Starch quality, starch genes, malt quality, beer foam proteins and fungal contamination in barley and other cereals.

The Australia Africa Connection: research in barley related to malt quality (and the other big four cereals)

Dr Glen Fox
Senior Research Fellow

Adjunct Professor
Stellenbosch University
Working together with the Queensland Government
Working together with the Queensland Government
Outline

• Who’s QAAFI
• Starch quality and genes
• Fungi contamination of cereals
• Foam quality
Who is QAAFI

- Queensland Alliance for Agriculture & Food Innovation
- Joint Partnership between Queensland Govt and University of Queensland (UQ) in 2010
- 200 staff and PhD student in three years

- UQ top 100 universities in the world
- No. 1 in Australia for agricultural research outcomes
- Top 10 in world for agriculture research outcomes
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Titilayo - Aflatoxin

Starch, *Fusarium*, *Aspergillus*
Wheat, barley, triticale, maize
Sandra (South Sudan), Irene (Kenya)

Phytochemicals in sorghum - Boitumelo

Sorghum – food and beverages

Armelle – Fermented milk

Aflatoxin - maize

Ben - Aflatoxin

Collaboration Australia
ICARDA germplasm exchange
Outline

- Who’s QAAFI
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Why starch quality

- Australian barley and malt requirements
- Domestic vs export
- Liquid adjuncts vs solid adjuncts
- Infusion mashing vs ramping
- Content vs composition
- Amylose and amylopectin

- Understanding starch substrate (and gene alleles) impact on malt and beer quality:
  - Sugar profile / fermentability
  - Flavour
  - Filtration/haze
Starch
What’s in starch?

Amylose chains

Amylose

Amylopectin
Hierarchical levels of starch structure in cereal grain

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
<th>Level 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylose branches</td>
<td>Amylopectin molecule</td>
<td>The clusters of double helices.</td>
<td>Crystalline</td>
<td>Amorphous shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amorphous</td>
<td>semi-crystalline shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crystalline</td>
<td></td>
</tr>
<tr>
<td>Amylopectin branches</td>
<td>Amylopectin molecule</td>
<td></td>
<td>The alternating layer of crystalline and amorphous lamellae.</td>
<td></td>
</tr>
</tbody>
</table>
Understanding starch - Predicting fermentability

Using RVA under high gravity mash profile to predict quality

Fox G, Visser J, Meijering I & Manley M (2014) Effect of different analysis conditions on Rapid Visco Analyser malt viscosograms in relation to malt of varying fermentability J. Institute of Brewing 120: 183-192,
What affects starch quality

- The changing environment
- Effect of environment on structure heat and or changes in protein
- Using SEC and FACE to characterise what’s happening to the starch when barley (and other cereals) grown under adverse conditions
Abiotic stress

• Drought & heat tolerance
• A number of physiological mechanisms for tolerance
• Flagship*ND population
• Stay-green – trait where head drying down, plant staying green
• Stay-green QTLs (doing synteny comparison with sorghum SG genes)
• Maintaining quality (grain and malt potential)
• Field trials in 2015
Working together with the Queensland Government

Flagship

ND line

A

B

Control  50%  30%  10%

Control  50%  30%  10%

Centre for Nutrition & Food Sciences
Starch quality maintained with SG-like characteristic

Change in structure due to moisture stress and high soil nitrogen

- High heat and drought stress site
- Shorter chain lengths in amylose and amylopectin
- “Environment” impacted on starch composition

Yallaroi

Hermitage

- Non-stressed site
- Longer chain lengths in amylose and amylopectin
- “Environment” no impact on starch composition
- Higher starch content
Let’s go to Ethiopia
More starch stuff (sorghum)

- Does your company have breweries in an African country?
- Does it only use barley? If not, what other grains, perhaps sorghum?
- Sorghum – gluten-free, high starch, poor digestibility, variation in colour
The Matrix – breaking down the starch:protein matrix
Starch quality: a product of biosynthesis

Starch biosynthesis pathway

- ADP-glucose
  - GBSS
    - Amylose
      - SBEI
        - Moderately branched Amylopectin
        - Highly branched Amylopectin
  - SSI, SSII, SSIII
    - Amylose
      - SBEI
        - SBEII
          - ISO
            - PULL
              - DP
                - GWD

SS  starch synthase
GBSS  granule bound SS
SBE  starch branching enzyme
PULL  pullulanase
Differences in starch

- Differences in starch structure and size

The fully branched distribution of starch \cite{Li2014}

Amylopectin peak
Shift to the right = Larger molecules

Amylose peak
Shift upwards = More branched

\( SbPUL-RA \) (Pullulanase mutant)
Changes in amylopectin expression impacts on endosperm structure

SbPUL-GD loosely packed starch

SbPUL-RA tightly packed starch (higher starch content)
A few problems at harvest time
Other starch gene mutations

Barley SSIIa mutation linked to improve bowel health – increased amylose

Wheat SSIIa mutation linked to increased pasting properties of flour

Variation in rice SSII changes gelatinisation temperature
SSIIa SNPs in rice

Figure 1 Schematic representation of the gene that codes for rice starch synthase IIa (SSIIa) showing the positions of exons, introns and single nucleotide polymorphisms (SNPs).
SSIIa SNPs in rice change gelatinization temperature

Figure 2: Relationship between haplotype and gelatinization temperature (GT) for 70 rice genotypes. Mean (M) GT (°C) and standard deviation (SD) for each haplotype (from left to right) were as follows: haplotype 1 (G/G/GC), M = 78.50, SD = 2.976; haplotype 2 (A/G/GC), M = 77.96, SD = 2.246; haplotype 3 (A/G/TT), M = 69.00, SD = 2.966; haplotype 4 (A/A/GC), M = 70.00, SD = 2.000. Bars represent the 95% confidence interval around the mean for each haplotype.
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The Stellenbosch connection – where it all started for me

- Fungal contamination
- *Fusarium* on single maize kernels
- Hyperspectral imaging (3D NIR) – very cool
- X-ray imaging – very, very cool
- 2014 starting on *Aspergillus* including aflatoxin expression
Mycotoxin producing fungi

25% of the world’s agricultural crops are contaminated with mycotoxins (WHO, 1999)

• *Aspergillus* spp.
  – *A. parasiticus*: Aflatoxins
  – *A. ochraceus* & *A. carbonarius*: Ochratoxins

• *Penicillium* spp.
  – Patulin & Ochratoxins

• *Fusarium* spp.
  – Fumonisins
  – Trichothecene type A: (e.g. T-2 toxin, HT-2 toxin, diacetoxyscirpenol)
  – Trichothecene type B: (e.g. deoxynivalenol, nivalenol, 3- and 15-acetyldeoxynivalenol)
  – Zearalenones

• *Claviceps* spp.
  – Lysergic acid and ergotamine
Mycotoxins

- Natural chemical produced by plants, algae, etc
- Hundreds of toxins, new toxins still being identified
- Recorded toxin poisoning in BC period
- Salem witch trials - now linked to ergot toxin
- Some mycotoxins - bioweapons
- Aflatoxin - potent carcinogen
- Australian Department of Defence permit to use aflatoxin standard
Hyperspectral Imaging 3D NIR
X-ray imaging
Bumper Kenya maize harvest contaminated by toxins

There is growing alarm among Kenyan farmers about a government announcement that 2.3m bags of maize were unfit for human consumption.

Health experts say the maize contained high levels of lethal aflatoxins, which have killed at least one child.

The government has pledged to buy and destroy the contaminated maize.

The crop was harvested in the drought- and famine-prone Eastern Province and went bad because farmers lacked the appropriate storage facilities.

The east of Kenya is regularly hit by drought and food shortages.

But the BBC's Anne Wathera in Maukeu, eastern Kenya, said heavy rains last year prompted a bumper harvest.

Farmers were not expecting so much maize and did not know how to store it properly, our correspondent says.

Maize can be hit by a toxic fungus if it is not stored properly.

There have reportedly been more cases of maize-related food poisoning, and farmers in areas where the maize was harvested have told the BBC they are still not sure what is safe to eat.

One farmer told the BBC the government was offering to buy the maize for much less than it was worth.
Table 1. Economic, aflatoxin exposure, and health characteristics of selected nations.

<table>
<thead>
<tr>
<th>Country</th>
<th>% population living below national poverty line (WHO, 2010b)</th>
<th>GDP per capita, 2010 USD (PPP) (IMF, 2010)</th>
<th>Aflatoxin exposure, ng/kg bw/day (Liu and Wu, 2010)</th>
<th>% stunted children (WHO, 2010b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>NA</td>
<td>15 030</td>
<td>0–4</td>
<td>8</td>
</tr>
<tr>
<td>China</td>
<td>5</td>
<td>7 240</td>
<td>17–37</td>
<td>22</td>
</tr>
<tr>
<td>France</td>
<td>NA</td>
<td>34 250</td>
<td>0.3–1.3</td>
<td>NA</td>
</tr>
<tr>
<td>The Gambia</td>
<td>58</td>
<td>1 479</td>
<td>4–115</td>
<td>28</td>
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<tr>
<td>India</td>
<td>29</td>
<td>3 176</td>
<td>4–100</td>
<td>48</td>
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<tr>
<td>Kenya</td>
<td>52</td>
<td>1 783</td>
<td>3.5–133</td>
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<td>Nigeria</td>
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<td>2 357</td>
<td>139–227</td>
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<td>0.3–1.3</td>
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<td>Tanzania</td>
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<td>1 484</td>
<td>0.02–50</td>
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<tr>
<td>Thailand</td>
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<td>8 479</td>
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<td>USA</td>
<td>NA</td>
<td>47 702</td>
<td>0.26</td>
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</tr>
</tbody>
</table>

*Note.* GDP = gross domestic product per capita; NA = not available; PPP = purchasing power parity.

Effects of aflatoxin poisoning

- Chronic
- Low level, long term exposure
- Liver cancer
- Undernourishment
- Growth impairment
  - Stunting
  - Underweight
  - Wasting

- Acute
- Exposure to high levels
- Death within days
- Other risks factors
  - Adult/child
  - Immuno-suppressed
  - Previous exposure
3-Year-Old Children

Normal

Extreme Neglect

©1997 Bruce D. Perry, M.D., Ph.D., Child Trauma Academy
Factors affecting *A. flavus* infection and aflatoxin contamination of Maize
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Beer Foam’omics

- Collaborative project with Joe White Maltings (Cargill) and Lion (Kirin – Japan)
- Foam positive and negative proteins from barley (LTP, Z4 & Z7) and yeast
- Full genome sequence of a number of yeast strains
- Proteomics and metabolomics profiling
- Comparing infusion (IBD) and ramping (EBC) mashing
- In-house foam tester (Lion)
Working together with the Queensland Government
Acknowledgements

• The University of Queensland
• Australian Govt
• B&M Gates Foundation
• Stellenbosch University Professor Marena Manley (+ students)
• SAB Maltings - South Africa
• Biosciences eastern central Africa - Kenya
• Ethiopian Institute Agricultural Research – Ethiopia
• Professor Ian Godwin for sorghum slides
Dream as you ‘ll forever
Live as if you will die today

Those who have the opportunity and capacity have the responsibility to have those who can’t help themselves

Questions?