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Autofluorescence Multispectral Image Analysis at the Macroscopic Scale for Tracking Tissues from Plant Sections to Particles. Wheat Grain as a Case Study

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In the cereal milling industry, wheat grains are fractionated at a histological scale for recovering the starchy endosperm into flour or semolina and collecting the peripheral tissues in bran fractions. The proportions of each tissue in the resulting fractions impact their nutritional quality or their end-use properties. The evaluation of tissue dissociation at the particle scale is required in order to understand, control and optimize the fractionation processes. If the identification of tissues in wheat grain is commonly performed, it remains challenging for powders. In powders, methods are mainly based on their specific biochemical composition or their specific spectral properties, in particular their autofluorescence. These methods were developed to give quantitative assessments of a bulk tissue composition in flour or bran fractions. None of these methods allowed the estimation of tissue dissociation at the particle scale even when an imaging system has been used.

Recent equipments are available to acquire multispectral fluorescence images at the macroscopic scale using filters with specific excitation/emission wavelengths. These fluorescence macroscopes allow obtaining images of a representative number of particles together with a spatial resolution of less than 3 μm . In such images, the intensities measured for each pixel are not spectra, but are spectral profiles relevant to identify tissues (Baldwin *et al.*, 1997). To identify the tissular origin from this information, we propose to develop a prediction model on particles using calibration data coming from the observation of tissue sections. This approach is based on several assumptions. The first one is that the multispectral autofluorescence of plant tissues is specific and the second is that it is possible to measure fluorescence intensities in a reproducible way. The objective of the present work was to check the fluorescence microscope as an efficient device for measuring and comparing fluorescence intensities. Wheat was retained as a model plant for which two major tissues of the grain had specific autofluorescence properties: the aleurone layer with mainly a UV fluorescence response and pericarp that fluoresce using both UV and visible excitation wavelengths (Jensen *et al.*, 1982; Symons *et al.*, 1993). Moreover particles of pure tissue can be obtained after hand isolation or fractionation process (Hemery *et al.*, 2007). The autofluorescence properties of tissues in sections and particles were compared in two mounting media (air and water) using a multispectral fluorescence microscope.

The variability of fluorescence profiles was studied by selecting pixels in cross-section or in particles mounted in air or in water. The statistical variations were studied by principal component analysis and variance analysis. The first effect, mainly described by principal component 1, was to differentiate the two tissues, aleurone layer and pericarp. The differences between each tissue came from UV and visible filters as expected. The second effect, mainly described by component 2, was a difference between the two mounting media. The differences between sections or powders were not correlated to the other factors and were considered as not significant. Our results show that profiles extracted from multispectral images of cross-sections or particles are similar and allow the identification of plant tissues. Hence tracking the tissues by predicting them on images of particles from profiles found in images of cross-section should be possible. The choice of the mounting media is flexible, multiples options are viable, but the adopted solution must be strictly applied to all the samples analyzed. If implemented, the prediction from cross-section could be less tedious than other methods requiring dissection and lead to the identification of more tissues.

Keywords: process control,wheat,aleurone layer,pericarp,image analysis,tissue dissociation,multispectral autofluorescence