Programme & Book of Abstracts

FOOD INTEGRITY

5th FOODINTEGRITY CONFERENCE

Nantes, France

14-15 November 2018

Assuring the integrity of the food chain: Delivering real world solutions



FOOD INTEGRITY

eurofins

5th FOODINTEGRITY CONFERENCE

Nantes, France
14-15 November 2018

Assuring the integrity of the food chain:
Delivering real world solutions

EDITORS
JEAN-FRANÇOIS MORIN – MONIKA TOMANIOVA
JAMES DONARSKI - PAUL BRERETON

ORGANISED BY

eurofins

&

Ensuring the Integrity of the European food chain (FoodIntegrity)

The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.

https://www.foodintegrity2018-nantes.eu/

ISBN 978-2-9566303-2-6

P7.6
ENTOMOLOGICAL AUTHENTICATION OF HONEY BASED ON DNA MARKERS: DIFFERENTIATION OF HONEY PRODUCED BY *APIS MELLIFERA* AND *APIS CERANA*

Amaral, J.S.[1,4,*], Soares, S.[1], Grazina, L.[1], Mafra, I.[1], Costa, J.[1], Pinto, M.A.[2], Duc, H.P.[3], Oliveira, M.B.P.P.[1]

1-REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Portugal
2-CIMO, Instituto Politécnico de Bragança, Bragança, Portugal
3-Bee Research Centre, Hanoi, Vietnam
*corresponding author e-mail: jamaral@ipb.pt

Keywords: *apis cerana*, *apis* mellifera, authenticity, real-time PCR

According to the European Union legislation, honey is the natural sweet substance produced by *Apis mellifera*, also known as European honeybee. However, in other regions of the world, honey is traditionally obtained from other bee species. Among those, *A. cerana* (also known as Asian honeybee) is also of economic importance since it is used in apiculture. Due to the decline of the wild populations of the *A. cerana* in some countries, such as Japan and parts of China, there is an increasingly interest in preserving the native Asian honeybee, being its honey increasingly valued. Owing to the growing demand for this traditional product, the honey produced by *A. cerana* attains a much higher market value compared to that of *A. mellifera*, thus being prone to adulteration. So far, only a few protein-based methods have been proposed to assess honey entomological origin [1], which in fact is related to its geographical origin since bee species generally occupy different geographical ranges according to their evolutionary lineages [2].

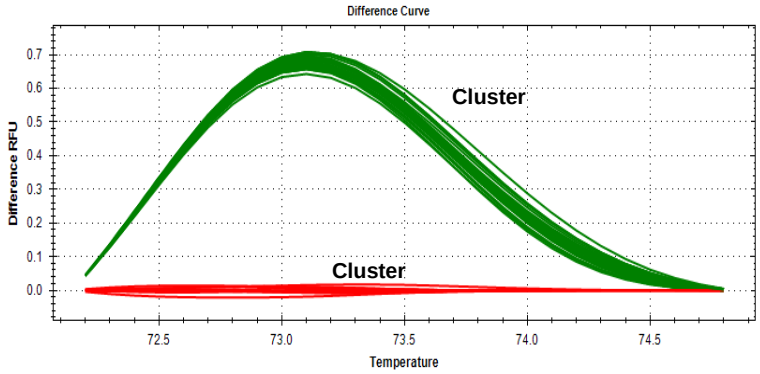

Fig. 1. Difference curves obtained by real-time PCR amplification with EvaGreen dye and HRM analysis targeting 16S rRNA gene (n=3 replicates). Legend: cluster 1, A. cerana from Vietnam and authentic honey samples produced by A. cerana from Vietnam; cluster 2, A. m. carnica; A. m. iberiensis; A. m. ligustica, authentic honey samples produced by A. mellifera from Vietnam, and commercial honey samples from Italy, France and Portugal

In this work, DNA methods were developed for the specific identification of *A. mellifera* and *A. cerana* DNA in honey. For this purpose, bees of *A. cerana* from Thailand, China and Vietnam and honeybees of 4 different subspecies of *A. mellifera* (*iberiensis*, *mellifera*,

ligustica and *carnica*) from EU countries were used. Different sets of primers were designed targeting the $tRNA^{leu}$ - COII intergenic region and the 16S rRNA gene. For both cases, the specificity and sensitivity of the designed primers were assayed by qualitative polymerase chain reaction (PCR). DNA was extracted from honey samples as previously described [3]. PCR with primers targeting the $tRNA^{leu}$ - COII intergenic region allowed the specific detection of *A. cerana*. The applicability of the proposed new PCR method was assayed with authentic *A. cerana* and *A. mellifera* honey samples, which enabled the identification of *A. cerana* honey. PCR targeting the 16S rRNA gene successfully amplified both honeybee species, but without being able to differentiate them. However, the use of real-time PCR with 16S rRNA primers coupled with High Resolution Melting (HRM) analysis allowed the differentiation of both species in distinct clusters (Fig. 1). The developed new HRM methodology was further applied to the analysis of authentic honey samples from Vietnam (produced from *A. cerana* and *A. mellifera* honeybees) and from Portugal (produced from *A. mellifera* honeybees), as well as commercial samples of honey labelled as produced in the EU, allowing its successful entomological origin identification [4]. Both developed techniques proved their effectiveness for establishing the entomological origin of honey and can be considered as useful tools for authentication/control purposes.

Acknowledgements

This work was supported by FCT (Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2013 – POCI/01/0145/FEDER/007265 with financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020, and project NORTE-01-0145-FEDER-000011. S. Soares, L. Grazina and J. Costa are grateful to FCT grants (SFRH/BPD/102404/2014, SFRH/BD/132462/2017 and SFRH/BD/75091/2010) financed by POPH-QREN (subsidized by FSE and MCTES).

References

[1] Won, S.R., Li, C.Y., Kim, J.W., Rhee, H.I. (2009). Immunological characterization of honey major protein and its application. Food Chemistry, 113, 1334-1338.
[2] Pinto, M.A., Henriques D., Neto M., Guedes H., Muñoz I., Azevedo J., Rúa P. (2013). Maternal diversity patterns of Ibero-Atlantic populations reveal further complexity of Iberian honeybees. Apidologie, 44, 430-439.
[3] 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, 130-136.
[4] Soares, S., Grazina, L., Mafra, I., Costa, J., Pinto, M. A., Duc, H. P., Oliveira, M.B.P.P., Amaral, J.S. (2018). Novel diagnostic tools for Asian (Apis cerana) and European (Apis mellifera) honey authentication. Food Research International, 105, 686-693.