

IDENTIFICATION OF GLUTEN IN CONDIMENT FOODSTUFFS BY THE IMMUNOCHROMATOGRAPHIC TEST

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Abstract

Here we report the results of a qualitative immunochromatographic assay used to detect gluten in some flavourings/additives and condiment foodstuffs. From a selected lot of ten samples, the following were found to contain gluten above 2 ppm: malt extract powder, textured vegetable protein TVP and mustard. These ingredients are often used in food products that are not always recognized as exempt of gluten by celiac disease patients. Celiac disease is an intestinal disorder caused by a permanent intolerance to gluten.

Keywords: *celiac disease, gliadin, gluten, immunochromatography*

Introduction

Literature data from some National Institutes of Health describe a 1% percentage of the total Europe and USA population suffering from celiac disease. Celiac disease is a food-induced disorder characterised by a permanent intolerance to wheat gluten and related proteins from rye and barley. Gluten is a wide component of our daily diet being spread not only in cereal and baking products but also in some ingredients including thickeners, stabilizers, flavourings, malt vinegar, condiments, yeast extracts, etc.

It has been shown that celiac disease is induced by some toxic serine-containing peptides and tyrosine-containing peptides, which are fragments of the A-gliadin (Cornell, 1992).

The ethiology of celiac disease is caused by digestive disorders of intestinal mucosa. The pathogenesis of the disease develops under an immunological mechanism driven by human leukocyte antigens-(HLA-DQ2 or DQ8)-restricted T cells (Sollid, 1989).

At present the only efficient treatment of celiac disease in both symptomatic and asymptomatic patients is a strict gluten-free diet during their lifetime (plain meats, vegetables, fruits). The great majority of the Health Associations of Celiac Disease agrees on the requirement for a correctly labelling of foods regarding the gluten content. The label *gluten-free* means that no gluten is detected by a sensitive assay. Research was intensified in the way of developing novel assays to detect toxic gluten peptides in food products. The modern and available detection methods used at present are the immunological ELISA test and the immunochromatographical test (Valdes, 2003; Ribes-Koninckx, 2003). The latest stick gluten method is in good correlation with the ELISA assay (Sorell, 1998). The Operon stick gluten assay is a qualitative and semi quantitative method for the detection of gluten in foods.

The analysis of gluten in foodstuff is sometimes a difficult approach because of the heterogeneity of the gluten, the diversity of the products and the interference with some treatments applied to food (heat, enzymatic processes).

In this paper we present the results of the immunochromatographic analyses of gluten in some flavourings and condiment foodstuffs as sometimes gluten is present as a "hidden" ingredient of foods.

Experimental

Sample extraction: The following food samples were analysed: flavouring mixture, malt extract powder, caramel syrup, textured vegetable protein (TVP), soy hydrolysed protein, mono- and diglycerides; commercial mustard, mayonnaise, ketchup and garlic sauce.

In case of solid samples these were first disintegrated and then extracted. The extraction was made in ethanol at 60%. After 30 minutes incubation at room temperature under stirring, the samples were diluted in test tubes with PBS pH 7.4 such as the final concentration in ethanol does not exceed 10%. The phosphate buffer saline was made from 10 mM phosphate pH 7.4, sodium chloride and sodium azide.

Analysis: The extracted samples were filtered on red 388 filter paper. The immunochromatographic test was assayed with the gluten-strip purchased from *Operon*. Ten gluten-strips were introduced in ten test tubes and the results in the reaction zone were read after 5 minute of chromatography running.

Results and Discussion

As sometimes it is difficult for celiac patients to have a strict gluten-free diet because of less obvious sources of gluten and the lack of specification of wheat in the ingredient listing, celiac patients must check carefully the ingredients of the food products consumed daily. It is known that small amounts of gluten cause intestinal damages in these persons. Clinically there exist both symptomatic (chronic diarrhoea, abdominal pain, weakness) and asymptomatic celiac disease (non-gastrointestinal symptoms such as anaemia, osteoporosis or neurological disorders) (Weiser, 1994; Hadjivassiliou, 1999; Rupert, 2002).

In the present paper we have screened the presence of gluten in condiment food products that were not labelled as "gluten-free", products that are used either as ingredients in foodstuffs or as dressings/sauces. These samples are not always recognized as exempt of gluten by the celiac disease patients. The following samples were tested: a flavouring mixture, malt extract, caramel syrup, vegetable textured protein, soy hydrolysed protein, mono-,di-glycerides, commercial mustard, mayonnaise, ketchup and garlic sauce.

After the ethanol extraction and PBS dilution of the samples, a sensitive immunochromatographic test was used for a qualitative determination of gluten. The gluten strip consists of four zones: the inferior adsorbent zone that is submerged in the sample, the red microsphere zone, the reaction zone and the superior adsorbent zone. The gluten positive samples react first with the red particles from the second zone of the strip and then migrate in the reaction zone of the strip to react with the anti-gliadin specific antibodies, developing a red band. A control band (blue) appears in both positive and negative samples (figure 1).

Gluten identification from condiment foodstuffs by immunochromatographic test

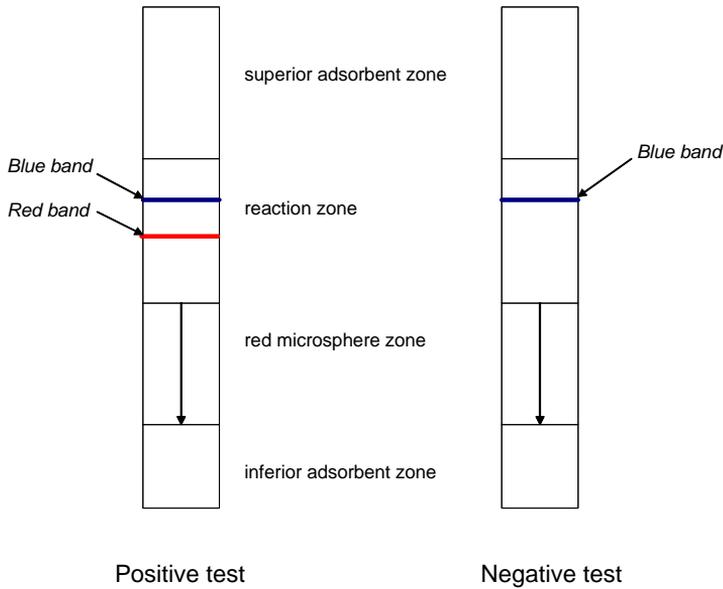


Fig. 1. Description of positive and negative reaction strip in gluten stick test

The sensitivity of the test is of 10 ng/ml of gliadins equivalent to 2 ppm of gluten in the sample. We have diluted the samples at 1/10 in PBS, dilution at which the calculated test sensitivity of the sample was 2 ppm (0.2 mg gluten/100 g).

Among the six flavourings/additives samples tested (flavouring mixture, malt extract powder, caramel syrup, textured vegetable protein, soy hydrolysed protein and mono-,diglycerides), the malt extract powder and the textured vegetable protein were found to contain gluten. The malt extract powder is an essential product used to prepare nutritious foods, malted milk foods, bakery products and beer. The textured vegetable protein (TVP) is a soy high quality protein used as a meat substitute and as ingredient for sausages and canned foods.

Among the four condiment foodstuffs tested (mustard, mayonnaise, ketchup and garlic sauce) only the mustard was found to contain gluten.

The results are presented in table 1.

Table 1. Stick-gluten test results of flavourings and condiment foodstuffs samples.

Sample	Test result (5 min.)	Estimated gluten in food
Flavouring mixture	-	
Malt extract powder	+	>2 ppm
Caramel flavouring	-	
Textured vegetable protein (TVP)	+	>2 ppm
Soy hydrolysed protein	-	
Mono-,diglycerides	-	
Commercial mustard	+	>2 ppm
Commercial mayonnaise	-	
Commercial ketchup	-	
Garlic sauce	-	

"+" = positive gluten test, "-" = negative gluten test.

The immunochromatographic test used in our gluten assay proved to be a sensitive, simple and reliable test for a rapid determination of gluten in food products.

Conclusions

Detection of gluten in raw and prepared foodstuffs is a very important task required for labelling of gluten-free food products and for the assurance of a correct diet of celiac disease patients. A diet exempt of gluten is needed for patients suffering of celiac disease, which is a permanent intolerance to gluten.

In the present paper, we described the results from the detection of gluten in some flavourings/additives and condiment foodstuffs by the rapid immunochromatographic test (Operon stick gluten). We have chosen a lot of ten condiment food samples. The results showed gluten content above 2 ppm in malt extract powder, textured vegetable protein and commercial mustard.

The recommendation for celiac disease patients and also for dieticians is to exclude these food components from the diet.

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