Digestion of raw banana starch in the small intestine of healthy humans: structural features of resistant starch

BY N. FAISANT[1], A. BULÉON[1], P. COLONNA[1], C. MOLIS[2], S. LARTIGUE[2], J. P. GALMICHE[2] AND M. CHAMP[1]*

[1] *INRA, BP 527, Rue de la Géraudière, 44026 Nantes, France*
[2] *Centre de Recherche sur Volontaires, Hôpital Laënnec, 44000 Nantes, France*

The digestion of freeze-dried green banana flour in the upper gut was studied by an intubation technique in six healthy subjects over a 14 h period. Of α-glucans ingested, 83·7% reached the terminal ileum but were almost totally fermented in the colon. Structural study of the resistant fraction showed that a small part of the α-glucans which escaped digestion in the small intestine was composed of oligosaccharides from starch hydrolysis, whereas the rest was insoluble starch in granule form with physical characteristics similar to those of raw banana starch. Passage through the small intestine altered granule structure by increasing susceptibility to further α-amylase hydrolysis. Compared with resistant starch values *in vivo*, those obtained with the *in vitro* methods tested were inadequate to estimate the whole fraction of starch reaching the terminal ileum.

Resistant starch: Banana: Small intestine: Human

It is well established that variable amounts of starch in food can escape digestion in the human small intestine and pass into the colon (McBurney, 1991). This fraction is referred to as resistant starch (RS).

There are several reasons for starch resistance to intestinal digestion, and a classification system has been proposed (Englyst *et al.* 1992). A first category is physically inaccessible starch composed of starch granules or macromolecules 'locked' within plant cells by the cell walls, as in legume seeds (Würsch *et al.* 1986). A second category is uncooked starches in the form of native granules. Depending on their botanical origin, native starch types can be differentiated by X-ray diffraction analysis (Katz, 1930). Type A is characteristic of cereal starches, type B of tubers and high-amylose starches, and type C of legumes. B-type starches are known to be less susceptible to amylolysis. The third RS category is retrograded starch. When starches are cooked in large amounts of water the granules are gelatinized and their constitutive molecules solubilized. Retrogradation occurs during cooling by reassociation of amylose and subsequently of amylopectin macromolecules in crystalline structures not easily susceptible to amylases.

In the case of retrograded starchy products such as potato flakes or bean flakes and retrograded or complexed high-amylose maize starch (Faisant *et al.* 1993*a*), the α-glucan fraction not digested in the upper gut was composed of crystallites of retrograded amylose, starch macromolecules and, to a lesser extent, oligosaccharides in different proportions depending on the foodstuff. However, it has not been determined whether these starch macromolecules are in a semi-crystalline or amorphous state. For resistant native starches no structural study has been done on RS *in vivo*. Although starchy foods are generally eaten after cooking, some native starches may be found in foodstuffs such as fruit (banana,

* For reprints.

chestnut) or biscuits in which starch is not totally gelatinized. Banana was chosen in this study as a model for this category of RS. Banana starch is known to present a low susceptibility to amylases *in vitro* (Duprat *et al.* 1980) and *in vivo*, in both rats (Sugimoto *et al.* 1980) and humans. Englyst & Cummings (1986) showed that up to 78 % of ingested α-glucans from banana escaped digestion in the small intestine of ileostomates. These authors observed that a large fraction (about 80 % of total α-glucans) was recovered as oligosaccharides in ileostomy effluent and that the rest was starch macromolecules. The presence of starch at the end of the ileum may be due to intrinsic resistance of the starch granules, factors related to the structure of the food itself, or physiological variables. Determination of the structural features of this resistant α-glucan fraction in the ileum could help explain why part of the starch ingested is not totally digested in the small intestine.

The purpose of the present study was to use the intubation technique in healthy individuals to determine what happens structurally to raw banana starch in the gastrointestinal tract, as an example of an uncooked or incompletely gelatinized starchy food.

SUBJECTS AND METHODS
Subjects

Six volunteers (three males, three females), 19–27 years of age, took part in the present study. All were in good health and had no history of gastrointestinal disease (except appendicitis), recent treatment with antibiotics, or use of laxatives.

Study design

The study took place over two periods. The first, located in the metabolic ward, involved intubation of volunteers for 2 d (days 1 and 2) to measure the ileal digestibility of banana flour. Subsequently, over an 8-d period, banana flour was ingested daily and stools collected over the last 4 d for total digestibility measurements.

Experimental diets

Raw banana flour was prepared from green bananas from Martinique purchased locally before the gas treatment used to induce ripeness, i.e. when they were totally green and inedible as sweet fruit. They were peeled, cut into small slices, freeze-dried and milled (3 mm sieve).

The evening before ileal effluent collection (day 1), subjects were given a meal free of plant polysaccharides and consisting of eggs, cheese, butter, yoghurt, sugar and water. The experimental meal for ileal effluent collection (day 2) consisted of yoghurt (200 g), ham (100 g), egg yolk (15 g), egg white (30 g), sunflower-seed oil (10 g), sugar (20 g), orange juice (200 ml) and coffee or tea (200 ml). Ham and eggs were ingested first. Banana flour (30 g; dry basis) was incorporated into the yoghurt together with sugar and oil. [111]In was incorporated into the orange juice as a meal marker and the subjects were asked to drink it while eating. Banana flour was the only source of starch and dietary fibre in the meal.

During the period of stool collection for total digestibility measurement, subjects were asked to eat 30 g banana flour/d (10 g at each meal) and to follow strictly a low RS content diet. They were given a list of all forbidden foods. The only source of starch allowed was instant potato flakes (no more than one portion per day) and four slices of French toast daily.

Effluent collection and treatment

On Day 1, each subject was intubated nasally using a triple lumen polyvinyl tube (8 mm external diameter) tipped with an inflated bag containing mercury at the tip to facilitate

passage into the gut. Once the bag reached the caecum (confirmed by fluoroscopy) it was deflated and the subject was required to remain in a semirecumbent position. It took about 6 h for the tube to reach the caecum. One lumen (2 mm internal diameter) was used to sample ileal content 50 mm above the ileocaecal junction, and another (1 mm internal diameter), 250 mm proximal to the aspiration port, for infusion of the recovery marker used to estimate water flow through the distal ileum. The solution infused, consisting of 9 g NaCl/l and 10 g polyethylene glycol (PEG) 4000/l as a flow marker, was maintained at 37° during infusion. On day 2, ileal infusion was begun at 08.00 hours at a rate of 2 ml/min. After 1 h equilibration, subjects were given the experimental breakfast plus 1850 kBq ^{111}In as a meal marker. Intestinal contents were then aspirated for 14 h while subjects continued to fast. Effluents were continuously collected by manual aspiration to obtain as much fluid as possible. Samples were pooled every 30 min. For each sample the pH was determined immediately in a portion subsequently used for ^{111}In and PEG analysis. The rest of the sample was frozen in liquid N_2, stored at $-70°$ and later freeze-dried. The maintenance of good tube position in the ileum was confirmed fluoroscopically at the end of the study.

The volume flowing through the terminal ileum during each 30 min sampling period was determined according to the dilution of the infused PEG marker. It was assumed that the solution was not absorbed in the 250 mm segment between the infusion and aspiration sites. Ileal flow of ^{111}In in the meal was calculated from the amount of ^{111}In aspirated, after correction for recovery of the ileal marker. The amounts of starch in each ileal sample were determined from the calculated ileal volume (without subtraction of the infused volume) and the corresponding starch concentrations. Total ileal content over 14 h was obtained by summation of the results for individual periods.

Stool collection

The first 4 d of the 8 d period were considered as an equilibrium period. Stools were collected from each subject during the other 4 d, weighed and stored immediately at $-20°$. After freeze-drying, faecal outputs during the 4 d collection were pooled for each subject. Stool data are expressed as mean wet 4 d outputs.

Preparation of pooled samples for biochemical studies

For each subject, portions of 30 min ileal samples, calculated to contain 5% of the starch which passed through the ileum during that period as estimated by marker dilution, were weighed and pooled. A total pool composed of the individual fractions collected during 14 h was considered representative of all the starch that reached the ileum during the experimental period. In addition, five partial pools for each subject were obtained by pooling fractions from periods lasting 2 or 2·5 h. Based on the starch recovery curve, these periods were respectively 0–2·5 h, 3–5 h, 6–8·5 h, 9–11 h and 11·5–14 h for the five partial pools.

Preparation of the amylase-resistant fraction from banana flour

Banana flour was subjected to *in vitro* pancreatic α-amylase (*EC* 3.2.1.1) hydrolysis for 16 h according to the modified Berry method (Champ, 1992), with isolation of the resistant residue which was recovered as previously described (Faisant *et al.* 1993*b*).

Preparation of lintnerized banana flour

Banana flour lintner was prepared by lintnerization according to a method previously described (Faisant *et al.* 1993*a*). The lintnerization consisted of the isolation of the crystalline fraction of the starch granules by acid hydrolysis of the amorphous fraction.

Chemical and physical analysis

Marker analysis. A scintillation counter was used to measure ^{111}In activity in each 30 min sample on the day following the experiment. PEG was measured by the turbidimetric method (Hyden, 1955).

Total α-glucan analysis. Total α-glucans in banana flour, ileal effluents and stools were determined by a method adapted from Englyst *et al.* (1992). Water (5 ml) was added to 50 mg sample. After gelatinization at 100° for 30 min, samples were cooled to 0° in ice and 5 ml 4 M-KOH added. After 30 min under mixing at 0°, 1 ml of the mixture was added to 10 ml 0·5 M-acetic acid containing $CaCl_2$ (4 mM) and 46·6 nkat amyloglucosidase (AMG; *EC* 3.2.1.3; 400 AGU, Novo Nordisk Bioindustries, UK) were then added before hydrolysis was performed at 70° for 30 min. After 10 min at 100° and subsequent cooling, samples were neutralized by 0·6 ml 4 M-KOH. Total glucose was determined using the GOD-PAP reagent (Merkotest, cat. no. 14365) after centrifugation (1000 *g*, 5 min).

Oligosaccharide extraction and analysis. Extraction of oligosaccharides (degree of polymerization (DP) 1 to approximately DP 10) with ethanol (800 ml/l) was performed on pooled samples (total and partial pools) as previously described (Faisant *et al.* 1993*a*). Free glucose was directly analysed in the extract by the enzymic NADP-ATP–hexokinase–glucose-6-phosphate dehydrogenase system (Boehringer, cat. no. 127825). Oligosaccharides (including free glucose) were first hydrolysed with amyloglucosidase (Merck, cat. no. 1332; 2217 nkat/mg glucose equivalent) for 90 min at 70° before measurement of total glucose released as above.

'Insoluble' starch. 'Insoluble' starch was defined as total α-glucans after removal of oligosaccharides.

Resistant starch analysis of banana flour. RS was measured by the method of Englyst *et al.* (1992) and the modified Berry method (Champ, 1992). This latter method was slightly modified, on the basis of the Englyst method for total starch analysis (Englyst *et al.* 1992), in order to improve total starch determination of the resistant residue in high RS products. After amylolysis and alcohol washings, 5 ml water were added to the residue. Samples were mixed, incubated for 30 min at 100° and then cooled in ice-water before 5 ml 4 M-KOH were added. After 30 min under magnetic stirring, 1 ml of the mixture was added to 10 ml 0·5 M-acetic acid containing 4 mM-$CaCl_2$. AMG (46·6 nkat) was then added, and the samples were incubated for 30 min at 70°. After neutralization and centrifugation, released glucose was determined using the GOD-PAP reagent.

Size exclusion chromatography on Superose® 12TM, X-ray diffraction analysis and differential scanning calorimetry (DSC) were performed as previously described (Faisant *et al.* 1993*a*).

Studies of raw banana flour and ileal samples were performed by light microscopy after staining with 0·02 M-I_2 + KI. Observations under polarization were performed on samples in water.

Proteins were measured by the Kjeldahl method, with 6·25 as conversion factor. Dietary fibre in banana flour was determined according to the Association of Official Analytical Chemists method (Prosky *et al.* 1988).

Values are expressed on a dry-matter basis, as means with their standard errors ($n = 6$).

Ethical considerations

The six subjects gave written informed constant to the protocol which was approved by the local Ethics Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Région Pays de Loire, Nantes, France).
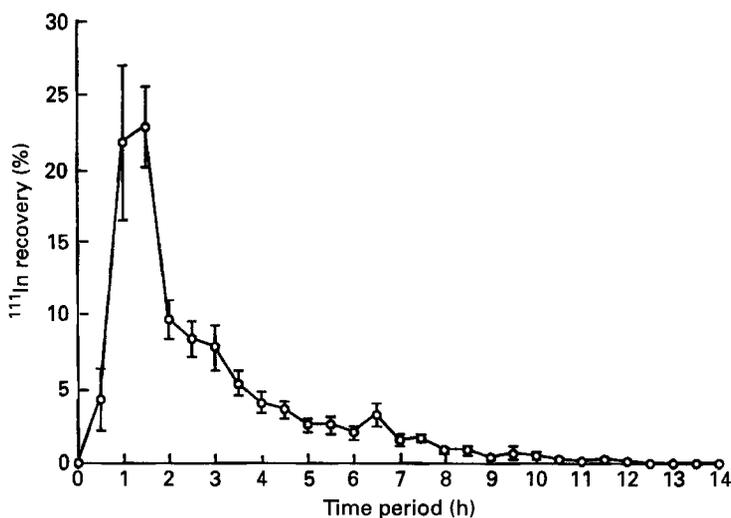
Fig. 1. Time course of the recovery of ^{111}In in the ileal contents of human subjects after ingestion of an experimental meal containing green-banana flour. Values are means for six subjects with their standard errors represented by vertical bars. For details of subjects and procedures, see pp. 112–113.

RESULTS
Composition of banana flour

The banana flour used was composed of (g/kg): protein 38, dietary fibre 92 (corresponding to cell materials) and total α-glucans 770, including oligosaccharides 60 (free glucose 47 plus oligosaccharides of DP 2 to 12 13) and 'insoluble' starch 710. Thus, 30 g banana flour contained 23·1 g α-glucans of which 1·8 g were oligosaccharides (including 1·4 g glucose) and 21·3 g 'insoluble' starch.

According to the method of Englyst *et al.* (1992), RS content was 542 g/kg, i.e. 16·3 g RS for 30 g banana flour. According to the modified Berry method (Champ, 1992) described above, it was 473 g/kg, i.e. 14·2 g RS for 30 g banana flour.

Volume flow in the ileum and marker recovery

Mean water flow through the distal ileum was 2·9 ml/min during the experiment, with an increase to about 3·5 ml/min within 1 h after ingestion of the meal and a rapid decrease 2 h after the meal.

The ^{111}In test-meal marker always appeared in ileal effluents within the first 30 min after the beginning of the meal (Fig. 1), with maximum recovery between 60 and 90 min after the meal. ^{111}In recovery decreased rapidly, and none was detected in ileal contents more than 11 h after the meal. The mean calculated percentage ^{111}In recovery during 14 h aspiration was 106·6 (SE 2·5)% (range 96·9–114·9%) of the amount ingested. Half the total ^{111}In recovery was always obtained within 2 h after the meal.

α-Glucan recovery from the distal ileum

The pattern of α-glucan recovery in the terminal ileum was different from that of ^{111}In (Fig. 2). α-Glucans appeared in ileal effluents within the first 30 min, reaching a maximum 2 h after the meal. Further reduction was progressive until baseline values were reached 13 h after the meal.

The mean amount of α-glucan not absorbed in the small intestine during the 14 h aspiration period was 19·3 (SE 0·7) g (range 16·7–21·5 g). Considering that total ingested α-
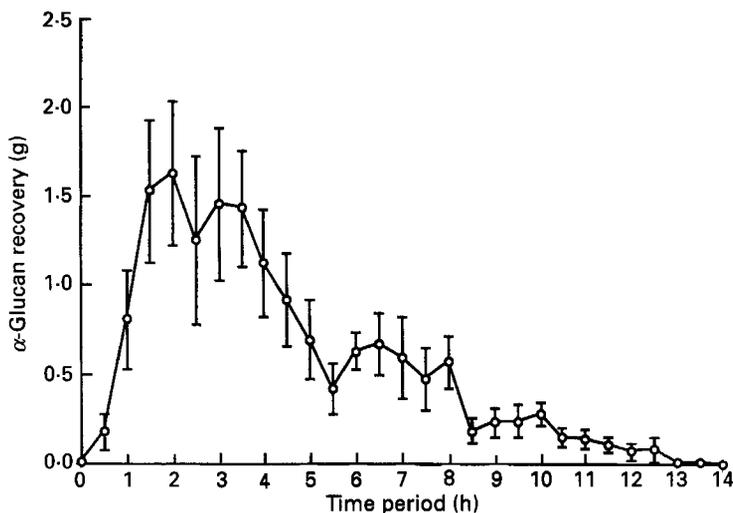
Fig. 2. Time course of the recovery of α-glucans in the ileal contents of human subjects after ingestion of an experimental meal containing green-banana flour. Values are means for six subjects with their standard errors represented by vertical bars. For details of subjects and procedures, see pp. 112–114.

glucans represented 23·1 g, the percentage unabsorbed was 83·7 (SE 3·1)% (range 72·1–93·0%).

Faecal outputs

Mean 4 d wet faecal weight was 497 (SE 85) g (range 254–762 g). Faecal outputs of α-glucans were 1·2 (SE 0·2) g (range 0·8–1·9 g) for a total ingestion of 92·4 g α-glucans from banana flour over 4 d. Thus, α-glucan recovery in faeces was 1·3 (SE 0·2)% (range 0·8–2·0%), i.e. the total digestibility of raw banana starch was 98·7 (SE 0·3)%. When these results are compared with the amount of starch recovered within 14 h from the terminal ileum after a load of 23·1 g α-glucans, it can be assumed that 98·4 (SE 0·4)% of the α-glucans reaching the caecum daily were fermented in the colon.

Composition of α-glucans in ileal effluents

Table 1 shows the mean composition of the total and partial effluent pools. The total α-glucan content of the different pools represented about 200 g/kg dry matter for total pools and pools 1 to 3, and was lower for pools 4 and 5. About 87% of the α-glucan recovered in total pools was 'insoluble' starch and 13% was oligosaccharides. The partial pools were used to study the kinetics of occurrence of the different α-glucan structures in the ileum. The 'insoluble' fraction of starch was about 82% of total α-glucans in pool 1 and about 90% in pools 2 to 5. Oligosaccharides contained about 40% free glucose (from 25% in pool 2 to 59% in pool 1). High interindividual variations were observed and no differences in α-glucan composition were found in the partial pools.

Structural characteristics of banana starch in vivo and in vitro

Microscopic studies of a banana flour suspension and a total pool of the ileal sample are shown in Fig. 3. In banana flour (Fig. 3a), starch granules were ellipsoid or spherical, varying in diameter from 10 to 50 μm. Some seemed to be associated with cell walls. Many granules were observed in the ileal sample (Fig. 3b). However, few showed signs of corrosion by enzymic attack. Under polarized light the Maltese cross feature could be observed clearly in both raw banana flour (Fig. 3c) and the ileal sample (Fig. 3d).

Table 1. *Recovery of α-glucans from raw banana flour in the ileal contents of human subjects**

(Mean values with their standard errors for six subjects)

	α-Glucan content (% dry matter)		Total α-glucans (g)		'Insoluble' starch (g)		Oligosaccharides			
							Dextrins† (g)		Free glucose (g)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fed (g/30 g banana flour)	77·0		23·10		21·30		0·39		1·41	
Recovered										
Total pool (g/14 h)	22·6	1·6	19·31	0·72	16·75	0·66	1·44	0·26	1·12	0·25
Pool 1 (0–2·5 h) (g)	24·2	2·2	6·32	1·00	5·21	0·99	0·44	0·07	0·65	0·37
Pool 2 (3–5·5 h) (g)	25·8	4·1	6·45	1·36	5·83	1·19	0·45	0·14	0·15	0·06
Pool 3 (6–8·5 h) (g)	23·9	4·2	3·66	0·91	3·38	0·88	0·20	0·04	0·07	0·03
Pool 4 (9–11 h) (g)	16·8	2·7	1·86	0·34	1·69	0·29	0·08	0·04	0·11	0·04
Pool 5 (11·5–14 h) (g)	4·3	2·1	0·37	0·15	0·34	0·15	0·02	0·00	0·01	0·00

* For details of subjects and procedures, see pp. 112–114.
† Comprising 2–10 glucose units.
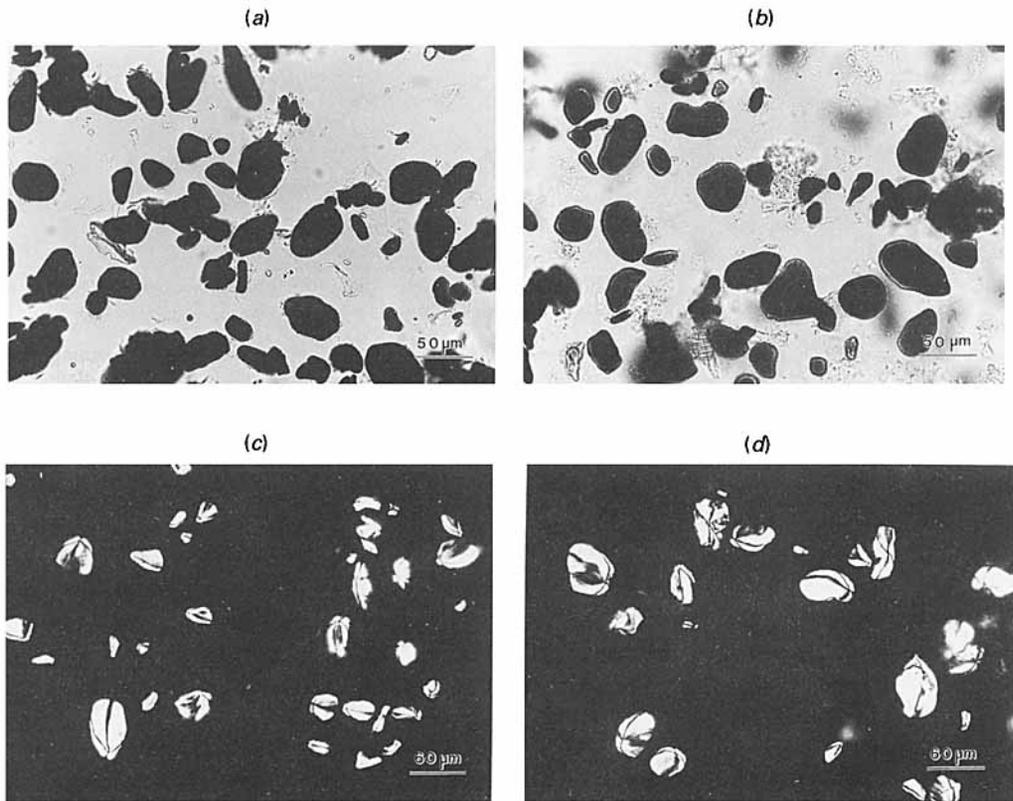
Fig. 3. Photographs of raw green-banana flour (*a* and *c*) and an ileal sample containing undigested banana starch (*b* and *d*) after iodine staining (*a* and *b*), or under polarized light (*c* and *d*).
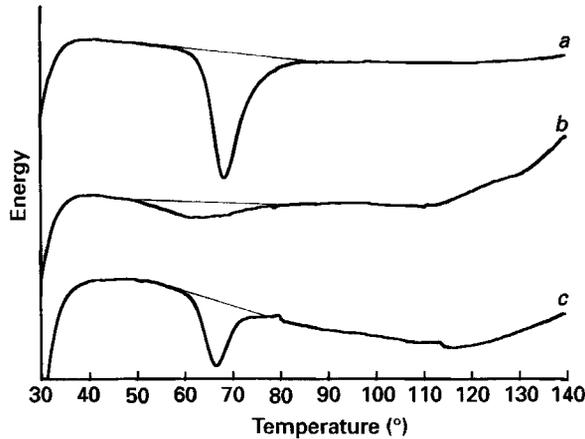
Fig. 4. Differential scanning calorimetry traces for (a) raw green-banana flour, (b) a pooled ileal sample containing undigested banana starch, and (c) a resistant starch residue prepared *in vitro* from green-banana flour.
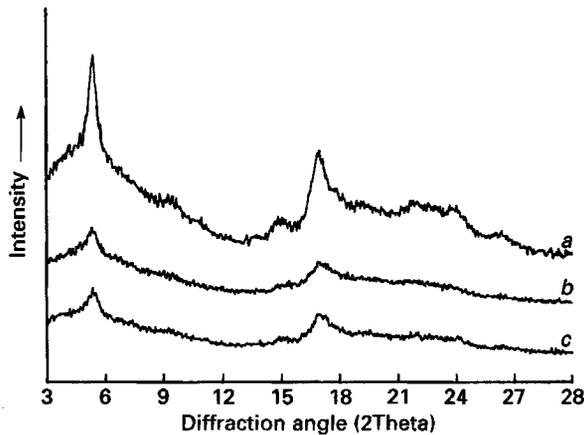

Fig. 5. X-ray diffraction patterns of (a) raw green-banana flour, (b) a pooled ileal sample containing undigested banana starch, and (c) a resistant starch residue prepared *in vitro* from green-banana flour.

Raw banana flour was characterized by a B-type X-ray diffraction pattern (Fig. 5a). The gelatinization temperature of starch granules was 69·4° with an enthalpy of 17·1 mJ/mg 'insoluble' starch (Fig. 4a).

Total pools of ileal contents exhibited a B-type X-ray diffraction pattern similar to that of raw banana flour (Fig. 5b). Endotherm was observed at about 61·1° with an enthalpy of about 11 mJ/mg 'insoluble' starch (Fig. 4b).

The partial pools also exhibited a B-type X-ray diffraction pattern and had the same endotherm as total pools (at about 61°) with an enthalpy of 7 to 13 mJ/mg 'insoluble' starch in pools with sufficient starch content (pools 1–3). In pools 4 and 5, starch content was too low to produce a detectable response in DSC and X-ray diffraction.

Size exclusion chromatography (SEC) on Superose 12TM was performed to assess the chain length of α-glucans in ileal samples. The elution profile of these samples (Fig. 6) showed one peak centred at Kav 0, indicating that all these molecules were excluded from the column. No molecule of intermediate molecular weight was eluted.
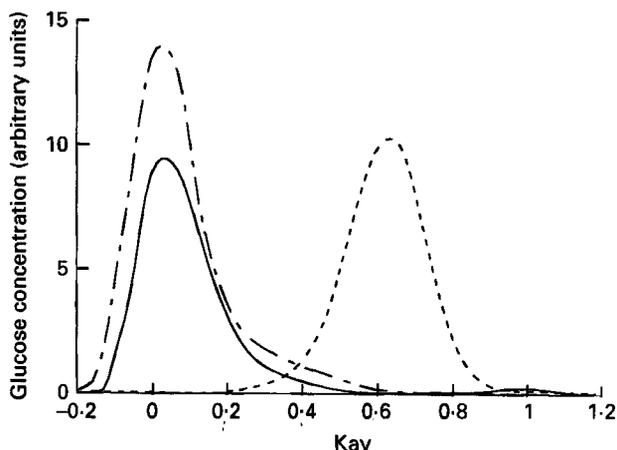
Fig. 6. Elution profiles on Superose 12TM of a pooled ileal sample containing undigested banana starch (——), banana flour lintner (-–-), and a resistant starch residue prepared *in vitro* from green-banana flour (-·—·). Samples were solubilized in 1 M-KOH and diluted 10-fold with water before analysis. Kav is defined as $(V_e - V_o)/(V_t - V_o)$, where V_e is the volume of the eluted molecules, V_o is the volume of the excluded molecules and V_t is the volume of the modules totally eluted by the column (such as glucose).

RS residue obtained *in vitro* from banana was characterized by a B-type pattern (Fig. 5*b*) similar to that of the *in vivo* fraction. An endotherm was observed at 66·5° with an enthalpy of 7·4 mJ/mg 'insoluble' starch (Fig. 4*b*). The SEC of this sample showed only one peak centred at Kav 0, similar to the pattern in *in vivo* samples (Fig. 6).

Banana flour lintner was used in isolating the crystallites constitutive of native starch granules by acid hydrolysis. The elution profile for this product (Fig. 6) showed one peak centred at Kav 0·6, corresponding to structures composed of DP_n molecules of about 16 glucosyl units.

Further RS amylolysis obtained in vivo *and* in vitro

Further hydrolysis with pancreatic α-amylase was performed on total pooled samples and RS residue from banana flour according to the modified Berry method (Champ, 1992). Hydrolysis of the total pools showed that only 16·9 (SE 2·1)% of the total α-glucans contained in ileal samples were resistant, indicating that about 83% were potentially digestible. Compared with ileal samples, only 19% 'insoluble' starch was resistant in these conditions.

In the case of *in vitro* resistant residue of banana flour, only 37·2% of the starch was still resistant to further hydrolysis with α-amylase.

DISCUSSION

Bananas are rarely eaten unripe except in tropical countries. The fate of banana starch in the human gastrointestinal tract depends on the ripeness of the fruit (Englyst & Cummings, 1986). In the present study green bananas were chosen as an example of native RS in a foodstuff. Although the bananas used were inedible directly as fruit, the desert prepared with freeze-dried banana flour, yoghurt and sugar was palatable.

RS has been defined as 'the sum of starch and products of starch hydrolysis not absorbed in the small intestine of healthy individuals' (Asp, 1992). According to that definition all α-glucans recovered in the terminal ileum should be considered as RS. As the term 'starch' could be confusing, fractions composed of both oligosaccharides and polysaccharides are referred to as α-glucans. Although the banana flour in our study contained a small amount

of oligosaccharides (i.e. α-glucans soluble in 800 ml/l ethanol), RS was calculated as the ratio of total α-glucans recovered in the terminal ileum to total α-glucans ingested from banana.

The RS value in the present study (83·7 %) is in the same range as that found *in vivo* by Englyst & Cummings (1986) in ileostomized subjects given unripe bananas (about 72 % when calculated on the same basis). The difference may be due to the degree of banana ripeness in the two studies. The structure of the α-glucans is even more important than the total amount reaching the terminal ileum since it could be indicative of the digestive mechanism involved and predictive of the fate of these polysaccharides in the colon. Table 1 shows that oligosaccharides comprised about 13 % of total α-glucans in the ileum. In a previous study, less than 10 % of oligosaccharides were observed with the intubation technique after ingestion of retrograded and complexed high-amylose maize starches (Faisant *et al.* 1993*a*). Conversely, Englyst & Cummings (1986) found a much larger amount (about 80 % for less ripe banana) of oligosaccharides in ileal effluents collected in ileostomized subjects. This was attributed to bacterial proliferation inside the ileostomy bag. With the intubation technique no bacterial fermentation could have occurred since samples were immediately frozen in liquid N_2. The differences between the ileostomy and intubation techniques emphasize the role of the method used for estimating starch digestion *in vivo* in humans (Stephen *et al.* 1983; Englyst & Cummings, 1986; Flourié *et al.* 1988; Faisant *et al.* 1993*a*). Although simple sugars are generally considered to be well absorbed in the small intestine, the results with banana flour confirm that a substantial fraction can escape digestion because of limited time and/or capacity for hydrolysis and absorption in the ileum (Faisant *et al.* 1993*a*).

Kinetically, the shape of the ileum starch arrival curve was similar to those for retrograded and complexed starches with the intubation technique (Molis *et al.* 1992). [111]In recovery was faster than for starch, mainly due to the fact that ^{111}In was a liquid-phase marker, and travelled faster than the solid phase of the meal. Further research to identify a solid-phase marker that could pass through the gut with the starch fraction is needed. However, ^{111}In is used as a recovery marker to validate each experiment but is not taken into account in the calculation of the flow rate at the end of the ileum. Although only one breakfast meal was consumed during the day, starch was still present in the ileum 12 h afterwards. These conditions can be considered as non-physiological since at least three meals are usually consumed per day. When Flourié *et al.* (1988) performed ileal aspiration for 24 h with three meals, starch levels in the ileum returned to basal levels only after a 12 h overnight fast. Since in normal conditions each meal during the day contains one or more starchy foods, it is evident that a substantial amount of starch or α-glucan is continually arriving in the terminal ileum and entering the colon throughout most of the day. Meal ingestion is known to induce higher small-intestinal motility by providing a net increase in fluid volume in the duodenum, with a first 'rush' of intestinal content (Bernier, 1984). This effect should limit the time for amylolysis by endogenous enzymes and increase RS. Moreover, the amount of unabsorbed starch is directly related to the quantity ingested (Chapman *et al.* 1985). These external factors affecting starch digestion *in vivo* are virtually impossible to study *in vitro*. Indeed, it is quite difficult to determine the time required for *in vitro* hydrolysis. A possible effect on transit time of the presence of the tube within the upper gastrointestinal tract has also been mentioned (Read *et al.* 1983), but its impact on starch digestion is still unknown. Since the solution perfused was isotonic and the flow rate (2 ml/min) was close to physiological, the perfusion should not have influenced the motility in the upper part of the gut. However, an influence of the intubation on the digestion of starch in such a study should not be excluded.

As shown in Table 1 and Fig. 6, α-glucans in the ileum, in addition to oligosaccharides,

were composed of high-molecular-weight molecules. The kinetic study of the structure of starch reaching the ileum showed that there were no significant differences between the partial pools, indicating that the proportions of the different structures were relatively constant over time. Microscopic studies showed that ileal samples contained many starch granules but that the surface of a few of these granules was altered, with pores indicative of enzymic attack. As attested by B-type X-ray diffraction and the Maltese cross feature, the ultrastructure and crystallinity of starch granules after transit through the small intestine appeared to be similar to those of raw banana flour. However, the melting temperature was lower compared with that of initial banana flour (61 v. 69°), suggesting that the structures may have undergone alteration during digestion, as in the case of wheat starch *in vitro* (Colonna *et al.* 1988). The decrease of enthalpy (from 17 to 11 mJ/mg 'insoluble' starch) may also have been due to the presence of other components such as proteins (Eliasson, 1983).

According to SEC analysis (Fig. 6) the molecules obtained by enzymic hydrolysis *in vivo* and *in vitro* were different from lintner, which represented the crystalline fraction of the granules. Therefore, native starches behaved differently from retrograded starchy products in which enzymic hydrolysis isolated crystallites of retrograded amylose, considered to be the most resistant fraction (Faisant *et al.* 1993 a).

Nevertheless, native banana starch appeared to be highly resistant to enzymic hydrolysis. How the resistance of some native starches is conferred remains obscure, but several factors have been proposed: the degree and type of crystallinity (Ring *et al.* 1988) related to amylose content (Behall *et al.* 1989; Cone & Wolters, 1990), and granule size (Franco *et al.* 1992). Morphology and ultrastructure, such as the specific area and porosity of granules (Colonna *et al.* 1992; Gallant *et al.* 1992), should also be considered. In addition, some residual cell walls present in banana flour may have entrapped starch granules, thereby protecting them from enzymic attack.

Further amylase hydrolysis of *in vivo* samples demonstrated that starch granules had undergone structural alterations, rendering them more susceptible to hydrolysis. In fact, the major part of the starch collected in the terminal ileum could have been hydrolysed by amylolytic enzymes. Transit through the small intestine might have improved the accessibility of some structures by changing the porosity and surface area without altering the integrity of the granules. The fate of RS upon entry into the colon depends greatly on its structure. Indeed, oligosaccharides and the easily digestible fraction of RS should ferment more rapidly than the resistant part. In the present experiment, total digestibility studies indicated that most of the starch that reached the colon was metabolized by the microflora, which is consistent with findings in other studies (Christl *et al.* 1993; Englyst & Cummings, 1993; Linnecar *et al.* 1993).

In vivo RS values were higher than *in vitro* determinations, which can be attributed to the potentially digestible starch fraction found in ileal samples but not taken into account in *in vitro* assays (Faisant *et al.* 1993 b). If we consider that the RS isolated *in vitro* was composed solely of 'insoluble' starch, 30 g banana flour would contain 16·3 g RS as determined *in vitro* by the Englyst method (Englyst *et al.* 1992) and 14·2 g by the modified Berry method (Champ, 1992). α-Glucan recovery *in vivo* in ileal samples was 19·3 g including 16·8 g 'insoluble' starch. In these conditions the Englyst method gave the closest value for the resistant 'insoluble' fraction of banana flour. Structural study of this insoluble fraction of *in vitro* RS showed features similar to those of *in vivo* samples. Further amylolysis of this resistant residue confirmed that a part could be rehydrolysed, indicating that, as *in vivo*, the accessibility to part of the granules had been improved.

The present study shows that the solid fraction of *in vivo* RS from banana starch can be accurately estimated by *in vitro* tests. Because of difficulties in mimicking the kinetic process

in the small intestine, the *in vitro* methods did not take into account the soluble fraction of α-glucans reaching the terminal ileum. Indeed, meal transit time and intestinal absorption capacity are contributing factors to the presence of oligosaccharides in the human ileum.

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