

NEW ADVANCES IN GLUTEN ANALYSIS: APPLICATION OF MALDI-TOF/TOF MASS SPECTROMETRY AND NANOLC-ESI-IT-MS/MS TECHNIQUES

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Introduction: Gluten is a group of proteins found in wheat, barley and rye. The ingestion of these proteins, in genetically predisposed people, induces the celiac disease. The only treatment for celiacs is a lifelong gluten-free diet. For this reason, new techniques are necessary to characterize and detect these proteins to monitor the absence of gluten in foods. Both, MALDI-TOF/TOF Mass Spectrometry and nanoESI-IT-MS/MS techniques are highly sensitive methods for detecting peptides. The aim of this work is to characterize prolamin proteins and their tryptic peptides from wheat, barley and rye and develop a new method for gluten analysis based on the detection of specific peptides.

Materials and methods: Prolamins are extracted from cereal seeds by using standard procedures. These proteins are directly off gel endoprotease digested or separated by SDS-PAGE electrophoresis and in gel endoprotease digested. Then, the yielded peptides are analysed and characterized by MALDI-TOF/TOF Mass Spectrometry or nanoLC-ESI-IT-MS/MS techniques. The resulting peptide masses were then searched against a protein data base (NCBI or Swiss Prot) for matching proteins using the Mascot Search. Sandwich R5-ELISA is used as an internationally reference standard technique (Codex Alimentarius ALINORM 08/31/26) to analyse prolamins from wheat, barley and rye.

Results: A large set of peptides were identified and assigned to different prolamins and specific proteotypic peptides for wheat, barley or rye as well as for common peptides corresponding to the three cereals. The results for both techniques were similar.

Conclusions: MALDI-TOF/TOF Mass Spectrometry and nanoESI-IT-MS/MS techniques can be used as non-immunological tools in gluten analysis of different wheat, barley and rye varieties. These proposed methodologies enabled us to set up a fast and accurate analysis for evaluating the gluten components.