Trends in wheat technology and modification of gluten proteins for dietary treatment of coeliac disease patients

F Cabrera-Chávez, AM Calderón de la Barca*

Coordinación de Nutrición. Centro de Investigación en Alimentación y Desarrollo, A. C. Carretera a la Victoria Km 0.6 P. O. Box 1735. Hermosillo 83000, Mexico

**Abstract**

Coeliac disease (CD) is a long-life intolerance to gluten proteins, with prevalence of 1–2% worldwide and health consequences if not treated. Currently, the treatment is the dietary gluten withdrawal, but commercial gluten-free foodstuffs present undesirable properties. Therefore, attempts are being made to modify the immunogenic sequences of gluten to avoid recognition by the immune system and to prepare safe and acceptable foods. These include long-time fermentation by sourdough and enzymic modification. The present article reviews our current knowledge of the pathogenesis of CD and the advantages and limitations of the current approaches to gluten modification and to develop safer foods for CD patients.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Coeliac disease (CD) is an enteropathy of the small intestine developed in genetically susceptible individuals. It is characterised by a life-long intolerance to undigested peptides derived from gluten proteins (the water-insoluble storage proteins), from wheat endosperm and similar proteins from taxonomically-related species such as rye and barley. Gluten is a mixture of related proteins which are classically divided into two groups, the monomeric gliadins which are soluble in alcohol–water mixtures (and hence defined as prolamin) and the gliutenins which are insoluble polymers stabilised by interchain disulphide bonds. However, the gluten subunits released from the polymers by reduction of the disulphide bonds have similar solubility properties to the native gliadins and it is now usual to define both groups as prolamins. Coeliac disease is an autoimmune T cell-mediated disease in which tissue transglutaminase (tTG) is the autoantigen and antibodies directed both against tTG and prolamin peptides, the exogenous anti-gliadin (Di Sabatino and Corazza, 2009).

CD is one of the most common human genetic disorders, with a prevalence of 1–2% worldwide, and is apparently increasing in incidence (Green and Cellier, 2007; Rodrigo, 2006). In addition to nutrient deficiencies, if not treated, CD can result in an increased risk of other autoimmune diseases and malignancy (Lohi et al., 2007; Rubio-Tapia et al., 2009). Currently, the only treatment for CD consists of a life-long gluten-free diet, although alternative treatments, such as oral doses of microbial endopeptidases to degrade wheat peptides, are under trial (Ehren et al., 2008; Mitea et al., 2008). Beyond therapeutic treatments, attempts are being made to modify the immunogenic sequences (epitopes) of gluten proteins to prepare foods for CD patients. These include long-time fermentation by sourdough, as well as enzymic modification to ensure that the epitopes are no longer recognised by the immune system of CD patients.

The key question is, why does a population previously naïve to gluten intake become intolerant to gluten proteins? Because the onset of CD depends on genetic and environmental factors, and genetic factors are stable over long periods, infections and dietary habits could be responsible. Dietary habits have changed dramatically in recent decades, and are affected by cereal food technology and processing (Rubio-Tapia et al., 2009). There have also been changes in wheat cultivars and in enzyme treatments used in processing, and gluten intake has increased because of its use in processed foods, especially fast foods. Thus, it has been suggested that higher gluten consumption is related to the increase in the prevalence of CD (Lohi et al., 2007). Therefore, in order to modify gluten proteins to produce foods for CD patients, it is necessary to consider changes in food technology in the last decades, as well as our current knowledge of CD pathogenesis. In this article we review current trends in wheat technology and modification of proteins to develop safer foods for CD patients.
2. Pathogenesis of coeliac disease

Genetic predisposition based on specific alleles of the human leucocitary antigen (HLA-DQ2 and HLA-DQ8) is a prerequisite for the mechanism of CD pathogenesis (Briani et al., 2008). This is consistent with the two-signal model (Bernardo et al., 2008), the first signal being generated by the innate immunity and the second one by the adaptive immunity. The innate response is probably mediated by DQ2- and DQ8-independent peptides that causes stress to intestinal cells releasing IL-15 (Meresse et al., 2004). Such peptides are defined as ‘toxic’ (Ciccocioppo et al., 2005). This response causes damage to enterocytes and increases intestinal permeability (Brandtzæg, 2006).

In the adaptive immune response, gluten peptides that are resistant to digestion by intestinal peptidases are transported across the epithelial barrier to the lamina propria due to the increased permeability. Once inside, these peptides are deamidated by tissue transglutaminase (tTG) which recognises the presence of a proline residue at two positions C-terminal to the target glutamine, or a large hydrophobic amino acid such as phenylalanine three positions C-terminal to the target glutamine (Koning et al., 2005). This generates new epitopes that are efficiently bound to HLA-DQ2 or DQ8 (Kim et al., 2004). HLA-DQ2 prefers negatively charged amino acids at the p4, p6, or p7 positions in the peptide (PQP\_E\_LPYPQ, PFPQP\_E\_LPY or PQQSFP\_E\_QE), while HLA-DQ8 prefers negatively charged residues at position p1 or p9 (EGSFQPSQE) (Koning et al., 2005). Such peptides, including the well-characterised 33mer derived from α\_gliadin (Shan et al., 2002), are defined as ‘immunogenic’ (Ciccocioppo et al., 2005), and recognised by intestinal T cells (Fig. 1 part A).

Deamidated peptides and tTG also form intermediate complexes which are internalised into B cells by binding to immunoglobulin on the surface of tTG-specific B cells, and deamidated peptides are released and bound by HLA-DQ2 or DQ8 molecules. After the complexes are transported to the cell surface, T cells recognise deamidated peptides and trigger in the proliferation of tTG-specific B cells and the production of antibodies (Briani et al., 2008). Finally, the activated intestinal T cells drive an inflammatory response that leads to the development of the characteristic coeliac lesions.

3. CD pathogenesis and wheat processing technology

Two features of CD pathogenesis are related to wheat processing in food production. One is the inability of gastrointestinal peptidases to digest the proline-rich gluten polypeptides to generate peptides smaller than nine amino acids. The other is the deamidation of glutamine residues of such peptides by tTG.

The exposure to dietary gluten peptides is essential to trigger the immune response in genetically predisposed individuals. Therefore, it is not surprising that CD has been recognised for many years in populations with high wheat intake. However, the consumption of wheat has spread throughout the world and it is one of the three most consumed cereals (Tatham and Shewry, 2008). In addition to making baked goods and pasta, gluten is used in many foods to confer properties such as emulsification, cohesiveness, viscoelasticity, gelation and foaming (Esteller et al., 2005; Maningat et al., 1999). These modern applications as well as traditional ones can decrease or increase epitopes in gluten proteins. For example, these processes can result in proteolysis, oxidation or enzymic modification by transglutaminases (Tatham and Shewry, 2008).

For example, traditional long fermentations in sourdough systems have been almost totally replaced by the use of leavening agents in very fast processes. These could result in gluten proteins remaining largely intact and their peptides immunogenic for CD sufferers (Rizzello et al., 2007). The gluten proteins contain about 15% of proline and 40% of glutamine (Hoseney, 1994). Proline is a cyclic imino acid which prevents cleavage of adjacent peptide bonds by most of mammalian peptidases (Hausch et al., 2002). Peptidases able to cleave proline-containing substrates are therefore required to digest coeliac-toxic sequences. It was formerly made by the acidic medium and peptidases activated during sourdough fermentation started with lactic acid bacteria (De Angelis et al., 2006; Rizzello et al., 2007).

---

**Fig. 1.** Adaptive immune pathogenesis mechanism in CD (part A) and different ways to avoid the T cell activation by gluten peptides modifications (parts B, C and D). Abbreviations: tTG: tissue transglutaminase; APC: antigen presenting cell.
Deamidation is another example of current gluten processing which could affect coeliac-toxic epitopes. This modification has been used to confer additional functional properties to gluten by removing the amid groups of glutamine (Agyare et al., 2009; Mimouni et al., 1994).

Over the last decade, microbial transglutaminase (mTG) has been included in many food processes in order to deamidate and/or cross-link (Malandain, 2005). However, most of the sequences of gluten peptides that trigger CD are preferred substrates for tTG so treatment with mTG may produce neoepitopes and enhance their T cell stimulatory potential. Consequently, the deamidated gluten peptides generated by mTG could be the same as those produced by tTG which trigger CD (Gerrard and Sutton, 2005; Malandain, 2005). In fact, it has been demonstrated that wheat prolamins treated in vitro by mTG, were recognised better by IgA from CD patients, than untreated prolamins (Berti et al., 2007).

mTG has also been used to improve the functional properties of gluten-free breads recommended for CD patients (Moore et al., 2006). One such gluten-free bread with improved quality properties used maize and rice flour as well as powered milk as an alternative to wheat flour. We showed that maize prolamins extracted from such bread, had higher in vitro reactivity of IgA (from some coeliac patients) than those without the mTG treatment (Cabrera-Chávez et al., 2008). The rationale for this could be that milk and perhaps rice proteins were cross-linked by mTG while maize prolamins (zeins) were preferentially deamidated. This is because zeins contain a peptide with three glutamine (Q) residues which can be deamidated by tTG (Koning et al., 2005). This is the QQQQPPSQQQQPSPPSQQQ sequence with glutamines at positions 3, 4 and 12 which are two positions C-terminal of proline residues. These glutamine residues may have been deamidated by mTG while maize prolamins (zeins) were preferentially deamidated. This is because zeins contain a peptide with three glutamine (Q) residues which can be deamidated by tTG (Koning et al., 2005). This is the QQQQPPSQQQQPSPPSQQQ sequence with glutamines at positions 3, 4 and 12 which are two positions C-terminal of proline residues. These glutamine residues may have been deamidated by mTG in vitro, allowing the recognition of the sequence by IgA antibodies from CD patients. However, this hypothesis has not been investigated experimentally.

These examples of trends in food technology which could affect the CD prevalence or morbidity emphasise the need to consider our current knowledge of the pathology of CD when modifying gluten to produce foods for CD patients.

4. Sourdough and its potential for degradation of gluten

Sourdough is produced using a culture of lactobacillus, frequently in combination with yeast. Sourdough is the oldest method for leavening bread and is still used for some applications. For example, in making bread from rye, perhaps because the dough made from rye flour needs a low pH to be appropriate for baking (Arendt et al., 2007). In comparison with yeast-leavened doughs for wheat- or rye-based breads, sourdough produces a distinctively tangy or sour taste, mainly as a result of lactic acid produced by the lactobacilli. Moreover, during sourdough fermentation, proteolysis provides compounds that are precursors for the aroma volatiles and amino acids which are converted by microbes to compounds which are precursors of flavours (Gänzle et al., 2008).

Traditionally sourdough is added as an ingredient to unmodified flour of wheat or rye for breadmaking. However, some authors (Rizzello et al., 2007) have proposed sourdough as the major ingredient and the only source of proteins for making gluten-free bread.

4.1. Mechanisms of degradation of gluten in sourdough

Lactobacilli produce a complex system of peptidases, including proline-specific peptidases (Cerez et al., 2008). However, no single strain produces the whole spectrum of peptidases required for hydrolysis of the proline-rich proteins involved in CD (Gobbetti et al., 2007). Therefore, it is necessary to consider the capacity to hydrolyse wheat prolamins when selecting lactobacilli for sourdough (Di Cagno et al., 2004; Rizzello et al., 2007). Thus, a pool of Lactobacillus sanfranciscensis, L. alimentarius, L. brevis and L. hilgardii, was added as a starter for sourdough of wheat flour. The ethnolic extract from such sourdough reduced the activation of peripheral blood mononuclear cells from CD patients, compared to the activation that occurred when cells were challenged by native prolamins (Rizzello et al., 2007). Fig. 1B summarises the effect of high proteolysis due to sourdough.

Proteolysis by sourdough is not only due to bacterial peptidases but also to the grain enzymes, with the activities of endogenous acidic enzymes, such as aspartic peptidases and carboxypeptidases in wheat and rye flours, being activated under the fermentation conditions (Gänzle et al., 2008; Wieser et al., 2008).

Another important effect of sourdough fermentation is to disrupt the gluten protein network. The highest molecular weight proteins in gluten are glutenins which are polymers stabilised by disulphide bonds. When glutenins are partially hydrolysed, the depolymerisation and solubilisation of the polymers occurs (Thiele et al., 2004). In addition, glutathione is an endogenous reducing agent in dough that can cleave disulphide bonds particularly when the pH is slightly acidic as during the first hours of sourdough fermentation (Grosch and Wieser, 1999; Wieser et al., 2008). Furthermore, the activity of glutathione reductase is increased due to the effect of the lactobacilli on the redox potential (Jänsch et al., 2007). Finally, proline-rich polypeptides released by disruption of the gluten network, are exposed to the action of proline-specific peptidases from lactobacilli.

4.2. Limitations of sourdough technology

In spite of the advantages of sourdough fermentation for hydrolysis of the wheat proteins responsible for CD (De Angelis et al., 2006; Di Cagno et al., 2002; Rizzello et al., 2007), it has some limitations. A very long sourdough fermentation (24 h) is required to reduce the intolerance by CD patients. Under such conditions, stability and dough resistance are decreased as a result of the disruption of the gluten network. In breadmaking, the optimum fermentation time using sourdough is 4 h (Gocmen et al., 2007). Therefore, it is still a challenge to prepare bread for CD patients by sourdough fermentation. Furthermore, some highly consumed wheat goods such as pastas, tortillas, pita and middle eastern breads are produced without fermentation.

5. Modification of gluten proteins by derivatisation of amino acid functional groups

Because gluten proteins contain relatively few ionisable groups, the -NH₂ groups and non-polar side chains have a greater opportunity to react with other compounds to form covalent bonds. This characteristic has been used to good effect in studies focused on chemical modifications such as the amide-ester conversion of glutamine in gluten proteins (Beckwith et al., 1963).

Kapoerchan et al. (2008), selected a gluten peptide with high affinity to HLA-DQ2 and confirmed the amino acid residues recognised by HLA-DQ2 and the other residues were still available for modification. The authors then substituted proline residues for the corresponding azide-modified amino acids. This modification caused abolition of T cell recognition due to the introduction of steric hindrance. Other researchers (Siegel et al., 2007; Xia et al., 2006) have also investigated the modification of gluten peptides, showing that it is feasible to modify immunodominant peptides to T cell activation in CD by steric hindrance. This is summarised in Fig. 1C.
Table 1

<table>
<thead>
<tr>
<th>Modification</th>
<th>Technological advantages on application</th>
<th>Technological limitations on application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sourdough fermentation</td>
<td>It could be applied in wheat process involving fermentation steps, just with the addition of sourdough starts with a selected pool of lactobacilli.</td>
<td>It has limitations for applying in non-fermented foodstuffs. In fermented products, it could be necessary the structuring agent addition because of the degradation of whole gluten. The long-time fermentation could be other disadvantage. Depending on the amino acids used as steric bulk, the functionality could be modulated.</td>
</tr>
<tr>
<td>Modification of gluten amino acids residues</td>
<td>Inclusion of steric bulk trough amino acids residues preserves the integrity of proteins.</td>
<td></td>
</tr>
</tbody>
</table>

5.1. Enzymatic derivatisation of immunogenic gluten peptides

Gianfrani et al. (2007) reported an interesting and novel approach to reduce gluten immunogenicity by transamidation using mTG. This method takes advantage of the same substrate specificity of tTG that creates coeliac-active gluten peptides via deamidation.

TG can catalyse either protein cross-linking, binding of free amines or the deamidation of glutamine residues to form glutata, depending on the available substrates and conditions in the system (Gerrard and Sutton, 2005; Malandain, 2005). TG crosslinks glutenine and lysine present in proteins or amine groups available from free amino acids, and it has no effect on the recognition of gluten proteins by the immune systems of CD patients. The γ-glutamyl-c-lysyl isopeptide bonds formed can be hydrolysed in the small intestine after the protein is completely hydrolysed (Seguro et al., 1996). Amine groups present on free lysine may also bind to the amino group from glutamine if insufficient protein-bound lysine is present. If no lysine is available in proteins or as free amino acids, glutamine residues present in gluten proteins are deamidated (Zhu and Tramper, 2008). The deamidation by tTG gives a negative charge to gluten peptides conferring high affinity to HLA-DQ2 or DQ8 (Fig. 1A).

Using mTG in a controlled system to bind free amino acids to glutamine amino groups in gluten proteins may prevent the immune recognition by HLA-DQ2 molecules in CD (Fig. 1D), due to steric hindrance by amino acid residues neighboring the negatively charged glutamic acid residues (Gianfrani et al., 2007). Thus, it may be possible to abolish the stimulatory activity of gluten proteins whilst still preserving some of their properties. Although the final functional properties of the modified proteins were not evaluated, their molecular weight was preserved, which is the major advantage compared with hydrolysis. Other transpeptidases have been tested for binding amino acids to side chains of proteins. For example, γ-glutamyl transpeptidase catalyses the addition of γ-glutamyl moieties to other amino acids and peptides, with similar mechanisms to TG (Suzuki et al., 2003).

On the other hand, under specific conditions, peptidases can also catalyse transpeptidation. The hydrolysis reaction by peptidases is reversible in principle, and the thermodynamic barriers to the reversal of hydrolysis can be allowed to proceed in the direction of peptide bond formation (Kumar and Chand Bhalla, 2005). In contrast to hydrolysis, transpeptidation requires low water activity, where free amino groups react as nucleophiles instead of water molecules and transpeptidation occurs (Goepfert et al., 1999). This principle may be also applied to incorporate steric bulk into gluten proteins for technological modification. Under the appropriate conditions, the unprotonated polar groups (NH2) induce to ε-amino from glutamine to act as a nucleophile for covalent binding of free amino acids.

5.2. Perspectives for gluten modifications to reduce coeliac toxicity

Whatever mechanism is selected to introduce steric bulk into gluten proteins, the modification will also influence the functional properties of the proteins. Therefore, the selection of compounds to be bound should take into account the properties that they may impart to the modified protein. For example, ionised amino acids increase protein solubility, while hydrophobic amino acids may decrease it. Modification by binding amino acids to gluten proteins is also more readily carried out on isolated gluten than in whole wheat flour. For breadmaking, modified gluten could be mixed with starch from wheat or another source using a longer mixing time than for unmodified wheat flour to allow starch-gluten interactions to be established. Therefore, technological assays are required to optimise the conditions to produce wheat goods.

6. Conclusions

Table 1 summarises advances that have been made in gluten protein modification to produce safer wheat products for CD patients. Firstly, sourdough degradation of gluten proteins is an option for food processing that includes fermentation. In other processes, the addition of structuring agents shall be required.

The incorporation of steric bulk into gluten proteins in order to avoid immune recognition (Gianfrani et al., 2007) is the most promising way to obtain wheat-based products that are tolerated by CD patients. This is because it can be applied to the whole wheat flour in order to modify all of the coeliac-active gluten proteins. However, further studies are required to optimise the processing of the modified flour or gluten to prepare foodstuffs with acceptable properties. The introduction of modified products into diets for CD patients must also be exhaustively evaluated, not only by using serological and cellular assays but also in dietary intervention studies to ensure that CD symptoms do not occur.

Acknowledgements

We thank the excellent commentaries on this manuscript by Noé Ontiveros Apodaca, Elvira Rojas Martínez and Alma Rosa Islas Rubio. This review was done as a part of a project financially supported by Consejo Nacional de Ciencia y Tecnología (CONACyT) through the grant CB-2008-01-106227 given to Ana María Calderón de la Barca.

References
