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Resistant Starch
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Physiological Implications of the Consumption of
Resistant Starch in Man

Edited by Nils-Georg Asp

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Classification and measurement of nutritionally important starch fractions

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For nutritional purposes, starch in foods may be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RS may be further divided into three categories according to the reason for resistance to digestion. A method is reported for the measurement of total starch, RDS, SDS, RS and three RS fractions in starchy foods, using controlled enzymic hydrolysis with pancreatic and amylglucosidase. The released glucose is measured by colorimetry, using a glucose oxidase kit. Values for RDS and SDS in foods obtained by the method reflect the rate of starch digestion in vivo. Values for RS are similar to the amounts of starch escaping digestion in the small intestine of ileostomates, and are a guide to the amounts of starch likely to enter the colon for fermentation. Results are given for a number of starchy foods.

In the last decade two widely held assumptions concerning the digestion of starch have been shown to be invalid. The first is that all starch, because it exists as large complex polymers, is hydrolysed and absorbed more slowly than simple sugars or disaccharides. A number of studies in vitro and in vivo have shown that the physical form of food is the major determinant of the rate of digestion of both starches and sugars (Wahlquist et al., 1978; Jenkins et al., 1987). The second assumption is that starch is completely hydrolysed and absorbed within the small intestine. It is now widely known that the extent of starch digestion within the small intestine is variable, and that a substantial amount of starch, depending again on physical form, escapes digestion in the small intestine and enters the colon.

The development of amylase-resistant starch in food was first observed in the process of developing a technique for the measurement of non-starch polysaccharides (NSPs). It was shown that some types of food processing rendered a small proportion of the starch resistant to hydrolysis with pancreatic amylase and pullulanase (Englyst, Wiggins & Cummings, 1982) and that this starch (mainly retrograded amylose) resisted digestion in the small intestine of man (Englyst & Cummings, 1985). Subsequently, it was shown that retrograded amylose is only a small proportion of the starch resisting digestion in the small intestine of man (Englyst & Cummings, 1986, 1987). The reasons for the incomplete digestion of starch may be separated into intrinsic factors (i.e. properties of the food) and extrinsic factors (chewing, transit through the bowel, etc.).

Intrinsic factors

Starch digestion is slowed in the small intestine if the physical form of the food hinders access of pancreatic amylase. This occurs if starch is contained within whole or partly disrupted plant structures such as grains or seeds; if rigid cell walls inhibit swelling and dispersion of starch, as in legumes (Warsch, Del Velasco & Koehler, 1980); or if starch is very densely packed.
in a food such as spaghetti (Hermansen et al., 1986). When the rate of starch digestion is decreased, postprandial glucose and insulin responses are reduced or delayed. Heaton et al. (1985) have demonstrated that these responses are smaller if wheat, maize or oats are given as whole or coarsely ground grains than if given when finely milled. When hydrolysis is grossly retarded, physically inaccessible starch may enter the colon and be fermented, or in some cases, appear in faeces (Englyst, 1985).

In the plant, starch is stored as partially crystalline intracellular bodies known as starch granules. The shape of these granules and the crystal structure within them are characteristic of the plant source and may be one of three types, A, B or C, as distinguished by their X-ray diffraction patterns (Katz, 1937). Cereal starches are usually of the A type. Starches from tubers such as potatoes tend to exhibit B-type crystallinity, and C-type starch, found in some legumes, is a combination of the A and B patterns. In general, starch granules showing X-ray diffraction patterns of the B or C type tend to be more resistant to digestion by pancreatic amylase, although the degree of resistance is dependent on the plant source (Fuwai, Takaya & Sugimoto, 1980). This resistance to hydrolysis with pancreatic amylase affects the digestibility of starch foods normally eaten raw such as banana, and of processed foods, such as biscuits, where the starch has been incompletely gelatinized.

When starch granules are fully gelatinized and dispersed, the starch becomes easily digestible. However, as the gel cools and ages, the polymers once more form a partially crystalline structure. Recrystallization or retrogradation depends on the formation of interchain hydrogen bonds and occurs most rapidly for the linear amylase. Retrogradation of amylpectin is limited by its branched structure and the polymers of retrograded amylpectin are less firmly bound than those of retrograded amylose. Retrograded starch characteristically forms the B-type pattern.

Other factors intrinsic to starchy foods that have been shown to affect α-amylase activity in vitro include amylase-lipid complexes (Holm et al., 1983), native α-amylase inhibitors (Shanmugan & Birk, 1976) and NSPs, which may have a direct effect on enzyme activity (Durai & Sheneeman, 1981). It is not clear to what extent these factors affect the digestibility of starch in vivo.

Extrinsic factors

Whilst the effect of intrinsic factors upon digestion of starchy foods may be measured in a reproducible way, this will not necessarily predict exactly the rate and extent of digestion of these foods by individuals. This is because eating exposes food to variable external influences that may alter the susceptibility of the starch to hydrolysis by pancreatic amylase. For example, the extent of chewing will determine the physiological accessibility of starch contained within rigid structures. Other factors are the transit time of the food from mouth to terminal ileum, the concentration of amylase in the gut, the amount of starch present, and the presence of other food components that might retard enzymatic hydrolysis. Any measurement of the digestibility of starchy foods should take account of both intrinsic and extrinsic factors, but should not include the large degree of variation that extrinsic factors introduce. Such a method allows a classification and comparison of starch foods according to their potential digestibility in man.

Classification of starches

A classification of starchy foods for nutritional purposes based on the intrinsic factors affecting starch digestibility is shown in Table 1. The aim of the method described here is to classify starches according to this scheme. The method gives reproducible estimates of the proportions of starch that are likely to be digested rapidly in the small intestine, likely to be digested slowly, and likely to pass into the colon.

Method

Principle

The various types of starch are determined by controlled enzymic hydrolysis and mea-
Table 1. In vitro nutritional classification of starch

<table>
<thead>
<tr>
<th>Type of starch</th>
<th>Example of occurrence</th>
<th>Probable digestion in small intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapidly digestible starch (RDS)</td>
<td>Freely cooked starchy food</td>
<td>Rapid</td>
</tr>
<tr>
<td>Slowly digestible starch (SDS)</td>
<td>Meat raw cereals</td>
<td>Slow but complete</td>
</tr>
<tr>
<td>Resistant starch (RS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Physically inaccessible starch</td>
<td>Partially milled grains and seeds</td>
<td>Resistant</td>
</tr>
<tr>
<td>2. Resistant starch granules</td>
<td>Raw potato and bananas</td>
<td>Resistant</td>
</tr>
<tr>
<td>3. Retrograded starch</td>
<td>Cooked, cooked potato, bread, and corn flakes</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Assessment of the released glucose using glucose oxidase. Total starch (TS) is determined as the glucose released by enzymatic hydrolysis following gelatinization in boiling water and treatment with potassium hydroxide to disperse retrograded amylase. TS is corrected for free glucose but includes maltose and maltodextrins. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) are measured after incubation with pancreatic amylase and amyloglucosidase at 37°C for 20 min and a further 100 min, respectively. Resistant starch (RS) is the starch not hydrolysed after 120 min incubation. If required, separate values may be obtained for physically inaccessible starch (RSₐ), resistant starch granules (RS₉) and retrograded amylase (RSₐ) (see Table 1).

Reagents

**Enzymes.** Amyloglucosidase solution EC 3.2.1.3, 400 AGU/ml (Novo Nordisk, Copenhagen). Invertase solution EC 3.2.1.26, 3000 EU/ml (BDH, cat. no. 35020). Pancreatin, amylase activity 30,000 BPU/g (Pancrex V, Paines & Byrne, Greenford, Middle, UK). Pullulanase, 2000 PNU/g (Protamzyme, Novo Nordisk). Heat stable α-amylase, 300 KNU/g (Termamyl, Novo Nordisk).

**Enzyme solution 1.** Dilute 2.8 ml amyloglucosidase to 8 ml with water to make a solution containing 140 AGU/ml. Weigh 3.0 g pancreatin into each of four centrifuge tubes and suspend each portion in 20 ml water. Add stirrer and stir magnetically for 10 min, then centrifuge for 10 min at 1500 g. Take 13.5 ml supernatant from each tube (54 ml total) and mix with 6 ml diluted amyloglucosidase and 4 ml invertase. Prepare immediately before use. The quantity given is sufficient for 12 samples.

**Glucose oxidase colorimetric kit.** Merck-o-test glucose (GOD-PAP), BDH cat. no. 25456, or Boehringer Glucose (GOD-PAP) Test Combination, cat. no. 166391.

**Glucose standard solution.** Weigh 5000 mg glucose (dried to constant weight over P₂O₅) to the nearest 0.1 mg. Make up to 200 ml with sodium acetate buffer to give a 25 mg/ml solution.

**Sodium acetate buffer (0.1 M).** Dissolve 13.6 g sodium acetate trihydrate (CH₃COONa·3H₂O) in 250 ml saturated benzoic acid solution, and make to 1 l with water. Adjust to pH 5.2 with 0.1 M acetic acid. To stabilize and activate enzymes, add 4 ml 1 M CaCl₂ per litre of buffer.

**Apparatus**

**Mincer.** With plate of 0.9 cm diam. holes (Kitchen Basics range, Boots, Nottingham).

**Polypropylene centrifuge tubes, 50 ml, with screw-caps.**

**Glass balls** of approximately 1.5 cm diam., sufficient to provide 5 per sample.

**Water-baths.** Shaking water-bath with maximum shaking capacity of not less than
160 strokes per min and a stroke length of 35 mm (e.g. Grant Instruments, 271, temperature range 0–80°C, shaking 0–200 strokes/min, cat. no. GT SS40). Boiling water-bath. Water-bath capable of maintaining 35–60°C.

Spectrophotometer.

General Measurement of glucose. Prepare the GOD–PAP reagent as directed in the kit. Pipette 100μl water (blank), samples and standards in duplicate into labelled test tubes. Add 2 ml GOD–PAP reagent and mix. Place tubes in water-bath at 37°C for 20 min. Measure absorbance of standards and samples against reagent/enzyme blank at 510 nm.

\[
\text{% glucose} = \frac{A_s \times V_t \times C \times D}{A_s \times W_t} \times 100,
\]

Where

- \( A_s \) is absorbance of test solution,
- \( V_t \) is total volume of test solution,
- \( C \) is concentration (in mg glucose/ml) of standard,
- \( A_s \) is absorbance of standard,
- \( W_t \) is weight (in mg) of sample taken for analysis, which may be corrected for moisture,
- \( D \) is a dilution factor.

\( V_t \) is the total volume of the hydrolysate from which the subsample taken for glucose determination originates. \( C \) is the concentration of glucose in the standard solution treated identically to the hydrolysate from which the subsample is taken, unless the samples and standards are diluted differently, in which case a dilution factor \( (D) \) is introduced. The calculation of these constants takes account of the subsamples taken during the procedure.

Determination of dry matter (DM). Dry matter of the sample should be determined at the time of weighing. Weigh accurately 1–3 g sample into a glass beaker and dry to constant weight in an air oven at 100°C.

<table>
<thead>
<tr>
<th>Dry matter (%)</th>
<th>Examples</th>
<th>Sample weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>75–100</td>
<td>Flour, cereals, biscuits</td>
<td>800 mg</td>
</tr>
<tr>
<td>55–75</td>
<td>Bread, cakes</td>
<td>1–2 g</td>
</tr>
<tr>
<td>35–55</td>
<td>Rice, pasta, rice</td>
<td>1.5–2 g</td>
</tr>
<tr>
<td>15–35</td>
<td>Canned foods, sauces</td>
<td>3–4 g</td>
</tr>
</tbody>
</table>

Sample weight. The sample weight required for the analysis depends on the water and starch contents of the food, which may be estimated from food table values. The amount of sample taken should contain between 700 and 900 mg DM, and the total starch content of the sample should not exceed 800 mg. Examples of suitable weights are given in Table 2.

Rapid measurement of TS in a finely divided sample

If measures of RDS, SDS and RS are not required, a finely milled sample (0.2 mm) may be used to determine total starch within approximately 4 h.

Sample preparation. Samples should be finely divided by milling or homogenization. Weigh, to the nearest mg, between 0.8 and 4.0 g, depending on the water and starch content of the sample (to give 700–900 mg DM) into 50 ml screw-cap tubes. Add a magnetic stirring bar to each. Into two extra screw-cap tubes, pipette 25 ml glucose standard. A blank tube, containing 25 ml acetate buffer, is required to correct for glucose present in the amylace solution. Treat these tubes exactly as the samples after the addition of acetate buffer.

Measurement of free glucose (FG). Pipette 25 ml of sodium acetate buffer into each sample tube, cap and vortex-mix. Place tubes in water-bath at 100°C for 30 min, mixing at the start to prevent lumps forming. Cool tubes to room temperature. Remove 1 ml of each sample, standard or blank into a test-tube containing 2 ml ethanol and vortex-mix. At this point it is best to continue with the measurement of total glucose in the main sample tubes and return to the FG determination when
Analysis of starch fractions

Centrifuge the FG portions at 1500 g for 5 min. Dilute 1 ml supernatant in 5 ml water (samples) or 20 ml water (standards), mixing well by inversion. Measure the glucose as described, using the following values: \( V_s = 25 \) plus 1 ml per gram wet weight of sample used, \( C = 0.397 \) and \( D = 18 \).

**Measurement of total glucose (TG).** To each tube containing sample, standard or blank, add 0.1 ml Termamyl, mix and replace in the boiling water-bath for 15 min. Transfer the tubes to a bath of ice-water and chill for 15-20 min. Add 10 ml 7 M potassium hydroxide (KOH), cap, and mix by inversion. Keep the tubes in ice-water, stir for 15 min.

Prepare in advance a second set of 50 ml screw-cap tubes capable of withstanding centrifugation. Into each tube, pipette 10 ml 0.5 M acetic acid. Remove samples from stirrer and immediately take 1 ml of the contents into the 10 ml acetic acid. Cap, and mix well. Take 0.5 ml of amylglucosidase and make to 4 ml with water to give a solution containing 50 AGU/ml. Add 0.2 ml of this to each sample. Mix and incubate at 70°C for 30 min. Place tubes in boiling water for 10 min, then cool to room temperature and add 40 ml water. Centrifuge at 1500 g for 5 min to remove the precipitate.

Measure the total glucose in these samples as described using the following values: \( V_s = 35.5 \) plus 1 ml per gram wet weight of sample used, \( C = 17.6 \) and \( D = 1 \).

\[ TS = (TG - FG) \times 0.9 \]

**Measurement of TS in sample as eaten**

This procedure allows the measurement of TG and FG using a sample identical with that used for the measurement of RDS, SDS and RS.

**Sample preparation.** Foods are analysed with minimal pretreatment. Those with a recognizable structure that would normally require mastication (e.g. pasta, rice, sweetcorn) should be passed through the mincer before weighing. For most foods, no further pretreatment is necessary. However, very hard materials, e.g. whole wheat grains, may require milling or homogenization to obtain an accurate TS measurement.

Weigh, to the nearest mg, between 0.8 and 4.0 g depending on the water and starch content of the sample into a 50 ml polypropylene screw-cap tubes, and add five glass balls to each. Into two extra screw-cap tubes containing glass balls, pipette 25 ml glucose standard. A blank tube containing glass balls and 25 ml acetic buffer is required to correct for glucose present in the amylglucosidase solution. Treat these tubes exactly as the sample tubes after the addition of acetic buffer.

**Measurement of FG.** Pipette 25 ml 0.1 M acetic buffer into each sample tube, and shake or vortex-mix vigorously to begin disrupting large particles. Place the tubes into a water-bath at 100°C for 30 min, shaking occasionally to prevent aggregation. Remove the tubes to a bath at 37°C, shaking vigorously again. When equilibrated, add 0.2 ml invertase, cap, and immerse horizontally in the shaking water-bath at 37°C, securing firmly. Incubate, with shaking, for 30 min.

Take the tubes out of the bath and shake vigorously. Remove 1 ml of the contents into a test tube containing 2 ml ethanol, and vortex-mix. (At this stage, continue with the main sample tube and return to the FG portions when convenient.) Centrifuge the FG portions at 1500 g for 5 min. Dilute 1 ml supernatant in 5 ml water (samples) or 20 ml water (standards), mixing well by inversion. Measure the free glucose (which in this case includes the glucose part of sucrose) as described using the following values: \( V_s = 25.2 \) plus 1 ml per gram wet weight of sample used, \( C = 0.394 \) and \( D = 18 \).

**Measurement of TG.** To the sample, standard and blank solutions add 0.1 ml Termamyl, cap, shake vigorously, and place in a boiling water-bath for 15 min. Vortex-mix or shake again. Transfer the tubes to ice-water and chill for 15-20 min. Add 10 ml 7 M KOH, cap the tube and mix by inversion. Immers horizontally in the shaking water-bath containing ice-water (0°C), securing firmly. Incubate, with shaking, for 30 min.
Prepare in advance a second set of 20 ml centrifuge tubes and pipette 10 ml 0.5 M acetic acid into each. Remove the sample tubes from the ice-water and immediately take 1 ml of the contents into the acetic acid. Mix well. Add 0.2 ml amyloglucosidase (diluted to 50 AGU/ml), mix and incubate at 70°C for 30 min. Transfer the tubes to a boiling water-bath for 10 min, then cool to room temperature and add 40 ml water. Centrifuge at 1500 g for 5 min to remove the precipitate. Measure the TG in these samples as described, using the following values: V = 35.7 plus 1 ml per gram wet weight of sample used, C = 17.55 and D = 1.

\[
TS = (TG - FG) \times 0.9
\]

**Measurement of RDS, SDS and RS fractions**

**Sample preparation.** Foods are analysed as eaten. Thus, cooked foods should be freshly prepared immediately before analysis and kept warm until the buffer is added. Foods with a recognizable structure that would normally require mastication (e.g. rice, pasta, sweetcorn) should be passed through the mincer before weighing. Weigh, to the nearest mg, between 0.8 and 4.0 g, depending on the water and starch content of the sample, into 50 ml polypropylene screw-cap tubes. Add to each tube 50 mg guar gum powder and five glass balls. Into two extra tubes weigh approximately 50 mg guar gum and add five glass balls. Pipette 20 ml glucose standard into each and shake well to disperse the gum. A blank tube containing glass balls, guar gum and 20 ml acetate buffer is required to correct for glucose present in the amyloglucosidase solution. Treat these tubes exactly as the samples after the addition of acetate buffer.

**Measurement of RDS and SDS.** First prepare enzyme solution 1. Pipette 20 ml 0.1 M acetate buffer into each sample tube and mix thoroughly to disperse the guar gum. Equilibrate the samples, standards and blank at 37°C in a water-bath. Taking the tubes one at a time, immediately add 5 ml enzyme solution 1, timing the analysis carefully from this point. (It is best to add enzyme to the samples at 1 min intervals to allow accurate timing later on.) Cap the tube and immerse horizontally in the shaking water-bath at 37 ± 0.5°C, securing firmly. The water-bath should be adjusted to a stroke speed of 160 strokes per min and a stroke length of 35 mm to ensure a crushing action of the balls, which is essential to the analysis.

After 20 min, remove 0.5 ml of the hydrolysate into a labelled tube containing 20 ml 65% ethanol and mix well. (The shaking action of the water-bath should preferably not be stopped during the sampling period.) Immediately replace the sample tube in the shaking water-bath. After a further 100 min, remove a second 0.5 ml sample in the same way, but this time do not replace the tube in the water-bath. The portion taken after 20 min is designated G20 and that taken after 120 min, G120. (At this stage continue with the main sample and return to the G20 and G120 portions when convenient.) Centrifuge the G20 and G120 portions for 1-2 min to obtain a clear supernatant. Measure the glucose in these portions as described, using the following values: V = 35.7 plus 1 ml per gram wet weight of sample used, C = 20 and D = 1.

**Measurement of TG.** Having removed the tubes from the shaking water-bath, vortex-mix or shake vigorously to break up any large particles. Place tubes in a boiling water-bath for 30 min. Vortex mix or shake again. Cool the tubes in ice-water for 10-20 min.

Add 10 ml 7 M KOH and mix well. Immerse tubes horizontally in the shaking water-bath containing ice-water (0°C), securing firmly. Incubate, with shaking, for 30 min. Prepare in advance a second set of 50 ml centrifuge tubes and into each pipette 10 ml 0.5 M acetic acid. Remove the sample tubes from the ice-water and immediately take 1 ml of the contents into the acetic acid. Mix well. Add 0.2 ml diluted amyloglucosidase (50 AGU/ml), mix and incubate for 30 min at 70°C. Transfer the tubes to a boiling water-bath for 10 min. Cool to room temperature then dilute with 40 ml water and centrifuge at 1500 g for 5 min to remove the precipitate. Measure the total glucose as
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Analysis of starch fractions

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5 min to ensure a
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well. (The shak-
should preferably
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and that taken after
stage continue with
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and G120 portions for
clear supernatant.
These portions as
allowing values: V4 =
et weight of sample

Having removed the
water-bath, vortex-
to break up any
ubes in a boiling
rrest mix or shake
as in ice-water for

30 and mix well.
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g ice-water (0°C),
re, with shaking, for
ance a second set of
and into each pipette.
Remove the sample
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nto the acetic acid-
diluted amylase-
ix and incubate for
fer the tubes to a
0 min. Cool to room
ite with 40 ml water
for 5 min to remove
re the total glucose as
described, using the following values: V4 =
35.4 plus 1 ml per gram wet weight of
sample used, C = 14.2 and D = 1. (If no
G20 portion is taken, V4 = 35.2, C = 14.20
and D = 1).

Calculation of RDS, SDS and RS. The
values obtained for G20, G120, FG and TG
are combined to obtain values for TS, RDS,
SDS and RS.

\[ TS = (TG - FG) \times 0.9, \]
\[ RDS = (G20 - FG) \times 0.9, \]
\[ SDS = (G120 - G20) \times 0.9, \]
\[ RS = TS - (RDS + SDS) \]
or
\[ (TG - G120) \times 0.9. \]

Separate measurement of RS1, RS2 and RS3

Measurement of RS1. This measurement is
appropriate for foods having a dense or rigid
particulate structure, or for foods containing
such structures, e.g. rice, pasta, sweetcorn,
legumes, softgrain bread.

Two portions, (a) and (b), of the same
sample are required. Portion (a) should be
passed once through the mincer; portion (b)
should be finely divided by milling (0.2 mm)
or homogenizing, and accurate dry matter
determinations should be made for both.
Measure G120 for each portion as described
for the determination of RDS and SDS. RS1
is calculated as the difference between the
glucose released from the minced sample
and that released from the milled sample
after 120 min incubation with pancreatin
and amylglucosidase:

\[ RS1 = (G120(b) - G120(a)) \times 0.9. \]

Measurement of RS2. This method is
appropriate for foods that are eaten raw or
that have been cooked with very little water
and might have retained some granular
structure, e.g. muesli, bananas, and biscuits
made with banana or potato flours.

Two portions, (c) and (d), of the same
sample are required. Both portions should
be finely divided (but should not be
subjected to bulk milling). Weigh portions
(c) and (d) as described for RDS and SDS,
including addition of guar gum and glass
bells. To portion (c) add 20 ml acetate
buffer, mix and place in a boiling water-bath
for 30 min, inverting occasionally. Cool
portion (c) to 37°C. To portion (d) add 20 ml
acetal buffer, mix and equilibrate at 37°C.
Measure G120 in these samples as described
previously. RS2 is calculated as the differ-
ence between the glucose released from
the boiled sample and that released from the
unboiled sample after 120 min incubation
with pancreatin and amylglucosidase:

\[ RS2 = (G120(c) - G120(d)) \times 0.9. \]

Measurement of RS3. Most cooked foods
contain a small amount of retrograded
amylose. Food most likely to contain larger
than average amounts of RS3 are those
cooked by moist heat, particularly at ele-
vated temperatures such as autoclaving, and
those heated more than once.

Samples should be finely divided by
milling or homogenizing. Weigh, to the
nearest mg, between 300 and 1000 mg
depending on the water content of the
sample (to give not more than 300 mg dry
matter) into a 30 ml screw-cap centrifuge
tube, and add a magnetic stirring bar.
Suspend the sample in 10 ml acetate buffer
and place the tube in a boiling water bath
for 1 h.

Prepare enzyme solution 2: weigh 1.0 g
pancreatin into a centrifuge tube and
suspend in 12 ml water. Add a magnetic
stirring bar and mix for 10 min. Centrifuge
(1500 g, 10 min) and take 10 ml of the
supernatant into a flask with 0.2 ml Promo-
yzeme and 1.8 ml water. Mix well. Cool the
sample tubes to 40°C. Add 0.6 ml enzyme
solution 2 and vortex-mix. Incubate over-
night at 40°C mixing continuously or vortex-
mix at 1 h after commencement of the
incubation.

Add 40 ml absolute ethanol and mix well
by repeated inversion. Centrifuge at 1500 g
to obtain a clear supernatant (5-10 min).
Remove by aspiration as much of the
supernatant liquid as possible without dis-
turbing the residue, and discard it. Add
approximately 5 ml 85% ethanol, mix to
suspend the sample, then make to 50 ml
with 85% ethanol. Mix by inversion and use
the magnetic stirrer to form a suspension of
the residue. Centrifuge and remove the
supernatant as before. Repeat this stage using absolute ethanol. Add 20 ml acetone to the residue and vortex mix, then use the magnetic stirrer to form a suspension. Centrifuge and remove the supernatant liquid as before. Place the tube in a beaker of water at 70–80°C on the hot-plate stirrer and mix the residue until dry. (It is essential that the residue and tube are completely free of acetone.) Use a fume cupboard or hood to remove the acetone vapour. If aggregation occurs during drying, disperse the sample using the vortex mixer. This is best done before the sample is completely dry.

Suspend the residue in 2 ml water and add 2 ml 4 M KOH. Stir magnetically for 30 min. At this stage prepare a blank tube containing water and 4 M KOH but no sample. Treat this tube in the same way as the sample tubes. Neutralize the suspension by adding 1.2 ml glacial acetic acid and 10 ml water. Mix and immediately add 0.2 ml diluted amyloglucosidase (140 AGU/ml). Cap the tube, mix by inversion, and incubate at 60°C for 60 min.

Cool the tubes to room temperature, add 3 ml 4 M KOH, and centrifuge at 1500 g for 5 min. Use the hydrolysate without dilution for the measurement of glucose by glucose oxidase as described. (Samples containing abnormally high levels of RS may require a five- or tenfold dilution.) Measure the absorbance against the enzyme blank and calculate the percentage RS directly from the equation using an external standard and multiplying by 0.9 to convert the value for monosaccharides to a value for polysaccharide ($V_r = 18.4$).

Results and discussion

Disruption of particulate structures

To measure total starch in a food by enzymic hydrolysis within a reasonable time there must be complete disruption of all particulate structures within which starch may be trapped. This would normally mean using a finely milled or homogenized sample. On the other hand, the rate and extent to which the starch in a food is digested in vivo is influenced by the particulate structures present in the food when eaten. Thus to measure the rate and extent of digestion in vitro demands that a sample should be analysed as eaten, without extensive milling or other preparative treatment. These two determinations of starch therefore have quite different objectives and would normally require different pre-treatment and analytical techniques. However, the aim of the present method is, where possible, to satisfy both objectives whilst starting with the same portion of sample, so that relevant comparisons between the measurements may be made.

For foods with a well-defined particulate structure, the rate and extent of starch digestion are critically dependent on the way the food sample is divided. This is the case both in vitro and in vivo. Chewing is a highly individual and variable method of dividing food. The extent to which a food is chewed will depend on its texture and on such factors as dental health, the degree of hunger of the consumer, the presence of other foods, and individual chewing habits. It is neither possible nor is it necessary to imitate chewing too closely when preparing foods for analysis in vitro. The method chosen should reflect the average division of the food achieved by chewing, whilst exhibiting a greater degree of reproducibility.

In an experiment designed to test the applicability of mincing as a means of dividing particulate foods, a range of products was either minced as described in the Methods section, or chewed by volunteers. The volunteers were asked to chew the food according to their usual habit, but to spit it out at the point where they would normally have swallowed it. Samples of chewed food were analysed immediately to minimize the effect of salivary amylase. A value for O$_{120}$ was obtained for each sample using the methodology described here, but without the addition of guar gum or glass balls, and this was compared with a value for total starch obtained from a milled or homogenized sample of the food. The difference between O$_{120}$ and TS was taken as the amount of starch resisting hydrolysis and expressed as a percentage of the dry matter (Table 3).

Both chewing and mincing resulted in a
Table 3. Starch resisting hydrolysis by pancreatic and amylglucosidase following mincing or chewing

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pretreatment</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long grain brown rice</td>
<td>Minced</td>
<td>12</td>
<td>20.8</td>
<td>0.8</td>
<td>19.5-22.2</td>
</tr>
<tr>
<td></td>
<td>Chewed</td>
<td>12</td>
<td>21.5</td>
<td>5.2</td>
<td>17.7-20.2</td>
</tr>
<tr>
<td>Parboiled rice</td>
<td>Minced</td>
<td>12</td>
<td>15.0</td>
<td>1.6</td>
<td>13.5-16.0</td>
</tr>
<tr>
<td></td>
<td>Chewed</td>
<td>12</td>
<td>12.3</td>
<td>1.9</td>
<td>8.8-15.1</td>
</tr>
<tr>
<td>White spaghetti</td>
<td>Minced</td>
<td>11</td>
<td>5.7</td>
<td>0.9</td>
<td>4.5-7.1</td>
</tr>
<tr>
<td></td>
<td>Chewed</td>
<td>10</td>
<td>5.4</td>
<td>3.7</td>
<td>0.8-13.2</td>
</tr>
<tr>
<td>Sweetcorn</td>
<td>Minced</td>
<td>12</td>
<td>14.0</td>
<td>1.9</td>
<td>9.7-17.7</td>
</tr>
<tr>
<td></td>
<td>Chewed</td>
<td>14</td>
<td>10.2</td>
<td>3.7</td>
<td>5.4-14.9</td>
</tr>
</tbody>
</table>

range of values for starch resisting hydrolysis, but mincing gave a smaller range and standard deviation in each case (Table 3). The mean values obtained by mincing were similar to the mean values obtained by chewing, provided that the food samples were minced in an appropriate way. For example, soft, starchy samples like spaghetti, once cooled, tended to become sticky and resist passage through the mincer so that they were extruded rather than chopped. Best results were achieved by mincing the sample immediately after cooking because this preserved the more authentic chopping action of the mincer.

Whilst in this test mincing mimicked chewing in a satisfactory way, the results of investigations carried out in this laboratory, into the digestion of rice by human ileostomates indicated that the amount of starch in this type of food resisting digestion after 2 h incubation in vitro far exceeded that resisting digestion in the small intestine. For example, using our method, 11-12% of the starch in polished rice was measured as RS, whereas only 1-3% of the starch from polished rice fed to ileostomy subjects was recovered in the effluent. This suggested that further degradation of particles was taking place after swallowing, and that this was not being accounted for in the method in vitro. It was considered that further reduction of the particle size before hydrolysis would be inappropriate, leading to falsely elevated values for RDS. Thus, a method of disrupting particles during incubation was sought. The introduction of glass balls into the sample tube during incubation in the shaking water-bath provided a gentle milling action, the degree of which could be adjusted using the speed and stroke-length controls on the bath. "Ball milling" in situ under the conditions given in the Methods section reduced the amount of starch resisting hydrolysis due to particulate structure.

However, in an experiment with raw potato starch, which is normally highly resistant to enzymic hydrolysis, the inclusion of glass balls in the sample tube led to a fourfold increase in the amount of glucose released from the sample over a 2 h incubation period. The increase was found to be dependent on the stroke speed of the shaking water-bath. Much smaller differences were seen with freeze-dried green banana flour, which also contains starch granules of high resistance. This was thought to be due to the presence of other polysaccharides that increased the viscosity, causing suspension and therefore reduced milling of the starch granules during shaking. It was considered that the disruption of potato starch granules might be reduced by increasing the viscosity artificially. The addition of 50 mg guar gum to increase the viscosity of the sample mixture markedly reduced the hydrolysis of the potato starch granules but had little effect on the disruption of particles in other foods (Table 4), and was found not to interfere in any other aspects of the method.
Table 1. Effect of increased viscosity due to the addition of gaur gum, on the hydrolysis of starchy foods

<table>
<thead>
<tr>
<th>Sample</th>
<th>% glucose released after 2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without gaur</td>
</tr>
<tr>
<td>Potato starch</td>
<td>35.1</td>
</tr>
<tr>
<td>Potato flakes</td>
<td>51.1</td>
</tr>
<tr>
<td>Banana flour</td>
<td>28.4</td>
</tr>
<tr>
<td>Banana biscuit</td>
<td>31.1</td>
</tr>
<tr>
<td>Boiled rice</td>
<td>84.1</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>78.8</td>
</tr>
</tbody>
</table>

For the determination of total glucose, glass balls facilitate the break-up of material within the sample tube following boiling and/or enzyme treatment. Vigorous shaking and vortex mixing of a tube containing glass balls provides an effective means of degrading large particles, and in most cases is sufficient to allow access of enzymes to all starch. For a few foods, for instance some legumes, the milling action of the glass balls may not be adequate for the dispersion of individual cells with strong walls, inside which starch may be trapped. However, in most cases the solubilizing action of the subsequent treatment with 2 M potassium hydroxide, coupled with shaking, is enough to release any unhydrolysed starch before the final incubation with amyloligosidase. Only in a few special cases of very hard particles or strong cellular structures, e.g. whole wheat grains and some legumes, is the treatment inadequate. In such cases, it is necessary to determine total starch using a finely milled or homogenized portion of the sample. For the separate determination of RS1, RS2 and RS3, the availability of a finely milled or homogenized sample is essential.

Gelatinization of granular starch
Gelatinization of starch granules is important in the determination of total starch, and in the methods for the separate measurement of RS2 and RS3, because of the resistance of certain granular starches to enzyme attack. In the present procedure, gelatinization of starch granules is adequate following 30 min boiling. Experimentation with white wheat flour, banana flour, potato flour and three legumes flours has shown that increasing the time of boiling from 30 min to 1 h does not increase the release of glucose from these samples during enzyme incubation. The gelatinization of granular starches inevitably causes an increase in the viscosity of the sample solution. This cannot be avoided during the determination of free glucose and may make sampling slightly difficult. However, the addition of Termaanyl to the sample solution following the removal of the free glucose portion quickly reduces this viscosity by dextrinizing the starch, and makes subsequent sampling easier.

Solubilization of retrograded amylose
Treatment with 2 M KOH has been shown to solubilize retrograded amylose (Englyst et al., 1982). However, strong alkali can lead to rapid destruction of free sugars, particularly at elevated temperatures. In the present method dispersion of retrograded amylose takes place in the presence of high concentrations of free glucose, maltose and dextrins as a result of enzymic hydrolysis during earlier stages of the procedure. To minimize the destruction of free glucose, the treatment with KOH is done at 0°C. When standard glucose solutions were shaken for 30 min at 0°C with either 2 M KOH or an equivalent volume of water, identical results were obtained by the two treatments, indicating that these conditions do not result in the destruction of glucose.

The length of treatment with KOH is not critical when the sample is finely milled or homogenized. In an experiment with a highly retrograded but finely milled maize starch, no benefit was obtained by extending the treatment time from 15 to 30 min. Thus, 15 min incubation is sufficient to solubilize retrograded amylose during the rapid method for measuring total starch. However, when the sample used is not finely milled, the treatment time is extended to 30 min to allow greater disruption of cellular structure, and hence more effective dispersion of starch.

Enzymic hydrolysis of starch
Using the method of Dahlqvist (1962), the amylase activity of enzyme solution 1 was shown to be approximately 7000 U/ml at 25°C, equivalent to a final amylase activity...
of 1400 U/ml in the reaction medium. At this concentration, the measurement of TS, RDS, SDS and RS is reproducible over a range of sample weights from 700 to 900 mg, as shown for white flour in Table 5. Reducing the amylase activity by a factor of 2 or 4 does not affect the determination of total starch, but influences the rate of starch digestion, so that the percentage of RDS decreases and that of RS increases with increasing sample weight (Table 5).

In this system, the rate of starch hydrolysis is determined by the amylase activity and is not significantly influenced by the presence of amylglucosidase (13 AGU/ml in enzyme solution 1), which degrades the α-limit dextrins left by amylase to glucose. No significant difference in the glucose released from a rapidly digestible food (corn flakes) or a slowly digestible product (raw potato starch) after 20 or 120 min was observed when the amylglucosidase activity of the enzyme solution was increased to 20 or decreased to 9 AGU/ml. In the absence of pancreatein, raw potato starch was poorly hydrolysed by AMG, but the rate of hydrolysis of corn flakes was inhibited only slightly.

The choice of 20 min to divide RDS from SDS is based on the observed rates of hydrolysis of starchly foods in vitro (Fig. 1).

![Fig. 1. Starch hydrolysis curves for seven foods, showing that starches from different sources or in different food forms are digested at variable rates.](image)

For rapidly digested foods such as white bread and cooked, homogenized potato, more than 89% of the starch present is converted to glucose within 10 min. Subsequently, the rate of hydrolysis declines so that only a further 5–10% of the starch is

### Table 5. Influence of sample weight and amylase activity on the measurement of total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in white wheat flour

<table>
<thead>
<tr>
<th>Amylase activity (U/ml)</th>
<th>Sample weight (mg)</th>
<th>% of dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TS</td>
</tr>
<tr>
<td>1750</td>
<td>300</td>
<td>72.7</td>
</tr>
<tr>
<td>1750</td>
<td>500</td>
<td>72.7</td>
</tr>
<tr>
<td>1750</td>
<td>700</td>
<td>72.6</td>
</tr>
<tr>
<td>1750</td>
<td>900</td>
<td>72.0</td>
</tr>
<tr>
<td>1750</td>
<td>1100</td>
<td>72.7</td>
</tr>
<tr>
<td>3500</td>
<td>300</td>
<td>71.0</td>
</tr>
<tr>
<td>3500</td>
<td>500</td>
<td>71.1</td>
</tr>
<tr>
<td>3500</td>
<td>700</td>
<td>71.7</td>
</tr>
<tr>
<td>3500</td>
<td>900</td>
<td>71.8</td>
</tr>
<tr>
<td>3500</td>
<td>1100</td>
<td>71.6</td>
</tr>
<tr>
<td>7000</td>
<td>300</td>
<td>72.0</td>
</tr>
<tr>
<td>7000</td>
<td>500</td>
<td>72.3</td>
</tr>
<tr>
<td>7000</td>
<td>700</td>
<td>72.4</td>
</tr>
<tr>
<td>7000</td>
<td>900</td>
<td>72.5</td>
</tr>
<tr>
<td>7000</td>
<td>1100</td>
<td>73.1</td>
</tr>
</tbody>
</table>
converted to glucose in the next 10 min. Complete hydrolysis of the starch is achieved within approximately 60 min. The RDS category includes all starch hydrolysed within 20 min and therefore incorporates the major part of the starch in the easily digestible white bread and potato, a substantial part of the starch in haricot beans, white flour and spaghetti, but only a small proportion of the highly resistant starch in raw potato and banana flours.

The 120 min cut-off point between SDS and RS is based on the results of a series of studies in vivo and in vitro. This length of incubation was found to be adequate to obtain an extent of starch digestion similar to that observed in ileostomy subjects (see Table 6). Figure 1 shows that raw white wheat flour is digested slowly but completely within approximately 120 min using our in vivo technique. White spaghetti reaches a plateau at this time but is not completely hydrolysed, indicating the presence of a small amount of the highly resistant retrograded amylose (RS). Raw potato starch, banana flour and haricot beans continue to be digested after 120 min, indicating the presence of RS fractions 1 and 2. The shape of the curve suggests that haricot beans contain some RS.

Englyst et al., (1982) have demonstrated that the pancreatic used in this method is free from contamination with NSP-degrading enzymes. The amylolucosidase used is also without significant activity towards NSP, but does have a significant invertase activity. To overcome this problem, and the possible problem of natural invertase activity being present in some foods, invertase was incorporated into the procedure to convert all sucrose present into its constituent monomers. In this way, an accurate correction for free glucose could be made. The invertase used did not show any amylase activity when incubated with wheat flour, wheat starch or maize starch. None of the enzymes used was able to hydrolyse inulin.

**Colorimetric determination of glucose**

During the initial stages of development of this method, end-point glucose analysis was carried out by gas-liquid chromatography (GLC) and quantified by comparison with an internal standard (galactose). This allowed direct and accurate determination of glucose without the risk of interference from other monosaccharides. However, the use of GLC for determination of glucose was considered too unwieldy for routine work, so a simple alternative was sought. Using the GOD-PAP kit, glucose in the hydrolysate can be determined rapidly by colorimetry, with no loss of accuracy compared with the GLC method (Table 7). The reagent gives a linear response over the range of glucose concentrations that would normally be encountered in the analysis of starch foods using the procedure described and is not subject to interference from any of the reagents used.

**Comparison with studies in vivo**

Resistant starch values obtained by the present method compare favourably with the results of studies in vivo using ileostomy

Table 6. Recovery of resistant starch from the effluent of ileostomy feed various biscuits.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Recovered (g)</th>
<th>Mean (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat biscuit</td>
<td>0.3</td>
<td>0.1-0.4</td>
<td></td>
</tr>
<tr>
<td>RDS biscuit</td>
<td>8.5</td>
<td>10.1-10.5</td>
<td></td>
</tr>
<tr>
<td>Potato biscuit</td>
<td>11.7</td>
<td>12.5-15.5</td>
<td></td>
</tr>
<tr>
<td>Banana biscuit</td>
<td>13.7</td>
<td>11.1-16.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Comparison of GLC and colorimetry for the measurement of glucose in the determination of starch fractions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fraction</th>
<th>GLC (%)</th>
<th>Colorimetry (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana flour</td>
<td>TS</td>
<td>76.0</td>
<td>75.5</td>
</tr>
<tr>
<td>RDS</td>
<td></td>
<td>5.3</td>
<td>5.1</td>
</tr>
<tr>
<td>SDS</td>
<td></td>
<td>17.1</td>
<td>17.4</td>
</tr>
<tr>
<td>Potato flour</td>
<td>TS</td>
<td>96.8</td>
<td>96.0</td>
</tr>
<tr>
<td>RDS</td>
<td></td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>SDS</td>
<td></td>
<td>5.9</td>
<td>6.2</td>
</tr>
</tbody>
</table>
subjects. In a recent study involving nine ileostomates (H. Englyst, S. Kingman & J. Cummings, unpublished), subjects received biscuits containing from 6 to 15 g of resistant starch (either RS₂ or RS₃) as determined by this method, and the excretion of starch in ileostomy effluent was assessed. For each type of biscuit, the mean recovery of RS was close to 100% (range 91–114%; Table 6), although there was variation between subjects due to extrinsic factors. Further comparisons may be made with the results of previous ileostomy studies (Englyst & Cummings, 1985), which have shown the recoveries of starch from white bread, corn flakes and raw oats to be 1.5, 3.7 and 1.3 g per 100 g, respectively, compared with values obtained in vitro of 1.2, 2.9 and 1.6 g per 100 g for similar products measured by the present method.

Results for foods
Table 8 shows values for RDS, SDS, RS and TS expressed as a percentage of the dry matter for 30 starchy foods. The starch content of the foods analysed ranged from 20% in frozen garden peas to 98–99% for the potato and maize starches, which are virtually free of NSP and protein. Three non-starchy foods, cabbage, red pepper and

Table 8. Digestibility in vitro of some carbohydrate-containing foods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry matter (%)</th>
<th>Types of starch (g/100g dry matter)</th>
<th>Starch digestion index (SDI)</th>
<th>Rapidly available glucose (RAG) (g/100 g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread</td>
<td>54.5</td>
<td>69 7 1 77 90 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wholemeal bread</td>
<td>52.0</td>
<td>56 4 1 60 92 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ready Break</td>
<td>92.4</td>
<td>62 3 1 68 93 64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shredded Wheat</td>
<td>91.3</td>
<td>66 4 1 71 94 68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice Krispies</td>
<td>95.6</td>
<td>74 2 1 78 95 84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn flakes</td>
<td>95.8</td>
<td>73 2 3 78 94 81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porridge oats</td>
<td>90.7</td>
<td>57 6 2 69 88 58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive biscuit</td>
<td>94.2</td>
<td>37 10 1 48 77 47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice bran biscuit</td>
<td>96.0</td>
<td>42 9 1 52 80 54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linseed biscuit</td>
<td>95.6</td>
<td>23 3 7 46 50 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbey Crunch biscuit</td>
<td>96.1</td>
<td>22 13 1 35 61 41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ryvita crispbread</td>
<td>94.3</td>
<td>52 6 3 61 83 56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato biscuit</td>
<td>94.0</td>
<td>23 17 1 55 42 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instant potato</td>
<td>16.7</td>
<td>72 1 1 73 98 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled potato (boil)</td>
<td>22.8</td>
<td>65 5 5 74 87 46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled potato (cold)</td>
<td>23.8</td>
<td>53 11 10 75 71 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato starch (raw)</td>
<td>81.8</td>
<td>6 19 75 90 6 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana flour</td>
<td>59.1</td>
<td>3 15 57 75 4 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-amylase maize starch²</td>
<td>95.2</td>
<td>70 1 17 98 71 74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat RS product²</td>
<td>92.5</td>
<td>55 9 34 98 56 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole wheat flour</td>
<td>89.7</td>
<td>40 39 2 81 49 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti (freshly cooked)</td>
<td>28.3</td>
<td>41 33 5 79 52 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti (cooked)</td>
<td>34.7</td>
<td>33 42 4 78 42 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearl barley (boiled 1 h, cold)</td>
<td>23.3</td>
<td>34 30 9 75 47 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milled (boiled 20 min, cold)</td>
<td>27.1</td>
<td>42 28 6 75 55 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peas (boiled, 5 min)</td>
<td>18.3</td>
<td>12 2 5 40 60 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentils (boiled 30 min, cold)</td>
<td>28.3</td>
<td>24 22 9 54 44 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley (boiled 40 min)</td>
<td>41.4</td>
<td>9 18 18 45 15 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bean flakes</td>
<td>93.6</td>
<td>27 16 6 49 53 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble starch</td>
<td>100.0</td>
<td>100 100 100 100 110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>100.0</td>
<td>- - - 100 110 110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White cabbage (raw)</td>
<td>8.9</td>
<td>- - - - - 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red pepper (raw)</td>
<td>7.0</td>
<td>- - - - - 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple (Granny Smith, raw)</td>
<td>11.8</td>
<td>- - - - - 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Samples from the EURESTA trial, described in text.
RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; TS, total starch.
apple, were analysed for comparative purposes. Separate measurements of resistant starch fractions 1, 2 and 3 were made for ten of the starchy food samples (Table 9). In general, the sum of these RS fractions agreed well with the overall value for total RS measured directly by the main procedure.

The highest levels of RDS were found in breakfast cereals, particularly Rice Krispies and corn flakes (74% and 73%, respectively). Breads and instant or freshly cooked potato also contained substantial amounts (50–69%) of RDS. The lowest values (3–8%) were found for banana flour, raw potato starch and haricot beans.

The amount of SIDS is inversely related to the amount of RDS and was therefore low for breads and breakfast cereals and relatively high for foods such as spaghetti, pearl barley and legumes in which the physical form of the food inhibits the access of pancreatic amylase to the starch.

The products containing the most RS were raw potato starch (75%), banana flour (57%) and a maize RS product, high-amylose maize starch that had been heated and cooled (34%). The large amounts of RS in raw potato starch and banana flour reflect their content of starch granules with the B-type crystal structure, which is highly resistant to pancreatic amylase (RS2 in Table 9). Potato biscuits made from 50% wheat flour and 50% potato starch also contained a substantial amount of RS2, indicating incomplete gelatinization of starch granules during baking. In comparison, the RS in the maize RS product was mainly retrograded amylose (RS3, in Table 9). The small amounts of RS in breads and breakfast cereals are mainly retrograded amylose and partly physically inaccessible starch (RS2). Of the common foods analysed, haricot beans (soaked overnight and boiled 40 min) contained the most RS (18%), which constituted 40% of the total starch, and consisted of all three RS fractions (Table 9).

A measure of the relative rate of starch digestion is given by the starch digestion index (SDI).

\[
SDI = \frac{\text{Rapidly digestible starch}}{\text{Total starch}} \times 100
\]

The highest SDI values (Table 8) were obtained for heat-processed breakfast cereals (93–95) and boiled beans (90–92) in breads and effective gelatinization of the starch in these products. In comparison, the SDI value for raw white wheat flour was only 49. The rate of starch digestion was considerably lower for cold potatoes than for freshly cooked potatoes, the SDI values being 71 and 87, respectively. Depending on the source of starch and the baking conditions used, the digestibility of biscuits was highly variable, with SDI values ranging from 42 (potato biscuits) to 80 (Rich Tea biscuits). The relatively low SDI values for spaghetti, pea the...
pearl barley, millet and the legumes reflect the fact that the physical form of these products renders the starch partly inaccessible to digestive enzymes. In a study with 62 starchy foods, it was shown that the SDI was highly correlated with the glycaemic index (J. Veenstra & H. N. Englyst, unpublished).

During the measurement of RDS and SDS, a value is obtained for rapidly available glucose (RAG) as the glucose measured after 20 min incubation with pancreatin, amyloglucosidase and invertase (G90).

\[\text{RAG} = \text{Free glucose} + \text{glucose from sucrose} + \text{glucose released from starch within 20 min incubation.}\]

The RAG value thus represents the amount of glucose (in g/100g food) that can be expected to be rapidly available for absorption after a meal. Since the RAG value relates to the food as eaten and includes both RDS and free sugar glucose, it should be a better indicator of blood glucose and insulin response than the SDI. Furthermore, unlike the SDI, the RAG value is applicable to fruit and vegetables as well as to starchy foods and mixtures of these. For the 62 starchy foods mentioned above, RAG (expressed as a percentage of total starch + free sugars) showed a slightly stronger correlation with the glycaemic index than did the SDI (J. Veenstra & H. N. Englyst, unpublished). Like the glycaemic index, the SDI reflects the rate of digestion of the starch (or carbohydrates) present in the food, but it does not give any information about the actual amount of glucose likely to be rapidly available from the food.

Because RAG is expressed in units of g/100g on a wet weight basis, it gives an easy-to-use guide to the amount of glucose rapidly available from different foods after consumption. RAG values could be used as a supplement to the glycaemic index and may be particularly useful to diabetic patients and dietitians. The foods in Table 8 show a wide range in their RAG content. For example, the RAG in a 35g portion of corn flakes (28g glucose) corresponds to that in 68g of white bread, 88g of wholemeal bread, 175g of freshly cooked potato, 700g of haricot beans (cooked as described) or 934g of apple.

**Collaborative trial**

Eight laboratories have participated in a collaborative trial of the procedure described here. Participants were supplied with a kit containing reagents in sufficient quantity to perform approximately 50 analyses, and two reference samples. The participants were asked to analyse two pre-trial samples of unknown composition, and to send the results to the MRC Dunn Clinical Nutrition Centre. If these were considered to be satisfactory, the participant was then asked to analyse six unknown test samples, A–F, and to report the results. The laboratories were obliged to report values for total starch and resistant starch for each sample. The measurement of RDS and SDS was optional, and these were not reported by all participants. One laboratory reported results for RS only.

The samples analysed were as follows:

- **Reference 1**: Maize RS preparation made at the Dunn by cooking, cooling and freeze-drying a high-amyllose maize starch;
- **Reference 2**: Potato starch granules;
- **Pre-trial X**: 64 mixture of corn flakes and maize RS;
- **Pre-trial Y**: 64 mixture of white flour and potato starch granules;
- **Sample A**: Bean flakes;
- **Sample B**: Modified high-amyllose maize starch (pre-gelatinized and freeze-dried);
- **Sample C**: Milled corn flakes;
- **Sample D**: Potato starch granules;
- **Sample E**: Banana flour;
- **Sample F**: Potato biscuit, made with 50% potato starch and 50% wheat flour.

Results from two laboratories were not included in the calculation because they failed to send results for the pre-trial samples. The results received from the remaining eight laboratories are reported in full in Table 10. These results were presented at the EURESTA meeting on Crete, May 1991, and results received after this
Table 10. Values for total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) obtained by eight laboratories participating in a collaborative trial of the method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TS</th>
<th>RDS</th>
<th>SDS</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference 1</td>
<td>90.7</td>
<td>51.0</td>
<td>8.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Reference 2</td>
<td>80.7</td>
<td>4.6</td>
<td>13.0</td>
<td>61.0</td>
</tr>
<tr>
<td>Pre-trial X</td>
<td>83.0</td>
<td>61.0</td>
<td>7.7</td>
<td>43.3</td>
</tr>
<tr>
<td>Pre-trial Y</td>
<td>75.4</td>
<td>19.0</td>
<td>32.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Boza flakes</td>
<td>45.8</td>
<td>25.3</td>
<td>14.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Maine RS preparation</td>
<td>93.2</td>
<td>66.2</td>
<td>10.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>74.7</td>
<td>70.0</td>
<td>11.8</td>
<td>19.9</td>
</tr>
<tr>
<td>Potato starch granules</td>
<td>81.7</td>
<td>58.8</td>
<td>17.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Banana flour</td>
<td>74.3</td>
<td>32.4</td>
<td>14.8</td>
<td>56.3</td>
</tr>
<tr>
<td>Potato biscuit</td>
<td>52.5</td>
<td>22.1</td>
<td>15.9</td>
<td>14.1</td>
</tr>
</tbody>
</table>

* Value outside 1.96 standard deviations of the group mean, and not included in subsequent statistics.
Table 11. Preliminary results from the collaborative trial of the method (g/100 g as 100)

<table>
<thead>
<tr>
<th></th>
<th>TS</th>
<th></th>
<th></th>
<th>RDS</th>
<th></th>
<th></th>
<th>SDS</th>
<th></th>
<th></th>
<th>RS</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Reference 1</td>
<td>5-6</td>
<td>88.8</td>
<td>2.2</td>
<td>46.7</td>
<td>9.6</td>
<td>11.4</td>
<td>9.0</td>
<td>30.6</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference 2</td>
<td>5-6</td>
<td>79.6</td>
<td>1.8</td>
<td>4.2</td>
<td>1.3</td>
<td>11.9</td>
<td>5.2</td>
<td>57.7</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prerat X</td>
<td>6-7</td>
<td>81.0</td>
<td>3.4</td>
<td>32.2</td>
<td>4.8</td>
<td>8.1</td>
<td>6.0</td>
<td>13.4</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prerat Y</td>
<td>6-7</td>
<td>73.4</td>
<td>1.9</td>
<td>19.4</td>
<td>0.9</td>
<td>20.3</td>
<td>4.0</td>
<td>22.0</td>
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<tr>
<td>Bean flakes</td>
<td>3-5</td>
<td>45.7</td>
<td>0.6</td>
<td>30.6</td>
<td>6.3</td>
<td>9.8</td>
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<td></td>
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<td>Maize starch</td>
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<td>4.5</td>
<td>57.0</td>
<td>4.3</td>
<td>9.6</td>
<td>1.6</td>
<td>11.5</td>
<td>3.8</td>
<td></td>
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<td></td>
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<tr>
<td>Corn starch</td>
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<td>74.4</td>
<td>2.1</td>
<td>38.3</td>
<td>1.0</td>
<td>1.6</td>
<td>0.5</td>
<td>4.4</td>
<td>2.4</td>
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<tr>
<td>Potato starch</td>
<td>3-1</td>
<td>82.0</td>
<td>0.3</td>
<td>7.0</td>
<td>1.3</td>
<td>18.3</td>
<td>4.2</td>
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<td>3.3</td>
<td></td>
<td></td>
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<tr>
<td>Banana flour</td>
<td>4-6</td>
<td>74.0</td>
<td>1.7</td>
<td>3.8</td>
<td>0.5</td>
<td>16.7</td>
<td>3.7</td>
<td>52.2</td>
<td>3.7</td>
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<tr>
<td>Potato juice</td>
<td>4-6</td>
<td>51.7</td>
<td>1.0</td>
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<td>1.6</td>
<td>18.0</td>
<td>2.6</td>
<td>11.6</td>
<td>1.2</td>
<td></td>
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</tr>
</tbody>
</table>

Date are therefore not included in the trial. Values that fell outside 1.96 standard deviations from the mean are marked by an asterisk and were excluded when preparing the final statistics given in Table 11. For the two pre-trial samples and the six trial samples, the mean reproducibility, \( R = \text{SD} \times 2.8 \) is 5.3 for total starch and 8.2 for RS. Taking into account the small number of participants, we regard this as satisfactory. The trial should be taken as a preliminary study, and plans are in progress for a new trial involving more participants.

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References


