Changes in starch physical characteristics following digestion of foods in the human small intestine

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Factors controlling the concentration of resistant starch (RS) in foods are of considerable interest on account of the potential for this type of fibre to deliver health benefits to consumers. The present study was aimed at establishing changes in starch granule morphology as a result of human small-intestinal digestion. Volunteers with ileostomy consumed six selected foods: breakfast cereal (muesli), white bread, oven-baked French fries, canned mixed beans and a custard containing either a low-amylose maize starch (LAMS) or a high-amylose maize starch (HAMS). Analysis showed that digesta total RS (as a fraction of ingested starch) was: muesli, 8·9 %; bread, 4·8 %; fries, 4·2 %; bean mix, 35·9 %; LAMS custard, 4·0 %; HAMS custard, 29·1 %. Chromatographic analysis showed that undigested food contained three major starch fractions. These had average molecular weights (MW) of 43 500 kDa, 420 kDa and 8·5 kDa and were rich in amylopectin, higher-MW amylose and low-MW amylose, respectively. The low-MW amylose fraction became enriched preferentially in the stomal effluent while the medium-MW starch fraction showed the greatest loss. Fourier transform IR spectroscopy showed that absorbance at 1022 per cm decreased after digestion while the absorbance band at 1047 per cm became greater. Such changes have been suggested to indicate shifts from less ordered to more ordered granule structures. Further analysis of amylose composition by scanning iodine spectra indicated that the MW of amylose in ileal digesta was lower than that of undigested amylose. It appears that high-MW amylose is preferentially digested and that MW, rather than amylose content alone, is associated with resistance of starch to digestion in the upper gut of humans.

Resistant starch: Structure: Morphology: Ileal digesta: Human subjects

Controlling the rate and extent of small-intestinal digestion of starch has important implications for human health. Slowing the rate of amylolysis lowers the glycaemic response to foods and is of established benefit in improving blood glucose control(1). However, the extent of human small-intestinal starch digestion is emerging as an important potential contributor to lower risk of diseases such as colorectal cancer. Resistant starch (RS) is that fraction of starch (and the products of starch digestion) that escapes from the human small intestine into the large bowel of healthy individuals(2). RS contributes to dietary fibre by virtue of its small-intestinal indigestibility and exerts its beneficial actions largely through the SCFA, especially butyrate, produced during its fermentation by the large-bowel microflora(3).

The RS content of a food is subject to a number of influences, including processing, the physical accessibility of the starch granule architecture and the degree to which any gelatinised starch has been allowed to retrograde(4). The molecular and structural properties of starches are also important primary determinants of their resistance to amylolysis. For example, high-amylose starches are generally slower to gelatinise on cooking and quicker to retrograde (and thus exhibit higher resistance to amylolysis) on subsequent cooling than conventional starches. Up to the present, attention has mostly been focused on the quantity of RS in a food, possibly because of an assumption that net SCFA production and the profile of the acids produced were the same for RS from different sources. However, there is some evidence that this is not the case and starches differ markedly in their capacity to produce different amounts and proportions of individual SCFA(5,6). While RS in general favours butyrate production(7) it has been reported that the microflora of some individuals cannot metabolise some types of RS(8), which raises the issue of identifying the factors that cause the resistance of starch to bacterial degradation.

Quantitative data on the relationship between dietary starches and structural characteristics of RS, specifically at the molecular level, are still very limited. In part this reflects the difficulty of accessing the human intestine to obtain starch post-digestion. The objective of the present study was to obtain information on the differences in starch morphology and molecular characteristics in a number of...
Table 1. Test foods used in feeding trials and ileal starch digestibility (Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Test food</th>
<th>Serving size (g, as-is)</th>
<th>Moisture content (%)</th>
<th>Total food consumed (g, dry)</th>
<th>Total starch ingested (g, dry)*</th>
<th>Starch collected in digesta (g, dry)†</th>
<th>Starch digestibility (g/100 g starch)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muesli§</td>
<td>90</td>
<td>7.2</td>
<td>83.5</td>
<td>41.4</td>
<td>0.1</td>
<td>3.7</td>
</tr>
<tr>
<td>White bread†</td>
<td>141</td>
<td>32.8</td>
<td>94.8</td>
<td>72.8</td>
<td>0.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Potato fries (oven-baked)</td>
<td>250</td>
<td>52.0</td>
<td>120.0</td>
<td>73.8</td>
<td>1.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Mixed beans (canned)¶</td>
<td>100</td>
<td>69.9</td>
<td>30.1</td>
<td>13.2</td>
<td>0.5</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Custard containing conventional maize starch (LAMS)</td>
<td>132</td>
<td>64.7</td>
<td>46.6</td>
<td>18.6</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Custard containing HAMS</td>
<td>132</td>
<td>65.7</td>
<td>45.3</td>
<td>19.7</td>
<td>0.1</td>
<td>5.7</td>
</tr>
</tbody>
</table>

LAMS, low-amylose maize starch; HAMS, high-amylose maize starch.
* Values of triplicate determinations for each food.
† Eight observations.
‡ Calculated as the difference in the amount of starch consumed and subsequently recovered in ileal digesta divided by the amount of the starch consumed.
§ Commercial breakfast cereal containing multiple cereal grains and other sources of starch.
¶ Canned product containing four different varieties of beans. Beans were drained before consumption.

Test foods, study design and protocol

Six different food products (four commercially processed foods: muesli, white bread, oven-baked potato fries and canned bean mix; two custards containing either conventional maize starch or high-amylose maize starch; HAMS) were chosen in the present study, as they are among the most common foods in Western diets. The foods were consumed in random order in the two trials (Table 1). In trial 1, locally available commercially processed foods were consumed after preparation as instructed by the manufacturer. The amounts consumed exceeded the recommended serving sizes to ensure an adequate level of starch in ileal digesta. For trial 2, volunteers consumed milk-based chocolate custards (about 120 g) containing 20 g of one of two commercially available low-amylose maize starch (LAMS) and HAMS (3401C and Hylon VII, respectively; National Starch and Chemical Company, Seven Hills, NSW, Australia). In addition to these ingredients the custards also contained a small amount of honey for flavouring (12 g). The custards were made to industry standards in an experimental kitchen.

The study comprised a total of eight feeding periods of either two (trial 1) or three (trial 2) consecutive days’ duration in which all volunteers consumed a low (~60 and 5 g/d, respectively) starch basal diet comprising mostly staple food items (for example, various red and white meats, dairy foods, eggs, fruits, fruit juices and non-starchy vegetables) consumed as three main meals and several snacks each day. All foods for the trial were supplied by CSIRO. Using Australian food composition databases, protein, fat and carbohydrate were calculated to provide about 27, 37 and 35 % of energy, respectively. Total dietary fibre intake was estimated to be 14 g/d. For some individuals the composition of the basal diet was varied slightly to meet individual preference and appetite. However, for each individual the same diet was eaten on each day of a given feeding period. Volunteers were asked to refrain from drinking alcoholic beverages during the feeding phases of the study. The weights of any uneaten foods were recorded. Meals were eaten at specified times and test foods (see Table 1) were consumed at breakfast on either 1 d (trial 1) or 2 d (trial 2) of the feeding periods. Each volunteer thereby served as his or her own control.
Ileal digesta collection and sampling

All ileostomy effluent was collected at 2 h intervals during each feeding period. Collection was from 07.00 hours in both studies and finished at either 19.00 hours (trial 2) or 23.00 hours (trial 1) on each day. The final collection was made at 07.00 hours on the morning following the final full collection day of each feeding period. At each collection, the entire contents of each ileostomy bag were drained into sterile plastic pots, which were capped and immediately frozen to await analysis.

Ileostomy samples collected during each 24 h period were thawed, pooled and homogenised. Duplicate portions of digesta and each test food were taken immediately, lyophilised and analysed later for glucose and starch.

Analytical procedures

Starch and starch morphology. A sample (30 ml) of aqueous ethanol (80:20, ethanol–water, v/v) was added to 80 mg of freeze-dried food and digesta in separate 50 ml polypropylene centrifuge tubes. The contents of each tube (capped) were mixed by hand shaking for 5 min, and then centrifuged at 2095 g for 10 min. The supernatant fraction was aspirated to waste and the pellet was re-suspended in 25 ml acetone. The shaking and centrifuge procedures were repeated. The final pellet (in a 50 ml tube) was air dried in a fume hood and sprinkled onto circular aluminium stubs containing double sticky tape. The stubs were coated with gold in a Hitachi 1B-3 ion coater. Starch granules and fragments were visualised using a scanning electron microscope (Autoscan Systems Pty Ltd, Brighton, Vic, Australia). The image of the selected area was recorded on a black and white high-speed photographic film with the help of an attached camera assembly. Each food and digesta sample was examined in triplicate.

Fourier transform IR spectroscopy. Fourier transform IR spectroscopy (FTIR) spectra were recorded on a spectrophotometer (Excalibur 3100; Varian Australia Pty Ltd, Melbourne, Vic, Australia) equipped with a cooled deuterated triglycine sulfate (DTGS) detector. The measurement was performed on a MIRacle™ attenuated total reflectance (ATR) crystal plate with Digital Readout High Pressure Clamp (Pike Technologies, Madison, WI, USA). Freeze-dried food products and digesta were directly loaded on the plate and scanned in the range of 3600–600 cm⁻¹ at a resolution of 4 cm⁻¹. Before recording, the spectra were transformed against an empty cell as background. Finally the spectra were deconvoluted using Resolutions Pro software (Varian, Inc., Palo Alto, CA, USA). A half-bandwidth of 15 cm⁻¹ were deconvoluted using Resolutions Pro software (Varian, Inc., Palo Alto, CA, USA). A half-bandwidth of 15 cm⁻¹ were applied.

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Absorbance values at 1047 and 1022 cm⁻¹ were obtained and a K factor of 1.5 with triangular apodisation were applied.

Inc., Palo Alto, CA, USA). A half-bandwidth of 15 cm⁻¹ were deconvoluted using Resolutions Pro software (Varian, Inc., Palo Alto, CA, USA). A half-bandwidth of 15 cm⁻¹ were applied.

The starch precipitate (approximately 20 mg), prepared as described earlier, was dissolved in 0.5 ml of 0.2 M-NaOH and mixed vigorously for approximately 10 s. The solution was neutralised by the addition of sodium acetate buffer (0.5 ml; 0.05 M, pH 4.0) before adding ion-exchange resin (0.20 g, BioRad AG 50-X8; Bio-Rad, Hercules, CA, USA) and incubating at 50°C for 1.5 h with occasional shaking. After centrifuging at 10000 rpm for 10 min (Centrifuge 5415D; Eppendorf, Hamburg, Germany), the clear supernatant fractions were collected for SE-HPLC analysis. The HPLC system comprised a GBC pump (LC 1150; GBC Instruments, Dandenong, Vic, Australia) equipped with an Auto Sampler (LC1610; GBC Instruments) and Evaporative Light Scattering Detector (ELSD) (Alltech Associates, Deerfield, IL, USA). The Ultrahydrogel™ linear column, Ultrahydrogel™ 250 column and guard column (7.8 mm × 300 mm; Waters, Osaka, Japan) were used and maintained at 35°C during HPLC operation. Ammonium acetate buffer (0.05 M; pH 5.2) was used as the mobile phase at a flow rate of 0.8 ml/min. A sample (50 μl) of the supernatant fraction was used for injection. Conditions for ELSD operation were: tube temperature, 115°C; N₂ gas flow rate, 2.0 litres/min; gain, 16; impactor, on. Dextran standards with molecular weights (MW) of 4900, 670, 150, 80, 25 and 5 kDa were used for column calibrations.

Starch–iodine spectrum. The iodine-complexing spectrum of starch was used to examine the changes in the amylose compositions before and after the digestion. The clear supernatant fraction, prepared for HPLC analysis as described earlier, was also used for the study of the amylose–iodine spectrum by UV-visible spectrophotometry. A quantity of 25 μl of I₂–KI solution (1.27 g I₂ + 3 g KI per litre) was added into 0.5 ml of the starch solution. After dilution using distilled water, the absorbance was recorded at 1 nm intervals from 350 to 800 per cm using a Varian (Model Cary 1E; Varian, Inc., Palo Alto, CA, USA) scanning UV-visible spectrophotometer and spectral analyses performed in triplicate.

Statistical analyses

RS content of a given test food was calculated as the difference between ileal starch output for the test food and that of the control (i.e. low-starch) diet. RS values are expressed on either a food or total starch basis. Values are expressed as means with their standard errors of three replicates for test foods and digesta samples from eight individuals. Both trials were considered to be completely randomised designs and the experimental data were subjected to one-way ANOVA using Genstat 5 (version 4.1, 1998; VSN International, Hemel Hempstead, Bucks, UK). Differences among test foods were assessed using the least significant difference procedure. Effects were considered significant at P<0.05.
Results

The quantities of food and starch consumed by the volunteers are shown in Table 1. The apparent digestibility of the starches ranged from 64 % for canned beans to 96 % for custard containing LAMS (Table 1). The RS content (expressed as a percentage of ingested starch) was: for muesli, 8·9 %; for bread, 4·8 %; for fries, 4·2 %; for beans, 35·9 %; for LAMS custard, 4·0 %; for HAMS custard, 29·1 %, respectively. The starches were fractionated by SE-HPLC into high-MW (HMW), medium-MW (MMW) and low-MW (LMW) fractions (Fig. 1). The peak materials in these fractions were eluted at the same elution time as MW standards of approximately 43 500 kDa, 420 kDa and 8·5 kDa, respectively. HMW was assumed to be principally amylopectin, because its MW was reported to vary from $10^7$ to $10^9$ Da\(^{14,15}\), whereas amylose has a relatively lower MW compared with amylopectin, ranging from $10^5$ to $10^6$ Da. Accordingly, MMW was considered to be mostly (but not exclusively) higher-MW amylose whereas LMW was considered to comprise mostly lower-MW amylose. Using a dual-column system, a previous study on the molecular structure of starches with different amylose contents by Shi et al.\(^{16}\) also suggested that starch molecules could be separated into these fractions: a HMW amylopectin fraction, a LMW amylose fraction, and an intermediate MW fraction which contains both linear and branched starch molecules.

Representative SE-HPLC chromatograms for starch in one product (muesli) before and after passage through the small intestine of one volunteer are presented in Fig. 1. A LMW fraction of approximately 4·1 kDa was found to predominate in starch that had escaped digestion. In a similar study using human ileostomates who had consumed a chick pea starch gel, chromatographic analysis of ileal effluent demonstrated that a fraction of lower MW with weight-average degree of polymerisation of 25 was found to be the major peak\(^{17}\).

Canned beans and HAMS custard contained starches of lesser digestibility consisting mostly of LMW and MMW fractions and, consequently, relatively little HMW starch (accounting for just 9 % and 16 % of starch, respectively). Conversely, the HMW fraction predominated in highly digestible starches. For instance, 65 % and 58 % of starch in LAMS custard and muesli, respectively, was the HMW fraction (see Table 2).

The MMW fraction accounted for between just 12 % and 20 % of the starch present in ileal effluent for the different food products that were consumed (Table 2) whereas the LMW fraction predominated at 45–68 % of total starch. Muesli and LAMS custard contained proportionately less of the LMW and more of the HMW fractions than the RS in beans and HAMS custard.

The HMW starch fraction in muesli, bread, LAMS custard and fries was digested extensively (between 93 % and 98 %) whereas that in beans (only 35 % of HMW starch digested) and to a lesser extent HAMS custard (67 % digested) was not. The MMW starch fraction appeared to be highly susceptible to amylolysis \textit{in vivo} regardless of the food product. For all test foods, < 10 % of this fraction was present in ileal digesta relative to that in the food consumed. Although a recent study of starch digestion \textit{in vitro} has shown that molecular reorganisation was likely to take place during the enzyme-digestion process\(^{13}\) (i.e. formation of recrystallised amyloseic fragments during digestion), molecular reorganisation does not seem to happen during \textit{in vivo} digestion (ZK Zhou, AR Bird, DL Topping and MK Morell, unpublished results). Thus, much more of the LMW starch fraction was assumed to be retained during the passage of starch through the upper gut. For instance, in the case of beans, more than 76 % of the LMW fraction appeared to be resistant to amylolysis; however, it is possible that some of the MMW and LMW fractions were derived from incomplete digestion of higher-MW starches (see Discussion).

From a quantitative perspective, the LMW fraction accounts for the greatest yield of RS for all six foods. For instance, consumption of 100 g mixed beans would result in 3·3 g LMW starch reaching the terminal ileum compared with just 0·7 and 0·8 g MMW and HMW fractions, respectively. Muesli was unusual in that this food yielded a comparatively substantial amount of HMW RS (1·6 g/100 g food).

Scanning electron micrographs of starch granules in the test foods and stomal effluent of volunteers after test food ingestion are shown in Fig. 2. Granules in all foods had an irregular shape, with the size distribution ranging from 8 to 40 μm, and surface cracks clearly evident, whereas the morphology of starch in ileal digesta (Fig. 2) was markedly different. The granules were generally smaller, the size distribution being between 2 and 7 μm according to the objective observations, and the surface relatively smooth compared with granules present in the foods.

FTIR spectral absorbances at 1047 and 1022 cm$^{-1}$ were measured for starches in each food and in corresponding...
Table 2. Molecular profile of starch in food and ileal digesta
(Mean values with their standard errors*)

<table>
<thead>
<tr>
<th>Test food</th>
<th>Food</th>
<th>Ileal digesta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Muesli</td>
<td>58·4</td>
<td>0·4</td>
</tr>
<tr>
<td>Bread</td>
<td>33·9</td>
<td>1·7</td>
</tr>
<tr>
<td>Fries</td>
<td>19·9</td>
<td>0·9</td>
</tr>
<tr>
<td>Beans</td>
<td>9·8</td>
<td>0·2</td>
</tr>
<tr>
<td>Custard, LAMS</td>
<td>65·3</td>
<td>1·6</td>
</tr>
<tr>
<td>Custard, HAMS</td>
<td>16·0</td>
<td>0·2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test food</th>
<th>Food</th>
<th>Ileal digesta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMW</td>
<td>MMW</td>
</tr>
<tr>
<td>Muesli</td>
<td>26·86</td>
<td>0·17</td>
</tr>
<tr>
<td>Bread</td>
<td>17·49</td>
<td>0·87</td>
</tr>
<tr>
<td>Fries</td>
<td>5·87</td>
<td>0·25</td>
</tr>
<tr>
<td>Beans</td>
<td>1·30</td>
<td>0·87</td>
</tr>
<tr>
<td>Custard, LAMS</td>
<td>0·79</td>
<td>0·05</td>
</tr>
</tbody>
</table>

LMW, low molecular weight; MMW, medium molecular weight; HMW, high molecular weight; LAMS, low-amylose maize starch; HAMS, high-amylose maize starch.
* Values of duplicate determinations for food and ileal digesta samples from eight ileostomates (each ileal sample was analysed in duplicate).
† Proportion of each starch molecular fraction in food or digesta was calculated as the peak area relative to total peak area (aggregate of HMW, LMW and MMW).
‡ Starch molecular profile presented as the quantity of each fraction contained in either 100 g of food or ileal digesta.
§ Ileal digestibility of starch molecular fractions expressed as a proportion of starch consumed.
digesta samples (for representative spectra, see Fig. 3). Absorptions at these wavelengths are thought to be suggestive of whether starch structures are more or less ordered, respectively, and the ratio 1047:1022 per cm provides an indication of the relative structural organisation of the starches in these samples (Table 3). It is evident for bread (Fig. 3(A)) that the intensity of absorbance at 1022 per cm is much less in the digesta sample than in the food, whereas at 1047 per cm the converse was true. Consequently, the 1047:1022 per cm ratio was larger for starch resistant to digestion than for that in the food. A similar pattern was observed for the other foods and digesta samples (Table 3), indicating that the more ordered starch was less susceptible to amylolysis.

Iodine spectra for one food (bread) and a corresponding digesta sample are shown in Fig. 3(B). The wavelength for maximum absorption ($\lambda_{\text{max}}$) was greater for the starch in food compared with that remaining in ileal digesta and this pattern was common to the other products that were tested (Table 3).

**Discussion**

The aim of the present study was to determine the impact of human small-intestinal digestion on the physical attributes of starch in convenience foods. To our knowledge this is the first study of its kind. Previous studies in intact human subjects, individuals with ileostomy, intubated human subjects or surgically modified animals have focused principally on the absolute amounts of RS in foods. Of the few studies that have investigated the structural properties of starch, the foods used have been either experimental or not commonly eaten or the study has been of limited scope. The data we have gathered show that there were not only the expected substantial losses of starch in the small intestine but also changes in particle size distribution and internal order of the starches that escaped from the terminal ileum. The foods that we tested were chosen to provide a representative sample of convenience products consumed in Australia. The custards are not commercial products but...
Of the convenience foods examined, ileal starch digestibility was greatest for the cereal foods and chips and least for the beans. This accords with the view that legume starches are much less digestible than those in cereals\(^{30-32}\). This is borne out also by animal and human studies that show relatively high large-bowel SCFA production following legume consumption\(^{33,34}\). The high digestibility of the LAMS in custard and the much lower digestibility of HAMS were expected from animal and human studies\(^{20,21,26,29,35}\). This is consistent with the view that starch amylose content seems to play an important role in determining the RS content of foods\(^{4,36}\).

Before digestion, the starch granules had an irregular appearance with cracked and rough surfaces. In contrast, starches recovered from the ileal effluent were smaller and smoother. Previous human, animal and \textit{in vitro} studies have shown similar changes that reflect the progressive erosion of granules by amylase\(^{37}\). As expected, there was a marked reduction in granule size from 8–40 \(\mu\)m before digestion to about 7 \(\mu\)m in the digesta. The relative contributions of the three major starch fractions were separated on the basis of MW using SE-HPLC. The largest of these was composed principally of amylopectin, a highly branched polymer. The medium fraction was mostly (but not exclusively) higher-MW amylose while the smallest fraction was lower-MW amylose. Amylose is a smaller polymer than amylopectin with a less branched structure and amylose molecules have a high tendency to aggregate and crystallise during retrogradation compared with other starch fractions\(^{38}\). Consequently, this amylose-dominated structure would contribute to the formation of regions containing aggregates of aligned amylose chains following starch retrogradation\(^{39}\) within which there is a relatively high resistance to amylolysis. Although the individual test foods showed different starch MW distributions, all showed a consistent trend on digestion, i.e. a disproportionate loss of the MMW fractions and a relative enrichment of the LMW fractions in digesta. Between 91 and 99 % of the MMW fractions of starch were lost on digestion, while there was retention of the LMW fraction, indicating that it is either resistant, or replenished from the products of breakdown of the MMW fraction during digestion. While the MMW fraction could be considered as amylose, it is clearly of a higher digestibility than the LMW fraction. The results from HPLC analysis support the previous suggestion\(^{17,40}\) that molecular

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Changes in the spectra before and after digestion, represented by bread (——) and its corresponding digesta (······). (A) Fourier transform IR spectrum ranging from 1200 to 850 per cm; (B) starch–iodine spectrum scanning from 800 to 350 nm. Lines designated a and b represent the slopes of the left \textquoteleft shoulder\textquoteright region of the spectrum relating to complexation of iodine and shorter chains of amylose molecules; slopes c and d relate to complexation between iodine and longer chains of amylose molecules; e represents the slope of the right \textquoteleft shoulder\textquoteright region indicative of complexation between iodine and the longest amylose chains.}
\end{figure}

<table>
<thead>
<tr>
<th>Table 3. Structural characteristics of starch in the six test foods and corresponding ileal digesta samples (Mean values with their standard errors)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FTIR ratio 1047:1022 per cm</strong></td>
</tr>
<tr>
<td><strong>Food</strong></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Muesli</td>
</tr>
<tr>
<td>Bread</td>
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<tr>
<td>Fries</td>
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<tr>
<td>Beans</td>
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<tr>
<td>Custard, LAMS</td>
</tr>
<tr>
<td>Custard, HAMS</td>
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</tbody>
</table>

\textsuperscript{FTIR}, Fourier transform IR spectroscopy; LAMS, low-amylose maize starch; HAMS, high-amylose maize starch.

\textsuperscript{*} Values of triplicate determinations for each food.

\textsuperscript{†} Values of eight ileostomates for each test food (each sample measured in triplicate).
size, rather than amylose content alone, is an important determinant of starch digestibility and, hence, RS. The small contribution of the HMW fraction to RS might be due to the reassociation of shorter double-helices of amylopectin branches formed during the retrogradation process\(^{(41,42)}\) or starch which is physically inaccessible to digestive enzymes as a consequence of the presence of plant cell walls (i.e. RS1)\(^{(43)}\) or resistant granular architecture.

Both the FTIR data and the iodine spectra support the above suggestions. Absorptions at 1047 and 1022 per cm are indicative of more and less organised starch structures, respectively\(^{(11–13)}\). Using the absorbance ratio 1047:1022 per cm, the FTIR results are consistent with a previous study\(^{(13)}\) showing that starch in the ileal effluent was more ordered than in the food. This differential spectrum indicates that less organised starch was much more sensitive to digestion, contributing less to RS than the more ordered fraction. However, the limitations of using FTIR spectroscopy to quantify starch molecular order must be acknowledged\(^{(10)}\). Unlike NMR and X-ray diffraction, FTIR data provide an indirect measure of molecular order in starches. Nevertheless, the iodine spectra for the food and its corresponding digesta sample are consistent with the FTIR data. The lower absorption maximum wavelength (\(\lambda_{\text{max}}\)) of starch in digesta compared with that of the starch in the food indicates that the MW of the major amylose component of starch present in ileal digesta was smaller than that of amylose in the starch of the food. The narrower peak width of amylose in the HPLC chromatogram also indicates that digestion altered the amylose compositions before and after digestion.

To our knowledge, this is the first study to examine the effects of passage through the human small intestine on the structure of starch in commonly consumed foods. Previous reports, using microscopic visualisation, showed that digestion had altered the physical appearance of the granules. The present data show that there were also substantial changes in size distribution and an enrichment of RS in smaller, more ordered granules, higher in amylose. New cereal cultivars, high in amylose, are being developed to produce foods that improve human health\(^{(44,45)}\).

**Acknowledgements**

We thank Dr Julie Clarke for providing some of the digesta samples and for helpful discussion, Debbie Davies, Caroline Cooke, Julie Dallimore and Ben Scherer for their assistance with the conduct of the ileostomy studies and with food preparation, sample collection and pre-processing of ileal digesta, and Sylvia Usher for analysis of starch in food and digesta samples. The authors are indebted to the ileostomates who participated in the studies referred to in this paper.

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All authors contributed to designing the experiment, interpreting the data and preparing, revising and approving the final version of the manuscript. Z. Z. also processed and analysed the samples, and collated and summarised the data.

None of the authors has any conflict of interest.

**References**