Foods 2024 Conference

The 5th International Electronic Conference on Foods

28–30 October 2024 | Online

MDPI

Honey fraud detection: Use of electrochemical genosensors to determine the safety and quality of honeys

Stephanie L. Morais[1], Michelle Castanheira[1], Marlene Santos[2], Valentina F. Domingues[1], Cristina Delerue-Matos[1] & M. Fátima Barroso[1*]

[1] REQUIMTE|LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal; stlom@isep.ipp.pt, up200604160@edu.fc.up.pt, vfd@isep.ipp.pt, cmm@isep.ipp.pt, *mfb@isep.ipp.pt

[2] REQUIMTE|LAQV, Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal; mes@ess.ipp.pt

INTRODUCTION & AIM

Food fraud has long been an issue for the food and beverage industry [1,2]. These fraudulent practices have heightened the awareness of food safety and quality and led to the implementation of new laws to minimize their impacts on the economy and human health [2]. Nevertheless, many adulterated products still find their way into the global market ever year. A prime example is honey [3]. Honey is a natural sweetener with a rich nutritional profile and several health benefits often consumed as a healthy alternative to sugar [3,4]. Therefore, it is important to develop an analytical tool that can help determine adulterated honeys.

In this study, a disposable electrochemical genosensor based on the hybridization reaction between two complementary DNA probes of *Calluna vulgaris* (the heather flower) was developed. A "sandwich" format for the DNA-target probe was designed using a complementary fluorescein isothiocyanate-labelled DNA-signaling probe and the DNA-capture probe. Chronoamperometric measurements of the electrochemical signals obtained a concentration range of 0.13 to 2.00 nM. The optimized genosensor was then employed to detect the amplified genomic DNA from real *C. vulgaris* samples.

The aim of this work was to create an effective and affordable DNA-based analytical technique capable of evaluating the purity and quality of honeys and, hopefully, help minimize the impacts of honey fraud. As the genosensor was able to detect the hybridization reaction from real heather samples, it is a promising tool to authenticate the genomic DNA found in the honey and, therefore help prevent further distribution of adulterated honeys in the international market.

METHODS

❖ *Calluna vulgaris* oligonucleotide sequences and samples

A 98 base pair DNA-target probe for *C. vulgaris* and its complementary probe were specifically designed analyzing public databases. The complementary probe was cut in two, originating the DNA-capture and DNA-signaling probes. These oligonucleotides were purchased as a lyophilized salt and their stock solution resuspended with ultrapure water. Real *C. vulgaris* plant samples were acquired from the Natural Park of Montesinho, Portugal. Those samples were then frozen using liquid nitrogen and milled to break the cellular wall and extract their DNA.

❖ Apparatus and electrodes

DNA amplifications were carried out using a conventional PCR (MyCycler™ thermal cycler, Bio-Rad Laboratories). DNA concentration were measured using a NanoDrop spectrophotometer (NanoDrop, Lit, ThermoScientific). Screen-printed gold electrodes (DRP-C223BT, ΩMetrohm) were used as the electrochemical transductors. Measurements were performed on an AutoLab potentiostat (ΩMetrohm) and the data processed by a NOVA 1.11.2 software. Chronoamperograms were recorded at -0.1V for 60s.

❖ Electrochemical genosensor design

The construction of the electrochemical genosensor involved essentially three phases (Fig. 1):

- Sensing phase: formation of a self-assembled monolayer interface between the DNA-capture probe and the mercaptohexanol (MCH) spacer;
- Sandwich hybridization reaction: two-step hybridization reaction; and
- Electrochemical detection: visualized by chronoamperometry.

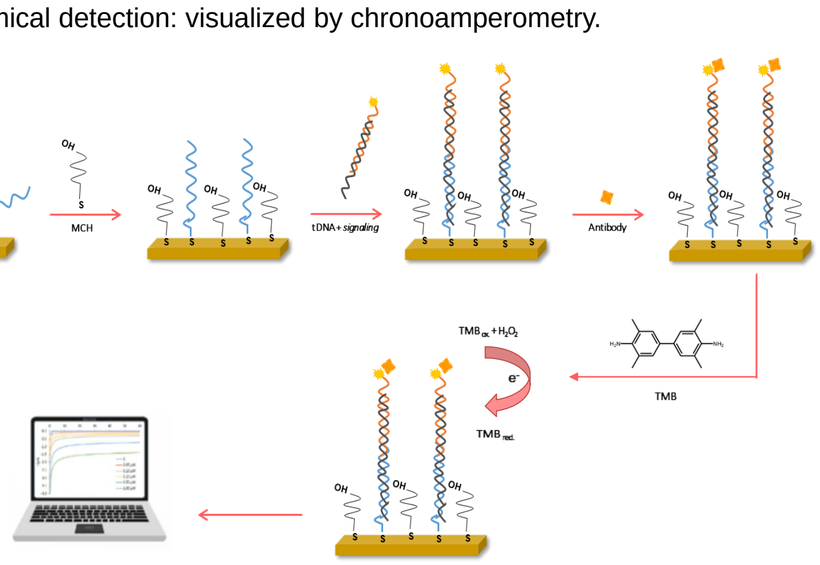

Figure 1. Representation of the electrochemical genosensor design.

RESULTS & DISCUSSION

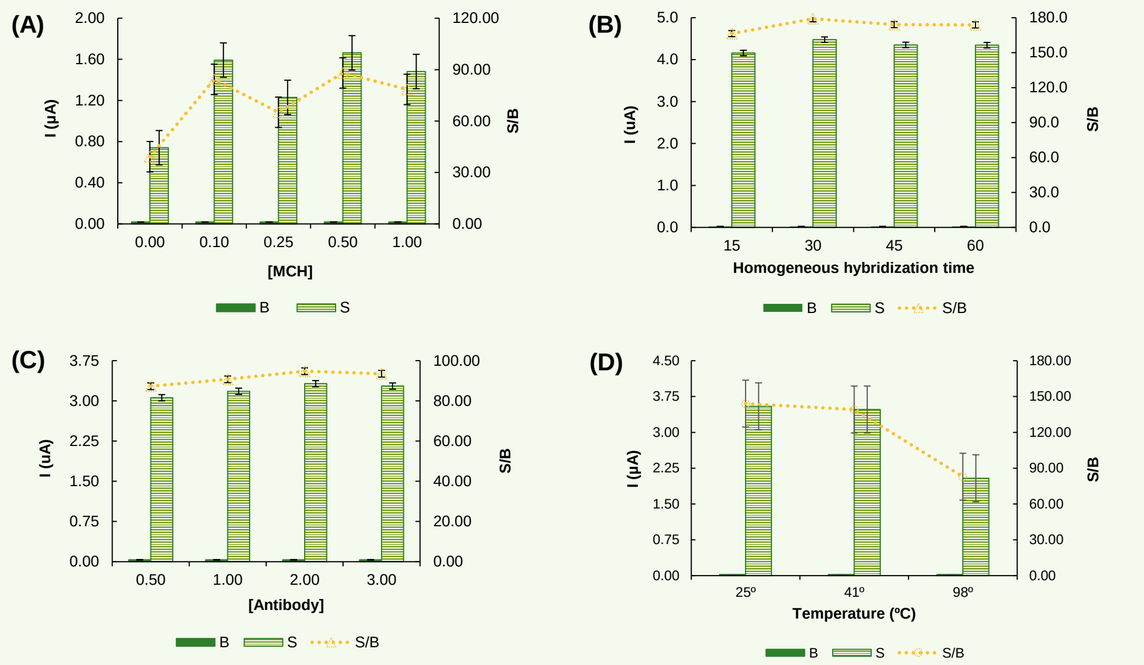

Figure 2. Influence of the (A) concentration of the MCH spacer, (B) homogeneous hybridization incubation time, (C) concentration of the antibody and (D) temperature on the signal intensity. The blank (B) values of *Calluna vulgaris* are represented in dark green, the electrochemical signal (S) in light green and the corresponding S/B ratios in orange, respectively. Error bars estimate the standard deviation of three replicates.

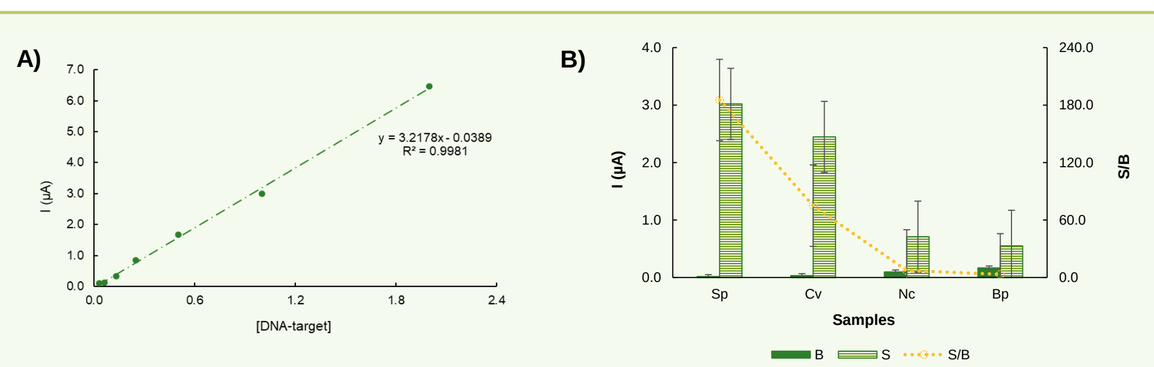

Figure 3. A) Calibration curve of the *Calluna vulgaris* and B) comparison of the registered electrochemical signal from the amplified DNA of *Calluna vulgaris* (Cv) to the synthetic DNA-target probe (Sp) and noncomplementary (Nc and Bp). Current values of the blank assays (B) in dark green, signal (S) in light green. All measurements were performed with a DNA concentration of 1 nM. Error bars estimate the standard deviation of three replicates

- All optimizations contributed to enhance the sensor's sensitivity (Fig. 2A - 2D);
- A linear relationship (R^2 = 0.9981) between the intensity current and the DNA concentration was obtained in the 0.13–2.00 nM range (Fig. 3A);
- The highest S/B value (S/B = 185.00), as well as the utmost current intensity (3.02 µA), were obtained with the synthetic DNA-target probe at 1 nM (Fig. 3B);
- As for the genomic DNA extracted from the *C. vulgaris* samples, a current intensity of 2.45 µA, with a S/B value of 75.00 were registered (Fig. 3B).
- Additionally, there is a clear difference in current intensity of the complementary (Sp and Cv) and non-complementary (Nc and (Bp) samples (Fig. 3B).

CONCLUSION & FUTURE WORK

- A disposable electrochemical genosensor capable of detecting *Calluna vulgaris* DNA, with high selectivity and sensibility was developed.
- The high sensitivity of the electrochemical genosensor were accomplished by employing a mixed thiol-DNA-capture probe and MCH spacer self-assembled interface. While the selectivity of the developed sensor resulted from the design of the sandwich format hybridization and of the heather-specific synthetic sequences.
- The amplification of the electrochemical signal conducted by the (POD) enzyme also influenced the genosensor's performance.
- The developed sensor was successfully employed for the detection and quantification *of C. vulgaris* samples, i.e., the genosensor was able to detect with great selectivity both the synthetic and genomic DNA of the *C. vulgaris* at different concentrations. Moreover, the developed sensor was able to distinguish the real *C. vulgaris* samples from the non-complementary samples.
- In the future, real honey samples will be applied to the developed electrochemical genosensor to detect the botanical origin of the samples and, hopefully, be used to combat honey fraud.

ACKNOWDEGMENTS

This work received financial support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through project MTS/SAS/0077/2020—"Honey+—New reasons to care honey from the Natural Park of Montesinho: A bioindicator of environmental quality & its therapeutic potential" and through the projects UIDB/50006/2020 and UIDP/50006/2020.

MFB thanks Fundação para a Ciência e a Tecnologia (FCT) for the FCT Investigator (2020.03107.CEECIND). Stephanie Morais (2023.028929.BD) and Michelle Castanheira (2023.05159.BDANA) are grateful to FCT and the European Union (EU) for their grants financed by POPH-QREN-Tipologia 4.1-Formação Avançada, funded by Fundo Social Europeu (FSE) and Ministério da Ciência, Tecnologia e Ensino Superior (MCTES).

REFERENCES

[1] Bannor, R.K.; Arthur, K.K.; Oppong, D.; Oppong-Kyeremeh, H. A comprehensive systematic review and bibliometric analysis of food fraud from a global perspective, *Journal of Agriculture and Food Research*, **2023**, *14*, 100686.

[2] Brooks, C.; Parr, L.; Smith, J.M.; Buchanan, D.; Snoich, D.; Hebishy, E. A review of food fraud and food authenticity across the food supply chain, with an examination of the impact of the COVID-19 pandemic and Brexit on food industry. *Food Control*, **2021**, *130*, 108171.

[3] Euroactiv. Honeygate: How Europe is being flooded with fake honey, **2020**.

[4] Ramanauskiene, K.; Stelmakiene, A.; Briedis, V.; Ivanauskas, L.; Jakštas, V. The quantitative analysis of biologically active compounds in Lithuanian honey, *Food Chemis*., **2012**, *132*, 1544-1548