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Compositional Equivalence of Barleys Differing Only in Low- and Normal-Phytate Levels

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Supporting Information

ABSTRACT: Recent breeding advances have led to the development of several barley lines and cultivars with significant reductions (50% or greater) in phytate levels. Low-phytate (LP) grain is distinguished by containing not only a reduced level of phytate P but also an increased level of inorganic P, resulting in greater bioavailability of P and mineral cations in animal diets. It is important to determine whether other nutritional characteristics are altered by breeding for the low-phytate trait. This study was designed to investigate if breeding for reduced phytate content in barleys had any effect on the contents of other attributes measured by comparing mean and range values of the levels of protein, oil, ash, total carbohydrate, starch, and β-glucan, fatty acid composition, and levels of tocopherols and tocotrienols between five LP and five normal-phytate barleys grown in three Idaho locations. Results show that only the phytate level in the LP group was substantially lower than that of the normal-phytate group and that all other attributes measured or calculated were substantially equivalent between the two groups of barleys. Therefore, the phytate level had little effect on the levels of protein, oil, ash, total carbohydrate, starch, and β-glucan, fatty acid composition, and levels of tocopherols and tocotrienols in barley seeds.

KEYWORDS: *Barley, barley oil, phytate, phytic acid, tocopherols, tocotrienols, β-glucan, Hordeum vulgare*

INTRODUCTION

Barley, one of the earliest cultivated cereal grains in the world, is gaining renewed interest for its use in food and as a bioethanol feedstock. Phytate (*myo*-inositol hexaphosphate) is the dominant phosphorus storage compound in grain.[1,2] Phytate cannot be efficiently digested by nonruminant animals such as pigs, chickens, fish, and humans, and it is an effective chelator of several nutritionally important mineral cations (zinc, iron, and, to a lesser extent, calcium and magnesium). Diets high in phytate are associated with mineral deficiencies and high levels of phosphorus excretion into the environment that contributes significantly to water quality problems. Recent breeding advances at the United States Department of Agriculture Agricultural Research Service (USDA-ARS) have led to the development and release of several hulled and hulless low-phytate (LP) germplasm lines and cultivars, including the hulled germplasm lines LP1, LP2, LP3, and LP4[3] and the hulless cultivar Clearwater.[4] LP grain is distinguished by containing not only a reduced level of phytate P but also an increased level of inorganic P, resulting in greater bioavailability of P and mineral cations in animal diets.[3−8] Breeding for LP barley has thus provided an effective genetic approach to reduce the discharge of phosphorus into the environment by reducing the levels of fecal phosphorus.[5,6] Previous studies have shown that barley oil contains high levels of valuable health-promoting tocotrienols and other functional lipids[9,10] and that the level of phytate has little effect on mineral contents in whole or abraded barley kernels.[7] The current study was conducted to compare levels of phytate, protein, oil, ash, total carbohydrate, starch, and β-glucan, fatty acid composition, and levels of tocopherols and tocotrienols in five LP barley lines or cultivars with five related cultivars or lines having normal phytate (NP) contents. The overall objective was to investigate if breeding for low phytate content had any effect on the contents of the above attributes measured or calculated.

MATERIALS AND METHODS

Materials. Ten two-rowed barley germplasm lines and cultivars adapted to the western barley growing regions of North America were selected as the experimental materials. Five lines represented the spectrum of available low-phytate barleys: germplasm lines LP1, LP2, LP3, and LP4 (hulled)[3] and the cultivar Clearwater (hulless).[4] The low-phytate trait in each of these lines is derived from a different mutation generated by treatment of hulled Harrington[11] barley with sodium azide.[12] LP1, LP2, LP3, and LP4 were derived by multiple backcrosses to Harrington. Clearwater was derived from crosses to multiple parents, including the hulless breeding line HB317 (Crop Development Centre (CDC), University of Saskatchewan, Canada) and the widely grown hulled cultivar Baronesse (Plant Variety Protection No. 9300211). The five normal-phytate barleys selected for this study included the three noted above in the Clearwater breeding program, Harrington (hulled),[11] Baronesse (hulled, Plant Variety Protection No. 9300211), HB317 (hulless, breeding line from the CDC, University of Saskatchewan, Canada), and two additional barleys with normal levels of phytate, CDC McGwire (hulless, developed and released in 1999 by the CDC)[13] and CDC Alamo

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(hulless, high β-glucan, developed and released in 1999 by the CDC). These normal-phytate barleys were chosen to represent a wide spectrum of two-rowed barleys and are diverse with respect to pedigree, hull type, and end-use qualities (malt, feed, or food use).

The 10 lines and cultivars were grown in 2009 at three different Idaho locations: Aberdeen (sprinkler irrigated, 1338 m), Filer (furrow irrigated, 1064 m), and Tetonia (rain fed, 1794 m). These environments are typical of commercial production environments, yet offer diversity with respect to the timing and length of the growing season, temperatures, and water availability. The growing seasons for Filer and Aberdeen, respectively, were late March to mid-July and mid-April to early August. For both locations, daily low to high temperatures, respectively, during anthesis (flowering) and grain filling generally ranged from 10−15 to 25−35 °C. The growing season for Tetonia was mid-May to late August, with temperatures generally 3−5 °C cooler than those of the other two locations. At each location, two replicates of each line were grown as small plots, 2.4 m in length, consisting of seven rows planted on 17.8 cm centers, with approximately 36 cm between plots. The planting rate at Aberdeen and Filer was approximately 112 kg/ha, and that at Tetonia was approximately 90 kg/ha. Grain was harvested with a small-plot combine. For this study, composite samples consisting of equal quantities of seed from each replicate were combined, passed through a screen to remove broken kernels and any foreign material, and stored in a cold room.

Chemical Analyses. Barley kernel samples were ground to pass through U.S. Standard No. 20 mesh (~0.850 mm in diameter) using a Wiley Mill (Thomas Scientific, Philadelphia, PA) immediately before analysis. Moisture and ash were determined by Association of Official Analytical Chemists (AOAC) Methods 930.15 and 942.05, respectively.[14] The total nitrogen/protein content was measured by a combustion method, [14] using a model FP-528 protein analyzer (Leco Corp., St. Joseph, MI). The protein content was calculated with a conversion factor of 6.25. The oil content was determined by American Oil Chemists' Society (AOCS) Official Procedure AM 5-04,[15] using a model XT-10 fat analyzer (Ankom Technology, Macedon, NY). However, instead of petroleum ether, hexane was used as the extracting solvent. β-Glucan was measured according to the Approved AACC Method 32-23[16] (AACC = American Association of Cereal Chemists) using a β-glucan enzymatic assay kit (Megazyme International, Wicklow, Ireland). Total starch was measured using AOAC Method 996[14] for the measurement of total starch in cereal flours and food products, with the Megazyme Total Starch Enzyme kit. Total carbohydrates (percent dry matter basis) were estimated by subtracting the sum of protein + oil + ash (percent dry matter basis) from 100%.

Phytate was measured according to the procedures described previously.[7] Briefly, aliquots of ground barley kernel samples (0.5 g) were extracted in 0.4 M HCl and 0.7 M Na_2SO_4. Phytic acid phosphorus (P) was obtained as a ferric precipitate, wet-ashed, and assayed colorimetrically for the P concentration. The phytate content was obtained by multiplying the phytic acid P content with the conversion factor of 3.5484 (molecular weight of phytic acid/molecular weight of phosphorus in phytic acid, 660/(31 × 6)).

For measuring the contents of tocopherols and tocotrienols, the first step was extraction of oil using a model 200 accelerated solvent extractor (Dionex, Sunnyvale, CA). The extracted oil was then analyzed for several isomers of both tocopherols and tocotrienols, using an Agilent 1100 high-performance liquid chromatography (HPLC) system with an Agilent 1100 fluorescence detector and an Agilent 1200 evaporative light scattering detector. The methods for both extraction and HPLC analysis were as previously reported.[17]

Fatty acid composition was measured according to a previously reported method,[18] which involved preparing fatty acid methyl esters by direct transesterification and analyzing them with a gas chromatograph (Agilent 6890N, Agilent Technologies, Santa Clara, CA). The percentage of individual fatty acids relative to the total fatty acids was expressed as the area percentage of the total peak area for each sample. Duplicate analyses were performed separately on each sample.

Data Treatment and Statistical Analysis. Data generated on the samples were measured on a fresh weight basis. On the basis of the moisture content determined for each sample in this study, data were then converted to a dry weight basis. Sample means based on the separate extraction and analysis of duplicate samples and ranges for the five LP and five NP barley lines or cultivars were determined for each attribute measured or calculated on the basis of each location and all locations. The standard error (SE) of the mean was calculated as the standard deviation divided by the square root of the number of values.

RESULTS AND DISCUSSION

To determine the effect of the phytate level on all other attributes (a total of 24) measured or calculated, Tables 1−3 were constructed to examine the range and mean values of the phytate content and 24 other attributes analyzed or calculated in seeds for the group of five LP barley lines or cultivars as compared to the group of five NP barley cultivars or lines, grown in three locations. This type of data analysis and comparison was previously used to demonstrate that the levels of 5 important phytonutrients (lectin, trypsin inhibitor, three isoflavone isomers) in 25 cultivars of glyphosate-tolerant (Roundup Ready) soybeans grown three years in a row were compositionally equivalent to those in 25 cultivars of conventional soybeans.[19]

Phytate Content, Proximate Composition, Starch, and β-Glucan Content. The range and mean values for the phytate level show some variation between Aberdeen and Filer and between Aberdeen and Tetonia for the NP group, but location by location the phytate range values in the LP group were all outside of the ranges for the NP group (Table 1). In addition, the mean values of phytate in the LP group were all less than half of those of the NP group. For example, at the Aberdeen location, the range and mean of the LP group were 0.05−0.60% and 0.40 ± 0.06%, respectively, while the range and mean value of the NP group were 0.75−1.21% and 0.93 ± 0.05%, respectively. This was also true when data were combined for each group across all three locations; the range and mean of the LP group were 0.05−0.67% and 0.41 ± 0.04%, respectively, while the range and mean value of the NP group were 0.75−1.29% and 1.00 ± 0.03%, respectively. These observations indicate that the phytate level in the LP barley group was substantially lower than that of the NP barley group. These data were consistent with phytate measurements in previous studies,[3−6,11] with an overall mean of 0.71% (Table 1).

The proximate composition (protein, oil, ash, and carbohydrate) and starch and β-glucan contents show some variations across all three locations (Table 1), but variation in mean values between the two groups was generally within the SE except for protein and β-glucan at all locations, where the LP group showed lower β-glucan and protein contents than the NP group. However, this apparent difference is probably not due to the low phytate level but is probably due to the fact that CDC Alamo in the NP group is a specially bred cultivar that is high in protein and β-glucan contents.[13] More importantly, unlike the phytate range, which was distinctly different and nonoverlapping for the two groups, the range values of the proximate composition and starch and β-glucan contents for the two groups were similar and were overlapping for each location and combined locations. For example, the β-glucan range for the LP group was 3.93−5.24%, which was within the range value of 3.39−7.40% from the NP group. Furthermore, the mean or range values of these attributes were similar to those reported in previous literature.[7,20] This indicates that variation in these attributes was within natural variation. Therefore, the proximate

Table 1. Phytate Content, Proximate Composition, and Starch and β-Glucan Contents in Seeds of Five LP and Five NP Barleys Grown in Three Locations[a]

	Aberdeen		Filer		Tetonia		all locations		combined
component	LP group	NP group	LP group	NP group	LP group	NP group	LP group	NP group	all samples
phytic acid	0.05–0.60	0.75–1.21	0.06–0.67	0.92–1.24	0.06–0.66	0.82–1.29	0.05–0.67	0.75–1.29	0.05–1.29
	0.40 ± 0.06	0.93 ± 0.05	0.42 ± 0.07	1.05 ± 0.04	0.41 ± 0.07	1.02 ± 0.05	0.41 ± 0.04	1.00 ± 0.03	0.71 ± 0.06
protein	9.96–12.35	10.08–14.33	12.34–14.09	11.69–14.60	10.2–13.69	10.81–16.96	9.96–14.09	10.08–16.95	9.96–16.95
	10.96 ± 0.21	12.01 ± 0.50	13.02 ± 0.19	13.45 ± 0.41	11.63 ± 0.34	12.66 ± 0.65	11.87 ± 0.21	12.77 ± 0.32	12.32 ± 0.26
oil	1.91–2.52	1.96–2.93	2.03–2.53	2.01–2.89	2.04–2.42	1.96–2.74	1.91–2.53	1.96–2.93	1.91–2.93
	2.23 ± 0.06	2.35 ± 0.11	2.23 ± 0.04	2.29 ± 0.09	2.26 ± 0.04	2.31 ± 0.08	2.23 ± 0.03	2.32 ± 0.05	2.28 ± 0.04
ash	1.68–2.51	1.70–2.44	1.92–2.76	2.01–2.90	1.66–2.49	1.88–2.53	1.66–2.76	1.70–2.90	1.66–2.90
	2.25 ± 0.10	2.12 ± 0.09	2.48 ± 0.10	2.36 ± 0.11	2.22 ± 0.08	2.17 ± 0.08	2.32 ± 0.06	2.22 ± 0.05	2.27 ± 0.06
carbohydrate	86.17–85.56	80.70–85.53	81.82–82.72	80.27–83.65	82.38–85.11	78.07–84.67	81.82–85.56	78.07–85.53	78.07–85.56
	84.56 ± 0.20	83.53 ± 0.53	82.28 ± 0.11	81.90 ± 0.30	83.90 ± 0.20	82.56 ± 0.69	83.56 ± 0.21	82.69 ± 0.33	83.14 ± 0.27
starch	51.98–66.35	52.83–61.66	45.85–59.89	48.85–60.54	52.79–61.61	52.40–64.54	45.84–66.35	48.85–64.55	45.84–64.55
	56.66 ± 0.94	57.64 ± 0.74	52.13 ± 1.43	55.91 ± 0.97	56.50 ± 0.71	57.65 ± 1.10	55.10 ± 0.68	57.07 ± 0.55	56.0 ± 80.76
β-glucan	4.04–5.24	3.97–7.40	3.93–4.89	4.32–7.26	4.04–5.08	3.39–6.70	3.93–5.24	3.39–7.40	3.39–7.40
	4.75 ± 0.07	5.09 ± 0.21	4.41 ± 0.06	5.17 ± 0.18	4.42 ± 0.06	4.56 ± 0.19	4.53 ± 0.04	4.94 ± 0.11	4.73 ± 0.15

[a]Data are expressed on a percent dry matter basis, with duplicate measurements for each sample. LP = low phytate, and NP = normal phytate. The first row of data for each component gives the range and the second row for each component the mean ± standard error.

Table 2. Fatty Acid Composition (Percentage Relative to Total Fatty Acids) in Seeds from Five LP and Five NP Barleys Grown in Three Locations[a]

	Aberdeen		Filer		Tetonia		all locations		combined
component	LP group	NP group	LP group	NP group	LP group	NP group	LP group	NP group	all samples
C16:0	23.26–26.77	23.24–25.91	23.52–25.57	23.46–25.56	22.27–26.96	23.72–27.20	22.77–26.96	23.24–27.20	22.77–27.20
	24.33 ± 0.26	24.68 ± 0.31	24.03 ± 0.20	24.46 ± 0.23	24.54 ± 0.42	25.36 ± 0.41	24.39 ± 0.17	24.83 ± 0.19	24.61 ± 0.18
C18:0	1.40–1.83	1.53–2.09	0.99–1.40	1.32–1.96	1.12–1.36	1.14–1.59	0.99–1.83	1.14–2.09	0.99–2.09
	1.58 ± 0.04	1.83 ± 0.07	1.15 ± 0.04	1.62 ± 0.07	1.26 ± 0.03	1.42 ± 0.04	1.33 ± 0.04	1.62 ± 0.05	1.47 ± 0.05
C18:1	13.40–15.45	12.76–15.65	12.23–14.19	12.16–14.51	11.50–15.61	10.20–14.47	11.50–15.61	10.20–15.65	10.20–15.65
	14.39 ± 0.20	14.02 ± 0.38	12.89 ± 0.22	12.97 ± 0.28	13.33 ± 0.43	12.43 ± 0.50	13.64 ± 0.20	13.14 ± 0.26	13.34 ± 0.23
C18:2	52.00–54.13	52.69–54.77	53.60–57.60	53.51–56.20	52.68–56.12	52.94–55.37	52.00–57.60	52.69–56.20	52.00–57.60
	53.28 ± 0.23	53.46 ± 0.22	55.75 ± 0.40	54.85 ± 0.28	53.61 ± 0.28	54.42 ± 0.26	54.21 ± 0.27	54.24 ± 0.16	54.23 ± 0.22
C18:3	3.25–3.86	2.86–3.83	2.90–3.42	2.45–3.96	3.73–4.35	3.18–4.15	2.90–4.35	2.45–4.15	2.45–4.35
	3.57 ± 0.06	3.28 ± 0.10	3.16 ± 0.05	3.25 ± 0.16	3.98 ± 0.06	3.64 ± 0.10	3.57 ± 0.07	3.39 ± 0.06	3.48 ± 0.07
other	2.21–3.64	2.45–3.06	1.97–4.04	2.26–3.39	2.88–3.92	2.31–3.11	1.97–4.04	2.26–3.39	1.97–3.39
	2.86 ± 0.15	2.72 ± 0.06	2.75 ± 0.22	2.85 ± 0.12	3.28 ± 0.10	2.73 ± 0.08	2.96 ± 0.10	2.77 ± 0.06	2.87 ± 0.08

[a]Duplicate measurements for each sample. LP = low phytate, and NP = normal phytate. The first row of data for each component gives the range and the second row for each component the mean ± standard error.

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Table 3. Tocopherol and Tocotrienol Compositions in Seeds of Five LP and Five NP Barleys Grown in Three Locations[a]

	Aberdeen		Filer		Tetonia		all locations		combined
component	LP group	NP group	LP group	NP group	LP group	NP group	LP group	NP group	all samples
αT	46.81−61.97	43.25−65.77	77.65−104.01	81.12−106.31	62.25−90.37	64.61−92.63	46.81−104.01	43.25−106.31	43.25−106.31
	55.68 ± 1.38	49.65 ± 2.3	89.74 ± 3.37	92.32 ± 2.44	73.94 ± 2.53	74.8 ± 2.31	73.12 ± 2.94	72.26 ± 3.57	72.69 ± 3.24
αT3	147.54−190.01	143.64−176.19	166.75−202.55	176.88−220.72	129.71−171.60	129.71−163.42	129.71−202.55	129.71−220.70	129.71−220.70
	160.5 ± 4.16	163.24 ± 3.36	183.8 ± 3.58	194.28 ± 5.03	147.46 ± 3.91	144.70 ± 3.70	163.92 ± 3.53	167.41 ± 4.43	165.66 ± 3.93
βT	2.65−3.49	1.97−4.49	2.47−2.96	2.19−4.56	1.35−2.21	1.32−2.84	1.35−3.49	1.32−4.55	1.32−3.49
	3.12 ± 0.09	2.86 ± 0.24	2.72 ± 0.05	2.94 ± 0.27	1.84 ± 0.08	1.96 ± 0.15	2.56 ± 0.11	2.59 ± 0.15	2.58 ± 0.13
γT	11.03−15.83	10.03−25.2	7.53−9.05	9.54−16.59	6.25−8.85	7.12−12.89	6.25−15.83	7.12−25.20	6.25−25.20
	12.99 ± 0.5	15.29 ± 1.31	8.25 ± 0.19	12.38 ± 0.73	7.42 ± 0.30	9.92 ± 0.63	9.55 ± 0.5	12.53 ± 0.66	11.04 ± 0.63
βT3	15.63−27.7	11.07−46.68	13.68−30.10	14.54−46.78	16.91−30.73	9.31−44.13	13.68−30.73	9.31−46.78	9.31−30.73
	22.45 ± 1.28	27.45 ± 3.92	21.67 ± 1.61	27.89 ± 3.63	23.24 ± 1.56	25.59 ± 3.72	22.45 ± 0.84	26.98 ± 2.1	24.71 ± 1.64
γT3	20.40−34.06	25.13−35.63	16.56−37.34	21.30−31.77	22.54−36.32	21.28−32.12	16.55−37.34	21.28−35.63	16.55−37.34
	29.48 ± 1.46	30.12 ± 1.09	26.29 ± 2.18	24.48 ± 1.09	29.25 ± 1.56	26.29 ± 1.20	28.34 ± 1.02	26.96 ± 0.77	27.65 ± 0.90
δT	1.84−3.18	1.32−6.68	3.59−5.14	4.09−7.24	3.25−4.96	3.11−6.06	1.84−5.14	1.32−7.24	1.32−7.24
	2.31 ± 0.17	2.72 ± 0.47	4.38 ± 0.18	5.54 ± 0.37	4.04 ± 0.17	4.93 ± 0.31	3.57 ± 0.19	4.43 ± 0.32	4.00 ± 0.26
δT3	3.69−5.92	2.84−9.09	3.11−4.82	2.60−5.26	3.79−6.53	2.71−7.10	3.11−6.53	2.60−9.09	2.60−6.53
	4.67 ± 0.28	5.03 ± 0.65	3.91 ± 0.18	3.85 ± 0.30	4.98 ± 0.26	4.60 ± 0.51	4.52 ± 0.16	4.49 ± 0.3	4.51 ± 0.23
total T	64.15−80.6	57.73−93.84	93.34−119.97	102.74−129.99	74.42−104.23	77.41−113.41	64.15−119.97	57.73−126.99	57.73−126.99
	74.1 ± 1.56	70.52 ± 3.82	105.08 ± 3.64	113.28 ± 2.53	87.23 ± 2.55	91.64 ± 3.68	88.8 ± 2.8	91.81 ± 3.75	90.31 ± 3.30
total T3	205.12−254.39	193.95−264.13	203.08−264.27	219.94−294.12	179.82−241.33	167.03−241.90	179.82−264.27	167.03−294.12	167.03−294.12
	217.1 ± 4.85	225.83 ± 7.49	235.67 ± 6.68	250.50 ± 8.97	204.93 ± 6.14	201.17 ± 7.94	219.23 ± 4.06	225.84 ± 5.89	222.53 ± 5.01
T + T3	273.05−335	264.1−354.09	316.91−363.24	329.08−421.11	254.24−331.72	252.16−353.48	254.24−363.24	252.16−421.11	252.16−421.11
	291.21 ± 5.77	296.36 ± 10.6	340.75 ± 5.91	363.78 ± 10.17	292.16 ± 6.95	292.81 ± 11.09	308.04 ± 5.52	317.65 ± 8.47	312.84 ± 7.13
percent T3	72.69−76.51	73.39−79.43	62.86−73.30	65.12−72.14	64.72−72.72	66.01−71.79	62.86−76.51	65.12−79.43	62.86−79.43
	74.53 ± 0.42	76.27 ± 0.67	69.09 ± 1.18	68.14 ± 0.76	70.09 ± 0.80	68.67 ± 0.58	71.24 ± 0.65	71.23 ± 0.76	71.23 ± 0.70

[a]Data are expressed as milligrams per 100 g of oil, except for the percent T3 in total tocols, with duplicate measurements for each sample. LP = low phytate, NP = normal phytate, T = tocopherol, and T3 = tocotrienol. The first row of data for each component gives the range and the second row for each component the mean ± standard error.

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composition and starch and β-glucan contents in the LP group were substantially equivalent to those of the NP barley group.

Fatty Acid Composition. Similar to oilseeds and other cereals, all 10 barley lines or cultivars contained 5 major fatty acids, including palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids (Table 2). Several other fatty acids were also detected, but at much lower concentrations, including myristic (C14:0), palmitoleic (C16:1), arachidic (C20:0), eicosanoic (C20:1), behenic (C22:0), erucic (C22:1), lignoceric (C24:0), and nervonic (C24:1) acids. Collectively, they were grouped and termed as "others" in Table 2. Like many oilseeds and other cereals, linoleic acid was the most abundant fatty acid in barley, but the order of the other five major fatty acids varies with the species. Data in Table 2 showed that the content of linoleic acid was followed by palmitic, oleic, linolenic, and stearic acids in a decreasing order. This finding confirmed an early report.[21]

Unlike other attributes, the fatty acid composition in either the LP or NP group did not show much variation among growing locations (Table 2). The mean and range of the LP barley fatty acid composition were similar to the mean and range of the NP barley fatty acid composition within each given location as well as across all the locations. This is shown by the fact that the variation in mean values between the two groups was generally within the SE. In addition, the mean values of fatty acid composition were similar to those reported previously for barley.[21] Therefore, in overall fatty acid composition, the LP group was substantially equivalent to the NP barley group.

Tocopherols and Tocotrienols in Barley Lines or Cultivars Grown in Three Locations. In all lines and locations, α-tocopherol was by far the most abundant tocopherol (80.5% of total tocopherols) for all barleys and locations, followed by γ-tocopherol (12.2%), δ-tocopherol (4.4%), and β-tocopherol (2.9%) (Table 3). Similarly, in all lines and locations, α-tocotrienol was by far the most abundant one (74.4% total), followed by γ-tocotrienol (12.4%), β-tocotrienol (11.1%), and δ-tocotrienol (2.0%). The relative proportions of tocopherols and tocotrienols found in this study are similar to those reported previously for barley.[17,22]

Like the proximate composition and starch and β-glucan contents, tocopherols and tocotrienols and their composition showed some variations among the three locations within either the LP or NP group (Table 3). Again, however, except for the γ-tocopherol at the Filer location, the mean and range values of the other tocols in the LP barleys were similar to the mean and range values in the NP barleys within each given location. The range values of the two groups were either inclusive or overlapping, while variation in mean values between the two groups was generally within the SE. Combining the three locations, the mean and range were also similar between the two groups. For example, the range and mean of α-tocopherol for the LP group were 46.81−104.01% and 73.12 ± 2.94%, respectively, while the α-tocopherol range and mean in the NP group were 43.25−106.31% and 72.26 ± 3.57%. Note that the LP range of 46.81−104.01% was within the NP range of 43.25−106.31%.

For γ-tocopherol, at the Filer location, the range was distinctly different and nonoverlapping between the two groups, 7.53−9.05 of the LP group vs 9.54−16.59% of the NP group, while the means were significantly different from each other (8.25 ± 0.19% vs 12.38 ± 0.73%). However, data from the other two locations as well as all locations show that even though the mean values of LP barleys were substantially lower than those of NP barleys, the range values were overlapping. This indicates that, like other tocols, the variation in γ-tocoperol was within a natural variation. In addition, the range values of tocopherols and tocotrienols found in this study for both LP and NP barleys are similar to those reported previously.[9,10,17,22] All above observations indicate that tocopherols and tocotrienols in the LP group were substantially equivalent to those of the NP barley group.

In summary, by comparing the range and mean values of phytate content, proximate composition, fatty acid composition, starch and β-glucan contents, and tocopherol and tocotrienol composition between five LP and five NP barleys grown in three Idaho locations, we found that only the phytate level in the LP group was substantially lower than that of the NP group and that all other attributes measured or calculated were substantially equivalent between the two groups of barleys. It should be noted that this study was limited to one growing season and the three locations were all within the state of Idaho, so additional studies may be needed to confirm that normal- and low-phytate barleys are compositionally equivalent when grown in other locations and in multiple growing seasons.

Previous studies showed that LP grain is distinguished by containing not only a reduced level of phytate P but also an increased level of inorganic P[3,4,7] and that the level of phytate has little effect on mineral contents in whole or abraded barley kernels.[10] Results of the current study further indicate that the phytate level had little effect on all attributes measured or calculated, including proximate composition, starch and β-glucan levels, fatty acid composition, and tocopherol and tocotrienol levels in barley seeds. It is concluded that low- and normal-phytate barleys are compositionally equivalent except for the phytate content. This new information provides additional evidence that low- and normal-phytate barleys can be expected to have comparable nutritional value when used for normal food and feed applications.

■ ASSOCIATED CONTENT

Supporting Information

Individual values (means and standard deviations) for the analytical data (Tables S1 and S2), with Table S1 including analytical data for phytate, protein, oil, ash, total carbohydrate, starch, β glucans, and fatty acids (C16:0, C18:0, C18:1, C18:2, C18:3, and other fatty acids), Table S2 including analytical data for tocopherols (α, β, γ, and δ) and tocotrienols (α, β, γ, and δ), total tocopherols, total tocotrienols, total tocopherols + total tocotrienols, and tocotrienol concentration (%; total tocotrienols/(total tocopherols + total tocotrienols)), and both tables including mean values for each of the three locations, the mean value for all locations, and analysis of variance letter designations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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