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A COMPREHENSIVE PEPTIDOMIC APPROACH TO CHARACTERIZE THE PROTEOMIC PROFILE OF SELECTED DURUM WHEAT GENOTYPES AND ITS IMPLICATION FOR COELIAC DISEASE AND WHEAT ALLERGY

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In the last decades, the varietal selection undertaken by breeders tailored to improve technological and productivity related traits, caused a considerable impoverishment of the genetic diversity of wheat varieties present on the market. Starting from this, the researchers are encouraged to investigate the natural diversity of available wheat genotypes in light of their potential to encode a lower number of celiac disease epitopes [1].
In a recent investigation we presented the detailed characterization of a tetraploid wheat collection containing 38 accessions of durum wheat (*Triticum turgidum*) selected from a wider list of 240 genotypes, developed at University of Bari Aldo Moro, including both wild and cultivated accessions [2]. The collection was investigated by a multidisciplinary approach including conventional proteomic profiling focused on the gliadin fraction (HPLC-UV and R5-ELISA), yield and quality traits of the whole grains [2]. A statistical evaluation of the acquired data set allowed the identification of a short list of candidate genotypes combining reduced gluten content with satisfactory rheological properties required for their perspective usability in bread or pasta [2].
As follow-up of that work, in the present communication an in-depth analysis of the proteomic profile of the selected wheat genotypes will be presented. Advanced proteomic approach was carried out combining proteins/peptides sequence information retrieved by specific enzymatic digestions (single and dual enzymes) with protein digestibility information provided by in-vitro simulated human gastroduodenal digestion experiments (see Figure 1 for details). The latter was applied to raw flours according to the standardized static protocol proposed by Minekus et al. in 2014 [3]. In both cases, the peptide pools were analysed by liquid chromatography high resolution tandem mass spectrometry in *data dependent*^{TM} acquisition mode. The instrumental method was customized in order to increase the amount of information retrieved and a dual-round software-based sequence identification with exclusion list was applied. The raw data were processed by the commercial software Proteome Discoverer 2.1 relying on the Sequest HT searching algorithm against a customized database containing all *Triticum* (Tax ID 4564) sequences available on UniProt DB.
The full list of enzyme specific peptides and gastroduodenal resistant peptides were filtered according to specific criteria of reliability for the highest identification confidence and finally, the refined list was screened for in-silico toxicity/immunogenicity risk assessment. Given the global information provided by the designed proteomic approach the risk assessment was

carried out not only tracing for potential toxicity for celiac disease patients, but also scouting for immunogenic sequences relevant for wheat allergic patients, achieving a comprehensive characterization of the selected genotypes. Various open-source bioinformatics tools were used for epitopes matching (*www.allergenonline.org/celiachome.shtml*, www.iedb.org).
The selected genotypes were assessed to encrypt a lower number of number of toxic/immunogenic epitopes for celiac disease and wheat allergy, and as such they could represent convenient bases for breeding practices and for the development of new detoxification strategies.

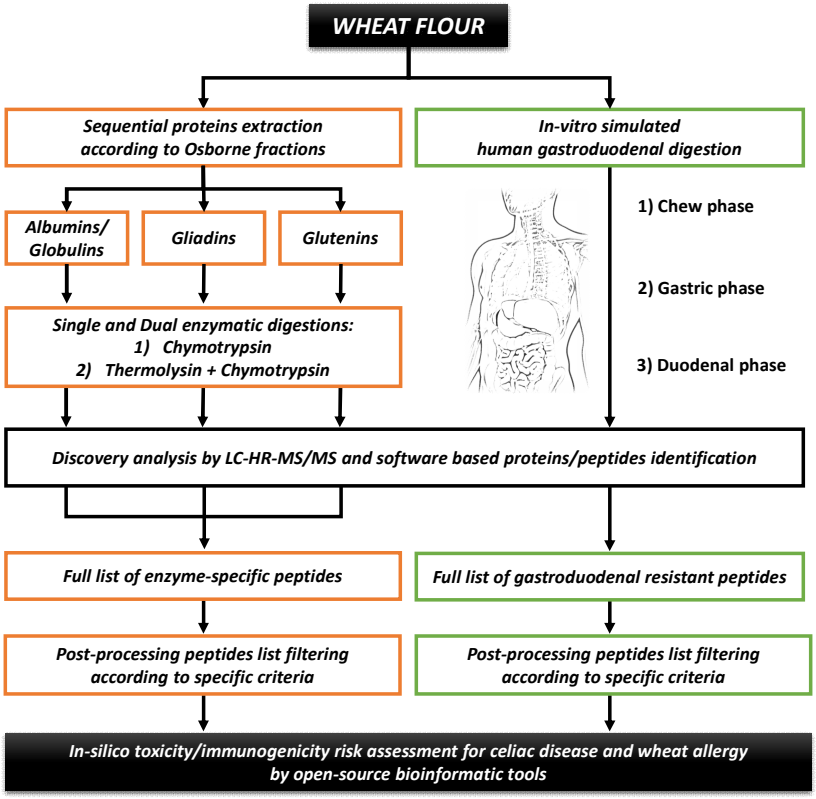

Figure 1. Scheme of the comprehensive peptidomic approach carried out to characterize systematically the proteomic profile of selected durum wheat genotypes.

The research was funded by the Ministry of Education, Universities and Research (MIUR-Italy), program SIR 2014 “S. Wheat Pro. - Proteomic characterization of Selected durum Wheat cultivars for PROduction of low toxicity-food products towards celiac disease patients (RBSI14QQ1W)”.

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