GPC/SEC-Light scattering for starch analysis

Application Note Food Analysis

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The study of native starches from different natural sources shows that GPC/SEC-MALLS can be used efficiently to perform molecular characterization and degradation monitoring.

Introduction
Starches are polysaccharides produced by all green plants to store energy. They are the most important carbohydrates in the human diet, present in potatoes, wheat, maize (corn), and rice. Pure starch is a white powder insoluble in cold water. It consists of two types of molecules: the predominantly linear helical amylose (20-25%) and the lightly branched amylopectin (5-80%).

GPC/SEC-Multi Angle Laser Light scattering (GPC/SEC-MALLS) is the method of choice to characterize starches given their high molar masses and branching. MALLS allows measurement of absolute molar masses and radius of gyration, which can provide additional structural information.

Sample preparation is a critical step and requires dedicated procedures, especially under aqueous conditions. The use of columns with large particle sizes and porosities ensure separations for molar masses up to several millions. Generally, native starches dissolve in DMSO (Dimethylsulfoxide) and separate well in medium polar PSS GRAM columns. Alternatively, if aqueous conditions are required, SUPREMA columns can be used.

Experimental Conditions
Native waxy maize, tapioca, potato, and rice starch were wet-milled in order to determine the effect of milling on average molar mass (Mw) and molar mass distribution. The molar mass distributions of the original pre-process and milled products were characterized by aqueous GPC/SEC with MALLS detection, using the following conditions:

<table>
<thead>
<tr>
<th>Conditions</th>
<th>PSS SEccurity GPC1200 isocratic pump</th>
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</thead>
<tbody>
<tr>
<td>Pump</td>
<td>• flow rate [mL/min]: 0.5</td>
</tr>
<tr>
<td></td>
<td>• mobile phase: aqueous, NaNO₃, pH=10</td>
</tr>
<tr>
<td>Injection system</td>
<td>PSS SEccurity GPC1200 Autosampler</td>
</tr>
<tr>
<td></td>
<td>• injection volume variable</td>
</tr>
<tr>
<td>Columns</td>
<td>PSS SUPREMA 20µ 30 000Å (8*300mm)</td>
</tr>
<tr>
<td></td>
<td>• exclusion limit &gt; 50 Mio Da &gt; 300 nm</td>
</tr>
<tr>
<td>Loading</td>
<td>Samples:</td>
</tr>
<tr>
<td></td>
<td>• 0.5 mg/mL, 100µL injection volume</td>
</tr>
<tr>
<td>Detectors</td>
<td>• Refractive index PSS SEccurity 1200 RI</td>
</tr>
</tbody>
</table>
|                                | • PSS SLD7000 7-angle MALLS optional:
|                                | • PSS ETA2010 Differential Viscometer |
| Software                       | PSS WinGPC Unity plus light scattering module |
**Results & Discussion**

Figure 1 shows the raw data of a native and a wet-milled starch for the refractive index detector RI and for the 90° signal of the MALLS detector. The light scattering detector is a molar mass sensitive detector that detects high molar masses efficiently while the sensitivity in the low molar mass region is less. Every molar mass sensitive detector needs a concentration detector (in this case the refractive index detector, RI) to measure the molar mass on-line without the need of a calibration curve.

![Fig. 1: Raw data for the refractive index and the 90° light scattering detector signal of a milled and native starch.](image)

Figure 2 shows an overlay of the measured molar mass distributions for native and wet-milled starches from different sources.

![Fig. 2: Overlay of the molar mass distributions of several milled and native starches.](image)
Figure 3 shows a summary of the molar mass loss when milling, which is 14-23% for all starches with the exception of maize starch. Here milling reduces the molar mass by 73%.

![Sample Degration by Milling](image)

Fig. 3: Comparison sample degradation for several native and milled starches.

The investigation of the radius of gyration, Rg, shows a similar picture. While it stays mainly constant for other types of starches, Rg decreases 40% for maize starch.  

**Conclusion**

The molecular characterization of native and milled starches from different natural sources shows that GPC/SEC-MALLS can be used efficiently to study the degradation. If sample preparation has been performed properly and proper GPC/SEC columns with wide pores and large particles are used, sample recovery is close to 100% and starch molar masses separation covering monomers to several 10 million g/mol are possible.

**Literature**