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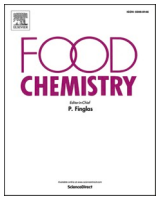

Analytical Methods

Colorimetric analysis platform based on thin layer chromatography for monitoring gluten cross-contamination in food industry

A. Martinez-Aviño, Y. Moliner-Martinez *, C. Molins-Legua, P. Campins-Falcó

MINTOTA Research Group, Departament de Química Analítica, Facultat de Química, Universitat de Valencia, 46100 Burjassot, Spain

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ABSTRACT

Monitoring of the accidental presence of gluten (Glu), resulting from cross-contamination, is imperative in different industries, in particular food industry. The objective of this study was the development of an analytical platform utilizing thin-layer chromatography (TLC) with colorimetric read-out for making binary (yes/no) decisions on surfaces and/or point of these industries. The composition of the extractive phase was optimized with commercial products used in cleaning processing lines. Subsequently, an exploration of TLC separation and detection was undertaken. CN-modified nanosilica plates and 30:70 acetonitrile:water were used to achieve a selective signal for Glu residues. The study of the detection performance showed that both spectroscopic measurement and image analysis were resulted in satisfactory results for quantitate analysis (RSD = 5 %, LOD = 0.12 mg). The practical application of the proposed methodology on surfaces of the food processing lines. This work demonstrated the operational feasibility in detecting gluten cross-contaminations within the food processing industry.

1. Introduction

A crucial food safety issue in food industry is the safeguarding of consumers to food containing target allergens, and in particular, to gluten (Glu). Glu is a mixture of proteins, mainly gliadins and gluteins, present in wheat, barley, rye oats and related grains. The consumption of this compound is associated with celiac disease (CD), a chronic autoimmune disorder that induces an inflammatory response resulting in different symptomatic manifestations such as gastrointestinal problems, nutrient deficiencies, anaemia, reduced bone density, dermatitis and liver and biliary disease, among others (Scherf et al., 2020). CD affects a percentage of 0.6 to 1 % of the world population, however, these values could be higher taking into account the underdiagnose of the disease (Falcomer et al., 2020).

Despite the high prevalence of CD, the only treatment consists of a gluten-free diet (GFD) to ensure the symptoms minimization, antibodies control and recovery of the normal intestinal activity. With this aim, notification of Glu content is crucial in food labelling for individual protection. Indeed, Codex Standard established a maximum of 20 mg Kg^{-1} for food labelled as "gluten-free". However, food cross-contamination can accidentally occur as a consequence of inadequate procedures in food processing industries (Parsons et al., 2021). Some examples are the shared use of production or packaging lines and their inadequate cleaning in companies that generate products containing gluten, which would result in the inadvertent presence of this allergen in gluten-free products (Atasoy et al., 2020; Fuciños et al., 2019). Despite the relevance of this cross-contamination, very few methods devoted to identify the presence of Gluten contamination in critical points of food processing industry have been reported. From the perspective of the food processing industry, analytical strategies to determine Glu cross-contamination must fit within the concept of user-friendly analytical devices taking into account mainly four aspects: analytical, environmental, cost and operational performance, since these industries need rapid response in real time, cost-effective and easy-handling devices to assess an efficient decision making process.

Basically, the analytical methods proposed for Glu determination are based on immunological devices, such as ELISA kits and genosensors based on DNA-based methods (Panda & Garber, 2019; Rzychon et al., 2017; Taylor et al., 2018; Yu et al., 2021). Both have shown a good performance for Glu detection in food samples, however, they are limited regarding to their application in cross-contamination processes mainly because of operational and economic costs.

On the other hand, proteomics has also been reported to exhibit excellent analytical performance. However, it is evident that in-situ and

* Corresponding author.
E-mail address: Yolanda.moliner@uv.es (Y. Moliner-Martinez).

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real-time measurements are not applicable. In addition, they require high cost instrumentation (Bromilow et al., 2017). Aptamer-based sensors have been recently proposed as an efficient alternative to determine Glu in food samples for point of care allergen testing. Accordingly, optical and electrochemical sensors have been proposed as analytical devices following different strategies (López-López et al., 2017; Pla et al., 2021, Tertis et al., 2023). In both cases, satisfactory results have been obtained, however, instrumentation is still required, hence, limiting the routine analysis.

Nowadays, commercial tests are available for the detection of gluten on food processing surfaces. These tests are primarily based on immunochromatographic assays, mainly enzyme-linked immunosorbent assays (ELISA) in lateral flow formats (Le et al., 2023). Although these tests are widely employed, they have some limitations that have not yet been resolved (Güven et al 2023, Rzychon et al., 2017). Basically, sample pretreatment procedure and compatibility of the extractive phases with the detection system, antibody specificity, need of more than one test to perform quantitative analysis and oversaturation are some of the drawbacks that need to be addressed.

In this context, the development of colorimetric probes, which overcome the limitations of immunoassays, for the detection of Glu through visual inspection and/or semi-quantitative determination in a single test, would be of real interest to the food processing industry. Specifically, it could facilitate routine assessment of the efficacy of cleaning practices to prevent potential cross-contamination (Tsagkaris et al., 2019).

Among the different strategies for Glu determination, thin layer chromatography (TLC) has been resurged as an attractive analytical technique that allows separation of target analytes in complex matrices by using cost effective and high-throughput procedures. TLC can be coupled to more sophisticated detectors such as DART and SERS to perform direct analysis. (Morlock, 2021; Xu & Liu, 2021) However, although the use of these detectors has been proved to provide satisfactory results, they present shortcomings for in situ analysis.

Due to the recent progresses in portable instrumentation and the implementation of smartphones as analytical devices the combination of TLC with colorimetric probes has emerged as a valuable alternative tool to perform in situ analysis. The chromatographic separation avoids false positive and false negative results and detection can be performed by image analysis with portable devices, including smartphones, used as analytical detectors. The chromatographic performance, together with the simplicity in detection, make this strategy especially attractive to develop fast, reliable and cost-effective detection methods, in particular for a binary (yes/no) response previously establishing a cut-off value.

Taking into account these characteristics, our hypothesis was that, Glu detection on surfaces of food industry could be performed by combining TLC with colorimetric detection using copper-based complex compound with bicinchoninic acid as derivatizing reagent. Therefore, the objective of this work was the development of an analytical platform based on thin layer chromatography combined with colorimetric readout to make yes/no decisions in gluten cross-contamination in critical points of a food processing industry. To this end, sampling in different critical points was carried out using cotton swabs and extraction was performed with different compounds used for cleaning-in-place. Chromatographic separation and detection conditions were optimized. The approach is based on Glu detection by derivatization with bicinchoninic acid after separation of gluten in commercially available silica TLC plates, for both qualitative and quantitative estimation. From our knowledge, this strategy could simplify Glu estimation on food industry surfaces, and hence help to avoid involuntary alimentary transgressions derived from undesirable contaminations.

2. Experimental section

2.1. Reagents and materials

Gluten from wheat, gliadin, casein, albumin from chicken egg white, sodium carbonate, sodium bicarbonate and bicinchoninic acid disodium salt hydrate were purchased from Sigma Aldrich (Steinheim, Germany). Betelene USP Plus, Tephex CB54, Dectocide A30, Dectocide CDA, Dectocide CDB, Ultra VF7, Ultra SDN surfactants were obtained from Betelgeux (Gandia, Spain), sodium tartrate, sodium hydroxide and acetonitrile were obtained from VWR Chemicals (Fontenay-sous-Bois, France) copper sulphate pentahydrate was purchased from Merck (Darmstadt, Germany) and ultrapure water type I was obtained using Adrona Connect system (Riga, Letonia).

Standard solutions were prepared by dilution of corresponding weight of analyte in a 1 % Betelene USP Plus solution in water for a concentration range from 2 to 6 $mg{\cdot}mL^{-1}$ of gluten. These solutions were used in order to optimize experimental procedure and to obtain analytical parameters. The reaction solution was prepared mixing 5 mL of a 6.7 mM bicinchoninic acid in carbonate buffer solution with 100 μL of 0.44 M $CuSO_4$ aqueous solution.

Silica gel, silica gel nanoparticles layers (20 x 20 cm) and CN modified nanosilica gel (modified with ciano propyl) and NH_2-modified nanosilica gel layers (3x5 cm) were obtained from Macherey-Nagel (Düren, Germany). All layers used Silica 60 as absorbent, they had a specific surface of 500 $m^2{\cdot}g^{-1}$, 60 Å of pore size, a pore volume of 0.75 $mL{\cdot}g^{-1}$ and thickness of 0.20 mm. Silica gel were of 5–17 μm particle size, Nano silica gel, and CN and NH_2-modified nanosilica gel were of 2–10 μm particle size.

2.2. Equipment

UV–Vis spectra were recorded using a Xiaomi mi8 lite Smartphone (Andriod 9) coupled to a GoSpectro portable spectrophotometer equipped with a diffuse reflectance probe from Goyalab (Bordeaux, France). Halogen lamp of 10 W were used as light source for spectra registering. Digital images were obtained using a Samsung Galaxy A70 smartphone and GIMP program was used to process images in order to obtain RGB parameters (Kim et al., 2017; Martínez-Aviñó et al., 2022). SX 1200 Power Hairdryer diffuser from Philips (Amsterdam, Netherlands) was used to dry silica gel layers.

2.3. Samples

Samples from critical points of different food companies were collected using sterilized cotton swabs for Glu analysis. Samples from cutting and mincing point (S1-S4), Filling and extruder machines (S5-S7), refrigerating and cooling tunnels (S8-S10), conveyor belts (S11-S16) and different devices (S17-S21) were analysed. Sample collection was performed by using sterilized in tube cotton swabs (Deltalab, Spain).

2.4. Methodology

2.4.1. Separation of different proteins by TLC optimization

Standard and samples solutions of gluten, gliadin, albumin and casein were deposited with a Camag 10 μL Syringe (Hamilton, Switzerland) on 3 x 5 cm HPTLC plates using CN modified silica gel as stationary phase. A volume of 1 μL of each standard solution was applied on a plate and dried for 45 s. Application process was repeated 10 times in order to increase signal response. Separation of different proteins was performed using a 30:70 Acetonitrile: H_2O solution as the mobile phase. The plate was developed in a chromatographic chamber using 5 mL of mobile phase by ascending chromatography over 4.5 cm at room temperature (T = 20 °C).

2.4.2. Colorimetric reaction optimization

The plate was sprayed with 5 mL of the copper sulfate bicinchoninic acid derivatization solution and left at room temperature for 15 min. Subsequently, the absorbance was measured by using a smartphone spectrometer (GoSpectro) equipped with a diffuse reflectance probe. RGB parameters were also obtained from digital images taken with the camera of the smartphone following optimized conditions from previous studies (Martínez-Aviño et al., 2022). Briefly, all measurements were performed using a white light box in order to control illumination conditions. Spectral analysis was performed placing the TLC plates at 1 cm of the reflectance probe at 90°. The response of the colorimetric reaction was studied in the temperature interval 20 ± 5 °C. Similarly, digital images were taken placing the TLC plates at 5 cm of the smartphone camera. Color analysis was performed processing the digitalized images using GIMP software. I.

2.4.3. Sample preparation

Extraction of gluten was performed prior to protein separation and its subsequent determination. For the extraction step each cotton swab sample was placed into an eppendorf tube filled with 400 μL of Betelene USP Plus and vortexed for 20 *sec*. Subsequently, 1 μL of the extracted sample was applied to the plate and then dried for 45 s. This process was repeated 10 times to increase signal response following the procedure described in 2.4.1. Finally, detection was carried out with the procedure described in 2.4.2. Control experiments were performed with cotton swabs fortified with Glu standard at a concentration of 1 mg mL^{-1}.

3. Results and discussion

3.1. Glu solubilisation studies

Glu determination is limited due to its low solubility in aqueous media. This is mainly caused by its large molecular size and intermolecular aggregation that results in non-covalent interactions, mainly involving hydrogen bonds and hydrophobic interactions. However, solubility can be improved taking advantage of the ionisable groups at Glu structure by pH variations and by adding different additives such as salts or surfactants. Since the practical application of the proposed strategy is Glu detection in food processing industries, the solubility performance of NaOH and NaOH:EtOH solutions was compared with the solubility of different commercial products commonly used in the cleaning of processing lines. An advantage of using these products as extractive phases is that no additional reagents or reagent mixtures are necessary, simplifying the detection process, reducing time, and costs. Fig. 1A qualifies the performance of the different solvents as a function of solubilisation performance. As can be seen, NaOH and NaOH:EtOH, mainly dispersed Glu and only NaOH provided an stable dispersion. Stable dispersions were also achieved with Ultra SDN, Ultra VF7, Detocide CDB y TEPHEX CB 34. However, Detocide CDA and Detecodide A30 resulted in a poor Glu dispersion stability. Only, Betelene USP plus gave rise to a complete dissolution of Glu at percentages lower than 3 %. This result indicated the possibility to obtain a homogeneous solution that was interesting from two different point of view, to extract Glu from the critical points in the processing chain and to remove residual Glu to avoid cross-contamination.

As expected, pH and composition were key factors in Glu solubilisation. As can be seen in Fig. 1A, basic pH favours solubilisation, however it was necessary the presence of surfactants to achieve a homogeneous solution. Under the proposed conditions, basic hydrolysis of Glu takes place facilitating its dissolution due to the decreasing of protein–protein interactions.

It should be noted that the previous study was performed using 10 min as agitation time. However, this time is a key parameter in terms of homogeneity and solubility. Fig. 1B shows the variation in solubility as a function of the agitation time. As can be seen, agitation improved Glu solubilisation, in particular when using Betelene as solvent. This solvent, was then employed for further studies.

Glu is a set of proteins mainly composed of gliadins. Gliadins are proteins known as prolamines, rich in prolines and glutamines. The IR spectrum for Glu, is shown in Fig. S1a. As expected, the amide I and II bands were observed at 1652 cm^{-1} (C═O) and 1534 cm^{-1} (N—H), respectively. Under the proposed conditions, the solubilisation of Glu in Betelene USP, gave rise to a basic hydrolysis of Glu, this process increased the solubility of Glu since protein–protein interactions decreased reducing aggregation and /or precipitation. This effect can be observed in Fig. S1b where the IR spectra of the solubilised Glu is shown. As can be seen, there was a shift of the amide I and II band, more likely due to protein hydrolysis. It should be remarked that the IR spectrum of Betelene USP Plus, as well as the spectra for the dissolved species in this solvent were also gathered. IR bands for Betelene USP plus were observed at 1585 and 1120 cm^{-1}, meaning that analyte identification was not possible at these wavenumbers. However, characteristic bands, typically found for amino acids were identified in the IR spectra of Glu solution (see Fig. S1b). Accordingly, two strong bands, almost overlapped, corresponding to the O—H and C—O bending of the carboxylic acid bond were observed at n = 1394 cm^{-1} and n = 1286 cm^{-1}, respectively. These bands were also seen when proline was dissolved in the selected solvent. A band in the IR fingerprint regime at 880 cm^{-1} result from C—H out-of-plane vibrations common to the investigated amino acids from Glu hydrolysis. In addition, a band at 1062 cm^{-1} resulting from C—N vibration was also observed. Hence, these results demonstrated the hydrolysis of the primary Glu, facilitating its

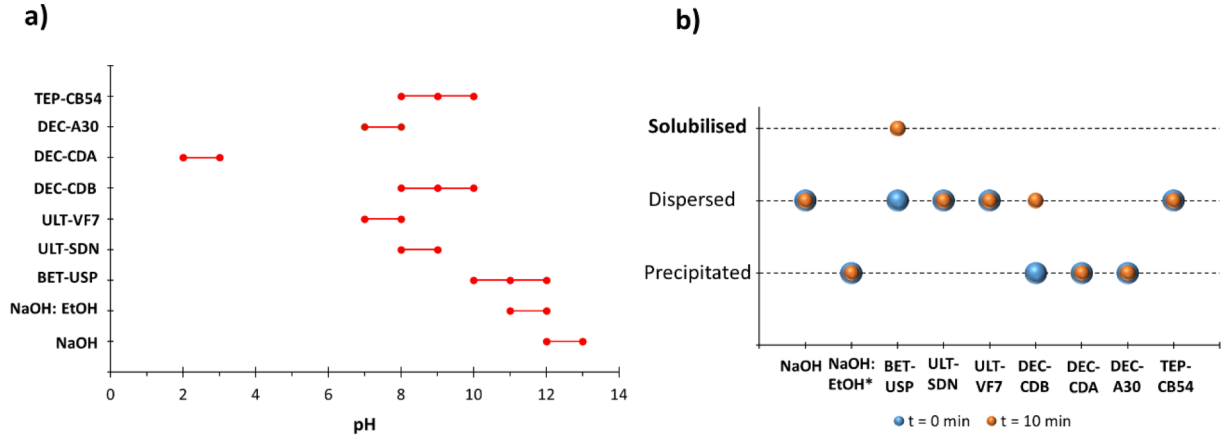

Fig. 1. A) Comparison of the pH working ranges of each different solvent used in the Glu solubility study B) Plot of the different solubility grades (precipitated, dspersed and solubilised) for each solvent when using two different agitation times (t = 0 and t = 10 min).

dissolution in the proposed medium.

3.2. TLC separation and detection optimization

The analytical response for Glu was obtained following the procedure described in the experimental section. Glu solution was spotted onto the nanosilica plates and subsequently, different compositions of the mobile phase were studied in order to achieve satisfactory Rf values. Stationary phase was selected taking in consideration the different chemical properties of the analyte of interest and its possible interactions with the adsorbent material. As above mentioned, gliadin is composed by a mixture of carbonaceous rings, polar and neutral functional groups. It implies that the separation should be directed by a moderately polar stationary phase to achieve satisfactory detection analyte spot detection, especially when spectral analysis or image analysis was carried out, which was the case with this strategy, since the quality of the analytical signal depends on the analyte spot homogeneity. As it as expected, the polarity of silica gel and NH_2-modified nanosilica gel layers was too high to achieve a good resolution for the analyte, indeed, an ion exchange mechanism governed the interaction analyte-NH2-modified nanosilica layer, and the spot detection was inefficient. Hence, CN modified nanosilica gel was studied, since the presence of the cyano propyl group provides the stationary phase with a dual interaction mechanism (Kulhmann et al., 2019) with the gliadin structure and functional groups, the selectivity of the separation. In this case, a homogeneous spot for the target analytes was observed. Therefore, CN modified nanosilica gel Glu was visualized by spraying BC staining solution over the plate, Fig. 2A shows the nanosilica plates at the studied compositions after the separation.

As can be seen, the elution depended on the percentage of acetonitrile in the mobile phase, the higher percentage of acetonitrile, the lower Rf value. As it was expected, the interaction between Glu and CN-modified nanosilica plates was favoured at high percentage of acetonitrile, showing low Rf values. The results indicated that 30:70 AcN:H_2O provided satisfactory results in terms of Rf and image analysis. It should be noted that higher percentage of water, did not improve the

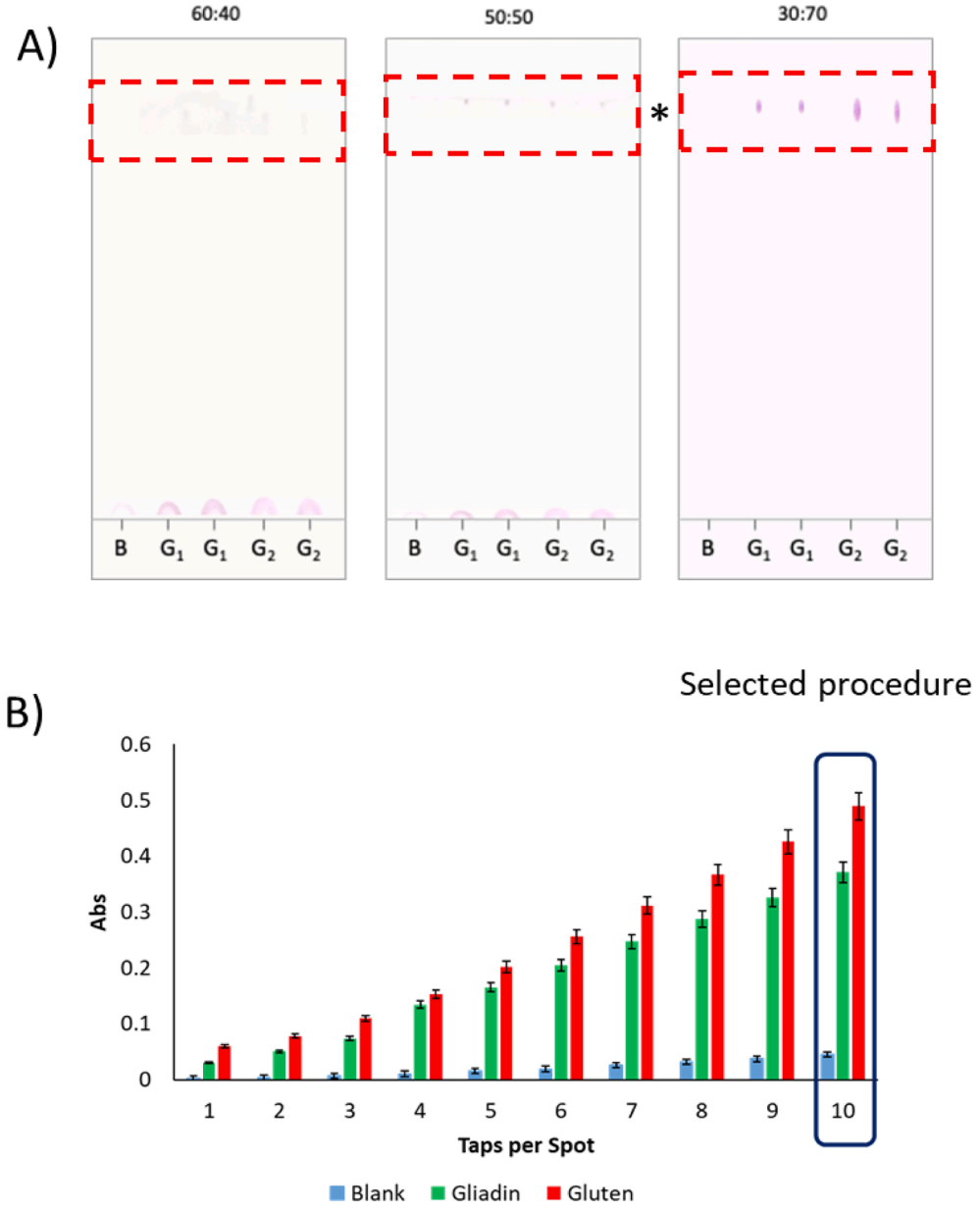

Fig. 2. A) Glu separation in CN modified nanosilica plates as for different proportions of mobile phase (acetonitrile:H_2O, 60:40, 50:50 and 30:70) B) Variation of the analytical response for the blank, gliadin and glu as a function of the number of taps applied per each spot (Glu and Gli concentration 2 mg mL^{-1}).

separation.

Taking into account the previous results, the possibility to improve sensitivity was studied by increasing the number of taps per spot. To avoid diffusion, the layer was dried for 45 s between each sample application. Fig. 2B depicts the variation of the responses as a function of the number of spots. As it was expected, a higher number of taps per spot resulted in a signal increase since Glu preconcentration was carried out in each spot before the chromatographic development. Moreover, the blank signals remained almost unreacted. In this sense, a number of taps per spot of ten was selected for the ongoing experiments.

Under this above described experimental conditions, the selectivity of the process was also studied since the presence in the processing chain of residues of other proteins is very common. Accordingly, the procedure was applied to a mixture of several proteins composed of Glu, casein and albumin. In this work, the colorimetric response was obtained by using the bicinchoninic assay. This assay is a routine test widely used to for proteins determination, mainly due to its simplicity, wide working concentration range, rapidity and compatibility with detergents, which is the case of these study. The experimental conditions under which the reaction has been applied, depends on the sample requirements not only in terms of analytical parameters but also considering the potential application in real analysis scenarios.

The bicinchoninic derivatization reaction occurs at room temperature, although depending on the required sensitivity, it is possible to increase the temperature to enhance the response (Cortes-Rios et al 2020; Rogatsky, 2021). In this study, the premise was to develop the colorimetric response at room temperature, taking into account the expected concentration ranges in real samples (mg mL^{-1}), simplicity and energy-cost-efficiency towards a sustainable in-situ analytical strategy. It should be noted that smartphone spectrophotometer and imagine analysis was proposed as detection systems. Nevertheless, we studied the analytical response as a function of slight temperature variations in the interval 20 ± 5 °C. Under these conditions, the absorbance measurements varied in the interval [0.057, 0.064] (1 mg mL^{-1}), which meant a variation interval, in terms of RSD lower than 10 %. These results demonstrated the temperature independent response within the experimental conditions established in this procedure.

The derivatized plate after the chromatographic development using the optimized methodology and the chromatogram obtained, measuring the absorbance at different Rf can be seen in Fig. 3. As can be seen, the studied proteins did not interfere in the Glu and Gli determination, since they showed a low Rf.

The results indicated satisfactory separation, as gluten and gliadins were isolated from casein and albumin. Moreover, suitable separation was achieved when a mixture of proteins was used as sample. Hence, satisfactory selectivity and detection by visual inspection can be carried out with the proposed procedure for the analysis of the surfaces. If more specificity would be required, an ELISA based test would be necessary in order to determine the food from which the contamination originates.

3.3. Analytical performance

In order to assess the quantitative capacity of the stablished method, spectra for Glu solution in the concentration range 0 to 6 mg mL^{-1} were measured using two different portable instrumentations in order to esure the in-situ routine analysis. It should be noted a wide variety of the commercial test, only provide qualitative measurement, and the quantitative estimation would require more than one test. In this work, the proposed methodology was focused on both analysis, visual inspection and quantitative analytis using spectral analysis by means of a smartphone spectrophotometer equipped with a fiber optic probe and image analysis by digitalization with the camera of the smartphone. Image analysis was carried out measuring the RGB parameters from the colored spots after image processing. The performance of this detection strategy has been previously validated in (Martinez-Aviño, et al, 2022). The registered spectra and the linear curves obtained for the three RGB parameters of the image analysis are shown in Fig. 4. Due to the small signals produced on the chromatographic plate, absorbances achieved were relatively low, however, good spectra in terms of shape and suitable sensitivity and linearity were achieved as can be seen in Fig. 4.

The linear curves for the RGB parameters clearly showed that best results were obtained using the green component. In this sense, the figures of merits of the gluten determination in HPTLC plates by using the two different approaches are shown in Table 1. The analyte quantification was carried out following the optimized procedure at a wavelength of 510 nm. The limits of detection (LOD) and limits of quantification (LOQ) were calculated as $3s_{yx}/b$ and $10s_{yx}/b$, respectively, where s_{yx} and b are the standard deviation and the slope of the regression, respectively.

As can be seen in the Table 1, good correlations and appropriated detection and quantification limits were obtained when using portable instrumentation equipped with the fiber optic probe. When using the processed images from the smartphone, the G (Green) component also provided a good correlation and the obtained analytical parameters (LODs and LQDs) were suitable for the analysis of gluten in surfaces. The analytical parameters, LOD, LOQ and precision were similar with both

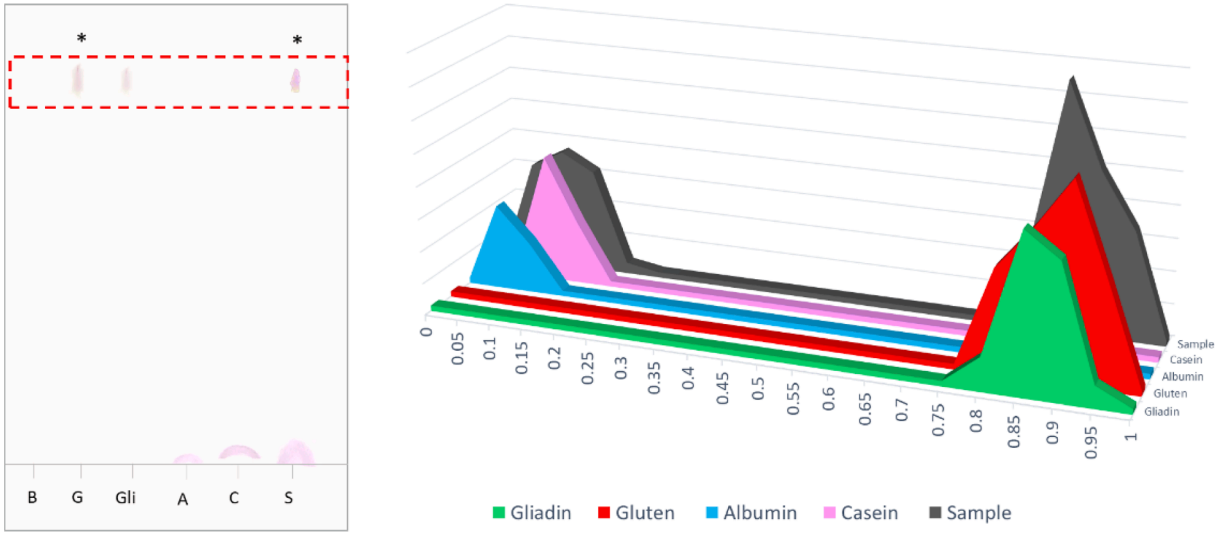

Fig. 3. Picture of a HPTLC plate after separation and derivatization of Gluten (G), Gliadin (Gli), Albumin (A) Casein (C) and a Sample or mixture of proteins (S) at a concentration of 1 mg·mL^{-1} when using optimized conditions and its correspondent chromatogram.

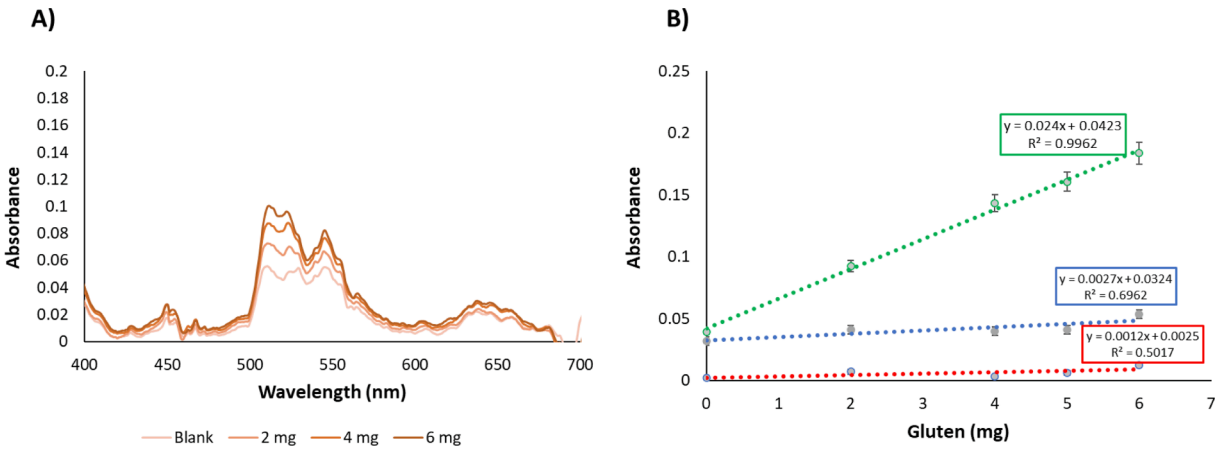

Fig. 4. A) Spectra obtained with the smartphone spectrometer coupled to a fiber optic probe for 0, 2, 4 and 6 mg of Gluten B) Calibration curve for each value of the obtained RGB parameters for different amounts of gluten (working interval 2–6 mg).

Table 1
Figures of merit for the studied method using smartphone spectrometer coupled to a fiber optic probe and smartphone captured image processing.

	Smartphone Spectrometer	RGB
$a \pm s_a$	0.0075 ± 0.0003	0.042 ± 0.003
$b \pm s_b$ (mL mg^{-1})	0.056 ± 0.0012	0.0240 ± 0.0008
R^2	0.996	0.996
LOD (mg mL^{-1})	0.5	0.53
LOQ (mg mL^{-1})	1.66	1.79
RSD (%)	4.25	4.83

spectral analysis and image analysis. Hence, the proposed Glu determination in surfaces of the food processing industry can be considered a screening strategy that provided a binary response using the LOD as cut-off value where positive responses would be indicated by the coloration of the spots at the studied Rf = 0.85. In addition, quantification can be also performed above the quantification method.

3.4. Application to real samples

The application of the proposed test was evaluated to estimate Glu cross contamination at different critical points in a food industry. The preliminary studies were performed by comparing difference in Glu residue from Glu-free zones and zones where Glu is present. For this aim, sampling was performed by using swabs and following the procedure described in section 2.4.3. Fig. 5A shows the estimation of Glu content in both studied zones. As can be seen, the Glu-free point, were below the cut-off value (0.12 mg) and then, it can be concluded that theses samples were negative. However, zones were Glu was present showed a value higher than 0.12 mg, it meant, positive responses.

Finally, the propose procedure was used to estimate the presence/absence of Glu at different critical point of the processing chain. Fig. 5B shows the results obtained at these points (cutting and mincing point (S1-S4), filling and extruder machines (S5-S7), refrigerating and cooling tunnels (S8-S10), conveyor belts (S11-S16) and different devices (S17-S21)) It should be noted that quantification was only carried out when the concentration level was higher than the quantification level. As can be seen in Fig. 5B, variations in Glu content were obtained as a function of the sampling point. As can be seen, positive responses were estimated in two meat mincers, in two filling machines and in a refrigeration and cooling tunnel. In the case of conveyor belts, positive responses were also obtained in two of the analysed belts. Finally, only in the case of a rough stainless steel device, Glu was observed above the cut-off limit.

The positive responses obtained at the different points demonstrated the potential cross-contamination in the food processing chain by using a simple and cost effective strategy. The proposed tool allowed the estimation by visual inspection and image analysis, by means of portable equipment thata provided in-place quantitative analysis. Therefore, the making decision process can be performed at real time without the need of laborious analytical procedure and training personal. Nevertheless, if more specificity is requirered inmunoassays would be necessary to detect the contamination origin.

4. Conclusions

In this work, a colorimetric analysis platform based on TLC and

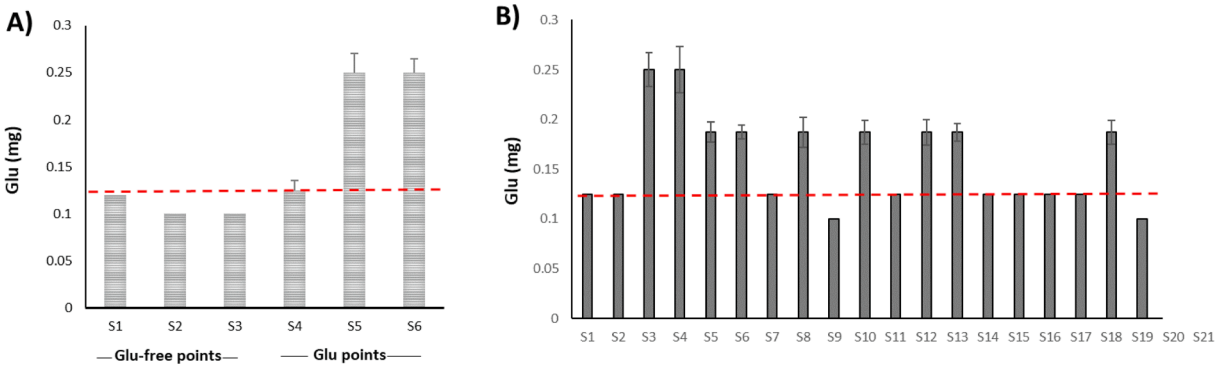

Fig. 5. A) Glu comparison in Glu-free point and Glu points in a food industry. B) Glu estimation in different samples from critical points at the food industry. Cutting and mincing point (S1-S4), filling and extruder machines (S5-S7), refrigerating and cooling tunnels (S8-S10), conveyor belts (S11-S16) and different devices (S17-S21).

bicinchoninic acid for the screening of Glu cross-contamination in critical point of food processing industries is proposed. The colorimetric response obtained, after extraction with a cleaning-in-place product, was registered by smartphone-supported spectrometric measurements and by digital image obtaining the RGB parameters of processed images, both in an attempt to reach a portable detection system that allowed not only the screening, but also the cross contamination quantification. It was demonstrated that quantification can be performed at levels higher than 1.7 mg, when using CN-modified nanosilica plates since homogeneous analyte spot was achieved. The figures of merit: linearity, LODs and LOQs, obtained by using the portable smartphone instrumentation were satisfactory proving this method to be a promising alternative for in place detection on food industry surfaces. In terms of precision RSD values were lower than 5 % for both approaches. The practical application of the proposed approach was demonstrated by evaluating surfaces of critical points of food industries showing satisfactory results, the use of the TLC plate provided satisfactory selectivity to isolate the analyte for a yes/no response, and in positive samples, the analyte level was directly estimated. Hence, the proposed procedure shows some relevant advantages in terms of sample pretreatment simplification, rapid response, quantitative and qualitative analysis performance when routine analysis is required. Nevertheless, if more specificity in the protein detection is needed, inmunoassays methods would be required.

CRediT authorship contribution statement

A. Martinez-Aviño: Writing – original draft, Validation, Methodology, Investigation. **Y. Moliner-Martinez:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **C. Molins-Legua:** Supervision, Methodology. **P. Campins-Falcó:** Resources, Project administration, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139025.

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