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DNA barcoding for the botanical species differentiation in honey

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Honey is a natural product widely consumed for its well-known medicinal properties. In general, monofloral honeys have higher economic value since they are perceived as better quality products owing to their specific and well-defined flavor and aroma and, consequently, most appreciated by consumers. Considering that these products are very prone to fraudulent practices and misleading labeling, the development of methodologies for the assessment of their botanical origin and authentication is of utmost importance. For this purpose, traditional methods based on pollen identification by microscopic analysis are presently used. However, this approach is time consuming and greatly depends on the experience/skill of trained analysts (Laube *et al.*, 2010). Recently, due to its high specificity and sensitivity, DNA-based methods are emerging as alternative tools for food authentication since they allow the unequivocal identification of different species. Currently, DNA barcoding has been regarded with increasing interest for the taxonomic identification of species, being recently suggested for the identification of plant species in honey (Bruni *et al.*, 2015). Among several barcode regions, two plastidial genes (*matK* and *rbcL*) have been proposed for the differentiation of plants.

The aim of this work was the botanical species differentiation in honey using mini-barcode regions coupled to high resolution melting (HRM) analysis. For this purpose different plant species, three different monofloral honeys, namely heather (Ericaceae spp.), rosemary (*Lavandula* spp.) and eucalyptus (*Eucalyptus* spp.), and one multifloral honey were used. Three DNA barcoding loci, namely the plastidial coding genes *rbcL* and *matK* and the noncoding intergenic *trnH-psbA* region, were used to design primers targeting Ericaceae spp. (GenBank accession no.: HM849840.1, HE966889.1 and HE966527.1) and *Lavandula* spp. (GenBank accession no.: Z37408.1, KJ196360.1 and HQ902822.1). DNA from plants and honeys was extracted with NucleoSpin Plant (method A) kit, according to Soares *et al.* (2015). The specificity and sensitivity of the designed primers targeting Ericaceae spp. and *Lavandula* spp. were assayed by qualitative polymerase chain reaction (PCR) and real-time PCR. Prior to the specific amplifications, DNA extracts were positively tested targeting an universal eukaryotic sequence (18S rRNA gene). The application of the specific PCR assays was successful in the detection of the target species in all samples. The results were further confirmed by real-time PCR amplification using EvaGreen fluorescence dye. The application of HRM analysis allowed the discrimination of the plant species at the genus level, enabling their discrimination into distinct clusters with high level of confidence (>98.7%). To our knowledge, this is the first study using HRM analysis for the rapid discrimination of plant species in honey.

Keywords: Honey, DNA barcoding, HRM analysis, authenticity, botanical identification.

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References:

Bruni, I., De Mattia, F., Martellos, S., Galimberti, A., Savadori, P., Casiraghi, M., Nimis, P. L., & Labra, M. (2012). DNA Barcoding as an Effective Tool in Improving a Digital Plant Identification System: A Case Study for the Area of Mt. Valerio, Trieste (NE Italy). PLoS ONE, 7(9), e43256.

Laube, I., Hird, H., Brodmann, P., Ullmann, S., Schöne-Michling, M., Chisholm, J., & Broll, H. (2010). Development of primer and probe sets for the detection of plant species in honey. Food Chemistry, 118 (4), 979-986.

Soares, S., Amaral, J. S., Oliveira, M. B. P. P., & Mafra, I. (2015). Improving DNA isolation from honey for the botanical origin identification. Food Control, 48(0), 130-136.