Influence of Cultivar and Environment on Mineral and Protein Concentrations of Wheat Flour, Bran, and Grain[1]

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Cereal Chem. 63(3):183–186

ABSTRACT

Protein, Mg, P, S, Cl, K, Ca, Mn, Fe, Cu, and Zn concentrations were determined in wheat flour, bran, and grain from 27 cultivars in the 12th International Winter Wheat Performance Nursery grown at six locations. Variations in mineral and protein concentration of flour, bran, and grain were demonstrated and could be attributed to differences in growing locations, cultivars, and their interaction. Variations in environment and genotype had a somewhat different influence on flour and bran composition. Mineral concentrations in bran were more strongly influenced by environmental conditions than were flour mineral concentrations. Concentrations of P and Mn in flour were influenced more by genotype than environment. Whole grain composition tended to reflect an average of the responses of flour and bran. The interaction of genotype and environments also contributed to mineral and protein variability. Protein content of grain had a positive influence on concentrations of all minerals except Cl and K.

Key words: Grain composition, Nutritional quality, *Triticum aestivum* L.

Although many researchers (Bassiri and Nahapetian 1977, Dikeman et al 1982, El Gindy et al 1957, Kleese et al 1968, Koivistoinen et al 1974, Murphy and Law 1974, Nahapetian and Bassiri 1976, Pomeranz and Dikeman 1983, Rasmusson et al 1971, White et al 1981, Wolnick et al 1983) have studied variability in mineral concentrations of wheat (*Triticum aestivum* L.), the relationships among flour, bran, and grain composition as affected by genotype and environments have not been established. Variation in mineral levels of wheat flour and bran have been attributed to genetic and environmental effects, their interaction, and variation in protein levels (Peterson et al 1983). We suggested that the mineral composition of whole grain, flour, and bran each may be influenced to a different degree by variation in protein content and by other genetic and environmental factors. Vogel et al (1976) found that while wheat grain and endosperm protein contents were correlated ($r = 0.98$), the correlations of bran and endosperm protein content ($r = 0.67$) and bran and grain protein content ($r = 0.75$) were lower. Little relationship was shown between endosperm and bran or grain and bran for lysine content (as percent of protein).

Whole grain analyses, used in many wheat studies, may not adequately reflect variation in the composition of endosperm or bran. Mineral concentrations in bran are much higher than in endosperm; therefore, variation in seed size and the proportion of bran to endosperm may influence whole grain mineral composition with little effect on composition of flour and bran components. The degree of independence in flour and bran mineral concentrations also may affect the usefulness of whole grain analyses.

This study investigated the relative influence of genotype, environment, and their interaction on the mineral and protein concentrations of wheat flour, bran, and grain. Natural variability in flour mineral concentrations is compared with nutrient addition levels appropriate for U.S. and Canadian flour fortification and enrichment programs.

MATERIALS AND METHODS

Grain of 27 cultivars from 14 countries grown in the 12th International Winter Wheat Performance Nursery in 1980 at six locations was studied (Table I). (Cultivars sampled, and their places of origin, were: NE 7060, USA, Nebraska; Odessa 4, USSR; GK-Tiszataj, Hungary; Atlas 66, USA, North Carolina; Lovrin 24, Romania; Clement, Netherlands; Hachiman-Komugi, Japan; JO 3057, Finland; Bezostaya 1, USSR; Lethbridge 1327, Canada; Martonvasari 5, Hungary; NSR-1, Yugoslavia; Doina, Romania; Adam, Austria; Downy, USA, Indiana; Blueboy, USA, North Carolina; Houser, USA, New York; Jana, Poland; Kopara, New Zealand; Trakia, Bulgaria; WWP 4394, Austria; Alcedo, East Germany; Aura, Finland; Bastion, Netherlands; Martonvasari 6, Hungary; TX 71A562-6 and TAM W-105, USA, Texas.) The cultivars differed in seed size, kernel texture, productivity, and agronomic characteristics. The locations were environmentally diverse for wheat production.

Samples of grain (10 g) were milled on a Brabender Quadraplex experimental mill, and bran and endosperm were separated by sifting samples on a Strand shaker using a brass U.S. standard sieve no. 70 as previously described (Peterson et al 1983).

Flour extraction rates averaged 60% in this study, with flour ash contents comparable to a 70–75% extraction commercial flour. With the Brabender micromill, flour ash content and mineral levels are highly correlated with extraction rate only above 70% extraction. Differences in milling characteristics of varieties in this study, which could influence extraction rates, should not have had a large effect on endosperm mineral or ash concentrations when a 60% extraction level was used. Therefore, a better estimate of endosperm mineral variability could be obtained.

The term "bran" as used in this study refers to all kernel components except the white flour fraction (<70 mesh) at 60% extraction rate. The bran resulting from the 60% extraction rate contains some flour. Because of the diluting effects of the flour, estimates of mineral variation in bran in this study were conservative estimates.

Mineral element measurements were made on 0.5-g samples by energy dispersive X-ray fluorescence spectrometry as described by

TABLE I
Growing Locations and Mean Grain Yield

Locations	No. of Replications	Grain Yield (q/ha)
Lincoln, NE	2	36.8
Stillwater, OK	4	31.6
Ithaca, NY	3	43.3
University Park, PA	2	···
Davis, CA	4	54.4
Monsheim, West Germany	4	73.7

[1]Contribution of the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) and the Department of Agronomy, University of Nebraska-Lincoln as Journal series paper 7649, Nebraska Agricultural Experiment Station.

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Peterson et al (1983). Standard curves were developed by adding mineral salts in ethyl alcohol to control flour and bran samples for instrument calibration. National Bureau of Standards wheat and rice flour standards were used to evaluate calibration curves (Peterson et al 1983). Mineral values are expressed on a dry weight basis (db). One gram of each sample (db) was analyzed in duplicate for nitrogen using the macro-Kjeldahl procedure, AACC method 46-12 (1969). Protein was calculated using the factor 5.7 to convert N to protein expressed as percentage (db).

Statistical Analyses

Whole grain mineral and protein data discussed in this study were calculated from flour and bran analyses and flour extraction rate for each sample as follows:

$$\text{Grain concentration} = \text{bran concn.} \times (1 - \text{extraction}) + (\text{flour concn.} \times \text{extraction}),$$

where extraction = flour wt/(flour wt + bran wt). The validity of this calculation was indicated by the high correlation of calculated with actual grain protein content ($r = 0.99$). The calculated whole grain mineral values correspond well to ranges in mineral concentrations for wheat grain in the United States reported by Wolnik et al (1983).

A weighted analysis of variance was performed for each variable based on the method of Steel and Torrie (1960), because unequal replications were used at the various locations. A standard ANOVA model was used with cultivar and location as main effects, and cultivar by location as the interaction term. Variance components were estimated by the method of Steel and Torrie (1960) using Statistical Analysis System (Helwig and Council 1979) procedures and programs. Genetic correlations were calculated according to Falconer (1960).

RESULTS AND DISCUSSION

Ranges in Mineral Concentrations

The concentrations of minerals and protein in flour, bran, and whole grain were highly influenced ($P<0.01$) by production site, cultivar differences, and the interaction of location and cultivar effects. Ranges in mineral concentrations indicate that genetic variation in mineral concentrations was comparable to, or larger than, variation associated with environmental factors (Table II). The significant influence of genotype by environment interaction contributes to the relatively wide overall variation in each of the minerals examined in this study.

Ratios of Genetic to Environmental Variance Components

A ratio of the variances associated with environmental effects (σ_e^2) to the variances associated with genetic effects (σ_g^2) provides a means for examining the relative influences of genotype and environment on mineral composition of wheat. A ratio larger than 1.0 indicates the greater influence of environmental factors on variability; a ratio of less than 1.0 indicates the relatively greater influence of genetic factors.

Differences are apparent when comparing the ratios for flour, bran, and grain for each element and protein (Table III). The ratios for flour protein (3.22) and bran protein (21.75) show that the effect of environment was greater than the effect of genotype on bran protein levels. The ratio for grain protein of 7.34 was intermediate

TABLE II

Ranges in Mineral Element and Protein Concentrations in Flour, Bran, and Grain over 27 Cultivars of Winter Wheat and Six Locations

	Protein (%)	Mg (%)	P (%)	S (%)	Cl (%)	K (%)	Ca (μg/g)	Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	Zn (μg/g)
Whole grain											
Locations											
Max	18.4	0.202	0.349	0.243	0.089	0.582	653	61.7	54.5	6.5	38.2
Min	11.2	0.182	0.289	0.166	0.057	0.456	367	44.9	33.9	4.2	24.0
Cultivars											
Max	17.1	0.209	0.372	0.235	0.085	0.561	606	65.2	55.8	6.8	39.5
Min	13.2	0.172	0.281	0.187	0.062	0.441	423	44.5	35.1	4.7	25.8
Overall											
Max	22.6	0.259	0.469	0.276	0.110	0.678	897	94.5	68.6	8.9	53.2
Min	8.5	0.158	0.243	0.128	0.042	0.359	237	33.5	25.5	3.4	19.5
Mean	14.7	0.191	0.325	0.208	0.072	0.514	518	54.5	44.0	5.4	31.5
LSD (0.05) for cultivars	1.1	0.013	0.031	0.014	0.007	0.042	65	7.3	4.2	0.6	4.2
C.V., %	3.9	5.6	5.4	3.7	5.4	5.2	7.3	9.2	9.0	9.8	8.4
Flour											
Locations											
Max	17.1	0.088	0.089	0.218	0.084	0.196	244	6.9	12.2	3.1	8.7
Min	10.2	0.078	0.080	0.145	0.053	0.148	179	4.6	8.2	2.2	5.3
Cultivars											
Max	16.6	0.091	0.110	0.215	0.079	0.200	251	8.8	12.7	3.3	9.3
Min	10.8	0.076	0.065	0.149	0.054	0.127	176	3.3	8.0	2.2	5.2
Overall											
Max	21.1	0.110	0.150	0.256	0.101	0.246	320	13.3	20.1	4.3	14.6
Min	7.1	0.063	0.056	0.103	0.041	0.101	114	1.9	6.0	1.9	3.6
Mean	13.3	0.084	0.086	0.180	0.068	0.162	211	5.8	10.3	2.7	6.6
LSD (0.05) for cultivars	1.2	0.007	0.011	0.015	0.006	0.020	30	1.7	2.0	0.4	1.4
C.V., %	4.6	10.8	5.6	4.6	4.8	8.2	13.0	31.2	22.8	20.5	14.0
Bran											
Locations											
Max	20.0	0.383	0.741	0.278	0.095	1.135	1310	148.0	116	11.9	80.7
Min	12.6	0.335	0.595	0.197	0.061	0.929	626	105.0	72	7.2	51.1
Cultivars											
Max	18.3	0.419	0.840	0.262	0.092	1.193	1173	156.0	109	11.3	84.9
Min	15.4	0.294	0.571	0.228	0.064	0.908	778	106.0	72	8.3	57.0
Overall											
Max	24.2	0.521	1.030	0.321	0.125	1.450	1680	237.3	146	16.1	120.9
Min	10.8	0.269	0.471	0.165	0.044	0.701	443	80.4	54	5.3	42.0
Mean	16.4	0.353	0.681	0.241	0.079	1.026	974	128.0	93	9.9	66.8
LSD (0.05) for cultivars	1.1	0.029	0.064	0.015	0.009	0.087	133	18.0	10	1.2	9.5
C.V., %	3.7	6.7	6.4	3.4	7.7	6.4	7.9	9.7	9.8	10.2	8.9

to the ratios for flour and bran protein. Variance ratios for Mg of 0.48 for bran and 0.66 for grain indicate a larger influence of genotype on Mg variability. Ratios for P show that genotype had a much more important influence on flour P levels than environment (0.03), but in the bran and grain both effects appear to have near equal influence.

The relative influence of environment on Ca levels was much more pronounced in the bran and whole grain than in flour. The ratios for Fe, Cu, and Zn in flour show a slightly larger environmental component, but in bran the environmental component was as much as five times larger than the variance component associated with genetic factors. Ratios for these elements in whole grain lie near the average of the flour and bran ratios.

With the exception of ratios for Mg and P in whole grain and bran, and Mg, P, and Mn in flour, ratios were all larger than 1.0, indicating that environmental factors had more of an influence on mineral variability than the genotype. This is in apparent contradiction with the ranges in mineral contents associated with locations and cultivars. Ranges in mineral contents indicated that the influence of cultivar on variability was similar to, or larger than, the influence of environment. The variance component ratios may indicate differences in the influence of genotype and environment on the distribution and deviation of mineral concentrations around the mean levels that, when examining the ranges alone, are not readily apparent.

The differences among the flour, bran, and whole grain variance ratios indicated some of the problems that may be associated with attempts to extrapolate analyses of whole grain to the composition and variability of flour, the major commercial product of wheat. Flour and bran mineral components tended to be influenced to a different degree by environmental and genetic variation. Bran composition appeared strongly influenced by environmental conditions, with a lesser influence of genotype. Genotype had a larger influence on flour or endosperm composition. Estimates of environmental and genetic effects on whole grain were an average of the individual influences on flour and bran composition.

Ratios of Genetic to Genotype by Environment Variance Components

The magnitude of the genotype by environment (G × E) interaction in relation to genetic effects can be shown through the use of the variance component ratio $\sigma_g^2 / \sigma_{g\times e}^2$ (Table III). A ratio larger than 1.0 indicates greater influence and stability of genetic factors relative to the variability associated with the interaction of genotype and environment.

The ratios for minerals in flour, bran, and grain range from only 0.41 to 2.17. The stronger influence of genetic factors on flour protein compared to bran protein was again shown. The ratios for Mg in flour (1.44) and bran (1.68) show a larger influence on variability by genotype than the G × E interaction, but in whole grain the components appear to have equal effects. Ratios for Ca, Mn, and Zn all were less than 1.0, indicating the important influence of the G × E interaction on concentrations of these elements. Iron ratios were larger than 1.0, indicating the greater influence of genotype.

Correlations of the Composition of Flour and Bran with Whole Grain

Phenotypic correlations of mineral and protein concentrations in flour and bran with those in whole grain were highly significant

TABLE III
Ratios of Environmental to Genetic Variance Components (σ_e^2/σ_g^2) and Genetic Variance to Genotype by Environment Interaction Components ($\sigma_g^2/\sigma_{g\times e}^2$) for Mineral and Protein Concentrations in Wheat Flour, Bran, and Grain

	Protein	Mg	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn
σ_e^2/σ_g^2											
Flour	3.22	1.35	0.03	2.38	4.15	1.50	1.83	0.46	1.36	1.67	1.62
Bran	21.75	0.48	0.89	16.81	5.79	1.78	6.51	1.22	3.29	4.96	4.34
Grain	7.34	0.66	0.87	5.67	4.82	2.34	6.76	1.43	2.28	2.81	3.73
$\sigma_g^2/\sigma_{g\times e}^2$											
Flour	2.04	1.44	1.44	2.17	1.34	1.02	0.65	0.95	1.37	1.98	0.80
Bran	0.41	1.68	1.18	0.44	0.77	1.07	0.96	0.86	1.37	0.64	0.41
Grain	1.09	0.95	0.68	1.21	1.12	1.00	0.73	0.61	2.08	1.22	0.48

TABLE IV
Correlations Among Wheat Grain, Flour, and Bran Concentrations of Protein and Mineral Elements

Correlation[a]	Protein	Mg	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn
Grain											
Flour											
r_p	0.98**[b]	0.69**	0.76**	0.96**	0.96**	0.72**	0.80**	0.69**	0.69**	0.83**	0.83**
r_g	0.96	0.97	0.93	0.95	0.98	0.76	0.72	0.93	0.85	0.86	0.87
Bran											
r_p	0.96**	0.81**	0.82**	0.94**	0.96**	0.83**	0.96**	0.89**	0.91**	0.92**	0.91**
r_g	0.67	0.88	0.83	0.67	0.98	0.80	0.90	0.84	0.81	0.93	0.75
Bran											
Flour											
r_p	0.88**	0.48**	0.65**	0.82**	0.85**	0.60**	0.71**	0.65**	0.59**	0.67**	0.76**
r_g	0.49	0.92	0.85	0.47	0.88	0.80	0.55	0.85	0.84	0.76	0.79

[a] r_p = Phenotypic correlation, and r_g = genotypic correlation.
[b] ** Significant at $P = 0.01$.

TABLE V
Correlations Between Mineral Element Concentrations in Whole Grain and Protein Content in Wheat Grain

Correlation[a]	Mg	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn
Grain										
r_p	0.39**[b]	0.13	0.90**	0.26**	−0.36**	0.76**	0.68**	0.78**	0.17*	0.62**
r_g	0.82	0.96	0.90	−0.44	−0.03	0.07	0.84	0.84	0.73	0.90

[a] r_p = Phenotypic correlation, and r_g = genotypic correlation.
[b] *, ** Significant at $P = 0.05$ and 0.01, respectively.

TABLE VI
Variation in Natural Levels of Mineral Elements (μg/g, db) in Wheat Flour and Concentrations Needed to Meet Flour Fortification Standards

Element	Overall Range in Flour	Range of Variation Among Varieties	Proposed U.S. Fortification Standards[a]	Canadian Expanded Enrichment Standards[a]
Fe	6.0–20.1	8.0–12.7	51	34–50
Ca	114–320	176–251	2,307	1,279–1,628
Zn	3.6–14.6	5.2–9.3	26	···
Mg	633–1,098	757–906	513	1,744–2,209

[a] From Ranum (1980).

($P<0.01$), ranging from 0.69 to 0.98 (Table IV). The correlations for grain with flour were lower than those with bran for all minerals except S and Cl. Whereas whole grain analysis does reflect variation in both flour and bran composition, it is somewhat more reflective of bran variation. Phenotypic correlations of flour minerals with bran minerals were highly significant but not as high as correlations of grain composition with composition of the two fractions. Flour and bran mineral compositions again appear somewhat independently influenced by genotype and environment, as indicated by variance component ratios.

Correlations of Mineral Concentrations with Protein

Peterson et al (1983) found that a significant portion of the variation in mineral content was related to, or a by-product of, variation in protein levels. Correlations between protein and mineral concentrations in bran were, in general, lower than those found in flour. The high correlations of several minerals with protein in flour suggested an added nutritional advantage of high-protein cultivars.

Correlations of minerals with protein in whole grain tend to reflect the correlations and relationships found in flour rather than the weaker associations in bran (Table V). High positive genotypic correlations were found for Mg, P, S, Mn, Fe, Cu, and Zn with protein. Little relationship of Cl or K with protein was indicated, and although the phenotypic correlation of Ca with protein was high at 0.76, no genetic relationship was shown. Sulfur is present primarily in sulfur amino acids in wheat grain, and correlations with protein in grain were very high.

Comparison of Natural Variability to Flour Fortification Standards and Procedures

For the proposed U.S. fortification standards (NAS/NRC 1974) and expanded Canadian optional enrichment program (1978; Ranum 1980) to be effective and economical, they must account for the natural variation in mineral concentrations of flour. Ranum (1980) presented nutrient addition levels appropriate for the two enrichment programs based on natural concentrations and variability in commercial flour samples. Table VI shows the flour fortification and enrichment standards for the United States and Canada in relation to the overall variability and genetic variability in flour Mg, Ca, Fe, and Zn concentrations determined in this study. Natural concentrations of Ca account for only about 10% of the U.S. fortification standard and less than 20% of the Canadian standard. Natural variation will not greatly affect addition procedures for this element. A natural range of 14 μg/g in Fe levels is wide in relation to the allowable ranges in the Canadian standards, and mean Fe levels satisfy approximately 20–25% of the standards.

Ranum (1980) found that there is enough natural variation in the natural levels of Zn and Mg to alter the addition rates needed in the United States based on flour type or ash content. This study is in agreement and indicates that natural variation in Mg and Zn concentrations may cover a significant portion of their respective fortification standards. Fortification programs must be aware of this potential variation, as addition procedures may need to be adjusted to allow for natural concentrations of these elements in flour. The problems of mineral bioavailability, not addressed in this study, will be a further concern in determination of addition procedures.

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[Received April 5, 1985. Revision received October 31, 1985. Accepted November 1, 1985.]