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Characterization of Mature Cladodes *of Opuntia ficus-indica* L. Using Morphological and Colorimetric Descriptors

Juan Arredondo-Valdez[1], Alejandro Isabel Luna-Maldonado[1,*], Ricardo David Valdez-Cepeda[2], Humberto Rodríguez-Fuentes[1], Juan Antonio Vidales-Contreras[1], Uziel Francisco Grajeda-González[1], Héctor Flores-Breceda[1]

[1]Department of Agricultural and Food Engineering, Faculty of Agriculture, Autonomous University of Nuevo Leon, General Escobedo, N.L., México
[2]Centro Regional Universitario Centro Norte, Universidad Autónoma Chapingo, Calle Cruz del Sur 100, Col. Constelación, CP 98085 Zacatecas, Zac., México

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Image processing

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Linear and nonlinear modeling

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ABSTRACT

Mexico is the world's leading producer of *Opuntia ficus-indica*. This kind of prickly pear is the most widespread and most commercially important cactus in Mexico. Morphological and colorimetric descriptors are among the most important agronomic traits because these parameters affect the yield, in such a way, the objective of the present research was to present a fast and reliable methodology to obtain the functional relationship in shape and color parameters of *O. ficus indica* cladodes, using a smartphone, a color meter, and open-access software. The acquisition and processing of images discovered interesting relationships between the *Opuntia* cladode's morphological characteristics, as well as colorimetric parameters of the cladodes. The non-linear data behaviors were fitted using deterministic models and CurveExpert software. Results of the study revealed that the best morphological descriptors were Circularity *vs.* Perimeter (r= 0.9815) and Aspect ratio *vs.* Roundness (r= 0.9999). In addition, mean values of the L*, C, and H color parameters were displayed in a window of a computer program online. It was found that the a-C relationship of the color parameters had the highest correlation coefficient (0.999). Therefore, it can be concluded that the morphological descriptors Circularity *vs.* Perimeter, Aspect Rate *vs.* Roundness, and a*-C color parameter can predict quickly and precisely the quality of *O. ficus-indica*.

* Corresponding author

E-mail: alejandro.lunaml@uanl.edu.mx (Alejandro Isabel Luna-Maldonado)

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1 Introduction

Mexico is a world leader in the production of *Opuntia ficus-indica* (prickly pear). In 2019, Mexico produced 863,000 tons of fresh *Opuntia* prickly pear in an area of 12,471.09 hectares; 34,000 tons of this prickly pear annually were exported to other countries, mainly to USA and Canada. In Nuevo León state the prickly pear average yield was 38 t ha^{-1} (SIAP 2021). However, the production of fresh *O. ficus-indica* prickly pear in Mexico could be affected by the selection of mature cladodes.

The quantitative evaluation of the shapes of the mature cladodes of the nopal is required in phenotyping research. The morphological descriptors describe the specific characteristics concerning the geometry of a particular trait, in such a way that measuring the physical characteristics of plants (the phenotype), allows researchers to relate them to the genetic makeup of the crops as Farina (2020) works on soybean, or Gutiérrez (2020) working on the morphological characterization of white and purple cocoa. The characterization of agricultural varieties has been based on morphological descriptors, including shape, color, and seed size, in addition to quantitative attributes such as mineral content (Morales-Morales et al. 2019).

Although the shape of an *Opuntia* cladode cannot be reconstructed by knowing the morphological descriptors, these can be discriminating variables in the shape of cladodes (Wirth 2004; Bober 2001; Hyun et al. 2015). Morphological descriptors have been successfully applied for the evaluation of various biological shapes in animals and plants (Žunić 2010; D'Silva and Bhuvaneswari 2015) such as the study of Laouadi et al. (2020) who provides a working basis on the morphology of local goats in Laghouat region by phenotypic characterization.

According to Iwata et al. (2004), the quantification of shapes is a prerequisite for assessing the inheritance of morphological features in quantitative genetics. Morphological characterization is necessary because it provides information about the features and structure of objects (Cheesa 2010). The identification of highly discriminant descriptors is important to obtain an efficient and reproducible classification of the mature cladodes of *O. ficus indica*. The potential taxonomic importance of plant and fruit morphology has been recognized by biologists, geneticists, and farmers (Visa et al. 2014). Morphological characterization has been advanced with the modern technologies of acquisition, processing, and analysis of the images of the plants and has acquired much importance for selection, and taxonomic studies (Brewer et al. 2006; Newton and Kendrick 1990; Ahmad et al. 2020).

On the other hand, color is an important sensory attribute to provides the basic quality information for human perception and has a close association with freshness, maturity, variety, convenience, and food safety. Therefore, colorimetric descriptors are important classification parameters for most agricultural and food products (McCaig 2002). In measuring food color, L * a * b * color space is the most widely used due to the uniform distribution of colors and because it is perceptually uniform (McGuire 1992; Leon et al. 2006). The objective of the present research was to present a fast and reliable methodology to obtain the functional relationship in shape and color parameters of *O. ficus indica* cladodes, using a smartphone a color meter, and open-access software.

2 Materials and Methods

2.1 Plant materials

One-year-old cladodes of *O. ficus indica* of the Villanueva cultivar were visually selected and collected by personnel experience in the management of *Opuntia* plants at the Alejandra ranch in Zuazua, Nuevo León; geographically located at 25 ° 52´ 03" North Latitude and 100 ° 05´18" West Longitude, located 30 km from the experimental site. The mature cladodes were disinfected with a solution of 1 kg of lime plus 1 kg of copper sulfate diluted in 98 L of water (Bordeaux broth) (Canseco-Guzmán and Canseco-Guzmán 2013). Subsequently, they were sown in 19 L pots, with clay loam soil.

2.2 Acquisition of images and morphological descriptors

Thirty mature cladodes were photographed individually, and all the obtained images were processed with the ImageJ version 1.51J8 platform to estimate the shape descriptors. ImageJ can display, edit, analyze, process, save, and print 8-bit, 16-bit, and 32-bit, grayscale, and 8-bit and 24-bit color images. As a first step, each jpg extension image was opened from of the corresponding directory in a personal computer and transformed into an 8-byte image. The threshold was set, converted to a mask, and transformed into a binary image. Subsequently, the binary image was inverted and the scale of cm to pixels was established, the desired morphological descriptors were configured, and again the image was inverted and finally, the *Opuntia* mature cladodes were analyzed with ImageJ software (Figures 1 and 2). A total of 12 morphological descriptors (Area, Length, Minor axis length, Perimeter, Feret Diameter, Aspect ratio, Compactness, Roundness, Strength, Centroid, Center of mass, and Kurtosis) were used to construct the data set and statistical analysis to characterize *Opuntia* mature cladodes and determine the potential use of these descriptors for its classification.

The shape descriptors of the mature cladodes with the Image J platform: Image formats including TIFF, GIF, JPEG, BMP, DICOM, FITS, and 'raw' can be imported and read as single images or stacks and incorporates several useful tools for image

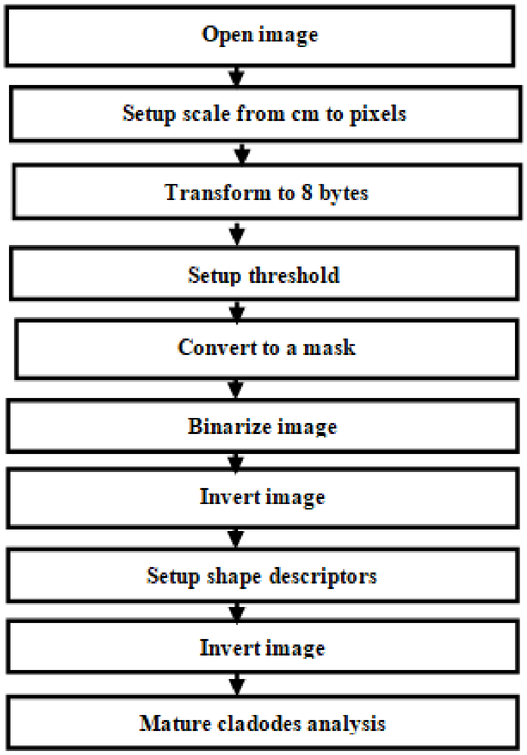

Figure 1 Flow diagram of the image processing operations.

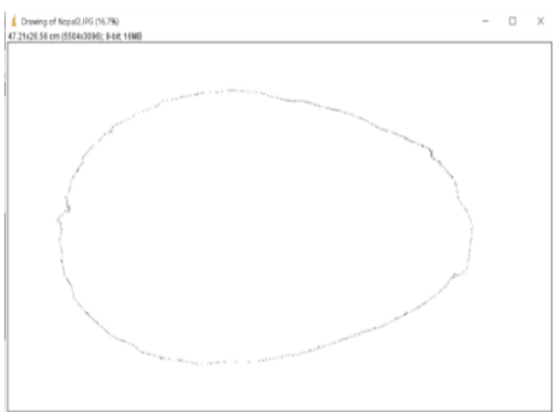

Figure 2 Mature cladodes processed with ImageJ.

processing (Hartig 2013) and incorporate an efficient way of image preprocessing using histogram equalization to properly select the region of interest (Veena Divya et al. 2016). The shape descriptors were:

Area: The area of the mature paddle was measured as the number of pixels in its outline silhouette.

a. Length of the major axis: The length of the longest line that can be drawn through the mature paddle was measured as the distance in pixels at the extreme points.

b. Minor axis length: The length of the longest line that can be drawn through the mature paddle perpendicular to the major axis, measured as the distance in pixels at the extreme points.

c. Perimeter: Length that corresponds to the closed contour of n vertices in a figure, that is the sum of the sides that form the polygon (polygonalized or vectorized limit).

d. Feret Diameter: Diameter of a circle that has the same area as the object, calculated with the formula: FD = square root of $[(4 * \text{area}) / \pi]$.

e. Aspect ratio (AR): The relationship between the length of the *Opuntia* mature paddle and its width.

f. Compactness: It provides a measure of the roundness of the mature paddle: if it is 1 the mature paddle is approximately circular, when it decreases by 1, the mature paddle is less circular, calculated as C = FD / Length of the major axis.

g. Roundness or circularity: A measure of roundness or circularity (area-perimeter ratio) that excludes objects from the area of a circle with the same convex perimeter. If the ratio is equal to 1, the object is a perfect circle, when the ratio decreases by 1, the object comes out from a circular shape, calculated as $R = [(4\pi \text{ X area}) / \text{perimeter}^2]$.

h. Strength: Measures the density of an object.

i. Centroid: Average of the x and y coordinates of all pixels in the image or selection.

j. Center of mass: Weighted average of the brightness of the x and y coordinates of all pixels in the image or selection. Use the XM and YM headers. These coordinates are the first-order spatial moments.

k. Kurtosis: The fourth-order time was above the average.

In addition, the mature cladodes' thickness and weight were measured.

2.3 Color descriptors

30 *Opuntia* cladodes were sown in 19-liter pots and the color parameters were measured every week, over 16 weeks from October 2019 to February 2020. The parameters a*, b*, C, L*, and H, were obtained using a Konica Minolta Chroma Meter CR-410 color meter, with a standard Illuminant C, 2° standard observers, and a field of view with a diameter of 8 mm, aperture 50 mm. The color was taken in the color space L* a* b*; where L* is the luminosity; a* is the chromaticity of green to red, and b * is the chromaticity from blue to yellow. The color hue (°H) was also measured (Figure 3).

Figure 3 Measuring the color parameters of the mature cladodes.

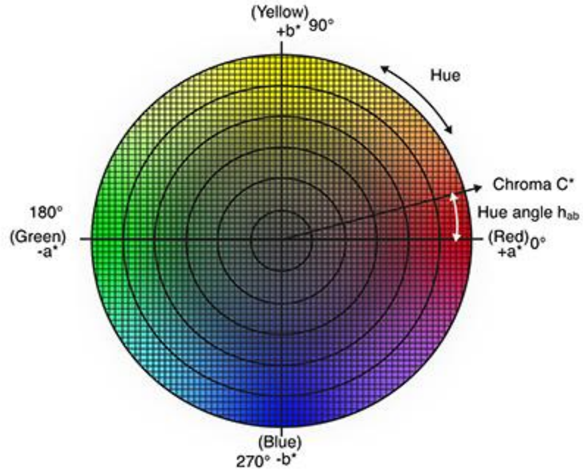

Figure 4 L*a*b* color space (Grajeda-González et al., 2015).

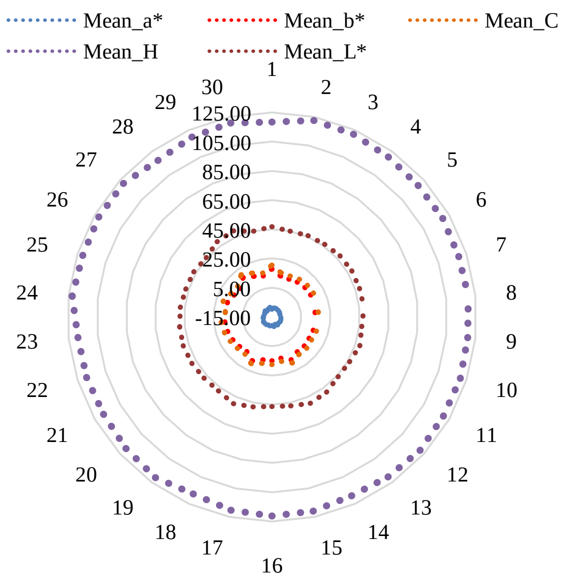

Figure 5 Charts showing mean values of color attributes.

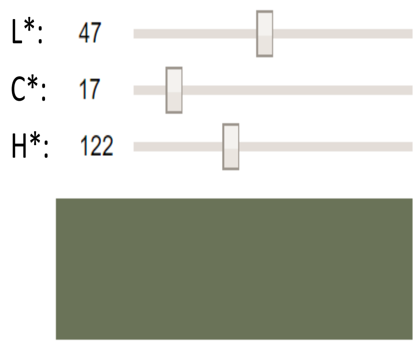

Figure 6 Mean L*, C, and H color parameters of cladodes.

The relationship between the color parameters, a*, b*, C, and H, are shown in the color sphere (Figure 4) where $C^* = (a^{*2} + b^{*2})^{1/2}$ and H = arc tan (b* / a*) (Grajeda-González 2015). The parameters L *, a *, and b * were obtained by the average of two measures carried out on two opposite sides of the prickly pear cactus cladodes (figure 5). Johnston online program was used to display the colors corresponding to the measured color parameter mean values of L*a*b*, (Figure 6) (Johnstone 2019).

2.4 Statistical analysis

Descriptive statistics were performed and a total of 12 quantitative variables were analyzed, and parameters such as mean, minimum, maximum, and coefficient of variation were obtained. The analysis of variance (ANOVA) was applied indicating significant differences with a low probability of error.

Moreover, a correlation analysis between morphological parameters was done to search for a relationship between them. Because of data distribution, heavy-duty nonlinear regression analysis was carried out using CurveExpert Pro: 2.6.5 freeware.

Moreover, the mean values of the color parameters of the 30 cladodes were obtained. An online program was used to visualize the measured L*a*b* parameters (Figure 6). The ANOVA was carried out (Table 7). The correlation coefficient r^2 and the nonlinear mathematical model were obtained (Table 9).

3 Results and Discussion

With the nonlinear regression analysis, we obtained the mathematical models for the combination of seven pairs of the most important morphological parameters, with their corresponding determination coefficient at a 95% confidence level.

The time to analyze images of mature *O. ficus-indica* cladodes to obtain quantitative variables related to their external morphology was reduced up to 9 significant digits' accuracy. The weight of the mature cladodes ranged between 682.95g and 1299.4g, the surface area of the mature cladodes ranged between 337.941 and 618.512 cm^2, and the width of the cladode ranged between 28.086 cm and 38.511 cm (Table 1). The obtained results of analysis of variance showed a significant difference with a 95% of confidence level in *Opuntia* mature cladodes for all treatments. The mean values, standard deviation, and amplitude of the other variables are also shown in Table 1, as well as the coefficient of variation, which ranged from 0.75% to 70.6%; however, most of the variables showed a variation coefficient of less than 10%.

The results obtained for the shape parameters from the correlation analysis showed correlation factors of 0.99 for Aspect Ratio and Roundness and 0.96 for Perimeter and Circularity (Table 2). The results of the non-linear regression analysis, using Curve Expert, indicated a strong relationship between the following parameters: Perimeter *vs.* Area (r= 0.8975), Circularity *vs.* Area (r= 0.9388), Feret Diameter *vs.* Area (r= 0.9394), Circularity *vs.* Perimeter (r= 0.9815), Feret Diameter *vs.* Perimeter (r= 0.9493), Roundness *vs.* Circularity (r= 0.8735), Aspect Ratio *vs.* Circularity (r= 0.8637), Aspect Ratio *vs.* Roundness (r=0.9999). There were other parameters with a less strong relationship as such as Solidity *vs.* Area (r= 0.679), Solidity *vs.* Perimeter (r= 0.640), Feret Diameter *vs.* Circularity (r= 0.764), Solidity *vs.* Roundness (r= 0.763), Solidity *vs.* Aspect Ratio (r= 0.753) and the rest of pairs showed a low relationship, with a lower correlation coefficient (Table 3). The parameters of the highest correlation coefficient, as well as the proposed type of mathematical model, are shown in Table 4.

Table 1 Mean, minimum, maximum values, standard deviation, and coefficient of variation of mature cladodes morphological parameters.

	Area	Xm	Ym	Perimeter	Width	Height
Mean	451.891	17.638	14.706	87.807	33.401	18.720
Mín. Value	337.941	15.012	11.747	73.048	28.086	15.848
Máx. Value	634.531	21.91	18.611	144.918	38.511	22.797
Std .Desv.	71.916	1.668	1.723	18.349	3.050	1.779
Variation Coefficient%	15.900	9.457	11.718	20.89	9.130	9.502
	Circularity	Feret Diameter	Kurtosis	Aspect Ratio	Roundness	Solidity
Mean	0.768	33.494	1.977	1.807	0.557	0.986
Mín. Value	0.329	28.372	-0.021	1.510	0.488	0.956
Máx. Value	0.870	38.653	5.624	2.050	0.662	0.993
Std. Desv.	0.132	3.028	1.395	0.149	0.047	0.007
Variation Coefficient %	17.142	9.040	70.600	8.274	8.456	0.751

Table 2 Correlation analysis between mature cladodes morphological parameters.

	Area	Perimeter	Circularity	Feret Diameter	Aspect Ratio	Round	Solidity
Area	1						
Perimeter	0.844	1					
Circularity	-0.688	-0.963	1				
Feret Diam.	0.862	0.679	-0.570	1			
Aspect Ratio.	-0.033	-0.101	0.031	0.453	1		
Round	0.0279	0.087	-0.011	-0.454	-0.996	1	
Solidity	-0.405	-0.378	0.344	-0.476	-0.171	0.160	1

Table 3 Nonlinear regression equation models and coefficient of determination r^2, with 95% of confidence for shape parameters.

Related variables	Equation model	r^2
Perimeter vs. Area	$\mathrm{Perimeter} = 119.5606 + 43.4425\mathrm{Cos}(0.008214\mathrm{Area} - 0.01434)$	0.805
Circularity vs. Area	$\mathrm{Circularity} = 0.3824 + \frac{0.4292\mathrm{Area}^{-115.092}}{542.744^{-115.092} + \mathrm{Area}^{-115.092}}$	0.881
Feret Diameter vs. Area	$\mathrm{Feret\ Diameter} = \frac{38.1158}{(1 + e^{(19.8949 - 0.0373\,\mathrm{Area})})^{\frac{1}{25.5921}}}$	0.882
Aspect Ratio vs. Area	$\mathrm{Aspect\ Ratio} = 1.7963 + 0.099\cos(.0984\mathrm{Area} - 6.8176)$	0.169
Roundness vs. Area	$\mathrm{Roundness} = 0.5559 + 0.03\cos(0.03875\mathrm{Area} + 2.7462)$	0.177
Solidity vs. Area	$\mathrm{Solidity} = 0.983 + 0.00836\cos(0.0235\mathrm{Area} + 8.9817)$	0.462
Circularity vs. Perimeter	$\mathrm{Circularity} = 2.152 - 0.01166\mathrm{Perimeter} + \frac{2558}{\mathrm{Perimeter}^2}$	0.963
Roundness vs. Perimeter	$\mathrm{Roundness} = 0.547 + 0.04\cos(0.0871\mathrm{Perimeter} + 0.4945)$	0.158
Aspect Ratio vs. Perimeter	$\mathrm{Aspect\ Ratio} = e^{11.626 - }\frac{195.75}{\mathrm{Perimeter}} - 1.96\ln(\mathrm{Perimeter})$	0.147
Solidity vs. Perimeter	$\mathrm{Solidity} = 0.977 + \frac{0.0123\mathrm{Perimeter}^{-8.51}}{88.6^{-8.51} + \mathrm{Perimeter}^{-8.51}}$	0.410
Feret Diameter vs. Perimeter	$\mathrm{Feret\ Diameter} = \frac{37.83}{(1 + e^{177.83 - 1.94\mathrm{Perimeter}})^{1/134}}$	0.901
Roundness vs. Circularity (Circ)	$\mathrm{Roundness} = 0.949 + (1 - 0.949)[1 - e^{1.82\mathrm{Circ} - 58.7\mathrm{Circ}^2 + 82.7\mathrm{Circ}^3 - 41.68\mathrm{Circ}^4}]$	0.763
Aspect Ratio vs. Circularity (Circ)	$\mathrm{Aspect\ Ratio} = 1.04 + (1 - 1.04)[1 - e^{26\mathrm{Circ} - 87.8\mathrm{Circ}^2 + 128\mathrm{Circ}^3 - 66.6\mathrm{Circ}^4}]$	0.746
Solidity vs. Circularity	$\mathrm{Solidity} = 0.987 + .0049\cos(189\mathrm{Circularity} - 2.629)$	0.244
Feret Diameter vs. Circularity	$\mathrm{Feret\ Diameter} = 37.885 - 7.374e^{-0.0002\mathrm{Circ}^{-34.287}}$	0.584
Aspect Ratio vs. Roundness	$\mathrm{Roundness} = 151.293 \Big/ (1 + \frac{\mathrm{Aspect\ Ratio}}{0.0066})$	0.999
Solidity vs. Roundness	$\mathrm{Solidity} = 0.987e^{-5025.24 - 1030.468\mathrm{Roundness}}$	0.582
Feret Diameter vs. Roundness	$\mathrm{Roundness} = 0.538 + \frac{1 - 0.538}{1 + e^{-66.594 + 20.208\ln(\mathrm{Feret\ Diameter})}}$	0.315
Solidity vs. Aspect Ratio (AR)	$\mathrm{Solidity} = \frac{0.969 - 0.473\mathrm{AR}}{1 - 0.497\mathrm{AR} + 0.0046\mathrm{AR}^2}$	0.568
Feret Diameter vs. Aspect Ratio (AR)	$\mathrm{Feret\ Diameter} = 278.281\cos(\mathrm{AR} + 40.579) + 81.885\cos(2\mathrm{AR} + 40.579) + 92.281\cos(3\mathrm{AR} + 40.579)$	0.347
Feret Diameter vs. Solidity	$\mathrm{Feret\ Diameter} = \frac{(32.599)(5541785617429.7) + 37.474\mathrm{Solidity}^{-1803.857}}{5541785617429.7 + \mathrm{Solidity}^{-1803.857}}$	0.314

Table 4 Pairs of shape parameters and the model with the highest correlation coefficients and Root Mean Square Error (RMSE).

Related variables	Proposed model	R value	RMSE
Perímeter vs. Area	Sinusoidal Regression	0.897	21.583
Circularity vs. Area	DR. Hill. Regression	0.939	--------
Feret Diameter vs. Area	Richards Regression	0.939	2.869
Circularity vs. Perimeter	Heat capacity Regression	0.981	0.787
Feret Diameter vs. Perimeter	Richards Regression	0.949	33.359
Roundness vs. Circularity	DR multistage-4 Regression	0.873	0.440
Aspect Ratio vs. Circularity	DR multistage-4 Regression	0.864	0.182
Aspect Ratio vs. Roundness	Weibul Model Regression	0.999	0.060
Solidity vs. Area	Sinusoidal Regression	0.679	0.005
Solidity vs. Perimeter	DR Hill Regression	0.640	0.004
Feret Diameter vs. Circularity	Weibul Model Regression	0.764	2.65
Solidity vs. Roundness	Exponential Decline Regression	0.763	0.984
Solidity vs. Aspect Ratio	Gaussian Model Regression	0.753	1.366

Table 5 Color parameter average values.

	Chromaticity a*	Chromaticity b*	Value C	Value H	Luminosity L*
Mean	-9.059	14.592	17.234	121.930	47.084

Table 6 Summary of ANOVA Opuntia cladodes color parameters mean values.

Groups	Number	Sum	Means	Variance
Mean_a*	30	-266.94	-8.90	0.25
Mean_b*	30	461.56	15.39	1.96
Mean_C	30	531.15	17.70	2.24
Mean_H	30	3606.40	120.21	1.92
Mean_L*	30	1425.56	47.52	1.66

Table 7 ANOVA of Opuntia cladodes color parameters.

Source	SS	df	Ms	F	Probability	$F_{Critical.}$
Between	299150	4	74787.415	46541.39487	2.9711E-224	2.434
Within	233	145	1.607			
Total	299383	149				

Moreover, descriptive statistics were performed for the color variables; mean color parameters values in 30 cladodes (Table 5) and parameters such as mean, and variance were obtained (Table 6). The mean values were obtained, showing very little variability over time (Figure 5). An online program was used to visualize the measured L*a*b* parameters, observing a high similarity to human eyesight (Figure 6). An analysis of variance (ANOVA) was also performed for the color parameters L*, a*, b*, C, and H. The ANOVA analysis indicated highly significant differences in the color variables at 1% confidence levels (Table 7). Subsequently, a correlation analysis was performed between the color parameters of *Opuntia* cladodes (Table 8) and finally, a nonlinear regression analysis was carried out using CurveExpert software (Hyams 2010), obtaining the equations and mathematical models. The correlation coefficient r^2 and the nonlinear mathematical model were obtained, being the highest between a* *vs.* C (0.999) as well as between b* *vs.* H (0.821) (Table 9).

Table 8 Correlation matrix of *Opuntia* cladodes color parameters.

Correlation matrix	Mean_a*	Mean_b*	Mean_C	Mean_H	Mean L*
Mean_a*	1				
Mean_b*	-0.759	1			
Mean_C	-0.819	0.993	1		
Mean_H	0.222	-0.789	-0.723	1	
Mean_L*	-0.244	0.577	0.554	-0.672	1

Table 9 Mathematical models between *Opuntia* cladodes color parameters.

Related Variables	Mathematical Model	R^2	RSME
a*-C	$a* = \frac{-2.341 * C}{1 + 0.292 * C - 0.00482 * C^2}$	0.999	0.403
b*-H	$b *= 16.59 + 2.356 * \cos(0.489 \cdot H + 87.914)$	0.821	1.176
C-b*	$C = 36.967e^{-e^{(0.963 - 0.0826\,b*)}}$	0.807	1.461
H-C	$H = 108.375e^{\left(\frac{1.823}{C}\right)}$	0.724	0.987
L*-H		0.682	0.860

Changes in the coloration of *O. ficus indica*, as well as the shapes and sizes of cladodes, are factors of interest to researchers. Here we presented a methodology using the freeware Image J, which it had not been used in *Opuntia* but has been used successfully for microscopy data extraction by Hartig (2013), as well as in cyst delineation for cyst/injury identification in dental studies by Veena Divya et al. (2016). On the other hand, Curve Expert software with good results in prediction models for runoff using rainfall as a single predictor obtaining r^2 of 95%, (Shah et al. 2017). In addition, Aponte (2017) estimated the productivity of *L. laevigatum* (biomass, carbon, and protein production) in the laboratory, establishing a logistic mathematical model with Curve Expert, and obtained an r^2 of 99%. These researchers obtained determination coefficients as to those we obtained using Curve Expert.

Conclusions

The obtained results of the research show that image processing and linear and nonlinear modeling allowed to characterize the morphology and color of *O. ficus-indica* mature cladodes (Villanueva cultivar cladodes) of a year-old using ImageJ and CurveExpert freeware. Significant correlation coefficient values with a confidence level of 95% for the shape descriptors of the non-linear regression models were obtained between 8 pairs of parameters. From the color features, we obtained very good correlation coefficient values for a* *vs*. C and b* *vs*. H parameters. The results of this research will be a helpful protocol for those who wish to carry out similar color and morphology characterization work with mature cladodes of different varieties or other seeds or products.

Conflicts of Interest

Authors would hereby like to declare that there is no conflict of interests that could arise.

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