

# Detection of protein and DNA from wheat in oat products

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## Background

Gluten enteropathy or coeliac disease is a common disorder among the population in many countries. The lifelong illness is caused by a protein fraction present in the cereals wheat, rye, barley, triticale and oats. These patients have to build their diet on naturally gluten free foods like maize, rice and buckwheat or on products based on wheat- or barley starch, which has been rendered gluten free. However, recent studies among grown-up patients in Finland and Sweden have shown that oat is well tolerated by most in this patient group. One reason might be that the prolamin of oat, avenin, has a different amino acid sequence compared to the wheat prolamin fraction, gliadin. Therefore oat has become allowed by clinicians in Sweden in the gluten-free diet for grown-ups.



## Gliadin specific EIA

The products were analysed with a commercially available enzyme immunoassay using a monoclonal antibody to  $\omega$ -gliadin. The antibody recognises a heat stable epitope in wheat, rye and barley but not in oat. The EIA is quantitative with a detection limit of 20 ppm.

Nine samples gave a positive signal among the oat products thus indicating the presence of wheat, barley or rye. The levels varied between just above the detection limit and as high as 4000 ppm.

## Immunoblotting

Samples, positive in the EIA, were further examined by blotting with an anti-serum to gliadin. Proteins in the samples were separated by electrophoresis in a polyacrylamide gel 4-12% (NuPAGE®, Bis-Tris gel) and then transferred to a nitrocellulose filter. The gliadin reactive bands were visualised with an antibody to gliadin (rabbit antiserum to wheat-gliadin, Riedel de Haen) followed by an enzyme-linked second antibody and finally with a substrate for the enzyme.

In eight out of the nine analysed samples wheat-specific protein bands were seen. One of the products, oat-drink (sample 6), did not give any reaction with the antiserum.

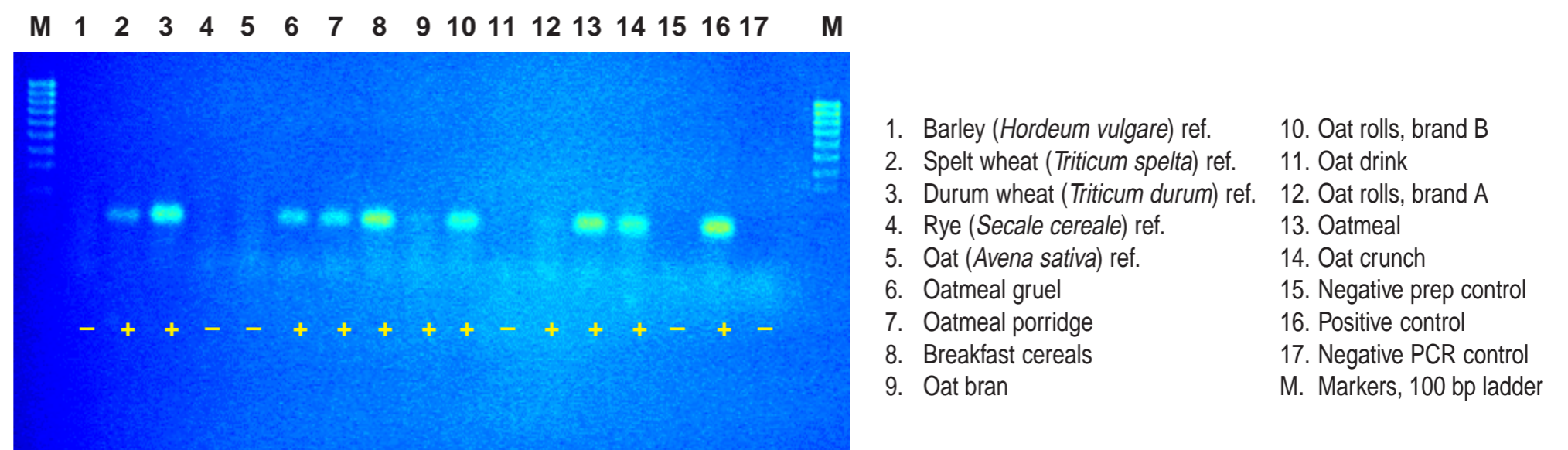
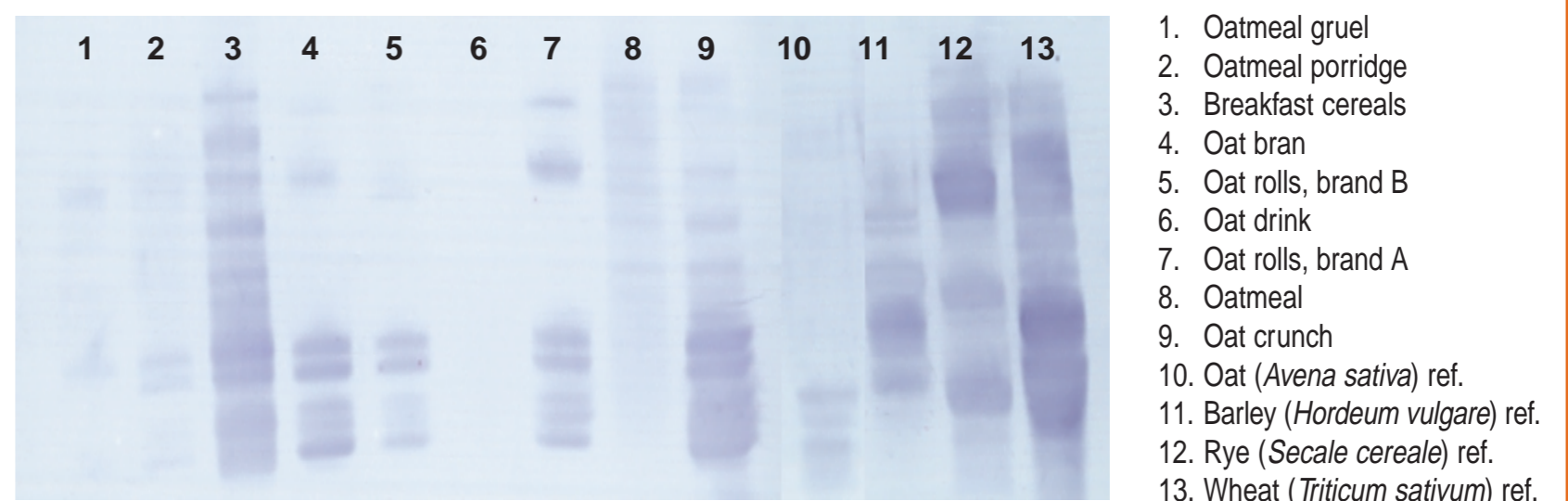
## Wheat-specific PCR

Positive samples were also examined for the presence of wheat DNA by polymerase chain reaction (PCR). The reactions were performed with primers for a wheat specific sequence as well as primers for an eucaryotic sequence as control. Following the PCR all samples were analysed by gel electrophoresis and the DNA was visualised by ethidium bromide staining.

The result matched the result from the immunoblotting indicating wheat contamination in 8 out of the 21 oat products.

Product	Gluten concentration
Oat rolls, brand A	52 ppm
Oat rolls, brand B	4097 ppm
Oat bran	48 ppm
Oatmeal	4128 ppm
Oatmeal porridge	85 ppm
Oatmeal gruel	156 ppm
Oat crunch	195 ppm
Oat drink	36 ppm
Breakfast cereals*	2775 ppm*

\* The product contains wheat flour as indicated on the label



## Results & Conclusion

Twenty-one oat products were analysed for contamination with wheat, rye or barley. A gliadin specific EIA showed that nine products contained gluten, varying from 36 ppm up to 4128 ppm. The contamination was shown to consist of wheat by using specific antibodies to wheat gliadin. In one sample, oat drink, no blotting pattern was observed. The contamination with wheat was further confirmed by a wheat specific PCR in eight of the nine samples.

Product	Gluten EIA	Blotting	Wheat-specific PCR
Oat rolls, brand A	52 ppm	+	+
Oat rolls, brand B	4097 ppm	+	+
Oat bran	48 ppm	+	+
Oatmeal	4128 ppm	+	+
Oatmeal porridge	85 ppm	+	+
Oatmeal gruel	156 ppm	+	+
Oat crunch	195 ppm	+	+
Oat drink	36 ppm	Not detected	Not detected
Breakfast cereals	2775 ppm	Not analysed	Not analysed

The highest level of wheat was found in one brand of oat rolls and in one oatmeal. These levels would have been harmful to a person suffering from coeliac disease.