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# BOOK OF ABSTRACTS

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## Comparison of DNA extraction methods for the determination of botanical origin of Portuguese honey

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Honey can be classified based on the type of flowers used by the bees to collect pollen as unifloral honey, if arising predominantly from a single botanical origin, which generally represents more than 45% of the total pollen content, or as multifloral honey, if it is produced from various plant species. A single plant origin should assure a product of better quality because it guarantees a specific and well-defined flavor and aroma. For this reason, monofloral honeys generally attain higher commercial value, especially those labeled as PDO (Protected Designation of Origin). Thus, the botanical authentication of honey is of high importance. To date, microscopic analysis of pollen (melissopalynology) is the technique used to assess the origin of honey. However, this type of analysis is time consuming and dependent on the experience and skill of trained analysts. In contrast, DNA-based methods are less dependent on the analyst, highly specific and easily applied to laboratories with the suitable equipment [1].

The aim of this work is to extract pollen DNA from honey samples for further analysis to exploit molecular markers for honey authentication. Considering the complexity of honey matrix, different extraction methods were tested and optimized, namely the commercial kit NucleoSpin Plant (Macherey-Nagel), the classical CTAB-based and the Wizard methods, as described by Mafra *et al.* [2] with modifications. Prior to DNA extraction, three different pretreatments were applied to three unifloral honey samples (*Ericaceae*, *Rosmarinus officinalis* and *Eucalyptus* spp.) and one multifloral honey. The protocols were evaluated by UV spectrophotometry to determine of yield and purity of DNA extracts. The amplifiability was tested by polymerase chain reaction (PCR) targeting *matK* and *rbcl* genes, as candidate loci for barcoding. The obtained honey extracts revealed low DNA yields with all the extraction protocols, but adequate purity for PCR was achieved using the Wizard method. The amplification of both *loci* was successfully attained with the Wizard method when applied to all honey samples, while the other two protocols exhibited lower reproducibility of results. The proposed method proved to be faster, simpler to implement and more robust than classical protocols, and therefore suitable to develop new methodologies based on the use of DNA markers for analyzing the botanical origin of honey.

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