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

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A B S T R A C T

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.

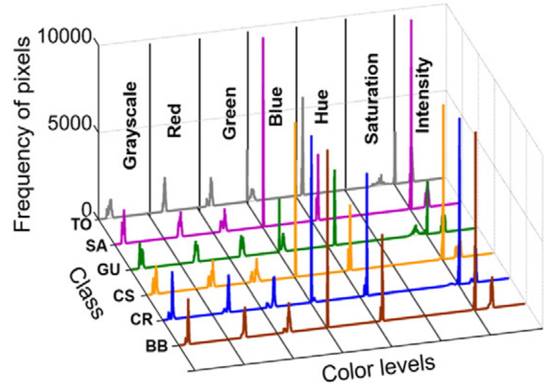


**Fig. 2.** Mean histograms of the propolis samples from six geographical origins of the province of Buenos Aires, Argentina. BB — Bahía Blanca; CR — Coronel Rosales; CS — Coronel Suarez; GU — Guamini; SA — Saavedra; TO — Tornquist.

## 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

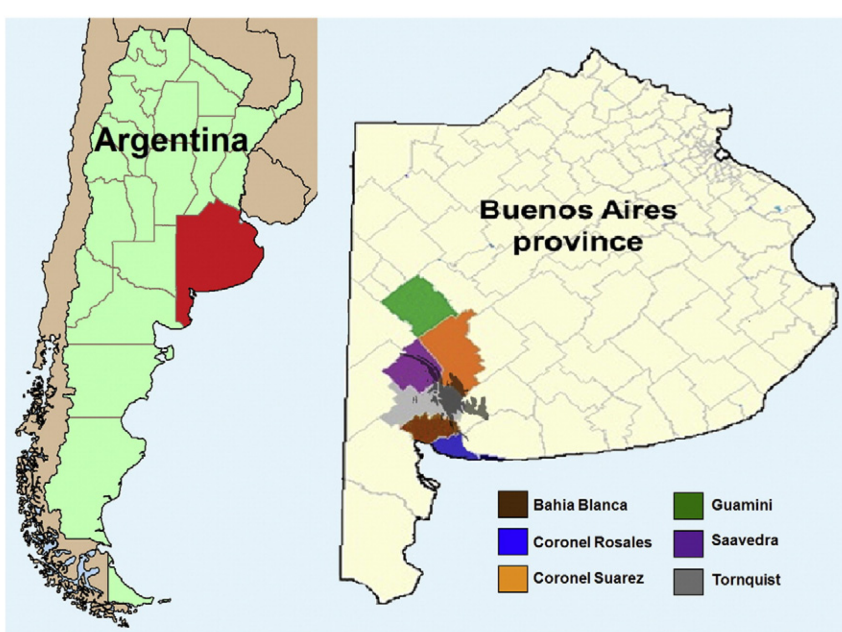


**Fig. 1.** Geographical location of the studied propolis samples.