Analysis of genetically modified soya using the Agilent 2100 bioanalyzer

Application

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Abstract

In this Application Note we describe how the Agilent 2100 bioanalyzer was used to analyze multiple PCR products from Roundup Ready soya DNA. The multiplex assay assessed the effects of heat and low pH on subsequent amplification of genetically modified DNA and estimated levels of Roundup Ready soya within a sample.

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Introduction

Genetically modified organisms (GMOs) and derived food ingredients are regulated throughout the European Union (EU). Legislation requires appropriate labeling of products containing GM DNA. Whilst DNA methods based on the polymerase chain reaction (PCR) are suitable for monitoring known GMOs in raw materials and processed foods, there is concern that the analytical methods are less reliable for quantification purposes. Particular concern is for processed foods, where soya and maize ingredients, which are most likely to contain GMOs, are only a minor component of the finished product.

Food processing has a significant effect on the quality of DNA. Physical and chemical factors such as shear forces, heat treatment, nuclease activity and low pH will lead to degradation of the DNA. Soya is a common component of a wide range of foods, used as flour, protein isolate or concentrate. Soybeans are usually defatted by pressing and/or solvent extraction. In both processes, the soybeans are heated to 60–80 °C and the resulting protein meal can then be concentrated by extraction using weak acid (pH 4.5). These processes combined with further processing during product manufacture significantly reduce the quality of the soya DNA in the final product. This fragmentation of DNA reduces the probability of PCR detection particularly if the fragment sizes are smaller than the DNA sequence that is amplified by the primers.

Studies indicate that small sequences of DNA remain detectable following all but the most extreme processing conditions. In routine screening analysis for GMOs the use of small targets (<200 bp) is common. However, if there is differential degradation in these small targets (some sequences will be more susceptible to degradation than others) quantifying GMOs in processed foods using amplification of two similar but slightly differently sized targets may affect the accuracy of the results.

The aim of this project was to study the effect of food processing on PCR-based amplification and quantification of GM DNA. The approach was to develop a simple model assay system to observe differences in detection when using small targets in Roundup Ready (RR) soya heat treated at low pH. The Agilent 2100 bioanalyzer was used in post-PCR analysis to measure the concentration and number of differently sized PCR products.

Results

Assay development

The aim was to develop a model assay that could be used to assess the quality of DNA extracted from heat-processed soya flour samples, in particular, to investigate differences in PCR amplification between small DNA targets. A single multiplex PCR assay was developed that enabled four GM soya targets to be analyzed in a single reaction mix. Primer concentration was optimized in order to obtain four PCR products resolved by gel electrophoresis which corresponded in size to the soya lectin gene target of 80 bp, and the EPSPS (5-enolpyruvyl-shikamate-3-phosphate synthase) gene targets of 117 bp, 150 bp and 202 bp respectively. These latter targets are only found in Roundup Ready GM soya (Monsanto).

Although gel-based analysis enables sizing of PCR products, it cannot be used to provide accurate information on the quantity of a PCR product. Therefore, post-PCR analysis was performed using the Agilent 2100 bioanalyzer, which can accurately size and quantify PCR products. Initially, this was carried out using the DNA 7500 LabChip® kit. Four peaks were observed, corresponding to the PCR products within the lectin and EPSPS genes. However, the 80 bp peak from the lectin gene was not completely resolved from the alignment marker so quantification was not possible. Subsequently, post-PCR analysis was performed using the DNA 500 LabChip® when it was made available and resolution of all four peaks was observed (figure 1).

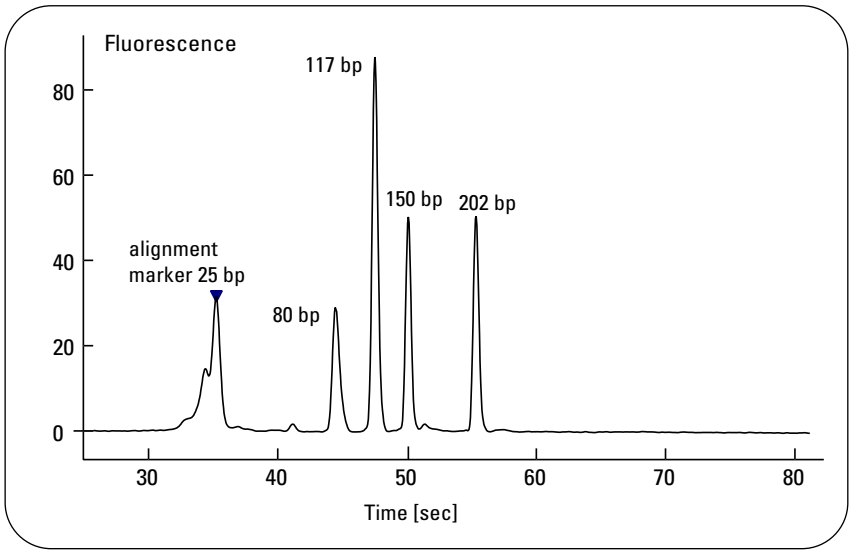

Figure 1
Multiplex assay for GM soya. Peaks produced by the four PCR products when analyzed with the Agilent 2100 bioanalyzer and DNA 500 LabChip kit.

The multiplex PCR assay was applied to DNA from RR soya flour reference materials (figure 2). The results show that there is an increase in PCR product concentration of the 117 bp, 150 bp and 202 bp products and little change in the concentration in the 80 bp product. This increase corresponds to the increase in RR content of the soya flour. No increase in the product from the lectin gene was expected, as it is common to both the GM- and non-GM soya. It should therefore be possible to estimate levels of GM soya in an unknown material by applying the multiplex assay and comparing the ratio of lectin product to the other products in the sample with ratios produced from certified reference materials (CRMs). The assays would have to

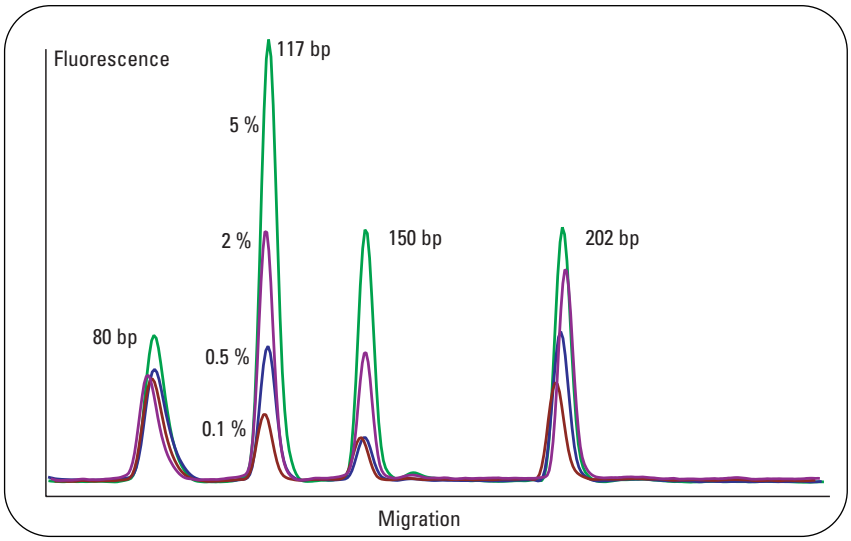

Figure 2
Peaks produced by the Agilent 2100 bioanalyzer using the multiplex assay on CRMs containing different levels of GM soya

Sample	Ratio 117/80	Ratio 150/80	Ratio 202/80
Extract 1a	1.50	0.68	1.04
Extract 1b	1.45	0.68	1.04
Extract 2a	1.40	0.46	0.71
Extract 2b	1.54	0.65	1.10
5% CRM	2.10	1.00	0.94
2% CRM	0.74	0.21	0.68
1% CRM	0.41	0.18	0.43

Table 1
Analysis of a soya flour

be performed using a limited number of PCR cycles in order to perform the end-point detection during linear stages of amplification. The reference materials and the unknown samples would also have to be similar in nature.

Analysis of a soya flour containing GM soya

The multiplex assay was applied in duplicate to two DNA extracts prepared from a soya flour sample which had given a positive result when screened for a common GM promoter sequence (CaMV 35S promoter). The assay was also applied to 1 %, 2 %, and a 5 % CRMs. Ratios of each EPSPS product compared to the lectin product were calculated (table 1). The same extracts were analyzed using a real-time PCR method for quantitative determination of GM soya. Results from the real-time analysis indicated that the sample contained approximately 5 % GM soya while the multiplex assay gave ratios indicating that the sample contained between 2 % and 5 % GM soya.

The effect of heating time and pH on detection and quantification of GM-DNA

The multiplex PCR assay was applied to soya flour samples containing approximately 1.3 % GM soya and boiled at either pH 3.3, 4.3 or 6.7 for up to 21 minutes. For accurate determination of the quantity of each PCR product, the samples were applied to the DNA 500 LabChip. The concentration of each PCR product was calculated using the Agilent 2100 bioanalyzer software. At pH 3.3 where an effect of heating time was observed, the amount of each PCR product at each time point was compared to the amount of each product at 0 minutes (table 2). At pH 3.3, the relative amount of the 80 bp product was reduced to 48 % after 15 minutes and no product was detected at 18 or 21 minutes. After 15 minutes, the relative amounts products of 118 bp and 150 bp were reduced to 27 % and 16 % respectively and the 202 bp product was not detected. None of the products were detected after 18 or 21 minutes.

Time at 100 °C and pH 3.3 (min)	Amount of PCR product*			
	80 bp	118 bp	150 bp	202 bp
0	100	100	100	100
3	74	77	73	67
6	57	58	21	6
9	36	23	24	15
12	67	33	47	21
15	48	27	16	0
18	0	0	0	0
21	0	0	0	0

* % product determined relative to the amount at 0 minutes

Table 2
The effect of heating time on RR flour held at pH 3.3, determined using the multiplex PCR method.

To eliminate any variation due to amount of DNA in each PCR reaction, the ratio of the lectin 80 bp product to each of the other three products was determined for all experiments (table 3), that is, normalized with respect to the 80 bp product. The ratios of each would be expected to remain constant if no degradation of the target DNA occurred or if the degree of degradation between the 80 bp target and the other targets was comparable. At pH 3.3 the ratios tended to increase with increasing heating time. This suggests that at low pH there were differences in the detectability of the three EPSPS targets compared to the smaller lectin target, with the

Time at 100 °C (min)		Ratio lectin 80bp/ RR-117bp	Ratio lectin 80bp/ RR-150bp	Ratio lectin 80bp/ RR-202bp
pH 3.3	0	1.8	3	1.9
	3	1.8	3	2.1
	6	1.7	7.8	17.5
	9	3	5	5
	12	3.6	4.4	6
	15	3.8	9	NP
	18	NP	NP	NP
pH 4.3	0	2	4.4	1.9
	3	2.2	2.9	1.8
	6	1.3	2	1.9
	9	1.3	2.2	2.3
	12	1.5	2.6	2.6
	15	1.8	3.7	2.7
	18	1.9	3.9	3
pH 6.7	0	1.8	4.2	1.7
	3	1.7	3.9	1.6
	6	1.2	2.3	1.5
	9	1.5	2.4	1.7
	12	1	1.9	1.3
	15	1.2	2.1	1.3
	18	1.4	2	1.4
	21	1.6	2.4	1.4

NP= no PCR products observed

Table 3
The effect of heating time on RR flour held at pH 3.3, 4.3 and 6.7, determined using the multiplex PCR method

80 bp target being degraded at a slower rate compared with the other targets. At pH 4.3, the 80/118 bp and 80/145 bp ratios decreased during the first 3–9 minutes of heating, then increased returning to the their original value, whereas the 80/202 bp ratio increased with heating time. Similar trends were observed at pH 6.7 except for the 80/202 bp ratio where little change occurred. However, further analyses are required to replicate these observations and focus around the pH where an effect is observed. These initial results indicate that the different targets used in PCR are not detected equally in these experiments.

Other studies show similar results. In 1998 Hüpfer et al.[1] demonstrated that PCR detection of GM maize in polenta could be influenced by pH during thermal treatment of the product. They showed that detection of a 1,914 bp segment of the cry1A(b) gene was not possible after boiling at neutral pH for 30 minutes, whereas a 211 bp fragment was detected after boiling for 105 minutes. At pH 2-3, the larger segment was not detected after boiling for 5 minutes and the smaller fragment was not detected after 15 minutes. As a result of such observations, it is common practice to use small target sequences in screening methods for GMOs. However, the work reported here suggests that at low pH, degradation of DNA results in differences in detection of very small target sequences. This may not be important for qualitative analysis, however, it is likely to have significance for the accuracy of quantitative analysis of processed foods with low levels of GM-DNA, when two target sequences are analyzed simultaneously as in real-time PCR.

Conclusion

The RR multiplex assay was used to quantify the amount of GM soya in a soya flour and assess the effects of pH and heat on the detection of GM soya DNA. A key component of the assay is the Agilent 2100 bioanalyzer which is used to accurately quantify the four PCR products simultaneously. This user-friendly instrument replaces gel based analysis and offers enormous potential for the routine screening of raw materials for levels of genetically modified organisms.

References

1.
Hüpfer C, Hotzel H., Sachse K., Engelk E.H. “Detection of the genetic modification in heat treated products of Btmaize by polymerase” *Zeitschrift für Lebensmittel Untersuchung und Forschung A.*, *206*, *203-201*, **1998**

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Published November 1, 2001
Publication Number 5988-4070EN

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