

Supplementary Material

Electrochemical Immunosensor for the Simultaneous Determination of Two Main Peanut Allergenic Proteins (Ara h 1 and Ara h 6) in Food Matrices

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Table S1. Optimized experimental parameters, tested range, and selected values.

Experimental variable	Tested range	Selected value
[CAb] ($\mu\text{g}\cdot\text{mL}^{-1}$)	10 – 25	25
[DAb] (Dilution Factor)	100 – 500	250
BSA addition to DAb (%)	0.0 – 1.0	1.0
BSA addition to STR-ALP (%)	0.1 – 1.0	0.1
t (Ara h 1 & Ara h 6) (min)	30 – 60	60
t (DAb) (min)	30 – 60	30
Temperature ($^{\circ}\text{C}$)	25 – 30	30

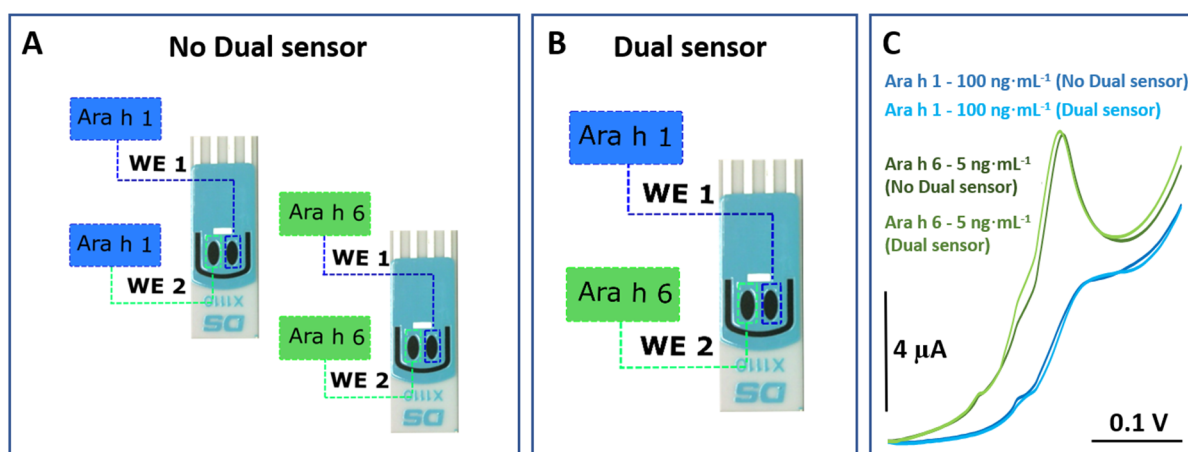


Figure S1. Schematic representation of the detection of Ara h 1 and Ara h 6 using a (A) No Dual sensor and (B) Dual sensor. (C) LSV voltammograms of the 'No Dual' and the Dual sensor (Ara h 1: 100-ng mL⁻¹ and Ara h 6: 5 ng·mL⁻¹).

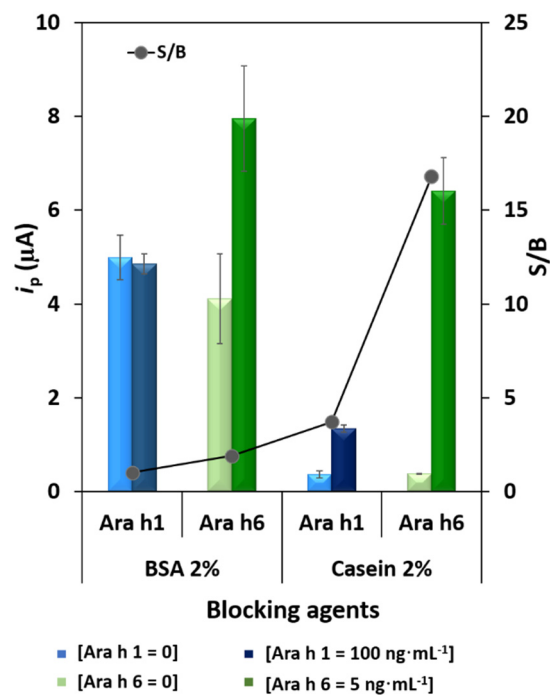


Figure S2. Study of the blocking agents BSA (2% (w/v)) and casein (2% (w/v)) and evaluation of non-specific adsorptions. (Ara h 1: 0 (blank) and 100 ng·mL⁻¹; Ara h 6: 0 (blank) and 5 ng·mL⁻¹; error bars correspond to the standard deviation of 3 replicates).

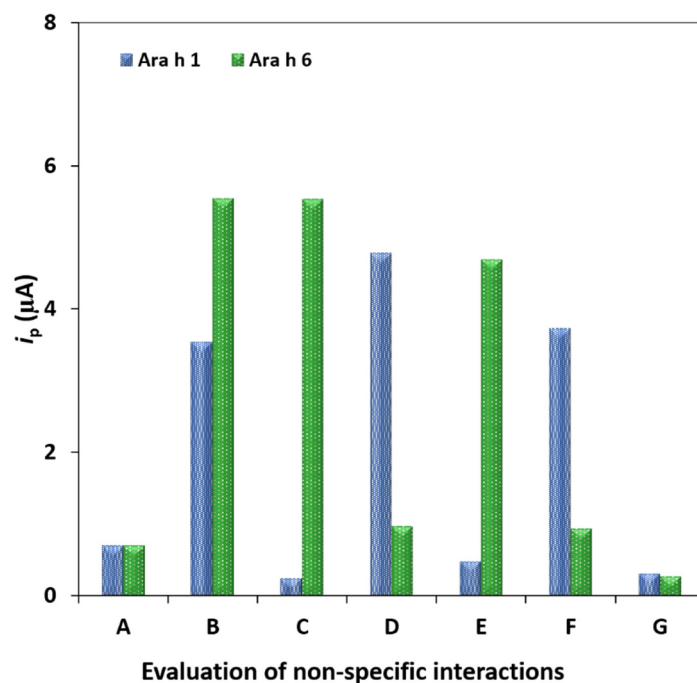


Figure S3. Effect of non-specific adsorptions of the target proteins and the capture and detection antibodies. (A) Blank and (B) 250 ng·mL⁻¹ Ara h 1 and 2.5 ng·mL⁻¹ Ara h 6 (control assays), Assays without the use of: Ara h 1 CAb (C), Ara h 6 CAb (D), Ara h 1 DAb (E), Ara h 6 DAb (F), Ara h 1 DAb and Ara h 6 DAb (G). (Ara h 1: 250 ng mL⁻¹; Ara h 6: 2.5 ng·mL⁻¹).

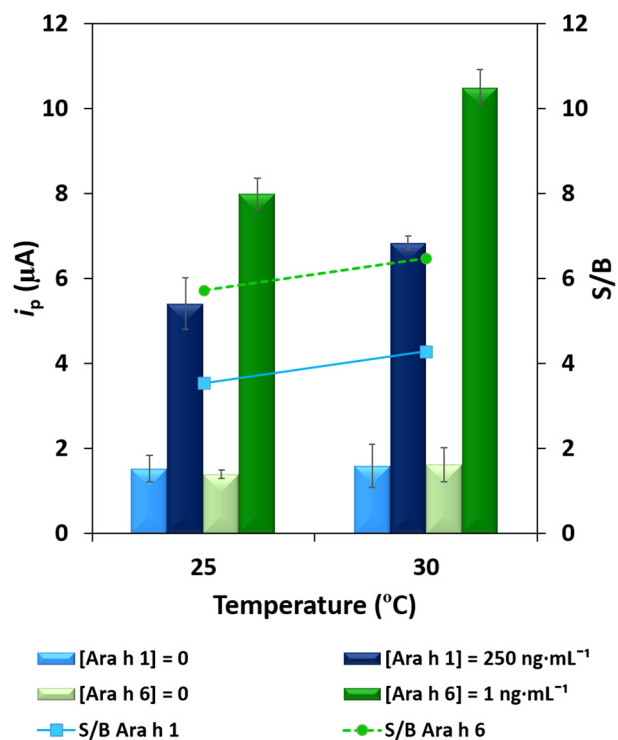


Figure S4. Current intensities obtained at 25 °C and 30 °C. (Ara h 1: 0 (blank) and 250 ng·mL⁻¹; Ara h 6: 0 (blank) and 1 ng·mL⁻¹; error bars correspond to the standard deviation of 3 replicates).

Table S2. Figures of merit of the immunoassay

Figure of merit	Ara h 1	Ara h 6
Concentration interval (ng·mL ⁻¹)	25 - 1000	0.050 - 1.0
Correlation coefficient (r)	0.997	0.998
Slope (m) (μA mL ng ⁻¹)	0.0111	7.10
Standard deviation of the slope (S_m) (μA mL ng ⁻¹)	0.0004	0.28
Intercept (b) (μA)	0.72	0.28
Standard deviation of the intercept (S_a) (μA)	0.21	0.17
Standard deviation of the method (S_{x0})	33.7	0.032
Coefficient of variation of the method (V_{x0}) (%)	10.5	3.31
Limit of detection (LOD) (ng·mL ⁻¹)	5.2	0.017
Limit of quantification (LOQ) (ng·mL ⁻¹)	17	0.055

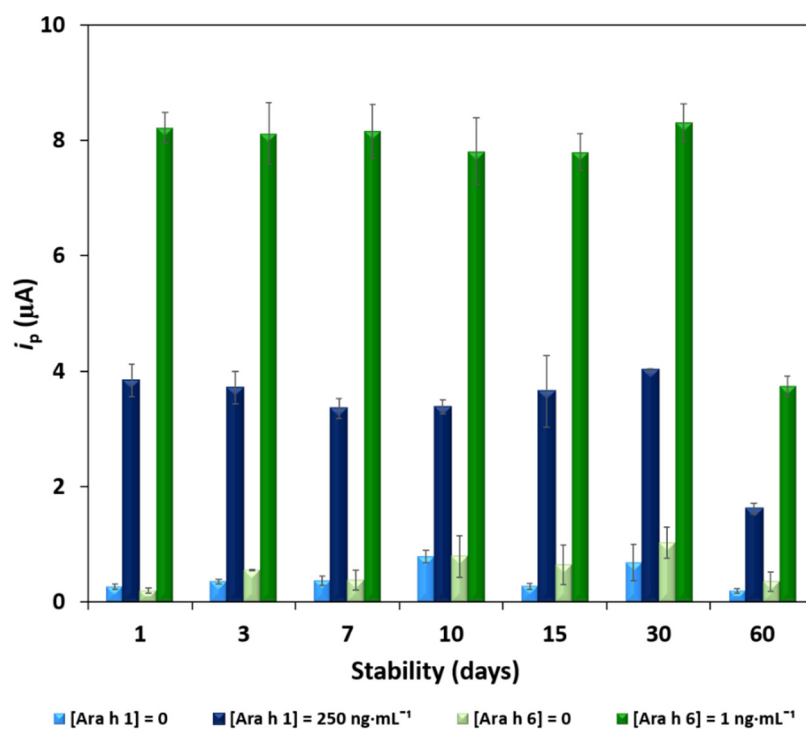


Figure S5. Storage stability of the immunosensor (Ara h 1 (0 (blank) and 250 ng·mL⁻¹) and Ara h 6 (0 (blank) and 1 ng·mL⁻¹); error bars correspond to the standard deviation of 3 replicates).

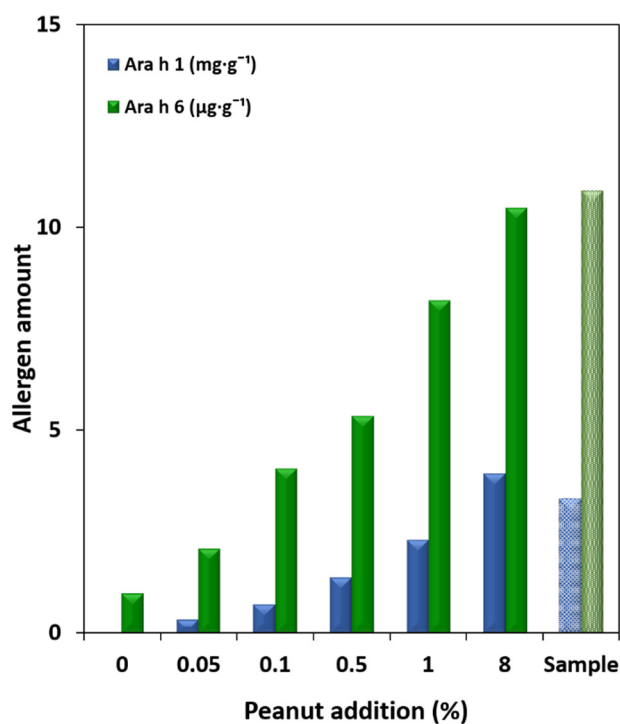


Figure S6. Quantification of Ara h 1 (mg·g⁻¹) and Ara h 6 (mg·g⁻¹) extracted from an allergen-free cookie sample spiked with increasing amounts of peanut and a peanut cookie.

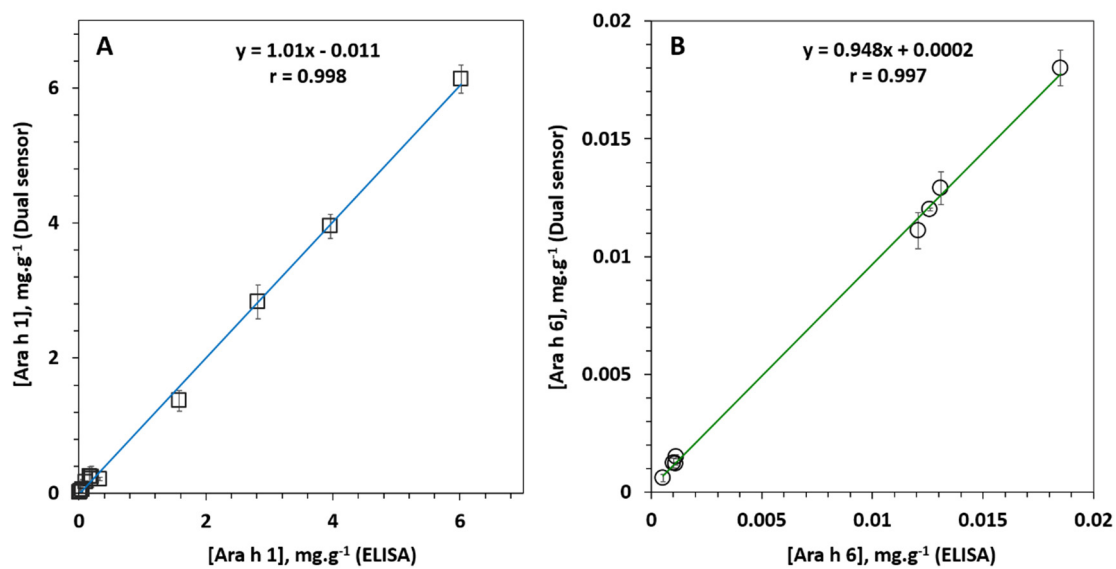


Figure S7. Correlation plots for the analysis of (A) Ara h 1 and (B) Ara h 6 in the tested food products using the developed immunosensor and ELISA kits. Error bars correspond to the standard deviation of 3 replicates.