

GMO detection in food and feed: not an easy task

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INTRODUCTION

European legislation requires labeling of GMOs in food and feed, in raw material as well as in processed food. The GMO laboratories are therefore faced with a wide spectrum of different samples such as simple ones like grains, flour, groats and soybean skins, and more complex ones, like flaps, maize porridge, corn flakes, tofu, cookies, bread, lecithin and various feed mixtures. Food products from different food producers differ in composition, causing great variability in DNA content and quality within the same type of food matrix. The different substances present in the sample can cause inhibition of qPCR reaction. In the present work we evaluated some parameters affecting detection and quantification of GMOs in different matrixes.

MATERIALS AND METHODS

We have chosen different often analyzed soybean and maize samples that represent either raw plant material, feed or processed food matrixes.

Samples were homogenised with Ultra Centrifugal Mill ZM100 (Retsch) or a coffee grinder. DNA was isolated with commercial kits, either NucleoSpin® Food (MACHEREY-NAGEL) or GENESpin (GeneScan Analytics). DNA concentration was measured with PicoGreen® dsDNA Quantitation Kit (Invitrogen™) and DNA was diluted to 25 ng/μL in order not to add more than 100ng of DNA in reaction to prevent the inhibition caused by high DNA amount. Samples that gave predictable amount of DNA were diluted 1:5 (maize flour, grains and groats, gluten, feed) or 1:10 (soybean flour and grains) to give Ct value for species specific gene (invertase for maize and lectin for soybean) about 23. Samples were analysed by Real-time PCR (ABI 7900HT) and the effect of matrix on PCR efficiency was assayed. PCR efficiency was calculated as follows: PCR efficiency (%) = $(e^{(ln10/slope)} - 1) \times 100$.

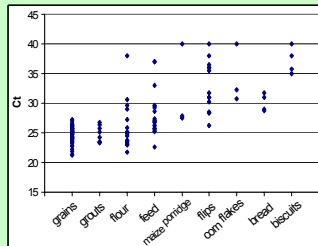
Soybean samples in which Roundup Ready® soybean was detected were quantified by interpolation of Ct values generated from Roundup Ready® soybean standard reference material in a standard regression curve.



RESULTS

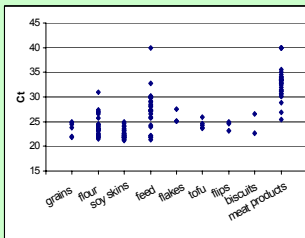
Ct values of species specific genes obtained by different food matrixes

MAIZE



Obtained Ct values are lower in raw materials, as expected, indicating higher DNA content in those materials. In the feed samples DNA is usually present at high content, except for some feed samples, which may have been processed. Less DNA is obtained from processed food matrixes, especially from heat treated food (flaps and bread), making GMO detection in these samples a difficult task.

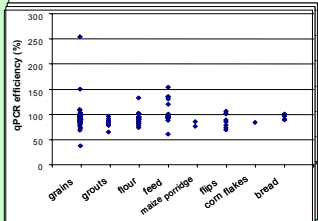
SOYBEAN



The same trend in DNA presence as with maize samples is seen in soybean samples where DNA of high quality and concentration is obtained from grains, flour and soy skins. Feed usually contains sufficient DNA for successful quantification but presents a variable matrix with Ct values ranging from 22 up to 33 and in some soybean DNA is not detected. The DNA content from most evaluated processed matrixes was sufficient for successful GMO detection. However we observed a great variability in meat products that can contain either soybean flour or proteins and can have different levels of processing.

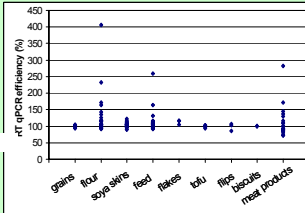
PCR efficiency in different food matrixes

MAIZE



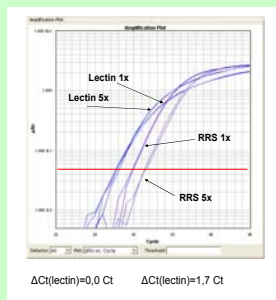
The efficiency of the PCR reaction was evaluated from the dilution series of the samples. Although the majority of samples reach efficiencies close to 100%, some samples show lower or seemingly very high efficiencies indicating inhibition. Although containing high amounts of DNA, grains and feed samples are the most variable and difficult to quantify in regard to PCR inhibition.

SOYBEAN



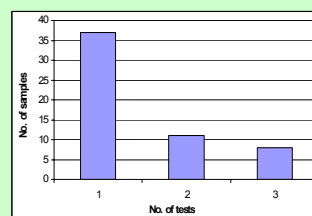
In the soybean samples the greatest variability in efficiency is observed in flour, feed and meat products. Some matrixes that contain lower amounts of DNA, like tofu and flakes, on the contrary do not inhibit the PCR reaction.

Differential PCR inhibition from the same DNA



Sometimes different PCR amplicons can be differently influenced by PCR inhibitors. A case of GMO quantification is shown where the same amount of DNA was added to reactions, but lectin amplicon was severely inhibited while RRS amplicon was not affected.

Number of tests needed for quantification of Roundup Ready® Soya samples



During our work we have identified matrixes that have low DNA content or often inhibit the PCR reaction. 66% of soybean samples were quantified with one test, while for 34 samples quantification had to be repeated once (20%) or twice (14%). Due to a high variability of food matrixes the quantification of GMOs remains a nontrivial task.

CONCLUSIONS

The behaviour of amplicons in qPCR reaction varies in different matrixes, as well as in different samples of the same matrix. Sufficient quantity of DNA for reliable analysis is normally obtained from raw materials, while DNA quantity is low in processed foods. Feed samples vary in composition and level of processing, therefore it is difficult to estimate the DNA amounts prior to analysis.

The PCR efficiency also greatly varies among the samples because of many inhibitors that are present in plant and food matrixes. Therefore it is necessary to include dilution controls for each individual sample. We have also observed that sometimes inhibition can only occur at one of the tested amplicons. In our routine testing we could determine the quantity of GMOs in 66% of the cases with the first real-time PCR run, while in other cases analysis had to be repeated with different dilutions to avoid PCR inhibition.

Studying the effects of food matrixes on successfulness of GMO quantification enables us to estimate the DNA concentrations and identify problematic matrixes in regard to PCR inhibition, therefore reducing the number of samples for which quantification must be repeated.