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A novel approach for honey pollen profile assessment using an electronic tongue and chemometric tools

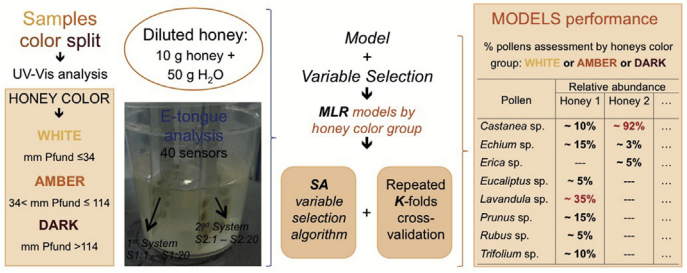
Luís G. Dias [a, b, **], Ana C.A. Veloso [c, d], Mara E.B.C. Sousa [e], Letícia Estevinho [e], Adélio A.S.C. Machado [f], António M. Peres [g, *]

[a] Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal
[b] CQ-VR, Centro de Química – Vila Real, University of Trás-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal
[c] Instituto Politécnico de Coimbra, ISEC, DEQB, Rua Pedro Nunes, Quinta da Nora, 3030-199 Coimbra, Portugal
[d] CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[e] CIMO-Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal
[f] LAQUIPAI – Laboratório de Química Inorgânica Pura e de Aplicação Interdisciplinar, Departamento de Química, Faculdade de Ciências da, Universidade do Porto, Rua Campo Alegre nº. 687, 4169-007 Porto, Portugal
[g] LSRE-Laboratory of Separation and Reaction Engineering-Associate Laboratory LSRE/LCM, Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal

HIGHLIGHTS

- Honey's floral origin labeling is a legal requirement.
- Melissopalynology analysis usually used to evaluate pollens profile is laborious.
- A novel E-tongue based approach is applied to assess pollens relative abundance.
- MLR models using SA variable selection and repeated K-fold cross-validation.
- The approach may be used as a preliminary tool for pollinic evaluation.

GRAPHICAL ABSTRACT



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ABSTRACT

Nowadays the main honey producing countries require accurate labeling of honey before commercialization, including floral classification. Traditionally, this classification is made by melissopalynology analysis, an accurate but time-consuming task requiring laborious sample pre-treatment and high-skilled technicians. In this work the potential use of a potentiometric electronic tongue for pollinic assessment is evaluated, using monofloral and polyfloral honeys. The results showed that after splitting honeys according to color (white, amber and dark), the novel methodology enabled quantifying the relative percentage of the main pollens (*Castanea* sp., *Echium* sp., *Erica* sp., *Eucaliptus* sp., *Lavandula* sp., *Prunus* sp., *Rubus* sp. and *Trifolium* sp.). Multiple linear regression models were established for each type of pollen, based on the best sensors' sub-sets selected using the simulated annealing algorithm. To minimize the overfitting risk, a repeated K-fold cross-validation procedure was implemented, ensuring that at least 10–20% of the honeys were used for internal validation. With this approach, a minimum average determination coefficient of 0.91 ± 0.15 was obtained. Also, the proposed technique enabled the correct classification of 92% and 100% of monofloral and polyfloral honeys, respectively. The quite satisfactory performance of the novel procedure for quantifying the relative pollen frequency may

* Corresponding author. Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal.
** Corresponding author. Escola Superior Agrária, Instituto Politécnico de Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal.
E-mail addresses: ldias@ipb.pt (L.G. Dias), peres@ipb.pt (A.M. Peres).

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envisage its applicability for honey labeling and geographical origin identification. Nevertheless, this approach is not a full alternative to the traditional melissopalynologic analysis; it may be seen as a practical complementary tool for preliminary honey floral classification, leaving only problematic cases for pollinic evaluation.

1. Introduction

Honey is a very complex natural product that contains sugars, organic acids, amino acids, proteins, minerals, vitamins, lipids, aroma compounds, flavonoids, vitamins, pigments, waxes, pollen grains, enzymes and other phytochemicals [1–3]. Honey distinct and unique flavor, aroma, color and composition depend on a lot of variables: nectar composition of the flora source, bees' species, climate, environmental and seasonal conditions, agricultural practices, geographical origin as well as of the techniques used during honey extraction and storage [3–9]. The occurrence of pollen grains in honey can be explained either by their presence in the floral nectar or due to exogenous sources [10,11]. Honey pollen profile reflects forest vegetation, floral diversity and species composition of the plants foraged by honey bees. The relative pollen frequency is used for label purposes and to guarantee the geographical origin, factors that greatly influence honey's commercial value, being also used as a traceability tool by food control institutions [10,12]. Honeys may be classified as monofloral or polyfloral depending on whether a dominating pollen grain, originated from only one particular plant (monofloral honey) or no dominant pollen type in the sample (polyfloral honey) is found [8]. Monofloral honeys are usually more expensive and appreciated than polyfloral honeys [13–16]. Nevertheless, polyfloral honeys may contain higher levels of flavonoid and phenolic compounds than monofloral honey [17], therefore providing higher antioxidant activity. In fact, honey consumption may lead to potential health benefits due to the antioxidant and antimicrobial properties of honey [17,18]. Some studies [17,19] report a positive correlation between honey color intensity and phenolic or flavonoid contents, and consequently antimicrobial activity. Also, it has been described that dark colored honeys show, in general, higher phenolic levels and antioxidant activity than light colored honeys [17,20–22]. However, consumers still prefer lighter honeys mainly due to the smooth sensory attributes [13,23,24]. In summary, honey color classification is also of major concern, not only since physico-chemical, sensory and healthy attributes may differ from light to dark honeys, but also due to consumer's preference. Usually, honey color is evaluated using a qualitative level scale [25], ranging from extra-white to dark amber, which is established by applying a millimeter Pfund scale calculated from the absorbance values recorded, at a specific wavelength (625 nm), from an aqueous diluted solution of honey. For honey floral classification, traditionally a melissopalynology analysis is used. This method consists of counting down the number of pollens grains of a honey sample and calculating the respective percentages of nectariferous pollens. These are then used to identify the botanical origin and the overall pollen spectrum, which may allow determining the geographical origin of honeys [12]. This technique is quite laborious, time-consuming and requires a high-skilled and trained technician. Nevertheless, so far no alternative or complementary analytical methodology has been reported for honey's pollinic analysis.

In the last years several studies reported the application of potentiometric electronic tongues for the classification of honeys according to botanical or geographical origins [13,14,26–30]. The successful results achieved may be explained by the variations found in honeys with different pollinic profiles (including monofloral to polyfloral honeys) and colors (white, amber and dark), which usually exhibit different sensory attributes, leading to overall different taste perception.

In this work, a novel quantitative application of a potentiometric E-tongue is evaluated, namely its potential use for assessing the relative abundance of the main pollens identified in Portuguese monofloral and polyfloral honeys: e.g., *Echium* sp., *Erica* sp., *Eucaliptus* sp., *Lavandula* sp., *Prunus* sp., *Rubus* sp. and *Trifolium* sp. The procedure includes a preliminary step where honey samples are split according to three main color groups (white, amber and dark), as suggested in a previous work [13]. Then, multiple linear regression models, based on the best sub-sets of electrochemical sensors (E-tongue), selected using a simulated annealing (SA) algorithm, are established, for the first time, to estimate pollen relative frequencies, using a repeated K-fold cross-validation procedure to reduce the possible risk of overoptimistic fitting. Finally, based on the predicted pollens relative percentage abundance, the capability of correctly classify each honey according to its floral origin as monofloral or polyfloral honey was also evaluated. The work carried out aimed to verify the potential of merging electronic tongue data and chemometric tools, as a novel approach for the quantification of pollens relative abundances in honeys, reducing the use of the traditional time-consuming melissopalynology analysis.

2. Materials and methods

2.1. Reagents

All the reagents used were of analytical grade and used as purchased. For pollinic analysis the following reagents were used: anhydride acetic (Panreac), sulfuric acid (M&B), acetic acid (Merck), KOH solution (Merck), fuchsin solution (Merck) and glycerine (Absolve). For construction of the electronic tongue the following reagents from Fluka were used as purchased: octadecylamine, oleyl alcohol, methyltrioctylammonium chloride and oleic acid as additives; bis(1-butylpentyl)adipate, dibutylsebacate, 2-nitrophenyl-octyl-ether, tris(2-ethylhexyl)phosphate, dioctyl phenyl-phosphonate as plasticizers; and, poly(vinyl chloride) polymer (PVC).

2.2. Honey samples: color and pollinic analysis

Portuguese honeys, collected during 2010 and 2011 throughout the main honey production regions, kindly supplied by Federação Nacional de Apicultores de Portugal (FNAP), were studied. All 89 honey samples were analyzed and classified according to color and pollinic profile. The color of each sample was determined according to the quantitative millimeter Pfund (mmPfund) scale, calculated from absorbance data of aqueous diluted honey samples (635 nm, UV/vis spectrophotometer – Jenway, Genova model) according to [31]: