MSRG
Medical Sciences
Research Group

RELATING THE (SUPRA)MOLECULAR STRUCTURE OF STARCH IN RICE TO ITS DIGESTIBILITY

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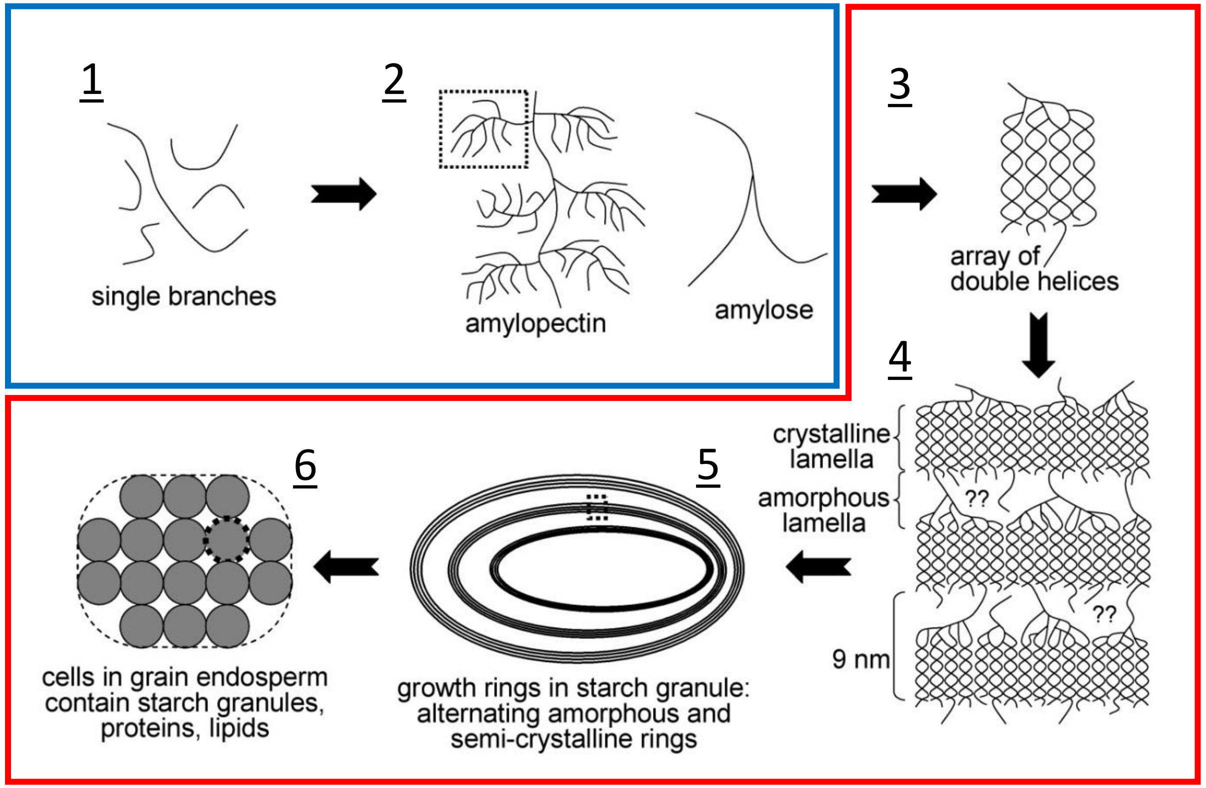
- Objectives
  - New method to characterise amylose
  - Examine starch structure at an organisational level of the same size domain as digestive enzymes operate
  - Relate these to digestibility

- Benefits to Partnership
  - Revised structure used to understand digestibility (and other market requirements such as texture)
  - Capacity to better target markers that can explain amylose structure
  - Provide tools to predict GI

Starch structure affects digestibility

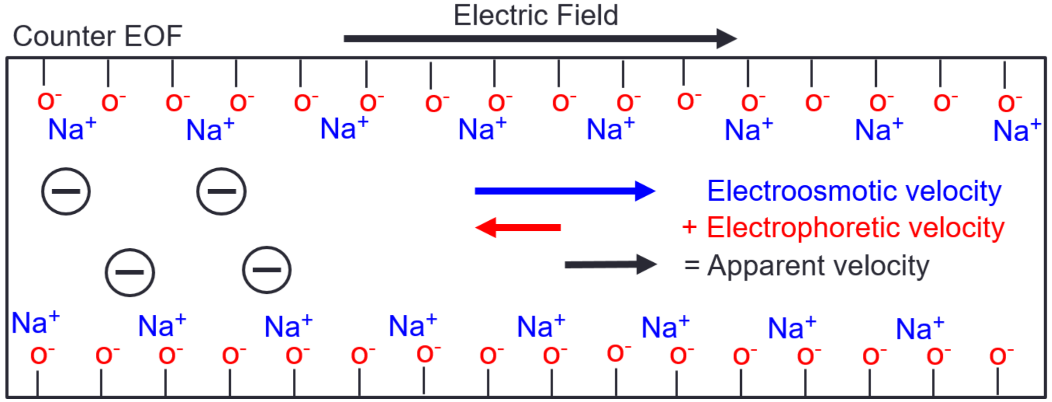
- Molecular structure
  - Amylose content
  - Branching
  - Distributions of branching structures
- Supramolecular structure
  - Crystallinity
    - Short–range → Formation of helices
    - Long-range → Overall order
  - Semi-crystalline lamellar structure



The six hierarchical structural levels of starch *(adapted from 1)*

1. M Gaborieau, P Castignolles, Caractérisation de l'amidon et de ses matériaux composites. *Les Annales des falsifications de l'expertise chimique et toxicologique (Société des Experts Chimistes de France)* **2009,** *9710*, 23-32.

Amylose content by capillary electrophoresis (CE)



Separation mechanism in CE (counter-EOF mode); EOF – Electroosmotic flow(velocity)

- Traditionally measured as a mixture of both components
  - Overlapping absorbance bands and poor reproducibility
- More accurate determination by separation of amylose (Am) and amylopectin (Ap) in CE
  - Possible by iodine binding[1]

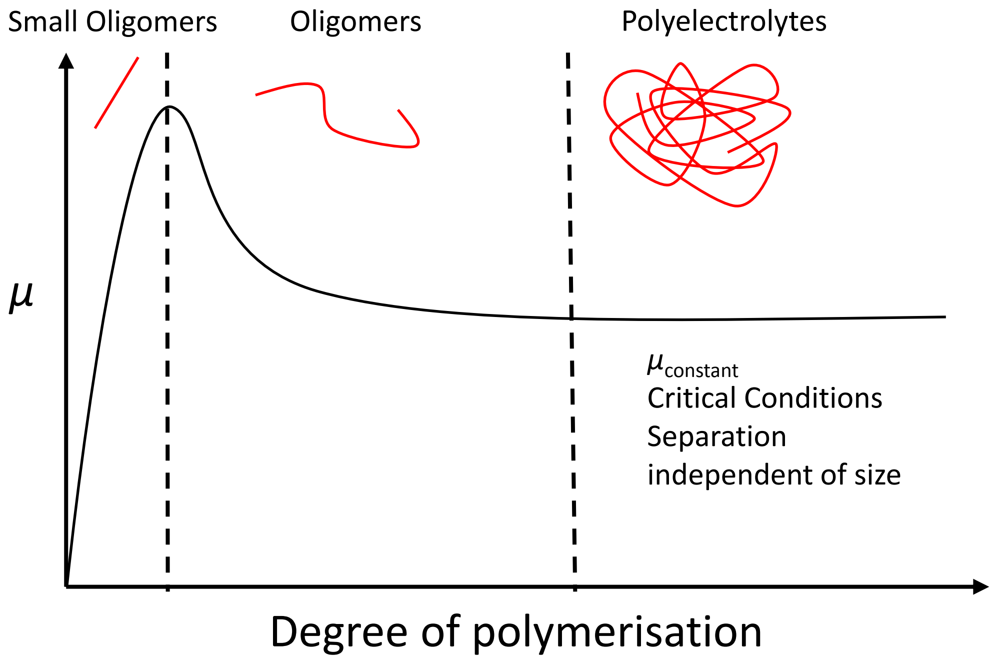
Electrophoretic velocity $\mu = \frac{l_d l_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eof}}\right)$

1. JM Herrero-Martínez, PJ Schoenmakers, WT Kok, Determination of the amylose–amylopectin ratio of starches by iodine-affinity capillary electrophoresis. *Journal of Chromatography A* **2004,** *1053*, 227-34.

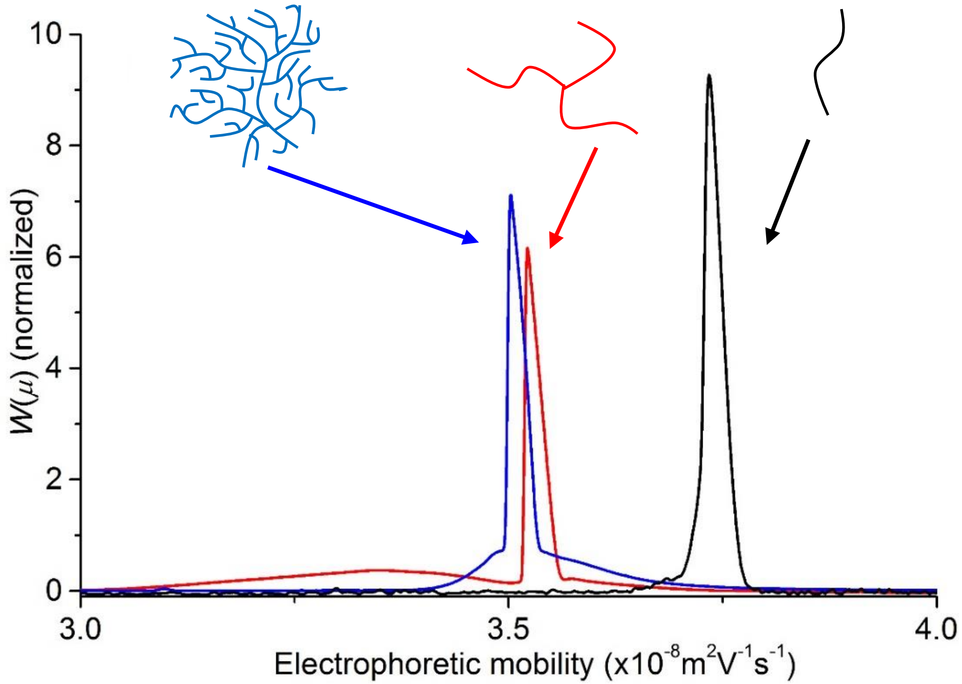
Heterogeneity of branching

- The mode “critical conditions” (CE-CC) allows large polyelectrolytes to be separated by factors other than molar mass such as branching or composition[1]

Evolution of electrophoretic mobility with size[2]



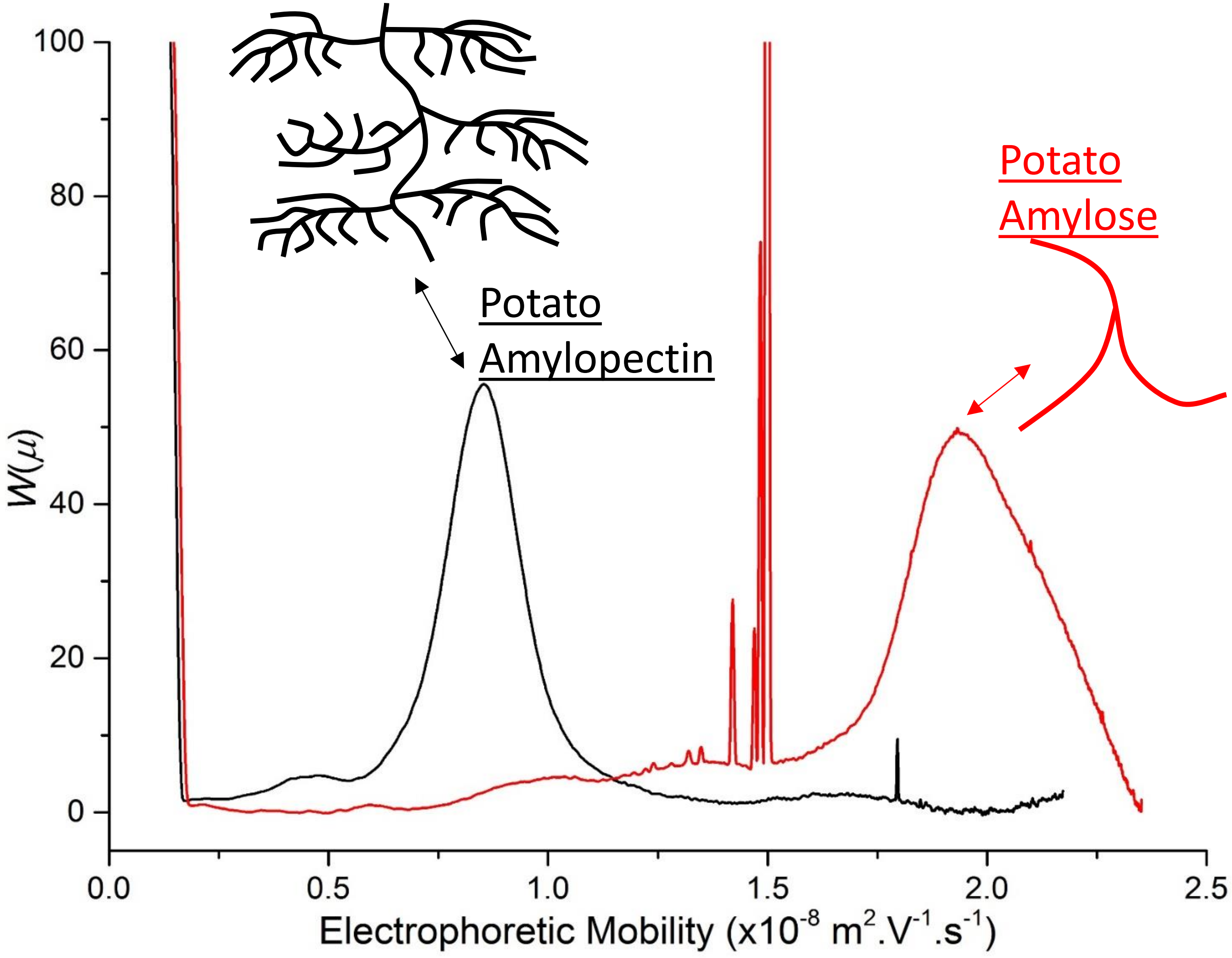
Separation of poly(sodium acrylate)s with different branching structures by capillary electrophoresis [3]



1. Thevarajah, J.J., Gaborieau, M., and Castignolles, P., *Separation and Characterization of Synthetic Polyelectrolytes and Polysaccharides with Capillary Electrophoresis.* Advances in Chemistry, 2014, **2014** Article ID 798503.
2. Cottet, H., Gareil, P., Theodoly, O., and Williams, C.E., *A semi-empirical approach to the modeling of the electrophoretic mobility in free solution: application to polystyrenesulfonates of various sulfonation rates.* Electrophoresis, 2000, **21**(17) 3529-40.
3. Thevarajah, J.J., Sutton, A.T., Maniego, A.R., Whitty, E.G., Cottet, H., Castignolles, P., and Gaborieau, M., *Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions.* Analytical Chemistry 2016, **88**(3) 1674-1681.

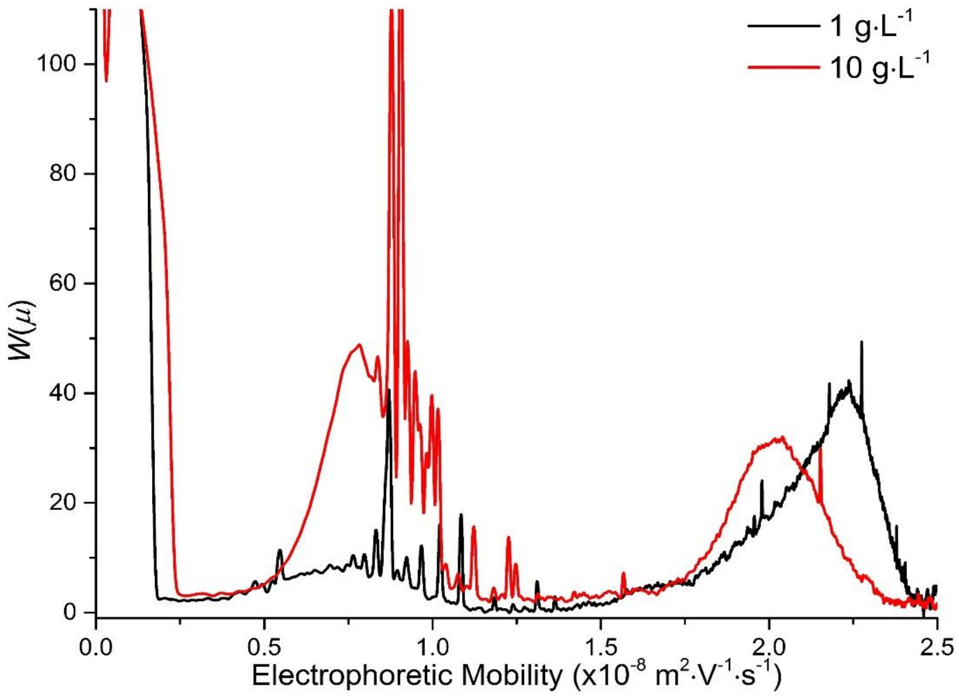
Heterogeneity of branching

- Transformation into weight distribution of electrophoretic mobilities $W(\mu)$
- In these conditions the 'broadness' of a peak represents a distribution of mobilities
    - These mobilities represent different structures
- For amylose and amylopectin this allows us to investigate the heterogeneity of branching

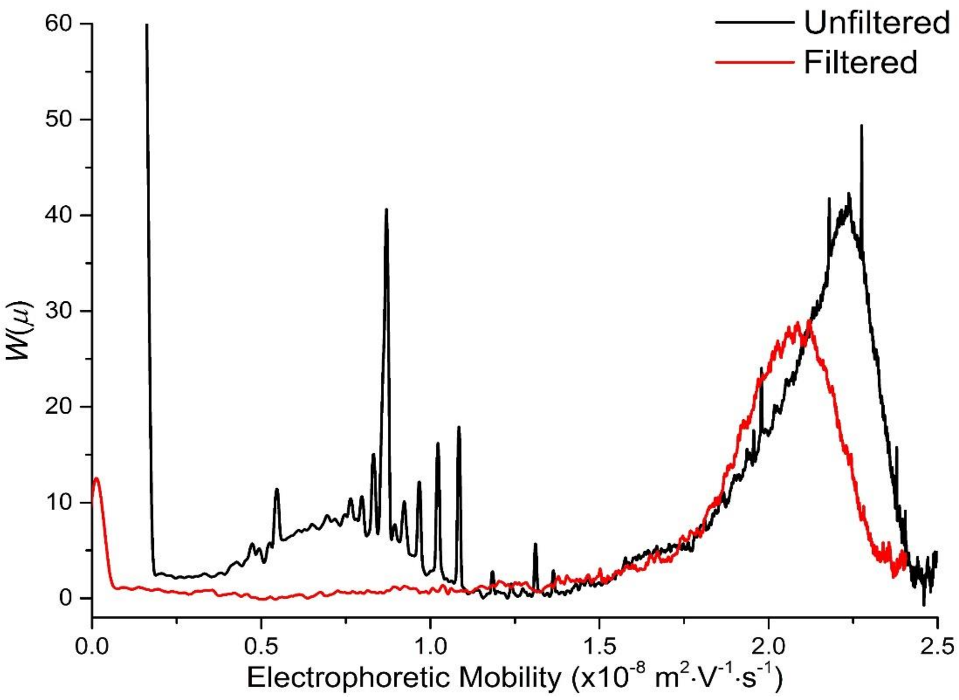


1. Thevarajah, J.J., Sutton, A.T., Maniego, A.R., Whitty, E.G., Cottet, H., Castignolles, P., and Gaborieau, M., *Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions.* Analytical Chemistry 2016, **88**(3) 1674-1681.

Dissolution is important !

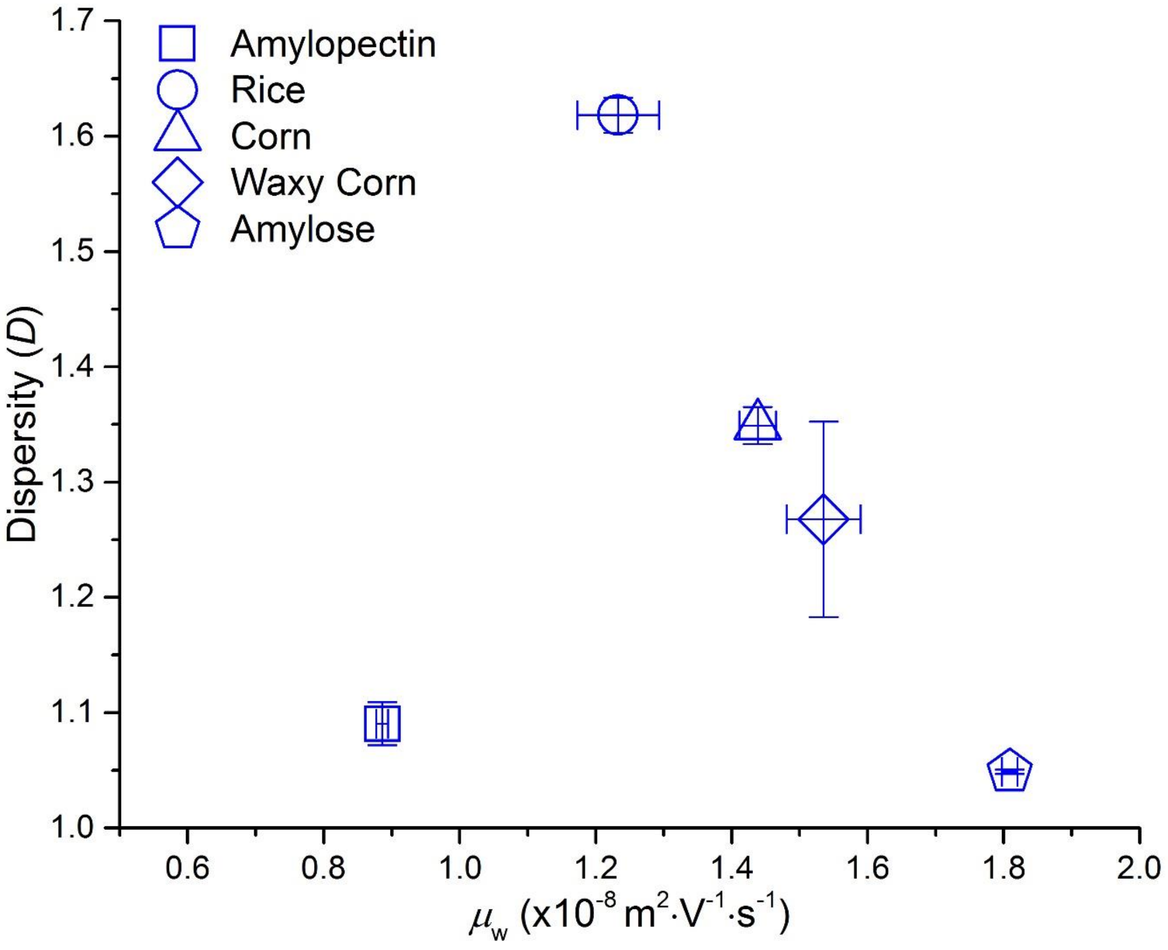


- Low concentration – prevent aggregation
- DO NOT FILTER – significant sample loss incurred



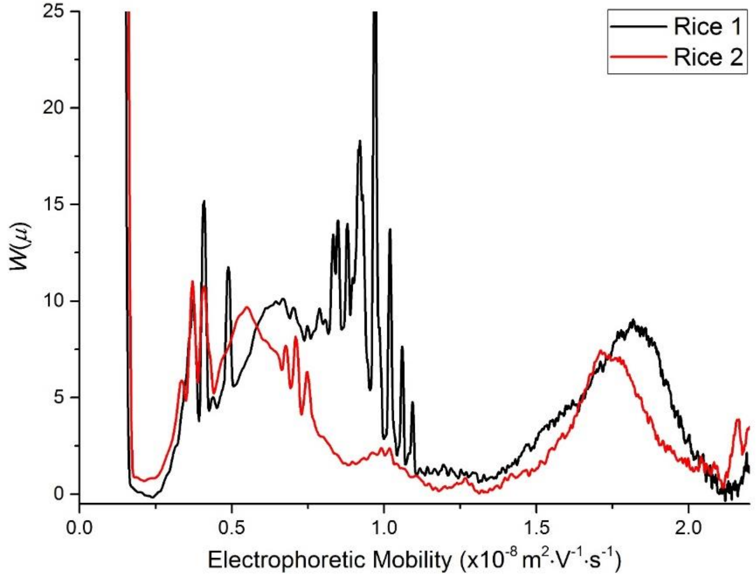
- Anhydrous DMSO with LiBr (H-bond disruptor)
- High dissolution temperature
- Maintain high solution temperature

Dispersity of distributions



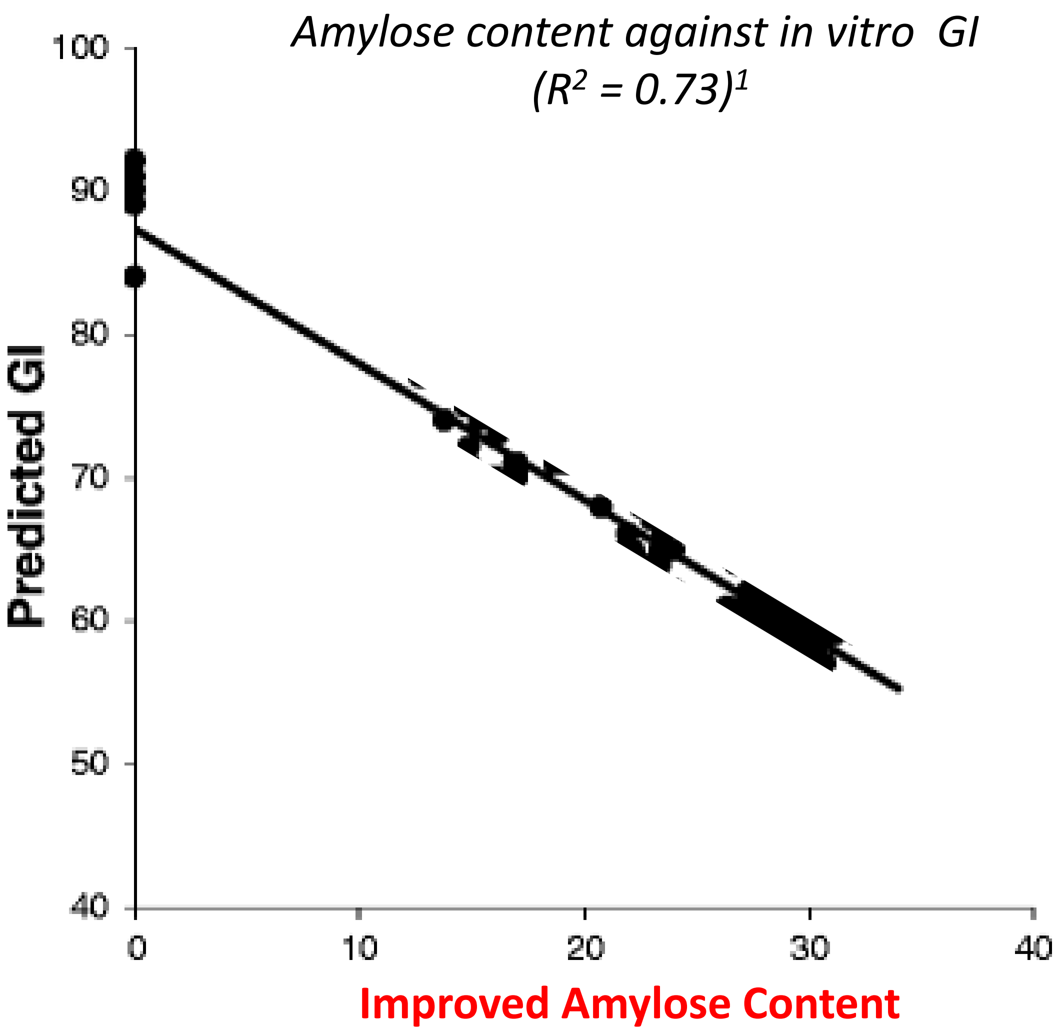
- Very high dispersity values[1] – extremely heterogeneous
- Dispersity values tell us about branching structure

In applying this to individual components we can determine the bias in branching of these components



1. JJ Thevarajah, AT Sutton, AR Maniego, EG Whitty, H Cottet, P Castignolles, M Gaborieau, Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions. Anal Chem 2016, 88, 1674-81.

Improving amylose content



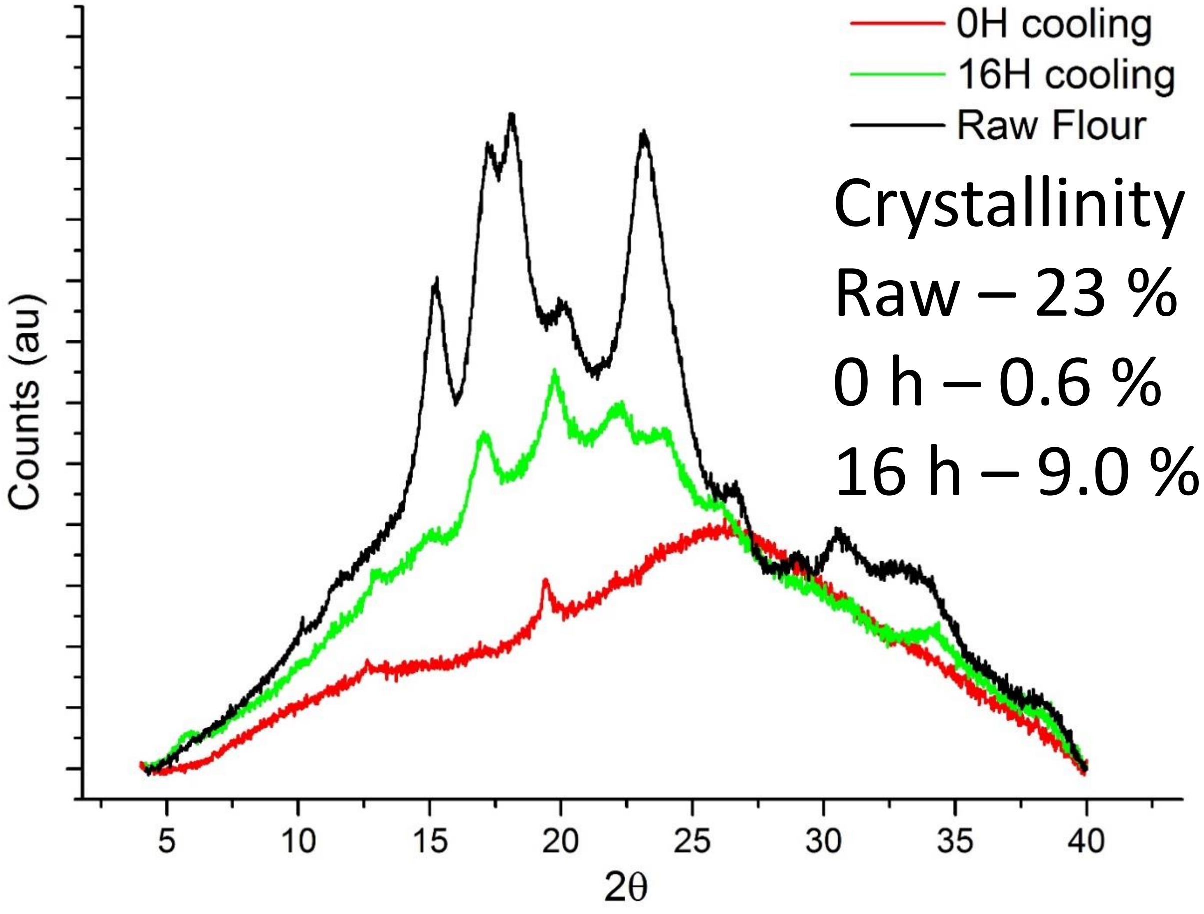
More accurate determination

Possibility for a new definition to better correlate with GI

By accounting for the branching of different amyloses – Better correlation?

1. Fitzgerald, M.A., Rahman, S., Resurreccion, A.P., Concepcion, J., Daygon, V.D., Dipti, S.S., Kabir, K.A., Klingner, B., Morell, M.K., and Bird, A.R., *Identification of a Major Genetic Determinant of Glycaemic Index in Rice.* Rice, 2011, **4**(2) 66-74.

Kinetics of starch



Crystallinity
Raw – 23 %
0 h – 0.6 %
16 h – 9.0 %

Crystalline structure is lost in cooked rice

- Crystalline structure partially reforms with cooling
- Monitoring kinetics by XRD is limited due to:
  - Measurement time
  - Damage to starch by X-ray exposure

Crystallinity by XRD

- Crystallinity by XRD relies on peak fitting[1]
  - Compared software packages – different algorithms, different background subtraction, different results
  - Important factor to consider in future experiments!

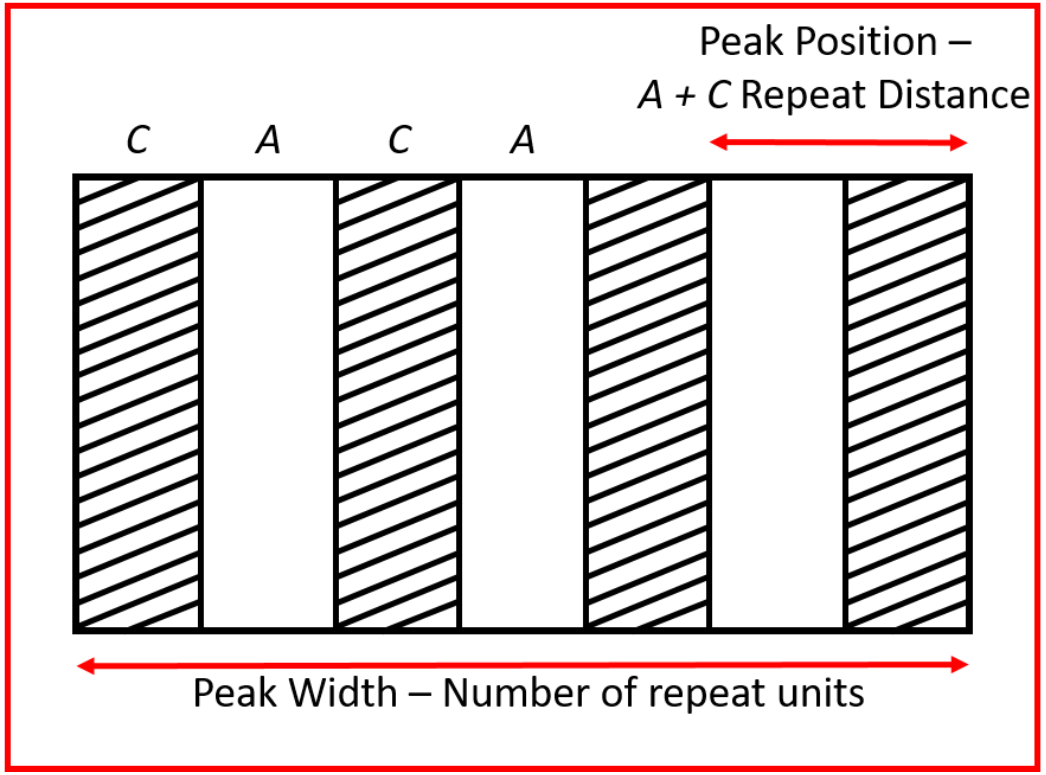
		Topas		Igor		Literature[1]	
	Type	Crystallinity	V-Type	Crystallinity	V-Type	Double helix	V-type
Gelose 80	B	24.5%	3.4%	29.9%	3.2%	18.0%	14.0%
Regular Maize	A	25.9%	2.4%	25.2%	0.9%	33.0%	3.0%
Waxy Maize	A	28.4%	1.8%	30.9%	0.2%	47.0%	0.0%

1. A Lopez-Rubio, BM Flanagan, EP Gilbert, MJ Gidley, A novel approach for calculating starch crystallinity and its correlation with double helix content: a combined XRD and NMR study. Biopolymers 2008, 89, 761-68.

Small angle X-ray scattering

- Investigate the lamellar structure of starch[1]
  - Crystalline and amorphous lamellae in the semi-crystalline lamellar structure
  - Size of the semi-crystalline lamellae
  - Total amount of semi-crystalline structure

Peak Intensity– Percentage of sample with this arrangement

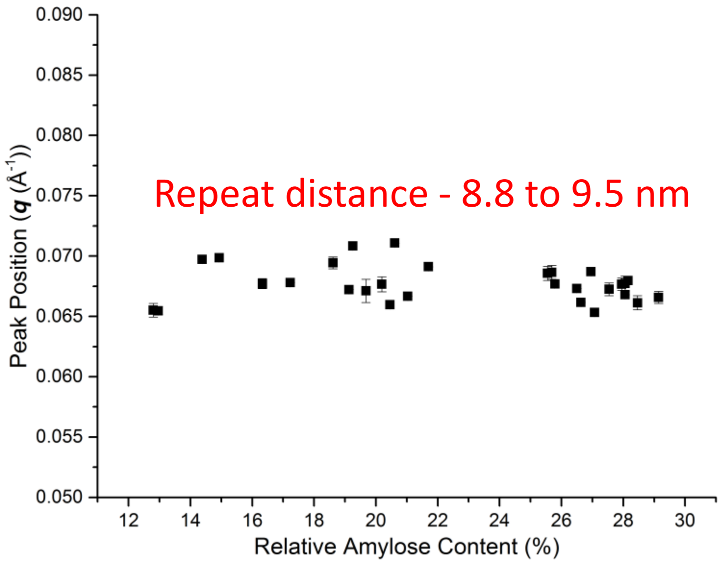


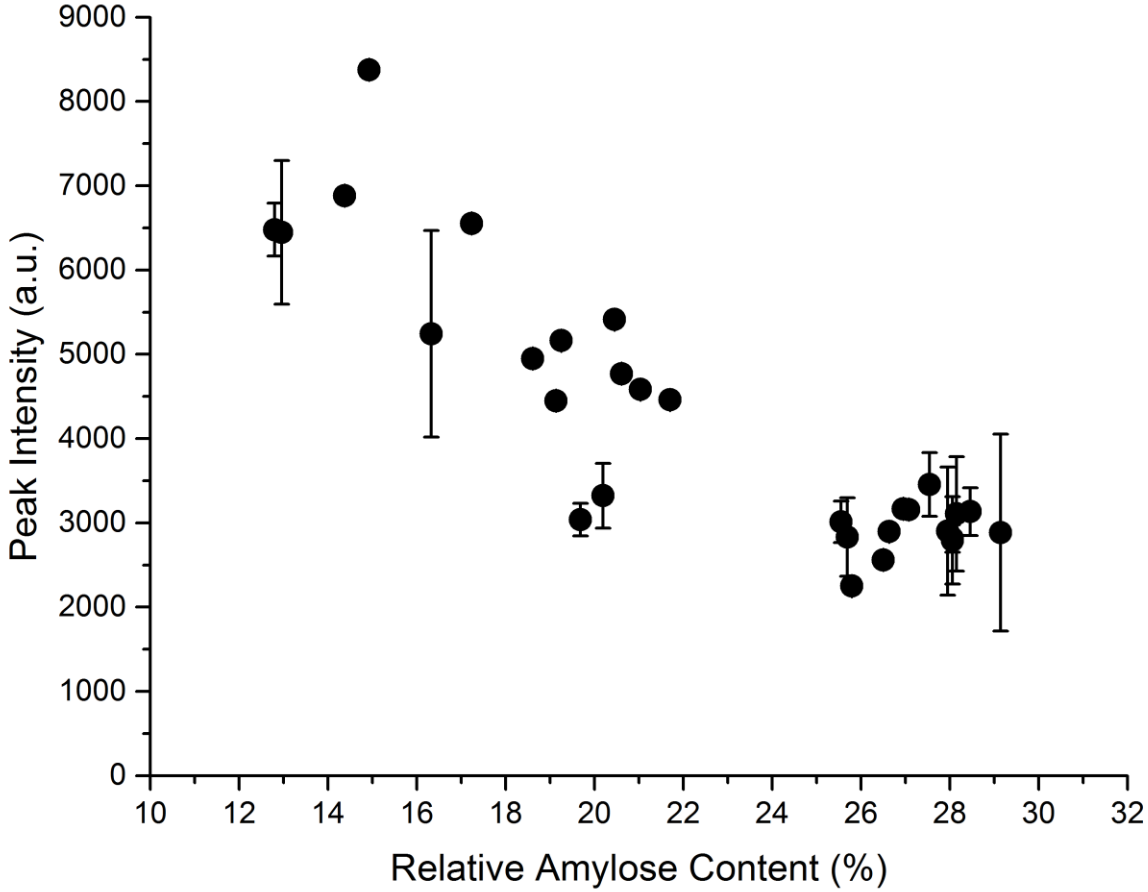
Relationship of peak parameters with real space lamellar features

1. J Blazek, EP Gilbert, Application of small-angle X-ray and neutron scattering techniques to the characterisation of starch structure: A review. Carbohydrate Polymers 2011, 85, 281-93.

SAXS of Rice Flour

- Amylose content influences semi-crystalline structure but not in a consistent way
  - Decrease of total semi-crystalline structure[1]
  - Repeat distance (and lamellar size) not apparently influenced
- This can be confirmed by other methods

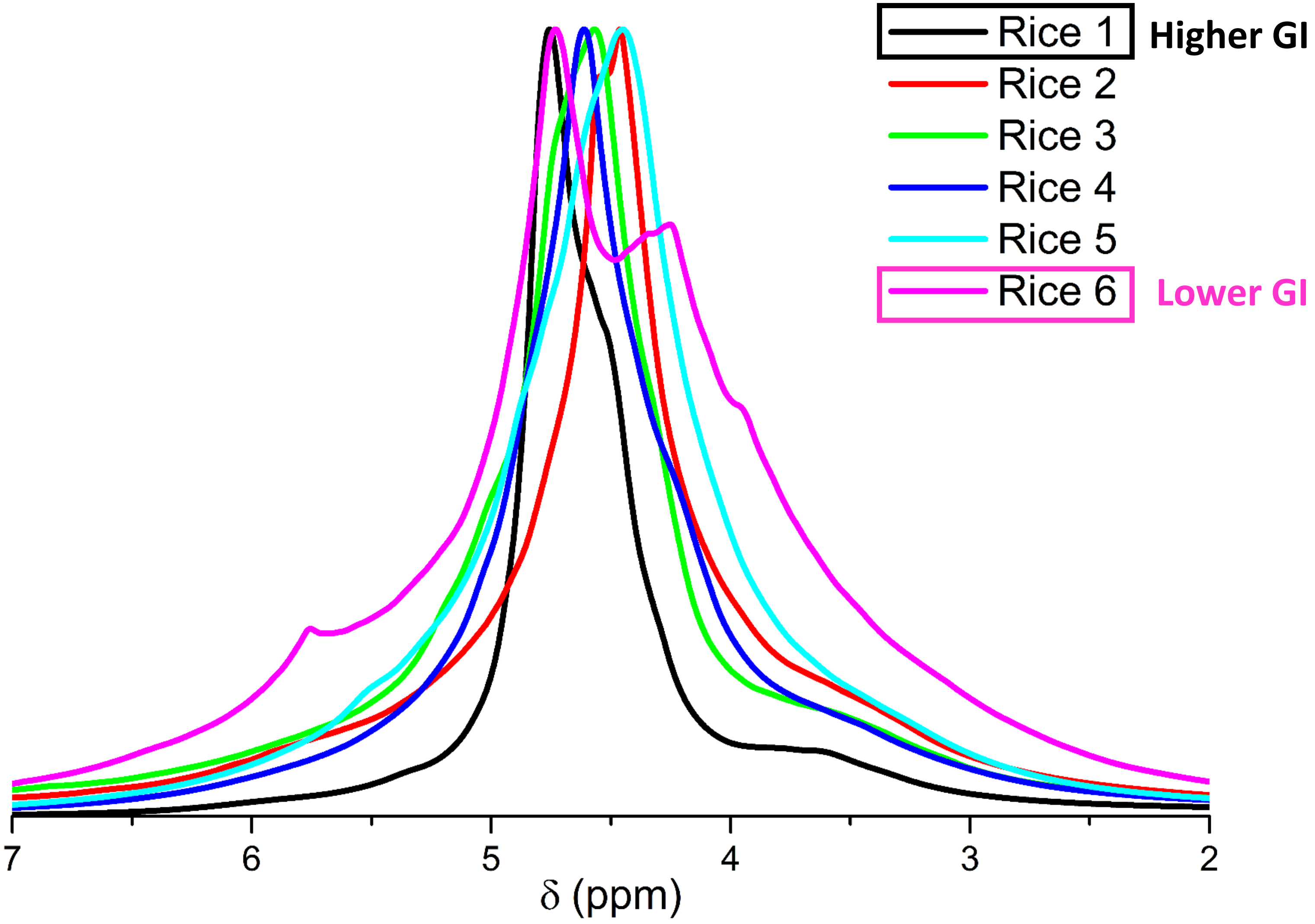


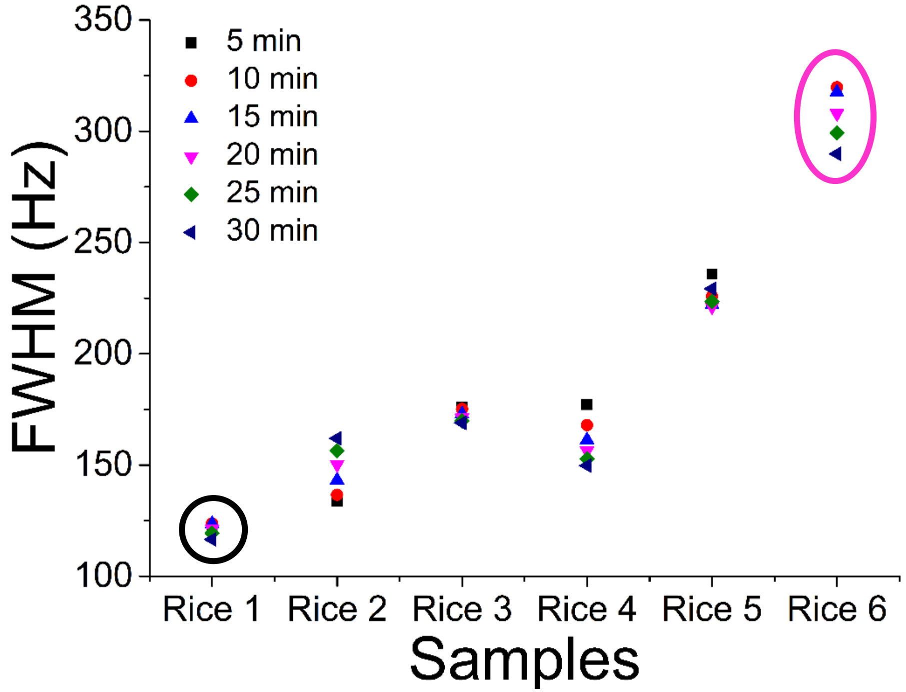


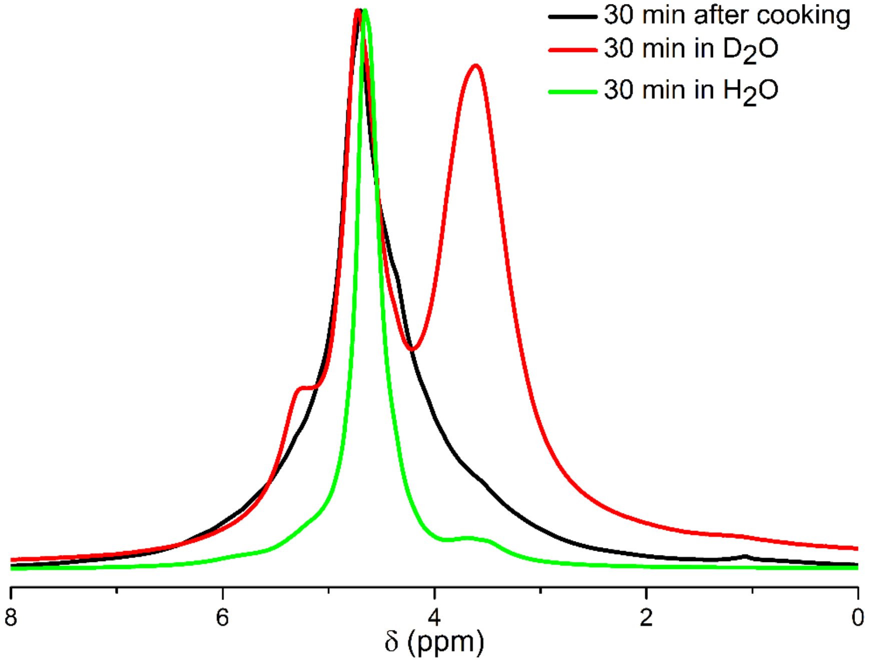
- <u>SANS for further analysis!</u>

1. J Blazek, EP Gilbert, Application of small-angle X-ray and neutron scattering techniques to the characterisation of starch structure: A review. Carbohydrate Polymers 2011, 85, 281-93.

Solid-state NMR spectroscopy







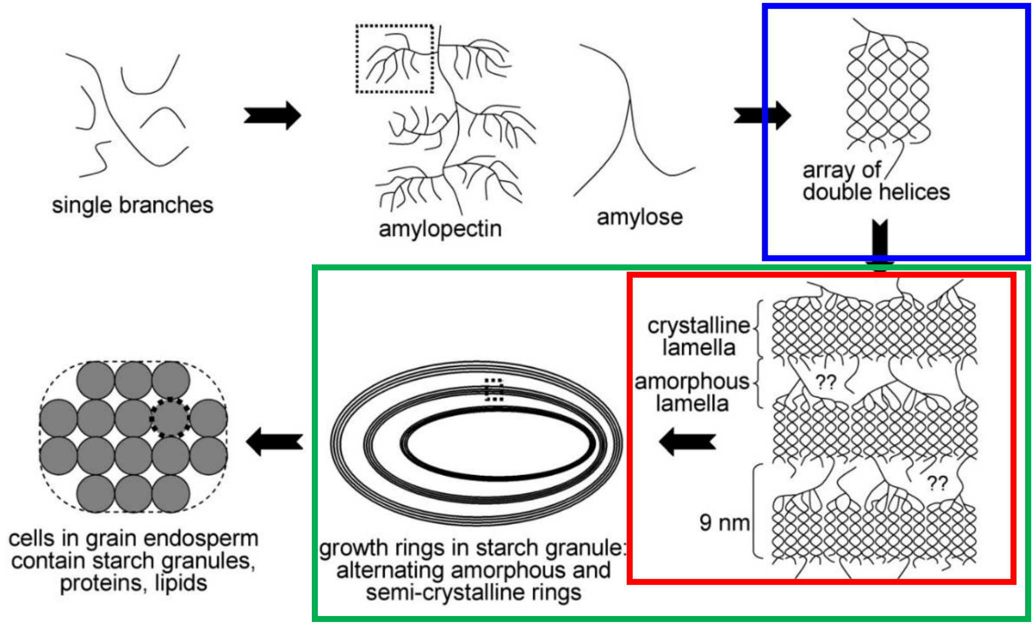
Conclusions

- A more accurate determination and characterisation of amylose content by CE
  - We can incorporate branching characteristics to account for different types of amylose
- Crystalline structure is an important factor to consider in predicting digestibility
  - Application of XRD to overall crystallinity is limited
  - Investigation of semi-crystalline lamellar structure (SAXS) shows promising relationships
- ^{1}H solid state NMR spectroscopy shows potential in predicting digestibility

Perspectives

Next steps

- Assessing the viability of XRD with shorter experimental times
- ^{13}C solid-state NMR spectroscopy to assess crystallinity on the helix level
- Small angle neutron scattering (SANS) opens the door to greater flexibility in sample
  - Monitor cooking of rice grains
  - Supramolecular structure of starch in cooked rice grains



Longer term

- Relate molecular features of amylose, higher levels of structure of rice starch to digestibility
- Provide new tools to predict digestibility

Acknowledgements

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