Original article[1]

Standardization and quantification of gluten in semi-processed and processed quinoa products (*Chenopodium quinoa Willd*)

Eric Maldonado, J. Mauricio Peñarrieta, José A. Bravo and Patricia Mollinedo*

Instituto de Investigaciones Químicas IIQ, Universidad Mayor de San Andrés UMSA, Av. Villazón N° 1995, La Paz, Bolivia, 0201-0220, iiq@umsa.bo

Keys: *Gluten, Gliadins, Prolamins, Glutenins codex alimentarius;* **Claves:** *Gluten, Gliadinas, Prolaminas, Gluteninas codex alimentarius.*

ABSTRACT

According to the Codex Alimentarius, gluten-free foods must not contain wheat, oats, barley or rye, and their gluten content must be less than 20 mg/kg. In addition, the amount of gluten in foods must be determined by an immunological method or another method that guarantees the same precision. The gluten content in foods made from native products was determined, to be certified using the standardized AOAC method number 2012.01. The results show that in products made from pure quinoa and cañahua, gluten was not detected even below 20 ppb, making them suitable for inclusion in the diet of celiac patients. Processed products are reported if they present gluten content values of around 26.85 ppb, due to other added products.

RESUMEN

Estandarización y cuantificación de gluten en productos semiprocesados y procesados de quinua (Chenopodium quinoa w.). Según el Codex Alimentarius, los alimentos libres de gluten no deben contener trigo, avena, cebada o centeno, y su contenido de gluten debe ser menor a 20 mg/kg. Además, la cantidad de gluten en los alimentos debe determinarse mediante un método inmunológico u otro que garantice la misma precisión. Se determinó el contenido de gluten en alimentos elaborados a partir de productos nativos para ser certificados empleando el método AOAC numero 2012.01 estandarizado. Los resultados samplen que en productos elaborados a base de quinua y cañahua pura, no se detectó gluten incluso por debajo de 20ppb, siendo aptos para su inclusión en la dieta de pacientes celíacos. Se reportan también productos procesados si presentan valores de contenido de gluten del orden de 26,85 ppb, a causa de otros productos añadidos.

Revista Boliviana de Química, 2024, 41, 173-180
ISSN 0250-5460, Rev. Bol. Quim. *Paper edition*
ISSN 2078-3949, Rev. boliv. quim. *e-edition*, *Sep-Dec*
30 diciembre 2024, https://doi.org/10.34098/2078-3949.41.3.5

https://bolivianchemistryjournaliiq.umsa.bo; https://bolivianchemistryjournal.org

[1]Received December 4, 2024, accepted December 18, 2024, published December 30, 2024. *Mail to: pattymollinedo@gmail.com

INTRODUCTION

Gluten is the most important storage protein in cereals wheat, oat, barley and rye (TACC, in Spanish: Trigo, Avena, Cebada y Centeno). It is composed of prolamines[1] and glutelins[2], responsible for the properties that allow the production of products such as bread, pasta and biscuits. Prolamines receive names as gliadin, avenin, hordein and secalin, in wheat, oat, barley, and rye respectively[3]. Gluten is the cause of celiac disease, an autoimmune condition. People with the genetic predisposition generate an immune response that damages the small gut after gluten ingestion, causing symptoms such as diarrhea, poor absorption and nutritional deficiencies.

Gluten is used as thickener in the food industry for products such as yogurt, flan, mayonnaise, pasta and canned foods. It is also used as a gelling agent in ice cream and candy, and as a drying agent in yerba mate and aromatic herbs.

The properties of elasticity, cohesion and viscosity are due to the prolamine (80%) and glutelin (20%) of wheat gluten[4]. These physicochemical properties give wheat a unique characteristic that is not found in any other cereal and that is important at a culinary level, allowing the production of bread and other processed foods such as pasta, cookies, cakes, sauces, etc.[4] Gluten is present in many foods and its elimination from the diet is really complicated. Gluten proteins are degraded with great difficulty by the enzymes of the gastrointestinal tract due to their high proline content[5], when the two proteins (gliadin and glutenin) come into contact with water, they join together forming a network, trapping the carbon dioxide produced during fermentation, as can be seen in Figure 1.

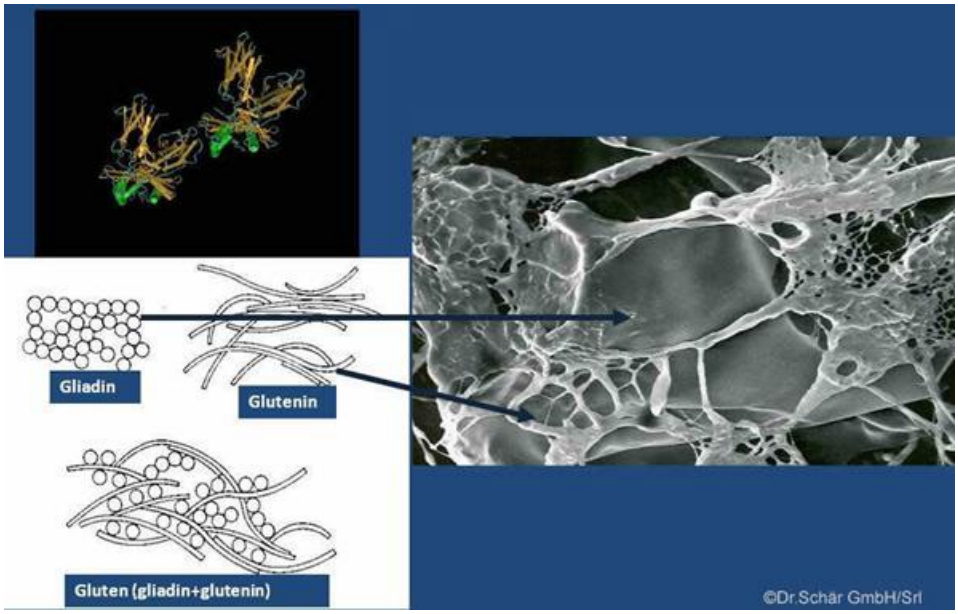

Fig. 1**.** Behavior of gluten proteins present in wheat. Source**:** Authorization pending from Dr. Schaer Institute, (2012).

It is precisely this network made up of hydrated glutenins and gliadins (Fig. 1) formed during kneading (wheat flour mixed with water) that gave rise to the term 'Gluten'; 80-85% of the total proteins in the dough correspond to gluten. Gliadins are highly polymorphic monomeric proteins with molecular weights of between 30-50 kDa (kilo Daltons), soluble in alcohol, which constitute almost 50% of gluten. They are classified into three groups: α/β-gliadins, γ-gliadins and ω-gliadins, which when hydrated form a viscous, extensible, fluid, but not very elastic dough, responsible for the expansion of the dough during bread making. Glutenins are a heterogeneous mixture of polymers linked by disulfide bonds, with molecular weights ranging from 500,000 to more than 10 million Da (Dalton), made up of high and low molecular weight subunits, which are linked by disulfide bonds, are insoluble in neutral salt solutions and 70% ethanol, but soluble or dispersible in acids and weak bases, when hydrated they produce an elastic and cohesive mass[6].

Celiac disease

Definition

Celiac disease (CD) is a chronic, systemic, immune-mediated enteropathy of the small intestine, caused by exposure to gluten and related prolamines, in genetically susceptible individuals[7]. When people with this condition consume gluten, an immune reaction occurs that damages the lining of the small intestine. Gluten is broken down into peptides, such as gliadin, which are modified by the enzyme tissue transglutaminase (tTG). These deaminated peptides are recognized by the immune system, triggering an inflammatory response that causes damage to the intestinal mucosa, villous atrophy, and a range of clinical symptoms both intestinal and extraintestinal. It is one of the most frequent chronic intestinal pathologies worldwide, with an estimated prevalence of 1:100[8] and a higher proportion of women to men (2:1), occurring at any age[9].

The pathophysiological process of gluten in celiac disease

This process is given by:

1. Gluten ingestion: When you eat foods that contain gluten, this protein reaches the small intestine.
2. Gluten fragmentation: In the intestine, gluten is broken down into peptides, one of the most important being gliadin[10].
3. *Tissue Transglutaminase (tTG):* The enzyme tTG modifies these gliadin peptides through a process called deamidation, which increases their immunogenicity[11].
4. Innate and Adaptive Immune Response: In people with celiac disease, the immune system recognizes deaminated gliadin peptides as a threat. Antigen-presenting cells present these peptides to immune system T cells, triggering an inflammatory response[12].
5. Damage to the Intestinal Mucosa: Activation of T cells triggers the release of cytokines and activation of intraepithelial lymphocytes, leading to inflammation and damage to the lining of the small intestine. This includes villous atrophy, which reduces the surface area for nutrient absorption[12] [12].
6. Clinical Symptoms: Damage to the intestinal mucosa causes symptoms such as diarrhea, malabsorption, weight loss, abdominal distension, and nutritional deficiencies. Extraintestinal symptoms such as anemia, osteoporosis, and neurological disorders may also occur[11] [13].

Therapy

At present, there is no cure for celiac disease, it is only possible to attenuate and regulate it so that it does not cause biochemical imbalances leading to severe metabolic disorders, with consequences such as osteoporosis, development of digestive tumors, short stature, infertility of patients, among others[14]. The only option to stabilize and improve the celiac condition is a strict gluten-free diet (GFD) for life[5] [15] [17] . With the consequent disappearance of clinical symptoms, the alterations in the intestinal mucosa are reversed, allowing serological markers to normalize and thus avoiding long-term complications[9]. With a strict GFD, symptoms improve after approximately two weeks, serological normalization occurs between 6 and 12 months and intestinal villi recover around 2 years after starting treatment[16], and possible late complications derived from gluten consumption in a celiac patient are avoided[8]. However, in recent years other possible therapeutic strategies other than the gluten-free diet have been investigated, which are still at different levels of development[15].

The gluten-free diet (GFD) consists of the exclusion of foods such as wheat cereals (especially gliadins and glutenins), and their homologues in barley (hordeins), rye (secalins), and oats (avenins), as well as hybrids of these cereals (such as kamut and triticale), and their derivatives (starch, flour, etc.)[15].

The concept of celiac disease is broader, since there are different levels at which gluten can affect the body. Patients with classic symptoms of celiac disease are only the “top of the iceberg” compared to all the subjects who are really affected without knowing it and for whom the disease presents itself in a silent way and sometimes with difficulty in reaching a true diagnosis[17].

Uses of gluten in processed foods

In the food industry, gluten is used as a thickener for products such as yogurt, flan, mayonnaise, pasta and canned foods such as corn and tomato puree, among others. It is also sometimes used as a gelling agent in ice cream, milk sweets, candy or as a drying agent in the case of yerba mate or other aromatic herbs[5].

The Codex Alimentarius considers gluten-free foods that are made up of or are produced only with one or more ingredients that do not contain wheat, oats, rye and barley whose gluten content does not exceed 20 mg/kg[18].

The objective of this work is to determine the gluten content in the different processed foods and in the native products of the department of La Paz using a standardized AOAC method.

EXPERIMENTAL AND METHODOLOGY

The ELISA method was used, which is based on the use of specific antibodies that bind to gluten present in the sample, allowing its detection and quantification in a precise manner[19 20].

Some of the advantages of the ELISA method for gluten analysis are:

- It is a sensitive and specific method for the detection of gluten, even at low concentrations[19 20 21].
- It allows the analysis of a wide variety of food matrices, such as processed foods, flours, etc.[19 22].
- There are various commercial ELISA kits available for the detection of gluten, which facilitates its application[19 22].

The ELISA kit (Enzyme-Linked ImmunoSorbent Assay)[23 24]
A commercial ELISA kit (RIDASCREEN® gliadin, art. No. R 7001) from R-Biopharm was used. It consists of a sandwich enzyme immunoassay for the quantitative analysis of prolamins from wheat (gliadins), rye (secalins) and barley (hordeins). It is used for the quantitative detection of gliadin in foods.

This kit is mainly used for:

Food analysis: Allows the detection and quantification of the presence of gliadin in foods, which is important for people with celiac disease or gluten sensitivity, as they can safely consume these products.

Quality Control: Food companies use this kit to verify that their gluten-free products comply with established limits for gliadin content.

Scientific research: Researchers employ this kit to study the presence of gluten in different food matrices and to develop new detection methods. The RIDASCREEN Gliadin kit from R-Biopharm has a high sensitivity and specificity for the detection of gluten in foods[25]. "The sensitivity of this method is 3 ppm of gluten, making it particularly suitable for the detection of traces of gluten in foods labeled as "gluten-free". The Ridascreen Gliadin kit is used by the National Food Institute (INAL) of Argentina to evaluate the reliability, sensitivity and specificity of gluten-free foods.

Sample preparation
0.25 g of homogenized sample was weighed, 2.5 ml of cocktail solution (RIDASCREEN ® gliadin, art. no. 7006) was added, the sample was stirred in a 50 ºC water bath for 40 minutes, allowed to cool to room temperature, then 7.5 ml of 80% ethanol was added and stirred for 1 h at room temperature.
It was transferred to Eppendorf tubes, centrifuged for 10 min at 8000 RPM at room temperature, and the supernatant was diluted with 20 µl + 980 µl of sample diluent.

Methodology of the assay
100 µl of the standard and sample solutions are placed in each cell or well, incubated in the dark for 30 min. at room temperature, then the liquid is removed from the cells, washed with the wash buffer solution, any bubbles remaining in the cells are removed, then 100 µl of the diluted conjugate solution is added to each cell, incubated in the dark for 30 min. at room temperature, then the liquid is removed from the cells, washed with the wash buffer solution, any bubbles remaining in the cells are removed, 50 µl of substrate is added to each cell, 50 µl of chromogen is added to each cell, incubated for 30 min. in the dark, then 100 µl of the stop solution is added, then the readings are taken in a spectrophotometer at a wavelength of 450 nm.

RESULTS

a) Quinoa grains

Table 1: Quantification of gluten concentration in different varieties of quinoa (*Chenopodium quinoa*), IIPN – 2018

Sample	Gluten ppm	Remarks
Quinoa grain A	1.20	G.F.
Quinoa grain B	1.69	G.F.
Quinoa grain C	1.43	G.F.
Quinoa grain D	1.56	G.F.
Quinoa grain Challapata Mix red-black OBOOK	18.87	G.F.
Quinoa grain Challapa MIX red-black	17.59	G.F.
Quinoa grain organic RC0216	< 5 ppm	G.F.
Quinoa grain organic TS	< 5 ppm	G.F.
Quinoa grain organic red Chojñacota	16.13	G.F.

G.F. gluten free

b) Quinoa flour

Table 2: Quantification of gluten in quinoa flour

Sample	Gluten ppm	Remarks
Quinoa flour organic red royal AVSA-L1 AV-7	1.13	G.F.
Quinoa flour precooked royal AVSA-L AV-4	4.23	G.F.

G.F. gluten free

c) Quinoa flakes

Table 3: Quantification of gluten in quinoa flakes

Sample	Gluten ppm	Remarks
Quinoa flake royal organic red AV-12	2.07	G.F.

G.F. gluten free

d) Royal quinoa Burger

Tabla 4: Quantification of gluten in royal quinoa burger

Sample	Gluten ppm	Remarks
Quinoa burger organic royal AV-10	0.36	G.F.
Quinoa burger organic royal AV-8	0.32	G.F.
Quinoa burger organic royal AV-9	5.02	G.F.

G.F. gluten free

e) Determination of gluten in quinoa products

Table 5: Quantification of gluten in quinoa-based products

Sample	Gluten ppm	Remarks
Banana quinoa	< 5 ppm	G.F.
Mango quinoa	< 5 ppm	G.F.
Organic pea, carrot and quinoa strained	0.27	G.F.
Organic carrot, pumpkin and quinoa strained	< 5 ppm	G.F.
Papaya, sweet potato and quinoa	< 5 ppm	G.F.
Quinoa, spinach and potato	< 5 ppm	G.F.
Quinoa sweet mango with chia	< 5 ppm	G.F.
Quinoa sweet strudel with apple and ciannamon	3.61	G.F.
Quinoa sweet fruits with pineapple and mango	< 5 ppm	G.F.
Quinoas vegetables with cañahua	< 5 ppm	G.F.
Quinoa cañahua with olive oil	< 5 ppm	G.F.
Quinoa brown rice with cañahua and spices	0.98	G.F.
Quinoa italian style pesto	< 5 ppm	G.F.
Quinoa mix with rice noodles	< 5 ppm	G.F.
Quinoa style curry	26.85	G.L.
Quinoa hummus dip	< 5 ppm	G.F.
Quinoa mango chutney dip	< 5 ppm	G.F.

G.F. gluten free G.L low in gluten

DISCUSSION

The Argentine Food Code considers gluten-free foods without TACC as dietary foods or foods for special diets in Chapter XVII. It establishes that plants must be equipped to produce these products that meet the needs of people with celiac disease.

In particular, gluten-free foods must be prepared with ingredients of natural origin and by applying good manufacturing practices that prevent cross-contamination. These products must be labeled with the name “gluten-free” and must also include the legend “TACC-free” in a good size and visibility.

Likewise, the Codex Alimentarius considers a food gluten-free when it contains less than 20 mg/kg. In this work, this point was taken into account, in addition to considering the gluten measurement parameters shown in table 6.

Table 6: Gluten level parameters

Gluten level ppm	Remarks
< 20	Gluten free
20-100	Low gluten
> 100	High gluten

From the results obtained and shown in Table 1, of the nine quinoa grain samples analyzed, none of the samples exceeds 20 mg/kg, which indicates that it is gluten-free.

In Table 2, where quinoa flour was used, a PCC is observed which shows that there is a very important contamination in the gluten. In baking, gluten is responsible for the fermentation gases being retained inside the dough, causing it to rise, pushing it upwards. After cooking, the coagulation of gluten is responsible for the bun not deflating once cooked.

The quantification of gluten in quinoa flakes observed in Table 3, the results show that its production is free of contamination, therefore, it is a very important data for the gluten parameter, which allows establishing control points in the production of gluten-free foods.

In Table 4, we observe that the place where the quinoa burgers were made, although the product is gluten-free, also indicates that there is contamination, which can be considered as a critical point in the production of the product.

In Table 5, it can be seen that cinnamon powder is no longer a generic product (it increases its flavour and texture) and this increases the gluten level, but if the cinnamon stick is used in the finished product, it does not influence the gluten content, while in curry, since it is not a spice, but is made from a mixture of spices and to give it its texture, starch is added, this makes the product have gluten and is not suitable for a celiac person[26].

CONCLUSIONS

The conclusions of the analysis indicate that the gluten content in foods made with quinoa can vary significantly depending on the manufacturing process and the ingredients used. In the samples analyzed, the quinoa flour does not present gluten contamination, which highlights the importance of critical control points (CCP). Likewise, the quinoa flakes are shown to be free of contamination, highlighting their potential as a gluten-free food. However, in products such as quinoa burgers, even though the final product is gluten-free, possible contaminations were identified at the manufacturing site, showing another critical CCP. In addition, the use of additional ingredients, such as cinnamon powder or mixes such as curry, can increase gluten levels due to additives such as starch, making such products unsuitable for people with celiac disease. These findings underline the need for strict controls during production to ensure that foods are gluten-free.

REFERENCES

[1] P. R. Shewry and N. G. Halford, *J Exp Bot*., 2002, **53**, 947.
[2] W. A. Kumar, S. K. Agarwal, Y. M. Reddy, A. D. Chintagunta, K. V. Saritha, G. Pal and S. P. J. Kumar, *Biocatalysis and Agricultural Biotechnology*, 2019, **17**, 710.
[3] V. Aguirre and M. de los Á. Guarino, 'La cocina de la salud', Arquetipo Grupo Editorial, Montevideo, 2014.
[4] R. Hoseney, 'Principles of Cereal Science and Technology', Published by American Assn. of Cereal Chemists, St. Paul MN, 1986.
[5] L. Shan, Ø. Molberg, I. Parrot, F. Hausch, F. Filiz, G. M. Gray, L. M. Sollid and C. Khosla, *Science*, 2002, **297**(5590), 2275.
[6] S. Badui Dergal, Química de Alimentos, 4th edition, Pearson, Addison Wesley press, 2006, pp 84,182,224-226.
[7] S. Husby, S. Koletzko, I. R. Korponay-Szabó, M. L. Mearin, A. Phillips, R. Shamir, R. Troncone, K. Giersiepen, D. Branski, C. Catassi, M. Lelgeman, M. Mäki, C. Ribes-Koninckx, A. Ventura and K. P. Zimmer, *J Pediatr Gastroenterol Nutr*, 2012, **54**, 136.
[8] M. Miranda Díaz, L. Alonso Romero, M. De Castro Ochoa and A. Millán Jiménez, *Vox Paediatrica*, 2012, **19**, 28.
[9] I. Polanco-Allué, 'Libro Blanco de la Enfermedad Celíaca: ¿Qué es la enfermedad celíaca?', ed. by I. Polanco-Allué, C. Ribes Koninckx, L. Rodrigo Sáez, et al., ICM Agencia Literaria, Madrid, 2008, pp. 7-17.
[10] J. Kumar, M. Kumar, R. Pandey and N. S. Chauhan, *Journal of Food Science*, 2017, **82**, 241.
[11] S. S. Kupfer and J. Bana, *Gastrointestinal Endoscopy Clinics of North America*, 2012, **22**, 639.
[12] I. Parzanese, D. Qehajaj, F. Patrinicola, M. Aralica, M. Chiriva-Internati, S. Stifter, L. Elli, and F. Grizzi, *World J Gastrointest Pathophysio,* **8**, 27.
[13] J. Barker and E. Liu, *Advances in Pediatrics*, 2008, **55**, 349.
[14] L. S. Sciarini, M. E. Steffolani, and A. E. León, *Agriscientia*, 2016, **2**, 61.

[15] M. Roca Llorens, **2018**, Estudio de mediadores humorales inmunológicos y factores dietéticos de riesgo en la enfermedad celiaca (PhD thesis), Universitat Politècnica de València, Valencia, España, retrieved from https://riunet.upv.es/handle/10251/110083
[16] I. Polanco Allué, 'Enfermedad celíaca y sensibilidad al gluten no celíaca: Enfermedad celíaca en la infancia', ed. by L. Rodrigo y A. S. Peña, OmniaScience, Barcelona, 2013, pp. 219-232.
[17] I. Polanco-Allué, *Evid. Pediatr.*, 2011, **7**, 52.
[18] Codex Alimentarius, 2009. CODEX STAN 118-1979, enmendado (1983**)**, revisado (2008**)**. Disponible en: htpp:// www.codexalimentarius.net/web/more_info.jsp?id_sta=291
[19] J. M. Yu, J. H. Lee, J.-D. Park, Y.-S. Choi, J.-M. Sung and H. W. Jang, *Foods*, 2021, **10**, 108.
[20] I. D. Bruins Slot, M. Bremer, G. E. G. Klerx, I. van der Fels and R. J. Hamer, *Cereal Chemistry*, 2015, **92**, 513.
[21] P., Amnuaycheewa, L. Niemann, R. E. Goodman, J. L. Baumert, and S. L. Taylor, *Foods*, 2022, **11**, 706.
[22] K. A. Scherf, *Food Analytical Methods*, 2016, **10**, 234.
[23] A. V. Lin., *Methods Mol Biol,* 2015, **1318**, 61.
[24] A. V. Lin, *Methods Mol Bio*l. 2015, **1318**, 51.
[25] M. E. García, *Revista de Investigación Académica*, 2006, **16**, 123.
[26] C. Molina-Rosell, *Omnia Science*, 2013**, 1**, 447.