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# Multivariate Correlation between Color and Mineral Composition of Honeys and by Their Botanical Origin

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The mineral content and color characteristics of 77 honey samples were analyzed. Eighteen minerals were quantified for each honey. Multiple linear regression (MLR) was used to establish equations relating the colorimetric CIELAB coordinates to the mineral data. The results obtained shown that lightness ($L^*$) was significantly correlated with S, Ca, Fe, As, Pb, and Cd for the dark honey types (avocado, heather, chestnut, and honeydew). For the light and brown honey types (citrus, rosemary, lavender, eucalyptus, and thyme), $C_{ab}^*$ and $b^*$ showed the lower correlation with the mineral content of the honeys; their regression functions involve a few independent variables (Mg and Al for $b^*$ and only Al for $C_{ab}^*$). Furthermore, by means of application of linear discriminant analysis to the mineral content, it was possible to obtain a model that classifies the honeys by their lightness. The prediction ability of the built model, determined with the test set method, was 85%.

**KEYWORDS:** ICP−OES; minerals; honey; color; multiple linear regression (MLR); discriminant analysis

## INTRODUCTION

Honey, a natural sweet product elaborated by honeybees, contains mainly simple sugars or monosaccharides, of which fructose and glucose are the main components (65%) and 18% of water content, approximately. Proteins, flavonoids, flavor and aroma, phenolic compounds, free amino acids, organics acids, and vitamins constitute minor components of honeys, but they contain all of the minerals that are essential to health.

The quantity and variety of minerals are present in honeys depending on the nutrients that have been absorbed by the plants, their availability in the soil, and the soil and environmental contaminations. Thereby, the excess or deficiency of certain chemicals in the soil or water will have repercussions in the chemical compositions of the plants and then in the nectar (*1*).

On the other hand, the appearance of foods is one factor that defines their quality and it is the first impression the consumer gets directly from the food. Color, as a part of the appearance, has to be within an expected range for consumer acceptance, and the degree of acceptability is judged within that range.

Honeys show very different colors, varying from white or pale yellow to dark red or even black. Many studies have dealt with the relation of honey color to the floral origin, industrial processing methods, and the temperature and/or time of storage (*2*−*6*). Other authors have considered the influence that the pollen grains (PGs) (their morphology and color) may have on the honey color (*7*).

The mineral content influences the color and the taste of honeys: The higher the quantity of metals and the darker color is, the stronger taste they will have (*8*). However, there are no studies of those estate correlations between mineral composition and chromatic characteristics of honeys by applying objectives methods such as spectrophotometric techniques.

The goal of this work is to study the relationship between honey color and mineral content in several honeys by the use of multivariate statistical techniques such as multiple linear regression (MLR). Thus, the present study is aimed at obtaining mathematical equations that permit us to decide which mineral from those present in the honey has more weight and allows the prediction of the different color parameters. Furthermore, classification of honeys, according to their lightness, by means of application of linear and stepwise discriminant analysis (LDA and SDA) to the mineral content was carried out.

## MATERIALS AND METHODS

**Samples.** The present study made use of 77 honey samples collected in Spain, between 2002 and 2003. All samples were unpasteurized and were taken no more than 3 months after extraction, stored in holders, and immediately transferred to the laboratory and kept at 0 °C. Analyses were made within a 6 month time period after harvesting.

**Pollen Analysis.** The quantitative analysis of the samples was carried out using the light microscope on slides prepared without any chemical treatment, according to the method described by Maurizio (*9*); all of

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the PGs and honeydew elements (HDE) were counted for each honey sample in four different slides, covering the whole surface of each slide. The botanical elements represent the addition of the PGs and the HDEs. The qualitative analysis was carried out using acetolyzed slides prepared according to the method described by Erdtman (*10*) and always using a subsample of 10 g of honey.

Nine certified monofloral honey classes were included as follows: eucalyptus (*Eucalyptus* sp.), rosemary (*Rosmarinus officinalis*), orange (*Citrus* sp.), heather (*Erica* sp.), thyme (*Thymus* sp.), lavender (*Lavandula* sp.), avocado (*Persea americana*), honeydew (*Quercus* sp.), and chestnut (*Castanea sativa*).

**Mineral Content.** The minerals were determined using a Jobin-Yvon Ultima 2 ICP optical emission spectrometer and an Ultrasonic nebulizer (U6000 AT$^+$, Cetac). The instrument was operated in the following conditions: radio frequency, 27 MHz; operating power, 1200 W; plasma argon flow rate, 2 L min$^{-1}$; auxiliary gas flow rate, 2 L min$^{-1}$; nebulizer gas flow rate, 0.02 L min$^{-1}$; nebulizer pressure, 1 bar; rinsing time, 35 s; rinsing pump speed, high; transfer time, 60 s; stabilization time, 20 s; and transfer pump speed, high.

Distilled, deionized water of 18 MΩ cm$^{-1}$ resistivity, obtained from a Milli-Q system (Millipore), was used to prepare all solutions. A 10% v/v solution of nitric acid (Panreac, Spain) was used for digestion of the samples. Spex plasma standard (1000 mg L$^{-1}$) was used to prepare Al, As, Ba, Be, Ca, Cd, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, S, Si, and Zn reference solutions. The ashes were obtained by calcinations (600 °C) of 5 g samples honey to constant weight (*11*). Nitric acid (0.1 M, 5 mL) was added to the resultant ashes, and the mixture was stirred on a heating plate to almost complete dryness. Then, 10 mL of the same acid was added and the mixture was brought up to 25 mL with distilled water. Results were expressed as mg of metal per kg of honey.

**Color Analysis.** The reflectance measurements were made using a CAS 140 B spectroradiometer (Instrument Systems, Munich, Germany), equipped with a Top 100 telescope optical probe and a Tamron zoom model SP 23A. For this purpose, a plastic cell for reflectance measurements was used (475 mm × 350 mm × 10 mm).

A subsample of 20 g of honey was heated again up to 45−50 °C to dissolve the sugar crystals and then poured into a 10 mm path length spectroradiometer cell, and the reflectance spectrum was measured. Blank measurements were made with the cell filled with distilled water, with a $BaSO_4$ pressed plate as reference white background (USRS-99-010, Labsphere Inc., North Sutton, NH). The reflectance spectra of the samples were registered directly onto the honey, with the same white background. The cells were placed inside a cabin with gray ($L^* = 50$) walls to which the external illumination source of the spectroradiometer was attached. The zoom, to which the probe was attached, was held at a fixed distance of 50 cm in a straight line from the sample. As far as geometry of presentation, 45° incident illumination was used throughout the experiment. The spectroradiometer was set to take three consecutive measurements of each sample, so color coordinates obtained were averages of three measurements. The whole visible spectrum (380−770 nm) was recorded ($\Delta\lambda = 1$ nm). Illuminant $D_{65}$ and 10° Observer were used in the calculus. The color parameters corresponding to the uniform color space CIELAB (*12*) were obtained directly from the apparatus. Within the approximately uniform space CIELAB, two color coordinates, $a^*$ and $b^*$, as well as lightness, $L^*$, are defined. Coordinate $a^*$ takes positive values for reddish colors and negative values for greenish ones, whereas $b^*$ takes positive values for yellowish colors and negative values for bluish ones. $L^*$ is an approximate measurement of lightness, which is the property according to which each color can be considered as equivalent to a member of the gray scale, between black and white, taking values within the range of 0−100, respectively. From the CIELAB space, other parameters are defined, such as chroma ($C_{ab}^* = [(a^*)^2 + (b^*)^2]^{1/2}$) and hue angle [$h_{ab} = \arctan(b^*/a^*)$].

The samples were submitted to a classification by experts. The panel consisted of nine people in the age group of 24−40 years, comprising both male and female, who regularly participated in sensory evaluations, and they had good experience in odor and flavor profiling of a number of food products. Testing was performed in sensory laboratory with individual booths under fluorescent lighting with a correlated color temperature to a daylight illuminant. Fifteen milliliters of honey samples, at room temperature (25 °C), was served in 25 mL labeled beakers. Two sets of samples were established according to their lightness: dark honeys and light honeys.

**Statistical Methods.** For the statistical treatment of the data, the Statistica computer package (*13*) was used. For this study, forward stepwise MLR was used. The general purpose of multiple regression is to learn more about the relationship between several independent (or predictor) variables and a dependent (or criterion) variable. In general, multiple regression allows the researcher to know what is the best predictor in a model. In multiple regression, more than one variable is used to predict the criterion, and procedures will estimate a linear equation of the form $Y = a + b_1 \times X_1 + b_2 \times X_2 + ... + b_p \times X_p$ where the regression coefficients ($b$) represent the independent contributions of each independent variable to the prediction of the dependent variable. The standardized versions of the $b$ coefficients are the $\beta$ weights, and the ratio of the $\beta$ coefficients is the ratio of the relative predictive power of the independent variables.

The multiple regression can establish that a set of independent variables explains a proportion of the variance in a dependent variable at a significant level. Furthermore, it can establish the relative predictive importance of the independent variables by means of comparing the $\beta$ weights. The estimates ($b$ coefficients and constant) can be used to construct a prediction equation and generate predicted scores on a variable for further analysis.

Discriminant analysis was carried out. This statistical technique required a qualitative variable (dependent variable) and two or more quantitative variables (independent variables). It is a method of classification whose aim is to estimate through linear functions (discriminant functions) of the independent variables (*14*, *15*) the probability that one of the cases belongs to each of the groups defined by the categories of the dependent variable. There will be as many groups as categories with the aforementioned dependent variable. This classification will be made according to the properties given by the independent variables common to each case. The aim of this analysis was to evaluate the capacity for classification and prediction of the obtained functions in order to check which of the variables were better for discriminating.

LDA obtains discriminant functions calculated to maximize distances between predefined groups. Its purpose is to calculate class models giving a rule of classification based on a set of known objects (training set). This rule can be applied to define the classification of unknown objects (test set).

In SDA, a model of discrimination is built step-by-step incorporating variables to the model in successive steps. Specifically, at each step, all variables are reviewed and evaluated to determine which one will contribute most to the discrimination between stages. That variable will then be included in the model, and the process starts again.

## RESULTS AND DISCUSSION

**Mineral Content.** In the analysis of individual mineral contents, 18 minerals were identified and quantified as follows: aluminum, arsenic, barium, beryllium, calcium, cadmium, copper, iron, potassium, lithium, magnesium, manganese, sodium, phosphorus, lead, sulfur, silicon, and zinc. **Table 1** shows the mean, range, and standard deviation for the mineral contents of honeys. Potassium is the mineral with the highest concentration present in the honeys, with a mean value of 634 mg/kg (58%). This is according with other authors that affirm that potassium is the majority mineral in honeys (*16*−*18*), followed by phosphorus (115 mg/kg) and sodium (106 mg/kg), with percentages around 10%; silicon, 8%; and calcium, 6%. Other minerals are present in quantities less than 47 mg/kg: magnesium (4%) and sulfur (2%). The rest of the minerals are less than 0.5% of the total quantified minerals.

As it was expected, the mineral content was higher (between 1340 and 1879 mg/kg) in those darker honeys (avocado, chestnut, honeydew, and heather). A high quantity of potassium in these types of honeys is remarkable (from 60 to 70% of the

**Table 1.** Mean, Range, and Standard Deviation for the Mineral Contents (mg/kg) of Honeys by Their Botanical Origin

| mineral | avocado ($n = 2$) | chestnut ($n = 4$) | citrus ($n = 10$) | eucalyptus ($n = 4$) | heather ($n = 5$) | honeydew ($n = 33$) | lavender ($n = 4$) | rosemary ($n = 11$) | thymus ($n = 4$) | mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Al | 12.41 ± 13.58 | 5.20 ± 3.13 | 2.26 ± 1.00 | 4.12 ± 1.52 | 3.25 ± 1.68 | 3.04 ± 1.94 | 2.73 ± 1.14 | 3.66 ± 3.63 | 2.65 ± 1.66 | 4.38 |
| | 2.81–22.01 | 1.85–7.90 | 1.02–4.71 | 2.61–6.03 | 0.96–4.85 | 0.92–9.98 | 1.90–4.40 | 1.27–14.07 | 1.24–5.01 | |
| As | 0.06 ± 0.09 | 0.04 ± 0.05 | 0.10 ± 0.08 | 0.03 ± 0.04 | 0.05 ± 0.04 | 0.05 ± 0.05 | 0.05 ± 0.05 | 0.06 ± 0.03 | 0.02 ± 0.03 | 0.05 |
| | 0.00–0.13 | 0.00–0.10 | 0.00–0.25 | 0.00–0.07 | 0.00–0.10 | 0.00–0.21 | 0.00–0.10 | 0.00–0.15 | 0.00–0.06 | |
| Ba | 0.00 ± 0.00 | 0.00 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.01 | 0.02 ± 0.09 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0 |
| | 0.00–0.00 | 0.00–0.01 | 0.00–0.01 | 0.00–0.01 | 0.00–0.01 | 0.00–0.51 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| Be | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0 |
| | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | 0.00–0.00 | |
| Ca | 68.65 ± 24.36 | 102.46 ± 33.14 | 50.72 ± 20.50 | 90.22 ± 42.65 | 50.83 ± 25.41 | 69.47 ± 25.56 | 50.37 ± 26.75 | 47.51 ± 27.26 | 69.18 ± 14.28 | 66.6 |
| | 51.42–85.87 | 66.37–135.25 | 23.36–93.26 | 27.63–118.24 | 19.10–85.26 | 39.23–138.77 | 21.79–86.43 | 27.42–108.51 | 56.41–84.49 | |
| Cd | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.01 |
| | 0.00–0.02 | 0.00–0.02 | 0.00–0.03 | 0.0–0.01 | 0.00–0.01 | 0.00–0.04 | 0.00–0.01 | 0.00–0.04 | 0.00–0.00 | |
| Cu | 1.60 ± 0.67 | 0.69 ± 0.11 | 0.52 ± 0.21 | 0.57 ± 0.33 | 0.80 ± 0.30 | 0.94 ± 0.33 | 0.44 ± 0.07 | 0.42 ± 0.26 | 0.82 ± 0.48 | 0.76 |
| | 1.13–2.07 | 0.53–0.79 | 0.05–0.76 | 0.18–0.96 | 0.49–1.14 | 0.31–1.72 | 0.33–0.48 | 0.11–0.89 | 0.43–1.49 | |
| Fe | 5.60 ± 3.55 | 4.68 ± 2.30 | 2.79 ± 1.13 | 1.84 ± 1.27 | 4.47 ± 4.58 | 4.26 ± 2.55 | 2.76 ± 0.51 | 2.41 ± 0.66 | 2.95 ± 3.94 | 3.53 |
| | 3.08–8.11 | 2.92–7.89 | 0.96–4.30 | 0.00–2.84 | 1.41–12.54 | 1.41–13.09 | 2.13–3.25 | 1.54–3.36 | 0.00–8.32 | |
| K | 1130.18 ± 777.94 | 1090.14 ± 466.60 | 237.00 ± 51.25 | 476.71 ± 193.77 | 870.05 ± 250.29 | 824.38 ± 338.50 | 325.87 ± 91.82 | 274.88 ± 141.10 | 484.83 ± 178.99 | 634 |
| | 580.09–1680.27 | 683.79–1738.01 | 151.83–335.45 | 334.41–763.06 | 528.41–1168.91 | 307.25–1502.61 | 203.73–397.99 | 169.02–639.76 | 295.4–677.30 | |
| Li | 1.32 ± 0.52 | 0.88 ± 0.07 | 0.81 ± 0.24 | 0.73 ± 0.11 | 0.70 ± 0.09 | 0.85 ± 0.14 | 0.78 ± 0.08 | 0.79 ± 0.15 | 0.89 ± 0.30 | 0.86 |
| | 0.95–1.68 | 0.80–0.96 | 0.47–1.38 | 0.65–0.90 | 0.62–0.85 | 0.64–1.14 | 0.70–0.84 | 0.62–1.04 | 0.58–1.29 | |
| Mg | 91.92 ± 72.86 | 75.99 ± 32.74 | 10.94 ± 3.56 | 29.12 ± 5.98 | 57.73 ± 20.72 | 94.63 ± 47.95 | 23.48 ± 14.24 | 14.32 ± 17.20 | 27.43 ± 12.74 | 47.2 |
| | 40.40–143.44 | 47.40–110.81 | 5.67–17.82 | 21.52–36.13 | 36.67–84.23 | 18.53–176.11 | 6.31–40.78 | 4.69–64.72 | 15.74–40.18 | |
| Mn | 2.96 ± 2.13 | 8.51 ± 3.84 | 1.23 ± 0.36 | 3.62 ± 1.48 | 6.08 ± 4.66 | 8.76 ± 4.84 | 1.57 ± 0.54 | 1.46 ± 1.07 | 1.50 ± 0.57 | 3.97 |
| | 1.45–4.47 | 2.89–11.28 | 0.63–1.92 | 1.53–4.92 | 1.91–13.81 | 1.60–26.96 | 1.18–2.36 | 0.83–4.41 | 0.85–2.05 | |
| Na | 128.27 ± 37.57 | 93.90 ± 16.51 | 83.94 ± 23.65 | 144.42 ± 117.05 | 77.18 ± 9.11 | 87.91 ± 12.94 | 81.20 ± 7.35 | 80.81 ± 17.33 | 175.93 ± 85.93 | 105 |
| | 101.70–154.84 | 77.10–116.65 | 47.49–134.27 | 79.40–319.86 | 68.05–91.41 | 65.65–18.39 | 70.72–87.19 | 63.28–119.09 | 83.15–277.22 | |
| P | 258.53 ± 131.64 | 104.66 ± 36.21 | 49.21 ± 11.74 | 74.05 ± 34.68 | 154.36 ± 107.20 | 156.21 ± 64.60 | 75.80 ± 22.08 | 61.12 ± 24.20 | 107.79 ± 44.21 | 115 |
| | 165.44–351.62 | 65.65–142.76 | 31.61–65.42 | 53.52–125.84 | 77.06–335.74 | 41.46–296.02 | 43.10–91.68 | 40.77–124.07 | 70.15–170.63 | |
| Pb | 0.22 ± 0.05 | 0.23 ± 0.09 | 0.31 ± 0.21 | 0.14 ± 0.10 | 0.14 ± 0.08 | 0.28 ± 0.17 | 0.21 ± 0.08 | 0.28 ± 0.26 | 0.14 ± 0.17 | 0.22 |
| | 0.19–0.26 | 0.11–0.33 | 0.04–0.77 | 0.00–0.24 | 0.05–0.21 | 0.05–0.66 | 0.15–0.31 | 0.10–0.79 | 0.00–0.33 | |
| S | 28.54 ± 22.46 | 28.03 ± 12.24 | 10.89 ± 2.23 | 14.87 ± 10.76 | 35.10 ± 8.55 | 29.72 ± 11.08 | 22.21 ± 14.79 | 14.89 ± 7.83 | 12.82 ± 10.65 | 21.9 |
| | 12.66–44.42 | 10.50–38.46 | 6.98–13.61 | 0.00–25.53 | 25.01–42.71 | 13.84–55.40 | 7.75–42.70 | 7.91–34.73 | 0.00–25.90 | |
| Si | 140.46 ± 0.60 | 90.75 ± 11.20 | 86.81 ± 9.41 | 77.38 ± 16.47 | 74.87 ± 9.77 | 83.43 ± 21.21 | 80.27 ± 7.88 | 81.05 ± 19.05 | 91.93 ± 34.04 | 89.6 |
| | 104.68–176.24 | 77.43–104.54 | 44.97–157.87 | 65.13–101.45 | 63.65–89.15 | 0.00–128.12 | 70.82–88.63 | 58.64–115.96 | 60.51–140.34 | |
| Zn | 8.82 ± 3.81 | 6.10 ± 3.54 | 4.92 ± 2.35 | 3.86 ± 2.48 | 4.80 ± 2.23 | 4.73 ± 2.14 | 4.34 ± 1.86 | 4.92 ± 2.23 | 4.20 ± 0.71 | 5.19 |
| | 6.13–11.51 | 2.27–9.90 | 2.24–9.94 | 2.07–7.53 | 2.98–8.53 | 2.09–11.05 | 2.52–6.19 | 2.40–10.42 | 3.23–4.95 | |
| total | 1879 | 1612 | 542 | 921 | 1340 | 1368 | 672 | 588.59 | 983.08 | 1101 |

**Table 2.** Mean, Range, and Standard Deviation for CIELAB Parameters by Their Botanical Origin

| variable | avocado ($n = 2$) | chestnut ($n = 4$) | citrus ($n = 10$) | eucalyptus ($n = 4$) | heather ($n = 5$) | honeydew ($n = 33$) | lavender ($n = 4$) | rosemary ($n = 11$) | thymus ($n = 4$) |
|---|---|---|---|---|---|---|---|---|---|
| $L^*$ | 41.88 ± 7.01 | 39.40 ± 3.10 | 78.65 ± 6.43 | 60.75 ± 4.68 | 38.55 ± 7.68 | 41.52 ± 6.14 | 67.20 ± 5.47 | 72.86 ± 12.29 | 54.73 ± 4.08 |
| | 36.92–46.83 | 37.59–44.02 | 65.57–87.42 | 55.97–66.89 | 28.68–46.37 | 31.01–62.39 | 59.79–72.98 | 44.28–86.47 | 50.96–59.46 |
| $a^*$ | 27.23 ± 3.59 | 23.76 ± 1.63 | 7.29 ± 3.54 | 25.09 ± 3.69 | 17.60 ± 11.75 | 21.15 ± 5.96 | 20.97 ± 4.40 | 11.73 ± 7.89 | 27.63 ± 1.74 |
| | 24.69–29.77 | 21.89–25.60 | 0.81–12.86 | 20.29–29.19 | 1.77–31.14 | 5.61–29.52 | 16.60–26.07 | 0.66–27.77 | 25.65–29.67 |
| $b^*$ | 23.94 ± 9.68 | 20.38 ± 3.64 | 52.21 ± 7.44 | 51.92 ± 5.56 | 16.22 ± 12.11 | 22.12 ± 10.18 | 59.48 ± 5.79 | 49.58 ± 10.27 | 43.29 ± 7.06 |
| | 17.09–30.78 | 17.28–25.65 | 38.09–61.42 | 45.89–57.82 | 1.59–31.37 | 5.11–52.99 | 53.17–66.12 | 24.90–59.81 | 36.46–50.30 |
| $C_{ab}^*$ | 36.42 ± 9.04 | 31.37 ± 3.26 | 52.81 ± 7.57 | 57.87 ± 3.57 | 23.98 ± 16.79 | 30.87 ± 11.07 | 63.25 ± 4.69 | 51.82 ± 8.32 | 51.54 ± 5.18 |
| | 30.03–42.82 | 29.39–36.24 | 38.49–62.75 | 54.39–61.28 | 2.38–44.20 | 7.59–58.67 | 59.21–68.17 | 37.30–61.33 | 46.16–57.03 |
| $h_{ab}$ | 40.33 ± 7.96 | 40.40 ± 4.16 | 82.13 ± 3.66 | 63.97 ± 5.59 | 41.20 ± 4.57 | 44.33 ± 7.39 | 70.41 ± 5.06 | 76.11 ± 12.74 | 57.04 ± 5.70 |
| | 34.70–45.95 | 35.18–45.05 | 76.39–89.05 | 57.54–70.66 | 33.79–45.21 | 29.39–64.58 | 63.88–75.91 | 41.88–89.11 | 52.02–62.07 |

total minerals). This is according with the results obtained in other studies carried out with Spanish, Italian, and Moroccan honeys (*17*, *19*−*21*).

On the other hand, the lighter honeys (citrus, rosemary, and lavender) have the lower total quantities of minerals (542 and 672 mg/kg). Potassium, silicon, sodium, calcium, and phosphorus are the majority in these types of honeys. Low levels of iron (<2.79 mg/kg) are remarkable in relation with the levels of this metal in the dark honeys (>4.26 mg/kg), and this is in agreement with the results obtained by Lynn et al. (*22*) that confirm that the ferrum salts are the principal compounds responsible for the darkening of honeys. Finally, the amber light honeys (eucalyptus and thyme) show middle quantities of total minerals (921 and 983 mg/kg, respectively) and especially iron (2.84 and 2.95 mg/kg).

**Color Characteristics. Table 2** shows the mean, range, and standard deviation for the different color parameters in the color space CIE 1976 ($L^*$, $a^*$, and $b^*$) for the different types of honeys included in the study. As it can be observed, the honeys with the highest levels of total minerals (avocado, heather, chestnut, and honeydew) show quite low mean values of lightness ($L^*$), between 38 and 41 CIELAB units, coinciding with dark color. This is in agreement with results obtained by Terrab et al. (*23*) and Mateo and Bosch (*24*) from Moroccan and Spanish honeys. The honeys that show the lower quantities of total minerals (citrus, lavender, and rosemary) have the highest values of this colorimetric parameter, with means higher than 67 CIELAB units.

The chroma ($C_{ab}^*$) represents the amount of color, and it is measured according to the distance to the coordinates origin. The lower values are shown for the dark honeys (means lower than 36 units), and the highest values are shown for the light honeys (means upper than 52 units), corresponding with vivid colors.

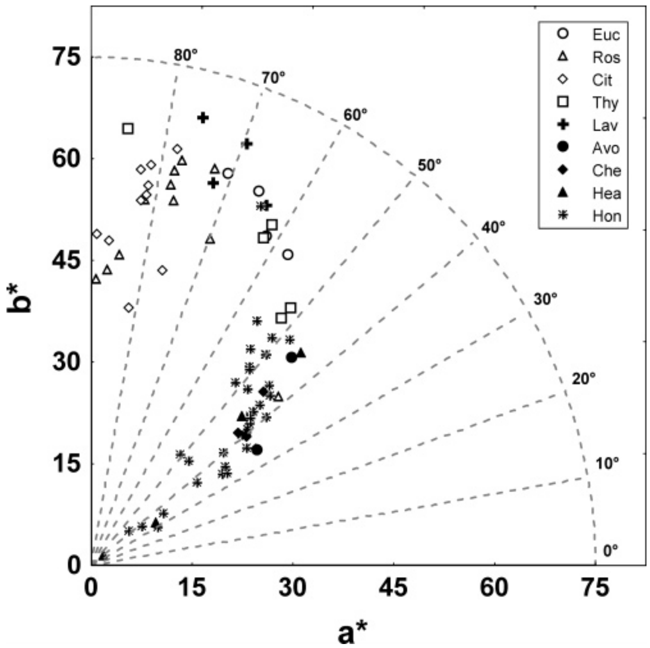


**Figure 1.** CIELAB color space. Location of the honey samples in the ($a^*b^*$) plane, grouping by their botanical origin.

**Table 3.** Regression Summary for Dependent Variables Defined by CIELAB Color Space[a]

| CIELAB parameter | | standardized coefficient ($\beta$) | *p* level |
|---|---|---|---|
| $L^*$ | K | −0.30 | 0.01 |
| $R = 0.875663$ | S | −0.32 | 0.00 |
| $p < 0.00000$ | As | 0.19 | 0.00 |
| | Cu | −0.21 | 0.06 |
| | Al | 0.10 | 0.13 |
| | Si | 0.14 | 0.11 |
| | Na | −0.14 | 0.06 |
| | Fe | −0.14 | 0.07 |
| | Mn | −0.13 | 0.20 |
| $a^*$ | Ca | 0.34 | 0.01 |
| $R = 0.574049$ | Pb | −0.31 | 0.01 |
| $p < 0.00006$ | Cu | 0.40 | 0.00 |
| | Fe | −0.26 | 0.03 |
| | Zn | −0.18 | 0.16 |
| | As | −0.12 | 0.27 |
| $b^*$ | Mg | −0.33 | 0.02 |
| $R = 0.852020$ | Fe | −0.20 | 0.01 |
| $p < 0.00000$ | K | −0.29 | 0.03 |
| | As | 0.14 | 0.03 |
| | S | −0.19 | 0.05 |
| | Al | 0.13 | 0.06 |
| $C_{ab}^*$ | Mg | −0.38 | 0.01 |
| $R = 0.823509$ | Fe | −0.30 | 0.00 |
| $p < 0.00000$ | Al | 0.14 | 0.05 |
| | S | −0.18 | 0.09 |
| | As | 0.10 | 0.13 |
| | K | −0.17 | 0.25 |
| $h_{ab}$ | K | −0.46 | 0.00 |
| $R = 0.873078$ | As | 0.19 | 0.00 |
| $p < 0.00000$ | S | −0.25 | 0.00 |
| | Mn | −0.12 | 0.19 |
| | Cd | 0.09 | 0.14 |
| | Cu | −0.14 | 0.10 |
| | Al | 0.10 | 0.13 |

[a] All samples included.

**Figure 1** shows the projection of the points given by the honey samples on the ($a^*$,$b^*$) plane, grouped by their botanical origin. The samples are located in a wide range of hue angles ($h_{ab}$ from 30 to 90°) and chroma. The lower hue values corresponded with the lower chroma values. Furthermore, it can be observed that the darker honeys (honeydew, heather, chestnut, and avocado) are perfectly separated from those light and brown (rosemary, citrus, lavender, thyme, and eucalyptus), except for two samples of thyme that are located on the dark honeys zone.

**Table 4.** Regression Summary for CIELAB-Dependent Variables[a]

| CIELAB parameters | | standardized coefficient ($\beta$) | *p* level |
|---|---|---|---|
| $L^*$ | Mg | −0.51 | 0.01 |
| $R = 0.766374$ $p < 0.00000$ | Fe | −0.34 | 0.01 |
| | S | −0.25 | 0.05 |
| | P | 0.29 | 0.12 |
| | K | −0.17 | 0.28 |
| $a^*$ | Fe | −0.67 | 0.00 |
| $R = 0.787639$ $p < 0.00002$ | Mg | −0.57 | 0.03 |
| | Li | 0.40 | 0.05 |
| | Be | 0.07 | 0.56 |
| | S | −0.39 | 0.03 |
| | Na | −0.54 | 0.00 |
| | Al | 0.08 | 0.60 |
| | As | 0.18 | 0.11 |
| | Ca | 0.47 | 0.02 |
| | Pb | −0.26 | 0.07 |
| | Zn | −0.21 | 0.16 |
| | P | 0.28 | 0.24 |
| $b^*$ | Mg | −0.45 | 0.02 |
| $R = 0.784074$ $p < 0.00000$ | Fe | −0.37 | 0.00 |
| | S | −0.29 | 0.03 |
| | P | 0.26 | 0.15 |
| | Al | 0.15 | 0.16 |
| | K | −0.21 | 0.18 |
| $C_{ab}^*$ | Mg | −0.38 | 0.00 |
| $R = 0.790482$ $p < 0.00000$ | Fe | −0.43 | 0.00 |
| | S | −0.32 | 0.01 |
| | Al | 0.19 | 0.05 |
| | As | 0.11 | 0.27 |
| $h_{ab}$ | K | −0.39 | 0.01 |
| $R = 0.748992$ $p < 0.00000$ | S | −0.19 | 0.16 |
| | Mn | −0.22 | 0.10 |
| | Na | 0.13 | 0.24 |
| | Be | −0.11 | 0.30 |

[a] Dark honeys.

**Statistical Approach.** A forward stepwise multiple regression technique has been applied in order to determine the correlations between color of honeys and their mineral content. CIELAB color parameters have been considered ($L^*$, $C_{ab}^*$, $h_{ab}$, $a^*$, and $b^*$) as dependent variables, and mineral concentrations have been considered as independent variables.

*All Samples Included (n = 78).* When multiple regression is applied to all samples, it can be observed (**Table 3**) that As, Fe, K, S, and Al are present in most of the regression equations with significant coefficients ($p < 0.05$). Although the regression functions for variables $a^*$ and $b^*$ present the higher number of significant coefficients, the equation for lightness ($L^*$) involves the higher number of independent variables. Except for $a^*$, all of the regression coefficients ($R$) are higher than 0.85.

According to the classification (dark and light honeys) made by the panelists, multiple regression techniques were applied to both sets of samples and regression equations with regression coefficients between 0.5 and 0.8 were obtained (**Tables 4** and **5**).

**Table 5.** Regression Summary for CIELAB-Dependent Variables[a]

| CIELAB parameter | | standardized coefficient ($\beta$) | $p$ level |
|---|---|---|---|
| $L^*$ | K | −0.68 | 0.00 |
| $R = 0.768017$ $p < 0.00026$ | As | 0.35 | 0.02 |
| | Zn | −0.21 | 0.17 |
| $a^*$ | Mg | 0.57 | 0.00 |
| $R = 0.745923$ $p < 0.00057$ | Mn | 0.25 | 0.16 |
| | As | −0.17 | 0.27 |
| $b^*$ | Pb | −0.24 | 0.34 |
| $R = 0.602894$ $p < 0.17523$ | Cu | 0.71 | 0.02 |
| | Zn | −0.35 | 0.16 |
| | Al | 0.19 | 0.33 |
| | Mn | 0.43 | 0.09 |
| | Na | −0.48 | 0.15 |
| $C_{ab}^*$ | Pb | −0.21 | 0.40 |
| $R = 0.594509$ $p < 0.11322$ | Cu | 0.55 | 0.04 |
| | Mn | 0.86 | 0.02 |
| | Na | −0.60 | 0.08 |
| | Ca | −0.45 | 0.14 |
| $h_{ab}$ | Mg | −0.61 | 0.00 |
| $R = 0.766698$ $p < 0.00027$ | Mn | −0.23 | 0.17 |
| | As | 0.17 | 0.25 |

[a] Light and brown honeys.

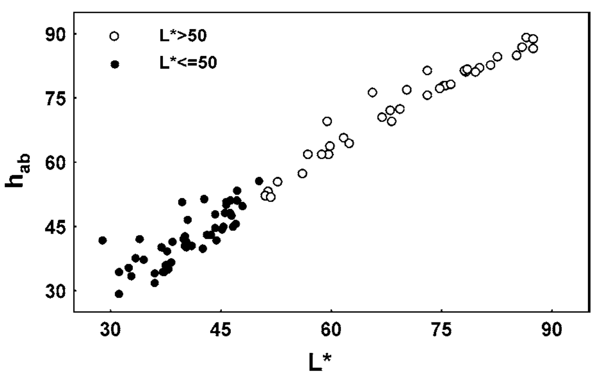


**Figure 2.** CIELAB color space. $L^*$ vs $h_{ab}$ representation of the honey samples, grouping by their lightness values.

To evaluate the accuracy of the grouping made by the experts, a new classification was undergone by means of instrumental analysis. Thus, two groups of samples, with regard to their lightness value, were established as follows: $L^* \leq 50$ CIELAB units (corresponding to dark honeys) and $L^* > 50$ CIELAB units (corresponding to light honeys). Nine honeys with $L^*$ values near 50 units were misclassified using visual analysis; all of them were light samples ($L^* > 50$) classified as dark honeys.

With regard to instrumental analysis, in **Figure 2**, it can be seen that most of the light honeys shown have hue angles ($h_{ab}$) above 55°, in the yellowish-orange zone, and the dark honeys had hue angles ($h_{ab}$) from 25 to 55°, in the orange-red zone. As it can be seen, a clear distribution toward low values of hue angles in dark honeys ($L^* < 50$) exists. In light honeys ($L^* > 50$), this distribution shows a direction toward higher hue angles and chroma values.

*Samples with $L^* \leq 50$ ($n = 44$).* This group of samples includes the darker honeys. As it is shown in **Table 6**, the regression equations obtained generally include a higher number of independent variables (minerals) than in the group of samples that show $L^*$ values higher than 50 CIELAB units. Furthermore, most of the cases have significant coefficients ($p < 0.05$). S, Ca, and Fe, followed by As, Pb, and Cd, are the minerals that show higher correlations with the CIELAB color parameters.

**Table 6.** Regression Summary for CIELAB-Dependent Variables[a]

| CIELAB parameters | | standardized coefficient ($\beta$) | $p$ level |
|---|---|---|---|
| $L^*$ | K | −0.35 | 0.05 |
| $R = 0.788448$ $p < 0.0001$ | Fe | −0.36 | 0.01 |
| | S | −0.36 | 0.02 |
| | As | 0.31 | 0.01 |
| | Be | 0.04 | 0.73 |
| | Ca | 0.34 | 0.04 |
| | Pb | −0.32 | 0.05 |
| | Cd | 0.25 | 0.07 |
| | Mg | −0.33 | 0.14 |
| | P | 0.24 | 0.30 |
| $a^*$ | Fe | −0.69 | 0.00 |
| $R = 0.818563$ $p < 0.00001$ | S | −0.43 | 0.00 |
| | Be | 0.12 | 0.32 |
| | Al | 0.18 | 0.18 |
| | Pb | −0.35 | 0.03 |
| | Ca | 0.34 | 0.03 |
| | As | 0.17 | 0.13 |
| | Cd | 0.17 | 0.19 |
| | Mg | −0.34 | 0.10 |
| | P | 0.28 | 0.23 |
| $b^*$ | K | −0.28 | 0.08 |
| $R = 0.772525$ $p < 0.00004$ | Fe | −0.36 | 0.01 |
| | S | −0.34 | 0.01 |
| | Mn | −0.20 | 0.15 |
| | Ca | 0.32 | 0.03 |
| | As | 0.26 | 0.04 |
| | Pb | −0.30 | 0.06 |
| | Cd | 0.25 | 0.07 |
| $C_{ab}^*$ | Fe | −0.56 | 0.00 |
| $R = 0.803332$ $p < 0.00004$ | Mg | −0.38 | 0.09 |
| | S | −0.40 | 0.01 |
| | Al | 0.15 | 0.29 |
| | As | 0.23 | 0.05 |
| | Ca | 0.38 | 0.02 |
| | Pb | −0.37 | 0.02 |
| | Cd | 0.24 | 0.08 |
| | P | 0.30 | 0.22 |
| | K | −0.20 | 0.25 |
| $h_{ab}$ | K | −0.67 | 0.00 |
| $R = 0.802985$ $p < 0.00004$ | Cd | 0.34 | 0.01 |
| | As | 0.27 | 0.02 |
| | S | −0.26 | 0.05 |
| | Ba | −0.17 | 0.13 |
| | Fe | 0.13 | 0.25 |
| | Pb | −0.30 | 0.06 |
| | Cu | 0.32 | 0.07 |
| | Si | −0.25 | 0.09 |
| | Ca | 0.17 | 0.22 |

[a] Samples with $L^* \leq 50$ CIELAB units.

*Samples with $L^* > 50$ ($n = 33$).* **Table 7** shows that the regression equation for lightness ($L^*$) involves the higher number of independent variables with significant coefficients ($p < 0.05$). $C_{ab}^*$ and $b^*$ show a lower correlation with the mineral content of honeys, and their regression functions involve a few independent variables (Mg and Al for $b^*$ and only Al for $C_{ab}^*$), and their coefficients are not significant. The iron is not included in these equations for light honeys, so Fe influences only darker honey colors.

**Table 7.** Regression Summary for CIELAB-Dependent Variables[a]

| CIELAB parameters | | standardized coefficient ($\beta$) | *p* level |
|---|---|---|---|
| $L^*$ $R = 0.876267$ $p < 0.00000$ | Mg | –0.44 | 0.00 |
| | As | 0.22 | 0.06 |
| | Na | –0.23 | 0.04 |
| | Zn | –0.32 | 0.02 |
| | Mn | –0.34 | 0.02 |
| | Ba | 0.29 | 0.04 |
| | Cd | 0.19 | 0.20 |
| $a^*$ $R = 0.868345$ $p < 0.00000$ | Mg | 0.35 | 0.03 |
| | As | –0.13 | 0.26 |
| | Mn | 0.24 | 0.05 |
| | Na | 0.37 | 0.01 |
| | S | 0.24 | 0.11 |
| | Cd | –0.15 | 0.23 |
| $b^*$ $R = 0.311553$ $p < 0.00000$ | Mg | –0.24 | 0.17 |
| | Al | 0.21 | 0.23 |
| $C_{ab}^*$ $R = 0.211712$ $p < 0.22937$ | Al | 0.21 | 0.23 |
| $h_{ab}$ $R = 0.877539$ $p < 0.00000$ | Mg | –0.34 | 0.04 |
| | Na | –0.62 | 0.00 |
| | Mn | –0.25 | 0.04 |
| | Si | 0.39 | 0.01 |
| | Cu | –0.29 | 0.06 |
| | S | –0.28 | 0.06 |
| | Ca | 0.19 | 0.15 |

[a] Samples with $L^* > 50$ CIELAB units.

**Table 8.** Classification Matrix for Learning and Test Sets

| | | % correct | honeys dark | honeys light |
|---|---|---|---|---|
| training set | dark honeys | 96.7 | 29 | 1 |
| | light honeys | 95.5 | 1 | 21 |
| | total | 96.2 | 30 | 22 |
| test set | dark honeys | 85.7 | 12 | 2 |
| | light honeys | 83.3 | 2 | 10 |
| | total | 84.6 | 14 | 12 |

**Table 9.** Forward SDA[a]

| | dark honeys | light honeys |
|---|---|---|
| Mg | –0.03 | –0.05 |
| Fe | 0.69 | 0.10 |
| Mn | 1.04 | 0.39 |
| Cu | 8.36 | 3.84 |
| Ca | 0.01 | 0.06 |
| As | 18.73 | 38.55 |
| constant | –10.25 | –4.94 |

[a] Coefficients of the classification functions.

LDA was performed to classify the honeys according to their lightness ($L^*$) value. Samples were randomly split into a training set, formed by 66% of the samples, to develop a discriminant model and a validation set, formed by the remaining 33% of the samples, on which the model was tested. To get a good estimate for the stability of the model (goodness of classification in the training set) and the prediction ability (goodness of the classification in the test set), this validation test was repeated three consecutive times using a random different subset each time. **Table 8** includes the best model set, showing a 96% of classification ability: about 97% for dark honeys ($L^* \leq 50$) and about 95% for light honeys ($L^* > 50$). The test set showed that 85% of the samples were assigned correctly: the percentages of prediction were about 86 and 83% for dark and light honeys, respectively.

In **Table 9** are given the coefficients for the classification functions when a forward SDA was carried out. It can be seen that the model only included Mg, Fe, Mn, Cu, Ca, and As as the variables needed to explain the 95% of the variance between both groups of samples (dark and light honeys).

**Conclusions.** In this study, the correlation between color of honeys and their mineral content has been clearly stated by means of the application of multivariate statistical techniques such as MLR and LDA. Thus, the color of the dark honeys (avocado, chestnut, honeydew, and heather) is greatly correlated with the concentration of the trace elements such as (As, Cd, Fe, S, and Pb) and with the Ca. On the other hand, the light and brown honeys (citrus, rosemary, lavender, thyme, and eucalyptus) are highly correlated with only Al and Mg.

## LITERATURE CITED

(1) Guizelan, G.; Guizelan, R. Données concernat les éléments mineraux dans quelques sortes de miel. *Proceedings of 23rd International Congress on Apiculture*; Moscow, Apimondia Publishing: Bucharest, Romania, 1971; pp 592−593.

(2) Free, J. B.; Williams, I. H.; Pinsent, R. J. F. H.; Townshend, A.; Basi, M. S.; Graham, C. L. Using foraging honey bees to sample an area for trace metals. *Environ. Entomol.* **1983**, *9*, 9−12.

(3) Crane, E. Bees, honey and pollen as indicators of metals in the environment. *Bee World* **1984**, *65*, 47−49.

(4) Féller-Demalsy, M. J.; Vincent, B.; beaulieu, F. Teneur en minéraux et origin géographique des miel du Canada. *Apidologie* **1989**, *20*, 77−91.

(5) Varju, M. Mineral content of Hungarian acacia honeys and its relationship with the plant and soil. *Z. Lebensm. Unters. Forcsh.* **1970**, *144*, 308−312.

(6) Pereyra, A. G.; Burin, L.; Buera, M. P. Color changes during storage of honeys in relation to their composition and initial color. *Food. Res. Int.* **1999**, *32*, 185−191.

(7) Terrab, A.; Escudero, M. L.; Gonzalez-Miret, M. L.; Heredia, F. J. Colour characteristics of honeys as influenced by pollen grain content: A multivariate study. *J. Sci. Food Agric.* **2004**, *84*, 380−386.

(8) Sancho, M. T.; Muniategui, S.; Sánchez, P.; Huidrobo, J. F.; Simal, J. Mieles del País Vasco XI. Evaluación de los distintos tipos de cenizas y su alcalinidad en función de la conductividad eléctrica en mieles. *Anal. Bromatol.* **1991**, *43*, 311−324.

(9) Maurizio, A. *Microscopy of Honey, in Honey. A Comprehensive Survey*; Crane E. Heinemann: London, 1979.

(10) Erdtman, G. The acetolysis method. A revised description. *Svensk Bot Tidskr* **1960**, *54*, 561−564.

(11) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990.

(12) Commission Internationale de l'Éclairage. *Colorimetry*, 2nd ed.; CIE 15.2.: 1986.

(13) Statsoft. *Statistica for Windows [Computer Program Manual]*; Statsoft: Tulsa, OK, 1999.

(14) Johnson, D. E. *Métodos Multivariados Aplicados al Análisis de Datos*; International Thomson Editores, S. A.: Madrid, 2000.

(15) Tabachnick, B. G.; Fidell, L. S. *Using Multivariate Statistics*; Harper & Row Publishers: New York, 1983.

(16) White, J. W. Honey. *Adv. Food Res.* **1978**, *24*, 287−375.

(17) Poiana, A.; Fudo, S.; Manzin, E.; Postarino, S.; Mincione, B. Ricerche sui mieli commercializzati in Italia: la componente minerale. *Ind. Aliment.* **1996**, *35*, 522−530.

(18) LaTorre, M. J.; Peña, R.; García, S.; Herrero, C. Authentication of Galician (N. W. Spain) honeys by multivariate techniques based on metal content data. *Analyst* **2000**, *125*, 307−312.

(19) Salinas, F.; Montero, V.; Osorio, E.; Lozano, M. Determinación de elementos minerales en mieles de diversos orígenes florales por análisis de inyección en flujo acoplada a la espectroscopía atómica. *Rev. Esp. Cienc. Tecn. Alim.* **1994**, *34*, 441−449.
(20) Terrab, A.; Recamales, A. F.; González-Miret, M. L.; Heredia, F. J. Contribution to the study of avocado honeys by their minerals content using the inductively coupled plasma optical emission spectrometry. *Food Chem*. **2005**, in press.
(21) Terrab, A.; González, A. G.; Díez, M. J.; Heredia, F. J. Mineral content and electrical conductivity of the honeys produced in north-west morocco and their contribution to the characterisation of unifloral honeys. *J. Sci. Food Agric.* **2003**, *83*, 637−643.
(22) Lynn, E. G.; Englis, D. T.; Milum, V. G. Effect of processing and storage on composition and color of honey. *Food Res*. **1936**, *1*, 23−27.
(23) Terrab, A.; Díez, M. J.; Heredia, F. J. Chromatic characterisation of Moroccan honeys by diffuse reflectance and tristimulus colorimetry. Nonuniform and uniform colour spaces. *Food Sci. Technol. Int*. **2002**, *8*, 189−195.
(24) Mateo, C.; Bosh-Reig, F. Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars and pH. *J. Agric. Food Chem.* **1998**, *46*, 393−400.

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