieties. *Food Chemistry*, v. 357, 2021.  
<https://doi.org/10.1016/j.foodchem.2021.129752>
- LENI, GIULIA; SOETEMANS, LISE; CALIGIANI, AUGUSTA; SFORZA, STEFANO; BASTIAENS, LEEN. Degree of Hydrolysis Affects the Techno-Functional Properties of Lesser Mealworm Protein Hydrolysates. *MDPI Journal Foods*, v. 9, 2020, p. 381.  
<https://doi.org/doi.org/10.3390/foods9040381>
- LOMPONG, VILAILAK; BENJAKUL, SOOTTAWAT; KANTACHOTE, DUANGPORN; SHAHIDI, FEREIDON. Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry*, v. 102, 2007, p. 1317-1327.  
<https://doi.org/10.1016/j.foodchem.2006.07.016>
- MAHDAVI-YEKTA, MINA; NOURI, LEILA; AZIZI, MOHAMMAD. The effects of hydrolysis condition on antioxidant activity of protein hydrolyzate from quinoa. *Food Science & Nutrition*, v. 7, n. 3, 2019, p. 930-936.  
<https://doi.org/10.1002/fsn3.871>

- MIR, NISAR; RIAR, CHARANJIT; SINGH, SUKHCHARN. Improvement in the functional properties of quinoa (*Chenopodium quinoa*) protein isolates after the application of controlled heat-treatment: Effect on structural properties. *Food Structure*, v. 28, 2021.  
<https://doi.org/10.1016/j.foostr.2021.100189>
- MONTOYA-RODRÍGUEZ, ALVARO; OSUNA-GALLARDO, EVELYN-ISABEL; CABRERA-CHÁVEZ, FRANCISCO; MILÁN-CARRILLO, JORGE; REYES-MORENO, CUAUHTÉMOC; MILÁN-NORIS, EVELIA-MARÍA; CUEVAS-RODRÍGUEZ, EDITH-OLIVA; MORA-ROCHÍN, SARAID. Evaluation of the in vitro and in vivo antihypertensive effect and antioxidant activity of blue corn hydrolysates derived from wet-milling. *Revista de Ciencias Biológicas y de la Salud*, v. 2, 2020, p. 155-162.
- MUCHEKEZA, JANE; JOMBO, TALKNICE; MAGOGO, CHARLES; MUGARI, AMIEL; MANJERU, PEPUKAI; MANHOKWE, SHEPHER. Proximate, physico-chemical, functional and sensory properties OF quinoa and amaranth flour AS potential binders in beef sausages. *Food Chemistry*, v. 368, 2021.  
<https://doi.org/10.1016/j.foodchem.2021.130619>
- PAKKAWAT, DETCHEWA; PATCHAREE, PRASAJAK; CHANTHIMA, PHUNGAMNGOEN; WICHEN, SRIWICHAI; ONANONG, NAIVIKULC; ANUCHITA, MOONGNGARM. Substitution of rice flour with rice protein improved quality of gluten-free rice spaghetti processed using single screw extrusión. *LWT- Food Science and Technology*, v. 153, 2022.  
<https://doi.org/10.1016/j.lwt.2021.112512>
- PAL-SING, TAJENDRA; SIDDIQI, RAASHID-AHMAD; SINGH-SOGI, DALBIR. Enzymatic modification of rice bran protein: Impact on structural, antioxidant and functional properties. *LWT- Food Science and Technology*, v. 138, 2021.  
<https://doi.org/10.1016/j.lwt.2020.110648>
- PERCZEL, A.; PARK, K.; FASMAN, GERALD D. Deconvolution of the circular dichroism spectra of proteins: The circular dichroism spectra of the antiparallel  $\beta$ -sheet in proteins. *Proteins: Structure, Function, and Bioinformatics*, v. 13, n. 1, 1992, p. 57-69.  
<https://doi.org/10.1002/prot.340130106>
- ROA-ACOSTA, DIEGO-FERNANDO; BRAVO-GÓMEZ, JESUS-EDUARDO; GARCÍA-PARRA, MIGUEL-ANGEL; RODRÍGUEZ-HERRERA, RAÚL; SOLANILLA-DUQUE, JOSE-FERNANDO. Harina hiperproteica de quinua (*Chenopodium Quinoa Wild*): Seguimiento y estudio de propiedades estructurales y reológicas. *Food Science and Technology*, v. 121, 2019.  
<https://doi.org/10.1016/j.lwt.2019.108952>
- ROA-ACOSTA, DIEGO-FERNANDO; SOLANILLA-DUQUE, JOSE-FERNANDO; AGUDELO-LAVERDE, LINA-MARCELA.; VILLADA-CASTILLO, HÉCTOR-SAMUEL; TOLABA, MARCELA-PATRICIA. Structural and thermal properties of the amaranth starch granule obtained by high-impact wet milling. *International Journal of Food Engineering*, v. 16, 2020, p. 10.  
<https://doi.org/10.1515/ijfe-2020-0024>
- RODRÍGUEZ-GÓMEZ, JOSÉ; PRIETO, JAVIER-MATÍAS; CRUZ-SOBRADO, VERÓNICA; CALVO-MAGRO, PATRICIA. Nutritional characterization of six quinoa (*Chenopodium quinoa Willd*) varieties cultivated in Southern Europe. *Journal of Food Composition and Analysis*, v. 99, 2021.  
<https://doi.org/10.1016/j.jfca.2021.103876>
- SAAVEDRA, PONCE-DE LEÓN; VALDEZ, ARANA. Nutritional and functional evaluation of 17 quinoa (*Chenopodium quinoa Willd*) accessions cultivated in the Andean area of Peru. *Scientia Agropecuaria*, v. 12, n. 1, 2021, p. 15-23.  
<https://doi.org/10.17268/sci.agropecu.2021.002>
- SUN, WENJUN; ZHAN, JUNYI; ZHENG, TIANRUN; WU, GUOMING; XU, HAISHEN; CHEN, YING; YAO, MIN; ZEN, JING; YAN, JUN; CHEN, HUI. Involvement of several putative transporters of different families in  $\beta$ -cyclocitral-induced alleviation of cadmium toxicity in quinoa (*Chenopodium quinoa*) seedlings. *Journal of Hazardous Materials*, v. 419, 2021.  
<https://doi.org/10.1016/j.jhazmat.2021.126474>

- VÁZQUEZ-FRIAS, R.; ICAZA-CHÁVEZ, M.; RUIZ-CASTILLO, M.; AMIEVA-BALMORIG, A.; ARGÜELLO-ARÉVALOR, I.; CARMONA-SÁNCHEZ, M.; FLORES-BELLO, V.; HERNÁNDEZ-ROSILES, G.; HERNÁNDEZ-VEZ, I.; MEDINA-VERA, E.; MONTIJO-BARRIOS, I.; NÚÑEZ-BARRERA, B.; PINZÓN-NAVARRO, C.; SÁNCHEZ-RAMÍREZ, C. Technical opinion of the Asociación Mexicana de Gastroenterología on soyplant-based beverages. *Revista de gastroenterología de México*, v. 85, n. 4, 2020, p. 461-471. <https://doi.org/10.1016/j.rgm.2020.07.005>
- VILLANUEVA, ALVARO; VIOQUE, JAVIER; SÁNCHEZ-VIOQUE, RAÚL; CLEMENTE, ALFONSO; BAUTISTA, J.D.; MILLÁN, FRANCISCO. Production of an extensive sunflower protein hydrolysate by sequential hydrolysis with endo- and exo-proteases. *Grasas y Aceites*, v. 50, n. 6, 1999, p. 472-476. <https://doi.org/10.3989/gya.1999.v50.i6.697>
- VILLANUEVA, ALVARO; VIOQUE, JAVIER; SÁNCHEZ-VIOQUE, RAÚL; CLÉMENTE, ALFONSO; PEDROCHE, JUSTO; BAUTISTA, JUAN; MILLAN, FRANCISCO. Peptide characteristics of sunflower protein hydrolysates. *Journal of the American Oil Chemists' Society*, v. 76, n. 12, 1999, p. 1455-1460. <https://doi.org/10.1007/s11746-999-0184-2>
- WU, QI; BAI, XUE; WU, XIAOYONG; XIANG, DABING; WAN, YAN; LUO, YIMING; SHI, XIAODONG; LI, QIANG; ZHAO, JUNMING; QIN, PEIYOU; YANG, XIUSHI; ZHAO, GANG. Transcriptome profiling identifies transcription factors and key homologs involved in seed dormancy and germination regulation of *Chenopodium quinoa*. *Plant Physiology and Biochemistry*, v. 151, 2020, p. 443-456. <https://doi.org/10.1016/j.plaphy.2020.03.050>
- WOUTERS, ARNO; DELCOUR, JAN. Cereal protein-based nanoparticles as agents stabilizing air-water and oil-water interfaces in food systems. *Current Opinion in Food Science*, v. 25, 2019, p. 19-27. <https://doi.org/10.1016/j.cofs.2019.02.002>
- XING, BAO; TENG, CONG; SUN, MENGHAN; ZHANG, QINPING; ZHOU, BANGWEI; CUI, HONGLIANG; REN, GUIXING; YANG, XIUSHI; QIN, PEIYOU. Effect of germination treatment on the structural and physicochemical properties of quinoa starch. *Food Hydrocolloids*, v. 115, 2021. <https://doi.org/10.1016/j.foodhyd.2021.106604>
- XIAOXUE, LU; RONGRONG, MA; HONGWEI, QIU; CHUNRUI, SUN; YAOQI, TIAN. Mechanism of effect of endogenous/exogenous rice protein and its hydrolysates on rice starch digestibility. *International Journal of Biological Macromolecules*, v. 193, 2021, p. 311-318. <https://doi.org/10.1016/j.ijbiomac.2021.10.140>
- YANG, HUAYAN; YANG, SHOUNING; KONG, JILIE; DONG, AICHUN; YU, SHAONING. Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy. *Nature Protocol*, v. 10, n. 3, 2015, p. 382-396. <https://doi.org/10.1038/nprot.2015.024>
- ZANG, XIAODAN; YUE, CHONGHUI; WANG, YUXIN; SHAO, MEILI; YU, GUOPING. Effect of limited enzymatic hydrolysis on the structure and emulsifying properties of rice bran protein. *Journal of Cereal Science*, 85, 2019, p. 168-174. <https://doi.org/10.1016/j.jcs.2018.09.001>