Marzo 411
Alimentaria 2010

Gluten detection method on surfaces

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Summary

One of the most important diseases related to food intolerances is Celiac Disease or Celiac Sprue, also known as intolerance to the gluten protein in some cereals. The presence or absence of these proteins in food must be clearly indicated according to European Union labeling directives. To ensure absence of gluten, methods are required to monitor gluten in ingredients and surfaces that enter en contact with food. Analytical laboratories, usually offsite from food manufacturing companies' facilities, can estimate the concentration of gluten. Estimating the level of gluten on surfaces is not easy to externalize in a classic way, however, it could be of great interest for companies that manufacture products for the celiac community. This paper describes an analytical technique that allows determining possible gluten contamination on surfaces where food is handled in a quick, reliable, and easy way.

Introduction

Celiac Disease is caused by intolerance to gluten from wheat, barley, rye and some types of oat. This autoimmune disease causes atrophy in the mucosa of the small intestine decreasing the nutrients absorption capacity resulting in symptoms that are not only gastrointestinal. Nowadays we estimate more than 400,000 celiacs in Spain, of which only 10% to 20% are already diagnosed. Currently, the only treatment for celiac disease sufferers is a strict gluten-free diet.

The Spanish food industry is in the process of improving quality and innovation, being aware of the problems celiac patients are facing and also labeling regulation. It is increasing its interest in guaranteeing the absence of gluten in food products.

The commitment of the food industry is crucial to increase safety of the food consumed by people suffering this disease and therefore improve their quality of lives.

For the control of food there is an European regulation in place: REGULATION (EC) No. 41/2009 of 20 January / 2009 for the composition and labeling of foodstuffs suitable for people suffering gluten intolerance (Ref.1), that stipulates that the accepted amount of gluten in foods that can be labeled "gluten-free" must be below 20 ppm (or mg / kg), while the "very low gluten" label is used for food that has been specially treated to reduce gluten, and that cannot exceed 100 ppm.

To verify compliance with EU rules on the amount of gluten in foods, companies use analytical techniques to analyze from raw materials to finished products, using mainly immunological methods such as ELISA or immunochromatographic strips that detect gluten peptides directly, generally from prolamins (wheat gliadin, barley hordein or rye secalin), but also methods such as PCR to detect the presence of DNA from these non-tolerated cereals.

One of the main problems for food companies is controlling cross contamination when foods both suitable and unsafe for coeliacs are produced in the same company, since a main cause for cross contamination is surfaces that have been exposed to food with gluten. Our research group has developed a suitable method that allows surface analysis using immunochromatographic strips containing a specific antibody raised against the most harmful gluten fraction for celiacs. This highly sensitive method can reliably detect the presence or absence of harmful gluten for celiacs.

Material and Methods

Immunochromatographic Strip (Stick)

The analytical method is performed using innovative immunochromatographic lateral flow strips available in the GlutenTox Sticks kit (Biomedal, Sevilla).

The immunochromatographic strip contains an absorption zone where the red colloid bound to the monoclonal antibody (Ref 2.) specific to the immunotoxic peptides from gluten (gliadin, secalin and hordein) is retained. Once the chromatography starts the colloid-antibody complex is dragged, and if there is gluten in the sample it will be bound to the specific antibody. In parallel, the blue colloid is also shifted to control the assay. When the sample is positive, the colloid-antibody-gliadin complex is retained in the middle of the strip through the binding of gliadin (gluten) to the monoclonal antibody attached to the strip, leading to the appearance of a red line through the accumulation of the red colloid. When the sample is negative, the colloid-antibody-gliadin complex is not retained, due to the absence of gliadin in the sample, because the attached antibody cannot recognize the gliadin, and therefore the red line does not appear (Figure 1).

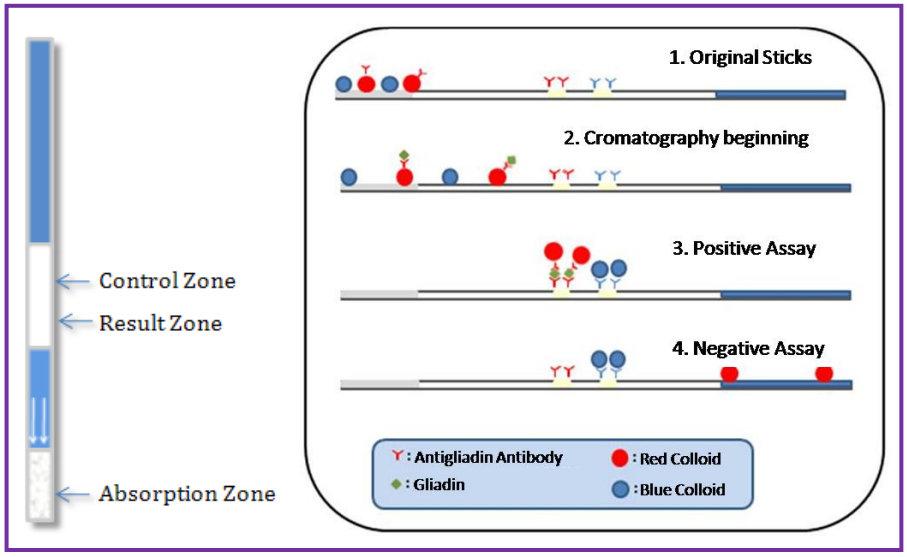

Figure 1. Scheme of the Immunochromatographic process and drawing of the immunochromatographic strip used in the method (stick).

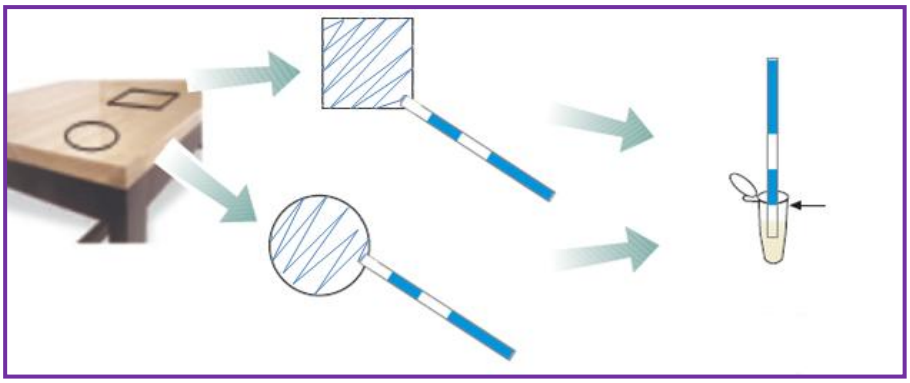

Figure 2. Samples collection process and analysis by the immunochromatographic strips

The Spanish food industry is in the process of improving quality and innovation, being aware of the problems celiac patients are facing and also labeling regulation. It is increasing its interest in guaranteeing the absence of gluten in food products

Determination of the minimum surface area to analyze

To determine the sensitivity of the method for surfaces, a non-rough (smooth) plastic surface (SP) is used, a surfacesimilar to those used in food manufacturing. Different amounts of gliadin (Sigma, St. Louis, Missouri, U.S.A.) are uniformly added to this surface. The gliadin was prepared with ethanol-water solution at 60% (w/v) to final concentration of 50 µg/ml. Different areas were contaminated with this gliadin control solution. The tested conditions were areas contaminated with gliadin at 200 ng/cm², 100 ng/cm², 50 ng/cm², 25 ng/cm², 10 ng/cm² and 5 ng/cm², and areas of 8cm², 16 cm², 32 cm² y 54 cm² were examined. Every condition was analyzed with a stick three different times.

To analyze the estimated area, the part of the strip intended for absorption is swabbed across the surface, and then is placed in a test vial with 100 µl of dilution solution (provided in thecommercially available kit) (Figure 2).

Validation of analysis of different surfaces

Different amounts of gliadin:
200 ng/cm², 100 ng/cm², 50 ng/cm², 25 ng/cm², 10 ng/cm² and 5 ng/cm² are added to four different type of working areas: Smooth Plastic (SP), Rough Plastic (RP), Smooth Cutting Table (SCT) and Rough Cutting Table (RCT). To analyze the different type of working areas the immunochromatographic strip is used in the same way described in the previous section for different type of surfaces.

Validation of washing protocols

To eliminate the cereal protein contamination, three cleaning protocols with the following parameters were established:

- Washing A: Water-soap + Drying with Absorbent paper.
- Washing B: Water-soap + Ethanol-Water 60% (v/v) + Drying with Absorbent paper.
- Washing C: Water-soap + 2 x Ethanol-Water 60% (v/v) + Drying withAbsorbent paper.

Conditions in the four different types of working areas were realized:
200 ng/cm², 100 ng/cm², 50 ng/cm², 25 ng/cm², 10 ng/cm² and 5 ng/cm². Each zone was analyzed in triplicate using a surface of 16 cm² and previous analysis of the working area were performed without contamination as a negative control.

Results

Determination of the minimal surface to analyze

Results obtained after analyzing the different amounts of gliadin in relation to the analyzed surface, a positive result was observed with gliadin contamination of more than 10 ng and surface analysis of16 cm² (see Table 1).

Table 1. Estimation of the minimal surface for analysis. (N: negative, P: positive, SP: smooth plastic working area).

		Analyzed Surface			
		8 cm²	16 cm²	32 cm²	64 cm²
SP	5 ng/cm²	N	N	N	N
	10 ng/cm²	N	P	P	P
	25 ng/cm²	N	P	P	P
	50 ng/cm²	P	P	P	P

Validation analysis for different surfaces

A positive result is observed with the stick on each working surface analyzed starting from a surface of 16 cm² and when the contamination was at least 10 ng/ (see Table 2).

Validation of washing protocol

A study was performed on the efficiency of different washing methods for removing the gluten from working areas. The presence of gliadin on surfaces was determined with the developed method after several washing steps. The results obtained reflected the importance of washing with the water-ethanol 60% (v/v) to eliminate the remaining gliadin in the working zone. The type of working surface also significantly influences the cleaning (see Table 3).

Table 2. Analysis of different working areas. (N: negative, P: positive, SP: Smooth Plastic, RP: Rough Plastic, SCT: Smooth Cutting Table and RCT: Rough Cutting Table. Results: in all cases when surface of 16 cm^2 are analyzed, 10 ng/cm^2 of gluten is detected with the sticks.

		Analyzed Surface			
		8 cm^2	16 cm^2	32 cm^2	64 cm^2
SP	5 ng/cm^2	N	N	N	N
	10 ng/cm^2	N	P	P	P
	25 ng/cm^2	N	P	P	P
	50 ng/cm^2	P	P	P	P
RP	5 ng/cm^2	N	N	N	N
	10 ng/cm^2	N	P	P	P
	25 ng/cm^2	P	P	P	P
	50 ng/cm^2	P	P	P	P
SCT	5 ng/cm^2	N	N	N	N
	10 ng/cm^2	N	P	P	P
	25 ng/cm^2	N	P	P	P
	50 ng/cm^2	P	P	P	P
RCT	5 ng/cm^2	N	N	N	N
	10 ng/cm^2	N	P	P	P
	25 ng/cm^2	P	P	P	P
	50 ng/cm^2	P	P	P	P

Discussion

In this study we have described a method to detect the presence of gluten on working surfaces. This method is suitable forthe food manufacturing industry and also for any kind of company dedicated to food: catering, cafeterias, restaurants which would like to offer gluten free products to their customers and control potential contaminations.

In order to standardize the method, we proposed a **minimal surface of analysis of 16 cm^2**,which always obtained a positive result under minimal conditions of 10 ng/cm^2. The standard surface can be obtained by cutting this area (4x4 cm^2) out of a plastic or a paper sheet and rubbing this part of the surface with the immunochromatographic stick by the cotton wool side. With this dataand considering, for instance, a working area of 1000 cm^2 (40 cm x 25cm) and food mass of 1 kg, we can ensure that the final product would have **less than 0,01 ppm of gluten** (0,01 mg of gluten / kg of food). This amount is around **2000 times less than the amount of 20 ppm** (20 mg of gluten / kg of food) established by the European regulation and around 1000 times less than the amount of 10 ppm (10 mg of gluten / kg of food) established by Federation of Associations of Celiacs of Spain, *Federación de Asociaciones de Celíacos de España* in Spanish (F.A.C.E.) [Ref.3].

Table 3. Cleaning efficiency according to washing protocol used. (N: Negative, P: Positive).

		Washing A			Washing B			Washing C		
Smooth Plastic		16 cm^2			16 cm^2			16 cm^2		
	0 ng/cm^2	N	N	N	N	N	N	N	N	N
	5ng/cm^2	N	N	N	N	N	N	N	N	N
	10ng/cm^2	P	P	P	N	N	N	N	N	N
	25ng/cm^2	P	P	P	N	N	N	N	N	N
	50ng/cm^2	P	P	P	N	N	P	N	N	N
	100ng/cm^2	P	P	P	N	P	P	N	N	N
	200ng/cm^2	P	P	P	N	P	P	N	N	P
		Washing A			Washing B			Washing C		
Rough Plastic		16 cm^2			16 cm^2			16 cm^2		
	0 ng/cm^2	N	N	N	N	N	N	N	N	N
	5ng/cm^2	N	N	N	N	N	N	N	N	N
	10ng/cm^2	P	P	P	N	N	P	N	N	N
	25ng/cm^2	P	P	P	P	P	P	N	N	N
	50ng/cm^2	N	P	P	P	P	P	N	N	N
	100ng/cm^2	P	P	P	P	P	P	P	P	P
	200ng/cm^2	P	P	P	P	P	P	P	P	P
		Washing A			Washing B			Washing C		
Smooth Cutting Table		16 cm^2			16 cm^2			16 cm^2		
	0 ng/cm^2	N	N	N	N	N	N	N	N	N
	5ng/cm^2	N	N	N	N	N	N	N	N	N
	10ng/cm^2	P	P	P	N	N	N	N	N	N
	25ng/cm^2	P	P	P	N	N	N	N	N	N
	50ng/cm^2	P	P	P	N	P	P	N	N	N
	100ng/cm^2	P	P	P	P	P	P	N	N	P
	200ng/cm^2	P	P	P	P	P	P	N	N	P
		Washing A			Washing B			Washing C		
Rough Cutting Table		16 cm^2			16 cm^2			16 cm^2		
	0 ng/cm^2	N	N	N	N	N	N	N	N	N
	5ng/cm^2	N	N	N	N	N	N	N	N	N
	10ng/cm^2	P	P	P	N	N	N	N	N	N
	25ng/cm^2	P	P	P	N	P	P	N	N	N
	50ng/cm^2	P	P	P	P	P	P	N	N	N
	100ng/cm^2	P	P	P	P	P	P	P	P	P
	200ng/cm^2	P	P	P	P	P	P	P	P	P

This means that the method has a high safety margin and using it should give customers, celiac associations and food safety inspectors peace of mind.

This method was used in this study to elaborate efficient washing protocols in order to remove proteins which cause intolerance to coeliacs, also suggesting the use of ethanol-water 60% (v/v) solution to decontaminate working surfaces.

About Biomedal

Biomedal is a company dedicated to the development of biotechnological processes and bioanalytical tools for laboratories and industry. The company has put a great effort in the development of latest generation analytical technology for detection and quantification of gluten based on the detection of the most immunogenetic peptides of gluten. Part of their gluten detection methods developed are marketed in the line of products branded GlutenTox® product line and are used by researchers at Stanford University (Ref. 4) and in biotechnology companies dedicated in the development of therapies for celiacs (Ref. 5).

To verify compliance with EU rules on the amount of gluten in foods, companies use analytical techniques to analyze from raw materials to finished products, using mainly immunological methods such as ELISA or immunochromatographic strips

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GlutenTox