MOLECULAR BASIS OF THE WHEAT GRAIN KERNEL HARDNESS DETERMINED BY CONFOCAL RAMAN MICROSPECTROSCOPY

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Aims

Few things are known about the molecular basis which are involved in the wheat grain fractures induced during the first transformation of the wheat (milling process). The aim of our work is to use spectroscopic technique, particularly Raman scattering spectroscopy, in order to characterise the nature and the structure of the molecular species responsible of the *Triticum aestivum* wheat grain cohesion. The present work is more particularly focused on the kernel hardness. Indeed a better understanding of the molecular basis involved in the kernel cohesion could lead to an improved control of fragmentation during the milling process, and therefore to an increase of the milling value of wheat grains.

Nature of the study

Raman spectroscopy permits to identify in situ molecules and to characterise the binding between the molecular components of a sample. It is a non destructive analytical technique and is rapidly performed. Moreover, the coupling between a Raman spectrometer and an optical microscope with respect of confocality brings to the technique a spatial resolution at the micrometer scale. For instance, such analytical technique permits to determine the composition of the kernel and of the aleurone cell layer, and reveals molecular heterogeneity within the starchy endosperm and between aleurone cell walls.

Several hypothesis have been emitted about the molecular species responsible of the kernel cohesion and about the factors which influence the hardness. Indeed, the level of hardness would depend not only on the nature of the protein matrix, but also on the interface between starch granule and protein matrix. Specific protein, such as puroindoline-b or friabiline, and/or lipid component are likely to be involved in the kernel cohesion. Moreover, the role of endosperm cell walls has not yet been determined in the grain grinding ability.

Materials and methods.

Experiments were carried out on wheat (*Triticum aestivum*) samples supplied by INRA (Montpellier, France) and Champagne Céréales (Reims, France).

Investigations were led on wheat varieties of different levels of hardness and at different maturation stages, in order to underline differences in structure between *soft* and *hard* varieties. Raman spectra were recorded on 50 µm thick sections of wheat grain. Various reference products such as arabinoxylans and protein fractions were extracted and purified by INRA Montpellier.

Investigations were conducted using a Labram microspectrometer (Dilor, France) equipped with He/Ne laser as excitation source. The choice of a red excitation (632.8 nm) permits to obtain an intense Raman scattering of the wheat components and similarly by avoiding parasite fluorescence. It is also possible to adapt a moving XY plate in order to construct spectral imaging.

Results.

The protein content of the starchy endosperm has been characterised in situ. It appears a more important protein quantity in the subaleurone endosperm than in the central part of the kernel. The protein distribution within the starchy endosperm has been mapped by constructing spectral images. We have determined not only the primary structure of the protein matrix in various amino acid residues (phenylalanine, tyrosine, tryptophan) but also its secondary conformation. It has been underlined that the distribution of the protein α-helical structure, β-sheet or random coil is a good indicator of the kernel hardness. Indeed, it appears that α-helical structure gets more important during the kernel ripening, and that the *hard* variety has a proportion in α-helix much greater than the *soft* variety at the same maturation step. In order to go further in the molecular determination of the interface between the starch granules and the protein matrix, lipid content has also been investigated by using specific Raman vibrations of lipids. Preliminary results shows that the lipid content is localised at the starch granules contour. Concerning the role of the endosperm cell walls in the hardness criterion, the structure of the endosperm cell walls has been determined by comparing with reference arabinoxylans chains, of which the number of xylose, arabinose and ferulic ester is well controlled. It is therefore possible to determine the length of the arabinoxylans chain and the binding between the chains and the neighbouring molecules, for grains of different levels of hardness.

Conclusion

The use of spectroscopy in cereal science had already permitted to determine the protein and lipid contents of a cereal grain. The development of microspectroscopic technique offers the advantages of an in-situ and non destructive analysis, at the micrometer scale. It is now possible to characterise the molecular nature of the interface starch granule – protein matrix and of the endosperm cell walls. Moreover, vibrational spectroscopy such as FTIR or Raman gives information about the secondary structure of the protein. For instance, the distribution in α helical structure has been correlated with the hardness of the wheat grain kernel. In further investigations, we will extend our work to the fracture zones of the milling products at each grinding step of the mill. The aim is to achieve a 3D characterisation of fracture zones by Raman spectral imaging.

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