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Assessment of biochemical markers identified in wheat for monitoring barley grain tissue

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ABSTRACT

The possible use of specific biochemical compounds identified in wheat grains was evaluated for monitoring barley grain tissues during fractionation. First barley grain anatomy was studied through microscopic observation and quantification of the relative proportion of each anatomical part in four distinct barley samples from both hulled and hulless genotypes. As expected from cereal phylogeny and irrespective of the possible presence of hull, common features were observed between barley and wheat grains, but the aleurone layer predominated in the outer layers. The specific location of the compounds identified in wheat was established. Phytic acid was specifically localized in the aleurone layer and alkylresorcinols in the composite layer containing the testa, even if their concentration differed from that observed in wheat grain tissues. Thus, these two markers identified in wheat can be used to monitor the corresponding barley tissues, independent of the presence of hulls. Conversely, phenolic compounds, either ferulic acid trimer or *p*-coumaric acid, cannot be used to monitor respectively the outer pericarp or the aleurone cell walls in barley grains. *p*-coumaric acid was identified as an efficient marker of the hull and could be used to distinguish hulled or hulless barley grains and to help monitor the dehulling process.

1. Introduction

Barley (*Hordeum vulgare* L.) belongs to the grass family of Poaceae, more specifically to the Triticeae tribe, like the main small cereal grains, wheat (*Triticum* spp.) and rye (*Secale cereale*). Barley is closely related to these two cereal species although it diverged approximately 11.6 million years ago and has further diversified into several subspecies (Chalupska et al., 2008). The major anatomical parts of the barley plant are typical of other members of Triticeae, in particular the kernel, which is an indehiscent fruit type, called caryopsis. In this grain, the future plant is accompanied by the endosperm comprising a storage tissue, called the starchy endosperm, and the aleurone layer. Both the embryo and the endosperm are surrounded by protective layers of maternal origin (the residue of nucellar epidermis, the testa and the pericarp). As also observed in wheat and rye, the outer layers, i.e. from the aleurone layer to the pericarp, are partly enclosed in the grain ventral side and create a crease dividing the grain in half longitudinally, but the crease is shallower in barley grains (Evers et al., 1999). The outermost layers of the barley grain were composed of structures resulting from spikelet differentiation, the palea and lemma. They are usually tightly stuck to the pericarp, which is considered desirable for selection of varieties for malting (Hoad et al., 2016). In the specific case of barley genotypes classified as hulless, this hull could be easily removed by combining threshing and cleaning of the grain.

Barley is primarily used for animal feed (60–70% of total production), then for malting and only small amounts (<1%) are used for human food (Sahlstrøm and Knutsen, 2010). But today there is a growing interest in completely or partially replacing wheat with barley flour given the potential nutritional health benefits of barley grains (Baik and Ullrich, 2008). Barley grains are relatively rich in β-glucan, a soluble fibre whose ability to reduce cholesterol and blood glucose after a meal, allowed a health claim to be recently approved by the European Food Safety Authority. Moreover, like other whole

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List of abbreviations

p-CA	para-coumaric acid
d.m.	dry matter
FAt	dehydrotriferulic acid (8-O-4′,5′-5″ form)
AL + NE	composite isolated layer made of the aleurone layer and the residue of nucellar epidermis
T + P	composite isolated layer made of the testa and pericarp

cereal grains, they are rich in mineral, fibres or micronutrients. The introduction of barley as an ingredient is not straightforward and the use of barley in palatable and acceptable food products implies the use of different barley types (hulless, proanthocyanidin-free grains) or a different method of processing the grain (Baik, 2014). Since the constituents are not evenly distributed in the grain, dry milling could be used to produce fractions rich in starch and protein, fibres or other bioactive compounds (as reviewed in Baik (2014)). Dry processing of barley grains is mainly based on abrasion/pearling in order to sequentially remove the hulls and the outer layers before possible grinding steps. If wheat milling facilities are to be used, barley grain specificities, e.g. grain hardness, bran brittleness and endosperm composition, will need to be taken into account (Baik, 2014). The rational development of efficient processes to obtain value added ingredients requires the ability to monitor the abrasion/pearling process and the fate of grain tissues, in the same way as for wheat grain (Hemery et al., 2007). This strategy relies on the identification of tissue-specific compounds whose concentration measured in mill streams could be directly linked to the proportion of a tissue, knowing their concentration in pure isolated tissues (Hemery et al., 2009). In wheat grains, phenolic compounds (a ferulic acid trimer and *p*-coumaric acid), alkylresorcinols and phytic acid have been shown to be specifically located in the outer pericarp, aleurone cell walls, testa and aleurone cell content, and were successfully used to monitor tissue distribution during traditional milling (Hemery et al., 2009; Raggiri et al., 2016), pearling process (Hemery et al., 2009) or innovative dry processing (Chen et al., 2013; Hemery et al., 2011). The close genetic relationship between barley and wheat grains and hence the similarity in grain structure and composition led to the hypothesis that the same biochemical tools previously identified for wheat grain tissue could also be used to monitor barley tissues.

The objective of this study was to evaluate the feasibility and the limits of this strategy. First, the barley (hulled or hulless) grain anatomy was studied through microscopic observation and the relative proportion of each anatomical part was measured. Second, distribution and quantification of the biochemical markers identified in wheat were studied in barley grain tissues to assess the efficiency of these markers for monitoring the barley grain tissue.

2. Experimental

2.1. Materials

Four different norwegian produced 2-rowed barley varieties were used in this study, including two hulled barley genotypes (Olve and Marigold) and two hulless barley genotypes (Pihl and Pirona). Their thousand kernel weight (TKW) was determined by weighing a 30 g-sample and counting the grains using an electronic seed counter. Results are reported as mean mass (g) of thousand kernels (ISO 108 International Standard 520:2010). At least three determinations were performed for each sample.

Barley grain tissues were isolated from these grains for chemical analyses and microscopic observations. Grains were cut to remove the germ and the brush. They were soaked in distilled water overnight to facilitate separation of the endosperm. For hulled grains, the loosely attached hulls were first removed and palea and lemma easily differentiated and separated into different samples. The crease was then removed and starchy endosperm scraped away. The aleurone layer + the residue of nucellar epidermis (AL + NE) were isolated from the testa + pericarp (T + P) layer with a scalpel. Dissected tissues were dried at 25 °C over phosphorus pentoxide (P_2O_5). The tissues were then ground in liquid nitrogen with a Spex CertiPrep 6750 laboratory impact grinder and further dried before chemical analysis.

2.2. Determination of the relative amount of main tissues within barley grains

The relative proportion of the main barley grain tissues was determined through hand-isolation and gravimetric measurements according to the procedure of Raggiri et al. (2016) slightly modified. The following tissues were hand-isolated: the embryonic axis, the scutellum, the starchy endosperm, the outer layers (made of the aleurone layer, the residue of the nucellar epidermis, the testa and the pericarp), and the hull (if present). Twenty barley grains were hand dissected in quadruplicate and the recovered barley tissues were weighed after drying on P_2O_5 until a constant mass was obtained or freeze-drying (in the case of the starchy endosperm). The relative proportion of each tissue was calculated by dividing the weight of the tissue by the weight of the initial grains (each in dry matter). The composition of the outer layers was deduced, as detailed by Barron et al. (2007), from the combined weight of AL + NE and T + P layer, dissected from outer layers especially prepared without the crease. To compare the means, Fisher's least significant difference (LSD) test was conducted at the 5% significance level.

2.3. Chemical analyses

2.3.1. Phytic acid

The phytic acid content was measured at 500 nm from acidic extracts according to a colorimetric method (Hemery et al., 2009). A standard curve was obtained with corn phytate (P-8810, Sigma) solutions of known concentrations. Samples were analysed in duplicate with a relative mean deviation <5%.

2.3.2. Alkylresorcinols

Total alkylresorcinols were extracted with two successive extractions with n-propanol, for 2 h each at 20 °C and an aliquot fraction measured through a colorimetric method (Hemery et al., 2009), using the coupling of alkylresorcinol to fast blue reagent and evaluation of the absorbance changes at 520 nm. The total amount of alkylresorcinols was estimated by comparison with a calibration curve (0–100 μg mL^{-1}) prepared with olivetol (5-pentyl-1,3-benzediol) as the reference molecule. Samples were analysed in duplicate with a relative mean deviation <5%.

2.3.3. Phenolic acids

Ester-linked phenolic acids were saponified under argon (oxygen-free) at 35 °C in 2N sodium hydroxide. An internal standard (2,3,5-trimethoxy-(E)-cinnamic acid (TMCA), T-4002, Sigma Chemical Co., St Louis, USA) was added before adjusting the pH to 2. Phenolic acids were then extracted with diethyl ether and quantified by RP-HPLC as described by Hemery et al. (2009). The response factors of the para-coumaric acid (*p*-CA) and the 4-O-8′, 5′-5″ dehydrotriferulic acid (ferulic acid trimer, FAt) relative to the