



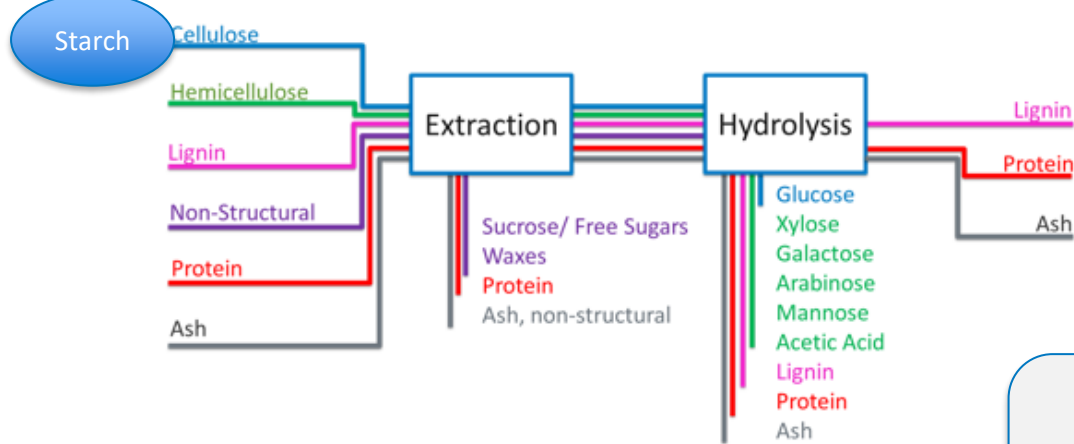
Fiber and the Past

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FELC 2024

Mandate from EPA/DOE

EPA had evaluated the methods available for cellulosic assay of corn grains and they were concerned about **starch related biases** and methods that were not **publicly available**

- Speciate starch and cellulose (and hemicellulose)
- Validated or VCSB method
- Neutral/third party method
- Developed for a natural substrate



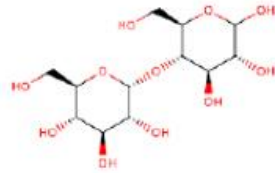
Multiple weighing steps add to the variability. Minimize those steps

Method Selection

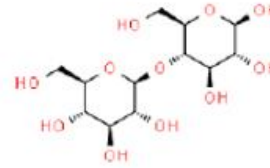
Method must be usable in a commercial lab. No NMR/MBMS/Xray

Existing methods hydrolyzed all carbohydrates to monomers for measurement. This causes a loss of the alpha-linkage that defines starch

- ASTM E1758-24 test method for measurement of carbohydrates by HPLC
- NREL Laboratory Analytical Procedures (LAPs)



α -Glucan
Maltose



β -Glucan
Cellobiose

Hemicellulose removed from the sample by NaOH prevents quantification

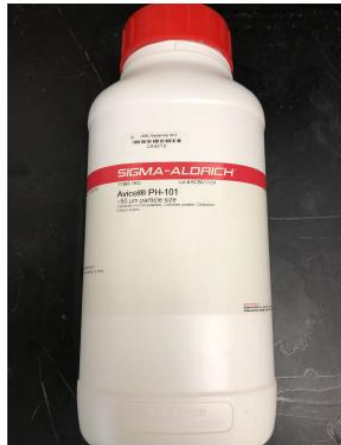
Boiling was hard to handle, and we found the DMSO method more variable

Starch Removal Prior to Hydrolysis

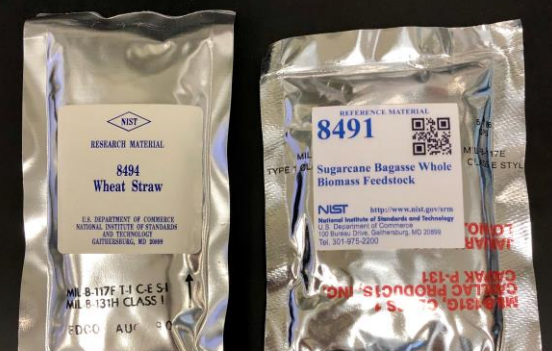
Enzymatic assays are very specific to bond linkages, while acid is not.

The Megazyme starch assay or AOAC 996.11 is well accepted in the industry. The official method uses DMSO to access the resistant starch.

- AOAC 996.11 Determination of total starch with resistant starch
 - DMSO boiling
 - Alternate NaOH cold



- Avicel Cellulose
 - Sigma Aldrich cellulose powder
 - 95.4% glucan by NREL acid hydrolysis
- Resistant Starch
 - Ingredion Hylon VII resistant starch
 - High amylose Starch
 - 97.4% glucan by NREL acid hydrolysis



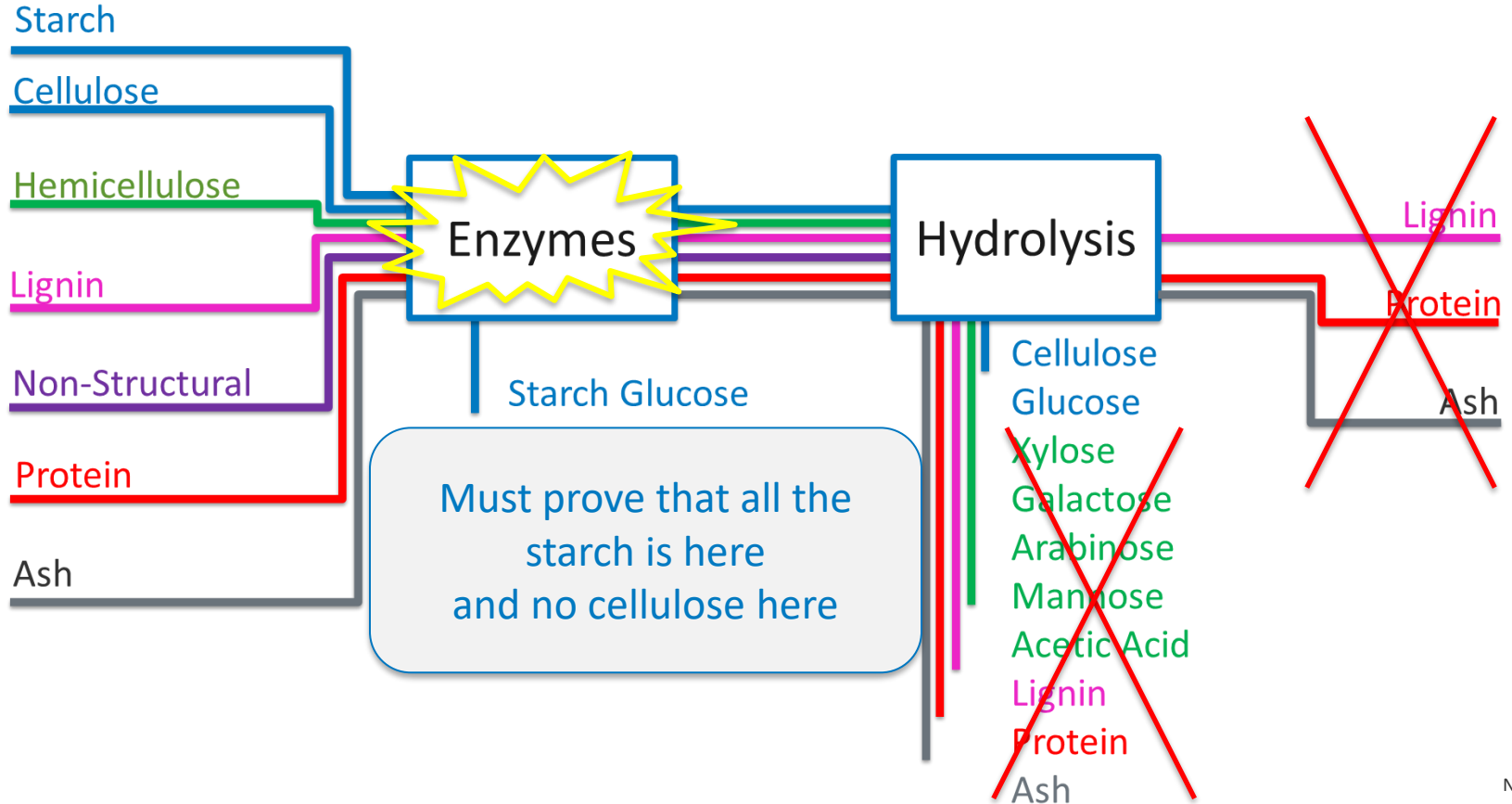
NIST materials have additional resistant starches due to preparation

Reference materials

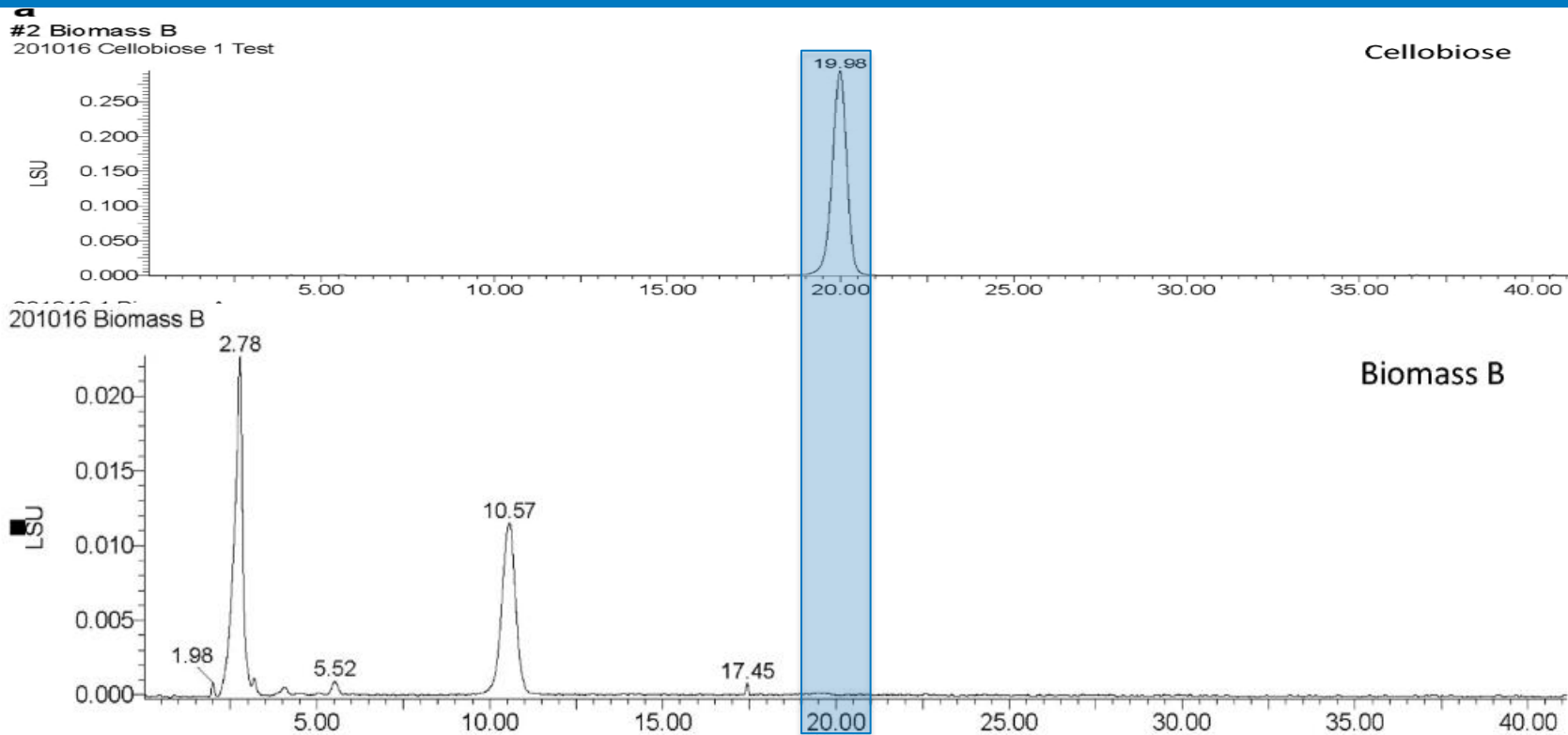
EPA felt that matrix effects were significant when determining starch and cellulose content. Work was restricted to native biomass samples.

- To ensure method responses, we did spike with Avicel and a resistant starch material

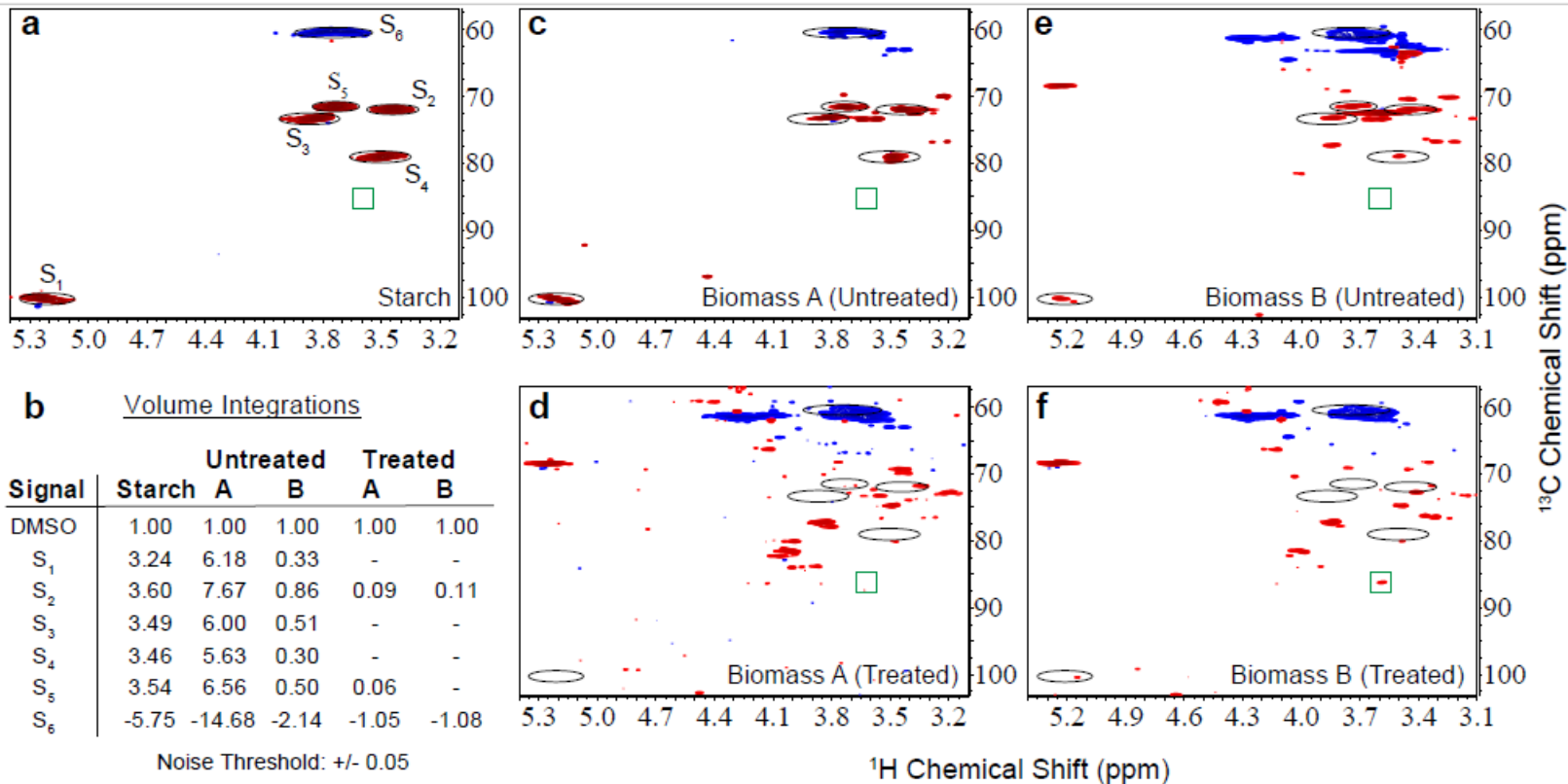
Method diagram



Validation of No Cellulose Removal



Validation of Starch Removal




Publication


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Direct determination of cellulosic glucan content in starch-containing samples

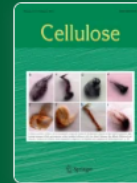
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