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Relative amounts of tissues in mature wheat (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition

C. Barron, A. Surget, X. Rouau*

INRA, UMR IATE (INRA, UMII, Agro.M, CIRAD), 2 Place Viala, 34 060 Montpellier, Cedex1, France

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Abstract

Hand dissection of mature grains from two common wheats (*Triticum aestivum* L., cv. Caphorn and cv. Crousty) were performed to quantitatively assess their tissue composition and to obtain homogeneous samples of embryonic axis, scutellum, starchy endosperm, aleurone layer, hyaline layer, outer pericarp and a composite layer made up of testa + hyaline layer + inner pericarp. Polymeric neutral sugar and phenolic acid contents of the samples were determined and used to identify specific composition patterns in each tissue irrespective of the cultivar. The scutellum and embryonic axis showed the lowest amount of carbohydrates with similar relative amounts of arabinose and xylose (Ara + Xyl), but the scutellum differed from the embryonic axis in its high phenolic acid, in particular ferulate dehydrodimer, content. The peripheral layers of the grains were mainly composed of cell wall polysaccharides, chiefly arabinoxylans but with differing structures. The hyaline layer was mostly composed of arabinoxylan with extremely low Ara/Xyl ratio (0.1), with high amounts of ferulic acid monomers and hence very weakly crosslinked. The aleurone layer differed from the outer pericarp by its much lower Ara/Xyl ratio and lower amounts of ferulic acid dimers and trimers. High proportions of ether-linked phenolic acids (released by alkali at 170 °C) were detected specifically in the seed coat and tissues in the crease region. The possible application of biochemical markers found in the various tissues to monitor wheat grain fractionation processes is discussed.

Keywords: Wheat grain; Aleurone; Germ; Scutellum; Pericarp; Arabinoxylan; Phenolic acids

1. Introduction

Traditionally, wheat grain fractionation is performed using a dry milling process which leads to the separation of flour and semolina (from the starchy endosperm) from the germ and bran (which are recovered either in a single fraction or as two separate fractions, depending on the milling procedure). However valuable nutritional constituents including micronutrients, phytochemicals and fibres are particularly concentrated in the germ and bran fractions. These milling fractions are not homogeneous with respect to tissue and biochemical composition. Some of the components of these fractions being potentially detrimental to food processing or pose nutritional problems should not be included into the final flours. Monitoring grain dry fractionation processes to recover all the potential nutritional benefits while discarding unwanted fractions, requires that the fate of the different tissues be tracked during processing. Biochemical markers have been used successfully to follow the behaviour of the aleurone and pericarp fractions in peripheral layers from grains of soft wheat (Antoine et al., 2004) and durum wheat (Peyron et al., 2002) during fractionation. To increase the efficiency of fractionation monitoring tools, the compositional heterogeneity of each of the grain tissues must be clearly assessed.

The different tissues constituting the wheat grain are generally described in terms of their embryogenic origin and structure (Bradbury et al., 1956a; Evers and Bechtel, 1988). The peripheral tissues of the grain overlying the

Abbreviations: Ara, arabinose; Ara/Xyl, arabinose to xylose ratio; ESEM, environmental scanning electron microscopy; FAm, *cis*- and *trans*-ferulic acid; FAd, dimeric forms of ferulic acid; FAt, 4-O-8′, 5′-5″ ferulic acid dehydrotrimer; Gal, galactose; Glc, glucose; Man, mannose; p-CA, *para*-coumaric acid; PCA, principal component analysis; Rha, rhamnose; Rib, ribose; SA, sinapic acid; Xyl, xylose

*Corresponding author. Tel.: +33 4 99 31 04; fax: +33 4 99 61 30 76.
E-mail address: rouau@ensam.inra.fr (X. Rouau).

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starchy endosperm, are made up successively from the outer to the inner surface, comprise the outer pericarp (beeswing bran), the inner pericarp (comprising cross cells and tube cells), seed coat (testa), hyaline layer (nucellar epidermis) and the aleurone layer. Thus the peripheral layers of mature grain are multilayered with layers of each tissue type of different thickness and closely associated with one another. The aleurone layer is the thickest (up to 65 μm), the outer pericarp is of intermediate thickness (15–30 μm) and the seed coat the thinnest (5–8 μm). The germ comprises the embryonic axis and the scutellum. Global compositions have been recorded for germ (mostly embryonic axis), the aleurone layer and outer pericarp (reviewed by Fincher and Stone, 1986; Pomeranz, 1988). The carbohydrate and phenolic acid compositions of the outer pericarp and aleurone layer (Antoine et al., 2003; Rhodes et al., 2002) indicated the presence of high amounts of cell wall polysaccharides, especially highly feruloylated arabinoxylans showing different structural features. More recently, Parker et al. (2005) focused on the composition of the bran layer of mature wheat grain, and in particular the inner pericarp and seed coat plus hyaline layer. However, due to the difficulty of completely dissecting tightly associated tissues, there are no compositional data, e.g. for the hyaline layer or scutellum. The isolation and determination of the complex compositional patterns of carbohydrates and phenolic acids for each tissue, which has already revealed their potential as tissue markers, is essential for the selection of improved markers for monitoring grain fractionation.

We have established the composition of each tissue of common wheat grains by first, determining the relative amounts of each tissue within the grain and then establishing their neutral sugar and phenolic acid compositions. The chromatographic methods used allowed the recording of the many different sugars and phenolic acids present by performing only two analyses per sample, which is advantageous with respect to the development of a tissue marker methodology.

2. Experimental

2.1. Tissues isolation

Two common wheat cultivars differing in kernel hardness were used: Caphorn (hard) and Crousty (soft). Non-adhering tissues (pericarp and modified aleurone layer (Bradbury et al., 1956b, c)) surrounding the embryonic axis were first removed and then the embryo was separated from dry grains with a needle. To facilitate endosperm dissection and to avoid losses of cell wall polysaccharides by dissolution, all the following steps were performed on grains soaked in 50% aqueous ethanol for 16 h at room temperature. To make the pretreatments comparable, the dissected embryos were also soaked in 50% aqueous ethanol for 16 h after which the scutellum was removed with a scalpel. The endosperm and peripheral layers were dissected from degerminated grain. First the crease was removed and the starchy endosperm was scraped away from the aleurone layer inner surface with a scalpel. Clean peripheral layers were further dissected with a scalpel: first the loosely attached and easily removed outer pericarp was removed, then the aleurone layer was dissected from the intermediate layer (composed of inner pericarp, seed coat and hyaline layer). In some cases, the composite intermediate layer was imbibed in water to allow specific recovery of the hyaline layer on its inner side. Dissected tissues were dried at 25 °C over phosphorus pentoxide (P_2O_5) and ball-milled for 5 min before chemical analysis.

To allow comparison of the effects of soaking in water and 50% ethanol prior to dissection, the aleurone, intermediate layer and outer pericarp were also dissected from degerminated grains after hydration in water for 16 h.

2.2. Determination of the relative amount of tissues within the grain

Similar hand dissections of 20 grains were performed in quadruplicate to quantify the relative amounts of the different tissues. All recovered tissues were weighed after drying over P_2O_5. Bran composition was deduced from the combined weights of the aleurone, intermediate layer and outer pericarp, dissected from peripheral layers without the crease.

2.3. Chemical analysis

2.3.1. Phenolic acids

Ester-linked phenolic acids were saponified under oxygen-free Ar at 35 °C in 2 M sodium hydroxide (Antoine et al., 2003). Ether-linked phenolic acids were released at 170 °C in 4 M sodium hydroxide (Grabber et al., 1995). An internal standard (2,3,5 trimethoxy-(E)-cinnamic acid (TMCA), T-4002, Sigma Chemical Co., St. Louis, USA) was added before adjusting pH to 2. Phenolic acids were then extracted with diethylether and quantified by RP-HPLC (Antoine et al., 2003). The response factors of the following forms of ferulic acid dehydrodimers (FAd) (8-O-4′, 8,5′ benzofuran, 5,5′) and ferulic acid dehydrotrimer (FAt) relative to the internal standard were determined at 320 nm with purified samples. The ferulic acid monomer (FAm) content was calculated from the amount of *cis*- and *trans*-ferulic acid (FAm) and the total amount of ferulic acid dehydrodimers (8-O-4′, 8,5′, 8-5′ benzofuran, 5,5′ forms) (Fad). All analyses were performed at least in duplicate unless otherwise stated, and the standard error of the mean was less than 10%.

2.3.2. Neutral sugars

Neutral sugars were quantified as anhydro-sugars determined by gas–liquid chromatography (DB 225 capillary column) of their alditol acetates obtained after sulphuric acid hydrolysis (1 M, 2 h, 100 °C) of the samples (Blakeney et al., 1983). A prehydrolysis step (13 M