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Unlocking the malting potential of new Canadian hulless barley varieties, CDC Armstrong and CDC Pristine: effects of steeping and germination time

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Abstract

Why was the work done: Interest in hulless barley for malting and brewing continues to grow due to advantages in sustainability, higher extract, and potentially enhanced flavour. This work evaluates two new Canadian hulless varieties, CDC Armstrong and CDC Pristine, and assesses their performance under resource efficient malting conditions.

How was the work done: Both barley varieties were grown in 2024 at two locations in Western Canada. Grain composition, physical traits, and properties of isolated starch granules were analysed. Both cultivars were malted using varying steeping (11, 13, 19 hours) and germination times (72, 96 hours). Routine malt quality parameters and glycosidic nitrile concentration were measured in malt, and wort was analysed for arabinoxylan, amino acids, and fermentable sugars.

What are the main findings: Both cultivars exhibited low β-glucan and high starch content. CDC Armstrong had a higher proportion of A type starch granules and lower starch gelatinisation temperatures. Under shortened steeping regimes, both cultivars hydrated efficiently. CDC Armstrong had low β-glucan and wort viscosity, while CDC Pristine showed the highest α- and β-amylase activities and fermentable sugar concentration. Both cultivars produced worts with high soluble protein, FAN, together with a favourable amino acid profile and concentration. Extract levels were > 86%, exceeding the values for 2-row hulled barley genotypes (79–82%). Analysis identified CDC Armstrong as a low glycosidic nitrile producer.

Why is the work important: CDC Armstrong and CDC Pristine represent significant advances in hulless barley breeding, offering benefits in sustainability through improved malting quality, higher extract potential, and strong performance under reduced input malting conditions. CDC Armstrong shows strong suitability for both brewing and distilling applications.

Keywords

hulless barley, hulless malt, β-glucan, water absorption, extract, glycosidic nitrile.

Introduction

The breeding of husk free or hulless barley in Canada began in the 1970s, with the primary objective of developing feed cultivars with enhanced digestible energy from the absence of hulls for monogastric animals and poultry. Subsequent breeding expanded toward cultivars with improved nutritional profiles for human consumption. Several recent hulless barley cultivars from the Crop Development Centre (CDC) and Agriculture and Agri Food Canada (AAFC) exhibit high dietary fibre and β-glucan concentration (CDC Valdres, CDC Henrick, AAC Beckett), elevated amylose content (CDC Hilose), or increased anthocyanin levels (AAC Magenta), making them valuable ingredients for nutrient dense and functional foods. Despite these nutritional advantages, widespread adoption of hulless barley has been constrained by limited consumer demand and insufficient processing infrastructure. Nevertheless, recent advances in cultivar development, combined with growing interest in health oriented products and sustainable feed options, are creating renewed opportunities for hulless barley in Canada.

Interest in hulless barley for malting and brewing applications arose from potential gains in malting efficiency, including higher extract yields (Edney and Langrell 2004). The absence of the husk can reduce process losses with reduced polyphenols limiting the formation of beer haze (Siebert et al. 1996) and less polysaccharides implicated in premature yeast flocculation (Herrera and Axcell 1991). Further, the leaching of polyphenolic compounds (van Waesberghe 1991), and the presence of organic radicals and iron reduce shelf life (Cortés et al. 2010). More recently, interest in hulless barley for brewing has increased due its potential contribution to a flavour profile more conducive to younger consumers. Here, beers are cleaner and less grainy, with a reduced astringent flavour profile that reflects the absence of hulls. The recognition that hulls are a negative flavour component is reflected by products such as 'Maltgems'®, a pre-ground malt with the hulls removed, and the Kubessa method of hull separation prior to mashing, which is known to produce smoother tasting beer with better flavour stability (Pieczonka et al. 2024).

The potential to use hulless barley in distilling has also been recognised, with benefits including higher alcohol yield and more efficient production of malt, which in turn, can decrease both water and energy use (Agu et al. 2002). Despite this, commercial development has been limited. Further, since the 1980s, distillers have been aware that whisky made from barley malt is potentially a source of ethyl carbamate, a known carcinogen, with a limit of 150 µg/L in beverages in Canada, France and Czechia (European Food Safety Authority 2007), and in Scotch whisky (Bringhurst 2015). However, this can be mitigated by natural barley mutants which lack the ability to produce glycosidic nitrile (GN) (Cook and Oliver 1991), the precursor to ethyl carbamate during the distilling process. The subsequent identification of a cytochrome P450 biosynthetic gene family on chromsome 1H linked to epiheterodendrin production (Ehlert et al. 2019) and the development of an associated molecular marker has allowed barley breeders to incorporate this trait into barley cultivars intended for distilling.

Early hulless barley cultivars intended for brewing, such as CDC ExPlus (registered in 2009), Taylor (registered in 2009) and CDC Clear (registered in 2012), did not develop sufficient hydrolytic enzyme activity during malting to effectively convert starch into fermentable sugars or to adequately degrade β-glucans, resulting in wort with undesirably high viscosity. Higher protein content and hardness were also characteristics that have been previously noted in hulless barley which result in poor modification (Evans et al. 1998; Agu et al. 2002). Recent breeding programs have therefore focused on improving these quality traits, particulaly enzyme activity and β-glucan degradation, to enhance the suitability of hulless barley for the brewing and distilling industries. Pre-registration quality assessments of two newly released Canadian hulless barley varieties, CDC Pristine and CDC Armstrong (both registered in 2024), indicated promising malting potential with improvements in starch and β-glucan hydrolysis compared with CDC Clear.

The objectives of this study were to (i) identify and quantify any unique chemical and physical attributes of these new Canadian hulless barley varieties that contribute to higher extract potential and malting processability, and (ii) evaluate the effects of reduced steeping and germination

times, representing lower water and energy input, on hydration behaviour, enzymatic development, extract yield, and overall malt modification.

Methods and materials

Hulless barley

Canadian hulless barley cultivars, CDC Armstrong and CDC Pristine, were used in this study. Both were grown in 2024 at two locations in Saskatchewan (Saskatoon and Birch Hills). For comparative purposes, CDC Clear, an older two-row hulless barley malting variety, and AAC Synergy, the most widely grown two-row hulled malting variety in Canada, were included as controls.

Physical and analytical tests

Barley kernel characteristics (kernel weight, hardness, kernel diameter) were evaluated using the Single Kernel Characterisation System (SKCS 4100, Perten Instruments). Germination energy was measured using ASBC Method Barley-3 and protein content was determined by Near Infrared Transmittance (NIT) (Infratec 1241, Foss). Total starch and ß-glucan content in grain were determined enzymatically using enzyme kits (Megazyme International, Bray, Ireland) according to AACC International Approved Methods 76-13.01 and 32-23.01.

Starch was isolated according to the method of You and Izydorczyk (2002). The diameter of isolated starch granules was determined using a Mastersizer 2000 instrument equipped with Hydro 2000S dispersion unit (Malvern Panalytical) with water as the dispersant. Thermal analysis of isolated starch and barley and malt was performed using differential scanning calorimetry with a TA instrument DSC2500 Discovery Series equipped with an RCS 90 cooling unit (TA Instruments, New Castle, DE, USA). Material (3-4 mg) was placed in the DSC pans and water added to give dispersions (ratio of solids to water 25:75, w/w). The hermetically sealed pans were heated from 20 to 120°C at a rate of 10°C/min. The TA Analysis software TRIOS was used to determine the onset (T_o), gelatinisation (T_g) and conclusion (T_c) temperatures (°C) along with enthalpy of gelatinisation (ΔH, J/g).

Malting and malt analyses

Barley was malted in a Phoenix Automated Micromalting System. All grain samples were subjected to intermittent steeping, consisting of four wet immersions alternating with air rests, (Table 1). The total steeping time was progressively reduced from 19 to 13 hours and to 11 hours by systematically shortening the duration of the first three steeps. Steeping was at 15°C. Following the three different steeping times, grain was germinated at 18°C for 96 hours (4 days) or 72 hours (3 days) and kilned according to the schedule outlined in Table 1.

Table 1. Steeping, germination, and kilning schedules.

Malting stage	19 hours steep	13 hours steep	11 hours steep
1st steep	**7**	**5**	**3**
1st air rest	8	8	8
2nd steep	**6**	**4**	**4**
2nd air rest	8	8	8
3rd steep	**6**	**4**	**4**
3rd air rest	8	8	8
Temperature	15°C	15°C	15°C
Germination	4 or 3 days at 18°C		
Kilning	16 hours at 55°C, 8 h at 55-65°C, 10 h at 65°C, 2 h at 65-80°C, 6 h at 80-82°C, 2 h at 60°C, 2 h at 40°C		

Malt samples (50 g) were ground (fine setting) using a Buhler laboratory mill (ASBC Malt 4, 2011) and mixed with distilled water in an automated mash bath with continuous stirring. Congress mashing involved mixing the ground malt with distilled water at 45°C for 30 minutes, then increasing the temperature by 1°C per minute for 30 minutes and ending at 70°C for one hour. The total mashing time was 120 minutes (ASBC Malt 4, 2011) and the total volume of water was 400 mL. Water was added after mashing to correct for evaporation (total sample and water weight equalled 450 g). The wort was separated from the grist by filtering through pleated filter paper (Whatman 802) at room temperature. Malt samples were analysed for moisture content (ASBC Malt 3, 1958). Protein content of malt was measured by nitrogen combustion with a LECO protein analyser (AACC 46-30.01, 1999). Diastatic power was analysed via segmented flow analysis (Skalar, Netherlands) using an automated neocuproine assay for reducing sugars (ASBC Method Malt-6A), calibrated with malt

standards. analysed by ASBC Method Malt-6C. Alpha amylase activity was determined using segmented flow analysis (Skalar, Netherlands) using β-limit dextrinised starch as the substrate (ASBC Method Malt-7C) and calibrated with standards (ASBC Method Malt-7A). β-Glucan was quantified by measuring calcofluor induced fluorescence of β-glucan polymers using segmented flow analysis (ASBC Method Wort-18B). Wort viscosity was determined with an Anton Paar Lovis ME rolling-ball viscometer (ASBC Wort 13B, 2013). The wort free amino nitrogen (FAN) concentration was determined by segmented flow analysis (ASBC Wort 12, 2011). Wort soluble protein was determined by spectrophotometry based on the differing UV absorption of protein at 215 and 225 nm (ASBC Wort 17, 2010).

Arabinoxylan and arabinoxylo-oligosaccharides in wort were estimated by hydrolysing the polysaccharides and then reducing and derivatising the monosaccharides to alditol acetates. These were measured by gas chromatography with an Agilent 6890 series gas chromatograph (Santa Clara, California, USA) equipped with a Supelco SP 2380-column (30 m × 0.25 mm × 0.2 µm film thickness) with an injection volume of 1 µL and a split ratio of 1:20. The separation was carried out at 225°C, while injection and detection occurred at 270°C. Detection was performed with a flame ionisation detector with hydrogen gas (Izydorczyk et al. 2014). The arabinoxylan content is expressed as the concentration of arabinose and xylose (Ara + Xyl) x 0.88.

Fermentable sugars in wort (diluted 200 fold) were determined using high-performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) using a disposable gold working electrode and a pH-Ag/AgCl reference electrode (ICS 5000 DC, Thermo Scientific). Gold standard PAD waveform was used for sugar detection. A CarboPac PA-100 column along with a CarboPac guard column were used to achieve the separation. A gradient of water (A), 250 mM NaOH (B) and 1 M NaAc in 250 mM NaOH (C) were used as the mobile phase at a flow rate of 0.25 mL/min for 80 minutes. These conditions achieved the separation of glucose, fructose, sucrose, maltose, isomaltose, maltotriose, isomaltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose (Neogen, Sigma Aldrich) in wort.

Free amino acids in wort were quantified using ultra high performance liquid chromatography (UPLC) using the Water AccQ-Tag derivatisation kit (*https://www.manualshelf.com/manual/waters/accq-tag-ultra-derivatization-kit/instruction-manual-english.html*). This uses a pre-column derivatisation technique with 6-Aminoquinolyl-N-hydroxysucciminidy carbamate (AQC), which reacts with both primary and secondary amines. Wort was filtered through 0.22 µm GHP membranes and diluted with deionised MilliQ water containing novaline as an internal standard. The diluted wort samples and amino acid standards (Waters amino acids cell culture standards) containing 26 amino acids were derivatised in sodium borate buffer. Chromatographic separation and detection were performed using Waters ACQUITY UPLC-H plus class with photodiode array detector at 260nm. A Waters AccQ-Tag Ultra reversed phase C18 column (1.7um, 2.1 X 100mm) set at 43°C with an injection volume of 1 µL. The mobile phases for separation were A; Waters AccQ-Tag Eluent A, B; Waters AccQ-Tag Eluent B diluted 10 fold in MilliQ water, C; MilliQ water, D: Waters AccQ-Tag Eluent B. Individual amino acids were identified and quantified using external calibration curves using the Waters amino acid standards. The uptake of wort amino acids by yeast (Jones and Pierce 1964; Stewart et al. 2013) was Group A (fast uptake: glutamic acid, aspartic acid, asparagine, glutamine, serine, threonine, lysine, arginine, methionine, isoleucine, leucine), Group B (intermediate uptake: valine, histidine, tryptophan, tyrosine, phenylalanine), Group C (slow uptake: alanine, glycine), and Group D (proline with little or no uptake).

The concentration of β-glucan, α-amylase, and β-amylase during mashing was determined using a different program. Mashing was performed using a mashing apparatus (Lochner, Germany) with twelve 400 mL stainless steel cylinders stirred throughout the process. Each cylinder contained 50 g ground malt and 360 mL of water with mashing at 45°C which was maintained for 20 min then ramped to 65ºC at 0.5°C per min for 40 min, and held at 65°C for 20 min. The temperature was increased at 0.5°C per min to 70°C and held for 20 min.

Duplicate samples of each malt cultivar were used in each mashing experiment. At 20, 40, 50, 65, 80, and 105 min of the mashing process - corresponding to 45, 55, 60, 65°C, again 65 and 70°C - 5 mL of suspension was removed from each mash and centrifuged to obtain clear wort/supernatant, which was used to determine β-glucans, and α-amylase. β-Amylase activity was measured using the Megazyme Betamyl-3® assay (Megazyme, Bray, Ireland).

Quantification of glycosidic nitrile in malt used an enzymatic extraction, distillation, and colorimetric determination workflow (European Brewing Convention method 4.21). Finely milled malt (50 g) was extracted in 200 mL sodium acetate buffer (pH 5.0) containing β-glucosidase (2000 U/L) at 60°C for 1 h, followed by distillation with antifoam to collect 100 mL of distillate. Calibration standards (50–500 ug/L) were prepared from a potassium cyanide stock solution. For colorimetric analysis, 10 mL of each standard or 5 mL distillate (diluted 1:1) with water was reacted with chloramine T and 1,3-dimethyl barbituric acid, pyridine reagent at 25°C, and the absorbance measured at 590 nm. Glycosidic nitrile concentrations were calculated from the calibration curve and expressed as ug/L and converted to g/tonne malt by dividing by 250.

Results and discussion

Grain properties

Small but notable differences in grain composition and physicochemical properties were observed between CDC Armstrong and CDC Pristine grown in 2024 in two locations in Saskatchewan. CDC Pristine exhibited a slightly higher average grain protein concentration (12.9%) compared with CDC Armstrong (12.4%) (Table 2). For hulled malting barley, the desirable protein content is considered to range from 10.5 to 12.5% (Izydorczyk and Edney 2017, BMBRI 2025); however, an equivalent benchmark has not yet been defined for hulless barley. Protein concentration in hulled barley would be expected to be lower than in hulless due to the dilution of the hull, which contributes no protein to the grain.

The average thousand kernel weight of CDC Armstrong was 49.3 g, which was 4.4 g greater than that of CDC Pristine (44.9 g) (Table 2). In addition to differences in weight, kernel shape varied between the two cultivars. CDC Armstrong kernels were slightly longer than those of CDC Pristine (Table 2), with a more elongated shape, supported by lower roundness values.

The β-glucan content of both hulless cultivars was relatively low, averaging 4.0% (Table 2). Whilst growing conditions can influence β-glucan levels in barley, the trait is primarily governed by genetic factors. Among the current two row hulled Canadian malting genotypes, AAC Synergy has the highest β-glucan content (4.2–4.4%), whereas CDC Copeland has the lowest (3.8%) (Izydorczyk and McMillan 2025).

Table 2. Composition and physical properties of CDC Armstrong and CDC Pristine barley.

	CDC Armstrong	CDC Pristine
Protein (%, db)	12.4 ± 0.2	12.9 ± 0.3
β-glucans (%, db)	4.00 ± 0.02	3.98 ± 0.08
Hardness Index	38.0 ± 2.2	44.8 ± 2.8
1000 Kernel weight (g)	49.3 ± 1.5	44.9 ± 2.2
Kernel length (mm)	7.63 ± 0.09	7.35 ± 0.06
Kernel width (mm)	3.44 ± 0.04	3.45 ± 0.02
Kernel roundness	0.57	0.59

Data represent the mean of two growing locations. db = dry basis

The kernel hardness index for both hulless cultivars was low, averaging 38.0 for CDC Armstrong and 44.8 for CDC Pristine (Table 2). Kernel hardness influences the rate of water absorption during steeping, with hard, steely kernels absorbing water more slowly than soft, mealy kernels. Although the hardness index can be affected by environmental conditions, it is primarily governed by genetic factors (Mohammadi et al. 2014). Among Canadian hulled malting varieties, CDC Copeland exhibits the lowest hardness index. According to the 2024 annual harvest report of western Canadian malting barley (Izydorczyk and McMillan 2024), the average hardness index was 48 for CDC Copeland and 54 for AAC Synergy. The lower hardness index for the new hulless cultivars compared to the leading hulled barley varieties, highlights an improvement in this quality trait for these hulless barleys. While kernel

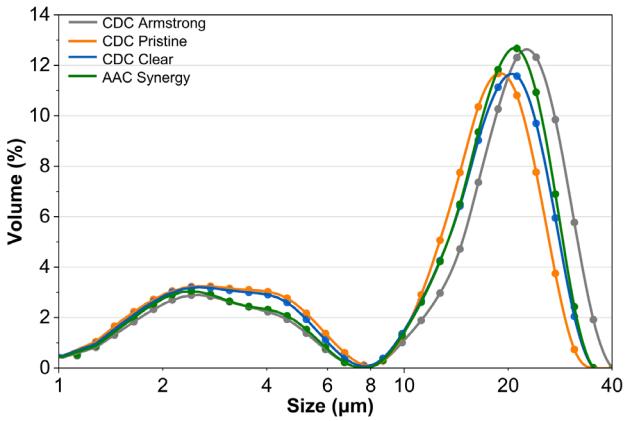
hardness remains the dominant factor, kernel shape may also contribute to differences in steeping behaviour by influencing diffusion pathways, although direct evidence for this effect is limited.

Further analyses on the content and characteristics of starch of AAC Synergy - the most popular Canadian hulled barley variety (Izydorczyk and McMillan 2025) - and CDC Clear, an older Canadian hulless variety. The results showed the average starch content in CDC Armstrong (63.5%) and in CDC Pristine (62.1%) was 3-4% higher than AAC Synergy (59.2%) reflecting the diluting effect of hulls in the latter variety (Table 3). The starch content of CDC Clear was 63.7%; the same as CDC Armstrong.

The diameter of starch granules isolated from CDC Pristine and CDC Armstrong ranged from 0.5 to 45 µm with a typical barley bimodal size distribution (Figure 1). When classified into small (<7.6 µm, B-type) and large (>7.6 µm, A-type) granules, differences emerged between the two varieties (Table 3, Figure 1). CDC Pristine contained a higher proportion of small granules and a lower proportion of large granules compared to CDC Armstrong (Table 3). Moreover, the large A-type granules in CDC Armstrong were larger than those in CDC Pristine, with a higher median size (22.7 µm vs 18.7 µm) and a shift of A-type granules toward a larger diameter (Figure 1). The distribution of granule size of CDC Armstrong resembled that of AAC Synergy, whereas CDC Pristine exhibited a pattern similar to that of the older hulless variety CDC Clear.

Small granules in barley starch negatively impact malt quality due to their higher gelatinisation temperature (MacGregor 1991) with slow hydrolysis rates compared with larger A-type granules (MacGregor and Balance 1980; Langenaeken et al. 2019). The difference in the volume distribution of small and large granules between CDC Armstrong and CDC Pristine were reflected in the gelatinisation temperature (*Tg*). The average gelatinisation temperature of starch from CDC Armstrong was 1.8°C lower than that of CDC Pristine (Table 4). In contrast, the difference in gelatinisation temperature between CDC Armstrong and CDC Clear or AAC Synergy was minor.

Figure 1. Distribution of starch granules using laser diffraction analysis of isolated starch from the three hulless cultivars compared to the hulled barley AAC Synergy.



The variation in gelatinisation temperature of isolated starch explained the differences in gelatinisation temperatures observed at the whole grain level. Table 4 shows the gelatinisation temperature of barley was higher than that of purified starch, reflecting the competition with other grain constituents (e.g., proteins) competing for water during the gelatinisation processes.

The average gelatinisation temperature of CDC Armstrong barley was 62.3°C, which was lower than that of CDC Pristine (64.1°C). These cultivar specific

Table 3. Starch content and relative volume of starch granules isolated from CDC Armstrong, CDC Pristine, CDC Clear and AAC Synergy.

Barley variety	Barley starch content	Isolated starch granules			
		Median size of all granules	B-type granules (<7.64 um)	A-type granules (>7.64 um)	Median size of A-type granules
	% dry basis	µm	%	%	µm
CDC Armstrong	63.5 ± 1.4	30.2	29.3	70.7	22.7
CDC Pristine	62.1 ± 0.2	25.1	37.6	62.4	18.7
CDC Clear	63.7 ± 1.0	26.8	35.6	64.4	21.2
AAC Synergy	59.2 ± 1.8	27.3	31.0	69.0	21.2

Table 4. Gelatinisation temperature (*Tg*) and enthalpy of gelatinisation (*ΔH*) of isolated starch granules and barley grain of CDC Armstrong, CDC Pristine, CDC Clear and AAC Synergy.

	Gelatinisation of isolated starch		Gelatinisation of barley grain	
	Tg	*ΔH*	*Tg*	*ΔH*
Barley Variety	°C	J/g	°C	J/g
CDC Armstrong	59.6 ± 0.1	11.00 ± 0.52	62.3 ± 0.3	7.05 ± 0.09
CDC Pristine	61.4 ± 0.1	10.96 ± 0.01	64.1 ± 0.1	6.51 ± 0.32
CDC Clear	59.9 ± 0.3	11.07 ± 0.24	62.7 ± 0.2	6.48 ± 0.23
AAC Synergy	60.6 ± 0.4	10.46 ± 0.81	63.2 ± 0.6	6.60 ± 0.43

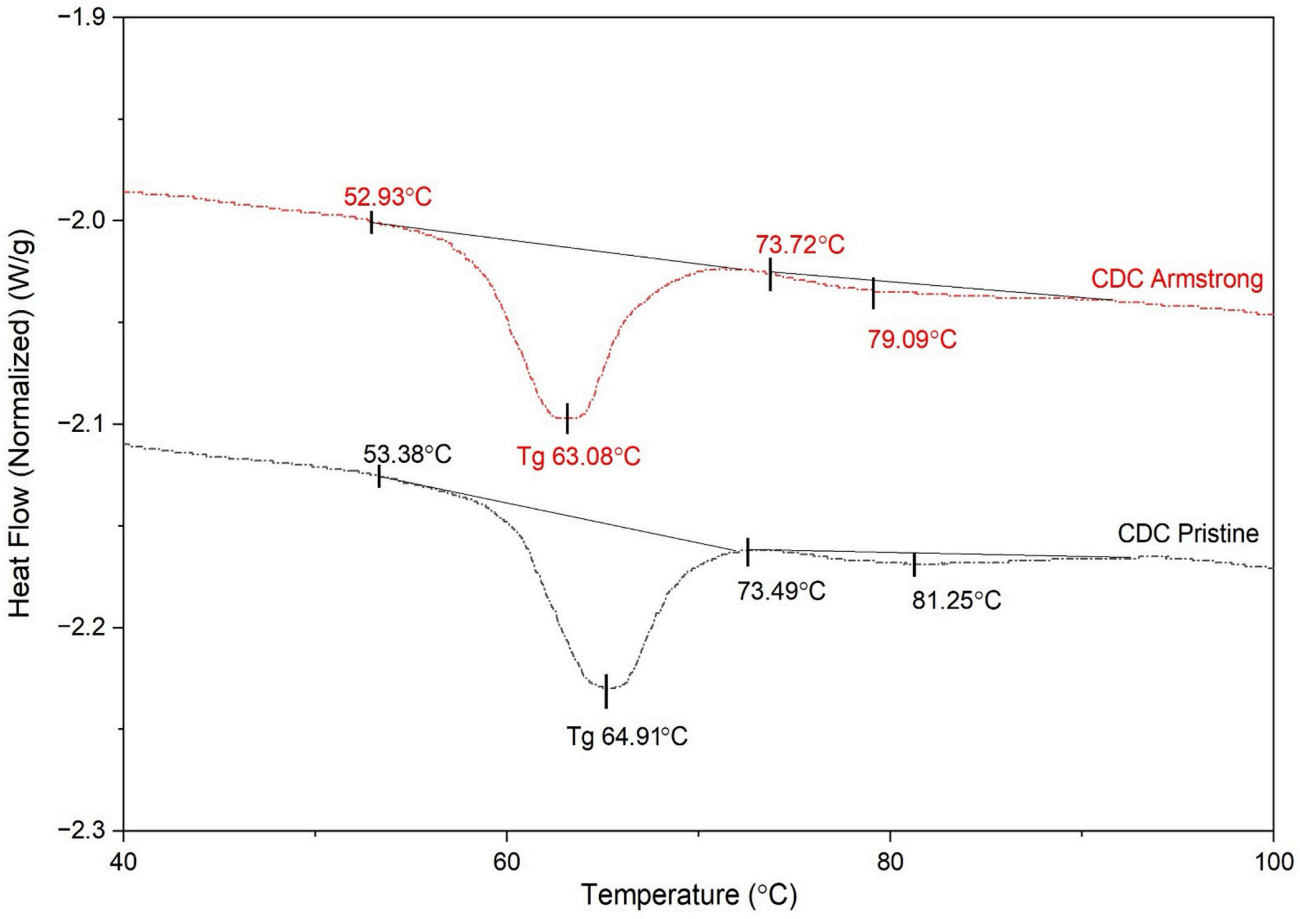
differences in gelatinisation temperature were also evident in the corresponding malts with CDC Armstrong showing a lower gelatinisation temperature than CDC Pristine (Figure 2).

Malting performance under varying steeping and germination time

Hydration behaviour

All grain samples were subjected to intermittent steeping, with three wet immersions alternated with three air rests, with the total immersion time reduced from 19 to 13 to 11 hours by shortening the duration of each of the three steeps. The impact of the reduced steeping time on the final grain moisture (steep-out moisture) for CDC Armstrong and CDC Pristine is shown in Figure 3. For comparison, CDC Clear was also included in these trials. As expected, a shorter steeping time led to a lower steep-out moisture across all cultivars with CDC Armstrong and CDC Pristine exhibiting 4-5% higher steep-out moisture at each steeping compared to CDC Clear.

Figure 2. Differential scanning calorimetry of CDC Armstrong and CDC Pristine malt to measure starch gelatinisation temperature.



Cytolytic modification

To assess the effects of germination length, grains steeped to three different moisture levels were germinated for three or four days. Germination was conducted at a constant temperature of 18°C, with identical kilning conditions. The impact of reduced steeping and germination times on cytolytic modification was evaluated by measuring parameters, including wort viscosity and β-glucan concentration, and by determining the levels of the other major cell wall polysaccharide, arabinoxylan. Overall, the β-glucan concentration in wort increased with reduced steeping and shorter germination periods (Figure 4). Although this pattern was consistent across all three cultivars, CDC Armstrong demonstrated increased β-glucan degradation, yielding a low wort β-glucan concentration (43-74 mg/L) under the most restrictive steeping and germination conditions. CDC Pristine also exhibited a lower concentration of β-glucan in worts from malt prepared at each malting conditions compared to CDC Clear (Figure 4). The friability values ranged from 45-55% for CDC Armstrong and 60-75% for CDC Pristine. Although these values are lower than typically observed for hulled barley malt, the low friability in hulless barley reflects the mechanical behaviour of the huskless kernel, as the absence of a hull causes kernels to flatten rather than fracture during friabilimeter testing. Consequently, friability does not reliably indicate endosperm modification in hulless barley. As demonstrated by Izydorczyk et al (2022), hulless barley malts may exhibit low friability yet show normal biochemical parameters of modification (high extract, low β glucan, good solubility), suggesting that modification should be assessed using biochemical rather than mechanical metrics. The ability of CDC Armstrong to extensively degrade β-glucans was further demonstrated by monitoring β-glucan concentration throughout the mashing process. As shown in Figure 5, mash from CDC Armstrong malt produced using a 13 hour steep and three day germination exhibited only a small increase in β-glucans (40 to 70 mg/L), whereas a mash from four day germinated malt maintained a consistently low concentration of β-glucan (~40 mg/L) across the process. These observations indicate that β-glucans in CDC Armstrong are effectively hydrolysed during malting and in the early stages of mashing. In contrast, β-glucan in mashes from CDC Clear and CDC Pristine rose once the mash temperature reached 65°C and continued to increase thereafter, reflecting the enhanced solubilisation of cell wall β-glucans at elevated temperatures coinciding with thermal inactivation of β-glucanases. CDC Pristine consistently exhibited lower β-glucan levels than CDC Clear at all mashing stages, suggesting more efficient β-glucan modification in this cultivar, although still substantially less complete than that for CDC Armstrong.

Wort viscosity is strongly correlated with the concentration of β-glucans (Jin et al. 2004). This relationship was confirmed with CDC Clear and CDC Pristine, where viscosity values increased proportionally with increasing

Figure 3. Different steeping time and steep-out moisture content of CDC Armstrong, CDC Pristine, and CDC Clear.

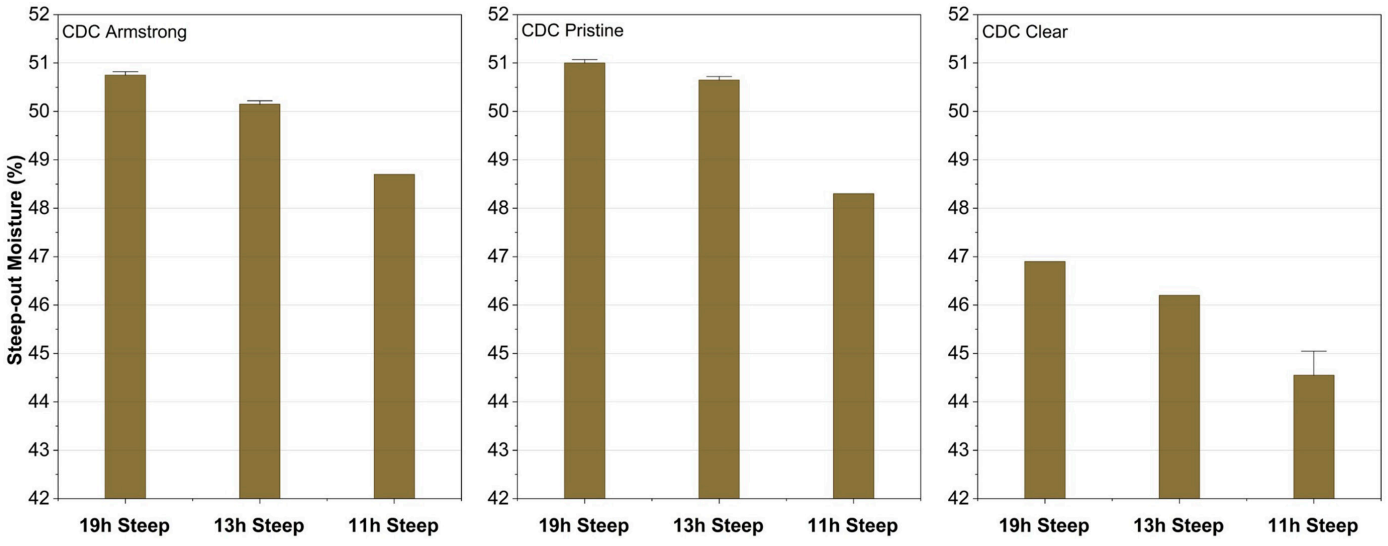
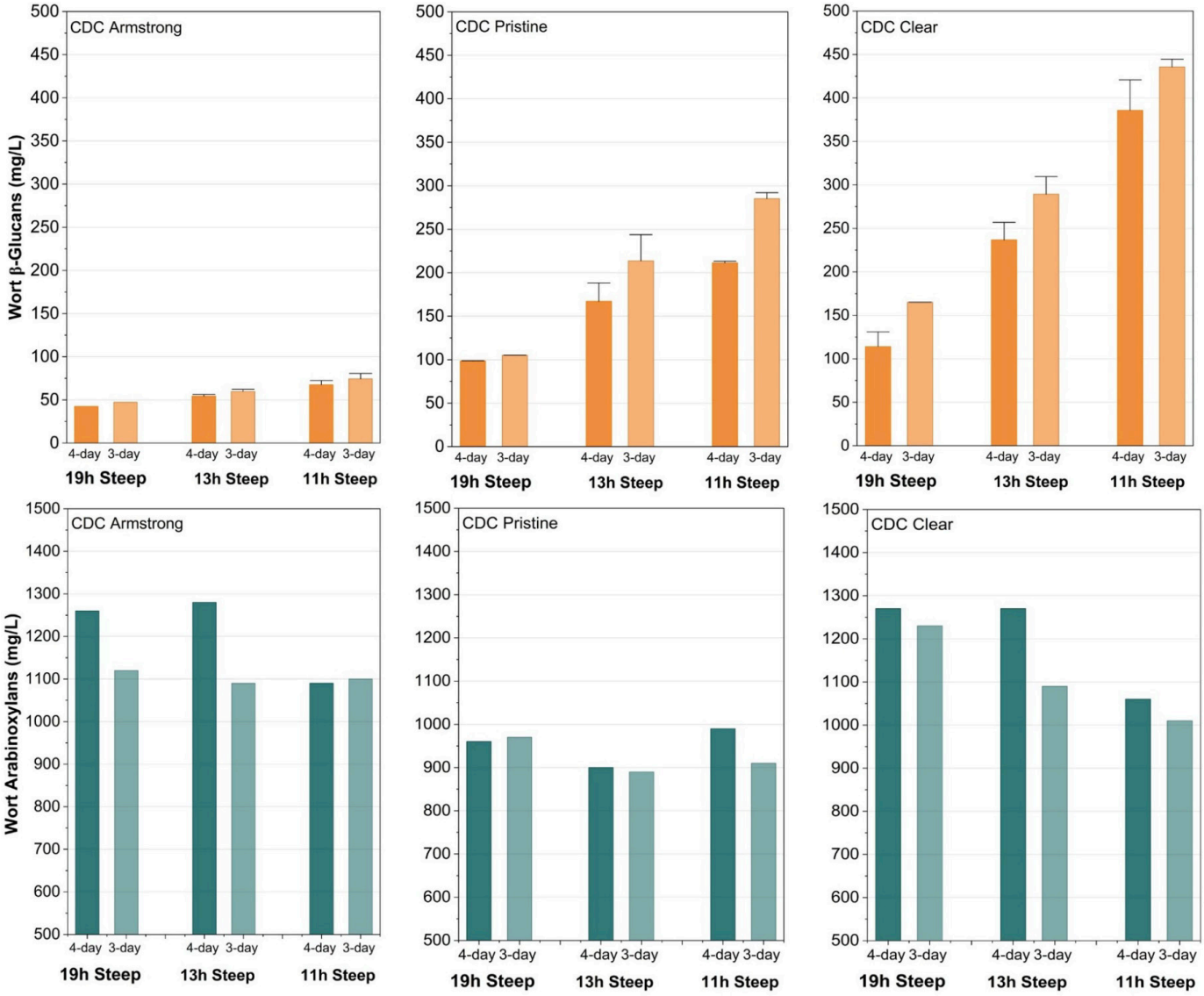


Figure 4. Steeping and germination time (4 or 3 days) and the concentration of β-glucans and arabinoxylans in wort from malts of CDC Armstrong, CDC Pristine and CDC Clear.

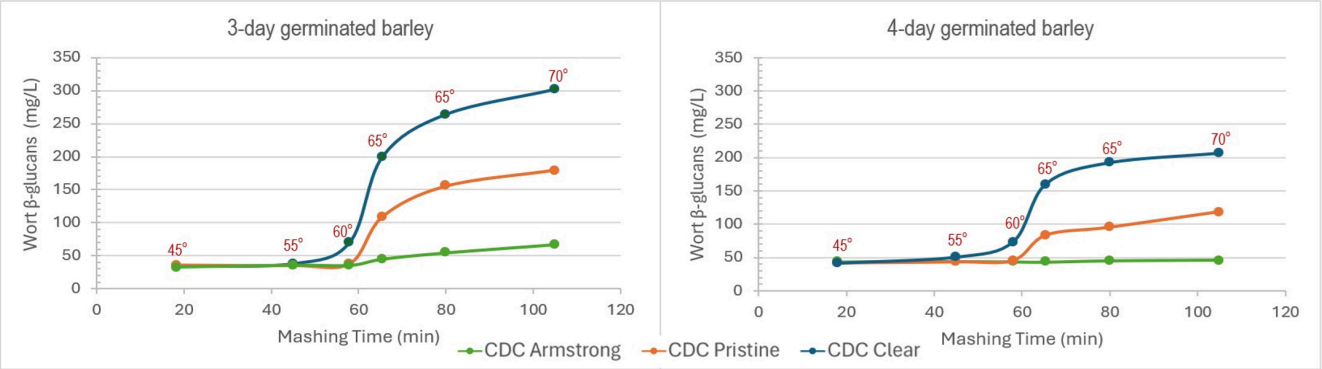


β-glucan concentrations under restricted steeping and germination conditions (Figure 6). Consistent with the low β-glucan concentration, CDC Armstrong exhibited the lowest wort viscosity among the three cultivars and showed minimal sensitivity to malting conditions. Interestingly, the viscosity values for CDC Armstrong - which ranged from 1.48 to 1.52 cP - were higher than those typically observed in wort from common 2-row hulled malting varieties at equivalent β-glucan concentrations (Izydorczyk and McMillan 2024). According to the 2024 annual harvest report of western Canadian malting barley (Izydorczyk and McMillan 2024), the β-glucan concentration in wort of AAC Synergy varied between 32 and 117 mg/L resulting in viscosities from 1.37 to 1.43 cP.

The concentration of arabinoxylans in wort, as influenced by duration of steeping and germination, exhibited an opposite trend to that observed for β-glucan. In general, arabinoxylan concentrations decreased with reduced steeping and shorter germination periods (Figure 4), suggesting that restrictive malt modification limits the solubilisation of this polysaccharides during mashing. In worts of CDC Armstrong, arabinoxylan concentrations ranged from 1090 to 1290 mg/L and were comparable to those measured in worts of CDC Clear.

In contrast, CDC Pristine consistently showed lower arabinoxylan concentrations (890–990 mg/L), which were relatively unaffected by malting conditions. The relatively high arabinoxylan

Figure 5. Concentration of β-glucan during mashing of malt from CDC Armstrong, CDC Pristine, and CDC Clear from barley steeped for 13 hours and germinated for three or four days.



content in CDC Armstrong wort may partly account for its elevated viscosity despite low β-glucan concentrations. However, additional wort constituents - fermentable and non-fermentable carbohydrates - may also contribute to the overall higher viscosity observed in worts prepared from hulless barleys.

These results demonstrate that, compared with CDC Clear, both new hulless cultivars exhibit traits that support a more complete enzymatic degradation of cell wall polysaccharides, particularly β-glucans, during malting. In CDC Armstrong, this enhanced cytolytic modification was evident, allowing steeping and germination times to be reduced without compromising wort viscosity, supporting efficient filtration and high extract yield in brewing.

Amylolytic modification

Figure 7 illustrates the diastatic enzyme activity in malts of all three hulless cultivars under varying steeping and germination conditions. As expected - since α-amylase is synthesised exclusively during germination (MacGregor et al. 1984) - its activity was more influenced by shortening the germination period (from four to three days) than by reducing the steeping time. Across all malting conditions, CDC Pristine exhibited higher α-amylase activity compared to CDC Armstrong and CDC Clear.

Diastatic power (DP), reflects the combined activities of β-amylase, α-amylase, and limit dextrinase, with β-amylase the dominant contributor (Duke and Henson 2011), was reduced when germination time was shortened. Interestingly,

Figure 6. Effect of steeping and germination time (4 or 3 days) on viscosity of wort from malt of CDC Armstrong, CDC Pristine and CDC Clear.

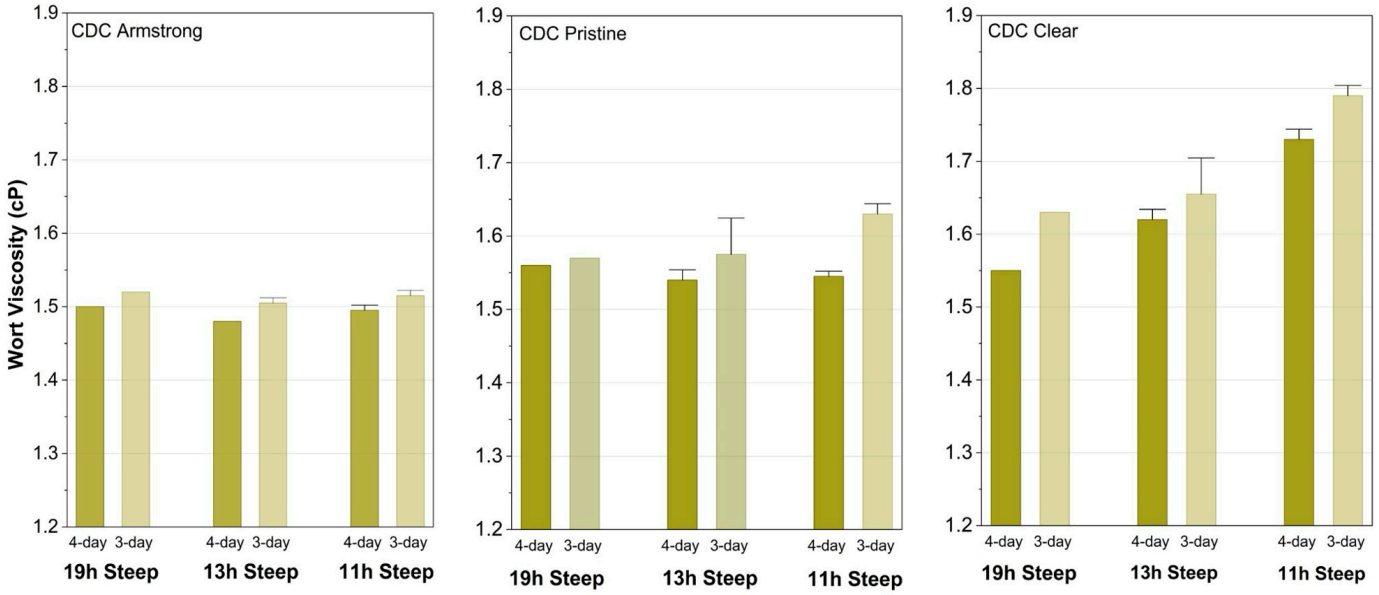
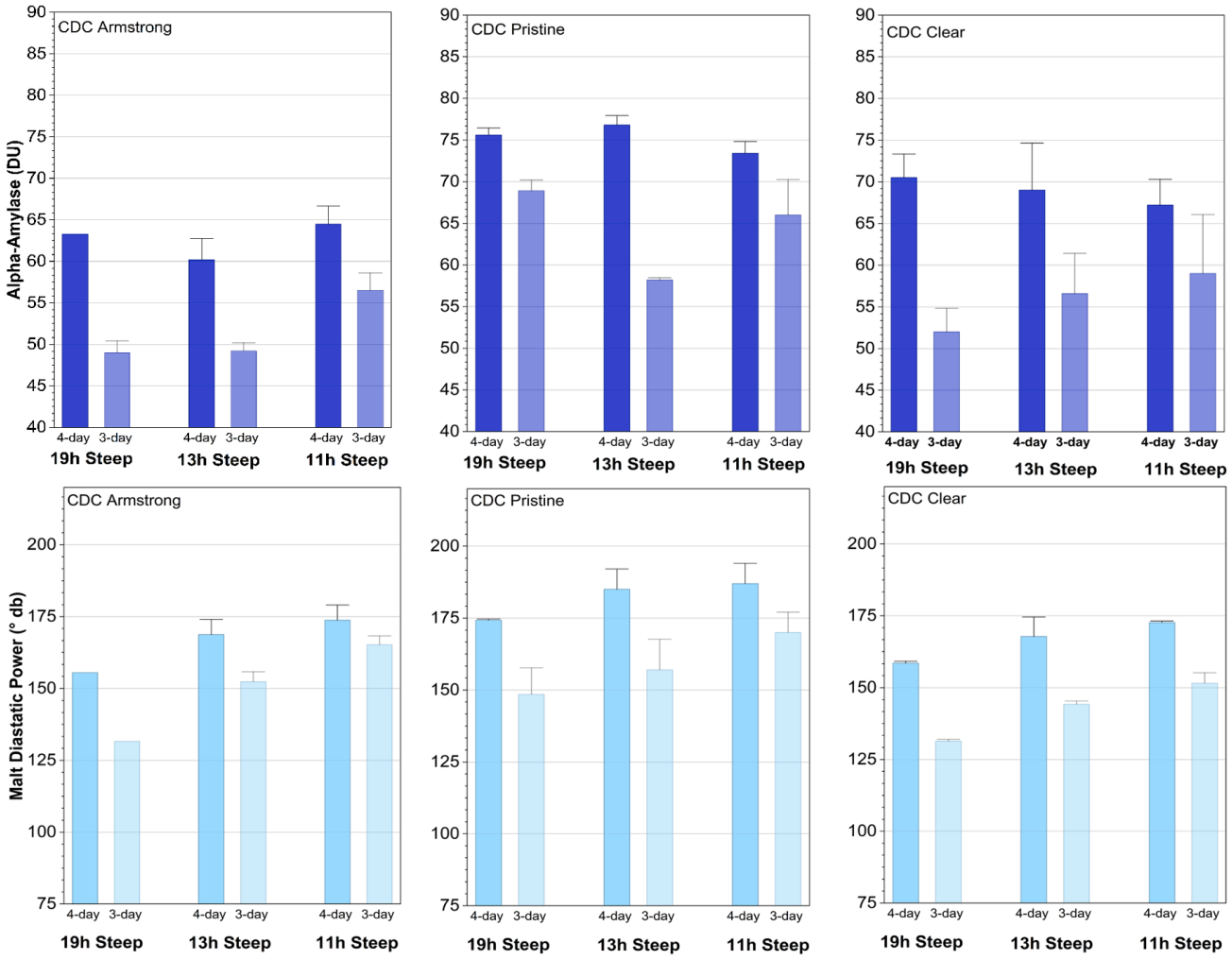


Figure 7. Effect of steeping and germination time (4 or 3 days) on the activity of α-amylase and diastatic power in malt of CDC Armstrong, CDC Pristine and CDC Clear.



DP values were not negatively impacted by reduced steeping duration; indeed malts produced with the shortest steeping period exhibited higher diastatic power activity (Figure 7). This outcome may be attributed to the contribution of β-amylase, which is present in the barley kernel prior to malting and requires only activation during the malting process (MacGregor and Fincher 1993).

Despite some negative effects of shortening germination time on diastatic enzyme levels, the overall enzyme activity remained sufficient to generate high malt extract yields from these hulless cultivars (Figure 8). Extract levels for all three hulless cultivars were above 86%, exceeding that reported for 2-row hulled barley cultivars (79–82%) (Izydorczyk and McMillan 2024). In most cases, reducing germination time from four to three days decreased extract yield by less than 1% (0.6–0.9%). A similarly small reduction was observed when steeping time was shortened from 19 to 11 hours. These results indicate that extract yield was only mildly affected by reduction of steeping and germination time. Among the hulless cultivars, CDC Armstrong produced slightly higher extract levels (87.2-88.8%) compared with CDC Pristine (86.2-87.7%) and CDC Clear (86.2-88.0%). Despite lower levels of diastatic enzymes relative to CDC Pristine, the higher malt extract of CDC Armstrong may be explained by favourable grain characteristics such as lower protein content, lower hardness index and higher starch content.

Fermentable sugars were measured in worts from barley steeped for 13 hours and germinated for either four or three days and compared to wort from AAC Synergy malt, a hulled barley cultivar. All hulless cultivars produced worts with a higher concentration of fermentable sugars (70.8-76.8 g/L) than AAC Synergy (67.6 g/L) (Table 5), reflecting the greater starch content (Table 3) and levels of diastatic enzymes (Figure 6). Elevated diastatic

Table 5. Fermentable sugars in worts from barley from CDC Armstrong, CDC Pristine, CDC Clear and AAC Synergy steeped for 13 hours and germinated for four or three days.

Sugars (g/L)	CDC Armstrong		CDC Pristine		CDC Clear		AAC Synergy	
	4 day germinated malt	3 day germinated malt	4 day germinated malt	3 day germinated malt	4 day germinated malt	3 day germinated malt	4 day germinated malt	3 day germinated malt
Glucose	10.4	9.2	10.7	10.4	9.7	8.9	8.0	7.2
Fructose	1.4	1.3	1.5	1.8	0.9	1.0	0.8	0.8
Sucrose	1.6	1.6	2.4	1.5	2.8	2.5	3.5	3.3
Maltose	48.2	48.0	50.5	49.6	49.7	49.1	45.7	46.5
Maltotriose	10.9	10.7	11.8	11.5	10.6	10.2	9.4	9.8
Total	**72.4**	**70.8**	**76.8**	**74.8**	**73.5**	**71.7**	**67.5**	**67.6**

enzyme levels in CDC Pristine contributed to the higher levels of fermentable sugars in wort compared to the other hulless cultivars (Table 5). Data from both gemination time periods, showed CDC Pristine wort to have 4.2 g/L more fermentable sugars than CDC Armstrong and about 3.3 g/L more than CDC Clear. These differences in CDC Pristine worts were attributable mainly to increased levels of maltose, maltotriose and glucose (Table 5). Further, worts from hulless barley cultivars contained higher fructose but lower sucrose levels compared with AAC Synergy, suggesting lower invertase activity in the latter.

The greater diastatic enzyme activity in CDC Pristine malt was further demonstrated by the activity of α- and β- amylase activity over the course of mashing. As shown in Figure 9, β-amylase activity for all cultivars peaked after 45 minutes of mashing as the temperature reached around 55°C. As the temperature rose to 65°C, β-amylase activity decreased slightly, and then declined during the 15 minute holding period before diminishing further as the temperature increased to 70°C. This pattern of β-amylase activity was consistent among malts of all cultivars reflecting the known thermolability of this enzyme. However, within the three hulless cultivars, CDC Pristine exhibited the highest β-amylase activity, comparable to that observed in AAC Synergy.

The activity of α-amylase in the mash of CDC Pristine and AAC Synergy was broadly constant between 45 and 60°C, increasing between 60 to 65°C, and then declining at 70°C, reflecting the recognised thermostability profile of this enzyme (Figure 9). The activity of α-amylase in the mash

Figure 8. Effect of steeping and germination (4 or 3 days) on the extract of malt CDC Armstrong, CDC Pristine, and CDC Clear.

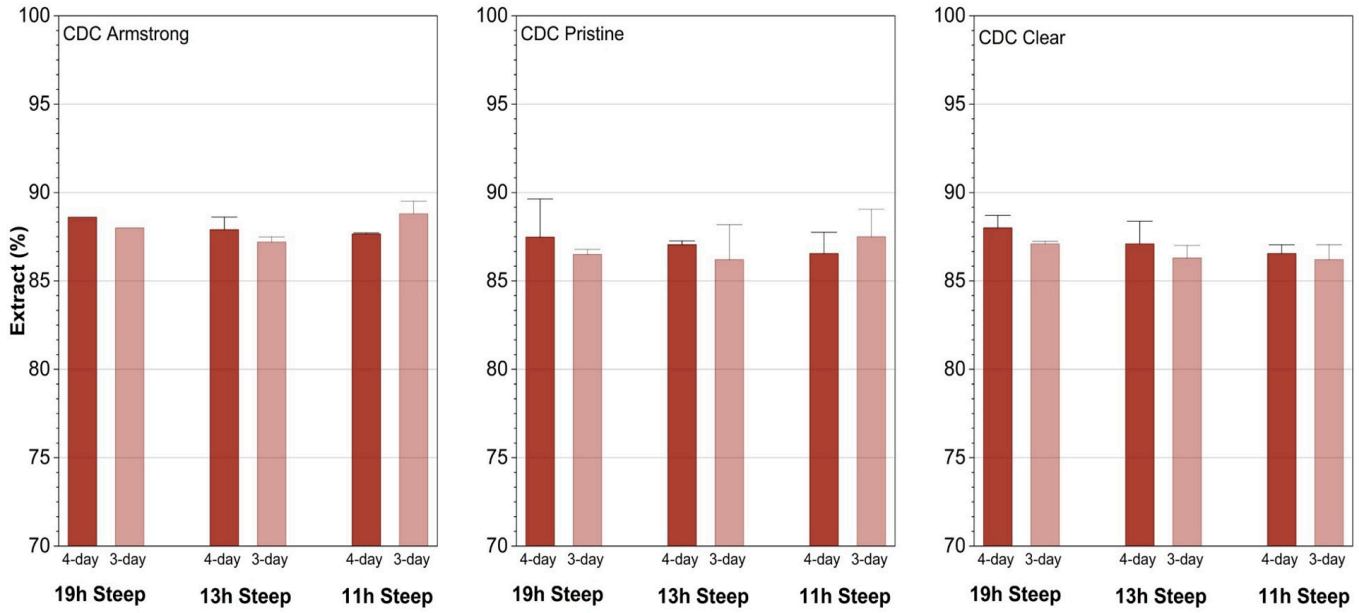
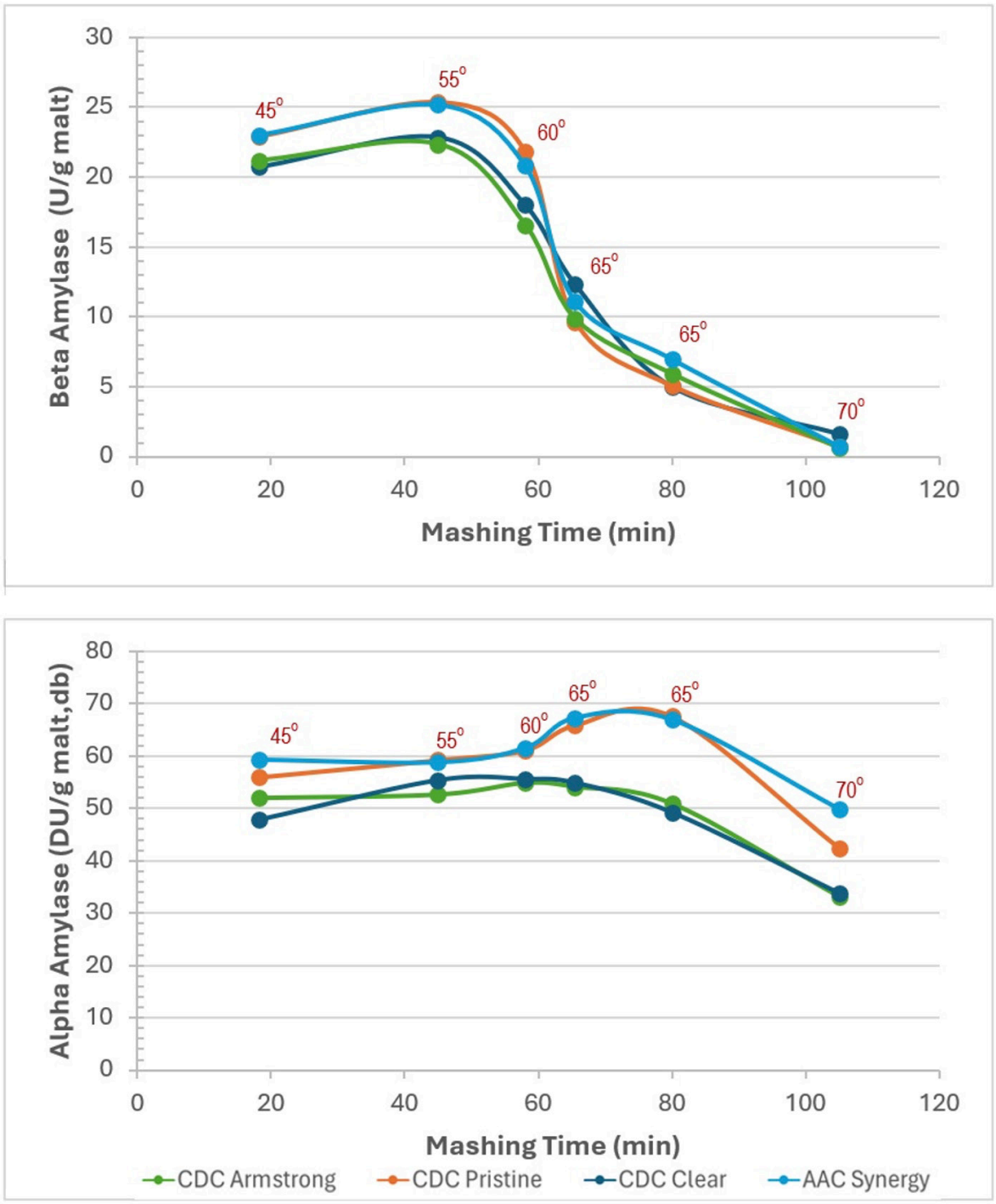


Figure 9. Activity of α- and β-amylases during mashing of malt from CDC Armstrong, CDC Pristine, CDC Clear and AAC Synergy steeped for 13 hours and germinated for three days.

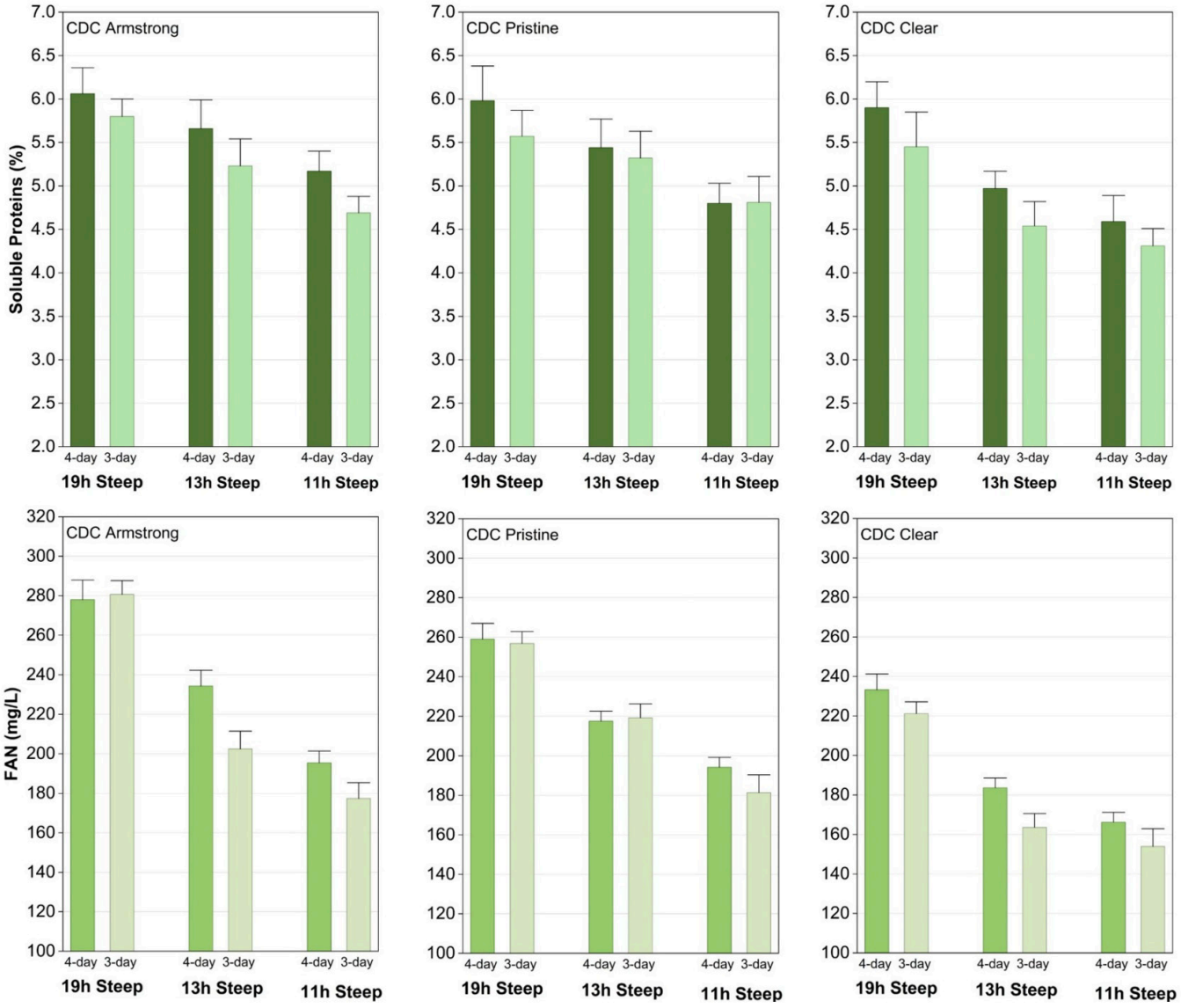


of CDC Armstrong and CDC Clear remained relatively stable between 45 and 65°C but also showed a substantial decline above 65°C (Figure 9). At each stage of the mashing process, α-amylase activity in the mash of CDC Pristine was consistently higher than with CDC Armstrong and CDC Clear, in agreement with the enzyme activity in the corresponding malts (Figure 6).

Proteolytic modification

The concentration of soluble proteins and free amino nitrogen (FAN) in worts produced from malts of the three hulless barley cultivars with varying steeping and germination regimes decreased as steeping and germination times were reduced, indicating a suppression of proteolytic modification under restrictive malting conditions (Figure 10).

Figure 10. Effects of steeping and germination (4 or 3 days) on the concentration of soluble proteins and free amino nitrogen (FAN) in wort produced from malts of CDC Armstrong, CDC Pristine and CDC Clear.



Despite this, CDC Armstrong and CDC Pristine generated worts with high levels of soluble protein (> 4.7 %) and FAN (>180 mg/L) that exceeded those of CDC Clear, even under the most constrained conditions (Figure 10). This suggests that CDC Armstrong and CDC Pristine possess greater proteolytic activity than CDC Clear, making them more robust candidates for efficient malt production under suboptimal processing conditions.

Analysis of amino acids in wort from malts using a 13 hour steep and four day germination showed hulless malts to have an appropriate profile for brewing. Amino acids constitute a major fraction of wort nitrogen and are essential for yeast nutrition and fermentation performance with free amino acids serving as the most readily assimilable nitrogen source (Stewart et al. 2013). As yeast utilise groups of amino acids in sequence (Jones and Pierce 1964), the relative abundance of amino acids in Group A (fast uptake) and B (intermediate uptake) is important. Table 6 shows the concentration of Group A and B amino acids in worts from CDC Pristine and CDC Armstrong were comparable to those in AAC Synergy and higher than in CDC Clear. Moreover, proline - the poorly assimilated amino acid by yeast - was present at lower concentration in worts of the new varieties compared to CDC Clear. These results suggest that worts from CDC Pristine and CDC Armstrong have a favourable amino acid composition, supporting their suitability for high quality malt production.

Table 6. Amino acids in worts from barley of CDC Armstrong, CDC Pristine, CDC Clear and AAC Synergy steeped for 13 hours and germinated for four days.

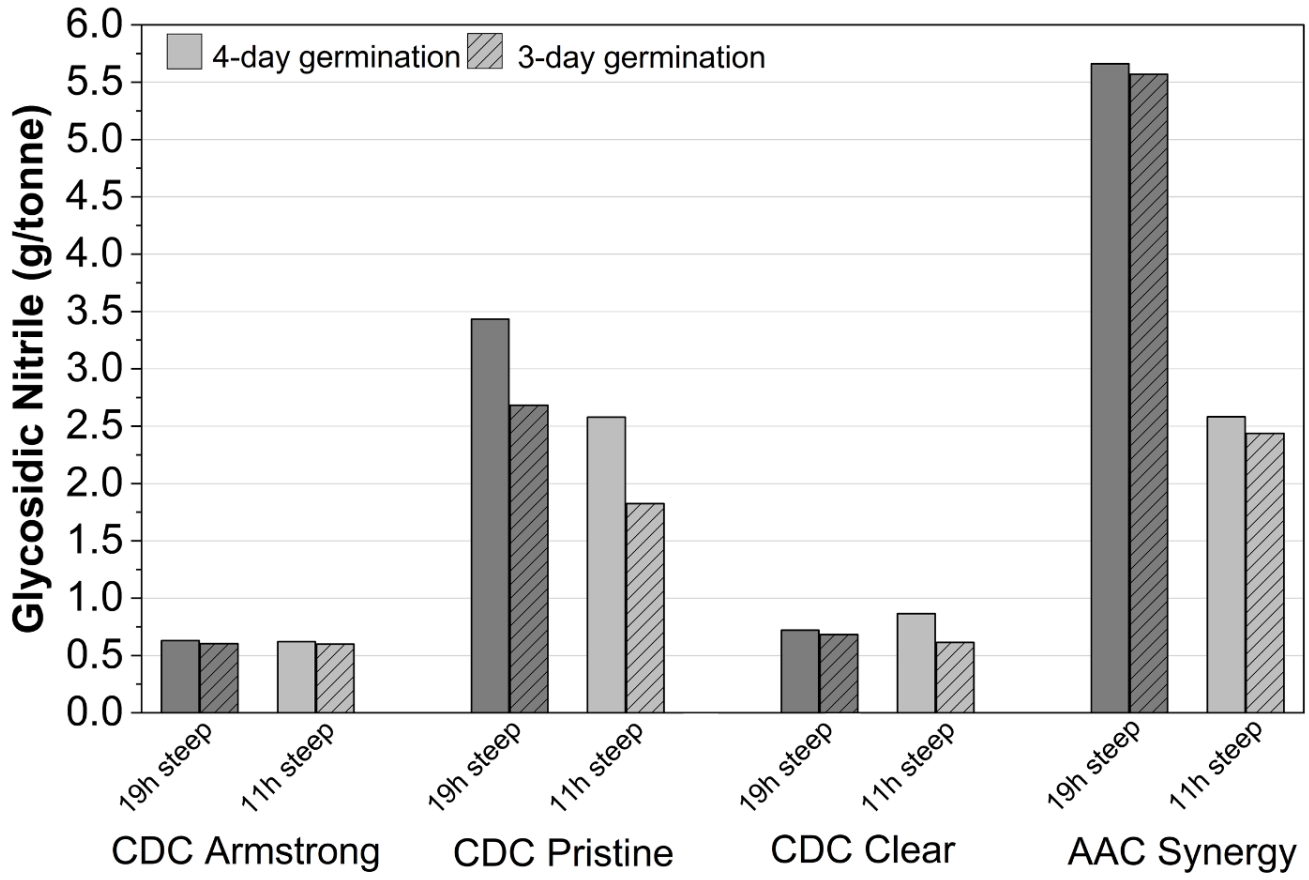
Amino Acids	CDC Armstrong		CDC Pristine		CDC Clear		AAC Synergy	
	Concentration mg/L	Distribution %	Concentration mg/L	Distribution %	Concentration mg/L	Distribution %	Concentration mg/L	Distribution %
Group A	1,147 ± 15	37	1,206 ± 20	38	999 ± 18	36	1,240 ± 20	41
Group B	485 ± 8	20	512 ± 9	20	424 ± 9	19	497 ± 11	19
Group C	164 ± 9	19	167 ± 10	19	130 ± 8	18	132 ± 9	18
GABA	104 ± 4	4	111 ± 5	5	85 ± 4	4	92 ± 5	4
Proline	437 ± 8	19	458 ± 9	19	496 ± 11	23	415 ± 9	17
Total	**2,338 ± 30**		**2,453 ± 37**		**2,134 ± 37**		**2,376 ± 40**	

Glycosidic nitrile

Hulless barley malts may also offer advantages for distilled spirit production due to high extract levels and elevated concentration of fermentable sugars, which together have the potential to increase alcohol yield. However, an important consideration when evaluating hulless barley malts for distilling is their content of glycosidic nitrile (GN), the primary precursor of ethyl carbamate, a probable human carcinogen that can form during fermentation and distillation (European Food Safety Authority 2007; Bringhurst 2015). Consequently the distilling industry uses barley varieties with inherently low glycosidic nitrile production and seeks to optimise malting, mashing, fermentation, and distillation to further limit ethyl carbamate formation. Barley genotypes are typically classified into three glycosidic nitrile production categories: (i) GN-null (<0.5 g/tonne), (ii) low GN producers (0.5–1.5 g/tonne), and (iii) high GN producers (>1.5 g tonne).

Analysis of GN concentration in hulless barley malts showed that CDC Armstrong and CDC Clear are low GN producers, whereas CDC Pristine is a high glycosidic nitrile producer, similar to AAC Synergy (Figure 11). As epiheterodendrin, the barley specific glycosidic nitrile, is concentrated in embryo and acrospire tissues, the easier detachment and loss of acrospires in hulless barley reduces the embryo derived fraction entering the finished malt

Figure 11. Effect of steeping and germination (4 or 3 days) on levels of glycosidic nitrile in malts of CDC Armstrong, CDC. Pristine, CDC Clear, and AAC Synergy.



and contributes to lower GN potential. However, acrospire loss is not the only factor influencing GN in hulless malt. CDC Pristine, a hulless cultivar, produced GN levels comparable to hulled AAC Synergy, indicating that cultivar specific biochemistry and germination are also implicated. Indeed, glycosidic nitrile levels can be moderated through shortened steeping and germination regimes, and modest reductions in glycosidic nitrile concentration were observed in CDC Pristine and AAC Synergy when steeping was reduced from 19 to 13 hours and germination from four to three days. These adjustments had minimal impact on CDC Armstrong and CDC Clear, which exhibited low glycosidic nitrile concentrations even under more extensive malting conditions.

Conclusions

The comparative assessment of hulless cultivars CDC Armstrong and CDC Pristine revealed several different physicochemical attributes in grain composition, kernel morphology, and starch functionality that are likely to influence their malting performance. Although both cultivars exhibited favourable β-glucan levels and low kernel hardness, CDC Armstrong had larger kernels, higher starch content, and greater proportion of large A-type starch granules; traits associated with lower gelatinisation temperatures and more efficient starch modification during malting. In contrast, CDC Pristine's slightly higher protein concentration and greater proportion of small granules may contribute to its higher starch gelatinisation temperature.

The study demonstrated that both CDC Armstrong and CDC Pristine possess improved hydration efficiency compared with the older hulless cultivar CDC Clear. Even under progressively shortened steeping regimes, the two new cultivars achieved higher steep-out moisture, indicating faster and more effective water uptake. This highlights their suitability for malting processes requiring reduced steeping times. This extended into cytolytic performance, where CDC Armstrong demonstrated enhanced degradation of β-glucans and improved wort viscosity under restrictive malting conditions.

Amylolytic assessments confirmed that both varieties possess strong diastatic potential, supporting high extract yields even with reduced germination time. CDC Pristine exhibited the strongest amylolytic profile. This was characterised by higher α- and β-amylase activities and elevated fermentable sugar concentration similar to that of the hulled malting standard AAC Synergy. CDC Armstrong, despite lower enzyme levels, maintained excellent extract yields, reflecting its favourable grain properties and high starch content. Together, these results show both new hulless cultivars possess robust amylolytic capacity, with CDC Pristine showing strength in enzymatic activity and CDC Armstrong with efficient starch conversion supported by advantageous grain traits.

Proteolytic performance was similarly strong, with both cultivars maintaining high soluble protein and FAN levels under accelerated malting conditions and producing wort amino acid profiles comparable to those of leading hulled malting cultivars, an important indicator of fermentation efficiency and flavour stability.

The assessment of glycosidic nitrile production highlighted important distinctions among the hulless cultivars with respect to their suitability for distilling applications. CDC Armstrong produced very low glycosidic nitrile levels across all malting conditions, positioning it as a strong candidate for minimising the ethyl carbamate risk during spirit production. CDC Pristine behaved similarly to AAC Synergy as a high glycosidic nitrile producer, although its glycosidic nitrile concentrations could be modestly reduced through shortened steeping and germination. These findings indicate that, while malting adjustments can partially mitigate glycosidic nitrile formation in high producing cultivars, inherent genetic differences remain a dominant factor. Consequently, CDC Armstrong represents a better option for use in distilling, whereas CDC Pristine would require additional process control to manage glycosidic nitrile.

Overall, the results of this study demonstrate that CDC Armstrong and CDC Pristine represent significant advancements in hulless barley breeding, offering improved malting quality relative to the older cultivar CDC Clear, with CDC Armstrong showing a balanced combination of favourable grain traits, strong modification capacity, and low glycosidic nitrile production. These findings

underscore the potential of modern hulless barley cultivars to meet the quality demands of both the brewing and distilling industries, particularly under accelerated or resource efficient malting regimes.

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CRediT author contributions

Marta S. Izydorczyk: Conceptualisation, funding acquisition, writing (original draft).
Tricia McMillan: Methodology, investigation.
Arzoo Sharma: Methodology, investigation.
Aaron D. Beattie: Conceptualisation, funding acquisition, writing (review and editing).

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