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ARTICLE

Application of Digital Imaging Allied to Chemometrics in the Use of Non-destructive Phenotyping of Sesame Seeds

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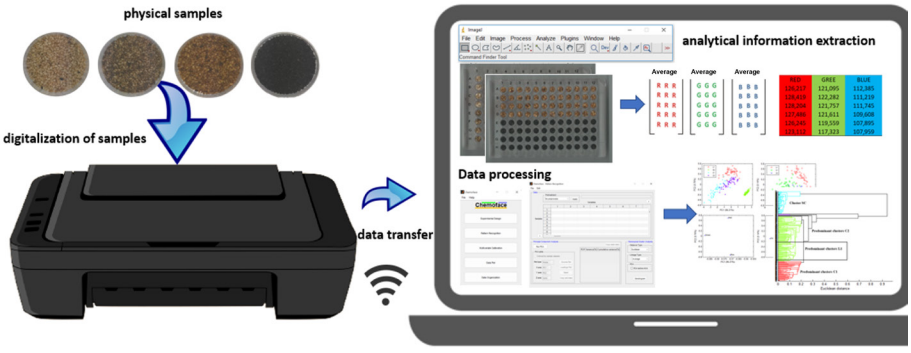

In this article, digital image processing and analysis (DIPA) combined with chemometric methods, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used to discriminate sesame seeds through their digitized images. For this purpose, four groups of seeds were used: BRS Anahí and BRS Seda cultivars, a lineage and a commercial sample. The images were scanned using an HP officejet 7610 scanner and, for extraction of the red-green-blue channels and colorimetric profile, the ImageJ software was used. The DIPA combined with chemometric methods allowed us to discriminate the four groups of sesame seeds efficiently, and a minimum accumulated variance of 89.03% of the total variance was obtained. The trends observed via the PCA were confirmed through the dendrograms obtained using the HCA. The results achieved in this work indicate that the proposed methodology can be a simple analytical alternative for the non-destructive phenotypic discrimination of seeds, with their color as an attribute.

Keywords: Digital image processing and analysis, pattern recognition methods, non-destructive phenotyping, colorimetry, multivariate image analysis

INTRODUCTION

New technological platforms have emerged in chemistry that enable professionals in this area to use new resources.[1,2] When applied to analytical chemistry, digital image processing and analysis (DIPA)

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enables the characterization of compounds and their physicochemical properties through a set of variables related to the color that a given sample presents.[3,4]

The use of digital images as a means to perform chemical, clinical, nutritional analysis, in quality control and to determine properties in substances, has been explored in recent years.[3,5] This methodology applied to chemistry, more specifically to analytical chemistry, deserves to be highlighted in regards to the determination, identification or discrimination of substances or properties based on colorimetric measurements.[5]

Despite the evolution of several instrumental techniques, colorimetric methods that use wavelength in the visible region are widely used as a tool to discriminate various physical-chemical parameters because they are simple, cheap and fast for obtaining results,[6,7] thus making it possible to find it in use in various areas of science such as medicine, engineering, chemistry and agriculture.[8-11]

The digital image processing of seeds presents itself as a non-destructive method that is automated and computer-assisted.[12] It is used for quality control of several crops, and the method seeks to optimize the classification of seeds to assess their physical and physiological quality and minimize the existing limitations of traditional techniques.[13,14]

In this sense, seeds are presented as species of agronomic interest since they enable the implantation of crops and are a raw material for the industry. Good quality seeds and improved cultivars determine the yield and productivity of a crop. Therefore, these seeds represent a technology that involves, in the case of cultivars, an intellectual property right that may have a high market value. Thus, there is a need for the discrimination or phenotyping of seeds, as the same species of agronomic interest may have several cultivars with characteristics that meet a certain need.[15,16]

Morphological descriptors are used to differentiate cultivars as well as in the certification of genetic purity, which can also be carried out via molecular markers, which is performed by the study of DNA. These discriminative methods involve time-consuming procedures in order to obtain the results, which are destructive, in addition to requiring expensive reagents that generate waste.[16-19]

However, digital image colorimetry (DIC), using cell phones, cameras, scanners, webcams to perform quantitative and qualitative determinations together with multivariate analysis methods, has shown adequate performance when compared to traditional instrumental techniques.[6,7,9] The DIC method is intrinsically linked to parameters related to Green Chemistry, which aims at the creation, development and application of chemical products and processes to reduce or eliminate the use and generation of toxic substances, and seeks the development of technologies and processes incapable of causing pollution to the environment.[20]

This method uses simple information based on a red-green-blue (RGB) color system that, with the aid of multivariate statistics, correlates a large number of variables simultaneously. This allows the extraction of information from the digitized images of the samples and enables their discrimination. Therefore, the absence of scientific studies using digital image colorimetry to differentiate cultivars reinforces the need for studies to define a methodology that is less costly, faster and that presents reliable results. As such, the objective of this work was to evaluate DIPA combined with DIC-chemometrics for the differentiation between two cultivars and a sesame seed lineage.

MATERIALS AND METHODS

Experimental

Acquisition of samples

For this study, samples of two cultivars and one sesame lineage, provided by the Brazilian Agricultural Research Corporation (Embrapa) of Roraima, were used. All sesame varieties were cultivated by Embrapa, at the Água Boa Experimental Field, under the same conditions and soil type, harvested in the mature stage, and stored at 25 ± 3 °C with approximately 75% relative humidity. The cultivars were BRS Seda (C1) and BRS Anahí (C2). A commercial black sesame (SC) sample was acquired from a local establishment, which was used as a colorimetric standard in the chemometric analysis performed in the pairings between the samples (Figure 1).

Figure 1. List of sesame seed samples. From the left to the right, the BRS Anahí (C1) and BRS Seda (C2) cultivars, lineage (L1) and the commercial sesame (SC).

Image acquisition system

About 20 seed samples (untreated) were placed in each microwell of a titration microplate, and pieces of plants, leaves and other interfering agents were removed before image acquisition. A microplate has 96 microwells, so, for each sample, 46 microwells were used, each with 20 seeds. (Figures 2 and 3). The samples were placed on the table of an HP officejet 7610 scanner and covered with a white EVA sheet as a background. The images were obtained with a resolution of 300 dpi (dots per inch) in JPEG format, and the software's automatic image correction options (HP Scanning) were disabled.[10,21]

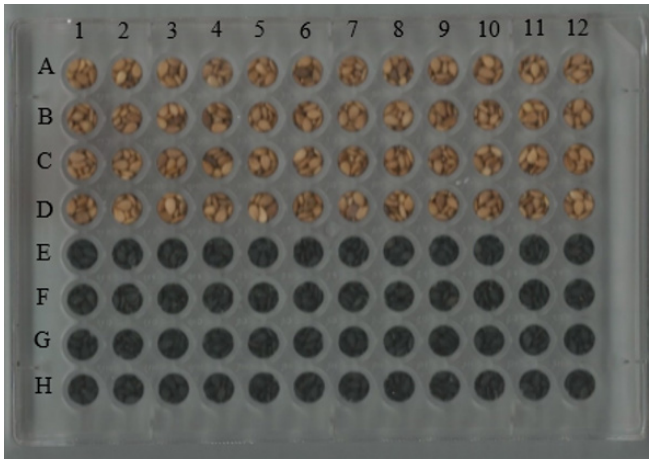


Figure 2. Layout of the sesame seed samples in the titration microplate. Lineage (L1) is in microwells A1 to D12, and the commercial sesame (SC) is in microwells E1 to H12.

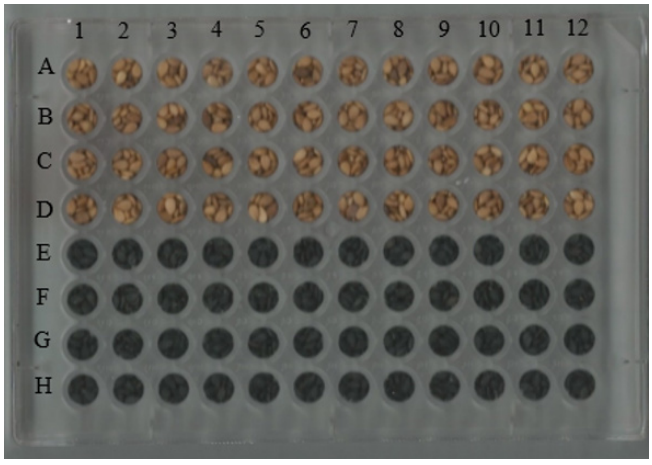


Figure 3. Layout of the sesame seed samples in the titration microplate. Cultivar (C1) is in microwells A1 to D12, and cultivar (C2) is in microwells E1 to H12.

Digital image processing software

There are several image processing software packages that perform quantitative analysis, either free software or not. Among the free ones, ImageJ (Figure 4) is one of the most commonly adopted by research institutions around the world. This software has several image processing resources that can be used for image analysis.

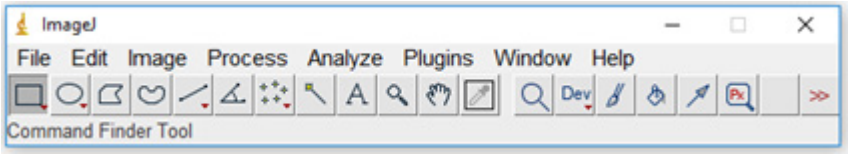


Figure 4. Opening layout of the ImageJ digital image processing and analysis software.

Acquisition of histograms (RBG)

RGB values were extracted from the images using the ImageJ software. The values of each channel (RGB) of all wells of the microplate were extracted simultaneously using the plugin “ReadPlate” from ImageJ.

To obtain the R values (RED) (Figures 2 and 3), in ImageJ, we clicked on “Plugins” and then “ReadPlate”. By clicking on “ReadPlate”, the “Measureme” window opened. In the “Measure window” (Figure 5), we selected the channel that we want to extract.

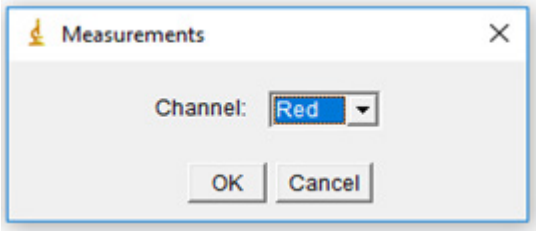


Figure 5. Measureme window. Selection of the channel to be extracted from a microplate.

After selecting the channel and clicking OK, the Grid Parameters window appears (Figure 6).

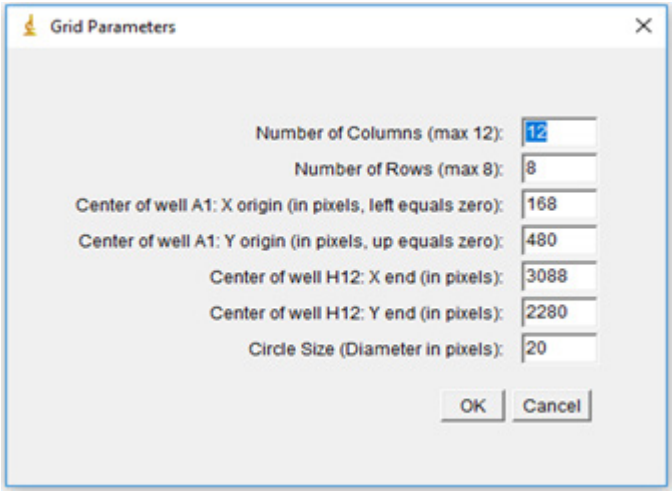


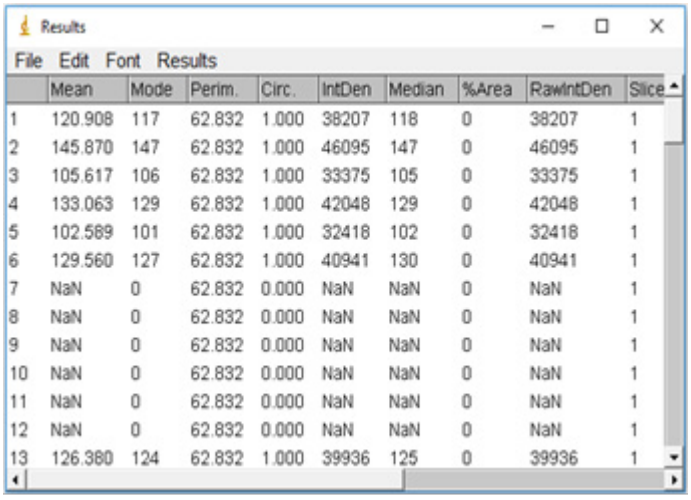
Figure 6. Grid Parameters window. Selecting the coordinates of the microplate wells.

In the “Grid Parameters” window, in “Number of columns”, the number of columns of the plate is selected, with a maximum of 12 columns allowed. In “Number of Rows”, the number of rows is selected, with a maximum of 8 rows allowed (from A to H). In “Center of Well A1:X” and “Center of Well A1:Y”, the coordinates in X and Y of the first well A1 are placed (Figures 2 and 3). In “Center of Well H12:X” and “Center of Well H12:Y”, the coordinates in X and Y of the last well H12 are placed. In ImageJ, the

coordinates of wells A1 and H12 are determined by positioning the cursor over the microplate, with “Circle size” being the diameter of the circle on the plate from which the RGB values were extracted.

In this work, 20 pixels were used. In the “Grid Parameters” window, after clicking OK, a window called “Results” (Figure 7) will appear, which contains, in the following order, the information: name, area, median, minimum, maximum, row, column.

In the “Results” window, click “Ctrl + A” to select all values, then click “Ctrl + C” to copy these values and, to paste these values into Microsoft Excel, click “Ctrl + V”. After extracting the RGB values and exporting the data to Microsoft Excel, the R, G and B values can have the chemometric data treatment applied.


Results

File Edit Font Results

	Mean	Mode	Perim.	Circ.	IntDen	Median	%Area	RawIntDen	Slice
1	120.908	117	62.832	1.000	38207	118	0	38207	1
2	145.870	147	62.832	1.000	46095	147	0	46095	1
3	105.617	106	62.832	1.000	33375	105	0	33375	1
4	133.063	129	62.832	1.000	42048	129	0	42048	1
5	102.589	101	62.832	1.000	32418	102	0	32418	1
6	129.560	127	62.832	1.000	40941	130	0	40941	1
7	NaN	0	62.832	0.000	NaN	NaN	0	NaN	1
8	NaN	0	62.832	0.000	NaN	NaN	0	NaN	1
9	NaN	0	62.832	0.000	NaN	NaN	0	NaN	1
10	NaN	0	62.832	0.000	NaN	NaN	0	NaN	1
11	NaN	0	62.832	0.000	NaN	NaN	0	NaN	1
12	NaN	0	62.832	0.000	NaN	NaN	0	NaN	1
13	126.380	124	62.832	1.000	39936	125	0	39936	1

Figure 7. Results window. Window that shows the RGB values for each microplate well.

Principal component analysis (PCA)

The multivariate PCA tool was used for image classification. The application of PCA consisted of changing the base of the multivariate data, which are represented by the matrix X. An algorithm that can be divided into three steps was applied to this matrix.[22]

1st step: This consisted of data pre-processing, in which the data from the original matrix (matrix X) were autoscaled.

2nd step: The data from the autoscaled matrix were decomposed into singular values, thus obtaining the matrix of loadings and scores.

3rd step: This consisted of determining the variance explained by each eigenvector or principal component.

Hierarchical cluster analysis (HCA)

The main objective of hierarchical analysis is to present data that emphasize natural groupings, since variables gathered in the same group have similar attributes. HCA was applied to identify clusters within a dataset, and also to test cluster hypotheses. To apply the HCA, we used the autoscaled data obtained through the PCA, Euclidean distance and the linkage type – nearest neighbor.

RESULTS AND DISCUSSION

Principal component analysis (PCA)

After the acquisition of the images, the processes of segmentation and extraction of attributes from the RGB profile of the samples were carried out. These were used to carry out the classification and recognition in order to discriminate the existence of similarities and/or differences between the cultivars, the lineage and the commercial sesame (SC) reference standard, with the aid of PCA (Figure 8).

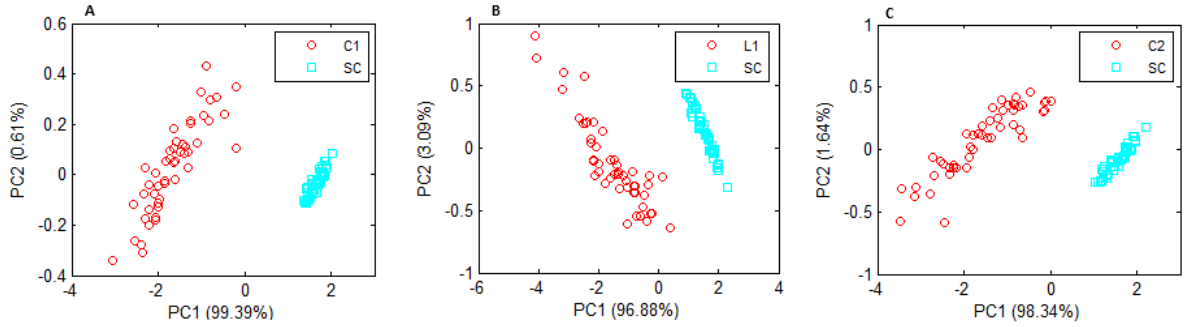


Figure 8. Graphs of the scores of the first two principal components, PC2 and PC1 (autoscaled) from the digitized images (300 dpi) of the sesame samples with the sesame standard (SC), saved in JPEG format.

Using PCA, we sought to describe, in geometric terms of PCs, the covariance of the variables (in this case, the RGB channels) using the smallest number of eigenvectors.[23] It was verified that the principal components PC1 and PC2 describe 100% of the full range of data and provide discriminatory information for sesame seeds. Figure 8 (A), (B) and (C) presents the graph of the scores, and it is possible to observe the formation of two large groups.

The graph of the scores makes it possible to assess the differences in behavior between the samples, and shows which ones have similarity and/or differences according to their colorimetric profile.[24] Analyzing PC1, it is possible to observe the separation between samples C1, C2 and L1 and the commercial sesame (SC), which in this work is being used as a standard, due to its colorimetric profile being different from other sesame samples.

The loadings graph (Figure 9) shows the influence of the variables (RGB channels) on the samples. The distinct behavior presented by them shows that color can be used as a pattern for discrimination or phenotyping in seed classification. This separation is explained via the RGB profile extracted from the digitized images, which, due to the lack of color in the commercial sesame standard (SC), presents an extremely distinct colorimetric profile. This fact can be proven because the RGB profile in the loadings graph is solely for the colorimetric profile of the C1, C2 and L1 seeds.

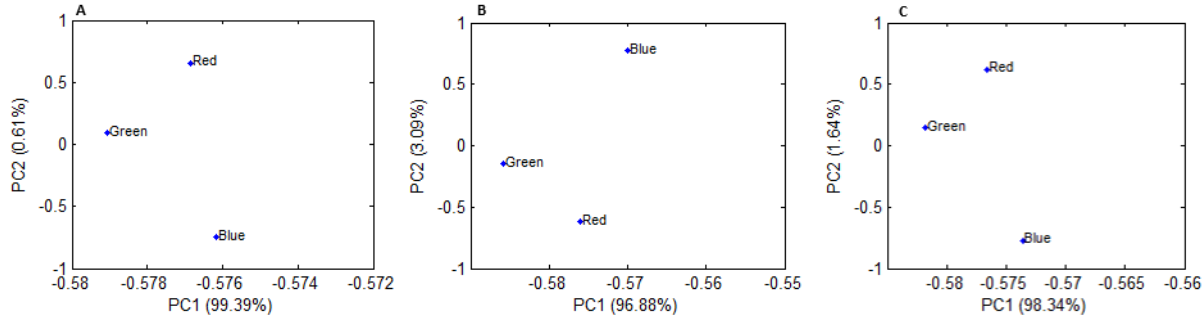


Figure 9. Graph of the loadings (A) C1 and SC, (B) L1 and SC and (C) C2 and SC of the first two principal components, PC2 and PC1 (autoscaled) from the digitized images (300 dpi) of the sesame samples with the sesame standard (SC), saved in JPEG format.

The application of digital images to define quality standards such as size, shape, color and texture in foods can be found in several studies on the classification of foods such as seafood, fruits, grains and vegetables. The shape and colors of foods can be evaluated and the mathematical treatment of the digitized images can be performed using tools such as multivariate analysis to discriminate them.[3,25]

Processing of digital images of seeds has been attributed to the quality control of different crops and for cultivar discrimination.[26,27] Currently, digital image processing has attracted great attention as an analytical

tool for colorimetric analysis due to its versatility, as it comprises simple, fast and low-cost procedures, and is a non-destructive method.[28]

The results for the discrimination between the samples and the standard (SC) were already expected, since the commercial sesame has a colorimetric profile that is significantly different from the other samples studied. Thus, seeking to evaluate the efficiency of PCA in the discrimination of samples, new pairings were made between the cultivars and the sesame lineage without the presence of the standard (Figure 10).

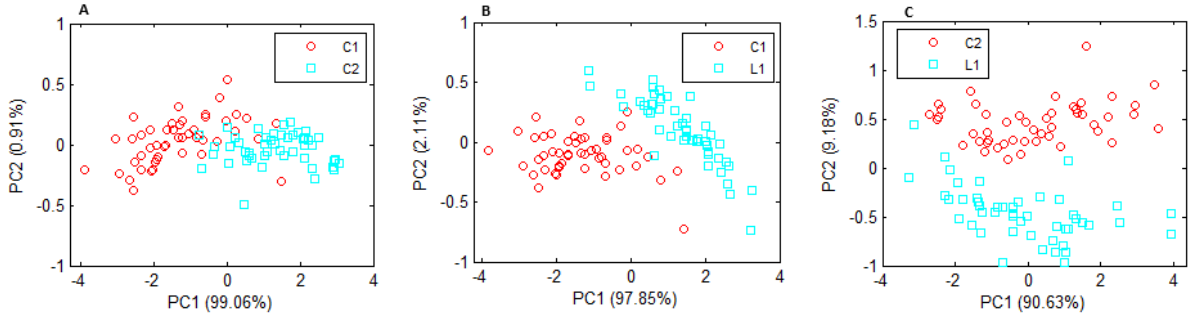


Figure 10. Graph of scores of the first two principal components, PC2 and PC1 (autoscaled) of the digitized images (300 dpi) of the sesame samples, without the presence of the commercial sesame standard, saved in JPEG format.

Based on the graphs of the scores, it is possible to observe the discrimination of the samples, which form two large groups. However, it can also be observed that within a group there is scattering between samples. This happens because the sesame seeds (Figure 1) do not present a uniform color, so their colorimetric profiles are distinct, which results in the displacement of points within the same cluster.

Analyzing PC2 in Figure 10 (C2 and L1), it is possible to observe a slight displacement of L1 in relation to C2. This was due to the colorimetric profile of the two samples (Figure 11) since L1 is discriminated by the red channel while C2 presents a profile associated with the blue channel.

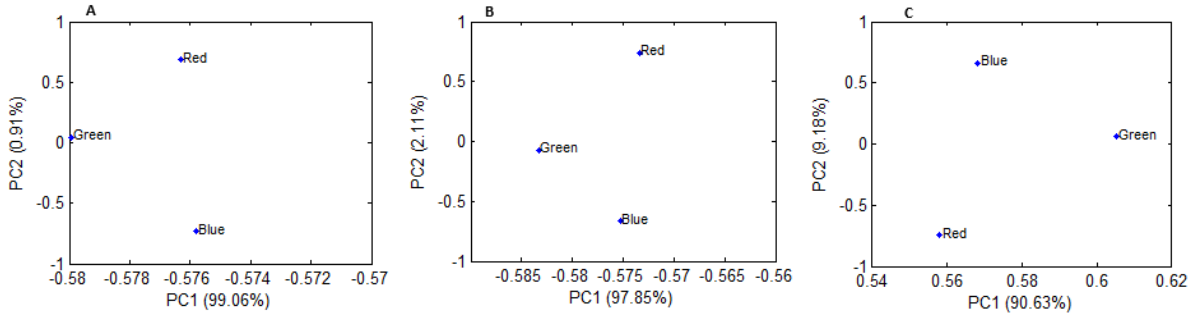


Figure 11. Graph of loadings (A) C1 and C2, (B) C1 and L1 and (C) C2 and L1 of the first two principal components, PC2 and PC1 (autoscaled) of the digitized images, with a resolution of 300 dpi, of the sesame samples with the sesame standard (SC), saved in JPEG format.

Information related to the colorimetric profile of digital images from their decomposition into a color diagram can be analyzed by chemometric procedures, as they generate a continuous spectrum. In this way, their data can be treated in the same way as in spectrophotometric measurements by multivariate calibration.[3]

In order to analyze the influence of the samples in relation to each other, a new PCA was generated. The three sesame samples were plotted with and without the presence of the standard (SC), as shown in Figure 12, to assess the efficiency of PCA in the non-destructive phenotyping process.

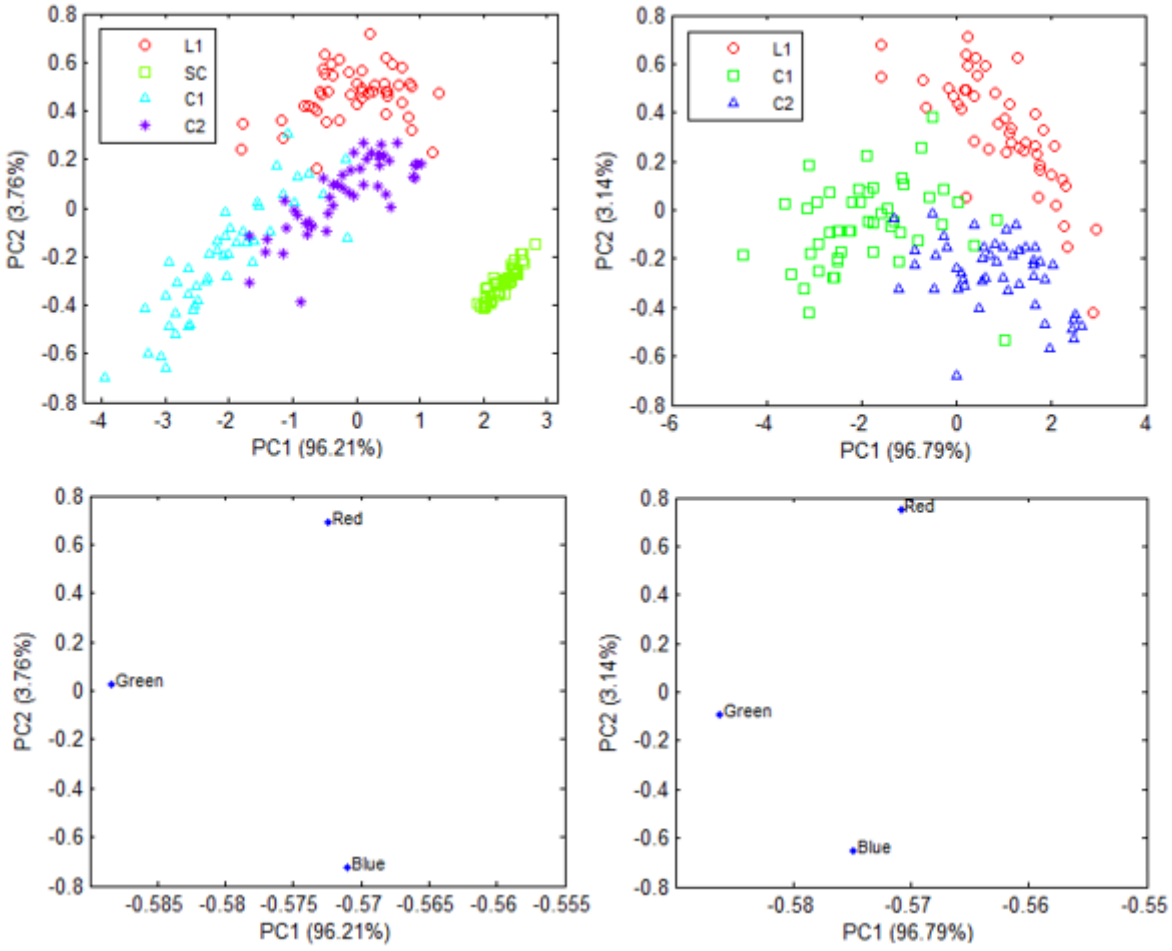


Figure 12. Graph of scores and loadings of the three sesame samples with the commercial sesame standard and without the commercial sesame standard, for the first two principal components, PC2 and PC1 (autoscaled) of the digitized images, with resolution 300 dpi, saved in JPEG format.

The graph of scores for the three samples with the standard (SC) shows the formation of four groups, with PC1 describing 96.30% of the total variance. It can be seen that the behavior of the samples remained constant, since the standard (SC) is to the right of PC1, and is isolated from the other samples, which was already observed in Figure 8. For the two cultivars, we can observe their separation via PC1, previously discriminated, whereas PC2 separates L1 and C2, a phenomenon already observed in Figure 10.

Without the presence of the standard (SC), the behavior is accentuated, confirming the discriminations previously seen. PC1 describes 96.85% of the total variance and separates the two cultivars efficiently. PC2 describes 3.10% of the total variation and separates L1 from C1 and C2. In this graph, the influence of the standard (SC) is evident, as its presence displaces C2. Thus, with the absence of the standard, one can see the similarity in the colorimetric profiles between C2 and L1, which are separated by PC2, but are found to the right of PC1.

Analytical methodologies that employ multivariate analysis in digital images have advantages such as speed, low cost, small waste generation and greater logistical ease when compared to conventional analytical methodologies such as titration and most spectroscopic methods.[4]

Several studies have demonstrated the use of this technique combined with chemometrics to determine various parameters in different samples, such as Damasceno et al. (2015)[4] in the determination of pH in drinking water, Colzani et al. (2017)[10] in the determination of manganese in piles and Dominguez and Centurión (2015)[21] in the discrimination of the color of honeys from different regions and Vilar et al. (2015)[29] in the classification of castor seeds using digital images combined with multivariate analysis.

Hierarchical cluster analysis (HCA)

The HCA was applied not only to identify the groups within the dataset, but also to test grouping hypotheses observed through the PCA. The dendrogram obtained via the HCA (Figure 13) refers to the pairing between the samples and the standard (SC), observing the formation of two large clusters.

These clusters occur because of the RGB profile of the digitized images, and inform us that the samples of the cultivars and the sesame lineage were discriminated from the standard (SC). However, it is observed that the branches in the dendrogram for the samples are more displaced, which indicates a variation in the colorimetric profile between samples in the same group.

Panero et al. (2009)[28] used HCA to display data from the analysis of metal content in okra from two states in Brazil in order to emphasize their natural clusters and patterns, and the study reflects the similarity of their properties.

According to Neto (2016),[30] when considering the analytical applications that can be conducted using digital images, those that involve the identification of substances or the discrimination/classification of samples based on colorimetric measurements deserve to be highlighted.

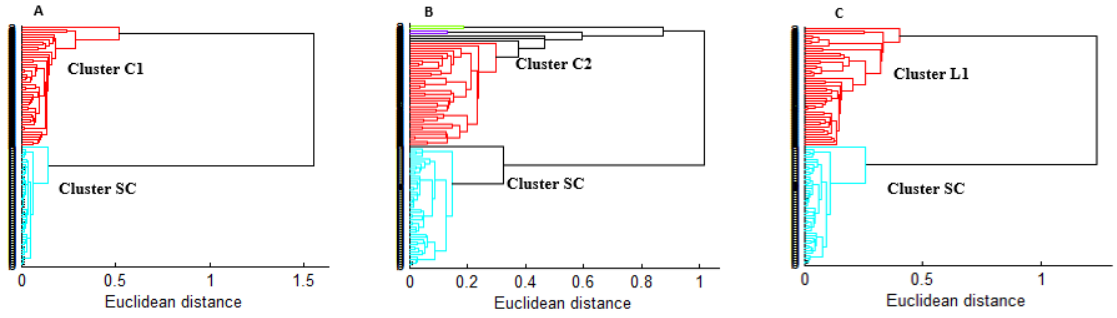


Figure 13. Sample dendrogram (autoscaled), Euclidean distance, linkage type - nearest, non-destructive phenotyping discrimination (A) C1 and SC, (B) C2 and SC and (C) L1 and SC.

We sought to evaluate the discrimination efficiency of the studied samples through the application of HCA, and made three pairings between the two cultivars and the sesame lineage (Figure 14). Based on the dendrogram (Figure 14A), we can observe the formation of two large clusters (C1 and C2). However, it can also be observed that within the cluster of C1 there are samples of C2. This happens because the sesame seeds (Figure 1) do not present a uniform color, so their colorimetric profiles may vary, which will directly reflect on their clusters. This same behavior can be observed in the other dendrograms.

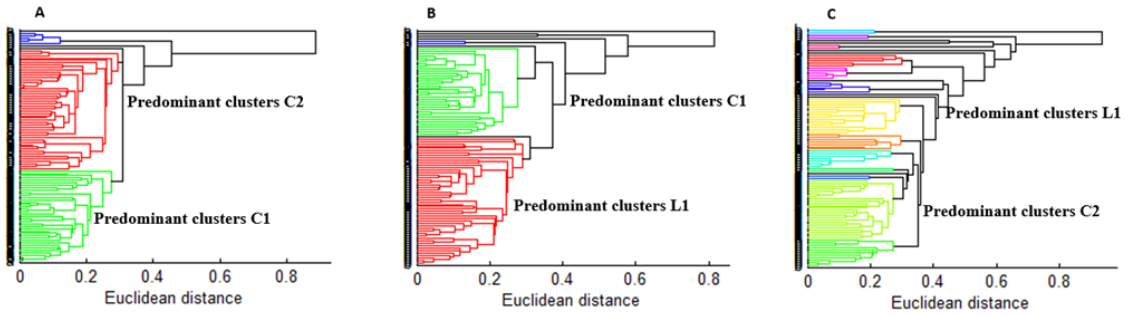


Figure 14. Sample dendrogram (autoscaled), Euclidean distance, linkage type - nearest, non-destructive phenotyping discrimination (A) C1 and C2, (A) C1 and L1 and (C) C2 and L1.

For the dendrogram in Figure 14C, which shows the clusters of C2 and L1, we can see that they were grouped in a similar way to the other pairings. However, in the upper part of the dendrogram, it was observed that within the L1 cluster there are several C2 samples. Because several samples of C2 are within the L1 cluster, we can infer that C2 is closer in color to L1 than to C1.

In order to analyze the influence of the samples, one in relation to the others, a new dendrogram was generated, as can be seen in Figure 15. The three sesame samples were plotted on a single graph with the presence of the standard (SC) in order to assess the efficiency of the HCA in the process of non-destructive discrimination.

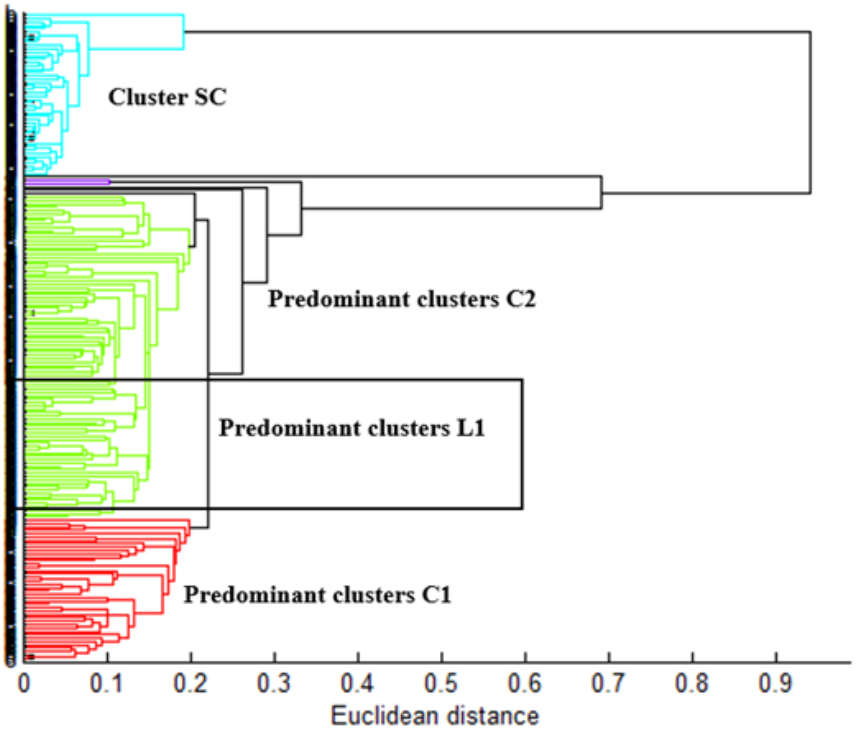


Figure 15. Sample dendrogram (autoscaled), Euclidean distance, linkage type - nearest, non-destructive phenotyping discrimination of the three sesame samples and the standard (SC).

The dendrogram for the three samples and the standard (SC) shows the formation of four clusters. It can be seen that the behavior of the samples remained constant, since the standard (SC) is found in an isolated cluster from the other samples, which was already observed in Figure 13. For the two cultivars (C1 and C2), it is possible to observe the formation of two distinct clusters, which directly reflects the discrimination between the samples by their collation.

For L1, its cluster is between the standard (SC) and C2, and this behavior may be associated with its colorimetric profile, since it presents, among the samples, a darker color than the cultivars C1 and C2, and is lighter than the standard (SC). Assessing the C1 clusters, it was possible to observe that it presents samples from the other groups (L1 and C2). In C2, it can be observed that it had samples from L1.

By presenting a distinct colorimetric profile, the commercial sesame standard (SC) can influence the process of discrimination of the samples, since it clearly differs from the others. As such, a new dendrogram was plotted without the presence of the standard (Figure 9) in order to discriminate the studied samples without possible interference.

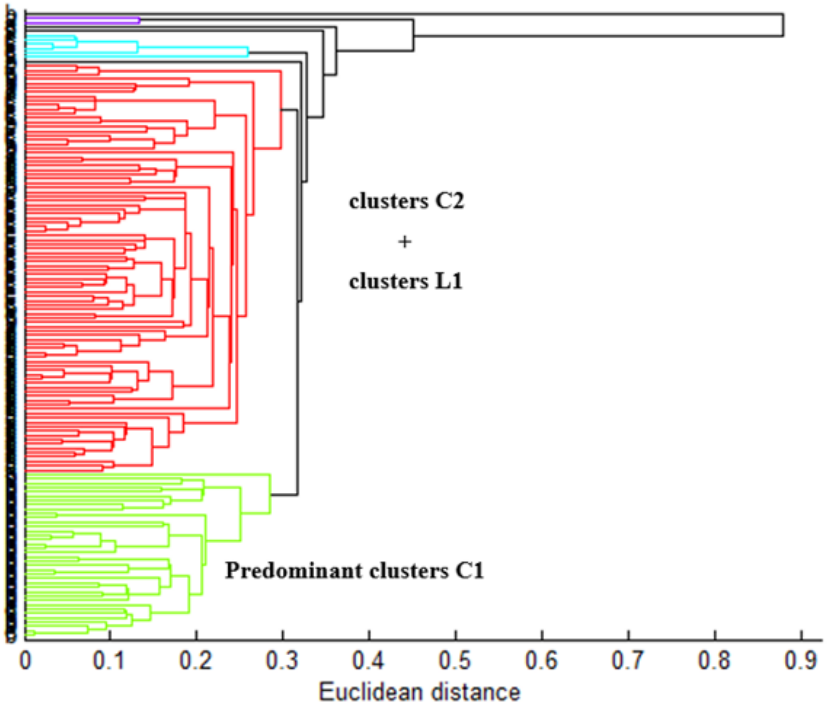


Figure 16. Sample dendrogram (autoscaled), Euclidean distance, linkage type - nearest, non-destructive phenotyping discrimination of the three sesame samples without commercial sesame standard.

Without the presence of the standard, the behavior of the samples is the same as was previously observed. However, the branches present a greater Euclidean distance, which is directly associated with the similarity between the samples and the variation in the colorimetric profile within the clusters. In the upper part of the dendrogram, samples from C2 and L1 were again observed within the C1 cluster.

Works such as Neto (2016),[30] which performed the classification of mineral waters based on digital images acquired by smartphones using hierarchical cluster analysis (HCA), reinforce the practicality of DIC when developed with the aid of chemometric techniques. Thus, it is important to emphasize that the results obtained through the HCA indicate the adequate discrimination of the studied sesame samples, which was achieved using an alternative instrumentation of low cost and a very simple procedure

CONCLUSIONS

The use of the alternative method proposed for the analysis of the seeds of the two cultivars and the lineage using color as an attribute, using simple and accessible equipment, such as a scanner, proved to be extremely effective, and highlights the method as an alternative methodology.

With the use of digital images combined with chemometric techniques, a non-destructive phenotypic discrimination was performed, employing digital image processing and analysis (DIPA) for the extraction of attributes from the digitized images of sesame seeds. It was possible to evaluate the colorimetric profile of the seeds by analyzing the digitized images using the RGB channels.

With the application of DIPA, the extraction of attributes from the digitized images was carried out, and they can be used for their discrimination via principal component analysis (PCA). Based on the results of the scores and loadings graphs, which successfully classified all the samples studied, their behaviors were basically discriminated by the PC1 in all the pairings performed, and described more than 89% of the total variance of the data.

With the application of hierarchical cluster analysis (HCA), it was possible to observe the formation of natural clusters of the samples, obtained using the Euclidean distance and the nearest linkage type technique. HCA confirmed the behavior observed in PCA through the natural patterns observed in the sample clusters.

The use of digital images to perform discriminative analyses or to quantify analytes has proved to be an alternative methodology for colorimetric determination, and has been used for non-destructive identification of rice seeds, classification of cereal grains, classification of beer, classification and determination of phosphate in soft drinks, determination of ascorbic acid, and classification of commercial tannin extracts among other matrices, though always supported by multivariate statistics.

Exploratory data analysis allowed us to obtain fast and efficient information about the similarity and/or difference between the samples through graphical visualization. The results obtained in this work indicate that the proposed methodology can be a simple and fast analytical alternative for the non-destructive phenotypic discrimination of seeds.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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