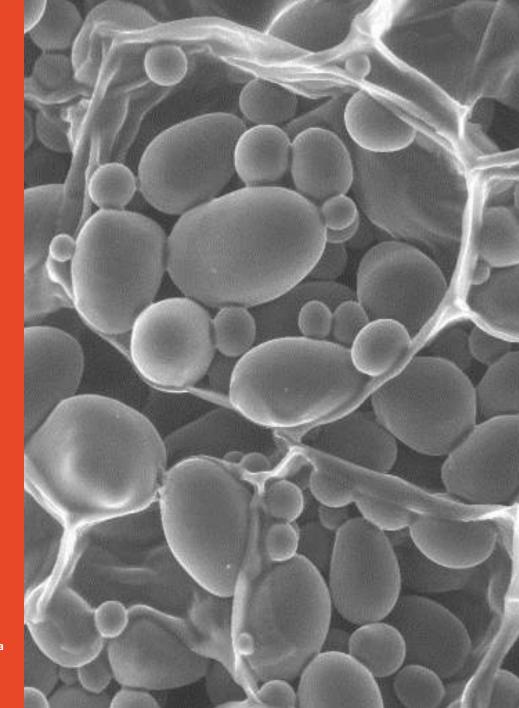
Digestibility of starch as a risk factor for life-style diseases

Les Copeland





University of Padova 28 Sep 2016

Grand challenges

- Food
- Resources
- Climate
- Agriculture as an instrument of public health
 - reduce illnesses due to hunger, nutrient deficiencies, over consumption
 - in 2014, >600 million obese adults, >400 million with T2D
 - increased risk of cardiovascular disease, inflammatory diseases, cancers, cognitive decline, ...
 - understand and improve functionality of foods and ingredients
 - modified carbohydrates for glycemic control, prebiotics
 - modified oils with better fatty acid profile, increase omega oils
 - increase bioavailability of micronutrients
 - nutraceuticals



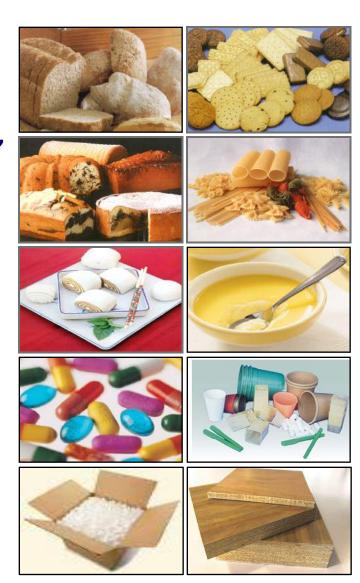






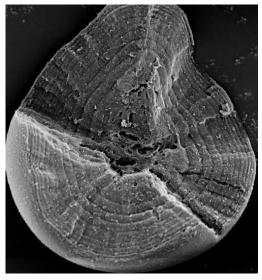
Starch

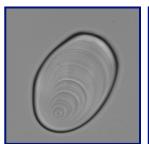
- Macro-nutrient in many foods
- Major industrial product
 - 60+ million t/yr extracted from wheat, corn, potato, rice, cassava, specialty sources
 - 60% used in foods
 - 40% for non-food uses
- Occurs in plants as semi-crystalline granules
 - crystallinity ranges from 15% (high AM starches) to 45% (waxy starches)
- Variability of granules between and within species
 - size and shape, degree of crystallinity
 - organization of starch molecules within granules
 - potential for specialty applications but unpredictability of functional performance

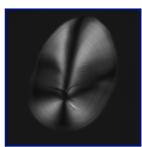


Molecular organization inside starch granules is complex

 Limited understanding of how amylose (lightly branched) and amylopectin (highly branched) are arranged into crystalline and amorphous growth rings

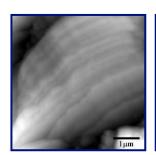






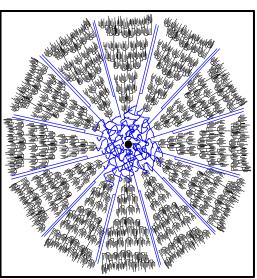


Ek et al. 2014. Brit J Nutr 111, 699-705 Ek et al. 2014. Food Funct 5, 2509-2515



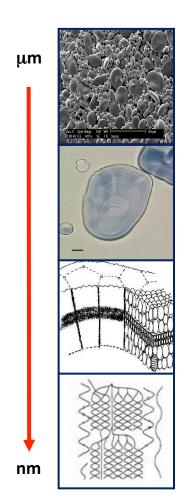
AFM of rice starch

Dang and Copeland 2004. JSFA 84, 707-713
Dang and Copeland 2006. J Microscopy 224, 181-186



Wang et al. 2012. Carbohydrate Polymers 87, 1941-1949 Wang and Copeland 2012. Food Chemistry 135, 1635-1642

Structure determines function



Structure

- Macro (1-100 μm)
 - granules
- Micro (0.1-0.4 μm)
 - growth rings, crystalline and amorphous lamellae
- Nano (10-100 nm)
 - organization of AM & AP
 - AP branching pattern
- Physicochemical properties





Function



- Viscosity
- Pasting
- Gelatinisation
- Retrogradation
- Gel properties
- Digestibility
- Collective properties





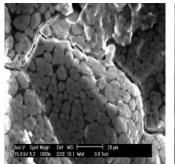
Structure is determined by biosynthesis

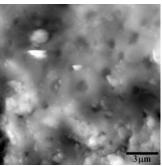
- multiple genes and enzyme forms
- developmental and environmental influences during growth

Difficult to assess contribution of specific properties to functionality of the whole

Cooking causes starch to gelatinize

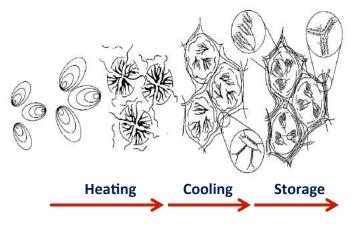
- Heat, moisture and shear disrupt granules irreversibly
 - granules absorb water and swell
 - H bonds break
 - AP crystallites melt, AM leaches out
- On cooling, dispersed starch molecules retrograde
 - aggregate into a new semi-ordered state
 - disrupted AP clusters entrapped in an AM matrix
 - min to hr for AM, hr to days for AP
- Gelatinization and retrogradation
 - control most functional properties of starch in foods
 - digestibility, texture, water absorption, shelf life,
 - extent depends on type of starch, temperature, moisture, shear, rate and duration of heating and cooling, composition of mixture, ...
- Most processed foods contain a mixture of intact and partially disrupted granules, retrograded AM and AP





Native and parboiled rice

Dang and Copeland L 2004 JSFA 84, 707-713



Goesaert et al. 2005

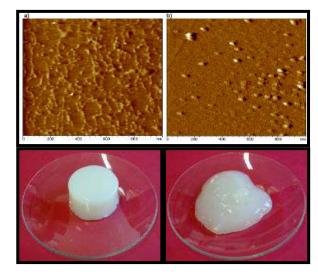
Starch gels







- Retrograded starch forms gels if concentration ≥ 4%
- Present in many cooked and cooled foods
- Affects digestibility
- Non-waxy starch gels are firm
 - strengthened by AM network
- Waxy starch gels and starch-lipid gels are soft
 - no AM network



Normal starch

Waxy starch



Starch



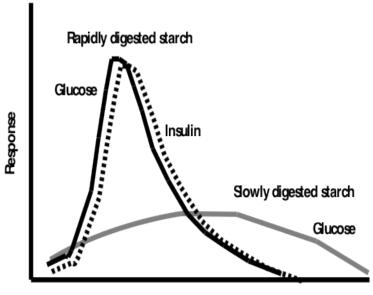
Starch + Monopalmitin



Starch + Tripalmitin

Starch contributes 50-70% of dietary energy

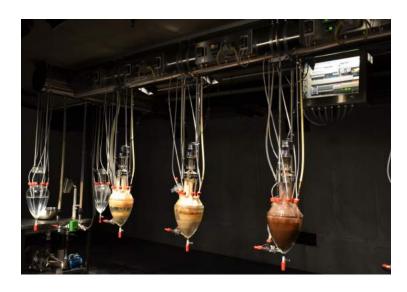
- Starch digestibility is linked to risk factors for disease
- Rapidly digested starch (highly-processed foods)
 - poor glycemic and insulin control
- Slowly digested starch (less-processed foods)
 - moderated glycemic and insulin responses
- Resistant starch (raw starch, cooked and cooled foods)
 - not digested in upper gut
 - prebiotic, no glycemic effect
- Slowly digested starch and resistant starch associated with health benefits
- Glucose released in the ileum triggers beneficial hormonal responses (ileal brake)
- Rapidly and slowly digested starch and resistant starch are kinetic phases of starch hydrolysis, not defined chemical entities



Time after meal

Glycemic effect of foods

- Caloric value of foods is an over-simplification
 - need to consider digestibility (bioavailability)
 - quality of calories
- Glycemic carbohydrates release glucose
 - starch, sucrose
- Glycemic Index indicates blood glucose-raising potential of a food
 - in vivo test of digestion, glucose absorption and clearance from bloodstream
 - low GI: <55 medium GI: 56-69 high GI: > 70
 - costly, slow, not always convenient, statistics
- In vitro testing methods are used as proxies
 - simple → complex
 - measure starch breakdown only
 - useful for high throughput and comparative studies
 - can be standardized



MONA, Hobart



NutraScan, Next Instruments

Enzyme hydrolysis of of starch

Access of enzymes to the substrate is the rate limiting step

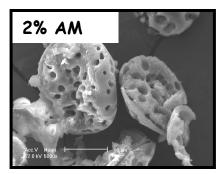
• form of starch (raw, encapsulated, structure, gelatinized/retrograded, complexes, ...)

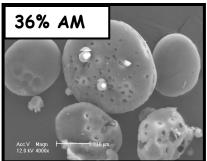
Raw starch (feeds and fermentations, uncooked foods)

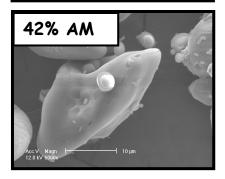
- hydrolysis is slow
- affected by varietal differences, amylose content
- rate determined by surface effects

Cooked starch (processed foods)

- hydrolysis much more rapid than raw starch
- no differences between freshly-gelatinised starches
- influenced by extent of cooking and cooling







Wheat starch granules after 2 h with α -amylase

Blazek and Copeland 2010. J Cer Sci 51, 265-270; Blazek J and Copeland L 2010. J Cer Sci 52, 295-302; Wang and Copeland 2013. Food Funct 4, 1564-1580

Genotype x environment effects on amylolysis of starch

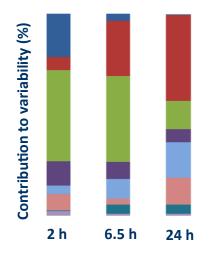
Native starch

- Genotype accounts for most of variance initially, but environmental factors become increasingly important
- Simple first order kinetics

Cooked starch

- **Conditions for gelatinization and retrogradation** accounted for much of the variance in digestibility
- For a specific set of conditions, genotype contributed about 60-70% of the variance in rapidly digested and resistant starch
- **Complex kinetics heterogeneity of substrate**

Heritability of susceptibility to amylolysis

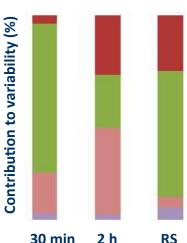


Year

Location

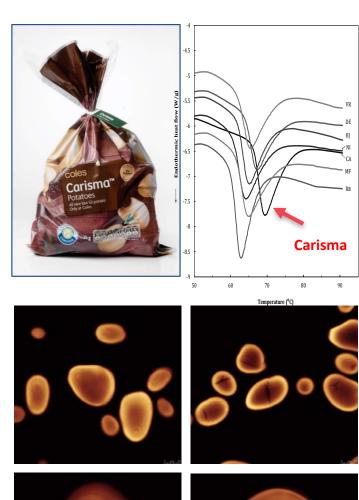
Variety

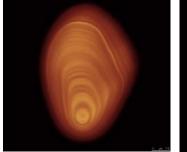
Residual

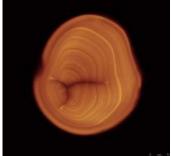


Genotypes for slow digestibility

- Carisma™ is a low GI potato
- Selected for firm cooking quality, size, agronomy
- Carisma starch does not gelatinize as readily as other potato starches
- Cell wall thickness may also be a factor







Ek 2014. British Journal of Nutrition. 111, 699-705. Ek 2014. Food and Function 5, 2509-2515.

Carisma

Russet Burbank

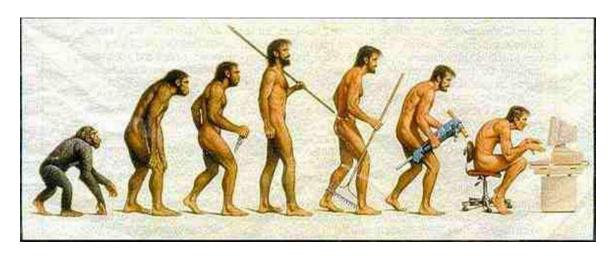
Carbohydrates are the main food for gut microbiota

- Carbohydrates that escape digestion in the upper gut provide essential dietary fibre, prebiotics
 - mostly resistant starch, non starch polysaccharides
- Gut microbiota strongly influence physiology
 - impact of diet on host metabolism
 - predisposition and resilience to disease
 - inflammatory and autoimmune conditions
 - cognitive abilities
- Complexity of species, taxonomic and functional diversity
 - influenced by diet, lifestyle, hygiene
 - resilience varies between individuals
 - changes in gut microbiota are associated with human disease – cause or effect?



MICHELLE POSSUM NUNGUARRAYI Indigenous Australian art www.aboriginal-art-australia.com

Role of nutrition in human evolution



- What is a healthy diet?
- Is our physiology adapted to the diet we evolved with?
- How and why did humans develop such large brains?

A new hypothesis

- Starchy plant foods in the Paleolithic were essential for the evolution of modern humans
- Essential for the increased metabolic demands of a growing brain and to support successful reproduction and increased aerobic capacity

Hardy K, Brand-Miller J, Browne KD, Thomas MG, Copeland L. 2015. The importance of dietary carbohydrate in human evolution. Quarterly Review of Biology 90, 251-268.

Early hominins

Brain

- increase in size began around 2 million years ago, accelerated from about 800,000 years ago
 - increased from 400 cc to 1800 cc
- required an increased supply of glucose

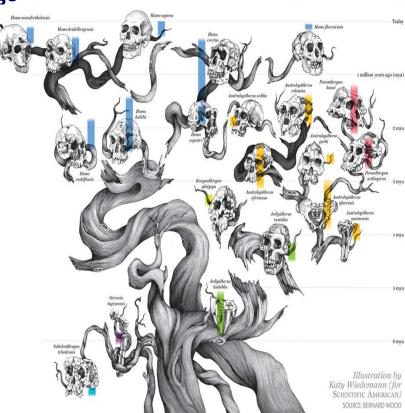
Gut

- smaller teeth and stomach, long small intestine around 1.8 million years ago
- shift from a high-volume, low-energy to low volume, high-energy diet

Aerobic capacity

increased around 2 million years ago

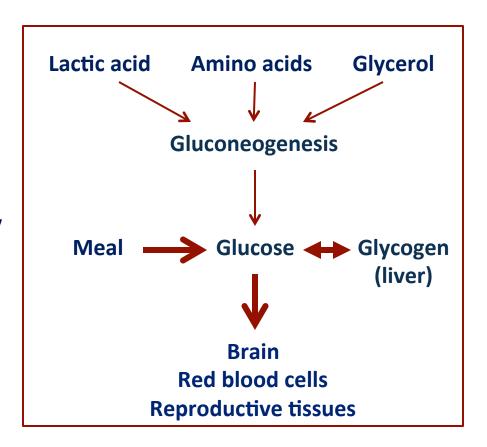
Earliest known date for *H. sapiens* is 195,000 years ago



Source: WHERE WE CAME FROM. Scientific American, 00368733, Sep2014, Vol. 311, Issue 3

Evidence from nutrition – humans need dietary glucose

- About 150 g/day of glycemic carbohydrate needed from the diet
 - additional requirements for pregnant and lactating females
- Protein ceiling humans can derive only 35-40% of energy from proteins
- Gluconeogenesis can meet 20-30% of the needs for glucose



Evidence from archaeology – dental calculus

Dental calculus contains information on early hominin diet

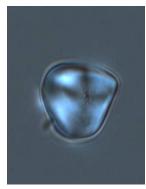


Starch in dental calculus



A, starch granules; B, microcharcoal; C, D, E, fungal spores; F, pine pollen grain: G, epidoptera wing scale; H, phytolith.





Neanderthal starch (ca 50,000 years old)



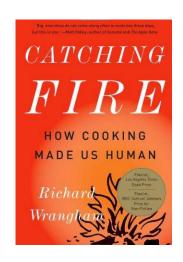


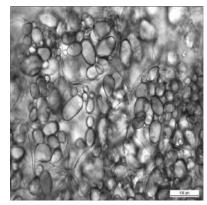
Amylolysis of ancient starch granules

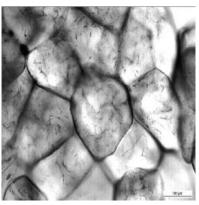
Hardy et al. 2012. Naturwissenschaften 99, 617-626 Hardy et al. 2016. Quaternary International 398, 129-136

Cooking

- Humans are the only species that cook food
 - transformational event in human evolution
 - linked to emergence of social behavior
- Firm evidence of fire for cooking 300,000 -400,000 years ago
 - may have occurred 800,000 years ago
- Improved palatability and digestibility of food
- Cooked starch greatly increased availability of energy and glucose for tissues with high glucose demand
 - large brain too costly energetically to function on a raw diet
 - earlier weaning and shorter inter-birth intervals







Starch granules in raw and cooked potatoes (From Kai Lin Ek 2014. Univ of Sydney PhD Thesis)

Amylases

- Humans have salivary (AMY1) and pancreatic (AMY2) α -amylases
 - only *H. sapiens* have between 2 and 20 gene copies of AMY1
 - other primates have only 2 copies
 - more amylase means greater ability to digest starch
- Multiplication of AMY1 genes
 - thought to have begun less than 1 million years ago
 - overlaps the time frame for hominin adoption of fire for cooking and enlargement of brain
- Cooking-driven increase in the availability of digestible starches would have provided evolutionary selection pressure for AMY1 and AMY2 copy number variation
- Individuals from populations with high-starch diets have, on average, more AMY1
 copies than those with traditionally low-starch diets
- Higher AMY 1 copy number results in higher blood glucose response (Fiona Atkinson and Jennie Brand-Miller)

Role of starch in human evolution

Co-evolution of cooking and copy number variation of *AMY1* (and possibly *AMY2*) gene(s) increased availability of glucose, permitting the acceleration in brain size increase observed from the Middle Pleistocene

- explains how our human ancestors were able to meet the greatly increased needs for glycemic energy
- unifies evidence from archaeology, prehistory, human physiology, nutrition and genetics

Thank you for your attention















Thank you for your attention

