BIOCHEMICAL CHARACTERIZATION OF ANCIENT WHEAT VARIETIES

Gianfranco Mamone

Principal investigator
Gianfranco Mamone

Other components of the research group
Luigia Di Stasio
Gianluca Picariello
Salvatore De Caro

Background

Gluten are the precipitating factor of Celiac Disease (CD). Gluten proteins are classified into gliadins and glutenins, which are present in approximately equal amounts and form 80% of the total storage protein content in the wheat kernel. Because of high percentage of proline residues, gluten proteins are resistant to gastrointestinal digestion so that long gluten fragments can reach high concentration levels in the gut epithelium stimulating either adaptive or innate immune responses in genetic predisposed subjects. To date, a strictly gluten-free diet represents the only medical treatment for CD patients. A fervent research is aiming at seeking wheat genotypes with reduced gluten content, with high digestibility and having naturally low amounts of epitopes toxic for CD patients. A diet based on wheat cultivar with reduced in T-cell stimulatory epitopes may help in the prevention of CD, as it has been observed that the amount and duration to gluten exposure are associated with the initiation of CD. The reduction of the amount of major T-cell stimulatory epitopes in food may especially benefit children, in which the onset of CD may be delayed or even prevented, and in non-diagnosed CD patients to strongly reduce their symptoms.

Main achievements

Ancient strains of wheat, are currently of special interest as candidate species at low toxicity for CD. In particular diploid Triticum monococcum (AA genome) wheat species are among the most promising, due to their diploid genomes, that are less complex compared to those of modern polyploid (tetraploid or hexaploid) wheat. The Triticum monococcum lacks the D genome that encodes for alpha-gliadin proteins containing

the 33-mer peptide, highly immunostimulatory in CD patients. We previously investigated the immunogenicity of two Triticum monococcum lines named ID331 and Monlis. Ours studies showed that only ID331 cultivar expresses a ω-gliadin harboring a protective peptide able to prevent the " toxic" effects induced by gluten proteins in in vitro systems. We have also investigated the digestibility of Triticum monococcum gluten proteins by in vitro system model mimicking the gastrointestinal digestive tracts. The immuno-stimulatory properties of gluten proteins extensively digested were further investigated on T cells highly responsive to gluten, and on mucosal biopsies from coeliac patients. Surprisingly, gluten proteins from both ID331 and Monlis revealed a marked susceptibility to be digested by gastro-intestinal proteases and gluten peptides, released by the extensive digestion, showed a lower T cell stimulatory capacity, compared to gluten protein from hexaploid wheat.

Future perspectives

We aim to carry out a comprehensive biochemical characterization of ancient wheat genotypes in order to identifying low gluten ones. To achieve our goals we combine proteomic and mass spectrometric technology with immunochemical assay. These investigations will provide a general comparison of the proteomic profiles, focusing mostly on the expression level of gliadin epitopes. In addition, the real bioavailability of peptides could be determined by using in vitro digestion model to investigate the digestibility after gastro-intestinal transit and to assess the impact of this process on immunogenicity reactivity in celiac patients. The cultivars which exhibit the lowest immunogenic potential could be selected for the production of wheat based food such as of bread and pasta. The effects of technological processing parameters such as mixing/leavening (bread) and extrusion/drying phases (pasta) will be investigated on the digestibility and immunogenicity. By this way, it is planned to assess the nature and the possible impact of the structural changes of gluten proteins resulting from the mechanical and thermal treatment. Simulated gastrointestinal digestion on the wheat based products will evaluate survival of immunogenic or harmful sequences and will address the link between the metabolic fate of proteins, the process parameters and the mechanical properties of dough and of the finished products.

Publications

1. *Iacomino G, Fierro O, D'Auria S, Picariello G, Ferranti P, Liguori C, Addeo F, Mamone G. Structural analysis and Caco-2 cell permeability of the celiac-toxic A-gliadin peptide 31-55. Journal of Agricultural and Food Chemistry 2013; 61 (5): 1088-1096.*
2. *Mamone G, Camarca A, Fierro O, Sidney J, Mazzarella G, Addeo F, Auricchio S, Troncone R, Sette A, Gianfrani C. Immunogenic peptides can be detected in whole gluten by transamidating highly*

susceptible glutamine residues: Implication in the search for gluten-free cereals. Journal of Agricultural and Food Chemistry 2013; 61 (3): 747-754.

3. *Picariello G, Mamone G, Cutignano A, Fontana A, Zurlo L, Addeo F, Ferranti P. Proteomics, Peptidomics, and Immunogenic Potential of Wheat Beer (Weissbier). Journal of Agricultural and Food Chemistry 2015; 63 (13): 3579-3586.*
4. *Mamone G, Nitride C, Picariello G, Addeo F, Ferranti P, Mackie A. Tracking the fate of pasta (T. Durum Semolina) immunogenic proteins by in vitro simulated digestion. Journal of Agricultural and Food Chemistry 2015; 63 (10): 2660-2667.*
5. *Gianfrani C, Camarca A, Mazzarella G, Di Stasio L, Giardullo N, Ferranti P, Picariello G, Rotondi Aufiero, V, Picascia S, Troncone R, Pogna N, Auricchio S, Mamone G. Extensive in vitro gastrointestinal digestion markedly reduces the immune-toxicity of Triticum monococcum wheat: Implication for celiac disease. Molecular Nutrition and Food Research 2015; 59 (9): 1844-1854.*
6. *Iacomino G, Di Stasio L, Fierro O, Picariello G, Venezia A, Gazza L, Ferranti P, Mamone G. Protective effects of ID331 Triticum monococcum gliadin on in vitro models of the intestinal epithelium. Food Chemistry 2016; 212: 537-542.*
7. *Gianfrani C, Mamone G, la Gatta B, Camarca A, Di Stasio L, Maurano F, Picascia S, Capozzi V, Perna G, Picariello G, Di Luccia A. Microwave-based treatments of wheat kernels do not abolish gluten epitopes implicated in celiac disease. Food and Chemical Toxicology 2017; 101: 105-113.*
8. *Mamone G, Iacomino G. Comparison of the in vitro toxicity of ancient Triticum monococcum varieties ID331 and Monlis. International Journal of Food Sciences and Nutrition 2018; 69 (8): 954-962.*
9. *Pilolli R, Gadaleta A, Mamone G, Nigro D, De Angelis E, Montemurro N, Monaci L. Scouting for Naturally Low-Toxicity Wheat Genotypes by a Multidisciplinary Approach. Scientific Reports 2019; 9 (1): art. no. 1646.*