Food Control 80 (2017) 401–410

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Development and in-house validation of a competitive ELISA for the quantitative detection of gluten in food

Nermin Sajic [a, *], Michalina Oplatowska-Stachowiak [a], Lucia Streppel [a], Jan-Wouter Drijfhout [b], Martin Salden [a], Frits Koning [b]

[a] EuroProxima B.V., Beijerinckweg 18, 6827 BN, Arnhem, The Netherlands
[b] Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 30 January 2017
Received in revised form
4 May 2017
Accepted 15 May 2017
Available online 15 May 2017

Keywords:
Gluten
Gliadin
Enzyme-linked immunosorbent assay
DQ2.5-glia-α3
Food

ABSTRACT

Wheat gluten contains peptide sequences, which activate specific T cells causing a chronic inflammation of the small intestine in celiac disease patients. It is well established that next to wheat gluten, the gluten-like proteins in barley and rye are similarly harmful to celiac disease patients whereas oat is generally considered safe. This study focuses on the development of an ELISA method for the detection of native and processed gluten proteins. The developed test utilizes a monoclonal antibody specific for the DQ2.5-glia-α3 T cell epitope present in the α-gliadins, which are part of wheat gluten that triggers celiac disease. The developed competitive ELISA uses a synthetic DQ2.5-glia-α3 peptide standard for calibration. The conversion from the measured DQ2.5-glia-α3 peptide concentration to gliadin content is achieved by using the experimentally determined multiplication factor of 250. The gluten content can be then calculated by multiplying the gliadin concentration by a factor of 2. A simple sample preparation method with 60% ethanol is used to extract the disease-causing proteins from cereals and processed foods. The assay was found to be specific for the detection of gluten from wheat, barley and rye with no cross-reaction with 8 tested oat varieties. The LOD and LOQ for gliadin were calculated based on the results obtained for 60 blank oats samples and they were 2.9 and 3.6 ppm, respectively. The assay could detect as little as 0.01% wheat gluten and gluten-like proteins from rye and barley in oats. The ELISA was also found to be applicable to the analysis of a range of processed food such as sauces, beers, soups and bread. In conclusion, the developed assay is a sensitive, specific and cost-effective tool for screening cereals and processed foods for the presence of harmful wheat gluten and gluten-like proteins from barley and rye.

1. Introduction

Celiac disease (CD) is an immune-mediated gastrointestinal disease. Pro-inflammatory T cells specific for particular gluten peptides bound to the disease-predisposing HLA-DQ2.5 and/or HLA-DQ8 molecules are typically found in patients. Upon consumption of the gluten-containing cereals wheat, barley and rye, complexes between the HLA-DQ molecules and gluten peptides form that trigger T cell responses, leading to inflammation in the small intestine and a variety of symptoms that are typically associated with celiac disease: diarrhea, stomach ache, failure to thrive in children. Gluten is a water-insoluble complex of proteins that are located in the endosperm of grains such as wheat, rye, and barley (Sollid & Jabri, 2013). Based on the difference in solubility of its components, gluten can be subdivided into the alcohol-soluble prolamins and the glutelins which are soluble in alkali or acid solutions (Osborne, 1907, 1924). Furthermore, prolamins and glutelins have different physico-chemical and structural properties depending on the type of grain. Prolamins from wheat, rye and barley are called gliadins, secalins and hordeins, respectively (Shewry & Tatham, 1990) and all of them are known to be harmful or toxic for CD patients. In contrast, the prolamins from oat – avenins – are characterized by lower toxicity and are considered safe for the large majority of patients with CD (Koning, 2015).

Abbreviations: CD, celiac disease; DTT, DL-dithiothreitol; ELISA, enzyme-linked immunosorbent assay; FAPAS, Food Analysis Performance Assessment Scheme; HRP, horseradish peroxidase; IC, inhibitory concentration; LOD, limit of detection; LOQ, limit of quantification; ppm, parts per million; PCR, polymerase chain reaction; PWG, Prolamin Working Group.

* Corresponding author.
E-mail address: nermin.sajic@europroxima.com (N. Sajic).

http://dx.doi.org/10.1016/j.foodcont.2017.05.021
0956-7135/

Scientifically agreed celiac disease relevant T-cell epitopes in gliadins from wheat, secalins from rye and hordeins from barley are not present in avenins from oats (Sollid, Qiao, Anderson, Gianfrani, & Koning, 2012), however small number of patients can be still sensitive to oats (Lundin et al., 2003). Some homologs of celiac disease epitopes with two and three amino acids substitutions have been recently identified in avenins but they are predicted to undergo proteolysis in gastro-intestinal tract (Londono et al., 2013). Several studies have shown that CD patients can safely add oats to their diet (Gilissen, van der Meer, & Smulders, 2016; Pulido et al., 2009) if oat containing products are free from contamination with wheat, rye and barley.

To prevent serious health problems celiac patients should avoid gluten in their diets. Gluten-free food is manufactured by using ingredients that do not contain gluten or by removing gluten from them before use. According to the Commission Implementing Regulation 828/2014 food labeled 'gluten-free' should contain no more than 20 mg/kg of gluten (20 ppm). A 'very low gluten' label can be used for food with less than 100 ppm of gluten. Several techniques are available for the detection of gluten including PCR, mass spectrometry and immunochemical methods, enzyme-linked immunosorbent assay (ELISA) in particular. ELISA is an immunological test which is based on antibodies recognizing the target structure. ELISA is a cost-effective screening method that is characterized by high specificity and sensitivity. Thus far the antibodies R5, Skerrit and G12 have been applied in developing ELISAs for the detection of gluten (Diaz-Amigo & Popping, 2012). The R5 Mendez ELISA is a current method recommended by Codex Alimentarius, 2006 for gliadin detection in food. The R5 antibody was raised against secalin and it detects the pentapeptide QQPFP present in gliadins, secalins and hordeins. A drawback of the sandwich ELISA based on the R5 antibody is that it can only be used for detection of non-hydrolyzed gluten as it requires the presence of at least two epitopes in a protein or peptide. If food processing leads to the formation of smaller gliadin fragments, containing only one epitope, the quantification is no longer accurate. Therefore, a competitive ELISA approach is required to analyze processed food (Mena, Lombardía, Hernando, Méndez, & Albar, 2012).

The Leiden University Medical Center (LUMC), the Netherlands has developed and characterized a range of monoclonal antibodies against gluten-derived T cell immune-stimulatory epitopes (toxic epitopes) involved in CD (Mitea et al., 2008), including an antibody specific for the DQ2.5-glia-α3 epitope. Based on this antibody the Gluten-TEC® ELISA was developed. This is a sensitive competitive assay which detects the well-characterized T cell stimulatory DQ2.5-glia-α3 epitope and homologous sequences present in barley, rye and their crossbred varieties. Importantly, the DQ2.5-glia-α3 epitope is located directly in C-terminal of the immunodominant DQ2.5-glia-α1 and DQ2.5-glia-α2 epitope in the α-gliadins and N-terminal of the immunodomiant DQ8-glia-α1 epitope so that the Gluten-TEC® ELISA reliably predicts the presence of these highly immunogenic epitopes. Like in gliadins, the DQ2.5-glia-α3 homologous sequence in hordeins (Entwistle, 1998) and secalins (NCBI protein database) is present in the N-terminal part of the protein. The Gluten-TEC® ELISA was successfully validated in an interlaboratory study (Mujico et al., 2012) where its performance was found to be comparable to the R5 sandwich ELISA. The Gluten-TEC® ELISA has an antigen-coated assay format with gliadin from wheat coated on a surface of microtiter plate. In this set-up the gliadin immobilized on the surface and gliadin from the samples compete for the binding site of the DQ2.5-glia-α3 specific monoclonal antibody labeled with biotin. In the next step horseradish peroxidase enzyme labeled with streptavidin is added to detect the antibody bound to the gliadin-coated surface. The calibration curve is prepared based on a DQ2.5-glia-α3 peptide standard.

This study presents the development and validation of a simplified ELISA for the detection of gluten in cereals and processed food. This new Gliadin ELISA uses a synthetic DQ2.5-glia-α3 peptide conjugated to HRP. The antibody is coated on the surface of the microtitre plates and gliadin from the sample competes with HRP labeled DQ2.5-glia-α3 peptide for the specific binding sites of the DQ2.5-glia-α3 specific monoclonal antibody. Synthetic DQ2.5-glia-α3 gliadin peptide is also used to prepare a standard curve. The new test uses an experimentally determined multiplication factor that allows direct conversion of measured DQ2.5-glia-α3 gliadin peptide to gliadin concentration in a sample. The performance of the developed assay was tested in a validation study that included a range of cereals and processed food. The developed ELISA offers the possibility of the analysis of both cereals and processed foods with one method. The antibody used has no cross-reaction with oat in contrary to G12 antibody used in other competitive assays. Despite availability of many commercial tests for gluten detection there is still a need for better tests for analysis of gluten in different matrices. In the recent publication by Bruins Slot, Bremer, Van Der Fels-Klerx, & Hamer, 2015, 14 commercial tests for gluten detection were compared. The kits tested were based on 401/21, R5, G12, DQ2.5-glia-α3 specific (called α-20 in the cited publication) and polyclonal antibodies. The authors found that there were still a lot of hurdles in the detection of gluten in food products, despite the availability of a type I method based on R5 antibody recommended by Codex Alimentarius Commission, 2015. The authors found that at the moment of publication there were no test kits available that could accurately detect and quantify the amount of gluten present in difficult food matrices. At the same time the study showed that the Gluten-Tec® ELISA developed by EuroProxima was unable to detect PWG gliadin reference material properly in spiked samples. Therefore, the Gluten-Tec® ELISA had to be improved. In order to meet these demands a new Gliadin ELISA was developed and validated in this study.

2. Materials and methods

2.1. Chemicals and reagents

DL-Dithiothreitol (DTT), Tris base, 1 M HCl and iodoacetamide were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Ethanol 96% p.a. was obtained from VWR (Amsterdam, the Netherlands). For the DTT/extraction two buffers were prepared. Buffer A contained 20 mM DTT, 50 mM Tris base and 60% ethanol in distilled water and its pH was adjusted to 8.0. Buffer B was composed of 110 mM iodoacetamide and 50 mM Tris base in distilled water and its pH was adjusted to 8.0.

2.2. Antibody, peptides and proteins

Synthetic DQ2.5-glia-α3 peptide (sequence PFRPQQPYPQP), its modified structure with an additional N-terminal cysteine residue (sequence CPFRPQQPYPQP) for conjugation with maleimide activated horseradish peroxidase (HRP) and the anti-DQ2.5-glia-α3 monoclonal antibody were obtained from the Leiden University Medical Center, The Netherlands. The PFRPQQPYPQP peptide was used to raise the antibody specific for this epitope. The minimal sequence required for optimal antibody reactivity is a 7 amino acid sequence right in the middle of this 11-mer peptide so flanked by PF at the N-terminus and QP at the C-terminus (Mitea et al., 2008).

The PWG-gliadin reference material was obtained from the Working Group on Prolamin Analysis and Toxicity. The DQ2.5-glia-α3 peptide at concentrations of 5.0, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/ml and the PWG-gliadin at concentrations of 1250, 625, 312, 156, 78, 39 ng/ml were used for the calibration curves in the