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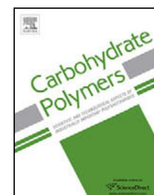

Relationships between protein content, starch molecular structure and grain size in barley

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ABSTRACT

Correlations among barley protein, starch molecular structure and grain size were determined using 30 barley samples with variable protein contents. Starch molecular structure was characterized by fluorophore-assisted carbohydrate electrophoresis and by size-exclusion chromatography (SEC, also termed GPC). The chain-length distributions of amylopectin were fitted using a mathematical model reflecting the relative activities of starch branching enzymes and starch synthase enzymes. Increased protein content significantly and negatively correlated with higher amounts of amylose with longer chains (degree of polymerization, DP 1600–40000) while barley grain sizes positively associated with starch contents. Protein content also positively correlated with the proportion of longer chains of amylopectin (DP 34–100). These results showed that the enzyme activities of starch synthases change with protein content, leading to altered starch contents, structures and grain sizes. From this perspective, selecting for large grain size (or low protein content) does not necessarily relate to starch structure, although may suggest long chains of amylopectin. Measuring starch structure could give a good indication of process performance in human food, animal feed and brewing, as all these structural features contribute to significant functional properties.

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1. Introduction

Barley (*Hordeum vulgare L.*) ranks fourth in the world in terms of cereal production, following maize, rice and wheat (Yangcheng, Gong, Zhang, & Jane, 2016). Barley is extensively used to feed animals, while it is also used as raw material for malting and subsequently the production of beer. Barley is also becoming appreciated as a component of a healthy diet, especially in some western countries (Baik & Ullrich, 2008), as a result of its high β-glucan content. In barley, starch is mostly stored in the endosperm and ranges from 62 to 77% of total grain dry weight (Asare et al., 2011; Bhatty & Rossnagel, 1998). The digestion rate of starch is nutritionally important when barley is used as human food and animal feed: a slow rate of digestion is important for human health (which can help in terms of obesity, diabetes and colo-rectal cancers), and a rapid rate for animal feed (leading to rapid weight gain in the growing animal and high energy availability during food production from the animal). Barleys for these uses are selected by various criteria which may not be directly related to the desired final outcome: for example, in brewing, barley is currently largely selected based on its grain size and protein content, even though it is actually the starch (not the protein) from which the fermentation sugars are derived during mashing (Asare et al., 2011). All of these processes involve enzymatic digestion of starch.

Starch enzymatic digestion is affected by many factors (Ahmed, Tetlow, Ahmed, Morell, & Emes, 2015; Celus, Brijs, & Delcour, 2006; Galvis, Bertinetto, Holopainen, Tamminen, & Vuorinen, 2015; Slack, Baxter, & Wainwright, 1979), including the size distributions of starch granules (Asare et al., 2011; Chiotelli & Le Meste, 2002), the diffusion of enzyme through the granule, the presence of enzyme inhibitors (Sancho et al., 2003) and starch-associated compounds including amylose-lipid complexes (Al-Rabadi, Gilbert, & Gidley, 2009), and the amylose/amylopectin ratio (Ahmed et al., 2015;

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Hang, Obert, Gironella, & Burton, 2007; Slack et al., 1979). Protein, which is typically 10–12% of total dry weight in barley can decrease the digestion rate of barley starch through the interaction with starch granules, thereby affecting the level of modification of starch during malting (Borén, Larsson, Falk, & Jansson, 2004; Fox et al., 2007; Slack et al., 1979). This is separate from any nutritional value of the protein when barley is used as human food or animal feed; high protein content is not preferable in brewing because it may not only affect the degradation rate of starch, but also affect the qualities of the malt and the wort, and affects beer quality (Jin, Du, Zhang, Xie, & Li, 2012).

Proteins can have some obvious effects on enzymatic degradation of starch, such as by physically hindering access of degradation enzymes to starch (Zou, Sissons, Gidley, Gilbert, & Warren, 2015). In barley, it is known that the starch granules can remain embedded within a protein matrix, reducing the digestion of barley starches by α-amylase, even when the endosperm cells have been broken during grinding (McAllister, Phillippe, Rode, & Cheng, 1993). Scanning electron microscopy showed that compared with good malting barley cultivars, the spaces between adjacent starch granules in poor malting barley varieties were filled with protein matrix in which small starch granules were completely embedded, indicating a high degree of starch-protein association, thus decreasing the degradation rate of starch during malting (Brennan, Harris, Smith, & Shewry, 1996). Hordein, which accounts for 40- 50% of total protein, is in protein bodies bound around starch granules in the barley endosperm, and can therefore inhibit the enzyme susceptibility of starch granules during digestion in the human and animal gastrointestinal tract, and during malting and mashing in brewing (Darlington et al., 2000; Slack et al., 1979).

In addition to protein-starch granule interactions, starch structural differences, including amylopectin and amylose chain length distributions (CLDs), can also affect starch digestion rate (Asare et al., 2011). For example, *in vitro* digestion experiments showed that barley varieties with lower amylose contents contain more rapidly digested starch (Asare et al., 2011). Also, like other cereals, barley starch digestion rate is strongly correlated with the fraction of short glucan chains (Shrestha et al., 2015). Despite a common error in many texts and papers, amylose contains a significant number of branches, although many less than in amylopectin (Hizukuri, Takeda, Yasuda, & Suzuki, 1981). This can be proved by comparing the weight distributions of fully branched and debranched amylose, which are significantly different. One of many examples in the literature is found in the present paper, comparing the maxima in these significant distributions given in the Supporting information ($R_{\text{h,AM(de)}}$ in Table S1 and $R_{\text{h,AM}}$ in Table S2).

There have only been limited reports of the molecular structure of barley starches (Asare et al., 2011), although the relations between starch structural features and other grain parameters, including protein content and grain size, are essential in determining end-use of barley products. Currently, commercial barley is chosen based on its protein content and/or grain sizes, two factors which may actually have no direct relationship to the final quality of utilization, especially in brewing. This work aims to find a better way to distinguish barley quality by taking the starch molecular structures into account through studying the relationship between these three factors. The molecular structure is characterized in our study by the chain length distributions (CLDs) and the molecular sizes of amylose and amylopectin, using fluorophore-assisted carbohydrate electrophoresis (FACE) and size-exclusion chromatography (SEC, also termed GPC), respectively.

Statistical methods will be used to relate these various grain features; this requires efficient parameterization of the data. In cereal grains, starch is synthesized in various organs (primarily in the endosperm), and its structure is determined by the coordinated action of a series of enzymes, granule-bound starch synthases

Table 1
Chemical composition of barley varieties.[a]

genotypes	Locations	protein content/%[b]	starch content/%[b]	moisture/%
Commander	Emerald	(14.6 ± 0.6)[c]	(53.7 ± 2.96)[ab]	(12.1 ± 0.6)[a]
Commander	Jimbour	(13.2 ± 0.4)[b]	(55.8 ± 0.8)[b]	(14.2 ± 0.5)[c]
Gairdner	Emerald	(15.1 ± 0.9)[c]	(53.9 ± 2.4)[ab]	(12.2 ± 0.1)[a]
Gairdner	Jimbour	(13.2 ± 0.7)[b]	(54.1 ± 1.5)[ab]	(13.7 ± 0.6)[c]
Grout	Emerald	(13.1 ± 0.5)[b]	(53.2 ± 1.3)[ab]	(12.3 ± 0.4)[a]
Grout	Jimbour	(11.6 ± 0.9)[a]	(54.3 ± 1.3)[ab]	(13.6 ± 0.8)[c]
Hindmarsh	Emerald	(14.6 ± 0.1)[c]	(51.1 ± 0.2)[a]	(11.8 ± 0.3)[a]
Hindmarsh	Jimbour	(13.0 ± 0.7)[b]	(53.4 ± 1.7)[ab]	(13.4 ± 0.5)[bc]
LaTrobe	Emerald	(15.0 ± 0.4)[c]	(53.0 ± 2.0)[ab]	(12.6 ± 0.2)[ab]
LaTrobe	Jimbour	(12.4 ± 0.4)[ab]	(53.8 ± 0.2)[ab]	(13.6 ± 0.5)[c]

[a]Data are the average of three replicates of each cultivar based on duplicate measurements. b, on dry basis. Samples with different letters in the same column are significantly different at $p < 0.05$.

(GBSS), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (DBE). Fitting the FACE data with a model developed by Wu, Morell, and Gilbert (2013), which is based on the biosynthetic processes involving these enzymes, gives the ratios of the activities of the various enzymes *in planta*, which in turn relates to the underlying genetics/environmental effects; this reduces the amylopectin CLDs to a small number of biologically meaningful parameters. Other structural data are parameterized empirically. This study also provide a new method for barley breeders to have a better understanding of granular starch biosynthesis during kernel development and to use this to select and develop varieties containing starch with improved functionality.

2. Materials and methods

2.1. Materials

Five cultivars of barley with three sample replicates from the 2013 National Variety Trials were grown in two different locations (Emerald and Jimbour, both in Queensland, Australia), as listed in Table 1. These five cultivars were a subset from a larger set grown in a complete randomized block design. Each field plot was 1.2 m wide × 6 m long. Plots were mechanically harvested and grain collected in cloth bags for later analysis. Pepsin from gastric porcine mucosa, protease from Streptomyces (type XIV) and porcine pancreatic α-amylase were from Sigma-Aldrich. Isoamylase (from *Pseudomonas*) and a total starch (AA/AMG) assay kit were from Megazyme International Ltd. Pullulan SEC standards with known peak molecular weights were from Polymer Standards (PSS) GmbH (Mainz, Germany); dimethyl sulfoxide (DMSO, GR grade) was from Merck Co. Inc. All other chemicals were of reagent grade and used as received.

2.2. Grain size

Barley samples were sieved using an Agtator with 2.2 mm and 2.5 mm sieves. Approximately 100 g of grain was sieved with 45 shakes in one minute. Grain that passed the 2.2 mm sieve were recorded as screenings (Scr) and grain that remained above the 2.5 mm sieve were recorded as retention (PG). Grain density was measured with a grain tester (Dickey-John) and recorded as kg/L (hectoliter weight, HLW).

2.3. Extraction of barley starch molecules for structural analysis

The extraction process was chosen to ensure complete molecular dissolution of the starch without degradation (Syahariza, Li, & Hasjim, 2010). 10 g barley seeds were ground using a cryogrinder (Freezer/Mill 6850 SPEX, Metuchen, NJ, USA) with liquid

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