Immunological Detection of Gluten

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Contributor: Eduardo Garcia-Calvo

Gluten is the general term for the ethanol-soluble proteins present in various cereal endosperms, including wheat, rye, barley, spelt, and kamut.

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1. Introduction

The definition by Codex Alimentarius also introduces some physical–chemical concepts: insoluble in water and 0.5 M sodium chloride solution [2]. Currently, this substance is slowly digested and presents a high permanence in the gut.

In 1924, Osborne introduced a classification method for plant proteins by extraction with different solvents that is still in use. After applying this classification (Table 1), wheat proteins are divided by their solubility behavior into the following fractions: globulins (soluble in a diluted salt solution), albumins (water soluble), gliadins (ethanol soluble), and glutelins (soluble in diluted acetic acid) [3].

Table 1. Protein fractions from cereal grains [4].

Osborne Fraction		Wheat	Rye	Oats	Barley	Corn
Globulin		Edestin				
Albumin		Leucosin				
Gluten	Prolamin	Gliadin	Secalin	Avenin	Hordein	Zein
	Glutelin	Glutenin	Secalinin	Aveninin	Hordeinin	Zeinin

Traditionally, gluten proteins have been separated into two fractions that are either soluble or insoluble in alcohol. This division, with some modifications, has remained in use to the present day, with the gluten proteins that are readily soluble in alcohol–water mixtures (e.g., 60–70% ethanol) being called gliadins and those that are insoluble being called glutenins. However, it is now know that the two fractions contain proteins that are structurally related, with the differences in solubility resulting from their presence as monomers that interact by non-covalent forces (gliadins) or as high molecular mass polymers stabilized by interchain disulphide bonds. When present as reduced subunits, the glutenin proteins are also soluble in alcohol–water mixtures and can therefore be defined together with gliadins as prolamins [5]. Glutelins are heterogeneous and can be separated using electrophoresis into over a dozen fractions that as categorized into high molecular weight (HMW) and low molecular weight (LMW) groups [6]. Glutelin subunits have been found to correlate with gluten properties that are related to baking quality [5]. Gliadins are represented as single chain polypeptides, and it is accepted that gliadins are divided, according to their electrophoretic mobility in Polyacrylamide gel Electrophoresis (PAGE) at low pH (lactate-PAGE), into four major groups (α-, β-, γ-, and ω-gliadins, from fastest mobility to slowest) [7]. Gluten proteins contain large repeat domains composed of homologous and repetitive sequences of six-to-eight amino acids rich in proline and glutamine [8]. In addition, when considering the alpha-gliadin structure, their central domain contains the proline- (P) and glutamine-rich (Q)

heptapeptide PQPQPFP and pentapeptide PQQPY. This domain contains the most characteristic immunogenic fragment, a 33-mer peptide comprising six overlapping epitopes significant for celiac disease pathogenesis [9], although this peptide is not present in every wheat cultivar [10].

Several fragments of gliadins and glutelins are associated with different types of gluten-related diseases, e.g., α and γ-gliadins in celiac disease [11]; γ-, α/β-, ω5-, and ω1,2-gliadins, as well as HMW and LMW subunits of glutenin, are involved in wheat allergies [12].

2. Gluten-Related Diseases

Several diseases related to the exposure to gluten of prone persons that can be classified with etiology into the three main groups of allergy, autoimmunity, and non-celiac gluten sensitivity have been described [13].

Gluten-related allergies, also known as wheat allergies, have a prevalence of 0.1% in the general population [14], and they have developed a well-known two-step pathological mechanism: the sensitization and effector phases [15]. Within this last phase, the onset of the main reactions occurs in minutes to hours after gluten exposure driven by an IgE response. This group includes the following pathologies classified by symptomatology: (a) a respiratory allergy, also known as baker's asthma, with bronchial symptoms as severe clinical presentation [16]; (b) a food allergy with major digestive presentation [17]; (c) wheat-dependent exercise-induced anaphylaxis (WDEIA), an inflammatory situation triggered by stress [18]; and (d) contact urticaria, with major dermatologic symptoms [14].

The second group of gluten-related diseases is associated with an autoimmune etiology. Celiac disease, with a prevalence of 1% in general population, is the most important [14].

Many molecular mechanisms leading to intestinal damage in celiac disease have been described, although not all have been discovered yet. The ingestion of gluten by sensitized people results in the partial digestion of gliadin (wheat prolamin), which interacts with CXCR3 (chemokine receptor 3) and stimulates the liberation of zonulin [19]. This leads to an increased intestinal permeability, facilitating the translocation of gliadin peptides from lumen to lamina propria. Then, the secretion of innate immunity mediators (Interleukins IL15 and IL8) is triggered, with consequent neutrophil recruitment [20]. The loosen gut barrier facilitates the engagement of toll-like receptor complex 4-M2-CD14 by trypsin and alpha-amylase inhibitors, thus releasing pro-inflammatory cytokines [21]. Following the innate immune-mediated apoptosis of intestinal cells with the subsequent release of intracellular tissue transglutaminase, gliadin peptides are partially deamidated [13]. These deaminated peptides are presented by DQ 2/8 (a class II Major Histocompatibility Complex or HLA cell surface receptor) antigen-presenting cells (APCs) to helper T cells that trigger the maturation and activation of B-cells producing IgM, IgG, and IgA against tissue transglutaminase [22] (for this reason, it is considered an autoimmune disease). Additionally, helper T cells produce pro-inflammatory cytokines like interferon-gamma and tumoral necrosis factor-alpha (TNF-α) [23]. This immune response, together with the function of killer T cells, initiates the enteropathy. Damaged enterocytes express the CD71 transporter to facilitate retrotranscytosis events [24] and further increase intestinal permeability; this spurs a pro-inflammatory and pro-growth environment, resulting in the development of hyperplastic crypts and affecting the absorption of nutrients [13].

In addition to celiac disease, gluten ataxia (a neurological disease [25]) and dermatitis herpetiformis [14] are considered gluten-related autoimmunity diseases.

Non-celiac gluten sensitivity (NCGS, also denominated non-celiac wheat sensitivity and, sometimes, gluten intolerance) is the third group of gluten-related diseases by etiological classification (non-autoimmune and non-allergic), with a prevalence of up to 7% in the general population [14]. Pathogenic mechanisms are still uncertain, but it seems that innate immunity plays a

major role [26]. The signs and symptoms are very similar to other gluten-related diseases, irritable bowel syndrome, and Crohn´s disease. DQ2/8 haplotypes and IgG/IgA anti-gliadin antibodies are present only in 50% of cases. Intestinal damage in this disease is lower than that observed in celiac disease [27].

There has been an intense research into the pharmacological treatment of these diseases, (especially celiac disease) including gluten neutralization agents, disruptors of mucosal transportation or antigen processing enzymes, modifications of the microbiome, immunomodulators, and anti-inflammatory drugs [28]. Notwithstanding, a gluten-free diet is the most recommended and has long been considered the only effective treatment [29]. When gluten consumption is eliminated, the exacerbated immune response is inhibited, leading to the partial (if not complete) healing of the duodenal mucosa along with the resolution of symptoms and signs of malabsorption [30].

3. Gluten Content Labeling Legislation in Different Countries

In contrast to other allergens, and following the recommendations included in Codex Standard 118-1979 [2], there is well-developed legislation about gluten presence in food in several countries.

In Europe, the Commission Implementing Regulation (EU) No. 828/2014 of 30 July on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food [31] rules that “The statement ‘gluten-free’ may only be made where the food as sold to the final consumer contains no more than 20 mg/kg of gluten” and “The statement ‘very low gluten’ may only be made where the food, consisting of or containing one or more ingredients made from wheat, rye, barley, oats, or their crossbred varieties which have been specially processed to reduce the gluten content, contains no more than 100 mg/kg of gluten in the food as sold to the final consumer”.

The U.S. Food and Drug Administration (FDA) has defined the term “gluten-free” for voluntary use in foods that are inherently gluten-free, or when they are not composed of gluten-containing grains—either raw or processed to remove gluten. Any unavoidable presence of gluten in the food must be less than 20 ppm (mg/kg), Food Allergen Labeling and Consumer Protection Act (FALCPA) [32].

Health Canada considers that gluten-free foods are those that contain levels of gluten not exceeding 20 mg/kg as a result of cross-contamination, and they must meet the health and safety intent of B.24.018 (2012). Regarding oats, on 19 May 20, Health Canada registered a marketing authorization 15 that permits the use of gluten-free claims for gluten-free oats [33].

The current legislation in Australia and New Zealand is the strictest. Australia and New Zealand Food Standard Code, standard 1.2.7, states that “for the food to be labeled as gluten-free, the food must not contain: detectable gluten; or oats or their products; or cereals containing gluten that may have been malted, or their products.” For the “not contain detectable gluten” statement, the limit was set at 3 ppm (mg/kg) [34].

In Mexico, the executive orders NOM-051-SCFI/SSA1-2010 and NOM-247-SSA1-2008 state that those foods that may produce any kind of allergy and intolerance must be labeled, and those containing grains and derivates must be labeled with “this product contains gluten” statement [35][36]. In Argentina, there is specific legislation for celiac disease (celiac law, passed by the Congress in 2009 (26.588), modified in 2015 (27.196)), claiming this pathology as “disease of national interest,” regulating not only food security issues but also social aspects. A gluten limit of 10 ppm (mg/kg) was set by this law for a product to be labeled as “gluten-free,” including a specific logo. The legislation also applies to medicines [37].

In Brazil, Federal Law 8543/1992, mandates that all industrialized foods that contain gluten must carry a warning that they contain gluten. It was updated by Federal Law 10674/2003, determining that all industrialized foods must indicate on their

labels and packaging the phrases “Contains Gluten” or “Does Not Contain Gluten.” Brazilian legislation follows the 20 ppm (mg/kg) limit included in Codex Alimentarius [38].

In China, Food Law GB/T23779 issued in 2009 by General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) “Allergens in prepackaged foods” includes gluten-containing grains and related products amongst the substances that may induce allergic reactions. A 2015 standard specifically applicable to the inspection of gluten allergen ingredients in prepackaged food for export made a clear reference to *Codex* standard STAN 118-1979 in order to verify the compliance of gluten-free claims. This regulation set a maximum limit for a gluten-free claim of 20 mg/kg [39]. However, this regulation does not apply for import or domestic trade.

By Japanese law, the labeling of allergens is designated as mandatory or recommended based on the number of cases of actual illness and the degree of seriousness. To standardize official methods, the Japanese government described the validation protocol criteria in the 2006 official guidelines and stated that any food containing allergen proteins at greater than 10 ppm (mg/kg) must be labeled under the current law [40].

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