

Appendix A

Extraction and determination of the amounts of glutenin and gliadin fractions

The amount of glutenin and gliadin in each plot, was obtained from 100 mg of wheat flour. First, we ground the wheat grains of each sample using a ball mill to obtain flour of a 100 μm particle size. The extraction of gliadin and glutenin proteins was done using a modified classical Osborne procedure based on protein solubility. The method is described in detail in Wieser et al. (1998) and Pistón et al. (2011). Gliadin protein was extracted stepwise three times, samples were centrifuged, and the supernatants were collected and pooled. The insoluble material from the previous step was used to obtain the glutenin fraction in a similar manner. Then, each of the extracts were filtered and they were applied to a 300SB-C8 reverse phase analytical column using a 1200 Series Quaternary LC System liquid chromatograph (Agilent Technologies) with a DAD UV-V detector. Absorbance was monitored with the DAD UV-V module at 210 nm. The amounts of both fractions were determined using bovine serum albumin as protein standard. Both fractions were expressed as $\mu\text{g}/\text{mg}$ flour and the glutenin to gliadin ratio was calculated by dividing the glutenins between the gliadins.

References:

- 44.- Pistón, F., Gil-Humanes, J., Rodríguez-Quijano, M., Barro, F., 2011. Down-regulating γ -Gliadins in bread wheat leads to non-specific increases in other gluten proteins and has no major effect on dough gluten strength. PLOS ONE 6, 1-10.
- 45.- Wieser, H., Antes, S., Seilmeier, W., 1998. Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. Cereal Chem. 75, 644-650.