Combining existing multiplex PCR with lab-on-chip capillary electrophoresis for detection of GMOs

Area of research interest: Novel and non-traditional foods, additives and processes
Study duration: 2006-09-01
Project code: G03022
Conducted by: Campden and Chorleywood Food Research Association
Back to top

Background

Our organisation is the competent authority for the Genetically Modified (GM) Food and Feed Regulation (EC) 1829/2003 which lays down labelling requirements for GMOs and products containing GM material. This stipulates that any food or feed product containing a GM ingredient must declare this on the label. A threshold of 0.9% is in place for the unintentional and technically unavoidable presence of authorised GM material below which labelling is not required. Unauthorised GM food or feed is not permitted at any level.

To determine whether the labelling regulations are working in practice, it is necessary for GM deoxyribonucleic acid (DNA) detection methods in foods to be available using high throughput, low cost methodologies. The method of choice for detecting GM DNA is termed the, 'real-time polymerase chain reaction (rt-PCR)'. Currently, rt-PCR requires expensive equipment, which is inaccessible to the majority of Public Analyst laboratories. This study therefore sought to develop alternative PCR DNA detection methods (or refine existing ones) for GM food (and feed) that can be used as simple screening methods for enforcement work.

Back to top

Research Approach

It was proposed that a lab-on-a-chip semi-quantitative Roundup Ready soya assay would be validated for use on DNA extracted from food and feed materials using a multiwell extraction instrument. In addition, multiplex assays for 35S, NOS terminator and lectin gene or 35S, NOS terminator and zein gene targets were developed and tested for general screening of soya and maize materials respectively. A cauliflower mosaic virus (CaMV) target was also included to allow the identification of false positives due to contamination with Brassica species, a common contaminant in some food samples. An assessment of published multiplex assays for the identification of a number of GM maize varieties was also undertaken. Where possible, PCR product or plasmid standards were used for semi quantitative analysis. This will enable the results to be expressed as the percentage GM-DNA copy numbers in relation to target taxon-specific DNA copy numbers calculated in terms of haploid genomes as this is the unit of measurement preferred by the European Community reference laboratory for GMOs. Finally, the full range of assays was subjected to external trial among a number of enforcement laboratories.

Back to top

Results

A novel multiplex GMO screening assay was developed to detect small target sequences (<130bp) found in the NOS terminator, lectin, zein, CaMV and the CaMV 35S promoter sequences. The assay was applied to certified reference materials, processed ingredients and animal feed samples. It was further validated in a trial amongst 10 local authority Public Analysts. The results indicated that the multiplex assay worked well with little variation in the number of targets detected by the laboratories for each sample.

A second multiplex assay was developed to detect the endogenous soya lectin gene and targets within the Roundup Ready (RR) soya transgene. This assay was used to estimate the levels of RR soya in some soya based ingredients including soya flour, textured vegetable protein (TVP) and animal feed. The relative levels of RR soya detected in these samples using the multiplex assay were in good correlation with the results obtained by real-time PCR. In addition, an evaluation of an automated DNA extraction instrument, the Maxwell 16 (from Promega), indicated it was suitable for the reliable extraction of DNA from a range of food matrices, although the amount of DNA obtained from some food matrices was low.

Finally, a third multiplex assay was used for the detection of 5 different GM maize varieties (Bt176, Bt11, Mon810, GA21, and T25) and the endogenous maize gene, zein. This used targets and PCR primer sets from existing and published assays. Results indicated that the individual assays detected the intended GM maize varieties with no-cross reactivity, but when primers were used together in a multiplex assay there was preferential amplification of some GM maize varieties. Further optimisation of this assay would therefore be necessary before being suitable for routine analysis by enforcement laboratories.

Research report

England, Northern Ireland and Wales

PDF
View GMO screening final report as PDF(Open in a new window) (3.08 MB)