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A digital image-based traceability tool of the geographical origins of Argentine propolis

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ABSTRACT

Propolis is a hive product prepared by honeybees (*Apis mellifera* L.) widely used in pharmaceutical and food preparations that plays beneficial roles beyond basic nutrition and therapeutic properties. These benefits are related with its quality, which depends on various factors, such as geographical origin, botanical sources, collecting seasons, races of honeybees, climatic conditions and also the method of harvest. In this sense, it would be helpful the implementation of a simple, fast and reliable analytical methodology for quality monitoring of propolis samples as a traceability tool of its geographical origin. Thus, this work proposes the use of digital images and chemometrics for the classification of raw propolis from six different geographical origins of the Buenos Aires Province, Argentina. For this purpose, different combinations between a color model (Grayscale, RGB and HSI) and a multivariate classifier (PCA–LDA, SIMCA, kNN, PLS-DA and SPA–LDA) were tested. The best analytical performance was achieved by SPA–LDA using Intensity histograms, classifying correctly a 100% of the samples in both training and test sets, taking in account the 27 variables selected by SPA. As a consequence, the proposed methodology serves to support local apiculturists, guaranteeing the offer of products with a clear indication of geographical origin, and enhancing regional capabilities.

1. Introduction

Propolis is a complex hive product prepared by honeybees (*Apis mellifera* L.) using beeswax and plant exudates, and it is generally composed of 50% resin and balsam, 30% wax, 10% aromatic oils, 5% pollen, 5% other organic substances and traces of inorganic salts. The composition and color of raw propolis varies according by various factors, such as geographical origin, botanical sources, collecting seasons, races of honeybees, climatic conditions and also the method of harvest [1–5].

Propolis has gained much importance in the world due to various applications in pharmaceutical and food preparations, because it plays beneficial roles beyond basic nutrition and therapeutic properties, including antibacterial, antiviral, anti-inflammatory, anticancer, antifungal, antioxidant, anti-inflammatory, immune system, antiulcer, hepatoprotective and antitumor activities. Such benefits have been mainly associated with the presence of polyphenols (flavonoids, phenolic acids and their esters), terpenoids, aminoacids and inorganic compounds. Propolis is therefore an important functional food/nutraceutical product, and be extensively used in complementary healthcare in Argentina [6–12].

In Argentina, the quality control of products containing propolis in its composition is regulated by the Argentine Food Codex [13]. This Code establishes several physicochemical and microbiological criteria. Within physicochemical determinations it can be mentioned loss on ignition (100–105 °C), ash (500–550 °C), foreign bodies, extractable waxes in n-hexane, oxidation rate, phenolic compounds (expressed as gallic acid), flavonoids, resins soluble in ethanol, absorption maximum between 270 and 315 nm (UV–VIS region), arsenic and lead contents, pesticide and antibiotics residues. Microbiological parameters are total coliforms, *Salmonella* spp., fungi and yeast contents. However, these methodologies employ several stages of pretreatment of the sample and generate chemical contaminants to the environment, besides using relatively simple instrumentation. This legislation authorizes the use of propolis only in the following products: candy with propolis, honey with propolis (may also contains pollen and/or royal jelly), hydroalcoholic extracts of ethanol or propylene glycol, and dietary supplements.

On the other hand, there is an increasing consumer interest in agricultural products and foodstuffs with a clear geographical origin,

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which is driven by the reputation of the country or specific cultivation area. Various analytical techniques combined with proper multivariate analysis have been proposed in the literature with the aim of assessing the geographical origin of propolis [1,5,14–27]. To the best of our knowledge, there are only two studies that classify Argentine propolis according to their geographical origins. Lima et al. [15] classified propolis samples from San Juan Province based on their metal content, qualitative and quantitative levels of selected phenolics compounds, flavonoids, and their free radical scavenger capacity. Cantarelli et al. [19] quantified fourteen trace elements by neutron activation analysis, and used suitable multivariate classification methods to discriminate raw propolis samples from three different regions of Argentina. However, all techniques abovementioned involves laborious sample preparation and time-consuming, for example to evaluate metal composition of propolis a sample mineralization with heating at 550 °C for 60 min should be used, besides requiring sophisticated instruments for detecting the analytes, which induce significant operational expenditures.

In order to overcome these disadvantages, digital imaging combined with proper chemometric tools has been emerged as a good alternative to conventional analytical approaches, mainly due to its versatility and low cost [28–31]. In this context, Linear Discriminant Analysis coupled with variable selection by the Successive Projections Algorithm (SPA–LDA) and digital images have been successfully proved to be useful for classifying food, fuel and microbiological samples [32–36], including the identification of the geographical origin of food samples, such as teas [32] and honeys [33].

Thus, the aim of this work is to develop a simple, reliable and fast methodology based on digital imaging and SPA–LDA for classifying raw propolis samples from the southwestern region of Buenos Aires province, Argentina. In order to find the best approach for this purpose, we compare different color models using Grayscale, Red-Green-Blue (RGB) and Hue-Saturation-Intensity (HSI) histograms, evaluating the performance of supervised pattern recognition techniques (in this case, k-Nearest Neighbors (kNN), Soft Independent Modeling of Class Analogy (SIMCA), Principal Component Analysis–Linear Discriminant Analysis (PCA–LDA), Partial Least Squares Discriminant Analysis (PLS-DA), and SPA–LDA) in terms of accuracy, sensitivity and specificity.

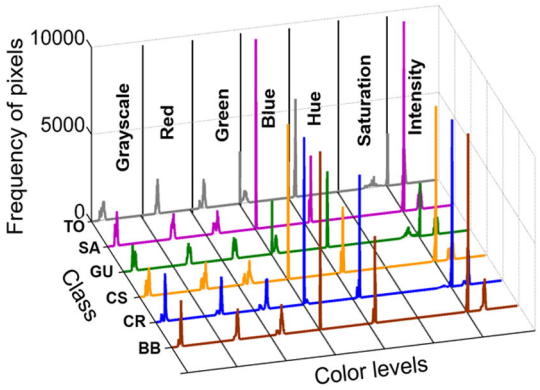

Fig. 2. Mean histograms of the propolis samples from six geographical origins of the province of Buenos Aires, Argentina. BB — Bahía Blanca; CR — Coronel Rosales; CS — Coronel Suarez; GU — Guamini; SA — Saavedra; TO — Tornquist.

2. Experimental

2.1. Samples preparation

A total of 78 samples of raw propolis were collected from apiaries of six districts (BB — Bahía Blanca, CR — Coronel Rosales, CS — Coronel Suarez, GU — Guamini, SA — Saavedra, and TO — Tornquist) of the province of Buenos Aires, Argentina, between September 2011 and May 2012. The geographic location of propolis samples are shown in Fig. 1. The ingathering was done with plastic meshes. In order to facilitate the harvesting of propolis samples the meshes were cooled at −20 °C. Sealed polyethylene bags were used to store the samples in the dark at room temperature. Before the analysis, ethanolic extracts of propolis were prepared using 0.10 g of raw propolis with 10 mL of ethanol (Carlo Erba). The extracts were then shaken in a vortex (IKA®) for one minute and following filtered with Whatman filter paper N° 41.

2.2. Apparatus and software

In order to obtain digital images, a Flow-Batch System coupled with a webcam (Philips VGA SPS900NC whit CCD sensor) was used, as described

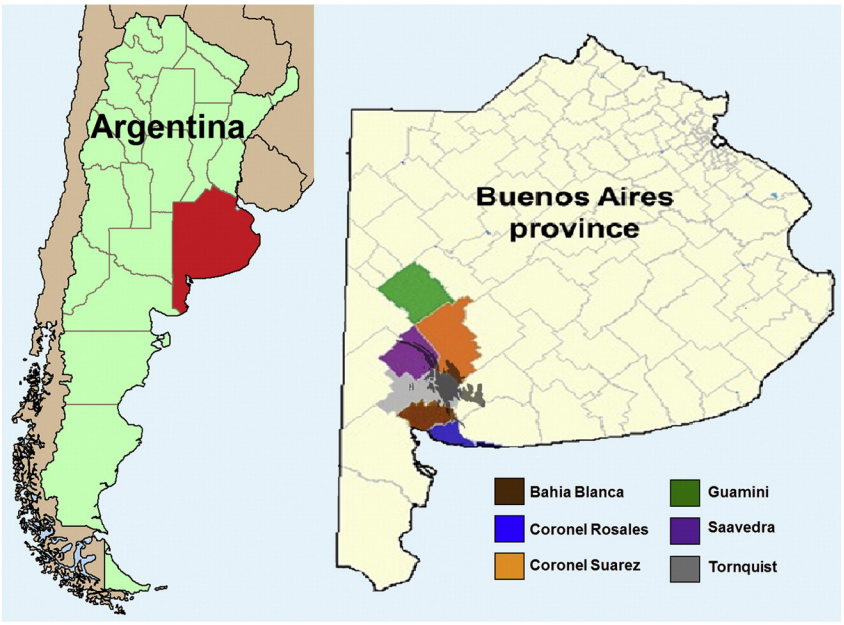

Fig. 1. Geographical location of the studied propolis samples.

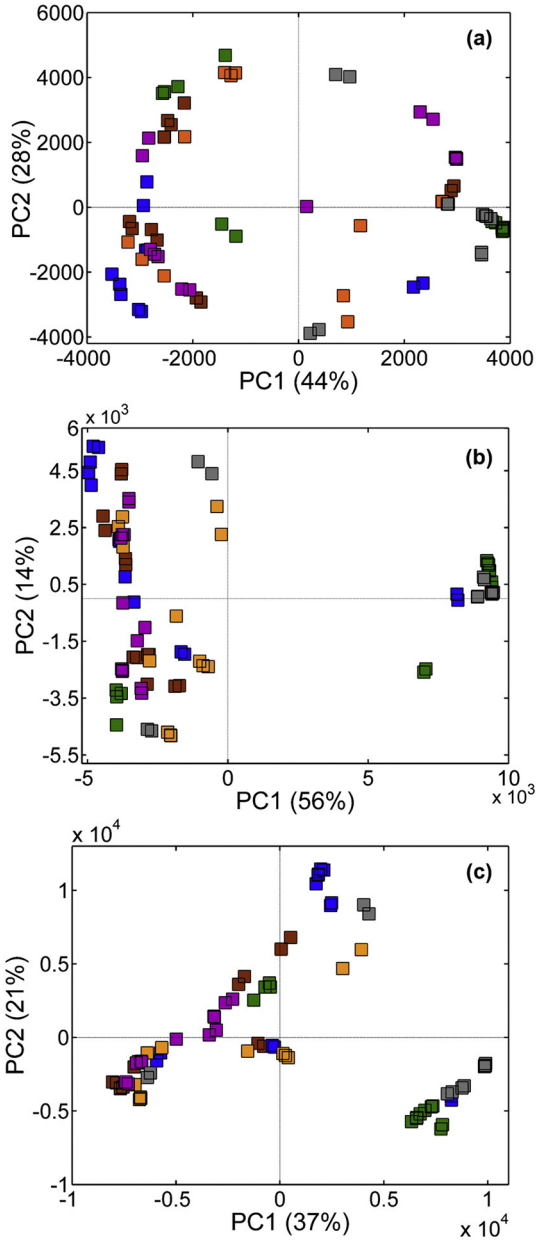

Fig. 3. PCA score plots obtained using the (a) Grayscale, (b) RGB and (c) HSI histograms of the studied propolis samples: Bahía Blanca (brown), Coronel Rosales (blue), Coronel Suarez (orange), Guamini (green), Saavedra (purple), and Tornquist (gray). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

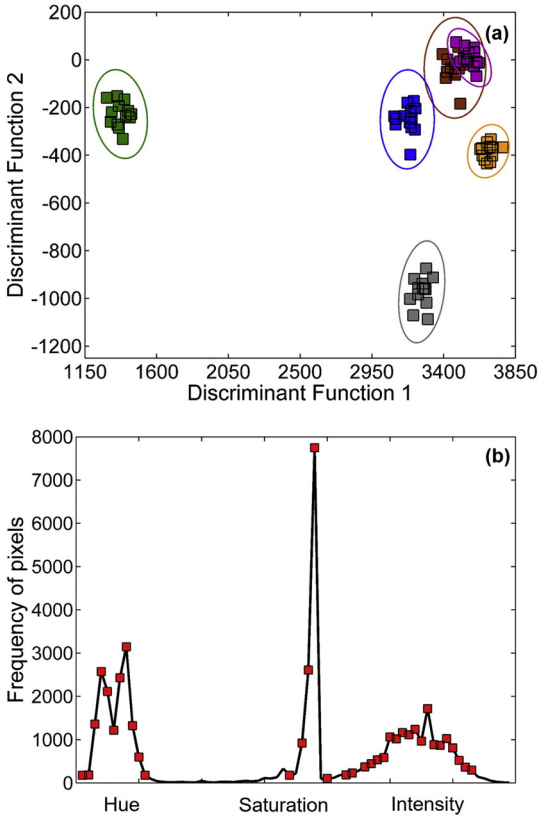

Fig. 4. (a) Score plot of the Fisher's discriminant functions obtained with (b) the 37 variables selected by SPA in the HSI histogram for the classification of the studied propolis samples: Bahía Blanca (brown), Coronel Rosales (blue), Coronel Suarez (orange), Guamini (green), Saavedra (purple), and Tornquist (gray). The selected variables are indicated with a red square. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by Dominguez et al. [33]. A white-light LED was suitably placed in front of a quartz window located in the lateral of the Flow-Batch chamber in order to generate a constant level illumination. The chamber of the Flow-Batch system is composed of polytetrafluoroethylene, which minimizes light scattering and fluorescence effects, and their effects on the obtained histograms. Additionally, the system maintains fixed positioning, homogeneous luminosity, sample-to-camera distance and focus in order to ensure reproducibility of the measurements. Thus, the proposed methodology dispensed with the need for further image manipulation, besides using low amounts of samples, minimizing waste generation. A Wilson Minipuls 3 peristaltic pump was employed to propel the solutions, which were directed to the chamber or to the waste by solenoid valves (model 137 161T031, NResearch). LabView 7.1 (National Instruments) software was used to control the system.

The obtained digital images were then converted into color histograms using the MatLab interface "Imagens_gui" freely downloadable at http://www.laqa.quimica.ufpb.br. Data analysis was performed in Matlab® 2010a (Mathworks Inc.).

2.3. Color histograms and data analysis

Ten digital images of each sample were obtained with a resolution of 640 × 480 pixels, saved in JPEG (jpg) format. The region of interest

Table 1
Classification accuracy obtained for the differing color histograms in the Grayscale, RGB and HSI channels using PCA–LDA, SIMCA, kNN, PLS-DA and SPA–LDA.

Histograms	Classification accuracy (%)									
	PCA–LDA		SIMCA		kNN		PLS-DA		SPA–LDA	
	Training	Test	Training	Test	Training	Test	Training	Test	Training	Test
Grayscale	58.0	89.0	56.7	83.3	80.0	88.9	61.7	88.9	83.3	88.9
RGB	36.7	61.0	66.7	88.9	21.7	100	86.7	100	100	94.4
HSI	76.7	94.4	68.3	77.8	35.0	100	86.7	94.4	100	100

(ROI), corresponding to 40% of the original image, was selected and converted in Grayscale, RGB and HSI histograms. RGB components and Grayscale levels vary from 0 to 255 (256 levels); S and I vary from 0 to 1°, and H varies from 0° to 360°. The average of ten histograms was calculated for each sample and following used as analytical signal. The data were organized in a matrix, where the samples were placed in lines, and the frequency of pixels for each color level was informed in the columns. Before using the chemometrics tools, color levels with frequency of pixels equal to zero simultaneously in all samples were removed to avoid compromising the results.

A first study of the data was performed using Principal Component Analysis (PCA). The data set was then separated into training set (60 samples) and test set (18 samples) using the Kennard–Stone algorithm [37]. Following, a supervised classification of the samples was performed using PCA–LDA, SIMCA, kNN, PLS-DA and SPA–LDA, whose mathematical principles of the modeling of each technique are done in [38]. The training set was used to construct the models, which were validated using full cross-validation. The test samples were used only for the final data evaluation, and comparison of the classification performance of the models in terms of accuracy, sensitivity and specificity. The accuracy rate was calculated as the number of correct classifications divided by the total number of samples in the set under consideration (training or test set). The sensitivity rate was calculated as the number of correct positive decisions divided by the total number of known positive cases. Finally, the specificity rate was calculated as the number of correct negative decisions divided by the total number of known negative cases [39,40].

KS and SPA–LDA algorithms were performed with Matlab® 2009b (Mathworks Inc.) software. PCA–LDA, SIMCA, kNN and PLS-DA were calculated by using the Classification toolbox for Matlab® (version 3.1) released by Milano Chemometrics and QSAR Research Group, which can be found at http://michem.disat.unimib.it/chm.

3. Results and discussion

Fig. 2 shows the mean histograms of the propolis samples from six geographical origins of the province of Buenos Aires, Argentina. As can be seen, samples from Tornquist and Guamini apart from the others when we take into consideration the profiles of mean histograms of both blue and saturation channels, which have low frequency of pixels. On the other hand, the profiles of mean histograms of grayscale, red and green channels for the samples from Bahía Blanca and Coronel Suarez are most intense than the samples from Coronel Rosales and Saavedra. In order to verify these observations, an unsupervised exploratory analysis of the data was performed, as discussed in the next section.

3.1. Exploratory analysis of the data

A screening analysis of all propolis samples was developed by using Principal Component Analysis (Fig. 3). As can be seen, information contained in the scores plot for the Grayscale histograms (Fig. 3a) is highly overlapped. By the other side, some samples from Tornquist and Guamini apart from the others in the score plots using RGB (Fig. 3b) and HSI (Fig. 3c) histograms. Despite this, a geographical discrimination of all samples from six different districts of Buenos Aires Province cannot be achieved, justifying the use of supervised pattern recognition techniques.

3.2. Classification

Table 1 presents the summary of the classification accuracy in both training and test sets into the six studied propolis classes using PCA–LDA, SIMCA, kNN, PLS-DA and SPA–LDA for Grayscale, RGB and HSI histograms.

Table 2
Confusion matrix, with the accuracy, sensitivity and specificity of the classification of the ethanolic extracts of propolis in both training and test sets for SPA–LDA modeling using Hue, Saturation and Intensity channels separately.

	Cross validation						Test					
Hue												
	BB	CR	CS	GU	SA	TO	BB	CR	CS	GU	SA	TO
BB	9	–	1	–	–	5	3	–	–	–	–	–
CR	–	5	1	–	2	2	–	2	1	–	–	–
CS	1	–	5	–	2	2	–	–	3	–	–	–
GU	–	–	1	10	–	–	–	–	–	3	–	–
SA	1	–	1	1	8	–	–	–	–	–	3	–
TO	–	1	–	–	1	8	–	–	–	–	–	3
Sensitivity (%)	90.0	50.0	50.0	100	80.0	80.0	66.7	66.7	100	100	100	66.7
Specificity (%)	96.0	98.0	94.0	98.0	92.0	92.0	100	100	86.7	93.3	100	100
Accuracy (%)	75.0						83.3					
Saturation												
BB	6	–	1	–	4	–	1	–	–	–	2	–
CR	1	7	1	–	1	2	–	2	1	–	–	–
CS	1	–	7	–	2	2	–	–	2	–	1	–
GU	–	–	–	6	–	4	–	–	–	0	–	3
SA	2	–	–	–	7	1	–	–	–	–	2	1
TO	2	–	–	–	–	8	–	–	–	–	–	3
Sensitivity (%)	60.0	70.0	70.0	60.0	70.0	80.0	33.3	66.7	66.7	66.7	66.7	100
Specificity (%)	88.0	100	98.0	100	86.0	90.0	100	100	93.3	100	83.0	73.3
Accuracy (%)	68.0						55.6					
Intensity (27 variables)												
BB	10	–	–	–	–	–	3	–	–	–	–	–
CR	–	10	–	–	–	–	–	3	–	–	–	–
CS	–	–	10	–	–	–	–	–	3	–	–	–
GU	–	–	–	10	–	–	–	–	–	3	–	–
SA	–	–	–	–	10	–	–	–	–	–	3	–
TO	–	–	–	–	–	10	–	–	–	–	–	3
Sensitivity (%)	100	100	100	100	100	100	100	100	100	100	100	100
Specificity (%)	100	100	100	100	100	100	100	100	100	100	100	100
Accuracy (%)	100						100					

The best result of PCA–LDA was obtained using HSI histograms, which classifies correctly 76.7 and 94.4% of the samples in both training and test sets, respectively, using an optimal number of 20 principal components. When SIMCA modeling was employed, a correct classification rate of 66.7 and 88.9% in both training and test sets, respectively, was achieved using RGB histograms. In this case, the optimal number of principal components was 2, 3, 3, 2, 1 and 3 for Bahía Blanca, Coronel Rosales, Coronel Suarez, Guamini, Saavedra and Tornquist classes, respectively. The best accuracy of kNN modeling was attained using Grayscale histograms, reaching 80.0 and 88.9% of classification in both training and test sets, respectively, using an optimal number of one nearest neighbor. PLS-DA modeling achieved its best result with RGB histograms, obtaining 86.7 and 100% of classification accuracy in both training and test sets, respectively, using an optimal number of 16 latent variables. Finally, the most successful result was obtained for SPA–LDA modeling using HSI histograms, which classified correctly all samples (100%) in their respective classes for both training and test sets.

Fig. 4a shows the score plot of the Fisher's discriminant functions obtained with the 37 variables selected by SPA in the HSI histogram (Fig. 4b) for the classification of the studied propolis samples.

As can be seen in Fig. 4b, most of informative variables selected by SPA–LDA were located in the Intensity channels. In this case, this occurs because the chromaticity (defined as the degree of color purity and is related to both hue and saturation [28]) of the samples is less discriminative than the Intensity. Thus, we investigated if SPA–LDA models using Hue, Saturation and Intensity channels separately can lead similar results to those obtained using complete HSI histograms. Table 2 presents the confusion matrix, with the accuracy, sensitivity and specificity for the six studied propolis classes in both training and test sets for SPA–LDA modeling using Hue, Saturation and Intensity channels separately.

As expected, the analytical information contained in the selected variables by SPA in the Intensity histogram is responsible for the complete discrimination between the studied propolis samples, reaching 100% of accuracy, sensitivity and specificity in both training and test sets (Table 2). Fig. 5a shows the score plot of the Fisher's discriminant functions obtained with the 27 variables selected by SPA in the Intensity histogram (Fig. 5b) for the classification of the propolis samples. Observing the groupings in Fig. 5b and relating them with the geographical locations in Fig. 1, the Guamini samples are located furthest from the others because it presents climate and vegetation characteristic of the *Pampas*. By the other side, the apiaries of Coronel Suarez and Saavedra are close together, because they are located in the boundaries of the *Sierra de la Ventana* area. Bahía Blanca and Coronel Rosales are coastal districts located in the south of the *Sierra de la Ventana* area, while Tornquist apiaries are located on top of the mountain region that has different climate and vegetation.

The low performance of PCA–LDA, SIMCA, kNN and PLS-DA when compared with SPA–LDA is due to the fact of color histograms have a large number of variables with a strong correlation over different analytical channels, compromising both precision and accuracy of the results. For this reason, it was necessary to find the better combination between the multivariate classifier and the color model. Thus, a suitable variable selection technique was required to improve the results by selecting variables minimally redundant, which helps to maximize the between-class separability, while minimizing the within-class variability when applying LDA.

4. Conclusion

In this work, a simple, fast and reliable analytical methodology based on digital images and chemometrics was developed for the classification of raw propolis from six different geographical origins of the Buenos Aires Province, Argentina. For this purpose, different combinations between a color model (Grayscale, RGB and HSI) and a multivariate classifier (PCA–LDA, SIMCA, kNN, PLS-DA and SPA–LDA) were tested. The best analytical performance was achieved by SPA–LDA using HSI histograms, classifying correctly a 100% of the samples according to their respective geographical origins taking in account the 37 variables selected by SPA. It was observed that most of these variables were selected in the Intensity channels. Then, new SPA–LDA calculations were performed to investigate the discrimination ability of Hue, Saturation and Intensity histograms separately. As expected, Intensity histograms contained information sufficient for the classification of all studied samples, being selected only 27 variables in this case, leading to a most simple analytical approach. Therefore, the proposed methodology is a good alternative to support local apiculturists, guaranteeing the offer of products with a clear indication of geographical origin and enhancing regional capabilities.

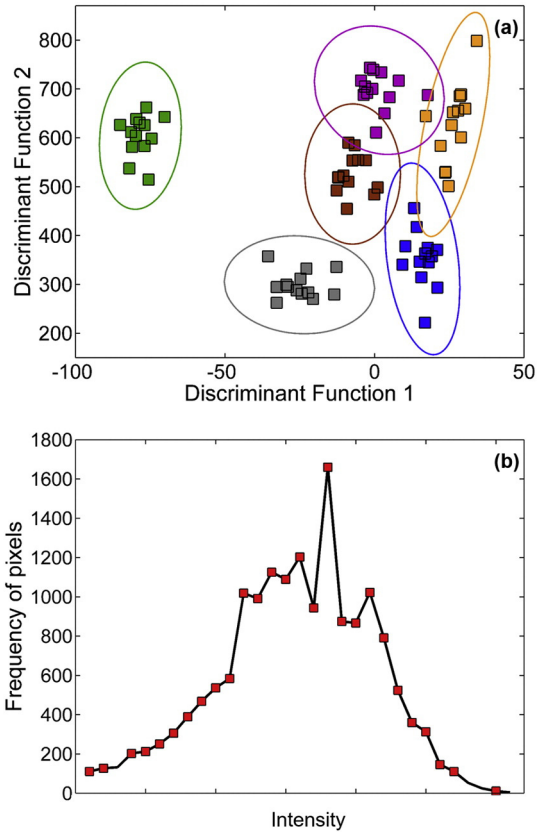

Fig. 5. (a) Score plot of the Fisher's discriminant functions obtained with (b) the 27 variables selected by SPA in the Intensity histogram for the classification of the studied propolis samples: Bahía Blanca (brown), Coronel Rosales (blue), Coronel Suarez (orange), Guamini (green), Saavedra (purple), and Tornquist (gray). The selected variables are indicated with a red square. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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