Exploring dry grain fractionation as a means to valorize high-protein malting barley

Marta Izydorczyk | Shin Nam | Arzoo Sharma | Jerry Kletke

Abstract

Background and objectives: Malting barley cultivars are potentially the most profitable commodities for producers; however, barley selected for malting purposes has to meet many stringent quality requirements. Barley with excessive grain protein concentration (>13%) is often the reason why it is rejected for malting grade and sold at a lower price on the feed market. The objectives of this study were to explore dry grain fractionation as a means to valorize high-protein malting barley by producing high fiber/protein fractions for human nutrition and starchy fractions for adjunct brewing.

Findings: Several malting barley varieties with grain protein concentration above 14% (db) were milled on a Buhler laboratory mill resulting in six flours streams. Coarse and fine shorts were further processed using a Buhler laboratory shorts duster resulting in coarse fiber fractions and two additional flour fractions. The total yield of combined flour fractions ranged from 50.2% to 51.6%, whereas the yield of fiber fractions ranged from 41.5% to 43.7%. The fiber fractions were enriched in β-glucans (9.3%–11.2%, db), arabinoxylans (9.9%–11.3%, db), and proteins (22%–26%, db) with average 2.2-, 1.7-, and 1.5-fold increase of these constituents, respectively, compared to the whole grain. The content of vitamin E in fiber fractions was higher than in the whole grain, ranging from 82 to 109 ug/g. The fiber fractions also exhibited an improved ratio of tocotrienol to tocopherols. The combined flour fractions were depleted of β-glucans (0.6%–0.7%, db) and arabinoxylans (0.5%–0.6%, db), contained acceptable levels of proteins (11.5%–13.5%, db) and high levels of starch (79%–82%, db). Mashing experiments with up to 40% replacement of malt with flour fractions showed significant improvements in malt extract without negative influence on malting quality parameters, such as wort beta-glucans, wort viscosity, and the average degree of polymerization of starch dextrins.

Conclusions: The study demonstrated that covered barley can be milled using milling equipment commonly used in wheat milling without the necessity of prior dehulling of the grain. The milling resulted in two high-yield fractions (flour and fiber/protein-rich fractions) with hulls effectively separated during the early stages of milling, making the fractionation process commercially feasible.

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1 | INTRODUCTION

Barley (Hordeum vulgare L.) is one of the most widely adapted cereal grain crops that can be grown under a wide spectrum of climatic conditions (Ullrich, 2011). Barley is also a very versatile crop and is used in animal feed, human food, and in the production of malt and beer. Barley varieties for the production of malt for brewing are potentially the most profitable commodities for producers; however, to be selected for malting purposes, barley grain has to meet stringent quality requirements. Barley varieties specifically bred for malting and brewing have the genetic potential to produce grain with an even and high rate of germination, high diastatic enzyme activities, and an ample supply of fermentable sugars. However, malting barley cultivars have to be grown under favorable environmental conditions to ensure the production of sound, plump, uniformly sized, grain protein concentration may absorb water more slowly during steeping leading to poor endosperm modification. In addition, high-protein barley tend to produce higher levels of soluble proteins in wort and beer which may cause filtration problems and haze formation (Izydorczyk & Edney, 2017). Conversely, barley with a high grain protein concentration may absorb water more slowly during steeping leading to poor endosperm modification. In addition, high-protein barley tend to produce higher levels of soluble proteins in wort and beer which may cause filtration problems and haze formation (Izydorczyk & Edney, 2017).

It is often difficult to produce malting barley with the right grain protein concentration. The protein content of the grain appears to be determined by a combination of genetic, environmental, and agronomic factors (Molina-Cano et al., 1997). In general, higher available nitrogen levels in soil and abiotic stresses, such as drought or high temperatures, may increase barley protein content. Malting barley cultivars with a grain protein content > 12.5% are usually disqualified for malting and brewing purposes and most often used as less profitable “feed grain” without fully exploring their potential value.

Barley is a wholesome and nutritious grain, naturally rich in β-glucans, dietary fiber, tocols, minerals, and other bioactive phytochemicals. Barley β-glucans, in particular, have been shown to offer a range of physiological benefits, including lowering cholesterol, reducing post-prandial glucose response, reducing blood pressure, and improving gut microbiota (Ames et al., 2019). Arabinoxylans, which offer the health benefits of soluble and insoluble fibers, have also been shown to play a role in modulating and supporting the immune system (Izydorczyk, 2019). Barley grain is also a rich and unique source of vitamin E because it contains relatively large amounts of all eight tocochromanols, with a favorably high ratio of tocotrienols to tocopherols, compared to other cereals and oilseeds (Badea et al., 2018). While past research into the benefits of vitamin E focused almost exclusively on alpha-tocopherol, unique biological functions of other vitamin E components have been also identified (Casal et al., 2006). For example, studies on tocotrienols demonstrated their positive role in suppressing proliferation of a wide variety of tumor cells, including those of the breast, colon, lung, and prostate, and lowering cholesterol in humans and animals (Cardenas & Ghosh, 2013; Sen et al., 2007). These findings have established the ratio of tocotrienols to tocopherols (T3/T) as an important criterion of the biopotential of grains and other foods considered as sources of vitamin E. Despite barley protein lacking certain essential amino acids, barley can still be considered a valuable source of proteins for human and livestock nutrition. Barley constituents, therefore, can be valuable food additives for the effective and economical control of many food-related health issues such as obesity, high blood pressure, heart disease, diabetes, and certain types of cancer. However, whole barley grain consists of approximately 50% to 60% starch and consumption of sufficient and recommended amounts of bioactive ingredients from barley grain is usually associated with consumption of large quantities of starch. Starch is often sought by breweries, however, as a fermentable sugar source to partially replace malt. Starchy adjuncts, such as rice, corn, and other unmalted grain products, are often used with the intention of cutting costs, but sometimes to create an additional feature, such as reduction of haze and off-flavor precursors, better foam retention, flavors, or nutritional value (Cooper et al., 2016).

Hull-less barley can be processed into fractions enriched in specific components (Izydorczyk and Dexter, 2016). The studies on roller milling of hull-less barley showed that high β-glucan/fiber fractions can be obtained by employing a relatively simple milling flow consisting of several break, sizing,
and shorts duster passages (Izydorczyk et al., 2014). The yield of these fractions was about 48%–49% with β-glucan content of 13%–18%, depending on the initial grain concentration of these polysaccharides. The earlier study on air classification of pin-milled barleys with waxy, regular, and high-amylose starch also resulted in β-glucan-enriched fractions (13%–24%); however, the yield of these fraction was relatively low (7.6%–20.9%) (Vasanthan & Bhatty, 1995). Better results were obtained by air classifying of double micronized barley. The approach was tested on two hull-less barleys with different starch types (Ferrari et al., 2009). Enriched fractions with 11.2% and 15.6% β-glucans and 29.8% and 28.4% yield were obtained from cultivars Priora and CDC Alamo, respectively. Fractionation of covered malting barley varieties has not been popular, likely due to the presence of hulls in these cultivars. The objectives of this study were to explore dry grain milling as a means to fractionate malting barley into fractions enriched in constituents with potential health benefits and starchy fractions for adjunct brewing.

2 | MATERIALS AND METHODS

2.1 | Material

Malting barley cultivars, AC Metcalfe, AAC Goldman, Lowe, CDC Bow, AAC Connect, and CDC Meredith were grown in Manitoba, Canada, in 2018.

2.2 | Milling

Six barley varieties with grain protein concentration ranging from 15.0% to 18.2% (dwb) were conditioned to 16.0% moisture content and rested overnight (16–18 hr) prior to milling. Milling was performed in duplicate in a climate-controlled room (21°C and 60% RH) with a Buhler MLU 202 Laboratory mill (Buhler Canada, Markham, Ontario) according to the milling flow shown in Figure 1.

The laboratory Buhler mill was equipped with 6 sets of corrugated rolls (B1-4 and S1-2). Flour streams obtained from each set of rolls were combined and sifted through a stack of five nylon sieves with apertures ranging from 475 to 63 μm to obtain a fraction designated as “B1-S2 flour.” The hulls were collected after the B4 passage. The coarse and fine shorts collected after the last sizing rolls passage (S2) were directed to the first shorts duster (SD1 equipped with 165 μm screen) to further separate starch granules adhering to the outer grain tissues and the endosperm cell walls. The floury fraction from the first SD passage was sifted and designated the “SD1 flour.” The material remaining on the sieves together with the SD1 coarse fraction was passed again through another shorts duster (SD2) equipped with a finer screen (100 μm). The floury fraction was sifted to obtain a fraction designated as “SD2 flour.” The material remaining on the sieves was designated as “fine fiber-rich fraction (FRF),” whereas the SD2 coarse fraction was designated as “coarse FRF.”

**FIGURE 1** Roller milling flow for the production of flour, fiber-rich fractions (FRF), and hulls from covered barley. B1-B4: break rolls; S1-S2: sizing rolls; SD: shorts duster [Colour figure can be viewed at wileyonlinelibrary.com]
2.3 | Physicochemical tests and composition analyses

All compositional analyses for whole grain and milling fractions were conducted in duplicates, and results are reported on a dry weight basis. Moisture content and ash contents were determined according to AACC International methods 44-15.02 and 08-01.01, respectively (AACC International, 1999). Protein content (% N × 6.25) was determined by combustion nitrogen analysis, method 46-30.01 (AACC International, 1999). Total starch and β-glucan contents were determined enzymatically (Megazyme International, Bray, Ireland) according to methods 76-13.01 and 32-23.01, respectively (AACC International, 1999). Arabinoxylan content of samples was determined after hydrolysis of polysaccharides to monosaccharides with 1 M sulfuric acid, followed by reduction and acetylation of the monosaccharides to alditol acetates. The samples were analyzed by GC-FID (Izydorczyk, 2014). Insoluble, soluble, and total dietary fiber was determined according to method AOAC 991.43 using the ANKOMTDF Dietary Fiber Analyzer. Tocots were extracted from whole grain and fiber-rich fractions by saponification under nitrogen according to the method of (Lampi et al., 2008; Nielsen and Hansen, 2008; Panfili et al., 2008), with some modifications. Chromatographic separation was achieved using Kromasil KR60-5-Diol column (250mm × 4.6mm, particle size 5 um, Supelco) normal phase column. The mobile phase was hexane/ethanol/acetate/acidic acid (94:6:3:6:1:8), with a flow rate of 1.0 ml/min using Waters® 2,695 Separations Module, Waters; Mississauga, ON) equipped with Fluorometric detection (Waters® 2,475 Fluorescence Detector, Mississauga, ON).

Flours (500 mg) were extracted sequentially with 0.5 M NaCl for 10 min at room temperature and 50% propanol containing 1% dithiothreitol (DTT) for 30 min at 60°C to obtain albumin/globulin and hordein fractions, respectively. The pellet remaining after the aqueous extractions contained gluatinins. All extracted fractions were freeze dried and quantified by LECO protein analyzer. All determinations were done in duplicates, and all results are reported on a dry weight basis.

Particle size measurements were carried out using a Mastersizer 2000 instrument equipped with Hydro 2000S dispersion unit (Malvern, UK) using ethanol as the dispersant. The development of viscosity of milling fractions in water was determined with a Perten Rapid Visco-Analyser 4,500 (Springfield, USA).

Beta-glucans were extracted by inactivating milling fractions (1g) by boiling in 70% ethanol for 60 min. The inactivated pellet was mixed with water and heated at 45°C C for 45 min followed by centrifugation. The supernatant was digested with alpha-amylase (A6255, Type I-A from Porcine, Sigma) in presence of CaCl2 overnight followed by precipitation with 4 volumes of 95% ethanol. The extracted β-glucans were analyzed by high-performance size-exclusion chromatography (HPSEC, Waters Alliance 2,695, Milford, MA) in line with UV, multangle laser light-scattering (Dawn Heleos II), refractive index (Optilab TrEX RI), and viscosity (ViscoStar II) detectors (Wyatt Technology, Santa Barbara, CA). The HPSEC system was comprised of a guard column (SB-G) and two OH-packed columns (SB 804 HQ, SB 806 M HQ; Shodex Denko K.K., Tokyo, Japan). Samples were dissolved in 0.075 M sodium nitrate containing 0.02% sodium azide) at 90°C and filtered through 0.45 μm GF/B glass fiber syringe filters(13 mm, Whatman Inc., Clifton, NJ). The determination of the average molar mass (Mw), intrinsic viscosity [η], polydispersity (Mw/Mn), radius of gyration (Rw), Mark–Houwink parameter (α) was performed using Astra software 7 (Wyatt Technology, Santa Barbara, CA).

2.4 | Mashing and wort analysis

Worts were produced by Congress mashing (ASBC method Malt–4) and were characterized by using the standard methods of American Society of Brewing Chemists (ASBC). Wort viscosity was determined with an Anton Paar Lovis ME rolling-ball viscometer (ASBC Wort-13B). Wort-free amino nitrogen (FAN) concentration was determined by segmented flow analysis (ASBC Wort-12B). The content of β-glucan in wort was determined by measuring increased fluorescence from calcofluor binding with β-glucan polymers by segmented flow analysis (Skalar, Netherlands) (ASBC Wort-18B). Protein in unhopped wort (soluble protein) was determined by spectrophotometry based on the differing UV absorption of protein at 215 and 225 nm (ASBC Wort-17). Malt protein content was measured by nitrogen combustion with a LECO protein analyzer (ASBC Malt-8B). Wort filterability was determined by the test described by Sjöholm et al. (1994).

2.5 | Statistical analysis

Statistical analysis was conducted in SAS Enterprise Guide Version 7.15. Analysis of Variance (ANOVA)-General Linear Model (PROC GLM) was conducted for the control malt and blends with barley flour/malt ratios of 20/80 and 40/60. Tukey’s all pairwise multiple comparison test was applied to determine significant differences among the control and different blends.

3 | RESULTS AND DISCUSSION

3.1 | Milling yield and composition of milling fractions

The average yield of milling fractions and concentration of β-glucans and proteins in individual fractions for the six barley
samples used in this study are shown in Figure 2. The initial milling of barley in the Buhler mill generated about 26.4% of B1-S2 flour, 6.5% of hulls, and about 67.1% of shorts. Two passages through the shorts duster effectively separated starchy fractions from the cell wall material in the shorts and generated two flour streams SD1 flour and SD2 flour with average yields of 16.5 and 7.8%, respectively. The average yields of coarse and fine fiber-rich fractions were 20.8 and 22%, respectively. All six barley cultivars used in this study exhibited similar milling behavior in terms of yield of individual fractions as indicated by narrow range and low standard variation values (Figure 2). These results clearly show that covered barley can be directly milled on a roller mill without prior dehulling. However, to effectively separate starchy flour from the fibrous cell wall material in barley, the compression, abrasion, and shearing forces in a roller mill need to be augmented by additional impact and shearing actions that occur in the shorts duster. The thick endosperm cell walls in barley contain high amounts of β-glucans, and due to their plasticity, it is difficult to effectively separate them from the adhering starch granules by roller milling. Also, unlike wheat bran, which is easy to remove from the endosperm tissues in large flakes, barley outer tissues are more brittle, prone to shattering, and result in production of fine particles mixed with flour fractions. The use of shorts dusters, commonly known in wheat milling as bran finishers, is necessary in barley milling to effectively separate starchy flour fractions from other grain tissues (Table 1).

The concentrations of β-glucans in the three flour fractions (B1-S2 flour, SD1 flour, and SD2 flour) were very low compared to their concentrations in both FRF (Figure 2). Among the flour fractions, the concentration of proteins was the lowest in the B1-S2 flour (11.4%), followed by SD1 flour (15.6%) and SD2 four (17.8%). Both FRF were enriched in proteins with average concentration of 21.8% and 25.4% in coarse and fine FRF, respectively. All six barley cultivars exhibited similar trend of protein and β-glucan partitioning among the milling fractions (Figure 2).

![Figure 2](image-url)  
**FIGURE 2** Average yield of various milling streams and concentration of β-glucans and proteins for six barley cultivars used in this study

| Barley variety | Protein (%dw) | BG* (%dw) | AX* (%dw) | Ash (%dw) | Total Starch (%dw) | 1,000 kwt (g) |
|---------------|---------------|-----------|-----------|-----------|-------------------|----------------|
| AC Metcalfe   | 18.2 ± 0.1    | 5.0 ± 0.1 | 6.9 ± 0.4 | 2.5 ± 0.1 | 52.7 ± 0.3        | 42.1           |
| AAC Goldman   | 15.2 ± 0.2    | 4.6 ± 0.1 | 6.5 ± 0.0 | 2.2 ± 0.0 | 57.3 ± 0.6        | 45.5           |
| Lowe          | 16.6 ± 0.2    | 5.2 ± 0.1 | 6.6 ± 0.2 | 2.4 ± 0.0 | 53.7 ± 0.1        | 45.4           |
| CDC Bow       | 16.2 ± 0.0    | 4.6 ± 0.0 | 6.3 ± 0.3 | 2.6 ± 0.1 | 54.2 ± 0.3        | 44.7           |
| AAC Connect   | 16.6 ± 0.0    | 4.6 ± 0.0 | 5.8 ± 0.0 | 2.4 ± 0.0 | 55.4 ± 0.1        | 48.3           |
| CDC Meredith  | 15.0 ± 0.0    | 4.5 ± 0.1 | 6.4 ± 0.3 | 2.2 ± 0.0 | 55.9 ± 0.1        | 48.9           |

*BG, β-glucans, AX, arabinoxylans.
3.2 Composition and properties of barley flour and fiber/protein-rich fractions

Based on the concentration of proteins and β-glucans in milling fractions, the three flours (B1-S2 flour, SD1 flour, and SD2 flour) were combined into one fraction designated barley flour, whereas the coarse and fine FRF were combined into a single barley fiber/protein-rich fraction (FPRF). The yield and physicochemical properties of the barley FPRF and barley flour for each cultivar are presented in Tables 2 and 3, respectively. Figure 3 illustrates the average enrichment and/or reduction of selected constituents in flour and FPRF in comparison with their concentrations in the whole grain. The FPRF showed significant enrichment of β-glucans, arabinoxylans, ash, proteins, and reduction in starch content compared to the whole grain. The flour fractions, in contrast, were

### Table 2 Yield and composition of fiber/protein-rich fractions (FPRF) obtained by Buhler milling of various barley cultivars

| Fiber/Protein-Rich Fractions (FPRF) | AC metcalfe | AAC goldman | CDC bow | AAC connect | CDC meredith |
|-----------------------------------|-------------|-------------|---------|-------------|--------------|
| Yield (%)                         | 43.7 ± 0.3  | 42.7 ± 0.3  | 41.5 ± 0.1 | 42.8 ± 0.6  | 43.0 ± 0.7    | 43.0 ± 0.3  |
| BG (%)                            | 10.3 ± 0.2  | 9.7 ± 0.1   | 11.2 ± 0.1 | 9.7 ± 0.1   | 9.3 ± 0.1     | 9.3 ± 0.1   |
| WE45 Mmass (kDa)                  | 1,475       | 1,595       | 1,444    | 1,067       | 1,011         | 1,044       |
| AX (%)                            | 11.2 ± 0.1  | 9.9 ± 0.2   | 11.3 ± 0.3 | 10.7 ± 0.1  | 9.9 ± 0.2     | 10.0 ± 0.2  |
| Total starch (%)                  | 30.2 ± 0.2  | 36.6 ± 0.6  | 31.6 ± 0.2 | 33.2 ± 0.1  | 33.1 ± 0.1    | 37.1 ± 0.2  |
| TDF (%)                           | 30.3 ± 1.5  | 32.9 ± 1.6  | 33.1 ± 1.2 | 30.2 ± 1.2  | 28.3 ± 1.5    | 29.1 ± 1.2  |
| SDF                               | 9.1         | 11.6        | 11.0     | 8.8         | 8.5           | 9.1         |
| IDF                               | 21.2        | 21.2        | 22.1     | 21.4        | 19.8          | 20.0        |
| Ash (%)                           | 4.4 ± 0.0   | 3.8 ± 0.1   | 4.3 ± 0.0 | 4.2 ± 0.0   | 4.2 ± 0.0     | 3.7 ± 0.0   |
| Protein (%)                       | 26.1 ± 0.1  | 22.1 ± 0.1  | 23.4 ± 0.2 | 23.7 ± 0.1  | 24.5 ± 0.1    | 22.0 ± 0.1  |
| Albumins & Globulins              | 5.2         | 5.6         | 4.8      | 5.3         | 5.3           | 5.7         |
| Hordeins                          | 8.4         | 7.0         | 7.0      | 7.2         | 7.7           | 6.6         |
| Glutelins                         | 12.5        | 9.6         | 11.6     | 11.1        | 11.5          | 9.6         |
| Vitamin E (ug/g)                  | 81.8 ± 2.7  | 116.1 ± 1.7 | 97.3 ± 0.8 | 109.2 ± 0.5 | 93.5 ± 1.9    | 103.3 ± 5.7 |
| Tocopherols (T)                   | 22.0        | 26.6        | 25.1     | 26.5        | 28.1          | 23.3        |
| Tocotrienols (T3)                 | 59.8        | 89.5        | 74.7     | 82.7        | 65.5          | 80.0        |
| Ratio T3/T                        | 2.7         | 3.4         | 2.9      | 3.1         | 2.3           | 3.4         |

Abbreviations: BG, beta-glucans; AX, arabinoxylans; TDF, total dietary fiber; SDF, soluble dietary fiber; IDF, insoluble dietary fiber.

### Table 3 Yield and composition of flour fractions obtained by Buhler milling of various barley cultivars

| Flour Fractions | AC metcalfe | AAC goldman | CDC bow | AAC connect | CDC meredith |
|-----------------|-------------|-------------|---------|-------------|--------------|
| Yield (%)       | 50.4 ± 0.4  | 50.6 ± 0.2  | 51.6 ± 0.1 | 50.2 ± 0.1  | 51.2 ± 0.7    | 50.6 ± 0.3  |
| BG (%)          | 0.9 ± 0.0   | 0.9 ± 0.0   | 1.0 ± 0.0 | 0.8 ± 0.1   | 0.9 ± 0.1     | 0.9 ± 0.1   |
| AX (%)          | 0.7 ± 0.1   | 0.8 ± 0.1   | 0.8 ± 0.1 | 0.7 ± 0.0   | 0.8 ± 0.0     | 0.8 ± 0.1   |
| Total starch (%)| 78.2 ± 0.5  | 80.1 ± 0.1  | 762 ± 1.1 | 79.2 ± 0.2  | 78.9 ± 0.1    | 79.3 ± 0.2  |
| Ash (%)         | 1.09 ± 0.00 | 0.97 ± 0.02 | 1.05 ± 0.01 | 1.03 ± 0.01 | 1.02 ± 0.00   | 0.96 ± 0.01 |
| Protein (%)     | 13.9 ± 0.1  | 12.1 ± 0.0  | 14.2 ± 0.1 | 12.6 ± 0.0  | 12.5 ± 0.1    | 11.9 ± 0.0  |
| Albumins & Globulins | 3.2 | 3.2 | 3.1 | 3.2 | 3.3 | 3.3 |
| Hordeins        | 7.5         | 6.5         | 7.7      | 6.6         | 6.5           | 6.3         |
| Glutelins       | 3.2         | 2.4         | 3.4      | 2.7         | 2.7           | 2.4         |

Abbreviations: AX, arabinoxylans; BG, beta-glucans.
depleted in cell wall polysaccharides, ash and proteins, but enriched in starch compared to the whole grain. The amount of β-glucans in the FPRF generated from different barley varieties represented 88%–91% of the total content of β-glucans originally present in the whole grain samples. The flour fractions contained the remaining small portion of β-glucans (9%–10%) present in the whole grain samples. Arabinoyxylans contained in the FPRF represented 66%–74% of the total arabinoyxylans present in the whole grain. The remaining portions of grain arabinoyxylans were partitioned between the flour fractions (5%–7%) and the hulls (25%–30%). These results indicate very efficient and desirable partitioning of the majority of β-glucans and arabinoyxylans into the FPRF during milling.

The efficiency of roller milling of covered barley was also determined by the distribution of starch in the milling fractions. The majority of starch present in the whole grain was contained in the flour fractions (70%–75%), with significantly smaller portions (25%–30%) remaining in the FPRF. The development of viscosity of barley FPRF slurries during a 30-min period of mixing at a constant shear rate and 65°C is shown in Figure 4. All FPRF exhibited a relatively fast evolution of viscosity during the initial 10-min period followed by relatively stable viscosity thereafter. Some differences in the attainable viscosity, observed among FPRF from different barley cultivars, were attributed to differences in β-glucans concentrations in the FPRF (Table 2). In addition to concentration, the molecular weight of beta-glucans is known to significantly affect the viscosity of solutions containing these polymers (Lazaridou et al., 2003). As shown in Table 2, substantial differences in molecular masses were observed among β-glucans extracted with water from FPRF of different barley varieties. These differences likely reflect genetic and environmental effects on the molecular mass of β-glucans. Despite some differences among varieties, all water-extractable β-glucans from FPRF were high molecular mass polymers. It has been shown that one possible mechanism by which β-glucans exert their cholesterol lowering effect is through formation of viscous environment in the small intestine, which interferes with the transport of cholesterol (Ames et al., 2019). Although very low activity of β-glucanase, an enzyme responsible for depolymerization of β-glucans chains, is expected in barley before germination, some activity of this enzyme has been shown in various barley cultivars (Izydorczyk & MacGregor, 2000). If barley fractions are to be incorporated into food systems, it will be important to suppress any β-glucanase activity by appropriate means (e.g., heat treatment) to ensure the efficacy of these polysaccharides.

Soluble dietary fiber constituted approximately 30% of the TDF in the FPRF and likely contributed to the viscosity building properties of these fractions.

FIGURE 3 Average enrichment and/or reduction of selected constituents in flour and fiber/protein-rich fractions (FPRF) in comparison to their concentrations in the whole grain of for six barley cultivars used in this study

FIGURE 4 Development of viscosity of fiber/protein-rich fractions (FPRF) slurries during a 30-min period of mixing at a constant shear rate and 65°C
All eight tocochromanols (α-, β-, γ-, and δ-tocopherols and α-, β, γ-, and δ-tocotrienols) were detected in the FPRF (Figure 5). The concentration of each tocochromanol in the FPRF was significantly higher than in the corresponding whole grain sample, resulting in the significantly higher overall concentration of vitamin E in the FPRF. Substantial differences in the total vitamin E content in the FPRF were observed and are likely related to the genetic differences between barley varieties (Table 2). Notably, there was a difference in the ratio of tocotrienols to tocopherols (T3/T) among varieties, with FPRF from AAC Goldman and CDC Meredith exhibiting the highest ratio of T3/T. Genetic and environmental factors are known to affect the levels and composition of tocochromanols in barley (Oliver et al., 2014).

The amount of vitamin E found in the FPRF generated from different barley varieties represented 56%–67% of the total content of vitamin E originally present in the whole grain. The recovery of vitamin E in the FPRF was therefore substantially lower compared to the recovery of β-glucans (88%–91%), arabinoxylans (66%–72%), and ash (60%–67%). These results suggest that a portion of vitamin E could have partitioned to other milling fractions (flour and hulls). Also, some losses of vitamin E may have occurred due to exposure to air and heat during milling. Food processing, especially the application of heat, is expected to affect the content, activity, and availability of natural antioxidants such as vitamin E (Casal et al., 2006).

The cumulative size distribution of particles contained in the flour and FPRF is shown in Figure 6. The particles in the flour fractions were significantly smaller than in the FPRF. The diameter of the majority of flour particles (90%) ranged from 3 to 40μm (Figure 6). The span of particle diameters in the FPRF was much wider, ranging from 51 to 416μm. While there were no significant differences in the mass median diameter (D50) values among varieties, FPRF from CDC Bow and Lowe exhibited slightly higher proportions of larger particles compared to other varieties.

The small diameter of particles constituting the flour fraction was in good agreement with its high starch content (76%–80%) (Table 3). Some differences in the starch content in the flour fraction obtained from different varieties were observed. The starch content was inversely related to the protein concentration in the flour fractions (Table 3). In general, the flour fractions contained significantly lower amounts of proteins (12%–14%) than the FPRF (22%–26%) (Tables 2 and 3). The proteins contained in the FPRF constituted about 60% of the total grain proteins with the remaining 40% being present in the flour fractions. The flour and FPRF fractions differed not only in the total concentration of proteins but also in the content of albumins, globulins, hordeins, and glutelins (Figure 7, Tables 2 and 3). On average, the proteins in FPRF consisted of 23% of albumins and globulins, 31% of hordeins, and 46% of glutelins. The proteins in the flour fractions consisted of 25% albumins and globulins, 53% of hordeins, and 22% of glutelins. The FPRF contained similar amounts of hordeins, but significantly higher amounts of glutelins compared to the flour fractions.

### 3.3 Barley flour as a high starch adjunct in malting

The control malt used in this study was from cv. CDC Copeland, the most common malting barley variety grown in Western Canada since 2015. This variety is known for its moderate grain
proteins and moderate levels of starch and protein hydrolyzing enzymes (Izydorczyk et al., 2018). The control malt was partially replaced with barley flour fractions at 20% and 40% levels prior to mashing. The control malt and resulting blends with barley flour/malt ratios of 20/80 and 40/60 were mashed in the same manner according to the Congress schedule. The brewer-desired parameter, extract yield, was significantly increased by the addition of unmalted barley flours (Table 4). The use of 20% barley flour as adjunct increased the extract by 2.2 to 3%. With 40% addition of adjunct flour, the extract increased by 4.9 to 5.7%. The average degree of polymerization of glucose dex- trins in wort produced from 20% and 40% adjunct mashes was generally not significantly different (except for Lowe) from that in the control wort (Table 4). These results indicate that the concentration and activity of starch degrading enzymes (α-amylase, β-amylase, limit dextrinase, and amyloglucosidase) produced by the malt were sufficient to effectively hydrolyze the malt and barley flour starch into fermentable sugars and into dextrins with a low degree of polymerization.

The concentration of β-glucans in wort was not affected by 20% adjunct flours, but increased slightly with 40% adjunct flours (Table 4). The concentration of arabinoxylans in wort decreased with increasing adjunct ratio because the arabinoxylan concentration in barley flour adjunct was lower than that of malt. The viscosity of wort was slightly lower for both adjunct mashes compared to the control mash. The slightly higher concentration of β-glucans in wort from 40% adjunct mash was compensated by substantially lower concentration of wort arabinoxylans, resulting in the overall low wort viscosity.

The concentration of soluble proteins and free amino nitrogen (FAN) decreased with increasing percentage of adjunct flour, indicating that the proteins in adjunct flours were not effectively solubilized and hydrolyzed by the malt proteolytic enzymes during mashing and did not significantly contribute to the overall FAN level in wort (Table 4). The

![Flour Fractions and Fibre/Protein-Rich Fractions](image)

**FIGURE 7** Average concentration of albumins and globulins, hordeins, and glutelins in flour and fiber/protein-rich fractions obtained by milling of six barley cultivars used in this study [Colour figure can be viewed at wileyonlinelibrary.com]

**TABLE 4** Effects of addition of adjunct barley flour (20 and 40%) on the wort quality parameters

|                                 | Fine Extract (%) | Malt protein (%) | Wort soluble protein (%) | Wort KI (%) | Wort FAN (mg/L) | Wort Color (°) | Wort β-glucan (mg/L) | Wort AX (g/L) | Wort viscosity (cP) | Wort dextrin | DP Wort dextrin |
|---------------------------------|------------------|------------------|--------------------------|-------------|----------------|-----------------|----------------------|----------------|---------------------|--------------|------------|
| 100% malt                       |                  |                  |                          |             |                |                 |                      |                |                     |              |            |
|                                | 81.0a            | 12.8ab           | 5.00c                    | 39.1e        | 215c           | 2.38            | 113a                 | 1.31c          | 1.45a               | 2.33b        |
| 80% malt / +20% barley flour    |                  |                  |                          |             |                |                 |                      |                |                     |              |            |
| AC Metcalfe                     | 83.4b            | 12.9b            | 4.44b                    | 34.5cd       | 171b           | 2.04            | 114b                 | 1.05b          | 1.43a               | 2.30b        |
| AAC Goldman                     | 83.6b            | 12.5a            | 4.39b                    | 35.1d        | 170b           | 2.06            | 117b                 | 1.06b          | 1.43a               | 2.32b        |
| Lowe                            | 83.2b            | 12.9b            | 4.37b                    | 33.8c        | 173b           | 2.13            | 119b                 | 1.11b          | 1.43a               | 2.36b        |
| CDC Bow                         | 83.6b            | 12.6a            | 4.49b                    | 35.7d        | 172b           | 2.08            | 115b                 | 0.97b          | 1.44a               | 2.16a        |
| AAC Connect                     | 83.5b            | 12.6a            | 4.44b                    | 35.4d        | 174b           | 2.01            | 119b                 | 0.97b          | 1.43a               | 2.13a        |
| CDC Meredith                    | 84.0b            | 12.4a            | 4.46b                    | 35.9d        | 171b           | 1.99            | 117b                 | 0.97b          | 1.44a               | 2.18a        |
| 60% malt / +40% barley flour    |                  |                  |                          |             |                |                 |                      |                |                     |              |            |
| AC Metcalfe                     | 86.7d            | 12.9b            | 3.6a                     | 27.8a        | 135a           | 2.11            | 131c                 | 0.85a          | 1.43a               | 2.35b        |
| AAC Goldman                     | 86.7d            | 12.2a            | 3.57a                    | 29.2b        | 131a           | 2.08            | 127c                 | 0.83a          | 1.43a               | 2.31b        |
| Lowe                            | 85.9c            | 13.1b            | 3.65a                    | 27.9a        | 131a           | 2.49            | 123bc                | 0.85a          | 1.43a               | 2.41bc       |
| CDC Bow                         | 86.5d            | 12.4a            | 3.64a                    | 29.4b        | 136a           | 2.17            | 132c                 | 0.83a          | 1.43a               | 2.35b        |
| AAC Connect                     | 86.5d            | 12.3a            | 3.59a                    | 29.2b        | 134a           | 1.93            | 133c                 | 0.73a          | 1.43a               | 2.22a        |
| CDC Meredith                    | 86.7d            | 12.07a           | 3.59a                    | 29.6b        | 132a           | 2.03            | 122b                 | 0.74a          | 1.43a               | 2.28ab       |

**Abbreviations:** KI, Kolbach Index, FAN, free amino nitrogen, AX, arabinoxylan, DP, degree of polymerization.

Mean values in each column followed by a different letter are significantly different ($p<.05$, Tukey’s test).
lower FAN levels in wort from adjunct mashes observed in this study are consistent with previously published observations. Adjuncts, such as rice or unmalted whole grain barley, were reported to contribute little FAN to wort compared with traditional malt mashing (Evans et al., 2014; Goode & Arendt, 2006). However, it was also reported that lower FAN levels in adjunct mashing might be sufficient for yeast nutrition during brewing due to the presence of a higher proportion of absorbable amino acids and/or the availability of other types of assimilable nitrogen, including ammonium sulfate and small peptides (Cooper et al., 2016). Other solutions for overcoming the nitrogen limitations associated with the low FAN levels include the use of malt with high FAN potential and/or through supplementation of wort with proteases.

The lautering performance of 20 and 40% adjunct mashes is presented in Figure 8. Significant increases in the lautering volumes were observed for both adjunct mashes indicating that the use of barley flour as an adjunct not only poses no risks to wort processing, but even improves filtration. The improved lautering performance of wort from adjunct mashes could be related to their lower viscosity (likely associated with lower concentrations of arabinoxylans) and lower content of soluble proteins.

4 | CONCLUSIONS

The results of this study clearly showed that high-protein barley, often used as less profitable feed grain, can be valorized by dry fractionation into health beneficial fiber/protein fractions and starchy flour fractions that can used as adjunct in brewing. The study demonstrated that covered barley was efficiently milled into various streams using milling equipment commonly used in wheat milling without necessity of prior dehulling of the grain. Ultimately, the milling streams were combined into two high-yield fractions, flour (50.2 to 51.6% yield), and fiber/protein-rich fractions (with 41.5% to 43.7% yield), thus making the fractionation process commercially feasible. All six barley varieties used in this study exhibited good milling performance and characteristics, indicating that similar outcomes are conceivable with many barley varieties. The fiber/protein-rich fractions enriched in β-glucans, arabinoxylans, ash, proteins and vitamin E can be used as valuable food ingredients for prevention and/or control of many food-related health issues such as obesity, high blood pressure, heart disease, diabetes, and certain types of cancer. The flour fractions substantially enriched in starch proved to be valuable adjunct material for a partial replacement of barley malt. Mashing experiments with 20% and 40% replacement of malt with flour fractions showed a significant improvement in malt extract without any negative effects on viscosity or filterability of wort. The reduced free amino nitrogen (FAN) in worts from adjunct mashes could be mitigated by common brewing practices (e.g., use of a high FAN malt). While the wort quality parameters measured in this study indicated that consistent quality outcomes were possible with many barley varieties, some differences among varieties might indicate an advantage to combining particular barley and malt varieties. Furthermore, optimal barley flour/malt ratios could be established to tailor to specific brewer’s requirements with additional brewhouse trials. Further laboratory or pilot scale experiments are also needed to determine the effects of barley flour adjuncts on the flavor of the resulting beer.

ORCID
Marta Izydorczyk https://orcid.org/0000-0003-3364-3603
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