Supplementary Information
"Measurement of Starch"; McCleary, Charmier and McKie

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2.2.2 Measurement of starch using AOAC Method 996.11 (McCleary *et al*.)

Assays were performed exactly as described in AOAC Method 996.11 [10]. Add ~ 100 mg of sample, weighed accurately, into a 16 x 120 mm glass text tube and wet with 0.2 mL of aqueous ethanol (80 % v/v). Stir on a vortex mixer to ensure that the sample is completely wet. Add 3 mL of diluted thermostable α-amylase (E-BLAAM; 100 Ceralpha U/mL at pH 7.0) in 50 mM MOPS buffer (pH 7.0) in 5 mM calcium chloride to the tube and incubate it in a boiling water bath for 2 min. Remove the tube and mix the contents vigorously for 20 sec on a vortex mixer. Return the tube to the boiling water bath for a further 4 min. Stir the contents vigorously after 2 and 4 min. Remove the tube, mix the contents on a vortex mixer and place the tube in a water bath set at 50^{o}C and allow to equilibrate over 5 min. Add 4.0 mL of 200 mM sodium acetate buffer, pH 4.5 and 0.1 mL of AMG solution (330 U on starch), stir the tube contents thoroughly on a vortex mixer, cap the tube and incubate at 50^{o}C for 30 min. Quantitatively transfer the contents of the tube to a 100 mL volumetric flask using distilled water from a wash bottle to effect complete transfer. Adjust the volume to the mark and mix the contents thoroughly. Centrifuge a portion of the solution at 4,000 g for 10 min, or filter at least 10 mL through Whatman No. 1 filter paper. For samples containing less than 10 % w/w starch content, after incubation with AMG, adjust the volume to 10 mL before centrifugation or filtration. Carefully and accurately transfer duplicate 0.1 mL portions of each supernatant (or filtrate) to the bottoms of two glass test tubes (12 x 120 mm). Add 3.0 mL of GOPOD reagent to each tube and incubate at 50^{o}C for 20 min. Concurrently, incubate duplicate reagent blanks (containing 0.1 mL of water in place of sample), and quadruplicate glucose standards (0.1 mL containing 100 μg of glucose) with 3.0 mL of GOPOD reagent. Prepare sample blanks by incubating sample with MOPS buffer and acetate buffer devoid of α-amylase and AMG. For most samples analysed, these values were negligible (less than 0.1 % w/w). Measure all absorbance values against the reagent blank value and calculate starch content as:

Calculations:

Total starch, % = A x F x 1000 x 1/1000 x 100/W x 162/180

= A x F/W x 90

where: A = absorbance of reaction solution read against reagent blank; F = factor to convert absorbance values to μg glucose (= 100 μg glucose divided by the absorbance value obtained for 100 μg of glucose); 1000 = volume correction, i.e. 0.1 mL taken from 100 mL; 1/1000 = conversion from μg to mg; 100/W = conversion to 100 mg sample; 162/180 = factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch.

2.2.3 Measurement of starch using AOAC Method 2014.10 (Hall)

Assays were performed exactly as described in AOAC Method 2014.10 [3]. Accurately weigh 100 to 500 mg of dried test sample or 500 mg of semi-moist or liquid sample containing < 100 mg of dietary starch into screw-cap glass tubes. Dispense 30 mL of 100 mM sodium acetate buffer (pH 5.0) into each tube. Add 0.1 mL of heat-stable α-amylase (Novozymes 120 L or Genencor Multifect AA 21 L; ca 1800 to 2100 Liquifon units; 8200 to 8300 BAU; equivalent to 135 Ceralpha Units at pH 5.0). Incubate tubes at 100°C for 60 min in a forced air oven, vortexing tubes at 10, 30 and 50 min of incubation. Cool tubes to room temperature on a bench for 30 min. Add 1 mL of diluted AMG [Megazyme cat. no. E-AMGDF (3300 U/mL) diluted 1:16; i.e. 206 U/mL on soluble starch] and incubate tubes for 2 h in a water bath at 50°C; vortex after 1 h of incubation. Add 20 mL of water, cap the tube and invert 4 times to mix contents thoroughly. Either remove a 1.5 mL of test sample and centrifuge in a microfuge at 1,000 g for 10 min, or filter the entire solution through a hardened filter paper with 22 mm retention with water rinses to 100 mL in a volumetric flask. Transfer duplicate 0.1 mL of aliquots of samples, either undiluted, or diluted further with water, into 16 x 100 mm test tubes and add 3.0 mL of glucose oxidase-peroxidase reagent. Incubate at 50°C for 20 min and measure absorbance at 505 nm. Concurrently incubate duplicate aliquots of glucose at 0, 25, 50, 75 and 100 mg per test with GOPOD reagent. Determine free glucose in each sample (sample blanks) concurrently by extracting test samples under identical conditions with acetate buffer but without inclusion of either α-amylase or AMG. Zero the spectrophotometer with the GOPOD reagent blank (no glucose) and measure all other absorbance values against this. Calculate starch content on a % (w/w) basis.

Calculate starch content as described in AOAC Method 2014.11 employing a quadratic equation that fits the values of the glucose standard solutions (0-100 μg).

2.2.4 Assay of α-amylase and AMG and definitions of enzyme activity.

The activities of thermostable (*Bacillus* sp. Megazyme cat. no. E-BSTAA) and heat stable α-amylase (*Bacillus licheniformis* in Novozymes 120L and Genencor Multifect AA 21L) were measured using the Ceralpha® assay procedure employing benzylidene blocked *p*-nitrophenyl maltoheptaoside in the presence of excess levels of thermostable α-glucosidase. Incubations were performed either in 50 mM MOPS buffer (pH 7.0) containing 5 mM $CaCl_2$, or 100 mM sodium acetate buffer (pH 5.0) containing 5 mM $CaCl_2$ at 40°C as described in the Ceralpha® kit booklet (Megazyme cat. no. K-CERA; AOAC Official Method 2002.01). One Unit of enzyme activity is defined as the amount of enzyme that releases one μmole of *p*-nitrophenol per minute under the defined assay procedure. AMG was assayed by incubating 0.2 mL of suitably diluted enzyme in 100 mM sodium acetate buffer (pH 4.5) with 0.5 mL of soluble starch (10 mg/mL) in 100 mM sodium acetate buffer (pH 4.5) at 40°C. At various time intervals, reaction tubes were heated to ~ 100°C in a boiling water bath to terminate the reaction and released glucose was measured using GOPOD reagent (Glucose Assay Kit; Megazyme cat. no. K-GLUC). One Unit of AMG is defined as the amount of enzyme required to release one μmole of glucose per minute at pH 4.5 and 40°C. AMG was also assayed using AMG Assay Reagent (Megazyme cat. no. R-AMGR3) and Units of activity on starch were calculated using a conversion factor.

2.2.5 Preparation of a D-glucose standard solution.

Purified D-glucose (> 99.5 %; Sigma-Aldrich, Inc.) was dried for 15 h under vacuum at 110°C and then allowed to cool to room temperature in a desiccator containing fresh silica gel. A solution of 1 mg/mL was prepared by adding 1.1 g of the dry solid to 1.000 Kg of deionized water. The exact concentration of this solution was then determined by use of the hexokinase-glucose 6-phosphate dehydrogenase glucose (HK/G6P-DH) determination method by measuring the increase in absorbance at 340 nm and calculating the amount of NADPH produced. The concentration was then adjusted to 1.000 mg/mL by adding the appropriate weight of water. Benzoic acid to give a final concentration of 0.2 % w/v was added and dissolved and the solution was divided into lots of 100 mL and stored in well sealed Duran® bottles. These solutions were stable at room temperature for >5 years.

2.2.6 Determination of D-glucose with GOPOD reagent.

2.2.6.A Preparation and storage of GOPOD reagent.

GOPOD (a) – Standard preparation: Prepare GOPOD reagent according to the procedure described in AOAC Method 996.11 [10]. Dissolve 13.6 g potassium dihydrogen orthophosphate, 4.2 g of sodium hydroxide, 3.0 of 4-hydroxybenzoic acid and 162 mg of 4-aminoantipyrine in 1.8 L of distilled water. Adjust the pH to 7.4 with either 2 M HCl or 2M NaOH and adjust the volume to 2 L. Add 0.4 g of sodium azide and dissolve. Then add 24,000 U of glucose oxidase and 1300 U of horse radish peroxidase. Mix the contents well and store in well-sealed 1 L bottles and protect the reagent from light by covering the bottles with aluminium foil. Use the reagent as a cold solution directly from a refrigerator or cold room. With this type of use, the reagent is stable for at least 3 months at 4^{o}C.

GOPOD (b) – Karkalas formulation: Alternatively, GOPOD reagent was prepared according to the procedure of Karkalas [7] with glucose oxidase (7,000 U/L), peroxidase (7,000 U/L), 4-aminoantipyrine (0.74 mM) and phenol (1.0 g/L) in phosphate buffer, pH 7.0.

2.2.6.B Linearity of glucose-GOPOD standard curve.

A range of solutions of increasing glucose concentration were prepared by mixing set weights of glucose standard solution (1 mg/mL) in 0.2 % (w/v) benzoic acid with a solution of 0.2 % (w/v) benzoic acid i.e. for 0.9 mg/mL, 90.0 g of glucose standard solution (1 mg/mL) in 0.2 % (w/v) benzoic acid was added to 10.0 g of 0.2 % (w/v) benzoic acid solution. A similar procedure was employed to prepare solutions of concentrations of 0, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 and 0.90 mg/mL. An aliquot (0.10 mL) of each of these solutions were incubated in duplicate with 3.00 mL of GOPOD reagent for 20 min at 50^{o}C. The absorbance at 510 nm was measured with a Shimadzu UV-1800 Spectrophotometer (cat. no. 206-25400-59) connected to SWA-2 Sample Waste Unit (cat. no. 206-23820-91) (Figure 2). With the MegaQuant™ Wave colorimeter, aliquots (0.1 mL) of glucose standard solution (1.00 mg/mL) were incubated with 3.0 mL of GOPOD reagent in spectrophotometer tubes sealed with Parafilm® in a hot block set at 40^{o}C. Absorbance readings were measured at 505 nm after 20 min (Supplementary Figure 1).

2.2.6.C Stability of GOPOD colour complex

Determination of the stability of the GOPOD complex was performed using a recording spectrophotometer [Biochrom Libra S22 UV/Vis Recording Spectrophotometer (cat. no. 80-2115-20), by incubating aliquots (0.1 mL) of glucose standard solution (1.00 mg/mL) with 3.0 mL of GOPOD reagent in a cuvette in the spectrophotometer (in the dark). Absorbance readings were recorded continuously (Figure 3). Determinations of GOPOD stability were also performed manually using a spectrophotometer with a sipper cell attachment [Shimadzu UV-1800 Spectrophotometer (cat. no. 206-25400-59) connected to SWA-2 Sample Waste Unit (cat. no. 206-23820-91)]. Aliquots (0.2 mL) of glucose standard solution (1.00 mg/mL) were added to a 16 x 120 mm test tube. GOPOD reagent (6.0 mL) was added and mixed thoroughly and tubes were incubated in the light at 40ºC or 50ºC. At various time intervals the tubes were removed from the water bath and aliquots removed using the sipper device on the spectrophotometer and absorbance values recorded (Supplementary Figure 2).

2.2.7 Gelatinisation of starch and hydrolysis by α-amylase.

2.2.7.A Effect of pH and presence of $CaCl_2$ on the stability of α-amylase at 100ºC.

Aliquots (10 mL) of 100 mM sodium acetate buffer (pH 5.0) were added to Corning screw-cap culture tubes (20 x 125 mm). An aliquot (0.1 mL) of either Novozymes 120 L or Genencor Multifect AA 21L heat stable α-amylase or Megazyme thermostable α-amylase (cat. no. E-BSTAA) was added to each tube and these were placed (at 1 min intervals) into a boiling water bath and the contents of each swirled for a few seconds every few minutes. At various time intervals (5, 10, 15, 20, 40 and 60 min) aliquots (0.2 mL) were removed from each of the tubes with a Eppendorf HandiStep® dispenser with a 5.0 mL Combitip® and transferred to 5 mL of 100 mM sodium maleate buffer (pH 6.5) containing 2 mM $CaCl_2$ (26-fold dilution). These solutions were then further diluted by transferring aliquots (0.2 mL) to 5.0 mL of 100 mM sodium maleate buffer (pH 6.5) containing 2 mM $CaCl_2$. (a total dilution of the original enzyme preparation of 68,276-fold). Enzyme activity was assayed using the Ceralpha method. An aliquot (0.2 mL) of diluted enzyme was incubated with 0.2 mL of Ceralpha reagent at 40ºC for 3 min. The reaction was terminated by the addition of

3.0 mL of 2 % w/v tri-sodium phosphate solution (pH 11) with thorough mixing. The absorbance was measured at 400 nm against a reagent blank. The activity remaining was calculated as a percentage of the activity in the non-heated original enzyme preparation (Figure 4).

This experiment was also performed using 100 mM sodium acetate buffer (pH 5.0) containing 5 mM $CaCl_2$ and also using 50 mM MOPS buffer (pH 7.0) both containing and not containing 5 mM $CaCl_2$.

2.2.7.B Stability of maltose under the incubation conditions used for the hydrolysis of starch by α-amylase.

Maltose (1 g) was dissolved in 100 mL of each of the following buffers; 100 mM sodium acetate, pH 5.0 plus containing 5 mM $CaCl_2$; 50 mM MOPS buffer, pH 7.0 containing 5 mM $CaCl_2$; 100 mM Tris-HCl buffer, pH 8.0 containing 5 mM $CaCl_2$; and 100 mM Tris-HCl buffer, pH 9.0 containing 5 mM $CaCl_2$. Aliquots (10 mL) of each of these solutions were transferred into four Corning screw-cap culture tubes (20 x 125 mm) and the tubes were placed into a boiling water bath. After 1 min one tube of each buffer mixture was removed from the boiling water bath and placed into an ice water bath. An aliquot (1 mL) of 2 M sodium acetate buffer, pH 4.0 was added to each tube immediately it was placed in the ice-water bath (to lower the pH to ~ 4.5) and the solution was mixed thoroughly. Additional tubes of each buffer mixture were removed from the boiling water bath after 10, 20 and 60 min and were immediately placed into the ice-water bath. An aliquot (1 mL) of 2 M sodium acetate buffer, pH 4.0 was added to each tube to lower the pH to ~ 4.5. When all tubes had been removed from the boiling water bath and cooled, the contents were mixed thoroughly on a vortex mixer and an aliquot (1.0 mL) was transferred to 10 mL of 100 mM sodium acetate buffer, pH 4.5 and mixed. Aliquots (0.1 mL) in duplicate of each of these solutions were transferred to the bottoms of glass test tubes (16 x 120 mm) and 0.1 mL of AMG (300 U/mL) in 100 mM sodium acetate buffer, pH 4.5 was added with mixing and the solutions were incubated at 50°C for 30 min. GOPOD reagent (3.0 mL) was added and the solutions incubated at 50°C for 20 min. The absorbance was measured at 510 nm (Supplementary Figure 3).

Aliquots (5 mL) of each of the above maltose incubation solution were transferred to 16 x 120 mm polypropylene tubes and 1.5 g of Amberlite® FPA53 (OH^-) resin and 1.5 g of Ambersep® 200 (H^+) were added. The tubes were capped and inverted regularly over 5 min to remove most of the salt in the sample. Samples were analysed by ion chromatography on a Thermo Fisher Dionex ICS-5000+SP ion chromatography system with a Dionex AS-DV autosampler. The column employed was a CarboPac PA200 Analytical (3 x 250 mm) with a CarboPac PA200 Guard column (3 x 50 mm). The gradient employed A. 100 mM sodium hydroxide and B. 120 mM sodium acetate plus 100 mM sodium hydroxide. Time 0 min, 100 % A plus 0 % B; time 5 min, 55 % A plus 45 % B; time 9 min, 30 % A plus 70 % B; time 10 min, 0 % A plus 100 % B for a further 8 min. Then washing with 100 % A plus 0 % B for 12 min (Figure 5).

2.2.8 Hydrolysis of sucrose under α-amylase incubation conditions.

Sucrose (400 mg) was dissolved in 150 mL of 200 mM sodium acetate buffer, pH 5.0 and the volume was adjusted to 200 mL. Aliquots (10 mL) of this solution were transferred into each of six 20 x 120 screw cap tubes. Aliquots (0.1 mL) of thermostable α-amylase (Megazyme cat. no. E-BSTAA) were added into 6 of these tubes and one tube was transferred to an ice-water bath and the other 5 tubes were placed into a boiling water bath. Tubes were removed from the boiling water bath at 5, 10, 20, 40 and 60 min and placed into an ice-water bath. Aliquots (0.1 mL) were removed in duplicate from each tube, 3.0 mL of GOPOD reagent added and the tubes incubated at 50°C for 20 min. Absorbance at 510 nm was measured (Supplementary Figure 4).

2.2.9 Hydrolysis of starch dextrins by AMG at 40°C and 50°C

Wheat starch (100 mg) was accurately weighed into two screw cap test tubes. An aliquot (10 mL) of 200 mM sodium acetate buffer (pH 5.0) and 0.1 mL of thermostable α-amylase (cat. no E-BSTAA) were added to each and these were incubated in a boiling water bath for 15 min. One tube was transferred to a water bath at 40°C and the second to a bath at 50°C and allowed to equilibrate over 10 min. Amyloglucosidase (0.1 mL, 330 U) was added to each tube and the contents mixed. Aliquots (1 mL) of solution were removed at 10, 20, 30, 40 and 60 min and heated in

a boiling water bath for 5 min to inactivate AMG. The tubes were cooled in an ice-water bath and 9 mL of distilled water was added to each tube and mixed thoroughly. Aliquots (0.1 mL) were removed in duplicate and 3.0 mL of GOPOD reagent added with mixing. The tubes were incubated at 40°C for 20 min and the absorbance measured at 510 nm (Supplementary Figure 6).

2.2.10 Purity of AMG

2.2.10.A Hydrolysis of cellulose and β-glucan.

The standard starch assay (2.2.1.F) from steps 1 to 7 was performed. An aliquot of the solution was centrifuged and this solution was analysed directly (without dilution) for glucose according to 2.2.1.F steps 8 to 12.

2.2.10.B Hydrolysis of sucrose by AMG.

Solutions of sucrose at 10 mg/mL were prepared by adding 10 g of sucrose to 900 mL of 100 mM sodium acetate buffer, pH 5.0 and dissolving. The volume was adjusted to 1 L with 100 mM sodium acetate buffer, pH 5.0. A solution of sucrose at 1 mg/mL was prepared by adding 100 mL of sucrose (10 mg/mL) to 900 mL of sodium acetate buffer, pH 5.0. Aliquots (10 mL) of each solution were transferred to Corning culture tubes (20 x 125 mm) and 0.1 mL of *A. niger* AMG (E-AMGDF; 330 U); high purity *A. niger* AMG (330 U) (purified by chromatography on anion and cation exchange resins and gel permeation chromatography) or *Rhizopus* sp AMG (330 U) were added and mixed. An aliquot (1.0 mL) was immediately removed from each solution and transferred into a 12 x 160 mm test-tube and heated in a boiling water bath for 5 min to give a zero time reference. The remaining sucrose solution was incubated at 50°C and aliquots (1.0 mL) were removed at 30 and 60 min and transferred to glass tubes and heated for 5 min in a boiling water bath to inactivate AMG. Sucrose hydrolysis was measured by removing 0.1 mL aliquots in duplicate from each tube and adding 3.0 mL of GOPOD reagent with mixing. The tubes were incubated at 50°C for 20 min and the absorbance at 510 nm (Supplementary Figure 5) measured. In separate experiments, sucrose cellulose controls containing ~ 10% and ~ 20% sucrose were analyzed according to the standard starch analytical procedure (2.2.1.F) with measurement of released glucose (see Table 1)

2.2.11 Accuracy and reproducibility of starch assay procedures.

The accuracy and reproducibility of the starch analytical procedure (2.2.1.F) was studied by analysing ten animal feeds/pet foods plus a reference maize starch sample in duplicate on four separate days. These same samples were also analysed in duplicate using AOAC Method 996.11 [10] and 2014.10 [3] and the values compared. A range of other samples were analysed for starch content, including breakfast cereals (Supplementary Table 1), cereal grains and legume seeds (Table 5), vegetables and fruits (Supplementary Table 2), a range of food products including beverages and food products (Supplementary Table 3), dairy products, spreads and sauces and pure starch samples (Table 4).

Supplementary Tables

Supplementary Table 1. Starch contents of breakfast cereals determined using the RTS method.

Starch type and lot number	Moisture content % w/w	Free glucose % w/w	Starch plus free glucose % w/w "as is"	Starch % w/w "as is"	Starch % w/w "dwb"
Kellogg corn flakes	5.48	1.4	72.6	71.2	75.2
Kellogg Special K Original	3.69	0.5	64.5	64.0	66.5
Nestle Shreddies	4.04	1.0	56.7	55.7	58.1
Kellogg All Bran Original	2.51	0.4	26.6	26.2	26.8
Kellogg Frosties	4.05	1.0	50.8	49.8	51.8
Weetabix	6.09	0.3	62.0	61.7	65.6
Maceroni pasta (Roma)	8.57	0.04	69.1	69.1	75.5
Regular maize starch (control)	13.7	0.03	85.5	85.5	99.0

[a] all values are the mean of duplicate analyses.

Supplementary Table 2. Starch content of a selected range of vegetables and fruits determined using the RTS method.

Sample	Starch content % w/w "dwb"	Moisture content, % w/w	Starch content % w/w of original material
Sweet potato	32.3	74.0	8.4
Potatoes (Rooster variety)	67.4	77.3	15.3
Cauliflower	0.1	90.6	0.01
Celery	0	95.0	0.0
Brochelli	0.1	88.5	0.01
Mushroom (*Agaricus bisporus*)	0.5	91.6	0.04
Red onions	0.6	87.7	0.07
Carrots	1.6	92.5	0.12
Swede	10.6	82.8	1.8
Red pepper	0.5	89.7	0.05
Ripe banana	18.9	74.1	4.9
Ripe mango	8.6	81.8	1.6

[a] all values are the mean of duplicate analyses.

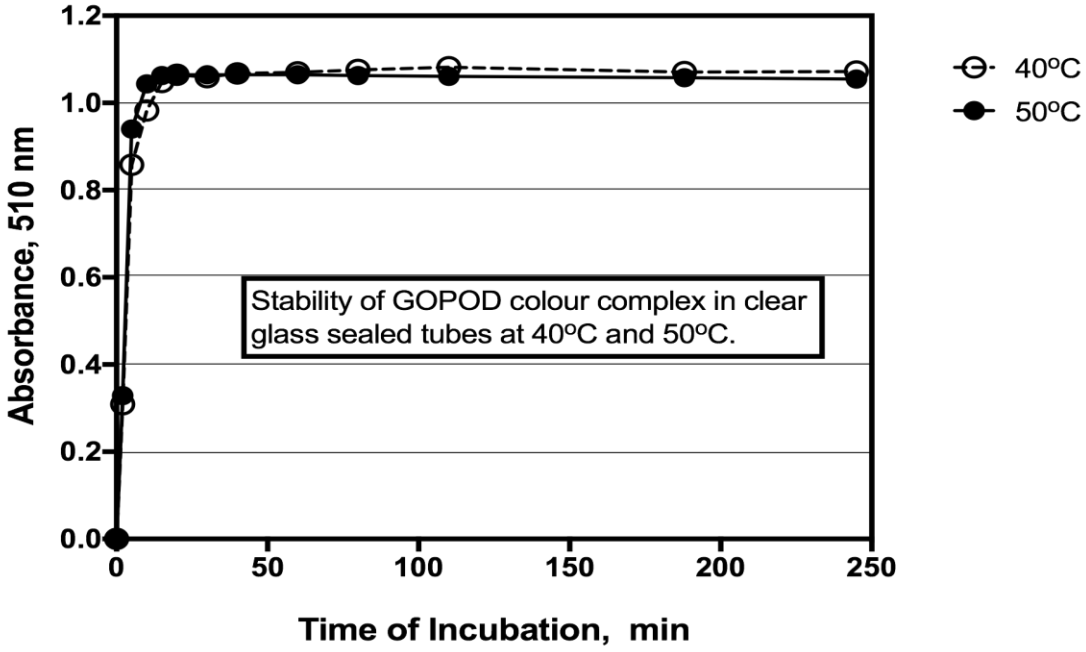
Supplementary Table 3. Starch contents of a range of beverage and food products determined using the RTS method.

Product	Starch, % w/v[a] or w/w[b] "as is" Replicate 1	Starch, % w/w "as is" Replicate 2	Average
Guinness draught beer	15.2 [a]	14.8	15.0
Smithwicks red ale	15.2 [a]	14.6	14.9
Heineken lager	18.0 [a]	18.1	18.0
Bulmers cider	1.1 [a]	1.2	1.2
Erdinger wheat beer	25.1 [a]	25.8	25.4
Innocent smoothie (mandarin, carrot, ginger, flax seed)	2.1 [a]	2.3	2.2
Naked vitamin (apple pineapple, kiwi)	3.1 [a]	2.9	3.0
The Original HP brown sauce	3.3[b]	3.7	3.5
Heinz tomato ketchup	0[b]	0	0
Tesco French dressing	1.0[b]	1.1	1.0
Heinz salad cream original	3.2[b]	3.3	3.1
Amoy dark soy sauce	2.8[b]	2.8	2.8
Tesco hot salsa dip	1.0[b]	0.9	1.0
Heinz soup (potato/leek/chives)	2.6[b]	2.8	2.7
Tesco sweetened condensed milk	0.34[b]	0.27	0.3
Avonmore luxury custard	2.0[b]	2.1	2.0
Lindt white chocolate with vanilla	0.32[b]	0.37	0.35
Tesco 85% cocoa plain chocolate			
Nutella	1.2[b]	1.4	1.3
Tesco peanut butter	3.7[b]	3.8	3.8
Tesco milk chocolate with caramel and sea salt	2.5[b]	3.5	3.0
Rudd's traditional white pudding sausage	8.8[b]	8.7	8.8
Rudd's traditional black pudding sausage	14.0[b]	13.4	13.7

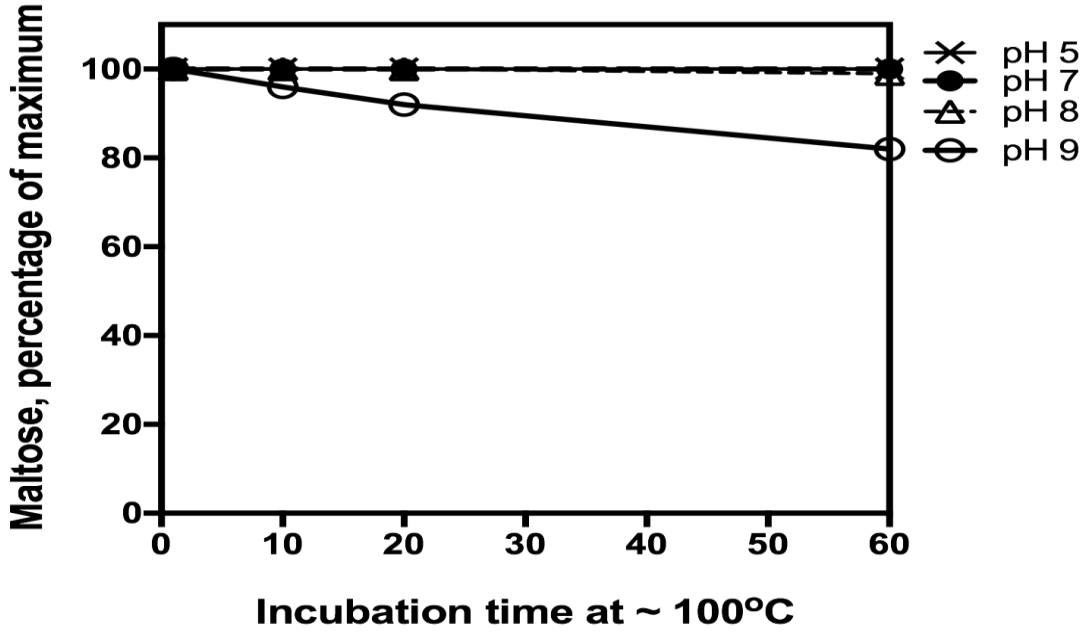
Supplementary Figures

Supplementary Figure 1. Linearity of glucose determination with GOPOD reagent containing *p*-hydroxybenzoic using the MegaQuant Wave Spectrophotometer.

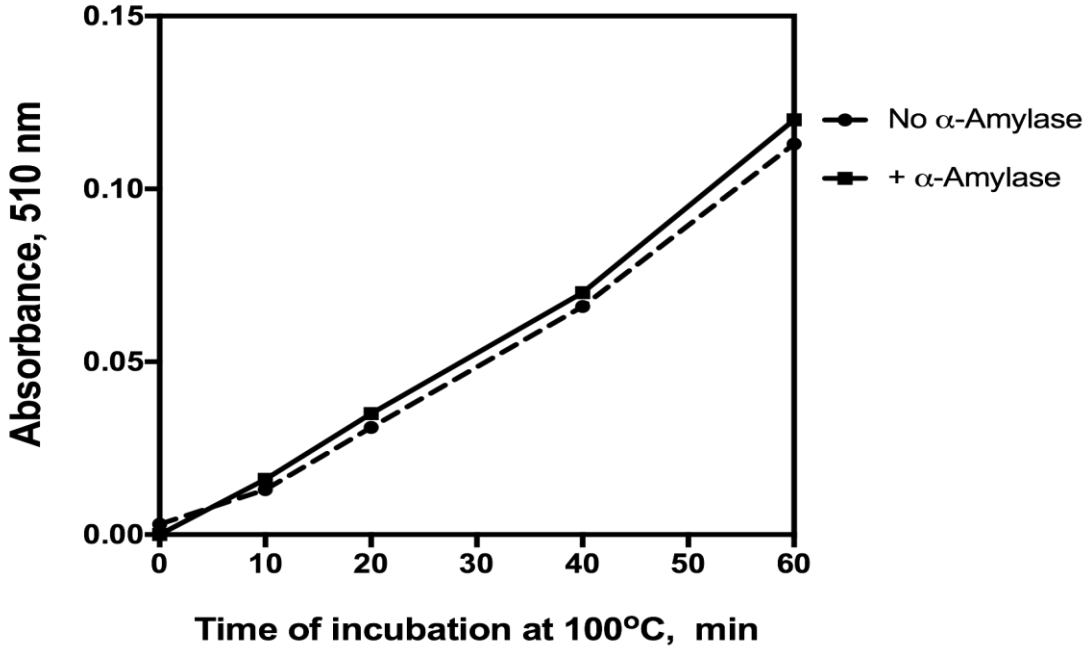
Supplementary Figure 2. Time course of colour development (and colour stability) on incubation of glucose (100 µg/assay) with 3.0 mL of GOPOD reagent (type) at 40ºC or 50ºC (in the light).



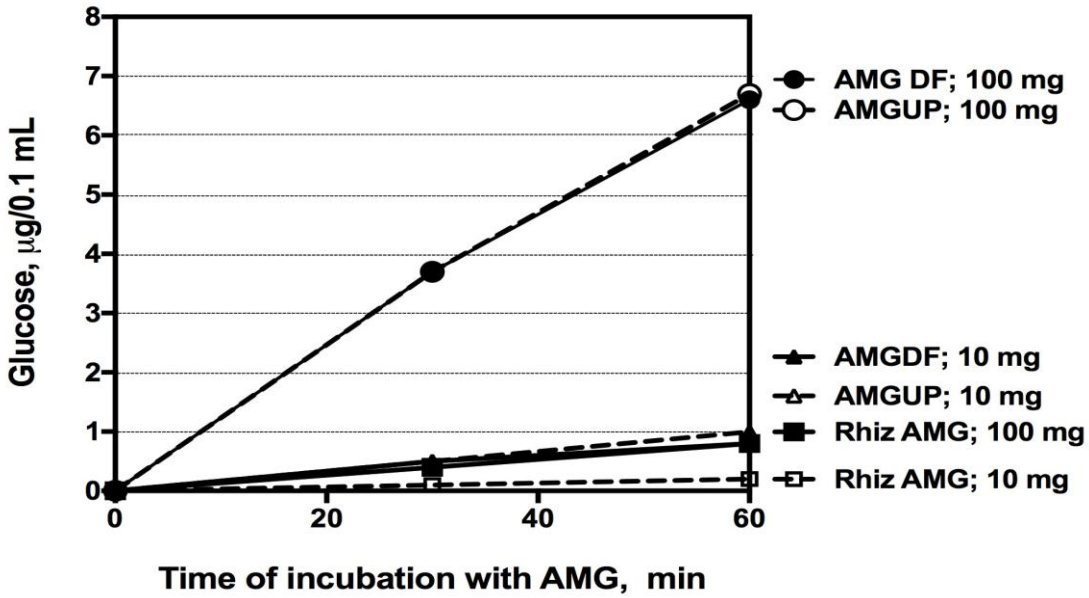
Supplementary Figure 3. Stability of maltose at 100°C at pH 5, 6, 7 and 8. Remaining maltose measured by hydrolysis to glucose with AMG and glucose determination with GOPOD reagent.



Supplementary Figure 4. Hydrolysis of sucrose at 100°C in the presence or absence of thermostable α-amylase.



Supplementary Figure 5. Hydrolysis of sucrose by various AMG preparations (330 U/incubation) at 50°C. Released glucose measured with GOPOD reagent. AMGDF is the *Aspergillus niger* AMG routinely used in the starch analytical methods; AMGUP is highly purified form of E-AMGDF. RhizAMG is an ultrapure amyloglucosidase from *Rhizopus* sp.



Supplementary Figure 6. Degree of hydrolysis of starch dextrins to glucose after 10 to 50 min incubation with AMG under standard incubations conditions at either 40°C or 50°C.