Dry Fractionation Methods to Produce Barley Meals Varying in Protein, Beta-Glucan, and Starch Contents

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ABSTRACT: Barley contains several valuable nutrients including beta-glucan (BG), protein, and starch. Each has additional value when concentrated. Dehulled and hulless barleys were sequentially pearled for 1 to 6 cycles, each with 8% removal. The 6 pearled kernels and the initial kernel were subjected to impact or abrasive milling, followed by sieving with a series of U.S. standard sieves. Results of pearling fines show that protein was most concentrated in the outer area, and decreased all the way toward the core area (near 100% surface removal). Starch showed an opposite trend. BG followed the starch trend, but reached a peak at about 60% surface removal. Upon milling and sieving of kernel samples, genotype and particle size had significant effects on nutrient contents in sieved fractions. The pearling cycle had significant effects on protein and starch contents but little effect on BG content, while the milling method had significant effects on protein and BG contents but little on starch content. Abrasive milling produced sieved fractions with much higher variation in protein content than impact milling, but the opposite effect was observed for shifting BG content. Mass frequency influenced more on recovery rates of nutrients than their concentrations in individual fractions. When the recovery rate was also taken into consideration, pearling alone was found to be the most effective way to enrich protein in barley kernels. However, a combination of pearling with the method of milling and sieving was needed for maximally shifting BG and starch contents.

PRACTICAL APPLICATIONS: Although dry fractionation is the method of choice for separating barley into fractions with varying levels of protein, BG, and/or starch, selection of a specific single or combined method is needed for achieving maximum shifts of a particular nutrient. Such information is significant to those who use dry fractionation methods to enrich protein, BG, and/or starch.

Keywords: barley pearling, beta-glucan, milling and sieving, protein, starch

Introduction

Barley is the 4th most important cereal in the world in terms of total production after wheat, rice, and corn. It is a versatile cereal adapted to, and produced, over a wider range of environmental conditions than any other cereals (Jadhav and others 1998). On average, barley contains about 64% starch, 11% protein, and 5% beta-glucan (BG). The remaining 20% includes moisture, fiber, ash, and other minor components (MacGregor and Fincher 1993). Worldwide, a significant amount of barley is consumed as livestock feed, a relatively lower amount for brewing, and very little amount is used directly as human food. In North America, the greatest use of barley is for malting purposes, most specifically for the brewing industry. However, in recent years, there has been a growing interest in incorporating barley into the human diet since it is naturally healthy, readily available, and relatively inexpensive. More importantly, BG in barley has been shown to have hypocholesterolemic effects (Keenan and others 2007).

A key strategy in increasing the value-added utilization of barley is to produce fractions with unique composition. In particular, there is considerable interest in producing fractions enriched in protein, BG, starch, and/or functional lipids. Because of the heterogeneous distribution of various components throughout the kernel of a cereal grain, dry fractionation has been a method of choice for enriching a particular nutrient. Researchers have reported several methods of dry fractionation of barley with varying levels of success. These include: (1) pearling (Knudsen and Eggum 1984; Wang and others 1997; Klaczynski and others 1998; Marconi and others 2000; Yeung and Vasanthan 2001; Izydorczyk and others 2003; Lampi and others 2004; Liu and Moreau 2008), (2) roller milling (Sundberg and Aman 1994; Izydorczyk and others 2003), (3) milling followed by air classification (Wu and others 1994; Knuckles and Chiou 1995; Vasanthan and Bhatty 1995; Andersson and others 2000), and (4) milling followed by sieving (Wu and others 1994). Pearling is a process to remove outer layers of cereal grains, typically by use of an abrasive dehulling device, such as a barley pearler, which provides gentle surface abrasion to grains with minimum breakage of residual kernels. Milling disintegrates grains into fine particles. Roller milling employs a special mill that allows grains to pass between rotating rollers for grinding and flaking and then through sifters for fraction separation. Sieving separates milled flours on the basis of particle size, while air classification separates flours based on differences in density, mass, and projected area in the direction of flow. The advantages of dry fractionation over a conventional wet separation process (Wu and others 1979; Mohamed and others 2007) is that it is relatively energy efficient, requires no solvent removal and recovery, and thus has lower capital investments.

Among the reported studies on dry fractionation of barley, some focused on the enrichment of a single component (Knuckles
Barley dry fractionation . . .

and Chiu 1995; Wang and others 1997; Izydorczyk and others 2003), while others evaluated only a single processing method (Klamczynski and others 1998; Yeung and Vasanthan 2001; Liu and Moreau 2008). There are no reports on the effects and interactions of 2 or more dry fractionation methods on the efficiency of nutrient enrichment. Furthermore, most previous studies reported only nutrient concentrations and neglected nutrient recovery. In this study, successive pearling and 2 milling methods (abrasive or impact) followed by sieving were compared and combined for dry fractionating hulled and hulless barley. The objectives were to determine the effects of barley type, pearling cycle, milling method, and particle size and their interactions on concentrations and recovery rates of 3 key nutrients, protein, BG, and starch in resulting fractions.

Materials and Methods

A total of 2 barley genotypes were used, 1 hulled cultivar (“Baronesse”) with normal protein and BG contents, and 02HR4586, a hulless breeding line with high protein and high BG contents. Baronesse is the most widely grown feed barley in the United States and Canada while 02HR4586 is a 2-rowed line with the pedigree “Azhul”/“Thuringa.” Azhul is the progenitor of most high BG lines in North American and Canadian breeding programs. Both were grown in Aberdeen (Idaho, U.S.A.) in 2007 under irrigation as 1.6 × 5 m strips. Fertilization was as would be followed for growing malting barley, and, therefore, nitrogen levels were moderate to keep protein at acceptable industry levels. Seed samples were passed through a screen to remove broken kernels and any foreign material. The cleaned barley grains were not tempered before processing.

Dehulling of hulled barley

Seed of Baronesse was dehulled with a Strong–Scott barley pearler (Seedburo Equipment Co., Chicago, Ill., U.S.A.) fitted with a 30-grit carborundum stone, an 8-mesh screen (8 slots per inch [25.3807 mm]) and a 1/2 hp motor providing a fixed standard speed of 1725 rpm. The hull fraction, about 11% of total kernel weight, was further separated into 2 fractions by passing through a sieve with a U.S. standard mesh size of nr 18 (1 mm opening). The material on the top of the screen (approximately 8% of the total kernel weight) was a light fraction consisting mainly of hulls. The material that passed the screen (making up about 3%) was a heavy fines fraction, consisting mainly of fine hull, germ, pericarp, and testa.

Successive pearling of dehulled and hulless barleys

The dehulled kernel of Baronesse barley or the whole kernel of the hulless barley was then processed according to a scheme in Figure 1. The experiment was duplicated at this stage. The kernels were first subjected to 6 cycles of pearling, with each cycle removing about 8%, by an electric seed scarifier (Forsberg, Thief River Falls, Minn., U.S.A.). The machine came with a standard 1/3 HP motor. It was replaced with a new motor, which had dual hp (1/3 and 1/6) corresponding to dual speeds. The high speed equaled the original factory motor speed of 1725 rpm. The low speed was 1140 rpm, and was chosen throughout the study due to an early report showing that the low-speed motor significantly reduces kernel breakage compared to the regular motor speed (Liu 2007).

For each abrading cycle, 100 g of a sample was put into the scarifier chamber. After abrading for a targeted removal level of 8%, pearled kernels (PK), mixed with surface layer material, were brushed into a container. The surface layer, termed pearled fines (PN) fraction, was then separated from PK by sifting the mixture through an 18 mesh (1 mm opening) screen. After several repeats of pearling for the same sample with the same charge size, fines were combined and retained. A portion of combined pearled kernels was subjected to another cycle of abrading. After the 6th cycle of pearling, 6 bran factions (PN1-6), and corresponding 6 pearled kernels (PK1-6), and the original dehulled kernel or whole hulless kernel (PK0) were obtained.

Further pearling of the PK6

For a portion of the PK6 sample, many additional cycles of pearling were conducted, but the procedure was modified from the previous pearling cycles. This was to study the relationship between nutrient-content and surface-removal levels over the entire 0% to 100% removal range. Basically, 100 g of PK6 sample were abraded sequentially until the kernel was reduced to the final core area of endosperm and no further pearling was possible due to the greatly reduced sample charge size. For each cycle of pearling, duration was fixed at 3 min. After the surface material was removed by the same screen and retained, the newly abraded kernel underwent another cycle of pearling and so on. Thus, for the same cycle, no repeats were made and sample charge size was reduced as pearling cycles progressed. The surface layer fraction for each pearling cycle was kept separately, and named sequentially as PN7, PN8, and so on.

Milling of kernel fractions (PK0-6)

Each surface-layer fraction obtained from the previously mentioned successive pearling was fine enough to pass through the nr 18 U.S. standard sieve. No further milling or sizing by sieving was performed on these bran fractions. However, each of the 7 kernel samples (PK0-6) for each barley genotype was subjected to 1 of the 2 milling methods, impact milling and abrasive milling. Impact milling was carried out by a Cyclone Sample Mill (UDY Corp., Fort Collins, Colo., U.S.A.) with mill enclosures, a vacuum system, and a sieve with 1-mm round openings. Abrasive milling was done by the same seed scarifier and followed the same procedure previously described for pearling the PK6 sample. One exception is that after a kernel sample had reached the final core area of the endosperm, all surface materials for all cycles of pearling were combined and mixed for subsequent sieving.

Sieving of milled flours from PK0-6

Flours, resulting from either impact milling or abrasive milling of kernel samples, were finally sized with a series of 5 U.S. standard sieves (mesh nr 60, 100, 200, 270, and 400, having opening of

![Figure 1 — Outline of barley dry fractionation methods.](image-url)
Barley dry fractionation...

250, 150, 75, 53, and 38 μm, respectively) and a pan, fitted into a sieve shaker (Ro-Tap, RX-29, W.S. Tyler Inc., Gastonia, N.C., U.S.A.). Sieving was carried out based on a reverse sieve procedure because compared with the conventional stacked sieve method the reverse sieve procedure offers improved sieving efficiency and performance (Liu 2009). Basically, each particulate sample (100 g initially) was sifted with a single sieve, from fine to coarse order, by multiple sieving steps. The time distribution for each sieve of 400, 270, 200, 100, and 60 mesh size was 30, 25, 20, 15, and 10 min, respectively. Material that passed through the sieve but retained on the pan was determined while the material retained on the sieve proceeded to the next sieving step. The procedure continued until the sieve with the largest opening was completed. The mass frequency (%) for material retained on each sieve (including the pan) of increasing size was calculated. A total of 6 fractions of different particle size ranges were finally collected for each sample, representing < 38, 38 to 53, 53 to 75, 75 to 150, 150 to 250, >250 μm, respectively.

Chemical analysis

All particulate samples, which included PN fractions and sieved kernel fractions after milling, were weighed and measured for moisture, protein, BG, and starch contents. Moisture was determined according to an official method (AOAC 2002) and was used to convert the contents of other components into a dry matter basis. The total nitrogen/protein content was measured by a combustion method (AOAC 2002), using a protein analyzer (Model FP-528, Leco Corp., St. Joseph, Mich., U.S.A.). The protein content was calculated with a conversion factor of 5.75. BG was measured according to the Approved Method 32-23 (AACC 2000), using the BG enzymatic assay kit supplied by Megazyme Intl. Ireland Ltd. (Wicklow, Ireland). Starch was measured according to an enzymatic method using a starch test kit (R-Biopharm Inc., Marshall, Mich., U.S.A.). Samples were treated with dimethylsulfoxide and HCl to solubilize starch, which was then hydrolyzed to D-glucose in the presence of amylglucosidase. The resulting D-glucose reacted with hexokinase and glucose-6-phosphate dehydrogenase. The amount of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) formed in the reaction was determined colorimetrically, which was stoichiometric to the amount of D-glucose.

Data treatments and statistical analysis

Data (mass frequency of sieved fractions and nutrient contents of all fractions) were treated with the JMP software, version 5 (JMP, a Business unit of SAS, Cary, N.C., U.S.A.) for analysis of variance (ANOVA) to determine effects of genotype, pearling cycle, milling method, and particle size and their interactions on the attributes measured. The Tukey’s honestly significant difference (HSD) test was also conducted for pair comparison of least square means.

Results and Discussion

Nutrient distribution within a barley kernel

Through pearling sequentially by the seed scarifier, relationship between the contents of protein, BG, and starch in the kernel surface layer abraded off at each cycle of pearling and the level of surface removal over the entire 0% to 100% range could be established (solid lines, Figure 2). This relationship essentially describes...
the distribution of each nutrient within a barley kernel. Data points corresponding to 0% removal level refer to nutrient contents of the whole grain, while the data points corresponding to 100% removal level refer to nutrients content of the inner core of the kernel.

The hulless barley had higher protein (16.42%) compared to 10.92% for the hulled barley (Figure 2A). The protein in hulless barley was the highest (25.38%) at the outermost area, about 55% higher than the whole kernel. It decreased almost linearly toward the inner core region (7.38%). In contrast, protein in hulled barley was lowest (4.98%) at the initial surface removal level, corresponding to the coarse hull fraction, and increased sharply to a peak at about 20% surface layer removal. The peak value was 20.14%, being about 84% higher than the whole kernel value. For the remainder of the kernel, as the surface removal levels increased, the protein content also decreased linearly, reaching to 4.99% at the inner core area. It is interesting that the change patterns of hulled and hulless samples were almost parallel except for the initial surface area, even though the hulless barley had significantly higher protein.

The whole kernel of hulless barley also had significantly higher BG than the hulled barley (5.62% compared with 3.65%, Figure 2B); yet, both types showed similar BG distribution patterns across the kernel. At the surface area, BG had the lowest concentration, close to zero for hulled barley, indicating that hulls contained little BG. As the surface removal level increased, BG content increased progressively, and reached a peak at about 60% removal levels. At the peak, BG content was at 27% increase over that of the original kernel for both genotypes. From here, BG decreased slightly toward the inner core area. The net effect is that BG content in the inner core area was similar to (hulled) or slightly higher (hulless) than that in the whole kernel.

Like BG, starch was lowest at the surface area, almost zero for the hulled barley, indicating that hulls contained little starch also (Figure 2C). As the surface removal levels increased, there was a progressive increase in starch content in both barley types. Unlike BG, the peak of which was reached at 60% removal, starch reached its highest level at the innermost core area. Also, the extent of this increase was slightly higher than that of whole kernel. Although the curve for hulled barley was steeper than that of hulless barley, the differences in the starch content between the 2 types in the whole kernel and in layers pearled off were much less than those in protein and BG contents. Also, unlike protein and BG, the 2 lines for the starch content crossed each other at about 35% removal level.

Previous investigators have also reported concentrations of protein, BG, and/or starch in surface material pearled off as a function of removal levels (Knudsen and Eggum 1984; Wang and others 1997; Klamczynski and others 1998; Marconi and others 2000; Yeung and Vasanthan 2001; Izydorczyk and others 2003). For example, Klamczynski and others (1998) reported that successive removal of the outer parts of barley kernels from 10% to 40% by abrasion caused significant increase in starch and BG contents, but significant decrease in protein content, independent of hull type and cultivar. Yeung and Vasanthan (2001) pearled 2 hulless varieties up to 80% removal levels, using a Satake testing mill and found that in pearling fines, BG and starch increased, while protein decreased. In pearled grains, starch content increased rapidly, indicating that starch was more concentrated in the inner tissues. BG also increased with pearling levels, but there was a gradual decrease in protein content.

Our study confirmed most previous reports. However, some of the previous studies abraded barleys only to a maximum removal level of 40%, while others obtained surface materials by a continuous abrading until a targeted percentage of removal was reached. Increasing levels of surface removal were achieved by abrading the same kernel with increasing abrading time. Thus, the composition of surface material pearled off for each run did not represent true nutrient distribution. In contrast, in this study, a sequential abrading mode was used, where kernels were abraded for many cycles. All cycles used a similar short abrading time but newly abraded kernels were used for next cycle of pearling. Thus, the level of surface removal was cumulative, and the composition of surface material pearled off for each cycle represented true nutrient distribution across a kernel.

Although the present study used the successive pearling mode, compositional data for surface materials pearled off by a theoretically continuous mode could be obtained through calculation (Figure 2, dotted lines). Results show that for all 3 nutrients in both barley genotypes, if the composition data were obtained by continuous pearling, the curves (dotted lines) would have been much flatter as compared with their corresponding curves obtained by sequential pearling (solid lines). Also, BG would have increased to a peak at the most inner layer instead of at about 60% removal. The difference in the 2 types of curves can be explainable by the different pearling modes as layers were made and collected differently. Because the dotted line curves do not represent true nutrient distribution, data interpretation based on them should be made with caution.

**Mass frequency of PK flours upon sieving**

A total of 6 pearled kernels (PK1-6) and the original kernel (PK0) (which is referred to as dehulled kernel for hulled barley or whole kernel for hulless barley) were subjected to either impact milling or abrasive milling. A 2 × 2 factorial treatment design with 2 genotypes and 2 milling methods resulted in 4 treatments. For each treatment, there were 7 PKs for milling. This led to a total of 28 PK flours. Each PK flour was sieved by the same set of selected U.S. standard sieve and pan. The mass frequency of each particle size category for each PK flour was calculated and adjusted to the whole kernel basis. This adjustment simplified the calculation of nutrient recovery rates discussed in later sections. By plotting mass frequency over the entire particle size range, particle size distribution (PSD) of individual PK flours could be obtained (Figure 3).

Results show that genotype, milling method, pearling cycle, particle size, and some of their interactions all significantly (P < 0.05) affected the PSD of PK flours (Table 1). When PK0-6 of hulled barley were milled by impact milling (Figure 3A), the resulting 7 PK flours had a similar bimodal PSD. A mode is the center of the size class that contains most of the material. A major mode was in the center of size class of 38 to 53 μm (the material retained in nr 400 sieve but passed through nr 270 mesh). The minor mode was in the center of size class of 75 to 150 μm. As a result, the size class of 53 to 75 μm gave the lowest mass frequency. However, when PK0-6 of hulled barley were milled by abrasive milling, the resulting PK flours had unimodal PSD (Figure 3B) instead of bimodal PSD observed for impact milling (Figure 3A). For PK0-2 and PK4-5 flours, the mode was in the center of 53 to 75 μm. For PK3 and PK6, the mode was in the center of 75 to 150 μm.

When the hulless barley was milled using impact milling, the resulting PK flours also had unimodal PSD (Figure 3C). For PK0-3 flours, the mode was in the center of 53 to 75 μm. For PK4-6 flours, the mode was in the center of 38 to 53 μm. This is in a sharp contrast to hulled barley milled by the same impact milling method. When the hulless barley was milled by abrasive milling, the
Barley dry fractionation... resulting PK flours had similar unimodal PSD (Figure 3D) to that of abrasive milling of PK0-6 from hulled barley (Figure 3B). For PK0 flour, the mode was in the center of 53 to 150 μm. For the rest of PK flours, the mode was in the center of 75 to 150 μm.

Protein content in sieved fractions
Protein content varied greatly among 7 kernel samples (Figure 4). This was observed with both dehulled (7.60% to 10.92%) and hulless barleys (11.73% to 16.42%). Increases in

![Image](image.png)

Figure 3—Mass frequency of pearled kernel flours retained on sieve sorted according to their particle sizes. (A) dehulled barley, impact milling followed by sieving; (B) dehulled barley, abrasive milling followed by sieving; (C) hulless barley, impact milling followed by sieving; and (D) hulless barley, abrasive milling followed by sieving.

Table 1 — F-values based on ANOVA for mass frequency, protein, BG, and starch contents and their recovery rates in sieve sized fractions as affected by many factors.*

<table>
<thead>
<tr>
<th>Source</th>
<th>Mass Frequency</th>
<th>Protein content</th>
<th>BG content</th>
<th>Starch content</th>
<th>Protein recovery</th>
<th>Glucan recovery</th>
<th>Starch recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>0.0055</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0060</td>
<td>0.0156</td>
<td>0.5177</td>
</tr>
<tr>
<td>Milling method</td>
<td>0.0114</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.9308</td>
<td>0.1514</td>
<td>0.0194</td>
<td>0.0067</td>
</tr>
<tr>
<td>Genotype × millling method</td>
<td>0.9881</td>
<td>0.6242</td>
<td>&lt;0.0001</td>
<td>0.4212</td>
<td>0.9702</td>
<td>0.9802</td>
<td>0.8497</td>
</tr>
<tr>
<td>Pearling cycle</td>
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<td>&lt;0.0001</td>
<td>0.1059</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype × pearling cycle</td>
<td>1.0000</td>
<td>0.4361</td>
<td>0.1692</td>
<td>0.6869</td>
<td>1.0000</td>
<td>0.9956</td>
<td>0.9921</td>
</tr>
<tr>
<td>Milling method × pearling cycle</td>
<td>1.0000</td>
<td>0.3247</td>
<td>0.7944</td>
<td>0.4908</td>
<td>1.0000</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Genotype × Milling method × Pearling cycle</td>
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<td>0.4564</td>
<td>0.4328</td>
<td>0.2516</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
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<tr>
<td>Particle size</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype × particle size</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>Milling method × particle size</td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype × milling method × particle size</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>Pearling cycle × particle size</td>
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<td>0.1175</td>
<td>0.9949</td>
<td>0.7969</td>
<td>0.0004</td>
<td>0.0053</td>
<td>0.6564</td>
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<tr>
<td>Genotype × pearling cycle × particle size</td>
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<td>0.9851</td>
<td>0.9866</td>
<td>0.9994</td>
<td>0.7427</td>
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<td>0.9981</td>
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<tr>
<td>Milling method × pearling cycle × particle size</td>
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<td>0.7638</td>
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<td>0.9997</td>
<td>0.3192</td>
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<td>0.9032</td>
</tr>
<tr>
<td>Genotype × milling method × pearling cycle × particle size</td>
<td>0.9584</td>
<td>0.8346</td>
<td>0.9994</td>
<td>0.9610</td>
<td>0.7526</td>
<td>0.8559</td>
<td>0.9867</td>
</tr>
</tbody>
</table>

*Bold numbers indicate significant effect at P < 0.05.
the degree of pearling resulted in lower protein content of the pearled kernels. These observations are supported by the protein distribution curve observed within a barley kernel in Figure 2. Furthermore, genotype, pearling cycle, milling method, particle size, and some of their interactions all showed significant effects ($P < 0.05$) on protein content of sieved fractions (Table 1). Among all the PK flours, the changing patterns were rather similar since the curves in each subfigure were almost parallel to each other (Figure 4). The order of protein content in sieved fractions among PK flours was the same as that of original PK samples, that is, the higher the pearling cycle, the lower the protein content was.

Impact milling of dehulled barley increased the protein content in the flour of the largest particle size class ($>250 \ \mu m$) relative to the original PK (Figure 4A). The variation in protein content among sieved PK flours was enlarged (except for finer particle size categories), as compared with the original PKs. With decreasing particle size, protein content showed an overall trend of decreasing, although the decrease did not follow straight lines. In the flour fractions of finest particle size ($<38 \ \mu m$), protein content approached to that in the original PK sample. In contrast, when the same PK0-6 from hulled barley were milled by abrasive milling, protein in flours of the largest particle size decreased substantially to a narrow range (around 6%) compared with that in the original PK0-6 (Figure 4B). As the particle size decreased, protein increased sharply and reached a 1st peak in the size classes of 75 to 150 $\mu m$ and 150 to 250 $\mu m$, where the content ranged from 9% to 16% among 7 PK flours. From here, as the particle size further decreased, the protein content decreased and then increased to a 2nd peak, corresponding to the finest particle size class. Therefore, for the same PK samples, impact milling brought about less variation in protein content than abrasive milling (6.17% to 13.34% compared with 5.90% to 15.76%). This was particularly noticeable for individual PK samples.

When hulless barley was used instead of hulled barley that had been dehulled, protein content in sieved PK flours showed similar but slight different patterns over the particle size range. Upon impact milling or abrasive milling, there was less variation in protein content among PK flours from the hulless barley. Again, for the same PK samples, impact milling resulted in less variation in protein content than abrasive milling (10.68% to 18.86% compared with 7.57% to 23.21%).

**Protein recovery in sieved fractions**

Figure 4 describes protein concentration in sieved fractions. Since each fraction had different mass frequency adjusted to the whole grain basis (Figure 3), recovery rate, which is a function of

![Figure 4](image-url)

*Figure 4—Protein content in sieved fractions (retained on sieve) of pearled kernels as a function of particle size. (A) dehulled barley, impact milling followed by sieving; (B) dehulled barley, abrasive milling followed by sieving; (C) hulless barley, impact milling followed by sieving; and (D) hulless barley, abrasive milling followed by sieving.*
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both nutrient content and mass frequency, becomes another factor to consider. Often, the fraction with the highest nutrient content has the lowest mass, resulting in a lower recovery rate, while the fraction with a higher recovery rate does not necessarily mean that it has a higher nutrient concentration. Therefore, there needs to be a balanced consideration between nutrient concentration and recovery rate during dry fractionation.

Since each subfigure in Figure 5 more closely resembles the corresponding subfigure in Figure 3 than in Figure 4, it can be concluded that mass frequency had a greater influence on recovery rate than protein content itself in a particular fraction. This observation indicates the difficulty in obtaining a fraction with both higher protein content and higher recovery rate. For example, upon abrasive milling PK0-6 of hulless barley, the sieved fractions of 150 to 250 μm size class contained the highest protein content (Figure 4D) but had a very low recovery rate (Figure 5D). However, in some cases it was possible to obtain fractions that contained the highest protein content with medium recovery rate. An example would be the sieved fractions of 75 to 150 μm class after abrasive milling PK0-6 of hulled barley (Figure 4B compared with Figure 5B).

Furthermore, careful examination of Figure 4 and 5 shows that the degree of pearling influenced protein content and recovery rate in the same manner. Regardless of genotype and milling methods, pearled kernels with lower cycles of pearling resulted in sieved flour fractions with higher protein content as well as higher recovery rate.

Beta-glucan contents in sieved fractions

Variation in BG content among PK0-6 and sieved PK flour fractions of the same particle size category were much lower than the variation observed in protein content (Figure 6). There was a significant effect of genotype, milling method, particle size, and some of their interactions on BG content. Interestingly, unlike protein content, BG content in sieved fractions was not significantly affected by pearling cycle (Table 1).

For dehulled barley, the original PK0-6 had BG content of approximately 4%. Upon impact milling, the BG content in flours of the largest particle size class increased to about 5% (Figure 6A). As the particle size decreased, BG increased and reached a peak in the 53- to 75-μm particle size class where the content ranged from 8.29% to 9.79% among 7 sieved PK flours. Further decrease in particle size resulted in a dramatic decrease in BG content. In the finest flours (<38 μm), BG content was reduced to less than 1%, the lowest observed value. In contrast, for the same PK0-6 of hulled barley, upon abrasive milling, the BG content in flours of the largest...
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particle size decreased slightly (less than 4%) compared with that in original PK0-6 (Figure 6B). With decreasing particle size, the BG content increased and reached to a peak in the 75 to 150 μm class where the content ranged from 5.08% to 7.61% among the 7 sieved PK flours. From there, as the particle size further decreased, the BG content decreased. In flours of finest particle size, the BG content ranged from 2.14% to 2.72%. Therefore, for the same PK samples, impact milling brought about much larger variation in BG content than abrasive milling (0.75% to 9.79% compared with 2.14% to 7.61%), the opposite effect of that shown for protein content, with regard to the effect of milling method.

For hulless barley, BG contents in sieved PK flours showed similar but slightly different patterns over the particle size range, in terms of peak value and variation among PK flours. Impact milling of hulless PK0-6 led to larger variation in BG content among sieved PK flours than impact milling of PK0-6 from dehulled barley. The peak values were in the 75- to 150-μm particle size class for hulless instead of 53 to 75 μm class for dehulled barley. Abrasive milling of hulless PK0-6 resulted in a peak value of BG content for some PK flours in the 150 to 250 μm class. As with the dehulled barley, for the same PK samples, impact milling brought about much larger variation in BG content than abrasive milling (0.42% to 15.88% compared with 2.04% to 8.80%).

**Beta-glucan recovery in sieved fractions**

As discussed for protein, for each sieved fraction, nutrient content is of interest, but its mass is another parameter to consider as it affects recovery rate. This is true for BG. As Figure 7 resembles Figure 3 more than Figure 6, and mass frequency again had a greater influence on BG recovery rate than its content in a particular fraction. For example, after impact milling PK0-6 of dehulled barley, although the sieved fractions of 53 to 75 μm class had the highest BG content (Figure 6A), they had the lowest recovery rates (Figure 7A) due to their lowest mass (Figure 3A). In contrast, after impact milling hulless PK0-6, the sieved fractions of 75 to 150 μm class (the material retained in nr 200 sieve but passed through nr 100 mesh) had the highest BG concentration (Figure 6C), while the recovery rate of BG in these fractions was moderately high (Figure 7C). Therefore, if BG content and recovery rate are both considered, impact milling PK0-6 of hulless barley is preferable over impact milling PK0-6 of dehulled barley.

Although abrasive milling of PK0-6 from either dehulled or hulless barley gave higher recovery rates for BG than impact milling, the concentration in sieved fractions was not ideal. Therefore, if BG content is of major interest, impact milling is preferable over abrasive milling. Furthermore, a comparison of Figure 6 and 7 shows that variation in BG recovery rate was much higher than in BG protein content.
content among sieved PK fractions of the same particle size fraction. The reason is that although there was less variation in BG content among the 7 unmilled PK samples, the mass frequency of each size class, when adjusted to the whole kernel basis, varied greatly among PK0-6.

**Starch content in sieved fractions**

Like protein, starch varied greatly among intact PK0-6 samples from either dehulled (64.23% to 75.12%) or hulless barley (54.17% to 68.99%, Figure 8). Yet, unlike protein, with increasing pearling cycle, the starch content increased. The observations are explainable by starch distribution within a barley kernel (Figure 2). Genotype, pearling cycle, particle size, and some of their interactions showed significant effects on starch content of sieved fraction. However, unlike protein and BG contents, starch content in sieved fractions was not significantly influenced by the milling method (Table 1).

For both dehulled and hulless barleys, upon impact milling, starch in flours of the largest particle size class (>250 μm) decreased compared to the original PK samples (Figure 8A and 8C). With decreasing particle size, starch further decreased to a minimum and then increased in classes of finer particle size. There were some minor differences between the 2 genotypes. The minimum was reached in the 53 to 75 μm class for dehulled barley and the 75 to 150 μm class for hulless.

Upon abrasive milling, both genotypes exhibited similar patterns in starch content with decreasing particle size to those with impact milling, that is, decreasing to a minimum and then increasing to a peak. However, there were some minor differences between the 2 genotypes upon abrasive milling: the valley was broader for dehulled barley (in the 75 to 250 μm classes) than that of hulless barley (in the 150 to 250 μm class). With regard to the effect of pearling cycle, the higher the degree of pearling, the lesser the variation in starch content among sieved fractions of the same PK sample. This was similar to the trend observed for protein content. However, sieved fractions from PKs with higher degree of pearling were lower in protein content but higher in starch content.

Unlike impact milling, abrasive milling led to an increase in starch content in the sieved fraction of the largest particle size class as compared with that of intact PK samples. Impact milling also caused larger variation in starch content among sieved fractions of the same PK sample than impact milling. This was true for both genotypes.

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**Figure 7—** BG recovery in sieved fractions (retained on sieve) of pearled kernels as a function of particle size. (A) dehulled barley, impact milling followed by sieving; (B) dehulled barley, abrasive milling followed by sieving; (C) hulless barley, impact milling followed by sieving; and (D) hulless barley, abrasive milling followed by sieving.
Starch recovery in sieved fractions

Again, the mass frequency of a particular sieved fraction had more influence on starch recovery rate than starch content, as Figure 9 more closely resembles Figure 3 than Figure 8. This was also observed for protein and BG. Yet, comparison of each subfigure in Figure 8 to the corresponding one in Figure 9 (for example, Figure 8A compared with Figure 9A, and so on) shows that for certain classes of particle size, higher starch content in one figure also corresponded to a higher recovery rate in another one. Thus, it is possible to achieve higher starch content while maintaining a higher starch recovery rate.

Careful examination of Figure 8 and 9 also shows that the pearling cycle influenced starch content and recovery rate in the opposite direction. Regardless of genotype and milling methods, pearled kernels with higher cycles of pearling led to sieved flour fractions with higher starch content but lower recovery rate. This is just opposite with the effect of pearling cycle on protein observed in Figure 4 and 5.

Correlations between measured parameters

To determine relationships between measured parameters (mass frequency, nutrient contents, and recovery rates), correlation coefficients ($r$ values) were calculated for each possible pair of all measured parameters over the entire particle size range (Table 2). Results show that for all 4 combinations (2 genotypes × 2 milling methods), protein content and mass frequency had very weak negative correlations ($r$ ranged from $-0.024$ to $-0.214$) and this could be partially responsible for a very weak correlation observed between protein content and protein recovery ($r = 0.042$ to 0.093). BG content and its recovery rate had positive correlation ($r = 60.381$ to 0.641). This relationship was influenced more by genotype than milling method; hulless barley gave higher $r$ values than dehulled one (0.641 compared with 0.381 by impact milling and 0.612 compared with 0.301 by abrasive milling). Starch content and its recovery rate also had positive correlation. The relationship was more influenced by milling method than genotypes; impact milling gave higher $r$ values than abrasive milling (0.499 compared with 0.281 for dehulled barley and 0.564 compared with 0.03 for hulless). These results indicate that it is difficult to obtain a sieved fraction with high protein content and high protein recovery, but for BG or starch, it is possible to collect a fraction with both high content and high recovery rate.

Furthermore, in terms of nutrient concentrations, starch contents had strong negative correlations with protein and BG...
Barley dry fractionation...

contents, while protein contents had a weak positive correlation with BG contents (except for the hulless-abrasive samples). The results show difficulty of producing a sieved fraction higher in protein or BG content and at the same time higher in starch content. However, it could be possible to have a sieved fraction that was higher in protein content and at the same time higher in BG content. With respect to recovery rate, starch had strong positive correlations with protein and BG (except for impact milling), while protein had a positive correlation only with BG.

Method comparison and optimization

As shown in Figure 1, in this study, dehulled or hulless kernels were first pearled for 6 cycles to obtain 6 bran fractions and corresponding pearled kernels. The pearled kernels and the original kernel were then subjected to milling followed by sieving. Two milling methods were used, impact and abrasive milling. Results show that there were great variations in mass and nutrient content among pearled fines and sieve sized fractions. Thus, if an objective was to produce a fraction or combined fractions (either pearled fines or sieved fractions or both) that had the highest concentration for a particular nutrient, selection of a fractionation method or a combined one would vary with a particular nutrient.

For protein, as the cycle of pearling progressed, the protein content in pearled fines as well as pearled kernels decreased since it was concentrated in the outer layers of barley seeds (Figure 2). Upon milling followed by sieving, there was a progressive decrease in protein content for the same sized fractions from the kernels with increased cycles of pearling. There was also a decrease in the number of sized fractions in which protein content was higher than that in the initial kernel. As a result, for abrasive milling followed by sieving, PK6 did not produce any fraction that was enriched with protein, while for impact milling followed by sieving only PK0 and PK1 could be used for protein enrichment. Therefore, by comparison, pearling alone was found to be the most effective way to enrich protein in barley seeds, while impact milling followed by sieving was least effective. Abrasive milling followed by sieving fell somewhat in between and became less effective as kernels were further pearled. For example, by pearling dehulled barley, the fraction with the highest protein content was PN1, which had 20.14% protein and 8% of total seed mass (Figure 2A, the protein content corresponding to 19% surface removal in the hulled, sequential line). This represented a 79.18% increase over the protein content (11.24%) of the initial kernel. When dehulled barley was fractioned by abrasive milling and sieving, the fraction with the highest protein content

![Figure 9](image-url)
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was PK0 (150 to 250 μm), which had 15.76% protein and 16.15% seed mass, representing a 40.21% increase in protein content. Furthermore, when processed by impact milling and sieving, the fraction with the highest protein content was PK0 (>250 μm), which had 13.34% protein and 7.44% of seed mass, resulting in an increase in protein content of only 16.68%.

Since BG and starch were lower in the outer surface area, pearling concentrated their contents in the remaining kernels. This was just opposite to what was observed for protein. After milling and sieving, there was a progressive increase in starch and BG contents for the same sized fractions from the kernels with increased cycles of pearling. This was especially true for starch. Therefore, unlike protein for which pearling alone was the best method to enrich it, for BG and starch, to achieve maximum nutrient shifting, pearling alone was not enough. An additional method of milling followed by sieving was required to further process pearled kernels. In other words, a combination of pearling with milling/sieving methods was needed for maximally shifting their contents. Furthermore, because impact milling gave a larger shifting effect on BG than abrasive milling, for enriching this nutrient, impact milling followed by sieving is the method of choice.

Only by pearling and then impact milling followed by sieving, was it possible to produce a single or combined fraction with the highest possible concentration of protein, and another single or combined fraction with highest possible concentration of starch. However, by any combination of fractionation methods, it was nonachievable to produce 3 types of fractions (either single or combined) with each having the highest possible concentration of protein, BG and starch, respectively. Note that here we are not talking about a single or combined fraction enriched with 2 or more nutrients. This was discussed in the previous section. Here we are talking about different fractions, each being enriched with a different nutrient.

**Further discussion**

Several groups of researchers reported dry fractionation of barleys through milling followed by sieving or by air classification and achieved varying levels of success in enriching nutrients. After milling and sieving a hullless barley that was high in protein, BG, and oil, Wu and others (1994) found that the fraction with smallest particle size (<64 μm) had higher protein, higher starch, and lower BG contents than the starting flour. The remaining 5 fractions all had lower protein and lower starch, but higher BG contents as compared with the starting flour. In the same study they also air classified the milled barley flour instead of sieving, and found a similar conclusion; that is, protein and starch were enriched in the smaller particles whereas BG was enriched in the fractions with larger particles. Furthermore, relative change in protein content was lower than that of BG upon either of the 2 fractionation methods.

Knuckles and Chiu (1995) milled and fractionated several barley samples with sifters (sieves) or an air classifier, and found that milling followed by sieving yielded fines fractions containing 88% to 90% starch and a coarse fraction enriched with BG (16% to 19%), while milling followed by air classification was less effective in producing fractions enriched with BG. After pin-milling and air classifying 3 barleys into 3 fine (F1 to F3) and 3 coarse (C1 to C3) fractions, Vasanthan and Bhatty (1995) showed that protein was enriched in F1 and F2 fractions (17% to 29%), BG in C1 to C3 fractions (13% to 24%), and starch in only in F3 fraction (77% to 78%). Anderson and others (2000) impact milled and air classified 7 barleys and

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<th>Table 2 — Correlation coefficient (r-value) for several measured parameters in all sieved fractions of barley flour.</th>
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<td>Glucan content</td>
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<td>Starch content</td>
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<td>Protein recovery</td>
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<td><strong>Hulled-abrasive milling</strong></td>
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obtained sized fractions (F1 to F5 and C5) with increasing particle size for each type of barleys. They found that for all the barley’s protein was enriched in F1 (14% to 26%) for the different barleys and BG was enriched in F5 and C5 (7% to 23%). However, starch was enriched in F4 for normal and waxy barleys (72% to 79%) and in F3 for high amylase barleys (72% to 75%).

The previous reports did not investigate the effect of peeling cycles on nutrient shifting by subsequent dry fractionation, or the effect of milling method. Although some had data on fraction mass, none provided information on recovery rate for each nutrient on the basis of individual fractions. Furthermore, the present study showed that regardless of genotype and milling method, starch tended to be enriched in finer fractions, while BG tended to be enriched in fractions with intermediate particle sizes. The pattern varied greatly for protein since it was largely influenced by genotype, milling method, and their interactions. Apparently, the observed differences among studies with regard to enrichment of a particular nutrient in fractions with certain particle sizes on a relative basis were due to differences in genotype, milling, and sizing methods used.

**Conclusions**

The present study shows that peeling had significant effects on the efficiency of subsequent milling methods followed by sieving, in terms of nutrient enrichment and recovery rates. The milling method also had significant effects on efficiency of sieving for nutrient enrichment and recovery rates, as did the genotype. For protein, peeling alone was the best method to enrich it, but for BG and starch, to achieve maximum nutrient shifting, a combination of peeling and milling followed by sieving was necessary. Therefore, although dry fractionation is the method of choice for separating barley into fractions with varying levels of protein, BG, and/or starch, selection of a specific single or combined method is needed for achieving maximum shifts of a particular nutrient.

**Acknowledgment**

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**References**


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