Journal of Chemical Health Risks

www.jchr.org

Review of Recent Advances in Treatment of Celiac Disease Using Enzymatic Gluten Degradation

Mansooreh Hooshiyar[1], Gholam Hossein Ebrahimi Pour[1], Mohammad Rostami-Nejad[*2], Fahimeh Sadat gholam-Mostafaei[2], Kaveh Baghaei[2], Alireza Emadi[3], Vahid Khalaj[*4]

[1] *Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran*

[2]*Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[3] *Deputy of Research and Technology, Semnan University of Medical Sciences, Semnan, Iran*

[4] *Fungal Biotechnology Group, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran*

(Received: 22 August 2020 Accepted: 17 April 2021)

KEYWORDS

Celiac disease;
Enzymatic gluten degradation,
Aspergillus niger;
Prolyl endopeptidase;
Treatment;
Glutenase;
Wheat;
Enzyme therapy

ABSTRACT: Celiac disease (CD), a chronic inflammatory disorder, is triggered by the ingestion of gluten peptide. Wheat gluten contains gliadins and glutenins fractions, where gliadin peptides are the main cause of CD and nonceliac gluten sensitivity development. Keeping a strict gluten-free diet is the only effective treatment for CD. In recent years, lactic acid bacterial and fungal prolyl endopeptidases (PEP) have been proposed as the sources of proteolytic enzymes for the complete elimination of gluten peptides, and have also proved as a potential common therapeutic agent for CD treatment. Considering these indications, a special focus was devoted to AN-PEP-secreted PEP. Herein, we review the proteolytic enzymes produced by microorganisms, especially by the fungal strain, *Aspergillus niger* (AN), and discuss its beneficial properties against toxic effects of α-gliadin digestion in affected patients. The present review reveals the importance of proteolytic proteases in industrial applications; from an economic perspective, AN-PEP protease is an appropriate choice for making high-quality gluten-free products.

Abbreviations

AN, *Aspergillus niger*; AO, *Aspergillus flavus* var. *oryzae*; CD, celiac disease; FM, *Flavobacterium meningosepticum*; GFD, gluten-free diet; LAB, lactic acid bacteria; MX, *Myxococcus xanthus*; RM, *Rothia mucilaginosa*; SC, *Sphingomonas capsulata*; TG2, human tissue transglutaminase; GLUFR, Friendly Gluten; ssdf, special sourdough-fermented;

INTRODUCTION

Celiac disease (CD) is a multifactorial and chronic inflammatory disorder with global prevalence of about 1% [1]. This disorder occurs in genetically susceptible cases and is mostly linked to the ingestion of gluten from the wheat, barley, and rye. Glutenins and gliadins are core factors affecting the CD development and non-celiac gluten sensitivity. Evidence has disclosed that the susceptibility to CD arises from the intestinal damage by interactions between specific gliadin oligopeptides and the human leukocyte antigen (HLA)-DQ2 (or DQ8) molecules [2], as well as macrophages, dendritic cells, and B-cells

*Corresponding author: m.rostamii@gmail.com ; v_khalaj@yahoo.com (M. Rostami-Nejad; Vahid Khalaj)
DOI: 10.22034/jchr.2021.1907514.1167

expressing these HLA class II molecules [3]. These cells are essential components of antigen-presenting cells and induce proliferation of T lymphocytes in the subepithelial layers [4].

The most frequent intestinal and extra-intestinal symptoms of CD include diarrhea, constipation, anemia, infertility, liver transaminase elevation, weight loss, osteoporosis, lymphoma, and carcinoma (Figure 1). For diagnosing CD, serological evaluations and antibody testing such as antibodies against deamidated gliadin peptides (ADPG), IgA anti-TG2, endomysial antibody (EMA) are required in addition to endoscopy and small intestine biopsies [5].

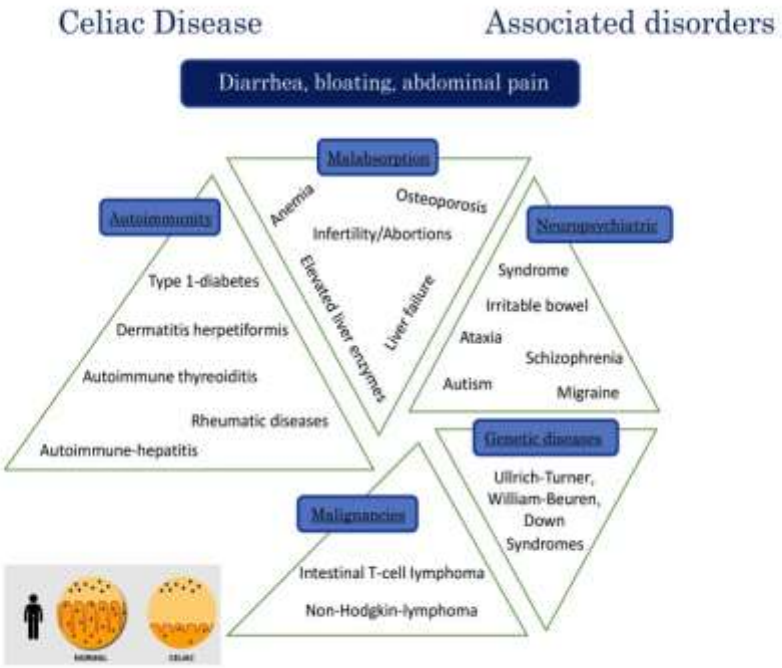

Figure 1. Diseases and clinical symptoms associated with CD.

The main treatment for CD is the lifelong adherence to a gluten-free diet (GFD), which often limits social activities and nutritional variety. It is also expensive and difficult to preserve in many countries. Owing to the economic pressure, nutrition deficiency in gluten-free products, unpalatable taste, weak functional features, and unfavorable textural and sensory properties, the production of high-quality gluten-free products is challenging [6-8]. Thus, a strict, long-lasting GFD can ameliorate the clinical symptoms in CD patients. Accordingly, alternative or adjunctive treatments are desired and necessary. To treat this disorder, researchers have proposed various strategies that may be promising in the near future [9].

The present survey reviews a possible enzyme therapy strategy for the treatment of CD, for which the only current therapeutic option is strict exclusion of gluten-containing foods. Also, the role of hydrolyze immunogenic peptides such as enzymatic hydrolysis with fungal peptidases e.g. AN-PEP has been discussed [10]. The main aim of the present review is to investigate the proteolytic enzymes produced by microorganisms especially *Aspergillus niger* and its effect on toxic a-gliadin digestion product, namely the 33-mer.

Search method

The literature search was restricted to English language, included all articles published up to November 2020 using data recorded in the PubMed, Scopus, and google scholar. Combinations of the following terms were used in the search strategy: celiac disease; enzymatic gluten

degradation, *Aspergillus niger* prolyl endopeptidase, treatment; glutenase; wheat; enzyme therapy. The eligible articles were used, and their reference lists were further checked for potentially additional articles. The duplicated references were found and removed by Mendeley software.

Wheat gluten

According to Osborne classification, glutenin and prolamin are two major classes of wheat protein [11]. Combination of glutenin (a glutelin) and gliadin (a prolamin) is defined

as gluten. The most substantial CD-immunogenic sequence within gluten is the 33-mer peptide from α2-gliadin (Figure 2) [12]. Today, wheat is extensively consumed due to the pleasant taste and unmatched protein content, and this widespread use can develop CD in various parts of the world.

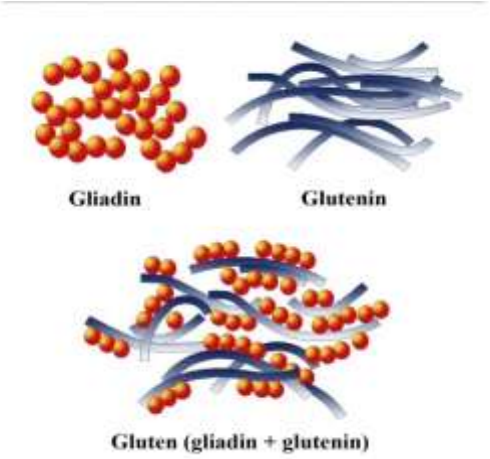

Figure 2. Structure of gluten (gliadins + glutenins)

Therapeutic approaches for CD

As GFD is a lifelong challenge for CD patients, to treat and prevent this disorder, researchers have conducted numerous studies to reveal the potential of nutritional, immunomodulatory, and biochemical strategies and have offered alternative solutions [9, 11]. Different approaches such as prevention of downstream immune activation after gluten exposure, tissue transglutaminase-2 inhibition in intestinal mucosa, prevention of gliadin epitopes uptake , intraluminal sequestration of gluten immunogenic epitopes, reduction of immunogenic epitopes in gluten, oral enzyme therapy, low FODMAP diet, probiotics, and finally vaccines have been proposed in various studies [11]. Enzyme therapy is one of the most successful approaches introduced as an attractive and a promising option for curing CD patients (Figure 3)[9,15].

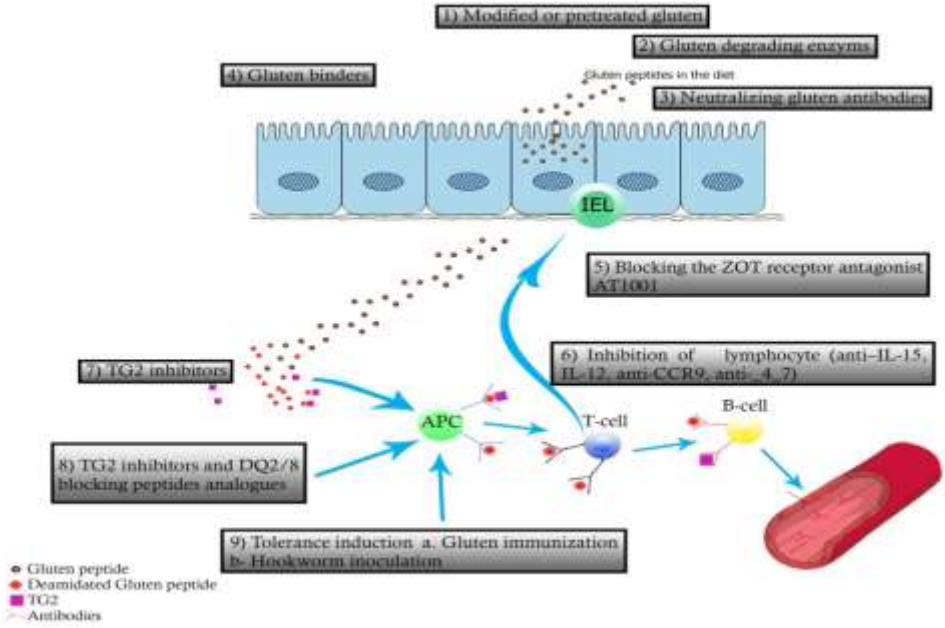

Figure 3. Novel therapeutic approaches for celiac disease .

Enzyme therapy was basically suggested to make modified gluten products, which can be tolerated by celiac-susceptible individuals [16]. This strategy has focused on applying prolyl endopeptidase (PEP), as a therapeutic option, with optimum activity at a wide pH and temperature ranges. Enzyme therapy with PEP benefits from maintaining nutritional value and safety. The use of bacterial proteases in baking processes and gluten modification is recognized as a therapeutic method for treating CD. The major realistic aim of enzyme therapy is "neutralization" of the low amounts of gluten (e.g. <20 mg/kg) to protect patients from minor unintentional or unavoidable gluten ingestion [17]. In the following, we discuss novel therapies tested by in vitro or in vivo models of CD and PEPs, which may be promising in the near future.

Recently, lactic acid bacterial and fungal proteases have been used as sources of proteolytic enzymes for complete degradation of gluten peptides to less than 10 ppm during bread and pasta processing or drug preparations [18]. Combination of *lactobacilli* and fungal proteases has proved to be highly efficient in eliminating gluten toxicity during wheat bread processing. This approach is a novel perspective for the treatment of CD [17, 19-21].

In the late 1950s, PEP enzymes were used to treat CD. Other enzymatic approaches that have been investigated were based on fungal peptidases for gluten modifications [22]. The *Aspergillus niger* (AN) produces protease enzymes to eliminate the immunogenicity of gluten. In this regard, AN-secreted PEPs have come under focus as they have potential of industrial usage [23].

Prolyl endopeptidases (PEPs)

For the first time in 1998, the basic structure of PEPs was identified [24]. PEPs are enzymes hydrolyzing substrates on the carboxyl site of proline residues in a peptide (or ester). These enzymes are a member of the serine protease subfamily and a significant target for the treatment of numerous diseases, including CD. As an enzyme, PEP family preferentially cleaves peptide (P-Q) bonds on the carboxyl side of internal proline residues. PEPs originate from various sources such as plants, bacteria, animals, and viruses [13]. Edible PEPs are used for the incomplete digestion of prolamin proteins to peptides smaller than 30 residues (about 3 kDa), through detoxifying the immunogenic peptide [21, 25]. PEPs are active in gastric environment and have a great effect on the degradation of allergic proteins.

Microorganisms represent an excellent source of PEPs because of their biochemical diversity and genetic susceptibility. Microbial proteases are superior to other plant and animal sources as they possess almost all the properties required for their biotechnological applications [13]. Various different gluten-degrading PEPs identified in bacteria and fungi have proved to be highly effective in digesting gluten.

Alkaline and neutral proteases derived from bacteria, particularly *Bacillus licheniformis*, *B. amyloliquefaciens*, and *B. subtilisare*, are major commercial enzymes [25]. Further, fungal proteases are active over a wide pH range and are stable between pH 2.5 and 6. These proteases have a broad application in industries such as food, tanneries, and pharmaceuticals. Fungal PEPs are known as a potential therapeutic agents for the treatment of CD [22]. Aspergillopepsins from *Aspergillus oryzae* (AO) and AN are food-grade enzymes extensively used in the food industry and food processing. This enzyme has been approved by the United States Food and Drug Administration (FDA) as a safe enzyme [25, 26].

Sources of PEP enzymes for gluten degradation

Bacterial PEPs

Over the past decade, nonhuman proteases, *Flavobacterium meningosepticum* (FM PEPs), have been recommended for gluten detoxification in clinical trials [22]. FM PEPs have been shown to degrade the peptides PQPQLPYPQPQLP at the PQPQLPYP↓QPQLP site[21]. Recently, these enzymes have been isolated from archaeal, eubacterial, and eukaryotic sources, such as the hyperthermophilic archeon *Pyrococcus furiosus*, *Myxococcus xanthus* (MX PEP), and *Sphingomonas capsulata* (SC PEP), due to their ability to cleave a long peptide (33-mer; LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF) at the PQPQLP↓YPQPQLP site [21]. This enzyme is also able to break the α-gliadin 13-mer peptide to PQPQLPYP↓QPQLP and PQPQLP↓YPQPQLP small peptides.

Using genetic engineering methods, the SC PEP encoding gene has been cloned where the recombinant enzyme was found to be effective in gluten detoxification under acidic conditions [27]. However, the combination therapy using both SC and MX FM PEPs have not been used for human consumption yet. Note that the co-administration of these two endopeptidase results in proline-rich gluten peptides digestion under the harsh condition of GI tract. ALV003 (a mixture of SC PEP and EP-B2)_ has been used as a novel oral therapy for CD, through decreasing immunogenic epitopes in the gluten. PEPs from *Rothia mucilaginosa* and *Rothia aeria*, with activity between pH 4 and 10, were highly effective in degrading XPQ↓X- and -LPY↓X peptide bonds [6].

Wheat, oat, and rye gliadins are digested and detoxified efficiently with enzymatic modification using bacterial (*B. licheniformis*, *B. subtilis*, *B. stearothermophilus*, *B. thermoproteolyticus*, and *B. Streptomyces griseus*) extracellular endopeptidase, which are capable of degrading the 33-mer peptides with low molecular weights [27, 28]. The other PEPs that have the ability to degrade α-gliadin peptide have been isolated from *Pseudomonas aeruginosa*, *Bacillus sp.*, *Lactobacillus sp.*, and *Bifidobacterium sp. Alicyclobacillus sendaiensis* (endopeptidase kumamolisin-As), and AO [28]. The hyperthermophilic archeon *Pyrococcus furiosus* produces a thermostable PEP enzyme (A 71-kDa thermophilic PEP) that can cleave azocasein [24]. *Lactobacillus alimentarius 15M*, *L. brevis 14G*, *L. sanfranciscensis 7A*, *L. hilgardii*, and *L. sanfranciscensis* release enzymes that digest the high gluten fractions [23]. It has been reported that all gluten proteins can be hydrolyzed by lactobacilli strains during sourdough fermentation for improving bread quality [29].

Fungal peptidases

Fungal proteases have been utilized as sources of proteolytic enzymes for complete degradation of gluten peptides to less than 10 ppm during bread and pasta processing, as well as drug preparations [19].

Metalloproteinase of *Penicillium citrinum* has cleavage specificity for internal proline residues [24]. Peptidases from *Aspergillus flavus var. oryzae* (AO) cleave NH2-XP↓X- peptide bonds and are capable of hydrolyzing various food proteins [14].

A number of investigations have attempted to address the issue of degradation of gluten and replaced it with other compounds by adding PEP[11]. Fungal peptidases, especially AN or AO, are able to hydrolyze immunogenic peptides to small peptides by a secondary proteolysis via lactic acid bacteria [25]. Another study found similar results where fungal peptidases, including prolyl endopeptidases, AO, and AN can cleave the human digestion-resistant gluten peptides both in vitro and in vivo [10].

AN-PEPs

Aspergillus is a spore-forming genus of filamentous fungi which is naturally found in the air, soil, and fruits.

About 180 species of the *Aspergilli* are known, some of which are harmful to humans or human interests [26, 30-32]. Some species may be pathogenic to humans and animals by invading the living tissue and cause aspergillosis while some may give rise to diseases in onions and ornamental plants, peanuts, and grapes. AN is less likely to create human diseases as compared to other *Aspergillus* species; however, in extremely rare instances, subjects may become ill due to a serious lung disease, aspergillosis. Certain strains of this genus can induce allergic reactions ranging from congestion to bleeding in the lungs. Further, some aspergillus toxins can harm people with weak immune system. AN is one of the most common causes of otomycosis (aspergillosis of the ear canal) and often accompanied with pain and temporary hearing loss [33]. AN fermentation is "generally recognized as safe (GRAS)" by FDA under the Federal Food, Drug, and Cosmetic Act and commonly applied in the pharmaceutical industry [34]

Aspergillus niger, as a powerful enzyme secretor, is widely used in commercial enzymes production. The main proteolytic activities of *AN* is assigned to acidic extracellular proteases [36]. In recent scientific investigations, an extracellular PEP has been found in *AN* (produced by *AN* Van Tieghem strain with a molecular weight of around 66 kDa) which can efficiently cleave proline residues, intact α-gliadins, and γ-gliadins present in wheat gluten (Figure 4). Aspergillopepsin (ASP) is an extracellular proline-specific protease and a member of the family S28 of serine peptidases. Attractive features of this protease are active at pH 2–8 and stable within pH 4–5 and resistant to stomach pepsin [22].

Proline-specific endopeptidase from AN CBS513.88 (EMBL; AX458699) was proposed as a potential therapeutic candidate because of having the ability to efficiently digest proline residues located internally in gluten. Additional details are provided in Table 1 [35].

Table 1. Classification of the AN-PEP

Name	Acid prolyl endopeptidase
Synonyms	Prolyl endoprotease; Proline-specific endoprotease; Proline endopeptidase; Endoprotease; Endo-protease; Protease; AN-PEP; Tolerase G.
IUBMB Enzyme Commission (EC) numbe	The classification of the enzyme according to the IUBMB is as follows: EC 3 Hydrolyases EC 3.4 Acting on peptide bonds (peptidase) EC 3.4.21 Serine endopeptidases EC 3.4.21.xx Acid prolyl endopeptidase AC AX458699;

Enzyme function	Hydrolysis of proteins and peptides in an endo-fashion, with a preference for cleavage of peptide bonds at the carboxyl site of proline residues and to a lesser extent alanine residues
Enzyme substrates	Aspergillus niger expressing multiple copies of the Aspergillus niger acid prolyl endopeptidase gene
Source organism	Aspergillus niger expressing multiple copies of the Aspergillus niger acid prolyl endopeptidase gene.
Amino acid sequence The peptide chain of acid prolyl endopeptidase from Aspergillus niger contains 526 amino acids (Sequence 1581), including a signal sequence of 22 and a pro-sequence of 19 amino acids, which are cleaved off during Secretion of the enzyme.	MRAFSAVAAAALALSWASLAQAARPRLVPKPVSRPASSKSAATTGEA YFEQLLDHHNPEKGTFSQRYWWSTEYWGGPGSPVVLFTPGEVSAD GYEGYLTNETLTGVYAQEIQGAVILIEHRYWGDSSPYEVLNAETLQYL TLDQAILDMTYFAETVKLQFDNSTRSNAQNAPWVMVGGSYSGALTA WTESVAPGTFWAYHATSAPVEAIYDYWQYFYPIQQGMAQNCSKDVS LVAEYVDKIGKNGTAKEQQALKELFGLGAVEHFDDFAAVLPNGPYLW QDNDFATGYSSFFQFCDAVEGVEAGAAVTPGPEGVGLEKALANYAN WFNSTILPDYCASYGYWTDEWSVACFDSYNASSPIYTDTSVGNAVDR QWEWFLCNEPFFYWQDGAPEGTSTIVPRLVSASYWQRQCPLYFPET NGYTYGSAKGKNAATVNSWTGGWDMTRNTTRLIWTNGQYDPWRDS GVSSTFRPGGPLASTANEPVQIIPGGFHCSDLYMADYYANEGVKKVV DNEVKQIKEWVEEYYA
Molecular mass	about 66 kDa by SDS PAGE, due to glycosylation of the protein

In 2005, Edens and coworkers first identified AN-PEP as a bittering agent in beer brewing [36]. The glutenase activity of AN-PEP has been investigated and it has been demonstrated that this enzyme targets the typical proline-rich regions of gluten [11, 14]. AN-PEP from AN is an exopeptidase that releases an N-terminal dipeptide from polypeptides and preferentially cleaves (-P↓X-) peptide bonds. It is also capable of hydrolyzing CD-active peptides, intact α-gliadins, γ-gliadins, and high- and low-molecular-weight glutenin subunits [14].

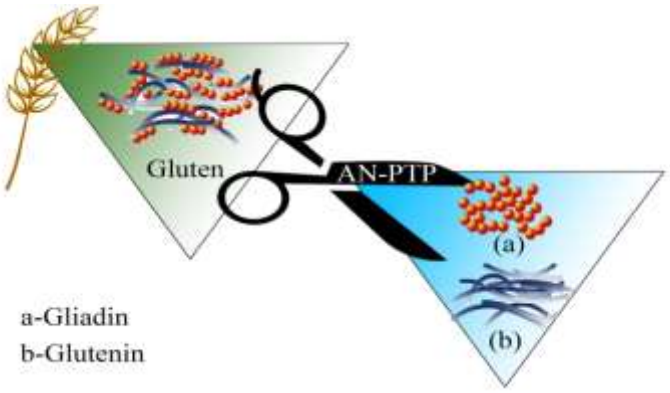

Figure 4. Schematic representation of degradation of the gluten macropolymer with AN-PEP

a. Wheat- and barley-based products: Food-grade proteases, such as the fungal DPPIV (AO) from AO and ASP from AN have the ability to extensively hydrolyze dietary gluten within the wheat bread [37, 38]. AN-PEP is a proline-specific peptidase commercially applied as a beer stabilizer. It is also used to ferment wheat and added to

sourdough flour made with wheat. Wheat- and barley-based products made from this sourdough are called "Friendly Gluten" or GLUFR [11]. In gluten-free bread production, AN-PEP, at pHs between 4.0 and 5.0, can degrade gluten in gluten-containing flours without inactivating the microorganisms in the sourdough [39]. This enzyme can also degrade gluten epitopes present in beer [34] and break down gluten immunoreactivity under special temperatures and pHs, without affecting the sourdough [33]. In bakery products, it can also minimize gluten immunogenicity to a concentration of <20 mg/kg[39]. AN-PEP is originally used under the name Brewers Clarex® (DSM Food Specialties) to prevent chill haze in beer and to degrade gluten in wheat bran as well as bread drink, rye flour, and sourdough [18]. At present, the Italian company Giuliani (Milan, Italy) produces a gluten-free wheat bread called “Giusto Sapori Tradizionali Bontà di Pane”. AN-PEP and LAB peptidases effectively remove gluten to levels below 20 mg/kg. In general, gluten-free bread made from special sourdough-fermented wheat flour offers better preservability [14]. In addition, AN-PEP improves dough functionality, bread immunoreactivity, and quality [23].

***b. AN*-PEP enzyme as drug targets:** AN-PEP is resistant to pepsin and acidic conditions in the stomach of celiac patients and is able to break down gluten in a gluten-sensitive body. Further, it prevents the protein from reaching the small intestine and decreases the side effects of gluten protein [13]. The gluten-degrading activity of this enzyme in a gastrointestinal model resulted in the complete disappearance of CD-active peptides. Promising results of a pilot study have demonstrated AN-PEP efficiency in eliminating CD immunoreactivity. The coadministration of AN-PEP and a slice of bread could accelerate the degradation of gluten in the stomach [40]. Recent data have suggested that AN-PEPs may serve as a drug candidate plus oral supplementation and can be active in degradation of toxic peptides in GI tract . AN-PEP has also been affirmed to be safe for use in food processing, especially for cases with CD, which is in accordance with the legislation of the US Food and Drug Administration[6]. An in vivo study has revealed that AN-PEP is highly efficient in the complete elimination of T-cell stimulatory peptides in gastric aspirates of CD patients [14].

DSM Company (DSM Food Specialties) provided AN-PEP enzyme tablets for degradation of gluten toxic peptides to non-toxic gluten products [41]. Tolerase®G is a commercial form of AN-PEP, which is used as a dietary supplement, is now marketed by DSM Nutritional Products, and can effectually digest α-gliadins in a gastrointestinal model [41]. SpectraZyme® and GlutnGo™ are two known dietary supplements now marketed in the USA [14]. It should be noted that all tablets used to inactivate hidden gluten contaminants in food products are not applied as a replacement for a gluten-free food [6, 24, 31]. There is little doubt that AN-derived PEPs can be considered as key tool in food industry [36]. Further, glutenase of AN has been proposed as an alternative strategy for the treatment of CD. Using AN, the development of enzyme therapy to detoxify wheat gluten proteins is an evolving therapy for CD[11].

CONCLUSIONS

Currently, the only efficient treatment for CD is a strict lifelong gluten-free diet, but patients with CD have a considerable risk of developing nutritional deficiencies. Since all other treatment modalities are only in the preliminary stages of research, enzyme therapy, as an ideal therapeutic agent, allows CD patients to consume products made from wheat flour, without compromising their quality of life. Various strategies have hitherto been developed for enzyme therapy, in order to degrade wheat gliadin and minimize celiac allergic symptoms. So far, only fungal proteases have extensively been investigated and demonstrated to have the potential for helping CD patients achieve their ideal status.

Results of preliminary studies on AN-PEP have revealed that this enzyme, in comparison to other enzymes studied so far, may hold more promise in future. AN-PEP protease is an important tool in enzyme therapy. These enzymes, as drug candidates, have been suggested to accelerate gluten

digestion. Currently, enzyme supplement therapy using AN-PEP has been recognized as a superior therapeutic agent for celiac cases. In the near future, without compromising the quality of life in celiac subjects, only GLUFR seems to be a novel option for CD therapy. AN-PEP glutenases are an appropriate candidate for producing GLUFR products in many industries, including, pasta, feed, bakery, and non-alcoholic beer processing because of maintaining nutritional value and safety. Economically, GLUFR offers a cheaper alternative to GFD. Expression of this treatment protease in recombinant yeast can certainly reduce immunogenic α-gliadin oligopeptides involved in CD. However, further studies on AN-PEPs would be required to fully prove the safety of fermented wheat flour in GLUFR.

ACKNOWLEDGEMENTS

The authors thanks the celiac disease department and Pasteur staffs for their sincere assistance in this project.

Conflict of interests

The authors declared no conflict of interest

REFERENCES

1. Ashtari S., Pourhoseingholi M.A., Rostami K., Aghdaei H.A., Rostami-Nejad M., Busani L., Tavirani M.R., Zali M.R., 2019. Prevalence of gluten-related disorders in Asia-Pacific region: a systematic review. Journal of Gastrointestinal & Liver Diseases. 28 (1), 95-105.

2. Rostami-Nejad M., Hejazi S.H., Peña A.S., Asadzadeh-Aghdaei H., Rostami K., Volta U., Zali M.R., 2018. Contributions of HLA haplotypes, IL8 level and Toxoplasma gondii infection in defining celiac disease's phenotypes. BMC Gastroenterology. 18(1), 66-71.

3. Schuppan D., Gisbert-Schuppan K., 2019. [How to recognize inflammatory wheat sensitivities]. MMW Fortschr Med.162(13):47-52.

4. Asri N., Rostami-Nejad M., Barzegar M., Nikzamir A., Rezaei-Tavirani M., Razzaghi M., Zali M.R., 2020. Suppressive Mechanisms Induced by Tregs in Celiac Disease. Iranian Biomedical Journal. 24(3), 140-147.

5. Taraghikhah N., Ashtari S., Asri N., Shahbazkhani B., Al-Dulaimi D., Rostami-Nejad M., Rezaei-Tavirani M., Razzaghi M.R., Zali M.R., 2020. An updated overview of spectrum of gluten-related disorders: clinical and diagnostic aspects. Bmc Gastroenterology. 20(1), 1-12.

6. Dvorak J., Horn M., 2018. Serine proteases in schistosomes and other trematodes. International Journal for Parasitology. 48(5), 333-344.

7. Jayedi A., Soltani S., Abdolshahi A., Shab-Bidar S., 2020. Healthy and unhealthy dietary patterns and the risk of chronic disease: an umbrella review of meta-analyses of prospective cohort studies. British Journal of Nutrition. 124(11), 1133-1144.

8. Jayedi A., Emadi A., Khan T.A., Abdolshahi A., Shab-Bidar S., 2020. Dietary fiber and survival in women with breast cancer: A dose-response meta-analysis of prospective cohort studies. Nutrition and Cancer. 1-11.

9. Tye-Din J.A., Galipeau H. J., Agardh D., 2018. Celiac disease: a review of current concepts in pathogenesis, prevention, and novel therapies. Frontiers in Pediatrics. 6, 350-60.

10. Wei G., Helmerhorst E. J., Darwish G., Blumenkranz G., Schuppan D., 2020. Gluten degrading enzymes for treatment of celiac disease. Nutrients. 12(7), 2095.

11. Yoosuf S., Makharia G.K., 2019. Evolving therapy for celiac disease. Frontiers in Pediatrics. 7, 193-201.

12. Balakireva A.V., Zamyatnin A.A., 2016. Properties of gluten intolerance: gluten structure, evolution, pathogenicity and detoxification capabilities. Nutrients. 8(10), 644-660.

13. Bascuñán K.A, Vespa M.C, Araya M,. 2017. Celiac disease: understanding the gluten-free diet. Eur J Nutr. 56(2):449-459.

14. Scherf K.A., Wieser H., Koehler P., 2018. Novel approaches for enzymatic gluten degradation to create high-quality gluten-free products. Food Research International. 110, 62-72.

15. Sharma N., Bhatia S., Chunduri V., Kaur S., Sharma S., Kapoor P., Kumari A., Garg M., 2020. Pathogenesis of celiac disease and other gluten related disorders in wheat and strategies for mitigating them. Frontiers in Nutrition. 7, 6-32.

16. Kaukinen K., Lindfors K., 2015. Novel treatments for celiac disease: glutenases and beyond. Digestive Diseases. 33(2), 277-281.

17. Schuppan D., Junker Y., Barisani D., 2009. Celiac disease: from pathogenesis to novel therapies. Gastroenterology. 137(6), 1912-1933.

18. Akeroyd M., van Zandycke S., den Hartog J., Mutsaers J., Edens L., van den Berg M., Christis C., 2016. AN-PEP, proline-specific endopeptidase, degrades all known immunostimulatory gluten peptides in beer made from barley malt. Journal of the American Society of Brewing Chemists. 74 (2), 91-99.

19. Rizzello C.G., Curiel J.A., Nionelli L., Vincentini O., Di Cagno R., Silano M., Gobbetti M., Coda R., 2014. Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate content of gluten. Food Microbiology. 37, 59-68.

20. Eugster P.J., Salamin K., Grouzmann E., Monod M., 2015. Production and characterization of two major Aspergillus oryzae secreted prolyl endopeptidases able to efficiently digest proline-rich peptides of gliadin. Microbiology. 161(12), 2277-2288.

21. Tanner G.J., Colgrave M.L., Howitt C.A., 2014. Gluten, celiac disease, and gluten intolerance and the impact of gluten minimization treatments with prolylendopeptidase on the measurement of gluten in beer. Journal of the American Society of Brewing Chemists. 72(1), 36-50.

22. Stepniak D., Spaenij-Dekking L., Mitea C., Moester M., de Ru A., Baak-Pablo R., van Veelen P., Edens L., Koning F., 2006. Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. American Journal of Physiology-Gastrointestinal and Liver Physiology. 291 (4), G621-G629.

23. Heredia-Sandoval N.G., Valencia-Tapia M.Y., Calderón de la Barca A.M., Islas-Rubio A.R., 2016. Microbial proteases in baked goods: modification of gluten and effects on immunogenicity and product quality. Foods. 5(3), 59-69.

24. Gass J., Khosla C., 2007. Prolyl endopeptidases. Cellular and Molecular Life Sciences. 64(3), 345-355.

25. Badgujar S.B., Mahajan R.T., 2010. Badgujar S.B., 2010. Biological aspects of proteolytic enzymes: a review,2015. J Pharm Res. 3(9),2048-2068

26. Abdolshahi A., Marvdashti L.M., Salehi B., Sharifi-Rad M., Ghobakhloo S., Iriti M., Sharifi-Rad J., 2019. Antifungal activities of coating incorporated with Saccharomyces cerevisiae cell wall mannoprotein on Aspergillus flavus growth and aflatoxin production in pistachio (*Pistacia vera* L.). Journal of Food Safety. 39 (2), 126-31.

27. Ehren J., Govindarajan S., Morón B., Minshull J., Khosla C., 2008. Protein engineering of improved prolyl endopeptidases for celiac sprue therapy. Protein Engineering, Design & Selection. 21(12), 699-707.

28. Siegel M., Bethune M.T., Gass J., Ehren J., Xia J., Johannsen A., Stuge T.B., Gray G.M., Lee P.P., Khosla C., 2006. Rational design of combination enzyme therapy for celiac sprue. Chemistry & Biology. 13(6), 649-658.

29. Rizzello C.G., De Angelis M., Di Cagno R., Camarca A., Silano M., Losito I., De Vincenzi M., De Bari M.D., Palmisano F., Maurano F., 2007. Highly efficient gluten degradation by lactobacilli and fungal proteases during food processing: new perspectives for celiac disease. Applied and Environmental Microbiology. 73(14), 4499-4507.

30. Etxebeste O., Espeso E.A.,2020. Aspergillus nidulans in the post-genomic era: a top-model filamentous fungus for the study of signaling and homeostasis mechanisms. Int Microbiol.23(1):5-22.

31. Gänzle M.G., Loponen J., Gobbetti M., 2008. Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. Trends in Food Science & Technology. 19(10), 513-521.

32. Abdolshahi A., Tabatabaiee Yazdi F., Shabani A.A., Mortazavi S.A., Mohammadi Nafchi A., 2016. Antifungal properties of gelatin-based coating containing mannoprotein from Saccharomyces Cerevisiae on Aspergillus flavus growth in pistachio. Journal of Mazandaran University of Medical Sciences. 26(139), 93-102.

33. H Walter T., Wieser H., Koehler P., 2015. Degradation of gluten in rye sourdough products by means of a proline-specific peptidase. Eur Food Res Technol. 240(3):517-524.

34. van Munster J.M., van der Kaaij R.M., Dijkhuizen L., van der Maarel M.J.E.C., 2012. Biochemical characterization of Aspergillus niger CfcI, a glycoside hydrolase family 18 chitinase that releases monomers during substrate hydrolysis. 158(Pt 8), 2168-2179.

35. Edens L., Dekker P., Van Der Hoeven R., Deen F., de Roos A., Floris R., 2005. Extracellular prolyl endoprotease from Aspergillus niger and its use in the debittering of protein hydrolysates. Journal of Agricultural and Food Chemistry. 53(20), 7950-7957.

36. Gaynor and Nutrition. Summary of Information Supporting the Generally Recognized as Safe (GRAS) Status of Acid ProlylEndopeptidase Produced by a Genetically Engineered Strainof Aspergillus niger 2019. (avalibale at: "G h RAS Notice (GRN) No. 832 https://www.fda.gov/food/generally-recognized-safe-gras/gras-notice-inventory).

37. Montserrat V., Bruins M.J., Edens L., Koning F., 2015. Influence of dietary components on Aspergillus niger prolyl endoprotease mediated gluten degradation. Food Chemistry. 174, 440-445.

38. Ehren J., Morón B., Martin E., Bethune M.T., Gray G.M., Khosla C., 2009. A food-grade enzyme preparation with modest gluten detoxification properties. PloS One. 4(7), e6313-323.

39. Walter T., Wieser H., Koehler P., 2015. Degradation of gluten in rye sourdough products by means of a proline-specific peptidase. European Food Research and Technology. 240(3), 517-524.

40. Mitea C., Havenaar R., Drijfhout J.W., Edens L., Dekking L., Koning F., 2008. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. Gut. 57(1), 25-32.

41. König J., Holster S., Bruins M. J., Brummer R.J., 2017. Randomized clinical trial: Effective gluten degradation by Aspergillus niger-derived enzyme in a complex meal setting. Scientific Reports. 7(1), 13100-110.