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Plant honey origin authentication: use of electrochemical genosensors for food safety and quality control

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INTRODUCTION & AIM

Honey is a naturally sweet high-quality food product consumed worldwide because of its unique taste, fragrance, nutritional profile and beneficial health properties [1]. These rare characteristics, which make honey of the most popular ingredients for a healthy diet, are intrinsically linked to the local flora from which the bees collect nectar. Therefore, the botanical and geographical origin of a honey plays an important role in the composition of the final product [2].

Nevertheless, honey fraud has become a serious problem for consumers and the food industry as an increasing number of adulterated honeys are being distributed in the market [3]. Mislabeling of a honey's geographic origin and unethical mixing with low-grade honeys, sugars and other substances are some of the common fraudulent practices. These acts can present various impacts on the economy, public health, and in the environment [4]. Hence, it is imperative to develop analytical tools to quickly, cheaply and successfully identify fraudulent products.

In this work, an electrochemical genosensor for the detection of two different plant species: *Calluna vulgaris* (the heather flower) and *Castanea sativa* (the European chestnut tree) was developed and optimized. To improve the genosensor's selectivity and avoid strong secondary structures, a sandwich format for both DNA-target probes was designed using a complementary fluorescein isothiocyanate-labelled DNA-signaling probe. Chronoamperometry measurements were performed in a 0.06 to 2.00 nM range for both species.

The goal of this work is to develop an innovative and cost-effect analytical device to facilitate the consumers' and the producers' assessment about the purity and, consequently the safety and quality of honeys. The developed genosensor was able to detect the hybridization reaction between the synthetic strands of each plant. Therefore, electrochemical genosensors are a promising and cost-effective analytical tool to authenticate the botanical origin of honey, guaranteeing honey safety, quality control and authenticity for both industries and consumers to combat fraud.

METHOD

Apparatus and electrodes

The electrochemical genosensors were constructed on disposable screen-printed gold electrodes (C223BT, DropSens, Ωmetrohm, Switherland). These devices functioned as a transducer along an Autolab potentiostat (PGSTAT101, ΩMetrohm) equipped with the NOVA 1.11.2 software to measure all the electrochemical signals. Chronoamperograms were recorded at -0.1 V for 60 s.

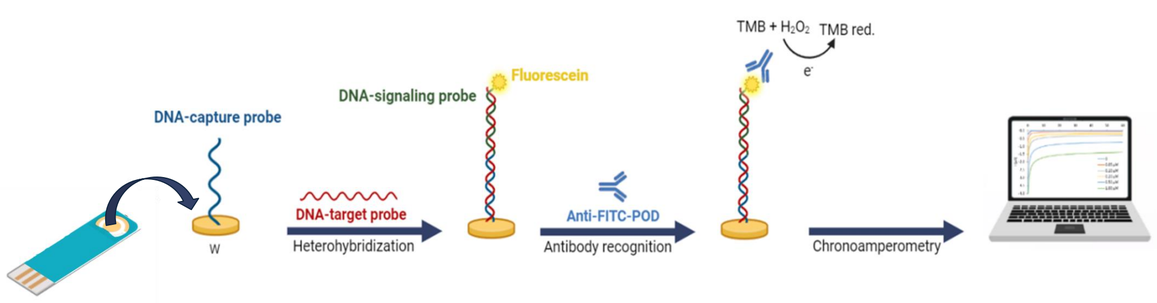
Oligonucleotides sequences

Analyzing public database platforms, a synthetic 98 base pair DNA-target probe for *C. vulgaris* and a 103 base pair DNA-target probe for *C. sativa* were selected and designed. The sequences were then purchased as a lyophilized salt, so their stock solution was resuspended with Milli-Q ultrapure water and stored at – 20 ºC. The working oligonucleotides were prepared daily by diluting the required concentration in the 2x SSPE buffer.

Electrochemical genosensor design

The construction of the electrochemical genosensor involved key four steps:

1. Pretreatment: cleaning of the electrodes surface;
2. Sensing phase: formation of a self-assembled monolayer (SAM) interface between the DNA-capture probe and the mercaptohexanol (MCH) spacer;
3. Sandwich hybridization reaction: design of a two-step hybridization format reaction to increase the sensor's selectivity; and
4. Electrochemical detection: visualized by chronoamperometry.



Scheme 1. Design of developed electrochemical genosensor.

RESULTS & DISCUSSION

Table 1. Selected parameters for the *Calluna vulgaris* and *Castanea sativa* electrochemical genosensors.

Experimental variables	*Calluna vulgaris* (heather flower)		*Castanea sativa* (chesnut tree)	
	Tested range	Selected value	Tested range	Selected value
DNA-capture concentration (µM)	0.25–10.00	1.00	0.25–1.00	0.25
MCH concentration (µM)	0.00–1.00	0.50	0.00–1.00	1.00
MCH incubation time (min)	5–30	30	15–60	30
Homogeneous hybridization time (min)	15–60	30	15–60	30
Homogeneous hybridization temperature (°C)	25–98	25	25–98	98
DNA-signaling concentration (µM)	0.13–0.50	0.50	0.13-0.50	0.25
Heterogeneous hybridization time (min)	30–120	60	15-60	30
Antibody concentration (U/mL)	0.50–3.00	2.00	0.25–2.00	2.00
Antibody incubation time (min)	15–45	30	15–60	60

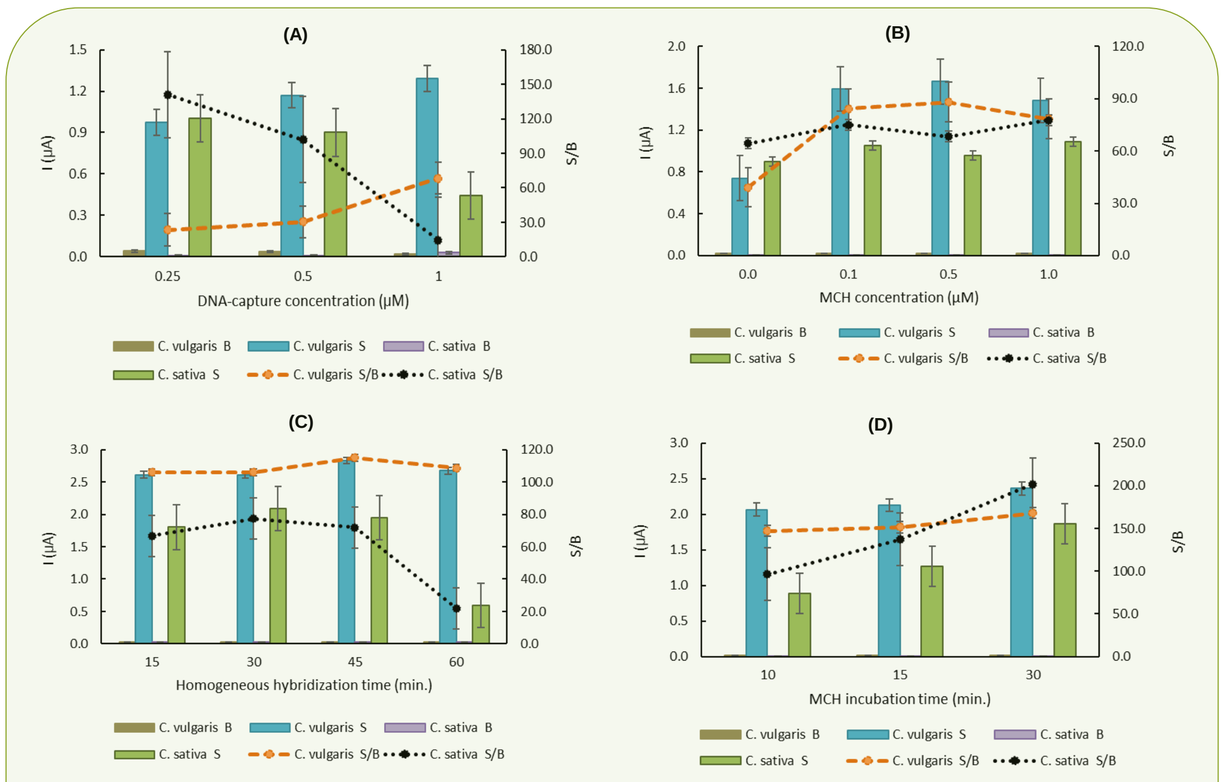


Figure 1. Chronoamperometric response registered when studying the influence of the increasing (A) concentration of the DNA-capture and (B) MCH spacer, as well as the incubation time of (C) the homogeneous hybridization reaction and (D) MCH spacer of both *Calluna vulgaris* and *Castanea sativa*. The blank (B) values of *C. vulgaris* and *C. sativa* are represented in brown and violet, the electrochemical signal (S) in blue and green and the corresponding S/B ratios in orange and black, respectively. Error bars estimate the standard deviation of three replicates.

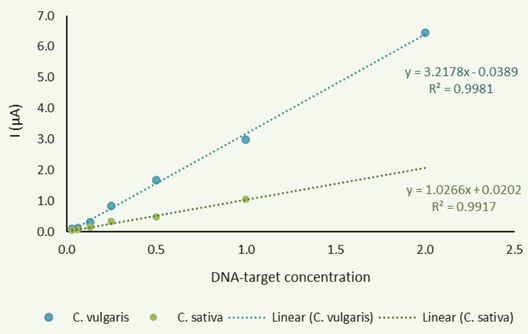


Figure 2. Calibration curve of the *Calluna vulgaris* in blue and *Castanea sativa* in green.

- *C. vulgaris* presented the highest current intensities during the optimizations.
- A linear relationship (R^2 = 0.9981) between the intensity current and the target concentration was obtained in the 0.06–2.00 nM range for *C. vulgaris*, with a slope and intercept value of 3.22 ± 0.03 (µA/nM) and 0.04 ± 0.01 (µA), respectively.
- As for *C. sativa*, a linear relationship (R^2 = 0.9917) was recorded in the 0.06–1.00 nM range, with a slope and intercept value of 1.03 ± 0.02 (µA/nM) and 0.02 ± 0.01 (µA), respectively.

CONCLUSIONS / FUTURE WORK

A disposable electrochemical genosensor capable of detecting the *C. vulgaris* and *C. sativa* synthetic DNA probes, with high selectivity and sensibility and in various concentrations, was developed. To increase the sensor's selectivity, a sandwich hybridization format was adopted, the electrochemical signal amplification conducted by the POD enzyme and chronoamperometry was utilized to measure the electrical currents. All optimizations contributed to enhance the sensor's sensitivity. In the future, the optimized electrochemical genosensor well then be applied for the detection of the botanical origin of real honey samples.

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