Grain and Nutritional Quality Traits of Southwestern U.S. Blue Maize Landraces

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ABSTRACT

Anthocyanin-rich Southwestern blue maize (*Zea mays* L.) landraces are receiving interest as functional foods, and commercial production is increasing. We determined variation in kernel color, anthocyanin content, texture, and selected compositional traits of representative varieties. In 2013, eight varieties were grown at four locations in New Mexico. Total kernel anthocyanin content (TAC) and component pigments were measured with spectrophotometry and HPLC, respectively. Oil, protein, starch, and kernel density were determined using NIR spectroscopy and amino acid concentrations using wet chemistry. An average of 49.6 mg/100 g of TAC with a range of 17.6–65.1 mg/100 g was observed. Cyanidin and pelargonidin were major components, and peonidin and succinyl 3-glucoside were minor components. Low levels of disuccinyl glucoside were detected. Blue kernels were higher in anthocyanin than purple or red kernels. Floury kernels displayed the highest protein and oil contents and the lowest starch content and kernel density. The highest starch and kernel density levels were observed in small flint/dent and pop-flint/dent kernels. Amino acid content was variable across genotypes and locations.

Historically, landrace varieties of maize in the U.S.-Mexico borderland region have been a mainstay in the crop-production systems and diets of traditional Native American Indian and Hispanic communities (Adams et al. 2006; Werth 2007; Uriarte-Aceves et al. 2015). Today, heirloom varieties of maize are still utilized, primarily in the production of nixtamalized (masa) food products such as tamales and atole (porridge), soft and hard flat breads (e.g., tortillas and tortilla chips, respectively), and whole kernel traditional foods such as pozole (de la Parra et al. 2007; Nuss and Tanumihardjo 2010; Frank 2011). In addition, health and nutrition issues in many Native American Indian communities are also focusing increased attention on reestablishing traditional food crops in the diet (Notah Begay III Foundation 2015).

In northern Mexico, diverse types of kernels are utilized for the production of food products from local landraces. In the southwestern United States, floury kernel types predominate, especially in New Mexico and Arizona (Ryu et al. 2013; Nankar et al. 2016b). Traditional food preparation techniques in the borderlands region may or may not include nixtamalization, and processing techniques have been optimized to deliver the best end products that can be achieved from diverse local landrace varieties. In contrast, the U.S. dry-milling industry requires specific kernel properties (i.e., hard endosperm) to achieve targeted milling and end-product qualities (Guelpa et al. 2016).

Commercial blue maize products derived from both nixtamalization and dry-milling processing are becoming increasingly recognized by consumers. Consumption of soft tortillas and (hard) tortilla chips, pancakes, and cornbread mixes (Kuleshove 1930; Betrán et al. 2000; de la Parra et al. 2007) continues to increase in the United States. Tortilla chips made from selected blue, white, and yellow maize cultivars grown outside the borderlands region of the United States are now available in commercial markets nationwide. Increased demand for local and heritage foods is also stimulating a growing interest in commercial production of southwestern landrace varieties.

Anthocyanins are water-soluble pigments that accumulate in the aleurone layer and result in kernel color from light to dark blue or purple. Some pigmented kernels may also display a red or reddish purple color (Sánchez G. et al. 2000; Nankar et al. 2016b). Previous research has examined total anthocyanin content (TAC) of southwestern maize land race varieties, but the presence of secondary metabolites that also contribute to the total anthocyanin pigments has not been ascertained (Ryu et al. 2013; Nankar et al. 2016a).

The abundance of red, purple, and blue anthocyanin pigments and other polyphenolic and flavonoid compounds in maize kernels (Dickerson 1999; Betrán et al. 2000) provides a rich source of antioxidants, which contribute to essential functions in both plants and humans (Steyn et al. 2002; Gould et al. 2009; Ryu et al. 2013). Anthocyanins play several physiological roles throughout plant growth and development. They protect plants from biotic and abiotic stresses through their antioxidant properties and help to attract the entomophilous (pollinator) insects (Steyn et al. 2002; Gould et al. 2009). Anthocyanins also contribute to cold stress tolerance by accumulating in cell clusters (Treutter 2006). Anthocyanins may also contribute to human health through prevention of cardiovascular disease and obesity (de Pascual-Teresa et al. 2002; Jones 2005).

Maize is considered an important source of carbohydrate energy for human and animal nutrition and also for biofuel production, but it also contributes approximately 12% of the global plant protein supply in addition to cooking oils (Millward 1999; Rosegrant et al. 1999). Kernel compositional values of protein, oil, and starch, as well as essential amino acids, have been described in southwestern maize germplasm (Nankar et al. 2016b). The varieties examined displayed higher protein and oil values than those typically observed in Corn Belt dent maize. These elevated antioxidant and compositional values could contribute favorably to human nutrition.

The diverse physical, biochemical, and compositional traits of southwestern maize germplasm have not been fully elaborated. The overall objective of this research was to extend the characterization of blue maize landrace kernel traits to include color, polyphenol secondary metabolites, additional essential and conditionally essential amino acids, and density. We also wished to determine the efficacy of more economical and expedient analytical procedures and instrumentation for determination of TAC, and the relative proportion of anthocyanin components compared with those utilized in prior studies of blue maize.

MATERIALS AND METHODS

Germplasm. We evaluated eight accessions of blue maize including six landraces representative of the southwestern United States (Navajo Blue, Santa Clara Blue, Flor del Rio, Yoeme Blue, Hopi Blue, and Taos Blue) and two selected open-pollinated blue maize varieties. The variety Ohio Blue is representative of U.S. Corn Belt dent, and Los Lunas High was selected for improved

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(darker) blue kernel color and larger ears. The addition of these varieties extended the range of kernel traits for comparison. The origin of these accessions was described in Nankar et al. (2016b).

Experimental Field Locations. All genotypes were evaluated during 2013 at the New Mexico State University (NMSU) Agricultural Science Centers at Las Cruces, Los Lunas, Alcalde, and Farmington. Las Cruces and Los Lunas are located at low to moderate elevations of 1,217 and 1,479 m, respectively. Farmington and Alcalde are located at higher elevations of 1,615 and 1,741 m, respectively. Each accession of blue maize was hand-planted in triplicate replications at all locations in a randomized complete block design. Each experimental plot was 6.1 m long with 45.7 cm between rows at all locations except Alcalde, where 7.3 m length plots were used. Organic production practices were utilized at all locations except at Farmington, and all but the Las Cruces location are certified organic. Plots were harvested manually at all locations, and samples were air-dried between 35 and 38°C.

Kernel Color Determination. Samples containing 100 representative kernels of each accession were visually rated using a Royal Horticultural Society color chart. Color class determinations were made indoors in indirect (northern) natural light conditions during the morning.

Milling and Extraction of Samples for Pigment Analysis. A sample containing 100 representative kernels of each accession was milled for 1 min with a 0.5 mm sieve using a Polymix micro hammer cutter mill (Glenn Mills, Clifton, NJ, U.S.A.) (Ryu et al. 2013). Flour samples (150 mg) were extracted with 1.5 mL of 1% HCl/methanol solvent as per the protocol of Li et al. (2008) with the exception that all extracts were refrigerated for 60 min versus 24 h. The tissue/solvent mixture was vortexed (Lab Line Instruments, Melrose Park, IL, U.S.A.) for 1 min. Samples were then immediately refrigerated (60 min) and then centrifuged twice with a microcentrifuge (HBI Centrifuge, Trappe, PA, U.S.A.) at 12,000 × *g* for 20 and 15 min, respectively. Final samples for TAC analysis were prepared from extracted supernatants as described in Nankar et al. (2016a).

TAC Analysis. TAC was analyzed following two methods: one at NMSU and another at the Experiment Station Chemical Laboratory (ESCL) of the University of Missouri (Columbia, MO, U.S.A.). The analytical method used at NMSU utilized the SpectraMax M2 (MDS Analytical Technologies, Sunnyvale, CA, U.S.A.) UV/vis 96-well-plated spectrophotometer, and all analyzed samples were screened at 520 nm wavelength. Spectrophotometric readings were taken in absorbance mode. TAC was quantitated using Beer's law with an extinction coefficient of 26,900 L mol^{-1} cm^{-1}. Anthocyanins analyzed at the ESCL were estimated from blue corn cookie samples according to the method of Li et al. (2011).

Qualitative Analysis of Anthocyanin Components. Relative proportions of anthocyanin components were estimated for each accession and are reported based on the peak area of individual anthocyanin components identified with HPLC. The percentage of individual anthocyanin components was estimated using percent area of the detected peak at a specific retention time. The protocol for HPLC analyses was adapted from Sigma-Aldrich (2017) using modified gradient times as described in Nankar et al. (2016a). HPLC instrumentation included the following: Waters 996 photodiode array detector, Waters 600-MS system controller, Waters 717plus autosampler, and Waters in-line degasser AF (Waters, Milford, MA, U.S.A.). An Ascentis C18 column was used with column dimension of 25 cm × 4.6 mm (Sigma-Aldrich, Supelco Analytical, Bellefonte, PA, U.S.A.). Binary solvents were utilized for qualitative analysis of anthocyanin components; solvent A was 9:1 water/formic acid, and solvent B was 5:4:1 acetonitrile/water/formic acid.

Near Infrared (NIR) Spectroscopy. Compositional traits of protein, oil, starch, and the physical property of kernel density were measured in whole grain samples with NIR spectroscopy using a Foss 1241 grain analyzer and a calibration developed by the Iowa Grain Quality Lab at Iowa State University. Protein, oil, and starch contents were reported in percentage of tissue mass (%) on a dry weight basis.

Amino Acid Analysis. Four essential amino acids (threonine, valine, isoleucine, and leucine) and two conditionally essential amino acids (proline and glycine) were analyzed at the ESCL, University of Missouri, from samples produced at all four New Mexico locations in 2013. Amino acids were measured following AOAC International method 994.12.

Statistical Analysis. Statistical analysis was conducted with SAS software version 9.3 (SAS Institute, Cary, NC, U.S.A.). Analysis of variance for anthocyanin content was performed for each location separately as well as all locations together. PROC GLM was used to estimate the mean squares for analysis of variance; landrace accessions were considered as fixed effects, and locations were considered as random effects. Student's *t* test was used to estimate the mean comparisons, and PROC CORR was used to estimate the correlations between kernel compositional traits and kernel density. Peak area comparisons between different accessions were calculated using Excel 2011 for Mac (Microsoft, Redmond, WA, U.S.A.).

RESULTS

The most predominantly visible kernel colors were violet blue 92A and blue 103A. Only Navajo Blue maize displayed consistent color (violet blue 92A) across and within locations. The most frequently visible color class seen in Flor del Rio was red purple 59A.

Across all accessions and locations, an average of 49.6 mg/100 g of TAC was recorded, and accessions showed statistically significant differences in TAC amounts. Differences in overall TAC mean values occurred at all four locations of Las Cruces, Los Lunas, Alcalde, and Farmington (Tables I and II). There were no significant interactions between location and accession main effects. Navajo Blue and Ohio Blue consistently showed the highest TAC, and Flor del Rio showed the lowest TAC across all locations. Average TAC reported across all locations and accessions was 49.6 mg/100 g with a range of 17.6–65.1 mg/100 g, with the highest TAC seen at Farmington and lowest at Las Cruces.

The results reported for TAC across different analytical methods were strongly correlated and consistently similar across instrumentation and year. The average TAC reported by Nankar et al. (2016a) from 2012 samples was 43 mg/100 g, whereas TAC reported in this paper was 49.6 mg/100 g and the correlation between both methods was 0.93 (Fig. 1). The average TAC reported across accessions following the University of Missouri ESCL method was 45.2 mg/100 g (Nankar et al. 2016b), and the correlation between ESCL and Nankar et al. (2016a) was 0.94. The correlation between ESCL and 2013 analysis was 0.92. The strong correlations between both methods suggest that TAC estimates were consistent across years and analytical methodology.

Individual components of anthocyanin among all accessions were identified by HPLC and mass spectrometry. Five anthocyanin components were consistently identified in all accessions. Based on the mass spectra, cyanidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, succinyl 3-glucoside, and disuccinyl 3-glucoside were eluted in the sequence. Considerable variation was observed in the anthocyanin components of the eight varieties. Relative proportions of these anthocyanin components are given in Table III. On average, 63.5% of total anthocyanins were contributed by cyanidin and pelargonidin, followed by peonidin and succinyl 3-glucoside with levels of 7.9 and 8.2%, respectively. Disuccinyl glucoside was found in the lowest abundance. The relative proportion of cyanidin and pelargonidin was highest in Los Lunas High and lowest in Flor del Rio. Peonidin 3-glucoside was highest in Yoeme Blue and Flor del Rio and lowest in Taos Blue. Yoeme Blue and Taos Blue displayed the highest levels of succinyl glucoside and Flor del Rio the lowest. The relative proportion of disuccinyl glucoside was fairly close across genotypes with the exception of Los Lunas High. The relationship between coeluted cyanidin and pelargonidin appeared to be inversely proportional to succinyl glucoside and disuccinyl glucoside.

On average, 83% of peak area of each chromatogram was from identified anthocyanins, and 17% of area was owing to potential unknown anthocyanins or other flavonoid components. Several other compounds unrelated to anthocyanins such as shikimic acid, quercetin, malic acid, tagatose, citramalic acid, terpinyl acetate, and picolinic acid N-oxide were detected (data not shown). Among these compounds, shikimic acid is a precursor of the anthocyanin biosynthetic pathway, and quercetin is closely related to flavonoids.

Across all environments, average protein, oil, and starch values were 12.0, 6.2, and 65.6%, respectively (Table IV). Accessions differed for mean protein, oil, and starch contents across all locations. Protein and oil contents were highest for Santa Clara Blue and Flor del Rio, and Ohio Blue was observed to have the lowest (Table IV). Flor del Rio and Ohio Blue displayed the highest starch and kernel density, whereas Santa Clara Blue was lowest in starch and kernel density. Locations also differed for mean protein, oil, and starch values. The highest protein and oil values were observed in Los Lunas and the lowest amounts in Las Cruces. Starch content was highest in Alcalde and lowest in Los Lunas.

Across all environments, average kernel density was 1.24 g/cm^3 (Table IV). Kernel density was similar at all locations and varied from 1.21 to 1.32 g/cm^3 across accessions. Kernel density was lowest in accessions with floury-type endosperm and higher in popflint Flor del Rio (1.32 g/cm^3), with Corn Belt dent Ohio Blue being

TABLE I
Total Anthocyanin Content (TAC) of Blue Maize Landraces Grown in Four Locations in New Mexico in 2013[a]

Statistic	Las Cruces	Los Lunas	Alcalde	Farmington	Across Locations
Mean (accessions)	38.8	53.2	51.8	55.6	49.6
Range among accessions	15.3–52.8	17.2–82.1	15.9–71.4	21.9–70.4	17.6–65.1
$LSD_{0.05}$	17.1	23.8	27.02	13.8	9.47

[a] TAC is expressed in mg/100 g.

TABLE II
Analysis of Variance of Total Anthocyanin Content of Blue Maize Landraces Grown in Four Locations in New Mexico in 2013[a]

		F Values				
Source of Variation	DF	Las Cruces	Los Lunas	Alcalde	Farmington	Across Locations
Rep	2	1.07	4.81**	0.65	1.57	2.30*
Accession (A)	7	3.99**	6.62***	4.81**	13.06***	20.12***
Location (L)	3					10.26***
A×L interaction	21					1.62

[a] *, **, and *** indicate significant at the 0.1, 0.05, and 0.01 probability level, respectively. DF = degrees of freedom.

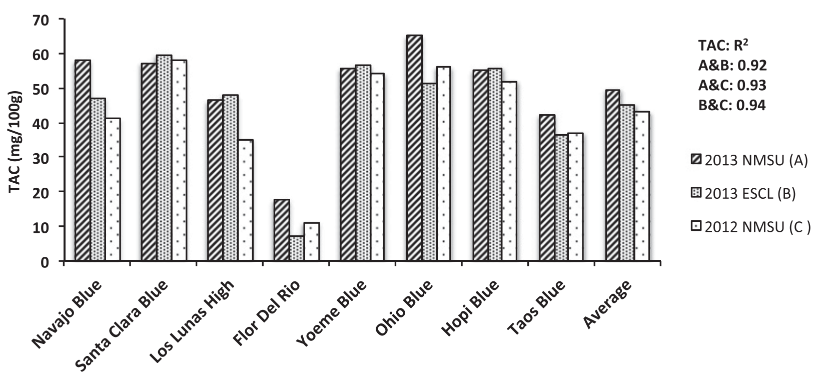

Fig. 1. Comparison of total anthocyanin content (TAC) among different analytical methods. NMSU = New Mexico State University; ESCL = Experiment Station Chemical Laboratory of the University of Missouri; A and C = kernel-based TAC; and B = cookie-based TAC.

TABLE III
Relative Proportion of Diverse Anthocyanin Components from Blue Maize Landraces Grown at Las Cruces in 2013[a]

Accession	Cyanidin and Pelargonidin	Peonidin	Succinyl Glucoside	Disuccinyl Glucoside	Total Anthocyanins	Other Compounds
Navajo Blue	61.8	8.1	10.1	5.0	85.1	14.9
Santa Clara Blue	76.1	6.2	4.8	2.9	90.0	10.0
Los Lunas High	83.7	5.2	4.7	1.7	95.4	4.6
Flor del Rio	15.9	11.3	3.9	4.4	35.4	64.6
Yoeme Blue	65.0	11.9	12.3	4.6	93.8	6.2
Ohio Blue	68.1	9.5	8.9	3.7	90.2	9.8
Hopi Blue	72.0	8.5	9.0	3.1	92.5	7.5
Taos Blue	65.0	2.5	12.2	3.9	83.6	16.4
Mean	63.5	7.9	8.2	3.7	83.2	16.8
Range	15.9–83.7	2.5–11.9	3.9–12.3	1.7–5.0	35.4–95.4	4.6–64.6
Bulk/composite	75.4	7.1	6.8	2.4	91.7	8.3

[a] Bulk/composite estimates are based on equal proportions of representative sample tissues. Relative proportion of different anthocyanin components is expressed in %.

1.26 g/cm³ (Table IV). The relationship between kernel density and starch was directly proportional, whereas the protein content was inversely proportional except pop-flint endosperm accession Flor del Rio, which displayed protein content similar to the floury varieties. Correlations between kernel density and both oil and protein were negative, whereas the correlation between kernel density and starch was positive (Table V). Across all environments, the interaction between accession and environment was significantly different, and accessions were also significant, although the locations displayed nonsignificant differences (Table VI).

TABLE IV
Kernel Compositional Traits and Kernel Density of Blue Maize Landraces Grown Across Locations of New Mexico in 2013[a]

Accessions	Protein (% db)	Oil (% db)	Starch (% db)	Kernel Density (g/cm^3)
Navajo Blue	11.6	6.0	65.8	1.23
Santa Clara Blue	13.1	8.1	63.6	1.21
Los Lunas High	12.3	7.6	63.8	1.22
Flor del Rio	11.6	4.4	68.0	1.32
Yoeme Blue	11.9	6.5	65.3	1.22
Ohio Blue	10.6	4.7	67.6	1.26
Hopi Blue	12.7	6.9	64.7	1.21
Taos Blue	12.4	5.6	65.6	1.23
Average	12.0	6.2	65.6	1.24
Range	10.6–13.1	4.3–8.1	63.6–68.0	1.21–1.32
$LSD_{0.05}$	0.78	0.52	0.89	0.01

[a] Data collected from Las Cruces, Los Lunas, Alcalde, and Farmington in 2013.

TABLE V
Correlation Analysis of Kernel Compositional Traits and Kernel Density of Blue Maize Landraces Grown Across Locations in New Mexico in 2013

Trait	Protein	Oil	Starch	Kernel Density
Protein		0.79*	–0.82**	–0.58
Oil	0.79*		–0.98***	–0.82**
Starch	–0.82**	–0.98***		0.87**
Kernel density	–0.58	–0.82**	0.87**	

Amino acid contents were variable at all locations (Table VII), and all amino acids showed significant differences among accessions except proline. Leucine and proline were the most abundant, and threonine and isoleucine were the least abundant (Table VII). Amino acid values were highest in Santa Clara Blue and Taos Blue, whereas Los Lunas High and Ohio Blue displayed the lowest. No significant interaction was seen between accession and location for amino acid content. Threonine displayed significant differences among accessions at all locations except Alcalde, whereas alanine and valine did not show any significant differences between accessions at any location.

DISCUSSION

Classification of kernel samples based on the Royal Horticultural Society color chart showed a direct relationship between color classification and relative proportions of cyanidin and pelargonidin. Blue kernels appeared to be higher in anthocyanin than purple red kernels. Santa Clara Blue, Los Lunas High, and Hopi Blue (highest proportions of violet blue 92A and blue 103A colored samples) displayed the highest amounts of cyanidin and pelargonidin, and Flor del Rio (primary classification red purple 59A) displayed the lowest. Similar observations have been made in other studies (Cevallos-Casals and Cisneros-Zevallos 2003; Moreno et al. 2005; Jing et al. 2007; Lopez-Martinez et al. 2009; Nankar et al. 2016a). Conversely, Yang et al. (2009) reported that purple maize kernels contained more anthocyanin than blue and red kernels. Pelargonidin 3-glucoside and peonidin 3-glucoside have been previously reported in studies of purple corn (Aoki et al. 2002; de Pascual-Teresa et al.

TABLE VI
Analysis of Variance of Kernel Compositional Traits and Kernel Density of Blue Maize Landraces Grown in Four Locations in New Mexico in 2013[a]

Trait	Effect	DF	Las Cruces	Los Lunas	Farmington	Alcalde	Across Locations
Protein	Accession (A)	7	5.57**	5.0***	1.05	1.73	11.06***
	Location (L)	3					7.51***
	A×L interaction	21					0.86
Oil	A	7	28.53***	12.96***	4.69**	11.64***	42.14***
	L	3					7.66**
	A×L interaction	21					1.59
Starch	A	7	32.53***	8.77***	3.41**	5.41*	26.36***
	L	3					13.61***
	A×L interaction	21					0.60
Kernel density	A	7	9.87***	18.17***	30.27***	37.67***	83.84***
	L	3					0.75
	A×L interaction	21					1.88*

[a] *, **, and *** indicate significant at the 0.1, 0.05, and 0.01 probability level, respectively. DF = degrees of freedom.

TABLE VII
Essential and Conditionally Essential Amino Acid Contents of Blue Maize Landraces Evaluated Across Locations of New Mexico in 2013[a]

	Essential				Conditionally Essential	
Accessions	Threonine	Isoleucine	Leucine	Valine	Proline	Glycine
Navajo Blue	0.40	0.41	1.56	0.56	1.04	0.45
Santa Clara Blue	0.41	0.43	1.68	0.60	1.08	0.46
Los Lunas High	0.39	0.41	1.51	0.56	1.01	0.44
Flor del Rio	0.39	0.43	1.62	0.57	1.07	0.43
Yoeme Blue	0.39	0.42	1.58	0.57	1.02	0.45
Ohio Blue	0.38	0.41	1.55	0.55	1.05	0.43
Hopi Blue	0.40	0.42	1.61	0.59	1.02	0.45
Taos Blue	0.42	0.46	1.75	0.62	1.08	0.46
Average	0.40	0.42	1.61	0.58	1.05	0.45
Range	0.38–0.42	0.41–0.46	1.51–1.75	0.55–0.62	1.01–1.08	0.43–0.46
$LSD_{0.05}$	0.03	0.03	0.13	0.04	0.07	0.02

[a] Data collected from Las Cruces, Los Lunas, Alcalde, and Farmington in 2013. All amino acids are expressed in % db.

2002; Jing et al. 2007; Li et al. 2008; Zhao et al. 2009). The findings of Fossen et al. (2001) with maize and reed canarygrass are in agreement with our study in regard to the presence of peonidin. Anthocyanin content from our study was higher than that reported in studies by Mora-Rochin et al. (2010), Pozo-Insfran et al. (2007), and Ryu et al. (2013).

Anthocyanin variability might be owing to the presence of genetic variation for kernel color from genotypes used to represent generic blue and purple designations. Environmental diversity in sample origin may also have contributed to differences across studies. Samples that were produced at higher elevations displayed higher anthocyanin content than those from lower elevation locations. Similar results were also observed by Chalker-Scott (1999), in which UV radiation exposure at higher altitude was assumed to result in increased anthocyanin biosynthesis. This relationship between anthocyanin content and elevation warrants further study.

TAC values in this study were similar to those reported by Nankar et al. (2016b), in which different anthocyanin analysis methods were used. Results reported for TAC from this study were relatively higher but were not significantly different from an analysis of samples obtained in 2012 Nankar et al. (2016a). These results clearly show the consistency and stability in TAC across different years and analytical methods. Both methods were considered to be cost effective for anthocyanin analysis. The ESCL method is an established method that is widely used by cereal chemists.

The negative correlation of starch with other compositional and biochemical traits indicates that the smaller kernels of southwestern landraces could have higher embryo-to-endosperm or pericarp-to-endosperm proportions than other races of corn. In comparison with commercial U.S. hybrids, the unimproved landraces might be expected to display higher protein and oil values because hybrids have been mainly selected for grain yield, which has been correlated with increases in kernel starch and decreases in protein and oil (Ridley et al. 2002; Belyea et al. 2004; Scott et al. 2006). Our results clearly showed that the blue maize landraces were lower in starch and higher in oil and protein. The oil and protein values expressed by the blue maize landraces were also significantly higher than those reported in high-oil maize varieties: 6% oil and 9.6% protein (Han et al. 1987) and 2.7–5.4% range of oil (Bauman et al. 1963). Landraces identified for high oil and protein contents were lowest in starch and kernel density.

The significant differences in kernel density of the varieties were consistent with the visual appearance of the kernels. Hard endosperm accessions such as pop-flint and dent type displayed high kernel density, and density values were lower in accessions with floury endosperm. Similar trends were seen in hard and soft maize by Fox and Manley (2009). The endosperm-specific variability for kernel density has been previously documented by Correa et al. (2002) and Taboada-Gaytan et al. (2010), who demonstrated the kernel density of Brazilian flints exceeded that of U.S. dent hybrids (1.29 and 1.23 g/cm^3, respectively). The low kernel density reported for floury blue maize was consistent with other research (Fox and Manley 2009; Uriarte-Aceves et al. 2015).

Kernel hardness can be an important parameter for different end uses. Lower density maize is suitable for wet-milling processing (Taboada-Gaytan et al. 2010; Uriarte-Aceves et al. 2015), and hard endosperm semi-dent and flint corns with high kernel density are widely used in dry milling (Guelpa et al. 2016). Another important food processing characteristic associated with kernel density is cooking time. Floury maize with soft endosperm would be ideal for nixtamalization, because the time needed for cooking is lower than for flint or dent maize (Betrán et al. 2000). The documented use of heirloom varieties of blue maize for multiple culinary uses among Native American and Hispanic communities reflects their suitability (Betrán et al. 2000; de la Parra et al. 2007; Salinas-Moreno et al. 2012) for nixtamalized food products.

In maize, research on analysis of individual amino acids has been little reported except for the essential amino acids associated with floury and opaque endosperm mutations. Results for individual essential amino acids cysteine, lysine, and methionine have been reported previously for these accessions in Nankar et al. (2016b). Threonine, isoleucine, leucine, and valine are considered nutritionally essential amino acids in the human diet under all conditions, whereas glycine and proline are conditionally essential amino acids only during specific physiological and pathological conditions (Young and Pellett 1994). Values of the nutritionally essential amino acids threonine, isoleucine, leucine, and valine were higher in this study than those reported by Zarkadas (1997). Conditionally essential amino acids proline and glycine also appeared higher than those previously reported (Zarkadas 1997; Zarkadas et al. 2000). Nutritionally and conditionally essential amino acids are of high importance in human and livestock nutrition because these amino acids play a key role in determination of protein quality (Young and Pellett 1994).

CONCLUSIONS

Inherent kernel color variation within blue maize populations was modest, and as expected, cyanidin 3-glucoside was the predominant form of anthocyanin present. Anthocyanin diversity was also in evidence, with the presence of peonidin, pelargonidin, and succinyl and disuccinyl glucosides of cyanidin derivatives. The relative proportion of different components was fairly variable among all accessions, except disuccinyl 3-glucoside. Kernels produced at higher altitude locations appeared to display higher anthocyanin content than those produced at lower altitude locations. Navajo Blue and Ohio Blue displayed the highest anthocyanin values. Anthocyanin content across all accessions was stable across different analytical instrumentation.

Compositional traits of protein and oil were higher in floury landraces, whereas starch appeared to be higher in pop-flint and dent kernels. Clear associations were observed relating high kernel density and starch with low protein and oil. Physical traits of kernel density and other compositional traits were observed to be highest in pop-flint and dent landraces and lowest in floury types. Significant diversity has been seen for different nutritional and conditional essential amino acids.

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