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Mini Review

Gluten Free Detection – A Recent Insight

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Abstract

Gluten can be described as a complex mixture of proteins found in wheat, rye, barley and oats that pose a health risk to people affected by conditions such as celiac disease and non-celiac gluten sensitivity. A standardized method of analysis is needed to quantitatively determine the gluten content of food and provide the basis for enforcing regulations regarding use of the term gluten-free in food labeling. The Association of Analytical Communities and the Codex Alimentarius Commission endorse different methods. This paper presents potential new approaches in support of industry and enforcement activities to ensure compliance with the gluten-free claim under the current regulatory framework.

Keywords: *Gluten; Celiac Disease; Detection; Technique; ELISA; Gluten Sensitivity; Regulation*

Abbreviations

CD: Celiac Disease; Pro: Proline; Gln: Glutamine; IEF: Isoelectric Focusing; HPCE: High-Performance Resolution Capillary Electrophoresis; ESI: Electrospray Ionization; ELISA: Enzyme-Linked Immunosorbent Assay

Introduction

Gluten is the collective term that describes the complex mixture of proteins present in wheat, rye, barley and oats that are rich in proline (Pro) and glutamine (Gln) and are as such called the prolamins [1,2]. The gluten proteins are responsible for imparting viscoelastic properties in different food products like pasta, bread and Noodles. The presence of high proline content of gluten also renders these proteins resistant to gastrointestinal digestion. Proline is the only amino acid whose side-chain links to the backbone α-amino group thereby hindering protein cleavage by most proteases. As a result relatively long proline and glutamine-rich gluten fragments can reach the small intestine where they elicit an autoimmune response in susceptible individuals.

The ingestion and limited proteolytic processing of gluten proteins in the gastro-intestinal tract are involved in the onset of celiac disease (CD), a condition that affects large number of population globally. For Example, Independent studies show that the prevalence of CD in Europe ranges from; 1:100, with differences between some countries. In the United States, the expected prevalence is from 1 in 111 to 1 in 141 patients. Indeed, we know that most patients with CD have not been detected. It is of critical importance to the health of those affected by CD or non-coeliac gluten sensitivity (NCGS) at the food industry establish accurate methods for gluten measurement [3,4].

The main methods for the detection of gluten in foods are based on directly targeting the gliadin (allergenic proteins in the gluten) or its peptide fragments. The detection can occur by isoelectric focusing (IEF), A-PAGE, SDS-PAGE, reversed-phase (RP)-HPLC, size exclusion HPLC (SE-HPLC), high-performance resolution capillary electrophoresis (HPCE), the combination of HPLC with electrospray ionization (ESI), tandem mass spectrometry detection (LCeMS/MS) and enzyme-linked immunosorbent assay (ELISA) [5,6]. This latter is the

currently accepted method for gluten determination in native and processed foods. However, these gliadin analysis methods are time intensive, expensive and require trained operators. Availability of fast, cheap but sensitive methods for gluten detection are necessary for an effective gluten-free products labelling and thus protecting celiac people from the unaware content of gluten in food higher than the official limit (20 ppm) set by the European regulation.

Immunoassays techniques like ELISAs (enzyme-linked immunosorbent assays) and LFDs (lateral flow devices) are recurrently used in the food industry to track allergen contamination owing to its advantages. However, one major concern in allergen quantification is that results vary from kit to kit. There are different factors like antibody specificity, target analytes, sample extraction buffers, extraction time and temperature, calibration standards, and the unit of measurement that can result into the technical differences. Furthermore, different matrixes and their different processing statuses add to the additional complexity of testing allergen. For example, thermal processing during production can lead to altered protein extraction efficiencies and antibody binding affinities.

In the present scenario, the best studied detection method with the most scientific data published is the one based on R5. The current Type I Codex method for gluten analysis is the ELISA R5 Mendez method, which is calibrated against the Prolamin Working Group (PWG) Gliadin standard and used by official control systems throughout Europe [7,8]. In addition, the assay was provided for calibration and validation of the noncommercial available gliadin reference material produced by the PWG. If we compare the method based on Skerritt antibodies, the R5 method has overcome major disadvantages. However, it has to be noted that R5 and Skerritt antibodies are not being compared under the same conditions. The Skerritt-based method was developed more than two decades ago and there was limited knowledge on the toxicity, impact of processing and recovery of gluten proteins as compared to present time. At this point, it would be a good idea to re-evaluation of the method of Skerritt using better extraction techniques so that assay could be improved in the processed food. Moreover, use of different extraction technique might further improve the cross-specificity to barley, another aspect for which the Skerritt method has been highly criticized.

Information about the true gluten content in foods samples is still doubtful using any of the above methods. This is one of the major controversial point about the recognition of the R5 Mendez method as type I for gluten detection. Additionally, the kit only targets prolamins, which are only one fraction of gluten. In future, we certainly need to identify considerations relative to current analytical approaches. The availability of a standardized and harmonized validation protocol would be very valuable since data generated by assays validated under this protocol would have global acceptance; therefore, facilitating trade activities.

Over the year it has been noticed that gluten detection has lacked of a appropriate confirmation method. In this regards, it can said that DNA-based technologies could be one of the potential candidate. The DNA –Based technologies can in fact discriminate between the cereal spices apart from adding multiplexing capabilities. We need to keep in mind that the gluten estimation as protein equivalent can commence a high degree of uncertainty in the analytical result since protein expression is very variable depending on cultivar and growing conditions. Food processing type and conditions may alter the ratio of DNA to protein, as well. Because of these reasons and the fact that the proteins are the actual trigger of CD, protein-based detection methods are always preferred over DNA applications.

In the past few years, it has been seen that latest developments in Mass spectrometry (MS) have allowed development of new application in the proteomics [11]. MS being very versatile, the specificity, and the fact that it targets the protein or peptides directly without the use of intermediaries (antibodies), this technology is a promising candidate for gluten analysis in the coming future. MS can offer solutions and additional benefits in those areas where antibody-based technology reaches its limitations. Multiplexing is one of the most attractive features of MS applied in context to the gluten analysis. MS can target selected multiple gluten peptides; it can, therefore, discriminate among wheat, rye, and barley. This is especially pertinent to countries like Canada where the source of gluten, when present, needs to be identified on the label [9,10].

In case of ELISA, the discrimination of the source of gluten is impossible until new developments lead to production of assays targeting gluten from individual grains. In this case, three independent ELISAs, for wheat, rye, and barley. This would further increase the cost per sample. The identification of the source of gluten can be a very important piece of information in monitoring and tracing the source of contamination, which can be included in risk assessment and risk management activities. Multiplexing also brings added benefits. Since peptides from different sources can be identified, it is possible to use standard materials consisting of mixtures of wheat, barley, rye, and other grains, like spelt and kamut. Since using MS, cereals can be discriminated, a standard curve for each cereal can be obtained from running a single standard curve and, if necessary, applying individual conversion factors more appropriate for each cereal. As a result, MS would be able to provide a more accurate estimation of the total content. In general, MS is opening a wide avenue that may help solve not all, but certainly many, problems associated with current detection systems. Very rapid developments in the technology itself along with ongoing research activities aimed at reducing sample preparation offer a promising future for this MS in this application.

Conclusion

There have been a number of major improvements during the last three decades. The advances in the regulatory and analytical environment have further led to enhancement of the methods of screening and declaration of label in gluten free products. It would definitely bring in an increase of food choices for celiac patients and their quality of life. Additionally, the more we understand about the uncertainties, the community is aware of new issues that were not considered before. These uncertainties may have a greater impact on food manufacturers since it is their responsibility to have all measures in place to ensure that the food is safe for the consumer. We need to ask more questions to helps us improve and fine tune our capabilities to establish more appropriate tolerance levels and more accurate and reliable detection methods.

Conflict of Interest

There is no conflict of Interest.

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