Microchemical Journal 118 (2015) 110–114

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

# Application of digital images to determine color in honey samples from Argentina

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ARTICLE INFO

*Article history:*
Received 28 April 2014
Received in revised form 4 August 2014
Accepted 4 August 2014
Available online 10 August 2014

*Keywords:*
Honey
Color
Digital image
Multivariate calibration

ABSTRACT

Honey color is the first quality attribute evaluated by consumers and is an important sensory property in the beekeeping market. The most commonly used method for color determination is based on optical comparison, employing a Pfund colorimeter. This method is laborious, time consuming and is based on a subjective assessment. In order to obtain a fast and objective measurement of honey color, an analytical method based on digital images analysis combined with multivariate calibration (Partial Least Squares) was developed. Three color models, RGB (red–green–blue), HSB (hue–saturation–brightness) and Grayscale, were used to analyze the digital images. The optimum results were obtained by using HSB color model ($r^2 = 0.97$ and RMSEP = 2.46), indicating that the digital image analysis combined with multivariate calibration is an excellent strategy for quantifying color in honey samples.



## 1. Introduction

In terms of production Argentina is positioned is in the sixth place, being simultaneously the second exporter of honey in the world [1]. For its extensive natural grasslands, its geographical location and climate, Argentina has a wide variety of floral resources that allow these activities to be carried out in most of the territory. However, the Pampas region located in the province of Buenos Aires concentrates more than 50% of national production [2]. In Argentina the internal market is underdeveloped due to low consumption of this product. However, Argentine honey is internationally appreciated for its organoleptic characteristics, its chemical composition and its wide variety of colors and purity [3–5]. In fact, the 95% of national production is destined for export markets which require good quality of products.

Color is the most important feature from the commercial standpoint and encourages honey export. This attribute varies according to the geographical origin of honey and depends on the pigment (carotenes and xanthophylls) and polyphenol (flavonols) contents [6–9]. The appropriate color measurement allows exporters to choose the most advantageous trading market for their products and is the only sensory examination with accurate coding within current regulations.

Internationally honeys are classified for commercialization using the Pfund color scale. The Pfund colorimeter is a simple instrument by which an observer compares the color of the honey sample with a standard colored glass [10]. The reference unit is the Pfund scale, which ranges from 0 to 140 mm, beginning with very light-colored honey and increasing up to the darkest honey.

However, this method does not distinguish between small variations of color and the determination is laborious, time consuming, requires large amounts of sample and depends on the person performing the analysis since different observers get different measurements.

Nowadays, there are few articles published in order to obtain other methods to determine honey color. Gallez et al. propose a nonlinear quadratic polynomial regression between data recorded with the Pfund and the CIELAB methods by means of multivariate analysis [11]. Other authors proposed a method to the reconstruction of honey reflectance spectra by using a characteristic vector analysis to obtain the color of honeys [12]. A potentiometric electronic tongue was used for the determination of color and other parameters for the discrimination of honey according to the botanical origin [13]. However, these methods have certain disadvantages such as they are not quite as simple as other methods, require expensive instrument which are not available in routine laboratories, tests need to be carried out by specialized analysts and required complex mathematical procedures. Therefore, it is necessary to develop alternative methods for color determination in honey samples to achieve objective measures. In a previous research work we described that digital images combined with chemometric techniques are an adequate tool for geographic origin classification of honey samples. These digital images were analyzed using different color models as RGB (Red–Green–Blue), HSB (Hue–Saturation–Brightness) and Grayscale [14].

The aim of this study was to apply digital image analysis combined with multivariate calibration for determining color in honey samples. From color data obtained by the reference method (Pfund) and color

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http://dx.doi.org/10.1016/j.microc.2014.08.002
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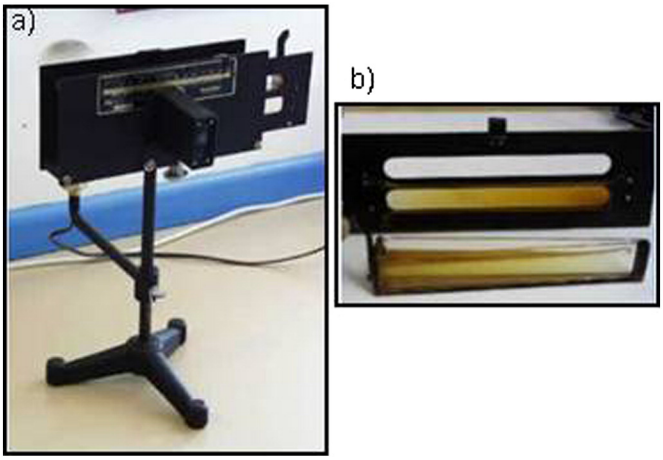


**Fig. 1.** a) Pfund color grader, b) wedge of amber-colored glass and wedge-shaped cell filled with the honey sample.

histograms, a multivariate calibration model using partial least squares (PLS) were constructed. By this way a simple method for color measurements, with less time analysis and human participation to assist the quality control of honey samples, was proposed.

## 2. Materials and methods

### 2.1. Samples

Eighty honey samples from southwest of the Buenos Aires province, Argentina were obtained from local markets. Fresh honey samples were stored at 4 °C in the dark until analysis. All samples were allowed to reach room temperature before analysis.

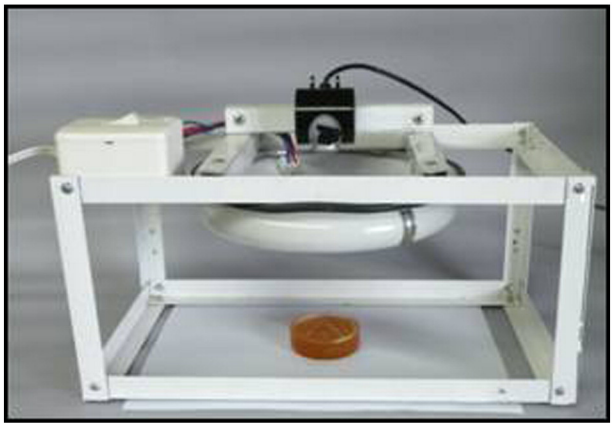

**Fig. 2.** Honey image capturing device.

**Table 1**
Calibration models results.

| Model | LV[a] | RMSEC | RMSECV | $r^2$ | Slope | Bias | Correlation |
|---|---|---|---|---|---|---|---|
| RGB | 6 | 2.60 | 4.73 | 0.97 | 0.97 | $7.15 \times 10^{-7}$ | 0.98 |
| Grayscale | 4 | 3.14 | 4.33 | 0.97 | 0.97 | $5.88 \times 10^{-7}$ | 0.98 |
| HSB | 4 | 2.06 | 3.63 | 0.98 | 0.98 | $-1.28 \times 10^{-6}$ | 0.99 |

[a] Latent variables.

### 2.2. Apparatus and software

A Philips Webcam SPC900NC VGA with a CCD sensor was used to obtain digital images. These images were processed to acquire the corresponding color histogram with the ImageJ1.44p program (a free internet download). Chemometric data treatment was implemented with The Unscrambler_9.7 (CAMO S/A), and Matlab® 6.5 software (The Mathworks). A Pfund color grader Koehler (New York, USA) was employed to determine color, according to Argentina regulations.

### 2.3. Reference method

Color determination was carried out according to Argentina regulations using a Pfund color grader Koehler (New York, USA). This device visually compares standard amber-colored glass wedge with liquid honey contained in a wedge-shaped cell placed in a sliding panel, as can be seen in Fig. 1a). Color measurement is done by sliding the panel until the color of the honey sample matches the color of the calibrated amber glass prism. The color intensity is expressed as a distance (in mm) along the amber-wedge and usually ranged between 1 and 140 mm beginning with very light-colored honey and increasing up to the darkest honey. Ten readings must be taken for each honey sample, five from each side of the glass wedge. Pfund values were obtained by a trained analyst.

### 2.4. Digital image acquisition

Approximately 10 g of honey sample was heated up to 40 °C to dissolve sugar crystals and was allowed to stand to remove air bubbles. The honey sample was placed in a Petri dish and using a Philips Webcam SPC900NC VGA the image was obtained. This procedure was performed in triplicate for each sample.

Fig. 2 shows the image capturing device [15]. The webcam was placed in a fixed position in the center of a circular daylight fluorescent lamp (22 W, temperature color 6400 K) over the honey sample. By this way the illumination and the distance between the sample and the camera remained constant. The sample holder used in this study was placed in a sealed box to prevent the passage of light. In order to avoid light scattering the interior walls of the box were covered with white paper.

In Fig. 3 digital images obtained from four different honey samples can be observed.

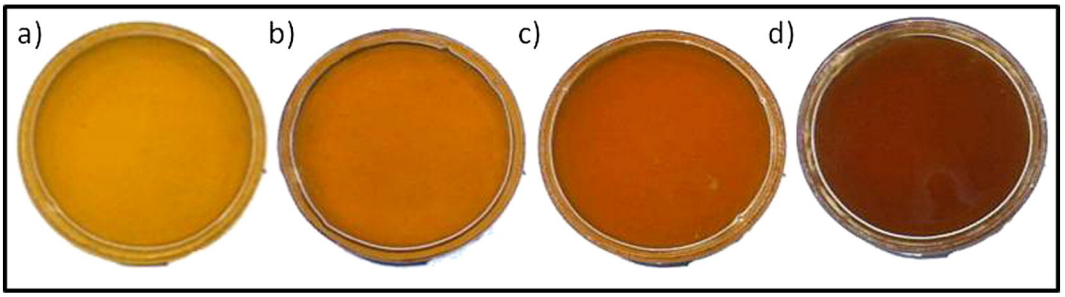


**Fig. 3.** Digital images obtained from four samples of honey with different color values. a) 14.7 mm Pfund, b) 35.0 mm Pfund, c) 57.1 mm Pfund and d) 95.0 mm Pfund.