Effects of a Novel Barley, Himalaya 292, on Rheological and Breadmaking Properties of Wheat and Barley Doughs

G. Mann,[1,2] E. Leyne,[1] Z. Li,[1] and M. K. Morell[1]

ABSTRACT

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A barley mutant with high-amylose starch, Himalaya 292, combines the potential cholesterol reducing effects of barley with the gastrointestinal benefits of high-amylose resistant starches. Himalaya 292 has alterations in the content and composition of a range of grain constituents, thus conditions for successful addition to foods need to be defined. In this study, the rheological and breadmaking properties of doughs prepared by combining wheat flours (with various gluten protein compositions) with various barley genotypes (Himalaya 292 and the control cultivars Himalaya and Torrens) have been determined. The effects of barley addition on the rheological properties of the admixtures differed. While addition of Himalaya 292 increased the strength and reduced the extensibility of admixture doughs, addition of the Himalaya and Torrens barley flours to the wheat flours reduced both strength and extensibility. The addition of Himalaya and Torrens barley flour reduced water absorption levels. However, addition of Himalaya 292 whole grain flour increased the water absorption of the admixtures significantly ($P < 0.01$). The baking data showed that selection of an appropriate wheat flour with a combination of strength and extensibility allows higher levels of incorporation of barley, facilitating an increased delivery per serving of constituents with positive health attributes in β-glucan and resistant starch.

There is continuing interest in providing dietary options that deliver increased fiber and reduced glycemic index for consumers with conditions such as type II diabetes, but also for those consumers who are mindful of the linkages between diet and health. The incorporation of barley into the diet has attracted interest because it can offer the benefits of a whole grain cereal, plus the cholesterol-lowering benefits associated with the high β-glucan content of barley (Behall et al 2004). In the present study, barley lines were sought that deliver not only high levels of β-glucan but also deliver higher amylose starches with the potential to formulate foods that are less rapidly digested and contain increased levels of resistant starch. Morell et al (2003) isolated a novel barley, Himalaya 292, that contained 70% amylose in the starch compared to 45% amylose in other increased amylose cultivars that carry the *amo*-1 mutation. Himalaya 292 has the potential to deliver a combination of β-glucan and resistant starch into the diet through a low glycemic index product (Morell et al 2003; Topping et al 2003; Bird et al 2004a,b).

Two mechanisms for incorporating barleys with increased health potential into the diet have been extensively explored. First, differing genetic stocks of barley have been utilized in a range of studies to examine the effect on health indices. Comparisons have been made of the composition and nutritional properties of hulled and hull-less barleys, and of barleys containing waxy and *amo*-1 alleles that lower amylose (waxy mutants) or increase amylose (high-amylose glacier, AC38). A broad range of characteristics of the variation in starch structure and properties of waxy, normal amylose, and *amo*-1 barleys has been described (Banks et al 1971; Schondelmaier et al 1992; Oscarsson et al 1997; Czuchajowska et al 1998; Song and Jane 2000; Yoshimoto et al 2000). Second, the effects of fractionation technologies such as pearling have been examined to develop processed grains or grain fractions that can be incorporated into diets and deliver health benefits (Vasanthan and Bhatty 1995; Yeung and Vasanthan 2001).

Himalaya 292 was isolated following a chemical mutagenesis program and shown to contain about half the normal level of starch compared with the parental line, Himalaya. However, the Himalaya 292 starch had a high-amylose content derived not from increased amylose synthesis but through a reduction in amylopectin synthesis (Morell et al 2003). This starch had an unusual combination of properties compared with other high-amylose starches. Because the genetic lesion affected a starch synthase rather than a branching enzyme, as is the case for maize and rice high-amylose starches, the external chain length of the amylopectin fraction was reduced in length. This reduced amylopectin chain length distribution is consistent with DSC observations that this starch has a reduced gelatinization temperature. While the initial focus of the research was on the Himalaya 292 starch, it was also noted that there were profound effects of this mutation on other components of the grain. For example, protein, β-glucan, lipid, and free-sugar components are all increased significantly above the levels expected from the proportional effect of simply reducing starch content (Bird et al 2004a). This suggests that carbon entering into the grain that cannot go into starch synthesis because of the loss of the starch synthase IIa enzyme is diverted into a range of other biosynthetic processes. This combination of a novel starch component along with increases in the levels of other grain constituents provides additional interest in the nutritional properties of the grain. However, given the unusual composition of the grain, methods for incorporating effective levels of the grain and grain-derived products into consumer foods have to be revisited.

One of the most effective means of delivering a grain such as Himalaya 292 into the diet is through breads. In this study, the factors that influence the levels of barley that can be incorporated into a standard rapid dough breadmaking process while retaining an acceptable bread quality have been explored. Factors investigated were composition of the various barleys added and the breadmaking characteristics of the wheat flour component of the blend.

MATERIALS AND METHODS

The barley Himalaya 292 is a chemically induced mutant of hull-less cultivar Himalaya that was selected on the basis of shrunken grains and possesses a unique starch and grain composition as reported by Morell et al (2003). Himalaya is the parent line to mutant Himalaya 292 and Torrens is a commercial hull-less barley cultivar. The wheat cultivar Glenlea is an extra strong Canadian cultivar, Chara is a strong Australian cultivar, and Janz is a medium to strong Australian cultivar. The protein profiles are summarized in Table I.

Milling

Wheat grains were tempered to 16.5% moisture and Himalaya and Torrens were tempered to 15.5% moisture (Approved Method 26-95, AACC International 2000) overnight and milled with a

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[1] CSIRO Plant Industry, GPO BOX 1600, Canberra, ACT 2601, Australia.
[2] Corresponding author. Phone: +61 2 6246 5380. Fax: +61 2 6246 5000. E-mail: Gulay.Mann@csiro.au

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Buhler laboratory scale mill at BRI Australia Ltd. to a final particle size of 150-µm straight-grade flour. Himalaya 292 and additional samples of Himalaya and Torrens were milled using Quadrumat Jr. mill and further sieved to achieve 150-µm particle size. No tempering regime was applied before Quadromat milling.

Barley and Wheat Flour Samples

Admixtures of wheat and barley were prepared in wheat-barley ratios of 80:20, 60:40, 50:50, 40:60, and 20:80. Himalaya 292 was used as a whole meal only; Himalaya and Torrens admixtures were prepared from both whole meal and Buhler mill-derived flours.

Biochemical Composition Analysis

β-Glucan content was determined using Megazyme mixed linkage β-glucan assay (Approved Method 32-23, AACC International 2000). Amylose and amylopectin contents were measured using an iodine blue value method (Regina et al 2004) or Sepharose CL2B chromatography (as indicated) (van de Wal et al 1998).

Reversed-Phase and Size-Exclusion HPLC

Gliadins and glutenins of the admixtures were analyzed by RP-HPLC according to Larroque et al (2000). SE-HPLC was performed to assess the proportion of the main classes of storage proteins (glutenins, gliadins, albumins/globulins). Total proteins were extracted according to Batey et al (1991).

The total polymeric protein was separated into extractable and unextractable (residue) fractions based on extractability in 0.5% (w/v) SDS-phosphate buffer (without sonication). To solubilize the remaining protein (unextractable protein), the residue was sonicated for 30 sec. Both extracts were filtered through 0.45-µm PVDF filters before chromatography (Gupta et al 1993).

Micro Z-Arm Mixing

Optimum water absorption values of wheat and barley flours were determined with the Micro Z-arm mixer which uses 4 g of test flour per mix (Gras et al 2000; Bekes et al 2002). Constant angular velocity (with shaft speeds for the fast and slow blades of 96 and 64 rpm, respectively) was used during all mixes. Mixing was done in triplicate for 20 min each. Before adding water to the flour, the baseline was automatically recorded (30 sec) by mixing only the solid components. The water addition was done in one step using an automatic water pump. The parameters were determined from the individual mixing experiments by taking the averages. WA% is water absorption determined at 500 BU dough consistency. DDT is dough development time determined as the time to peak resistance (sec).

Mixograms

Samples with variable water absorption corresponding to flour protein (Approved Method 54-40, AACC International 2000) were mixed in a 10-g CSIRO prototype mixograph keeping the total dough mass constant. Parameters recorded for each of the flour samples were mixing time (MT, sec); mixograph peak resistance (PR, AU); bandwidth at peak resistance (BWPR, AU); resistance breakdown (RBD, %); bandwidth breakdown (BWBD, %); time to maximum bandwidth (TMBW, sec); and maximum bandwidth (MBW, AU).

Micro Extension Testing

Doughs with a final mass of 17.5 g were mixed to peak dough development in a 10-g prototype mixograph for extension testing on the TA.XT2i texture analyzer, using a Kieffer rig. Extension at 1 cm/sec was done in five replicates on a TA.XT2i texture analyzer with a modified geometry Kieffer dough and gluten extensibility rig (Mann et al 2003). Dough samples for extension testing (≈1.0 g/test) were molded with a Kieffer molder and rested at 30°C and 90% rh for 45 min before extension testing. The R_{max} and Ext_{Rmax} were determined from the data with the help of Exceed Expert software (Smewing 1995; Mann 2002).

10-g Rapid Dough Breadmaking Process

Flour (13.02 g) and ingredients were mixed to peak dough development time in a 35-g mixograph. The recipe used (based on the 13.02 g of flour) was flour 100%, salt 2%, dry yeast 1.5%, vegetable oil 2%, and improver 1.5%. The water addition level was based on the micro Z-arm water absorption values that were adjusted for the full formula. The molding and panning were done in two-stage proofing steps at 40°C and 85% rh. Baking was done in a Rotel oven for 15 min at 190°C and the loaf volume and the weight measurements were taken by the seed displacement method after cooling on a rack for 2 hr (Mann 2002).

Physical Characterization

The protein and moisture content of these samples was determined by infrared reflectance (NIR) according to Approved Method 39-11 (AACC International 2000). The protein (%N × 5.7) and moisture contents of the admixtures containing Himalaya 292 were also determined by Dumas method and air-oven method according to Approved Method 44-15A (AACC International 2000).

Statistical Analysis

Principal component, multiple regression, and analysis of variance (ANOVA) analyses were performed using GENSTAT. This method finds linear combinations of a complex set of variables, deriving a smaller number of new variables called principal components (PC). The PC are orthogonal to each other and give the best description of the variability in the data in decreasing order. The PC are used as axes in a plot of variables, which makes it possible to get an overview of the data and gives an insight into the variates that are related or distinguishing between the samples. Because the variates are measured on a range of scales and are of very different types, a correlation matrix was used throughout. In this analysis, two types of variables were used in the matrix: 1) response variables measured by rheological and baking methods were mixing time and energy (by pin mixer), optimum water absorption values (micro Z-arm mixer), extension at maximum resistance, area under the extension curves, and loaf specific volume; and 2) explanatory variables that were compositional parameters measured by SE-HPLC and RP-HPLC methods including percentage of the unextractable polymeric proteins, D-hordeins, HMW-

TABLE I

Composition of Wheat and Barley Flours[a]

	Protein	MC	Starch (as is)	Amylose	Amylopectin	β-Glucan
Glenlea	15.4	12.4	63.9	35.9	64.1	0.14
Chara	13.1	13.0	66.5	34.1	65.9	0.09
Janz	12.5	12.4	65.9	34.8	65.2	0.12
Himalaya	11.0 (11.9)	13.0 (9.0)	67.7 (55.0)	26.6	73.4	2.10 (5.00)
Himalaya 292	16.4	9.2	27.0	81.6	18.4	9.70
Torrens	11.1 (11.8)	12.8 (10.0)	67.6 (54.1)	31.2	68.8	1.24 (4.96)

[a] Values are mean of triplicates. Figures in parentheses are Himalaya and Torrens whole meal protein, moisture, starch, and β-glucan contents. All data is expressed as % of total whole meal and refined flour.

GS (Ax, Bx, By, Dx, and Dy), total gliadins (including α, β, γ, and ω) and the ratio of HMW-GS to LMW-GS.

RESULTS

Composition of Barley and Wheat Cultivars

Flours were obtained from each of the wheats used in the study, plus Himalaya and Torrens. Satisfactory yields of flour were not obtained from Himalaya 292 and therefore this material was used as a whole meal. Whole meal flours of Himalaya and Torrens were also used in these studies, where appropriate, to provide direct comparisons with Himalaya 292. Compositional analysis of the barley and wheat cultivars utilized in this study were conducted to define the major components of the refined and whole meal flours contributing to flour performance and baking quality (Table I). Of the three base wheat flours used, Glenlea had the highest protein content while Janz had the lowest. The protein content of Himalaya 292 was higher than the parent line Himalaya and the commercial barley cultivar Torrens; as noted before, this was a consequence of the pleiotropic effect of the ss2-292 allele.

The β-glucan contents of the refined barley flours from Torrens and Himalaya were significantly higher than that of the wheat flours. The β-glucan content of Himalaya 292 whole meal was significantly higher than the whole meals from Torrens and Himalaya, which in turn, were higher than the β-glucan contents of the refined flours produced from these cultivars.

The amylose content of starch from the wheat flours used in the study was similar and within the normal range expected for bread wheats (Rodriguez-Quijano et al 2003). Starch from Himalaya 292 had the highest amylose content among the wheat and barley flours used. Himalaya and Torrens flours had the lowest amylose content.

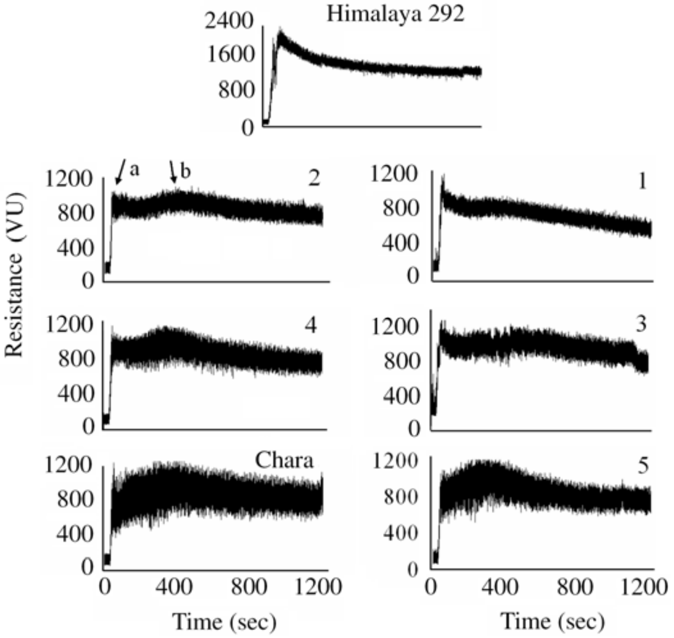

Fig. 1. A series of Z-arm mixing curves showing the effect of addition of Himalaya 292 (H292) into the base flour Chara. 1) 80% H292 and 20% Chara, 2) 60% H292 and 40% Chara, 3) 50% H292 and 50% Chara, 4) 40% H292 and 60% Chara, 5) 20% H292 and %80 Chara. Note that the scaling for the Himalaya 292 mixing curve is twice the scaling for the rest of the mixing curves. Starch hydration (a) and protein network development (b) phases are indicated.

Protein Allelic Composition of Pure Lines and Admixtures

Janz had the standard expression level Bx7+By8 allele at *Glu-1B*; however, both Glenlea and Chara had Bx7 over-expression alleles previously associated with increased dough strength (Radovanovic et al 2002; Vawser and Cornish 2004).

Table II summarizes the relative expression levels of HMW and LMW glutenins in the wheat and barley lines as obtained by the SE and RP-HPLC methods. Chara and Glenlea had similar HMW-GS subunit composition, which were both significantly ($P < 0.05$) higher than Janz. However, the LMW-GS content of Janz was significantly ($P < 0.05$) higher than Glenlea and Chara. Himalaya 292 did not have a detectable level of expression of D-hordeins, despite the parental line Himalaya expressing D-hordein. Torrens had twice the amount of the D-hordeins compared with Himalaya.

WA and Mixing Characteristics by Micro Z-Arm Mixer

Figure 1 illustrates the effects of the increasing levels of addition of the Himalaya 292 whole meal flour on the Z-arm mixing characteristics of the base flour Chara. Addition of Himalaya 292 affected three different phases of the Z-arm mixing: 1) Himalaya 292 increased the response on initial hydration of the samples; 2) bandwidth of the mixing curve of the dough was decreased on Himalaya 292 addition; and 3) dough development time and stability decreased. There are two prominent peaks on the Z-arm mixing curves, the first peak is attributed to starch and nonstarch polysaccharides (NSP) hydration and the second peak is attributed to protein hydration and network development (Sebecic and Sebecic 1996). For Himalaya 292, there is only one maxima at a position, indicating that the peak is related to starch and NSP hydration events. There is no detectable protein network formation in Himalaya 292. Three features of the altered composition of Himalaya 292 are likely to contribute to the large initial hydration peak. First, Himalaya 292 has smaller starch granules with convoluted surfaces in comparison to the smooth lenticular shape of the normal barleys or wheats, resulting in much increased starch granule surface area per unit mass (Morell et al 2003). Second, Himalaya 292 has double the content of NSP, largely composed of β-glucans and arabinoxylans (Topping et al 2003). Third, Himalaya 292 has a high protein content. As the wheat flour component of the admixtures increases, the starch and NSP hydration peak decreases and the protein network formation peak increases. Furthermore, the width of the curves becomes greater, suggesting that the resistance of dough to mixing is increasing and proportionate to the levels of the wheat flour proteins present in the admixtures. This trend was similar to that of the Himalaya and Torrens admixtures although less pronounced (data not shown).

TABLE II
Summary of Wheat and Barley Protein Composition[a]

	Ax	Bx	By	Dx	Dy	D Hordein	HMW	LMW	H/L Ratio
Himalaya	. . .	. . .	. . .	. . .	. . .	2.2	2.2	97.8	0.02
Himalaya 292	. . .	. . .	. . .	. . .	. . .	. . .	. . .	100	. . .
Torrens	. . .	. . .	. . .	. . .	. . .	5.4	5.4	94.6	0.06
Chara	4.7	16.2	3.6	7.6	2.0	. . .	34.1	65.9	0.52
Glenlea	4.0	15.4	3.6	7.9	2.7	. . .	33.6	66.4	0.51
Janz	5.6	9.9	3.0	6.0	1.7	. . .	26.1	73.9	0.35
LSD[b]	0.36	0.50	0.46	1.06	2.15	. . .	3.64	3.64	0.07

[a] Data obtained by RP-HPLC method. All data is expressed as % of total HMW and LMW glutenins of flour and whole meal samples.

[b] LSD, 5% least significant difference for comparison of the wheat flour group mean values.

Figure 2 shows the optimum water absorption predicted by the micro Z-arm mixer. The water absorption of whole meal barley flours differed from the refined barley flours. The whole meal flours for Himalaya 292, Torrens, and Himalaya showed high water absorption levels, presumably due to the presence of high levels of nonstarch polysaccharides and other water-binding compounds in the bran. For the refined Torrens and Himalaya flours, the trend is reversed, with the refined barley flours having lower water absorption levels than the wheat base flours (Fig. 2). The mechanistic reason for this difference between refined wheat and barley flours is outside the scope of this study; however, it should be noted that high water absorption is a target for wheat breeders and not for barley breeders, which indicates the differences may be genetically determined.

Pin Mixer Analysis of Flour Samples

The three barleys studied had significantly shorter mixing times than wheat flours, which was to be expected due to the lack of ability of barley to form a protein network on mixing (Table III). However, the addition of Himalaya 292, Himalaya, and Torrens (flours) to each of the base wheat flours resulted in pronounced increases in mixing time at levels of incorporation up to 50% (Fig. 3). This effect was similar whether the whole meal barley or the refined barley flours were used. Typical wheat flour dough mixing curve shapes were attained when wheat constituted 50% or greater of the admixture.

Uniaxial Extension Testing

Figure 4 shows the maximum resistance (R_{max}) and extension at R_{max} (Ext_{Rmax}) values for Himalaya 292 and Chara admixtures. Similar trends were obtained for the Himalaya 292 (whole meal), Himalaya, and Torrens (refined flour) admixtures in each base flour.

It is interesting to observe that addition of the Himalaya 292 reduced the extensibility and increased the resistance to extension (R_{max}) of the base wheat flour Chara, indicating that the structure was becoming more inflexible with increased amount of Himalaya 292 (Fig. 4).

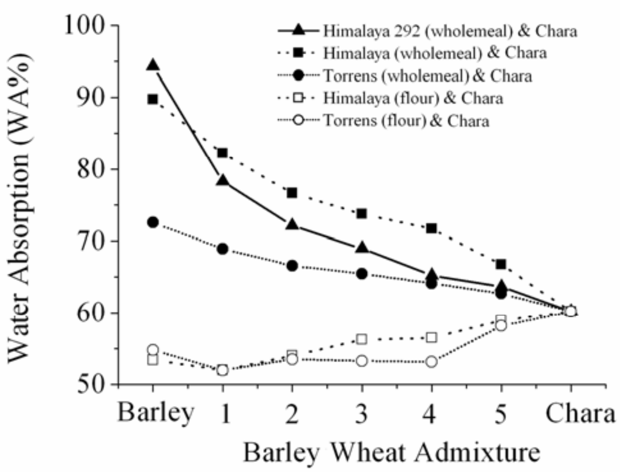

Fig. 2. Impact on Z-arm mixer derived water absorption characteristics of the addition of Himalaya (■), Torrens (●) and Himalaya 292 (▲) as whole meal and Himalaya (□), Torrens (○) as flour to wheat Chara base flour. 1) 80% barley and 20% Chara, 2) 60% barley and 40% Chara: 3) 50% barley and 50% Chara, 4) 40% barley and 60% Chara, 5) 20% barley and 80% Chara. SE of the method was ±1.4.

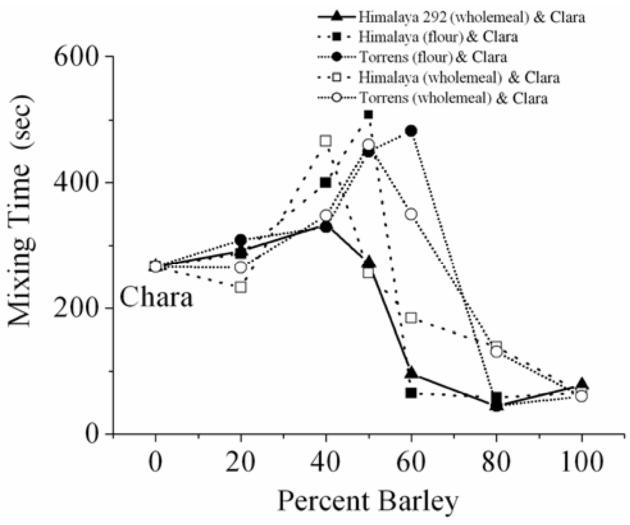

Fig. 3. Pin mixing characteristics after addition of Himalaya (■), Torrens (●) and Himalaya 292 (▲) as whole meal and Himalaya (□), Torrens (○) as flour to wheat Chara base flour. SE of the method was ± 20 sec.

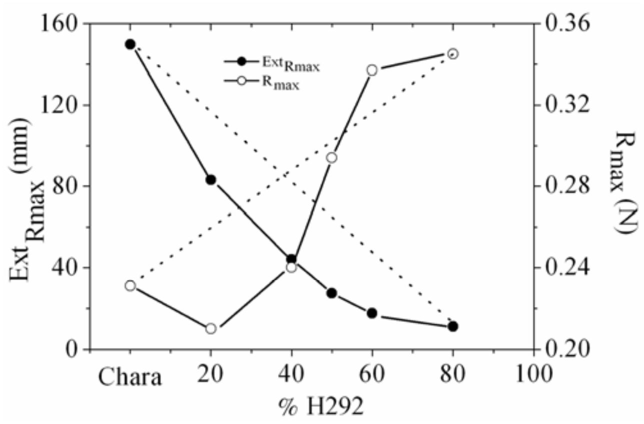

Fig. 4. Uniaxial extensional characteristics of Chara and Himalaya 292 (whole meal) admixtures measured using a modified Kieffer method. Maximum resistance (R_{max}) and extensibility at maximum resistance (Ext_{Rmax}) were measured. Dotted lines indicate a calculated linear response based on the properties of the component flours, measured data is connected by solid lines: Ext_{Rmax} (●), R_{max} (○). SE of the method was R_{max} ± 0.01 and Ext_{Rmax} ± 2.46.

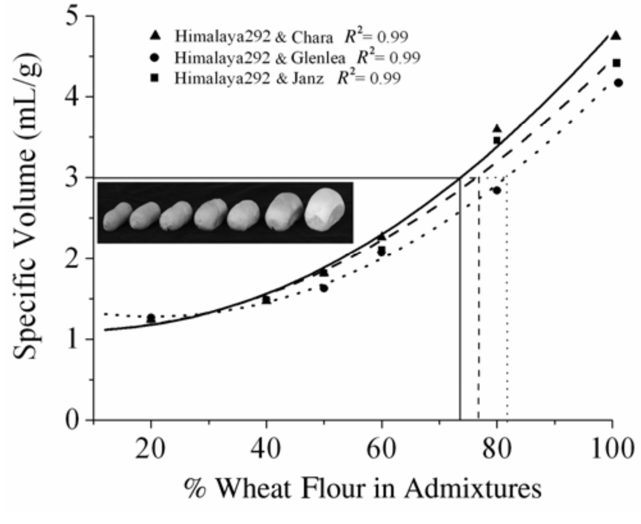

Fig. 5. Chara and Himalaya 292 (whole meal) admixture breads. Left to right is increased wheat flour and decreased Himalaya 292 levels in the admixture. SE of the method was ± 0.04. Solid line shows the exponential growth (first order) model fitted and dotted line shows unsuitability of the linear model.

Baking Properties

The breads made from Himalaya 292 blends with each base flour are shown in Fig. 5. Specific volumes of the breads decreased significantly ($P = 0.01$) as the level of H292 increased. This trend was similar for Himalaya and Torrens admixtures. Loaves of acceptable crumb structure, texture, and appearance were obtained when the specific volume was ≥3 mL/g. Of the three base flours used, a consistent order of performance in breadmaking attributes was noted: Chara was superior to Janz, which was superior to Glenlea. The effect of base flour on specific volume is shown in Fig. 5. Use of Chara as a base flour allowed incorporation of 27% H292 while attaining a specific volume of 3.0 mL/g. Use of Janz or Glenlea allowed lower levels of addition of H292 (24 and 18%, respectively, at a specific volume of 3.0 mL/g). Net water loss after baking was lower for H292 than for other barley flours or whole meals. One explanation is the high level of NSP in this line.

Principal Component Analysis

Principal component analysis was conducted to obtain some insight into the relationships between the compositional (explanatory) and rheological (response) data to give the best predictors of baking performance. Figure 6 shows results for Himalaya 292 and Chara admixtures. Similar results were obtained when Janz and Glenlea were used as the base flour. Figure 6A shows that the addition of barley at all levels had a significant impact on performance. Only barley admixtures containing 20 and 40% barley approached the performance of the undiluted base flour. Figure 6B and C shows that the response and explanatory variables associated with high breadmaking performance in these wheat-barley admixtures were Ext_{Rmax} and area under the extension curve and the HMW-GS, HMW to LMW glutenin subunit ratio, and total gliadins. Mixing time was a poor predictor of breadmaking performance.

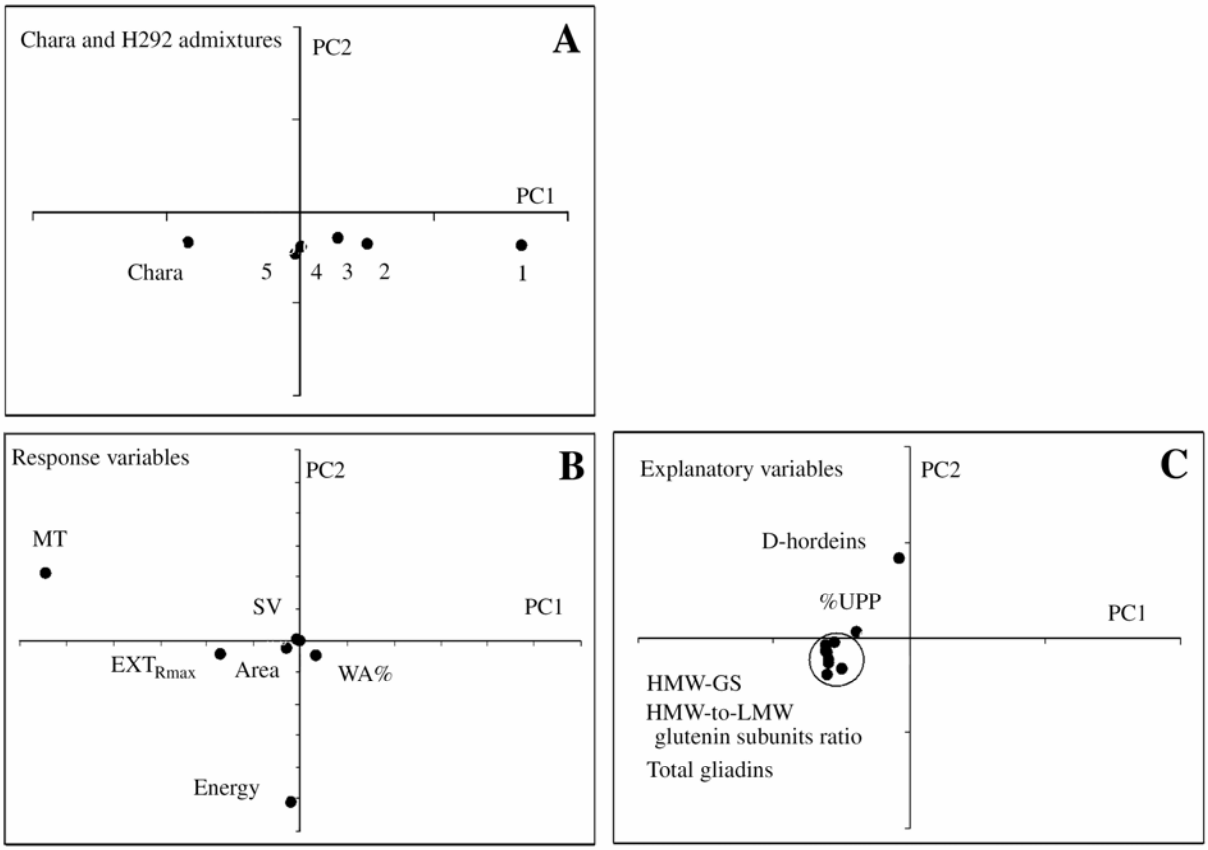

Fig. 6. Principal component analysis showing (**A**) score plot of Chara and Himalaya 292 (H292 whole meal) admixtures, (**B**) loading plot of response and (**C**) explanatory variables. Key to score and loading plots: 1) 80% H292 and 20% Chara, 2) 60% H292 and 40% Chara: 3) 50% H292 and 50% Chara, 4) 40% H292 and 60% Chara, 5) 20% H292 and %80 Chara. Response variables MT (mixing time obtained by pin mixer); energy (obtained from a mixing curve by pin mixer); WA% (optimum water absorption value obtained by micro z-arm mixer); Ext_{Rmax} (extensibility at maximum resistance), Area (area under the extension curve); SV (specific volume obtained by the small-scale rapid dough breadmaking process). Explanatory variables: total gliadins (including α, β, γ, and ω), D-hordeins, HMW-GS (Ax, Bx, By, Dx, and Dy), ratio of HMW-GS to LMW-GS measured by RP-HPLC method and UPP% (% unextractable polymeric proteins) measured by the SE-HPLC method.

TABLE III
Summary of Dough Rheological Properties of Wheat and Barley Lines

	R_{max} (N)[a]	Ext_{Rmax} (mm)[a]	MT (min)[b]	MBW (AU)[b]	WA (%)[c]
Himalaya	. . .	. . .	1.17	503	53.4
Himalaya292	. . .	. . .	1.05	750	94.3
Torrens	. . .	. . .	1.00	389	54.8
Chara	0.21	148.6	3.47	1,113	60.3
Glenlea	0.35	103.9	4.27	1,450	62.9
Janz	0.21	105.7	2.51	1,025	61.8

[a] Measured by TAXT2i with Kieffer rig.

[b] Measured with 10-g prototype mixograph.

[c] Measured with micro Z-arm mixer.

DISCUSSION

The objective of this study was to define factors that were important for the addition of barley flour into breads, with a particular emphasis on the addition of a novel barley, Himalaya 292, containing both a high-amylose starch and high levels of NSP. The composition of Himalaya 292 differs significantly from the parental line (Himalaya) and other comparison barleys in having low starch, higher protein, high NSP, increased lipid, and increased free sugar (Bird et al 2004). In addition, the study utilized Himalaya 292 as a whole meal because it could not be milled into a refined flour at high yields and, in this study, the objective was to retain the potentially positive health benefits of a whole grain derived product with high levels of dietary fiber. Because of the unusual nature of Himalaya 292, it was necessary to explore the nature of the base flour best suited to enhance the quality of barley-wheat admixtures. Three wheat lines were selected as base flours: Janz because it is Australian high quality bread wheat, Glenlea because its high dough strength characteristics may allow high levels of addition, and Chara, a line combining both high strength and good extensibility.

The rheological and baking behavior of barley-wheat flour admixtures gave strongly nonlinear responses relative to those that were predicted from the compositional, rheological, and baking characteristics of the barley and wheat base flours. The degree of nonlinearity is a function of both the proportion and the types of barley and wheat flour components. Water absorption (Fig. 2), $\mathrm{Ext}_{\mathrm{Rmax}}$ characteristics (Fig. 4), and baking performance (Fig. 5) can be described by the exponential decay and growth (first order) models, respectively, while the nonlinearity of $R_{\max}$ is sigmoidal. Mixing time also showed a strongly nonlinear response across admixture levels, with increases in mixing time observed in admixtures from 20 to 60% barley addition (Fig. 3).

Increases in the strength of the admixtures on the addition of barley were observed in the results from the $R_{\max}$ parameter derived from extensibility measurements. Conversely, increasing levels of barley in admixtures resulted in reduced extensibility. These increases in strength and reductions in extensibility were more pronounced with Himalaya 292 admixtures, and this may have been due to the increased levels of nonstarch polysaccharides in the admixtures derived from barley. Each of the barleys used in this study had significantly higher levels of β-glucan than wheat, with Himalaya 292 having significantly higher levels of β-glucan (9–10%) than the parent line Himalaya (5%). Wang et al (2004) demonstrated that addition of water unextractable solids (WUS) or water extractable pentosans (WEP) resulted in gluten with a higher maximum resistance to extension ($R_{\max}$) and a reduced extensibility. Increases in dough strength on addition of β-glucan were also observed by Izydorczyk et al (2001).

The protein contents of the admixtures were poor predictors of breadmaking quality, not only because of the lack of functionality of barley proteins in breadmaking but because of altered content and composition of protein in Himalaya 292. Compared with Himalaya and Torrens (11%), Himalaya 292 has a substantially higher protein content (16.4%) because of the concentration effect resulting from the low starch content of the grain. The expression of the D-hordeins was suppressed in Himalaya 292 (Table II). Genetic analysis showed that the genes from D-hordeins were present in Himalaya 292 and were expressed normally in outcrossed progeny of Himalaya 292 with normal starch content, indicating that they were not mutated in the mutagenesis procedure that lead to the isolation of the Himalaya 292 mutant (data not shown). This suppression of D-hordein expression may be due to pleiotrophic effects of the mutation in Himalaya 292. Because the single genetic change in Himalaya 292 results in pleiotrophic changes in grain composition (increased amylose, β-glucan, sugars, lipids, protein content, and decreased starch and D-hordeins content), it is not possible to attribute the impact of Himalaya 292 on rheology and breadmaking to changes in any one grain constituent. The effects noted may reflect the effect of one change or the combined effects of a number of alterations in grain composition. Further research is required to address this issue.

Principal component analysis was used as a method to define those parameters that were the best predictors of the baking quality in these wheat-barley admixtures. Two parameters that showed clear predictive ability were the content of HMW-GS and the extensibility ($\mathrm{Ext}_{\mathrm{Rmax}}$). Mixing time and $R_{\max}$ were poor predictors of baking performance. In this study, analysis of each of the HMW-GS and the HMW-to-LMW-GS subunit ratio gave essentially the same predictive power, indicating that some rationalization of analysis could be achieved. A further possibility for predicting breadmaking properties of barley-wheat admixtures is to determine the wet gluten index on admixtures; however, this possibility was not directly tested in this study. The use of this method may have advantages because it will quantify the nonlinear effects on functional parameters noted across admixture levels.

The interest in the use of barley in cereal-based products is likely to increase as further evidence is emerging about the potential to improve human health indices through consumption of barley. Because of the inability of barley to form a functional protein network equivalent to that of wheat proteins, technological challenges will be encountered in incorporating high levels of barley into food that require high levels of protein functionality, such as rapid dough breads. Hatcher et al (2005) demonstrated that the yellow alkaline noodle (YAN) quality characteristics such as YAN sheet color and texture was deteriorated by the addition of barley with different types of starches.

This study demonstrated that there were very significant differences in the behavior of Himalaya 292 in comparison to reference barleys that need to be taken into account in formulating products from this grain. The study also demonstrated that the properties of the wheat flour are critical to optimizing the product attributes of wheat-barley admixtures. Careful selection of the wheat flour component can increase the levels of barley that can be added.

CONCLUSIONS

In this study, we demonstrated that selection of the base flour can have a strong effect on the ability to formulate an acceptable bread product given various levels of barley addition in admixtures. For example, in achieving a specific volume of 3 mL/g, the base flour derived from the cultivar Glenlea allowed 18% addition of Himalaya 292, whereas Chara allowed 27% addition, a 50% increase in addition level. Increases in addition level are likely to be important to achieving population-wide benefits of increasing resistant starch levels and decreasing glycemic index using grains such as Himalaya 292. Other strategies to be explored in the future will be to survey additional wheat cultivars for their ability to add high levels of Himalaya 292 and retain functionality, and to explore the use of gluten addition and other bread improvers as a means of increasing addition levels.

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