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Digestion of Cooked Starches from Different Food Sources by Porcine α-Amylase

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ABSTRACT

The rate, limit and products of porcine α-amylase (EC.3.2.1.1) hydrolysis of cooked starches differ with starch source. The major controlling factors appear to be the amylose:amylopectin ratio and the gel age.

*The limit of hydrolysis of freshly prepared gels after 4 h treatment ranged from 70·5% for wrinkled pea (*Pisum sativum*) Cv. Scout to 92·2% for potato (*Solanum tuberosum*) starches. Ageing the gels before enzyme treatment reduced the limit of hydrolysis of the legume starches but not the cereal starches. The rate of hydrolysis of the α-amylase-susceptible fraction of the starches was unaffected.*

The maltose:maltotriose ratio in the hydrolysis products for the cereals was 1:0·90 whereas that for the legumes ranged from 1:0·84 to 1:0·60. The differing ratios appear to be related to the amylose:amylopectin ratio.

INTRODUCTION

It has been demonstrated that various high starch foods elicit different glycaemic and insulin responses when equivalent amounts of starch are ingested (Crapo *et al.*, 1977; O'Dea *et al.*, 1980; Jenkins *et al.*, 1981). From correlations between starch digestion rates *in vitro* and glycaemic responses *in vivo* it has been suggested that the digestion rate plays a major role in the glycaemic and hence insulin response (O'Dea *et al.*, 1980). Jenkins *et al.* (1981) reported the lowest blood glucose response for legumes and this has been interpreted to mean that the legume starches have slower digestion rates *in vivo* compared with other high starch foods. A classification of food

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starches has been proposed (Englyst & Farlane, 1986) based on their digestibility *in vitro*. This raises the question as to the mechanisms that regulate digestion rate *in vivo*. Factors such as dietary fibre (Snow & O'Dea, 1981), food form (Wong & O'Dea, 1983; Crapo & Henry, 1988), phytic acid (Yoon *et al.*, 1983), barrier effects of cell walls (Wursch *et al.*, 1986; Thorne *et al.*, 1983) and viscosity (Gee & Johnson, 1985) have all been shown to have an effect on the rate of digestion and these factors may be reflected in glycaemic response.

However, it is not possible to elucidate the factors that may play a regulatory role without considering whether there are differences in the food starches *per se* which may cause slower rates of hydrolysis or which may alter the limit of digestion and thereby give a conflicting result since the levels of 'available starch' (starch that will digest if exposed to α-amylase during transit through the small intestine) may not be similar. The reason for this is that cooked starches will retrograde to produce α-amylase-resistant structures both *in vitro* (Kerr, 1950) and *in vivo* (Bjorck *et al.*, 1986). Factors affecting the rate and extent of retrogradation include the source of the starch, amylose:amylopectin ratio, gel concentration, time, temperature, pH and the presence of inorganic salts (Collinson, 1968). Any comparison of the glycaemic response or rates of in-vitro or in-vivo digestion must therefore be made between strictly controlled systems that are truly comparable in terms of the potentially available starch.

This paper attempts to explain some of the effects observed *in vivo* by comparing the rates, limits and products of in-vitro digestion of freshly cooked and retrograded starches isolated from foods.

MATERIALS AND METHODS

All chemicals were AnalaR or HPLC grade except the starches which were isolated from food sources. Starch was isolated from rice (*Oryza sativa*), red kidney beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), red lentils (*Lens culinaris*), mung beans (*Phaseolus aureus*), wheat (*Triticum aestivum*), wrinkled (*Cv. Scout*) and smooth (*Cv. Birte*) seeded peas (*Pisum sativum*) by wet milling, sieving and centrifugation as previously described (Faulks *et al.*, 1989). Potato (*Solanum tuberosum*) starch (BDH, Poole, Dorset) and maize (*Zea mays*) starch (Corn Products Co. (UK) Ltd, Manchester) were commercial samples. The purity of the starches was checked by hydrolysing 200 mg by a modification of the method of Saeman *et al.* (1954) (2 ml 12M H_2SO_4 at 1°C for 18 h, dilute to 1M H_2SO_4 and heating in boiling water bath for 2 h) and found to be in the range 95–98% expressed as starch as measured by a glucose oxidase procedure (Boehringer Cat. No. 124036).

Gelation and digestion of starches

Duplicate 200 mg samples of dry starch were weighed into 50 ml glass screw cap centrifuge tubes and 40 ml of water and a 12 mm magnetic stirrer bar added. The tubes were tightly capped and heated in a boiling water bath for 2 h with constant stirring, quickly cooled to 37°C in a water bath and 10 ml 0·05M phosphate buffer (KH_2PO_4/K_2HPO_4) pH 6·9, containing 100 units of porcine pancreatic α-amylase (EC 3.2.1.1) (Sigma Cat. No. A4268) added immediately. The samples were incubated at 37°C with constant stirring, 5 ml subsamples being withdrawn into 50 ml screw cap glass centrifuge tubes at 15, 30, 60, 120, 180 and 240 min, capped and briefly heated to 100°C in a boiling water bath to inactivate the enzyme. The subsamples were cooled to room temperature, 20 ml of ethanol added, the tubes capped and the insoluble carbohydrate allowed to precipitate at 4°C for 18 h. The tubes were then centrifuged at 2000*g* for 10 min and the supernatants transferred to 50 ml volumetric flasks. The residue was resuspended in 20 ml 80%(v/v) aqueous ethanol, centrifuged as above and the supernatant made to volume with 80% aqueous ethanol, mixed and stored at 1°C until used for analysis. Gels made as described were aged (retrograded) for 24 and 48 h at 1°C, brought to 37°C and treated with α-amylase as above.

Determination of digestion products

Samples were prepared for HPLC analysis by placing 10 ml of the 80% ethanolic extract in a 50 ml round bottomed flask and evaporating to dryness under reduced pressure at 35°C. The dry residue was dissolved in 400 μl of water before being diluted with 1600 μl of acetonitrile. After mixing, the samples were transferred to 2 ml capped vials before being analysed for glucose and its oligomers (G2-G5) by comparison of peak areas with G2-G5 standards in the range 0–500 μg/ml.

HPLC conditions

Samples (100 μl) were injected through a Rheodyne valve fitted with a 100 μl loop (using loop filling) and separated on a carbohydrate column (Millipore-Waters Cat. No. 84038) using 70:30 acetonitrile:water pumped at 2 ml/min (Perkin Elmer 2B HPLC pump). The eluted components were detected by refractive index (Pye Unicam PU 4023) and the data collected and calculations executed by a data station (Pye Unicam PU 4850 chromatographic control centre). The total run time was 12 min.

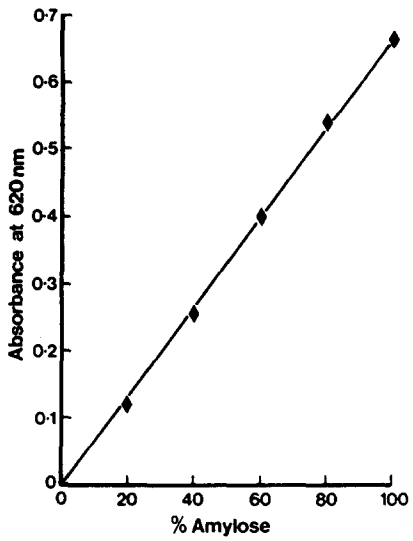

Fig. 1. Standard curve of amylose in amylose: amylopectin mixtures of constant total carbohydrate. Points are means of duplicate determinations.

Amylose:amylopectin ratios

The amylose:amylopectin ratios in the native starches were measured colorimetrically by a method adapted from that of Knutson (1986). Fifty milligram samples of dry starch were weighed into 50 ml screw cap test tubes containing a 12 mm PTFE-coated magnetic stirrer bar; 10 ml of 90% v/v dimethyl sulphoxide/water containing 6×10^{-2}M iodine was added, the tubes tightly capped and heated in a boiling water bath for 1 h with continuous stirring. The solutions were cooled to room temperature and aliquots diluted × 200 with distilled water. After 35 min the absorbances were measured at 620 nm. The amylose content was determined by comparison with a standard curve constructed from mixtures of pure amylose and amylopectin in different ratios between 0–100%. The absorbance response was linear over this whole range of 0–100% amylose (Fig. 1).

RESULTS

Limit of digestion

From the digestion curves, examples of which are given in Fig. 2, it can be seen that different starches had different limits and that little additional starch was hydrolysed after 60 min. However, since the hydrolysis was carried out for up to 240 min (approx. residence time in the human small intestine) and some slight additional hydrolysis occurred between these

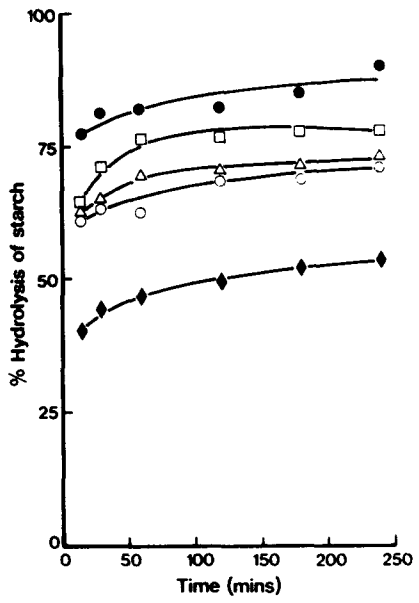

Fig. 2. Percentage hydrolysis of starches as the sum of the oligosaccharides up to maltopentaose. Points are the mean of duplicate determinations. Wrinkled pea ○; smooth pea ●; wheat △; maize □; wrinkled pea aged 48 h ◆.

times, the limit of hydrolysis was taken as the percentage of starch hydrolysed at 240 min (Table 1). For practical purposes this fraction was designated 'available starch', i.e. starch that is potentially digestible. In the freshly prepared gels the available starch ranged from 70% for wrinkled peas to 92% for potato, there being no clear division between the different starch sources. Retrogradation for 24 and 48 h had a marked effect in reducing the limit of hydrolysis in both wrinkled pea and red kidney bean but had no effect on maize or rice starch gels. The source of starch, and, in some cases, the age of the gel, are therefore crucial factors when considering the potentially available starch.

TABLE 1

Sum of the Hydrolysis Products G1-G5 after Treatment with α-Amylase for 240 min, expressed as a Percentage of the Total Starch

Sample	*Gel age (h)*	*% Hydrolysis*	*Sample*	*Gel age (h)*	*% Hydrolysis*
Wrinkled pea	0	70·5	Red kidney bean	0	84·1
	24	58·9		24	70·5
	48	53·4	Smooth pea	0	89·8
Maize	0	76·8	Mung bean	0	80·0
	24	78·4	Red lentil	0	90·4
	48	78·0	Wheat	0	72·1
Rice	0	72·9	Broad bean	0	80·0
	24	76·3	Potato	0	92·2

Rates of digestion

In these dilute gels the system is fully fluid and there is no restriction on the enzyme access to the starch. The rates of digestion of the available starch fraction are given in Table 2. From the data it is clear that 81–94% of the available starch in the fresh gels has undergone hydrolysis to low molecular weight oligosaccharides (< G5) within 15 min and that these figures rise to 91–99% at 120 min. In the case of the four retrograded gels the initial rates of digestion appear to be slower with 57–79% of the available starch being digested at 15 min. Thereafter the rate was slower, with the percentage of available starch hydrolysed reaching a value similar to that for the fresh gels at 120 min.

Amylose:amylopectin ratios

The amylose contents of the starches are shown in Table 3. No relationship was found between the amylose content and the percentage of starch susceptible to hydrolysis at 240 min in the fresh gels. However, retrograded wrinkled pea and red kidney bean starch gels, at 98% at 46% amylose

TABLE 2
Percentage Digestion of Available Starch[a]

Sample	*Gel age (h)*	*Digestion time (min)*				
		15	*30*	*60*	*120*	*180*
Wrinkled pea	0	88	90	88	96	97
	24	67	84	89	94	96
	48	75	82	88	92	96
Maize	0	88	92	99	99	100
	24	70	90	95	97	100
	48	79	88	91	99	95
Red kidney bean	0	92	93	94	94	100
	24	57	73	84	93	97
Rice	0	93	95	99	97	93
	24	60	91	92	95	97
Smooth pea	0	86	90	91	91	95
Mung bean	0	94	94	96	95	100
Red lentil	0	81	85	91	91	99
Wheat	0	92	90	95	97	98
Broad bean	0	88	93	96	98	93
Potato	0	89	90	99	98	97

[a] Percentage digestion calculated on the total starch digested after 240 min.

TABLE 3
Amylose Content and G2:G3 Ratios in the 120 min α-Amylase-Hydrolysates of Different Freshly Cooked Food Starches

Starch	*% Amylose*	*G2:G3*
Cereal		
Rice	26	1:0·90
Wheat	36	1:0·90
Maize	33	1:0·87
Legume		
Broad bean	48	1:0·84
Mung bean	43	1:0·80
Smooth pea	51	1:0·80
Red kidney bean	46	1:0·78
Red lentil	46	1:0·78
Wrinkled pea	98	1:0·60
Tuber		
Potato	30	1:0·80

respectively, did show a lower percentage of available starch than retrograded maize and rice starch gels at 33% and 26%, respectively.

Hydrolysis products

The patterns of hydrolysis products (Fig. 3) fall into two categories, those of the cereals and those of the legumes. The pattern for potatoes is similar to that of the legumes. In all, five products were detected by HPLC, glucose (Gl), maltose (G2), maltotriose (G3), maltotetraose (G4) and maltopentaose (G5). Glucose was present only in small amounts throughout, whereas maltotetraose, present at 10–20% at 15 min, rapidly declined as it was further hydrolysed. Maltopentaose, present at $<2\%$ at 15 min, was absent at 60 min. The sugar present in the greatest amount was maltose, maltotriose being present in smaller amounts. Differences were apparent in the ratio of G2 to G3 between the cereals and legumes (Fig. 3) and this appears to be related to the amylose content. As the percentage of amylose in the starches increased, the proportion of G3 fell. This is what might be expected since a lower proportion of amylose would mean a greater proportion of amylopectin and G3 (as isomaltotriose) might arise from the α-1-6 branch points of amylopectin. Potato starch, however, contained only 30% amylose but had a G2:G3 ratio similar to the legume starches which contained 40–50% amylose. The reason for this is unclear but potato starch was the most thoroughly hydrolysed (92%), possibly indicating a selective hydrolysis in those starch gels that were less well hydrolysed.

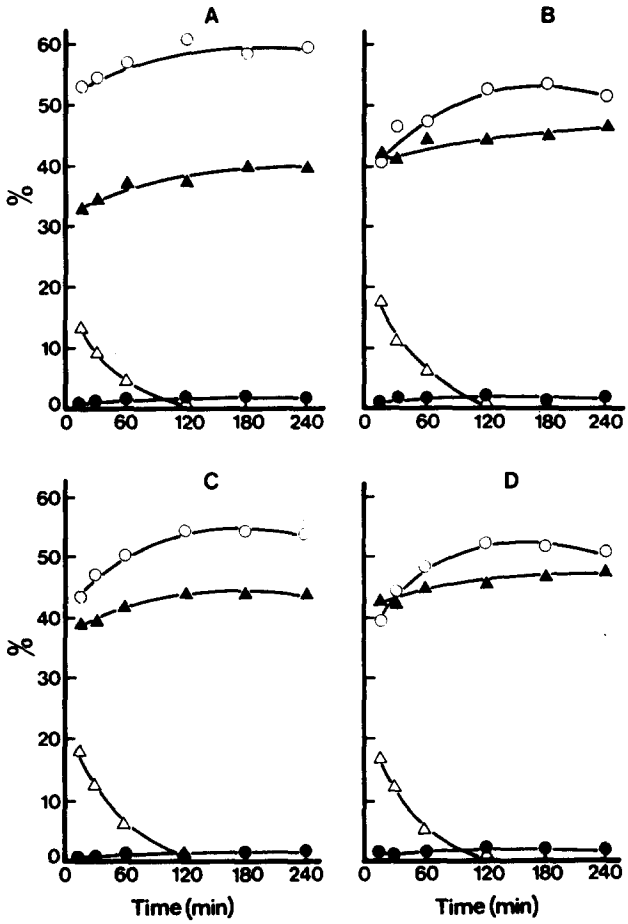

Fig. 3. Percentage composition of hydrolysates for some food starches treated with porcine α-amylose. Glucose ●; maltose ○; maltotriose ▲; maltotetraose △. A, wrinkled pea; B, maize; C, red kidney bean; D, rice.

DISCUSSION

Carbohydrate in 'western' diets is estimated to provide approximately 50% of energy intake (McMichael, 1975) and about half of this comes from starch. Starch is derived from a range of food sources where the composition and structure of the starch granules may be different, e.g. cereals, potato and may be consumed either as native starch, e.g. raw banana, or after varying degrees of heat processing at differing moisture levels, e.g. toasting, boiling. Additionally many cooked starchy foods may be kept for some time, e.g. bread, breakfast cereals, canned and frozen products and some may be reheated before consumption. Starches may also be modified to alter their

functional properties. What we consume as starch is therefore a variable dietary component in water content, composition and structure and is usually part of a complex food matrix. Two recent findings, differing glycaemic responses to the ingestion of starchy foods (Jenkins *et al.*, 1981) and 'resistant starch' (Englyst & MacFarlane, 1986), have resulted in a reassessment of how dietary starches are handled *in vivo* and the physiological consequences. Some studies of the energy value derived from different starches have also been undertaken (Livesey *et al.*, 1989). The commonly perceived wisdom that all dietary starch is digested and absorbed as carbohydrate, since it is predominantly an α-1-4 linked glucan and therefore susceptible to hydrolysis by mammalian α-amylases *in vivo*, has also been questioned (Englyst & MacFarlane, 1986). The variable glycaemic response to starch-containing foods has been ascribed to food form (Brand *et al.*, 1985; Wursch *et al.*, 1986; Crapo *et al.*, 1988) and to the rate of digestion of starch (Jenkins *et al.*, 1981). Because it is not clear which mechanism predominates, milling and blending of both dry and cooked foods have been used to try to eliminate differences in glycaemic response (Wong & O'Dea, 1983; Wursch *et al.*, 1986; Crapo *et al.*, 1988). The endosperm cell walls of the cereals are very thin and fragile so that both dry milling and blending of the cooked food will result in a food matrix where there is virtually no cell wall barrier to amylolytic enzymes. Blending of cooked potato, which has thin parenchyma cell walls, will result in considerable cellular disruption but the majority of the starch will remain inside intact cells. Particle size reduction in cooked potato mainly occurs by cell separation along the middle lamella (Hughes *et al.*, 1975) rather than through cell rupture as in the cereals. Milling of dry legumes will result in a high proportion of cellular disruption, but blending of cooked legumes will, as in the potato, result in a cell suspension with the starch contained within the cells. Furthermore, the cotyledon cell walls of legume seed may be up to ten times thicker than either cereal endosperm or potato parenchyma cell walls so that the blending of cooked legumes is unlikely to result in much cell disruption. In all these foods, however, the concentration of starch in the gel is high (approximately 20%) and as such will be set at normal eating temperature. Enzymic hydrolysis will therefore be restricted to the surface of the gel and, as such, the rate of hydrolysis may be surface area-dependent.

It is clear, therefore, that it is not possible to elucidate the roles of food form and differences in digestion rates of starch unless strictly comparable systems are used. In addition to comparing similar systems it is also important to consider whether the amylolysis limits of the starches are similar rather than depending on an analysis of total starch or food table data and assuming that all the starch will be digested and absorbed as carbohydrates. Although almost complete hydrolysis of starches can be

achieved *in vitro* by prolonged (24 h) exposure to amylase (Ring *et al.*, 1988) or enzyme mixtures (Englyst *et al.*, 1982), *in vivo* the starch is only briefly exposed to amylases in the mouth and for a few hours in the small intestine. The presence of lipases and proteases *in vivo* may serve to enhance the action of pancreatic amylases by hydrolysing other components of the food matrix.

In the freshly prepared dilute fluid systems used here the results showed that there were major differences in the limit of hydrolysis (Table 1) and that retrogradation had a marked effect, reducing the limit of hydrolysis in both wrinkled pea and red kidney bean but having no effect on maize or rice starch gels. In the aged gels, the percentage of amylose in the starch (Table 3) appears to alter the limit of hydrolysis, presumably because it is this fraction that rapidly forms the hydrogen bonded crystallite structures that are resistant to amylolysis (Miles *et al.*, 1984). Reduced digestibility of high amylose maize starches has also been observed in rats (Borchers, 1962). Since legume starches tend to have a higher proportion of amylose than cereals it is possible that the reduced glycaemic index found for this food (Jenkins *et al.*, 1980) may be partially due to retrogradation. It has been suggested that there is limited swelling of legume starches during cooking as a result of limited access of water (Wursch *et al.*, 1986) but experience in this laboratory has shown that partially swollen granules are present in both peas and beans even after prolonged cooking. It would appear more likely that limited water uptake by legume starch granules at 100°C is a property of the starch granule structure and composition rather than lack of water. Foods such as canned legumes may contain considerable amounts of retrograded starches which will not be made susceptible to amylolysis after reheating under normal conditions (Ring *et al.*, 1988). If these starches are not digested and absorbed as carbohydrate they will enter the large intestine where they will be at least partly fermented (Faulks *et al.*, 1989) and may contribute to flatus.

The appearance of glucose in the blood following ingestion of starchy foods will be dependent upon the rate of gastric emptying, the rate of hydrolysis of the starch present in the lumen of the gut, and the rate of absorption and clearance of glucose. It is believed that in normal healthy individuals the rate of secretion of pancreatic α-amylase is not a limiting factor in starch digestion (McMichael, 1975). From the data in this experiment where 0·5 units of α-amylase/mg starch was used (cf. Ring *et al.*, 1988) 81–94% of the starch was hydrolysed to low molecular weight ($<$G5) in the first 15 min in the fresh gels but only 57–79% in the retrograded gels. In these dilute systems there was no restriction on enzyme access to the starch. However, in the retrograded gels, where there are highly ordered structures, initial rates were only slightly lower (hydrolysis of unassociated starch) but continued slowly for some time (hydrolysis of more ordered structures) reaching parity with the fresh gels at 120 min. In retrograded gels the ordered structure would appear to restrict enzyme access so that it can

only work externally, thus reducing the rate. Similarly, in concentrated (set) gels, enzyme activity will be restricted to the surface of gel fragments, especially if the pore size of the gel is such as to restrict or preclude enzyme penetration.

It would seem probable, therefore, that (within gelled starches) there is a hierarchy of structures of differing susceptibility to amylolysis, and that ageing increases the degree of ordering (Miles *et al.*, 1984) and thus reduces digestibility.

If the starch is readily accessible to pancreatic α-amylase then differences in glycaemic index are likely to be as a result of differences in the limit of hydrolysis and it would appear probable that these differences can be increased if the hydrolysis rate is reduced by restricting the enzyme to surface activity as in retrograded, dilute systems or set gels. Following primary hydrolysis of starch in the lumen of the gut, the low molecular weight products move to the brush border by mixing and diffusion. The major products of this initial step are maltose and maltotriose, neither of which can be absorbed directly. They undergo further hydrolysis by brush border-bound enzymes, glucoamylase and α-dextrinase which hydrolyse α-1-4 and α-1-6 links, respectively, the resulting glucose being transported into the cells either by diffusion or by a sodium ion-dependent carrier system. In normal subjects, the ability to transport glucose is not limiting (McMichael, 1985). However, it is possible that there may be a reduction in glycaemic response between subjects or between starches if either glucoamylase or α-dextrinase are limiting. Differences in the hydrolysis product profiles were observed (Fig. 3), there being more G3 from those starches high in amylopectin. Since reduced glycaemic indices are not normally associated with cooked cereal starches it is unlikely that brush border enzymes are a limiting factor in the appearance of glucose in the blood irrespective of the pattern of amylolysis products.

CONCLUSION

These studies indicate that the type of food starch and how it has been treated have a direct bearing on the rate, limit and products of α-amylase digestion. These factors should be considered when assessing the glycaemic index of a food and how food starches are handled *in vivo*.

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