Emirates Journal of Food and Agriculture. 2016. 28(10): 713-724
doi: 10.9755/ejfa.2016-02-156
http://www.ejfa.me/

REGULAR ARTICLE

Chemical profiling combined with multivariate analysis of unfractionated kernel-derived extracts of maize (*Zea mays* L.) landraces from central Colombia

Yeraldine Velásquez-Ladino, Roberto Quiñones, Ericsson Coy-Barrera*

Bioorganic Chemistry Laboratory, InQuiBio Group, Faculty of Basic and Applied Science, Universidad Militar Nueva Granada, AA49300, Cajicá-Cundinamarca, Colombia

ABSTRACT

Unfractionated extracts from twenty-five colored and uncolored maize (*Zea mays* L.) kernel landraces collected in Colombia were characterized according to tristimulus colorimetry parameters (*L*, *C* and *h*), antioxidant capacity (DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP), total phenolic, flavonoids and anthocyanins contents (TPC, TFA and TAC, respectively), and RP-HPLC-MS-DAD analysis. Good correlations were found between TPC values and antioxidant capacity. All chemical data were also correlated using multivariate analysis in order to observe chemical variations and identify patterns. The multivariate analyses on quantitative and antioxidant activity data of all landraces separately resulted in differentiation regarding chemical data and origin. In addition, the clustering on chromatographic data using chemometrics was found to be correlated with quantitative values, antioxidant capacity, color and origin, indicating metabolite variability among extracts. The results therefore indicated that chemical composition can be considered a crucial factor to differentiate landraces as well as determine their variability regarding relative metabolite contents. TPC values and isoquercetin and cyanidin malonylglucoside relative contents were found to be the most discriminating factors among landraces. The present work constitutes the first study focused on chemical profiling combined with multivariate analysis as a tool for discriminating colored *Z. mays* kernels.

Keywords: Anthocyanins; Antioxidants; Multivariate data analysis; Phenolics; HPLC-DAD-MS

INTRODUCTION

In the last decade, there are a lot of studies focused on colored maize (*Zea mays* L.) (mostly purple or blue) related to the determination of anthocyanin content, antioxidant capacity, the technified production of some genotypes, and the use of its benefits for the human consumption (Žilić et al., 2012; Ramos-Escudero et al., 2012). Latin American countries such as Peru, Mexico and Bolivia have a meaningful trajectory in the study of corn-derived anthocyanins by working with native landraces, varieties, and phenotypes. Other countries such as China and Turkey, where the corn is introduced, have also developed several studies on colored *Z. mays* (Yang and Zhai, 2010; Tanyolaç et al., 2007).

Colombia is one of the small producers of corn in the world. Its main maize production is performed through traditional procedures instead technified systems, only focused on yellow and white corn genotypes (Garcia et al., 2014). However, although there also is a production of some varieties of colored corn (purple, blue, red, and brown), the divulgation of this kind of materials to Colombian consumers and customers is limited and unusual. Only very few research activities on chemical composition on Colombian colored corn landraces are performed. So far, there are no records of any previous study on colored corn (*Z. mays*) from cundiboyacense region in Colombia. Therefore, the present study is focused on contributing to the chemical characterization of colored kernel landraces produced in Colombia in order to divulgate their existence and discrimination in the country. The characterization of a collection of landrace kernels was performed through quantitative coloring parameters (tristimulus colorimetry). Additionally, above-mentioned characterization was complemented by quantitative metabolite analyses (phenolics, flavonoids and anthocyanins) of the kernel-derived unfractionated

***Corresponding author:**
Ericsson Coy-Barrera, Bioorganic Chemistry Laboratory, InQuiBio Group, Faculty of Basic and Applied Science, Universidad Militar Nueva Granada, AA49300, Cajicá-Cundinamarca, Colombia. E-mail: ericsson.coy@unimilitar.edu.co

Received: 12 February 2016; **Revised:** 15 August 2016; **Accepted:** 31 August 2016; **Published Online:** 31 August 2016

extracts as well as the determination of antioxidant capacity and LC-based fingerprinting in combination with chemometrics. The easily-acquiring parameters evaluated in the present study could be considered useful in the selection/authentication of maize phenotypes/landraces to benefit the expansion of such industry in Colombia and other producing countries for ensuring good-quality products. Thus, the results will serve as reference about chemical information for further analytical food chemistry studies directed to the authentication of colored *Z. mays* as previously mentioned in a review (Arvanitoyannis and Vlachos, 2009). The combination of profiling (i.e. chemical characterization) and multivariate analysis is firstly reported in the present work as an approach for differentiating colored *Z. mays* kernels.

MATERIALS AND METHODS

Reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), quercetin, gallic acid, cyanidin-3-glucoside, and potassium persulfate were purchased from Sigma-Aldrich Chemical Co. Folin-Ciocalteau reagent, BHT (2,6-di-tert-butyl-4-hydroxytoluene), formic acid, acetone, ethanol and methanol were purchased from Merck Chemical Co.

Sampling and classification

A random sampling of landraces was performed in several local farms (maize cultivated by local population) in the Santander, Cundinamarca and Boyacá departments (Colombia), collecting phenotypically different samples (n=23) related to the colored and uncolored maize. Among samples, a white corn (obtained from a local market) and a purple corn (from Perú) were used as negative and positive controls, respectively. These materials were classified according to the origin, phenotypic characteristics (such as color and grain shape as researcher perception), and the measurement of grain color through tristimulus colorimetry CIELCH, employing the colorimeter MiniScan Spectrocolorimeter Hunter Lab (Garcia-Noguera et al., 2014).

Extraction

Fifty grains of each raw corn landrace were pulverized with a grinder. The resulting flour was separately kept in capped glass flasks at -20°C prior to use. Different extraction systems were used in order to determine the best extraction system for phenolics removal, such as acetone, ethanol, water, 0.5% formic acid in ethanol, 0.5% formic acid in water. 5 g of flour were then disposed in erlenmeyers with the corresponding solvent (40 mL) for 24 hours in vortex, and then filtered, washed and diluted to the mark with the same solvent into a 50.00 mL volumetric flask, and mixed thoroughly.

Total phenolics content (TPC)

TPC in extracts were determined by the Folin-Ciocalteau method with some modifications (Bernal et al., 2013). An extract aliquot (200 µL) was mixed with freshly, diluted (1:10) Folin-Ciocalteau reagent (400 µL). The mixture was then kept during 3 min and 7.5% Na_2CO_3 (1500 µL) was added. After 2 h of incubation at room temperature in the dark, absorbance at 765 nm was measured using a Genesys 20 spectrophotometer. Each measurement was performed in triplicate. TPC was calculated as mg gallic acid equivalents per 100 g dried material (mg GAE/100 g dm) by employing a calibration curve.

Total flavonoids content (TFC)

TFC in extracts were determined by the aluminum(III) complexation method with some modifications (Bernal et al., 2013). An extract aliquot (1000 µL) was mixed with ethanol (800 µL), 10% aluminum chloride (200 µL), and 0.1 M sodium acetate (200 µL). The mixture was homogenized in vortex and kept in darkness for 40 min. Absorbance was measured at 420 nm using a Genesys 20 spectrophotometer. Each measurement was performed in triplicate. TFC was calculated as mg quercetin equivalent per 100 g dried material (mg QE/100 g dm) by employing a calibration curve.

Total anthocyanins content (TAC)

TAC in extracts was determined by the pH-differential method (Lee et al., 2005). Briefly, an aliquot (1500 µL) of each extract was separately mixed with pH 1 buffer (1500 µL, 0.025M potassium chloride). Another aliquot (1500 µL) of each extract was also separately added to pH 4.5 buffer (1500 µL, 0.4 M sodium acetate). Absorbance was measured at 520 and 700 nm, respectively. Each measurement was performed in triplicate. TAC was calculated as mg cyanidin 3-*O*-glycoside equivalent per 100 g dried material (mg C3GE/100 g dm) as follows:

$$\text{mg C3GE/100 g dm} = \frac{A \times MW \times DF \times V \times 10^3}{\varepsilon \times l \times SW}$$

Where $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin 3-*O*-glycoside (C3G); DF = dilution factor; V = total volume of sample solution after extraction, in L; 10^3 = factor for conversion from g to mg; ε (molar extinction coefficient) = 26,900 $L \cdot mol^{-1} \cdot cm^{-1}$, for C3G; l = path length, in cm; and SW = sample weight used for extraction, based on 100 g dried material.

Determination of antioxidant capacity

DPPH• free radical scavenging ability assay

The ability to scavenge DPPH• free radicals was determined based on a colorimetric method (Bernal et al., 2013). A DPPH stock solution (100 μM) was prepared. An aliquot (100 μL) of each extract was separately allowed to react with DPPH (1900 μL) for 1 h in the dark. Each measurement was performed in triplicate. Absorbance was then measured at 517 nm. BHT was used as positive control. The ability to scavenge DPPH• radical was expressed as μM Trolox per g dried material (μM TE/g dm) (Yang and Zhai, 2010).

ABTS•+ free radical scavenging ability assay

The ability to scavenge $ABTS^{\bullet+}$ free radicals was determined based on a colorimetric method method (Bernal et al., 2013). An ABTS solution (0.7 mM) was mixed with potassium persulfate (5.1 mM) at 1:1 ratio during 16 h in the dark at room temperature to produce the $ABTS^{\bullet+}$ stock solution. This solution was diluted to give an absorbance of 0.760 at 734 nm with ethanol prior to be used. An aliquot (100 μL) of each extract was separately mixed with $ABTS^{\bullet+}$ solution (1900 μL). The absorbance was then measured at 734 nm after 4-5 min of reaction in the dark at room temperature. Each measurement was performed in triplicate. BHT was used as positive control. The ability to scavenge $ABTS^{\bullet+}$ radical was expressed as μM Trolox per g dried material (μM TE/g dm) (Yang and Zhai, 2010).

Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power was determined based on a colorimetric method (Bernal et al., 2013). Briefly, FRAP reagent was prepared mixing acetate buffer (pH 3.6; 300 mM), TPTZ (10 mM) in HCl (40 mM), and $FeCl_3 \bullet 6H_2O$ (20 mM) at 10:1:1 ratio. Reagent was storage at 37°C before use. An appropriate dilution of sample solution (100 μL) was mixed with FRAP reagent (1900 μL). The absorbance was then measured at 593 nm after 30 min of reaction at 37°C. BHT was used as positive control. Ferric reducing antioxidant power was expressed as μM Trolox per g dried material (μM TE/g dm) (Yang and Zhai, 2010).

LC-UV-DAD-ESI-MS analysis

LC-UV-DAD-ESI-MS chromatograms were recorded on a Shimadzu LCMS-2020 Liquid Chromatograph coupled to a Mass Spectrometer equipped with a multiwavelength detector (DAD). A Shimadzu 110A C18 column (50 x 4.6 mm; 5 μm) was employed at flow rate of 0.3 mL/min. The mobile phase consisted of A (1% aqueous formic acid) and B (1% formic acid in acetonitrile) and the gradient varied as follows: 0% B from 0 to 5 min, 0 to 35% B from 5 to 23 min, 35% B from 23 to 28 min, 35 to 0% B from 28 to 33 min, and 0% B from 33 to 38 min. The monitoring wavelengths were selected at 270 and 520 nm. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative modes. Voltage detector of 1.5 kV was used. CDL and Heat Block temperature at 250°C was employed. Nebulization gas flow was set at 1.5 L/min. Samples were diluted in 5:100 ratio and then 5 μL was injected (Bernal et al., 2016).

Statistical analysis

All samples were analyzed by triplicate and the quantitative results were expressed as mean ± standard deviations. The results were analyzed by ANOVA and the Tukey test at the level of 95% of confidence level within a Randomization-based design. Correlation between the different assays was evaluated by a multiple correlations test using Pearson coefficients. All of these statistical analyses were performed using R 3.00 (R Development Core Team, 2013) software package.

In addition, quantitative and chromatographic data were analyzed by multivariate analysis. Chromatographic data were handled within a data set. Chromatographic profiles monitored at 270 nm as function of time (baseline-corrected using FastChrom algorithm, (Johnsen et al., 2013)) were exported as 2D ASCII files for each sample and a matrix of 1502 x 25 points was then built. Two-way array raw data were submitted to chromatographic peak alignment as first step of pretreatment prior to the statistical analysis. Correlation optimized warping (COW) algorithm was employed to the alignment process (Nielsen et al., 1998). Principal Component (PCA), partial least square discriminant (PLS-DA) and orthogonal partial least square (OPLS) analyses were carried out on the two-way array after corresponding above-mentioned pre-processes. Results were presented as score, loadings, and line plots. All data analyses were performed using MATLAB R2013a (The Mathworks, Inc., Natick, MA, USA) and SIMCA 13.0.3 (Umetrics Inc., San Jose, CA, USA) software packages on a PC with an Intel Core i5 processor containing 8 GB RAM and running under Microsoft Windows 7.

RESULTS AND DISCUSSION

Classification of samples

The collected corn landraces (n=23) were classified according to the origin and apparent color. A white and a purple corn were also included within the samples group. The landraces (coded as M1 to M25) exhibited different colors (from yellow to dark purple). The uncolored corn (M21) was taken as negative control for the anthocyanins content. The color of each kernel was measured through a Hunter Lab colorimeter on parameters of C (chroma), h (hue or tone) and L (luminosity). The obtained values of each measurement are presented in Table 1.

Table 1: Total phenolics, flavonoids and anthocyanins contents of 25 maize (*Zea mays* L.) kernel landraces including the tristimulus colorimetry measurements data (means±standard deviation)

Phenotype	M1	M2	M3	M4	M5
					
Origin	Boyacá	Boyacá	Boyacá	Boyacá	Boyacá
Cie *L*	34.36±0.73	34.77±1.12	33.38±3.18	66.25±7.09	28.66±0.91
C	1.31±0.31	2.03±0.52	13.38±4.58	36.46±4.28	7.20±0.65
h	63.72±28.43	75.65±5.34	282.62±51.96	71.48±1.10	59.90±3.52
TPC[1]	772.31±20.62^{lmn}	881.79±24.64^{ghi}	815.81±39.85^{jkl}	726.01±13.04^{n}	745.00±12.35^{mn}
TFC[1]	19.06±1.55^{hij}	20.54±2.57^{ghi}	15.83±1.25^{ijk}	8.86±2.21^{mn}	10.87±3.00^{lmn}
TAC[1]	14.69±0.01^{f}	13.6±0.01^{g}	22.55±0.02^{d}	1.88±0.03^{mn}	12.10±0.43^{hi}
Phenotype	**M6**	**M7**	**M8**	**M9**	**M10**
					
Origin	Cundinamarca	Boyacá	Perú	Boyacá	Boyacá
Cie *L*	55.22±4.68	88.78±1.56	14.17±1.23	37.48±11.55	34.28±3.46
C	28.09±12.72	25.33±2.15	4.33±0.63	5.43±1.34	2.45±0.73
h	327.52±9.27	88.32±0.95	8.17±2.44	77.49±3.93	207.37±56.94
TPC[1]	872.32±23.03^{hij}	1007.14±6.49^{f}	1633.43±32.97^{a}	830.42±23.41^{ijk}	891.68±30.97^{gh}
TFC[1]	9.69±2.86^{mn}	9.85±1.06^{mn}	67.19±0.36^{ab}	15.58±1.29^{ijk}	19.15±1.49^{hij}
TAC[1]	1.99±0.03^{mn}	n.d.	12.50±0.05^{h}	14.20±0.09^{fg}	11.80±0.08^{i}
Phenotype	**M11**	**M12**	**M13**	**M14**	**M15**
					
Origin	Boyacá	Boyacá	Boyacá	Boyacá	Boyacá
Cie *L*	32.86±1.67	27.40±0.89	29.32±0,65	57.65±1.88	61.44±1.77
C	3.59±0.30	30.90±1.45	30.55±1,28	37.37±1.81	44.21±2.11
h	118.05±9.51	27.30±1.48	28.69±1,55	58.12±0.99	63.32±1.89
TPC[1]	1187.14±84.31^{d}	1316.17±27.52^{c}	1539.84±71.42^{b}	1258.57±64.63^{c}	538.00±11.17^{o}
TFC[1]	27.48±3.57^{ef}	50.84±2.98^{c}	32.49±2.57^{d}	15.95±1.63^{ijk}	22.37±2.92^{gh}
TAC[1]	15.60±0.05^{e}	6.61±0.10^{j}	5.17±0.13^{k}	2.33±0.08$^{\mu}$	1.89±0.11^{mn}
Phenotype	**M16**	**M17**	**M18**	**M19**	**M20**
					
Origin	Santander	Santander	Santander	Santander	Cundinamarca
Cie *L*	17.83±0.85	21.57±1.51	21.47±0.81	19.98±1.05	92.30±1.35
C	3.17±0.77	9.64±0.36	9.16±1.82	8.78±0.78	58.31±8.27
h	22.62±13.13	25.46±6.80	10.93±2.61	15.26±3.27	50.45±6.00
TPC[1]	1163.11±34.43^{d}	853.59±13.56^{hij}	1130.19±24.98^{d}	1071.76±30.48^{e}	733.52±24.16^{n}
TFC[1]	68.08±2.99^{a}	24.23±1.14^{fg}	62.67±1.09^{b}	28.50±2.15^{def}	18.63±2.01^{hij}
TAC[1]	67.64±1.04^{b}	22.88±0.43^{d}	69.81±0.87^{a}	44.59±1.30^{c}	1.45±0.09^{n}

(Contd...)

Table 1: ***(Continued)***

Phenotype	M21	M22	M23	M24	M25
					
Origin	Cundinamarca	Cundinamarca	Cundinamarca	Cundinamarca	Cundinamarca
Cie *L*	72.49±0.71	58.98±1.94	66.25±6.24	58.52±3.12	82.16±1.86
C	21.29±2.07	36.44±2.41	66.77±2.10	49.85±4.19	21.01±1.62
h	90.17±0.37	57.78±0.94	68.46±4.87	63.00±1.03	84.62±0.34
TPC[1]	562.91±8.91^{o}	840.19±25.40^{hijk}	936.34±21.25^{g}	792.19±3.78^{klm}	749.70±10.79^{mn}
TFC[1]	6.63±0.71^{n}	11.55±0.74^{klm}	31.91±1.95^{de}	17.17±1.79^{ij}	9.81±2.52^{mn}
TAC[1]	n.d.	4.17±0.43^{l}	4.48±0.15^{l}	2.33±0.86^{m}	5.67±0.09^{k}

[1]TPC: Total phenolics content; TFC: Total flavonoids content; TAC: Total anthocyanins content in mg/100 g dry sample; *L*: Luminosity; *C*: Chroma; *h*: Tone. Values with the same letter expresses that they are not significantly different (p<0.05) according Tukey test; n.d., not detected.

Fig. 1 represents the CIELCH polar-coordinate system (parameters *C* as radial magnitude with values ranged from 0 to 100 and parameter *h* as angular magnitude ranged from 0° to 360°. Luminosity values, *L*, are described in Table 1, where 0 is black and 100 is white). From measured kernels, 21 of them are located in *h* values ranged from 0° (red) to 90° (yellow), and other 2 samples are positioned between 270° (blue) and 360° (red). Darker grains (having *C* <10) are situated in the purple oval (corresponding to M1, M5, M8-M11, and M16-M18). Less saturated grains (having *C* >50) were distributed along the above-mentioned two quadrants.

Prior to the chemical analyses and in order to properly compare the composition among samples, the optimal extraction system was firstly established. Five polar solvent systems were then tested to achieve the best phenolics removal. Significant differences between these TPC, TFC and TAC values were evaluated by ANOVA test at 95% level (data not shown). The presence of formic acid in the extraction system resulted in higher TPC and TAC values. However, this effect was more marked for water. Acetone, ethanol and water were found to be not able for extracting phenolics than that of acidulated water. Therefore, 0.5% formic acid was selected as extraction system.

Total phenolic, total flavonoid and total anthocyanin contents

Total phenolic, total flavonoid and total anthocyanin contents (TPC, TFC, and TAC, respectively) of different Colombian landraces of *Z. mays* kernels (colored, light colored, and uncolored) were measured, whose values are exposed in Table 1. Fig. 2 showed the behavior across all samples organized by origin and TPC values, including the TAC and TFC values. The maize landraces M8, M11-M14, M16 and M18 exhibited the higher TPC values (1100-1650 mg GAE/100 g dm) while the landraces M15 (light red) and M21 (white) showed the lower TPC values (< 600 mg

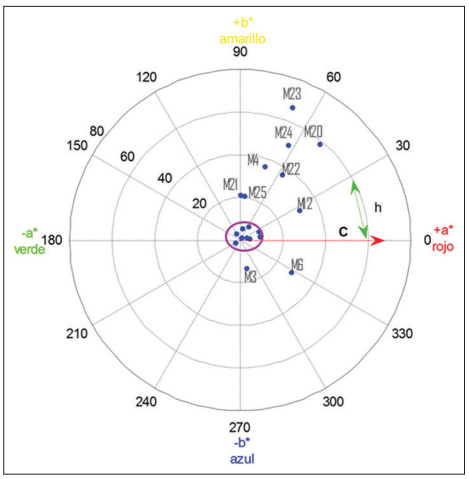

Fig 1. Chromatic characterization on parameters *C* (chroma) and *h* (tone) of colored grains of *Z. mays* in a CIELCH polar-coordinate system.

GAE/100 g dm). Some previous studies have described that red and blue-colored maize exhibited higher phenolic contents in comparison to that of light colored corns (Hu and Xu, 2011). This fact is partially in agreement with findings of this study since the M13 (red) and M8 (purple) samples demonstrated to have significantly different TPC values from the other samples (p<0.05). However, the colored samples M4, M5, and M20 exhibited lower TPC values in comparison with the other colored landraces. In general, the maize landraces from Boyacá exhibited higher TPC values compared to those of other origins.

Regarding TFC values, the dark colored maize landraces M8, M12, M16, and M18 presented higher TFC values (50-70 mg QE/100 g dm), while the M4-M7, M21, M22, and M25 had lower TFC values (< 12 mg QE/100 g dm). The results indicate that the flavonoids content is independent from coloration and origin, but the uncolored samples (yellow and white) presented the lowest flavonoids content.

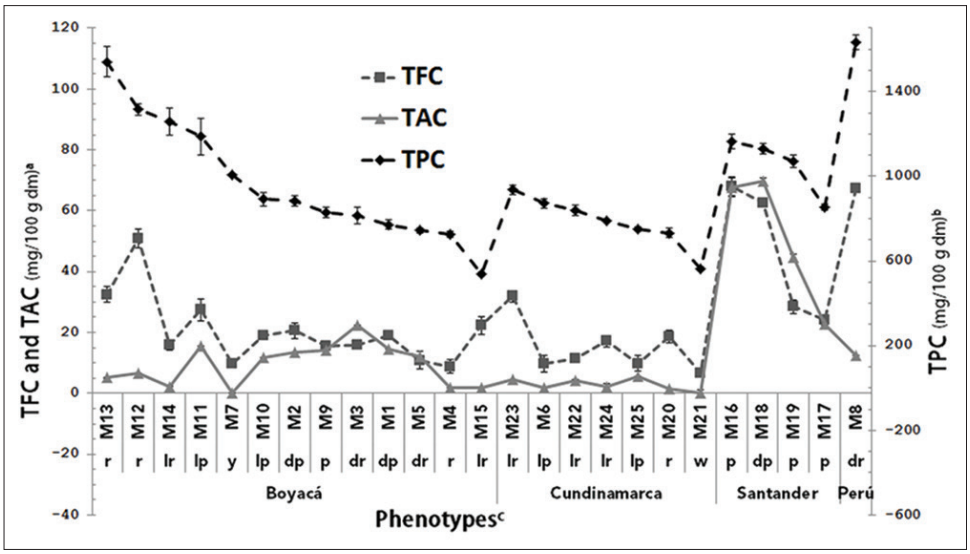

Fig 2. Total phenolics, flavonoids and anthocyanins contents (TPC, TFC, TAC, respectively) of unfractionated kernel-derived extracts of *Z. mays*; [a]TFC and TFC expressed as mg quercetin/100 g dried material and mg cyanidin 3-*O*-glycoside/100 g dried material, respectively; [b]TPC expressed as mg gallic acid/100 g dried material; [c]Landraces were organized according to the origin and the apparent color (r=red, dr= dark red; lr=light red; p=purple; dr= dark purple; lp=light purple; y=yellow; w=white). All measurements were done in triplicate.

The accumulation of anthocyanin-related compounds is one of the responsible of the pigmentation of maize kernel (Hallauer, 2001). Because of that, as expected, those kernels with dark color exhibited higher TAC values, such as M16, M18 and M19 (45-70 mg C3GE/100 g dm), and the light colored and uncolored showed the lowest anthocyanin contents (< 8 mg GAE/100 g dm). However, other colored landraces exhibited significant intermediate ($p<0.05$) TAC values (10-23 mg C3GE/100 g dm) in comparison to that of dark colored kernel landraces. In general words, the behavior among samples is similar between TPC and TFC values, but TAC values behave independent to those of TPC and TFC. These results are in agreement with data reported for uncolored and colored maize pheno- and genotypes (Žilić et al., 2012; Kuhnen et al., 2011; Lopez-Martinez et al., 2001).

Antioxidant capacity

Scavenging ability (against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals) and FRAP assays were used for the evaluation of the antioxidant capacity of all unfractionated extracts. The results indicate that extracts showed a similar behavior regarding the assay used to, as shown in Fig. 3a. In this sense, DPPH$^{\bullet}$ assay showed the highest values (expressed as Trolox equivalent antioxidant capacity, TEAC, in µM Trolox/g dm) compared to ABTS$^{\bullet+}$ and FRAP assays. Extracts with high antioxidant capacity by three methods were M8, M12, M13, M16, M18 and M19. However, landrace M13 and M8 exhibited the best DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ free radical scavenging abilities, respectively, while landrace M12 showed the best reducing power. Landraces with lowest antioxidant capacity were M4 and M21 for DPPH$^{\bullet}$ assay, and M4, M15 and M21 for FRAP and ABTS$^{\bullet+}$ assays, respectively. On comparing the antioxidant capacity of the positive control (butylated hydroxytoluene, BHT), whose values for DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP were 1438, 277 and 802 uM Trolox/g BHT, respectively, only landraces M8, M12, M13, M16, M18 and M19 showed the higher antioxidant capacity to that of BHT in the DPPH$^{\bullet}$ and FRAP assays, while only kernels M8 and M13 exhibited greater antioxidant activity to that of BHT in the ABTS$^{\bullet+}$ assay. The correlation analysis between TPC and antioxidant capacity by three assays showed a proportional relationship between them, with correlation coefficients of 0.801, 0.738 and 0.841 for TPC *vs* DPPH$^{\bullet}$, TPC *vs* FRAP and TPC *vs* ABTS$^{\bullet+}$, respectively (Fig. 3b), which is in agreement with previous studies (Das et al., 2014).

Chromatographic profiling

LC-DAD-UV profiles (monitored at 270 nm) revealed the presence of 19 main components with phenolic-related structure in the kernel-derived extracts (Fig. 4). In order to get structural information regarding these components, LC-ESI-MS analyses were achieved. Positive ion mode mass spectra for the main compounds exhibited the respective pseudomolecular ($[M+H]^+$) and fragments ion peaks as reported in previous studies for *Z. mays* (Yang and Zhai., 2010; Zhao et al., 2008; Lozovaya et al., 2006), being consistent with the phenolics, flavonoids and anthocyanins listed in the Table 2. In order to get more information on anthocyanin composition, the chromatogram at 520 nm was also recorded. In this chromatogram, nine main anthocyanins were detected, whose tentative identification is therefore showed in Table 2 as well. The

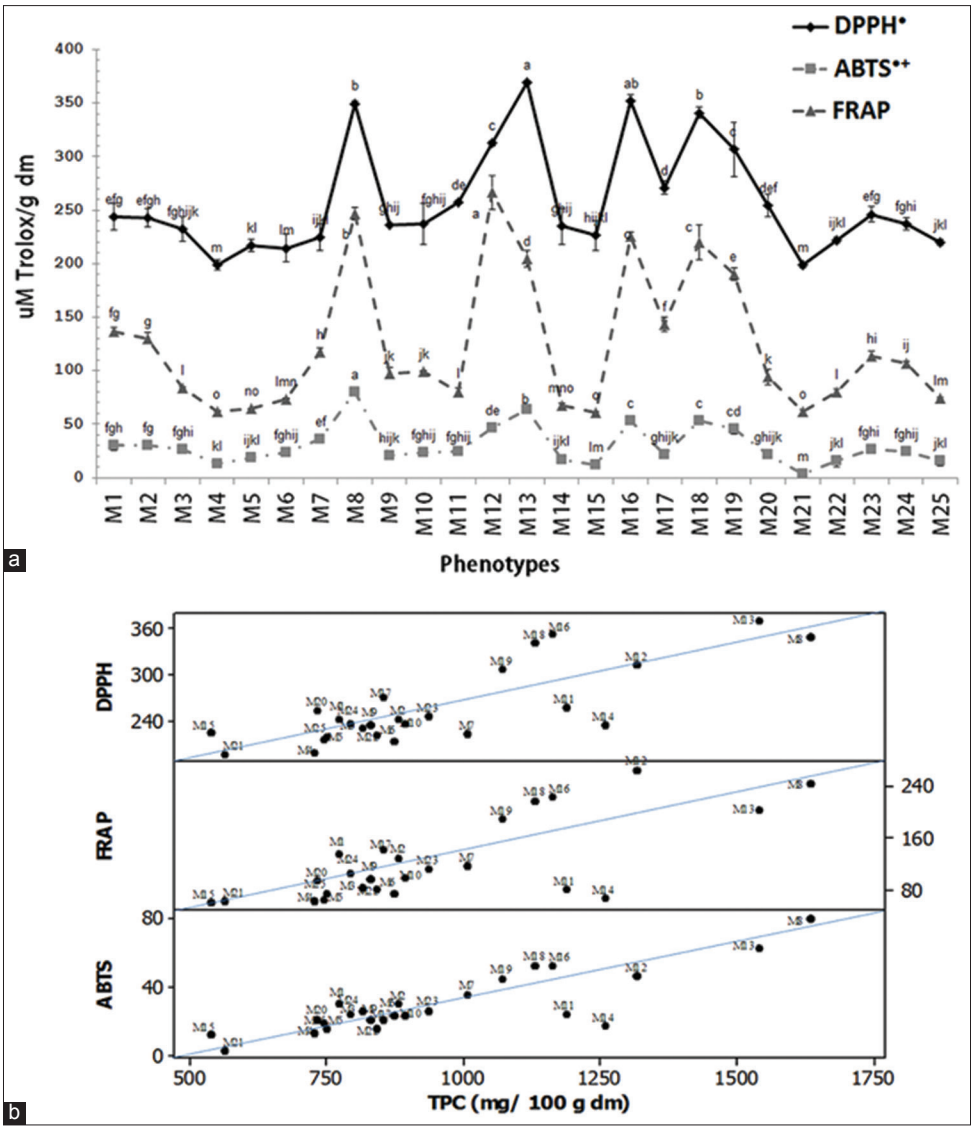

Fig 3. (a) Antioxidant capacity by DPPH• and ABTS•+ free radical scavenging ability and FRAP Ferric reducing antioxidant power (FRAP) assays of *Z. mays* kernels. All measurements were done in triplicate. Values with the same letter expresses that they are not significantly different ($p<0.05$) according Tukey test. (b) Correlation analysis for kernel-derived extracts between TPC values and antioxidant capacity results.

main compounds in the most extracts were found to be two flavonoid glycosides (isoquercetin 6 and astragalin 7) and two anthocyanins (cyanidin malonylglucoside 14 and cyanidin succinylglucoside 17). Furthermore, important relative variations are presented among all unfractionated extracts, but purple colored landraces (M1, M2, M3, M9-M11, M16-M18) exhibited anthocyanin-rich profiles.

Multivariate statistical analyses

In order to discriminate the variability of the chemical analyses of all unfractionated extracts, an unsupervised clustering study based on principal component analysis was performed using total contents (TPC, TFC, and TAC) and antioxidant capacity (DPPH•, ABTS•+, and FRAP). The resulting PC1 *vs* PC2 score plot (Fig. 5a) exhibited six clusters (Groups 1-6). The first cluster (Group 1) involves the landraces M13 and M8 (red colored kernels), whose TPC and antioxidant capacity values were found to be the highest among test samples, but between these values were found to be significant different ($p<0.05$). M8 and M13 landraces have different origin and apparent color, but these chemical properties influenced on their discrimination. Similar trend was observed in Group 2, including landraces M11 and M14, whose TPC and antioxidant capacity values were found to be close (although significant different ($p<0.05$) between them), with distinct apparent color. The above-mentioned landraces exhibited significant differences ($p<0.05$) in TAC and TFC values, indicating that the discrimination of these kernels is not produced by color-derived properties. This fact is confirmed according to the PCA-derived loading plot (Fig. 5c), which indicates the strong influence by TPC values

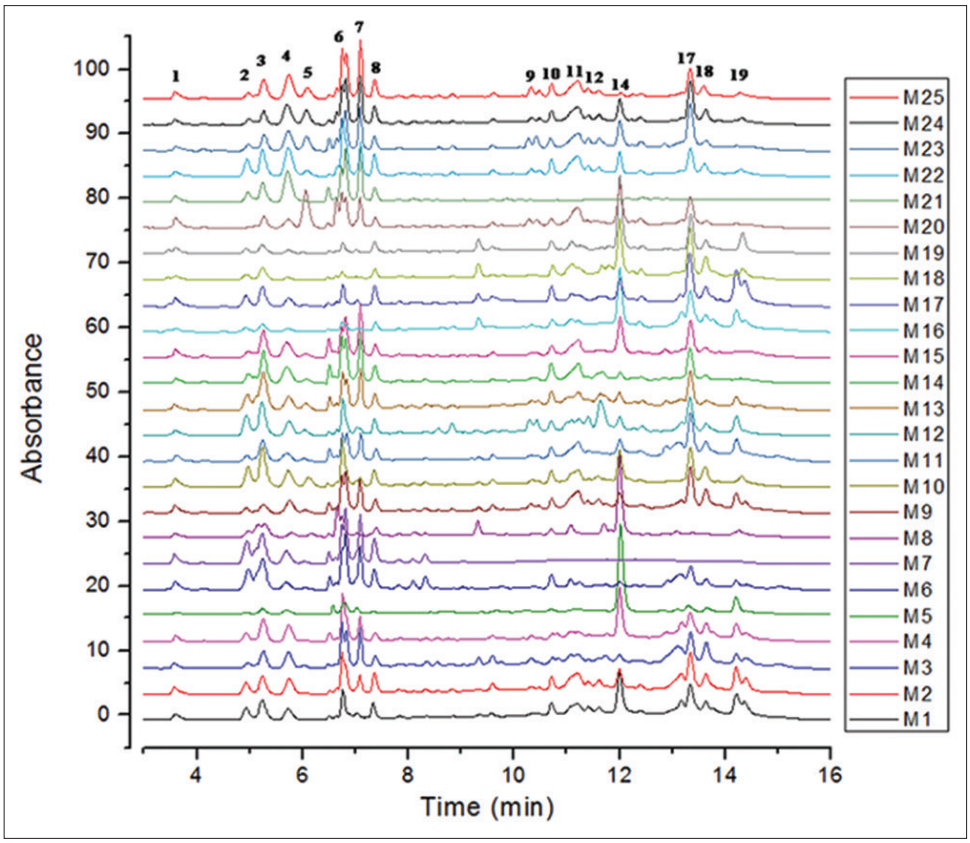

Fig 4. Chromatographic profiles of unfractionated extracts of *Z. mays* kernels monitored at 270 nm. Compounds: caffeic acid 1, chlorogenic acid 2, caffeoyl-Glu 3, feruloyl-Glu 4, sinapoyl-Glu 5, isoquercetin 6, astragalin 7, quercetin-Rut 8, cyanidin-Glu 9, isorhamnetin-Glu 10, pelargonidin-Glu 11, peonidin-Glu 12, quercetin-diGlu 13, cyanidin-Mal-Glu 14, pelargonidin-diGlu 15, pelargonidin-Mal-Glu 16, cyanidin-Suc-Glu 17, cyanidin-EthylMal-Glu 18, peonidin-Mal-Glu 19. (Glu: Glucoside; Mal: Malonyl; Rut: Rutinoside; Suc: Succinyl).

Table 2: Compounds identified in unfractionated *Zea mays* extracts by HPLC-ESI-MS

No	t_R (min)	Compound	Molecular formula	$[M+H]^+$ (*m/z*)	Fragment ions (*m/z*)
1	3.55	Caffeic acid	$C_8H_8O_4$	181.2	-
2	4.94	Chlorogenic acid	$C_{16}H_{18}O_9$	355.4	181.0
3	6.07	Caffeoyl-Glu	$C_{15}H_{18}O_9$	343.1	181.0
4	5.23	Feruloyl-Glu	$C_{16}H_{20}O_9$	357.4	195.1
5	5.70	Sinapoyl-Glu	$C_{17}H_{22}O_{10}$	387.3	225.1
6	6.74	Isoquercetin	$C_{21}H_{20}O_{12}$	465.5	303.2
7	7.06	Astragalin	$C_{21}H_{20}O_{11}$	449.3	287.2
8	7.33	Quercetin-Rut	$C_{27}H_{30}O_{16}$	611.5	303.2, 327.2
9	10.33	Cyanidin-Glu	$C_{21}H_{21}O_{11}$	449.2	287.2
10	10.68	Isorhamnetin-Glu	$C_{22}H_{22}O_{12}$	479.2	317.2
11	11.15	Pelargonidin-Glu	$C_{21}H_{21}O_{10}$	433.2	271.1
12	11.27	Peonidin-Glu	$C_{22}H_{23}O_{11}$	463.1	301.1
13	11.55	Quercetin-diGlu	$C_{27}H_{30}O_{17}$	627.2	303.2, 343.2, 465.1
14	11.98	Cyanidin-Mal-Glu	$C_{24}H_{23}O_{14}$	535.2	287.2, 449.2
15	12.28	Pelargonidin-diGlu	$C_{27}H_{31}O_{15}$	595.2	255.1, 343.2, 433.2
16	13.29	Pelargonidin-Mal-Glu	$C_{24}H_{23}O_{14}$	519.1	271.1, 433.2
17	13.33	Cyanidin-Suc-Glu	$C_{25}H_{25}O_{14}$	549.2	287.2, 449.2
18	13.60	Cyanidin-EthylMal-Glu	$C_{26}H_{27}O_{14}$	563.2	287.2, 449.2
19	14.20	Peonidin-Mal-Glu	$C_{25}H_{25}O_{14}$	549.1	301.1, 463.1

Glu: Glucoside; Mal: Malonyl; Rut: Rutinoside; Suc: Succinyl

for the samples clustered in the corresponding quadrant. Kernels clustered in Group 3, including samples M12, M16, M18 and M19, also exhibited high TPC and TFC values as well as the highest antioxidant capacity. Although landraces M16, M18 and M19 demonstrated to have high TAC values and the apparent color is similar, landrace M12

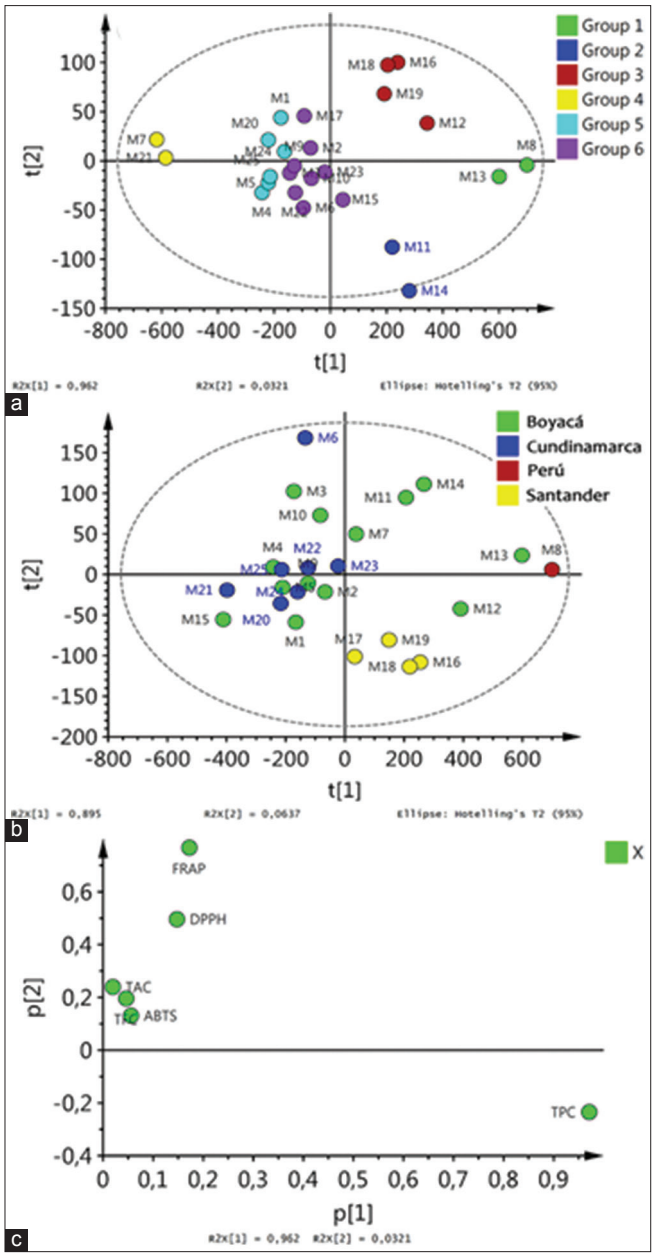

Fig 5. Multivariate analyses on total contents and antioxidant capacity data of *Z. mays* kernels; (a) PCA-derived PC1 *vs* PC2 score plot; (b) PLS-DA-derived PC1 *vs* PC2 score plot according the classification based on origin; (c) PCA-derived PC1 *vs* PC2 loading plot on total contents and antioxidant capacity data.

showed a very low TAC value. In this point, although M12 was gathered in group 3 by the statistical algorithm, the apparent color is similar to that of M13, but the chemical properties are significant different ($p<0.05$). Landraces clustered in Group 5 and 6 displayed different TPC values and TFC values, but the highest TAC values and moderate antioxidant activity. In these groups, the discrimination is most produced by the TAC values, since the loading plot (Fig. 5c) shows the influence of the TAC values for the samples clustered in Group 5 and 6 (TAC is located at right side of this loading plot). As expected, Group 4 (including the uncolored kernels M7 and M21) was separated from the other samples because of the absence of their TAC values.

With the aim to observe the behavior on the chemical analyses for all landraces regarding origin, a PLS-DA model was constructed with these data. The PC1 vs PC2 PLS-DA-derived score plot (Fig. 5b) exposed the differentiation of the landraces. Although M12 and M13 were collected in Boyacá, the statistical algorithm on evaluated chemical data correlated these kernels to M8 from Perú. Kernels from Santander were successfully clustered in a distinct group but samples from Boyacá and Cundinamarca were not totally discriminated. Therefore, the origin was not a factor to be correlated with the chemical data of these origins.

In addition, multivariate analyses were performed on chromatographic data for identifying patterns among profiles. Prior to the statistical analyses, several data pre-processing techniques were performed on the raw data matrix in order to reduce noise and inconsistency for improving the data quality. The 25 chromatographic profiles of unfractionated extracts were therefore pre-processed in baseline correction, peak alignment, normalization and Pareto scaling. The shifts of retention time in chromatographic data were thus perfectly corrected as well as the between-sample alteration of the variables and the undesired effects due to dissimilar concentrations in the injected samples were respectively removed after pre-processing.

PCA was then performed on the pre-processed chromatographic data matrix (two-way array) for expressing the data in a way to emphasize their similarities and differences. The PCA-derived score plot (Fig. 6a) displays the landrace discrimination in five Groups according to the chromatographic profile (at 270 nm) data. Samples M5 and M8 were grouped by the presence of compound 14 as main component, whereas Group 2 and 3, involved purple and light colored kernels, respectively, were gathered in relation to the anthocyanin profile. Landraces clustered in groups 4 and 5 exhibited differences in some anthocyanin and phenolic-related compounds. Group 4 includes the kernels with the lowest relative contents of compound 14 and Group 5 involves landraces with the highest relative contents of compounds 6 and 7. The influence of some compounds in the extracts on the PC1 (47% total variance) is observed in the corresponding loading line (Fig. 6b). PC1 is most positively influenced by compound 14, whose influence is presented for clustering of all groups by its high and low/absence relative content. Similar strong effect was observed with compounds 6 (isoquercetin) and 8 (quercetin rutinoside) but in negative way. Compounds 2 (chlorogenic acid) and 4 (feruloyl glucoside) exerted a lower negative influence.

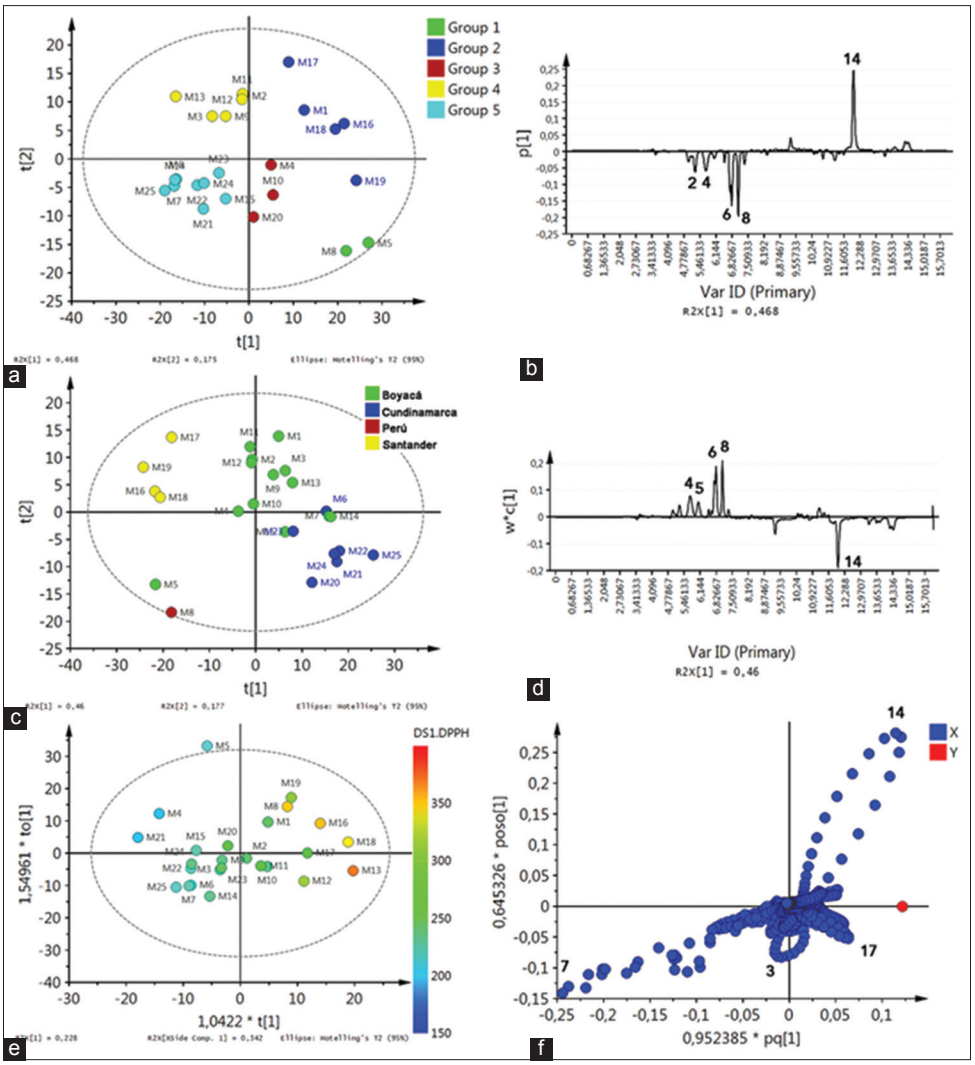

Fig 6. Multivariate analyses on chromatographic data (profiles at 270 nm) of *Z. mays* kernels; (a) PCA-derived PC1 *vs* PC2 score plot; (b) PCA-derived loading line; (c) PLS-DA-derived PC1 *vs* PC2 score plot according the classification based on origin; (d) PLS-DA-derived loading line; (e) OPLS-derived PC1 *vs* PC2 score plot (Y=DPPH•); (f) OPLS-derived PC1 *vs* PC2 loading plot.

In contrast, the PLS-DA using chromatographic data instead quantitative and antioxidant data afforded good differentiation according origin (Fig. 6c). Kernels from Santander were also differenced from the others, whereas landraces from Boyacá and Cundinamarca were mostly differenced. However, some landraces from Boyacá and Cundinamarca were found to be common between these origin classes. These kernels are related to the light colored samples collected in bordered zones between Boyacá and Cundinamarca. The compounds responsible of the PLS discrimination along PC1 (46% total variance) are exposed in the loading line (Fig. 6d), which were found to be 14 (negatively) and 4-6 and 8 (positively).

A supervised analysis was also performed on chromatographic data, using DPPH radical scavenging ability values as supervision variable. The OPLS-derived PC1 *vs* PC2 score plot (Fig. 6e) reveals a good regression along t[1] between chromatographic profile and antioxidant capacity of unfractionated extracts, starting from most potent extracts (M13, M18 and M16) until lowest active extracts (M4, M21 and M25). These results also demonstrated the close relationship between chemical composition and antioxidant capacity. The influence of the chemical components in extracts is exposed in the respective loading plot (Fig. 6f). Compound 14 was found to be the chemical constituent with the most influence on the DPPH scavenging ability for the most active extracts. Compound 17 exhibited a similar effect but in opposite way to that of 14. Astragalin (7) was found to be the constituent occurred in the extracts possessing the lowest DPPH scavenging ability. Finally, results indicated that the search of novel, holistic authentication methods allowing an adequate discrimination of edible samples is still required. Therefore, the present strategy resulted to be advanced on comparing the current differentiation procedures based on

few chemical properties/markers which commonly ignore minor changes in specific compounds. The differences in composition for Colombian *Z. mays* landraces could be interpreted/rationalized in significant quality differences in/for similar products. Therefore, a more effective quality assurance protocol for *Z. mays* landraces could be thus incorporated in further studies.

CONCLUSIONS

Tristimulus colorimetry parameters were compared to quantitative values but no good correlation was established. Whereas TPC values were found to be independent from kernel color, TFC values were not related with light colored and colored landraces, but keeping a relationship with uncolored samples. In addition, a close correlation was found between TPC and antioxidant capacity. The multivariate analyses on quantitative and antioxidant capacity data of all landraces resulted in good differentiation regarding quantitative data and modest discrimination concerning origin. TPC variable was found to be the most discriminating factor among landraces. The PCA on chromatographic data clustered five groups according the presence/absence of specific constituents. Isoquercetin and cyanidin malonylglucoside were found to be the compounds with the most influence in the PCA. Additionally, kernel origin could be used as a good classifying factor for discriminating the chromatographic profiles and cyanidin malonylglucoside was found to be the most responsible compound of the antioxidant capacity for the most active extracts. The multivariate analyses on the total raw data matrix from the LC-based fingerprints of Colombian *Z. mays* kernel landraces afforded a holistic strategy for analyzing selective differences in chemical components between landraces.

The present work demonstrates that the use of multivariate analyses combined with LC-based fingerprints offers an excellent method of analysis of kernel-derived unfractionated extracts, representing an excellent analytical tool for an easy, rapid, reproducible and accurate differentiation and authentication of grain-derived food samples as key step in quality control of maize and related samples as well as in chemometrics research.

Conflicts of interest

All authors declare no conflicts of interest

ACKNOWLEDGEMENTS

Authors thank MU Nueva Granada for the financial support. The present work is a product derived by the Project CIAS-939 financed by Vicerrectoría de Investigaciones at UMNG - Validity 2012.

Authors contributions

Authors contributed to the present work as described in the following: Kernel collection: RQ, ECB. Sample preparation, tristimulus colorimetry and quantitative analyses: YVL. HPLC and statistical analyses: ECB. Data analysis: YVL, ECB. Manuscript preparation and proofreading: YVL, RQ, ECB.

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