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Development of rapid *in vitro* GI testing for cooked milled grains

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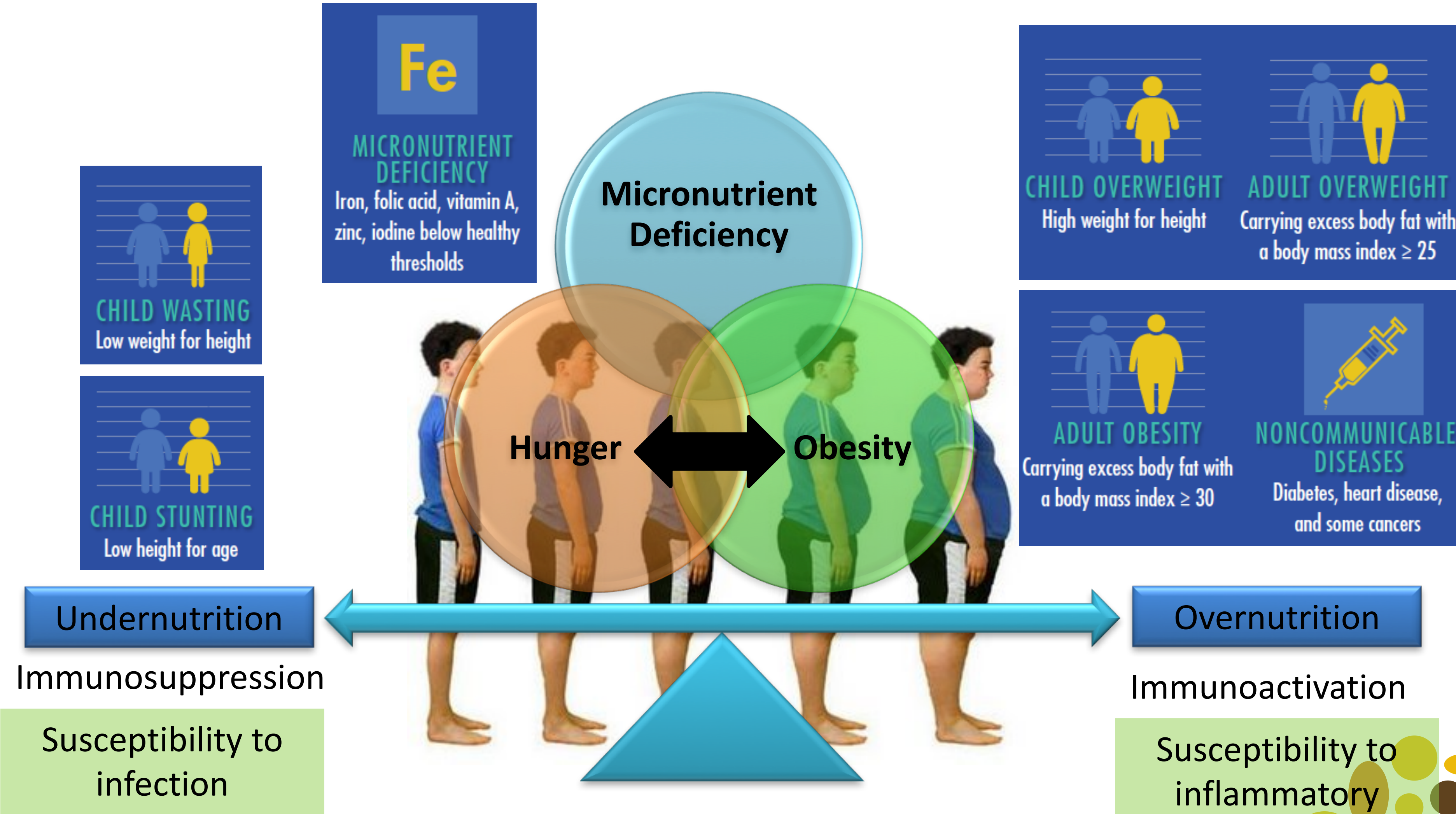
Postdoctoral Research Fellow

Functional Grains Centre

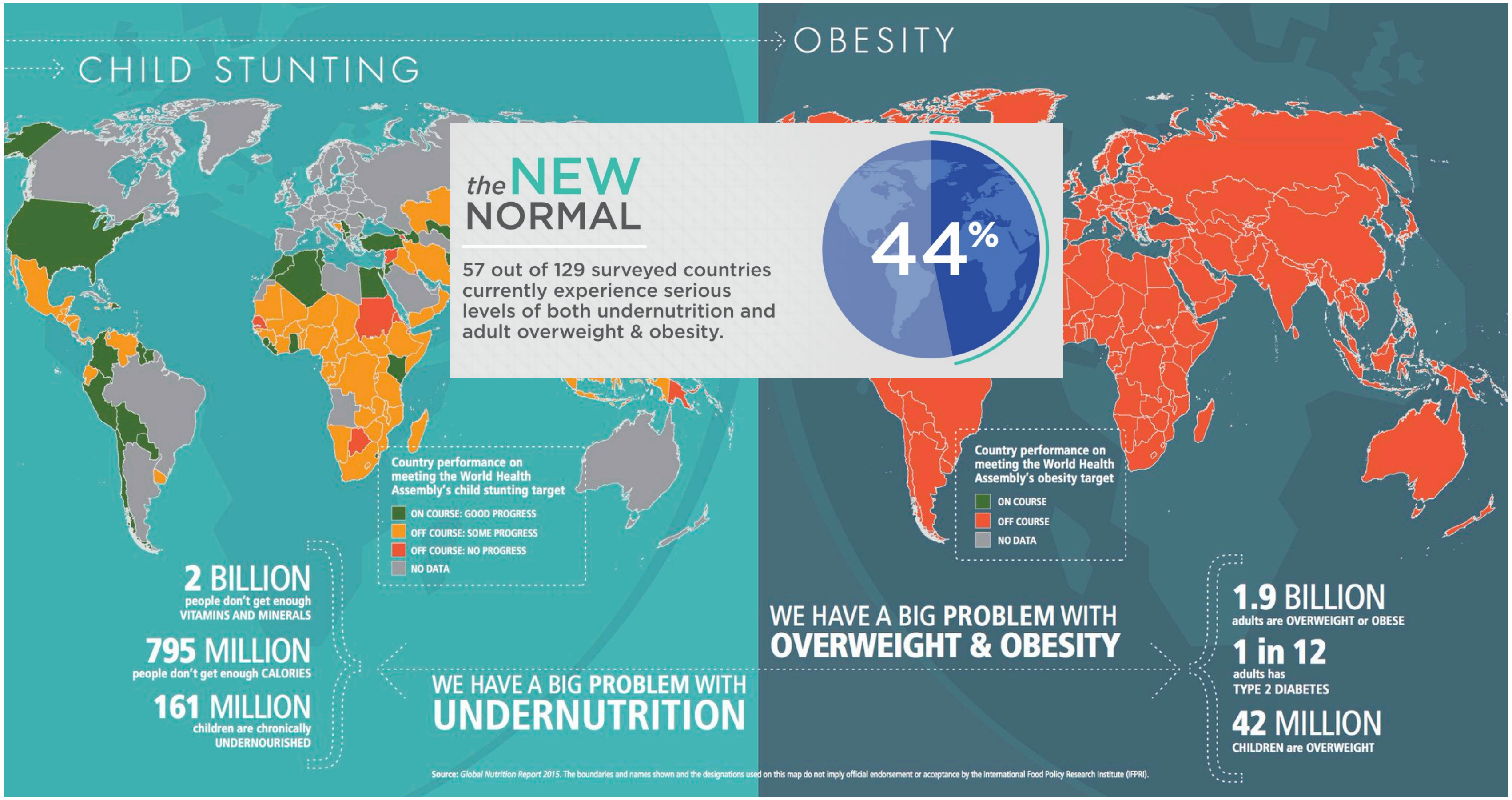
Australian Rice Quality Symposium
Wednesday 19 July 2017
National Wine and Grape Industry Training Centre
Building 412, Carpark 61, Mambarra Drive
Charles Sturt University, Wagga Wagga, NSW 2678
ARC Industrial Transformation Training Centre for Functional Grains

Malnutrition has many faces



Gardner and Halweil 2000; Meijers et al. 2010

Malnutrition affects all countries in the world



Global Nutrition Report 2016

Obesity, overweight and metabolic diseases are costly

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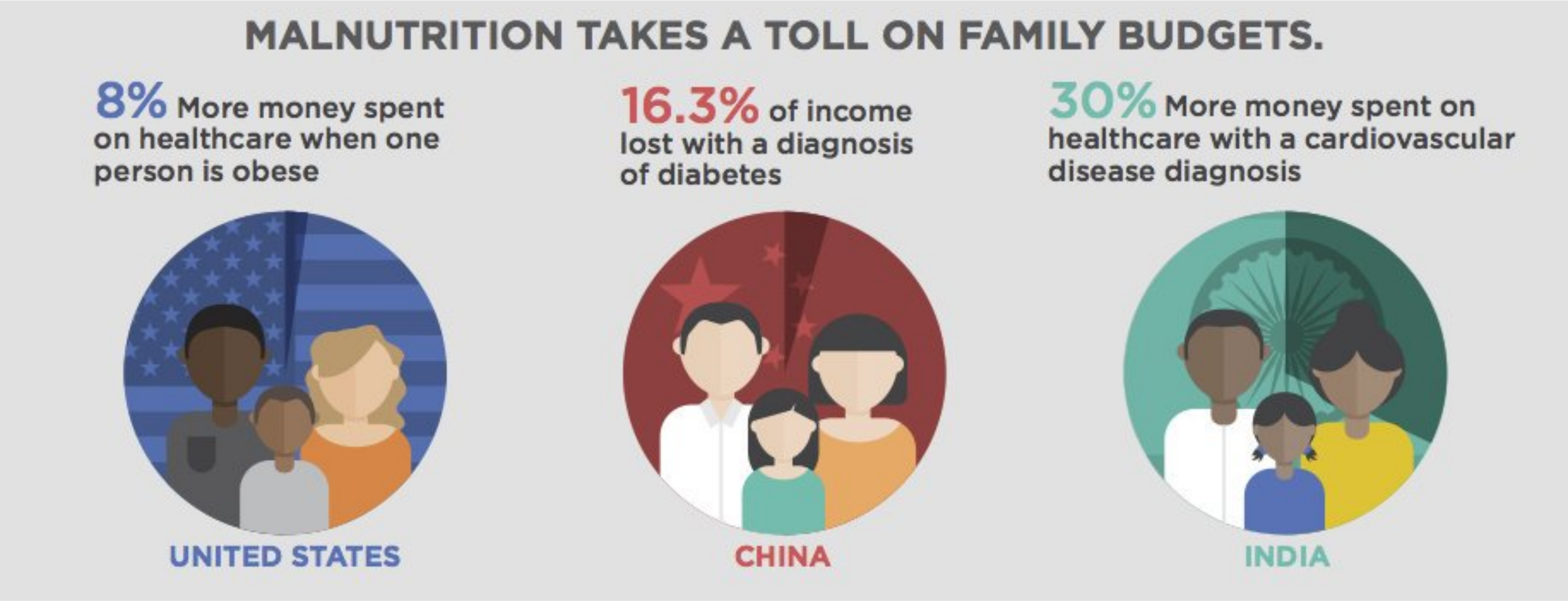
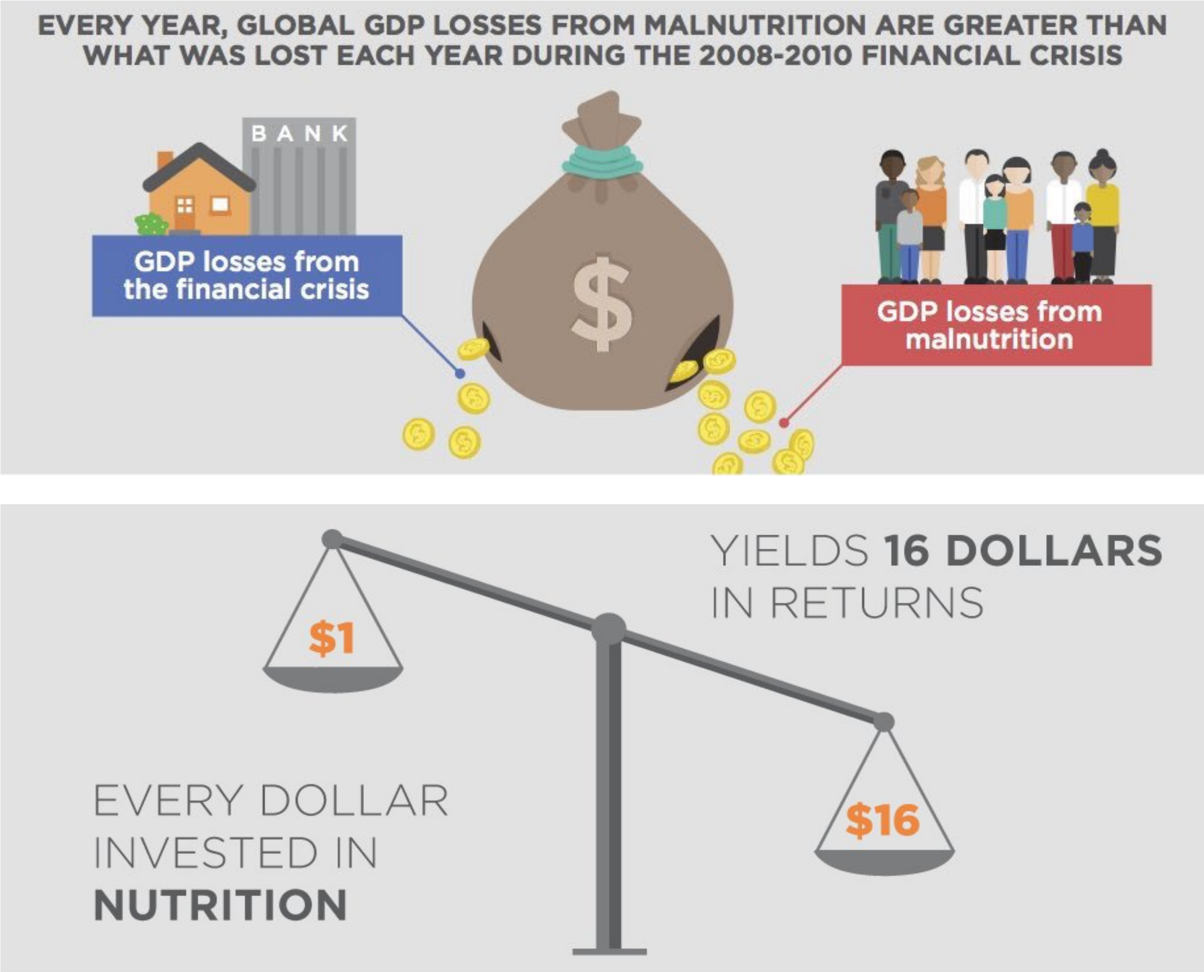


Significant impact on government spending on healthcare cost

Significant impact on countries' GDP

Investing on health and nutrition R&D is critical



Global Nutrition Report 2016

Mitigating the impact of global burden of obesity

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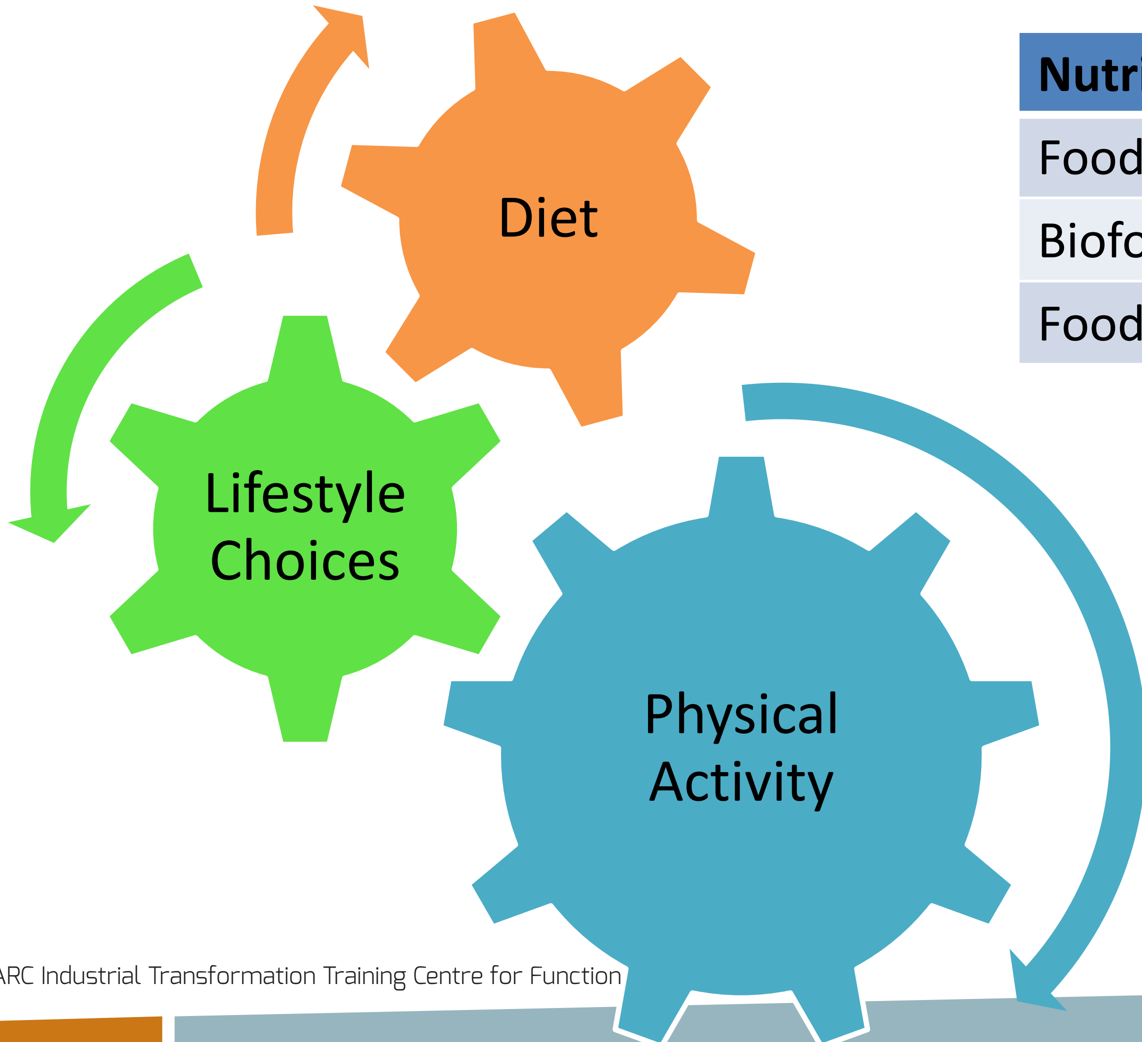
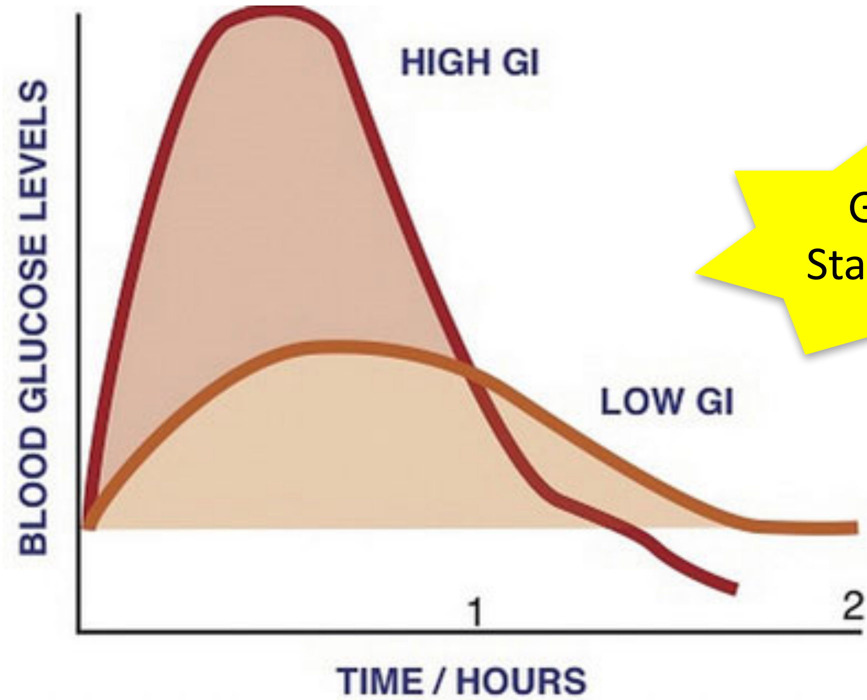


Nutritional Intervention
Food diversification
Biofortification & biomodification
Food processing

GLYCEMIC INDEX TESTED
LOW GI
GLYCEMIC INDEX TESTED

Reducing glycemic index as a dietary intervention



Gold Standard

High GI ≥ 70
Low GI ≤ 55

Glycemic Response (GR)

- the post-prandial blood glucose response upon carbohydrate consumption

Glycemic Index (GI)

- the GR elicited by a portion of food containing 50 g of available carbohydrate and is expressed as a percentage of the GR elicited by 50 g of the reference carbohydrate

Glycemic Load (GL)

- the product of GI and the total available carbohydrate content in a given amount of food

Agustin et al. 2015

Rice as a vehicle for nutritional intervention

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Whole grain rice is rich in nutraceuticals but milled rice is composed mostly of starch

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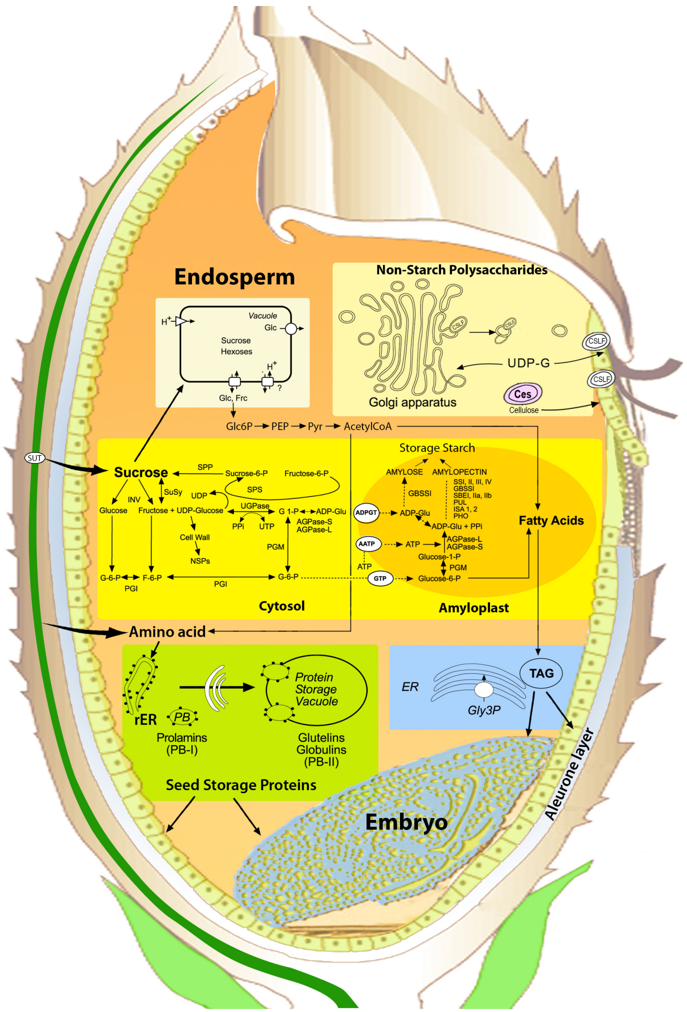
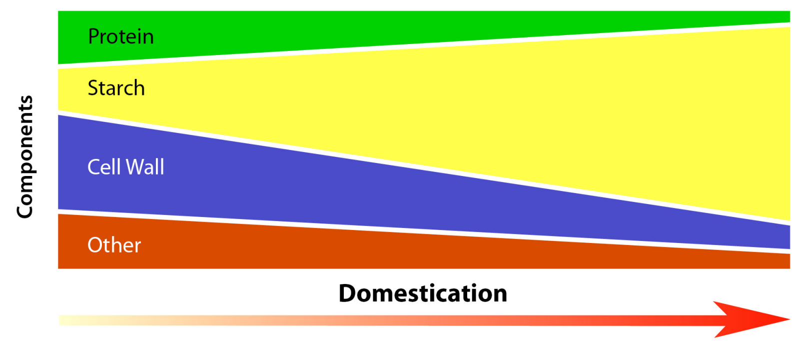
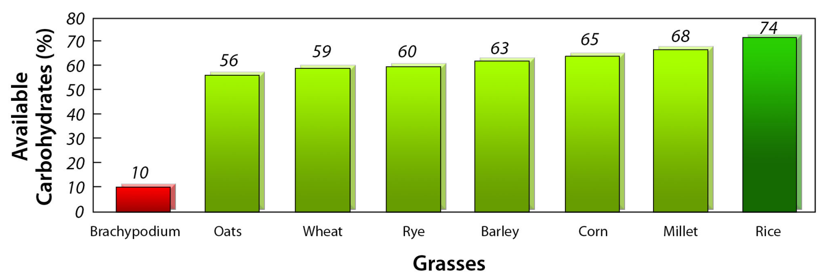


Components	Composition	Health Benefits
Seed Storage Protein	4-11%	Source of protein and amino acid
Storage Starch	85-90%	Source of calorie Source of resistant starch Influences glycemic response
Non-Starch Polysaccharides	Trace	Source of dietary fiber
Storage Lipids	0.3-0.5%	Source of lipids and fatty acids





Development of low GI rice is limited by available screening tools

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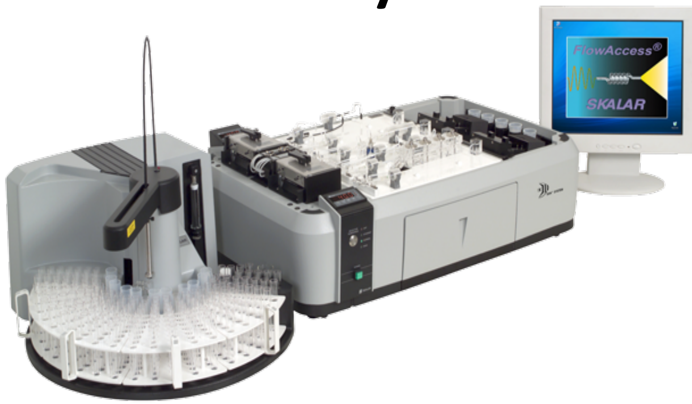
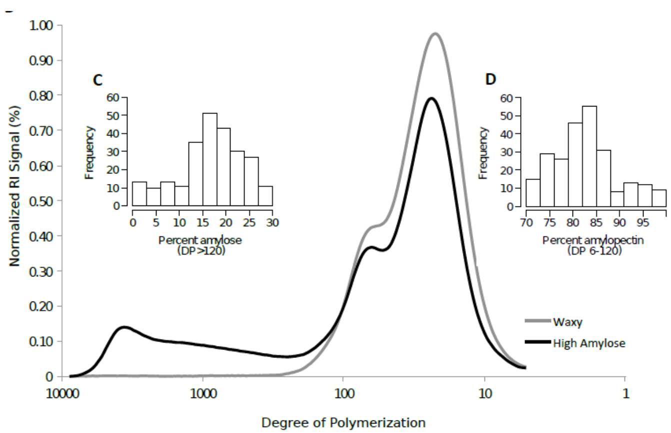


Apparent Amylose Content (AAC)

Dietary forms of starch	Hydrolysis Time (min)
Rapidly digestible	0-20
Slowly digestible	20-120
Resistant starch	>120 (16h)

RDS, SDS, RS



Debranched starch SEC

Hydrolysis Index

Butardo et al. 2017; Fitzgerald et al. 2009;
Butardo et al. 2011; Fitzgerald et al. 2011; Englyst 1992

Hydrolysis Index by NutraScan (Next Instrument)

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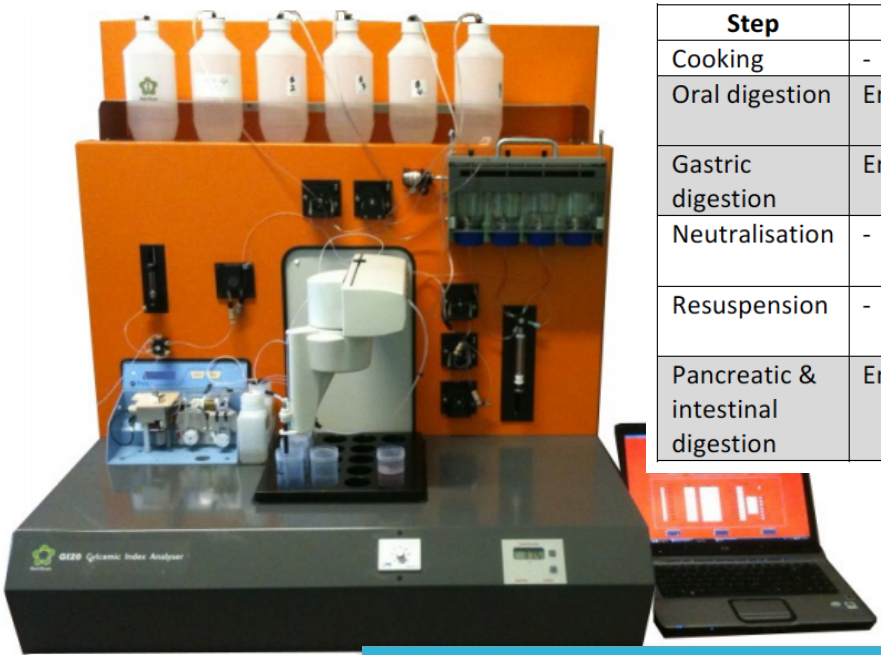

Step	Enzyme	Identity	Volume	Enz Conc	Time	Buffer/Blank
Cooking	-	Water	5 mL	-	30 min	-
Oral digestion	Enzyme A	α-amylase	2 mL	250 U/ml	5 min	Buffer 1 (carbonate)
Gastric digestion	Enzyme B	Pepsin	5 mL	1 mg/mL	30 min	Buffer 2 (HCl)
Neutralisation	-	Sodium hydroxide	5 mL	-	-	Buffer 5 (NaOH)
Resuspension	-	Acetate buffer	28 mL	-	-	Buffer 3 (Acetate)
Pancreatic & intestinal digestion	Enzyme C	Pancreatin AMG	5 mL	2 mg/mL 28 U/mL	3 hr	-

Advantage

- Semi-automated
- Correlated with clinical GI

Limitations

- Expensive
- Low throughput (n=20)
- Slow (5 hours)

Butardo et al. 2011; Fitzgerald et al. 2011

Testing NutriScan to simulate rice digestion *in vitro*

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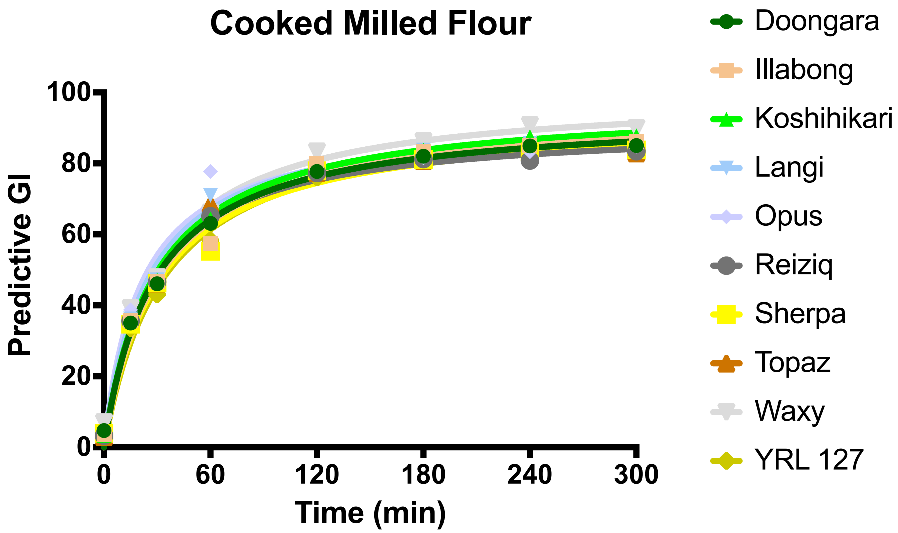
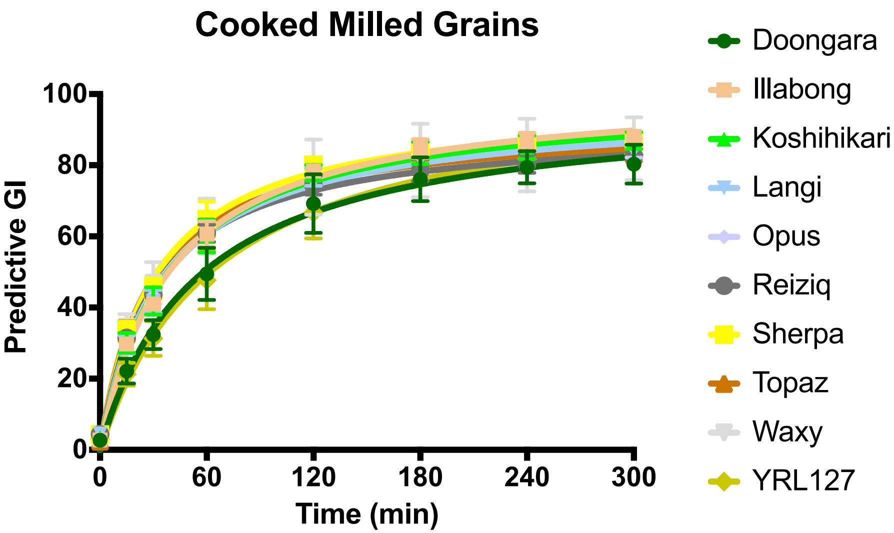
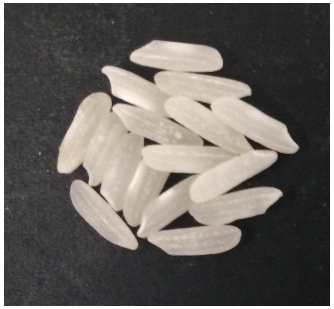




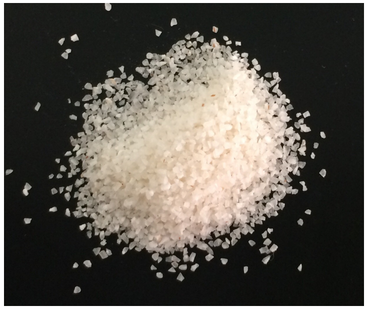
Time	Doongara	Illabong	Koshi	Langi	Opus	Reiziq	Sherpa	Topaz	YRL127	Waxy
300 min	80	88	87	84	85	83	87	83	83	85

eGI-60 = % starch hydrolysed at 60 minutes ???

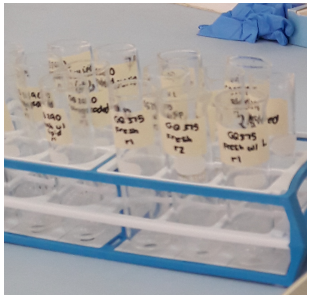
Amylolysis to characterise cooked grain digestibility



rice grains



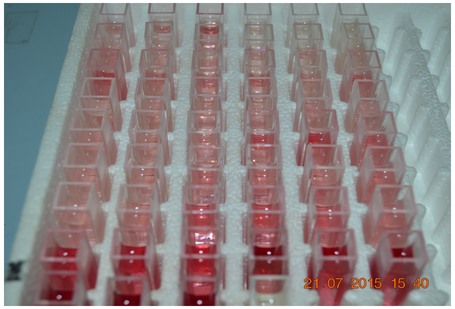
grinding to 400-600 microns



cooking

amylolysis

Sampling on ice
Centrifugation at 4^{o}C



Glucose quantification by GOPOD

Computations
glucose released
starch hydrolyzed
equilibrium concentration ($C\infty$)
starch hydrolysis rate (*k*-value)

Butardo et al. 2017

Differentiating digestibility of IRRI rice lines using amylolysis

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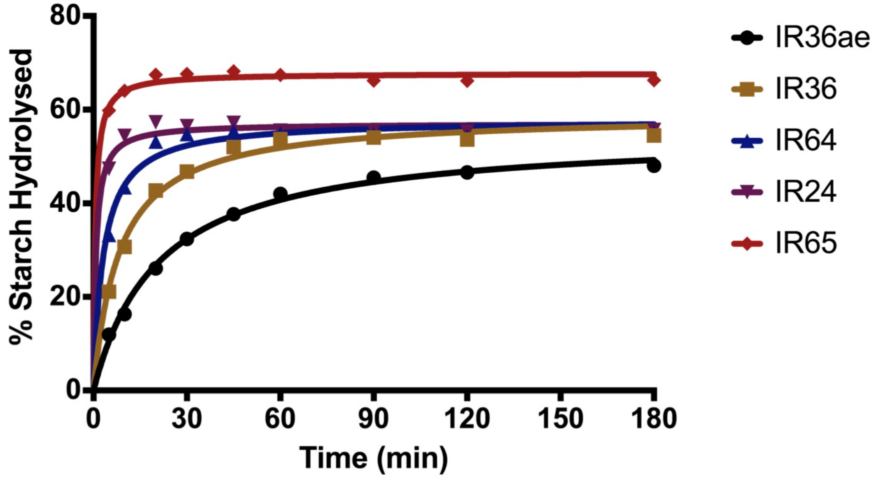

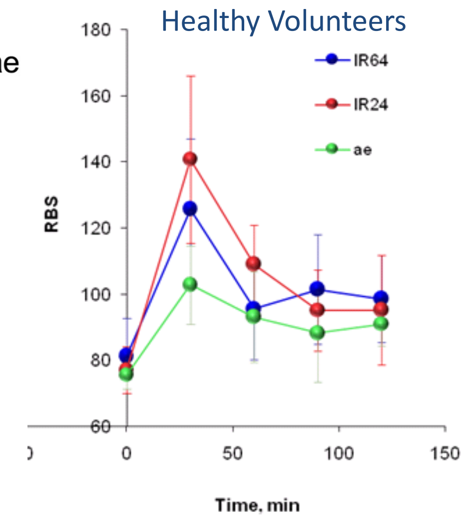

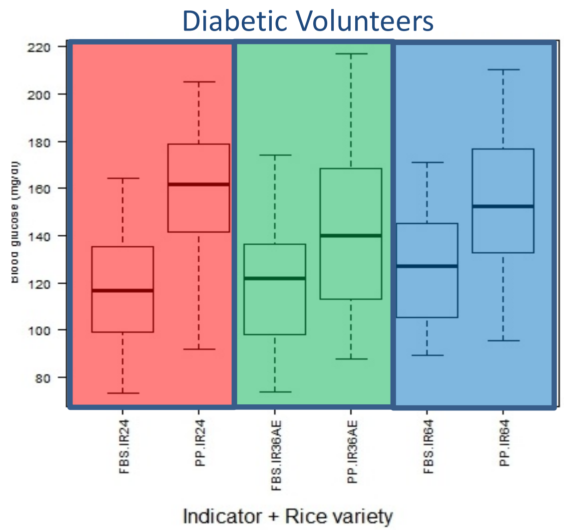

Advantage

- Cheaper
- Faster (3 hours)
- Correlated with AAC and HI

Limitations

- Low throughput (n = 6-10)
- Very manual method

Butardo et al. 2017; De Guzman et al. 2017; Fitzgerald et al. 2011

Developing *in vitro* digestibility assay for Australian rice industry

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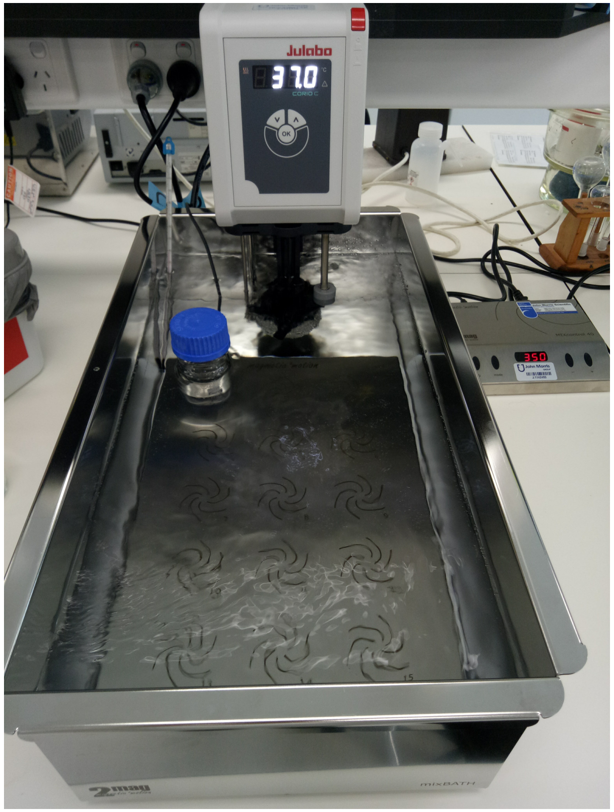
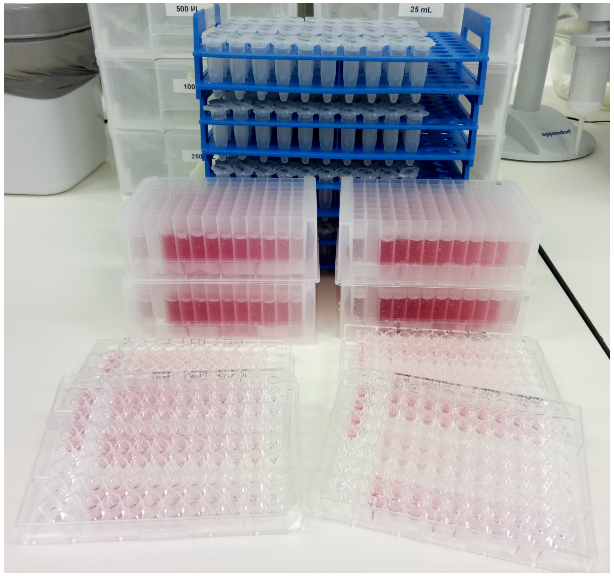
Individual magnetic stirring ensures stirring uniformity



Submersible magnetic stirring ensures temperature uniformity



Use of 96-well plate format for high throughput glucose assay

Optimising enzyme concentration: α-amylase

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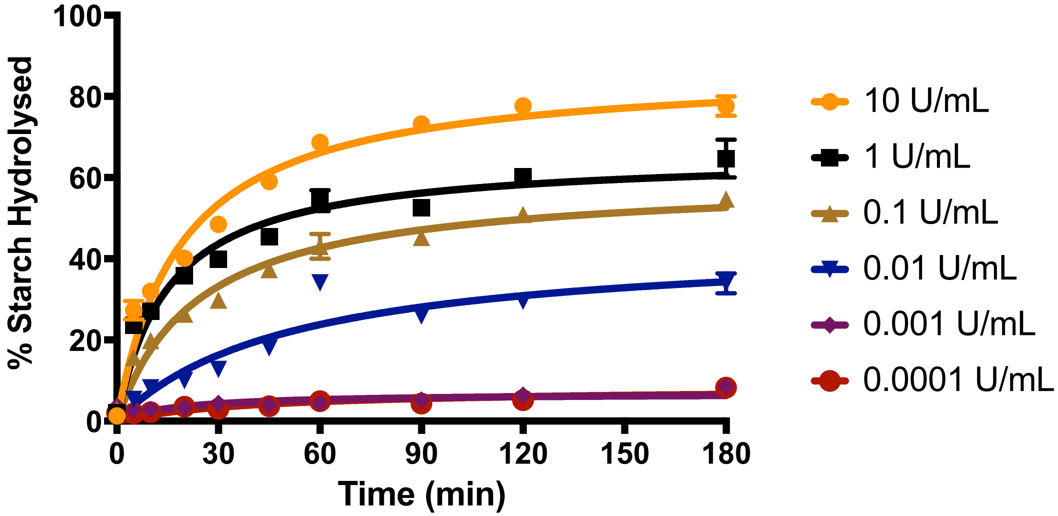

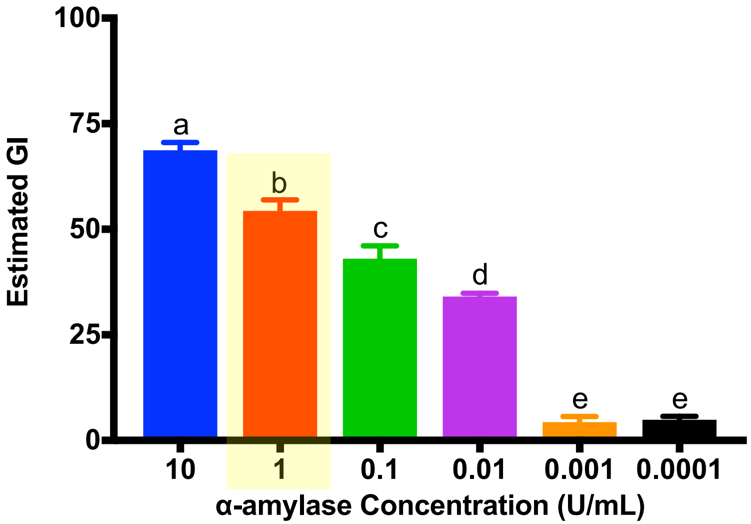

Amylolysis using 1 U/mL porcine pancreatic α-amylase

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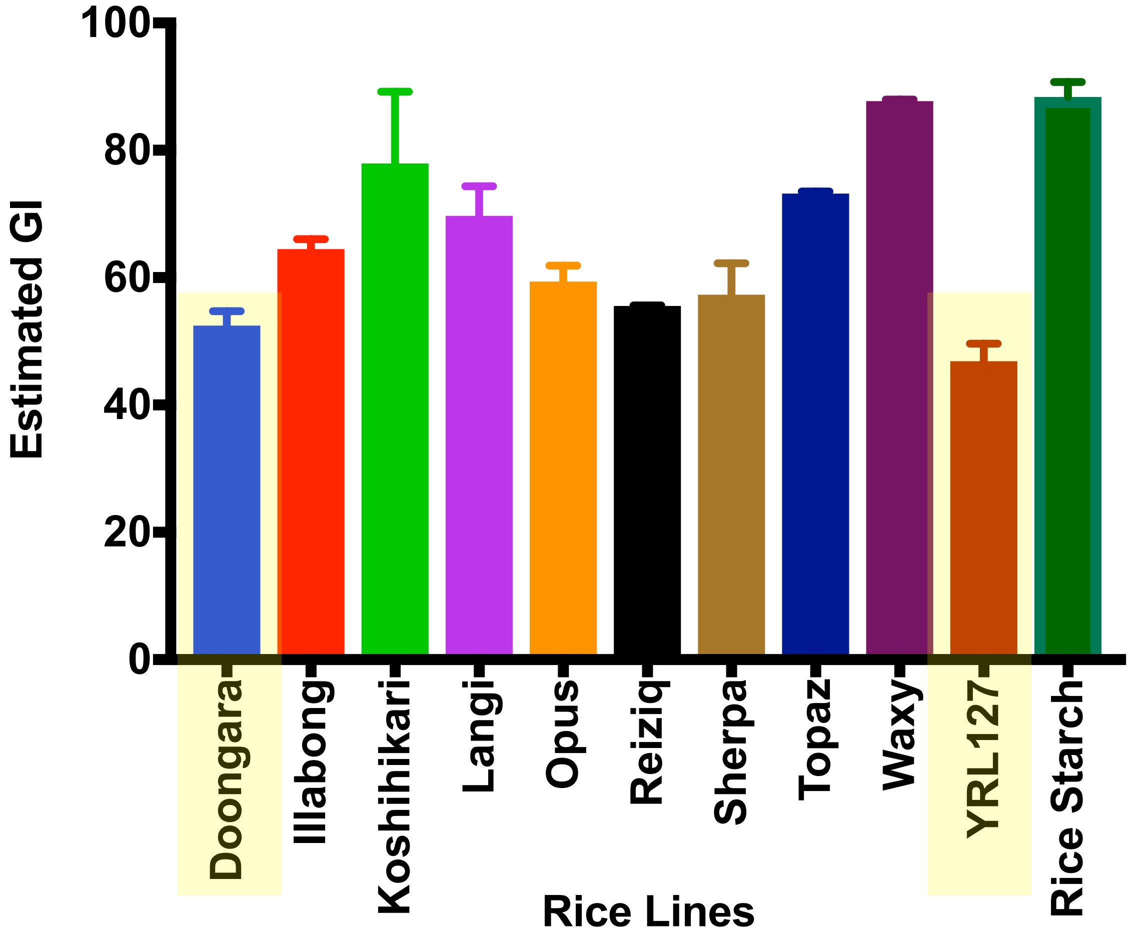
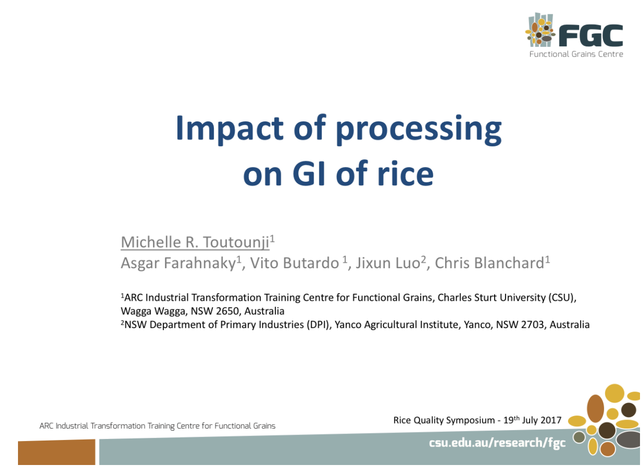


Actual research application: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Impact of processing
on GI of rice

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ARC Industrial Transformation Training Centre for Functional Grains
Rice Quality Symposium - 19th July 2017
csu.edu.au/research/fgc

Limitation
Indirect, sequential amylolysis

Optimising enzyme concentration: AMG

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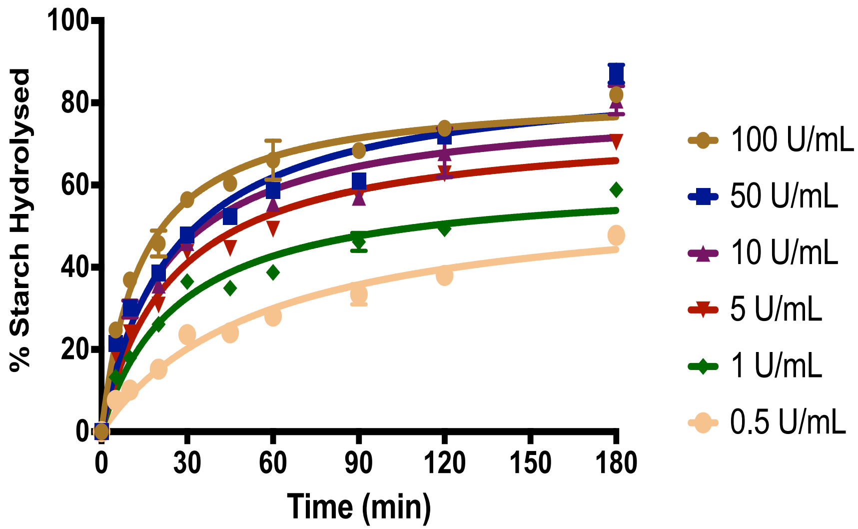
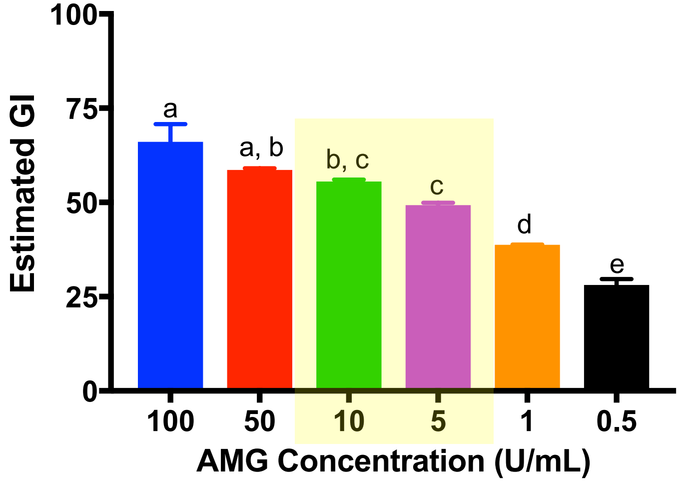
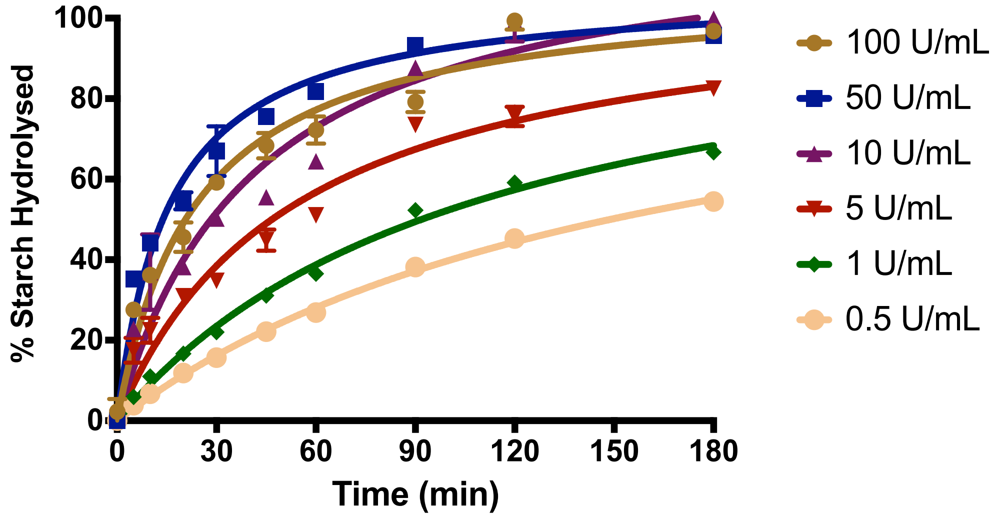
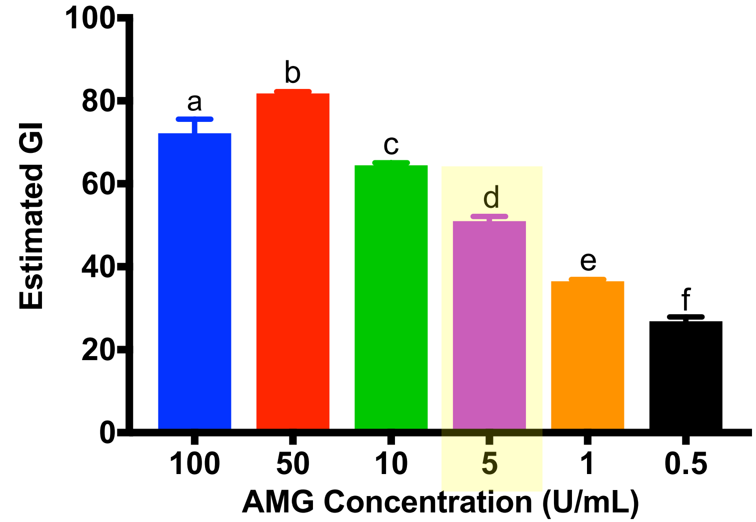


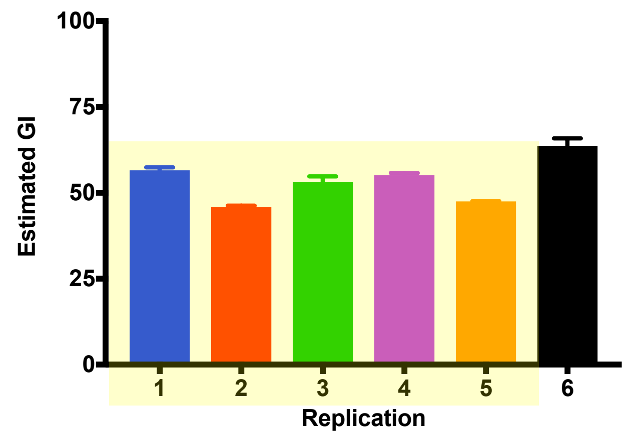


Butardo et al. unpublished CONFIDENTIAL

Optimising enzyme concentration: constant α-amylase + varying AMG

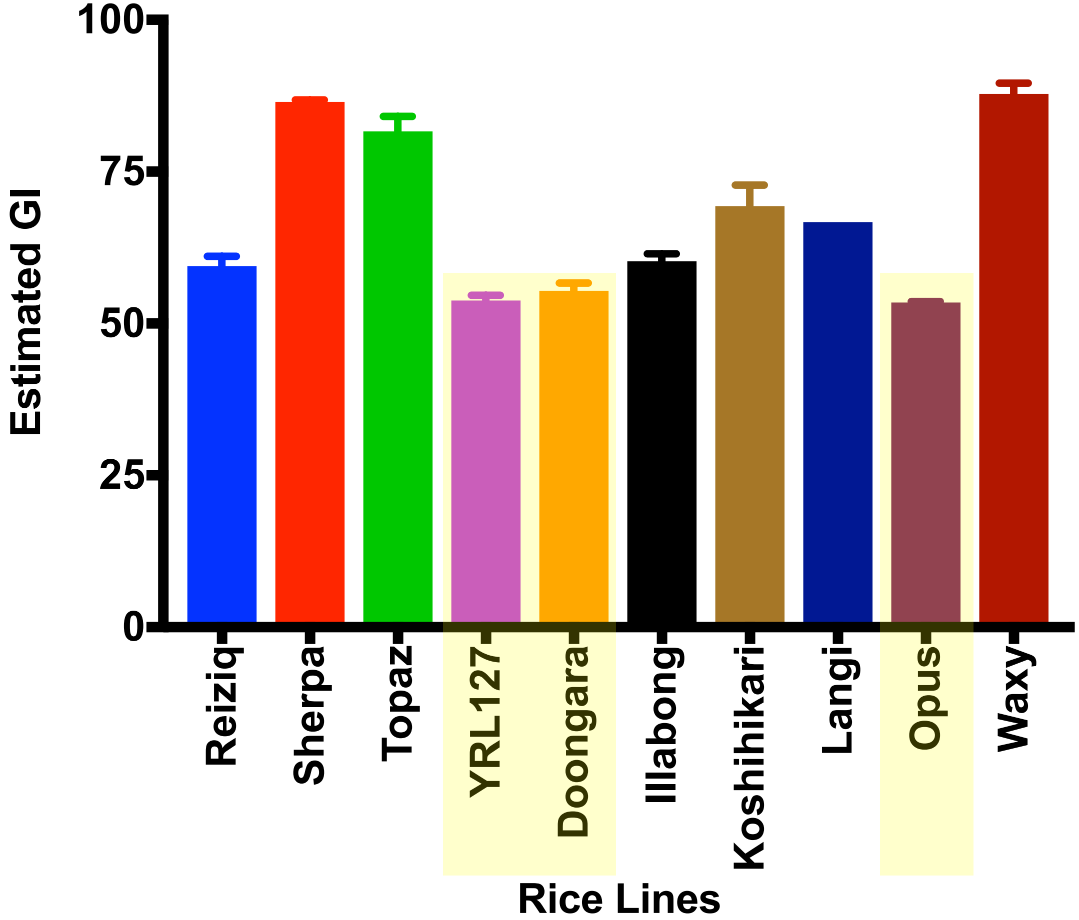






Butardo et al. unpublished CONFIDENTIAL

Amylolysis using 1 U/mL porcine pancreatic α-amylase + 5 U/mL AMG



Next activity: Screening of

1. Rice biparental mapping population
2. Rice diversity panel

Advantage
Direct amylolysis

Conclusion

The following can differentiate the digestibility of commercially-available milled Australian rice grains:

Proxy Measure of Digestibility	Limitation
NutriScan (Next Instrument)	Needs further optimisation, consider eGI-60
α-amylase (1 U/mL)	Needs sequential digestion with AMG
α-amylase (1 U/mL) + AMG (5 U/mL)	Cannot use existing enzyme kinetic model

Ongoing and Future Work

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Ongoing

1. Enzyme kinetic modeling using log of slope and nonlinear regression
2. Characterise the synergistic and/or antagonistic effects of mixing different enzyme cocktails

Future

1. Screening of rice diversity and biparental mapping populations (QTL mapping and GWAS)
2. Effect of non-starch components in digestibility
3. Clinical correlations

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THANK YOU…

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