Comment and summary in reference to reports of analysis

CD59558 through CD59605 from 06/20/06
CD96333 through CD96369, CD69376 through CD96399 from 04/17/07


Project 1
Various samples of sugar were analyzed for the presence of DNA. A polymerase chain reaction (PCR) test specific for DNA from ubiquitous plant cell organelles called plastids served as an indicator for the presence of plant DNA in general. Since this PCR procedure detects plastid DNA irrespective of the plant species, it was an appropriate procedure to examine sugar derived from various sources for the presence of DNA. PCR is generally more sensitive than other commonly used means of specific or unspecific detection of DNA. Therefore a PCR test appeared suitable to attempt the detection of residual trace amounts of DNA.

Forty-four samples described as commercially available sugar from different sources were analyzed:
- Six samples of organic cane sugar, from Europe, South America and the United States
- Seven samples of turbinado / muscovado sugar, from Africa, Mauritius, Mexico and the United States
- Sixteen samples of white beet sugar from Canada, Europe and the United States
- Fifteen samples of white cane sugar from Africa, Australia, Canada, Caribbean, Europe, Japan and the United States

In addition, four reference samples of analytical grade sucrose were tested. All forty-four samples of commercial sugar and the four reference samples tested negative. It was concluded that plant DNA could not be detected in any of these samples of sugar.

Project 2
From a commercial sugar production process using industrially grown and harvested H7-1 Roundup Ready® sugarbeets, samples of eight different initial, intermediate and final products were taken. These samples ranged from sliced, raw sugarbeets to commercial white sugar. Each different type of product was sampled at the beginning, half way through, and towards the end of Roundup Ready® sugarbeet processing (twenty-four samples total). Eurofins GeneScan inspection personnel on site verified the sampling process and chain of custody for samples submitted to the laboratory.

For control purposes, a respective set of twenty-four samples was taken from a production run using conventional sugarbeets. Thirteen of the sugar samples from Project 1 were used as additional control samples. All samples were analyzed for the presence of DNA sequences indicative of H7-1 Roundup Ready® sugarbeet. The respective real-time PCR method was developed and in-house validated by Eurofins GeneScan, together with KWS
SAAT AG, Einbeck Germany and Monsanto Company, St. Louis, MO. The method was validated in a collaborative trial conducted by the European Commission Joint Research Centre - Community Reference Laboratory\(^1\). All samples were also tested for the presence of the particular novel protein CP4-EPSPS, which confers Roundup tolerance to the H7-1 Roundup Ready\(^\circledR\) sugarbeet plant. A commercially available protein test kit for CP4-EPSPS was used\(^2\).

As expected, neither the 24 samples from processing of conventional sugar beets nor the 13 other sugar samples from Project 1 showed any detectable CP4-EPSPS protein nor H7-1 DNA sequences.

The results for the 24 samples of H7-1 Roundup Ready\(^\circledR\) sugarbeet and respective products are summarized in the table below. Results for the three samples of the same product taken at different times were identical in each case.

<table>
<thead>
<tr>
<th>Samples (set of 3 each)</th>
<th>Detection of H7-1 DNA</th>
<th>Detection of CP4-EPSPS Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sliced Sugarbeet</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pressed Pulp</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dried Pulp</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Raw Sugarbeet Juice</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Thin Sugarbeet Juice</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Thick Sugarbeet Juice</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>White Sugar</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Molasses</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

The CP4-EPSPS Roundup Ready\(^\circledR\) protein was detected in all three samples of sliced, raw H7-1 Roundup Ready\(^\circledR\) sugarbeets and all three samples of raw sugarbeet juice. CP4-EPSPS Roundup Ready\(^\circledR\) protein could not be detected in the respective sugar, molasses and pulp samples processed from the Roundup Ready\(^\circledR\) sugarbeets.

H7-1 DNA was detected in sliced, raw H7-1 Roundup Ready\(^\circledR\) sugarbeets as well as in raw sugarbeet juice and in pulp from H7-1 Roundup Ready\(^\circledR\) sugarbeets. However, H7-1 could not be detected in the samples of commercial white sugar and molasses made from H7-1 Roundup Ready\(^\circledR\) sugarbeets.

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\(^1\) Event-specific method for the quantitation of sugar beet line H7-1 using real-time PCR
[http://gmo-crl.jrc.it/summaries/H7-1-Protocol%20Validated.pdf](http://gmo-crl.jrc.it/summaries/H7-1-Protocol%20Validated.pdf)

\(^2\) TraitChek Lateral Flow Strip – Sugarbeet Seed Application Guide; Part Number 7000014
Strategic Diagnostics Inc., 111 Pencader Dr, Newark, DE 19702