Comparative study of phenolic profile, antioxidant capacity, and color-composition relation of roselle cultivars with contrasting pigmentation

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Abstract

Roselle is a plant that accumulates anthocyanins significantly, hence its importance as food coloring and as a source of antioxidant compounds for human health. This study was aimed to determine phenolic composition and antioxidant capacity of methanolic extracts, and beverages obtained from roselle cultivars native to México (Negra, Sudan, Rosa and Blanca) with different degrees of pigmentation, and to establish the color-composition relationship. Chromatographic methods were used to determine phenolic compounds: flavanols, flavonols, benzoic, hibiscus and phenolic acids and two main anthocyanins (cyanidin 3-sambubioside and delphinidin 3-sambubioside), and antioxidant capacity was evaluated by ABTS and FRAP assays. Tristimulus colorimetry showed to be a useful technique for determination of color-composition relationship, leading to equations that allowed predicting anthocyanin content of roselle (R>0.84). Also a stepwise linear discriminant analysis (SLDA) was developed in order to classify roselle cultivars. Obtained mathematical model could be an important tool that can be used for colorimetric characterization of functional compounds used in food processing

Keywords: Antioxidant capacity, color-composition, *Hibiscus sabdariffa*, multivariate statistical analysis, phenolic compounds, tristimulus colorimetry.

Abbreviations: DIA: digital image analysis**,** Dp3Sa: delphinidin-3-sambubioside, Cy3Sa: cyanidin-3-sambubioside, LDA: linear discriminant analysis, SLDA: stepwise linear discriminant analysis, TA: total anthocyanins.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is a plant belongs to family Malvaceae, their chalices are recognized as an important source of phenolic compounds [1, 2, 3]. Mexico is one the

main roselle producing countries, where it is widespread consumed in beverage. This beverage is obtained by thermal infusion of dehydrated chalices [3]. It has been used in traditional medicine for treatment of some diseases because of its antioxidant properties [1, 2]. Roselle chalices are used worldwide for drinks production, as additives in cosmetics and pharmaceuticals [4]. Currently consumers demand healthy products, hence the importance of studies about extraction and purification of bioactive compounds such as phenolics from natural sources in order to identify phytochemicals for processing of food supplements or nutraceuticals, functional food ingredients and food additives [3]. There are few studies on roselle cultivars having as characteristic different pigmentation degree. Christian and Jackson [1] studied roselle grown in Jamaica, reporting that dark roselle chalices presented the highest anthocyanins content and antioxidant activity, followed by red chalices, while in the white ones, these compounds were not detected; these results were similar to reported by other authors [2,5]. Mexico is one the main roselle producing countries, recently breeding programs have generated new cultivars with different pigmentation levels, volatile compounds of these plants have been evaluated [5]. The aim of this study was to determine phenolic profile and antioxidant capacity of both methanolic extracts and beverages, obtained from roselle cultivars with contrasting pigmentation (Negra, Sudan, Rosa and Blanca) and, to establish the color-composition relationship.

Materials and methods

Plant material

Roselle chalices were obtained from breeding programs and named according to pigmentation: three pigmented cultivars (Negra, Sudan and Rosa) and, one unpigmented cultivar (Blanca); chalices characteristics and storage conditions of raw material were previously described [5].

Extraction procedure

For methanolic extraction, samples (5 g) were homogenized in 25 mL of solvent (75% methanol, acidified with HCl 5% [1 N]), kept under shaking for 1 h and further centrifuged at 906 g for 5 min; supernatant was collected and the pellet was submitted to the same process three more times, supernatants were mixed. On the other hand, aqueous extraction was prepared in order to simulate the traditional beverage. For this purpose, 5 g of sample were macerated in 100 ml of water, according the method described by Sayago-Ayerdi et al. [6]. Extracts were carried out in triplicate.

Analysis of phenolics compounds

Determination was carried out in an Agilent 1260 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode-array detector (200-700 nm), and a C18 Poroshell 2.7 Micron (4x6x50 mm) [7]. The solvents were 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at the following gradient: 0–5 min, 5% B linear; 5–20 min 50% B linear; 20–25 min, washing and re- equilibration of the column. The flow rate was 1.5 mL/min, injection volume of 10 μL and the temperature of the column was set at 25°C. The wavelength of detection was 280 nm (flavanols and benzoic acids), 320 nm (phenolic acids), and 370 nm (flavonols). Anthocyanins determination was carried out following the methodology adapted from Ivanova et al. [8]. These compounds were separated using a Zorbax C18 column (250 x 4.6 mm, 5 μm particle size) maintained at 38 °C. A gradient of the mobile phase A (trifluoroacetic acid, 0.1%), and acetonitrile as solvent B, were used. The elution profile was as follows: 0-3.25 min 90% A - 10% B; 3.25-15.62 min 90% A - 10% B; 15.62-20.83 min 85% A - 15% B; 20.83-26.04 min 85% A - 15% B; 26.04-46.88 min 82% A - 18% B; 46.88-52.08 min 70% A - 30% B; 52.08-60 min 65% A - 35% B; 60-65 min 90% A -10% B. The flow rate was 0.8 mL/min, and the

injection volume was 50 µL. The wavelength of detection was 525 nm. The identification of phenolic compounds was achieved by comparison of the retention time with those of the available pure standards, our data library, and /or date in the literature. The quantification of phenolic compounds was done by external calibration from the areas of the chromatographic peaks recorded at 280, 320, 370 and 525 nm. Results were expressed as mg phenolic compound/100g of dry matter.

Antioxidant capacity

Antioxidant capacity was evaluated by FRAP and ABTS assays according to Benzie and Strain [9] and Re et al. [10], respectively. Different dilutions of each extract were assayed and the results were obtained by interpolating the absorbance on a calibration curve obtained with Trolox (30-1000µM). Three independent experiments in triplicate were performed for each assayed extracts and results were expressed as Trolox-equivalent antioxidant capacity (TEAC; mmols of Trolox with the same antioxidant capacity as 100 g of dry matter).

Color analysis

Digital image analysis of color was developed using a DigiEye®, the conditions of image capture and lighting were previously reported [11-13]. In order to obtain CIELAB coordinates from RGB color space, the software DigiFood® [15] and the average of 18 measurements, were used.

Statistical analysis

The statistical analysis was done using the Statgraphics Plus version 5.1® (Manugistics, Inc., Rockville, MA, USA) software. Univariate analysis of variance (LSD test) was applied to establish: if phenolic composition differed significantly between roselle cultivars. Moreover, pattern recognition (PR) techniques, like stepwise linear discriminant

analysis (SLDA), was performed in order to classify roselle cultivars according to phenolic compounds, antioxidant capacity and color parameters.

Results and discussion

Phenolic compounds in roselle cultivars

Chromatographic method allowed the separation of up to 24 compounds: two hibiscus acids, six phenolic acids, six flavanols, eight flavonols and two anthocyanins for all cultivars, except for Blanca cultivar, where anthocyanins were not found (Online Resource 1). Phenolic compounds of pigmented roselle extract have been widely studied [3, 14-16]; however, the phenolic compounds content in native Mexican cultivars with different levels of pigmentation have not been reported. Results indicate that Negra cultivar showed the highest content of hibiscus acid, phenolic acids and flavonols comparing to Sudan, Rosa and Blanca cultivars ($p < 0.05$). Sudan cultivar showed the highest anthocyanins content (3.02 ± 0.19 g/100g) followed by Negra (2.45 ± 0.43 g/100g) and Rosa (0.50 ± 0.06 g/100g) cultivars. Blanca cultivar showed higher flavanols content ($p < 0.05$) than the other analyzed cultivars.

Dp-3-Sa and Cy-3-Sa were identified as the only anthocyanins in the cultivars analyzed. Dp-3-Sa content was up to three times more than Cy-3-Sa in colored chalices; these pigments were also reported in red calyces of roselle from Mexico [16] and Senegal [16, 17]. The differences observed between our samples and previously reported in the number and concentration of the identified compounds may be due to the chemical complexity and/or the method of extraction and analysis [2], since the content of compounds is influenced by both internal (genotype) as external (environment, cultivation and storage) factors [17,18]. Online Resource 2 shows the average normalized area of extraction efficiency of chemical compounds in roselle beverages, anthocyanins showed the lowest

extraction efficiency, near of 4%, while phenolic acids presented an extraction efficiency in a range of 30 to 60%. Proportion of phenolics and flavonols in roselle beverages of Negra, Sudan, Rosa and Blanca cultivars was analyzed (Online Resource 2).Chemical data about roselle beverages showed high phenolic compounds content represented as hibiscus and phenolic acids, flavanols, flavonols and anthocyanins in pigmented cultivars, the significant amounts of phenolics can be an important issue to develop of functional foods and, benefits to human health.

Antioxidant capacity of roselle cultivars

Negra cultivar showed higher values *($p < 0.05$)* for FRAP assay than Sudan and Rosa cultivars; on the other hand, Negra and Sudan cultivars had the highest values for ABTS assay (Online Resource 1). Methanolic extracts include a closer determination of a total antioxidant activity present in roselle calyx, while aqueous extracts refer to the activity present in beverage. Beverages showed higher antioxidant properties compared to those found in calix, values were up to 50% and 60% for ABTS and FRAP assays, respectively (Online Resource 2). It is noteworthy that is not easy the comparison of antioxidant activity data between obtained in this study and other previously published, due that the content of these kind of compounds could be influenced by extraction conditions and, samples origin (geographic location) [18]. Antioxidant capacity of roselle extracts could be associated to efficiency of chlorogenic acid derivatives and anthocyanins as reducing agents [14, 15]. Correlation analysis showed a positive and significant relationship ($p < 0.05$, $r > 0.9$) between antioxidant capacity and chemical compounds, these results are similar to those reported by Mohd-Esa et al [2].

Color Analysis

The color data were plotted in a CIELAB diagram (Figure 1), pigmented cultivars overlap each other due to anthocyanins presence, color data representation converged to redness. Rosa cultivar showed higher value of chroma ($p < 0.05$) than the other cultivars. The color intensity of the pigmented samples was similar, however, anthocyanins content showed significant differences ($p < 0.05$), the co-pigmentation is suggested, as it naturally occurs in anthocyanins containing plants. Christian and Jackson [1] reported color values similar to found in this study using a Lab Scan XE 1669 Hunter colorimeter. We propose DIA as a useful, non-invasive and fast tool for color determination in roselle cultivars with different pigmentation degrees, moreover, minimal sample processing is required and it may be applied to other food and pharmaceutical products.

Color-composition relationships

At first, using simple correlations (Person coefficient), the relationship between color parameters of roselle cultivars and their anthocyanin content, was analyzed. The *p* value for anthocyanin content in methanolic extracts and, color parameters (a^*, b^* and C^*_{ab}), showed significant relationship ($p < 0.05$), but the same parameters in aqueous extracts were not significant. To achieve accurate evaluation of the correlation between color and pigments, multiple linear regressions were calculated. Color parameters were accounted as dependent variables while anthocyanin were considered independent variables, to determinate total and individual anthocyanin content, simple models were proposed ($p < 0.01$) (Table 1). Moreover, models to evaluate the extraction efficiency were designed; however, those models did not show good correlation coefficients (data not showed). It has been reported that the use of high number of variables allows a better predictive model [13]. The

correlation coefficients were similar to those reported by the literature for color-composition relationships in food products [11, 13].

Multivariate statistical analysis of roselle cultivars

In order to classify roselle beverages two multivariate analyses (LDA and SLDA) were used (Table 2). Color (polar coordinates), antioxidant capacity and chemical parameters, were included. Chromatographic and color data were enough to classify analyzed cultivars. Additionally, stepwise linear discriminant analysis (SLDA) was performance in order to plot data within the plane defined by the two corresponding canonical functions (Figure 2) and identifying the most important variables for the model were obtained. The function coefficients to classify samples according to chemical characteristics are showed in Online Resource 3. These functions were verified according to the success rate of the cases in their corresponding group. Discriminant function 1 is highly related to C^*_{ab}, with negative value; on the other hand, discriminant function 2 is highly linked to L^*, hab, anthocyanins and FRAP with negative values, thus the scatterplot showed a quite good separation among the samples as a function of each cultivar. The first function allowed samples classification (100%) into four groups (Negra, Sudan, Rosa and Blanca), some variables showed negative value, roselle samples were located at the bottom of the scatterplot, demonstrating that chemical and color data (L^*, C^*_{ab}, h_{ab}, anthocyanins and FRAP) of native Mexican roselle are useful parameters to discriminate cultivars with different pigmentation levels.

Conclusions

Twenty-four different phenolic compounds (benzoic and cinnamic acids, flavonols, flavanols and anthocyanins) were identified. Tristimulus colorimetry and multivariate statistical analysis allowed establishing models of color-composition relationship, which showed high correlation coefficients. Chromatographic, color and antioxidant parameters

were reliable tools to classify roselle cultivars according to chalice pigmentation. In this study, we successfully demonstrated the usefulness of a simple method to estimate anthocyanin levels based on image analyzes. This tool could be profitable in food and pharmaceutical industries for the use of these pigments as antioxidant in human health.

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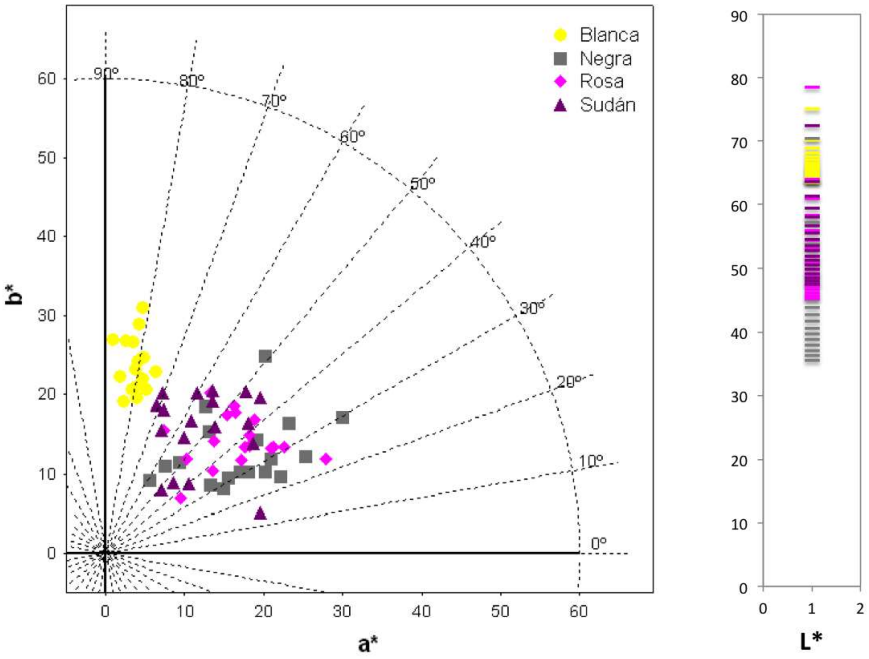

Figure 1. CIELAB coordinates for Blanca, Negra, Rosa and Sudan roselle cultivars

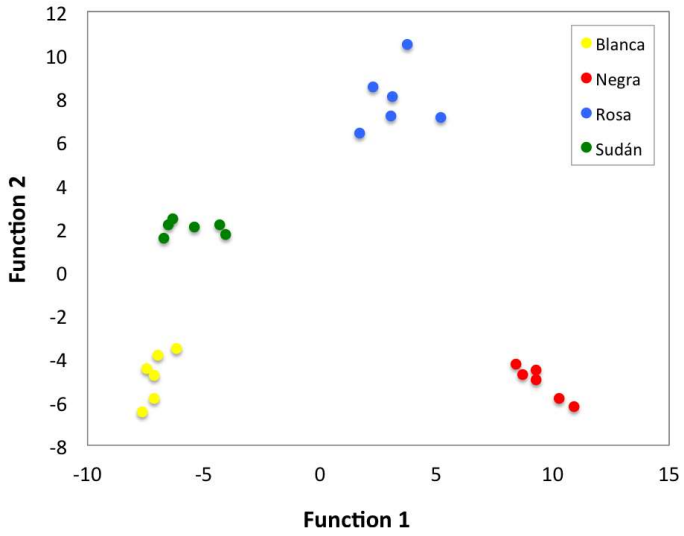

Figure 2. Representation of roselle beverages in the space defined by the first and second discriminant functions obtained from chemical and color data.

Table 1. Color composition relation between anthocyanins content and CIELAB (L^*, a^* and b^*) and polar coordinates (C^*_{ab} and h_{ab}) for roselle cultivars ($p<0.01$).

	Model	R
Total	$Dp3Sa = 1642.3 + 409.39L^* - 797.05a^* + 519.88b^*$	0.92
	$Dp3Sa = -190.90 + 414.98L^* - 431.59C^*_{ab} - 1.34h_{ab}$	0.86
	$Cy3Sa = 76.34 + 105.87L^* - 105.51a^* - 40.72b^*$	0.91
	$Cy3Sa = 273.30 + 90.14L^* - 103.06C^*_{ab} - 0.58h_{ab}$	0.90
	$TA = 1718.64 + 515.26L^* - 902.56a^* + 479.16b^*$	0.92
	$TA = 82.40 + 505.11L^* - 534.65C^*_{ab} - 0.76h_{ab}$	0.88
Beverage	$Dp3Sa = 28.28 + 14.90L^* - 25.74a^* + 14.76b^*$	0.90
	$Dp3Sa = -3.74 + 12.84L^* - 13.58C^*_{ab} + 0.13h_{ab}$	0.84
	$Cy3Sa = 22.38 + 7.73L^* - 5.25a^* - 5.82b^*$	0.92
	$Cy3Sa = 18.23 + 4.79L^* - 5.73C^*_{ab} + 0.18h_{ab}$	0.92
	$TA = 5.84 + 22.63L^* - 30.99a^* + 8.94b^*$	0.90
	$TA = 14.49 + 17.63L^* - 19.31C^*_{ab} + 0.31h_{ab}$	0.87

Dp3Sa: delphinidin-3-sambubioside and Cy3Sa: cyanidin-3-sambubioside; TA: total anthocyanins

Table 2. Linear discriminant analysis (LDA) and stepwise linear discriminant analysis (SLDA) to classify Negra, Sudan, Rosa and Blanca roselle cultivars.

Data	LDA	SLDA	
	% of classification	% of classification	Retained variables
Chromatographic [a]	100	100	Ha, Pa, Fa, An
Color [b]	100	100	L^*, C^*_{ab}, h_{ab}
Antioxidant [c]	75	75	ABTS, FRAP
Total [d]	100	100	L^*,C^*_{ab}, h_{ab}, An, FRAP

[a] Group of compounds: Hibiscus acids, phenolic acids, flavanols, flavonols and anthocyanins. [b] Polar coordinates (L^*, C^*_{ab} and h_{ab}). [c] FRAP and ABTS assays. [d] Chromatographic, color and antioxidant data. Abbreviations: Ha: hibiscus acids, Pa: phenolic acids, Fa: flavanols, An: anthocyanins.

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Conflicts of Interest Statement

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I certify that there is no actual or potential conflict of interest in relation to this article.

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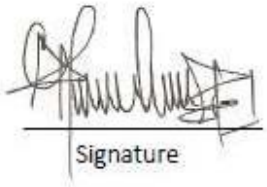

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Author	Signature	Date

Online Resource 1. Phenolic content (mg/100 g) in roselle extracts cultivars.

Peak	Compound	Acidified methanol				Beverage			
		Negra	Sudan	Rosa	Blanca	Negra	Sudan	Rosa	Blanca
1	Hibiscus acid	16.59±0.36^{a}	12.92±1.02^{b}	15.00±6.85^{a}	10.27±1.25^{c}	2.43±0.5^{a,b}	3.18±0.76^{a}	1.97±0.85^{b}	1.70±0.15^{b}
2	Protocatechuic acid derivate	14.47±0.27^{a}	9.16±0.37^{c}	9.60±2.70^{b,c}	10.93±0.43^{b}	6.78±1.59^{a}	4.44±0.98^{b}	6.91±1.32^{a}	2.78±1.77^{b}
3	Protocatechuic acid	302.80±8.57^{a}	134.90±4.47^{b}	167.69±68.50^{b}	126. 03±8.62^{b}	134.20±33.46^{a}	65.04±15.70^{b}	131.55±26.18^{a}	56.82±3.32^{b}
4	Chlorogenic acid	2.07±0.30^{d}	7.52±0.45^{a}	2.99±0.33^{c}	5.33±0.28^{b}	Nd	0.52±0.36^{a}	0.03±0.04^{b}	Nd
5	Chlorogenic acid isomer	269.51±8.98^{b}	334.69±8.10^{a}	191.51±40.45^{d}	228.13±10.37^{c}	102.47±31.05^{a,b}	124.50±33.91^{a}	84.26±26.18^{b,c}	67.47±8.69^{c}
6	Hibiscus acid derivate	29.89±1.90^{a}	22.86±0.72^{b}	16.94±2.17^{d}	19.87±0.87^{c}	7.53±2.79^{a}	7.35±2.06^{a}	7.54±1.70^{a}	5.64±0.46^{a}
7	Quercetin 3-sambubioside	335.60±14.83^{a}	93.83±7.36^{d}	195.11±15.37^{c}	235.00±13.94^{b}	108.41±42.44^{a}	28.33±8.71^{c}	82.20±19.11^{a,b}	68.72±5.02^{b}
8	Quercetin 3-rutinoside	4.24±3.36^{b}	7.44±9.97^{b}	22.08±21.59^{a}	12.94±0.38^{a,b}	3.12±2.30^{b}	12.81±2.89^{a}	11.78±12.01^{a}	2.61±0.44^{b}
9	Kaempherol 3-sambubioside	142.33±6.87^{a}	101.97±3.79^{b}	104.54±23.24^{b}	107.02±4.74^{b}	47.99±17.96^{a}	35.98±10.01^{a}	43.22±16.56^{a}	32.55±2.97^{a}
10	Kaempherol 3-rutinoside	45.95±4.25^{a,b}	62.74±2.19^{a}	42.60±43.66^{a,b}	22.32±1.29^{b}	17.85±5.51^{a}	22.35±6.52^{a}	19.25±15.97^{a}	5.50±0.32^{b}
11	Flavonol	27.87±4.79^{b}	30.26±2.43^{b}	27.95±6.31^{b}	44.79±8.43^{a}	Nd	Nd	9.27±13.20^{a}	0.75±0.28^{b}
12	Quinic acid isomer	0.83±0.40^{b}	2.20±0.27^{a}	0.21±0.06^{c}	2.44±0.05^{a}	0.01±0.01^{a}	0.25±0.29^{a}	0.18±0.46^{a}	Nd
13	Quinic acid isomer 2	5.71±0.81^{c}	15.20±1.80^{b}	3.67±0.20^{d}	19.39±0.32^{a}	1.64±0.99^{b}	4.15±1.83^{a}	0.96±0.40^{b}	1.80±1.41^{b}
14	Flavanol 1	30.19±21.88^{a,b}	18.81±17.43^{b}	28.92±1.32^{a,b}	39.55±1.95^{a}	5.15±1.60^{b}	8.23±2.43^{a}	10.19±2.43^{a}	7.98±0.93^{a}
15	Flavanol 2	9.52±2.68^{c}	20.77±2.86^{b}	12.37±0.65^{c}	48.65±3.13^{a}	2.84±1.12^{b}	5.63±2.17^{a}	5.10±2.70^{a,b}	5.54±1.45^{a}
16	Flavanol 3	71.15±12.45^{d}	105.99±7.90^{b}	89.16±4.10^{c}	125.77±2.87^{a}	18.55±6.30^{b}	31.99±11.02^{a}	30.02±6.65^{a}	25.63±3.01^{a,b}
17	Flavanol 4	21.87±6.10^{d}	35.17±5.40^{c}	40.88±1.32^{b}	52.71±1.49^{a}	8.50±2.70^{c}	11.46±4.09^{b,c}	15.71±3.23^{a}	12.68±1.56^{a,b}
18	Quercetin-3-	18.45±3.19^{d}	29.22±2.16^{b}	25.50±1.51^{c}	40.07±1.74^{a}	4.63±2.46^{b}	7.77±2.61^{a}	7.73±1.48^{a}	6.52±1.38^{a,b}

	glucoside								
19	Flavanol 5	4.79±1.66^{c}	9.68±2.46^{a}	6.67±0.56^{b}	7.67±0.22^{b}	1.42±0.51^{c}	2.86±0.65^{a}	2.21±0.64^{a,b}	1.79±0.26^{b,c}
20	Myricetin	26.00±15.78^{b}	30.28±13.38^{b}	15.08±6.38^{b}	60.31±20.67^{a}	0.60±0.93^{b}	1.65±1.06^{a}	Nd	Nd
21	Flavanol 6	20.08±8.26^{a}	16.96±6.68^{a}	9.56±1.39^{b}	8.73±0.92^{b}	1.50±1.29^{b}	3.17±1.06^{a}	1.85±0.67^{b}	Nd
22	Quercetin	34.40±14.68^{b,c}	25.51±11.56^{c}	48.61±21.57^{a,b}	63.03±24.25^{a}	0.51±0.80^{a}	0.84±0.95^{a}	0.64±0.37^{a}	Nd
23	Delphinidin-3-sambubioside	1818.27±318.71^{b}	2373.61±172.431^{a}	324.11±42.32^{c}	Nd	57.97±3.85^{b}	79.60±13.46^{a}	10.70±2.99^{c}	Nd
24	Cyanidin 3 sambubioside	629.913±110.62^{a}	655.50±49.94^{a}	170.56±16.85^{b}	Nd	36.87±3.60^{a}	40.55±6.14^{a}	10.31±2.67^{b}	Nd
	Σ hibiscus acids	46.48±2.12^{a}	35.78±1.33^{b}	31.94±7.65^{b,c}	30.14±1.68^{c}	9.96±3.33^{a,b}	10.53±2.82^{b}	9.52±1.08^{a,b}	7.33±0.47^{b}
	Σ phenolic acids	595.40±15.26^{a}	503.67±13.49^{b}	375.68±76.00^{c}	392.31±18.07^{c}	245.11±67.10^{a}	198.91±53.00^{a}	223.91±47.25^{a}	128.88±13.15^{b}
	Σ flavonols	618.71±37.22^{a}	340.60±37.66^{d}	456.93±10.72^{c}	540.19±55.81^{b}	183.64±66.93^{a}	110.21±29.49^{b}	167.30±33.39^{a}	117.37±9.50^{b}
	Σ flavanols	185.47±31.28^{c}	237.65±15.58^{b}	215.51±13.82^{b}	327.87±12.50^{a}	37.96±13.46^{c}	63.34±17.43^{a,b}	74.37±20.67^{a}	54.38±6.85^{b,c}
	Σ anthocyanins	2448.18±429.09^{b}	3029.11±191.87^{a}	494.68±59.12^{c}	Nd	94.83±7.43^{b}	120.15±19.49^{a}	21.00±5.62^{c}	Nd
	FRAP**	11.93±2.98^{a}	7.38±1.48^{b,c}	5.54±1.068^{c}	10.30±0.27^{a,b}	6.87±0.67^{a}	5.86±1.02^{a}	3.45±1.02^{b}	6.76±0.40^{a}
	ABTS**	26.09±1.51^{a}	24.98±0.7.6^{a}	16.74±1.73^{c}	20.46±0.54^{b}	14.38±0.93^{a}	13.17±1.40^{a}	10.53±0.78^{b}	13.29±0.52^{a}

*Express in mg/100 g DW

** Express in mmol Trolox equivalents /100g

Data expressed as mean ± SD (n=6). Different letters indicate significant differences, least significant differences (LSD) test.

Nd: No detected.

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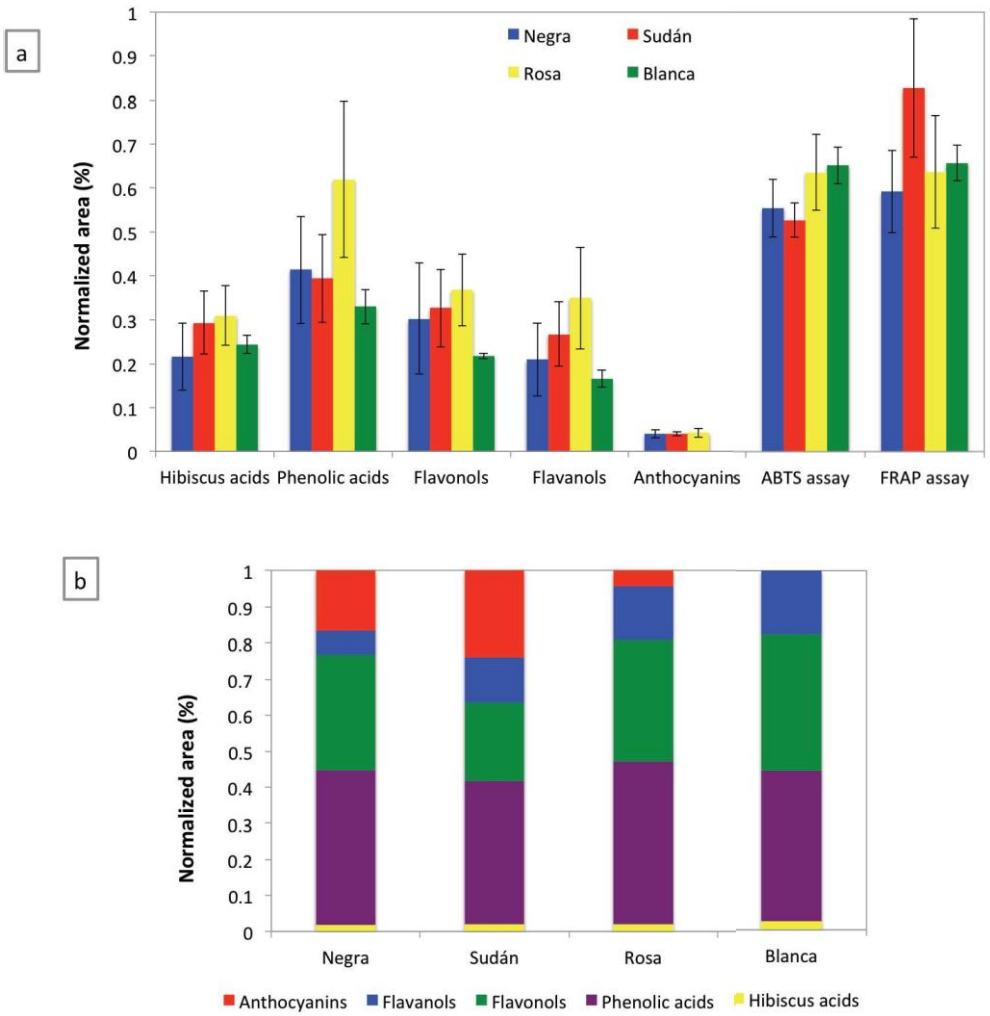

Online Resource 2. (a) Average normalized area of extraction efficiency of chemical compounds and antioxidant capacity in roselle beverages based on total present in chalices (b) proportion of chemical compounds in roselle beverages of Negra, Sudán, Rosa and Blanca cultivars.

Online Resource 2. Classification functions coefficients according to Mexican roselle cultivars.

	Blanca	Negra	Rosa	Sudán
L^*	1.458	3.769	-4.886	-3.255
C^*_{ab}	5.106	-10.492	15.639	12.963
h_{ab}	-0.973	0.638	-1.742	-1.378
Total anthocyanins	-0.579	1.582	0.497	-0.560
FRAP	1.223	2.580	1.009	0.218
Constant	-116.40	-136.194	-169.749	-53.705