INFLUENCE OF SAMPLE PARTICLE SIZE ON SUBSEQUENT STEPS IN REAL TIME PCR GMO ANALYSIS

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Introduction

Preparation of laboratory sample is a first and very sensitive step in GMO analysis. The aim of the present study is to demonstrate correlation of different sample particle size with isolated DNA quantity and quality. Repeatability of the results obtained from the two GMO Real Time PCR quantification was shown. This is especially important because threshold level for GMO contamination is 0,9% in accordance with the Regulation (EC) No. 1829/2003 and Serbian GMO law since 2009.

Materials and Methods

Routine laboratory sample of soybean seed was gravimetricaly prepared to obtain 1% genetic modification.

Grinded on the laboratory mill within different time spans

Six sample portions were prepared by sieving through sieves with different measuring range

Silica based DNA extraction in duplicate

DNA quantity and quality control by UV VIS spectrophotometer

Event-specific real-time quantitative TaqMan® PCR procedure for GMO quantification

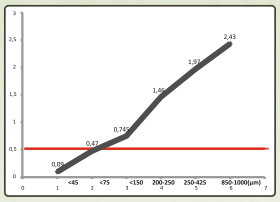
Results

- In rough grinded sample DNA concentration was 9,2ng/µl while in the finest milled sample <45µm was 100ng/µl as shown on Table 1.

Sample size (µm)	DNA concentration (ng/µl)	
	First isolation	Second isolation
850-1000	7,33	8,29
	11,3	9,9
x	9,20	
250-425	22,72	25,16
	23,64	25,14
x	24,16	
200-250	32,34	48,84
	39,27	38,7
x	39,79	
<150	49,74	50,1
	47,9	59
x	51,66	
<75	58,35	57,98
	68,44	67,87
x	63,16	
<45	100,87	95,79
	102,83	102,03
x	100,38	

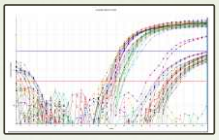
Table1.

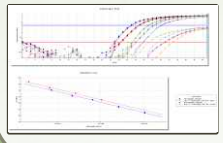
- Absolute difference in Cq value between double DNA isolations were above 0,5 in samples with average particle size higher than 150µm (Picture 1.)



Picture 1. Difference in Cq value

Aplication plots for taxon specific lectins and transgen Roundup Ready

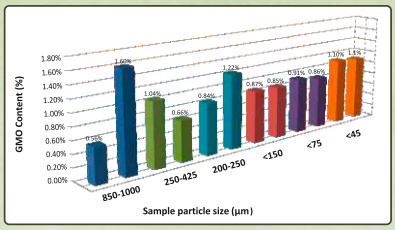




Relative quantification: Standard curve is based on DNA copy numbers

- Absolute values of estimated GMO content between two parallel probes were changed

- In hardly grinded samples difference in GMO concentrations where up to 1% of absolute value, unlike in samples with finest granulation, were distinction were negligible (Picture 2.)



Picture 2. Absolute difference in two paralel DNA isolations and impact on % GMO were accurate value is 1%

Conclusion

- This comparative analysis demonstrates the importance of correlation between sample particle size and isolated DNA quantity. The concentration of isolated DNA was increased with decreasing particle size of the sample.

- In addition, it enhances the importance of grinding and homogenization which leads to uniformity in GMO estimation, especially considering sensitive legal threshold of 0,9%.
- Heterogeneity in sample particle sizes distribution influences amplification efficiency taxon specific (lectins) and transgenes (Roundup ready gts 40-30-2), resulting in significant impact on Cq value.

- Average sample size of 150µm represents most common particle size obtained from laboratory mill and absolute difference in estimated percentages of GMO modifications between two paralel DNA isolations were satisfactory in accordance with acceptable measurement uncertainty +/- 30% for values greater than 0,2%.

- Insufficiently grinded sample consequently leads to under-or over-estimation of GMO content.

References

CRL-GMFF (2009) Event specific method for the quantification of soybean line 40-30-2 using Real-time PCR JRC, IHCP, Biotehnology&GMO's Unit
European Network of GMO Laboratories (2009) Definition of minimum performance requirements for analytical methods of GMO testing

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