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Natural Variability of Metabolites in Maize Grain: Differences Due to Genetic Background

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Understanding the impact of genetic diversity on crop biochemical composition is a prerequisite to the interpretation and potential relevance of biochemical differences experimentally observed between genotypes. This is particularly important in the context of comparative safety assessments for crops developed by new technologies such as genetic engineering. To interrogate the natural variability of biochemical composition, grain from seven maize hybrids grown at four geographically distinct sites in Europe was analyzed for levels of proximates (fat, protein, moisture, ash, and carbohydrates), fiber, amino acids, fatty acids, four vitamins, nine minerals, and secondary metabolites. Statistical evaluation of the compositional data at the $p < 0.05$ level compared each hybrid against every other hybrid (head-to-head) for all analytes at each site and then across all sites to understand the factors contributing to variability. Of the 4935 statistical comparisons made in this study, 40% (1986) were found to be significant. The magnitude of differences observed, as a percent, ranged between 0.84 and 149% when all individual sites and the combined sites were considered. The large number of statistically significant differences in the levels of these analytes between seven commercial hybrids emphasizes the importance of genetic background and environment as determinants of the biochemical composition of maize grain, reflects the inherent natural variability in those analytes across a representative sampling of maize hybrids, and provides a baseline of the natural range of these nutritional and antinutritional components in maize for comparative compositional assessments.

KEYWORDS: Maize (*Zea mays* L.); natural variability; composition; substantial equivalence

INTRODUCTION

Many studies have been conducted to evaluate the chemical composition of maize grain in order to understand and evaluate the effects of the genetic background, environmental factors, and agronomic practices on the chemical composition and nutritive value of the maize grain (*1*−*4*). Changes in composition during kernel development (*5*), between different parts of the grain (*6*), in different maize types such as starchy, sweet corn, or popcorn (*7*, *8*, *9*), and at different stages and types of processing (*10*) have been studied in detail. In addition, nutritionally enhanced varieties, such as the *opaque*-2 mutant, have been extensively characterized due to the enhanced levels of endosperm lysine content (*11*). This focus on maize grain composition is a reflection of the importance of maize as an animal feed, in human nutrition, and for industrial products worldwide. Consistently, these studies have shown that there is a considerable range in the values for maize composition. Specific analytes such as fatty acids in maize are influenced by hybrid genetics (*12*), fertilizers (*13*), temperature (*14*), position of the kernel on the ear (*15*), geographic location (*12*, *16*), and planting year (*12*). The concept of natural variability is important when one attempts to understand the role of analytes in crop physiology and the significance of changes in analyte levels.

This study was initiated to develop a better understanding of the variability present in nutritional and antinutritional components in conventional maize varieties with differing genetic backgrounds and grown in different locations. Although there is an inherent acceptance that levels of crop analytes can vary, the extent of this variability is rarely appreciated or considered when direct comparisons are made. The phenotypic and compositional properties of a biotechnology-derived (biotech) crop are often compared to those of a crop with an established history of safe use. The conclusion of comparable relative safety of the new biotech crop is often referred to as "substantial equivalence" (*17*−*25*). This comparative safety assessment process involves quantitative evaluation of crop agronomic/phenotypic characteristics and compositional levels of key nutrients and antinutrients that are relevant to human and animal health. The goal is to understand whether the composition of the biotech crop falls within the generally accepted definition or specification compared to the conventional crop variety.

A key aspect of compositional studies is the development of data for the nutritional and antinutritional components in

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conventional maize hybrids grown as references at the same site as the biotech and control hybrids. Data from the conventional hybrids can be used to calculate a tolerance interval that is a statistically determined range designed to represent, with defined confidence, a given percent of the population of commercial hybrids (*26*, *27*, *29*). Within the composition study, the range of the test values is compared to the tolerance interval. This tolerance interval is a critical aspect of the compositional analysis because it allows for differences found between the biotech and conventional crops to be placed within the contextual framework of the natural variability of analyte levels due to genetic and environmental factors (*26*). The purpose of this study was to evaluate the variation in composition of grain samples collected from maize hybrids grown in the European Union (EU) in 2001 to better understand natural variability.

MATERIALS AND METHODS

Maize Samples for Compositional Analysis. Grain samples were collected from field trials conducted in 2001. The maize was grown at four replicated trials in Germany, southern France, northern France, and Italy. Seven different maize hybrids were planted at each site in a randomized complete block design with three replicates of each hybrid at each site. The hybrid maize varieties in this study were selected on the basis of their diverse genetic backgrounds: a high-protein hybrid (H4817604), two closely related hybrids (DK427 and DK440), two exotic hybrids (H4817604 and DK281), flint hybrids (DK440, DK427, DK537, and MI8390), and flint × dent hybrids (DK281 and AW812). The hybrids DK427, DK440, DK537, and MI8390 would be considered adapted to southern France; hybrids DK281 and AW812 (AW812) adapted to northern France; hybrids DK440, DK537, and MI8390 adapted to Italy; and hybrids DK281 and AW812 adapted to Germany.

Compositional Analyses. Grain samples were ground to a fine powder in the presence of dry ice and maintained frozen until subjected to compositional analysis. Grain samples were analyzed for proximates (protein, fat, ash, carbohydrate, and moisture), acid detergent fiber (ADF) and neutral detergent fiber (NDF), amino acids, fatty acids (C8−C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), riboflavin (vitamin B_2), thiamin (vitamin B_1), folic acid, vitamin E, and phytic acid content. In addition, the carbohydrate content in grain was calculated by difference. All compositional analyses were performed at Covance Laboratories, Inc. (Madison, WI). The methods utilized were described previously (*26*, *27*).

Statistical Analysis of Composition Data. Statistical evaluation of the compositional data involved comparison of each hybrid against every other hybrid (head-to-head) for all analytes at each site and then across all sites to determine significant differences at $p < 0.05$. The following 14 analytes, for which >50% of observations were below the limit of quantitation (LOQ) of the assay, were excluded from statistical analysis: sodium, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 γ-linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. As a result, a total of 47 of the 61 initially tested components were statistically evaluated. Except for moisture, all component values were converted from a fresh weight (fw) to a dry weight (dw) basis and then into their respective units described in **Table 5**.

All maize compositional components were statistically analyzed using a mixed model analysis of variance. Each site utilized a randomized complete block study design. Data from each site were analyzed individually and in a combined site analysis. Individual site analyses used the model

$$Y_{ij} = U + T_i + B_j + e_{ij}$$

where Y_{ij} = unique individual observation, U = overall mean, T_i = line effect, B_j = random block effect, and e_{ij} = residual error.

Combined site analyses used the model

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = line effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by line interaction effect, and e_{ijk} = residual error. For cases when one or more random effects are estimated to be zero, statistical analyses for that analyte were based on a reduced model containing only nonzero random terms. Error degrees of freedom were estimated using the Satterthwaite approximation. Fisher's protected least significant difference (LSD) procedure, which first performs an overall analysis of variance and then conducts pairwise t tests only if this overall F test is significant, was done. For each analyte, all possible pairwise comparisons between lines were of interest only when the overall line effect F test was statistically significant ($p < 0.05$). The significant differences ($p < 0.05$) between hybrid comparators, the least-squares mean of each hybrid comparison, the percent difference from the mean, and the p value were determined for each site (data not shown).

RESULTS AND DISCUSSION

Because of the volume of data generated, the following summary tables are presented: (1) total number of significant differences ($p < 0.05$) based on analyte and site (**Table 1**); (2) total number of significant differences ($p < 0.05$) for all hybrids at each site (**Figure 1**); (3) number of significant differences ($p < 0.05$) for all analytes for each comparison for each site (**Table 2**); (4) summary of the magnitude of the mean difference of the significant differences ($p < 0.05$, as a percent of the comparator line) based on site, analyte, and hybrid (**Tables 3** and **4**); and (5) comparison of the range of values for each analyte for all hybrids from the combined site data for all hybrids compared to historical and literature data for each analyte (**Table 5**).

Contribution of Specific Analytes, Hybrids, or Sites That Contribute Significantly to the Total Number of Significant Differences. Data were developed and statistical analyses conducted for 21 sets of hybrid comparisons: each of the 7 hybrids was compared against every other hybrid at each site. These 21 sets of hybrid comparisons contained 47 analytes within each site for a total of 4935 comparisons (47 analytes × 21 head-to-head hybrid comparisons × 5 site comparisons = 4935 total comparisons). A summary of the significant differences by analyte and site is shown in **Table 1** and by hybrid in **Figure 1**.

At each of the sites, there were many significant differences ranging from 33% (323 of 987) of the total comparisons at the site in Germany to 47% (464 of 987) of the total comparisons at the southern France site. The analyte(s) with the greatest number of significant differences at any one site is (are) summarized as follows [analyte (number of differences)] and also shown in **Table 2**: Germany [calcium (17) and 18:0 stearic acid (17)]; southern France [18:0 stearic acid (17), 18:1 oleic acid (18), and glutamic acid (17)]; northern France [16:0 palmitic acid (20), 18:1 oleic acid (17), and 18:2 linoleic acid (19)]; Italy [18:2 linoleic acid (17) and 20:1 eicosenic acid (17)]; combined sites [18:0 stearic acid (17) and 20:1 eicosenic acid (18)].

In general, fatty acids represented the majority of significant differences and had greater levels of variability compared to other analyte groups. These differences were equally distributed among sites. Fatty acid composition in maize is influenced by factors such as hybrid genetics (*12*), fertilizers (*13*), temperature (*14*), kernel position on the ear (*15*), geography (*12*, *16*), and

Table 1. Total Number of Statistical Differences Based on Analyte and Site[a] ($n = 21$)[b]

analyte	Germany	southern France	northern France	Italy	combined sites	total[c] ($n = 105$)
proximate						
moisture	0	5	8	0	8	21
protein	13	11	15	11	10	60
total fat	16	13	16	9	17	71
ash	0	0	0	10	0	10
carbohydrates	13	14	15	12	14	68
fiber						
ADF	*0*	*0*	*0*	*0*	*0*	
NDF	0	7	9	0	6	22
vitamin/antinutrient						
folic acid	0	8	0	11	0	19
phytic acid	*0*	*0*	*0*	*0*	*0*	*0*
riboflavin	0	9	0	0	0	9
thiamin	11	9	13	8	9	50
vitamin E	8	15	11	12	11	57
mineral						
calcium	***17***	13	15	14	16	75
copper	0	9	6	11	6	32
iron	0	15	14	9	12	50
magnesium	0	8	11	14	8	41
manganese	8	14	11	0	8	41
phosphorus	0	7	11	11	6	35
potassium	0	7	10	6	5	28
zinc	8	9	14	12	13	56
fatty acids						
16:0 palmitic	10	8	***20***	7	14	59
16:1 palmitoleic	11	10	0	9	10	40
18:0 stearic	***17***	***17***	16	12	***17***	79
18:1 oleic	13	***18***	***17***	14	12	74
18:2 linoleic	11	15	***19***	***17***	13	75
18:3 linolenic	0	0	13	0	9	22
20:0 arachidic	14	14	13	9	14	64
20:1 eicosenoic	15	15	16	***17***	***18***	81
22:0 behenic	7	8	10	6	10	41
amino acids						
alanine	9	14	7	13	13	56
arginine	16	16	6	11	13	62
aspartic acid	14	12	14	8	7	55
cystine	0	10	0	7	12	29
glutamic acid	10	***17***	0	11	14	52
glycine	16	14	12	11	12	65
histidine	11	15	5	11	11	53
isoleucine	0	5	0	0	7	12
leucine	14	15	10	11	14	64
lysine	16	14	8	11	13	62
methionine	0	9	0	8	9	26
phenylalanine	10	15	0	7	8	40
proline	0	12	11	10	10	43
serine	0	0	0	0	6	6
threonine	6	0	5	6	11	28
tryptophan	9	11	0	9	8	37
tyrosine	*0*	*0*	*0*	*0*	*0*	*0*
valine	0	7	0	0	9	16
total ($n = 987$)	323	464	381	375	443	1986

[a] Boldface italic entries indicate analyte(s) with the most differences at that site, and lightface italic type indicates analyte(s) with no differences at any site. [b] $n = 21$ represents the 21 hybrid-to-hybrid comparisons. [c] $n = 105$ represents the 21 hybrid-to-hybrid comparisons at the four sites and the combined site ($5 \times 21 = 105$).

planting year (*12*). Significant differences between hybrid comparisons were observed for most analytes except ADF, serine, tyrosine, and phytic acid, which had no significantly different values for any of the four site comparisons. Ash, ADF, folic acid, phytic acid, riboflavin, and tyrosine also had no significantly different values for the combined site analysis. Serine, however, had six significant differences for the combined site comparison. The consistency of the analytes with no statistical differences between species may be related to complex mechanisms of pathway regulation that maintain levels of each of these compounds.

The number of significant differences for each hybrid, based on site, is shown in **Figure 1**. In general, the largest occurrence of significant differences for each hybrid was seen at the southern France site, and the least at the Germany site, confirming the totals cited earlier. The experimental hybrid H4817604 had more significant differences at any given site than the other hybrids. At one site (southern France) and for

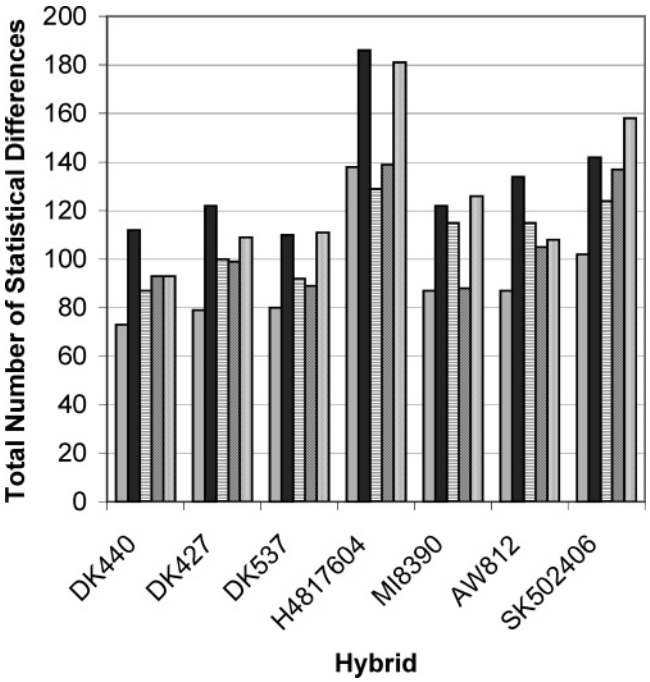

Figure 1. Total number of statistical differences for each individual hybrid at each site ($n = 282$). Bars represent, from left to right in each grouping, Germany, southern France, northern France, Italy, and combined site.

Table 2. Number of Statistically Significant Differences for All Analytes for Each Head-to-Head Comparison for Each Site[a]

comparison	Germany	southern France	northern France	Italy	combined site
DK440 vs DK427	***3***	12	13	11	7
DK440 vs DK537	4	***9***	***6***	***2***	***6***
DK440 vs H4817604	*26*	31	19	26	31
DK440 vs MI8390	13	15	13	10	9
DK440 vs AW812	12	20	17	17	15
DK440 vs DK281	15	25	19	*27*	25
DK427 vs DK537	9	16	12	9	14
DK427 vs H4817604	24	30	22	27	29
DK427 vs MI8390	11	18	13	13	21
DK427 vs AW812	14	26	18	15	14
DK427 vs DK281	18	20	22	24	24
DK537 vs H4817604	24	31	23	26	*33*
DK537 vs MI8390	13	17	17	8	20
DK537 vs AW812	15	17	14	17	13
DK537 vs DK281	15	20	20	*27*	25
H4817604 vs MI8390	20	29	21	22	27
H4817604 vs AW812	20	*33*	25	23	29
H4817604 vs DK281	24	32	19	15	32
MI8390 vs AW812	13	18	24	12	17
MI8390 vs DK281	17	25	*27*	23	32
AW812 vs DK281	13	20	17	21	20

[a] Boldface italic entries indicate comparison with the least differences at each site and the combined site. Lightface italic type indicates comparison with the most differences at each site and the combined site.

the combined site analysis, >50% of the comparisons between H4817604 and the other hybrids were statistically significantly different.

Specific Hybrids with Similar or Disparate Composition. At each of the four different sites and for the combined site, each individual hybrid was compared to every other hybrid for all 47 analytes. A summary of the total number of significant differences from each hybrid comparison at each site and for the combined sites is presented in **Table 2**. Many of the hybrids are, compositionally, very similar. For example, only two significant differences were observed between DK440 and DK537 at the Italy site, whereas 70% of the comparisons between other hybrids were significantly different ($p < 0.05$, H4817604 versus AW812 at the southern France site). The hybrid comparison of DK440 versus DK537 had the fewest number of significant differences ($p < 0.05$) for four of the five comparisons (southern France, northern France, Italy, and across the combined sites). At the Italy site, only two significant differences between DK440 versus DK537 were found: calcium and thiamin. At the site in Germany, the hybrid comparison DK440 versus DK427 had the fewest number of significant differences, that is, 3 of 47 comparisons.

At each site, it was evident that many hybrids were statistically quite different in composition from the other hybrids grown at the same site (italic type, **Table 2**). The hybrid comparisons with the greatest number of significant differences are summarized below and highlighted by the italic type in **Table 2**. The numbers of significant differences for specific hybrid comparisons at that site for 47 analytes were as follows: Germany, DK440 versus H4817604 (26); southern France, AW812 versus H4817604 (33); northern France, MI8390 versus DK281 (27); Italy, DK440 versus DK281 (27) and DK537 versus DK281 (27); combined site, DK537 versus H4817604 (33).

This indicates that virtually all of the hybrids contribute to the variability in analytes as all but one hybrid (DK427) is represented in the summary above. Therefore, the data are not skewed by one or two hybrids that have vastly different compositions from the other hybrids. The data accurately reflect the inherent natural variability across a representative sampling of maize hybrids.

Magnitude of Statistical Differences. To determine the magnitude of the differences that were statistically different (described above), a summary was prepared of the mean difference as a percent of the comparator for each site and for the combined sites analysis (**Table 3**). The magnitude of the differences, as a percent of the mean values, ranged between 0.84 and 149% when all individual sites and the combined site were considered. Most (69%) of the significant differences were between 0 and 20% of the mean difference indicating that, for these comparisons, the analyte values did not vary to a large extent. There were a total of 69 significant differences when the mean difference as a percentage of the comparator line was >50%. These differences were further analyzed with respect to the specific analytes and their distribution between sites and hybrids and are summarized in **Table 4**. The following analytes had significant differences in the head-to-head comparisons >50% of the time across sites: 16:1 palmitoleic acid, 20:0 arachidic acid, 20:1 eicosenoic acid, 22:0 behenic acid, calcium, copper, folic acid, manganese, protein, and vitamin E (**Table 4**). Many of these large differences for an analyte were found at only one site (20:0 arachidic acid, folic acid, manganese, and protein), whereas others were seen at every site and for the combined site (20:1 eicosenoic acid, 22:0 behenic acid, and vitamin E). Half of these differences were due to fatty acids that were described previously to vary widely due to many environmental and genetic factors. These large mean differences were distributed among all of the hybrids. H4817604 had the greatest number of significant differences in which the mean difference was >50%. The analytes 22:0 behenic acid and calcium had a mean difference of >50% in all hybrids.

Comparison of Values for Selected Hybrids to Literature and Composition Databases. The range for each analyte from all sites was compared to observed ranges within the literature

Table 3. Number of Statistical Differences at Each Site Based on the Mean Difference (as a Percent of the Comparator Line)

mean difference (% of comparator)	Germany	southern France	northern France	Italy	combined site	total number $n = 4935$ (% of total differences)
0–10	142	167	138	112	203	762 (38.4)
0–20	70	145	132	145	123	615 (31.0)
20–30	51	83	74	59	73	340 (17.1)
30–40	21	37	24	39	27	148 (7.5)
40–50	19	15	4	7	7	52 (2.6)
>50	20	17	9	13	10	69 (3.5)
total differences	323	464	381	375	443	

Table 4. Summary of Statistical Differences >50% of the Mean Difference Based on Site, Analyte, and Hybrid

	site ($n = 21$)				
analyte	Germany	southern France	northern France	Italy	combined site
16:1 palmitoleic acid	4	1	0	1	0
20:0 arachidic acid	1	0	0	0	0
20:1 eicosenoic acid	3	1	1	1	1
22:0 behenic acid	7	2	3	5	3
calcium	3	6	3	0	3
copper	0	1	0	1	0
folic acid	0	0	0	1	0
manganese	0	1	0	0	0
protein	0	2	0	0	0
vitamin E	2	3	2	4	3
total	20	17	9	13	10

	hybrid ($n = 30$)						
analyte	DK440	DK427	DK537	H4817604	MI8390	AW812	DK281
16:1 palmitoleic acid	2	0	3	4	1	0	2
20:0 arachidic acid	0	1	0	1	0	0	0
20:1 eicosenoic acid	1	5	1	7	0	0	0
22:0 behenic acid	1	16	5	7	1	5	5
calcium	4	5	1	3	1	2	14
copper	0	0	0	0	0	2	2
folic acid	0	0	0	1	0	0	1
manganese	0	0	0	1	0	0	1
protein	0	0	0	2	1	1	0
vitamin E	0	1	0	13	6	3	5
total	8	28	10	39	10	13	30

and the International Life Sciences Institute (ILSI) Crop Composition database (**Table 5**). Thirty-nine of the 47 analytes (83%) were found to overlap with literature and the ILSI Crop Composition database values, but several had values outside these ranges (**Table 5**). These values include aspartic acid, glutamic acid, histidine, isoleucine, lysine, proline, 18:3 linolenic acid, 22:0 behenic acid, and calcium. Generally, these differences between the literature data/ILSI Crop Composition Database and the experimental data are quite small and are distributed mostly within the amino and fatty acids. This confirms that the composition of hybrids chosen for this study is representative of the range of composition for varieties that have previously been analyzed.

The concept of natural variability is important when one attempts to understand the significance of changes in analyte levels. This study has clearly demonstrated that the level of analytes from maize hybrids grown in the same location can vary dramatically. Of 4935 comparisons, 40% were found to be statistically different. Typically >90% of the analytes had statistical differences for the hybrid comparisons for at least one site. Greater than 30% of the comparisons at any one site or across combined sites were statistically different. Consistently, H4817604 had the most statistical differences compared to the other maize varieties, indicating the tremendous impact that selection of desirable traits through breeding has on analyte levels in maize grain. DK440 had the least number of statistical differences from the other hybrids. Of all the head-to-head hybrid comparisons, no comparison consistently showed a large number of statistical differences, but the comparison of DK440 to DK537 consistently had the least differences at all sites. A third of the statistical differences were >50% different from the mean as a percent of the comparator line. These large differences in analyte levels highlight the importance of genetic background as determinants of biochemical composition and enable a better understanding of the natural range of these components.

Conclusions. The noted statistical differences between conventional hybrids emphasize the importance of genetic background and breeding as determinants of biochemical composition and enable a better understanding of the natural range of these components. This can aid in the interpretation of small, statistically significant differences that may be observed when one evaluates the compositional equivalence of a biotech product and can provide a context in which to interpret such changes. Defining the composition of biotech products is a key step in safety assessment, especially in the context of "substantial equivalence" or "comparative risk assessment." This study clearly demonstrates that the levels of specific analytes are not

Table 5. Comparison of Observed Ranges of Maize Analytes with ILSI Crop Composition Database and Literature Ranges for the Combined Site[a]

analytical component[b]	observed range	ILSI Crop Composition Database range[c]	lit. range[d]
proximate			
ash (% dw)	1.05–2.05	0.62–6.28	1.1–3.9;[4] 0.89–6.28[2]
carbohydrates (% dw)	79.3–88.0	77.4–89.5	77.4–87.2;[2] 82.2–88.1[1]
fat (% dw)	2.40–5.14	1.74–5.56	3.1–5.7;[4] 2.48–4.81[2]
moisture (% fw)	7.53–10.7	6.10–26.2	7–23;[4] 8.18–26.2[2]
protein (% dw)	8.03–15.6	6.15–15.0	6–12;[4] 9.7–16.1[3]
fiber			
ADF (% dw)	2.20–5.50	1.82–11.3	3.3–4.3;[4] 2.46–11.34[1,2]
NDF (% dw)	8.03–17.4	5.59–22.6	8.3–11.9;[4] 7.58–18.12[2,9]
amino acid[e]			
alanine	6.96–8.02	4.39–12.0	6.4–9.9[5]
arginine	3.51–5.64	2.58–6.23	2.9–5.9[5]
aspartic acid	***6.32–7.63***	***4.17–9.50***	***5.8–7.2[5]***
cystine	1.68–2.51	1.48–3.16	1.2–1.6;[5] 1.63–2.62[2]
glutamic acid	***16.9–20.4***	***10.4–30.4***	***12.4–19.6;[5] 18.61–20.26[9]***
glycine	3.06–4.36	2.80–4.98	2.6–4.7[5]
histidine	***2.53–3.80***	***1.97–4.18***	***2.0–2.8;[5] 2.72–3.21[9]***
isoleucine	***3.40–4.14***	***2.04–5.96***	***2.6–4.0[5]***
leucine	11.1–14.5	6.42–21.7	7.8–15.2[5]
lysine	***2.19–3.95***	***2.36–5.57***	***2.0–3.8[5]***
methionine	1.62–2.33	1.30–3.44	1.0–2.1;[5] 1.89–2.58[9]
phenylalanine	4.76–5.52	2.63–8.30	2.9–5.7[5]
proline	8.01–10.3	5.76–14.6	6.6–10.3;[5] 8.60–10.56[9]
serine	4.67–5.57	2.35–7.66	4.2–5.5;[5] 2.87–5.63[2]
threonine	3.01–3.78	2.24–6.50	2.9–3.9;[5] 2.61–3.89[2]
tryptophan	0.44–0.76	0.36–0.90	0.5–1.2;[5] 0.41–1.04[2]
tyrosine	2.30–4.55	1.10–5.95	2.9–4.7;[5] 1.93–3.82[9]
valine	4.41–5.19	3.16–7.23	2.1–5.2;[5] 3.93–5.40[2]
fatty acid (% total fatty acid)			
16:0 palmitic	9.16–15.8	8.51–17.5	7–19[5]
16:1 palmitoleic	0.05–0.25	0.10–0.33	1[5]
18:0 stearic	1.23–2.80	1.02–2.76	1–3[5]
18:1 oleic	18.7–35.2	18.6–40.1	20–46[5]
18:2 linoleic	48.8–64.7	43.1–65.6	35–70[5]
18:3 linolenic	***0.92–2.31***	***0.7–1.92***	***0.8–2;[5] 0.71–1.50[1]***
20:0 arachidic	0.28–0.59	0.28–0.72	0.1–2[5]
20:1 eicosenoic	0.20–0.42	0.17–1.92	0.19–0.45[2]
22:0 behenic	***0.06–0.32***	***0.11–0.35***	***0.073–0.22;[1] 0.13–0.24[2]***
mineral			
calcium (% of dw)	***0.0011–0.0059***	***0.0022–0.0208***	***0.01–0.1[4]***
copper (mg/kg of dw)	1.09–3.75	0.73–5.01	0.9–10[4]
iron (mg/kg of dw)	15.6–31.5	10.42–49.07	1–100[4]
magnesium (% of dw)	0.09–0.14	0.08–0.16	0.09–1[4]
manganese (mg/kg of dw)	3.65–8.80	2.61–11.3	0.7–54[4]
phosphorus (% of dw)	0.21–0.42	0.21–0.43	0.26–0.75[4]
potassium (% of dw)	0.28–0.48	0.27–0.53	0.32–0.72[4]
zinc (mg/kg of dw)	12.5–28.7	6.5–37.2	12–30[4]
vitamin			
folic acid (mg/100 g of dw)	0.032–0.11	0.015–0.12	0.03[4]
vitamin B_2 (μg/g of dw)	0.84–1.39	0.70–1.93	0.25–5.6[5]
vitamin B_1 (mg/100 g of dw)	0.35–0.50	0.13–0.85	0.3–0.86;[5] 0.23–0.33[9]
vitamin E (mg/kg of dw)	3.4–15.0	1.5–68.7	3–12.1;[5] 17–47[4]
antinutrient			
phytic acid (% of dw)	0.36–1.00	0.29–1.29	0.42–1.37[9]

[a] Boldface italic type indicates analytes for which differences exist between experimental results and literature values. [b] fw = fresh weight; dw = dry weight; vitamin B_1 = thiamin; vitamin B_2 = riboflavin. Conversions: % dw $\times 10^4$ = μg/g of dw; mg/g of dw $\times 10^3$ = mg/kg of dw; mg/100 g of dw $\times 10$ = mg/kg of dw. [c] International Life Sciences Institute (ILSI) Crop Composition Database, version 2.0, http://www.cropcomposition.org. Search criteria: corn grain, all locations, all years, all proximates, amino acids, fatty acids, bioactives, fiber; vitamins, dry weight other than moisture. Accessed Sept 14, 2005. [d] Literature range references: 1 (*28*); 2 (*29*); 3 (*30*); 4 (*31*); 5, amino acid values reported as percent of total protein and fatty acid values reported as percent of total fat (*32*); 9 (*36*). [e] Values for the observed and literature ranges for amino acids are % ot total amino acid. Values for the ILSI Crop Composition Database for amino acids are mg/g of dw.

static in maize grain. We conclude that the characterization of extant analyte variability is a prerequisite for comparative risk assessments. Natural variability will define the upper and lower boundaries of acceptable composition, that is, limits of analytes present in conventionally grown maize varieties that have a history of safe use and consumption. Thus, only those analytes that fall outside the boundaries of natural variability may be relevant to further investigation with respect to safety or nutritional impact.

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