Food Chemistry 175 (2015) 128–136

Contents lists available at ScienceDirect

Food Chemistry

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

Neural networks applied to discriminate botanical origin of honeys

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ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 19 November 2014
Accepted 20 November 2014
Available online 27 November 2014

Keywords:
Honey
Physical–chemical parameters
Botanical origin
Neural networks
Overfitting
Classification problem

ABSTRACT

The aim of this work is develop a tool based on neural networks to predict the botanical origin of honeys using physical and chemical parameters.

The managed database consists of 49 honey samples of 2 different classes: monofloral (almond, holm oak, sweet chestnut, eucalyptus, orange, rosemary, lavender, strawberry trees, thyme, heather, sunflower) and multifloral. The moisture content, electrical conductivity, water activity, ashes content, pH, free acidity, colorimetric coordinates in CIELAB space (L^*, a^*, b^*) and total phenols content of the honey samples were evaluated. Those properties were considered as input variables of the predictive model. The neural network is optimised through several tests with different numbers of neurons in the hidden layer and also with different input variables.

The reduced error rates (5%) allow us to conclude that the botanical origin of honey can be reliably and quickly known from the colorimetric information and the electrical conductivity of honey.

1. Introduction

Honey is a natural food product processed by honeybees by blending the sweetened sap collected from flowers with metabolic gastric enzymes. It is a very rich food product due to its composition, which contains water, saccharides (mainly fructose and glucose), minerals, amino acids and proteins (Anjos, Campos, Ruiz, & Antunes, 2014; Doner, 1977; Mesallam & El-Shaarawy, 1987). The differences observed in the honey composition depend on the region, season, nectar plant source, bee's breed (Cavaco, Miguel, Antunes, & Guerra, 2012).

The main quality parameters for honey are given by Codex Alimentarius (2001) as follows: moisture content, acidity, hydroxymethylfurfural content (HMF), diastase activity and sugars content (sum of glucose, fructose and sucrose). The honey composition is related to its characteristics, namely, the profiles of predominant sugars (glucose, fructose, sucrose and maltose) that have been associated with a wide variety of second-order quality properties such as viscosity, hygroscopic, granulation and energy value (Ouchemoukh, Schweitzer, Bachir Bey, Djoudad-Kadji, & Louaileche, 2010). Another important parameter regarding food, especially honey, is color, which is often related to the consumption patterns and consequently used to make judgments on its quality (Caivano & Buera, 2012; Wilczyńska, 2014).

Depending on the botanical origin of honey, two main types can be distinguished: monofloral or unifloral honey when there is a predominant plant species' nectar, and multifloral honey when the nectar is collected from more than one plant species. Unifloral honey types have higher market value due to their limited production and availability, and their geographical origin can also be an important economical factor due to the existence of protected designation of origin (PDO) and protected geographical indications (PGI) (Cajka, Hajslova, Pudil, & Riddellova, 2009). The determination of the geographical and botanical honey origin is regulated by specific legislation (Codex Alimentarius Commission FAO/OMS, 2001; European Union, 2002).

Melissopalynological analysis is the reference method to determine the botanical origin of honey. However, this method requires a specific background and very well trained technicians with a high expertise in the interpretation of results. It is also necessary to identify a large amount of pollen grain, which makes the process of the identification of botanical origin of honey relatively difficult and very time-consuming (Benedetti, Mannino, Sabatini, & Marcazzan, 2004). Due to this fact, there is a need for alternative methods to analyse the botanical origin of honey in a faster, easier

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http://dx.doi.org/10.1016/j.foodchem.2014.11.121
0308-8146/

way for sample screening in a quality control laboratory (Corvucci, Nobili, Melucci, & Grillenzoni, 2014; Escriche, Kadar, Domenech, & Gil-Sánchez, 2012).

Some methods are being developed for this proposes: electronic devices such as the electronic-tongue (e-tongue) or the electronic-nose (e-nose) with chemometric models have been proved to be useful tools for the discrimination of honeys' botanical origin (Benedetti et al., 2004; Cavaco et al., 2012; Dias et al., 2008; Escriche et al., 2012; Ulloa et al., 2013).

In this work, we use neural networks to determine the botanical origin of honey samples. Previous research works have proved they are an effective method for managing natural parameters due to their ability to find the complex relationships between the studied variables (Cilla, Martínez, Peña, & Martínez, 2012; Iglesias et al., 2014). Regarding honey, neural networks have also been applied: Benedetti et al. (2004) and Escriche et al. (2012) used an electronic nose to obtain the volatiles profile and neural networks to discriminate the origin of the samples; Shafiee, Minaei, Moghaddam-Charkari, and Barzegar (2014) characterised honey through ash content, antioxidant activity and total phenolic content, which were estimated using neural networks and color information.

Other research works have focused on the study of color to determine the botanical origin since it is related to the chemical composition of the product (González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005; González-Paramás, García-Villanova, Gómez Bárez, Sánchez Sánchez, & Ardanuy Albajar, 2007; Gámbaro, Ares, Giménez, & Pahor, 2007; Juszczak, Socha, Rożnowski, Fortuna, & Nalepka, 2009; Terrab, Diez, & Heredia, 2002; Tuberoso et al., 2014).

The aim of this work is to evaluate the performance of neural networks for the determination of the botanical origin of honey samples from some physical–chemical properties. For this purpose, the best input parameters are evaluated in order to achieve a successful classification with the lowest number of parameters. The objective is to propose an objective methodology to evaluate the botanical origin of honey based on the results of quality control analyses.

The importance of this study could be the application of this technique to a fast screening in a quality control laboratory in other to identify the botanical origin of honey. Given the specificity of the botanical analysis and their slowness, this technique could be helpful in this determination in the future, with great potential for the melissopalynology classification of honey samples.

2. Materials and methods

2.1. Honey samples

The monofloral honey samples produced in different Portuguese and Spanish regions were obtained from the commercial market. The samples were stored in the dark at a room temperature of 12 °C. A total of 49 samples were taken from 14 different botanical origins.

The preparation of honey samples for identification of botanical origin followed the standardized acetolyzed method of Erdtman (1960).

20 g of honey sample was dissolved in 40 mL of distilled water at 30 °C. After the samples were centrifuged (Biofuge 28 RS, Heraeus, Seportech) for 15 min at 3000 rpm, the supernatant was removed and mixtured with 10 mL of sulphuric acid (Merck, Darmstadt, Germany) and acetic anhydride (Sigma–Aldrich, Missouri, USA) (1:9) and placed in a thermostatic bath at 100 °C for 10 min (acetolysis). Then, the samples were centrifuged at 3000 rpm, the supernatant was removed and liquefied Kaiseŕs glycerol gelatine (Merck, Darmstadt, Germany) was added to the pollen grains sediment. Finally, the entire mixture was spread on a glass slide. Each analysis was carried out in triplicate.

At least, 500 pollen grains were counted per sample in order to confirm the botanical origin indicated on the label of the packaging. Pollen identification was based on the reference collection from the Laboratory of the School of Agriculture in Castelo Branco and some pollen atlases.

2.2. Physical–chemical analysis

A total of 10 parameters will be described: free acidity and pH, electrical conductivity, moisture content, water activity, ash content, total phenol content and colorimetric coordinates in CIELab space. Three measurements were done for each honey sample and parameter.

2.2.1. Free acidity and pH

Free acidity was determined by potentiometric titration and pH was estimated using a potentiometer (Radiometer PHM61, Hach Lange, USA), according to the harmonized methods of the European Honey Commission (Bogdanov, Martin, & Lullmann, 1997). Honey samples were homogenized in a water bath before analysis. 10 g of honey were dissolved in 75 mL of distilled water. The solution was titrated with 0.1 M NaOH (Sigma–Aldrich, Missouri, USA). Free acidity was expressed as milliequivalent of acid per kg of honey.

2.2.2. Electrical conductivity

The electrical conductivity was measured at 20 °C using a portable Conductivity meter Model WTW 315i (Weilheim, Germany). The instrument was calibrated using 0.01 M KCl (Merck, Darmstadt, Germany).

2.2.3. Moisture and water activity

The moisture content of the honey samples was determined in refractometer ATAGO-1T (Tokyo, Japan).

Water activity (aw) was evaluated in Rotronic Hygroscop DT equipment with a thermostatic WA-14TH probe (Zurich, Switzerland), where the sample is sealed in the cell apparatus, allowing the relative moisture in the honey sample and within the cell to remain in equilibrium. The sensor located at the top of the cell is constantly reading relative moisture, stabilizing when the variation is less than 0.02% HR/min to 0.02 °C/min.

2.2.4. Ash content

Total ash content was done according to the standard NP-1308 (1976), by incinerating honey samples in a muffle furnace (Nabertherm, Germany) at a temperature of 550 °C.

2.2.5. Phenol content

Total phenols were determined according to a modified Folin–Ciocalteu method (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005; Waterhouse, 2003). Each honey sample (5 g) was diluted to 50 ml with distilled water and filtered through Whatman No. 1 paper. This solution (0.1 mL) was then mixed with 1.5 mL of water, 0.1 mL of Folin–Ciocalteu reagent (Panreac Quimica, SA, Barcelona, Spain) for 5 min and 0.3 mL of 20% (w/v) sodium carbonate (Na_2CO_3) (Panreac Quimica, SA, Barcelona, Spain) was then added to a total volume of 2 mL. After incubation at room temperature for 1 h, the absorbance of the reaction mixture was measured at 765 nm (JASCO 7800 UV/VIS spectrophotometer, Elnor, Germany). Gallic acid (Panreac Quimica, SA, Barcelona, Spain) (0–25 mg/L) was used as standard to produce the calibration curve.

The mean of three readings was used and the total phenol content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey.