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

James Jaeyoung Lee

Dr Ben Liu, Dr Rachelle Ward,
Dr Vito Butardo Jr, Dr Dan Waters, Prof Chris Blanchard
Jonathan Tang, Dr Patrice Castignolles & Dr Marion Gaborieau
Consumers want healthier foods

- 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.

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2. Glycemic Index Foundation, *Glycemic Index Foundation*, Glycemic-Index-Foundation_logo. 2015
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
Can quantify sugars in breakfast cereals


Online *in vitro* monitoring of maltotriose sugar standard

Starch → → Oligosaccharides → → → 1 Maltotriose → 1 Maltose + 1 Glucose → 3 Glucose

**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
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 \textit{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

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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.


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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