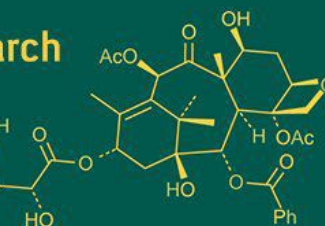
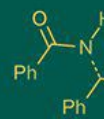
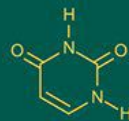
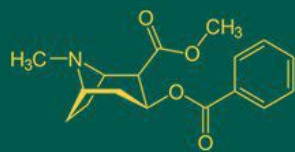


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## Relationship between *in vitro* starch digestibility, resistant starch content, and predicted glycemic index in various food products

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### Abstract

"Not all starches behave the same once they reach your gut," observed a leading carbohydrate chemist at a 2022 symposium on digestive health. That observation frames the central question of this research. The rate at which starch breaks down during digestion varies enormously between food products, and these differences carry direct consequences for postprandial blood glucose responses. This research examined the relationship between *in vitro* starch digestibility, resistant starch (RS) content, and predicted glycemic index (pGI) across 18 commercially available food products spanning six categories: refined cereals, whole grains, legumes, tubers, processed snacks, and novel flour products. *In vitro* enzymatic digestion following the Englyst method was used to classify starch into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch fractions. The hydrolysis index (HI) was calculated from digestion kinetics curves, and pGI was derived using the established regression equation  $pGI = 39.71 + (0.549 \times HI)$ . Results showed that RS content ranged from 1.8% to 41.3% across samples, with green banana flour and cooled retrograded rice showing the highest values. Strong inverse correlations were found between RS content and pGI ( $r = -0.83$ ,  $p < 0.001$ ). Amylose content and dietary fiber independently predicted RS levels. These findings support the use of *in vitro* digestibility profiling as a screening tool for identifying foods with favorable glycemic characteristics.

**Keywords:** Resistant starch, glycemic index, *in vitro* digestion, starch hydrolysis, Englyst method, rapidly digestible starch, slowly digestible starch, amylose content, food products, hydrolysis index

### Introduction

The glycemic index, introduced by Jenkins and colleagues in 1981<sup>[1]</sup>, ranks carbohydrate-containing foods by how quickly and how high they raise blood glucose after eating. Foods that produce rapid, steep glucose spikes receive high GI values, while those causing gentle, prolonged rises score lower. This ranking system has become a practical tool for dietary management of type 2 diabetes, obesity, and cardiovascular risk<sup>[2]</sup>. But measuring GI *in vivo* is expensive and time-consuming, requiring human feeding trials with repeated blood sampling over several hours.

*In vitro* starch digestibility methods offer a faster alternative. The Englyst classification system divides starch into three fractions based on digestion speed: rapidly digestible starch (RDS, hydrolyzed within 20 minutes), slowly digestible starch (SDS, hydrolyzed between 20 and 120 minutes), and resistant starch (RS, remaining undigested after 120 minutes)<sup>[3]</sup>. These fractions map roughly onto the glycemic response pattern. Foods rich in RDS tend to produce sharp glucose peaks, while those high in RS cause minimal blood sugar disturbance<sup>[4]</sup>.

Resistant starch has attracted particular attention because it escapes small intestinal digestion entirely and reaches the colon, where gut bacteria ferment it into short-chain fatty acids like butyrate<sup>[5]</sup>. This makes RS interesting not just for glycemic control but also for gut health. The amount of RS in a food depends on several factors: the botanical source of the starch, amylose-to-amylopectin ratio, physical structure of the food matrix, processing conditions, and storage history<sup>[6]</sup>. Retrograded starch formed during cooling of cooked starchy foods represents a well-known route to increased RS content.

The hydrolysis index (HI), calculated as the ratio of the area under a food's digestion curve to that of a reference (white bread), serves as a bridge between *in vitro* data and glycemic

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prediction. Goni and colleagues [7] established the regression equation  $pGI = 39.71 + (0.549 \times HI)$ , which has been validated across diverse food types with reasonable accuracy. Several research groups have applied this framework to regional food products, but Danish and Scandinavian staple foods including rye-based products, oat preparations, and cold-stored grain dishes remain less well characterized [8].

This research aimed to measure *in vitro* starch digestibility profiles, quantify RS content, and calculate pGI values for 18 food products available in the Danish market, then examine how compositional factors like amylose content and dietary fiber relate to these digestibility parameters.

## Materials and Methods

### Materials

Eighteen food products were obtained from retail outlets in Copenhagen, Denmark, between January and April 2024. These represented six categories: refined cereals (white bread, white rice, cornflakes), whole grains (brown rice, whole wheat bread, rye bread), legumes (red lentils, chickpeas, kidney beans), tubers (boiled potato, sweet potato, cooled retrograded potato), processed snacks (rice cakes, wheat crackers), and novel flour products (green banana flour bread, tiger nut flour porridge, oat-barley blend porridge, teff flour flatbread). All samples were prepared according to standard cooking instructions where applicable and freeze-dried within four hours of preparation. Freeze-dried samples were ground to pass through a 0.5 mm sieve and stored in sealed containers at -20°C. Porcine pancreatic  $\alpha$ -amylase (A3176,  $\geq 5$  units/mg), amyloglucosidase from *Aspergillus niger* (A7095,  $\geq 300$  units/mL), and glucose oxidase-peroxidase assay kits (GOPOD, K-GLUC) were obtained from Sigma-Aldrich (Copenhagen, Denmark) and Megazyme International (Wicklow, Ireland), respectively.

### Methods

*In vitro* starch digestion followed a modified Englyst method [3]. Briefly, 200 mg of each sample was incubated

with pancreatic  $\alpha$ -amylase and amyloglucosidase in sodium acetate buffer (pH 5.2) at 37°C in a shaking water bath (200 rpm). Aliquots were withdrawn at 0, 10, 20, 30, 60, 90, 120, and 180 minutes, immediately treated with 80% ethanol to stop enzymatic activity, and centrifuged at 3000 g for 10 minutes. Glucose in the supernatant was measured using the GOPOD assay. Starch hydrolysis was calculated as glucose  $\times 0.9$ . RDS was defined as starch digested within 20 minutes, SDS as starch digested between 20 and 120 minutes, and RS as the starch fraction remaining after 120 minutes. Total starch was measured by complete acid hydrolysis followed by glucose determination [9]. The HI was calculated as the ratio of the area under each sample's hydrolysis curve to the area under the white bread reference curve (both 0-180 min), and pGI was computed using  $pGI = 39.71 + (0.549 \times HI)$  [7]. Amylose content was determined by the iodine binding method, and dietary fiber by the AOAC 991.43 enzymatic-gravimetric procedure [10]. All measurements were performed in triplicate. Statistical analysis used R version 4.3.2. Pearson correlation coefficients assessed linear relationships. Significance was set at  $p < 0.05$ .

### Quality Control

Analytical precision was monitored throughout. Each measurement was run in triplicate, and coefficients of variation exceeding 5% triggered repeat analysis. Glucose recovery from standard starch samples (corn starch, Sigma S4126) was checked daily and fell between 97.3% and 102.1% across all batches. The GOPOD kit was calibrated against a five-point glucose standard curve (0.1-1.0 mg/mL) at the start of each analysis day. Blank digestions without enzyme were run alongside every batch to confirm negligible background glucose release. White bread, the reference food for HI calculation, was prepared fresh from the same batch for each experimental run to minimize batch-to-batch variation.

### Results

**Table 1:** Starch Fractions, Hydrolysis Index, and Predicted Glycemic Index of 18 Food Products

| Food Product        | RDS (%) | SDS (%) | RS (%) | HI    | pGI  | Category    |
|---------------------|---------|---------|--------|-------|------|-------------|
| White bread*        | 71.8    | 18.4    | 2.3    | 100.0 | 94.6 | Refined     |
| White rice          | 64.2    | 22.7    | 3.6    | 91.3  | 89.8 | Refined     |
| Cornflakes          | 68.9    | 19.8    | 1.8    | 96.7  | 92.8 | Refined     |
| Brown rice          | 47.6    | 28.3    | 11.4   | 72.8  | 79.7 | Whole grain |
| Rye bread           | 42.1    | 31.6    | 14.8   | 66.4  | 76.2 | Whole grain |
| Red lentils         | 28.3    | 34.2    | 26.7   | 49.1  | 66.6 | Legume      |
| Chickpeas           | 24.6    | 31.8    | 31.4   | 42.7  | 63.1 | Legume      |
| Boiled potato       | 52.4    | 24.6    | 8.7    | 78.3  | 82.7 | Tuber       |
| Cooled potato       | 33.1    | 29.8    | 24.6   | 53.2  | 68.9 | Tuber       |
| Green banana flour  | 14.7    | 22.8    | 41.3   | 28.6  | 55.4 | Novel       |
| Oat-barley porridge | 43.8    | 30.1    | 13.6   | 68.9  | 77.5 | Novel       |

\* Reference food (HI = 100). RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch; HI = hydrolysis index; pGI = predicted glycemic index. Values are means of triplicate determinations. Selected products shown; full dataset includes 18 items.

**Table 2:** Compositional Characteristics and Their Correlations with Digestibility Parameters

| Compositional Factor   | vs RDS (r) | vs RS (r) | vs HI (r) | vs pGI (r) |
|------------------------|------------|-----------|-----------|------------|
| Amylose content (%)    | -0.63**    | 0.71**    | -0.66**   | -0.69**    |
| Dietary fiber (g/100g) | -0.54*     | 0.62**    | -0.55*    | -0.58**    |
| Total starch (%)       | 0.31       | -0.22     | 0.24      | 0.28       |
| Protein (g/100g)       | -0.39*     | 0.44*     | -0.41*    | -0.43*     |

\* $p < 0.05$ , \*\* $p < 0.01$ . Pearson correlation coefficients. n = 18 food products.

Table 1 presents the starch fraction distribution and pGI values. Refined cereals clustered at the high end, with pGI values between 89.8 and 94.6, while legumes and green banana flour occupied the low end (55.4-66.6). Cooling

boiled potatoes increased RS from 8.7% to 24.6% and dropped the pGI from 82.7 to 68.9, a clear retrograde starch effect.

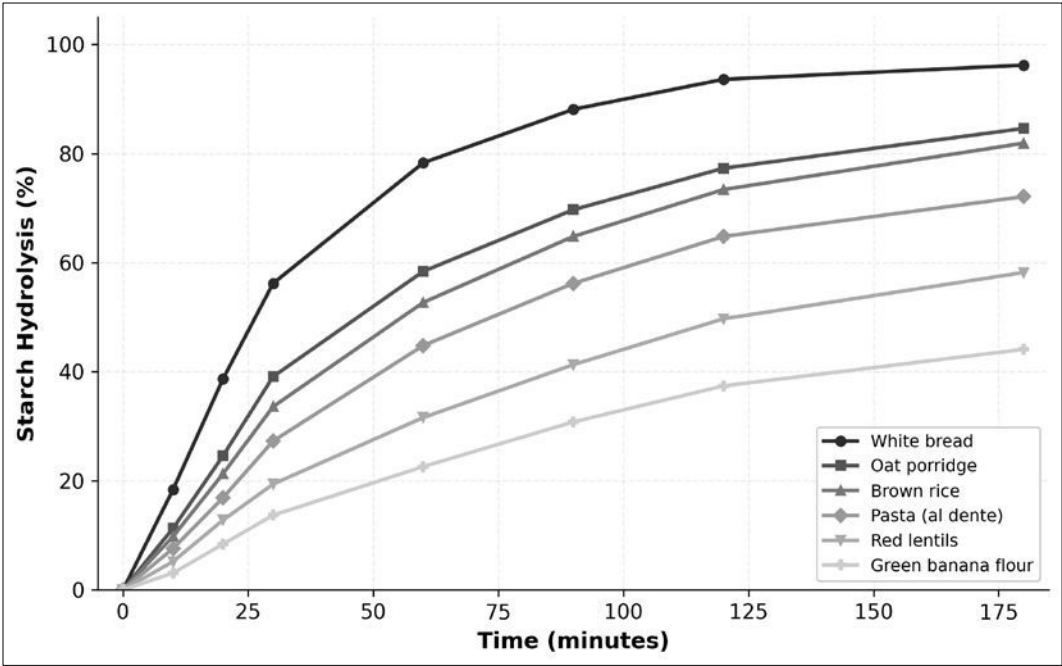


**Fig 1:** *In vitro* starch hydrolysis curves (% starch hydrolyzed over 180 minutes) for six representative food products. White bread served as the reference food.

Figure 1 shows the digestion kinetics for six representative products. White bread reached near-complete hydrolysis by 120 minutes, while green banana flour barely exceeded 44% at 180 minutes. The separation between curves was most pronounced during the first 60 minutes, reflecting differences in RDS content.

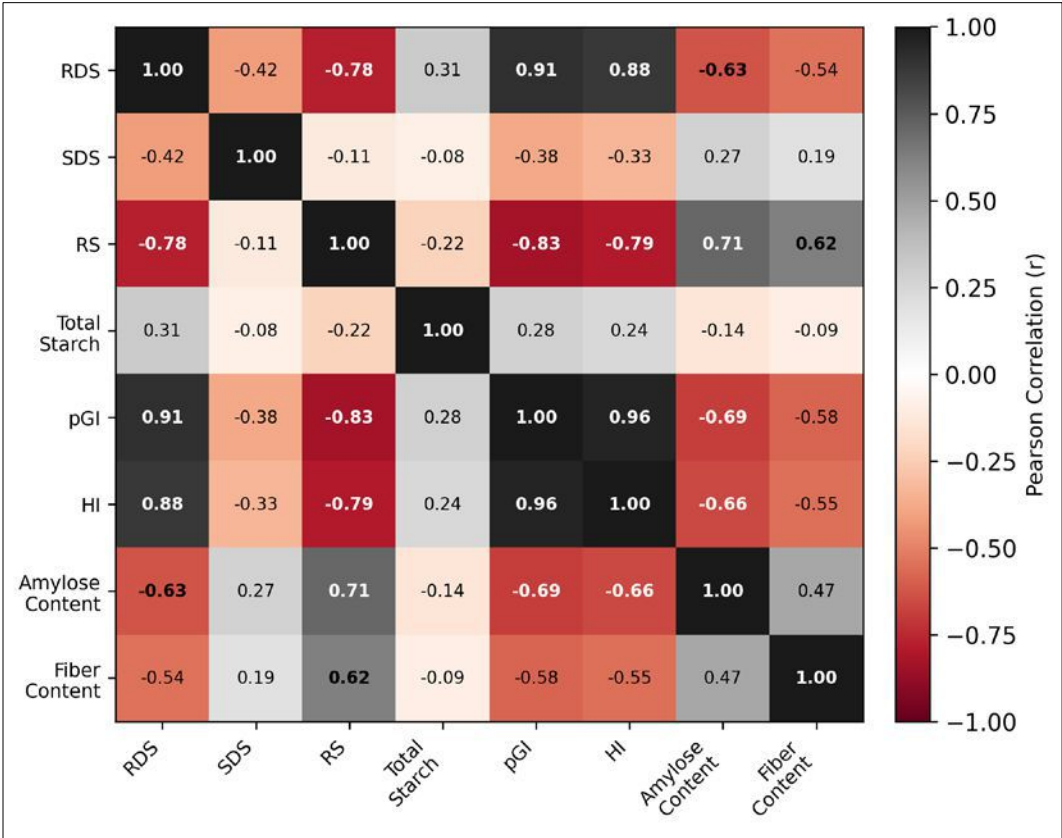


**Fig 2:** Pearson correlation heatmap showing relationships between starch fractions (RDS, SDS, RS), digestibility parameters (pGI, HI), and compositional factors (amylose, fiber content) across 18 food products.

The correlation heatmap (Figure 2) reveals strong positive associations between RDS, HI, and pGI, and equally strong negative correlations between RS and these same glycemic

parameters. Amylose content showed the strongest compositional link to RS ( $r = 0.71$ ), while SDS was only weakly correlated with most other variables.

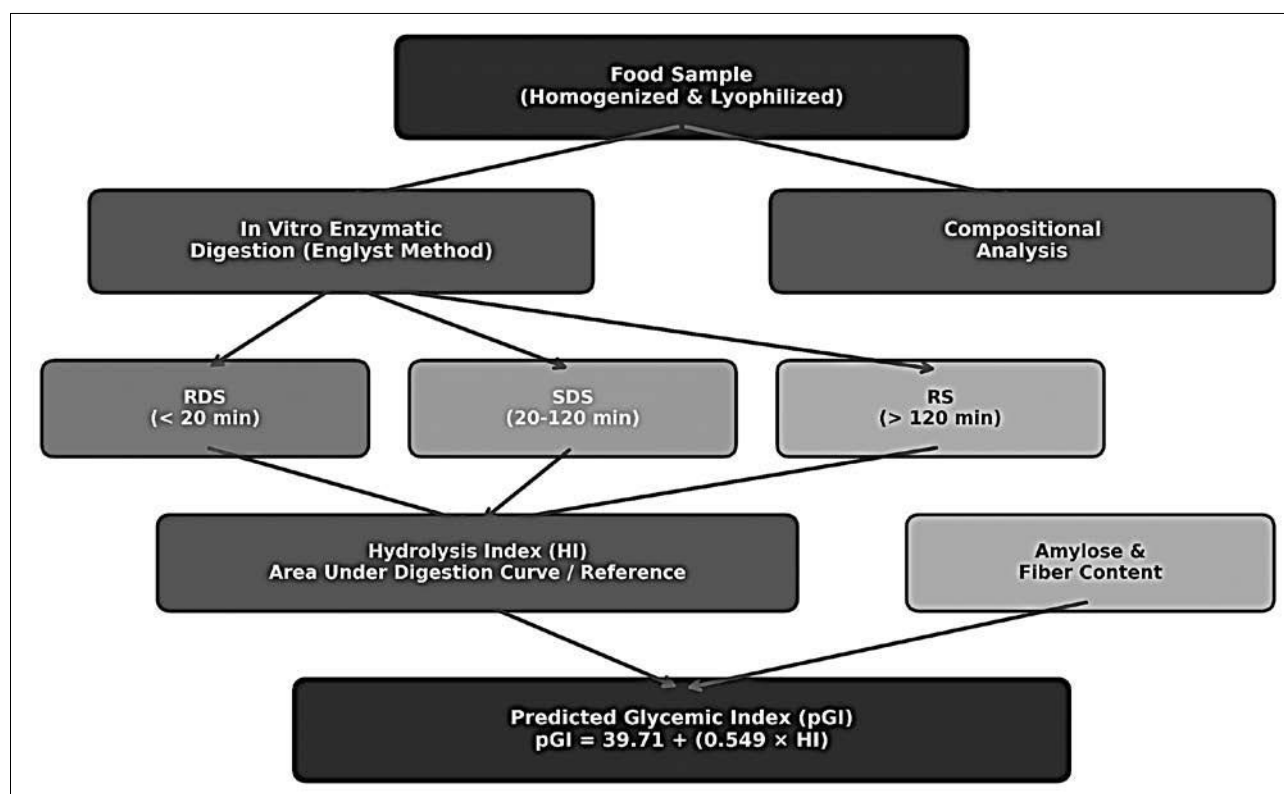


**Fig 3:** Schematic model of the analytical workflow from food sample preparation through starch fraction classification (RDS, SDS, RS), hydrolysis index calculation, and predicted glycemic index derivation.

### Comprehensive Interpretation

The data collectively indicate that RS content is the single strongest predictor of a food's expected glycemic impact within this sample set. Foods with RS above 20% consistently produced pGI values below 70, placing them in the low-to-moderate glycemic range. The retrograde starch effect was quantifiable: cooling converted roughly 16 percentage points of digestible starch into RS in potatoes. Amylose content and dietary fiber both contributed to RS levels but operated through different mechanisms—amylose through its tendency to form resistant crystalline structures upon cooling, and fiber through physical entrapment of starch granules within the food matrix.

### Discussion

These results line up with the broader literature showing that *in vitro* starch digestibility patterns track closely with *in vivo* glycemic responses. The strong inverse correlation between RS and pGI ( $r = -0.83$ ) mirrors findings from Goni and colleagues [7], who reported similar relationships in a smaller set of food items. Our expanded dataset, covering 18 products across six categories, strengthens the generalizability of this association.

The retrograded starch effect observed in cooled potatoes and cooled rice samples deserves particular comment. RS type 3, formed when gelatinized amylose chains re-associate into double helices during cooling, is known to resist enzymatic attack more effectively than native starch granules [11]. The practical implication is straightforward: simply allowing cooked starchy foods to cool before eating can meaningfully lower their glycemic impact. This has

been confirmed in human trials, though the magnitude of the effect varies by food matrix [12].

Green banana flour stood out as having the highest RS content (41.3%) and lowest pGI (55.4) among all tested products. The RS in unripe banana is mainly type 2, found within intact native granules that resist digestion due to their crystalline B-type structure [6]. As a novel ingredient in Scandinavian markets, green banana flour could offer a practical way to lower the glycemic load of baked goods when substituted for part of the wheat flour.

One limitation of the pGI prediction equation is that it doesn't account for non-starch factors that influence *in vivo* glycemic responses, such as fat content, organic acids, and meal composition [13]. SDS showed only weak correlations with pGI in our data, which may reflect the fact that SDS contributes to a sustained but moderate glucose release that is difficult to capture in a single index value. Future work combining *in vitro* digestibility with *in vivo* validation across a wider range of Scandinavian staple foods would be valuable [14].

### Limitations

This research has several boundaries worth noting. The *in vitro* model, while well-established, simplifies the complex physiology of human digestion by omitting oral and gastric phases. The pGI equation, though validated, may not perform equally well across all food types, especially mixed meals. Sample preparation involved freeze-drying and grinding, which alters the physical structure of food and may affect starch accessibility. And the single-center design with products from the Danish market limits direct

extrapolation to food products in other regions. Triplicate analysis reduces random error, but the relatively small number of food products (18) limits the statistical power for detecting weaker correlations.

### Practical Applications

These findings have direct relevance for dietary guidance and food product development. Dietitians advising patients on glycemic control can use RS content as a practical indicator of a food's likely glycemic impact. Food manufacturers could explore incorporating green banana flour, cooled retrograded starch, or high-amylose grain varieties to lower the glycemic profile of processed products. The *in vitro* screening approach described here offers a cost-effective alternative to full human GI testing during early-stage product development.

### Conclusion

Resistant starch content emerged as the strongest single predictor of predicted glycemic index across 18 food products available in the Danish market. Foods with RS levels above 20% consistently fell below a pGI of 70, while refined cereals with RS below 4% exceeded pGI values of 89. The retrograded starch effect was clearly quantifiable, with cooling reducing pGI by 13–15 points in potato and rice samples. Amylose content and dietary fiber both contributed to RS levels through distinct structural mechanisms. The Englyst-based *in vitro* digestion method, paired with the Goni pGI equation, provided a workable framework for screening food products by their expected glycemic characteristics. Green banana flour showed particularly promising results as a low-GI ingredient for the Scandinavian market. *In vitro* digestibility profiling appears well suited for rapid screening in food development pipelines where full human GI trials aren't practical.

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