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RESEARCH

# Roselle (*Hibiscus sabdariffa* L.) cultivars calyx produced hydroponically: Physicochemical and nutritional quality

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## ABSTRACT

The chemical constituents of roselle (*Hibiscus sabdariffa* L.) vary by environmental stimuli and cultivar. The effect of cultivars produced under hydroponic and greenhouse conditions on the mineral composition, chemical and nutraceutical quality of the calyx was evaluated. An experiment was conducted in greenhouse and hydroponic conditions, in a randomized complete design with three replicates and four plants per experimental unit. Treatments were formed by a six roselle cultivars: Cruza Negra, Criolla Huajicori, UAN25-1, UAN16-2, 4Q4, and UAN6-Puga. Principal component analysis (PCA) showed that 'Cruza Negra' is the best cultivar because it presents the highest values of total and insoluble fiber (395 and 260 g $kg^{-1}$ dry basis [db]), total phenols (29.178 mg $kg^{-1}$ db), anthocyanins (18.133 mg $kg^{-1}$ db), 2,2-diphenyl-1-picrylhydrazyl (DPPH; 645 mg $kg^{-1}$ db), and the absorption capacity of oxygen radicals (ORAC; 240 mg $kg^{-1}$ db). The highest contribution of minerals was observed in 'Cruza Negra' (K, Na, Zn, and Cu) and 'Criolla Huajicori' (Ca, Mg, S, and Mn), contributing significantly to the recommended daily intake (RDI). Variability was evident in all the quality characteristics evaluated in the greenhouse roselle calyx. Ash, total and insoluble fiber, phenols, anthocyanins, DPPH, and ORAC contributed the most to the nutritional and nutraceutical quality of the roselle calyx. These variables were influenced by the cultivar.

**Key words:** Anthocyanins, composition, fiber, *Hibiscus sabdariffa*, main components, quality.

## INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) production is conducted in countries with tropical and subtropical regions. This species is primarily cultivated because of the consumption of its calyx (sepals); they are commercially important in the food industry for the production of juices, jams, salads, pigments, and beverages (Borrás-Linares et al., 2015).

On a dry basis, roselle as a food species contains proteins, fats, carbohydrates, raw fiber, and ashes (Adanlawo y Ajibade, 2006), as well as vitamins, organic acids, and phytosterols (Ismail et al., 2008), which are useful for the health of consumers (Da-Costa-Rocha et al., 2014). Roselle is also an important source of minerals, with K, Ca, and Mg as well as trace elements (Fe, Mn, Zn, and Cu) that the play role in health by functioning as antioxidants or as components of antioxidant enzymes (Evans and Halliwell, 2001).

In addition to its nutritional value, the roselle calyx contains components that confer pharmacological, nutraceutical, and cosmetological properties. Within this group are the polyphenols including delphinidin and cyanidin (Borrás-Linares et al., 2015; Jabeur et al., 2017), that show antioxidant activity in the human body (Wang et al., 2011).

Crop management and genetic variation are determining factors of the components of the agricultural products that engender benefits to human health. Changes that have demonstrated the effects of the cultivar in terms of the proximal composition of roselle calyx on the contents of its ash, protein, total carbohydrates, and total soluble and insoluble fiber have been reported (Duarte-Valenzuela et al., 2016). Studies on the production of roselle under greenhouse conditions are scarce and designed to evaluate the effect of N and pathogens on plant development (Rhoden et al., 1993; Hassan et al., 2014); however, no studies have related this information to the quality of the calyx. However, the behavior of the chemical constituents of the roselle calyx obtained in protected agriculture remains unknown. This research evaluated the effect of the cultivar on the mineral composition, as well as the chemical and nutraceutical quality of the roselle calyx produced under hydroponic and greenhouse conditions.

## MATERIALS AND METHODS

The experiment was conducted from October 2015 to March 2016 by roselle calyx in a greenhouse at Culiacan, Sinaloa, Mexico. Maximum and minimum temperatures and relativity humidity were recorded using a data logger (HOBO H8 Onset, Computer Corporation Data Logger, Massachusetts, USA) throughout the development cycle of the crop inside the greenhouse (Figure 1).

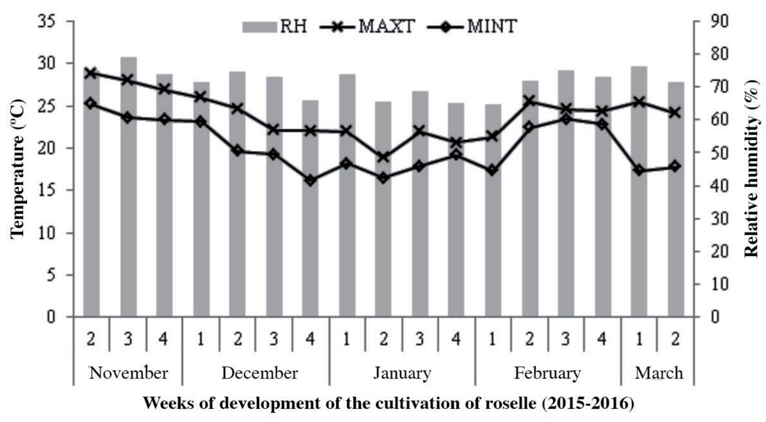
The roselle 'Cruza Negra', 'UAN16-2', 'Criolla Huajicori', 'UAN6Puga', 'UAN25-1', and '4Q4' were provided by the Autonomous University of Nayarit, Mexico. The seedlings were produced in 120-well germination trays with a mixture of peat substrate:perlite (70:30 v/v). The transplantation that occurred in the greenhouse was conducted with 17-d-old seedlings. Each seedling was placed in a polyethylene bag with 13 L substrate (60% Fluvisol soil and 40% coconut fiber). The distance between plants was 0.50 m and 1.0 m between rows. Through drip irrigation, the Steiner nutrient solution (Steiner, 1961) was supplied at a rate of eight daily 5 min-events, with 4 L $h^{-1}$ drippers.

The fruits were harvested at commercial maturity (capsule opening) 138 to 158 d after transplantation depending on the cultivar. The calyces were manually separated from the capsule and dehydrated to a constant dry weight in an oven (NAHITA 631, France) at 60 °C by 36 h. After drying, they were milled in a stainless-steel mill (Mod. 3383-L10; Thomas Scientific, Swedesboro, New Jersey, USA) and screened in an nr 40 mesh for quality determinations.

### Proximal and mineral composition

The humidity, ash, fat, protein (N × 6.25), and soluble, insoluble, and total fiber contents were determined using AOAC (1998). Total carbohydrates were obtained via the difference method, where carbohydrates = 100 - (humidity + protein + fat + total fiber + ash). Total N was measured via the semi-Kjeldahl method (Bremner, 1996), and P was measured via visible spectrophotometry (Spectrophotometer UV/Vis 6705, Jenway, Stone, UK). Ca, Mg, K, S, Na, Fe, Zn, Mn, and Cu were quantified via atomic absorption spectrometry (AA FS flame AA 280FS + SIPS 20, Agilent Technologies, Santa Clara, California, USA) according to the AOAC official methods (AOAC, 1998).

**Figure 1. Weekly average of relative humidity (RH) and maximum (MAXT) and minimum temperatures (MINT) during the cultivation of roselle.**



**Nutraceutical attributes**

The anthocyanins were analyzed via absorbance at 520 nm (Abdel-Aal and Hucl, 1999), using the standard curve of cyanidin-3-glucoside (Sigma-Aldrich, St. Louis, Missouri, USA) and the results were expressed as milligram equivalents of cyanidin-3-glucoside per kilogram dry basis (mg EC3G $kg^{-1}$ db). Total phenols were quantified according to the Folin-Ciocalteu method (Swain and Hillis, 1959) using the standard curve of gallic acid (Sigma-Aldrich) and the results were expressed as milligram equivalents of gallic acid per kilogram dry basis (mg EAG $kg^{-1}$ db). The antioxidant capacity (AC) was determined using two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Panchawat, 2011) and the absorption capacity of oxygen radicals (ORAC) (Huang et al., 2002). For both methods, the standard curve of Trolox (Sigma-Aldrich). The AC of both methods was converted into equivalent micromoles of Trolox (ET) per gram dry basis (μmol ET $g^{-1}$ db). The three response variables were evaluated using a Sinergy HT Microplate reader (BioTek, Winooski, Vermont, USA).

**Color analysis**

Color attributes of roselle calyx were obtained using the CIELCH scale using a spectrophotometer (CM-700d; Konica Minolta, Ramsey, New Jersey, USA). Luminosity (L), hue angle (Hue = arc tan b/a) and chromaticity (Chroma = $\sqrt{[a^2 + b^2]}$) were obtained with OnColor QC version 5 (CyberChrome, Stone Ridge, New York, USA) (Minolta Konica, 2007; Báez-Sañudo et al., 2017).

**Statistical analyses**

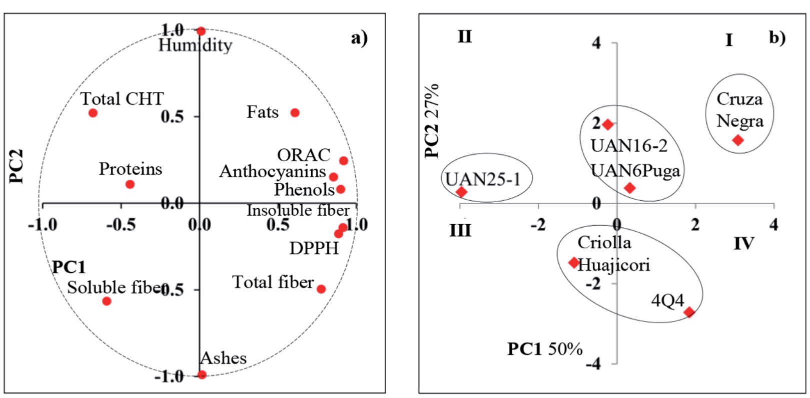
The results were analyzed using principal component analysis (PCA), ANOVA, and Tukey mean comparisons ($p \leq 0.05$) by SAS software for Windows, version 9.0 (SAS Institute, Cary, North Carolina, USA). The color was plotted in the CIELCH space with Sigma-Plot, version 11.0 (2008; Systat Software, San José, California, USA). The proximal (nutritional) and nutraceutical attributes of the roselle calyx were analyzed via the Princomp procedure using the correlation matrix, and the components were plotted to visualize the spatial distribution of the cultivars. After PCA, the variables selected for each component, mineral content, and color of the calyx were analyzed using ANOVA (one factor = cultivar), and the means were compared using the Tukey test ($p \leq 0.05$).

## RESULTS AND DISCUSSION

**Principal component analysis**

In this research, the first two components were chosen, which accounted for 77% (PC1:50% PC2:27%) (Figure 2b) of the total variance according to Kaiser's criterion, that mentions that with the sum of approximately 80% of the variation is sufficient for the choice of the main components (Braeken and Van Assen, 2017). The coefficients of the first eigenvector (50%) and correlations showed that the contents of total fiber (r = 0.78), insoluble fiber (r = 0.92), phenols (r = 0.90), anthocyanins (r = 0.85), DPPH (r = 0.89), and ORAC (r = 0.92) were positively associated with component 1 (PC1). Component 2 (PC2; 27%) was related to humidity (r = 0.99) and inversely related to ash (r = -0.99) (Figure 2a).

**Figure 2. Correlation of nutritional and nutraceutical variables of roselle calyx (a) and a scatterplot of different cultivars (b).**



According to PC1 and PC2, the contents of total fiber, insoluble fiber, phenols, anthocyanins, humidity, ash, DPPH, and ORAC presented the highest loads (positive and negative) and were therefore used to define the quality of roselle cultivars. Similar results were reported with regard to calyx obtained in open field cultivation, where total fiber, phenols, and antioxidant activity (ABTS and FRAP) contributed to greater variability and affected the quality of different cultivars (Duarte-Valenzuela et al. 2016).

The dispersion of the cultivars determined by PC1 and PC2 allowed the formation of four clusters (Figure 2b). In quadrant I, the first cluster included 'Cruza Negra', with the best quality in humidity, total fiber, insoluble fiber, total phenols, anthocyanins, DPPH, and ORAC; cluster 2 ('UAN16-2' and 'UAN6Puga') with intermediate values; cluster 3 ('UAN25-1'), showed the lowest contents of quality and cluster four was represented by '4Q4' and 'Criolla Huajicori', showing high values in total fiber and ash.

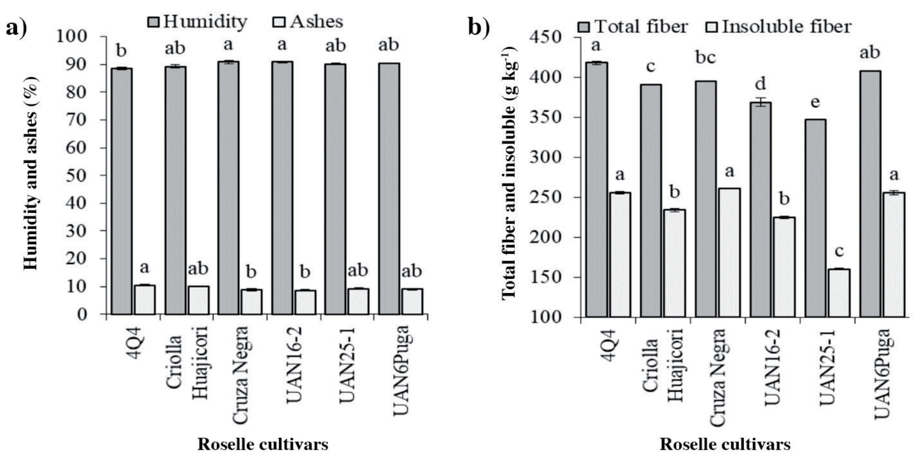
**Proximal variables selected in PC1 and PC2 and mineral composition**

The ANOVA revealed significant differences ($p \leq 0.05$) with regard to the cultivar in humidity, ash, total fiber, insoluble fiber, phenols, anthocyanins, DPPH, and ORAC.

In the six cultivars, ash content ranged from 8.6% to 10.5% db, with that in '4Q4' being higher than those in 'Cruza Negra' and 'UAN16-2' (Figure 3a). These results corroborate the reported values of 9.5% and 10.6% db in open field crops of white and red roselle, respectively, harvested in Sudan (Suliman et al., 2011). '4Q4' and 'UAN6Puga', but not 'UAN25-1', presented the highest total fiber content (Figure 3b), an attribute that can be exploited for industrial use. '4Q4', 'Cruza Negra', and 'UAN6Puga' obtained the highest contents of insoluble fiber. In this research, the total fiber content was slightly lower than the 550 g $kg^{-1}$ db reported by Sáyago-Ayerdi et al. (2014) for roselle in the open field.

The composition of the minerals of the roselle calyx was affected by the cultivar (Table1), where the highest content was observed for 'UAN25-1'. According to the mineral content and considering a consumption of 25 g dehydrated roselle, P, Ca, Mn, Zn, and Cu were the minerals with the highest nutritional intake based on the recommended daily intake (RDI) of each mineral (García-Gabarra, 2006). Of the most important macronutrients, P reached values greater than 30% RDI for all roselle cultivars, where 'UAN25-1' had the highest content with 45%. Calcium showed until 39% of the RDI for all cultivars, with 'Criolla Huajicori' having the highest content and the Mg too. Manganese was the most common micronutrient, surpassing 72% of its RDI for all cultivars, whereas Zn reached its maximum content in the 'Cruza Negra'. The Cu content was greater than 18% of its RDI for the six cultivars, with 'Cruza Negra' having the highest contribution. Comparatively, 'UAN25-1', 'Criolla Huajicori', and 'Cruza Negra' showed the highest ability to absorb and assimilate N in the calyx. The minerals play an important role in the health of consumers.

**Figure 3**. **Contents of humidity, ashes (a), total and insoluble fiber (b) of calyx of different roselle.**




Different letters on bars corresponding to the same response variable indicate significant differences between cultivars according to Tukey test ($P \leq 0.05$). Vertical bars correspond to standard deviation.

**Table 1. Mineral content (dry basis) of calyx from different roselle cultivars.**

| | 4Q4 | Criolla Huajicori | Cruza Negra | UAN 16-2 | UAN 25-1 | UAN6 Puga | HSD | CV | RDI |
|---|---|---|---|---|---|---|---|---|---|
| | mg kg$^{-1}$ | | | | | | | % | mg d$^{-1}$ |
| N | 18251b | 19540ab | 19042ab | 18512b | 22007a | 16598b | 2973 | 5.7 | * |
| P | 8099c | 10831b | 11115b | 10908b | 11954a | 11040b | 443 | 1.5 | 664 |
| K | 9074d | 14532c | 19605a | 17088b | 17317b | 15476c | 1358 | 3.2 | 4700 |
| Ca | 7264e | 14061a | 7914d | 11295b | 10049c | 6928e | 417 | 1.6 | 900 |
| Mg | 2379d | 3259a | 2840c | 3027b | 2870bc | 2323d | 173 | 2.3 | 248 |
| S | 173b | 263a | 92cd | 164b | 134bc | 68d | 61 | 14.8 | * |
| Na | 26d | 62bc | 92a | 51c | 80ab | 59c | 20 | 11.7 | 1500 |
| Fe | 28d | 59d | 187ab | 212a | 160bc | 130c | 33 | 9.3 | 17 |
| Mn | 66c | 178a | 63c | 86b | 58c | 71bc | 16 | 6.5 | 2 |
| Zn | 116d | 128d | 323a | 83e | 166c | 180b | 13 | 2.9 | 10 |
| Cu | 7b | 8b | 11a | 9b | 8b | 8b | 2 | 6.5 | 1 |
| Total | 45483 | 62921 | 61284 | 61435 | 64803 | 52881 | | | |

Values are mean ± SD (n = 3) different letters in the same row indicate significant differences between samples using Tukey test (P ≤ 0.05).
HSD: Honest significant difference; CV: coefficient of variation; RDI: recommended daily intake.
*No data available.

The mineral content in the cultivars studied was superior or similar to those in other studies conducted on roselle cultivated in the open field. Glew et al. (1997) reported P contents of 1630 mg kg$^{-1}$ db in the calyx of the criolla roselle; K has been quantified as up to 20 000 mg kg$^{-1}$ db in the green, red, and dark calyxes of roselle (Babalola et al., 2001). Jung et al. (2013) found Ca contents of 10707 mg kg$^{-1}$ db and Mg contents of 510 mg kg$^{-1}$ db in roselle calyx.

The literature is inconsistent concerning the chemical composition of the roselle calyx with regard to Na (55 to 450 mg kg$^{-1}$ db), whereas Mn (88 to 254 mg kg$^{-1}$ db), Fe (163 mg kg$^{-1}$ db), Zn (33 mg kg$^{-1}$ db), and Cu (4 mg kg$^{-1}$ db) were below the content reported in the literature (Babalola et al., 2001; Jung et al., 2013).

## Nutraceutical attributes

The total phenol content in the cultivars varied from 2941 to 29178 mg EAG kg$^{-1}$ db; 'Cruza Negra' showed the largest contribution (Figure 4a). In general, these results are low compared with those reported by Borrás-Linares et al. (2015), with 24 000 to 100 000 mg EAG kg$^{-1}$ db for the 25 Mexican varieties and 38000 mg EAG kg$^{-1}$ db for the roselle from Nigeria (Ifie et al., 2018) both of which were cultivated in the open field. In addition, these results are within the average to those reported by Zhang et al. (2018) for 19 mandarin genotypes. The cultivars of this study, 'UAN16-2' and 'UAN25', had phenol contents that were 64% and 95% lower than those of the same materials cultivated in the open field reported by Sánchez-Feria et al. (2017); possibly because of UV radiation (Neugart et al., 2012).

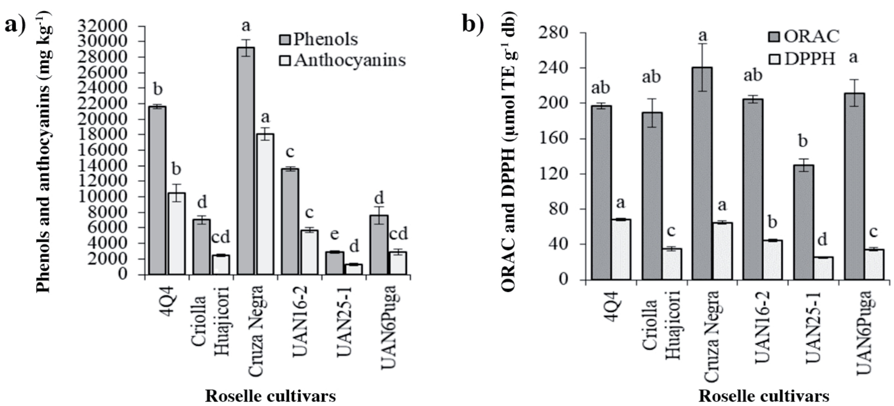
The anthocyanin content of the roselle calyx showed similar results to those of the phenols but in a smaller quantity (Figure 4a). In addition, the literature records anthocyanin contents that vary from 9290 to 44080 mg EC3G kg$^{-1}$ db for different roselle calyx cultivars under open field crops (Salinas-Moreno et al., 2012; Borrás-Linares et al., 2015). The content of anthocyanins in the calyxes presented here is higher than those reported by Zhang et al. (2016) for three onion varieties (7.5-299.9 mg 100 g$^{-1}$ FW).

In AC, five materials were significantly equal, with a maximum value at 2140 µg mol$^{-1}$ ET db (Figure 4b). DPPH radical was 62% less than the ORAC, and 'Cruza Negra' and '4Q4' exhibited higher values the others calices and the concentration was similar to Mexican roselle (Borrás-Linares et al., 2015). Regarding ORAC, no studies were found for roselle, however, reports of pigmented materials for amaranth flowers (*A. hypochondriacus*; 58 µmol ET g$^{-1}$ db) were reviewed, and the results for roselle were lower (Li et al., 2015).

## Roselle color

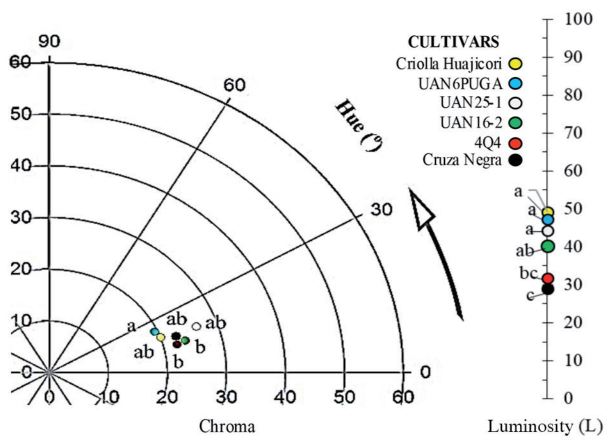
The cultivar had significant effects (p ≤ 0.05) on the color, L, and °Hue (H) attributes of the roselle calyx. Chroma (C) values were not affected. In the CIELCH color space (Figure 5), the L of the roselle calyx showed values of 29-49 among the cultivars. 'Cruza Negra' and '4Q4' showed the lowest L, which was related to the higher content of pigments (Figure 4a). Studies in Mexican and on imported roselles reported L values between 31 and 79 (Salinas-Moreno et al., 2012; Camelo-Méndez et al., 2016), with a similar variability as shown in this study.

**Figure 4. Contents of phenols and total anthocyanins (a), ORAC and DPPH (b) in the cultivars of roselle.**




Different letters on bars corresponding to the same response variable indicate significant differences between cultivars according to Tukey test (P ≤ 0.05). Vertical bars correspond to standard deviation.

**Figure 5. Color of the six roselle flower cultivars in the CIELCH color space.**




Different letters corresponding to the same response variable indicate significant differences between cultivars according to Tukey test (P ≤ 0.05).

Among the cultivars, the calyx tone angle varied from 14 to 24 °Hue, '4Q4' showing the more intense red color (°Hue = 14). These results ranged between 12 and 74 °Hue reported for Mexican roselle (Salinas-Moreno et al., 2012). The Chroma variable enables the differentiation of cultivars.

## CONCLUSIONS

It can be concluded that characteristics evaluated in the roselle calyx are affected by the cultivar. The roselle calyx was corroborated as having antioxidant compounds (e.g., phenols, anthocyanins, and antioxidant activity) in addition to its high content of total and insoluble fiber; however, through the mode of production under hydroponic and greenhouse conditions, this crop showed considerable increases in mineral content. The contents of total fiber, insoluble fiber, phenols, anthocyanins, humidity, ash, DPPH, and ORAC contribute the most to the nutritional and nutraceutical quality of hydroponically produced roselle calyx. The cultivar also influenced these contents.

Variations in the nutritional and nutraceutical composition of roselle allow the production company to select cultivars according to the production approach, where 'Cruza Negra' can be the cultivar chosen for commercial production given its high contents of phenols, anthocyanins, insoluble fiber and antioxidant capacity, components that are considered as desirable for their benefits to human health.

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