JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of the Phosphorus and Mineral Concentrations in Bran and Abraded Kernel Fractions of a Normal Barley (*Hordeum vulgare*) Cultivar versus Four Low Phytic Acid Isolines

KeShun Liu,* Kevin L. Peterson, and Victor Raboy

National Small Grain and Potato Germplasm Research Facilities, Agricultural Research Service, U.S. Department of Agriculture, Aberdeen, Idaho 83210

Phytic acid consists of 65−80% of the total phosphorus (P) in cereal grains. Its salts are concentrated in the germ and aleurone layers, which are typically removed during milling. We hypothesize that concentrations of different types of P and minerals in milled products will be greatly altered in low phytic acid (*lpa*) barleys. Seeds of cv. Harrington (control) and four *lpa* isolines—*lpa*1-1, *lpa*2-1, *lpa*3-1, and M955—were abraded by a laboratory method into five surface layer and four remaining kernel fractions. Results show that phytic acid in the four *lpa* lines ranged from 75% to 5% of the control. The decrease in phytic acid P concentration was matched almost equally by an increase in inorganic P, so that the rest of P (the sum of all P-containing compounds other than phytic acid P and inorganic P) and total P levels remained relatively unchanged among the five genotypes. These trends were also observed for the processed fractions. The major mineral elements in barley seeds were P, K, Mg, S, and Ca, while minor ones were Fe, Zn, Mn, Cu, and Ba. All types of P and other minerals measured were generally concentrated in the outer layers of the grain. Although there were substantial differences in mineral contents of bran fractions among genotypes, the level of phytic acid P had little effect on mineral contents in whole or abraded kernels. One major exception was Fe, which had the highest level in all tissues of M955 genotype. The above findings were all confirmed by analyzing another set of barley samples grown in a different environment. Thus, in general, breeding *lpa* barleys does not lead to reduced mineral contents in whole grains or elevated mineral levels in milled products.

KEYWORDS: Phytic acid; minerals; low phytic acid crops; barley; distribution; abraded grains

INTRODUCTION

Cereal grains form a significant portion of the food supply for humans and other animals, as they are a major source of carbohydrates, proteins, and lipids. A less widely recognized class of nutrients is minerals, which have major nutritional significance for human and animals. Deficiencies in elements, such as Ca, Fe, Mn, Zn, can lead to a variety of medical problems from anemia to osteoporosis. It is estimated that about a third of the human population, some two billion people, have dangerously low micronutrient intake. Worldwide, about 40% of women are anemic due to iron deficiency (*1*).

One important factor affecting mineral bioavailability of grains for food and feed is the presence of phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate). Phytic acid interacts with various dietary components, particularly certain minerals, to reduce their availability to humans and nonruminant animals (*2*−*4*) and contributes to increased phosphorus (P) discharge into environments (*5*). One strategy to solve the problem is to develop crops that are lower in seed phytic acid content as compared with conventional cultivars. The low phytic acid (*lpa*) grains have been shown to have improved bioavailability of P and other minerals (*6*, *7*) in humans and animals and reduced P pollution to environments (*8*).

Phytic acid is the main storage form of P in grains (cereal, legume, and oilseed), accounting for about 1% or more of the dry weight and 50−80% of the total P. It is deposited in mature seeds as phytate salts of mineral cations, such as K, Mg, Ca, Fe, Zn, and Mn, in spherical inclusions called globoids that are located within protein storage vacuoles (*9*). In cereal grains, phytates are concentrated in the germ and aleurone layers (*10*). Most cereal grains are consumed or utilized following milling. For example, most rice is consumed as white rice, and most wheat is utilized in bakery and pasta manufacture following milling to remove bran (surface layers). Although milling helps remove most phytic acid, the process also removes minerals and other nutrients concentrated in the bran fraction, thus reducing the nutritional value of the remaining kernel. A few studies reported higher concentrations of certain minerals in milled rice (*11*−*12*) or milled wheat flour (*13*) made from *lpa*

* Corresponding author. E-mail: keshunL@uidaho.edu. Tel.: 208-397-4162. Fax: 208-397-4165.

10.1021/jf0637776
Published on Web 05/09/2007

crops as compared with conventional ones, concluded that alterations of mineral distributions within a *lpa* kernel occurred, and suggested that development of *lpa* crops might improve the nutritional quality of not only whole grains and bran fractions but also the remaining milled products. This conclusion may be very significant since developing *lpa* crops might result in elevated minerals in the central endosperm that ultimately serves as a major food source worldwide.

Barley, one of the earliest cultivated cereal grains in the world, is now gaining renewed interest for food, feed, and industrial uses due to its hypocholesterolemic properties and other characteristics (*14*). Like other grains, for value-added utilization of this crop, its high phytic acid content presents a problem. Recent advances have led to the development of four *lpa* barley genotypes, namely *lpa*1-1 (formerly M422), *lpa*2-1 (formerly M1070), *lpa*3-1 (formerly M635), and M955, that produce grains with phytic acid P reductions in the range of 50−95% (*5*, *15*).

In this study, we used one conventional barley variety and its four *lpa* isolines with varying degrees of phytic acid reduction to test the hypothesis that greatly reducing the ability of grains to synthesize and accumulate phytic acid may alter both the amount and distribution of P and minerals in grain tissues. Such a study will discern the impact of the *lpa* mutation on the concentration of various P and minerals in whole grains as well as in different seed tissues or milled fractions.

MATERIALS AND METHODS

Barley Seed Materials. Two sets of barley samples with different growing locations and years were selected for this study. The first set of barley samples was grown at the University of Idaho Tetonia Research and Extension Center, Tetonia, ID, in 2003 (defined as Environment 1). It consisted of the cultivar Harrington (a control) and four near-isogenic lines (isolines) produced by backcrossing into Harrington four *lpa* alleles, representing at least three different loci, described in Dorsch et al. (*15*)—*lpa*1-1, *lpa*2-1, *lpa*3-1, and M955. These genotypes have been shown to have whole-grain phytic acid P reduction ranging from 50% to 95%, as compared with Harrington (*5*, *15*). This set of samples was analyzed in the main study. The second set of barley samples was grown in Saskatoon, Saskatchewan, Canada, in 2005 (defined as Environment 2), and was kindly provided by Dr. Brian Rossnagel, University of Saskatchewan. It consisted of one conventional cultivar—Valier—and three *lpa* genotypes—*lpa*1−1, *lpa*3−1, and M955. This second set of samples was used to provide an independent test of the findings obtained for the first set of samples. For each genotype within each set, duplicate samples were collected, processed, and analyzed for chemical composition.

Barley Seed Processing. The seed samples were passed through a screen to remove broken kernels and any foreign material. The cleaned barley grains were not tempered before dehulling and pearling. Barley grains were dehulled and pearled at a laboratory scale as follows. Seed samples were first dehulled by a Lab Huller (Model LH 5095, Codema, Inc., Minneapolis, MN). The hull fraction, about 11% of total kernel weight, was further separated into two fractions by passing through a sieve with a U.S. mesh size of #18 (1.0 mm opening). The material on the top of the screen (making up about 8% of the total kernel weight) was a light fraction mainly consisting of hulls, designated as CSH (coarse hulls). The material that passed the screen (making up about 3%) was a heavy fines fraction, designated as FNH (fine hulls).

The dehulled kernel was then subjected to three successive cycles of abrasive milling by an electric seed scarifer (Forsberg, Thief River Falls, MN) with a $^1/_3$ HP motor. Each abrading cycle removed about 8% of outer layers. Samples of bran were removed at each abrading stage (BN-1, BN-2, and BN-3). The corresponding abraded kernels (AK-1, AK-2, and AK-3), together with two hull fractions (CSH and FNH), the corresponding dehulled kernel (DHK), and the original whole or hulled kernel (HLK) were collected and milled into particulates by a cyclone sample mill (UDY Corp, Forth Collins, CO) with mill enclosures, a vacuum system, and a sieve with 1.0 mm round openings. Bran samples were not milled since the particle size was fine enough to pass the 1.0 mm sieve. All particulate samples (10 per genotype) were evaluated for moisture, phytic acid P, inorganic P, total P, and 19 minerals (Ar, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, V, and Zn). The "rest of P" was calculated by subtracting the sum of phytic acid P and inorganic P from total P. It represents the sum of all P-containing compounds in a sample other than phytic acid P (and other inositol phosphates) and inorganic P, including, for example, P found in DNA and RNA, and proteins, lipids, and starches. In addition, the original whole grain (HLK) samples were analyzed for protein and ash content. For the second set of samples produced in Canada, all fractions were measured for moisture and minerals, but only the original whole grain (HLK) samples were tested for phytic acid P, protein, and ash contents.

Chemical Analysis and Data Treatments. Moisture and ash contents were determined according to AOAC methods (*16*). The moisture content was used to convert concentrations of other components into a dry matter basis. The total nitrogen/protein content in seed samples was measured by a combustion method (*16*), using a protein analyzer (Model FT528, Leco Corp. St. Joseph, MI). The protein content was calculated with a conversion factor of 5.75. Total P, phytic acid P, and inorganic P were measured by the methods described in Dorsch et al. (*15*). Briefly, total P was determined after wet-ashing aliquots of samples (150 mg) and colorimetric assay of P in the digests (*17*). Inorganic P was determined colorimetrically after extraction of tissue samples (0.5 g) in 12% (w/v) TCA and 25 mM $MgCl_2$, according to the same method of Chen et al. (*17*). For phytic acid P, aliquots of samples (0.5 g) were extracted in 0.4 M HCl and 0.7 M Na_2SO_4. Phytic acid P was obtained as a ferric precipitate, wet-ashed, and assayed for P as in the total P analysis. All P-containing fractions are expressed as their P (atomic weight 31) content to facilitate comparisons. When necessary, phytic acid P was converted to units of phytic acid (MW 660) by multiplying the conversion factor of 3.5484 [$= 660/(31 \times 6)$].

Mineral elements were determined by the Analytical Sciences Laboratory, University of Idaho, Moscow, ID, using a Perkin-Elmer Optima 3200 ICP-OES (inductively coupled plasma-optical emission spectrometer) to quantify constituents in an aqueous solution following nitric acid digestion of the samples. Standard quality control measures, including blanks, check standards, reference materials, and duplicates were used for all chemical analyses.

Data were treated with the JMP software, version 5 (JMP, a Business unit of SAS, Cary, NC), expressed as the mean with standard deviation (SD) and compared by analysis of variance, followed by the Tukey's HSD (honestly significant difference) test.

RESULTS AND DISCUSSION

The material milled off (or abraded) during dehulling and three cycles of abrading, expressed as the percentage weight removed from the original kernel, is represented by five fractions (**Table 1**). During pearling, differences in the amount of fractions removed were found, as well as differences among the barley isolines. Presumably these were due to differences in hardness or resistance to pearling among tissue layers and among varieties. In order to produce similar portions of material to be removed during each pearling stage, pearling time was adjusted accordingly for each genotype as well as each pearling cycle. The removed five outer layer fractions, CSH, FNH, BN-1, BN-2, and BN-3 consisted of about 8%, 3%, 8%, 8%, and 8%, respectively, of total grain weight. The total accumulated surface material removed from barley seeds was about 35%. The residual kernels (one dehulled and three pearled kernels) represented about 89%, 81%, 73%, and 65%, respectively, of the original whole kernel weight (**Table 1**).

On the basis of germination tests conducted in our laboratory (data not shown), for hulled barleys, up to about 7.5% surface removal did not cause germ damage or removal. Beyond this level, damage to or removal of germs started to occur and peaked at about 12.5%. Above this level, germs were either damaged or removed. At about 20% removal, all germs are

Table 1. Description of Different Fractions after Dehulling and Abrading Barley Seeds by the Lab Method Described in Materials and Methods

fraction name	symbol	type of fraction	cumulative surface removal (%)[a]	% of original kernel[a]	major tissues[b]
coarse hulls	CSH	outer layer	8	8	hulls
fine hulls	FNH	outer layer	11	11	germ, pericarp, and testa
first layer bran	BN-1	outer layer	19	19	pericarp, testa, germ, and aleurone
second layer bran	BN-2	outer layer	27	27	aleurone and subaleurone
third layer bran	BN-3	outer layer	35	35	subaleurone and endosperm
hulled (whole) kernel	HLK	kernel	0	100	all seed tissues
dehulled kernel	DHK	kernel	11	89	endosperm, aleurone, testa, pericarp, germ
first abraded kernel	AK-1	kernel	19	81	endosperm, subaleurone, aleurone
second abraded kernel	AK-2	kernel	27	73	endosperm, subaleurone
third abraded kernel	AK-3	kernel	35	65	endosperm

[a] Approximate values of the average of several runs at each cycle, weight basis. [b] Major tissues that a fraction was mainly made of were based on reasoning.

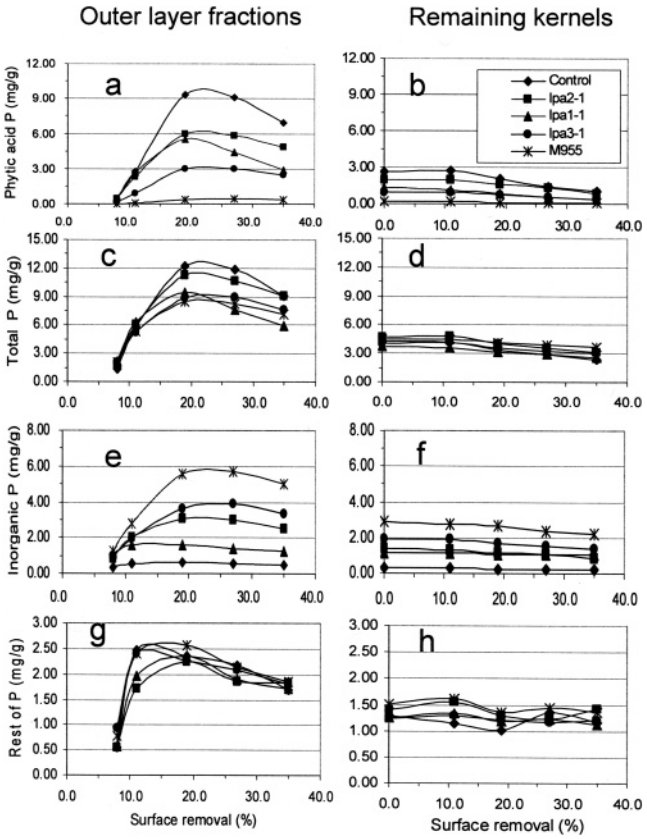

Figure 1. Four types of P in various fractions of cv. Harrington and its 4 *lpa* barley isolines. (**a**, **b**) Phytic acid P. (**c**, **d**) Total P. (**e**, **f**) Inorganic P. (**g**, **h**) Rest of P, which refers to the sum of all P-containing compounds other than phytic acid P and inorganic P. Error bars for this and other figures are equal to or smaller than legend markers.

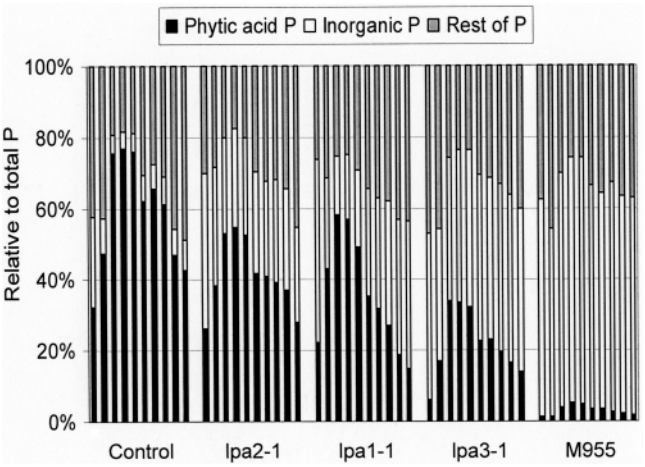

Figure 2. Relative percentages of phytic acid P, inorganic P, and rest of P in various fractions of Harrington and its four *lpa* barley isolines. "Rest of P" refers to the sum of all P-containing compounds other than phytic acid P and inorganic P. Under each genotype, 10 bars represent 10 fraction samples. Refer to the text for further explanation of each bar.

removed. On the basis of this observation and close microscopic examination of each fraction during dehulling and pearling process, we could reasonably determine what types of major tissues constituted each fraction (**Table 1**).

Among the 19 minerals measured, nine of them, including Ar, Cd, Co, Cr, Mo, Na, Ni, Pb, and V, had contents near or less than their detection limits based on the ICP method, and therefore that data for these nine minerals are not presented. The data for the element P by the ICP method was found to have no significant difference ($p < 0.05$) from that of total P by the wet chemistry method. Therefore, only the total P data were used.

Mean values (duplicate data set) for the contents of four different types of P in 10 different fractions (whole barley kernel and their fractions) for the cultivar Harrington and the four *lpa* isolines grown in Idaho were plotted against levels of cumulative surface removal (**Figure 1**). Mean values of major and minor minerals in 10 different fractions of barley seeds are presented in **Figures 3**−**6**. In these figures, the left graphs represent five samples of outer layers for each genotype. The right graphs represent hulled (0% removal level), dehulled, and three abraded kernels, with increasing amounts of endosperm tissue in these samples. Thus the left and right graphs represent distribution patterns of constituents measured from the outer layers toward the inner section of the barley seed.

The results indicate that the processed fractions varied greatly in chemical composition. This has direct effect on nutritional values as well as utilization of each fraction of barley grains. It is noteworthy that, for each observed constituent, the changing pattern among genotypes was consistent, an indication that the lab scale dehulling and abrading method used in this study is useful and reliable in separating different layers of cereal grains for compositional study. However, for barley seeds, the removed fractions do not necessarily reflect the same grain components because of differences in the kernel shape. In fact, some components of the external layers such as bran, aleurone, etc., remained in the creases of the kernels even after several fractions have been removed.

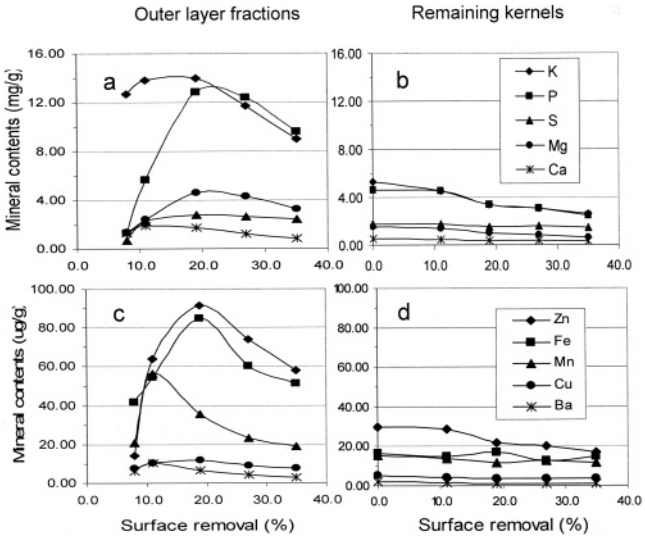

Figure 3. Major (K, P, S, Mg, and Ca) and minor (Zn, Fe, Mn, Cu, and Ba) minerals in various fractions of cv. Harrington. (**a**, **b**) Major minerals. (**c**, **d**) Minor minerals.

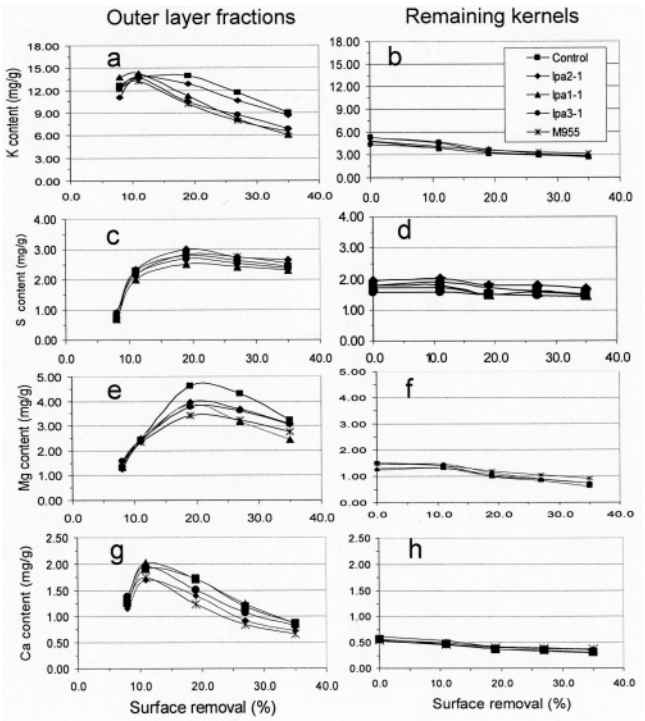

Figure 4. Major mineral (K, S, Mg, Ca) contents in various fractions of Harrington and four *lpa* barley isolines. (**a**, **b**) K content. (**c**, **d**) S content. (**e**, **f**) Mg content. (**g**, **h**) Ca content.

Phytic Acid and Proximate Composition of Seed Samples. Phytic acid reduction between the control and *lpa* isolines was again demonstrated from samples of different locations and crop years (**Table 2**). For seeds grown in Idaho, the order of increase in the percent reduction of phytic acid was as follows: *lpa*2-1 < *lpa*1-1 < *lpa*3-1 < M955, representing about 26%, 51%, 66%, and 94% reduction, respectively. However, the percent reduction was slightly different between seed samples grown at the two different growing environments in this study and from previous reports (*5*, *15*). For the same genotype, samples grown in Canada demonstrated a larger decrease in phytic acid concentration than the U.S. samples. The effects of genotype, growing locations, and crop year were also observed with protein

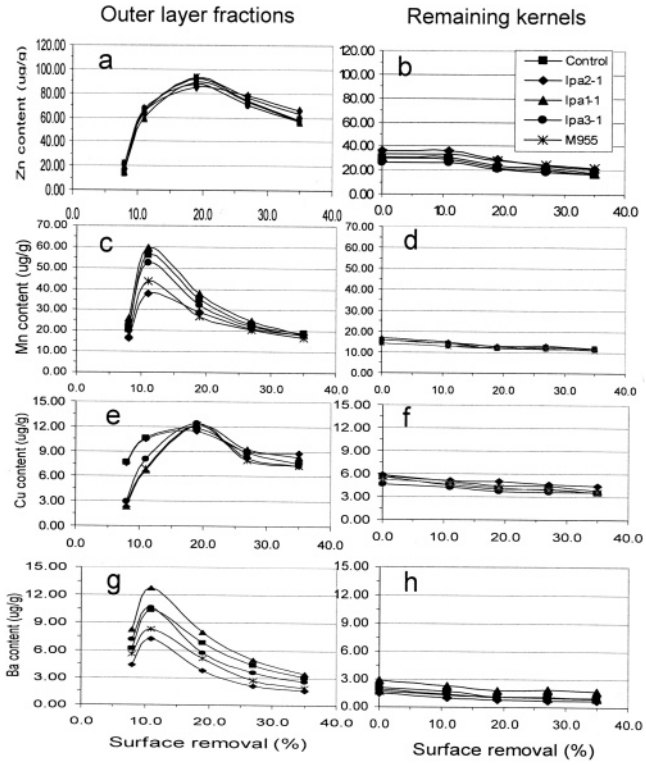

Figure 5. Minor mineral (Zn, Mn, Cu, and Ba) contents in various fractions of Harrington and four *lpa* barley isolines. (**a**, **b**) Zn content. (**c**, **d**) Mn content. (**e**, **f**) Cu content. (**g**, **h**) Ba content.

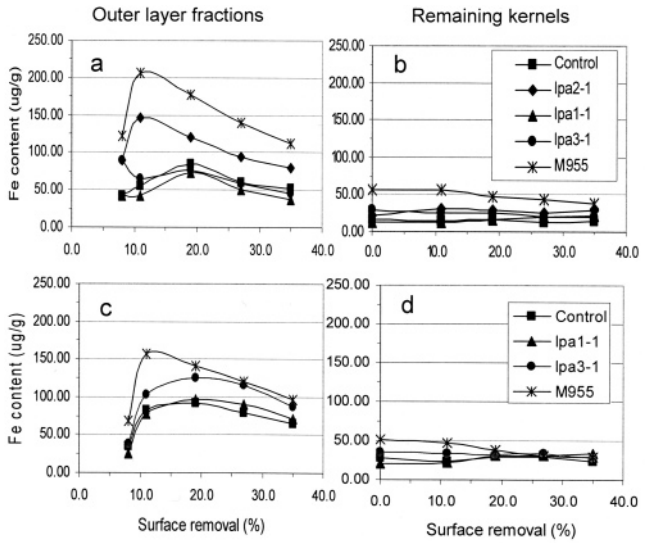

Figure 6. Iron content in various fractions of Harrington and its four *lpa* barley isolines grown in Idaho, 2003 (**a**, **b**), and of Valier and three *lpa* barley isolines grown in Canada, 2005 (**c**, **d**).

and ash contents. Similar to phytic acid, the protein and ash contents in the U.S. samples were higher than the Canadian samples. The moisture contents among samples were similar, ranging from 7.11 to 7.84%

Contents and Distributions of Various Types of P. One of the objectives in this study was to determine if *lpa* genotypes have any impact on the levels and distribution of inorganic P, total P, and rest of P (the sum of all P-containing compounds other than phytic acid P and inorganic P) in barley seeds. Therefore, for every attribute, we looked at three aspects: (1) the content in the original seeds, (2) the distribution pattern

Table 2. Moisture, Protein, Ash, and Phytic Acid (PA) Contents in Conventional and Low Phytic Acid Barley Seeds[a]

genotype	moisture (%)	protein (%)	ash (%)	phytic acid (%)	PA reduction from control (%)
		samples grown in Aberdeen, ID, 2003			
Harrington	7.32 ± 0.56	13.67 ± 0.25	2.28 ± 0.04	0.938 ± 0.006	0.00
*lpa*2-1	7.84 ± 0.43	15.60 ± 0.16	2.71 ± 0.19	0.696 ± 0.018	25.83
*lpa*1-1	7.39 ± 0.39	12.77 ± 0.31	2.24 ± 0.07	0.455 ± 0.069	51.45
*lpa*3-1	7.65 ± 0.42	13.67 ± 0.27	2.38 ± 0.01	0.323 ± 0.007	65.59
M955	7.33 ± 0.17	15.83 ± 0.19	2.75 ± 0.07	0.052 ± 0.003	94.48
means	**7.51**	**14.31**	**2.47**		
		samples grown in Guelph, Canada, 2005			
Valier	7.11 ± 0.32	12.36 ± 0.15	2.16 ± 0.02	0.582 ± 0.018	0.00
*lpa*1-1	7.35 ± 0.16	12.46 ± 0.10	2.04 ± 0.03	0.323 ± 0.006	44.41
*lpa*3-1	7.41 ± 0.29	12.02 ± 0.09	2.17 ± 0.04	0.296 ± 0.007	49.17
M955	7.16 ± 0.17	12.55 ± 0.25	2.10 ± 0.05	0.034 ± 0.006	94.10
means	**7.26**	**12.35**	**2.12**		

[a] Mean percentage ± standard deviation of duplicate measurements.

within a seed, and (3) the impact of *lpa* mutation on the content as well as the distribution pattern of these three types of P.

With regard to phytic acid P, there was a reduction in the four *lpa* isolines as compared to the control cultivar (**Figure 1**). The reduction in phytic acid in the four isolines was seen for all fractions. Regardless of total seed phytic acid content, the three bran fractions had the highest phytic acid P content. As the degree of pearling increased, there was a progressive decrease in phytic acid P of the remaining kernels. This distribution pattern of phytic acid P is expected since it is known to be localized in the aleurone layer of cereal grains (*10*). We also noticed that the pattern of phytic acid distribution within a barley seed was similar among genotypes even though the phytic acid P in the original seed varied.

In regard to individual genotypes, the distribution of total P content showed a similar pattern to phytic acid P: higher in bran fractions and progressively lower toward inner seed tissues. Among Harrington and the four *lpa* isolines, only bran fractions showed differences in the total P content. With decreasing phytic acid P, there was a decrease in total P in bran fractions. However, the decrease in total P was not as large as that for phytic acid P. Furthermore, for the whole kernel, dehulled kernel, and three pearled kernel fractions, the difference in total P was minimal. In other words, the total P was relatively unaffected by *lpa* genotypes.

Distribution of the inorganic P within a barley seed exhibited a pattern similar to that of phytic acid P: higher in bran tissues and lower in inner sections. As expected, among the five genotypes there were differences in inorganic P. The decrease in phytic acid P was almost matched with increasing inorganic P across all fractions of the seed. This explains why the total P remained relatively constant among genotypes with different levels of phytic acid P. The *lpa*2-1 genotype showed a slight deviation. Its increase in inorganic P did not surpass *lpa*1-1 even though the latter had higher phytic acid P. This is due to the fact that in barley *lpa*2-1, the reduction in phytic acid P is accompanied by increases in inorganic P and other nonphytic acid inositol phosphates such as inositol tetra and pentaphosphate (*15*).

Like total P, the rest of P remained relatively constant across the five genotypes, although M955 had a slightly higher value in all the kernel fractions. With regard to distribution within seed tissues, outer layers contained the highest concentration of rest of P, but just like total P, the difference between the outer layers and the inner section was not as large as that for phytic acid P.

The changing patterns of three forms of P (phytic acid, inorganic P, and rest of P) among seed fractions and genotypes were further described by their relative percentages with respect to total P in each seed fraction shown in **Figure 2**. Under each genotype, there are 10 bars. The first five bars represent the five surface fractions while the remaining five bars represent whole kernel, dehulled kernel, and three abraded kernel fractions. Again, we can clearly see that the rest of P in each fraction, like total P, remained relatively unchanged among five genotypes. Also, a decrease in phytic acid P was matched almost equally with an increase in inorganic P.

In respect to whole grains, previous researchers with *lpa* crops also noticed a decrease in phytic acid P with an increase in inorganic P and a conserved value of total P, not only in barley (*15*, *18*) but also in wheat (*19*), rice (*11*), and maize (*20*). With regard to effects of *lpa* mutation on distribution of different types of P, Ockenden et al. (*18*) measured levels of phytic acid P and total P in embryo and rest of grains (hand separated) of the four *lpa* barley genotypes, the same four isolines used in the present study but grown in different environments. They found that proportional reductions in phytic acid P of both embryo and aleurone layer contributed to whole-grain phytic acid P reduction in *lpa*3-1 and M955, with little effect on embryo or aleurone layer total P. With respect to total P, these mutations show no grain tissue specificity. In contrast, whole grain phytic acid P reduction in *lpa*1-1 and *lpa*2-1 was exclusively or mainly aleurone layer specific. In *lpa*1-1, the distribution of total P shifted in part from rest of grain to embryo, with a net reduction in total P. Data in the present study appears to confirm the findings of Ockenden et al. (*18*), since whole-grain total P was lowest in *lpa*1-1 and the relative difference in both phytic acid P and total P in BN-1 as compared with BN-3 was greatest in *lpa*1-1 (**Figure 1**).

Contents and Distributions of Major and Minor Minerals. In the whole barley kernel as well as their fractions, major minerals, which have concentrations in the mg g^{-1} range, were P, K, Mg, S, and Ca; while minor minerals, which have concentrations in the μg gm^{-1} range, were Fe, Zn, Mn, Cu, and Ba (**Figure 3**−**6**). For example, in the hulled kernel of the control cultivar, Harrington, the values for Ca, Mg, S, P, and K in decreasing order, were in the range of 0.5−5.8 mg/g level, while the values for Ba, Cu, Mn, Fe, and Zn, also in decreasing order, were in the range of 1−30 μg/g level (**Figure 3**).

Mineral distributions in the four *lpa* isolines (data not shown) were similar to that in the control variety (**Figure 3**). In general, similar to the distribution of the different forms of P, minerals

were concentrated in outer layers of barley seeds. As the degree of pearling increased, the content for each of these minerals decreased. However, different minerals had different distribution patterns across tissues regardless of phytate P content. For example, P, Mg, Zn, and Fe were richer in the BN-1 fraction, corresponding to the aleurone layer; Mn was concentrated in the FNH fraction, corresponding to germ, pericarp, and testa tissues; K, Ca, Cu, and Ba were concentrated in CSH, FNH, and BN-1 fractions, corresponding to hulls, germ, pericarp, testa, and aleurone tissues, respectively; and S was lowest in CSH and remained relatively flat for other fractions, indicating that it was equally distributed among tissues, including the endosperm tissue, except for the hull.

Liu et al. (*21*) determined 11 elements (P, K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Al, and Mo) for one single conventional barley variety in whole kernel, in caryopsis components separated by hand, in products of tangential abrasion of barley, in roller-milled flours, and in air-classified flours and in isolated starch. They found that the outer grain tissues (pericarp and aleurone) contained higher concentrations of minerals than the central section, but individual elements showed varying gradients of decrease in concentration from the pericarp to the central endosperm. They also found different patterns of distribution within seed fractions for individual mineral elements. For example, Ca was more uniformly distributed throughout the kernel than Mg. Furthermore, the whole barley, barley tissues, and fractions contained decreasing concentrations of P, K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Al, and Mo. The values of Na, Ca, Mg, K, and P were in the range of 0.25−5.6 mg/g; the values of Cu, Mn, Zn, and Fe were in the range of 15−36 μg/g; and the values of Al and Mo were in the range of 1.4−4.9 μg/g. Our data with cv. Harrington and its four *lpa* isolines generally confirmed the early finding of Liu et al. (*21*). The concentration of mineral elements as well as their general patterns of distribution also resembled those in wheat (*19*, *22*), and rice (*23*).

One of the key objectives in this study was to determine if the *lpa* genotypes altered mineral contents in whole grain or milled fractions. Another objective was to evaluate if the *lpa* barley genotypes altered the distribution of various elements inside the grains. On the basis of the data in **Figures 1** and **4**−**6**, we found that although there were substantial differences in mineral contents of bran fractions among genotypes, the differences in kernel fractions were very minimal. One major exception was Fe. It had the highest level in all tissue fractions of the M955 isoline, as compared with fractions of all other genotypes (**Figure 6**). This was true for seeds grown at two different environments. This observation could provide another positive impact on nutritional improvement of cereal crops beyond reduction of phytic acid, since a higher Fe content in food and feed is desirable. If the observation in this research is to be confirmed by additional studies, *lpa* crops could be an effective measure to combat anemia, a chronic disease that affects an estimated 40% of all women worldwide due to iron deficiency (*1*). Yet we must be cautious about this assertion, since an increase in Fe was observed only in the M955 genotype and not in the other three *lpa* genotypes. In addition, minor differences in S, Ba, Cu, and Zn contents of kernel fractions were also noticed among genotypes.

Regardless of some differences in mineral concentrations among genotypes when comparing fraction by fraction, as just discussed, the distribution patterns for all the minerals remained relatively unchanged (**Figures 5**−**6**); that is, they were concentrated in bran fractions and decreased toward the center endosperm tissue. More specifically, for each of the 10 minerals (including P), the distribution in *lpa* isolines was similar to that of Harrington shown in **Figure 4**. Minor deviations were noticed for Mg in *lpa*1-1, K, and Cu in *lpa 1-1*, *lpa-3-1*, and M955, and P in *lpa1-1*, but all were limited to the bran fractions.

Previous researchers have attempted to determine the effects of *lpa* mutation on mineral contents and distribution of various crops, including barley (*18*), rice (*23*), corn (*24*), and wheat (*24*), using energy dispersive X-ray (EDX) analysis to measure relative element contents of *lpa* dry grain tissues. EDX equipment, when coupled with a scanning electron microscope, allows analysis of tissues and cellular regions that require no specimen preparation beyond cutting the dry grain open, but the method does not provide absolute values of elements measured. Instead, the method can only provide peak-to-background ratios for relative comparison within a study. Results from these studies based on the EDX method suggested that with all the cereal *lpa* grain genotypes studied there was no evidence that *lpa* genotypes in cereals result in an increase or decrease in certain elements and no evidence that shift of mineral nutrients to the starch endosperm occurs.

In contrast to the findings by a majority of the studies, Bryant et al. (*11*) found an increase (25−40%) in total P, K, and Mg concentrations in *lpa1-1* milled rice products as compared with those from the control. Most recently, Ren et al. (*12*) reported significantly higher concentrations of Ca, K, Mg, Fe, and Zn in milled *lpa* rice than the control variety across three growing locations. Guttieri et al. (*13*) measured and compared mineral concentrations in milled fractions of a *lpa* wheat with that of a control wheat cultivar using a Brabender Quadrumat senior mill. They also found that milled flour from the *lpa* genotype had elevated concentrations of P and Mg. However, these three studies all used only one pair of comparisons (one *lpa* line vs one control variety). In one case, the increase of K in milled *lpa* rice was observed in one year crop but not in another year (*11*). In another case, the whole grain of the *lpa* rice happened to be higher in mineral concentrations than the control one (*12*). Still in another case, the concentration of whole grains was not measured and compared (*13*).

In the present study, we used a unique lab abrading method that could remove surface layers of cereal grains, layer by layer, and measured and compared the absolute contents of elements in several bran fractions as well as corresponding abrading kernels abraded from four *lpa* barley isolines with varying levels of phytic acid reduction and one normal cultivar, Harrington. This methodology allows us to compare not only the concentration of a particular mineral element fraction by fraction among genotypes but also the distribution pattern for an element within a seed and among the genotypes. Results from the present study provided direct yet stronger and more consistent evidence to conclude that the *lpa* mutation had some effect on mineral concentrations in certain surface fractions of barley seeds but little effect on mineral distribution within a barley kernel. This is an important finding, since it shows that breeding low phytic acid crops (or at least for *lpa* barleys) do not lead to reduction in minerals, which, unlike phytate P, are valuable micronutrients for humans and animals.

On the basis of the observation of this study and a few previous ones (*18*, *23*−*25*), there is no major shift of minerals nutrients among tissues, such as from tissues in the bran area to the endosperm in the center area or visa versa. Thus, the results of this study lead to a rejection of the hypothesis that reducing the ability of grains to synthesize and accumulate

phytic acid might alter both the amount and distribution of P and minerals in grain tissues.

Confirmation of Findings by Data from the Second Set of Samples. The above discussion is based on results made with the first set of barley samples grown at Tetonia, ID, in 2003. In order to confirm findings on mineral contents and distributions in barley seeds as well as any impact of the *lpa* mutation, we analyzed a second set of samples, which were grown at Saskatoon, Saskatchewan, Canada, in 2005.

The mineral concentration data from the second set of samples confirmed all the findings with the first set of samples, although there were differences in absolute values when comparing fraction by fraction of the same genotype (data not shown except for Fe shown in **Figure 6**). The differences were attributed to the effect of different growing environments.

Possible Associations of Minerals with Constituents Other Than Phytic Acid P. All seeds store minerals in the form of mineral deposits. These deposits are generally believed to be composed of phytin, a salt of phytic acid and its cations (mostly Mg, K, and Ca) (*9*). In mature seeds, phytin is located inside protein storage vacuoles in inclusions called globoids, which store up to 90% of the total P. During germination, phytin is decationized and then hydrolyzed sequentially by phytase to phosphate and a series of lower phosphoric esters of *myo*-inositol (*26*), which serve as important sources of P and cations for the germinating embryo (*9*).

The lack of large differences in mineral concentration and distribution between conventional varieties and the *lpa* genotypes observed in this study and many previous ones indicates that there is no direct or major role of the localization of phytic acid synthesis in mineral distribution. In other words, the localization of phytic acid synthesis in the germ and aleurone plays a minor role in mineral localization in these tissues, even though phytic acid serves as the site of binding of various cationic elements. Instead, an increase in inorganic P found in *lpa* grains is likely stored as salts of cations, as in phytic acid P in grains of conventional varieties.

Furthermore, the observation that high mineral fractions (surface layers) are also richer in protein (*21*, *27*), lipid, and dietary fiber (*27*, *28*) indicates that there might be some association of minerals with protein, lipid, fiber, and/or some other constituents not mentioned here.

Finally, from this and many previous studies (*11*, *27*−*30*) comes the realization that outer fibrous layers of the cereal grains contain factors that may interfere with the utilization of nutrients and/or cause poor functional or organoleptic properties in final products. These factors can be removed by certain processing steps, such as pearling or milling. However, through such processing, a considerable proportion of the protein, minerals, and many other nutrients are also removed. For value-added utilization, a concerted effort must be made in controlling the right amount of layers to be removed and exploring new uses of different milled fractions. For nutritional purposes, it is beneficial to expand the utilization of whole grains. If phytic acid is the major concern, processing to remove it is not the best option. Instead, the development of *lpa* crop varieties is crucial in addressing this problem.

ACKNOWLEDGMENT

The authors express thanks to Mike Woolman and Amanda Lewis of USDA-ARS at Aberdeen, ID, for their technical assistance; Jim Whitmore, University of Idaho Tetonia Research and Extension Center, Tetonia, Idaho, for growing the first set of barley seed samples; and Dr. Brian Rossnagel, University of Saskatchewan, Saskatoon, Canada, for providing the second set of barley seed samples.

LITERATURE CITED

(1) Welch, R. M.; Graham, R. D. A new paradigm for world agriculture: meeting human needs productive, sustainable, nutritious. *Field Crops Res.* **1999**, *60*, 1−10.

(2) McWard, G. W. Effects of phytic acid and ethyleneaminetetraacetic acid (EDTA) on the chick's requirement for magnesium. *Poult. Sci.* **1969**, *48*, 791−794.

(3) Zhou, J. R.; Fordyce, E. J.; Raboy, V.; Dickinson, D. B.; Wong, M.-S.; Burns, R. A.; Erdman, J. W. Reduction of phytic acid in soybean products improve zinc bioavailability in rats. *J. Nutr.* **1992**, *122*, 2466−2473.

(4) Sathe, S. K.; Reddy, N. R. Introduction. In *Food Phytates*; Reddy, N. R., Sathe, S. K., Eds.; CRC Press: Boca Raton, FL, 2002; pp 1−5.

(5) Raboy, V.; Young, K. A.; Dorsch, J. A.; Cook, A. Genetics and breeding of seed phosphorus and phytic acid. *J. Plant Physiol.* **2001**, *158*, 489−497.

(6) Mendoza, C.; Viteri, F.; Lönnerdal, B.; Young, K.; Raboy, V.; Brown, K. H. Effect of genetically modified low-phytate maize on absorption of iron from tortillas. *Am. J. Clin. Nutr.* **1998**, *68*, 1123−1127.

(7) Veum, T. L.; Ledoux, D. R.; Raboy, V.; Ertl, D. S. *Low-phytic acid* corn improves nutrient utilization for growing pigs. *J. Anim. Sci.* **2001**, *79*, 2873−2880.

(8) Spencer, J. D.; Allee, G. L.; Sauber, T. W. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *J. Anim. Sci.* **2000**, *78*, 675−681.

(9) Lott, J. N. A.; Greenwood, J. S.; Batten, G. D. Mechanisms and regulation of mineral nutrient storage during seed development. In *Seed Development and Germination*; Kigel, J., Galili, G., Eds.; Marcel Dekker: New York, 1995; pp 215−235.

(10) O' Dell, B. L.; de Boland, A. R.; Koirtyohann, S. R. Distribution of phytate and nutritional important elements among the morphological components of cereal grains. *J. Agric. Food Chem.* **1972**, *20*, 718−721.

(11) Bryant, R. J.; Dorsch, J. A.; Peterson, K. L.; Rutger, J. N.; Raboy, V. Phosphorus and mineral concentrations in whole grain and milled *low phytic acid* (*lpa*) 1−1 rice. *Cereal Chem.* **2005**, *82* (5), 517−522.

(12) Ren, X. L.; Liu, Q. L.; Fu, H. W.; Wu, D. X.; Shu, Q. Y. Density alteration of nutrient elements in rice grains of a low phytate mutant. *Food Chem.* **2007**, *102*, 1400−1406.

(13) Guttieri, M. J.; Peterson, K. M.; Souza, E. Mineral distribution in milling fractions of low phytic acid wheat. *Crop Sci.* **2006**, *46*, 2692−2698.

(14) Newman, R. K.; Newman, C. W. Barley as a food grain. *Cereal Foods World* **1991**, *36*, 800.

(15) Dorsch, J. A.; Cook, A.; Young, K. A.; Anderson, J. M.; Bauman, A. T.; Volkmann, C. J.; Murthy, P. P. N.; Raboy, V. Seed Phosphorus and Inositol Phosphate phenotype of barley *low phytic acid* genotypes. *Phytochemistry* **2003**, *62*, 691−706.

(16) AOAC (Association of Official Analytical Chemists). *AOAC Official Methods of Analysis*; AOAC International: Arlington, VA, 2002.

(17) Chen, P. S.; Toribara, T. Y.; Warner, H. Microdeterminations of phosphorus. *Anal. Chem.* **1956**, *28*, 756−1758.

(18) Ockenden, I.; Dorsch, J. A.; Reid, M. M.; Lin, L.; Grant, L. K.; Raboy, V.; Lott, J. N. A. Characterization of the storage of phosphorus, inositol phosphate and cations in grain tissues of four barley (*Hordeum vulgare* L.) *low phytic acid* genotypes. *Plant Sci.* **2004**, *167*, 1131−1142.

(19) Guttierri, M. J.; Bowen, D.; Dorsch, J. A.; Raboy, V.; Souza, E. Identification and characterization of a *low phytic acid* wheat. *Crop Sci.* **2004**, *44*, 418−424.

(20) Raboy V.; Gerbasi, P. F.; Young, K. A.; Stoneberg, S. D.; Pickett, S. G.; Bauman, A. T.; Murthy, P. P. N.; Sheridan, W. F.; Ertl, D. S. Origin and seed phenotype of maize *low phytic acid 1-1* and *low phytic acid 2-1*. *Plant Physiol.* **2000**, *124*, 355−368.

(21) Liu, D. J.; Robbins, G. S.; Pomeranz, Y. Composition and utilization of milled barley products, IV. mineral components. *Cereal Chem.* **1974**, *51*, 309−316.

(22) Waggle, D. H.; Lambert, M. A.; Miller, G. D.; Farrell, F. P.; Deyoe, C. W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. *Cereal Chem.* **1967**, *44*, 48−52.

(23) Liu, J. C.; Ockenden, I.; Truaz, M.; Lott, J. A. N. Phytic acid-phosphorus and other nutritionally important mineral nutrient elements in grian of wild-type and *low phytic acid* (*lpa1-1*) rice. *Seed Sci. Res.* **2004**, *14*, 109−116.

(24) Lin, L.; Ockenden, I.; Lott, J. N. A. The concentrations and distribution of phytic acid phosphorus and other mineral nutrients in wild-type and *low phytic acid 1-1* (*lpa1-1*) corn (*Zea mays* L.) grains and grain parts. *Can. J. Bot.* **2005**, *83*, 131−141.

(25) Joyce, C.; Deneau, A.; Peterson, K. L.; Ockenden, I.; Raboy, V.; Lott, J. N. A. The concentrations and distributions of phytic acid phosporus and other mineral nutrients in wild-type and *low phytic acid 1-1* (*lpa1-1*) wheat (*Triticum vulgare* L.) grain parts. *Can. J. Bot.* **2005**, *83*, 1599−1607.

(26) Loewus, F. A.; Murthy, P. P. N. *myo*-Inositol metabolism in plants. *Plant Sci.* **2000**, *150*, 1−19.

(27) Klamczynski, A.; Baik, B.-K.; Czchajowska, Z. Composition, microstructure, water imbibition, and thermal properties of abraded barley. *Cereal Chem.* **1998**, *75*, 677−685.

(28) Summer, A. K.; Gebre-Egziabher, A.; Tyler, R. T.; Rossnagel, B. G. Composition and properties of pearled and fines fractions from hulled and hull-less barley. *Cereal Chem.* **1985**, *62*, 112−116.

(29) Robbins, G. S.; Pomeranz, Y. Composition and utilization of milled barley products: III. Amino acid composition. *Cereal Chem.* **1972**, *49*, 240−246.

(30) Yeung, J.; Vasanthan, T. Pearling of hull-less barley: product composition and gel color of pearled barley flours as affected by the degree of pearling. *J. Agric. Food Chem.* **2001**, *49*, 331−335.

Received for review December 31, 2006. Revised manuscript received March 12, 2007. Accepted March 25, 2007.

JF0637776