Chromatic, Phenolic and Antioxidant Properties of *Sorghum bicolor* Genotypes

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Abstract

Chromatic, phenolic and antioxidant properties were evaluated in ten sorghum genotypes grown in Nuevo León, México. Lightness, Chroma and hue angle ranged from 64 to 83, 12 to 20 and 61 to 82 respectively, indicating that colour of the samples were located in the gray orange-yellow zone of the hue circle. Based on these results, samples were classified in three colour groups being Very Soft Orange, Slightly Desaturated Orange and Grayish Orange. Results in phenolics ranged from 796 to 15,949, 175 to 12,674 and 193 to 25,780 µgCE g⁻¹ in total phenolics by Folin-Ciocalteu, total flavonoids by Aluminum Chloride and condensed tannins by Vanillin-HCl respectively. On the other hand, antioxidant capacity ranged from 1.20 to 93.83, 30.25 to 156.08 and 2.62 to 98.50 μmolTE g⁻¹ in 2,2-diphenyl-1-picrylhydrazyl, 3-ethylbenzothiazoline-6-sulfonic acid and Ferric Reducing Antioxidant Power respectively. Significant differences (p≤0.05) were observed in statistical analysis for both individual and group colour samples in chromatic, phenolics and antioxidant activity evaluations, showing 'Rox Orange' genotype and Grayish Orange colour group the highest levels.

Keywords: ABTS, condensed tannins, DPPH, FRAP, total flavonoids, total phenolics, *Sorghum bicolor*

Introduction

*Sorghum (Sorghum bicolor (L.) Moench)* is native from Africa grown in tropical, subtropical and arid regions around the world, and is the fifth most produced cereals in the world, in addition is used as food in Africa and as feed in the western hemisphere (Dykes *et al*., 2013). According to the data of Food and Fisheries Statistics Service (SIAP) the annual production of sorghum grain in México increased from 6.1 millions of tonnes in 2009 to 8.3 millions of tonnes in 2014, being animal feed their main use (SIAP, 2015). New ways to use sorghum for food purposes are looking to promote its consume taking into account some technological and nutritional components which includes gluten free leavened breads, cakes, cookies, tortillas, snacks and noodles (Taylor *et al*., 2006). In addition, a great interest has focused on sorghum grain and its co-products as a source of functional and nutraceutical components with human health promoting actions including phenolics (Althwab *et al*., 2015), since they have a high antioxidant activity provided by phenolic acids, condensed tannins and anthocyanins (Awika and Rooney, 2004), that can induce phase II detoxifying enzymes and inhibit proliferation of carcinoma cells (Awika *et al*., 2009). The aim of this work was to evaluate and compare chromatic characteristics, the phenolic compounds content and the antioxidant capacity levels of sorghum genotypes Grown in Nuevo León, México.

Materials and Methods

Plant material

Seeds of ten sorghum genotypes with different geographic origins and varying pigmentation including ‘46038B’, ‘7B’, ‘FAUANL-3’ ‘WB’ from Nuevo León, México; ‘RB Norteño’, ‘RB Paloma’ from Tamaulipas, México; ‘Keller’ ‘Rox Orange’ from Kentucky, United States; ‘SPV4511-2’ from Telangana, India; and ‘Tanol-1’ (commercial genotype, origin unknown) were grown in the experimental field of the Agronomy Faculty of Universidad Autónoma de Nuevo León located in Marín, Nuevo León (25°52'13.5"N and 100°02'22.5" W) during 2014 spring summer cycle. The sowing was performed on March 5th on a clay soil and experimental units involved 4 furrows of 5 m
long and 0.8 m spaced from each other and it was established a 0.1 m distance between plants in simple line. A day before sowing fertilisation of soil was performed with poultry manure (3 tonnes ha\(^{-1}\)).

Four irrigations were carried out during whole cycle (March 7\(^{th}\), March 20\(^{th}\), April 15\(^{th}\) and May 5\(^{th}\)), all sorghum genotypes were harvested at physiological maturity (July 5\(^{th}\)) and complete grains (endosperm and bran) were milled and sieved to obtain flour with a particle size ≤ 0.5 mm (mesh 35).

**Chromatic measurements**

Sixty grams of every sorghum sample were placed in a Petri dish and colour was measured on the surface flour using a CR-300 Konica Minolta Chroma Meter (Tokyo, Japan).

Chromatic parameters were obtained using CIELAB (\(L^\ast, a^\ast, b^\ast\)) colour system where \(L^\ast\) defines Lightness (0=black, 100=white), \(a^\ast\) indicate red (positive \(a^\ast\)) or green value (negative \(a^\ast\)) and \(b^\ast\) indicate yellow (positive \(b^\ast\)) or blue value (negative \(b^\ast\)). In addition \(C^\ast\) (Chroma; saturation level of b) and (hue angle; \(0^\circ=\)red, 90°=yellow, 180°=green, 270°=blue) were obtained using CIELAB colour values as \(C^\ast= (a^\ast+b^\ast)^{1/2}\) and \(h=\arctan (b^\ast/a^\ast)\) (Commission Internationale de l’Eclairage, 2004).

Colour view was obtained by online software ColorHexa colour converter using \(L^\ast, C^\ast\) and \(b^\ast\) values (ColorHexa, 2015) and sorghum genotypes were grouped by colour based on \(b^\ast\) values.

**Phenolic extracts**

One hundred milligrams of sorghum samples were homogenized with 3 mL of 80% methanol in a screw cap culture tube and stirred for 4 h at 200 rpm. After that, samples were centrifuged at 2,600 g, supernatants were recovered and stored at -20 °C until they were used for phenolics and antioxidant capacity analysis.

**Phenolics**

The total phenolics content was determined using a colorimetric method reported by Chun and Kim (2004) based on the reaction of Folin-Ciocalteu reagent. Briefly, 0.2 mL of phenolic extract was placed in 2.6 mL of distilled water, oxidized with 0.2 mL of Folin-Ciocalteu reagent and after 5 min neutralized with 2 mL of 7% Na\(_2\)CO\(_3\) solution. The reaction was left for 90 min and finally absorbance of samples was measured at 750 nm.

The total flavonoids content was evaluated according to Ivanova et al. (2011) based on the reaction of aluminium chloride. Briefly, 0.2 mL of phenolic extract was placed in 3.5 mL of distilled water, followed by 0.15 mL of 5% Na\(_2\)CO\(_3\), after 5 min 0.15 mL of 10% AlCl\(_3\) was added and finally 5 min later 1.0 mL of 1M NaOH was added. Reaction was left for 15 min and finally the absorbance of samples was measured at 510 nm.

The condensed tannins content was determined as described by Sun et al. (1998) based in the reaction of vanillin-H\(_2\)SO\(_4\). Briefly, 0.25 mL of phenolic extract was mixed with 0.65 mL 1% vanillin solution and 0.65 mL of 25% H\(_2\)SO\(_4\) (both dissolved in methanol). Reaction was left for 15 min at 30 °C and finally the absorbance of samples was measured at 500 nm.

For total phenolics, total flavonoids and condensed tannins assays, catechin was used as standard (0 to 200 mg L\(^{-1}\)) and results were expressed as micrograms of catechin equivalents per gram of dry sample (\(\mu\)g CE g\(^{-1}\)) using a linear equation.

**Antioxidant capacity**

DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated using a working solution 60 \(\mu\)M with and absorbance adjusted to 0.7 at 517 nm; the assay was carried out by mixing 0.2 mL of phenolic extract with 3.3 mL of the DPPH working solution, the reaction was left for 30 min in the dark and the reduction of DPPH was determined. ABTS (3-ethyl-benzothiazoline-6-sulfonic acid) was carried out using a working solution obtained by mixing one mL 7.4 mM of ABTS and one mL of 2.6 mM of K\(_2\)S\(_2\)O\(_8\) and allowing them to react for 12 h in the dark, after that time absorbance of working solution was adjusted to 0.7 at 734 nm diluting with methanol; the ABTS assay was performed by mixing 0.2 mL of phenolic extract with 3.3 mL of ABTS working solution, reaction was left for 2 h in the dark and the reduction of ABTS was measured.

FRAP (Ferric Reducing Antioxidant Power) was determined using a working solution prepared by mixing 300 mM C\(_6\)H\(_5\)Na\(_2\)O\(_7\)3H\(_2\)O (pH 3.6), 10 mM TPTZ (2,4,6-triprydyl-s-triazine, in HCl 40 mM) and 20 mM FeCl\(_3\)-6H\(_2\)O in 10:1:1 proportion; the FRAP assay was prepared by mixing 0.2 mL of phenolic extract with 3.3 mL of FRAP working solution, reaction was left for 30 min in the dark at 37 °C and the absorbance of samples was taken at 593 nm. Antioxidant capacity assays were performed according to Thaipong et al. (2006) with modifications, Trolox was used as standard (0 to 200 \(\mu\)mol L\(^{-1}\)) and results were expressed as micromoles of Trolox equivalents per gram of sample (\(\mu\)mol TE g\(^{-1}\)) using a linear equation.

**Statistical analysis**

Analysis of variance was used to assess statistical differences among sorghum genotypes with a 5% confidence level. When significance difference was found, Tukey’s multiple range tests were carried out to separate means using Minitab 14.0 (Minitab Inc., 2004).

**Results and Discussion**

There were significant differences in \(L^\ast, C^\ast\) and \(b\) (p<0.05) between samples (Table 1). Lightness values ranged from 64 to 83 such indicate that all samples were more white than black;
values of $a^*$ and $b^*$ (data not shown) were positive in all samples which indicate that they are more red than green and more yellow than blue. According to McGuire (1992), to include $C^*$ and $h$, is a more appropriate measurement of colour since the first one is how we perceive colour and the second one is the level of saturation of the colour perceived. In general, colour saturation of all samples were low with $C^*$ values ranging from 12 to 20 located in the gray zone of the hue circle.

The hue angle ranged from 61 to 82 indicating that all values were in the orange zone (red-yellow) colour on the hue circle. The values of $C^*$ and $h$ of all genotypes analyzed in our study are in agreement with most of the data reported by Dykes et al. (2011) for lemon-yellow (obtained from $a^*$ and $b^*$) sorghum genotypes grown in two locations of Texas (United States), but $L^*$ values of these authors are different since they found a range from 44 to 63 being their genotypes darker than ours.

There are not a defined classification nomenclature colour of sorghum genotypes according to their chromatic values, but based on $L^*$, $C^*$ and $h$ values obtained we classified our sorghum genotypes in three colour groups: 1) Very Soft Orange (VSO), including ‘46038B’, ‘7B’ and ‘RB Paloma; 2) Slightly Desaturated Orange (SDO), including ‘FAUANL-3’, ‘Keller’, ‘RB Norterho’, ‘SPV4511-2’ and ‘WB’; 3) Grayish Orange (GO), including ‘Rox Orange’ and ‘Tanol-1’. When samples are grouped based on colour, VSO showed significant differences ($p \leq 0.05$) in $L^*$ being 1.12 and 1.18-fold higher than SDO and GO groups respectively; SDO was 1.08 and 1.18-fold higher than VSO and GO in $C^*$ respectively, showing significant differences ($p \leq 0.05$); in addition three colour groups showed significant differences ($p \leq 0.05$) in $h$ being VSO group 1.17 and 1.30-fold higher than SDO and GO respectively (Table 2).

## Phenolics
Significant differences ($p \leq 0.05$) were observed in total phenolics, total flavonoids and condensed tannins between samples (Table 3). In all phenolics analysis ‘Rox Orange’ showed the highest levels followed by ‘Tanol-1’ while the lowest levels were in ‘46038B’, in addition the first one was 20, 72 and 133-fold higher than the latter one in total phenolics, total flavonoids and condensed tannins respectively.

Total phenolics values obtained ranged from 796 to 15,949 µg g$^{-1}$ and were between data reported by Awika et al. (2005) that found from 1,000 to 21,000 µg g$^{-1}$ analyzing whole grains of brown, black and white sorghum genotypes grown in Texas (United States). Total flavonoids content ranged from 175 to 12,674 µg CE g$^{-1}$ and data obtained for most of the samples are in the range of those reported by Herald et al. (2012) who analyzed flours of tannin and non-tannin sorghums grown in Kansas (United States) and found a range from 500 to 6,810 µg g$^{-1}$ except for ‘Rox Orange’ and ‘Tanol-1’ that were higher than the data reported by these authors.

Condensed tannins levels ranged from 193 to 25,780 µg CE g$^{-1}$ and excepting ‘Rox Orange’ and ‘Tanol-1’ which had content higher than 20,000 µg CE g$^{-1}$, the rest eight samples were between values reported by Dykes et al. (2005) who analyzed sorghum grains of varying genotypes developed in Texas (United States) finding levels from 200 to 15,500 µg g$^{-1}$. According to Dykes et al. (2013), sorghum genotypes with values of condensed tannins less than 2,000 µg g$^{-1}$ measured by vanillin-HCl assay are considered tannin-free, taking that into account ‘46038B’, ‘7B’ and ‘RB Paloma’ can be classified as tannin-free sorghum genotypes.

When sorghum genotypes were grouped by colour (Table 4), the behaviour in all phenolics analysis was GO > SDO > VSO and significant difference ($p \leq 0.05$) between the colour groups was observed. GO group was 4.8 and 8.7, 5.9 and 33.4, 5.24 and 27.1-fold higher than SDO and VSO in total phenolics, total flavonoids and condensed tannins respectively.

There are several studies of phenolics in different sorghum genotypes but most of them did not report colour data and only described visual characteristics. Afify et al. (2012) analyzed phenolics of three white sorghum genotypes grown in Giza (Egypt) and found an average content of 1,121, 532 and 84 µg g$^{-1}$ in total phenolics, total flavonoids and condensed tannins respectively; the description of genotypes evaluated by these authors are similar to VSO colour group of the present study and the values in total phenolics and condensed tannins were lower than our results but data obtained in total flavonoids were higher. Kobue-Lekalale et al. (2007) evaluated containing-tannin (red) and tannin-free (white) genotypes developed in Botswana and South Africa, which had visual characteristics similar to ours VSO and GO colour groups respectively and they found a total phenolics average of 15,066 µg g$^{-1}$ for containing-tannin and 2,333 µg g$^{-1}$ for tannin-free sorghums. In addition, they found 52,300 µg g$^{-1}$ of condensed tannins in containing-tannin genotypes, being
their results higher than ours. Taking into account levels of condensed tannins, sorghum genotypes of the present study can be classified as tannin-free, moderate containing tannin and high containing-tannin for samples within VSO, SDO and GO groups respectively.

### Antioxidant capacity

Significant differences (p≤0.05) between samples were observed in DPPH, ABTS and FRAP antioxidant capacity assays. Results of antioxidant capacity were 1.20 to 93.83, 30.25 to 156.08 and 65.73 to 28.19 in DPPH, ABTS and FRAP respectively (Table 5). ‘Rox Orange’ showed highest levels in three antioxidant capacity assays followed by ‘Tanöl-1’, in addition ‘46038B’ showed the lowest levels in DPPH and FRAP and ‘RB Norreño’ obtained lowest level in ABTS. ‘Rox Orange’ was 78 and 37-fold higher than ‘46038B’ in DPPH and FRAP respectively, and 5.15-fold higher than ‘RB Norreño’ in ABTS.

Results of DPPH and ABTS assays are into the values reported by Awika et al. (2003) who found 6 to 202 μmolTE g⁻¹ in DPPH and 6 to 226 μmolTE g⁻¹ in ABTS analyzing sorghum of varied colours including white, red, brown and black genotypes grown in Texas (United States), although ‘Hi Tannin’ and ‘Sumac’ genotypes include in that study were higher than all our samples; in addition, Moraes et al. (2015) evaluated the antioxidant capacity of ‘SC21’ genotype (brown pericarp, pigmented testa) by FRAP assay reporting 90 μmolTE g⁻¹ respectively, which is around levels found in ‘Rox Orange’ of our study.

In samples grouped by colour, the behaviour in all antioxidant capacity assays was GO > SDO > VSO, significant difference (p≤0.05) was observed between GO in relation to VSO and SDO, but VSO and SDO did not present significant difference (p>0.05) between them (Table 6). GO group was 15.1 and 35.9, 3.1 and 2.7, and 10.4 and 16.3-fold higher than SDO and VSO in DPPH, ABTS and FRAP respectively.

Afify et al. (2012), evaluated the antioxidant capacity of white sorghums finding average values of 14.51 μmolTE g⁻¹ in DPPH and 15.54 μmolTE g⁻¹ in ABTS, these results were higher than SDO group of our study which had similar characteristics of genotypes analyzed by these authors. Four tannin-containing sorghum grains from Texas (United States) and Kari (Kenya) were analyzed by Awika et al. (2009) and they found lower average values than our GO group samples with 27 μmolTE g⁻¹ in DPPH and 103 μmolTE g⁻¹ in ABTS. In addition, Luthria et al. (2013) evaluated the antioxidant capacity of the breeding lines peeled kernels ‘PR6E14’ and ‘PR6E6’ grown in Texas (United States) by FRAP, reporting an average value around 80 μmolTE g⁻¹ which were higher than all our colour groups.

Although there are many reports about different sorghum genotypes from around the world in terms of phenolics and antioxidant capacity, there are not a colour system classification of sorghum genotypes based on CIELAB chromatic characteristics and so difficult comparisons among reports. In addition, data variation in phenolics and antioxidant capacity analysis among different reports using same plant material is mainly attributed to solvent and technique used for extraction of phenolic compounds and such topic is analyzed by Luthria (2006).

### Conclusions

‘Rox Orange’ showed the lowest values in b colour parameter and highest levels of phenolic compounds and antioxidant capacity evaluated. Although there are not a colour system classification of sorghum genotypes, we proposed to group our samples as Very Soft Orange (VSO), Slightly Desaturated Orange (SDO), Grayish Orange (GO) based on their L*, C and b values, and also genotypes were classified as non tannin-containing, moderate tannin-containing and high tannin-containing respectively, based on their condensed tannins content. In addition, behaviour in phenolics of samples group by colour were Grayish Orange ≥ Slightly Desaturated Orange ≥ Very Soft Orange while in antioxidant capacity was Grayish Orange ≥ Slightly Desaturated Orange = Very Soft Orange. Finally, the ten evaluated sorghum genotypes grown in Nuevo León, Mexico, were in agreement with most of the data available in literature for content of phenolics and levels of antioxidant capacity of sorghum genotypes from different regions around the world, although data variation among different reports is mainly attributed to solvent and technique used for extraction.

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### Tables

| Table 5. Antioxidant capacity of sorghum genotypes grouped by colour |
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| **Genotype** | **Antioxidant capacity (μmolTE g⁻¹)** |
| | **DPPH** | **ABTS** | **FRAP** |
| **GO** | 2.37±0.16 | 32.69±0.45 | 4.88±0.05 |
| **SDO** | 1.80±0.25 | 35.30±0.59 | 5.70±0.35 |
| **VSO** | 2.37±0.16 | 32.69±0.45 | 4.88±0.05 |
| **Values with different letters within column are significantly different (p≤0.05). Data are expressed as means values of three samples ± standard deviation** |

| Table 6. Antioxidant capacity of sorghum genotypes grouped by colour |
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| **Genotype** | **Number of genotypes** | **Antioxidant capacity (μmolTE g⁻¹)** |
| | **DPPH** | **ABTS** | **FRAP** |
| **VSO** | 5 | 1.83±0.52 | 44.21±18.79 | 3.94±1.03 |
| **SDO** | 5 | 4.33±3.90 | 38.51±6.99 | 6.16±2.96 |
| **GO** | 2 | 2.62±0.06 | 12.73±3.76 | 6.34±3.81 |
| **Values with different letters within column are significantly different (p≤0.05). VSO, SDO and GO are expressed as means value of nine, fifteen and six samples ± standard deviation respectively** |
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