Monitoring *in vitro* digestion of rice using capillary electrophoresis online

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ARC Industrial Transformation Training Centre for Functional Grains

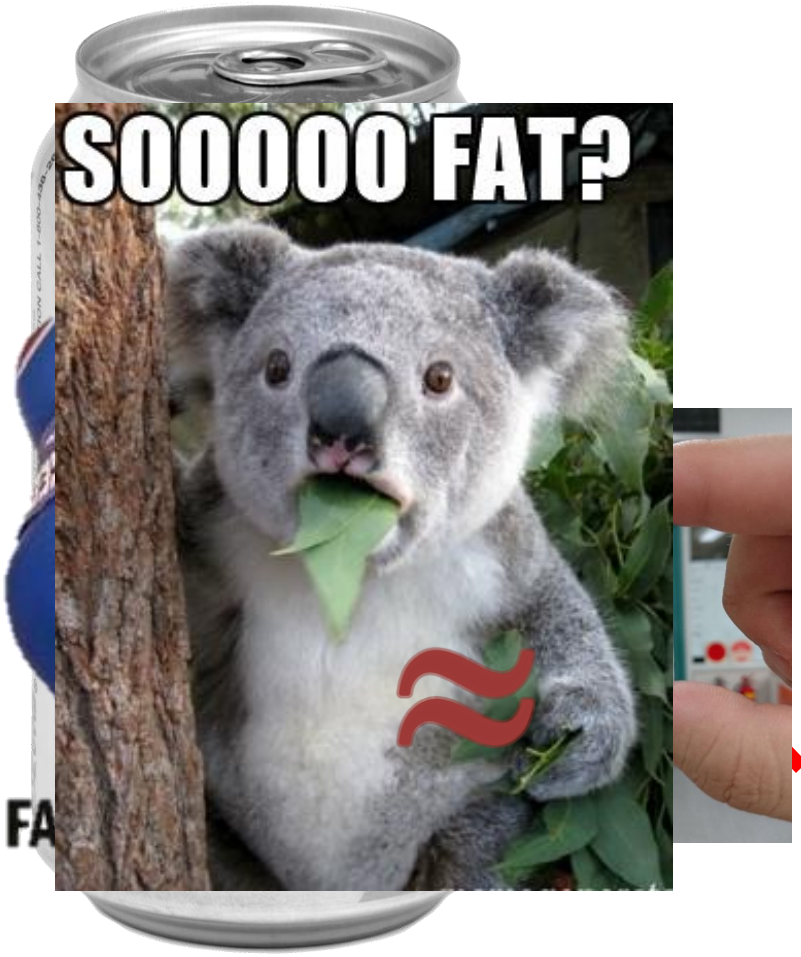
Consumers want healthier foods

FGC
Functional Grains Centre

- Obesity, dietary diseases getting more prevalent. Consumers demand healthier foods → industry produces “healthier” food alternatives.

- Food digestibility measured *in vivo*: costly, volunteers, time consuming, can be imprecise. Companies use it for: marketing > development.

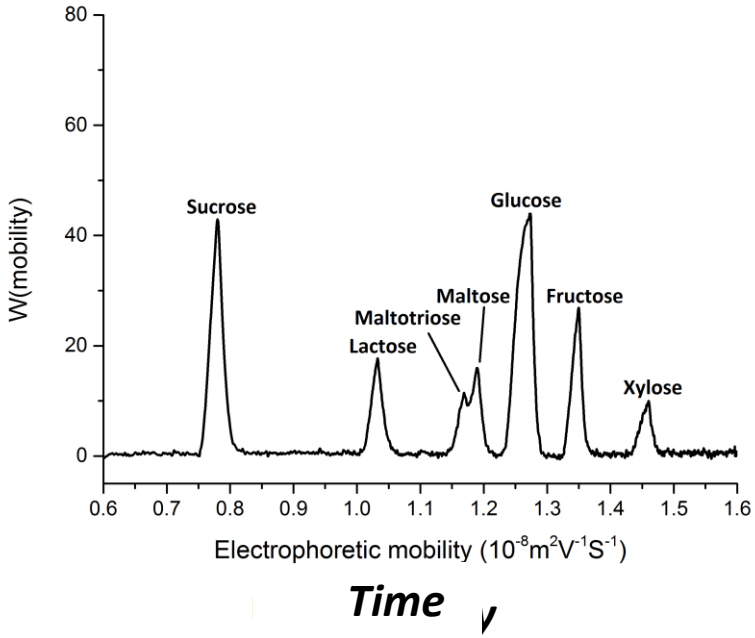
- No effective tool during product development cycle. Alternative: *in vitro* tests are accessible, no volunteers, affordable, quicker, precise.



[1]Jenkins, D., et al., *Glycemic index of foods: a physiological basis for carbohydrate exchange,* The American journal of clinical nutrition, 1981. 34(3): p. 362-366
[2]Glycemic Index Foundation, *Glycemic Index Foundation*, Glycemic-Index-Foundation_logo. 2015

Monitoring *In vitro* Digestion Of Rice Using Capillary Electrophoresis (CE) Online

Peak area



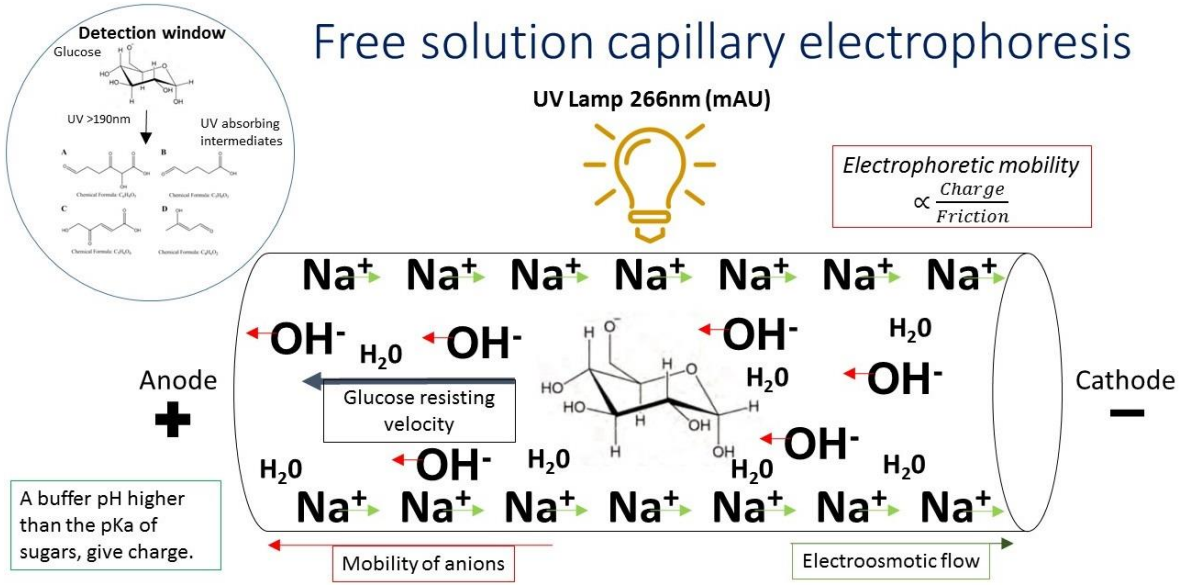
Time

Aim

Assess feasibility of new, rapid, accurate, online CE method to monitor rice digestion:

- Monosaccharides and oligosaccharides release
- Starch structural changes.

Capillary electrophoresis



Separation of sugars: high resolution and robustness

No sample prep necessary; minimise time, potential risks

Cost-effective

Smaller sample requirements

Can monitor fermentation for the production of ethanol[5]

Can quantify sugars in breakfast cereals[4]

[3]Altria, K.D., *Capillary electrophoresis guidebook : principles, operation, and applications*, ed. K.D. Altria. 1996, Totowa, N.J.: Totowa, N.J. : Humana Press. 1-13.

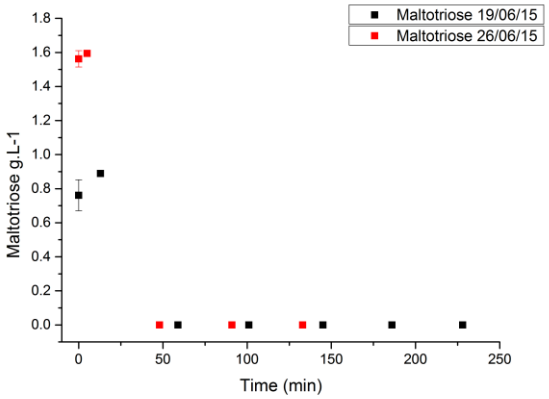
[4]MR Toutounji, MP Van Leeuwen, JD Oliver, AK Shrestha, P Castignolles, M Gaborieau, Carbohydrate Research, 2015. **408**: 134-141.

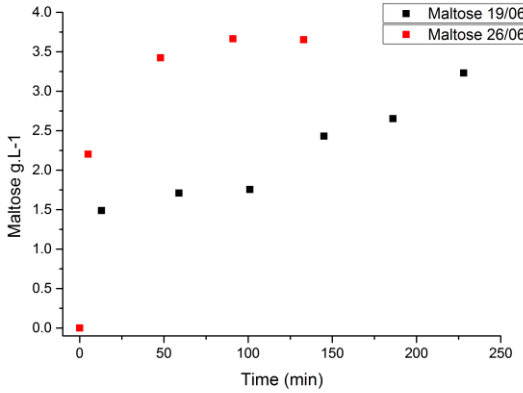
[5]*Oliver, J.D., et al. 2014. Simple and robust monitoring of ethanol fermentations by capillary electrophoresis: Capillary Electrophoresis to Monitor Fermentations.* Biotechnology and Applied Biochemistry.

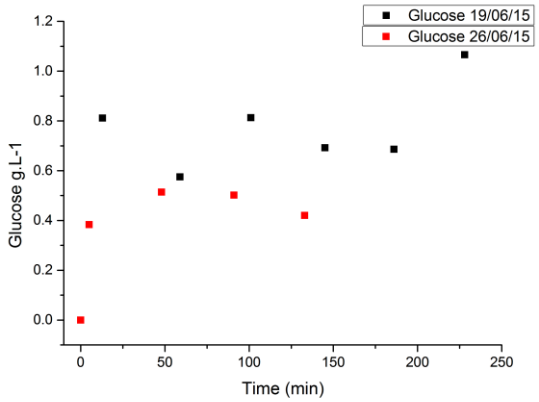
[6]T Schmid, M Himmelsbach, JD Oliver, M Gaborieau, P Castignolles, W Buchberger, Journal of Chromatography A, 2015. **1388**: 259-266.

Online *in vitro* monitoring of maltotriose sugar standard

Starch → → → Oligosaccharides → → → <u>1 Maltotriose → 1 Maltose + 1 Glucose → 3 Glucose</u>







Experimental Conditions

- Sodium acetate buffer 0.2 M at pH 6.0
- Thermomixer set at 37°C
- 1.66 g/L maltotriose concentration
- 0.466 U/mL amyloglucosidase concentration
- 45-50 minute per plot

Interpretation

- Shows clear maltotriose digestion
- Release of maltose and glucose
- Heat retention differences between dates shows clear enzymatic kinetic activity
- Proof of concept that online monitoring of *in-vitro* digestion is possible with CE
- Comparative to literature, underperforming.
- 3 year old enzyme

Monitoring online *in vitro* digestion of rice starch

Experiment aim:

To monitor and differentiate the digestibility of rice that underwent 2 different types of cooking.

- Doongara rice, 2 variables: 1 cooked in water and 1 cooked in water & oil mixture.
- Samples 6.25 mg or rice starch within 15 mL of *in vitro* digestion solution.
- Online experiment successful after feedback from Vito to try varying enzymatic conditions.
- Result: Rice cooked in water & oil mixture (D2A) lower glycaemic potential

Future work

Monitoring monosaccharide and oligosaccharide release in *in vitro* digestion of starchy food

- Free solution capillary electrophoresis
- High performance liquid chromatography
- High performance anion-exchange chromatography
- Solution state nuclear magnetic resonance spectroscopy (NMR)

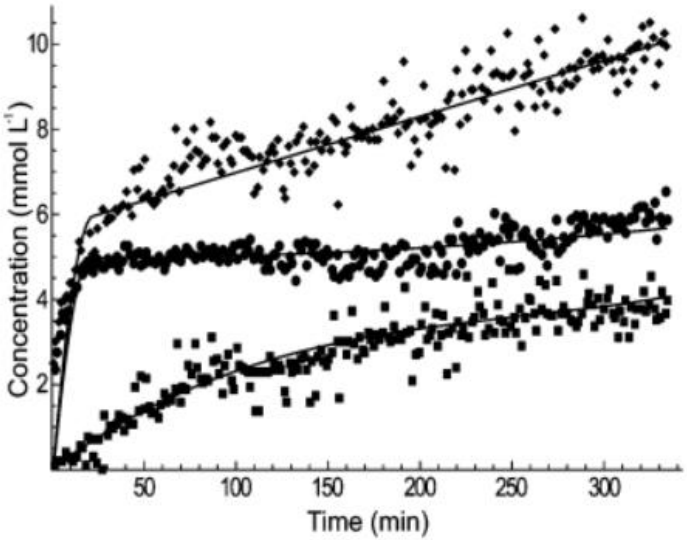
Monitoring starch structural changes during *in vitro* digestion of starchy food

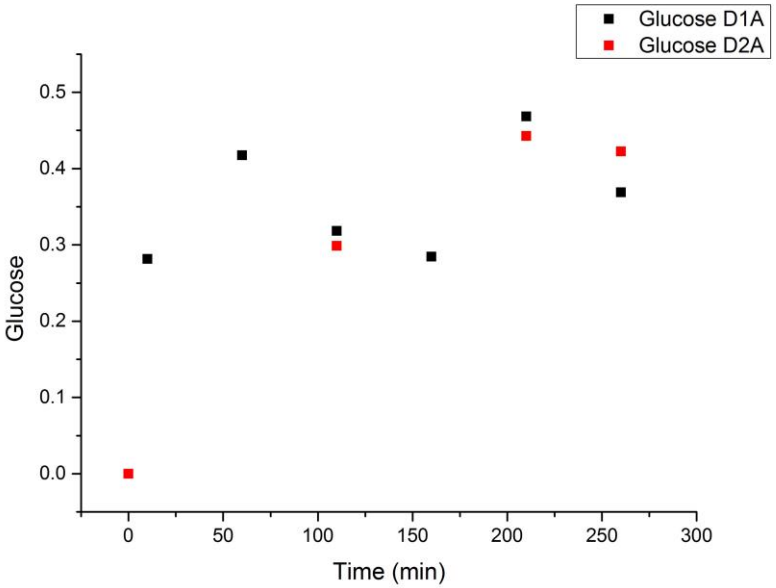
- Iodine affinity capillary electrophoresis (IACE)
- Scanning electron microscope
- X-ray powder diffraction
- Solution state nuclear magnetic resonance spectroscopy

***In vitro* digestion of starchy food**

- Testing varying biochemical parameters of *in vitro* digestion
  - Starch enzyme interactions: varying enzyme mixtures; inclusion/exclusions, concentrations, synergistic, antagonistic
  - Monitoring starch vulnerabilities; amylose vs amylopectin under various conditions
- Testing varying physical parameters of *in vitro* digestion
  - Effects of mastication, mixing? Differences between *in vitro* digestion within reaction vessel and in a CE vial? Experiment: modified CE instrument with a reaction vessel

Validation against solution state NMR





- Solution State NMR can measure every minute.
- CE measure every 45 minutes; requires optimisation.
- Promising preliminary CE results that show a kinetics curve comparable to NMR.

[7]AC Dona, G Pages, RG Gilbert, M Gaborieau, PW Kuchel, Biomacromolecules, 2009. **10**: 638-644.

Conclusion

- Method robust enough to monitor digestibility without sample preparation; minimise time and risks.
- Method can monitor various sugars including glucose precursors.
- Method good study basis for potential predictive glycaemic load in food → *in vitro* digestibility method.

Acknowledgements

Charles Sturt University, Functional Grain Centre for ARTP Scholarship

Western Sydney University, ACROSS facility

Department of Primary Industries

Macromolecular Characterisation Team

Functional Grain Centre

Primary Supervisor – Professor Chris Blanchard

Secondary Supervisor – Dr Patrice Castignolles, Dr Daniel Waters Dr Marion Gaborieau