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Gluten denaturation bioprocesses in raw materials used in Agribusiness. A Review

Jácome Pilco Carlos Rodrigo[1]; Verdezoto Guillin Joselyn Katherine[2]; Romero Villitanga Licia Nataly[3]; Iza Iza Sandra Patricia[4]

[1,2,3,4] Universidad Estatal de Bolívar
Facultad de Ciencias Agropecuarias, Recursos Naturales y del Ambiente
Carrera de Ingeniería Agroindustrial
Departamento de Bioingeniería
Laguacoto II, km 1 ½ Vía San Simón, Guaranda-Ecuador
[1]cjacome@ueb.edu.ec
https://orcid.org/0000-0002-9713-0228
[2]josverdezoto@mailes.ueb.edu.ec
https://orcid.org/0009-0003-3284-6115
[3]licia.romero@ueb.edu.ec
https://orcid.org/0009-0001-2814-4001
[4]siza@ueb.edu.ec
https://orcid.org/0000-0001-5388-7862

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Abstract: The bioprocesses of gluten denaturation in Agribusiness raw materials assume gluten consumption in people with celiac diseases, who face nutritional challenges and problems, are problematic. The pathogenesis of these diseases involves peptides from wheat and other cereal storage proteins (i.e., gliadins and glutenins) resistant to gastrointestinal hydrolysis. Gluten denaturation may be desired to improve the properties of the finished product or to meet specific requirements such as the production of gluten-free foods for people with intolerance. The processes include enzymatic modifications, modifications by microorganisms, heat treatments and enzymatic hydrolysis. Enzymatic modifications alter the structure of gluten, including hydrolysis of peptide bonds to reduce its immunogenicity. Modifications by microorganisms involve fermentation by Aspergillus strains, which degrade or modify gluten proteins. Heat treatments can partially denature gluten, decreasing its immunogenic capacity. Enzymatic hydrolysis uses enzymes such as peptidase to break down gluten into smaller fragments, reducing its ability to trigger adverse responses. These bioprocesses offer opportunities to develop agro-industrial products suitable for consumers with gluten-related dietary restrictions, thereby improving food quality and safety.

Keywords: Denaturation, gluten, celiac disease, bioprocesses, genetic modifications, hydrolysis, enzymatic activity.

1. INTRODUCTION

It is currently estimated that one-fifth of the world's population experiences adverse reactions that occur mainly after the ingestion of a food with a high gluten content. (Caminero, 2021)

Gluten is present in the main cereals that are part of our daily diet. People who suffer from celiac disease or non-celiac gluten sensitivity may have different discomforts depending on the degree to which their body is or is not able to digest it, in the most severe cases it is advisable to follow a gluten-free diet. (Güven & Onur Azizoglu, 2023)

Celiac disease (CD) is the most common chronic bowel disorder in the population, affecting around 1% of people worldwide and at all ages. (Jiménez Ortega, 2022). They present typical digestive symptoms such as chronic diarrhea, bloating, nausea, anemia, and fatigue, it is also recognized that the disease can manifest with symptoms or signs outside the gastrointestinal tract, called atypical symptoms. (Reme Troche, 2023)

Non-celiac gluten sensitivity is a pathology with both intestinal and extraintestinal symptoms, which usually manifest shortly after gluten ingestion and cease rapidly when this component is eliminated from the diet in individuals in whom celiac disease (CD) and wheat allergy (TA) have been excluded. (Moreno, 2020)

Bioprocesses are used to denature gluten in the production of gluten-free products, which involves altering its structure or conformation without modifying the original amino acid sequence. These changes can be induced by biotechnological processes such as genetic modifications, fermentation, heat treatments and enzymatic hydrolysis. The application of these methods alters the structure of gluten, affecting its elasticity, viscosity and ability to form protein networks. . (Sun, 2005)(Hellmann, 2020)

Bioprocesses for gluten denaturation include enzymatic modifications, modifications by microorganisms, heat treatments, and enzymatic hydrolysis. Enzymatic modifications alter the structure of gluten, including hydrolysis of peptide bonds to reduce its immunogenicity. (C. Wehrli & Kratky, 2021).

Modifications by microorganisms involve fermentation by strains of Aspergillus, which degrade or modify gluten proteins. Heat treatments can partially denature gluten, decreasing its immunogenic capacity. (da Silva Ramos & Maciel Rocha, 2023).

Enzymatic hydrolysis uses enzymes such as peptidase to break down gluten into smaller fragments, reducing its ability to trigger adverse responses. . These bioprocesses offer opportunities to develop agro-industrial products suitable for consumers with gluten-related dietary restrictions, thereby improving food quality and safety.(Ganesh & Widya Ningtyas, 2022)

Gluten-free raw materials, such as rice, corn, quinoa and sorghum, among others, are increasingly being used in agribusiness due to the growing demand for gluten-free products. These raw materials offer a safe and accessible alternative for people with celiac disease or non-celiac gluten sensitivity, as well as those who opt for a gluten-free diet for health reasons or preference. (C.M, 2019)

The main purpose of this review is to provide up-to-date information on the bioprocesses of gluten denaturation in raw materials used in agribusiness in order to improve the texture and quality of food products and adaptation to dietary requirements and consumer preferences.

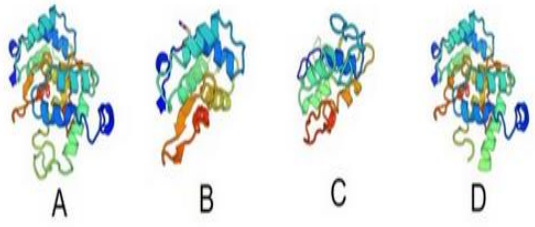
2. GLUTEN

Gluten is a complex protein found in some cereals, mainly wheat (gliadins and glutenins), barley (hordeins) and rye (secalins). Most of the protein content in these grains is made up of gluten (about 85 – 90% in wheat grains). .(Güven & Onur Azizoglu, 2023)

These proteins have the ability to confer viscosity and elasticity to the flour matrices where they are present, which has promoted their wide use in the manufacture of various processed products. (Jiménez Ortega, 2022). Exposure to gluten can create conditions conducive to the appearance of certain pathologies in humans, among which celiac disease is the best known. (Brizuela Labrada, 2020)

Gluten exhibits resistance to degradation by gastric, pancreatic and intestinal proteases due to the presence of high levels of proline. These peptides pass through the submucosa of the small intestine by mechanisms that are not yet fully understood, using both the transcellular and paracellular pathways, triggering an inflammatory response in susceptible individuals. (Mühlenbrock-Pinto, 2023)

Figure 1. *Graphical representation of the 3D structures of various inhibitors of the cereal family: Wheat amylase and trypsin inhibitor A) 0.53 B) 0.28 C) CM3 D) 0.19.*



Source: Swiss-Model Repository https://swissmodel.expasy.org/repository.

2.1.Determination of the percentage of gluten

Analytical procedures for the detection of wheat, rye and barley gluten should demonstrate sufficient sensitivity, specificity and suitability for application in routine analyses, as well as be supported by collaborative validation studies. (Xhaferaj, 2020)

Different analytical methods are used to detect traces of gluten and ensure food safety. These methods include enzyme-linked immunosorbent assay (ELISA), lateral flow devices (LFDs), polymerase chain reaction (PCR), mass spectrometry (MS), and surface plasmon resonance (SPR). (Sharma, 2015)

2.2.Gluten Composition

As Badui (1993) mentions, gluten generally has a composition of amino acids approximately 6% ionizable, 45% polar amino acids and 49% non-polar amino acids; It is also characterized by high levels of proline and glutamine (glutamic acid), 14% and 37% respectively. (Cerda Mejía, 2017)

Gluten is mainly made up of two proteins: glutenin (Glu) and gliadin (Gli). (Güven & Onur Azizoglu, 2023)

Glu forms the network structure through cross-chain disulfide (SS) bonds, which provide flexibility to the WG network. On the other hand Gliadin (Gli) is a small monomeric protein rich in cysteine residues that favor the composition of the WG network and provide viscosity and extensibility. (Liu & Liang, 2023)

Gliadin, the main antigenic stimulant in patients with genetic susceptibility to celiac disease, is a prolamine rich in glutamine and proline and is responsible for giving elasticity and texture to flours. (Quevedo, Hernández, & Remes, 2017)

2.3.Main Raw Materials Containing Gluten

2.3.1. Wheat

Wheat, being one of the most widely cultivated species globally, plays a crucial role as a source of protein. . Wheat gluten contains about 75% to 85% protein, 5 to 10% lipids, residual starch, carbohydrates, and water-insoluble proteins trapped in the dough (Zhang X. , 2021)(Li Q. , 2024)

The gluten content in wheat flour plays a critical role in generating a viscoelastic matrix that exhibits glue-like properties. This matrix gives elasticity to the dough and allows the bread to expand during the baking process, giving a chewy and satisfying texture to the final product. (Li Q. , 2024)

10,921 Proximal chemical composition of wheat grain and flour INIA – 418.

Component	**grain (g)**	**flour (g)**
Humidity	10,88	13,04
Protein	14,87	12,94
crude fat	1,74	1,25
crude fiber	2,64	2,32
Total ashes	1,72	0,94
Carbohydrates	68,15	69,41
wet gluten	---	32,76
dry gluten	---	10,92

Source: Ponce Ramírez & Málaga Juárez (2016)

2.3.2. Rye

Rye is a cereal grain of great importance in European agriculture, especially in the northern, central and eastern regions of the continent. This crop is used in the production of various food products both for human consumption and for livestock feed. (Ciudad Mulero, 2019)

The composition of rye is similar to that of other cereal crops, although it is distinguished by its remarkable concentration of dietary fiber, which ranges from 15.0 to 25.0 percent of its dry matter. (Szuleta, 2023)

1,92 Chemical Composition of Rye

(% Weight)	**Rye**
Water	13,7
Protein	11,6
Lipids	1,7
Starch	52,4
Other HCOs*	16,6
crude fiber	2,1
Minerals	1,9

Source: Troche, Cobos, & Hernández (2017)

2.3.3. Barley

Barley *(Hordeum vulgare L.)* It represents a cereal harvest of great global importance. Hordeins, which make up the primary storage proteins in barley, have the ability to elicit immune responses that can result in celiac disease or symptoms linked to food allergy. (Li Z. , 2023)

2,3

(% Weight)	**Barley**
Water	11,7
Protein	10,6
Lipids	2,1
Starch	52,2
Other HCOs*	19,6
component s	Grain (g)
flour (g)	Humidity

Source: Parada & Araya (2010)

2.3.4. Spelt

Spelt *(Triticum spelta)* It is a cereal that contains gluten in its structure and therefore is not suitable for people with celiac disease. When we ingest this protein, our body triggers an immune reaction, as it detects these protein fragments as toxic, secalins in rye and gliadins in the case of spelt.(Otero, Mañes, & Lara, 2016)

2.3.5. Kamut

Kamut is a cereal that was already cultivated in Ancient Egypt about 5,000 years ago. One of its main attractions is that, as it has not undergone major genetic manipulations, it is considered a pure durum wheat that is very rich in essential nutrients. Therefore, the WHO recommends its consumption. (Sciarini L.S., Steffolani , & A.E. León, 2016)

3. THE SPECTRUM OF GLUTEN-RELATED DISORDERS

Several conditions associated with the consumption of gluten and related proteins are known, the varieties of celiac diseases are:

1. **Asymptomatic, subclinical or silent celiac diseases:** It is an asymptomatic disease, they are usually diagnosed in disease screening programs and many of them are family members of patients with celiac disease or related high-risk diseases (e.g., autoimmune diseases). (Cobos - Quevedo & Hernández - Hernández, 2017)
2. **Symptomatic celiac diseases:** Individuals experience obvious clinical symptoms such as bloating, abdominal pain, diarrhea or fatigue caused by gluten intake. (Moscoso, 2015)
3. **Classic or typical celiac diseases:** They present gastrointestinal manifestations such as diarrhea, malnutrition, weight loss, steatorrhea, and other similar symptoms. (Rojas Vargas, 2021)
4. **Celiac resistant diseases:** This condition is characterized by persistent or recurrent symptoms and damage to the small intestine despite following a strict gluten-free diet. Two variants are currently recognized: type I is characterized by normal intraepithelial lymphocyte phenotypes and type II is differentiated by aberrant clones

(they do not express CD3, CD4 or CD8) and can then be associated with the appearance of lymphomas.

10,88	**13,04**	**Protein**
14,87	12,94	Crude fat
1,74	1,25	Crude Fiber
2,64	2,32	Total ash
1,72	0,94	Carbohydrates
68,15	69,41	Moist gluten
---	32,76	Dry gluten
---	10,92	Inmunización

Table 4. New treatments for celiac diseases.

Source: Vriezinga SL, (2015)

3.1.Gluten Extraction

Based on the combination and optimization of documented gliadin and glutenin extraction protocols, there is a fast and efficient method for the extraction of gluten proteins in raw materials (wheat, barley, rye, spelt and kamut).

Immunization (Broeck , 2009)

3.1.1. Conventional Method

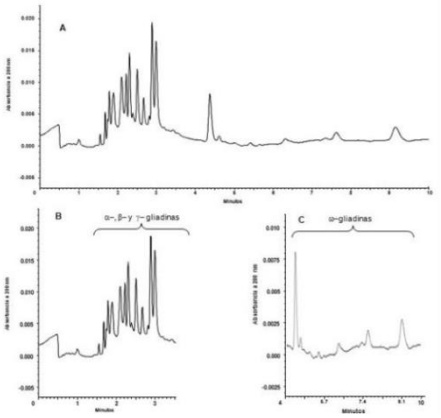
The conventional method for gluten extraction typically involves the use of 60% aqueous ethanol. (Güven & Onur Azizoglu, 2023)

3.1.2. Gliadin Extraction

It involves the removal of gliadin prior to a lateral flow immunoassay (LFIA). This method uses a non-ionic detergent, 1% Triton X-100, diluted in phosphate buffer for rapid extraction of gliadin from different food matrices specifically for LFIA application. (Güven & Onur Azizoglu, 2023)

As mentioned by Ece Güven (2023), the amount of gliadin in samples and the efficacy of extraction methods are evaluated using the internal sandwich RIDASCREEN® ELISA method. For the development of the ELISA sandwich, commercial antibodies, an anti-gliadin mouse monoclonal antibody (SAB42000864) and conjugated to HRP polyclonal antibody (Cat. No. A1052; Sigma-Aldrich, USA), two epitopes are used for the detection (Sensitivity and specificity) of these antibodies are initially evaluated by direct and indirect ELISA by coating 96-well microtiter plates with gliadin. (Broeck , 2009) Since gliadin is an alcohol-soluble protein, gliadin stock solution is prepared in 70% ethanol and then diluted in a coating buffer. For the coating buffer, 70% ethanol, carbonate-bicarbonate buffer (0.1 M, pH 9.6), and a combination of 70% ethanol and carbonate-bicarbonate buffer in direct ELISA were compared. (Ece Güven , 2023)

Figure 2. *Gliadin profile of the Buck Mataco cultivar: (A) complete electropherogram; (B) main body of gliadins; (C) ω−gliadin fraction profile.*

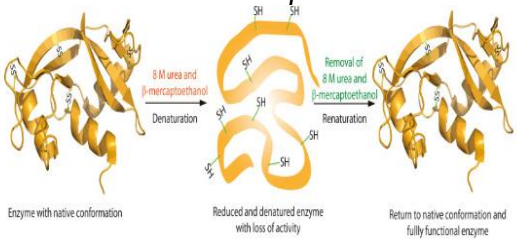


Source: Leon, Colombo & Ribotta (2008)

4. BIOPROCESSES FOR GLUTEN DENATURATION

The different enzymatic approaches used to hydrolyze or modify gluten, including specific proteases, transglutaminases, and other enzymes, are discussed. In addition, the effects of these bioprocesses on the structure and immunogenicity of gluten are explored, as well as potential applications in the food industry for the production of gluten-free foods. Therefore, they are determined by the conditions (e.g. denaturation concentration, temperature, pH, heat). (Bustamante-Rangel M, 2021)(Hellmann, 2020).

Figure 3. *Desaturation and renaturation of ribonuclease A PDB FILE ID: 1fs3.*



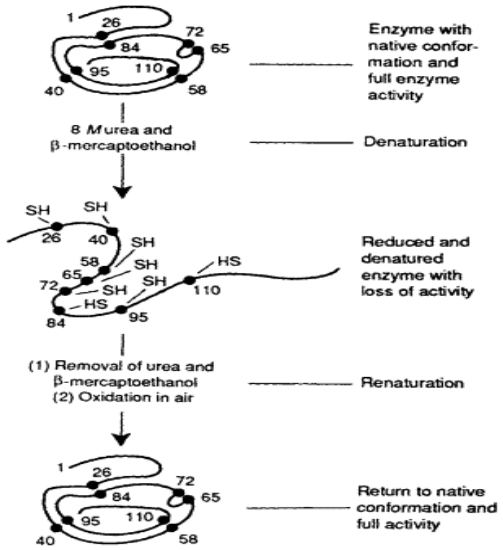
Source: Chung Eun Ha (2023)

A protein undergoes a transition from its primary structure to a specific three-dimensional configuration, known as the native state, to hydrolyze toxic peptides, heat treatments, and fermentation with microorganisms. The effects of these processes on the structure and functionality of gluten are analyzed. The denaturation of a native protein can be described as a change in its physical, chemical, or biological properties. Mild denaturation can destroy tertiary or quaternary structures, while more severe conditions can fragment the chain. Mild denaturation is usually a reversible process. Some of the changes in properties that can be caused by denaturation are as follows: (Ribeiro M., 2021)(BHAGAVAN, 2002)

1. Decreased solubility (often, but not invariably);
2. Alteration of the internal structure and arrangement of peptide chains that does not involve the breaking of peptide bonds (e.g., separation of oligomeric protein subunits);
3. Disturbed secondary structure (e.g., loss of helical structure);
4. Increased chemical reactivity of amino acid functional groups, in particular ilizable and sulfhidyl groups (e.g. change of pK values);
5. Increased susceptibility to hydrolysis by proteolytic enzymes;
6. Decrease or total loss of original biological activity, and

7. Loss of crystallability.

Figure 4. *Denaturation and renaturation of ribonuclease A. (BHAGAVAN, 2002)*



Source: Bhagawan (2002)

4.1.Enzymatic modifications

Enzymatic modification of proteins is gaining increasing importance both in the food industry and in other non-food sectors. This process is essential to improve various properties of gluten and other protein components, adapting them to the specific needs of different applications. . Enzymes are used with the aim of altering the structure of food, either to improve the functional properties of gluten or to reduce its presence, thus adapting to the needs of consumers with celiac diseases.(C. Wehrli & Kratky, 2021)

Enzyme modifications offer significant advantages for several reasons. First, enzyme technology, as a cleaning process, can result in low energy consumption, minimal waste production, and safer working conditions with reduced or no toxicity. Second, unlike chemical modifications, they do not affect the nutritional value of wheat gluten. Thirdly, enzymes, being biodegradable proteins, tend to denature during cooking, constituting a favourable alternative to pure additives and chemical agents.(Pourmohammadi & Abedi, 2021)

Studies have shown that limited enzymatic hydrolysis of the protein presents itself as an opportunity for the preparation of peptides with functional properties distinct from the parent proteins, such as emulsifying capacity and foaming function (Thakur N., 2021)

4.2.Protein modifications by microorganisms

4.2.1. Degradation of gluten by peptidases

In the initial breakdown of gluten, the peptides are subjected to the catalytic action of peptidases from native cereals, activated by the increase in acidity in the dough, resulting from the growth of lactic fermentation bacteria. Subsequently, the released peptides can be transported to bacterial cells, where they undergo further degradation at the hands of other peptidases.(Brzozowski & Stasiewicz, 2023)

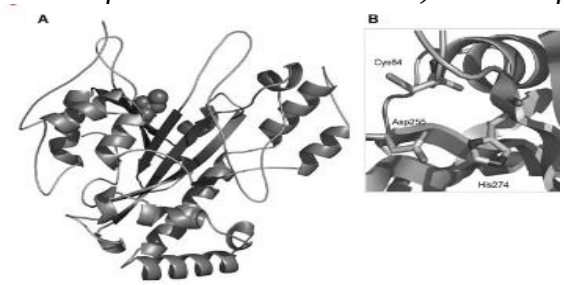
The process of hydrolysis of gluten, catalyzed by peptidases from native cereals and bacteria, carried out in a sour mass, can extend for a period of 24 to 48 hours. However, by

incorporating peptides from molds, caryopses from sprouted grains, or the cytoplasmic fraction of lactic acid-fermenting bacteria, it is possible to significantly reduce the time needed for gluten hydrolysis, while enhancing the breakdown of the released peptides (Zouari Ellouzi, 2014).

4.3.Heat Treatments

Transglutaminase, a naturally occurring enzyme, plays a significant role in modifying proteins by having the ability to catalyze the formation of bonds between proteins. This process reinforces the gluten network, giving it greater resistance and better water retention capacity. In the food industry, transglutaminase has been widely used as a tool to improve the texture and quality of food.(da Silva Ramos & Maciel Rocha, 2023)

Figure 5. *A) 3D structure of the microbial TGase. B) Details of the catalytic triad.*



Source: Aguilar Zárate (2012)

Microbial transglutaminase is characterized by a single domain with a disc-shaped configuration, as illustrated in Figure 1. The active site is located at the bottom of a deep groove at the edge of the disc. The structure of microbial transglutaminase belongs to the α + β folding category, composed of 11 α helices and 8 β strands.(Aguilar Zárate & Aguilar Zárate, 2012)

4.4.Enzymatic hydrolysis

4.4.1. Complete Hydrolysis

Complete hydrolysis breaks down into small fragments employing the addition of a large amount of water and results in a DH greater than 10, i.e., it involves the breaking of chemical bonds by adding water molecules to the molecular structure. Complete hydrolysis provides medicinal and nutritional properties, but produces bitter-tasting peptides and is therefore not suitable for use in the food industry. (Ganesh & Widya Ningtyas, 2022)

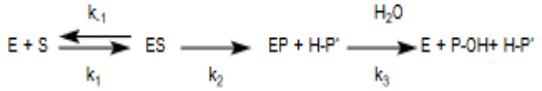
4.4.2. Partial hydrolysis

Enzymatic partial hydrolysis or limited proteolysis is a promising approach to modify the structure of proteins and improve their functional properties while keeping their nutritional value unaltered. (Ganesh & Widya Ningtyas, 2022)

4.4.3. Stages of Enzymatic Hydrolysis

Proteolytic hydrolysis refers to a set of simultaneous bond-breaking reactions with different charged species in equilibrium, making these processes very complex. The hydrolysis process includes three consecutive reactions. First, an enzyme-substrate (protein) complex is formed, and then the amide bond is broken, resulting in the release of a peptide. Finally, after nucleophilic attack of the water molecules, the remaining peptide is cleaved from the enzyme. The process can be restarted with two new peptides or probably just one of them. These three steps are schematically depicted in Figure 6. (Benítez , Ibarz, & Pagan, 2008)

Figure 6. *Catalytic mechanism of a protease* (Adler, 1993)



E: enzima, S: sustrato, P, P': péptidos resultantes, k_x: constante velocidad de reacción.

Source: Adler (1993)

4.4.4. Hydrolysis Conditions

The ratio between protein concentration and protease concentration should be established after any pre-treatment of the protein, if necessary. Then, it is crucial to define the conditions of the reaction in the hydrolysis process. The main variables that affect the outcome of the reaction are temperature, pH, enzyme-substrate ratio, and reaction time. The first three factors influence the rate of the reaction and can affect the specificity of the enzyme, while the reaction time determines only the final degree of hydrolysis. . To avoid changes in pH during hydrolysis, it is recommended to perform the reaction in a buffer system or in a pH-stad system, where the pH remains constant. (Benítez , Ibarz, & Pagan, 2008)

4.4.5. Determination of enzyme activity

When it is necessary to evaluate the proteolytic activity of an enzyme, one of the most common methods employed is the Anson procedure. In this technique, hydrolysis of denatured hemoglobin is performed using the enzyme of interest at a pH of 7.5 and a temperature of 25 °C for a period of 10 minutes. The hemoglobin that does not undergo hydrolysis is separated by precipitation with trichloroacetic acid (TCA), and the resulting supernatant, after filtering, is added the phenolic reagent of Folin-Ciocalteu, which produces a blue coloration in the presence of tyrosine, tryptophan and to a lesser extent cystine, cysteine and histidine. Absorbance is recorded at a wavelength of 750 nm. To establish a calibration curve, an enzyme of known Anson activity, usually pancreatic trypsin, is used, which undergoes the same procedure as the enzyme of interest. (Abellán-Victorio, 2020)

4.4.6. Degrees of hydrolysis

The degree of hydrolysis is considered critical for monitoring and regulating protein hydrolysis reactions. This indicates the proportion of hydrolyzed peptide bonds to the total number of bonds present. It is determined using equation 1, where h represents the number of hydrolyzed peptide bonds and denotes the total peptide bonds present in the parent protein. Both h-values and are expressed in milliequivalents per gram (meq/g). $h_{tot}h_{tot}$ (Escudero E., 2019)

$$DH = (H/h_{tot}).100\%$$

The techniques used to calculate DH are based on three main approaches: the quantification of α-amino free groups, the measurement of soluble nitrogen after protein precipitation with trichloroacetic acid, and the evaluation of proton release when a peptide bond is broken at specific pH values. (Benítez , Ibarz, & Pagan, 2008)

5. GLUTEN-FREE RAW MATERIALS, THEIR USE IN AGRIBUSINESS

Gluten-free raw materials and their use in the food industry. Various gluten-free alternatives, such as non-traditional cereal flours (maize, rice, quinoa, sorghum), modified starches, as well as plant and animal products, are being discussed. The technological properties of these raw materials and their applicability in the production of gluten-free

foods, including bakery, pastry, meat and dairy products, among others, are discussed. In addition, the challenges and opportunities associated with the use of gluten-free raw materials in agribusiness are explored. (Morreale, 2020)

5.1.Corn

Corn is a widely used raw material in agribusiness and is naturally gluten-free. It is used in the production of a variety of products, from food to biofuels. (C.M, 2019)

5.2.Rice

Rice is another gluten-free raw material used in agribusiness to produce a wide range of products, including cereals, flours, and beverages. (Barahona, Villareal, Gonzáles, & Itzel, 2019)

5.3.Sorghum

Sorghum is a gluten-free grain used in agribusiness for the production of food, fodder, biofuels, and more. (León, Mendoza, & Baladran, 2022)

5.4.Quinoa

Quinoa is a gluten-free pseudocereal that has become popular in agribusiness due to its unique nutritional profile and versatility in food production. (Jordy , Acosta, Paucar, & Universidad Nacional del Santa, 2022)

6. POTENTIAL OF GLUTEN-FREE PRODUCTS

The exploitation of plant-based by-products would represent an opportunity to improve both the nutritional profile and the overall quality of gluten-free foods, further improving the sustainability of the agri-food system. (Melini, 2019)

Gluten-free products have the potential to benefit people with gluten sensitivity or celiac disease, allowing them to enjoy a worry-free diet. The demand for gluten-free products is closely linked to health and wellness trends, these products can expand the options for those looking for healthier and more varied alternatives in their diet. However, it is important to note that the nutritional quality of these products can vary, so it is crucial to choose balanced options and be on the lookout for potential nutritional deficiencies. (Merino, 2020) (Gómez, 2022)

7. CONCLUSIONS

Gluten is a complex protein present in cereals such as wheat, barley and rye, composed mainly of gliadin and glutenin. Gliadin, an antigenic stimulant in celiac disease, and glutenins, previously considered harmless, may have adverse effects in some celiac patients. The denaturation of gluten involves changes in its structure, altering properties such as elasticity and viscosity.

The composition of gluten varies in different cereals, with wheat being the main source. Biotechnological processes, such as genetic modifications, fermentation, heat treatments and enzymatic hydrolysis, allow gluten to be denatured, affecting its properties. This review highlights the importance of these processes in agribusiness to improve the texture and quality of food products, adapting to dietary preferences and requirements.

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