Electrochemical immunosensor modified with carbon nanofibers coupled to a paper platform for the determination of gliadins in food samples.

ANALYTICAL METHODS

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***A. Table 1*** Summary of optimum conditions for gliadin determination

| Sequence	Conditions	Time (min)
Blocking procedure	1% of bovine serum albumin (BSA) in 0.01 M PBS pH 7.2	5
Washing step	PBS, pH 7.2	2
Samples	Sample	10
Washing buffer	PBS, pH 7.2	2
Enzymatic conjugated	HRP-conjugated (dilution of 1/1000)	5
Washing buffer	PBS, pH 7.2	2
Substrate	1 mM Q in 1 mM citrate-phosphate buffer pH 5 and 1 mM H_2O_2	1
Amperometric detection	Applied potential: -0.15 V	1
Assay time		28

B. Figure 1. A figure shows a comparison of CVs obtained in a solution of 1 mM Q in 1 mM citrate-phosphate buffer pH 5 at 0.075 V s^{-1} for CNFs/SPCE without paper platform (red line) and CNFs/SPCE with paper platform (black line).
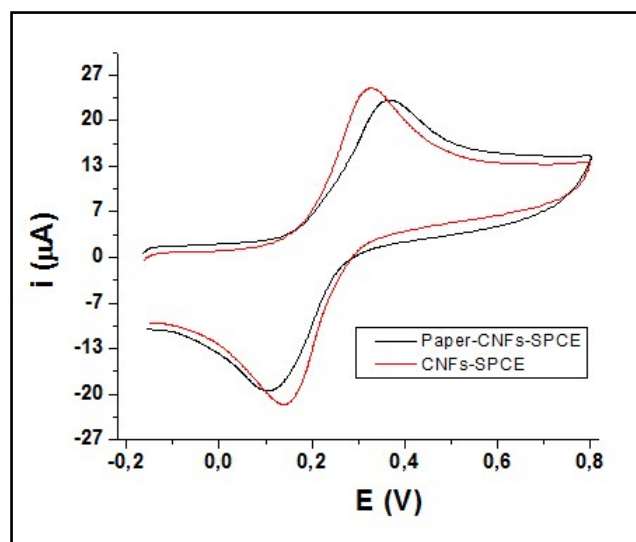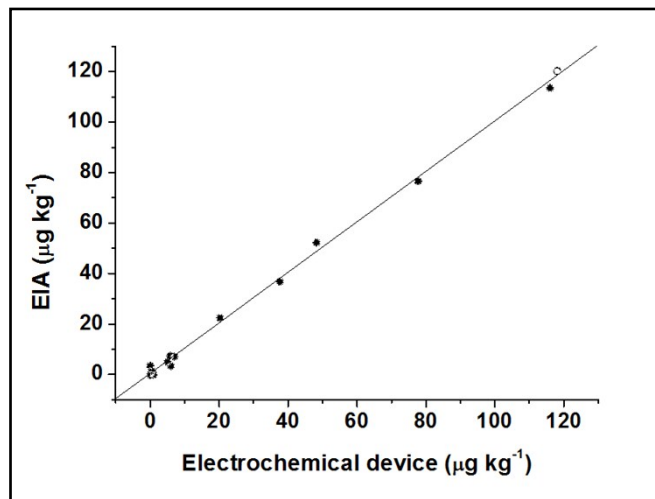

C. Figure 2. Parameters optimization: (a) concentration of immobilized anti-gliadin antibodies. (b) Current intensity as a function of reaction time for 20, 40 and 80 μg kg^{-1} of gliadin standard concentrations.
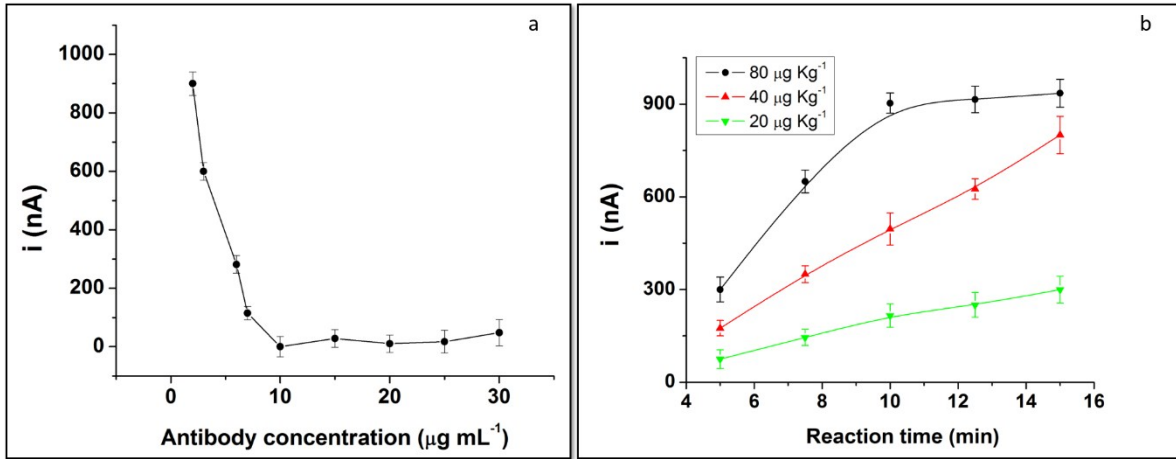
D. Dilution test for determination of accuracy.
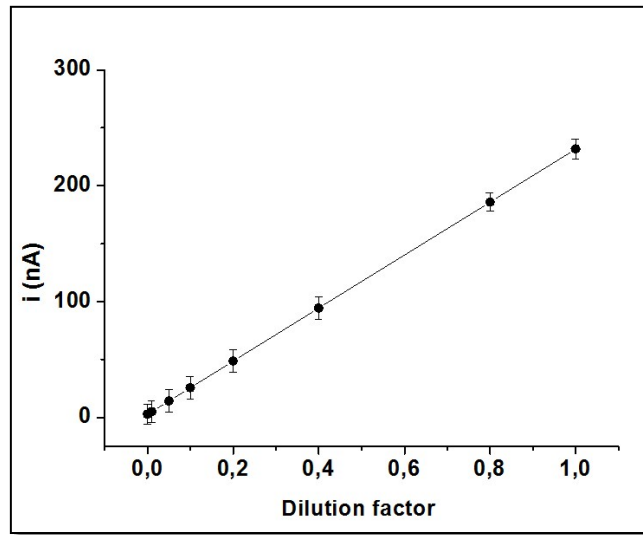
E. Correlation graph between the ELISA R5 method and the developed immunosensor.

F. Table 2. Comparison of the electrochemical immunosensor with the commercial ELISA kit for gliadin determination in food samples.

Samples N°.	Electrochemical Immunosensor[a] (mg kg^{-1})	ELISA Kit[a] (mg kg^{-1})
Manioc flour (2)	Nd	Nd
Rice flour (2)	Nd	Nd
Gluten free flour (3)	3.01	4.13
Common wheat flour (3)	59,06	57.43

a The data is given as average value±SD obtained from five independent experiments (n = 6).

G. Table 3. Within-assay precision (five measurements in the same run for each gliadin standard solution) and between-assay precision (five measurements for each gliadin standard, repeated for three consecutive days).

Standard[a]	Within-assay		Between-assay	
	Mean[a]	CV%	Mean[a]	CV%
5 µg kg^{-1}	5.62	3.87	5.98	5.80
20 µg kg^{-1}	19.42	5.13	19.23	5.23
80 µg kg^{-1}	80.32	4.11	82.12	6.56

a Gliadin concentration (µg kg^{-1})