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Barley Grain Constituents, Starch Composition, and Structure Affect Starch in Vitro Enzymatic Hydrolysis

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ABSTRACT: The relationship between starch physical properties and enzymatic hydrolysis was determined using ten different hulless barley genotypes with variable carbohydrate composition. The ten barley genotypes included one normal starch (CDC McGwire), three increased amylose starches (SH99250, SH99073, and SB94893), and six waxy starches (CDC Alamo, CDC Fibar, CDC Candle, Waxy Betzes, CDC Rattan, and SB94912). Total starch concentration positively influenced thousand grain weight (TGW) ($r^2 = 0.70, p < 0.05$). Increase in grain protein concentration was not only related to total starch concentration ($r^2 = -0.80, p < 0.01$) but also affected enzymatic hydrolysis of pure starch ($r^2 = -0.67, p < 0.01$). However, an increase in amylopectin unit chain length between DP 12−18 (F-II) was detrimental to starch concentration ($r^2 = 0.46, p < 0.01$). Amylose concentration influenced granule size distribution with increased amylose genotypes showing highly reduced volume percentage of very small C-granules (<5 μm diameter) and significantly increased ($r^2 = 0.83, p < 0.01$) medium sized B granules (5−15 μm diameter). Amylose affected smaller (F-I) and larger (F-III) amylopectin chains in opposite ways. Increased amylose concentration positively influenced the F-III (DP 19−36) fraction of longer DP amylopectin chains (DP 19−36) which was associated with resistant starch (RS) in meal and pure starch samples. The rate of starch hydrolysis was high in pure starch samples as compared to meal samples. Enzymatic hydrolysis rate both in meal and pure starch samples followed the order waxy > normal > increased amylose. Rapidly digestible starch (RDS) increased with a decrease in amylose concentration. Atomic force microscopy (AFM) analysis revealed a higher polydispersity index of amylose in CDC McGwire and increased amylose genotypes which could contribute to their reduced enzymatic hydrolysis, compared to waxy starch genotypes. Increased β-glucan and dietary fiber concentration also reduced the enzymatic hydrolysis of meal samples. An average linkage cluster analysis dendrogram revealed that variation in amylose concentration significantly ($p < 0.01$) influenced resistant starch concentration in meal and pure starch samples. RS is also associated with B-type granules (5−15 μm) and the amylopectin F-III (19−36 DP) fraction. In conclusion, the results suggest that barley genotype SH99250 with less decrease in grain weight in comparison to that of other increased amylose genotypes (SH99073 and SH94893) could be a promising genotype to develop cultivars with increased amylose grain starch without compromising grain weight and yield.

KEYWORDS: *Hordeum vulgare*, amylopectin chain length distribution, amylose, rapidly digestible starch, slowly digestible starch, resistant starch

INTRODUCTION

Barley (*Hordeum vulgare* L.) cultivation and utilization ranks fourth after maize, rice, and wheat.[1] However, barley is the least utilized cereal for human food consumption. It is poor man's food in underdeveloped countries. In contrast to this, in western countries it is fast becoming a part of a natural healthy diet.[2] Barley is an excellent source of complex carbohydrates and β-glucans, two important constituents of dietary fiber. This has led to the wide acceptance of barley as human food with significant human health benefits.[3−6] A growing number of health conscious consumers have increased demand for foods such as barley. Starchy food after ingestion is assimilated in the upper gastrointestinal tract. A variable portion of starch, not assimilated in the upper gastrointestinal tract, classified resistant starch (RS), reaches the large intestine where its fermented products, primarily short chain fatty acids (SCFA), help in maintaining healthy viscera.[7] In addition, SCFA also lower lumen pH creating a less conducive environment for cancer and other diseases.[8]

Digestion of starchy foods is a complex process, affected by the rate of starch digestion and absorption, including the source of food material, its components, physical nature, presence of enzyme inhibitors, antinutrients, and processing methods.[9,10] Rate of carbohydrate absorption in fiber rich food is low due to the high viscosity created in the upper digestive tract.[11,12] It is believed that enzymes responsible for carbohydrate hydrolysis are excluded by the fiber components in food thereby preventing/slowing the rate of hydrolysis.[10]

On the basis of plant source and processing methods, RS is classified into four major types. RS type 1 is trapped in plant architecture helping escape from amylolysis. Physical damage via chewing or milling makes them accessible. RS type 2 is derived

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from specific plant sources such as green banana or starch with poor gelatinization and hence slower hydrolysis (e.g., high amylose corn starches). Increase in the amylose content of starch granules favors increase in dietary fiber and resistant starch content.[13] RS type 3 is formed via retrogradation after cooking and RS type 4 includes chemically modified starches.[14]

In addition to 60−80% of carbohydrates in barley grain, it also contains 9−13% nitrogenous compounds, 1−2% fat, and 10−15% water.[15] The predominant carbohydrate in barley grain is starch, and it ranges from 62 to 77% of the grain dry weight.[16] Starch physicochemical properties and end use are significantly affected by the amylose to amylopectin ratio and other storage compounds.[17,18] On the basis of amylose concentration, barley starch can be classified into normal (∼25−27% amylose), waxy (nondetectable to <5% amylose), and increased amylose (>35% amylose).[16,18−20] Increased amylose starch food is not completely digested in the small intestine of monogastric animals and is, therefore, classified as resistant starch.[8,21] Waxy starch (95 to 100% amylopectin) finds its use in the food industry for improvement of properties including uniformity, stability, texture, and better freeze−thaw ability of the food products.[22]

Starch hydrolysis by α-amylases is influenced by various physical and structural features including granule size; phosphorus content; complexes between amylose and lipid;[23] distribution and perfection of crystalline region in both amorphous and crystalline lamellae;[24] starch crystallinity and packing;[25] porosity; structural inhomogeneity and degree of integrity;[26] connectivity between hilum and surface channels;[27] interaction of amylopectin chains during hydrolysis; and the extent of helix formation in amylose and amylopectin.[28,29] Inhibition of α-amylases by maltose and maltotriose may also be relevant.[30] During cooking, starch is gelatinized, and amylose molecules are leached out of the swollen starch granules as coiled polymers which on cooling associate as double helices and form hexagonal networks.[29,31] In waxy starches instead of this network, aggregate formation occurs, which is more susceptible to hydrolysis by amylases.[28] In brief, the factors that hide starch from amylases contribute to resistant starch content.

Amylopectin chain length distribution (CLD) and packing have been reported to play an important role in starch digestibility. In increased amylose rice, RDS is reported to be strongly correlated with short chains, while RS is associated with long and intermediate chain fractions.[32] On the contrary, amylopectins of increased amylose barley varieties did not show significantly larger proportions of long chains than that of normal and waxy barley starch.[33] In Cassava, longer chains of amylopectin form complexes with amylose and contribute to the starch high gelatinization group.[34] Srichuwong and co-workers[35] analyzed starches from different botanical sources and reported no significant correlation between the functional properties of starch with average granule size, shape, or apparent amylose content but strong correlation with amylopectin CLD. In cereals, both amylose and amylopectin contribute toward SDS formation.[36]

Endogenous and externally added lipid and proteins also affect starch digestibility and resistant starch formation.[26] Complexes with long chain fatty acids are more resistant than complexes with shorter chains as amylose becomes less available to form network aggregates.[37]

The differences in starch composition and structure can be utilized in food applications to reduce the risk of diabetes and/or digestive tract related diseases. In the present study, grain composition and starch properties were analyzed from ten different hulless barley genotypes with varying amylose concentration, an important feature for RS formation. The major objective of the study was to study the influence of grain constituents and starch properties on the rate of in vitro enzymatic hydrolysis of barley pure starch and meal samples with varied amylose concentration.

MATERIALS AND METHODS

Plant Materials. The study was conducted with ten hulless barley (*Hordeum vulgare* L.) genotypes, including one normal starch (CDC McGwire), six with waxy starch (CDC Candle, CDC Alamo, CDC Rattan, CDC Fibar, SB94912, and waxy Betzes) and three with increased amylose concentration (SH99250, SH99073, and SB94983). Three replicates of all the genotypes were grown in standard small plots at Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, Canada, in 2005, except for CDC McGwire which was grown in 2003, and waxy Betzes grains were taken from long-term storage. One thousand seeds from each genotype were weighed for thousand grain weight (TGW) determination. Ten grams (10 g) of barley seed was ground through a 0.5 mm sieve by UDY mill (UDY Corporation, Fort Collins, CO).

Protein Concentration. Protein concentration was determined by the combustion method with the FP-528 Protein/Nitrogen Analyzer (LECO Corporation, St. Joseph, MI). Meal samples (0.25 g ± 0.01 g) in duplicates were combusted for protein analysis. Percent protein concentration of samples was obtained using the formula % P = % N × C, where C is 5.7 for wheat, while it is 6.25 for all other crops.[38]

Crude Lipid Concentration. Crude lipid concentration was determined by the Goldfisch lipid extraction method, using hexane as the extraction solvent.[38] Barley flour (2 g) was placed in Whatman #2 filter paper, folded into a lipid extraction thimble, and then clamped into the lipid extractor (Goldfisch, Labconco Corporation, Kansas City, MO). Hexane (60 mL) was added to each preweighed beaker and connected to the extractor. The extraction was carried out for 5 h. The extracted lipid left behind in the beaker after hexane recovery and drying at 100 °C for 30 min was cooled and weighed. Percent lipid (% lipid) was expressed as the weight of lipid per gram dry weight of initial material used.

***β*-Glucan Determination.** β-Glucans were determined using enzymatic[38,39] as well as the flow injection method using Calcoflour and fluorescent detection.[40]For the enzymatic analysis, barley meal (100 mg) suspended in ethanol (50% v/v,1 mL) was mixed with sodium phosphate buffer (5 mL; 20 mM pH 6.5) and boiled for 5 min with intermittent vortexing. After cooling, it was digested with 200 units of lichenase (1000 U/mL, Megazyme) for 1 h at 40 °C. Total volume was adjusted to 30 mL with water. An aliquot (0.1 mL) taken in triplicate was mixed with 0.1 mL of sodium acetate buffer (50 mM, pH 4.0) in the first tube, while 4 units of β-glucosidase (40 U/mL, Megazyme) was added to the rest, followed by incubation at 40 °C for 15 min. The reaction mixture was treated with 3 mL of GOPOD reagent and reincubated for 20 min. The glucose control is used as a single calibration point standard curve. The amount of glucose was determined by comparing it against a glucose control at 510 nm using a spectrophotometer (DU800, Beckman Coulter), and the values were used to estimate β-glucan concentration in samples.[41]

Flow injection analysis (FIA) uses calcofluor as the binding probe. A concentration range (0−200 μg in 100 μL of water) of barley meal for each sample and a series of standards [0−1.75 μg of barley β-glucan (Sigma-Aldrich St. Louis, MO) in 100 μL] were prepared. Phosphate buffer (0.1 M, pH 8.0) was used as the carrier and for Calcoflour preparation. The working solution (100 μL) containing 35 mg/L Calcofluor in 0.01% Triton X-100 was rapidly added to each sample including the standards, mixed, and injected at a flow volume of

2.0 mL/min using an autosampler. Increase in fluorescence, proportional to the β-glucan concentration was determined by comparing sample peak area or peak height with a standard curve generated from injections of β-glucan standards.[41]

Total Dietary Fiber (TDF). Dietary fiber was determined by sequential enzymatic digestion of barley flour (1 g) with thermo stable α-amylase (3,000 U/mL, Megazyme International Ireland Ltd., Wicklow, Ireland) in 50 mL of 0.08 M phosphate buffer at pH 6.0 at 95 °C, followed by acidification and redigestion for 30 min in 35 units of protease (350 U/mL tyrosine) at 60 °C.[38] The final digestion step consisted of treatment with 40 Units of amyloglucosidase (200 U/mL Megazyme) for 30 min at 60 °C. Dietary fiber was finally precipitated with 4 volumes of ethanol (95% v/v), washed with 78% (v/v) ethanol and acetone, and air-dried. One part was used for protein determination by the Kjeldahl method and the other for ash determination (incinerate for 5 h at 525 °C). Total dietary fiber was the weight of residue less the weight of protein and ash.[42]

Total Starch Concentration. Total starch concentration was determined on the basis of the AACC approved method.[38] In brief, 100 mg (duplicate) samples of ground barley was weighed into 10 mL glass tubes and dispersed in 2 mL of 80% (v/v) ethanol. To each sample, 3 mL of α-amylase (240 U/mg, Megazyme) in 50 mM MOPS [3-(*N*-morpholino) propanesulfonic acid] buffer (pH 7.0) was added. Samples were vortexed and incubated in a boiling water bath for 8 min with 3 times of intermittent shaking. Samples were allowed to cool at room temperature and reincubated (50 °C, 30 min) with 330 U of amyloglucosidase (3300 U/mL, Megazyme) in 4 mL of sodium acetate buffer (200 mM, pH 4.5). After the reaction was complete, the sample volume was made up to 100 mL with distilled water, and three aliquots (100 μL) were transferred into different test tubes with 3 mL of glucose determination (GOPOD) reagent. Samples in duplicates were incubated including glucose standards at 50 °C for 20 min. Total starch concentration was determined as free glucose by measuring the absorbance at 510 nm.[43] Starch concentration was calculated on a percent dry weight basis.[44]

Starch Extraction. Coarsely ground barley meal from 4 to 6 grains was steeped in 0.02 N HCl (2 mL) overnight at 4 °C. After neutralization with 0.2 N NaOH and centrifugation at 4000*g*, the residue was crushed in 2 mL of 0.1 M Tris-HCl buffer containing 0.5% $NaHSO_3$ (pH 7.0), followed by enzyme treatments, proteinase (15 U/g barley), lichenase (2 U/g barley), and β-xylanase (8 U/g barley).[45] Samples were digested overnight and filtered though a 100 μm pore size nylon filter. Crude starch slurry was centrifuged for 10 min at 4000*g*. The precipitate was resuspended with 200 μL of water, layered over 1 mL of 80% (w/v) cesium chloride solution, and centrifuged at 13,000*g* for 30 min. The starch pellet was washed twice with water, followed by acetone washing and overnight air drying.

Starch Granule Size Distribution. Starch granule size distribution (by volume) of the starch slurries was determined using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Malvern,England). A 40 mg/mL starch solution was used for size analysis at a pump speed of 1700 rpm.

Amylose Concentration Determination. Amylose concentration was determined as described by Demeke et al.[46] with high performance size exclusion liquid chomatography (HPSEC). One milliliter of gelatinized starch (1 mg) was incubated for 4 h (40 °C) with 4 units of isoamylase (200U/mL, Megazyme) and 55 μL of sodium acetate buffer (1M, pH 4.0). Debranching by isoamylase was terminated by boiling the sample for 20 min. Freeze-dried debranched samples were suspended and vortexed in 200 μL of DMSO (99% v/v). The sample was then centrifuged at 15,000*g* for 10 min. An aliquot (40 μL) of supernatant was injected into a 5 μM PL gel MiniMix-C guard coloumn attached to a PLgel MiniMix 250 × 4.6 mm ID column (Polymer Laboratories, Inc. Amherst, MA) to separate amylose and amylopectin using a high performance liquid chomatography system (Waters 600 Controller, Waters 610 Fluid Unit, Waters 717 plus Autosampler, Waters 410 Differential Refractometer, Waters Corporation, Milford, MA). DMSO (99% v/v) with lithium bromide 4.4% (w/v) was used as eluent at a flow rate of 0.2 mL/min with similar time intervals of 30 min for injection and delay. Data was collected and analyzed using Empower 1154 Chomatography software (Waters Corporation, Milford, MA). Percent amylose was obtained by integrating the peak area under the amylose curve.

Starch Morphology by Atomic Force Microscopy (AFM). Starch extracted from barley grains, as described above, was suspended in water (1 mg/mL) and gelatinized (95 °C) and stored in a rapid visco analyzer (RVA) until deposition on mica. Sample temperature was reduced to 70 °C until 100 μL of gelatinized starch (30 μg/mL) was deposited onto preheated freshly cleaved mica as an aerosol spray with nitrogen gas.[47]

AFM images were taken using a PicoSPM instrument (Molecular Imaging, Tempe, AZ) which operates in intermittent contact mode. The force constant on the silicon cantilever (Nanoscience Instrument, Tempe, AZ, USA), the resonant frequency, and the curvature radius for AFM imaging were 48 N m^{-1}, 190 kHz, and <10 nm, respectively. The ratio of set-point oscillation amplitude to free air oscillation amplitude was 0.75:0.85, while resonance amplitudes ranged from 0.4 to 1.0 V.[47] The instrument was under ambient conditions and mounted in a vibration isolation system with a scan rate of 1−1.5 Hz (512 pixels per line). Analysis of images and measurements were done using SPIP V5.0.5 software (Image Metrology, Denmark).

Amylopectin Chain Length Distribution Analysis. Amylopectin chain length distribution was determined by fluorophore-assisted capillary electrophoresis (FACE)[48] using the Proteome Lab PA800 (Beckman Coulter, Fulerton, CA) equipped with a 488 nm laser module. Twenty milligrams of purified defatted starch samples in a microfuge tube (2 mL) were suspended in distilled water (750 μL) followed by the addition of 50 μL of NaOH (2 M). Samples were mixed vigorously and boiled for 5 min. Heated starches were allowed to cool at room temperature and neutralized with glacial acetic acid (32 μL). Sodium acetate buffer (1 M, 100 μL) and distilled water (1 mL) were added to gelatinized starches. Gelatinized starches were debranched (37 °C for 2 h) with 10 U of isoamylase (1000 U/mL) followed by boiling (10 min) and centrifugation (3000*g* for 10 min). The supernatant was deionized by filtration though an ion-exchange resin (20−50 mesh) in a microfuge tube. After deionization, 50 μL of aliquot was dried for 30 min under vacuum (SPD SpeedVac, Thermo Electron Corporation, Milford, MA, USA). Debranched chains were fluorescent labeled with 8-aminopyrene 1,2,6-trisulfonate (APTS) by overnight incubation of the reaction mixture at 37 °C. The N-CHO (PVA) capillary with a preburned window (50 μm ID and 50.2 cm total length) was used for separation of debranched samples. Maltose was used as an internal standard. Samples (stored at 10 °C) were injected at 0.5 psia for 3 s and separated at constant voltage of 30 kV for 30 min. Data was recorded and analyzed using 32-Karat software (Beckman Coulter). The degree of polymerization (DP) was assigned to peaks on the basis of the relative migration time of maltose used as an internal standard.

In Vitro Kinetics of Starch Enzymatic Digestion. Barley pure starch and meal samples were enzymatically hydrolyzed in vitro for kinetic analysis.[49] Meal and pure starch (100 mg, in triplicates) samples were incubated with constant agitation in a 4 mL solution of pancreatic α-amylase (10 mg/mL) plus amyloglucosidase (3 U/mL) in sodium maleate buffer (0.1 M, pH 6.0). For kinetic analysis of starch hydrolysis, separate reaction mixtures were incubated for 30, 60, 120, 240, and 480 min incubation. The treatment without incubation was taken as 0 min of control. Enzyme treatment was terminated with 4 mL of ethanol (99%, v/v). After amylolysis termination, the reaction mixture was centrifuged (3000*g*, 15 min), and the residue (isolated RS) was washed twice with

Table 1. Carbohydrate and Noncarbohydrate Contents from Ten Selected Hulless Barley Genotypes[a]

genotype	starch phenotype	starch (%)	amylose (%)	protein (%)	TGW (g)	β-glucan (%) calcofluor	β-glucan (%) megazyme	TDF (%)	crude lipid (%)
CDC McGwire	normal	72.2 ± 0.6 g	25.8 ± 0.7 d	13.5 ± 0.0 a	46.2 ± 0.7 d	5.8 ± 0.4 a	5.1 ± 0.4 a	11.8 ± 1.0 a	3.04 ± 0.4 a
SH99250	increased amylose	61.1 ± 2.2 bc	38.5 ± 0.3 e	14.5 ± 0.1 c	37.4 ± 0.3 c	11.3 ± 1.7 d	7.9 ± 0.6 d	17.4 ± 0.7 e	3.4 ± 0.2 ab
SH99073		59.7 ± 0.6 ab	40.8 ± 1.1 f	14.8 ± 0.0 d	32.4 ± 0.4 a	9.7 ± 0.5 bc	8.5 ± 0.3 de	18.2 ± 0.6 e	3.3 ± 0.2 ab
SB94893		61.9 ± 0.2 c	38.0 ± 0.1 e	15.0 ± 0.0 e	35.4 ± 2.0 b	8.9 ± 0.3 bc	7.9 ± 0.1 d	16.0 ± 0.7 d	3.2 ± 0.1 a
CDC Candle	waxy	68.5 ± 0.9 f	4.5 ± 0.4 c	13.6 ± 0.0 a	45.1 ± 0.1 d	6.8 ± 0.2 a	6.9 ± 0.1 b	13.6 ± 0.5 b	3.4 ± 0.1 ab
Waxy Betzes		66.5 ± 0.7 e	3.9 ± 0.1 c	14.2 ± 0.1 b	35.1 ± 0.4 b	8.7 ± 0.4 bc	7.1 ± 0.0 bc	15.13 ± 1.0 cd	3.4 ± 0.5 ab
SB94912		61.0 ± 0.7 bc	1.6 ± 0.5 b	15.2 ± 0.1 f	36.8 ± 0.2 c	9.8 ± 0.2 c	8.2 ± 0.2 de	15.0 ± 0.2 cd	3.8 ± 0.2 bc
CDC Alamo		64.2 ± 0.2 d	0 a	15.0 ± 0.0 e	37.8 ± 1.0 c	8.7 ± 0.4 bc	7.3 ± 0.1 bc	13.7 ± 0.4 b	4.4 ± 0.3 d
CDC Rattan		65.6 ± 1.1 de	0 a	15.0 ± 0.2 e	34.1 ± 0.3 b	8.6 ± 0.1 b	7.4 ± 0.1 c	14.2 ± 0.8 bc	4.2 ± 0.6 cd
CDC Fibar		58.1 ± 1.8 a	0 a	16.6 ± 0.2 g	37.1 ± 0.2 c	13.3 ± 0.1 f	9.7 ± 0.4 f	17.5 ± 0.6 e	3.6 ± 0.1 ab

[a] Data reported on a dry basis (db) and represent the means of three biological replications and two independent observations for each replicate ± standard deviation (SD). Mean values within a column followed by different letters are significantly different ($p < 0.05$).

ethanol (50%, v/v). The final residue after centrifugation at 1500*g* followed by drying was dispersed in 2 mL of potassium hydroxide (2 M) and incubated in an ice water bath for 20 min with constant shaking. It was further incubated with amyloglucosidase (3300 U/mL) in 8 mL of sodium acetate buffer (1.2 M, pH 3.8). Starch content (hydrolyzed and unhydrolyzed) at the end of each treatment was determined enzymatically by the GOPOD kit method.[38] Hydrolyzed starch was determined as mg of glucose × 0.9. Rate of starch digested (hydrolyzed) was expressed as the percentage of total starch (TS) at the end of each interval.

Statistical Analysis. All determinations were done in triplicate. The analysis of variance (ANOVA) of the means was performed with SPSS univariate analysis (version 10). Multiple means comparisons were determined with the Duncan's multiple range test at $p < 0.05$ confidence level. Cluster dendrogram analysis based on average linkage was performed using Minitab software (Version15) (Minitab, Inc. Pensylvania).

RESULTS

Thousand Grain Weight (TGW). TGW varied between genotypes. It was significantly reduced ($p < 0.05$) in atypical amylose genotypes (increased amylose or waxy) in comparison to that in normal amylose genotypes (46.2 g, CDC McGwire). TGW was more severely affected in the increased amylose genotypes (range 32.4−37.4 g) than the waxy genotypes (range 34.1−45.1 g) (Table 1).

Protein Concentration. Protein concentration differed among genotypes. The waxy genotype CDC Fibar recorded the highest percent protein (16.6%) (Table 1). The values for the increased amylose genotypes ranged from 14.5−15%. A strong negative ($r^2 = -0.80$; $p < 0.01$) correlation was observed between protein and total starch concentration (Table 5).

Lipid Concentration. Lipid concentration ranged from 3.04−4.4 and varied significantly ($p < 0.05$) between genotypes (Table 1). Lipid concentration in the waxy (3.4−4.4%) and the increased amylose (3.2−3.4%) genotypes was comparatively greater than that of the normal starch genotype (3.04%).

Total Dietary Fiber concentration. Total dietary fiber concentration ranged from 11.8% to 18.2% and differed significantly ($p < 0.05$) among genotypes (Table 1). CDC McGwire (normal starch) had the lowest TDF at 11.8%, while SH99073, an increased amylose genotype, had the highest (18.2%) TDF (Table 1). CDC Candle (13.6%) and CDC Alamo (13.7%) had the lowest TDF among the waxy genotypes with no significant ($p < 0.05$) difference between them. CDC Fibar (17.5%) had the highest TDF among the waxy genotypes. Increased amylose genotypes had significantly ($p < 0.05$) higher dietary fiber concentration compared to that of either normal or waxy genotypes. Among increased amylose genotypes, SH99073 had the highest (18.2%) TDF, followed by SH99250 (17.4%) and SB94893 (16.0%). Variation in dietary fiber concentration among waxy genotypes was 3.9%, while 2.2% was observed for the increased amylose genotypes. These results concur with an earlier observation that dietary fiber concentrations are higher in increased amylose genotypes.[50]

***β*-Glucan Concentration.** There were significant ($p < 0.05$) differences in β-glucan concentration among genotypes and between methods used to determine β-glucan concentration. However, the ranking of genotypes for β-glucan concentration was the same for both methods. The Calcofluor method gave slightly higher values (0.7 to 3.4%) than the Megazyme method (Table 1). The difference observed could be due to the interference from cellulosic components, (1→3)-β-D-glucans, xylans, etc.[51,52] Values for the Calcofluor method ranged from 5.8% to 13.3%, while the Megazyme method values ranged from 5.1% to 9.7%. The normal starch genotype, CDC McGwire, recorded the lowest β-glucan concentration in both methods at 5.8% and 5.1% for the Calcofluor and Megazyme methods, respectively. The waxy and increased amylose genotypes recorded significantly higher β-glucan concentration compared to that of the normal starch genotype. CDC Fibar had significantly higher β-glucan concentration either from the Calcofuor (13.3%) or Megazyme (9.7%) methods but lower starch concentration and nondetectable amylose (Table 1). Among waxy genotypes, CDC Candle had the lowest β-glucan concentration using both methods and varied by 0.1%. CDC Alamo had 8.7% and 7.3% β-glucan from the Calcofluor and Megazyme methods, respectively. β-Glucan concentration values for CDC Rattan and SB94912 varied by 1.2% and 1.6%, respectively, using the Megazyme and Calcofluor methods.

Values from the Calcofluor method for the increased amylose genotypes were 11.3%, 9.7%, and 8.9% for SH99250, SH99073 and SB94893, respectively. However, corresponding values from the Megazyme method were significantly lower (7.9%, 8.5%, and 7.9%, respectively).

Total Starch Concentration. The total starch concentration of the ten genotypes varied significantly ($p < 0.05$) and ranged

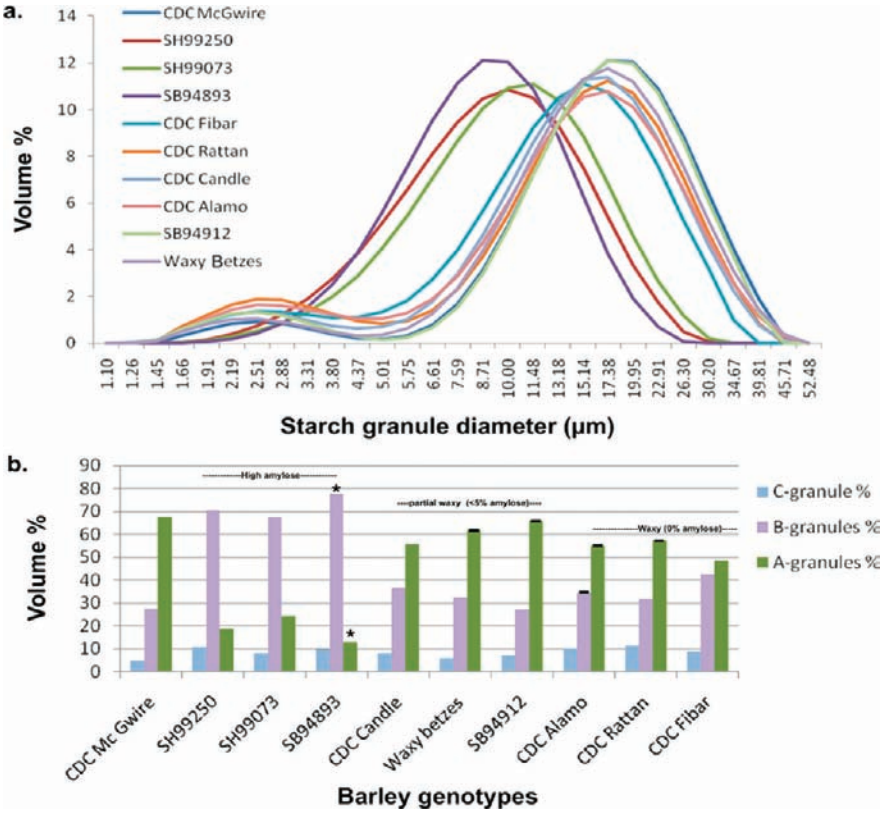

Figure 1. (a) Starch granule distribution in normal starch (CDC McGwire), increased amylose starch (SH99250, SH99073, and SH94893), and waxy starch (CDC Fibar, CDC Rattan, CDC Candle, CDC Alamo, SB94912, and Waxy Betzes) genotypes of barley. (b) Effect of amylose content on C-type (<5 μm), B-type (5−15 μm), and A-type (>15 μm) diameter ranges of starch granules. (* indicates maximal increase in B-type and decrease in A-type starch granule volume percent as compared to normal starch genotype CDC McGwire.)

from 58.1% to 72.2%. Similar to TGW, total starch concentration was significantly reduced in atypical amylose genotypes (Table 1).

Starch Granule Morphology (Granule Size). Starch granule diameter ranged from 1.3−45.7 μm (Figure 1a). Increased amylose genotypes (SH99250, SH99073, and SB94893) showed skewed starch granule distribution compared to that of CDC McGwire and the waxy genotypes. Mean surface diameter of the starch granules decreased with altered amylose concentrations for all genotypes versus CDC McGwire (16.3 μm). For waxy starch genotypes, mean starch granule size ranged from 12.9−15.9 μm, while for increased amylose genotypes, it was lower, ranging from 8.2−9.5 μm. Division of starch granules in the diameter range of >5 μm (C-type), 5−15 μm (B-type), and >15 μm (A-type) showed significant reduction ($p < 0.05$) in the volume percentage of A-type granules in all genotypes in comparison to that of CDC McGwire (Figure 1b). Increased amylose genotypes showed a dramatic increase in B-type starch granules with a corresponding significant reduction ($p < 0.05$) in A-type starch granules in comparison to that of waxy genotypes.

Amylose Concentration. Six genotypes, CDC Rattan, CDC Fibar, CDC Candle, Waxy Betzes, SB94912, and CDC Alamo, had less than 5% amylose and hence were classified as either waxy or partially waxy genotypes (Table 1). CDC McGwire, a normal starch genotype, had 25.8% amylose, while three barley genotypes (SH99250, SH99073, and SB94893) had an amylose concentration more than ∼40% and were therefore classified as increased amylose genotypes.

Starch Morphology. A previously developed spray deposition method,[47] applying hot starch suspensions (95 °C), deposited starch evenly on freshly cleaved mica to avoid multilayers and aggregation observed in the drop deposition method. Images for a waxy starch genotype (undetectable amylose) showed large biopolymer fibrils with an average height of 1.9 nm. Lengths run into several micrometer long single fibrils or are bundled together in an intertwining structure (Figure 2a). A partially waxy genotype (<5% amylose) showed small biopolymer strands with an average height of 0.7 nm and nm-scaled length (Figure 2b), indicating that these are amylose. The height and length of large biopolymer fibrils were similar to those observed in Figure 2a. The long biopolymer fibrils were considered to be amylopectin in starch granules that did not completely gelatinize during the starch preparation process. AFM for normal starch (∼25% amylose) and increased amylose starch (>38% amylose) genotypes showed large biopolymer fibrils with average height and length of 2.6 to 2.9 nm and 15 μm, respectively (Figure 2 c,d). These were also observed as bundled fibrils in a continuous network and were considered to be amylopectin. Significant ($P < 0.05$) differences were observed in large biopolymer fibril lengths from waxy genotypes (1.9 nm) and genotypes with ∼25% or increased amylose concentration (2.6 to 2.9 nm). Sections containing smaller fibrils (amylose fibrils) from normal and increased amylose genotypes were magnified and had nm-length fibrils with average height ranging from 0.7 to 0.8 nm, as also observed from amylose in partially waxy (<5% amylose) genotypes (Figure 2 c,d). McIntire and

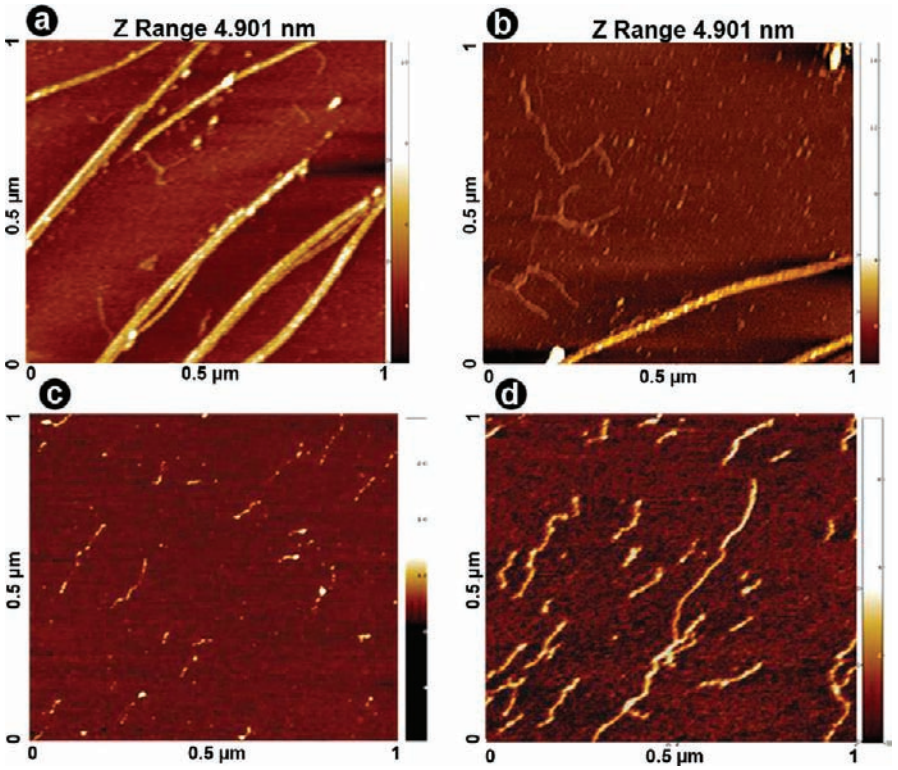

Figure 2. Atomic force microscopy (AFM) images from barley genotypes varying in amylose concentration accumulated in the endosperm. Biopolymer fibrils of amylose in waxy (a); partial waxy (<5% amylose) (b); normal (~25% amylose) (c); and increased amylose genotypes (d).

Table 2. Biopolymer Fibril Properties of Starch Amylose from Normal, Increased Amylose, and Waxy Hulless Barley Genotypes[a]

barley phenotype	amylose (%)	height (nm)	contour length (nm)	M_n ($\times 10^5$Da)	M_w ($\times 10^5$Da)	polydispersity index (M_w/M_n)	DP
normal	25.8	0.8	202 ± 141 b	2.46	3.64	1.48 b	1530 b
increased amylose	>38	0.8	214 ± 108 c	2.62	3.28	1.25 b	1620 c
waxy	<5.0	0.7	130 ± 60 a	1.59	1.92	0.33 a	985 a

[a] Values of amylose properties obtained from 36 or more different biopolymer chains. Means within the same column with different letters are significantly different ($p < 0.05$).

Brant[53] estimated a single amylose chain height as 0.54 nm by using noncontact AFM.

Amylose biopolymer fibril chains, produced using an aerosol spray method, were well-spaced and therefore allowed contour length estimation. Contour lengths were 130 ± 60, 202 ± 141, and 214 ± 108 for waxy, normal, and increased amylose starch genotypes, respectively. It has been proposed in earlier reports that amylose (a fairly linear glucose polymer) adopts a shape with six sugar residues in each turn (V-shape) and 1.32 Å rise per residue with a linear mass density of 1220 Da nm^{-1}.[54,55] On the basis of this, the degree of polymerization, the polydispersity index (distribution of molecular mass), weight average molecular weight (M_w), and the number average molecular weight (M_n) were estimated. Waxy starch had a very low polydispersity index and DP, while normal starch and increased amylose starch showed no significant difference in polydispersity index, but increased amylose starch had higher DP than normal starch (Table 2).

Amylopectin Chain Length Distribution (CLD). Depending on the changing slopes, FACE characterized chain length distribution (CLD) curve was divided into four fractions DP 6−11 (F-I), DP 12−18 (F-II), DP 19−36 (F-III), and DP >37 (F-IV)

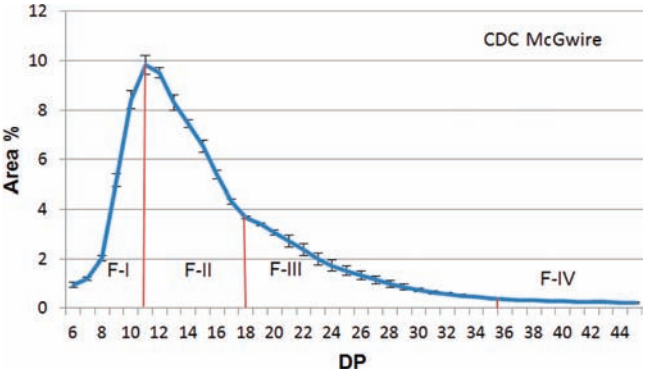

Figure 3. Amylopectin unit chains divided into four fractions F-I (DP 6−11), F-II (DP 12−18), F-III (DP 19−36), and F-IV (>DP 37), on the basis of changing slopes as depicted in normal starch barley genotype CDC McGwire.

as indicated in the CLD graph from CDC McGwire (Figure 3). More than 60% of the total DP area was occupied by DP 6−18 fractions. CDC Rattan and CDC Fibar were comparatively

Table 3. Amylopectin Chain Length Distribution (CLD) in Ten Hulless Barley Genotypes with Varied Grain Starch Amylose Concentration[a]

	distribution (%)				
genotype	DP 6−11 F- I	DP 12−18 F- II	DP 19−36 F- III	DP 37−45 F- IV	average DP[b]
CDC McGwire	27.55 ± 1.09 d	45.13 ± 1.27 b	24.86 ± 2.11 ab	2.46 ± 0.26 a	16.18 ± 0.35
SH99250	18.49 ± 1.75 a	43.36 ± 0.77 b	34.73 ± 2.38 e	3.43 ± 0.19 a	18.07 ± 0.40
SH99073	23.10 ± 2.86 bc	43.32 ± 4.05 b	30.42 ± 2.45 cd	3.15 ± 0.47 a	17.26 ± 0.32
SH94893	25.39 ± 0.87 cd	43.64 ± 1.96 b	28.32 ± 0.51 bcd	2.63 ± 0.58 a	16.72 ± 0.21
CDC Candle	23.37 ± 2.10 bc	43.36 ± 1.69 b	29.36 ± 2.65 cd	3.92 ± 0.91 a	17.32 ± 0.63
Waxy Betzes	22.08 ± 1.70 b	43.86 ± 1.40 b	30.37 ± 1.88 cd	3.70 ± 0.56 a	17.49 ± 0.44
SB94912	21.67 ± 1.54 b	42.70 ± 0.67 b	31.47 ± 1.16 de	4.15 ± 0.48 a	17.76 ± 0.38
CDC Alamo	26.93 ± 0.49 d	41.82 ± 0.07 ab	27.71 ± 2.95 bc	3.54 ± 2.67 a	16.95 ± 0.52
CDC Rattan	30.73 ± 2.86 e	43.95 ± 2.89 b	22.84 ± 0.60 a	2.48 ± 0.51 a	15.76 ± 0.25
CDC Fibar	32.23 ± 1.18 e	38.87 ± 0.07 a	24.86 ± 0.74 ab	4.04 ± 0.54 a	16.36 ± 0.30

[a] Means within the same column with different letters are significantly different ($p < 0.05$). [b] Average DP = $\Sigma(DP_n \times \text{peak area})/\Sigma(\text{peak area})_n$.

Table 4. Hydrolytic Analysis of Meal and Pure Starch Samples from Ten Selected Hulless Barley Genotypes[a]

genotype	RSM	RSPS	RDSM	RDSPS	SDSM	SDSPS
CDC McGwire	7.3 ± 0.7 de	8.3 ± 0.3 e	50.7 ± 1.0 ef	41.8 ± 0.6 c	42.3 ± 0.8 a	49.6 ± 0.6 g
SH99250	26.5 ± 0.8 g	28.1 ± 0.5h	27.6 ± 1.0 b	32.7 ± 0.5 a	46.0 ± 0.3 def	39.2 ± 1.0 f
SH99073	26.3 ± 0.3 g	24.3 ± 0.2 g	25.4 ± 0.8 a	35.3 ± 0.6 b	48.2 ± 0.9 g	40.5 ± 0.8 f
SB94893	22.4 ± 0.5 f	15.4 ± 1.7 f	33.1 ± 0.9 c	52.2 ± 0.4 d	44.2 ± 0.3 bc	32.3 ± 1.0 c
CDC Candle	4.4 ± 0.3 a	3.0 ± 0.1 d	51.8 ± 0.9 f	60.3 ± 0.5 e	43.9 ± 1.0 abc	36.7 ± 1.0 e
Waxy Betzes	5.7 ± 0.3 bc	1.2 ± 0.4 bc	51.2 ± 0.9 f	66.1 ± 0.2 g	43.2 ± 1.0 ab	32.8 ± 0.9 cd
SB94912	6.5 ± 0.5 cd	1.8 ± 0.7 c	46.8 ± 0.8 d	65.3 ± 0.3 fg	46.8 ± 1.0 efg	32.2 ± 1.4 c
CDC Alamo	5.6 ± 0.3 b	0.1 ± 1.1 a	49.4 ± 0.6 e	82.1 ± 0.4 i	45.2 ± 1.0 cde	17.9 ± 0.3 a
CDC Rattan	5.0 ± 0.1 ab	1.4 ± 0.3 c	47.9 ± 0.3 d	64.6 ± 0.9 f	47.1 ± 1.0 fg	34.3 ± 0.9 d
CDC Fibar	7.9 ± 0.7 e	0.5 ± 0.5 ab	47.8 ± 1.0 d	76.9 ± 1.0 h	44.4 ± 1.0 bcd	22.5 ± 0.9 b

[a] Hydrolysis data are based on the average of three replicates with two observations for each replicate ± standard deviation (SD). Mean values within a column followed by the same letter are not significantly different ($p < 0.05$).

enriched in F-I DP 6−11 fraction than in other genotypes. SH99250 was unique, having significantly higher ($p < 0.05$) F-II (DP 19−36) fraction and reduced F-I (DP 6−11) fraction (Table 3). CLD was significantly ($p < 0.05$) correlated with protein and starch concentrations. Amylose concentration affected F-I and F-III fractions of amylopectin in a significant but opposite manner. Significant increase in F-III chains ($r^2 = 0.37$, $p < 0.05$) of amylopectin was observed with increasing amylose concentration in selected genotypes.

Starch Enzymatic Hydrolysis. On the basis of the time required for starch enzymatic hydrolysis, starch can be divided into rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS). RDS can be defined as the starch fraction hydrolyzed within 20−30 min of incubation with RS, the fraction remaining unhydrolyzed even after 180 min of incubation. The difference between the two types is termed SDS. The barley starch enzymatic hydrolysis rate was analyzed for an 8 h incubation period; however, more than 80% starch was hydrolyzed within 4 h in all samples (both meal and pure starch). RDS values in pure starch samples ranged from 32.7−82.1% (Table 4). As expected, the RDS values in pure starch samples were higher than in the meal samples for the same genotype. RDS followed the order: waxy > normal > increased amylose. SDS content varied significantly among genotypes and between sample types. SDS values in meal samples ranged from 42.3−48.2%, while values for pure starch ranged from 17.9−49.6%. Differences in SDS content were higher in pure starch than in meal samples (Table 4). SDS is the most important dietary starch, and values in the pure starch samples followed the order increased amylose > normal > waxy genotype. In both meal and pure starch samples, RS concentration followed the order increased amylose > normal > waxy genotypes. RS concentrations were significantly higher in meal than in pure starch samples for all genotypes. Endogenous amylase inhibitors present in barley meal may be responsible for the observed difference in meal and pure starch hydrolysis.[56] In meal samples, RS concentration for increased amylose genotypes ranged from 22.4 to 26.5%, while it ranged from 15.4 to 28.1% in pure starch samples. However, increased amylose genotype SH99250 was an exception as RS in meal was 1.5% lower than the pure starch sample.

DISCUSSION

A balanced concentration of protein, fat, carbohydrate, vitamins, and minerals provided by cereal grains is essential for human development and healthy living. The correlation between increased amylose and resistant starch has increased its utility to develop diet-based solutions for disease prevention and healthy living. It has been observed that, similar to fiber, RS also plays a useful role in preventive health care in diseases such as colon

Table 5. Correlation Analysis between Barley Grain Components Affecting Starch Enzymatic Hydrolysis

	starch	amylose	protein	TGW	F-I	F-II	F-III	F-IV	C-granule	B-granule	A-granule	RSM	RSPS	RDSM	RDSPS	SDSM	SDSPS	BGC	BGM	TDF	lipid
starch	1																				
amylose	−0.15	1																			
protein	−0.80[b]	−0.25	1																		
TGW	0.70[a]	−0.11	−0.56[b]	1																	
F-I	0.1	−0.40[a]	0.45[a]	0.02	1																
F-II	0.46[a]	0.29	−0.60[b]	0.17	−0.36[a]	1															
F-III	−0.30	0.37[a]	−0.2	−0.12	−0.89[b]	0.10	1														
F-IV	−0.24	−0.29	0.16	0.04	−0.18	−0.44[a]	0.2	1													
C-granule	−0.48[b]	0.02	0.44[a]	−0.44[a]	0.12	−0.23	0.01	−0.05	1												
B-granule	−0.52[b]	0.83[b]	0.13	−0.40[a]	−0.31	0.01	0.39[a]	−0.14	0.42[a]	1											
A-granule	0.55[b]	−0.79[b]	−0.17	0.43[a]	0.28	0.02	−0.37[a]	0.14	−0.51[b]	−0.99[b]	1										
RSM	−0.50[b]	0.91[b]	0.05	−0.40[a]	−0.43[a]	0.09	0.48[b]	−0.15	0.30	0.94[b]	−0.93[b]	1									
RSPS	−0.31	0.94[b]	−0.17	−0.22	−0.51[b]	0.21	0.51[b]	−0.18	0.21	0.84[b]	−0.82[b]	0.96[b]	1								
RDSM	0.56[b]	−0.86[b]	−0.12	0.49[b]	0.41[a]	−0.07	−0.47[b]	0.13	−0.37[a]	−0.90[b]	0.91[b]	−0.98[b]	−0.93[b]	1							
RDSPS	−0.06	−0.89[b]	0.46[a]	−0.06	0.48[b]	−0.43[a]	−0.37[a]	0.27	0.12	−0.58[b]	0.54[b]	−0.75[b]	−0.89[b]	0.71[b]	1						
SDSM	−0.55[b]	0.13	0.36	−0.62[b]	−0.10	−0.06	0.15	0.02	0.50[b]	0.26	−0.30	0.36[a]	0.32	−0.50[b]	−0.15	1					
SDSPS	0.47[b]	0.57[b]	−0.67[b]	0.35	−0.32	0.56[b]	0.11	−0.31	−0.46[a]	0.11	−0.05	0.29	0.50[b]	−0.25	−0.84[b]	−0.09	1				
BGC	−0.90[b]	−0.01	0.82[b]	−0.54[b]	0.07	−0.52[b]	0.15	0.26	0.45[a]	0.36	−0.38[a]	0.32	0.18	−0.37[a]	0.14	0.33	−0.48[b]	1			
BGM	−0.91[b]	−0.05	0.84[b]	−0.64[b]	0.10	−0.55[b]	0.13	0.3	0.48[b]	0.40[a]	−0.43[a]	0.31	0.11	−0.39[a]	0.22	0.47[b]	−0.55[b]	0.87[b]	1		
TDF	−0.82[b]	0.40[a]	0.53[b]	−0.66[b]	−0.23	−0.27	0.38[a]	0.17	0.36	0.68[a]	−0.69[b]	0.68[b]	0.55[b]	−0.71[b]	−0.25	0.45[a]	−0.16	0.77[b]	0.80[b]	1	
lipid	−0.12	−0.58[b]	0.35	−0.25	0.27	−0.14	−0.29	0.22	0.48[b]	−0.36[a]	0.30	−0.40[a]	−0.46[a]	0.29	0.58[b]	0.30	−0.58[b]	0.16	0.17	−0.1	1

[a] Significance at $p < 0.05$. [b] Significant at $p < 0.01$.

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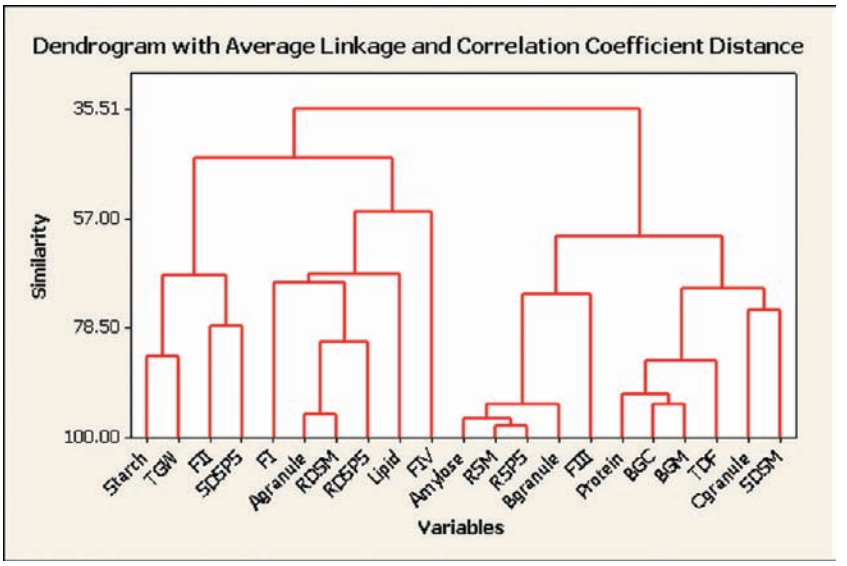

Figure 4. Average linkage dendrogram depicting the physical relationship between different components of barley grain related to starch enzymatic hydrolysis.

cancer, diabetes, obesity, osteoporosis, and cardiovascular diseases.[57] In comparison to amylopectin, amylose contributes toward a low glycemic index and also promotes bowel health, thereby, reducing colorectal cancer risk.

Analysis of the grain constituents and starch characteristics of ten hulless barley genotypes with varied carbohydrate composition revealed a high correlation between grain constituents and starch enzymatic digestibility indices. A negative correlation between amylose and starch enzymatic digestibility was observed. The normal starch genotype (CDC McGwire) had the highest starch concentration (72%) as compared to increased amylose, waxy, or partially waxy genotypes (range 58−68%). Total starch concentration was an indicator of higher grain weight as shown by significant positive correlation ($r^2 = 0.70$, $p < 0.05$) between TGW and total starch concentration (Table 5).

Starch granule size showed bimodal distribution in normal and waxy genotypes. For increased amylose genotypes, starch granule size distribution appeared unimodal (Figure 1). In CDC McGwire and waxy genotypes, the highest volume percentage was occupied by starch granules with a diameter between 15 and 17 μm. Increased amylose genotypes (SH99250, SH99073, and SB94893) showed altered starch granule distribution with the highest volume percentage occupied by granules between 8 and 11 μm in diameter. Decrease in the mean starch granule diameter observed in the present study concurs with the results observed in high amylose "Glacier" barley.[58] Amylose concentration was strongly correlated ($r^2 = 0.83$, $p < 0.01$) with B-type starch granule (5−15 μm) content, concurring with an earlier report.[45] Starch hydrolysis studies showed significant positive correlation of RS with B-type starch granule number ($r^2 = 0.84$, $p < 0.01$) and amylose concentration ($r^2 = 0.94$, $p < 0.01$) while showing negative correlation with A-type starch granule number ($r^2 = -0.82$, $p < 0.01$). Comparative analysis revealed that the medium sized B-type starch granule with increased amylose contributed most toward resistant starch formation.

Amylopectin structure and concentration contributes to starch uniformity, stability, texture, and better freeze−thaw abilities.[22] Amylopectin chain length distribution (CLD) and packing have been reported as an important characteristic of starch digestibility.[32] Amylopectin unit chains F-II (DP 12−18) were strongly correlated ($r^2 = 0.56$, $p < 0.01$) with SDS content of pure starch. This indicates a relationship between amylopectin unit chain length distribution and starch digestibility. However, no correlation was found in meal samples suggesting the interference of nonstarch components in starch digestibility. Increased amylose in barley genotypes affected CLD by influencing shorter chains F-I in negative and longer or intermediate F-III chains of amylopectin in a positive manner. In fact, F-III (DP 19−36) chains were synergistic with amylose concentration in RS formation for both meal and pure starch samples (Table 3).

Amylopectin biopolymers were common for all samples; however, amylose concentration was the only factor separating starch types. Amylose is the principal component in the formation of RS Types 2 and 3.[59] For this reason, we also estimated the polymer properties of amylose, which has unique features in different starch samples. Differences in contour lengths of biopolymer fibrils from normal, increased amylose, and waxy (<5% amylose) genotypes could explain the packaging of amylose within the amorphous matrix of an amylopectin molecule. The waxy (undetectable or <5% amylose) genotypes will have less compact structure compared to that of the normal and increased amylose starch genotypes. This could be one of the reasons for the ease of gelatinization and enzymatic hydrolysis of waxy starch in comparison with those of increased amylose starch. The polydispersity index was obtained from the ratio of M_w to M_n and showed significant differences between the genotypes (Table 2). The degree of polymerization (DP) was significantly higher in normal (1530) and increased amylose (1620) compared to that of waxy genotypes (985).

The kinetics of hydrolytic studies showed an initial rapid phase of RDS release followed by a slower phase of SDS release. The lowest starch digestion rate in all stages was observed for the increased amylose genotypes (SH99250, SH99073, and SB94893), while the highest was observed for waxy genotypes (Table 3). The AFM results for amylose morphology indicated

higher polydispersity indices for normal (1.48) and increased amylose starch genotypes (1.25) compared to that of the partially waxy starch genotype (0.33) (Table 4). This suggests that there were too many molecules within a defined length in the first two genotypes as compared to the latter. The expected resultant effect on starch gelatinization and enzymatic hydrolysis would be a faster rate in the latter than in the former. The observed effect corroborates the hydrolysis finding where the rate of enzymatic starch hydrolysis was faster for waxy genotypes compared with that of genotypes with >25% amylose. The energy required to gelatinize and completely hydrolyze starch into glucose will obviously be lower for waxy starch compared to that for either normal or increased amylose starch. This also makes waxy starch more susceptible to hydrolytic enzymes in comparison to starch granules with significant amylose concentration, which interferes with the access of hydrolytic enzymes to the amorphous zone of starch granules.

Waxy or partially waxy starch genotypes can be recommended for famine prone regions, while increased amylose types could be recommended for diabetic diets due to the associated slow release of glucose (low glycemic index). Total starch concentration was lower in waxy and increased amylose genotypes implying that the altered amylose to amylopectin ratio negatively affects starch concentration.

Barley genotypes with atypical amylose had higher β-glucan concentration compared to that of normal starch genotypes, in agreement with earlier reports on altered amylose and its effect on starch and β-glucans.[18] High β-glucan concentration in barley makes it more useful in human nutrition due to associated health benefits. Malting and feed barley (for monogastric animals) are selected for lower β-glucan concentration. β-Glucan concentration in hulless barley ranged from 3.0% to 7.0%.[18] This study indicates substantial improvement in β-glucan concentration especially with atypical amylose concentration. In the present study, β-glucans affected SDS in meal samples (r^2 = 0.47, $p < 0.01$), decreasing RDS concentration by inhibiting starch hydrolysis.

Cluster dendrogram analysis (Figure 4) also revealed patterns of relationships between grain constituents and starch characteristics related to starch digestibility. The dendrogram analysis based on average linkage showed a right branch−left cluster group consisting of RSM, RSPS, and amylose which is linked to B-type starch granule content and influenced maximally by the F-III fraction of amylopectin. The right subgroup of the above right cluster exhibited strong correlation between protein, BGC and BGM having >80% similarity with TDF, and this set has an average >55% similarity with both the C-type starch granule and SDSM (strongly intracorrelated) pair and with the left subgroup containing amylose. The left branch cluster group has the starch and TGW (>80% intracorrelation) pair and F-II and SDSPS pair, which depicts >55% similarity level and forms the left subgroup of this cluster. The right subgroup of the left branch cluster group exhibits strong correlation between A-granules and RDSM, which is further influenced by RDSPS (>80% similarity level) and F-I. This is linked with lipid at a >60% similarity level. This right subgroup also has F-IV at a 55% level of similarity with other variables of this right subgroup. Within the left branch, the two subgroups have 40% similarity. This analysis revealed that variation in amylose concentration significantly influenced resistant starch content in meal and pure starch. These factors are further affected strongly by B-type starch granules (5−15 μm) and the amylopectin F-III (19−36 DP) fraction (Figure 3). Amylopectin medium chains of DP 12−18 (F-II) influenced SDS in pure starch samples (r^2 = 0.56, $p < 0.01$).

The major challenge in the development of increased amylose genotypes is reduced grain size. The known increased amylose barley mutants Himalaya 292 (sex6 mutant) (65−70% amylose, *ssIIa* mutation), *sbe IIa* and *b* mutant (>65% amylose), and *Amo* etc. are reported to have reduced starch content with increased fiber, lipid, and phosphate.[60−62] This indicates the need to adopt a breeding approach to preferably use a specific genotype and also consider overall yield and large grain size. In the present study, TGW positively correlated with percent total starch but negatively with protein. Increase in protein could be the result of interference in carbon partitioning between protein and carbohydrates and hence decreased starch concentration as observed in altered amylose genotypes. The effect of atypical amylose on lipid concentration and its interaction with starch needs to be investigated.

This study indicates that barley cultivars with increased F-III chains of amylopectin and increased amylose could be selected for breeding lines with higher resistant starch content. The optimization of the data suggested that SH99250 with less decrease in grain weight and enriched DP 19−36 amylopectin in comparison to other increased amylose genotypes (SH99073 and SH94893) may be a promising genotype for developing cultivars with increased amylose concentration.

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ABBREVIATIONS USED

BGM, β-glucan with megazyme method; BGC, β-glucan with calcoflour method; CLD, chain length distribution; DP, degree of polymerization; GOPOD, glucose oxidase peroxidase; RDS, rapidly digestible starch; RDSM, rapidly digestible starch in meal; RDSPS, rapidly digestible starch in pure starch; RS, resistant starch; SDS, slowly digestible starch; SDSM, slowly digestible starch in meal; SDSPS, slowly digestible starch in pure starch; TDF, total dietary fiber; v/v, volume/volume.

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