Megazyme

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RESISTANT STARCH

ASSAY PROCEDURE
(Rapid Format)

K-RAPRS 01/19

(100 Assays per Kit)

An updated modification of:
AOAC Method 2002.02
AACC Method 32-40

INTRODUCTION:

By definition, resistant starch (RS) is that portion of the starch that is not broken down by human enzymes in the small intestine. It enters the large intestine where it is partially or wholly fermented. RS is generally considered to be one of the components that make up total dietary fiber (TDF).

The presence of a starch fraction resistant to enzymic hydrolysis was first recognised by Englyst *et al* (1982)[1] during their research on the measurement of non-starch polysaccharides (NSP). This work was extended by Berry[2] who developed a procedure for the measurement of RS incorporating the α-amylase/pullulanase treatment employed by Englyst *et al*,[1] but omitting the initial heating step at 100°C, so as to more closely mimic physiological conditions. Under these conditions, the measured resistant starch contents of samples were much higher. This finding was subsequently confirmed by Englyst *et al*.[3-6] through studies with healthy ileostomy subjects. By the early 1990's the physiological significance of RS was fully realised. Several new/modified methods were developed during the European Research Program EURESTA by Champ[7], Muir and O'Dea,[8] Faisant *et al*.,[9] Goni *et al*.[10] and Akerberg *et al*.[11]

In 2002, McCleary *et al*.[12,13] developed a robust and reliable method for the measurement of RS, which in many ways reflected *in vivo* conditions, and thus yielded values that are physiologically significant. The method allowed the measurement of the resistant starch, solubilised starch and total starch content of samples. This method was successfully subjected to interlaboratory evaluation and became AOAC Method 2002.02 and AACC Method 32-40.01.

In developing an Integrated total dietary fiber (INTDF) method (AOAC Method 2009.01; 2011.25)[14,15] the incubation conditions in the resistant starch method were mimicked, including the time of incubation (*i.e.* 16 h) with pancreatic α-amylase/amyloglucosidase (PAA/AMG). However, in more recent studies on the hydrolysis of "newer" resistant starch materials such as phosphate crosslinked starch (RS_4), it became clear that to obtain meaningful physiologically relevant values for RS, the time of incubation with PAA/AMG should be in-line with the time of residence of food in the small intestine, namely, 4 h[16] (not 16 h as used in AOAC Method 2009.01). To obtain RS values with a 4 h incubation period, that are in-line with ileostomy data, the concentrations of PAA and AMG had to be increased. This new method, termed the Rapid Integrated TDF method (RINTDF),[17] was successfully subjected to interlaboratory evaluation through AOAC International and the International

Association of Cereal Science and Technology (ICC) to become AOAC Method 2017.16[18] and ICC Method 185.

On the basis of these developments, it became clear that the original resistant starch method should also be updated to incorporate a 4 h incubation with PAA/AMG. This has now been done and the method is described in this booklet as the Rapid Resistant Starch (RAPRS) procedure.

PRINCIPLE OF THE METHOD:

Samples are incubated in a shaking water bath in linear motion with saturating levels of purified PAA and AMG for 4 hr at 37°C. During this time, non-resistant starch (digestible starch) is solubilised and hydrolysed to D-glucose by the combined action of the two enzymes.

$$\text{(1) Starch} + H_2O \xrightarrow{\text{(PAA + AMG, pH 6.0, 37°C, 4 h)}} \text{D-glucose + RS}$$

The reaction is terminated by the addition of an equal volume of ethanol or industrial methylated spirits (IMS, denatured ethanol), and the RS is recovered as a pellet on centrifugation.

$$\text{(2) Reaction mixture} \xrightarrow{\text{(Ethanol or IMS)}} \text{soluble D-glucose + RS pellet}$$

This RS pellet is then washed twice by suspension in aqueous IMS or ethanol (50% v/v), followed by centrifugation. Free liquid is removed by decantation. RS in the pellet is dissolved in 1.7 M NaOH by vigorously stirring in an ice-water bath over a magnetic stirrer. This solution is neutralized with acetate buffer and the starch is quantitatively hydrolysed to D-glucose with AMG.

$$\text{(3) Dissolved RS pellet} \xrightarrow{\text{(AMG, pH 4.5, 50°C, 30 min)}} \text{D-glucose}$$

D-Glucose is measured with glucose oxidase/peroxidase reagent (GOPOD), and this is a measure of the RS content of the sample.

$$\text{(4) D-Glucose} + O_2 + H_2O \xrightarrow{\text{(glucose oxidase)}} \text{D-gluconate} + H_2O_2$$

$$\text{(5) } 2H_2O_2 + \textit{p}\text{-hydroxybenzoic acid + 4-aminoantipyrine} \xrightarrow{\text{(peroxidase)}} \text{quinoneimine dye} + 4H_2O$$

Non-resistant starch (solubilised starch) is determined by pooling the original supernatant and the washings, adjusting the volume to 100 mL and measuring D-glucose content with GOPOD Reagent.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The assay is specific for D-glucose derived from resistant starch. Non-resistant (digestible) starch values would also include free D-glucose and/or maltodextrins if they are present in the sample.

The smallest differentiating absorbance for the assay is 0.010 absorbance units. This corresponds to 0.018 g/100 g resistant starch "as is", using a sample weight of 100 mg and extract volume of 10.3 mL. The detection limit is 0.036 g/100 g resistant starch "as is", which is derived from an absorbance difference of 0.020 with a sample weight of 100 mg and extract volume of 10.3 mL.

The glucose detection assay is linear over the range of 4 to 100 μg of D-glucose per assay.

APPLICABILITY AND ACCURACY:

The method is applicable to samples containing more than 2% w/w RS. With such samples, standard errors of ± 5% are achieved routinely. Higher errors are obtained for samples with RS contents < 2% w/w.

SAFETY:

The reagents used in the determination of resistant starch are not hazardous materials in the sense of Hazardous Substances Regulation. The general safety measures that apply to chemical substances should be adhered to. Both PAA and AMG can cause allergic reactions in some individuals. If an analyst is allergic to powdered PAA and/or AMG, another analyst who is not allergic should prepare the powdered enzymes as an ammonium sulphate suspension for use (see **CAUTION**, page 4).

KITS:

Kits suitable for performing 100 assays of RS are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: **Mixture of purified PAA (40 KU/g) and AMG (17 KU/g);** 3.0 g.
Stable for > 5 years stored dry below -10°C.

Bottle 2: **Amyloglucosidase** [12 mL, 3300 U/mL on soluble starch (or 200 U/mL on *p*-nitrophenyl β-maltoside)] at pH 4.5 and 40°C. AMG solution should be essentially free of detectable levels of free D-glucose.
Stable for > 3 years at 4°C.

Bottle 3: **GOPOD Reagent Buffer.** Buffer (50 mL, pH 7.4), *p*-hydroxybenzoic acid and sodium azide (0.09% w/v).
Stable for > 4 years at 4°C.

Bottle 4: **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder.
Stable for > 5 years below -10°C.

Bottle 5: **D-Glucose Standard Solution** (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.
Stable for > 5 years at room temperature.

Bottle 6: **Resistant Starch Control** (~ 5 g). Resistant starch content shown on the label.
Stable for > 5 years at room temperature.

A. PREPARATION OF REAGENT SOLUTIONS:

1. ***Stock PAA/AMG solution.***— PAA (4 KU/5 mL) plus AMG (1.7 KU/5 mL). Immediately before use, add 0.1 g of PAA/AMG powder mixture (**Bottle 1**) to 5 mL of sodium maleate buffer *[B(a)]* and stir on a magnetic stirrer for 5 min. Store on ice during use. Use within 4 h of preparation. **CAUTION:** If an analyst is allergic to powdered PAA and/or AMG, engage an analyst who is not allergic to prepare the powdered enzymes as an ammonium sulphate suspension as follows: gradually add 0.5 g of PAA/AMG powder mix (PAA 40 KU/g plus AMG 17 KU/g; **Bottle 1**) to 30 mL of cold, distilled water in a 50 mL beaker on a magnetic stirrer in a fume cupboard and stir until the enzymes are completely dissolved (approx. 5 min). Add 15 g of granular ammonium sulphate and dissolve by stirring. Adjust the volume to 50 mL with ammonium sulphate solution (50 g/100 mL).
Stable for 3 months at 4°C.

2. Use the contents of bottle 2 (Amyloglucosidase) as supplied. Stable for > 3 years at 4°C.

3. Dilute the contents of bottle 3 (GOPOD Reagent Buffer) to 1 L with distilled water. This is solution 3. Use immediately.

4. Dissolve the contents of bottle 4 in 20 mL of solution 3 and quantitatively transfer this to the bottle containing the remainder of solution 3. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is Glucose Determination Reagent (**GOPOD Reagent**). Stable for ~ 3 months at 2-5°C or > 12 months below -10°C.

   If this reagent is to be stored frozen, it should be divided into ~ 250 mL aliquots and stored in polypropylene containers. Do not apply these stored solutions to more than one freeze-thaw cycle.

   Freshly prepared reagent is either a light yellow or light pink colour. The solution will develop a stronger pink colour over 2-3 months at 4°C. The absorbance of this solution should be less than 0.05 when read against distilled water.

5. Use the contents of bottle 5 as supplied.
   Stable for > 5 years at room temperature.

6. Use the contents of bottle 6 as supplied.
   Stable for > 5 years at room temperature.

B. REAGENTS (not supplied):

a. ***Sodium maleate buffer (50 mM, pH 6.0) plus 2 mM calcium chloride dihydrate and sodium azide (0.02% w/v).*** Dissolve 11.6 g of maleic acid in 1600 mL of distilled water and adjust the pH to 6.0 with 4 M (160 g/L) sodium hydroxide. Add 0.6 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) and 0.4 g of sodium azide and dissolve. Adjust the volume to 2 L.
   Stable for 12 months at 4°C.

b. ***Sodium acetate buffer (1.2 M, pH 3.8).***—Add 68.6 mL of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water and adjust to pH 3.8 using 4 M sodium hydroxide. Adjust the volume to 1 L with distilled water.
   Stable for 12 months at room temperature.

c. ***Sodium hydroxide solution, 1.7 M.***— Add 68 g of sodium hydroxide to 800 mL of distilled water in a fume cupboard and dissolve by stirring. Adjust the volume to 1 L with distilled water and store in a Duran® bottle. Stable for > 4 years at room temperature.

d. ***Ethanol, 50% v/v or IMS 50% w/v.***— Add 500 mL of either ethanol (95% v/v) or industrial methylated spirits (IMS, 95% v/v) to 500 mL of distilled water. Store in a 1 L Duran bottle. Stable for > 4 years at room temperature.

C. APPARATUS REQUIRED:

a. ***Grinding mill.***— Centrifugal, with 12-tooth rotor and 0.5 mm sieve, or similar device. Alternatively, a cyclone mill can be used for small test laboratory samples provided the mill has sufficient air flow or other cooling to avoid overheating of samples.

b. ***Meat mincer.***— Hand operated or electric, fitted with a 4.5 mm screen.

c. ***Bench centrifuge.***— Capable of holding 101 x 65 mm polypropylene tubes, with rating of approx. 3250 rcf (~ 4,000 rpm), e.g. Sigma Laboratory Centrifuges 4-15 No.10730.

d. ***Shaking water bath.***— (Grant OLS 200) (Grant Instruments Cambridge Ltd.) (or similar) set in linear motion at 100 revolutions per minute on the dial (equivalent to a shake speed of 200 strokes/min), a stroke length of 35 mm and 37°C.

e. ***Polypropylene tube holder for shaking water bath.***— A specifically made polypropylene tube holder with clamps to hold 13 mL polypropylene tubes in horizontal orientation in a Grant OLS 200 shaking water bath (Figure 1).

f. ***Spectrophotometer.***— capable of operating at 510 nm, preferably fitted with flow-through cell (10 mm path length).

g. ***Analytical balance.***— 0.1 mg readability, accuracy and precision.

h. ***Freeze-drier.***— e.g. Virtis Genesis® 25XL or similar. Biopharma Process Systems, Biopharma House, Winchester, UK.

i. ***Magnetic stirrer.***— e.g. IKA KMO 2 basic stirrer.

j. ***Magnetic stirring bars.***— e.g. Fisherbrand™ PTFE Stir Bars 6 x 12 mm ridged.

k. ***Pipettor.***— capable of delivering 100 µL or 1.0 mL, e.g. Gilson Pipetman®, with disposable tips.

Figure 1. Attachment of 13 mL polypropylene tubes to a polypropylene tube-holder in a Grant OLS 200 water bath.

l. ***Positive displacement pipetter.***— e.g. Brand HandyStep®S
- with 25 mL Brand PD-Tip® (to dispense 0.5-2.5 mL aliquots).
- with 5 mL Brand PD-Tip® (to dispense 0.1 mL of α-amylase or AMG solution).

m. ***Sarstedt polypropylene tube.***— 13 mL, 101 x 16.5 mm (cat. no. 60.541.685 https://www.sarstedt.com/en/search/?id=77&L=1&q=60.641.685&x=2&y=3 (accessed 6th August, 2018).

n. ***Glass test tubes.***— 16 x 100 mm, 14 mL capacity.

o. ***Plastic "lunch box".***— large enough to hold test-tube rack and serve as an ice-water bath (see Figure 2).

p. ***Thermometer.***— Capable of reading 37 $\pm$ 0.1°C and 50 $\pm$ 0.1°C.

q. ***Volumetric flask.***— 100 mL capacity.

D. PREPARATION OF TEST SAMPLES:

Collect and prepare samples as intended to be eaten, *i.e.* baking mixes should be prepared and baked, pasta should be cooked etc. Defat per AOAC 985.29 if > 10% fat. For high moisture samples (> 25%) it is desirable to freeze dry. Grind ~ 50 g in a grinding mill *[C(a)]* to pass a 0.5 mm sieve. Transfer all material to a wide mouthed plastic jar, seal, and mix well by shaking and inversion. Store in the presence of a desiccant. Grind wet samples (e.g. wet pasta) in a meat mincer to yield an homogeneous paste. Remove a representative sample for analysis and record weight. Separately, determine the moisture content of the wet sample and allow for the liquid volume in the calculations.

E. ASSAY PROCEDURE:

(a) Hydrolysis and solubilisation of digestible (non-resistant) starch.

i. Accurately weigh a 100 ± 5 mg of sample directly into each screw cap polypropylene tube *[C(m)]* and gently tap the tube to ensure that the sample falls to the bottom.

NOTE: For wet samples such as minced canned beans or food product, the sample size is approx. 0.5 g (weighed accurately). With such materials, the moisture content is usually 60-80%.

ii. Add 3.5 mL of sodium maleate buffer (pH 6.0) *[B(a)]* using a Brand HandyStep®S with a 25 mL Brand PD-Tip®, cap the tube, mix the contents on a vortex mixer for 5 sec. Place the tube in a water bath at 37°C and allow the contents to equilibrate to temperature over 5 min.

iii. Uncap the tubes and add 0.5 mL of PAA/AMG solution *[A(1)]* to each tube, cap the tubes tightly and attach them horizontally, aligned in the direction of motion (Figure 1), in a shaking water bath *[C(d)]* set at 37°C. **NOTE:** if using the $(NH_4)_2SO_4$ suspension of this enzyme preparation *[A(1)]*, add 3 mL of sodium maleate buffer *[B(a)]* and 1 mL of enzyme suspension.

iv. Incubate tubes at 37°C with continuous shaking (200 strokes/min) for **exactly 4 h.** (**NOTE:** for linear motion, a setting of 100 on the water bath is equivalent to 200 strokes/min; 100 forward and 100 reverse).

v. Remove the tubes one at a time from the water bath and remove excess surface water with paper towel. Remove the tube caps and add 4.0 mL of 95% v/v ethanol or IMS to each tube using a Brand HandyStep®S dispenser with 25 mL Brand PD-Tip®, cap the tubes and mix the contents vigorously on a vortex mixer.

vi. When ethanol or IMS has been added to all samples, remove the caps and centrifuge the tubes at 4,000 rpm (approx. 3250 *rcf*) for 10 min.

vii. Immediately after the centrifuge stops, carefully decant the supernatant solution (ensuring that the pellets are not disturbed). Retain this solution for measurement of "digestible starch".

viii. Re-suspend the pellet in 2 mL of 50% v/v ethanol or IMS with vigorous stirring on a vortex mixer. Add a further 6 mL of 50% v/v ethanol or IMS, cap the tubes and mix the contents thoroughly by inversion. Tap the tubes so that all liquid moves

from the lid to the tube and then remove the caps and centrifuge the tubes at 4,000 rpm for 10 min.

ix. Decant the supernatant solutions, repeat the suspension and centrifugation step (viii) once more. Combine both of these supernatants with that obtained at "step vii" for determination of digestible starch.

x. Invert the tubes on absorbent paper to remove excess liquid. Ensure that the residue is not dislodged.

(b) Measurement of Resistant Starch.

i. Add a magnetic stirrer bar (6 x 12 mm) *[C(j)]* and 2 mL of 1.7 M NaOH *[B(c)]* to each tube and re-suspended the pellets (and dissolve the RS) by stirring for approx. 20 min in an ice/water bath over a magnetic stirrer (Figure 2).

NOTE:

1. Do not mix on a vortex mixer as this may cause the starch to emulsify.
2. Ensure that the tube contents are vigorously stirring as the NaOH solution is added. This will avoid the formation of a lump of starch material that will then be difficult to dissolve.

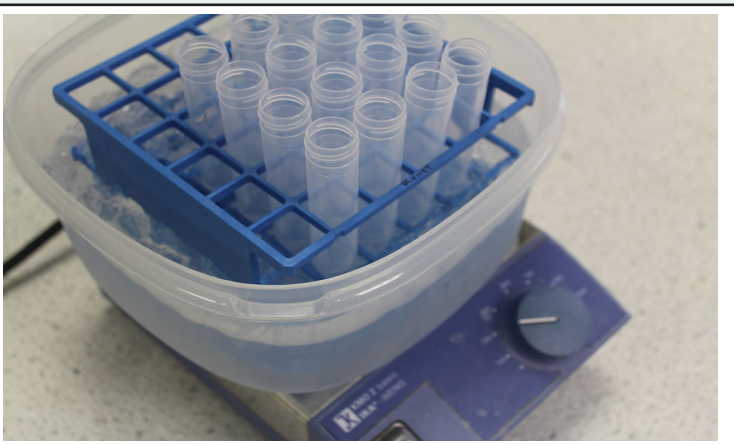

Figure 2. Arrangement of tubes in an ice-water bath over a magnetic stirrer for dissolution of resistant starch with 1.7 M NaOH.

ii. Add 8 mL of 1.2 M sodium acetate buffer (pH 3.8) *[B(b)]* to each tube with stirring on the magnetic stirrer. Immediately add 0.1 mL of AMG (3300 U/mL) *[A(2)]*, mix well and place the tubes in a water bath at 50°C.

iii. Incubate the tubes at 50°C for 30 min with intermittent mixing on a vortex mixer.

iv. ***For samples containing > 10% RS content;*** quantitatively transfer the contents of the tube to a 100 mL volumetric flask (using a water wash bottle). Use an external magnet to retain the stirrer bar in the tube while washing the solution from the tube with the water wash bottle. Adjust to 100 mL with distilled water and mix well. Centrifuge an aliquot of the solution at 4000 rpm for 10 min.

v. ***For samples containing < 10% RS content;*** directly centrifuge the tubes at 4000 rpm for 10 min (no dilution). For such samples, the final volume in the tube is approx. 10.3 mL (however, this volume will vary particularly if wet samples are analysed, and appropriate allowance for volume should be made in the calculations).

vi. Transfer 0.1 mL aliquots (in duplicate) of either the diluted (step iv) or the undiluted (step v) supernatants into glass test tubes (16 x 100 mm), add 3.0 mL of GOPOD Reagent *[A(4)]* and incubate at 50°C for 20 min.

vii. Measure the absorbance of each solution at 510 nm against the reagent blank.

viii. Calculate the content of resistant starch (Section F).

Prepare reagent blank solutions by mixing 0.1 mL of 100 mM sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD Reagent.

Prepare D-glucose standards (in quadruplicate) by mixing 0.1 mL of D-glucose (1 mg/mL) and 3.0 mL of GOPOD Reagent.

Incubate these solutions at 50°C for 20 min along with the sample solutions.

(c) Measurement of Digestible (Non-Resistant) Starch.

i. Combine the supernatant solutions obtained on centrifugation of the initial incubation *[E(a)vii]* with the supernatants obtained from the subsequent two 50% ethanol washings [*E(a)*ix] and adjust the volume to 100 mL with distilled water in a volumetric flask. Mix the contents well.

ii. Incubate 0.1 mL aliquots of this solution with 3.0 mL of GOPOD Reagent (Solution 4) and incubate the tubes for 20 min at 50°C.

iii. Measure the absorbance at 510 nm against a reagent blank.

iv. Calculate the content of digestible starch (Section F).

F. CALCULATIONS:

Calculate resistant starch, digestible (non-resistant) starch and total starch content (%, on a dry weight basis) in test samples as follows:

Resistant Starch (g/100g)

$$= \Delta_A \times F \times \frac{EV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta_A \times F \times \frac{EV}{W} \times 0.90$$

Digestible Starch (g/100g)

$$= \Delta_A \times F \times \frac{EV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta_A \times F \times \frac{EV}{W} \times 0.90$$

Total Starch (g/100g) = Resistant Starch + Digestible Starch

where:

ΔA = absorbance of sample 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).

EV = sample extraction volume (10.3 mL or 100 mL).

0.1 = volume of sample analysed.

1/1000 = conversion from μg to mg.

100/W = conversion to 100 mg sample.

W = sample weight in mg.

162/180 = factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch.

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

G. REFERENCES:

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Table 1. Comparison of RS values obtained with AOAC Method 2002.02 and the Rapid Resistant Starch method.

	Resistant starch (average of duplicate analyses) % w/w 'as is' basis	
Sample	**AOAC Method 2002.02**	**Rapid RS**
Brennans whole meal bread	**0.9**	**0.8**
U B Milled long grain rice	0.5	0.5
UB Express boiled rice	**2.4**	**2.4**
Heinz® baked beans (FD)	**3.6**	**3.8**
Wheat Starch (Sigma S512L)	**0.4**	**0.5**
Corn Flakes (20-12-10)	**2.2**	**2.1**
Tinned butter beans	3.1	3.3
Tinned chick peas	**5.0**	**5.1**
Tinned garden peas	8.2	7.7
Ryvita® dark rye crackers	1.7	1.9
Tinned kidney beans (20-7-11)	**4.3**	**4.3**
Kidney beans (2015)	3.5	4.00
UB Ready Extra White Rice	3.2	3.2
Regular maize starch 60401	0.9	1.8
Semi green banana	13.8	11.0
Native potato starch (Sigma)	**60.9**	**63.9**
High Amylose Maize starch	37.9	48.5
Hylon VII® (60901)	**41.5**	**52.3**
Novelose 240® (96LF10063)	**40.4**	**44.6**
Potato Amylose (Sigma A9262)	35.6	35.3
Actistar®	**46.3**	**49.3**

Table 2. Repeatability of the Rapid Resistant Starch Assay method for measurement of resistant starch.

Sample	Resistant Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD$_r$[c])				
	Day 1	Day 2	Day 3	Day 4	Interday mean, ±2 SD, (%RSD$_r$)
Regular Maize Starch Lot 60401	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	1.8 ±0.2
	5.07	5.09	1.52	3.23	4.33
Hylon VII	48 ± 0.2	48.7 ± 0.4	49.2 ± 0.2	48.6 ± 0.5	48.6 ±0.9
	0.25	0.45	0.25	0.48	0.92
Tinned Garden Peas	8 ± 0.3	8 ± 1.1	8.5 ± 0.4	8 ± 0.6	8.1 ±0.6
	1.79	6.74	2.48	3.50	3.94
UB Express Boiled Rice	2.6 ± 0.2	2.3 ± 0.1	2.5 ± 0	2.4 ± 0.2	2.5 ±0.3
	3.12	1.91	0.89	3.95	5.31
Native Potato Starch Sigma S4251	63.1 ± 0.3	66.2 ± 9.2	60.5 ± 18.5	66 ± 0.2	63.9 ±9.3
	0.23	6.96	15.27	0.13	7.24
Green Banana	45.6 ± 2.3	46.2 ± 2.1	48.7 ± 2.1	47.1 ± 3.7	46.9 ±3.2
	2.48	2.32	2.18	3.95	3.44
ActiStar	48 ± 0.5	50.4 ± 0.4	50.5 ± 0.3	48.8 ± 0.2	49.4 ±2.2
	0.57	0.38	0.28	0.23	2.26

The repeatability (%RSD$_r$) of the Rapid Resistant Starch assay method was assessed using 7 milled samples. For each sample, duplicate extractions were processed and applied to the assay on each day across 4 separate days. The resistant starch content of the samples tested covered a working range of 1.8 to 63.9% (w/w). The repeatability (%RSD$_r$) across this sample data set was extremely high, less than or equal to 7.24% for samples containing 10 to 100% (w/w) resistant starch and less than or equal to 5.31% for samples containing 0.2 to 10% (w/w) resistant starch. This level of repeatability and precision indicates that the Rapid Resistant Starch Assay method is reliable and repeatable, and therefore suited to the application of measuring resistant starch in various food samples including pulses, cereals, fruits and vegetables.

Table 3. Repeatability of the Rapid Resistant Starch Assay method for measurement of digestible (non-resistant) starch.

Sample	Digestible Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD,[c])				
	Day 1	Day 2	Day 3	Day 4	Interday mean, ±2 SD, (%RSD_r)
Regular Maize Starch Lot 60401	80.3 ± 2	81.1 ± 1	82.6 ± 0.5	82.7 ± 1.6	81.6 ±2.4
	1.25	0.61	0.31	0.99	1.50
Hylon VII	32.2 ± 0.6	33 ± 0.6	33.1 ± 0.1	33.5 ± 1.4	32.9 ±1.2
	0.93	0.98	0.12	2.08	1.79
Tinned Garden Peas	16.6 ± 0.2	16.6 ± 1.1	17.7 ± 0.5	16.9 ± 0.1	16.9 ±1.1
	0.53	3.19	1.40	0.28	3.11
UB Express Boiled Rice	69.7 ± 1.2	72.7 ± 0	71.6 ± 0.2	72 ± 1.6	71.5 ±2.5
	0.88	0.00	0.13	1.09	1.77
Native Potato Starch Sigma S4251	11.4 ± 0.4	11.2 ± 0.2	11.9 ± 0.4	11.3 ± 0.2	11.5 ±0.6
	1.79	0.96	1.51	1.10	2.71
Green Banana	17.3 ± 1.5	17.7 ± 0.2	14.8 ± 1.2	16.7 ± 4.3	16.6 ±3
	4.30	0.63	4.18	12.79	9.01
ActiStar	37.5 ± 1.1	36.7 ± 0.3	36.8 ± 0.9	37.2 ± 0.1	37.1 ±0.9
	1.53	0.48	1.20	0.13	1.20

The repeatability (%RSD_r) of the Rapid Resistant Starch assay method for the measurement of digestible starch was assessed using 7 milled samples. For each sample, duplicate extractions were processed and applied to the assay on each day across 4 separate days. The digestible starch content of the samples tested covered a working range of 11.5 to 81.6% (w/w). The repeatability (%RSD_r) across this sample data set was extremely high, less than or equal to 9.01%.

Megazyme

Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com