NOVEL THIOLOMICS APPROACH TO STUDY THE BIOCHEMICAL FEATURES OF PROTEIN NETWORK IN CEREAL-BASED COMPLEX MATRICES

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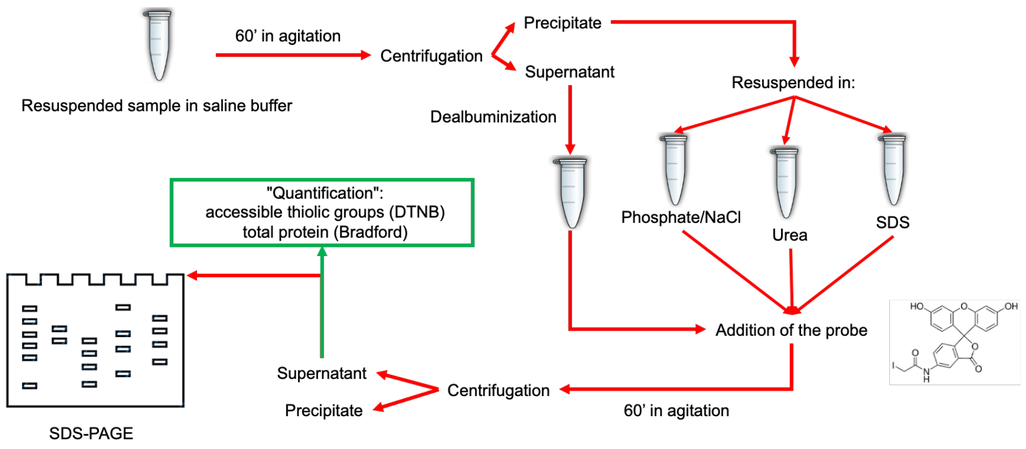
INTRODUCTION

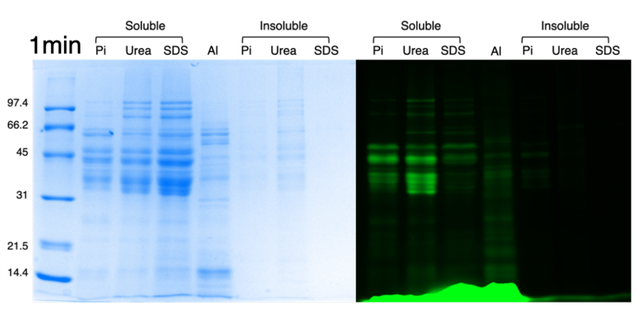
Research activities have been focused (1) on the development of a protocol to obtain a “thiolomic” description of cereal-based matrices. This approach was used (2) to study the structural evolution of proteins involved in the formation of protein networks (such as in dough) and (3) to highlight molecular differences in apparently similar matrices (e.g. wheat varieties with different technological properties).

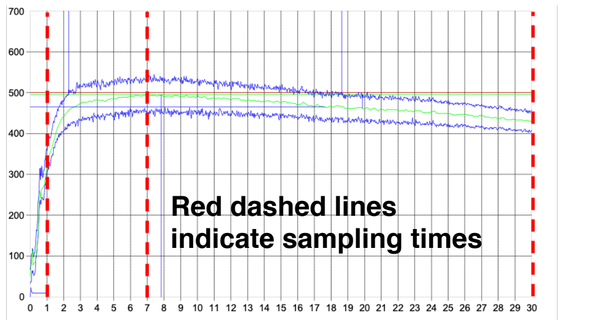
RESULTS



1) Set-up of a protocol for fluorescent labelling

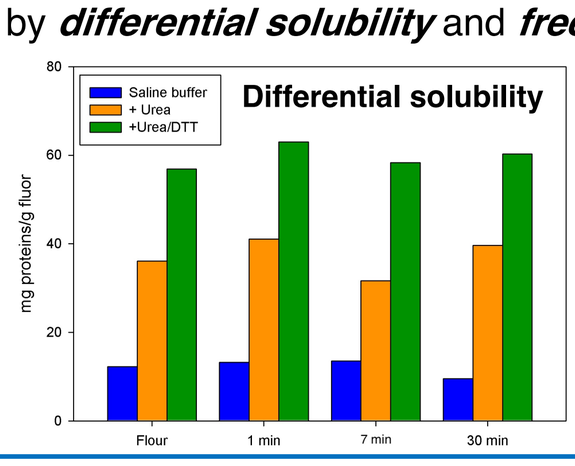
A fluorescence-based thiol-labeling protocol was set up on commercial flour and semolina. A first step of “dealbuminization” in saline buffer (to remove “non gluten” proteins, i.e. albumins and globulins) proved to be essential to clearly visualize gluten proteins. Urea and SDS (at least 2M and 0.2%, respectively) were found to be both effective in solubilizing gluten protein at room temperature. Nevertheless, in the presence of > 2M urea proteins are more efficiently labelled than in the presence of > 0.2% SDS.

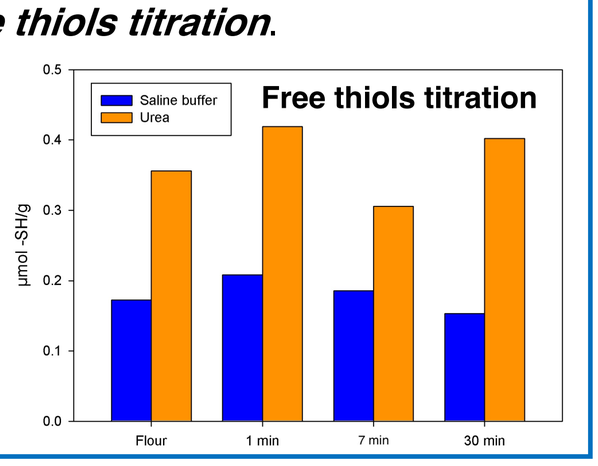




2) Evolution of free thiols during gluten development

The two commercial flour and semolina were kneaded in a ***farinograph***. Dough samples were taken before (***1 min***) and at full (***7 min***) development, as well as at overmixing (***30 min***), and labeled after liophylization. Preliminary results show that disulfide exchange reactions do not results in macroscopic difference in the protein pattern and in the pattern of accessible thiols, as confirmed by ***differential solubility*** and ***free thiols titration***.



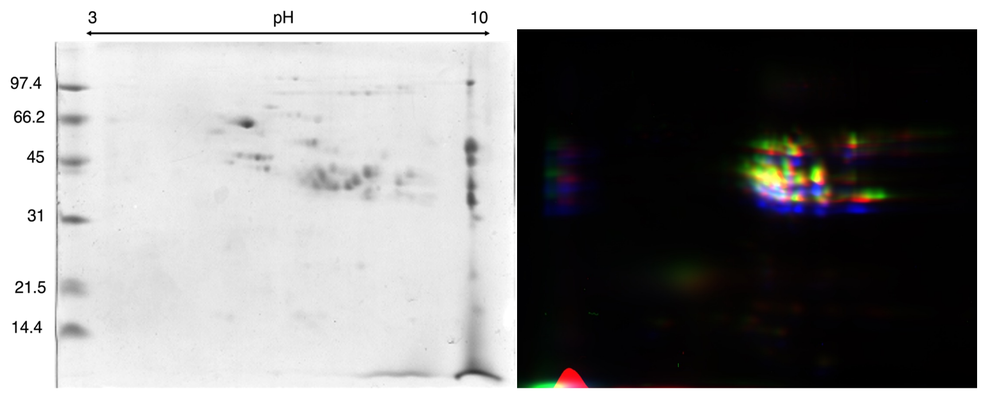


3) Thiolomic as a tool for samples comparation

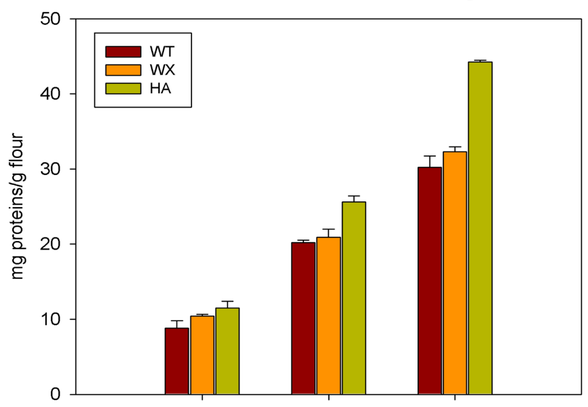
Three near isogenic wheat lines (control (WT); waxy (i.e., no amylose, WX); high-amylose line (HA)), sharing the same ***protein and thiols pattern***, have been compared to highlight “matrix effects” (i.e. starch composition) on the structural, functional and technological properties of gluten proteins.

Proteins in isogenic wheat flours show ***different solubility*** in the presence of chaotropes and reductants, suggesting a different stability of the protein aggregates, and difference in process-dependent changes in ***protein surface hydrophobicity*** upon solvation (40% humidity) and kneading.

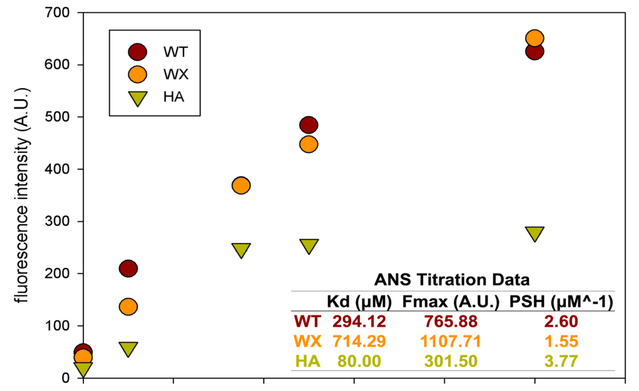
Protein and thiols pattern



Differential solubility



Protein surface hydrophobicity



Starch composition exerts a "matrix effect" that affects process-induced structural changes of gluten proteins (and their kinetics) and the organization of protein network. This evidence is a prerequisite to address the role of competition for water, played between proteins and starch, for the evolution of the thiols-disulfide process triggered by kneading.

FUTURE PERSPECTIVE

The fluorescent labelling information will be integrated by exploiting the reactivity of Au nanoparticles (AuNPs) with thiols to study the accessibility of thiols in proteins involved in the formation of the network. Protein “fished” by the AuNPs will be identified by mass spectrometry after proteolysis. Ideally, the use of AuNPs of different size will allow to study the geometrical features of the protein network.

The overall results will be an extensive comprehension of the structure of the proteins that play a central role in the formation of the gluten network, also in consequence of both the characteristic of the raw material and of the transformation process.

References

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