Food Control 147 (2023) 109624

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

# Lossy mode resonance-based optical immunosensor towards detecting gliadin in aqueous solutions

M. Benítez [a], P. Zubiate [a], A.B. Socorro-Leránoz [a,b,c], I.R. Matias [a,b,c,*]

[a] Department of Electrical, Electronic and Communication Engineering, Public University of Navarra, Ed. Los Tejos, Campus Arrosadía s/n, E-31006, Pamplona, Spain
[b] Institute of Smart Cities, Public University of Navarra, Ed. Jerónimo de Ayanz, Campus of Arrosadía s/n, E-31006, Pamplona, Spain
[c] Navarra Institute for Health Research (IdiSNa), Recinto de Complejo Hospitalario de Navarra, C/Irunlarrea, 3, E-31008, Pamplona, Spain

ARTICLE INFO

*Keywords:*
Gluten
Gliadin
Food safety
Lossy mode resonance
Biosensor
Planar waveguide

ABSTRACT

The development of accurate, intuitive, and easy-to-handle devices to detect different types of allergens is on the rise, as these are useful tools to guarantee consumer safety, which should be a priority for any food industry. Gliadin, one of the main proteins present in gluten, is the one responsible for triggering the immune system to produce autoantibodies in celiac disease, the most dangerous pathology related to gluten. Lossy Mode Resonance (LMR) based biosensors are lately known as a promising sensing technology and its implementation on planar waveguides has been shown to result in manageable, sustainable and robust structures. In this work, an LMR based microfluidic biosensor for gliadin detection is proposed, by coating a coverslip with Titanium Dioxide ($TiO_2$) by Atomic Layer Deposition (ALD) to generate the resonance phenomena and functionalizing the sensor surface with anti-gliadin antibody (AGA) through covalent bond. The sensor was exposed to different gliadin concentrations in ultrapure water, in the range of 0.1–100 ppm with an accuracy of ±0.14 ppm, for a sensitivity of 1.35 ppm/ml. The calibration curve was obtained from the experimental data corresponding to three repetitions of the assay and a limit of detection (LOD) of 0.05 ppm was achieved. Moreover, the sensor was exposed to commercial flour samples, some of them labeled as gluten free (GF) and the response agreed with the expected results according to product label. Biosensor specificity to gliadin was demonstrated by injecting chicken egg white albumin without obtaining any significant response.

## 1. Introduction

Gluten is the complex formed by a family of storage proteins, mainly gliadin and glutenin (Brouns et al., 2013). It can be found in cereal grains that include wheat, rye, and barley (Balakireva & Zamyatnin, 2016). A significant part of the calories consumed by humans globally comes from wheat. (Shewry & Hey, 2015) and a wide variety of processed foods contain wheat and other grains, due to the abundance and versatility of these ingredients.

Wheat main nutrients composition, which is shown in Fig. 1, includes 8%–15% of protein, from which 10%–15% is albumin-globulin and 85%–90% is gluten. The main gluten proteins have been classified by their solubility, the gliadins soluble in alcohol-water and the insoluble glutenins. Celiac disease is the most alarming and common disease associated with gluten consumption (Caio et al., 2019). This autoimmune disorder occurs because the immune system of celiac people produces autoantibodies when it detects the presence of gliadin. Since the treatment of this pathology is a gluten-free diet, gliadin detection has gained relevance (Malvano et al., 2017).

Food products can be labeled as "gluten-free" if gluten is present in a concentration below 20 ppm(European Commission, 2014). There are products that are naturally free of gluten but have been contaminated by it. Therefore, if they are labeled gluten-free, they also pose a risk to celiac patients (Verma et al., 2017) (Bascuñán et al., 2017). From the harvest field to the distribution point, products can be contaminated and consequently contain trace amounts of gluten (Thompson et al., 2010) (Falcomer et al., 2020). There is also a risk of cross-contamination in kitchens where foods that contain gluten and other supposedly gluten-free foods are made (Malvano et al., 2017).

Consequently, developing trustable methods for the detection of gliadin has become a crucial issue so that patients can ensure that the foods they eat do not exceed the levels of gluten that they can ingest, but

* Corresponding author. Department of Electrical, Electronic and Communication Engineering, Public University of Navarra, Ed. Los Tejos, Campus Arrosadía s/n, E-31006, Pamplona, Spain.
*E-mail address:* natxo@unavarra.es (I.R. Matias).

https://doi.org/10.1016/j.foodcont.2023.109624
Received 13 October 2022; Received in revised form 12 December 2022; Accepted 11 January 2023
Available online 12 January 2023
0956-7135/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

above all so that industries have tools to analyze both the raw material and the finished product quickly and reliably. The most versatile and commonly accepted methods for gluten detection are immunological assays, particularly ELISAs (Sajic et al., 2017). The AOAC (Association of Official Agricultural Chemistry) has approved a sandwich assay which uses anti ω-gliadin antibodies (Codex Alimentarius Commission, 2019). Moreover, the Codex Alimentarius Commission has approved it as an official method for gluten assays (Hahn, 2020). A limit of detection of gliadin of 0.5 ppm has been reached by a competitive ELISA that uses HRP-labeled G12 antibodies when testing hydrolyzed food. (Garcia--Calvo et al., 2021). Chromatography (Li et al., 2019) and mass spectrometry (Boukid et al., 2019) are among the non-immunological methods that are also based on the detection of gluten proteins. On the other hand, there are studies using PCR for gliadin detection in food products, being their main weakness the degradation of DNA after cooking process (Ahmed & Meng, 2019). Another technology that has been applied to gluten quantification is the electronic tongue (e-tongue) (Peres et al., 2011), achieving LOD values as low as 0.005 mg $kg^{-1}$ of gliadin (Daikuzono et al., 2017). These analytical devices that have been developed during the last decades use arrays of non-selective chemical sensors and pattern recognition methods to recognize and quantify composition of simple and complex solutions (Jiang et al., 2018).

In this sense, biosensor devices can be a useful tool to decentralize food analysis with sufficient precision and simplicity in real time and at low cost. Regarding optical biosensors, a highly sensitive SPR biosensor for the detection of gluten peptides in urine was reported as a promising method for gluten free diet monitoring (Soler et al., 2016). More recently, a nanostructured microarray platform based on LSPR was developed for gliadin detection with a LOD of 0.0075 ppm (Casari Bariani et al., 2022).

As it is well-known, Lossy Mode Resonances (LMR) based optical sensors have arised as a promising sensing technology (Del Villar and Matías, 2021). This phenomenon occurs when an optical substrate is coated with certain metal oxides and polymers. LMR-based optical fiber structures have been used to fabricate label-free refractometers with high sensitivity and adaptability (Arregui et al., 2016) (P. Zubiate et al., 2015). Moreover, LMR-based optical fiber sensors have been used for biosensing applications such as immunoglobulin G detection at femto-molar concentration in human serum (Chiavaioli et al., 2018), the development of a C-reactive protein (CRP) aptasensor which can be regenerated (P. Zubiate et al., 2017) and the detection of D-dimer, an essential diagnostic biomarker for venous thromboembolism (VTE) (Pablo Zubiate et al., 2019). Also, in the field of food safety, studies based on LMRs have recently been found (Jyoti & Verma, 2022). Furthermore, LMR-based devices developed on planar waveguides have reported as an alternative to optical fiber substrate (Fuentes et al., 2019) (Fuentes et al., 2020) (Dominguez et al., 2021).

Planar waveguides have also proved to be easy to manipulate, cheap and robust structures for the development of LMR based biosensors (Benitez et al., 2022). On the other hand, microfluidic devices have several advantages such as miniaturization, low sample volume required, fast response times, and the possibility to avoid cross-contamination (Preetam et al., 2022). From this perspective, a planar technology device as the one that is proposed, could be more suitable for industries to test gluten content in raw materials and even the processed products to guarantee consumers safety.

An LMR based microfluidic biosensor is proposed for gliadin detection, the main protein responsible for activating the immune system to produce autoantibodies in celiac disease. The first part of the manuscript describes the device fabrication process, which includes the resonance generation by coating the surface with a TiO2 thin-film and the bio-functionalization step as well as the used setup followed by the analysis of the sensor behavior for different gliadin concentrations, specificity test and concluding with the detection of gliadin in commercial flour samples.

## 2. Material and methods

### 2.1. Chemical and biological reagents

Methacrylic acid/methacrylate copolymer (Eudragit L100), purchased from Evonik Health Care. 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Thermo Fisher Scientific. Ethanol (EtOH), phosphate-buffered-saline (PBS, 10 mM, pH 7.4), bovine serum albumin (BSA), chicken egg white albumin, gliadin from wheat and anti-gliadin antibody (AGA) were purchased from Sigma Aldrich Inc.

### 2.2. LMR chip fabrication and experimental setup

A soda-lime glass coverslip was coated with a thin film of $TiO_2$ by Atomic Layer Deposition (ALD) following the protocols detailed in (Benitez et al., 2022). When coating with a metal oxide, the necessary conditions are created for resonance to be generated.

The experimental setup is described in Fig. 2. Light is transmitted from a TAKHI-HP tungsten-halogen broadband source (Pyroistech S.L.) through a multimode optical fiber (Ocean Optics, 200/225 μm of core/cladding diameter). This fiber is in alignment with a lateral of the

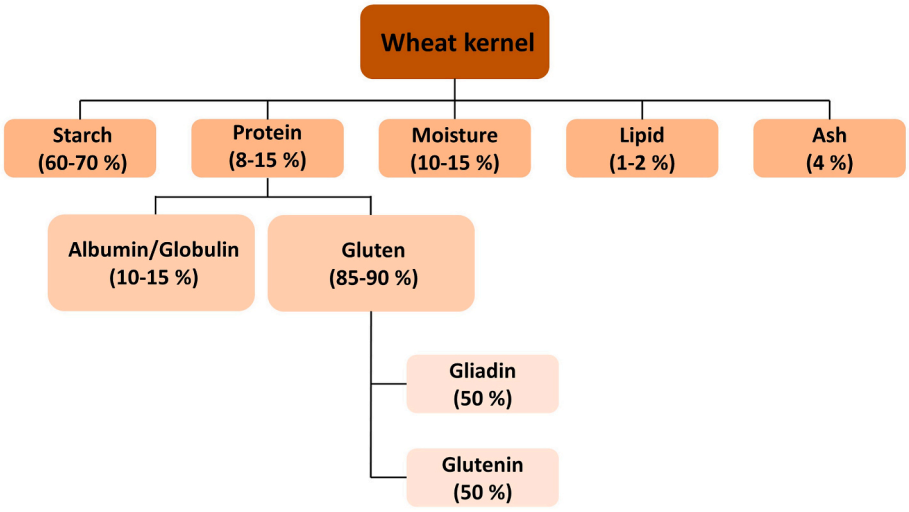


**Fig. 1.** Nutrients composition of a wheat grain. Gliadin supposes half the composition of the protein part corresponding to gluten.

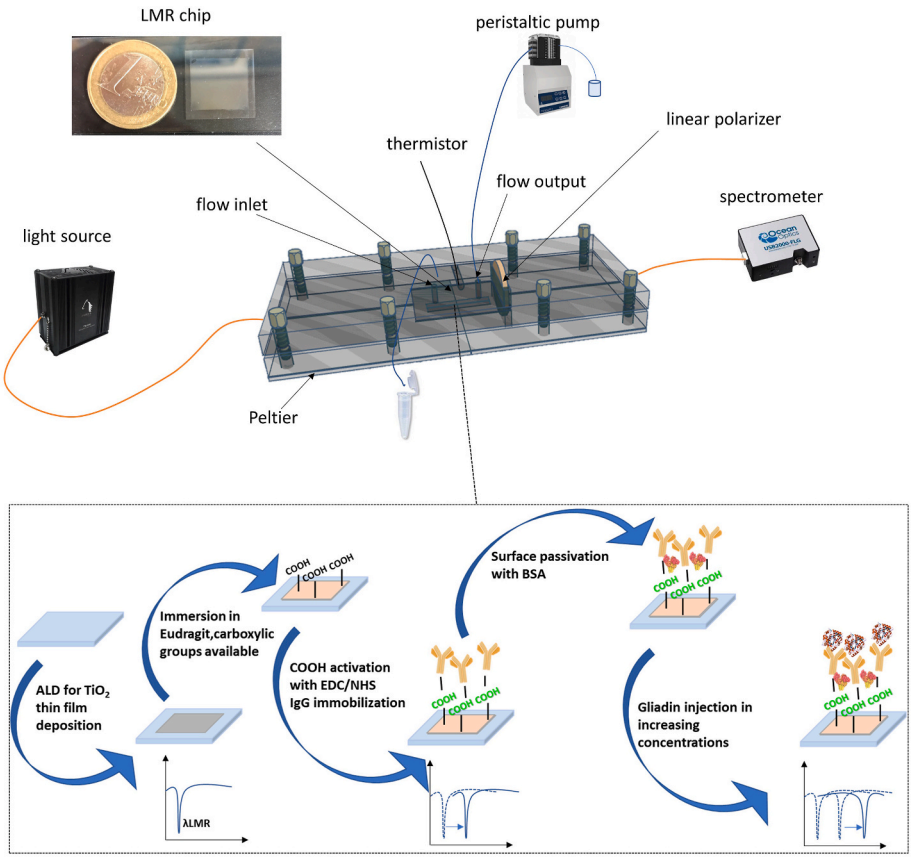


**Fig. 2.** Schematic representation of the experimental setup and the microfluidic cell. Inset: Step-by-step sketch of the sensor fabrication, biofunctionalization and gliadin detection.

coverslip. The output light of the waveguide passes through a polarizer to obtain resonance in TM polarization and then received by another multimode fiber, that is connected to a spectrometer USB2000 FLG (Ocean Optics Inc.), which operates in the visible-NIR wavelength range (400–1000 nm).

The chip was placed into a PMMA microfluidic cell. A temperature control system is used to keep this parameter at 26 °C during the biological assays (Chiavaioli et al., 2014). The microfluidic cell was connected to a peristaltic pump.

### 2.3. Biofunctionalization and detection assay

The AGA immobilization on the sensor surface was carried out by covalent binding between amino terminals that are present in antibodies and carboxylic groups provided by the Eudragit L100 (0.04% w/v in ethanol), a copolymer with which the surface is previously coated. The carboxylic groups were activated by injecting an EDC/NHS solution (2 mM/5 mM) (Sam et al., 2010) (Pablo Zubiate et al., 2019). After that, the AGAs (100 ppm in PBS) were injected during an hour. To passivate the surface, BSA (1% w/v) was flowed for 15 min, followed by an ultrapure water washing.

Five gliadin solutions were prepared (1) 0.1 ppm, (2) 1 ppm, (3) 10 ppm, (4) 50 ppm, and (5) 100 ppm, using ultrapure water supplied by a Barnstead nanopure Diamond water purification system with resistivity of 18 MΩ/cm. Solutions were incubated overnight while stirring at room temperature and then filtered using 0.45 μm PTFE filters (Acrodisc).

For the detection assay, each gliadin solution was flowed for 30 min, from lower to higher concentration, with an intermediate step of ultrapure water washing. The layers that make up the biosensor are shown in Fig. 2 (inset).

Specificity was evaluated by conducting all the AGAs immobilization steps and then injecting chicken egg white albumin diluted in PBS (100 ppm), which is the main protein of egg. During this experiment, the LMR wavelength shift was monitored.

### 2.4. Commercial flour samples testing

Seven commercial flours, some of them labeled gluten-free (GF), were purchased from a grocery store to test their gliadin content: wheat, buckwheat (GF), chickpea (GF), rice (GF), oats, corn (GF) and spelt flour. Each flour was diluted in ultrapure water at a concentration of 1 mg/ml. All solutions were incubated for 1 h under stirring and filtered with 0.45 μm PTFE filters. The AGAs functionalized sensor was used to test these samples and to check if the obtained results agreed with product information.

## 3. Results and discussion

The LMR generated with the deposition of the $TiO_2$ thin film is used to detect gliadin/AGA interactions. When the protein is captured by the bioreceptor, both the thickness of the coating and the effective RI of the sensing layer change, which can be accurately assess by detecting the resonance shift.

### 3.1. Detection of gliadin in ultrapure water

In order to demonstrate the ability of the LMR based microfluidic biosensor for a label-free detection of gliadin, the response to different concentrations of protein in ultrapure water is evaluated. For this purpose, the first step is to biofunctionalize the sensor surface with the capture antibody. AGAs immobilization process is monitored in real time and the sensor response is represented in Fig. 3 (a). After the

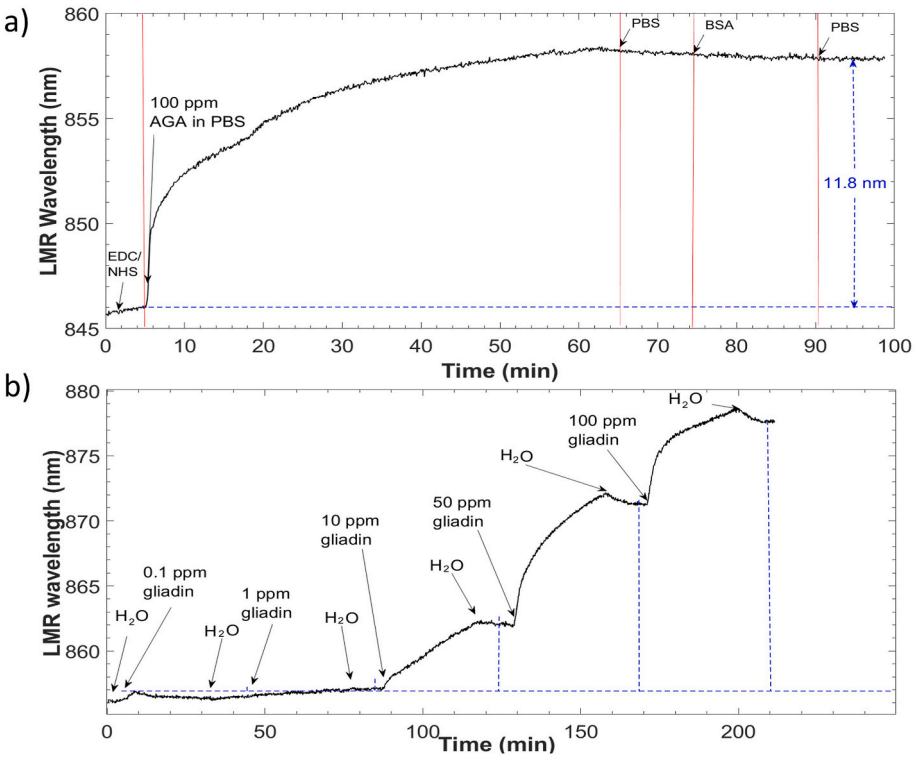


**Fig. 3.** Monitorization of the LMR position during experiments: a) LMR wavelength during AGA immobilization. b) Sensorgram obtained from the detection of different Gliadin solutions starting from the baseline (blank in ultrapure water) up to 100 ppm of protein.

carboxylic groups activation, AGAs in PBS buffer is circulated through the system, which produces an increase in the resonance wavelength. After an hour it is observed that the curve starts to stabilize, and this behavior remains after PBS washing with a total shift of 11.8 nm.

Considering that ultrapure water has been chosen as buffer for gliadin detection, and that the immobilization process is carried out using PBS buffer, to set the baseline in water is necessary before starting the gliadin detection assay. Therefore, all the gliadin measurements are carried out at pH 4.

The sensorgram shown in Fig. 3 (b) describe how the LMR central position changes over time, with respect to the baseline ($H_2O$), when gliadin solutions in the range of 0.1–100 ppm are circulated. The thickness of the structure deposited on the coverslip increases when gliadin adheres to the AGAs available on the surface of the sensor, what makes the LMR moves to longer wavelengths (Del Villar & Matías, 2021). The resonance wavelength position after washing with water is related to the concentration of gliadin that has flowed through the system.

Three repetitions of the experiment under the same criteria (n = 3) are considered to obtain the calibration curve that is shown in Fig. 4, which relates the resonance shift to each gliadin concentration. The Hill function is used to fit the experimental data and a correlation coefficient $R^2$ of 0.999 is obtained. The limit of detection (LOD) is calculated as indicated in literature (Chiavaioli et al., 2017) and the value achieved is 0.05 ppm, considering $\sigma_{blank}$ = 0.016 nm. This value is in the order of magnitude of those obtained in other methods recently reported for gluten detection (Svigelj et al., 2022). On the other hand, the sensitivities have been calculated from the slope of the tangent at the different concentrations using the first derivative of the Hill approximation and the values are 1.35, 0.60, 0.26, 0.15 and 0.11 nm/ppm for 0.1, 1, 10, 50

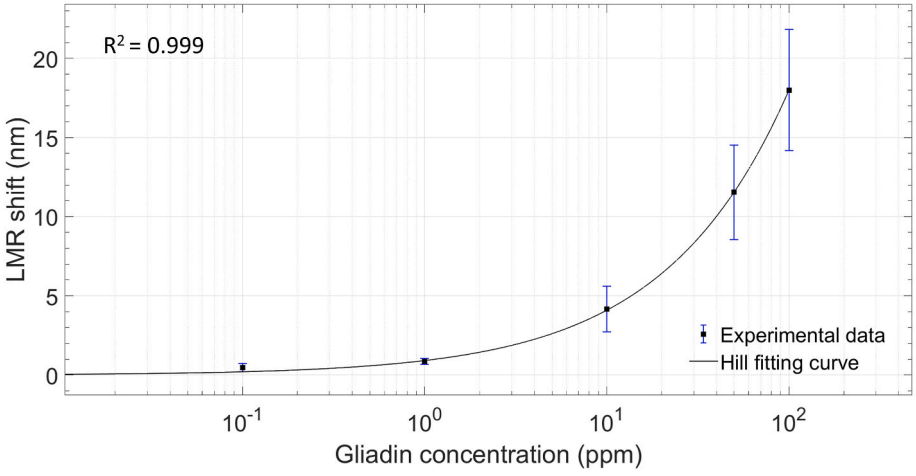


**Fig. 4.** Calibration curve of the gliadin biosensor, obtained from three identical and independent devices.

and 100 μg/ml respectively. This means that in the best case (concentration of 0.1 ppm), the spectrometer used in this work with a 0.38 nm resolution can distinguish concentrations with a difference of ±0.14 ppm of gliadin. The utilization of better resolution equipment and the improvement of the device sensitivity would allow us to improve the accuracy of the detection.

The average of the response time calculated for gliadin detection with the LMR based microfluidic biosensor is 20 min. This value depends on the antigen concentration since for gliadin concentrations from 0.1 to ppm the binding reaction takes between 25 and 30 min whereas for concentrations from 50 to 100 ppm the antibody-antigen binding takes between 11 and 18 min. Therefore, the proposed sensor shows a faster response than the approved immunoassays.

Finally, a specificity assay is conducted by injecting a 100 ppm solution of chicken egg white albumin in PBS followed by a gliadin in water solution at the same concentration and comparing the sensor response. This protein commonly appears in processed foods, which makes it a viable candidate to verify the specificity of the gliadin sensor. As shown in Fig. 5, the baseline set to measure the LMR shift due to albumin is PBS buffer whereas the one set for gliadin is ultrapure water. As observed, the shift produced by the interaction of the sensor with the chicken albumin is 0.24 nm, which is negligible if compared to the 11.8 nm shift that occurs when gliadin is injected, considering that both solutions have the same protein concentration. This behavior indicates that there is no binding interaction between albumin and AGAs that can interfere with the biosensor specificity to gliadin.

### 3.2. Detection of gliadin in commercial flour samples

Commercial flour samples were tested with the LMR based microfluidic gliadin biosensor and results are shown in Fig. 6. Considering the approximate nutrient composition of wheat detailed in Fig. 1, a cut-off value of 5.5 nm was estimated for the maximum wavelength shift produced by a sample that contains less than 20 ppm of gluten. Therefore, an LMR shift lower than the cut-off value is expected when the sensor is exposed to flour samples labeled gluten-free. In the case of cereal flour samples that are inherently gluten-free but have not been labeled as such, a wavelength shift below or above the threshold is acceptable, as the product may or may not be contaminated.

As shown, among the GF labeled flour samples, the highest LMR shift occurred when the sensor is exposed to corn flour. However, it is still situated within the range determined for GF samples. In the case of oat and spelt flour samples, which have not been labeled as GF, the wavelength shifts are lower than the one produced by corn flour, but they exceed the LMR shifts produced by the rest of the GF samples. Regarding the sensor response to wheat flour sample, a resonance shift of 12.6 nm was registered, which duplicates the cut-off value and indicates that the system was able to detect the gliadin naturally present in wheat flour. The results obtained in this assay were in good agreement with the expected behavior for all the analyzed flour samples.

On the other hand, the applied method uses a single recognition element, which means that it is not necessary for the protein to be in its intact form, unlike what occurs in sandwich assays.

Hence, it is possible to detect gluten content even in samples where the protein is in the hydrolyzed form (Svigelj et al., 2022). In this sense, the detection of gliadin in processed food samples employing the proposed system could be explored in future works.

Some features of the gliadin LMR based immunosensor are summarized in Table 1, as well as those corresponding to others commercially and not commercially available methods for gliadin immunodetection. Although only sandwich ELISA-based kits have been FDA and Codex-cleared for the detection of gluten, they fail to detect partially hydrolyzed protein in samples and there is risk of losing the antibody reactivity in ELISA tests when heat-processed food samples are analyzed. There is other approach available, as the competitive ELISA shown in Table 1, which has proved its utility when hydrolyzed samples are tested. However, this method has not been recommended by any organization for gluten detection. In the case of the immunosensor proposed in this work, it has both limit of detection and response time lower than the ELISA kits that appear below. Moreover, because it uses a label-free approach, it has potential to detect gliadin in processed or hydrolyzed food samples. Another study that has obtained lower LOD than ELISAs and also uses label-free detection, is the LSPR based immunosensor included in the table. Nevertheless, LMR based technology offers greater versatility in terms of manufacturing and materials that allow the phenomenon to be generated.

From the results below, the fabricated LMR based immunosensor is a good and competitive device when compared with previous works in the field of immunodetection methods, specifically with those that employ an optical approach, and presents a promising opportunity of improvement for future works.

## 4. Conclusions

An LMR based microfluidic gliadin biosensor has been fabricated and characterized. Planar waveguide LMR based sensors have proven to be manageable, cost-effective, and robust substrates that, integrated into a microfluidic platform, makes the proposed system suitable for fast and reliable gluten quantification by industries, to guarantee consumer safety. The resonance was generated by depositing a $TiO_2$ thin film onto

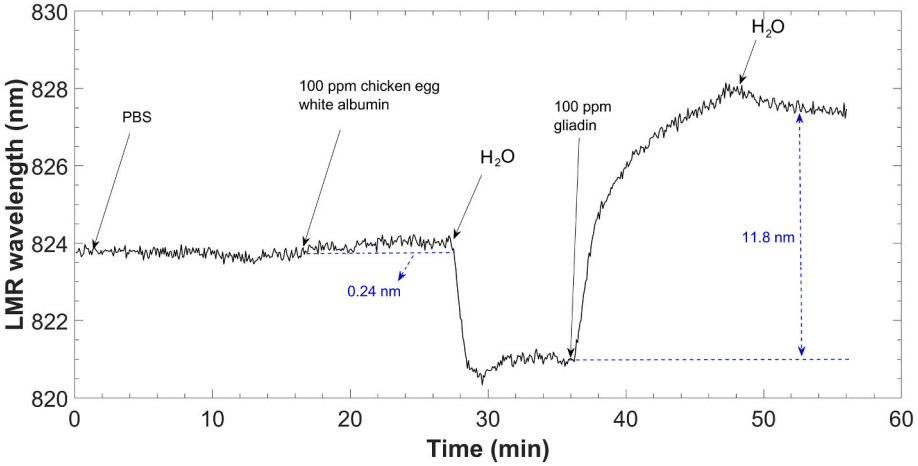


**Fig. 5.** Specificity test sensorgram with the LMR wavelength shift during injection of 100 ppm of chicken egg albumin in PBS and 100 ppm of gliadin in ultrapure water.

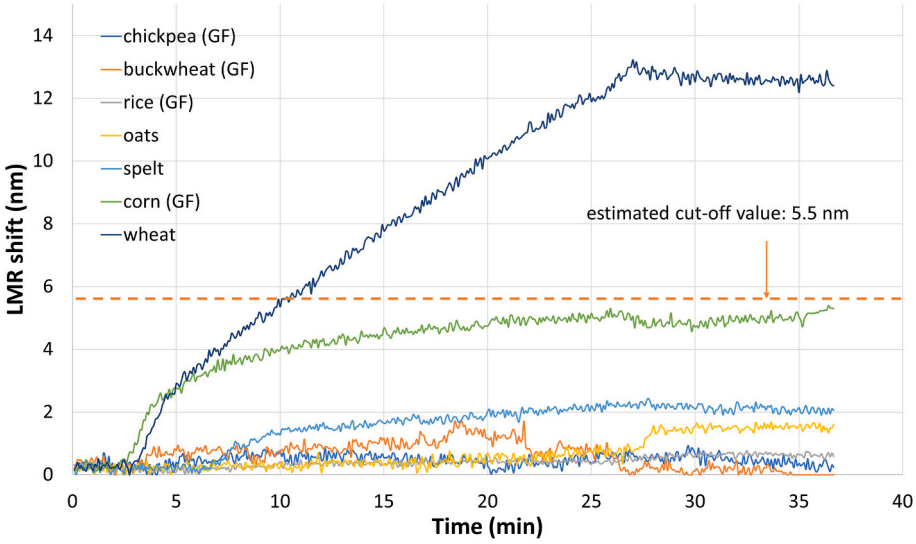


**Fig. 6.** Sensorgram of gliadin detection in commercial flour samples with the estimated cut-off value of the wavelength shift for being labeled as gluten-free.

**Table 1**
Comparative table of the results obtained with the LMR based immunosensor and with other gliadin immunodetection methods.

| | LoD (ppm) | Response time | Suitable for hydrolyzed food | Reference |
|---|---|---|---|---|
| **RIDASCREEN competitive ELISA** | 1.36 | 40 min | Yes | Osorio et al. (2019) |
| **RIDASCREEN Sandwich ELISA** | 3 | 1.5 h | No | Valdés et al. (2003) |
| **Veratox Sandwich ELISA** | n/a | 30 min | No | Osorio et al. (2019) |
| **Skerrit mAb Sandwich ELISA** | 1 | 30 min | No | Skerritt and Hill (1990) |
| **DQ2.5- glia-α3 competitive ELISA** | 2.9 | n/a | Yes | Sajic et al. (2017) |
| **LSPR immunosensor** | 0.0075 | n/a | Yes | Casari Bariani et al. (2022) |
| **LMR based immunosensor** | 0.05 | 20 min | Yes | This work |

a coverslip and the Eudragit L100 copolymer was used to immobilize the bioreceptor on the chip by covalent bond. In particular, the fabricated devices can detect concentrations of gliadin of 0.05 ppm in ultrapure water, a value that is comparable to other methods that have been reported recently for gluten detection. Although the accuracy of the detection with the current equipment is ±0.14 ppm of gliadin for a sensitivity of 1.35 nm/ppm, it could be improved in future works. The proposed biosensor shows a response time of 20 min, which overcomes the limitations of the well-established ELISA assays in terms of long response time. Furthermore, the sensor can detect gliadin in commercial flour samples, although gliadin detection in processed foods should be studied. In addition, the gliadin biosensor shows specificity to this analyte and negligible interaction with other target molecules was observed.

## Author Contributions

"Conceptualization, M.B., P.Z., A.B.S-L and I.R.M.; methodology, M.B., P.Z., software, M.B., P.Z.; validation, M.B., P.Z.; formal analysis, M.B., P.Z.; investigation, M.B.; resources, M.B.; data curation, M.B., P.Z.; writing—original draft preparation, M.B.; writing—review and editing, M.B., P.Z., A.B.S-L and I.R.M.; visualization, M.B; supervision, P.Z., A.B.S-L and I.R.M.; project administration, A.B.S-L and I.R.M.; funding acquisition, A.B.S-L and I.R.M. All authors have read and agreed the current version of the manuscript."

## Data availability

No data was used for the research described in the article.

## Acknowledgements

Authors would like to thank the partial funding by the Spanish Agencia Estatal de Investigacion (AEI) with references PID2019-106070RB-I00 and also by the Public University of Navarra, with the project BIOFOCEL (ref. PJUPNA2033) and a pre-doctoral research grant.

## References

Ahmed, N., & Meng, M. (2019). Detection of gluten-rich cereals in processed foods with enhanced sensitivity by targeting mitochondrial DNA using PCR. *Food Analytical Methods, 12*(3), 811–825.

Arregui, F. J., Del Villar, I., Zamarreño, C. R., Zubiate, P., & Matias, I. R. (2016). Giant sensitivity of optical fiber sensors by means of lossy mode resonance. *Sensors and Actuators, B: Chemical, 232*, 660–665. https://doi.org/10.1016/j.snb.2016.04.015

Balakireva, A. V., & Zamyatnin, A. A. (2016). Properties of gluten intolerance: Gluten structure, evolution, pathogenicity and detoxification capabilities. *Nutrients, 8*(10). https://doi.org/10.3390/nu8100644

Bascuñán, K. A., Vespa, M. C., & Araya, M. (2017). Celiac disease: Understanding the gluten-free diet. *European Journal of Nutrition, 56*(2), 449–459.

Benitez, M., Zubiate, P., Villar, I. D., Socorro-ler, B., & Mat, I. R. (2022). *Lossy Mode resonance based microfluidic platform developed on planar waveguide for biosensing applications*.

Boukid, F., Prandi, B., Faccini, A., & Sforza, S. (2019). A complete mass spectrometry (MS)-Based peptidomic description of gluten peptides generated during in vitro gastrointestinal digestion of durum wheat: Implication for celiac disease. *Journal of the American Society for Mass Spectrometry, 30*(8), 1481–1490. https://doi.org/10.1007/s13361-019-02212-8

Brouns, F. J. P. H., van Buul, V. J., & Shewry, P. R. (2013). Does wheat make us fat and sick? *Journal of Cereal Science, 58*(2), 209–215. https://doi.org/10.1016/j.jcs.2013.06.002

Caio, G., Volta, U., Sapone, A., Leffler, D. A., De Giorgio, R., Catassi, C., & Fasano, A. (2019). Celiac disease: A comprehensive current review. *BMC Medicine, 17*(1), 1–20.

Casari Bariani, G., Zhou, L., Poggesi, S., Manzano, M., & Ionescu, R. E. (2022). Patterning large-scale nanostructured microarrays on coverslip for sensitive plasmonic detection of aqueous gliadin traces. *Chemosensors, 10*(2). https://doi.org/10.3390/chemosensors10020038

Chiavaioli, F., Gouveia, C. A. J., Jorge, P. A. S., & Baldini, F. (2017). Towards a uniform metrological assessment of grating-based optical fiber sensors: From refractometers to biosensors. *Biosensors, 7*(2). https://doi.org/10.3390/bios7020023

Chiavaioli, F., Trono, C., Giannetti, A., Brenci, M., & Baldini, F. (2014). Characterisation of a label-free biosensor based on long period grating. *Journal of Biophotonics, 7*(5), 312–322. https://doi.org/10.1002/jbio.201200135

Chiavaioli, F., Zubiate, P., Del Villar, I., Zamarrenìo, C. R., Giannetti, A., Tombelli, S., Trono, C., Arregui, F. J., Matias, I. R., & Baldini, F. (2018). Femtomolar detection by nanocoated fiber label-free biosensors. *ACS Sensors, 3*(5), 936–943. https://doi.org/10.1021/acssensors.7b00918

Codex Alimentarius Commission. (2019). *Review of cereals, pulses and legumes methods, joint FAO/WHO food standards programme Codex committee on methods of analysis and sampling*. Budapest: 40th Session.

Daikuzono, C. M., Shimizu, F. M., Manzoli, A., Riul, A., Piazzetta, M. H. O., Gobbi, A. L., Correa, D. S., Paulovich, F. V., & Oliveira, O. N. (2017). Information visualization and feature selection methods applied to detect gliadin in gluten-containing foodstuff with a microfluidic electronic tongue. *ACS Applied Materials and Interfaces, 9*(23), 19646–19652. https://doi.org/10.1021/acsami.7b04252

Del Villar, I., & Matías, I. R. (Eds.). (2021). *Optical fiber sensors: Fundamentals for development of optimized devices*. Hoboken, New Jersey: Wiley-IEEE Press, ISBN 978-1-119-53476-1.

Dominguez, I., Del Villar, I., Fuentes, O., Corres, J. M., & Matias, I. R. (2021). Dually nanocoated planar waveguides towards multi-parameter sensing. *Scientific Reports, 11*(1), 1–8.

European Commission. (2014). Commission implementing regulation (EU) No 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. *Official Journal of the European Union, 228*, 5–8.

Falcomer, A. L., Santos Araújo, L., Farage, P., Santos Monteiro, J., Yoshio Nakano, E., & Puppin Zandonadi, R. (2020). Gluten contamination in food services and industry: A systematic review. *Critical Reviews in Food Science and Nutrition, 60*(3), 479–493. https://doi.org/10.1080/10408398.2018.1541864

Fuentes, O., Del Villar, I., Corres, J. M., & Matias, I. R. (2019). Lossy mode resonance sensors based on lateral light incidence in nanocoated planar waveguides. *Scientific Reports, 9*(1), 1–10. https://doi.org/10.1038/s41598-019-45285-x

Fuentes, O., Goicoechea, J., Corres, J. M., Villar, I. D., Ozcariz, A., & Matias, I. R. (2020). Generation of lossy mode resonances with different nanocoatings deposited on coverslips. *Optics Express, 28*(1), 288. https://doi.org/10.1364/oe.28.000288

Garcia-Calvo, E., García-García, A., Madrid, R., Martin, R., & García, T. (2021). From polyclonal sera to recombinant antibodies: A review of immunological detection of gluten in foodstuff. *Foods, 10*(1). https://doi.org/10.3390/foods10010066

Hahn, S. M. (2020). Food labeling; gluten-free labeling of fermented or hydrolyzed foods. *Rules and Regulations, 85*(157), 25–64. https://doi.org/10.4324/9781315164274-2

Jiang, H., Zhang, M., Bhandari, B., & Adhikari, B. (2018). Application of electronic tongue for fresh foods quality evaluation: A review. *Food Reviews International, 34*(8), 746–769. https://doi.org/10.1080/87559129.2018.1424184

Jyoti, K., & Verma, R. K. (2022). Selective detection of urea as milk adulterant using LMR based Fiber Optic Probe. *Journal of Food Composition and Analysis, 114*(April), Article 104825. https://doi.org/10.1016/j.jfca.2022.104825

Li, H., Bose, U., Stockwell, S., Howitt, C. A., & Colgrave, M. (2019). Assessing the utility of multiplexed liquid chromatography-mass spectrometry for gluten detection in Australian Breakfast food products. *Molecules, 24*(20), 1–14. https://doi.org/10.3390/molecules24203665

Malvano, F., Albanese, D., Pilloton, R., & Di Matteo, M. (2017). A new label-free impedimetric aptasensor for gluten detection. *Food Control, 79*, 200–206. https://doi.org/10.1016/j.foodcont.2017.03.033

Osorio, C. E., Mejías, J. H., & Rustgi, S. (2019). Gluten detection methods and their critical role in assuring safe diets for celiac patients. *Nutrients, 11*(12), 17–20. https://doi.org/10.3390/nu11122920.

Peres, A. M., Dias, L. G., Veloso, A. C. A., Meirinho, S. G., Morais, J. S., & MacHado, A. A. S. C. (2011). An electronic tongue for gliadins semi-quantitative detection in foodstuffs. *Talanta, 83*(3), 857–864. https://doi.org/10.1016/j.talanta.2010.10.032

Preetam, S., Nahak, B. K., Patra, S., Toncu, D. C., Park, S., Syväjärvi, M., Orive, G., & Tiwari, A. (2022). Emergence of microfluidics for next generation biomedical devices. *Biosensors and Bioelectronics, X*, 10. https://doi.org/10.1016/j.biosx.2022.100106

Sajic, N., Oplatowska-Stachowiak, M., Streppel, L., Drijfhout, J. W., Salden, M., & Koning, F. (2017). Development and in-house validation of a competitive ELISA for the quantitative detection of gluten in food. *Food Control, 80*, 401–410. https://doi.org/10.1016/j.foodcont.2017.05.021

Sam, S., Touahir, L., Salvador Andresa, J., Allongue, P., Chazalviel, J. N., Gouget-Laemmel, A. C., De Villeneuve, C. H., Moraillon, A., Ozanam, F., Gabouze, N., & Djebbar, S. (2010). Semiquantitative study of the EDC/NHS activation of acid terminal groups at modified porous silicon surfaces. *Langmuir, 26*(2), 809–814. https://doi.org/10.1021/la902220a

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security, 4*(3), 178–202. https://doi.org/10.1002/FES3.64.

Skerritt, J. H., & Hill, A. S. (1990). Monoclonal antibody sandwich enzyme immunoassays for determination of gluten in foods. *Journal of Agricultural and Food Chemistry, 38*(8), 1771–1778. https://doi.org/10.1021/jf00098a029.

Soler, M., Estevez, M. C., de Moreno, M. L., Cebolla, A., & Lechuga, L. M. (2016). Label-free SPR detection of gluten peptides in urine for non-invasive celiac disease follow-up. *Biosensors and Bioelectronics, 79*, 158–164. https://doi.org/10.1016/j.bios.2015.11.097

Svigelj, R., Zuliani, I., Grazioli, C., Dossi, N., & Toniolo, R. (2022). An effective label-free electrochemical aptasensor based on gold nanoparticles for gluten detection. *Nanomaterials, 12*(6). https://doi.org/10.3390/nano12060987

Thompson, T., Lee, A. R., & Grace, T. (2010). Gluten contamination of grains, seeds, and flours in the United States: A pilot study. *Journal of the American Dietetic Association, 110*(6), 937–940. https://doi.org/10.1016/j.jada.2010.03.014

Valdés, I., García, E., Llorente, M., & Méndez, E. (2003). Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol. *European Journal of Gastroenterology & Hepatology, 15*(5). https://journals.lww.com/eurojgh/Fulltext/2003/05000/Innovative_approach_to_low_level_gluten.2.aspx.

Verma, A. K., Gatti, S., Galeazzi, T., Monachesi, C., Padella, L., Del Baldo, G., Annibali, R., Lionetti, E., & Catassi, C. (2017). Gluten contamination in naturally or labeled gluten-free products marketed in Italy. *Nutrients, 9*(2), 1–10. https://doi.org/10.3390/nu9020115

Zubiate, P., Urrutia, A., Zamarreño, C. R., Egea-Urra, J., Fernández-Irigoyen, J., Giannetti, A., Baldini, F., Díaz, S., Matias, I. R., Arregui, F. J., Santamaría, E., Chiavaioli, F., & Del Villar, I. (2019). Fiber-based early diagnosis of venous thromboembolic disease by label-free D-dimer detection. *Biosensors and Bioelectronics X, 2*(August), Article 100026. https://doi.org/10.1016/j.biosx.2019.100026

Zubiate, P., Zamarreño, C. R., Del Villar, I., Matias, I. R., & Arregui, F. J. (2015). High sensitive refractometers based on lossy mode resonances (LMRs) supported by ITO coated D-shaped optical fibers. *Optics Express, 23*(6), 8045. https://doi.org/10.1364/oe.23.008045

Zubiate, P., Zamarreño, C. R., Sánchez, P., Matias, I. R., & Arregui, F. J. (2017). High sensitive and selective C-reactive protein detection by means of lossy mode resonance based optical fiber devices. *Biosensors and Bioelectronics, 93*, 176–181. https://doi.org/10.1016/j.bios.2016.09.020

**Melanys Benítez** received her MS degree in Biomedical Engineering (2016) and the master's degree in Biomedical Engineering (2021) from the Higher Polytechnic Institute Jose Antonio Echeverría (ISPJAE) and the Public University of Navarra (UPNA), respectively. She is currently pursuing the Ph.D. degree with the Department of Electrical, Electronic and Communication Engineering, Public University of Navarra (UPNA). Her current research interests include optical biosensors and microfluidics.

**Pablo Zubiate** received his MS degree in Electrical and Electronic Engineering, the master's degree in communications and his Ph.D. degree in 2013, 2015 and 2018, respectively from the Public University of Navarra (UPNA). He has been working as visiting scientist at the school of Engineering of the University of East Anglia (Norwich, England) in 2018. Since 2019, he has been a postdoctoral position at Public University of Navarra. His research interest includes optical fiber sensors and nanostructured materials.

**Abián B. Socorro-Leránoz** is a Telecommunications Engineer (2010), M.Sc. in Biomedical Engineering (2012) and Ph.D. in Engineering (2015) from the Public University of Navarre (PUN-UPNA), Pamplona, Navarre, Spain. He was at the Armani Research Lab (University of Southern California, Los Angeles, CA, USA) as a visiting Ph.D. student in 2014. Currently, he is working as Lecturer at PUN/UPNA, where he has published more than 75 scientific contributions both in JCR journals and conferences and he has been involved in more than 20 competitive research projects with both public and private funding. Most of them have been related to the development of optical fiber sensors and their application to biomedicine. His research interests are the development of photonic sensors (optical fiber sensors mainly), including biosensors and, in general, the development of biomedical applications based on photonics.

**Ignacio R. Matias** received the M.S. degree in Electrical and Electronic Engineering and his Ph.D. degree in Optical Sensors from the Polytechnic University of Madrid (UPM), Madrid, Spain, in 1992 and 1996, respectively. He became a Lecturer at the Public University of Navarra (Pamplona, Spain) in 1998, where he is a Full Professor from 2006. He has co-authored more than 250 journal and 300 conference papers related to optical sensors for different applications.