Novel and Efficient Techniques for the Detection of gluten traces in complex food matrices

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Abstract

According to Codex the products labeled "gluten-free" could not contain more than 20 mg gluten per kg. Keeping a true gluten-free diet is challenging, This requirement arrays the standard for analytical techniques for gluten detection. In this paper the currently used methods for gluten analysis and new advances are reviewed. At present, the most generally used methods are ELISA-based, but also PCR-based methods have been successfully employ-ed. Proteomics-based methods such as reversed-phase (RP-) or gel permeation (GP-) highperformance liquid chromatography (HPLC) have been commonly used for categorization of cereal proteins. Methods joining mass spectrometry and liquid chromatography (LC-MS/MS) are the most promising non-immunological approaches for accurate quantitation of gluten traces. However, due to its requirement of expensive equipment and expertise it is not widely used for routine analysis. New techniques include immunosensors, aptamers, microarrays, and multianalyte profiling. Despite the facts and challenges of the different approaches, the need for an independent reference method and a generally applicable reference material continue.

Keywords: gluten-free, food, analysis method, Novel methods

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Introduction

Gluten is a general term used to describe a mixture of wheat storage proteins (prolamins and glutenins). People who suffer from celiac disease need to remove this proteins from their diet. However other cereals have proteins that exert a toxic effect for CD patients; toxic prolamins include gliadin in wheat, secalin in rye and hordein in barley (Saturni et al, 2010).

Celiac disease is an inherited autoimmune disorder that affects the digestive process of the small intestine. When a person who has celiac disease consumes gluten the individual's immune system responds by attacking the small intestine and inhibiting the absorption of important nutrients into the body (Green, P.H. et.al, 2006).This chronic disease is recognized as long-life disease and the only solution is adherence stickiness to gluten-free products.

According to the legislation of EU (Regulation EU No 1169/2011 of the European Parliament and of the Council, 2011) gluten sources include "wheat , rye, barley, oats or their hybridized strains, and products thereof" with some exceptions. According to the Commission Regulation (EC) No 41/2009 (2009) products containing "ingredients made from wheat, rye, barley, oats or their crossbred varieties which have been especially processed to reduce gluten", which contain less than 100 mg of gluten per kilogram could be termed "very low gluten". The products labeled "gluten-free" could not contain more than 20 mg gluten per kg. Identical classification was adopted by US Food and Drug Administration limiting the level of gluten in GF products to 20 ppm (Witczak et al, 2016).In this review the most applied methods for gluten analysis and new approaches are discused.

Gluten analysis

The detection of gluten in heated or extruded products like bread and pasta (Mena et al, 2012) and in products containing partially hydrolysed gluten (Tanner et al,2013) is particularly challenging. As the first step, the extraction of gluten proteins and/ or peptides from the food matrix should be as complete as possible. The analysis itself needs to be accurate and adequately calibrated against a suitable, representative reference protein. Most methods are based on quantitating the alcohol-soluble prolamin fraction of gluten. The alcohol-insoluble glutelin fraction is often not targeted, although both prolamins and glutelins contain immunogenic peptides (Tye-Din et al, 2010). Because the prolamin content of gluten is taken as 50% (Codex Standard 118-1979, 2008) (Codex Standard 118-1979, 2008), the determined prolamin content is usually multiplied by a factor of 2 to obtain the gluten content. This is based on the assumption that the prolamin/glutelin ratio is 1. However, a comprehensive analysis of wheat, spelt, emmer, einkorn, rye, and barley flours as well as wheat starches showed that the true prolamin/glutelin ratios were highly variable ranging from 0.2 in wheat starch to 13.9 in einkorn. Therefore, the gluten content is overestimated, or, more seriously for CD patients, underestimated by duplication of the prolamin content (Wieser and Koehler, 2009).

Proteomics-based methods

Reversed-phase or gel permeation high-performance liquid chromatography (RP- or GP-HPLC) with UV detection and gel electrophoresis are most widely used for the characterisation of cereal proteins. While these one-dimensional (1D) separation techniques are proficient for the analysis of gluten profiles from flours, they are inadequate for detecting gluten traces in complex food matrixes due to low selectivity and sensitivity. Modern proteomics approaches combine high resolution 2D separation (isoelectric focussing and SDS polyacrylamide gel electrophoresis or chromatography) with mass spectrometry (MS) using soft ionization such as matrix-assisted laser desorption/ionisation (MALDI) or electrospray ionisation (ESI) followed by time-of-flight (TOF), ion trap or triple quadrupole detection (Ferranti et al, 2007). The identification of gluten proteins or peptides after MS analysis requires extensive database (e.g., UniProt Knowledgebase or National Center for Biotechnology Information) searching with powerful tools to predict protein/peptide masses, isotopic patterns, enzymatic cleavage sites and possible modifications (e.g., glycosylation, oxidation, ammonia loss).

LC-MS/MS

The most important points to consider are appropriate extraction of gluten proteins and peptides from the food matrix, choice of a suitable enzyme for gluten digestion, selection of specific gluten marker peptides, and calibration with a representative RM to allow the calculation of gluten contents based on the determined gluten marker peptide concentration. Food samples were digested directly with pepsin,

trypsin, and chymotrypsin without prior extraction and the six peptides derived from a- and g-gliadins could be reproducibly quantitated in a range from 0.01 to 100 mg/kg in various native and processed food samples. A calculation of gluten contents was not attempted and the LC-MS/MS method was seen as a complementary tool to ELISA to check for the presence of immunogenic peptides. (Van den Broeck, Cordewener and Nessen, 2015).

Currently, the analysis of gluten peptides by LC-MS/MS requires expensive equipment and expertise that are only available in specialized laboratories or research institutes. However, due to their high selectivity, sensitivity, versatility, and wide applicability also for heated and hydrolysed gluten, LC-MS/ MS methods are the most promising non-immunological approach for the accurate quantitation of gluten. (Gomaa and Boye, 2015).

Genomics-based methods

Genomics-based methods do not target gluten proteins themselves, but DNA or RNA indicative of the presence of gluten. DNA detection is more sensitive by several orders of magnitude compared to protein analysis, because DNA fragments can be amplified by polymerase chain reaction (PCR) generating thousands to millions of copies in a short time. Real-time PCR using fluorescent dyes such as SYBR Green I or probes such as TaqMan®, allows the detection and verification of the amplified target DNA sequences. Wheat, rye, barley and oats could be distinguished by real-time PCR. However, PCR is unsuitable for gluten detection in highly processed or hydrolysed samples such as beer, syrups or malt extracts due to massive DNA degradation. While not yet directly applicable to the analysis of gluten traces, NGS(next-generation sequencing) is a promising tool to differentiate between CD immunogenic epitopes and gluten sequences that are not CDactive (Ozuna et al, 2015).

Novel methods

In the past two years, a number of novel methods for gluten detection were reported that are based on recent developments, such as aptamers, magnetic beads, microarrays or multianalyte profiling. Aptamers are structured, single-stranded nucleid acid ligands chosen from a vast coll-ection through an in vitro selection process called systematic evolution of ligands by expo-nential enrichment. The binding of the aptamer to the target triggers an adaptive folding which is promoted and stabilised by the target through non-covalent interactions. Using gliadin as target molecule, a G33 aptamer (49 nucleotides) was identified that showed specific interaction with gliadin. This aptamer was used to develop a competitive real-time apta-PCR which showed no cross-reactivity to corn or oats, but it was not yet applied to the analysis of real food samples (Pinto, 2014). Another aptamer called gli4 against the 33-mer peptide was applied in a competitive electrochemical aptamer-based assay using magnetic particles. The analysis of one gluten-free and three gluten-containing food samples with known gliadin contents revealed a good agreement with the expected values and with the R5 ELISA (Amaya-Gonzalez et al, 2014).

A microfluorimeter device for the in situ detection of gliadin in foods with a disposable polymer chip was developed for a rapid control of raw materials. The performance characteristics of the indirect competitive assay were good and the results of the microfluorimeter correlated well with a laboratory-made ELISA when analysing three real food samples (Mairal et al, 2009).

Protein or peptide microarrays are multiplex lab-on-a-chip devices where the protein/peptide is spotted onto the surface of a glass or plastic chip. This chip can be incubated with capture agents such as fluorescent dye-labelled antibodies. The first application of this technology to gluten detection was based on the immobilization of gliadin and chymotrypsin-digested gliadin and subsequent detection using a fluorescently labelled glutamine-binding protein or 4F3 mAb raised against the 33-mer peptide. The first microarray results seemed promising, but real food samples were not analysed so far (Cimaglia et al, 2014). A new portable gliadin immunochip was recently developed and used to determine gliadin in beers and flours (Chiriaco et al, 2015).

Multianalyte profiling is based on magnetic particles that contain fluorescent dyes as colour code. Gluten proteins or peptides can be conjugated to the particle surface and detection is achieved via a specific antibody labelled with the fluorescent dye phycoerythrin (PE). Dual laser excitation is used to determine the colour code of the particle as well as the presence of PE (Cho et al, 2015). The advantages of multiplexed systems are the simultaneous detection of several analytes with high

sensitivity and specificity, high-throughput, short analysis duration and reduced cost and labour requirements.

Conclusion and future perspectives

Currently employed analytical methods in gluten-free management and legal compliance testing offer the sensitivity required to meet Codex recommended limits of 20 mg gluten/kg. ELISAs are most commonly used, which are appreciated for their specificity, sensitivity, and suitability for routine analysis. Many challenges remain with ELISAs, highly heat-treated and hydrolysed gluten and considerations for an optimised combination of extraction procedure and test system are necessary. Similar problems were reported with PCR-based assays. DNA-based methods can theoretically offer higher sensitivity and specificity than ELISAs, but they are unsuitable for gluten detection in highly processed or hydrolysed samples due to massive DNA degradation. Proteomics-based methods have complemented immunological methods to gain a better understanding of the processing effects on gluten and cereal proteins in general and to provide extensive information on cereal variety, protein/peptide masses, isotopic patterns, enzymatic cleavage sites and possible modifications. LC-MS/ MS methods are the most promising approaches for accurate quantitation of gluten traces to ensure the safety of gluten-free foods for CD patients, due to their high selectivity, sensitivity, versatility, and wide applicability also for heated and hydrolysed gluten. However, due to the required expensive equipment and expertise these methods have not extensively been used in routine analysis, but rather in specialised laboratories and research institutes.

As more analytical methods for gluten determination emerge, the comparison of analytical results and the standardisation/calibration of the analytical tools will become increasingly important. Novel methods aim at improved recovery of gluten, higher sensitivity and lower cross-reactivities with related species, and simple, fast, and on-site detection procedures. Described approaches include the use of immunosensors, aptamers, magnetic beads, microarrays, and multianalyte profiling. In the future, multiplexing analytes and more flexibility in “choosing” targets will be an important factor for success of newmethods for routine analysis. Considerations for the right choice of method in the future will include sensitivity, specificity, speed of analysis, efficiency and simplicity of extraction procedures, high throughput or rapid method, labour and infrastructure costs, as well as recommendations from standardisation bodies and Codex.

Refrences:

Letizia Saturni, Gianna Ferretti, Tiziana Bacchetti, The Gluten-Free Diet: Safety and Nutritional Quality Nutrients, 2010, 2, 16-34; doi:10.3390/nu2010016

Green, P.H. et.al, Characteristics of adult celiac disease in the USA: results of a national survey, 2006, American Journal of Gastroenterology.

Mariusz Witczak a, Rafał Ziobro b, Lesław Juszczak c, Jarosław Korus
, Starch and starch derivatives in gluten-free systems . A review, 2016, Journal of Cereal Science, (67): 46-57

Mena, M.C., Lombardía, M., Hernando, A., M_endez, E., Albar, J.P., 2012, Comprehensive analysis of gluten in processed foods using a new extraction method and a competitive ELISA based on the R5 antibody. Talanta, (91): 33-40.

Tanner, G.J., Colgrave, M.L., Blundell, M.J., Goswami, H.P., Howitt, C.A.,.. Measuring hordein (gluten) in beer e a comparison of ELISA and mass spectrometry. 2013, PlosOne 8, e56452.

Tye-Din, J.A., Stewart, J.A., Dromey, J.A., Beissbarth, T., van Heel, D.A., Tatham, A., Henderson, K., Mannering, S.I., Gianfrani, C., Jewell, D.P., Hill, A.V.S., McCluskey, J., Rossjohn, J., Anderson, R.P., Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. 2010, Sci. Transl. Med, (2) 41-51.

Codex Standard 118-1979, 2008. Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten. Codex Alimentarius Commission. Revision 1.

Wieser, H., Koehler, P., Is the calculation of the gluten content by multiplying the prolamin content by a factor of 2 valid? 2009, Eur. Food Res. Technol. (229), 9-13.

Ferranti, P., Mamone, G., Picariello, G., Addeo, F. Mass spectrometry analysis of gliadins in celiac disease., 2007, J. Mass Spectrom. (42), 1531-1548.

Van den Broeck, H.C., Cordewener, J.H.G., Nessen, M.A., America, A.H.P., van der Meer, I.M., Label free targeted detection and quantification of celiac disease immunogenic epitopes by mass spectrometry, 2015, J. Chromatogr.(1391), 60-71.

Gomaa, A., Boye, J.,. Simultaneous detection of multi-allergens in an incurred food matrix using ELISA, multiplex flow cytometry and liquid chromatography mass spectrometry (LC-MS). 2015, Food Chemistry, (175), 585-592.
Ozuna, C.V., Iehisa, J.C.M., Gimenez, M.J., Alvarez, J.B., Sousa, C., Barro, F. Diversification of the celiac disease a-gliadin complex in wheat: a 33-mer peptide with six overlapping epitopes, evolved following polyploidization, 2015, Plant J,(82), 794-805.
Pinto, A., Polo, P.N., Henry, O., Redondo, M.C.B., Svobodova, M., O'Sullivan, C.K., Label-free detection of gliadin food allergen mediated by real-time apta-PCR, 2014, Anal. Bioanal. Chem, (406), 515-524.
Amaya-Gonzalez, S., de-los-Santos-Alvarez, N., Miranda-Ordieres, A.J., Lobo- Castanon, M.J.,. Aptamer binding to celiac disease-triggering hydrophobic proteins: a sensitive gluten detection approach. 2014, Anal. Chem, (86), 2733-2739.
Mairal, T., Frese, I., Llaudet, E., Bermudo Redondo, C., Katakis, I., von Germar, F., Drese, K., O'Sullivan, C.K., Microfluorimeter with disposable polymer chip for detection of coeliac disease toxic gliadin, 2009, Lab Chip (9), 3535-3542.
Cimaglia, F., Potente, G., Chiesa, M., Mita, G., Bleve, G., Study of a new gliadin capture agent and development of a protein microarray as a new approach for gliadin detection. 2014, J. Proteomics Bioinforma, (7), 248-255.
Chiriaco, M.S., de Feo, F., Primiceri, E., Monteduro, A.G., de Benedetto, G.E., Pennetta, A., Rinaldi, R., Maruccio, G., Portable gliadin-immunochip for contamination control on the food production chain. 2015, Talanta ,(142), 57-63.
Cho, C.Y., Nowatzke, W., Oliver, K., Garber, E.A.E., Multiplex detection of food allergens and gluten, 2015, Anal. Bioanal. Chem, (407), 4195-4206.