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SEPARATION OF WHEAT PROTEINS ON A FRIT INLET CHANNEL BY ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION (AF4).

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INTRODUCTION:

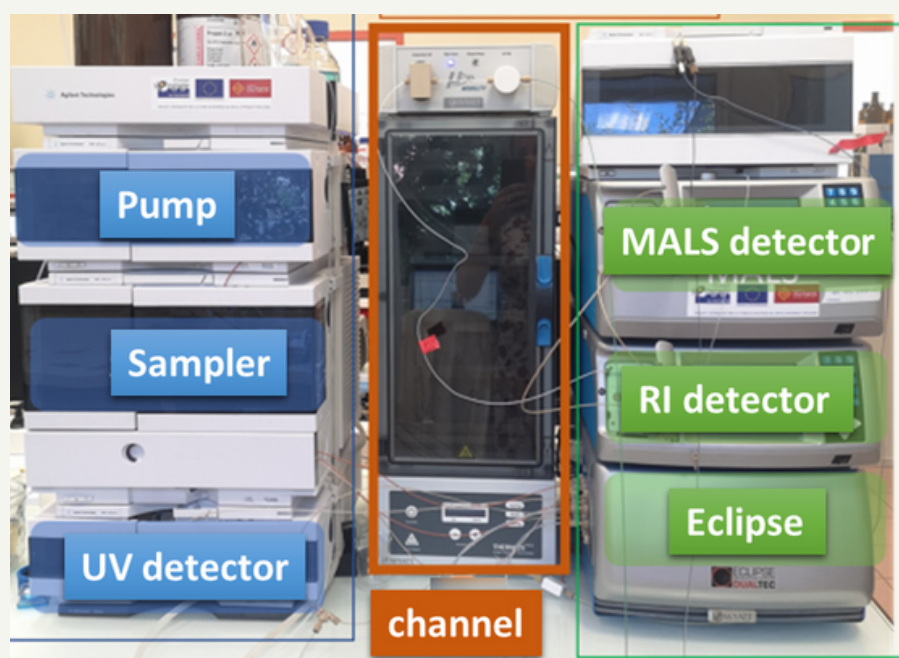
AF4 [1] is a sub-technique widely used of Field Flow Fractionation (FFF) that separates molecules based on their size. AF4 is based on an external force perpendicular to the flow of the channel applied in order to fractionate all analytes.

Food sector is the leading consumer of wheat, with segmented products and consumption patterns that meet specific requirements in terms of quantity and quality. Facing to this development, progress in characterization is essential and the use of common analytical instruments is necessary for the better understanding of protein composition in wheat.

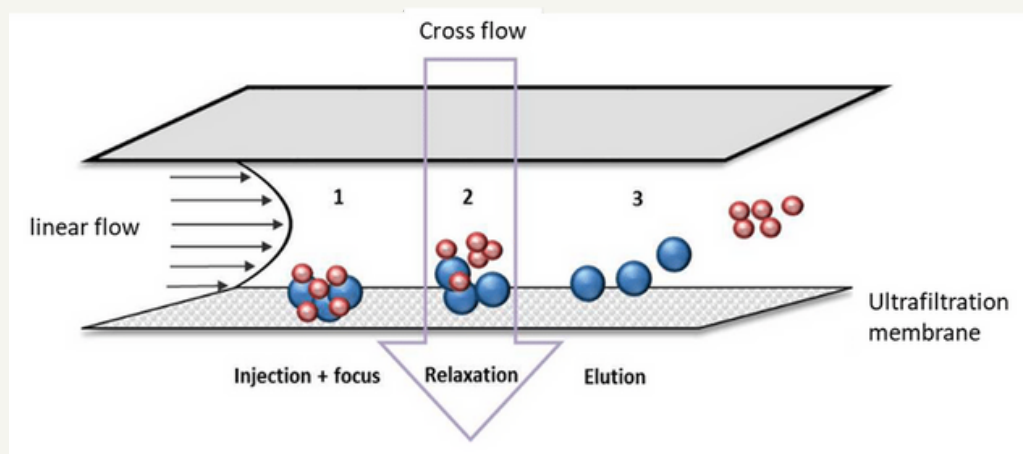
With this work, our objective was to answer to the following questions: Is AF4 able to give the protein composition of wheat samples? What type of information can it provide in addition to the SE-HPLC analysis ?

Our studies explore the use of a Frit-Inlet [2], channel. Frit-Inlet, has a channel design modified from AF4 by introducing a small inlet frit at the beginning end of the depletion wall so that sample relaxation can be made hydrodynamically by the use of the compressing action of the frit flow entering through the small inlet frit. Components are separated according to their size by their diffusion coefficient. Quantitative detector is UV (214nm) and MALS is used to determine molecular weight, size and conformation. We want to discuss in this work, that Frit-Inlet AF4 does not produce any artefact and is a preferred FFF method for the separation of wheat proteins.

MATERIALS AND METHODS:



Wyatt Eclipse System



Overview of the separation inside the channel

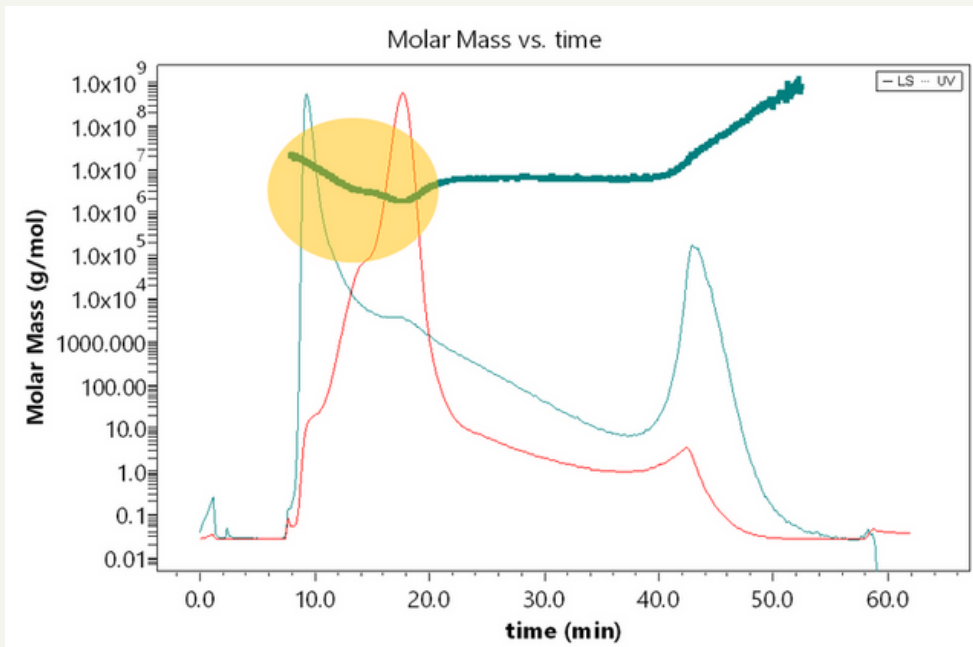
Flash the QR-code: method of AF4 analysis

Flash the QR-code: method of extraction of wheat proteins

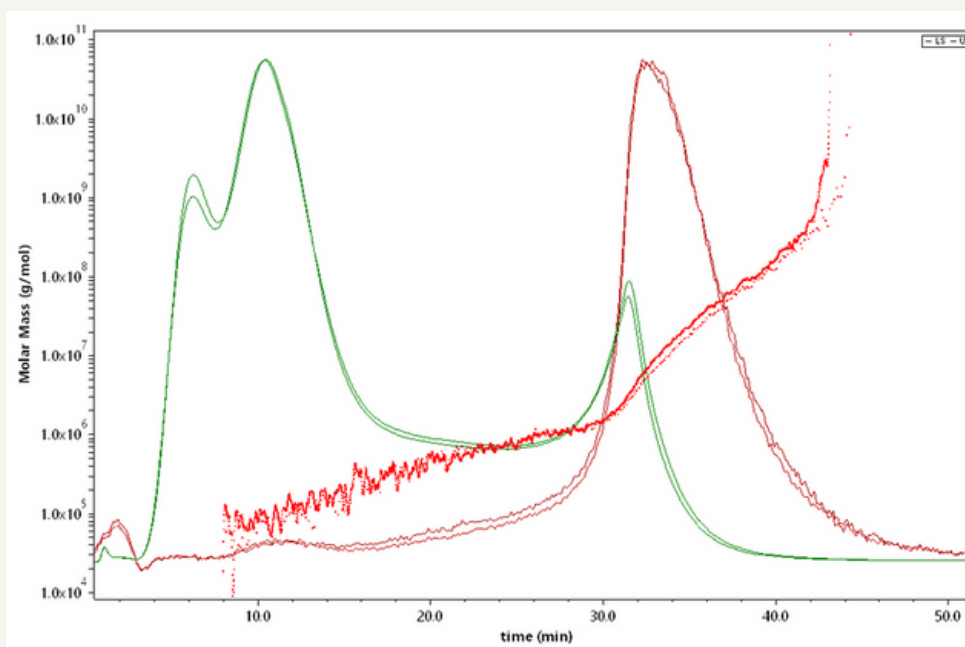
Flash the QR-code to learn more about the Frit Inlet (FI) channel

AF4-UV-MALS VS. SEC-UV-MALS:

AF4 analysis



FI analysis

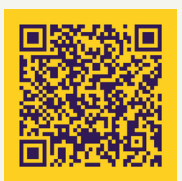


By AF4, we highlighting the elution of higher molar mass proteins **steric mode** while the use of an FI channel allows the steric mode to be overcome.

Steric elution is a phenomenon leading to a reversal of the normal mode of elution of the sample and leading to co-elution of particles of different sizes in the steric transition region. This results in a loss of information to determine the size of total protein samples

Small molecular weights are eluted first, then high molecular weights

CAN WE IDENTIFY THE VARIETY AND GROWING SYSTEM OF WHEAT PROTEINS BY AF4?



Soft Wheat Grains



Soft Wheat Flours



Hard Wheat Grains



CONCLUSION:

An AF4 analysis method was used to obtain profiles of soluble SDS total protein soluble SDS total proteins from wheat samples. Frit-Inlet, has a channel design modified from AF4 by introducing a small inlet frit at the beginning end of the depletion wall so that sample relaxation can be made hydrodynamically by the use of the compressing action of the frit flow entering through the small inlet frit. Components are separated according to their size by their diffusion coefficient. Quantitative detector is UV (214nm) and MALS is used to determine molecular weight, size and conformation and we show in this work, that Frit-Inlet AF4 does not produce any artefact and is a preferred FFF method for the separation of wheat proteins. AF4 is a complementary technique to SEC, the interest of which is to analyse protein fractions of higher molar mass.

[1] Lemelin, E., Aussenac, T., Violleau, V., Salvo, L., Lein, V. (2005). The impact of variety and environment on the size characteristics of wheat proteins using asymmetrical flow field-flow fractionation and multi-angle laser light scattering. Cereal Chemistry

[2] Fuentes, C., Choi, J., Zielke, C., Peñarrieta, J.M., Lee, S. & Nilsson, L. (2019). A comparison between conventional and frit-inlet channels in separation of biopolymers by asymmetric flow field-flow fractionation. Analyst, 144, 4559-4568.