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Electrochemical Platform Based on Modified Ti Electrodes to Test a Food Allergen Presence

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Abstract*. Celiac disease (CD) is an autoimmune disorder. For these patients, the only treatment is a gluten free diet. In this work we showed that there can be an easy and cheap method to test gluten presence. We used several modified Ti electrodes. All modified electrodes were prepared using electrochemical methods. Modified electrodes were tested using differential pulse voltammetry in solutions with or without gluten. Selected modified Ti electrode was tested using different known gluten concentrations and extracts from different aliments.*

Keywords: *Modified Ti, Graphene Oxide, Gluten, Differential Pulse Voltammetry*

1.Introduction

There are different agents affecting food safety: food residues, additives, allergens, and bacteria. Gluten is one of the allergens present in food. It is an allergen for a category of consumers presenting various forms of diseases such as: dermatitis herpetiformis, gluten sensitivity and celiac disease (*1*). Although for consumers with Celiac disease (CD) there are many strategies of treatment (*2*), up to date, the only treatment is lifelong and complete avoidance of gluten intake from food (*3*). Adherence of gluten-free diet may significantly reduce the rate of complications and death for these persons (*4*).

Gluten is found in many cereals like wheat, triticale, barley, rye and their crossbred varieties and derivatives (*1, 5*) Gluten is a complex mixture of at least 50 proteins, classified in two groups: prolamins - gliadins (soluble in alcohol) and glutenins (insoluble in alcohol, proteins with covalent disulphide bonds) (*1, 6, 7*).

European Community established limits of gluten in food. Food products containing gluten below 20 ppm are labeled “gluten-free” and the ones containing between 20 and 100 ppm are classified with “very-low gluten content” (*8*). To detect gluten from food products, the commercially available test is Enzyme-linked immunosorbent assay (ELISA). There has been an increasing interest to develop other methods, more practical and time saving (*5*). A proof of this are many research studies trying to replace Elisa. A fluorescent paper based immunosensor was consisting of a paper platform modified with a mesoporous material containing antibodies (*9*). Other gluten testing include more complex methods like: mass spectrometry (*10*), high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (*11*), quartz crystal microbalance (*12*) and FT-Raman spectroscopy (*13*). A research is using real-time polymerase chain reaction (PCR) (*14*). But many of these are not very easy or ready to be used by a consumer in his home. Surface plasmon resonance (SPR) was used for gliadin detection from urine samples from celiac patients (*15*).

Different electrochemical methods were also developed for gluten detection. Among used methods were: amperometry, impedance and differential pulse voltammetry (DPV). Amperometry was used in many research studies. One was using amperometric flow system of detection coupled to a competitive ELISA assay (*8*). Another was using chronoamperometry and screen-printed carbon electrodes as working electrodes (*16*). The third one was using an enzyme electrodeposited on carbon electrode modified with nanoparticles of fullerene (*6*). In other example, amperometric signal was obtained from gliadin immobilized to tosyl-activated magnetic beads by covalent coupling (*17*). Electrochemical test includes also the use of impedance. This was based the immobilization of an aptamer on the gold

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electrode (*18*). DPV was used with reduced graphene oxide modified electrodes with antigliadin antibody. At least 4 hours are needed to prepare the modified electrode (*19*). A research study was using both impedance and DPV. They were using modified electrodes with antibody based on the use of dithiols (*20*).

A simpler method was using a graphite mine and rapid differential pulse voltammetry (*5*).

In the present research, we used modified Titanium (Ti) electrodes and DPV. We used different surface modifications: nanotubes obtained by anodization, nanofibers and graphene oxide. We tested different food products with the modified electrodes.

2. Materials and methods

Reagents used: ethylene glycol (EG), polyvinyl pyrrolidone M=1300000, 2-propanol, N-N-dimethyl formamide (DMF) (Alpha Aesar), ammonium fluoride (NH_4F), graphene oxide powder (GO), $Na_2HPO_4 \cdot 2H_2O$, $NaH_2PO_4 \cdot H_2O$, titanium butoxide, glacial acetic acid, natrium acetate, dimethyl sulfoxide (DMSO) and gluten from wheat (Sigma Aldrich). All chemicals used were of analytical grade and were used as received, without further purification.

First, Ti round samples, with 8 mm diameter (99.99% purity, Alpha Aesar) were polished and cleaned with ultrasounds. Distiled water, ethanol and acetone were used (15 minutes each). Electrode was named E1.

The nanotubes (TiNT) were prepared at room temperature in a viscous electrolyte: EG: 0.5 wt% NH_4F + 2 vol.% H_2O at 50 V, 2 h. E1 was used as working electrode and a Pt wire as counter electrode. A Matrix MPS-7163 was used as power supply. After preparation, they were rinsed with distilled water, and subjected to ultrasonic treatment 30 seconds in distilled water. Electrode was named E2.

TiO_2 nanofibers (TiNF) were prepared at high voltages using a previous described method (*21*) with some modifications. Briefly, electrolyte was: 4 wt.% TBut + 8 wt.% PVP in DMF/isopropanol (mass ratio 1/1) + 2 wt.% acetic acid. Prior to electrodeposition, the samples were coated with 100 µl solution using a spin-coater Laurell WS-650-23 (Laurell Technologies). The dynamic mode was used, with 3000 rpm, 30 seconds. For nanofibers formation, solution was fed at a rate of 0.5 ml/h using a Legato180 pump (Kd Scientific) from a plastic syringe with a blunt tip needle. 20 kV were applied between the needle tip and Cu collector plate (at 10 cm distance) for 30 min. A high-power supply was used - PS/EJ30P20 (Glasmann). Samples were dried overnight and treated 4 h at 450°C. E1 was used as working electrode. Resulted electrode was named E3.

Graphene oxide (GO) was deposited using cyclic voltammetry (CV). Parameters used: scan rate 40 mV/s; applied between -1.5 V and +1 V versus the Ag/AgCl/KCl 3 M. Total number of cycles were 30. Electrolyte: 0.1 M phosphate buffer *p*H 9.32. We used Ti (E1) or modified Ti (E2 or E3) as working electrode. Electrolyte was 0.1 M phosphate buffer *p*H 9.32 (prepared from 0.2 M stock solutions of NaH_2PO_4 and Na_2HPO_4, adjusted with NaOH 0.1 M) with 0.5 mg/mL GO. Resulting electrodes were E4 30 TiGO, E5 (TiNTGO) and E6 (TiNFGO). On Ti, we also deposited GO during 40 cycles. Resulting sample was named E4 40 TiGO.

Gluten presence evaluation was made using DPV between 0 V and 1.2 V, with 50 mV amplitude at 20 mV scan rate. Electrolyte used was DMSO:acetate buffer (pH 4.8) 1:4. Acetate buffer was prepared from natrium acetate and glacial acetic acid; with or without gluten.

Gluten extraction: flour samples (1g) were mixed with distilled water (10 mL) for 1 h under ultrasound stirring. After centrifugation at 8000 rpm for 10 min a pellet was formed. This pellet was immersed into ethanol (60%, 50 mL) for 1 h, centrifuged (30 min, 8000 rpm). 5 mL of the supernatant was used for analysis dissolved in 25 mL buffer pH 4.8

Equipment: scanning electron microscopy (SEM) images for all samples were recorded with Thermo Scientific FEI Quanta 650 FEG (Hillsboro, OR, USA) high-performance scanning electron microscope. All electrochemical data were recorded with a potentiostat/galvanostat from Metrohm Autolab (PGSTAT 302N). Electrochemical cell was of three electrodes in a single compartment.

Samples have been used as working electrodes, platinum rod was the auxiliary electrode, and all values reported were measured vs. an Ag/AgCl/KCl 3 M reference electrode.

3. Results and discussions

Recorded curves during sample preparation and their corresponding SEM images for modified electrodes are presented in Figure 1. For GO deposition on Ti using CV (30 cycles) the recorded curve is seen in Figure 1 a. It is a typical CV curve for a redox process. In the SEM image (Figure 1b) it can be seen the graphene agglomerations. For sample Ti/NT, the curve recorded during anodization is presented in Figure 1 c. It is typical anodization curve with revealing the mechanism of nanotubes formation previously described (*22*). In the SEM image (Figure 1d), very ordered nanotubes are visible, having diameters around 100 nm. Using high voltage process, random aligned TiO_2 nanofibers were prepared (Figure 1 e). The CV curve for GO deposition on top of nanotubes (TiNTGO), the currents recorded during CV (Figure 1 f) were higher compared with the ones recorded in TiGO case (Figure 1a). It is visible that the current was increasing with each cycle. In the SEM image (Figure 1 g), it is visible that the nanotubes were covered with a film. Their diameters are smaller compared with Ti/NT sample. Most of the nanotubes remained open after GO deposition. The Ti surface modification influence is visible once again in the curve recorded during GO electrodeposition on top of nanofibers (Figure 1h). This curve is very different compared with the ones recorded for TiGO30 sample and TiNTGO sample. GO electrodeposition on top of nanofibers is clearly seen in Figure 1 i. Nanofibers diameters were changed after GO electodeposition (Figure 1 e and i).

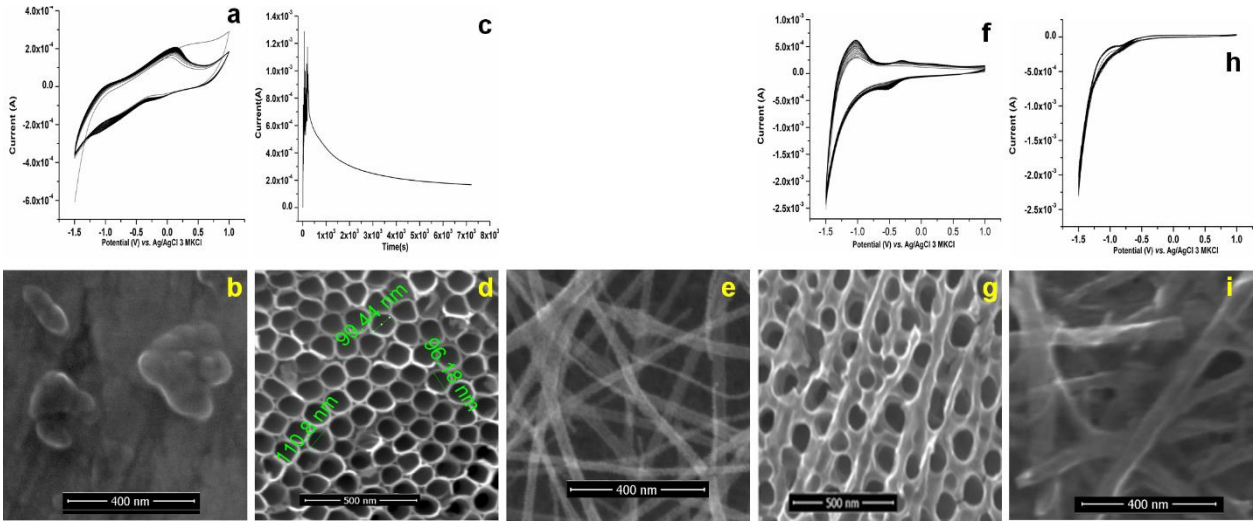

Figure 1. Curves recorded during preparation and corresponding SEM images for all modified Ti electrodes [a, b – E4 30 TiGO; c, d – E2 (TiNT), e – E3 (TiNF); f, g – E5 (TiNTGO); h, i - E6 (TiNFGO)]

To see which of the proposed electrodes it is desirable to evaluate the presence of gluten, we tested six electrodes using DPV. One electrode was E1 (polished and cleaned Ti) and the others were modified Ti electrodes. From the Figure 2, it can be seen that only for E3 (TiNF) and E4 30 TiGO we obtained a signal in the presence of gluten. Because the for E3 preparation was longer compared to E4 30 TiGO, we used E4 30 TiGO for further tests.

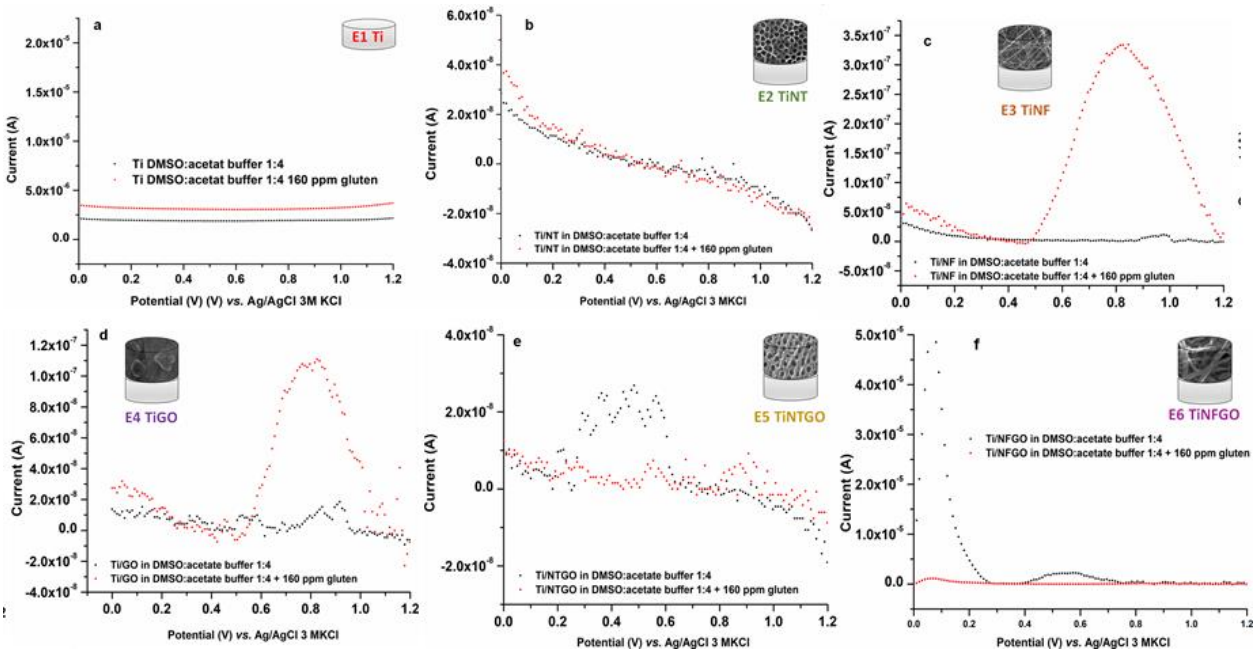

Figure 2. DPV curves is DMSO:acetate buffer solution (1:4) with or without gluten from wheat 160 ppm

Tests were conducted in DMSO:acetate buffer *p*H 4,8 with or without known gluten concentrations. To see influence of cycles number on TiGO performance in gluten solutions, we also tested E4 40 TiGO electrode. Results are presented in Figure 3. Only five concentrations were presented. At sixth concentration, probably Ti was oxidized and the signal was not proportional with the gluten concentration anymore. Gluten concentration was plotted against peak height. We calculated the limit of detection (LOD) and the limit of quantification (LOQ) in each case, using relations described in literature (*23*), with Excel program. In first case we obtained LOD 36 ppm and LOQ 119 ppm. In the second case, results were: LOD 66 ppm and LOQ 220 ppm. It can be seen that increasing the cycles number during GO electrodeposition did not increased the GO film conductivity, so the results were not improved.

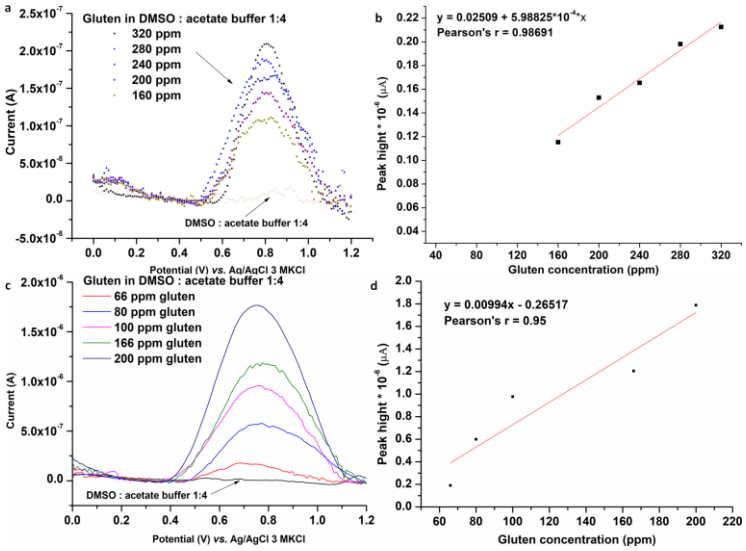

Figure 3. Electrochemical DPV curves and calibration curves recorded with E4 30 TiGO (a and b) and E4 40 TiGO (c and d)

To see if the E4 30 TiGO electrode can be used to analyze real samples, we prepared three kind of flour samples extracts. From Figure 4, it can be seen that with this modified Ti electrode, we were able to distinguish between flour samples. The higher signal was obtained for wheat flour. This is known to have gluten. A smaller signal compared with the one obtained in DMSO:acetate buffer with 66 ppm gluten was obtained for corn flour , labeled by manufacturer "may contain traces of gluten". A signal compared with the one obtained in DMSO:acetate buffer without gluten was obtained for flour labeled by manufacturer "gluten free".

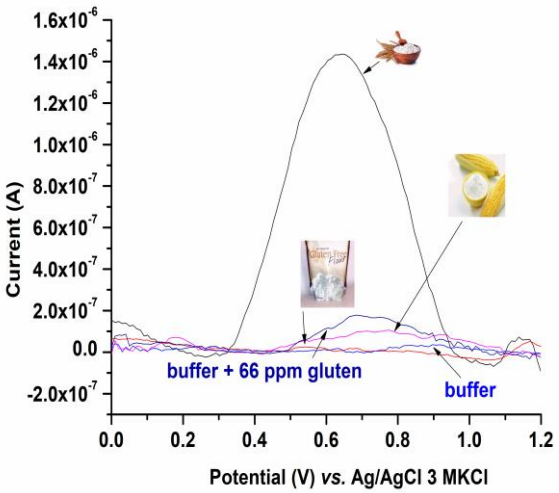

Figure 4. Flour samples testing with E4 30 TiGO

To verify the reproducibility and repeatability, we freshly prepared E4 30 TiGO at different times. We recorded the signal in DMSO:acetate buffer with 100 ppm gluten. From results presented in Figure 5 a. The result is similar. We also performed five consecutive scans in DMSO:acetate buffer with 100 ppm gluten. We obtained a good reproducibility (Figure 5b).

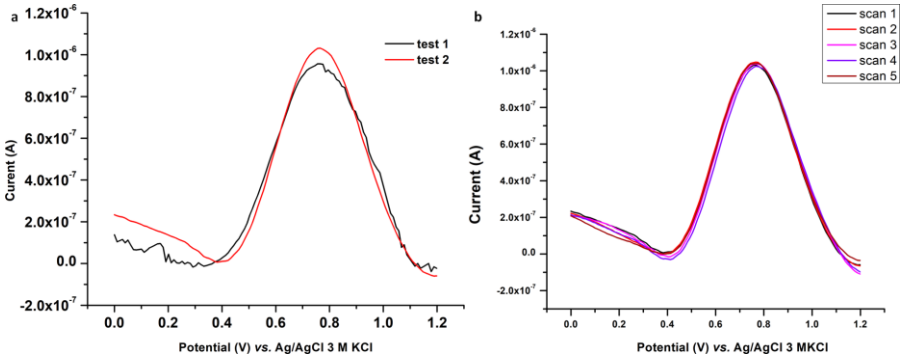

Figure 5. Repetability tests – DPV curves in DMSO:acetate buffer + 100 ppm gluten
(a – tests performed with E4 30 TiGO freshly prepared at different times;
b – consecutive scans with the same E4 30 TiGO electrode)

4. Conclusions

Different nanostructures (nanotubes, nanochannels, nanofibers) were prepared for a potential gluten detection application. GO was also used as titanium or nanostructured titanium surface modification method. Their structure was analyzed using SEM.

Modified electrodes were tested for gluten presence evaluation using an electrochemical method (DPV). Ti modified with graphene oxide and nanofibers gave a signal in DPV in solutions containing

known gluten concentrations. Increasing graphene oxide layer thickness (by increasing cycles number during electrodeposition), does not increased the conductivity.

We tested different kinds of flour with Ti modified with graphene oxide: wheat flour (known to have gluten), corn flour (labeled "may contain traces of gluten") and a mixed fluor labeled "gluten free". We were able to distinguish between them.

Future experiments need to be performed to try to lowed LOD and LOQ, but up to this point, the proposed electrode (Ti with graphene oxide) and proposed method (DPV) can be used to detect gluten from aliments which were contaminated with gluten during manufacturing process.

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5. References

1.TAYLOR, S.L., NORDLEE, J.A., JAYASENA, S., BAUMERT, J.L., Evaluation of a Handheld Gluten Detection Device, *J. Food Prot.*, **81**(10), 2018, 1723-1728.
2.NAIK, R.D., SEIDNER, D.L., ADAMS, D.W., Nutritional Consideration in Celiac Disease and Nonceliac Gluten Sensitivity, *Gastroenterol. Clin. North. Am.*, **47**(1), 2018, 139-154.
3.DHAWAN, A., AGARWAL, A., MULDER, C.J., MAKHARIA, G.K., Celiac disease in the East and the West: Bridging the gaps between the guidelines and their implementation in daily practice is mandatory, *Indian J. Gastroenterol.*, **38**(3), 2019, 185-189.
4.DEGEORGE, K.C., FRYE, J.W., STEIN, K.M., ROLLINS, L.K., MCCARTER, D.F., Celiac Disease and Gluten Sensitivity, *Prim. Care*, **44**(4), 2017, 693-707.
5.EKSIN, E., CONGUR, G., ERDEM, A., Electrochemical assay for determination of gluten in flour samples, *Food. Chem.*, **184**, 2015,183-187.
6.ONTIVEROS, G., HERNANDEZ, P., IBARRA, I.S., DOMINGUEZ, J.M., RODRIGUEZ, J.A., Development of a Biosensor Modified with Nanoparticles for Sensitive Detection of Gluten by Chronoamperometry, *ECS Transactions*, **76**(1), 2017, 103-107.
7.URADE, R., SATO, N., SUGIYAMA, M., Gliadins from wheat grain: an overview, from primary structure to nanostructures of aggregates, *Biophys. Rev.*, **10**(2), 2018, 435-443.
8.AMAYA-GONZÁLEZ, S., DE-LOS-SANTOS-ÁLVAREZ, N., LOBO-CASTAÑÓN, M.J., MIRANDA-ORDIERES, A.J., TUÑÓN-BLANCO, P., Amperometric Quantification of Gluten in Food Samples Using an ELISA Competitive Assay and Flow Injection Analysis, *Electroanalysis*, **23**(1), 2011,108-114.
9.MARÍN-BARROSO, E., MOREIRA, C.M., MESSINA, G.A., BERTOLINO, F.A., ALDERETE, M., SOLER-ILLIA, G.J.A.A., RABA, J., PEREIRA, S.V., Paper based analytical device modified with nanoporous material for the fluorescent sensing of gliadin content in different food samples, *Microchemical Journal*, **142**, 2018, 78-84.
10.DE STEFANO, L., Rossi, M., Staiano, M., Mamone, G., Parracino, A., Rotiroti, L., Rendina, I., Rossi, M., D'Auria, S., Glutamine-Binding Protein from Escherichia coli Specifically Binds a Wheat Gliadin Peptide Allowing the Design of a New Porous Silicon-Based Optical Biosensor, *J. Proteome Res.*, **5**(5), 2006, 1241-1245.
11.JIRA, W., MÜNCH, S., A sensitive HPLC-MS/MS screening method for the simultaneous detection of barley, maize, oats, rice, rye and wheat proteins in meat products, *Food Chem.*, **275**, 2019, 214-223.

12.FUNARI, R., TERRACCIANO, I., DELLA VENTURA, B., RICCI, S., CARDI, T., D'AGOSTINO, N., VELOTTA, R., Label-Free Detection of Gliadin in Food by Quartz Crystal Microbalance-Based Immunosensor, *J. Agric. Food Chem.*, **65**(6), 2017, 1281-1289.
13.CZAJA, T., MAZUREK, S., SZOSTAK, R., Quantification of gluten in wheat flour by FT-Raman spectroscopy, *Food Chem.*, **211**, 2016, 560-563.
14.MARTÍN-FERNÁNDEZ, B., DE-LOS-SANTOS-ÁLVAREZ, N., MARTÍN-CLEMENTE, J.P., LOBO-CASTAÑÓN, M.J., LÓPEZ-RUIZ, B., Challenging genosensors in food samples: The case of gluten determination in highly processed samples, *Talanta*, **146**, 2016, 490-495.
15.SOLER, M., ESTEVEZ, M.C., MORENO MDE, L., CEBOLLA, A., LECHUGA, L.M., Label-free SPR detection of gluten peptides in urine for non-invasive celiac disease follow-up, *Biosens. Bioelectron.*, **79**, 2016, 158-164.
16.AMAYA-GONZALEZ, S., DE-LOS-SANTOS-ALVAREZ, N., MIRANDA-ORDIERES, A.J., LOBO-CASTANON, M.J., Sensitive gluten determination in gluten-free foods by an electrochemical aptamer-based assay, *Anal. Bioanal. Chem.*, **407**(20), 2015, 6021-6029.
17.LAUBE, T., KERGARAVAT, S.V., FABIANO, S.N., HERNÁNDEZ, S.R., ALEGRET, S., PIVIDORI, M.I., Magneto immunosensor for gliadin detection in gluten-free foodstuff: towards food safety for celiac patients, *Biosens. Bioelectron.*, **27**(1), 2011, 46-52.
18.MALVANO, F., ALBANESE, D., PILLOTON, R., DI MATTEO, M., A new label-free impedimetric aptasensor for gluten detection, *Food Control*, **79**, 2017, 200-206.
19.CHEKIN, F., SINGH, S.K., VASILESCU, A., DHAVALE, V.M., KURUNGOT, S., BOUKHERROUB, R., SZUNERITS, S., Reduced Graphene Oxide Modified Electrodes for Sensitive Sensing of Gliadin in Food Samples, *ACS Sens.*, **1**(12), 2016, 1462-1470.
20.NASSEF, H.M., BERMUDO REDONDO, M.C., CICLITIRA, P.J., ELLIS H.J., FRAGOSO, A., O'SULLIVAN, C.K., Electrochemical immunosensor for detection of celiac disease toxic gliadin in foodstuff, *Anal. Chem.*, **80**, 2008, 9265-9271.
21.DUMITRIU, C., STOIAN, A.B., TITORENCU, I., PRUNA, V., JINGA, V.V., LATONEN, R.M., BOBACKA, J., Demetrescu I., Electrospun TiO_2 nanofibers decorated Ti substrate for biomedical application, *Mater. Sci. Eng. C Mater. Biol. Appl.*, **45**, 2014, 56-63.
22.DUMITRIU, C., PIRVU, C., DEMETRESCU, I., The Electrochemical Formation and Shielding Mechanism of TiO_2 Nanotubes in Organic Electrolytes with Different Viscosity, *J. Electrochem. Soc.*, **160**, 2013, G55-G60.
23.MILLER, J.N., MILLER, J.C., *Statistics and Chemometrics for Analytical Chemistry*, *Fifth Edition*, Pearson Education Linited, Edimburg, 2005

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