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Grain Hardness in Barley

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Key Words: barley, grain hardness, milling energy, SKCS, light reflectance, protein, beta-glucan

Abstract

Grain hardness is described as the resistance of the kernel to fracture or the extent of endosperm packing. In barley, it is a product of the complex interaction between compositional and structural endosperm components, including starch, protein and beta-glucan and the matrix formed between these components. Grain hardness may contribute significantly to barley quality. This research examined the relationship between grain hardness determination by milling energy, SKCS hardness, and endosperm light reflectance of eight Western Canadian feed and malting barley genotypes grown at multiple locations and the influence of protein and beta-glucan on hardness. Genotypes differed in milling energy, SKCS hardness, and endosperm light reflectance with all three hardness methods ranking genotypes similarly. All three hardness methods were significantly correlated. McLeod, CDC Dolly and Valier genotypes were consistently harder while CDC Bold was consistently softest. Grain hardness was influenced by protein and beta-glucan content in this small sample set.

Introduction

Grain hardness is the resistance of the kernel to fracture (Anjum and Walker, 1991) and is a product of the complex interaction between compositional and structural endosperm components, including starch, protein and beta-glucan and the matrix formed between these components. It is also described as the extent of endosperm packing (Holopainen et al., 2005). Mealiness (soft) describes loosely packed cells with air spaces between starch granules while steeliness (hard) describes densely packed cells in a dense starch-protein matrix (Chandra et al., 1999; Allison, 1986). Methods to evaluate grain hardness include measuring energy required to mill (milling energy) or crush (hardness) the grain, with harder grain requiring more force. It may also be measured by light transmission, with harder grain transmitting more light through the endosperm (Chandra et al., 2001). Grain hardness may contribute significantly to barley quality.

Milling energy is the measurement of electrical energy required to mill small (five grams) grain samples into flour (Allison et al., 1976). As samples are milled, the deceleration of a rotating flywheel driving the mill hammers is recorded and equated to milling energy (joules). Allison et al. (1976) established that milling energy differentiated good from poor malting barley varieties, with good varieties requiring less milling energy. Henry and Cowe (1990) reported malt modification correlated positively with milling energy ($r = 0.56$) with lower milling energy indicating more complete modification.

SKCS hardness is the measurement of force required to crush grain using the Single Kernel Characterization System (SKCS) developed by Martin et al. (1993). The SKCS crushes single kernels between a narrowing crescent-shaped gap and toothed rotor to obtain crush-response profiles and conductivity measurements. These factors are needed to algorithmically calculate kernel hardness, weight, diameter and percent moisture (Gaines et al., 1996).

Light transmission is the measurement of the quantity of light transmitted through a kernel, using the Light Transflectance Meter (LTm) developed by Brewing Research International (Chandra et al., 2001), with low and high LTm values indicating mealy and steely grain texture, respectively. Holopainen et al. (2005) found an association between LTm values and malting performance, with steely grains being less friable and slower to modify. Conversely, Nielsen (2003) measured total light reflectance of kernels using the GrainCheck™ 310 instrument along with the SKCS hardness to predict malting quality. He reported these factors were significantly positively correlated with malt beta-glucan ($r = 0.74$), wort beta-glucan ($r = 0.82$), malt friability ($r = 0.82$), and wort viscosity ($r = 0.71$).

This research examined the relationship between milling energy, SKCS hardness, and endosperm reflected light intensity of several Western Canadian feed and malting barley genotypes grown at multiple locations and the influence of protein and beta-glucan on grain hardness.

Materials and Methods

One malting barley breeding line and one malting and six feed varieties were grown in field trials at a total of twelve Western Canadian sites over two years, 2003 and 2004. To provide seed of relatively uniform size and shape, grain samples were sieved before analysis. 2003 samples were sieved such that seed passing through a 2.5 X 18.75 mm slotted sieve and remaining on a 2.3 X 18.75 mm slotted sieve were evaluated. 2004 samples were sieved such that seed passing through a 3.1 X 18.75 mm slotted sieve and remaining on a 2.9 X 18.75 mm slotted sieve were evaluated.

Milling Energy

Five-gram samples were milled in a 'Comparamill' flourmill at the Scottish Research Institute in Invergowrie, Dundee, Scotland to determine milling energy.

SKCS Hardness

Three hundred seed per sample were evaluated using the Perten Instruments SKCS™ to determine SKCS hardness.

Light Reflectance

Analogous to the GrainCheck™, to determine reflected light intensity, fifty seed per sample were pearled to 85% of initial weight (ie, to remove hull) and sliced longitudinally, with one seed half used for analysis. Two incandescent lights were placed adjacent to a Leica MZFLIII light microscope, equipped with a Q-Imaging Micropublisher 3.3 RTV camera. Seed halves were secured in plastercine with the exposed endosperm facing upward. Each illuminated endosperm was photographed (exposure 2.2 milliseconds, gain 50%, offset 0%, ROI sampling 2x2, size 1024x768 pixels) using Empix Imaging's Northern Eclipse V7.0™ microscope image acquisition and analysis software. Digital images were converted to inverted grayscale and

analyzed for endosperm reflected light intensity (light pixels per mm2) using Bio-Rad's Quantity One® 1-D analysis software. Reflectance data were reported as thousand light pixels/mm^2.

NIT Percent Protein
Samples were analyzed for % protein using Near Infrared Transmittance (NIT) (Infratec Food and Feed Analyzer™), based on the 2003 and 2004 Crop Development Centre hulled barley NIT protein calibrations.

FIA Percent Beta-glucan
Samples were analyzed for % beta-glucan using the calcofluor flow injection analysis method described by Aastrup (1988).

Statistical Analysis
Data for all traits measured were analyzed by ANOVA using PROC GLM (SAS Institute, 2006). Differences among genotypes were tested using Tukey's multiple comparisons procedure (significance level $P<0.05$).

Results
Significant differences were detected between genotypes (Table 1) and environments for all traits measured ($P<0.001$). No genotype by environment interaction was detected for milling energy, light reflectance or protein ($P>0.10$). Inconsequential (non-cross over) genotype by environment interaction was detected for SKCS hardness and beta-glucan ($P<0.05$). As a result, all environments were combined for final analysis.

Milling energy of genotypes ranged from 617 to 736 joules (SE=5.29) (Table 1). McLeod and CDC Dolly required significantly more energy to mill, followed by Valier, Newdale, CDC Helgason, CDC Trey and TR253. CDC Bold required the least energy to mill, indicating a softer endosperm. Milling energy ranged from 629 to 701 joules across environments (data not shown).

Table 1. Milling energy, SKCS hardness, light reflectance, protein, and beta-glucan of eight barley genotypes across twelve Western Canadian locations, 2003 and 2004.

Genotype	Use	Milling Energy*	SKCS Hardness~	Light Reflectance#	Protein (%)	Beta-glucan (%)
McLeod	Feed	736 a	56.1 a	19.2 a	12.3 a	3.98 cd
CDC Dolly	Feed	733 a	48.4 bc	21.4 bc	12.0 ab	4.72 a
Valier	Feed	691 b	50.4 b	21.1 b	11.9 ab	4.35 b
Newdale	Malt	675 bc	45.4 cd	22.5 cde	11.9 ab	4.12 c
CDC Helgason	Feed	663 cd	44.4 d	22.3 bcd	11.8 abc	3.88 d
CDC Trey	Feed	645 d	47.1 cd	21.6 bc	11.1 c	3.88 d
TR253	Malt	644 d	44.8 d	23.6 de	11.5 bc	4.01 cd
CDC Bold	Feed	617 e	38.2 e	23.8 e	11.4 bc	3.40 e
Mean		676	46.9	21.9	11.7	4.04

Means in same column followed by same letter are not significantly different (P=0.05).
*Milling Energy (joules) with higher values equaling harder grain.

~SKCS Hardness (0-100 score) with higher values equaling harder grain.
#Light Reflectance (thousand pixels/mm2) with lower values equaling harder grain.
SKCS hardness ranged from 38.2 to 56.1 (SE=0.73) (Table 1). McLeod was hardest, followed by Valier and CDC Dolly. CDC Trey, Newdale, TR253, and CDC Helgason, with CDC Bold softest. SKCS hardness ranged from 35.2 to 55.8 across environments (data not shown). Light reflectance ranged from 19.2 to 23.8 thousand pixels/mm^2 (SE=0.32) across genotypes (Table 1). McLeod least reflective (steeliest), with Valier, CDC Dolly and CDC Trey following. CDC Helgason, Newdale and TR253 were more mealy, with CDC Bold being mealiest. Light reflectance ranged from 20.0 to 24.5 thousand pixels/mm^2 across environments (data not shown).

Protein content of genotypes ranged from 11.1 to 12.3% (SE=0.15) (Table 1). McLeod, CDC Dolly, Valier, Newdale and CDC Helgason had more protein followed by TR253, CDC Bold and CDC Trey. Protein content varied from 8.9% to 15.5% across environments (data not shown). Percent beta-glucan of genotypes ranged from 3.4% to 4.7% (SE=0.04) (Table 1). CDC Dolly had the highest beta-glucan followed by Valier, Newdale, TR253 and McLeod. CDC Helgason, CDC Trey and CDC Bold followed, with CDC Bold having lowest levels. Beta-glucan content varied from 3.8% to 4.3% across environments (data not shown).

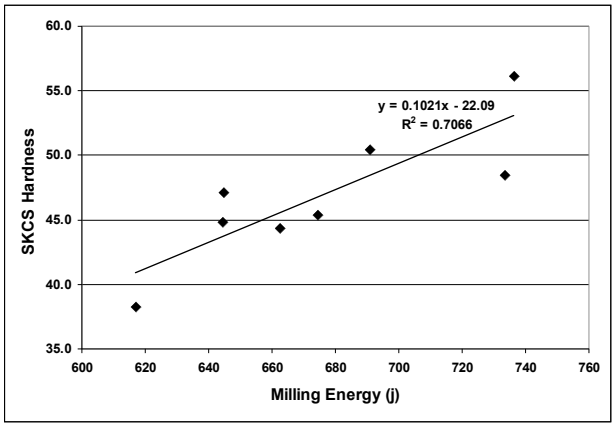

Figure 1. Relationship between milling energy (j) and SKCS hardness.

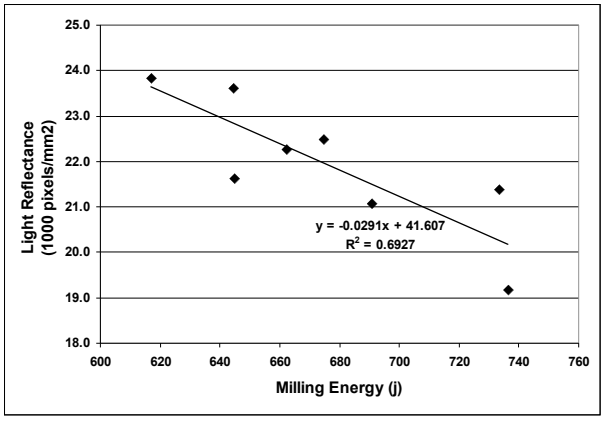

Figure 2. Relationship between milling energy (j) and light reflectance (thousand pixels/mm^2).

Milling energy was positively correlated (n=8) with SKCS hardness (r = 0.84, P<0.009) (Figure 1), protein (r = 0.86, P<0.006), and beta-glucan (r = 0.75, P<0.03) (Figure 4). However, the milling energy-beta-glucan correlation was not significant (P=0.57, n=6) when high (CDC Dolly) and low (CDC Bold) beta-glucan genotypes were removed from the analysis. This effect, caused by the small genotype sample size, was evident throughout for some trait relationships. Both milling energy (r = -0.83, P<0.01) (Figure 2) and SKCS hardness (r = -0.94, P<0.0005) (Figure 3) were negatively correlated with light reflectance. SKCS hardness and protein (P=0.08), SKCS hardness and beta-glucan (P=0.17), light reflectance and protein (P=0.08) and light reflectance and beta-glucan (P=0.29) were not correlated. However, the SKCS hardness-protein correlation was significant (r = 0.80, P<0.05, n=6) when high (McLeod) and low (CDC Trey) protein varieties were removed from the anlaysis. Similar results were found for light reflectance and protein (r = -0.92, P<0.0009).

Conclusions

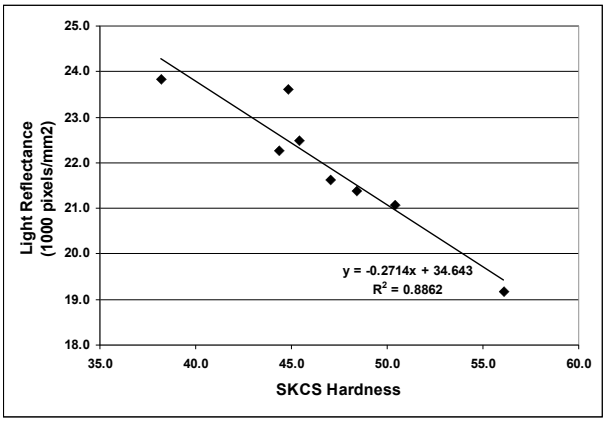

Figure 3. Relationship between SKCS hardness and light reflectance (thousand pixels/mm^2).

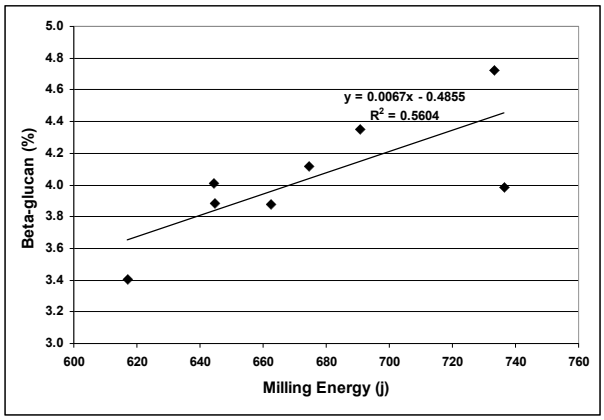

Figure 4. Relationship between milling energy (j) and beta-glucan content.

Genotypes differed in milling energy, SKCS hardness, and endosperm light reflectance. All three grain hardness methods ranked genotypes similarly and were significantly correlated. McLeod, CDC Dolly and Valier were consistently harder while CDC Bold was consistently softest. Grain hardness was influenced by protein and beta-glucan content in this small sample set.

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