Oats as a safe alternative to Triticeae cereals for people sufferring from celiac disease?

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Abstract

Oats represents a promising alternative to small-grain cereals from Triticeae group (wheat, barley, rye) for persons suffering from any form of gluten intolerance, especially celiac disease (CD), since oat-specific prolamins avenins reveal generally lower gluten content and immunoreactivity. Recent studies on avenin molecular structure revealed large genetic variability in avenin sequences affecting the spectrum of gluten peptides produced by hydrolases in human digestive tract. The aim of the present review is to summarise recent knowledge obtained in laboratory in vitro studies focused on the effect of avenin-derived peptides on reactivity of crucial components of human immune system such as dendritic cells (DC) and T-cells. The other part of the review summarises the results of clinical studies with CD patients including oat products in their diet. Since different clinical studies revealed contradictory results regarding potential safety of oats for CD patients, the focus has to be directed at genetic variability in oat avenins. Identification of avenin isoforms with minimum CD immunoreactivity will open up ways leading to designing novel oat cultivars suitable for CD patients. Knowledge on immunoreactivity of gluten peptides together with breeding new oat cultivars revealing minimum avenin immunoreactivity with respect to CD as well as application of food processing technologies leading to gluten content reduction should result in development of gluten-free oats safe for celiacs.

Keywords: celiac disease; avenins; genetic variability; immunoreactivity; clinical studies; oats (*Avena sativa*)

Introduction

Celiac disease (CD) is an autoimmune enteropathy triggered by dietary gluten in genetically predisposed persons carrying HLA-DQ2.2/2.5/8 receptors on the membranes of dendritic cells (DC). „Gluten" is a mixture of non-hydrolysable polypeptides rich in proline and glutamine which arises by hydrolysis of grain storage proteins belonging to prolamin family which are found in wheat (glutenins and gliadins), barley (hordeins), rye (secalins), and oats (avenins) [1]. Grain storage proteins including albumins, globulins, prolamins, glutelins, and unextractable protein fractions are estimated to amount around 15% of total kernel weight in small-grain cereals (wheat, barley, rye) out of which prolamins are estimated to contribute ca 15% of total grain storage protein weight, i.e., ca 6-8% of total kernel weight. The name „prolamin" is derived from „proline" and „amine/amide" referring to amide groups in glutamine residues. The joint proline and glutamine content can reach up to 50-60% of total amino acids content in wheat gliadins [2]. Prolamins differ in their solubility in ethanol and electrophoretic mobility as well as in their ability to form oligomers which is determined by the number of cysteine residues enabling formation of intra- and intermolecular disulphide bonds. Prolamins are

divided into several groups. In wheat, prolamins are distinguished into alcohol-insoluble oligomeric glutenin fraction (high-molecular weight glutenins, low-molecular weight glutenins) and alcohol-soluble monomeric gliadin fraction containing S-rich α,β, and γ-gliadins, and S-poor ω-gliadins. S-rich prolamins contain three conserved regions A, B and C interspersed by repetitive regions and variant regions in their molecules while S-poor prolamins lack the conserved regions. S-poor prolamins such as wheat ω-gliadins and barley C hordeins were suggested to had evolved from S-rich ones by deletion of the conserved regions and multiplication of repetitive regions [1].

Celiac disease represents a complex of human body responses to gluten intolerance which is underlied by the inability of human small intestine enzymes to cleave peptide bonds in gluten proteins from prolamin family which are rich in glutamine (Q) and proline (P) residues and can resist small intestine hydrolases [1;2]. CD is determined genetically by the presence of HLA-DQ2.2, HLA-DQ2.5 or HLA-DQ8 haplotypes of receptors on the plasma membrane of dendritic cells (DCs) which are important antigen-presenting cells. A crucial role in CD biogenesis is played by human enzyme tissue transglutaminase 2 (tTG2) which deamidates glutamine residues to glutamic acid in uncleaved gluten peptides thus forming gluten epitopes recognised by HLA-DQ2/8 receptors. Deamidation of glutamine residues (Q) to glutamic acid (E) by tTG2 introduces a negative charge into QXP motifs in gluten peptides which then function as epitopes recognised by HLA-DQ2/8 receptors. The interaction of HLA-DQ2/8 with deamidated gluten peptide leads to formation of HLA-DQ2/8-gluten complex whose interaction with T-cell receptor (TCR) on the surface of specific CD4+ T lymphocytes in lamina propria of proximal small intestine. The interaction leads to T-cell proliferation and enhanced release of γ-interferon (γIFN) and interleukin 21 (IL-21) leading to inflammatory processes, atrophia of small intestine villi, and others. These processes eventually lead to degradation of proximal small intestine (duodenum, jejunum) lamina mucosa, villous atrophy and crypt hyperplasia causing serious digestion problems. Moreover, celiac disease is also an autoimmune disease due to a formation of complexes between tTG2 and gliadin peptides bound to HLA-DQ2.2/2.5 due to transamidation reactions between tTG2 and side amino groups in glutamine residues. As a result of the occurrence of tTG2-gliadin complexes bound to HLA-DQ2.2/2.5 on dendritic cells, IgA-class autoantibodies against tTG2 were found in serum of celiac patients. The strongest immune response was found for 33-mer peptide (p57-89) derived from wheat α2-gliadin repetitive region against which a monoclonal antibody moAbG12 was raised. Currently, six different epitopes recognised by HLA-DQ2.5 type receptor were identified in α2-gliadin 33-mer [1;3]. Besides the 33-mer (p57-89), the 25-mer (p31-43) interacting with interleukin 15 (IL-15) was identified in α2-gliadin [1]. Only three epitopes recognised by HLA-DQ8 were identified; one in α-gliadins, another one in γ-gliadins, and the third one in glutenins. Moreover, HLA-DQ8-mediated CD does not seem to be dependent on deamidation by tTG2 [4].

Molecular markers of CD include serum antibodies against gluten-derived peptides (IgE, IgG); IgA class autoantibodies against tTG2 (tTG2-targeted IgA class autoantibodies), γIFN, interleukins IL-15, IL-21. Histological symptoms of CD include damage to lamina propria (mucosa) in duodenum leading to villous atrophy/atrophia of villi.

It is estimated that the amount of people suffering from gluten intolerance can reach up to 1% in Western countries population. The only live-long efficient treatment of celiac disease lies in consummation of gluten-free diet (GFD) which is a serious problem in Western countries due

to ubiqity of gluten as an additive in food industry. Moreover, contamination of gluten-free foods with gluten represents another important problem. Oats can represent a promising alternative to foods prepared from wheat (barley, rye) since the majority of people suffering from CD can tolerate oats without any clinical symptoms. Currently, oats is included into gluten-free ingredients, i.e., those ingredients which do not exceed the threshold of 20 ppm gluten, by European Commission Regulation 41/2009 [5] although its safety for celiac patients is still a matter of debate. In contrast, oat is not considered „gluten-free food" in Australia and New Zealand [6]. Recent studies have indicated significant genetic variability in oats with respect to oat avenins potential immunoreactivity and induction of clinical symptoms characteristic for CD. Thus, there is urgent need to characterise oat genetic variability in avenins molecular structure and potential immunoreactivity with respect to CD clinical symptoms.

The aim of the present review was to summarise recent knowledge on oats avenins molecular structure, genetic variability and potential immunoreactivity with respect to CD. Results of clinical studies aimed at determining the effects of oats on key steps in induction of CD symptoms are summarised. Currently, it is necessary to explore oat genetic variability with respect to avenin molecular structure and their potential immunoreactivity. Recommendations for oat breeders with respect to desired avenin structure inducing no (minimum) immunoractivity should be formulated. The knowledge of oat genetic variability with respect to their potential immunoreactivity will enable oat breeders to select suitable genotypes for their breeding programs aimed at gaining novel oat cultivars with no (minimum) CD immunoreactivity. The knowledge on the relationship between avenin molecular structure and their potential immunoreactivity opens up new ways for genetic modifications of avenins sequences.

Avenins molecular structure and potential immunoreactivity with respect to celiac disease

Oats (*Avena sativa* L.) belongs to gramineaceous group called Avenae which is related but distinct from Triticeae group including wheat, barley, and rye. In oats, prolamin proteins are called avenins. In comparison to Triticeae prolamins reaching 6-8% of kernel weight, avenins represent only ca 1.2-2% of oat kernel weight [2;7]. Sequence analysis of avenin protein sequences revealed three avenin groups termed avenins A, B, and C, respectively (Real et al. 2012). Avenins B and C are homologous to wheat alcohol-soluble S-rich α- and γ-gliadins, barley B hordeins and rye γ-secalins, respectively, while avenins A are alcohol-insoluble proteins homologous to wheat LMW-glutenins which thus means that they form oligomers which is enabled by disulfide bridges between cysteine residues. However, A-class avenins can be solubilised by using reduction agents indicating that their alcohol insolubility is underlied by intermolecular disulfide bonds leading to oligomeric structures. Oats does not contain S-poor prolamins with large repetitive regions rich in proline and glutamine. Avenin sequences belonging to groups B and C contain eight cysteine residues while avenins of group A have nine cysteine residues in their molecules [2]. However, in comparison to wheat prolamins, avenins can form only intermolecular disulfide bonds [1]. Analysis of protein sequences revealed that oat avenins reveal analogous molecular structure to other prolamins containing A, B and C conserved regions interspersed by repetitive regions and variant regions, but they contain relatively less proline (P) and glutamine (Q; joint content of P+Q up to 30% of total

amino acid content) in their protein sequences than compared to other prolamins, especially wheat α- and γ-gliadins (joint content of P+Q up to 50-60% of total amino acid content; data according to Real et al. 2012). However, unlike Triticeae prolamins which contain one single long P+Q rich repetitive region, avenins contain two shorter P+Q rich repetitive regions resulting in a lower total P+Q content with respect to Triticeae prolamins [1;8]. Thus, it can be expected that avenins are more easily hydrolyzable by duodenal enzymes than other prolamins. Moreover, it is becoming evident that different oat genotypes differ in their avenins composition and that some oat genotypes could lack some avenin structural types. In their study aimed at an analysis of avenin proteins in selected Spanish oat lines, Real et al. [2] identified avenins belonging to all A, B and C classes in all lines except for line OP722 which lacks avenins belonging to class A.

Expression pattern of avenin proteins during oat kernel development was also studied. Chesnut et al. [9] detected avenin mRNAs and proteins between 4 and 6 days after anthesis (DPA) with a maximum at 8 DPA while Real et al. [2] were able to detect avenin proteins at 8 DPA with a maximum expression level between 20 to 28 DPA. Greater expression levels were found for α- and γ-gliadin-like avenins of groups B and C than for LMW-GS (low-molecular-weight glutenins) avenins of group A.

Large genotypic diversity was found in avenin sequences. Comino et al. [10] studied avenin proteins, their gluten peptides and their potential stimulatory effects on DC in a set of oat cultivars from Spanish and Australian commercial resources. They observed significant polymorphism patterns in avenin proteins separated on 1D SDS-PAGE gels which was even greater than in globulin fraction. The large differences in avenin protein sequences (the size of avenin proteins ranged between less than 20 up to 50 kDa on 1D gels) were also reflected at the level of their hydrolysis products, i.e., gluten peptides. With respect to wheat gliadins producing long 33-mer proline- and glutamine-rich peptide inducing immune response, oat avenins were found to yield a series of shorter peptides (6, 10, 14, and 27 AA) which are easier to be cleaved or internalised to endosomes in dendritic cells (DCs) than larger peptides from wheat gliadins [10]. The short 6 AA avenin-derived gluten peptides did not trigger any DC stimulatory effects. Only the 27 AA avenin-derived peptides EF27 and QM27 triggered T-cell proliferative response similar to wheat gliadin-derived 33-mer peptide. However, a different way of endosomal internalization of shorter (6 AA) and intermediate (10 and 14 AA) peptides was proposed with respect to the larger ones. As a result, these peptides do not induce T-cell response leading to development of CD molecular symptoms such as T-cell proliferation, γIFN release, tTG2 autoantibodies production, etc [10;11].

Antibodies used for gluten peptides detection in oats include the two major kinds of anti-gluten antibodies R5 moAb and G12 moAb, respectively. The R5 mAb antibody binds to epitope variants QQPFP, QQQFP, LQPFP, and QLPFP, and, to a lesser extent, to QLPTF, QQSFP, QQTFP, PQPFP, and QQPYP, and which is mostly used for detection of gluten in wheat, barley and rye [12]. Another kind of anti-gluten antibody represents G12 mAb which binds to epitopes QPQLPY, QPQQPY, and QPQLPF which reveals limited cross-reactivity with avenins, and moAb 401.21 also referred to as Skerritt antibody which binds to PQPQPFPQE and PQQPPFPEE epitopes [13]. A brief overview of studies aimed at gluten detection in oat varieties using specific antibodies is given in Table 1.

Table 1. Oat varieties revealing enhanced gluten levels (above 20 ppm) detected by antibodies raised against gluten peptides.

Variety	Gluten level (ppm)	Antibody type (kit)	Reference
Dakar (husked)	80	R5-based ELISA (RIDASCREEN Gliadin kit)	[14]
Expander	20.91		
HJA76037N	20.59		
Kynon	19.29		
BG2	38.4		
BG4	41		
Nave (naked)	75.5		
Brazilian Check	195 (R5), 2568 (Skerritt)	R5-based sandwich ELISA; Skerritt S-ELISA	[15]
Kanota	99 (R5), 238 (Skerritt)		
Curt	50 (R5), 238 (Skerritt)		
OM719	1340	G12 moAb based ELISA	[11]
OH727	344		
OE717	Positive response to G12 moAb on immunoblot Immunoreactive peptides QL6, QQ6, PV10, QL14, EF27, QM27	G12 moAb	[10]
Nave (naked)	A positive reaction to TEER of T84 cell monolayer	Rabbit anti-wheat gliadin polyclonal antibody; R5-based ELISA RIDASCREEN	[18]

Immunoreactive peptides interacting with anti-33mer monoclonal antibody moG12 raised against 33mer gluten peptide from wheat α2-gliadin as detected in oat avenins by Comino et al. [10]:

Immunoreactive peptides derived from gliadin-like avenins (B- and C-type avenins): QL6 (QPQLQL), QQ6 (QPQLQQ), PV10 (PYPEQQEPFV), EF27 (EQYQPYPEQQEPFVQQQPPFVQQEQPF)

Immunoreactive peptides derived from glutenin-like avenins (A-type avenins):

QL14 (QQPFMQQQPFMQPL), QM27 (QYQPYPEQQPFMQQQQPFMQPLLQQQM).

Similarly, Ballabio et al.[14] found out differential response of 36 oat cultivars to anti-gliadin protein kit using RIDASCREEN ELISA test based on R5 moAb. The majority of studied oat cultivars revealed levels of gluten-like proteins well below the limit of 20 ppm although some varieties such as Expander, HJA76037N, Kynon and BG2 revealed concentration of gluten-like proteins very close to 20 ppm, and a few oat varieties such as commercial husked cv. Dakar, experimental naked genotype BG3, naked cultivar Nave and genotype BG4 significantly exceeded the 20 ppm limit of gluten proteins considered for gluten-free products, some of them (BG3, Dakar) even exceeding the limit of 80 ppm for the RIDASCREEN Gliadin ELISA kit.

Similarly, Benoit et al. [15] tested nineteen oat varieties given their response to R5 moAb antibody assay; R5 epitope LQPFP is present in highly immunogenic α-gliadin 33mer sequence. Most of the 19 oat cultivars tested revealed a negative response to R5 assay; however, three of them including Brazilian Check, Kanota, and Curt, revealed a positive response to R5 assay indicating presence of immunogenic peptides. Moreover, the three oat cultivars also revealed a positive response to Skerritt antibody which revaled even higher gluten levels when compared with R5 antibody (Table 1).

Arentz-Hansen et al. [16] studied T cell reactivity to synthetic avenin-derived peptides and found recognition by two T-cell lines for peptide 1490 with the following sequence „SEQYQPYPEQQEPFVQQQQ" which was dependent on peptide deamidation by tTG2. A 9-mer core peptide Av-α9A with sequence PYPEQEEPF arising from deamidation of glutamine to glutamic acid (E) at 6th position and revealing sequence homology to wheat DQ2-α-I gliadin epitope PFPQPELPY was predicted to be recognised as an epitope for HLA-DQ2 receptors on the surface of dendritic cells.

Oat genetic variability leads to a variability of avenin-derived peptides which can potentially induce CD response. A series of HLA-DQ2/8 interacting avenin-derived peptides were characterised; however, only longer peptides can induce inflammatory response similar to those induced by wheat gliadin 33-mer, i.e., T-cells activation, production of serum IgA anti-avenin and anti-tTG2 antibodies, enhanced levels of γIFN. It is becoming evident that besides sequence, conformation of gluten peptides plays an important role in determining peptide immunoreactivity. Longer gluten peptides acquire β-sheet conformation [10;17].

Besides qualitative aspects, i.e., molecular structure and sequence of prolamin molecules, and the length and sequence of gluten peptides, respectively, recent studies also indicate a quantitative aspect of gluten immunoreactivity, i.e., that the potential immunoreactivity of gluten peptides increases with their concentration. Recently, it was found out that the human body immune response is enhanced with an increasing concentration of gliadin 33-mer due to a formation of supramolecular oligomeric rod-like structures of polyproline II structure based on oligomerization of type II β-sheets formed by monomeric 33-mers [17].

Clinical studies on the effects of oats on CD clinical symptoms

Clinical studies aimed at the effects of oat consumption by persons suffering from CD revealed contradictory results with respect to the effect of oat-containing diet on CD symptoms.

Silano et al. [18] investigated the potential of different oat varieties to elicit tTG2-derived events in some in vitro models of CD. The following early gluten-induced TG2-dependent events were studied: K562(S) cells agglutination, transepithelial electrical resistance of T84-cell monolayers, intracellular levels of TG2 and phosphorylated form of protein 42-44 in T84 cells. The tests revealed that oat cultivar Nave elicited these events whereas Irina and Potenza varieties did not which correlated with the extent of pepsin-mediated proteolysis of the prolamin fraction of the cultivars. In addition, the ability of a cultivar to induce the above-listed events correlated with avenin electrophoretic pattern and their reactivity to anti-gliadin polyclonal antibodies.

Koskinen et al. [19] studied the effect of 2-year-long diet containing oats on 23 children with CD with respect to their levels of jejunal mucosal tTG2-targeted IgA-class autoantibodies deposits and found no significant change in the level of anti-tTG2 autoantibodies in children with CD diagnosis. Thus, they concluded that consumption of oats does not induce anti-tTG2 autoantibody production at jejunal mucosal level in children with CD.

Comino et al. [11] found a direct relationship between avenin reactivity to monoclonal AbG12 antibody raised against wheat α-2 gliadin-derived 33-mer (p57-89) and their potential immunotoxicity determined as peripheral blood mononuclear T cell proliferation and IFNγ release.

Kaukinen et al. [20] found no CD symptoms regarding the levels of small-bowel mucosal CD3+, αβ+ and γσ+ intraepithelial lymphocytes, small-bowel mucosal villous damage, inflammation or other gastrointestinal CD symptoms in a group of adult CD patients consuming a median of 20 g of oats per day for up to eight years whereas the consumed oat products come from general stores. Moreover, oats daily cosumption had beneficial effects since it provided enhanced fiber intake to CD patients consuming oats with respect to those who did not consume oats. In contrast, Lundin et al. [21] found enhanced levels of IFNγ mRNA after a challenge including 50 g of oats per day for 12 weeks in 19 adult CD patients who previously used gluten-free diet (GFD) only. One of the patients developed partial villous atrophy after a challenge.

Hardy et al. [7] found T cell reactivity to 20mers encompassing 9mer sequences closely related to avenin-derived epitopes DQ2.5-ave-1a (PYPEQEEPF where E results from deamidation of Q39 in several native avenin sequences) and DQ2.5-ave-1b (PYPEQEQPF, deamidation Q19 to E). Further immunogenic sequences include 9mer epitopes derived from partially deamidated avenin peptide QYQPYPEQEQPILQQQ (deamidation of Q34 to E) that included a 9mer amino-acid sequence differing from DQ2.5-ave-1b by substitution of isoleucine for phenylalanine at position 9. Moreover, they reported a crossreactivity between hordein-derived epitope DQ2.5-hor-3b and avenin epitopes DQ2.5-ave-1b and DQ2.5-ave-1c, respectively. Cross-reactive T cells specific for DQ2.5-hor-3b and avenin epitopes DQ2.5-ave-1b and DQ2.5-ave-1c are commonly mobilized in HLA-DQ2.5+ CD patients when they eat barley, and also oats, but less consistently and at lower frequencies than in barley. It could be concluded that hordein-derived peptides functioned as „priming" means, i.e., immunogenic response to avenin-derived peptides was induced by hordein-derived peptides since avenin peptides

themselves had reduced binding stability on HLA-DQ2.5 as compared to homologous hordein-derived peptides. The researchers thus demostrated that food antigen cross-reactivity has significant immunological relevance in vivo which opens the possibility that other peptides may induce pathogenic response in CD patients under some circumstances.

These contradictory results obtained by different clinical studies probably reflect genetic variability with respect to gluten content and its immunoreactivity in oats used in food industry. Variability in avenin composition including variability in avenin-derived gluten peptides and their immunoreactivity to anti-gliadin antibodies was found [10;18]. Some recent studies revealed differential gluten peptides with differential stimulatory capacity on DC which indicates that people with CD who want to include oats in their diet should pay attention to oat genetic resources used for food preparation [10]. It thus arises a challenge for oat breeders to select oat genetic materials with no (minimum) CD reactivity without any negative effects on other oat qualities. Some celiac patients can contain gluten-reactive (or avenin-reactive) intestinal T cells without any clinical symptoms (mucosal inflammation) due to remission.

However, breeding for CD-safe oat varieties with reduced gluten content can bring several obstacles due to the fact that avenin genes are distributed in the whole oat genome including a vicinity of several resistance genes. For example, Satheeskumar et al. [22] detected three avenin loci in the vicinity of *Pc68* gene conferring resistance to crown rust caused by *Puccinia coronata*.

Lerner [23] postulated the major problems associated with oat consumption in gluten-free diet (GFD) which include genetic variability in avenin-derived gluten peptides among different oat varieties as well as contamination of oat products by other gluten-containing cereals such as wheat, barley and rye resulting in exceeding the gluten limit of 20 ppm.

Regarding the potential breeding of gluten-safe oat varieties, it is necessary to describe the variability of avenin alleles with respect to the potential gluten peptides. In the next step, it will be necessary to exclude potential unsuitable avenin alleles from promising oat materials. Recently, a boom of gene editing techniques including CRISPR/Cas, TALENs, and others (reviewed in [24]) could facilitate the gain of prospective oat materials cumulating only suitable avenin alleles.

Conclusions and future perspectives

Generally, oat avenins represent a relatively lower portion of grain storage proteins when compared to wheat gliadins. Moreover, in comparison to wheat gliadins, oat avenins contain lower amounts of proline and glutamine residues resulting in shorter gluten peptides which can undergo endosomal internalisation in dendritic cells (DCs) without any T-cell-mediated immune response. However, oat avenins reveal large genetic variability resulting in a variety of gluten peptides with differential immunoreactivity in human gastrointestinal tract. The majority of avenin-derived gluten peptides tested are relatively short and do not induce immunogenic response; however, a few larger avenin-derived peptides revealing immunogenic reactivity were also reported. Based on the results of recent studies, it is becoming evident that some oat varieties accumulate avenins yielding immunoreactive peptides while many other oat varieties seem to be safe for CD patients. Given the genotypic variability in avenin-derived peptide sequences and their immunoreactivity, urgent need arises to test the individual oat

varieties used for preparation of oat-derived foods for GFD. Therefore, the following aims for the future research should be outlined:

1/ Clinical tests: The aim for clinical researchers (immunologists) is to describe clinical symptoms induced by various avenin-derived gluten peptides on CD patients in order to identify immnuoreactive peptides.

2/ Oat breeding: The aim for oat breeders is to select those oat genotypes accumulating avenins providing only short gluten peptides with no or minimum immune response in CD patients which would be safe for people suffering from CD. The basis for achievements of these aims lies in detailed description of genetic variability in avenin molecular structure among oat cultivars. Oat breeding can thus lead to development of CD-safe oat varieties with well-characterised avenin composition.

3/ Oat food processing: Currently, technologies of food processing based on dough fermentation using bacterial and fungal proteases can significantly decrease gluten amounts in gluten-containing foods such as wheat dough. For example, Rizzello et al. [25] demonstrated efficient reduction of gluten contents up to 12 ppm associated with hydrolysis of albumins, globulins, glutenins as well as 33mer peptide in wheatdough fermented by Lactobacillus (*L. brevis, L. sanfranciscensis, L. hilgardii*) and Aspergilus (*A. niger, A. oryzae*) proteases.

In conclusion, given the genetic variability in avenin protein sequences and their immunoreactivity in CD patients, it is necessary to carefully select and breed new oat varieties with minimum CD reactivity in order to include oats into GFD. To achieve this aim, characterization of variability and immunoreactivity of avenin-derived gluten peptides is a necessary prerequisite. In the next step, breeding programmes aimed at breeding novel oat varieties encoding only those avenin alleles providing gluten peptides with no (minimum) immunoreactivity should result in production of CD-safe oat foods suitable for GFD. Companies producing oat foods suitable for persons suffering from CD have to pay attention to the origin (genotype) of oats used for food production. An alternative way can lie in gluten reduction during food processing via bacterial and fungal fermentation. Considering all these aspects of gluten reduction in oats, CD safe oat products suitable for celiacs is a matter of control.

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