HPTLC Association

A Validated High-Performance Thin-Layer Chromatography Method for Monitoring Glucose and Malto Oligosaccharides with Multiple Degree of Polymerization During Bioethanol Production from Corn Biomass.

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Introduction

Fossil fuel

Is a hydrocarbon-containing material formed underground from the remains of dead plants and animals.

Coal

Petroleum

Natural gas

Introduction

Advantages

- Fossil fuels generate a large amount of electricity at a single location.
- Can be found easily and are cost-effective.
- Transportation of oil and gas can be done easily through pipelines.

Disadvantages

- Fossil fuels are the main driver of global warming, then contribute to climate change.
- Fossil fuels are non-renewable sources of energy.

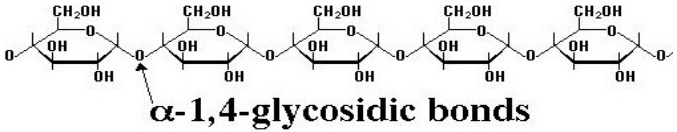
Renewable sources of energy

- Solar energy
- Geothermal energy
- Wind energy
- Hydropower from flowing water
- **Biomass from plants**

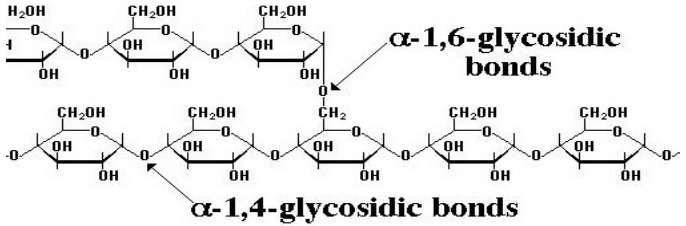
Biomass from plants

- Corn has been widely used to produce ethanol due to the large amount of carbohydrates (specifically starch) produced in the endosperm of the kernel.

Amylose



Amylopectin

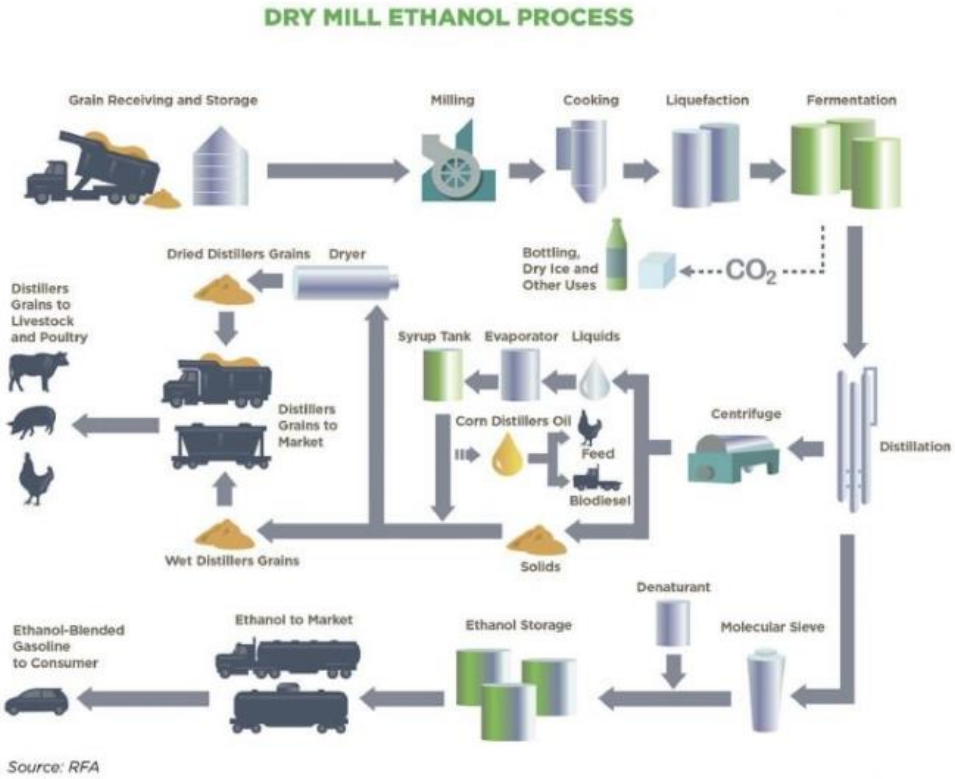


Biomass from plants

- Bioethanol production is a process of sugar fermentation to yield ethanol

- Corn requires from **60 to 100** days to reach harvest depending on the variety and warm weather.

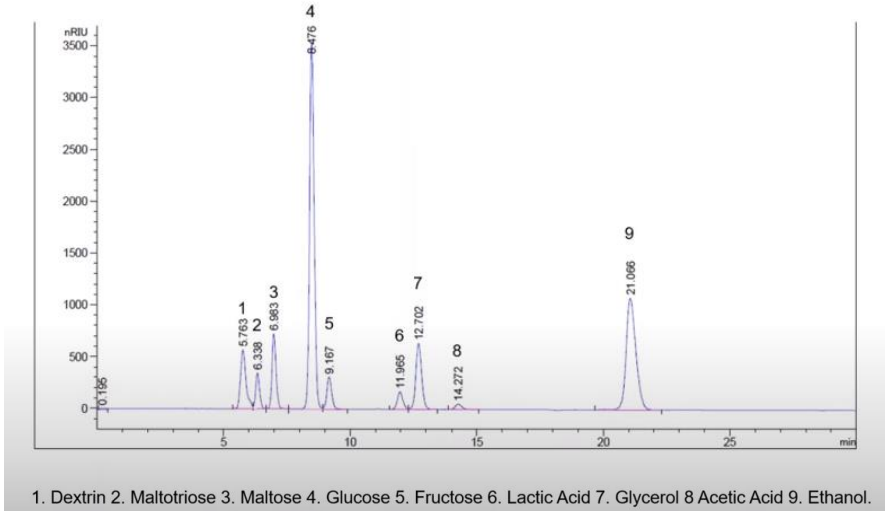
Ethanol production from corn



https://ethanolrfa.org/ethanol-101/how-is-ethanol-made

Monitoring sugars by HPLC

- The fermentation process of most bioethanol plants in U.S is monitored by HPLC.
- The HPLC methods range from 21 to 42 min and the detection is usually performed using a refractive index detector.
- The HPLC method can simultaneously detect ethanol, organic acids, glucose, maltose and maltotriose.
- Maltotetraose and sugars with the highest degree of polymerization are not resolved by HPLC.



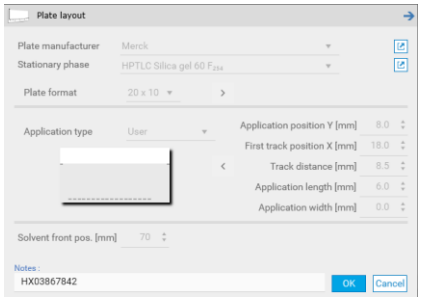
1. Dextrin 2. Maltotriose 3. Maltose 4. Glucose 5. Fructose 6. Lactic Acid 7. Glycerol 8 Acetic Acid 9. Ethanol.

The aim of this work is to develop an HPTLC method to quantify from glucose up to DP_8 and improve the turnaround of the process.

HPTLC instruments and step process

Glucose	6-48 hours= 100x dilution
	54-60 hours= 5x dilution
Maltose	No dilution
Maltotriose	No dilution
DP4	6, 36-60 = 2x dilution
	12-30 hours = 5x dilution
DP5	2x dilution
	6 &12 hours = 5x dilution
DP6	no dilution
	6 hours 5x dilution
DP7	No dilution
DP8	No dilution

HPTLC instruments and step process



Developing distance: 60 mm

Mobile phase: acetone: NP: water: formic acid (40:25:12:0.1 (v/w/v/v%)

diphenylamine, aniline and phosphoric acid, speed 5; time 3s

120 °C for 10 min

625 nm after derivatization

HPTLC validation

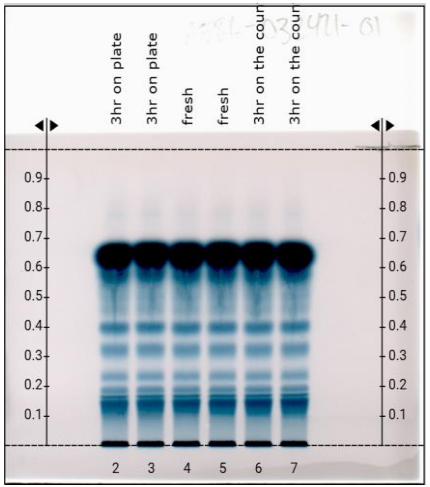
Stability

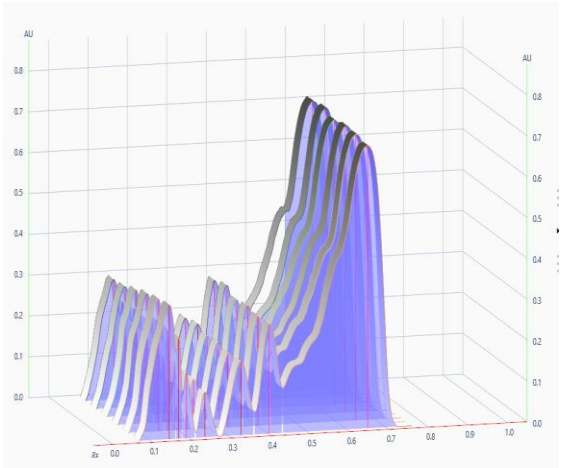
- Sample in solution (3h)
- Sample on plate (3h)
- 2D-Separation
- Evaluate chromatogram immediately and during 1 hour after derivatization or final drying.

Stability

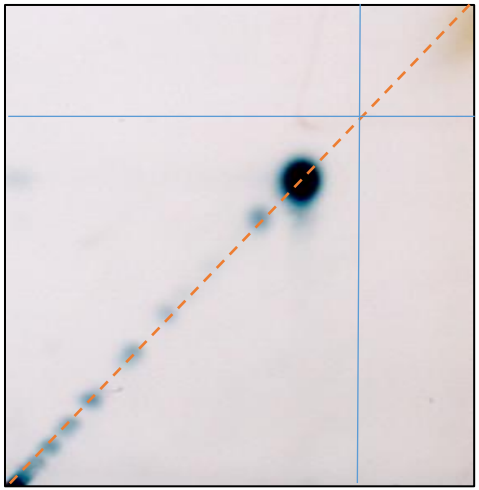
Stability

- Stability of the sample in solution and on the plate (3h)



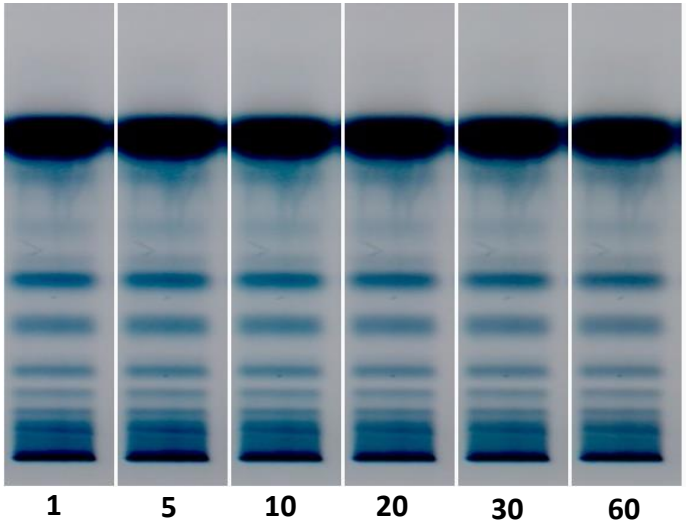


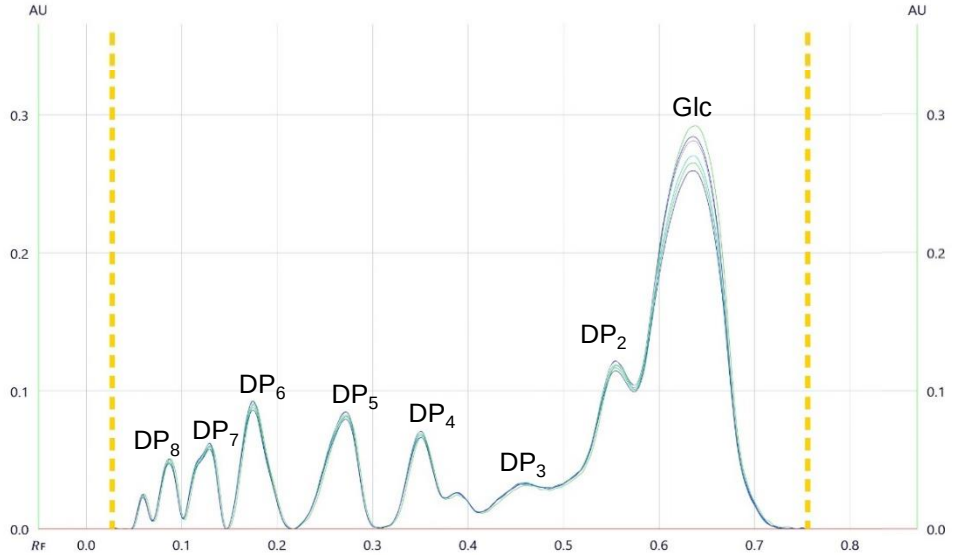
- 2D-Separation



Stability

- Evaluation of the chromatogram immediately and during 1 hour after derivatization.





Repeatability

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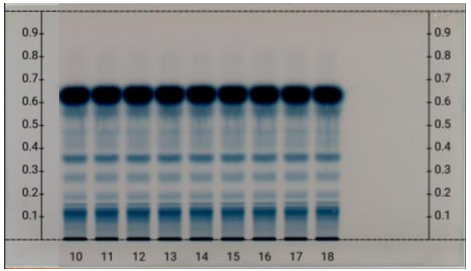

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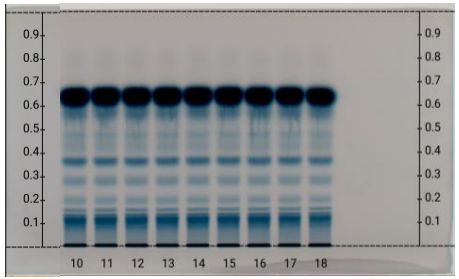

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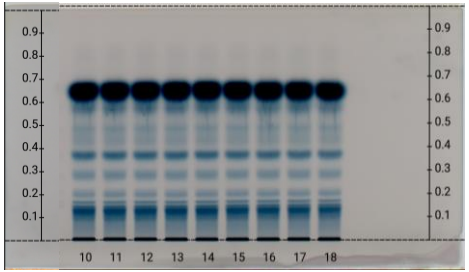
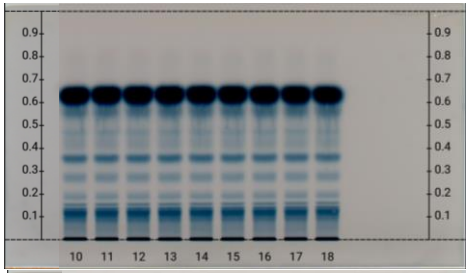
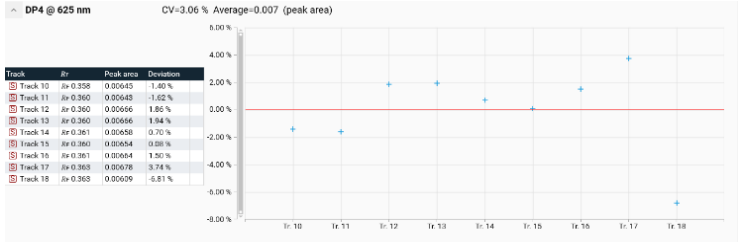


Sugars	A586-031921-01	A586-031921-02	A586-032021-01	ΔR_F
Glc	0.64	0.65	0.65	0.01
DP2	0.56	0.57	0.57	0.01
DP3	0.46	0.47	0.47	0.01
DP4	0.36	0.37	0.37	0.01
DP5	0.27	0.28	0.28	0.01
DP6	0.19	0.20	0.20	0.01
DP7	0.16	0.16	0.17	0.01
DP8	0.14	0.13	0.14	0.01

Repeatability

A586-031921-01





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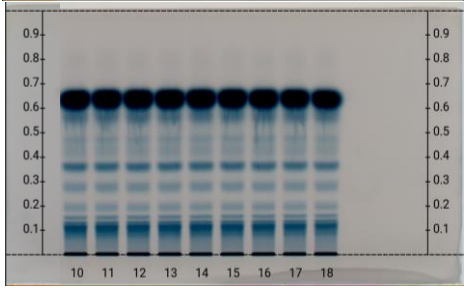

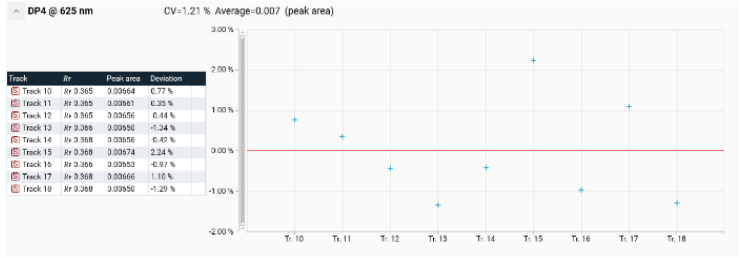

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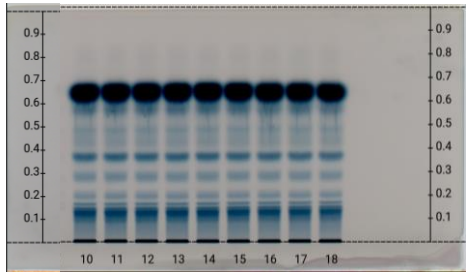
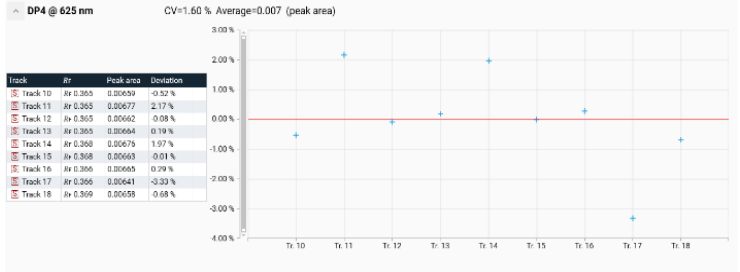
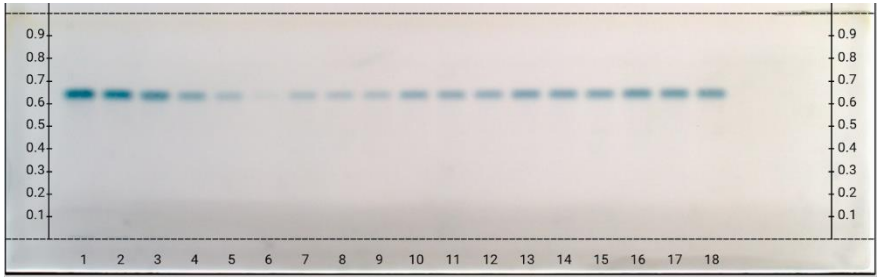
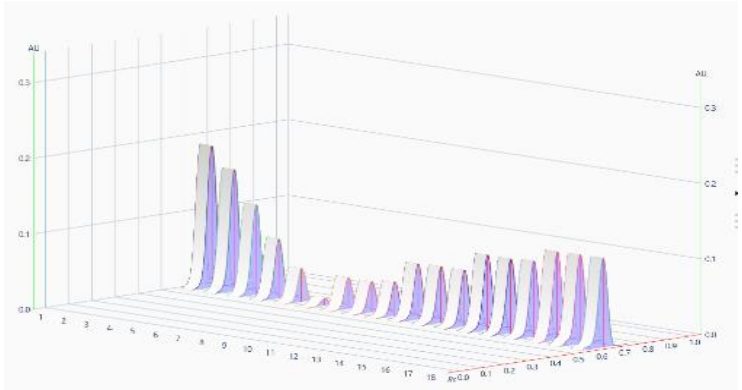




Recovery for glucose





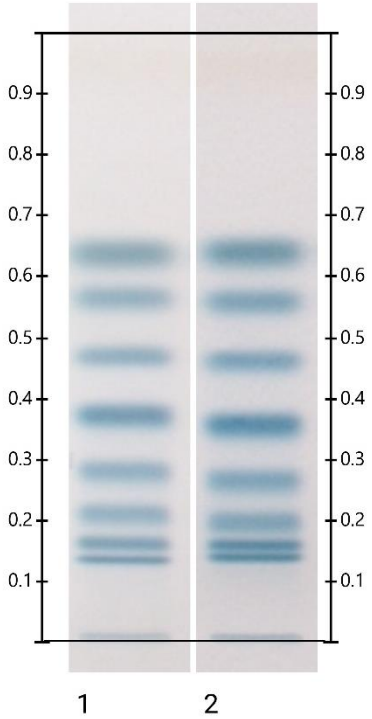
Sample 'Glu-040721-01-01'		23.64 µg/ml	CV=5.47 % (3 applications)
Volume: 3.0 µl		23.64 µg/ml	CV=5.47 % (3 replicas)
Track 7	Rf 0.640	22.91 µg/ml	68.72 ng
Track 8	Rf 0.642	22.89 µg/ml	68.67 ng
Track 9	Rf 0.642	25.14 µg/ml	75.41 ng
Sample 'Glu-040721-01-03'		50.17 µg/ml	CV=1.23 % (3 applications)
Volume: 3.0 µl		50.17 µg/ml	CV=1.23 % (3 replicas)
Track 10	Rf 0.644	49.49 µg/ml	148.5 ng
Track 11	Rf 0.644	50.70 µg/ml	152.1 ng
Track 12	Rf 0.646	50.33 µg/ml	151.0 ng
Sample 'Glu-040721-01-04'		73.08 µg/ml	CV=0.52 % (3 applications)
Volume: 3.0 µl		73.08 µg/ml	CV=0.52 % (3 replicas)
Track 13	Rf 0.646	72.64 µg/ml	217.9 ng
Track 14	Rf 0.648	73.35 µg/ml	220.0 ng
Track 15	Rf 0.648	73.25 µg/ml	219.8 ng
Sample 'Glu-041321-01-05'		95.19 µg/ml	CV=2.20 % (3 applications)
Volume: 3.0 µl		95.19 µg/ml	CV=2.20 % (3 replicas)
Track 16	Rf 0.650	92.82 µg/ml	278.5 ng
Track 17	Rf 0.650	96.80 µg/ml	290.4 ng
Track 18	Rf 0.650	95.95 µg/ml	287.9 ng

$$\text{Recovery (\%)} = \frac{S - U}{A} \times 100$$

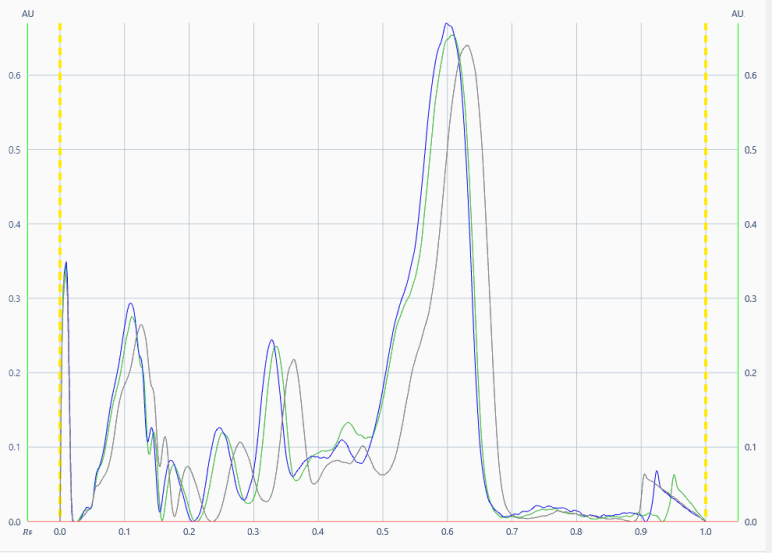
Spiked with 25 µg/mL= 106.1%
Spiked with 50 µg/mL= 98.9%
Spiked with 75 µg/mL= 95.4%

Robustness

- Activation with $MgCl_2$ and molecular sieves

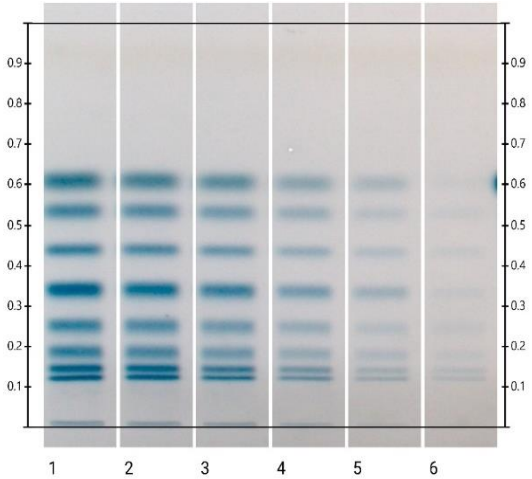


- Heating of different plates at 110 °C; 120 °C and 130 °C

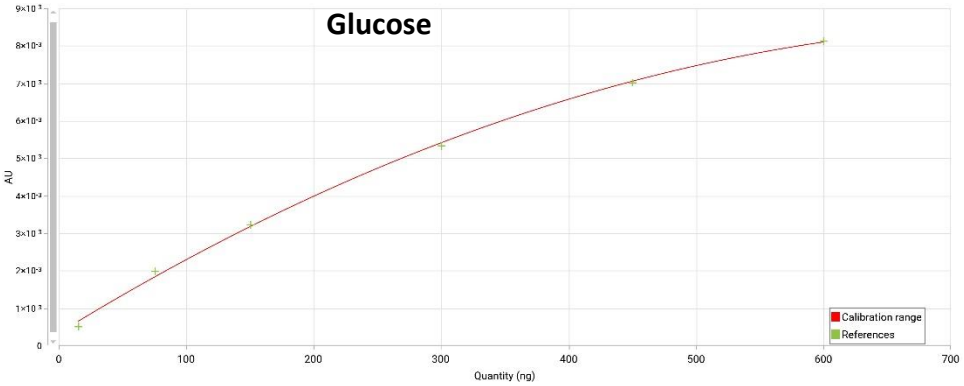


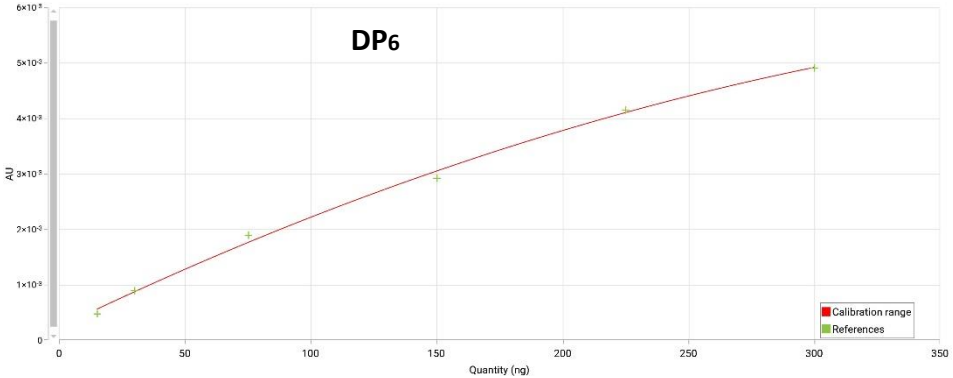
	#	Vial	Sample
120°C	1	T1376-031621-01-01	A4794 12 hours fermentation
130°C	2	T1376-031621-01-01	A4794 12 hours fermentation
110°C	3	T1376-031621-01-01	A4794 12 hours fermentation

Calibration curves

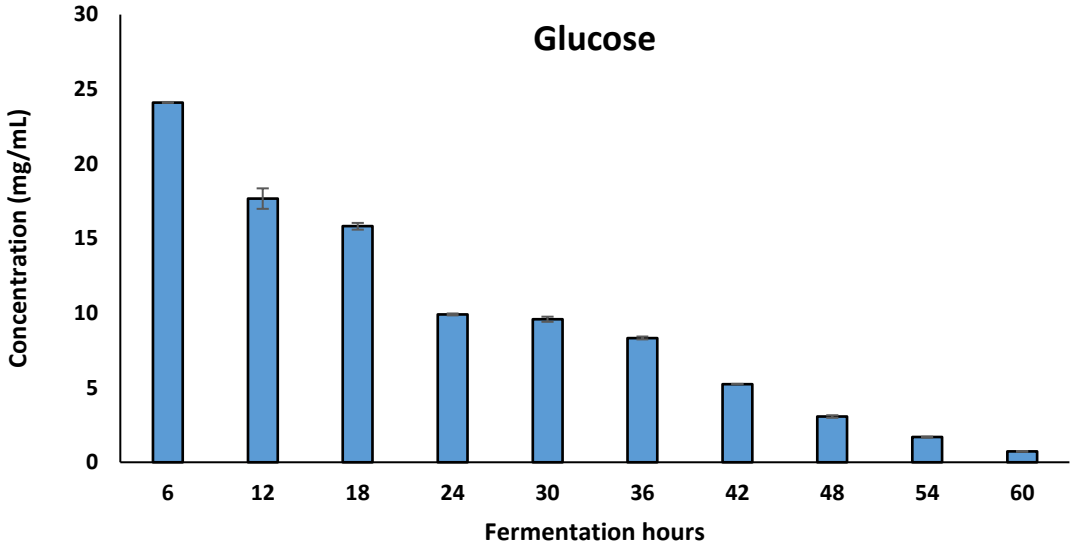


Glc and DP4: 200 µg/mL to 5 µg/mL
DP2, DP3, DP5-DP8: 100 µg/mL to 5 µg/mL

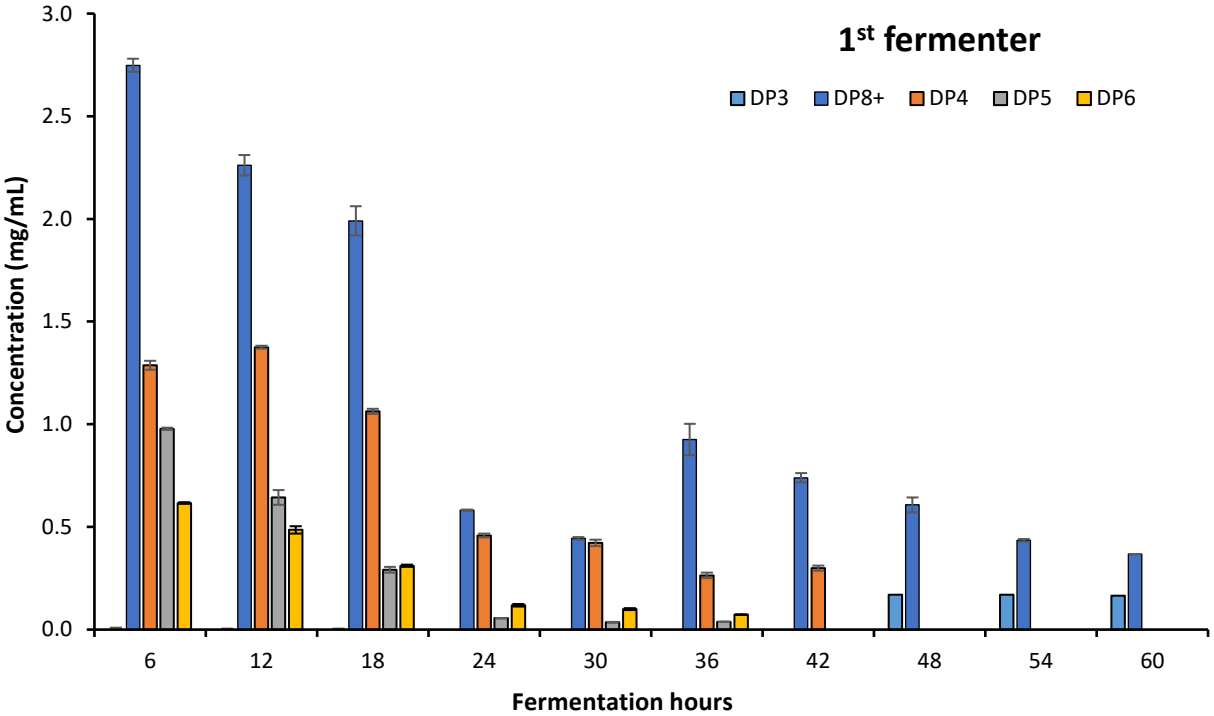




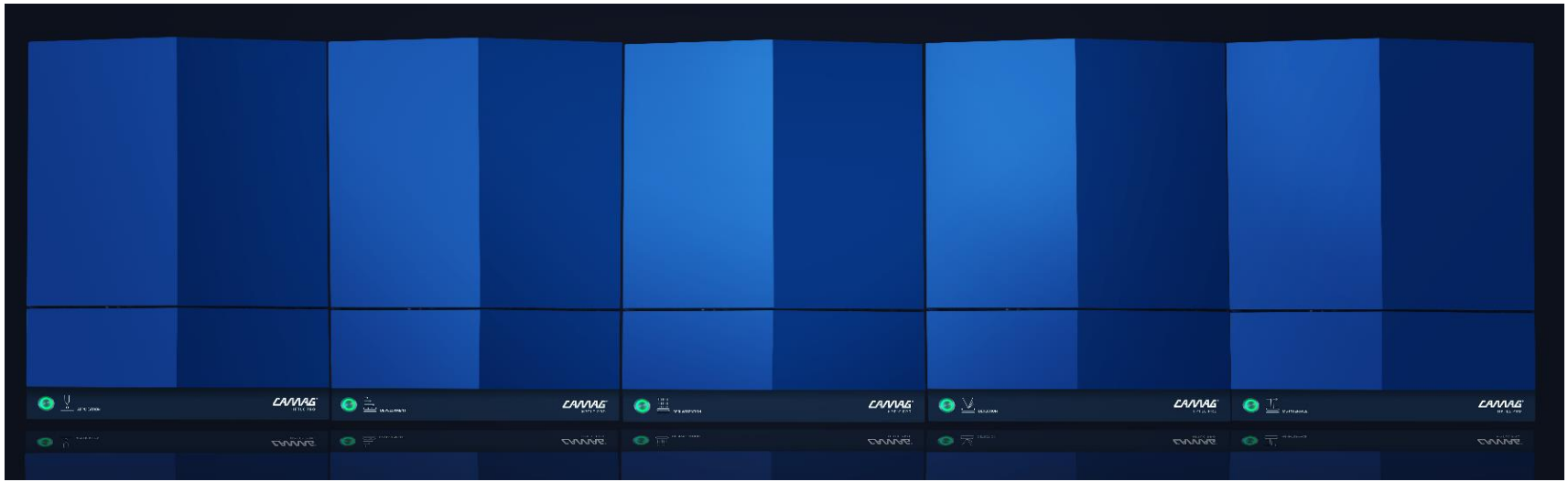
Analysis of the sugars in three fermenter vessels



Analysis of the sugars



Automation of the process



Comparison between HPLC and HPTLC method

Parameters compared	HPLC	HPTLC
Sugars separation	Glc, DP_2, DP_3 and DP_4^+	Glc, DP_2, DP_3, DP_4, DP_5, DP_6, DP_7 and DP_8^+
Time for 20 samples	~600 min	~120 min
Amount of solvent per 20 samples	~360 mL	~40 mL

For the comparison an HPLC method of 30 min and a flow rate of 0.6 mL/min was considered.

Thank you!