Impact of new tool development to study wheat grain fractionation and control the product quality

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Introduction

Wheat grains are known to be composed of a number of different tissues displaying distinct biochemical composition, structure and mechanical properties (*Evers et al., 1999*). Therefore, fractionation process appears as a critical step in the control of the composition and properties of the obtained products for food and non-food applications as well as polymer or potentially interesting valuable molecules isolation. However, wheat grain fractionation was primarily based on technical know-how rather than scientific knowledge. A few new approaches (pre-treatments, degerming, debranning and bran fractionation) have been introduced over the last 15 years, generally developed by the milling industry and described in patents, in order to produce either fractions of nutritional or technological interest, or to reduce contaminants (*Hemery et al., 2007*). However, the development of these new processes mainly resulted from empirical studies. Furthermore, yields are relatively variable and resulting products are generally not sufficiently described in order to meet new emerging objectives such as:

- increasing the nutritional properties of cereal food products without altering technological properties or taste;
- decreasing potentially detrimental biochemical compounds such as *Fusarium* mycotoxins, allergens, heavy metals, etc.;
- and exploring the potential use of under-exploited fractions or developing new uses for cereal fractions;

Thus, it is particularly important :

- to develop knowledge on each tissue fate along processing in order to better understand and predict the product quality obtained after fractionation ;
- to determine key factors involved in tissue fractionation and better characterize the composition and properties of the fractions related to their end-value.

Material and methods

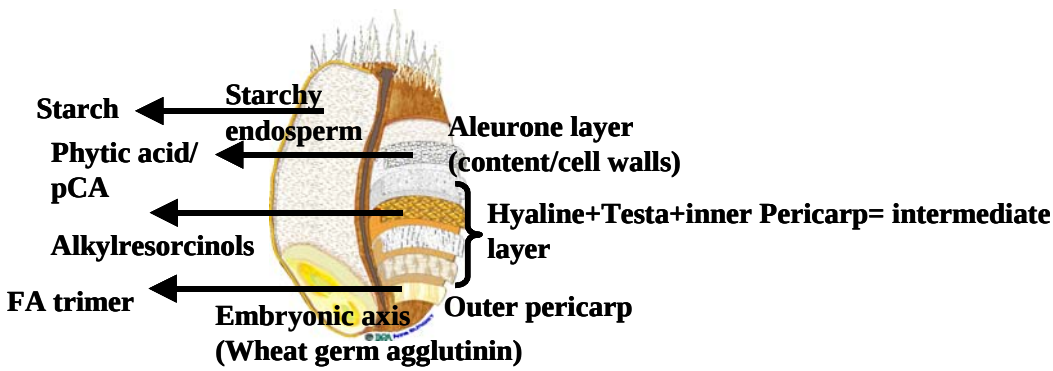
- **Wheat samples:** Samples of common wheat grains from pure cultivars differing by hardness were selected and characterized according to classical methods (thousand kernel weight, test weight, water and ash content, protein content). Near isogenic lines differing by hardness were developed from crossed between inbred lines with a high degree of homozygosity selected in the INRA breeding program as described in Greffeuille et al. (2006), two hard and soft F6 progenies lines were characterized and used as described in Greffeuille et al. (2007). Durum wheat grains contaminated in field by *Fusarium* and displaying high level (4203 $\mu g.kg^{-1}$ d. m.) of DON contamination were selected to study the mycotoxin distribution after fractionation.
- **Biochemical markers:** Total starch, phytic acid, alkylresorcinol and phenolic acid content of the analyzed samples were quantified in duplicate related to reference samples using respectively a Megazyme enzymatic kit (Megazyme International Ireland Ltd., Ireland, AACC method 76-13 (*AACC, 2000*), already described colorimetric methods (*Vaintraub and Lapteva, 1988, Tluscik et al., 1981*) which were adapted to the sample amount of the extracted compounds or chromatographic separation after alkaline hydrolysis in mild conditions of the samples (*Antoine et al., 2003*). Wheat germ agglutinin (WGA) was determined using an adapted enzyme-linked immuno-sorbent assay (ELISA) as previously described by *Vincenzi et al.* (2002).
- **Mechanical assays:** Uniaxial traction tests under controlled temperature and humidity were performed using Dynamical Mechanical Thermal Analysis DMTA on hand-isolated tissues to estimate the mechanical behaviour of each of the grain outer layers under stress. Observed stress-strain curves

generally corresponds to an elasto-plastic behaviour which could be described by the relevant measured parameters: *i.e.* linear strength to rupture (f_{max}) taking into account the measured tissue thickness; elastic strength (f_{ela}), maximum tensile strain (ε_{max}) and elastic strain (ε_{ela}), elastic modulus (*E'*), and rupture energy (W_{max}).

- **Fractionation studies:** Milling studies were undertaken with semi-industrial or laboratory conventional mills or with laboratory prototypes which helps for example to also measure the energy required along the process (*Pujol et al. 2000*). Debranning assays were undertaken using an abrasive laboratory mill (Satake Model TM-05C, Stockport, UK).

Results and discussion

- **Grain tissue monitoring along fractionation**: In the last years, our research team has pointed out the potential of some biochemical markers to monitor specific wheat grain tissue behaviour along fractionation. Of course, starch could be used as a marker of the endosperm, but we also demonstrated that low molecular mass specific molecules such as phytic acid, *p*-coumaric acid, alkylresorcinols and ferulic acid trimer allow to monitor the aleurone content and cell walls, the testa and the outer pericarp respectively (*Hemery et al., 2009a*). This characterization based on the quantification of the extracted targeted molecules present in the milling fractions is relatively simple (**Fig. 1**), involving only one enzymatic assay, two colorimetric assays and one chromatographic separation. Moreover a relatively high number of samples could be processed per day. Furthermore, wheat germ agglutinin was shown to constitute a possible marker of wheat germ axis even if relative amount was used at this step only to classify fractions. Thus, this monitoring method appears reliable to evaluate the proportion of each of the grain tissue in the fractions obtained after processing and more accurate related to the classical determination of fibres or ash content. It is also really feasible compared to other strategies as for example the use of a transgenic marker to monitor fate of the transformed tissues (*Shepherd et al., 2008*). If the relative amount of each biochemical marker was known from previous measurement on hand-isolated tissues, the distribution in percent of each of the grain tissues in fractionation products (obtained from 5-10 kg of grains after milling for example) could be quantified by solving a set of equations for each considered wheat cultivar.



$$[\text{Ferulic-acid trimer}]_f = \%p * [\text{FA-trimer}]_p$$

$$[p\text{-Coumaric acid}]_f = \%p * [p\text{-CA}]_p + \%i * [p\text{-CA}]_i + \%a_w * [p\text{-CA}]_{aw}$$

$$[\text{phytic acid}]_f = \%a_c * [\text{phytic acid}]_{ac}$$

$$[\text{starch}]_f = \%e * [\text{starch}]_e$$

$$[\text{alkylresorcinols}]_f = \%i * [\text{alkylresorcinols}]_i$$

$$[\text{wheat germ agglutinin}]_f = \%axis * [\text{WGA}]_{axis}$$

Figure 1: Concentration of specific biochemical markers which allow to monitor grain tissues were determined in fractions from grain processing. Percentage of each of the monitored tissue will be then determined using a set of equations taking into account the determined or mean quantity of the considered biochemical marker in the tracked tissue. Abbreviations in the equations are the following:

aleurone content (ac), aleurone cell walls (aw), endosperm (e), ferulic acid (FA), fraction (f), intermediate layer (i), p-coumaric acid (pCA), pericarp (p).

Particular focus was on the aleurone layer behaviour, taking into account its content in micronutrients and potential interest in increasing the nutritional value of fractionation products (*Antoine et al., 2002 ; Fenech et al., 2005 ; Buri et al. , 2004 ; Amrein et al., 2003*). Indeed, a simple quantification of the distribution of phytic acid in the different fractions from milling related to the grain could also be easily done and allow to estimate the fraction which contains the highest amount of aleurone cell content. This has allowed to demonstrate the distinct behaviour of the aleurone layer (*Greffeuille et al., 2005*) depending on the wheat hardness, *i.e.* a greater amount of phytic acid was found in flours obtained from hard wheat cultivars compared to soft wheats and this distinct behaviour was particularly observed at the breaking step. This difference in the aleurone tissue behaviour related to wheat grain hardness which is known to be controlled genetically was puzzling and could be either a consequence of the distinct mode of rupture of the starchy endosperm, or to differences in the starchy endosperm and outer layers separation or to intrinsic mechanical behaviour of the peripheral tissues.

- Grain tissue mechanical properties related to its fractionation behaviour and other physical characteristics of the wheat outer layers potentially interesting for their isolation: Therefore, intrinsic mechanical properties of grain outer layers from hard and soft common wheat cultivars displaying a distinct milling behaviour were analysed (*Greffeuille et al., 2006*). Uniaxial traction tests on grain outer layers (from the outer pericarp to the aleurone layer) clearly pointed out a distinct behaviour of tissues isolated by hand dissection from common wheat grains differing by hardness. Tissues isolated from soft wheat grains displayed a higher value of strain to rupture (**Fig. 2**) which was interestingly shown to be related with coarse bran size obtained after grain milling. Furthermore, these differences in mechanical properties of grain outer layers were also demonstrated using isolated tissues from near-isogenic lines of common wheat differing by hardness (*Greffeuille et al., 2007*).

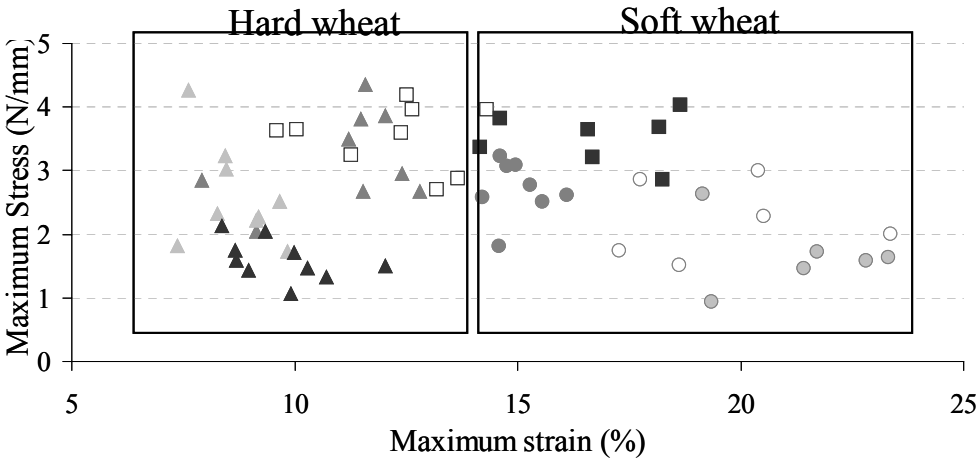


Figure 2: Maximum stress related to maximum strain values for isolated grain outer layers from six common wheat cultivars (o soft , Δ hard type) or from near-isogenic lines (■ soft, □ hard type) submitted to traction tests under controlled temperature and relative humidity conditions.

Thus, as the behaviour of each of the grain outer layers could play a key role in the difference of milling product composition, better characterization of their intrinsic mechanical properties and understanding of the factors involved in their mechanical resistance are essential. Each of the isolated tissues was already shown to display distinct properties (*Antoine et al., 2003*) and revealed the higher plasticity of the intermediate tissue composed of the inner pericarp, hyaline and testa. Furthermore the impact on the outer layer mechanical properties of physical parameters, such as water content and temperature was demonstrated (*Hemery et al., unpublished*). As these parameters play mainly a role in the plasticity and resistance to rupture of the material, they could be used in the process to target the

mechanical behaviour of these grain tissues either towards dissociation or tissue separation. In order to avoid the water soaking step of grains which is used to allow hand-isolation of the outer layers, original methods using pulse laser ablation was also developed to better assess the peripheral layer mechanical properties (*Martelli et al., 2009*). However, if the mechanical properties of the wheat tissues were mainly the determinant factors for their process behaviour using conventional process, it was already pointed out that innovative methods using electrostatic sorting of the particles after bran grinding could also be used to obtain better purified aleurone fractions (*Stone and Minifie, 1988 ; Bohm and Kratzer, 2005*). Thus, investigations of the tribo- and corona-charging properties of the ground bran and aleurone or pericarp enriched fractions were undertaken related to potentially important parameters as the particle size, the moisture content, or the sample composition (*Hemery et al., 2009b*). These studies further demonstrated the potential of electrostatic separation as distinct tribocharging properties of the pericarp and the aleurone enriched fractions were observed. Moreover, changes in these properties were obtained depending on the moisture content as well as the aleurone cell opening.

- Relationships between mechanical properties and cell wall structure

Mechanical properties of the grain tissues were characterised to better analyse their rupture behaviour and relationships with the corresponding tissue cell wall biochemical composition and structure (*Antoine et al., 2003; Greffeuille et al., 2007)*. But, if some relationships could be pointed out, clear involvement of a specific cell wall compound or compounds' interaction is rather difficult to determine. Therefore, grain tissue pre-treatments which are able to modify this composition or structure have been undertaken to better point out these relationships. For example, changes in the interaction between compounds of the outer layers' cell walls were obtained if peroxidase activities were increased via calcium soaking of wheat grains (*Desvignes et al., 2006*). These changes were found to correlate with strengthening of the mechanical properties of the corresponding tissues (**Fig. 3**).

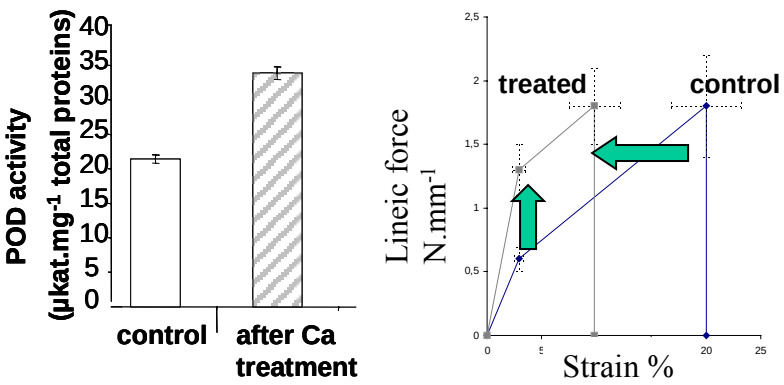


Figure 3: Changes in wheat grain peroxidase activities and wheat grain outer layers' mechanical properties after calcium soaking pre-treatment.

Changes in the mechanical behaviour of the wheat grain tissues were also observed after treatment with ozone which is known as a strong oxidant and a diffusible compound through the cell structure (*Desvignes et al., 2008*). The results demonstrate the impact of these modifications on the tissue mechanical properties and their effect on the tissue behaviour and the energy required for the fractionation process. However, more work is needed in order to better point out the involved factors and mechanisms.

- Relationships between grain tissue behaviour and fraction properties obtained by processing

Development of new tools to monitor each of the wheat tissue as well as to better characterize their properties is essential in order to control the fractions use-value. Moreover, increase in the knowledge of the relationships between composition and structure of all of the grain tissue, and particularly

tissues which either contain potentially interesting compounds for nutritional or technological properties or detrimental or even toxic molecules, also appears as a relevant objective.
For example, biochemical markers which were developed to monitor wheat tissue fate along fractionation were analysed in parallel with the removal of the contaminant *Fusarium* fungi and the corresponding produced mycotoxin, known as deoxynivalenol (DON), along durum wheat grain abrasion (*Rios et al., 2009a*). This study allowed to point out the efficiency of the pearling process towards milling for the decrease of the mycotoxin and microorganisms in the extracted products in accordance to other works showing the interest of debranning for the removal of wheat grain contaminants (*Aureli and d'Egidio, 2007 ; Fleurat-Lessard et al., 2007 ; Laca et al., 2006*). Indeed the pearling process allowed to reduce the mycotoxin content of the remaining grains of about 65-70 % whereas reduction of the DON level was only reduced at around 50-60 % by the milling process, for a similar 75 % extraction rate of the grain mass. Additionally, it showed a sharp reduction of *Fusarium* and mycotoxin amounts around 7-10 % of the dry mass removal which corresponds to the aleurone layer or the testa-aleurone interface (**Fig. 4**).

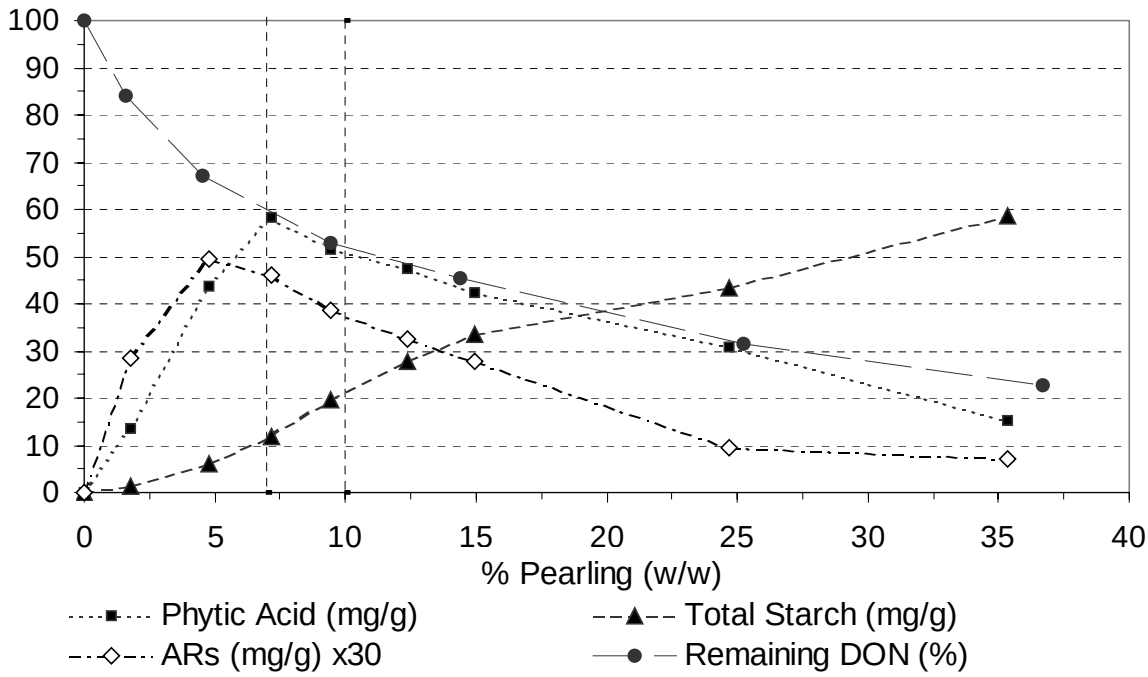


Figure 4: Monitoring of remaining DON according to mass removal by pearling. Alkylresorcinols (AR), phytic acid, and starch analysis allowed to monitor respectively the testa, the aleurone layer and the starchy endosperm.

These biochemical tools were also useful to monitor new grinding and separation technologies based on the better characterization of the tissue fate. Mechanical or physical tissue properties of the tissue or generated particles and knowledge of the process parameters effects will allow to better control the quality of the products obtained from fractionation or to develop new steps to increase the end-product value such as the nutritional properties.

Conclusions-Perspectives

Expected progress will be now more focused on the following points :

- demonstration of the relationships between cell wall compounds interaction and mechanical properties ;
- understanding of the relationships between corona- and tribo-charging and the nature of the tissue or the particle surface ;
- development of rapid screening methods for grain tissue monitoring that could be based potentially on the spectral tissue properties (*Barron and Rouau, 2008*) ;

- development of quick test to evaluate the mechanical resistance of grain and tissue and measurement of the minimum required energy for the tissue rupture ;
- elaboration of new diagram of fractionation which allow to lower the required energy for fractionation and adapt the process parameters in order to obtain the required end-value depending on the grain characteristics.

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