

RESEARCH ARTICLE

Effect of the dehydration method of *Hibiscus sabdariffa* L. calyces on the quality of their aqueous extracts

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ABSTRACT

Hibiscus sabdariffa L. is a plant from tropical climates, which produces, among other products, deep red calyces used to prepare refreshing drinks. The high humidity (85-87%) of the calyces at the time of harvest must be reduced to about 12% for handling and storage. The aim of the work was to evaluate the effect of the dehydration method of the calyces (sun drying, SD; hot air-drying, AD, at three temperatures: 50, 60 and 70 °C) of three varieties of *Hibiscus sabdariffa* L. on the quality of their aqueous extracts. The quality was measured as a function of color, and chemical variables (titratable acidity, TA, total soluble phenols, TSP; total anthocyanins content TAC, and organic acids). The varieties used were Negra Quiviquinta (NQ) with dark red calyces, UAN-9 with light red calyces, and UAN-16, with white calyces. The dehydration method affected the quality of the extracts. The SD was the one that most affected color and chemical variables. The color of the extracts was darker than that of the control while titratable acidity (TA) was reduced on average 40.6%. No effect of AD treatments was observed on these variables. On chemical composition, SD reduced TSP on average 15.3%, with UAN-16 as the most affected variety. In the varieties with red calyces, the average TAC reduction was 36.9%. Of the organic acids, malic and succinic were the most damaged. As occurred in physical variables, AD treatments showed little negative effect on chemical composition of the calyces. Of the dehydration methods evaluated, the AD_{70°C} rendered the best quality of aqueous extract, in base of the variables evaluated.

Keywords: *Hibiscus sabdariffa*, anthocyanins, acidity, color, total soluble phenols.

INTRODUCTION

Hibiscus sabdariffa L. is an annual herbaceous plant that belongs to the Malvaceae family, native to Sub-Saharan Africa (Wilson, 1994). It is cultivated in the tropical and subtropical regions of the world, to take advantage of its calyces in the preparation of culinary dishes, although its main use is for the preparation of refreshing drinks with a bright red color and a characteristic acid flavor (Cid-Ortega and Guerrero-Beltrán, 2015). In Mexico, the dehydrated calyces of this plant are known as “flor de jamaica” or “jamaica”, while in other countries they are called roselle, karkade or bissap. The jamaica extracts are used in traditional medicine due to their diuretic, digestive, laxative, anti-fever properties, as well as for the control of hypertension and cholesterol (Da-Costa-Rocha et al., 2014; Wahabi et al., 2010).

The medicinal qualities of the calyces of jamaica are due to the high content of phenolic compounds that they possess, mainly anthocyanins, and phenolic acids (Ife et al., 2016; Reyes-Luengas et al., 2015), which are related to the different biological activities of their extracts (Tsai et al., 2002). Postharvest handling of calyces, particularly the dehydration process, can alter the content of phenolic compounds. At the time of harvest, the mature calyces have a high humidity (85-87%), which must be reduced to around 12%, to facilitate their handling and storage (NMX-FF-115-SCFI- 2010).

The quality of jamaica calyx is defined by its attributes of color, acidity and safety. Color is the result of anthocyanin content, while acidity is a consequence of the content and balance of organic acids such as citric, malic, oxalic, succinic and tartaric (Ibrahim et al., 2015).

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In countries producing jamaica calyces such as Mexico, calyces are dried by placing them on a solid surface, directly under the rays of the sun, for the time necessary to reduce their humidity (Contreras et al., 2009). If the weather conditions are cloudy and rainy, the drying time is prolonged, and fermentation and staining of the calyces often occurs, causing losses and reducing quality.

In recent years, the presence of rains during the harvest season has made difficult to adequately dehydrate the calyces in some producing regions of Mexico. Therefore, the use of electrical or fuel dehydrators are options to avoid this problem. Although there are studies on various dehydration methods for jamaica calyces, most are aimed to evaluate technical parameters of the process (Meza-Jiménez et al., 2009; Saeed, 2010), or the proximal composition of the calyces (Amoasah et al., 2018). Recently, Ledesma-Valladolid et al. (2020) evaluated three dehydration methods on physical and chemical variables of *Hibiscus sabdariffa* calyces. Their results showed little effect of the dehydration methods on the pH and acidity of the calyces. Direct sun drying of the calyces did not cause anthocyanins losses higher than that induced by drying with hot air or solar drier. The antioxidant activity of the calyces was not affected by the drying methods used.

However, it is necessary to have a greater amount of information about the impact of dehydration methods on the quality of calyces and their bioactives to choose the one that best suits the possibilities that the producer has of dehydrating their calyces and that allows them to better preserve their quality. The objectives of the work were to evaluate the effect of dehydrating directly in the sun or using hot air on the quality of the aqueous extracts of *Hibiscus sabdariffa* calyces. The quality of the extracts was measured according to the color, titratable acidity and content of total soluble phenols, anthocyanins and organic acids.

MATERIALS AND METHODS

Vegetal material

The fresh calyces of three *H. sabdariffa* L. genotypes were used, with variation in the color of their calyces: dark red calyces (Negra Quiviquinta), light red calyces (UAN-9), and white calyces (UAN-16). Pictures of the calyces of the varieties used in the study are in Fig. 1, sections A, B, and C. The varieties were grown and harvested in the municipality of Acaponeta, Nayarit, Mexico (22° 29' 47" North, 105° 21' 32" West, 24 masl). The agronomic management applied in each variety was the one commonly practiced in the production region.

Calyx dehydration treatments (DTs)

Dehydration in the sun (SD). It was performed by placing the calyces (~ 10 kg) on a concrete surface exposed to solar radiation for 6 days (9 AM to 5 PM) (Figure 1D). With sunny days, dehydration is completed in two or three days, but due to the presence of rainy and cloudy days, this time it lasted for six days. The indicator to stop drying was the crunchy texture of the calyces. The dehydrated calyces were kept in plastic bags and maintained in darkness until they were processed in the laboratory.

Dehydration with air (AD). A commercial tray dehydrator (EBERHARD® MFG. Co.) was used. Three temperatures (50, 60 and 70° C) were evaluated. The experimental unit was 10 kg of fresh calyces and two repetitions were handled (Figure 1E and 1F). Completion of drying was established subjectively by palpating the crunchy texture of the calyces. A sample of fresh calyces from each variety was lyophilized, to be used as a control for dehydration treatments. The dehydrated samples were kept in 'ziploc' type plastic bags (USA) in dark conditions, until they were processed in the laboratory, that was around one month after being dehydrated. The quantification of humidity in the dehydrated calyces from the dehydration treatments (DTs) was subsequently performed (AOAC, 1998).

Preparation of the aqueous extracts

The method described by Galicia-Flores et al. (2008) was used. In brief, 2.5 g of whole calyces on a dry basis and 100 mL of distilled water were boiled for 15 min in a 600 mL beaker in a crude fiber system adapted to reduce water evaporation. After the boiling time, the extract was separated by decantation and the extraction repeated under the same conditions. The two extracts recovered were joined, and the volume was filtered with Whatman No. 4 paper, and the final volume adjusted to 200 mL with distilled water. From these extracts, all the determinations were made, except the analysis of organic acids in which were employed lyophilized calyces. Extracts from fresh calyces of each *Hibiscus* variety were prepared in order to be used as controls for the DTs.

Color of the extracts

The color of the extracts was measured with a Hunter Lab MiniScan XE Plus colorimeter (model 45/0-L) on the CIE $L^*a^*b^*$ scale, with illuminant D65 and a 10° angle. The values of the parameters L^* , a^* and b^* were obtained under the protocol described in the Hunter-Lab manual to measure color in translucent liquids (Leggett, 2008). A volume of 50 mL of extract was used. The color difference (ΔE) was calculated according to the CIE Guide 1976 (Sharma, 2003) using the expression: $\Delta E^* = [(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2]^{1/2}$. Variables with subscript 0 correspond to the color variables of the extract from fresh calyces (control); those of



Fig 1. Pictures of the calyces of the varieties used in the study and of the methods of dehydration in the sun and with hot air. A) Negra Quiviquinta variety, B) UAN-9 variety, C) UAN-16 variety, D) dehydration of the calyces directly in the sun, E) dehydration of the calyces in the hot air-dehydrator, F) the calyces in the trays of the hot air-dehydrator.

subscript 1 correspond to that of the extracts from different dehydration treatments of the calyces. Color squares were generated by converting the L^* , a^* , and b^* values to their corresponding R, G, and B values using an information converter (<http://colormine.org/convert/rgb-to-lab>) and PowerPoint software of Microsoft office 2010.

Titrateable acidity (TA)

It was determined with the method 942.15 described in the AOAC (1998), in a 10 mL aliquot that was diluted to 200 mL with distilled water. The percentage of acidity was expressed as a function of citric acid.

Phenolic composition

Total soluble phenolics (TSP). The Folin-Ciocalteu method (Singleton and Rossi, 1965) was used according what was describing by Galicia-Flores et al. (2008). A standard curve of gallic acid (20 to 100 ppm) was drawn up, and the results were expressed as mg equivalent of gallic acid per 100 g of dry sample (mg GAE 100 g⁻¹ DW).

Total anthocyanin content (TAC). The spectrophotometric method described by Galicia-Flores et al., (2008) was used. A standard curve was prepared with cyanidin 3-glucoside (Polyphenols[®], NW) in concentrations from 0 to 30 ppm. Results were expressed as mg equivalent of cyanidin 3-glucoside per 100 g of dry weight (mg CGE 100 g⁻¹ DW).

Anthocyanins analysis by HPLC

A volume of 2 mL of extract was filtered with a nylon acrodisk (0.45 μ m, Chromafil[®] Xtra) in an amber vial that

was kept at 4°C until analysis. The equipment used was a PerkinElmer[®] Series 200, with a C₁₈ Hypersil ODS column (200 x 4.6 mm, 5 μ m). A gradient system with two solvents was employed. Solvent A: formic acid (Merck[®]): water (JT Baker[®]) (1: 9 v/v); solvent B: formic acid: water: methanol (JT Baker[®]) (1: 4: 5 v/v/v). All solvents used were HPLC grade. The injected sample volume was 10 μ L, the run time was 21 min, a flow of 1.2 mL min⁻¹ and a column temperature of 25°C (Fossen et al., 2001). Anthocyanins were detected at 520 nm. Identification was performed by comparing UV-vis spectra and commercial standard retention times with separate peaks and supported by references on the subject.

Extraction of organic acids and their analysis by HPLC

The dehydrated calyces from the different DTs were ground in a hammer mill (IKA[®], model MF 10), equipped with a 0.5 mm mesh. Due to the extraction protocol, lyophilized calyces were used as control treatment in this determination. The extraction was made from 0.5 g of calyces flour in 25 mL of bi-distilled water. The sample was placed in an ultrasonic bath (Branson[®] 2510) for 15 min, agitated in a horizontal agitation shaker (Gyratory Shaker[®], model G10, USA) for 30 min, then refrigerated at 4°C for 90 min. The samples were filtered (Whatman No. 4) and their volume adjusted to 25 mL with bi-distilled water. A volume of 2 mL of extract from each sample was filtered on an acrodisk with a nylon membrane (0.45 μ m, Chromafil[®] Xtra), in amber vials and stored at 4°C until analysis by HPLC.

The analysis was carried out on the equipment already described for anthocyanins. The method of Cen et al. (2007), with some modifications, was used. The mobile phase was a mixture of 0.01M potassium phosphate (pH 2.4, adjusted with phosphoric acid) and HPLC grade methanol in a ratio of 95: 5 (v/v). A C₁₈ Hypersil ODS column (200 x 4.6 mm, 5 μm) was used; the run time was 10 min with a flow of 1.0 mL min⁻¹. The column temperature was maintained at 30°C. The injected sample volume was 10 μL. Detection was carried out at 214 nm. The standards used to develop standard curves for the identification and quantification of organic acids were: malic acid (100 to 500 ppm), citric, oxalic and succinic acids (100 to 300 ppm), and tartaric acid (20 to 100 ppm).

Statistical Analysis of Data

The results of the variable TSP, TAC, TA and color (luminosity, a* and b*) were subjected to an analysis of variance with a completely randomized design under a 3 x 4 factorial arrangement and two repetitions. The factors were the varieties (Negra Quiviquinta, UAN-9 and UAN-16), and the dehydrated treatments (SD, AD_{50°C}, AD_{60°C} and AD_{70°C}). The CF extract was incorporated as a control to estimate the effect of DTs on response variables. Additionally, a multiple comparison of means (Tukey, P ≤ 0.05) was performed to identify differences between dehydrated treatments on the variables TSP, TAC, TA, and the descriptive variables of color (L*, a*, b*). The statistical package used was SAS System Version 9.0.

RESULTS AND DISCUSSION

Dehydration of *Hibiscus sabdariffa* (Hs) calyces is a mandatory practice for their handling and preservation. Fresh ripe calyces have high humidity (85-87%), which must be reduced to values close to 12% to achieve a product that can be stored with low risk of damage by microorganisms. The humidity of the calyces from the different DTs was between 10 and 13%. The value for this variable established in the NMX-FF-115-SCFI-2010 is 12%.

The sums of squares from the analysis of variance, revealed that the phytochemical (TSP, TAC), acidity (TA) and color (L*, a*, b*) variables of the aqueous Hs extracts were affected by the variety (V), dehydration treatments (DTs), and by the interaction (V x DTs) of these factors, although in different proportions (Table 1). The main variation factor for TSP and TAC was variety, which explained 85% and 93% of their variation respectively; while the DTs of the calyces, and the interaction V x DTs explained 14% and 1% of the remaining variation in TSP, and 5% and 2% in the case of the TAC. The TA in the aqueous extracts was markedly affected by the DTs (66%); the effects of the

variety and the interaction V x DTs only contributed to explain a 33 and 1%, respectively, of its variability.

The color descriptor variables of the aqueous extracts (Luminosity, a* and b*) were also affected by the variety factor (66 to 99%); in the case of variable b*, the second factorial effect of importance was the interaction V x DTs, which caused 26% of the total effects.

The data on color in the aqueous extracts of the calyces of the different DTs are presented in Table 2. The effect of the significant interaction (P < 0.01) is reported in Table 1. The DTs caused darkening of the extracts by reducing the L* values in relation to the control extract (fresh calyces), particularly for the NQ variety. In the extracts of the UAN-9 variety, the reduction of L* occurred only in the DTs with hot air (AD_{50°C}, AD_{60°C} and AD_{70°C}) while for UAN-16 the darkening was observed both in the SD calyces, as in the AD_{60°C} and AD_{70°C} treatments (Table 2). The reduction of the L* value has been reported in dehydrated strawberry by means of hot air convection (Wojdyło et al., 2009), and it is attributed to the compounds formed during the Maillard reaction, of which many are water soluble (Kunz et al., 2013). So it is possible that in the case of the *Hibiscus* calyces, these compounds were originated during dehydration process and extracted and be presented in the aqueous extracts.

The a* values of the extracts were significantly reduced with the dehydration of the calyces in the red calyx varieties (NQ and UAN-9). This means that they were more intense red than that of the FC. In the NQ variety, the greatest decrease in a* was found in the extracts of the calyces from the SD treatment, which was lower than that in the AD_{60°C} and AD_{70°C} treatments. In the extracts of the UAN-9 variety, the a* values were the same in the sun-dried calyces and with the different hot air treatments, but lower than those of the FC extract. The a* values of the extracts of the UAN-16 variety of white calyces, in

Table 1: Sums of squares from the analysis of variance applied to chemical variables (TSP, TAC, and TA) and color variables (L*, a* and b*) in aqueous extracts of calyces of three varieties of *H. sabdariffa* subjected to different dehydration treatments (TDs)

Variable	Varieties (V)	%	DTs	%	Interaction (V x DTs)	%
	g/2		g/4		g/8	
TSP	10549126**	85	1736450**	14	138010**	1
TAC	5525902**	93	279936**	5	125605**	2
TA	311**	33	611**	66	10**	1
L*	26940**	99	130**	1	49**	0
a*	27856**	99	160**	1	115**	1
b*	2135**	69	165**	5	803**	26

TSP: total soluble phenolics; TAC: total anthocyanin content; TA: titratable acidity; L*= luminosity; DTs: dehydration treatments

Table 2: Parameters of color (L*, a* and b*) and ΔE of aqueous extracts of *H. sabdariffa* L. calyces fresh and dehydrated under different dehydration treatments

Varieties	Treatments	L* (%)	a*	b*	ΔE	Color
NQ	FC	18.3±0.5h	46.0±0.0b	31.0±0.0c	-	
	SD	12.8±0.5i	36.5±0.6f	18.5±0.6i	16.6±1.0	
	AD _{50°C}	12.5±0.6i	38.5±0.6e	19.5±0.6hi	14.9±1.1	
	AD _{60°C}	12.0±0.0i	39.8±0.5d	20.5±0.6gh	13.7±0.5	
	AD _{70°C}	12.3±0.5i	40.3±0.5d	21.0±0.0fg	13.0±0.2	
UAN-9	FC	22.0±0.0e	48.0±0.0a	37.5±0.6a	-	
	SD	21.5±0.6ef	44.5±0.6c	33.5±0.6b	5.4±1.3	
	AD _{50°C}	21.0±0.0f	46.0±0.0b	33.0±0.8b	5.0±0.5	
	AD _{60°C}	19.3±0.5g	46.0±0.0b	33.0±0.0b	5.7±0.4	
	AD _{70°C}	19.8±0.5g	46.5±0.6b	33.8±1.0b	4.7±1.5	
UAN 16	FC	60.0±0.0a	1.0±0.0g	16.0±0.0j	-	
	SD	56.0±0.0 d	1.0±0.0g	28.8±0.6d	13.4±0.5	
	AD _{50°C}	58.0±0.0 b	2.0±0.6g	20.5±0.0gh	5.0±0.5	
	AD _{60°C}	57.0±0.0c	1.5±0.6g	22.0±0.0ef	6.8±0.0	
	AD _{70°C}	57.0±0.0c	1.5±0.0g	23.0±0.5e	7.7±0.0	
HSD		0.9	1.0	1.3		

FC = fresh calyces; SD = sun dehydration; AD = air dehydration. ΔE = color difference between each extract from dehydrated calyces and the FC extract. Means with same letter by column are statistically equal, (Tukey, $P \leq 0.05$)

addition to being extremely low, were not modified by the dehydration treatments.

In red calyces varieties, the yellow hue of the extracts, associated with the b* values, was reduced by the DTs of the calyces. In the NQ variety, the lowest value ($P \leq 0.05$) of b* corresponded to the extract of the sun-dried calyces (SD), while, in the DTs with hot air, the value of this variable was equal ($P > 0.05$) in the extracts of AD_{50°C} and AD_{60°C}, but lower than AD_{70°C}. In the extracts of the UAN-9 variety, those from the DTs presented a lower value of b* than the FC extract, but the same ($P > 0.05$). The effect of the TDs in reducing the yellow hue (b*) of the extracts was less for UAN-9 than for NQ. Extracts of the calyces dehydrated by both SD and AD, of the UAN-16 variety (calyces without anthocyanins), presented higher values (P

< 0.05) of b*, indicative of greater yellow hue, than the FC extract. This result could be associated with a higher oxidation of its chemical components, possibly more susceptible to drying conditions, than those occurred in red calyces varieties. It is also possible that, since it lacks anthocyanins, the yellow tone of the extracts was less masked than it could be in the extracts of the red calyces varieties.

According to the ΔE values, calculated in relation to the color of the FC extract, the extracts of the NQ variety obtained from dehydrated calyces (SD, AD_{50-70°C}) had a different color (darker) than that of the FC extract. This difference would be easily perceived by the human eye, which registers color differences between samples from a ΔE greater than five (Obon et al., 2009). Color squares

allow appreciating such differences. In the UAN-9 variety, the color of the fresh and dehydrated calyx extracts could not be clearly differentiated, when presenting ΔE values similar to each other and close to five (Table 2). In the extracts of the variety with white calyces (UAN-16), the extract from dehydrated calyces in the sun (SD) was darker than the others, according to the color squares, and the $\Delta E = 13.4$. No differences by the human eye would be detected between the color of fresh calyces extract (control) and that of the calyces of the AD_{50°C} with a $\Delta E = 5.0$ (Obon et al., 2009).

Titrateable acidity (TA)

The acidity of Jamaica's beverages is an important quality criterion for the consumer, and it defines the characteristic smell of the drink (Bechoff et al., 2014). The calyx drying method affected the TA of the extracts (Fig. 2). The extracts from the SD calyces had the lowest TA, with reductions of 44, 37, and 41% in the varieties NQ, UAN-9 and UAN-16, in that order, in relation to the TA of the extract from FC. The affectation of the TA by the DTs with hot air was similar in the UAN-9 and UAN-16 varieties, while in the NQ variety, the greatest decrease in the TA was with the AD_{60°C}.

The acidity of the *Hs* calyx is due to the organic acids it contains (malic, succinic, hibiscus, citric, oxalic, among others), which may have been partially fermented by the natural microflora, in the SD treatment due to the longtime of drying (6 days). In ADs, the temperature $\geq 50^\circ\text{C}$ could inactivate the microflora, which in combination with the short exposure time (6 to 8 h), could favor the preservation of organic acids.

The drying temperature of the calyces has an important influence on their aroma and flavor characteristics. The aroma of the calyces is made up of more than 104 compounds, among which furans and aldehydes predominate (Farag et al., 2015), and in combination with the acidity, and color determine the acceptance of the product by consumers.

The average acidity of the fresh calyces was 24.2, 25.3 and 19.1%, in the varieties NQ, UAN-9 and UAN-16, respectively. These values are similar to those reported by Salinas-Moreno et al. (2012) in eight varieties of *Hs* that were 16.9 to 23.7%. Although the variety is the factor that much contributes to the acidity of the *Hs* calyces, others as the production site and agronomic management, also influence this characteristic (Sánchez-Feria et al., 2018).

Ledesma-Valladolid et al. (2020) did not observed changes of the TA in the aqueous extracts obtained from *Hs* dehydrated in the sun with respect to those calyces dehydrated in a hot air dehydrator. The differences with respect to our results could be due to the fact that Ledesma-Valladolid et al. achieved the dehydration of the calyces in the sun in about three days, while we required six days, because the days were cloudy.

Phenolic composition

Total soluble phenolics (TSP)

The SD treatment caused the greatest decrease in TSP in the *Hs* varieties analyzed. The losses were 12, 14 and 20%, for the varieties NQ, UAN-9 and UAN-16, respectively

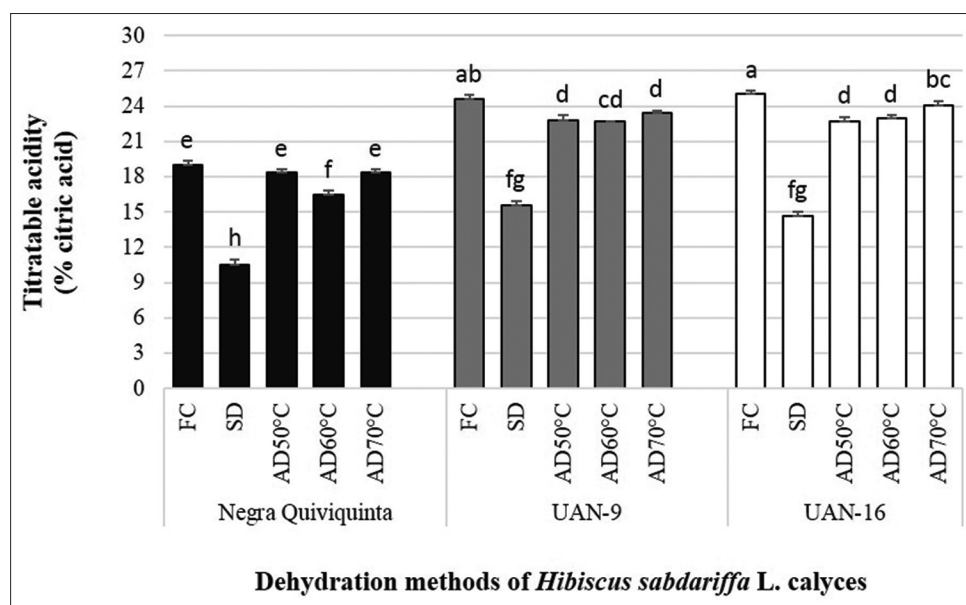


Fig 2. Effect of calyx dehydration treatments on the titratable acidity values of the aqueous extract of three varieties of *H. sabdariffa*. Means with the same letter are statistically equal (Tukey, 0.05). The vertical lines in each bar correspond to the standard deviations ($n = 4$).

(Fig. 3A), and they are attributed to the prolonged time of exposure to light, since, due to the prevailing rainy conditions, they required six days to complete the process. These climatic situations occur in some of the *Hs* calyces producing areas in Mexico and are the cause of losses for the producer, since the calyces are fermented, which affects their quality. Dehydration of calyces in the sun is the most widely used practice in most of the *Hs* calyces producing areas in countries such as Sudan (Mohamed et al., 2012), and Mexico (Contreras et al., 2009).

In the red calyx varieties, the dehydration treatments with air did not affect the TSP, since the values of this variable were the same ($P > 0.05$) as that of the control treatment (FC). However, in the UAN-16 variety, with white calyces, the extracts from the AD_{60°C} and AD_{70°C} treatments had

lower ($P \leq 0.05$) TSP values than the control. Only the extract from the AD_{50°C} treatment reported a TSP value equal to that of the control. Of the phenolic compounds present in the *Hs* calyces, which are extracted with water, anthocyanins are considered the most temperature sensitive group (Castañeda-Ovando et al., 2009). However, the UAN-16 variety, with a marginal amount of anthocyanins, showed reduction of the TSP in the AD treatments with the highest temperatures, which suggests the presence of compounds other than anthocyanins in the calyces of this variety, with high sensitivity to temperature.

The TSP values for the *Hs* extracts analyzed are within the variation reported by Borrás-Linares et al. (2015) for this variable in calyces dehydrated in oven with air convection of 25 varieties of *Hs* that was of 2400 ± 300 to $10,000 \pm 400$ mg GAE 100 g^{-1} DW.

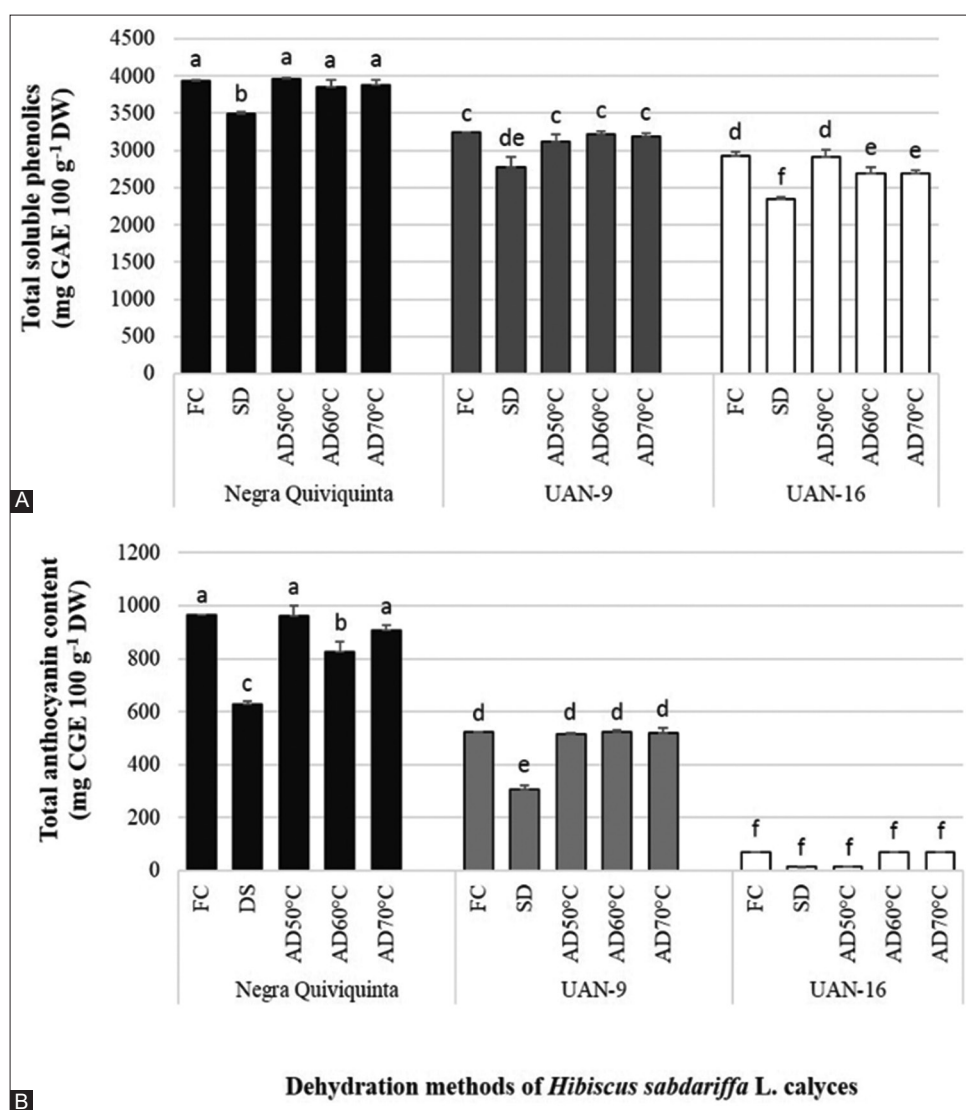


Fig 3. Effect of calyx dehydration methods on total soluble phenols (A) and total anthocyanin content (B) of three varieties of *Hibiscus sabdariffa*. DHS = 197 mg EAG 100 g^{-1} MS. Means with the same letter are statistically equal (Tukey, 0.05). The vertical lines in each bar correspond to the standard deviations ($n = 4$).

Total anthocyanin content (TAC)

Anthocyanins are the water-soluble pigments responsible for the red tones in the calyxes of *Hs* (Juliani et al., 2009). As observed for the TSP, the SD treatment was the one that most affected the TAC, with losses of 32.3 and 41.5%, for the NQ and UAN-9 varieties, in that order, in relation to the control. The TAC values in the control treatments were 964.5 and 522.5 mg EC3G 100 g⁻¹ MS, for the NQ and UAN-9 varieties, in that order. In the UAN-16 variety of white calyxes, with a marginal anthocyanin content, no differences among treatments were obtained for this variable (Figure 3B). In the aqueous extract of *Hs* calyxes, anthocyanins represent one of the predominant phenolic groups in TSP (Reyes-Luengas et al., 2015), so a similar effect would be expected from dehydrated calyx treatments, for TAC and TSP. However, this did not happen, since TAC was more affected than TSP. This is attributed to the fact that the products of thermal degradation of anthocyanins are phenolic of the phenolic acid type (Patras et al., 2010) and chalcones (Sadilova et al., 2007) that react with the Folin-Ciocalteu reagent and are quantified within the TSP.

During the dehydration of plant products, degradation of anthocyanins is lower when high temperatures are used for short periods, than when low temperatures are used for long periods of time (Červenka et al., 2018). According to the results obtained, *Hs* calyxes can be dehydrated at a temperature of 70°C without impact on the degradation of their anthocyanins, and in less drying time than if done at a temperature of 50°C. With the use of solar dehydrators, the most adequate temperature reported to dry the *Hibiscus* calyxes was 65°C (Saeed, 2010).

Anthocyanin analysis by HPLC

With the protocol of analysis applied, four peaks were separated, of which peaks 2 and 4, corresponding to delphinidin 3-sambubioside and cyanidin 3-sambubioside, respectively, that are the predominant anthocyanins

in calyxes of *Hs* (Tsai et al., 2002; Juliani et al., 2009). The dehydration treatments applied to the fresh calyxes of the red varieties of *Hs* evaluated did not affect the chromatographic profile of their anthocyanins (Fig. 4). In Table 3 is shown information on the content of each of the major anthocyanins, the relative proportion of both anthocyanins and percentages of losses of each anthocyanin in the calyxes of the different dehydration treatments.

The relative percentage (RP) of the main anthocyanins in the *Hs* calyxes is on average 70% D3S and 30% C3S, although these proportions may vary slightly depending on the production environment (Sánchez-Feria et al., 2018). Calyx dehydration treatments showed little effect on this variable. The RP of D3S varied between 64 and 70% for the NQ variety, and from 62 to 66% for UAN-9; while to C3S, the variation was 26 to 29% in NQ, and from 28 to 30% for UAN-9 (Table 3).

The effect of all the dehydration treatments was significantly less than that of the control. However, the lowest losses of D3S and C3S occurred with the AD_{50°C} treatment for the two varieties with red calyxes (NQ and UAN-9), and the results were statistically different from the AD_{60°C} and AD_{70°C} treatments. However, in the quantification of total anthocyanins, the three air drying treatments were statistically the same (Figure 3). Drying with the AD_{50°C} treatment required 8 hours, while with the AD_{70°C} it was completed in 4 h. If the energy expenditure in each treatment is considered, drying at a higher temperature is more convenient.

Quantification of organic acids by HPLC

Organic acid content varied among cultivars and dehydration treatments. The SD was the one that most affected the contents of the quantified organic acids. The UAN-9 variety was the most affected, showing a

Table 3: Contents of delphinidin 3-sambubioside (D3S) and cyanidine 3-sambubioside (C3S) quantified by HPLC in the aqueous extracts of calyxes of *H. sabdariffa* varieties subjected to different dehydration treatments.

Varieties	Treatments	D3S	RLA (%)	C3S	RLA (%)
NQ	FC	708±0a (69)		299±0a (29)	
	SD	474±2e (70)	33.1	180±1e (26)	39.8
	AD _{50°C}	656±2b (65)	7.3	279±1b (28)	6.7
	AD _{60°C}	582±3d (65)	17.8	255±1d (28)	14.7
	AD _{70°C}	592±2c (64)	16.4	262±1c (28)	12.4
UAN-9	FC	355±0f (66)		163±2f (30)	
	SD	211±0j (65)	40.6	92±0i (28)	45.5
	AD _{50°C}	339±4g (62)	4.5	153±0g (28)	6.1
	AD _{60°C}	294±2i (63)	17.2	134±1h (29)	17.8
	AD _{70°C}	305±2h (62)	14.1	149±1g (30)	8.6
HSD		8.3		4.1	

* The treatments were: FC: fresh calyxes; SD: drying in the sun; AD_{50°C}: dehydration with air at 50°C; AD_{60°C}: dehydration with air at 60°C; AD_{70°C}: dehydration with air at 70°C. RLA: relative loss of anthocyanins. HSD: honest significant difference. Values in parenthesis represent the relative percentage of area of each anthocyanin

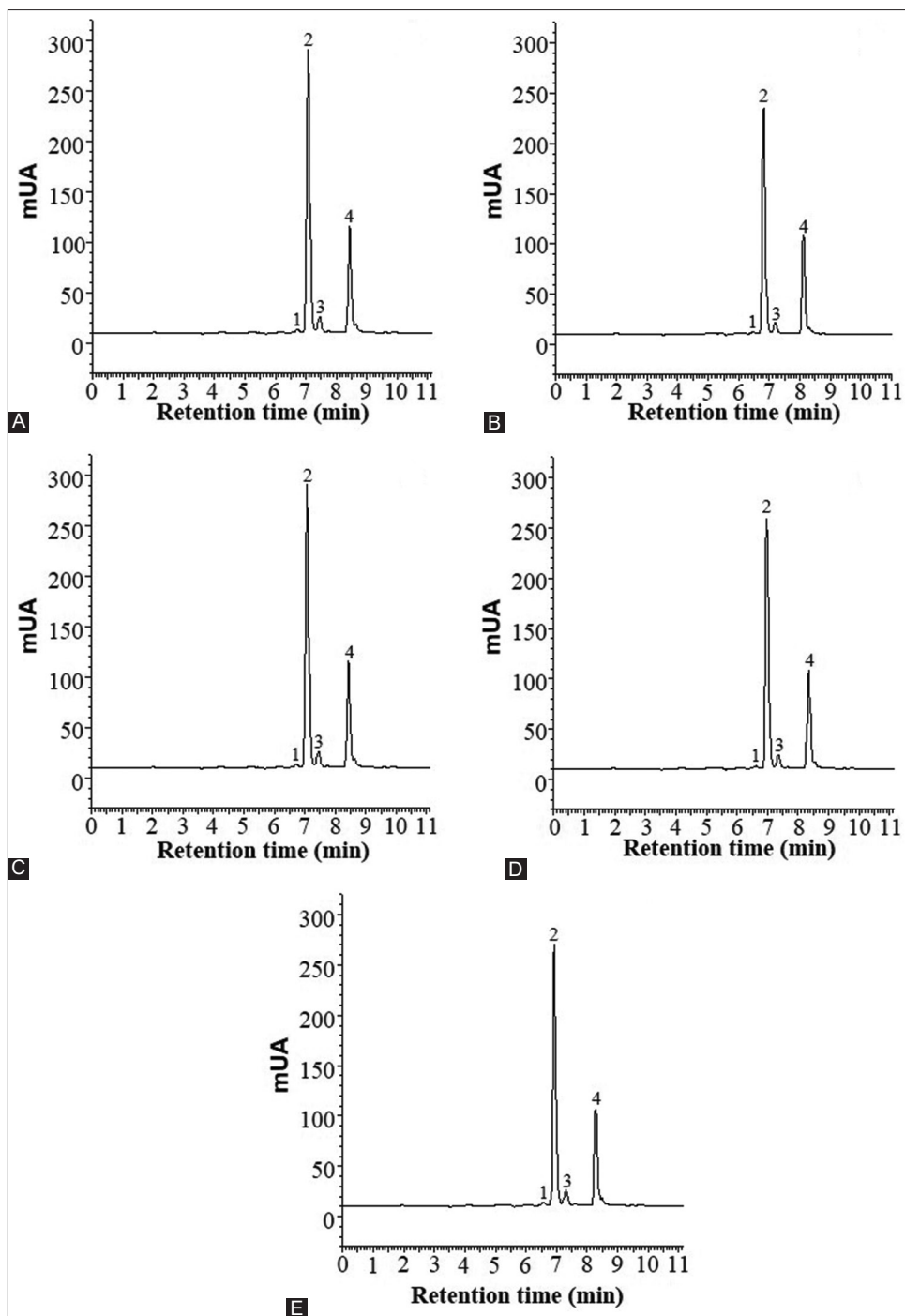


Fig 4. Profile of anthocyanins chromatograms by HPLC from the aqueous extract of calyces of *H. sabdariffa* from Negra Quiviquinta variety subjected to different dehydration treatments. The treatments were: 1) fresh calyces (FC), 2) drying in the sun (SD), 3) dehydration with air at 50°C (AD_{50°C}), 4) dehydration with air at 60°C (AD_{60°C}), and 5) dehydration with air at 70°C (AD_{70°C}). The identity of the anthocyanins was: 1) and 3) not identified, 2) delphinidin 3-sambubioside and 4) cyanidin 3-sambubioside.

reduction of the total acid content of 54.9% in relation to the lyophilized control. In NQ and UAN-16 varieties, the reduction was 44.9% in both. The analysis of the effect of the SD on each acid showed that in NQ the most affected were malic and succinic; in UAN-9 and UAN-16 all were affected, except citric acid, which was even greater in the calyces dehydrated in the sun (SD) than

in the lyophilized control and in some air dehydration treatments (Table 4).

The dehydration treatments with hot air had little or no impact on the levels of each of the acids, when comparing their values in relation to the control. Inclusively, the calyces of UAN-9 and UAN-16 varieties, from the AD_{70°C}

Table 4: Organic acid content in calyces of three varieties of *H. sabdariffa* subjected to different dehydration treatments.

Variety	Treatments	Oxalic	Tartaric	Malic	Citric	Succinic	Total
(mg 100 g ⁻¹ DW)							
NQ	FC	1.2±0.1gh	50.7±0.6gh	132.0±3.3c	7.5±0.6eg	325.2±7.0c	516.5±10.3bc
	SD	0.8±0.0h	45.1±0.4j	87.3±1.8d	21.6±3.4ac	129.5±4.0f	284.3±8.8h
	AD _{50°C}	0.9±0.0h	45.7±0.3ij	149.5±2.3b	5.9±1.3fg	409.0±9.1a	610.9±12.9a
	AD _{60°C}	1.0±0.0h	43.9±0.7j	151.4±4.4ab	28.3±1.8a	309.0±3.6cd	533.6±10.4bc
	AD _{70°C}	0.9±0.1h	43.1±0.4j	159.8±2.1ab	14.5±3.6cf	328.8±9.9c	547.0±9.0b
UAN-9	FC	0.9±0.1h	48.4±0.3hi	153.0±1.9ab	9.7±1.0dg	284.3±17.5d	496.3±17.1cd
	SD	0.2±0.0i	45.1±0.7j	74.9±3.8df	25.6±1.6ab	78.2±6.9g	224.0±12.9i
	AD _{50°C}	1.7±0.0fg	52.8±0.3eg	134.3±5.0c	17.3±6.3be	309.5±15.6cd	515.6±27.1bc
	AD _{60°C}	1.8±0.4f	56.6±0.8cd	120.7±4.3c	28.7±1.2a	239.8±9.8e	447.6±15.7de
	AD _{70°C}	0.8±0.0h	51.0±0.1fh	164.8±0.1a	19.0±0.6ad	371.9±4.7b	607.4±4.3a
UAN 16-1	FC	9.9±0.1a	59.6±0.8ab	122.0±8.2c	6.6±1.1fg	246.2±6.4e	444.2±3.8ef
	SD	4.7±0.0c	55.1±0.0de	64.3±0.1f	27.1±1.8ab	93.7±2.6fg	244.8±4.4hi
	AD _{50°C}	3.5±0.2d	53.9±1.0df	82.1±2.0de	9.3±2.4dg	245.0±11.2e	393.9±11.9fg
	AD _{60°C}	2.8±0.3e	58.2±2.0bc	71.9±2.4ef	4.2±1.2g	221.3±11.4e	358.5±15.1g
	AD _{70°C}	7.6±0.0b	61.8±0.5a	120.8±0.8c	19.2±2.7ad	303.1±4.3cd	512.3±0.3bc
HSD*		0.58	3.02	13.86	9.97	37.09	50.55

* The treatments were: FC: fresh calyces; SD: drying in the sun; AD_{50°C}: dehydration with air at 50°C; AD_{60°C}: dehydration with air at 60°C; AD_{70°C}: dehydration with air at 70°C. HSD: honest significant difference.

treatment presented higher total acid values. It was noteworthy that freeze-dried calyces (control) had a lower total organic acid content than that observed in some of the AD treatments. This result could be related to what has been reported by Ardestani et al. (2015) in relation that exposure to freezing temperatures can induce a decrease in the content of organic acids, such as malic and succinic. It is possible that the freezing of the calyces prior to the lyophilization process has caused losses of these acids in the calyces of the *H. sabdariffa* varieties.

The values obtained for the different organic acids are similar to those reported by Wong et al. (2002), who reported values of 510 and 120 mg 100 g⁻¹ for succinic and malic acids, in that order. The authors did not find citric acid in the calyces of the cultivar analyzed. The relative presence of organic acids in the aqueous extract of the calyces, in the *Hs* varieties studied was succinic > malic > tartaric > citric > oxalic, which corresponds to the order reported by Sánchez-Feria et al. (2018) in varieties of *Hs* grown in Mexico. However, the order of predominance of organic acids can vary depending on the variety and the place where it is grown. For Wong et al. (2002) the relative concentration order of organic acids was succinic > oxalic > tartaric > malic, in the calyces of a cultivar from Malaysia, while Ibrahim et al. (2015) reported this same order of predominance of the organic acids mentioned for a cultivar from Iran.

In the present investigation, the low presence of oxalic acid in the aqueous extracts of the *Hs* calyces analyzed stands out. This result would be favorable for the national varieties of *Hs* since oxalic acid can bind with Ca and prevent its absorption (Weaver et al., 1987). This opportunity does not occur as

reported by Wong et al. (2002) for the cultivar of Malaysia, nor for the cultivar described by Ibrahim et al. (2015).

CONCLUSIONS

Of the variables used to evaluate the quality of the aqueous extracts of the dehydrated calyces, the titratable acidity was the most affected by the dehydration methods. For the phenolic composition and color variables, the most important factor was the genotype. The interaction of the genotype x dehydration method factors had a low contribution in the variation of the quality variables evaluated. The method of dehydration in the sun was the one that most detracted from the quality of the calyces of *H. sabdariffa*, by reducing in a greater proportion the contents of total soluble phenols, total anthocyanins, and titratable acidity, while the method that best preserved the quality of the calyces was that of dehydration with air at 70°C. The chromatographic profiles of anthocyanins and organic acids in the calyces were not modified by the dehydration method used. The method of dehydration in the sun, reduced on average 49.9% the content of organic acids in the calyces, with malic and succinic as the most sensitive. The air dehydration method did not affect the level of organic acids in the calyces. The method of dehydration with hot air at 70°C was the one that best preserved the quality of the calyces.

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César Sánchez-Feria, Yolanda Salinas-Moreno and Víctor Arturo González Hernández participated in the design of the research proposal, search for funding and monitoring of the experimental work. In the case of the first author, he participated in the execution of the required field and laboratory analyzes. Yolanda Salinas-Moreno wrote the first draft of the document; María del Carmen Ybarra-Moncada supported the statistical analyzes carried out, while María Luisa Machuca-Sánchez supported the drying of the calyces with hot air.

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